

# Program and Abstract

## 11<sup>th</sup> Asia-Pacific Microscopy Conference

33<sup>rd</sup> Microscopy Society / 39<sup>th</sup> Anatomy Association - of Thailand Annual Conference



APMC11  
MST33/AAT39



Phuket, Thailand May 23-27, 2016



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## 11<sup>th</sup> Asia-Pacific Microscopy Conference

33<sup>rd</sup> Microscopy Society / 39<sup>th</sup> Anatomy Association - of Thailand Annual Conference



# APMC11

## MST33/AAT39



Phuket, Thailand May 23-27, 2016

Organized by:

Department of Anatomy, Faculty of Science, Mahidol University

Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University

Microscopy Society of Thailand (MST)

Anatomy Association of Thailand (AAT)

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**May 23 – 27, 2016, Phuket, Thailand**  
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## Message from the Vice President of Mahidol University



**Banchong Mahaisavariya, M.D.**  
Professor and Vice President

On behalf of the Mahidol University, I warmly welcome you to 11<sup>th</sup> Asia-Pacific Microscopy Conference, the 33<sup>rd</sup> Annual Conference of the Microscopy Society of Thailand and the 39<sup>th</sup> Annual Conference of the Anatomy Association of Thailand.

Mahidol University, first established as the medical school at Siriraj Hospital in 1888, is a multi-disciplinary and research-led institution with key competencies in the arts, medicine and science. Given this expertise, Mahidol University is a leading center of knowledge and understanding, rooted in its determination statement of being the "Wisdom of the Land". Understanding that the role of the university is not limited to creating new knowledge, Mahidol University aims to produce global professionals who are well equipped with "depth of knowledge" in their professional and academic fields and "breath of knowledge" in multiple life skills, including analytical and critical thinking, intercultural communication adaptability, information technology, entrepreneurship, and social contribution.

Microscopy is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye. Advances in microscopy over recent years are revealing some breathtaking new views. It is obvious that the rapid progress of new techniques in microscopy has led to outstanding contributions in a variety of scientific fields, especially Materials Science, Instrumentation, Life Sciences and Anatomical Sciences. Mahidol University has also encouraged some of its academic staff and students to be involved in these areas, both teaching and research.

I would like here to congratulate the academic staff from Departments of Anatomy, Faculty of Science, and Faculty of Medicine Siriraj Hospital, Mahidol University, together with the Microscopy Society of Thailand and the Anatomy Association of Thailand who have organized this conference successful. This conference will provide our next generation information to generate ideas or even lit a new light for education and research in microscopy.

I would like to take this opportunity to express my gratitude to all delegates and sponsors for their full support, cooperation and contribution to this conference.

In closing, I realize that you are fully dedicated to the sessions that will follow but I do hope you will also take time to enjoy fascinating Phuket, Thailand, with its tropical setting, friendly people and multi-cultural cuisine. I wish the participants a very fruitful and productive meeting and with that, I declare the 11<sup>th</sup> Asia-Pacific Microscopy Conference, the 33<sup>rd</sup> Annual Conference of the Microscopy Society of Thailand and the 39<sup>th</sup> Annual Conference of the Anatomy Association of Thailand open.

Thank you.

## Message from Dean, Faculty of Medicine Siriraj Hospital, Mahidol University



**Prasit Watanapa, M.D., Ph.D.**  
Professor and Dean

It's my great pleasure for Faculty of Medicine Siriraj Hospital to co-organize the 11th Asia-Pacific Microscopy Conference (APMC11) during 23-27 May 2016, at Graceland Resort & Spa, Phuket, Thailand.

The Faculty of Medicine Siriraj Hospital at Mahidol University, established in 1888 by H.M. King Chulalongkorn, is the first medical school in Thailand. Enriched with "SIRIRAJ" culture and traditions for more than a century. The Faculty has produced medical graduates and allied health personnel with distinctive character, vision and ability to serve the health care system around the country by pursuing the H.R.H. Prince Mahidol of Songkla's philosophy, "True success is not in learning but in its applications to the benefit of mankind".

The Faculty of Medicine Siriraj Hospital strives to advance medical education, patient care and research. The APMC11 meeting will provide the recent developments in microscopy, the sharing advances, scientific information and modern technology that give the progress in medical research.

Finally, I would like to extend our utmost gratitude to the participants, invited speakers, and organizing committee for making the conference a success. Special thanks also go to the companies for their generous contribution and support.

I wish everyone a fruitful and memorable congress.



## Message from Dean, Faculty of Science, Mahidol University



**Sittiwat Lertsiri, Ph.D.**  
Associate Professor and Dean

It is my great privilege, as the Dean of Faculty of Science, Mahidol University, to extend my appreciation to the distinguished keynote speakers, invited speakers, oral and poster presenters and all researchers who have gathered here for the 11<sup>th</sup> Asia-Pacific Microscopy Conference (APMC11), the 33<sup>rd</sup> Annual Conference of the Microscopy Society of Thailand (MST33) and the 39<sup>th</sup> Annual Conference of the Anatomy Association of Thailand (AAT39) to be held in Phuket from May 23 to 27, 2016.

On behalf of the Faculty of Science, Mahidol University, it is our great honor to be a host organizing the APMC11/MST33/AAT39 Conference with the support from the Council of Asia-Pacific Societies for Microscopy (CAPSM), the International Federation of Societies for Microscopy (IFSM),

As Mahidol University is the lead research university, Faculty of Science has placed strong emphasis on research for commitment to the national development and international scientific advancement for more than half a century. This institution is a community of scientists and teachers whose dedicative works have proven to be of great importance in improving the quality of life of our people. We are proud of these accomplishments, but in ever-changing world, the knowledge must be continuously updated and more research questions must be solved. Microscopy has come a long way in the last few years, but there are still many challenges it has yet to face. With the help of all participants gathered here, I believe that we are ready to come across new challenges in this evolving world.

I would like to thank all participants and look forward to seeing successful collaboration among us all in the near future.

## Message from the Governor of Phuket



**Mr. Chamroen Tipayapongtada**  
Phuket Governor

I am honored to have been invited to say a few words of welcome to you this morning at the opening of the 11<sup>th</sup> Asia-Pacific Microscopy Conference (APMC11), the 33<sup>rd</sup> Annual Conference of the Microscopy Society of Thailand (MST33) and the 39<sup>th</sup> Annual Conference of the Anatomy Association of Thailand (AAT39), I am especially pleased that Phuket has been chosen as the venue for this event, and on behalf of the city and people of Phuket I would like to extend a very warm welcome to each and every one of you, and I do hope your stay here with us will be an enjoyable and fruitful one

Microscopy is a part of science that may deliver us answers, or solutions, or cures, to all our problems. We all must also learn to think differently to make best use of new technologies, if we are to make real and sustainable differences to the lives of our citizens. And that's why once again I want to congratulate you all for bringing this event here to Phuket. I am sure that with your help, we can foster a culture of creativity and innovation which will allow us to deliver on our promises to our citizens. I wish you every success in your deliberations this week, and an enjoyable stay in Phuket.

Thank you.



## Message from APMC11/MST33/AAT39 Organizing Committee Chair



**Sukumal Chongthammakun, Ph.D.**  
Associate Professor

It is my great pleasure, on behalf of the entire Organizing Committee of the 11<sup>th</sup> Asia-Pacific Microscopy Conference (APMC11), the 33<sup>rd</sup> Annual Conference of the Microscopy Society of Thailand (MST33) and the 39<sup>th</sup> Annual Conference of the Anatomy Association of Thailand (AAT39), to welcome all of you from abroad and within Thailand, to Phuket to participate the APMC11/MST33/AAT39 Conference. In this 2016, it is the second time that Thailand takes turn to be the host to organize the APMC. The first one, the 4<sup>th</sup> Asia-Pacific Electron Microscopy Conference (APEM-4) was held in 1988, in Bangkok, Thailand.

Our Scientific program features a broad range of keynote, symposia, papers and posters that will engender an exciting exploration of important issues in the advances in the biological and materials sciences, anatomical sciences, techniques and instrumentation. Complementing the program is the exhibition of microscopy and nanotechnology related instrumentation.

We have more than 400 delegates joining us from Thailand and around the world. We are so grateful to all of you for joining us here. Over these five days of academic events, you will have valuable opportunities to attend many excellent sessions presented by experts from many countries. We also have oral and poster presentation sessions for investigators and students to present their researches and share experiences among participants. Prizes for best oral and poster presentation will be awarded. As part of the Conference, we invited the participants to select their best micrographs to enter a competition for the best micrograph. Selected micrograph will be showcased during the meeting.

The successful organization of this Conference has required the talents, dedication and time of many individuals. We have been most fortunate to collaborate with a dedicated conference organizing committee. In particular, I would like to thank the members from Departments of Anatomy, Faculty of Science and Faculty of Medicine Siriraj Hospital, Mahidol University, and also those from the Microscopy Society of Thailand and the Anatomy Association of Thailand.

Special gratitude and appreciation is due to our invited speakers for their invaluable contribution to the keynote lectures and symposia in their areas of expertise. I also wish to express my gratitude to the various sponsors for their kind support. Lastly, I would also like to thank all the delegates and presenters, Your attendance helps ensuring the Conference an outstanding success.

Welcome again to APMC11/MST33/AAT39. I do hope that you will seize this opportunity to join us at this exciting meeting and enjoy the wonders of Phuket, Pearl of the Andaman Sea.

Thank you.

## Message from CAPSM President



**Ze Zhang, Ph.D.**  
Professor

The 11th Asia-Pacific Microscopy Conference (APMC11) with the strong collaboration between Microscopy Society of Thailand, Anatomy Association of Thailand, Mahidol University and Committee of Asia Pacific Societies for Microscopy (CAPSM) will be held at Phuket Graceland Resort & Spa in the exotic island of Phuket from May 23 to 27, 2016.

The APMC is an international conference that is held every 4 years under the auspices of the CAPSM providing cutting-edge knowledge and communications in microscopy to the Asia- Pacific region, extending its scope to include TEM, SEM, confocal, X-ray, near-field microscopy, SPM, AFM and application of microscopy in life & materials science. The Organising committee have invited many international and regional renowned experts in their field of microscopy during this much anticipated conference.

On behalf of CAPSM and APMC11 Organising committee, I would like to take this opportunity to invite our microscopy colleagues from the Asia Pacific region and beyond to come together in extending collaboration and research in microscopy. APMC11 will be a platform for microscopists to deliberate and exchange ideas and research during this FIVE days conference. It is my wish and hope that some fruitful projects, research collaboration and strategies for the future of microscopy be created and developed. As Phuket Island is world renowned as the “Pearl of the Andaman Sea” for its clean white sandy beaches and crystal clear blue sea and one of the most beautiful islands in the Southeast Asia region, do take some times to explore the beauty of Phuket to make it more memorable. Phuket will be a place where you form a long lasting friendship, networking and leave with a deeper understanding of the field of microscopy. On behalf of the APMC11 Organising Committee and CAPSM, I would like to wish all the participants a fruitful and enriching conference with an everlasting memory of APMC11 in Phuket.

## Message from MST President



**Torranin Chairuang斯里, Ph.D.**  
Associate Professor

On behalf of the Microscopy Society of Thailand (MST), it is my great pleasure to invite you to Phuket, Thailand, where the 11th Asia-Pacific Microscopy Conference (APMC-11) will be hosted in concurrence with the 33rd Annual Conference of the Microscopy Society of Thailand (MST33) in May 2016.

Our kingdom of Thailand's east coast borders the Gulf of Thailand and the west coast adjoins the Andaman Sea. Among numerous offshore islands, Phuket, the biggest island of Thailand located in the Andaman Sea, is inevitably the most prominent attraction in the South. We are enthusiastic to welcome you at this one of the best known seaside resorts of the world.

By 2016, it will have been 28 years since the 4th Asia-Pacific Electron Microscopy Conference (APEM-4) was held in Bangkok, Thailand, in 1988. Consequently, we hope that the APMC-11 in Phuket, Thailand, will be a scientifically and socially memorable event.

We look forward to meeting you in Phuket, Thailand, in May 2016.

## Message from AAT President



**Supin Chompoopong, Ph.D.**  
Associate Professor

The 11th Asia-Pacific Microscopy Conference (APMC11) will for the second time, be organized in Thailand and held, from 23-27 May 2016, at Graceland Resort & Spa, Phuket, Thailand.

On behalf of the President of the Anatomy Association of Thailand, AAT, I am honored and delighted to welcome you to the 39th Annual Conference of the Anatomy Association of Thailand (AAT39), co-organized with APMC11 at Phuket. I believe we have chosen a venue that guarantees a successful conference amid the culture and the beautiful scenery, "Phuket", the Pearl of the Andaman. This island is rich in fresh food, traveling sites, several beautiful beaches and scenic waterfalls.

As we realize, the knowledge base in the microscopy is large, complex, and growing rapidly by researchers' reporting and journal publications. The APMC11- 2016 meeting will be a get-together for all participants in a friendly atmosphere. You will be inspired by the stimulating meeting that reflects recent developments in microscopy, the scientific information and technology. The meeting is your opportunity to share significant advances and idea, and to take the chance of interacting in a mutually profitable relationship. Meeting in Phuket is also for discovering one of Thailand's timeless city.

On behalf of the AAT, I would like to welcome you to the APMC11-2016.

## Message from Chair, Department of Anatomy, MUSC



**Somluk Asuvapongpatana, Ph.D.**  
Associate Professor

We are delighted to welcome you all at 11th Asia-Pacific Microscopy Conference (APMC11) which is jointly organized by the Microscopy Society of Thailand (MST33), the Anatomy Association of Thailand (AAT39) and Department of Anatomy, Faculty of Medicine Siriraj Hospital and Faculty of Science, Mahidol University. This year the meeting is held on May 23-27, 2016 at Phuket, Thailand.

This event bring the researchers in the fields of Material Sciences, Instrumentation and Development, Life Sciences, Anatomical Sciences to discuss and share the fruitful opinions as well as many advanced techniques that will be very helpful for all researchers in particular young scientists. We have organized many keynote lectures in all fields mentioned above, together with oral and poster sessions, which open up the opportunity in scientific exchanges. The highlighted extra-agenda activities include visiting around the most popular touristic town of Phuket with the appreciable scenic views either on land beaches and submarine view through the unbelievable clear sea water.

We wish you the most fruitful academic experience and the most joyful touristic experience in participating this APMC11 conference. I also hope that through your fruitful discussion, networking and collaborating researches will be established in the future.

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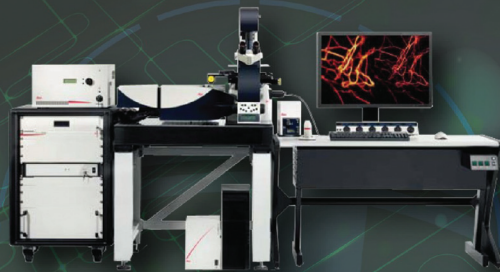
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## Hi - TECH INSTRUMENTS

### HI-TECH INSTRUMENTS SDN. BHD.

(Head Office) (388534-U)  
19, Jalan BP4/8,  
Bandar Bukit Puchong,  
47120 Puchong, Selangor,  
Malaysia.  
Tel: +603 - 8061 2228  
Fax: +603 - 8061 6668

(Penang Office)  
2D-1, Tingkat Kenari 5,  
11960 Sungai Ara,  
Bayan Lepas,  
Pulau Pinang, Malaysia.  
Tel: +604 - 643 8668  
Fax: +604 - 643 6226

(Kuching Office)  
1295B Lorong 5E,  
Jalan Keranji, Tabuan Jaya,  
93350 Kuching,  
Sarawak, Malaysia.  
Tel: +6082 - 369 203  
Fax: +6082 - 366 204

### HI-TECH INSTRUMENTS PTE. LTD.

(Singapore Office) (200103160-C)  
25 Bukit Batok Crescent,  
#05-03, The Ellitt,  
Singapore 658068  
Tel: +65 - 6899 9218  
Fax: +65 - 6899 0969

### HI-TECH INSTRUMENTS INC.

(Philippines Office) (A200110681)  
Unit 112, Prince Reiji Building,  
103 Gloria Diaz St cor L Bennet,  
BF Resort Village,  
Taion 2 Las Pinas City,  
Manila, Philippines.  
Tel: +632 - 873 3009  
Fax: +632 - 873 3010

### HI-TECH IMAGING CO. LTD.

(Thailand Office) (0105549128429)  
1/32 (Venice Di Iris, Block6)  
Watcharapol Rd., Tharang, Bangkok,  
Bangkok 10220,  
Thailand  
Tel: +662 024 1400  
Fax: +662 024 1401

General Email: [sales@htimail.com.my](mailto:sales@htimail.com.my), [service@htimail.com.my](mailto:service@htimail.com.my) Web Site: [www.htiweb.com](http://www.htiweb.com)

Monday, 23 May 2016

13:00	<b>Registration</b> (Graceland Lobby, First Floor)
14:00	
15:00	
16:00	
17:00	
18:00	
19:00	<b>Welcome Reception</b>  (sponsored by MICROSCOPY- official journal of The Japanese Society of Microscopy)
20:00	
21:00	

## Tuesday, 24 May 2016

8:00-17:00	<b>Registration</b> (Graceland Lobby, First Floor)
8:30	<p><b>Welcome and Congress Opening</b> (Orchid Ballroom)</p> <p>Welcome Address by <b>Associate Professor Sukumal Chongthammakun</b> Chairperson of APMC11/MST33/AAT39 and Deputy Dean of Faculty of Science, Mahidol University</p> <p>Address by <b>Professor Ze Zhang</b> President of CAPSM and Professor of Department of Materials Science, Zhejiang University</p> <p>Address by <b>Mr. Chamroen Tipayapongtada</b> Governor of Phuket</p> <p>Opening Remarks by <b>Professor Banchong Mahaisavariya</b> Vice President, Mahidol University</p> <p><b>Plaque and Certificate Presentation to Major Sponsors</b></p> <p><b>MST Honorary Membership Presentation</b></p>
9:30	<p><b>Keynote Presentation I</b> (Orchid Ballroom)</p> <p>“From Nano to PICO – the Next Generation of Aberration Corrected TEMs”</p> <p><b>Professor Joachim Mayer</b> RWTH Aachen University, and Forschungszentrum Jülich, Germany</p> <p><b>Chair: Ze Zhang</b> <b>Co-chair: Supin Chompoopong</b></p>
10:30	<b>Break</b>
11:00	



Tuesday, 24 May 2016

Orchid Room		Dalah-1 Room		Bu-nga Room		Dalah-2 Room		Dalah-3 Room	
M-1 Low-Dimensional Nanomaterials: Dots, Wires, and Sheets		M-9 Biomass Materials		I-1 Advances and Applications of Atomic Resolution Electron Microscopy		L-1 Cryo-Electron Microscopy of Single Particles		A-8 Cognitive Science	
Chair: Annon Klamcheun Co-chair: Jin Zou		Chair: Takayuki Takarada Co-chair: Suparin Chaiklangmuang		Chair: Nobuo Tanaka Co-chair: Wen-An Chiou		Chair: David Bhella Co-chair: Paula da Fonseca		Chair: Ingrid Liu Co-chair: Gavin Reynolds	
11:00	"Heterostructure of ZnO Nanowire Arrays on Ag thin film prepared by Gas-limiting RF Magnetron Sputtering and Its Photocatalytic Activity"	"Low temperature catalytic pyrolysis/gasification of biomass using natural products as catalysts"		"Research direction of environmental transmission electron microscopy for nano-materials"		"Towards <i>in situ</i> structure analysis of virus-host interactions"		"Using behavioral training paradigms and mouse models to delineate memory formation pathways and select novel drug targets"	
	Annon Klamcheun	Takayuki Takarada		Nobuo Tanaka		David Bhella		Ingrid Liu	
11:30	"Impact of Catalysts in Epitaxial Growth of III-V Semiconductor Nanowires"	"Plasma arc technology processing of destroying biomass materials"		"Recent Studies for Materials Characterization with High Resolution Analytical TEM/STEM"		"High resolution cryo-EM of proteasome complexes as a new tool for therapeutic drug development"		"Abnormal Protein Citrullination in Alzheimer's Disease"	
	Jin Zou	Pudji Untoro		Masahiro Kawasaki/ Makoto Shiojiri		Paula da Fonseca		Akihito Ishigami	
		"Arc Plasma Sintering: New Challenge for low emission bio-waste treatment"							
12:00	OM0101 "Exploring the configuration of atomic defects and the multi-path migration of Mo adatoms in atomically thin MoS <sub>2</sub> by statistic ADF-STEM"	OM0901 "Comparison of Bio-oil and Extracted Residue from Acid and Enzyme Hydrolysis Processes of Microalgae"		"Ultrafast <i>in-situ</i> and <i>ex-situ</i> heating of AL and TA nanoparticles"		"Direct detection and electron counting - A beginning of a new era for electron microscopy"		"Parvalbumin deficits in psychotic illness - from pathology to epigenetics"	
	Chuanhong Jin	Nuapon Duongbia		Wen-An Chiou		Ming Pan		Gavin Reynolds	
	OM0102 "Real-time Study of the Growth Behavior of InAs Nanorods Using in situ RHEED Transmission mode and TEM"								
12:30	Janghyun Jo								
Lunch Talk									

Tuesday, 24 May 2016

Orchid Room		Dalah-1 Room		Bu-nga Room		Dalah-2 Room		Dalah-3 Room	
M-1 Low-Dimensional Nanomaterials: Dots, Wires, and Sheets		M-2 Semiconductors and LSI Device Materials		A-10 Cell Biology		L-2 Cellular Electron Tomography		A-3 Stem Cell and Cancer Biology	
Chair: Takeshi Yanagida Co-chair: Luu Tien Hung		Chair: Chanchana Thanachayanon Co-chair: Sakuntam Sanorpin		Chair: Takashi Yashiro Co-chair: Apiwat Mutrangura		Chair: José López Carrascosa Co-Chair: Puey Ounjai		Chair: George Wai-Cheong Yip Co-chair: Sam Wah Tay	
13:30	"Material Design of Single Crystalline Metal Oxide Nanowires and Their Promises for Green-Innovation and Life-Innovation"	"TEM analysis of cubic GaN films on GaAs (311) oriented substrates grown by MOVPE"		"Analysis of Cell to Cell and Cell to Extracellular Matrix Communication in the Anterior Pituitary Gland"		"Correlation of cryo-soft X-ray tomography and electron microscopy to study nanoparticle-whole cell interaction"		"Heparan Sulfate 6-O-Sulfotransferase 2 in Breast Cancer"	
	Takeshi Yanagida	Sakuntam Sanorpin		Takashi Yashiro		José López Carrascosa		George Wai-Cheong Yip	
14:00	"Synthesis, Characterization, Photocatalytic and Bactericidal Activities of (Co, Ag) - Doped ZnO Nanoparticles"	"Depth Quantitative and Chemical State Analysis from Few nm to Several nm using Soft X-ray Emission Spectroscopy"		"Epigenetic Roles of Repetitive Sequences in Cancer"		"Structural Insights into Substrate Recognition and Surface Adhesion in <i>Chlamydomonas reinhardtii</i> "		"Mesenchymal Stem Cells Secretome Alters Microglial Proliferation via the Expression of the <i>ECT2</i> gene"	
	Luu Tien Hung	Hideyuki Takahashi		Apiwat Mutrangura		Puey Ounjai		Sam Wah Tay	
14:30	"Nanowires for extracellular vesicles analysis"	"TEM Characterization of microstructural evolution of Gallium Nitride grown by MOCVD on c-sapphire"		"Morphology of breast cancer cells overexpressing the Y-Box Binding Protein 1 as observed by immunofluorescence confocal microscopy"		OL0201 "Making the practically impossible 'merely difficult' -cryogenic FIB lift-out for damage free soft matter imaging"		"Modeling $\beta$ -thalassemia by using induced pluripotent stem cells (iPSCs)"	
	Takao Yasui	Kuttanellore Muralaeddharan		Boon Huat Bay		Christopher Parmenter		Yindee Kitiyanant	
15:00	"Controlled Nanostructures by Physical Vapor Deposition with Glancing Angle Deposition Technique for Sensor Applications"	OM0201 "Investigation on defect related luminescence in semi-polar InGaN/ GaN quantum well by using TEM-CL"		CAPSM Business Meeting		OL0202 "Actin-bundling formation of dimeric human fascin-1 was determined by IHRSR and tomography methods"		Jeong Min Chung	
	Mati Horprathum	Mi-Hyang Sheen							
	OM0103 "Exploring the Composition-related Stability for Layered Cathode Material for Lithium Ion Battery by Analytical STEM"	OM0202 "Strain analysis by STEM moiré method"							
	Wei Huang	Yukihito Kondo							
	OM0104 "Interacting magnetic nanoparticles of three dimensional magnetic vortex in different configuration assemblies"								
	Min-Kwan Kim								
15:30	Break								

Orchid Room		Dalah-1 Room		Bu-nga Room		Dalah-2 Room		Dalah-3 Room	
I-11 Teaching Microscopy: On-line, Hands-on & Remote/		M-3 Surface, Interfaces, Grain Boundaries, and Coatings		L-3 Cell and Tissues Structures I		L-5 Advanced Techniques in Biological Sample Preparation and Observation		A-7 Aging Science	
Chair: Supapan Seraphin Co-chair: Makoto Shiojiri		Chair: Yuttanant Boonyongmaneerat Co-chair: Sukanda Jansirisomboon		Chair: Pakorn Kanchanawong Co-chair: Kanokpan Wongprasert		Chair: Georg Ramm Co-chair: Keisuke Ohta		Chair: Thamthiwat Nararatwanchai Co-chair: Amornpun Sereemaspun	
15:30	"Teaching microscopy: sharing wondrous journey into the micro- and nano-world"	"Enhancing corrosion resistance of galvanized coating via microstructural engineering and microscopy analysis"		"Mapping the nanoscale architecture of cadherin-based adhesions by superresolution microscopy"		"Correlative light and electron microscopy techniques for live-cell imaging and for identifying rare structures in small organisms"		"Scientific method for antiaging research by using bioengineering measurement"	
	Supapan Seraphin	Yuttanant Boonyongmaneerat		Pakorn Kanchanawong		Georg Ramm		Thamthiwat Nararatwanchai	
16:00	OI0101 "Probing structure and electronic structure of battery materials at atomic scale by scanning transmission electron microscopy"	OM0301 "Challenges in HR-SEM: Direct observation of Micro-, Meso- and Macro-pore openings and fine structure details of nanostructured materials"		"Adhesion formation and transformation at the cell-matrix interface"		"Three-dimensional organization of mitochondria-associated membrane revealed by FIB-SEM combined with live cell imaging"		"Current biomarkers of cellular aging: from morphology to molecular evidences"	
	Lin Gu	Shunsuke Asahina		Cheng-tan Yu		Keisuke Ohta		Amornpun Sereemaspun	
16:30	OI0102 "3D Fourier transform analysis to evaluate a high-performance TEM"	OM0302 "Metallography Techniques for Microscopy of Electrogalvanized Coatings: Effectiveness of Mechanical, Electrochemical, and Ion polishing"							
	Kazuo Ishizuka	Narin Jantaping							
	OI0103 "Quantitative experimental determination of site-specific magnetic structures by transmitted electrons"	OM0303 "Characterization of the Interface between Metal and Glass Produced by Cold Spray"		OL0301 "Histological study of larval development of the blow fly, <i>Chrysomya megacephala</i> (Diptera: Calliphoridae)"		"Recent developments in preparation of biological samples for electron microscopy"		OA0701 "Cerebellar cortical layer atrophy in the aging human-A Post-mortem observation"	
	Xiaoyan Zhong	Minghui Song		Chutharat Samerjai		Wen An Chlou		Tahamida Yesmin	
17:00	OI1101 "Digital transformation in Microscopy Teaching"	OM0304 "In-situ Observation of Mutual Reduction between ceramics in TEM"		OL0302 "Ultrastructure of Immature Stages of <i>Lucilia shiensis</i> Aubertin, 1933 (Diptera: Calliphoridae) for Identification"				OA0702 "Degenerative Change in Lumbar Facet Joint Surface related with Age in A Thai Population"	
	Silvia Zenner-Gellrich	Nobuhiro Ishikawa		Sangob Sanit				Chanatporn Inthasan	
		OM0305 "Real time observation of the reconstructing TiO2 (001) surface via an Environmental TEM"						AAT Business Meeting	
		Yong Wang							
17:30		OM0306 In-situ TEM study of oxidation in a nickel-based single crystal superalloy"							
		Qingqing Ding							

Wednesday, 25 May 2016

8:00-17:00	<b>Registration</b> (Graceland Lobby, First Floor)
8:30	<b>Keynote Presentation II</b> (Orchid Ballroom)  “Innovating New Materials through Aberration-corrected Microscopy”  <b>Professor Stephen John Pennycook</b> National University of Singapore, Singapore  <b>Chair: Prasert Sobhon</b> <b>Co-chair: Kazuo Furuya</b>
9:30	<b>Keynote Presentation III</b> (Orchid Ballroom)  “Visualizing Lipid Regulation of Gene Expression Using New and Classical Imaging Tools”  <b>Professor Pavel Hozak</b> Institute of Molecular genetics ASCR, v.i., Czech Republic  <b>Chair: Prasert Sobhon</b> <b>Co-chair: Kazuo Furuya</b>
10:30	<b>Break</b>
11:00	

Orchid Room		Dalah-1 Room		Bu-nga Room		Dalah-2 Room		Dalah-3 Room	
M-4 Metals, Alloys and Steels		I-13 Advances and Applications of Electron Energy loss Spectroscopy I-4 Advances in Electron and X-ray Spectroscopy		A-4 Cancer Biology and Viruses		L-4 Cell and Tissues Structures II		A-1 Neuroscience I	
Chair: Jer-Ren Yang Co-chair: Torranin Chairuangsrri		Chair: Hiroki Kurata Co-chair: Chris Boothroyd		Chair: George Sai-Wah Tsao Co-chair: Christopher Beng Ti		Chair: Steeve Boulant Co-chair: Worawit Suphamungmee		Chair: Wei Yi Ong Co-Chair: Guo-Fang Tseng	
11:00		"Microstructure observation of Al-Mg-Ge alloy aged at 423K and 473K using TEM"		"Live cell imaging of visible Epstein-Barr virus in human epithelial cells"		"Dynamics and ultrastructural characterization of the membranous flat clathrin coated arrays"		"Distribution and Function of Phospholipases A2 in the brain"	
Kenji Matsuda		Hiroki Kurata		George Sai-Wah Tsao		Steeve Boulant		Wei Yi Ong	
11:30		"Atomically-Resolved Electron Spectroscopy for Emergent Phenomena at Oxide Interfaces"		"Precision Medicine in Neuro-Oncology"		"Focused Ion Beam Scanning Electron Microscopy Revealed Three-Dimensional Fine Structure of the Cells"		"Epigenetic regulation of microglia in the ischemic mouse brain"	
OM0401 "Aging Strengthening of Interphase-Precipitated Strengthened Copper-Bearing Dual-Phase Steels"		Ming-Wen Chu		Christopher Beng Ti		Depicha Jindatip		S Thameem Dheen	
Cheng-Han Li									
OM0402 "Multi-dimensional analysis of bainite in Cu-Al-Mn alloy by advanced electron microscopy techniques"									
Toru Hara									
12:00		"Determining the structure of carbon-based materials using electron energy-loss spectroscopy"		"Detection of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in Penaeus monodon by <i>In-situ Hybridization</i> at Transmission Electron Microscopic Level"		OL0401 The combination of atorvastatin and cyanidin-3-glucoside attenuated oxidative stress in vascular smooth muscle cells		"Cortical, hippocampal and striatal changes accompanied the behavioral alterations in a rat model of infantile hydrocephalus"	
OM0403 "Spinodal decomposition in CoCuFeMn Ni and CuFeMnNiZn high-entropy alloys observed by electron microscopy"		Chris Boothroyd		Kanokporn Chayaburakul		Rungsa Pantan		Guo-Fang Tseng	
Ruangdaj Tongsrri									
OM0404 Analytical investigation of rapidly solidified braze ribbons for transient liquid phase bonding						OL0402 Study of the interaction of Macrobtrachium rosenbergii Noda virus virus-like particles in Sf9 insect cells: the hijacking of caveolin-mediated endocytosis and the possible existence of a protruding VLP capsid ligand domain			
Riza Iskandar						Monsicha Somrit			
12:30									
13:30									

Lunch Talk

Orchid Room		Dalah-1 Room		Bu-nga Room		Dalah-2 Room		Dalah-3 Room	
M-4 Metals, Alloys and Steels I-13 Advances and Applications of Electron Energy Loss Spectroscopy		I-3 New Methods in Diffraction and Imaging		I-7 Advanced Light-Optical Microscopy		L-6 Confocal and other Microscopies in Biomedical Sciences		A-9 Surgical and Clinical Anatomy	
Chair: Kenji Matsuda Co-chair: Waraporn Piyawit		Chair: Kenji Tsuda Co-chair: Fu-Rong Chen		Chair: Suejitt Pechprasam Co-chair: Michael Somekh		Chair: David Becker Co-chair: Wattana Weerachatyanutkul		Chair: Meechai Srisai Co-chair: Pasuk Mahakkanukrauh	
13:30	"Substructures of lenticular martensite in high-carbon Hig-chromium stainless steel"	"Study of nanoscale local structures of perovskite-type ferroelectrics using STEM-CBED"		"Surface wave attenuation coefficient measurement using confocal surface plasmon microscopy"		"Targeting connexins to improve chronic wound healing"		"A continuing study model from preclinical, clinical, through postgraduate medical education"	
	Jer-Ren Yang	Kenji Tsuda		Suejitt Pechprasam		David Becker		Meschai Srisai	
14:00	OM0405 "Heat-treated microstructure and property of sintered of Fe-0.85Mo + 4 wt. % SiC + 0.4 wt. % C materials" Dhritti Tanprayoon	"Atomic Resolution Tomography of Nanoparticles Reconstructed from Exit Wave" Fu-Rong Chen		"Evanescent wave and confocal microscopy" Michael Somekh		"C-terminal domain on the outer surface of the <i>Macrobacterium rosenbergii</i> Nodavirus capsid is required for S19 cell binding and internalization"		"Surgical and Clinical Anatomy" Pasuk Mahakkanukrauh	
	OM0406 "Sintered Ductile Iron/Compacted Graphite Iron-like Fe-Mo-Si-C alloy" Kittikhun Ruangchai					Wattana Weerachatyanutkul			
14:30	OM0407 "Evidences of Sympathetic Nucleation and Ledgewise Growth Revealed by Scanning Electron Microscopy" Wananurat Srijanpan	OI0301 "Electron diffraction analysis of microcrystalline of organic molecules" Tetsuo Oikawa		OI0701 "Ultra-sensitive biosensor using double- metallic-layer-waveguide structure" Mengqi Shen		OL0601 "Active Targeted Delivery of PLGA- Liposome Nanoparticles Specific for Cervical Cancer Therapy" Suphawadee Bunthot		"Training and Education Center for Clinical Skills" Rossarin Rattanalekha	
	OM0408 "Characterization of CoCuFeMnNi alloy" Panita Choeychom	OI0302 "Quantification of crystallinity using energy filtered electron diffraction" Byeong-Seon An		OI0702 "Embedded interferometry with dynamic reference beam" Wai-Kin Chow		OL0602 "The Alyscon Detector from Zeiss: Confocal imaging with improved signal-to- noise ratio, faster speed and super- resolution" Xianke Shi			
15:00	OI0301 "Effects of nickel oxide underlayer on the catalytic properties of manganese oxide nanoparticles" Sangmoon Yoon	OI0303 "A Relativistic-energy femtosecond- pulse electron microscopy" Jinfeng Yang				OL0603 "Nanoscale approaches to characterize the dynamic and biophysical determinants of receptor-mediated virus internalization" Megan Stanifer		OA0901 "Stature Estimation from Skull using Craniometric Method in A Thai Population" Chirapat Inchai	
		OI0304 "MEMS Waveguide Sensor for Photoacoustic Detection" Supanee Learkthanakhon						OA0902 "Extensor tendons and variations of the medial four digits of hand: a cadaveric study" Athikhun Suwannakhan	
15:30									
Break									

Wednesday, 25 May 2016

15:30	<b>Poster Presentation</b>  All presenters should be present at their poster board
16:00	
16:30	
17:00	
17:30	<b>MST Business Meeting</b>
18:00	

18:30	<b>Conference Banquet (Orchid Ballroom)</b>  (sponsored by JEOL ASIA PTE LTD)
19:30	
20:30	
21:30	



Registration (Graceland Lobby, First Floor)					
8:30-17.00	Orchid Room	Dalah-1 Room	Bu-nga Room	Dalah-2 Room	Dalah-3 Room
	M-6 Amorphous Materials and Quasicrystals Chair: Eiji Abe Co-chair: Nattapol Laorodphan	I-8 Scanning Probe Microscopy I-9 Atom Probe Microscopy Chair: Michael Hietschold Co-chair: Tongjai Choolajorm	I-5 In Situ Dynamics in TEM and SEM, Environmental Microscopy Chair: Yoshitumi Oshima Co-chair: Masanori Koshino	L-7 Microscopy Applications in Pathology and Laboratory Medicine Chair: Suchin Worawichawong Co-Chair: Hing Hiang Lian	L-9 Reproductive Biology and Glycobiology Chair: Ken Kitajima Co-Chair: Krai Meemon
9:00	"Structure determination of quasicrystals: STEM imaging and hyperspace x-ray crystallography" Eiji Abe	"Scanning Tunneling Microscopy Investigations of Pattern Control in Molecular Self-Assembly" Michael Hietschold	"The Morphological Study of Wet Biopolymer Capsules with Living Cells by Environmental Scanning Electron Microscope AQUASEM II" Vilem Nedela	"Role of microscopy in diagnosis of transplant kidney" Suchin Worawichawong	"Effects of polyunsaturated fatty acids and prostaglandins on crustacean reproduction" Prasert Sobhon
9:30	"Real time observation of the reconstructing TiO <sub>2</sub> (001) surface via ETEM" Ze Zhang	"Development of nanomanipulator based on scanning probe microscopes for biological applications" Futoshi Iwata	"A novel approach for the scanning electron microscopy at ambient atmospheric pressures" Yusuke Ominami	"Studying Virus Trafficking in Cells using Immune Fluorescence Microscopy" Mark Douglas	"Glycan-mediated interactions on pig sperm lipid rafts during fertilization" Ken Kitajima
10:00	"Structural Characterization of Amorphous Materials via Electron Beam Radial Distribution Function Analysis" Manabu Ishimaru	"Analysis of Geological Materials by Atom Probe: Understanding the Mechano-chemical Behaviour of Zircon" Julie Cairney	OI0501 "In situ TEM Study of Vapor-Solid Nanotube Growth" Zhengfei Zhang  OI0502 "In situ electron holography of electric potentials inside a solid-state electrolyte: Effect of 3D electric-field leakage" Tsukasa Hirayama	"Evaluation of cells integrity using different fixation time using scanning electron microscopy" Hing Hiang Lian	"Chemical tools to image glycans by metabolic labeling" Yann Gueraedel
10:30		"Revealing the internal structures of nanostructured alloys using atom probe tomography" Tongjai Choolajorm			
11:00					
Break					

Orchid Room		Dalah-1 Room		Bunga Room		Dalah-2 Room		Dalah-3 Room	
	M-5 Ceramics and Inorganic Materials  Chair: Naoya Shibata Co-chair: Bralee Chayasombat	I-9 Atom Probe Microscopy I-10 Theoretical Modeling and Analysis in Electron Microscopy  Chair: Fu-Rong Chen Co-chair: Raynald Gauvin	I-5 In Situ Dynamics in TEM and SEM, Environmental Microscopy  Chair: Vilem Nedela Co-chair: Yusuke Ominami	L-8 Mass Spectrometry  Chair: Mitsutoshi Setou Co-chair: Scott F. Cummins	A-2 Neuroscience II  Chair: Sutisa Thanoi Co-chair: Sukumal Chongthammakun				
11:00	"Development of atomic-resolution scanning transmission electron microscopy for ceramic interfaces"  Naoya Shibata	"Atomic Scale Mechanical Microscopy"  Xiao Dong Han	"Operand' Transmission Electron Microscope Observation of Lithium Ion Battery--What happened during the Operation"  Yoshifumi Oshima	"Platform of functional metabolic imaging"  Mitsutoshi Setou	"Deficits in the GABAergic System after Exposure to Abused Drugs"  Sutisa Thanoi				
11:30	"Assessment of Strain-generated Oxygen Vacancies Using SrTiO <sub>3</sub> Bicrystals"  Si-Young Choi	"Quantitative X-Ray Microanalysis and High Resolution Imaging With Monte Carlo Simulations"  Raynald Gauvin	"Application of Low-voltage STEM With EELS and EDS for Soft Materials Analysis"  Masanori Koshino	"Mass spectral identification of aquatic 'excretomes' that dictate key ecological interactions"  Scott F. Cummins	OA0201 "Methamphetamine impairs the P-gp transporter in primary rat brain microvascular endothelial cells"  Pichaya Jumnongprakhon  OA0202 "Characterization of olfactory ionotropic glutamate receptors 25a in the giant freshwater prawn, <i>Macrobrachium rosenbergii</i> "  Natwalee Jakunta				
12:00	"TEM and STEM Investigations of Oxide Thermoelctrics"  Miran Čeh	OI0901 Improvement of mass resolution in atom probe tomography for oxide materials with surface modification  Chang Min Kwak	OI0503 "Improved heating stage for EBSD <i>In-situ</i> work"  Seiichi Suzuki	OL0801 "Multimodal approach to improving <i>Macrobrachium rosenbergii</i> aquaculture through in-depth studies into MrNV infection and sperm-egg interaction processes"  Atthaboon Watthammawut	OA0203 "Role of Fluoride ingestion in the pathogenesis of Alzheimer's Disease"  Raghu Jetti				
	OM0501 "In situ Studies of Heat Induced Precipitation of Yttria Precursor using Liquid-Cell Transmission Electron Microscopy"  Sašo Šturm	OI1001 "Quality enhancement and strain measurement in HAADF images using Super-Resolution techniques"  P. L. Galindo	OI0504 "In-situ TEM study of nano-scale conductive filament used for threshold selector device"  Byeong Gyu Chae		OA0204 "Entrapment of the Martin-Gruber anastomotic nerve in the forearm: a case report"  Anu V Ranade				
12:30	Lunch Talk								

Orchid Room		Dalah-1 Room		Bu-nga Room		Dalah-2 Room		Dalah-3 Room	
M-11 Microscopy and Microanalysis in Industry M-5 Ceramics and Inorganic Materials		M-7 X-ray Analysis of Geological / Mineralogical Materials M-8 Novel Applications of Material Science and Engineering		I-5 In Situ Dynamics in TEM and SEM, Environmental Microscopy I-12 Surface Microscopy with LEEM, PEEM, UHV-SEM, REM and SREM		A-11 Reproductive Biology		A-5 Applied and Clinical Anatomical Sciences	
Chair: Lynne Gignac Co-chair: Paul E. Fishchione		Chair: Nirawat Thammajak Co-chair: Syo Matsumura		Chair: Vilem Nedela Co-chair: Ing Eva Tihlaříková		Chair: Samur Thanoi Co-chair: Somluk Asuvapongpattana		Chair: Wojciech Pawlina Co-chair: Kittikun Vivapinyo	
13:30	"Imaging 8 µm thick semiconductor interconnects using 300-400 keV/SE, BSE, STEM and 3D FIB-SEM"	"X-ray absorption spectroscopy for gemology and mineralogy applications: a case study of fresh-water cultured pearls"	"In-situ preparation method for repetitive ESEM and SEM observation of plant samples"	"Observation of dynamic phenomena using Atmospheric Scanning Electron Microscope (ASEM)"	"Methamphetamine and Changes of Sperm Quality"	"Teaching Microscopic Anatomy in Medical Curriculum: Changes, Trends, and Competencies"	"Alpha-2 macroglobulin is involved in sperm activation and decapacitation factor of the blue swimming crab, <i>Portunus pelagicus</i> "	"Association between age and distal femoral articular surface of knee joint morphological changes in dry bone in a Thai population"	"Age Estimation from Pubic Symphysis in a Thai Population by Using Method of Boldsen <i>et al.</i> "
	Lynne Gignac	Nirawat Thammajak	Ing Eva Tihlaříková	Mitsuo Suga	Samur Thanoi	Wojciech Pawlina	Thanyaporn Senarai	Kan Wangpiriyapanitch	Treerat Gumpangseth
14:00	"Unlocking structure and chemistry at the atomic level through advanced TEM specimen preparation"	"Structural Investigation of Some Nanocomposite Powders for Li-ion Battery Anode Applications"	"Strong perpendicular magnetization of ferromagnetic multi-layers with high brightness and highly spin-polarized LEEM"	"Imaging and tailoring chiral magnetism"	"Alteration of GABA Concentrations in Rat Testis after Methamphetamine Exposure"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "	"Association between age and acetabular morphological changes in dry bones in a Thai population"	"Association between age and distal patellar articular surface of knee joint morphological changes in dry bone in a Thai population"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "
	Paul E. Fishchione	Makoto Shiojiri	Takanori Koshikawa	Andreas Schmid	Paweena Kaewman	Pornhatai Komuttrattanont	Suphawita Pliannuom	Suphawita Pliannuom	Treerat Gumpangseth
14:30	"Failure Analysis of Semiconductor Devices by FIB-SEM and TEM-EEL"	"Atomic Resolution Quantification of Elemental Ratio"	"Preparation of Electrospun Polystyrene Fibrous Membranes Containing Silver Nanoparticles"	"Imaging and tailoring chiral magnetism"	"Alteration of GABA Concentrations in Rat Testis after Methamphetamine Exposure"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "	"Association between age and acetabular morphological changes in dry bones in a Thai population"	"Association between age and distal patellar articular surface of knee joint morphological changes in dry bone in a Thai population"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "
	Yasufumi Yabuuchi	Mitsutaka Haruta	Takanori Koshikawa	Andreas Schmid	Paweena Kaewman	Pornhatai Komuttrattanont	Suphawita Pliannuom	Suphawita Pliannuom	Treerat Gumpangseth
15:00	OM0502 "Synthesis and characterization of mixed phase TiO <sub>2</sub> (B)/anatase TiO <sub>2</sub> thin films on a number of different substrates by LPCVD"	OM0801 "Preparation of Electrospun Polystyrene Fibrous Membranes Containing Silver Nanoparticles"	"Imaging and tailoring chiral magnetism"	"Imaging and tailoring chiral magnetism"	"Alteration of GABA Concentrations in Rat Testis after Methamphetamine Exposure"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "	"Association between age and acetabular morphological changes in dry bones in a Thai population"	"Association between age and distal patellar articular surface of knee joint morphological changes in dry bone in a Thai population"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "
	Yothin Chimupaia	Chutima Srisithirakul	Andreas Schmid	Andreas Schmid	Paweena Kaewman	Pornhatai Komuttrattanont	Suphawita Pliannuom	Suphawita Pliannuom	Treerat Gumpangseth
15:30	OM0503 "Microstructural evolution of hydroxy/fluorapatite in the different precursor solution under hydrothermal process"	OM0802 "Quick Evaluation of Electric Field Surrounding a Charged Particle"	"Imaging and tailoring chiral magnetism"	"Imaging and tailoring chiral magnetism"	"Alteration of GABA Concentrations in Rat Testis after Methamphetamine Exposure"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "	"Association between age and acetabular morphological changes in dry bones in a Thai population"	"Association between age and distal patellar articular surface of knee joint morphological changes in dry bone in a Thai population"	"Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen <i>et al.</i> "
	Upsorn Boonyang	Katsuhiro Sasaki	Andreas Schmid	Andreas Schmid	Paweena Kaewman	Pornhatai Komuttrattanont	Suphawita Pliannuom	Suphawita Pliannuom	Treerat Gumpangseth
Break									
16:00									

	Orchid Room	Dalah-1 Room	Bu-nga Room	Dalah-2 Room	Dalah-3 Room
	M-11 Microscopy and Microanalysis in Industry  Chair: Lynne Gignac Co-chair: Paul E. Fishchione  "Industrial-user-oriented Functionality of Transmission Electron Microscopy Application Software"  Hiromitsu Furukawa	M-9 Novel Applications of Material Science and Engineering  Chair: Nirawat Thammajak Co-chair: Thapinee Sarakonsri  "La ordering in epitaxial $\text{Li}_{3-x}\text{La}_{2/3-x}\text{TiO}_3$ films and its effects on Li-ion conduction"  Kazutaka Mitsuishi	I-5 In Situ Dynamics in TEM and SEM, Environmental Microscopy I-12 Surface Microscopy with LEEM, PEEM, UHV-SEM, REM and SREM  Chair: Takanori Koshikawa Co-chair: Sumet Sakulsersmuk  "Structure/Dynamics of Two-Dimensional Layers Studied by Low Energy Electron Microscopy and nano-Low Energy Electron Diffraction"  Michael S. Altman		A-6 Teaching Anatomy: On-line, Hands-on & Remote  Chair: Kem A. Rogers Co-chair: Kulathida Chaitirayanon  "Design and Implementation of Online Courses in Anatomy and Histology and the Perception of Both Students and the Faculty"  Kem A. Rogers
16:00					
16:30	OM1101 "Scanning electron microscopes with integrated Raman spectrometer revealing new complementary information"  Fang Zhou	OM0803 "Inclusion Complex between water-soluble quaternized $\beta$ -cyclodextrin grafted chitosan and $\alpha$ -mangostin: Influence of degree of $\beta$ -CD substituted on chitosan"  Sarunya Phunpee	OI0505 "In-situ TEM study of deformation behavior in a dual-phase high-entropy alloy AlCoCrFeNi"  Qiannan Wang		"Cognitive load and commercial Software Design for Teaching Anatomical Sciences"  Sonya Van Nuland
	OM1102 "Surface Sensitive Imaging using Energy Filtered SEM at low kV"  Fang Zhou		OI0506 "In-situ nanoengineering based on TEM"  Litao Sun		
17.00	OM1103 "Origin of dramatic oxygen solute strengthening effect in Titanium"  Qian Yu		OI0507 "Ultra-high resolution SEM for 3D analysis in biology"  Jarosav Jiruse		

Registration (Graceland Lobby, First Floor)					
Orchid Room		Dalah-1 Room	Bu-nga Room	Dalah-2 Room	Dalah-3 Room
M-10 Mineral Geochronology & Isotope Characteristics by X-ray & Ions		I-6 EBSD of Synthetic and Natural Materials			
Chair: Boontarika Srithai Co-chair: Richard Wirth		Chair: Chaiyasit Banjongprasert Co-chair: Yothin Chimupala			
9:00		"Reviews of geochronology of igneous rocks: implications for tectonic evolution of Thailand"			
Boontarika Srithai		Chaiyasit Banjongprasert			
9:30		<b>O10601</b> "Study of pseudo-symmetric misindexing in EBSD analysis of $\gamma$ -TiAl alloys with refined accuracy band detection" <b>Wu Jiang</b>			
Richard Wirth		<b>O10602</b> "Direct observation of low angle boundary migration during recrystallization using electron channeling contrast imaging and electron backscatter diffraction" <b>Jin-Su Oh</b>			
Break					
10:00					
10:30	Closing Ceremony and Award Presentation (Orchid Ballroom)				
11:00	APMC12 Announcement				
11:30					
12:00					
12:30	Lunch (Bua Luang Restaurant)				
13:00					
13:30					

**PROGRAM**  
**APMC11 / MST33 / AAT39 CONFERENCE**  
**Phuket Graceland Resort & Spa, Phuket, Thailand**  
**May 23-27, 2016**

**Monday, May 23<sup>rd</sup>, 2016**

- 13.00-17.00**      **Registration (Graceland Lobby, First Floor)**
- 19.00-21.00**      **Welcome Reception (sponsored by MICROSCOPY- official journal of The Japanese Society of Microscopy)**

**Tuesday, May 24<sup>th</sup>, 2016**

- 08.00-17.00**      **Registration (Graceland Lobby, First Floor)**
- 08.30-09.00**      **Welcome and Congress Opening (Orchid Ballroom)**
- Welcome Address by **Associate Professor Sukumal Chongthammakun**  
Chairperson of APMC11/MST33/AAT39 and  
Deputy Dean of Faculty of Science, Mahidol University
- Address by **Professor Ze Zhang**  
President of CAPSM and Professor of Department of Materials Science,  
Zhejiang University
- Address by **Mr. Chamroen Tipayapongtada**  
Governor of Phuket
- Opening Remarks by **Professor Banchong Mahaisavariya**  
Vice President, Mahidol University
- Plaque and Certificate Presentation to Major Sponsors**
- MST Honorary Membership Presentation**
- 09.30-10.30**      **Keynote Presentation I (Orchid Ballroom)**  
**From Nano to PICO – the Next Generation of Aberration Corrected TEMs**  
**Professor Joachim Mayer**  
*RWTH Aachen University, and Forschungszentrum Jülich, Germany*  
Chair: Ze Zhang  
Co-chair: Supin Chompoopong
- 10.30-11.00**      **Coffee Break**

Tuesday, May 24<sup>th</sup>, 2016

**11.00-12.30 M-1 Low-Dimensional Nanomaterials: Dots, Wires, and Sheets**

**Chair: Annop Klamcheun**

**Co-chair: Jin Zou**

**(Orchid Ballroom)**

- 11.00-11.30** Heterostructure of ZnO Nanowire Arrays on Ag thin film prepared by Gas-timing RF Magnetron Sputtering and Its Photocatalytic Activity  
**Annop Klamcheun**  
*National Nanotechnology Center, NSTDA, Thailand*
- 11.30-12.00** Impact of Catalysts in Epitaxial Growth of III-V Semiconductor Nanowires  
**Jin Zou**  
*University of Queensland, Australia*
- 12.00-12.15 OM0101**  
Exploring the configuration of atomic defects and the multi-path migration of Mo adatoms in atomically thin MoS<sub>2</sub> by statistic ADF-STEM  
Jinhua Hong, Zhixin Hu, Yuhao Pan, Wei Ji, **Chuanhong Jin**, Jun Yuan, Ze Zhang
- 12.15-12.30 OM0102**  
Real-time Study of the Growth Behavior of InAs Nanorods Using in situ RHEED Transmission mode and TEM  
**Janghyun Jo**, Youngbin Tchoe, Hwangsun Kim, Gyu-Chul Yi, Miyoung Kim

Tuesday, May 24<sup>th</sup>, 2016

**11.00-12.15 M-9 Biomass Materials**

**Chair: Takayuki Takarada**

**Co-chair: Suparin Chaiklangmuang**

**(Dalah-1 Room)**

- 11.00-11.30** Low temperature catalytic pyrolysis/gasification of biomass using natural products as catalysts  
**Takayuki Takarada**  
*Gunma University, Japan*
- 11.30-11.45** Plasma arc technology processing of destroying biomass materials  
**Pudji Untoro**  
*Surya Institute, Indonesia*
- 11.45-12.00** Arc Plasma Sintering: New Challenge for low emission bio-waste treatment  
**Arbi Dimiyati**  
*National Nuclear Energy Agency (BATAN), Indonesia*
- 12.00-12.15 OM0901**  
Comparison of Bio-oil and Extracted Residue from Acid and Enzyme Hydrolysis Processes of Microalgae  
**Nuapon Duongbia**, Nuapon Duongbia, Suraphon Chaiwongsar, Chatchawan Chaichana, Suparin Chaiklangmuang



**Tuesday, May 24<sup>th</sup>, 2016**

**11.00-12.30 I-1 Advances and Applications of Atomic Resolution Electron Microscopy**

**Chair: Nobuo Tanaka**

**Co-chair: Wen-An Chiou**

**(Bu-nga Room)**

- 11.00-11.30** Research direction of environmental transmission electron microscopy for nano-materials  
**Nobuo Tanaka**  
*Nagoya University, Japan*
- 11.30-12.00** Recent Studies for Materials Characterization with High Resolution Analytical TEM/STEM  
**Masahiro Kawasaki/ Makoto Shiojiri**  
*JEOL USA Inc., USA*
- 12.00-12.30** Ultrafast *in-situ* and *ex-situ* heating of AL and TA nanoparticles  
**Wen-An Chiou**  
*University of Maryland, USA*

**Tuesday, May 24<sup>th</sup>, 2016**

**11.00-12.30 L-1 Cryo-Electron Microscopy of Single Particles**

**Chair: David Bhella**

**Co-chair: Paula da Fonseca**

**(Dalah-2 Room)**

- 11.00-11.30** Towards *in situ* structure analysis of virus-host interactions  
**David Bhella**  
*MRC-University of Glasgow Centre for Virus Research, Scotland*
- 11.30-12.00** High resolution cryo-EM of proteasome complexes as a new tool for therapeutic drug development  
**Paula da Fonseca**  
*Cambridge Biomedical Campus, UK*
- 12.00-12.30** Direct detection and electron counting - A beginning of a new era for electron microscopy  
**Ming Pan**  
*Gatan, Inc, USA*

**Tuesday, May 24<sup>th</sup>, 2016**

**11.00-12.30 A-8 Cognitive Science**

**Chair: Ingrid Liu**

**Co-chair: Gavin Reynolds**

**(Dalah-3 Room)**

- 11.00-11.30** Using behavioral training paradigms and mouse models to delineate memory formation pathways and select novel drug targets  
**Ingrid Liu**  
*Tzu Chi University, Taiwan*
- 11.30-12.00** Abnormal Protein Citrullination in Alzheimer's Disease  
**Akihito Ishigami**  
*Tokyo Metropolitan Institute of Gerontology (TMIG), Japan*

**12.00-12.30** Parvalbumin deficits in psychotic illness - from pathology to epigenetics  
**Gavin Reynolds**  
*Sheffield Hallam University, UK*

**Tuesday, May 24<sup>th</sup>, 2016**

<b>12.30-13.30</b> <b>Lunch Talk</b>
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**Tuesday, May 24<sup>th</sup>, 2016**

<b>13.30-15.30</b> <b>M-1 Low-Dimensional Nanomaterials: Dots, Wires, and Sheets</b> <b>Chair: Takeshi Yanagida</b> <b>Co-chair: Luu Tien Hung</b> <b>(Orchid Ballroom)</b>
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**13.30-14.00**      Material Design of Single Crystalline Metal Oxide Nanowires and Their Promises for Green-innovation and Life-innovation  
**Takeshi Yanagida**  
*Kyushu University, Japan*

**14.00-14.30**      Synthesis, Characterization, Photocatalytic and Bactericidal Activities of (Co, Ag) - Doped ZnO Nanoparticles  
**Luu Tien Hung**  
*Vinh University, Vietnam*

**14.30-14.45**      Nanowires for extracellular vesicles analysis  
**Takao Yasui**  
*Nagoya University, Japan*

**14.45-15.00**      Controlled Nanostructures by Physical Vapor Deposition with Glancing Angle Deposition Technique for Sensor Applications  
**Mati Horprathum**  
*National Electronics and Computer Technology Center, Thailand*

**15.00-15.15**      **OM0103**  
Exploring the Composition-related Stability for Layered Cathode Material for Lithium Ion Battery by Analytical STEM  
**Wei Huang**, Chunyang Wu, Yuewu Zeng, Chuanhong Jin, Ze Zhang

**15.15-15.30**      **OM0104**  
Interacting magnetic nanoparticles of three dimensional magnetic vortex in different configuration assemblies  
**Min-Kwan Kim**, Prasanta Dhak, Ha-Youn Lee, Jae-Hyeok Lee, Myoung-Woo Yoo, Jehyun Lee, Kyoungsuk Jin, Arim Chu, Ki Tae Nam, Hyun Soon Park, Shinji Aizawa, Toshiaki Tanigaki, Daisuke Shindo, Miyoung Kim, Sang-Koog Kim

Tuesday, May 24<sup>th</sup>, 2016

**13.30-15.15 M-2 Semiconductors and LSI Device Materials**

**Chair: Chanchana Thanachayanon**

**Co-chair: Sakuntam Sanorpin**

**(Dalah-1 Room)**

- 13.30-14.00** TEM analysis of cubic GaN films on GaAs (311) oriented substrates grown by MOVPE  
**Sakuntam Sanorpin**  
*Chulalongkorn University, Thailand*
- 14.00-14.30** Depth Quantitative and Chemical State Analysis from Few nm to Several nm using Soft X-ray Emission Spectroscopy  
**Hideyuki Takahashi**  
*JEOL Ltd., Japan*
- 14.30-14.45** TEM Characterization of microstructural evolution of Gallium Nitride grown by MOCVD on c-sapphire  
**Kuttanellore Muraleedharan**  
*Central Glass & Ceramic Research Institute, India*
- 14.45-15.00** **OM0201**  
Investigation on defect related luminescence in semi-polar InGaN/GaN quantum well by using TEM-CL  
**Mi-Hyang Sheen**, Sung-Dae Kim, Jongjin Jang, Okhyun Nam, Young-Woon Kim
- 15.00-15.15** **OM0202**  
Strain analysis by STEM moiré method  
**Yukihito Kondo** and Noriaki Endo

Tuesday, May 24<sup>th</sup>, 2016

**13.30-15.00 A-10 Cell Biology**

**Chair: Takashi Yashiro**

**Co-chair: Apiwat Mutirangura**

**(Bu-nga Room)**

- 13.30-14.00** Analysis of Cell to Cell and Cell to Extracellular Matrix Communication in the Anterior Pituitary Gland  
**Takashi Yashiro**  
*Jichi Medical University School of Medicine, Japan*
- 14.00-14.30** Epigenetic Roles of Repetitive Sequences in Cancer  
**Apiwat Mutirangura**  
*Chulalongkorn University, Thailand*
- 14.30-15.00** Morphology of breast cancer cells overexpressing the Y-Box Binding Protein 1 as observed by immunofluorescence confocal microscopy  
**Boon Huat Bay**  
*National University of Singapore, Singapore*

**Tuesday, May 24<sup>th</sup>, 2016**

**15.00-15.30 CAPSM Business Meeting  
(Bu-nga room)**

**Tuesday, May 24<sup>th</sup>, 2016**

**13.30-15.00 L-2 Cellular Electron Tomography**  
**Chair: José López Carrascosa**  
**Co-Chair: Puey Ounjai**  
**(Dalah-2 Room)**

- 13.30-14.00** Correlation of cryo-soft X-ray tomography and electron microscopy to study nanoparticle-whole cell interaction  
**José López Carrascosa**  
*Centro Nacional de Biotecnología (CNB-CSIC), Spain*
- 14.00-14.30** Structural Insights into Substrate Recognition and Surface Adhesion in *Chlamydomonas reinhardtii*  
**Puey Ounjai**  
*Mahidol University, Thailand*
- 14.30-14.45 OL0201**  
Making the practically impossible 'merely difficult' – cryogenic FIB lift-out for 'damage free' soft matter imaging  
**Christopher Parmenter**, Michael Fay, Cheryl Hartfield, Hoda Eltaher
- 14.45-15.00 OL0202**  
Actin-bundling formation of dimeric human fascin-1 was determined by IHRSR and tomography methods  
**Jeong Min Chung**, Sangmin Lee and Jung, Hyun Suk

**Tuesday, May 24<sup>th</sup>, 2016**

**13.30-15.00 A-3 Stem Cell and Cancer Biology**  
**Chair: George Wai-Cheong Yip**  
**Co-chair: Sam Wah Tay**  
**(Dalah-3 Room)**

- 13.30-14.00** Heparan Sulfate 6-O-Sulfotransferase 2 in Breast Cancer  
**George Wai-Cheong Yip**  
*National University of Singapore, Singapore*
- 14.00-14.30** Mesenchymal Stem Cells Secretome Alters Microglial Proliferation via the Expression of the *ECT2* gene  
**Sam Wah Tay**  
*National University of Singapore, Singapore*
- 14.30-15.00** Modeling  $\beta$ -thalassemia by using induced pluripotent stem cells (iPSCs)  
**Yindee Kitiyanant**  
*Mahidol University, Thailand*

**15.00-15.30 Coffee Break**

Tuesday, May 24<sup>th</sup>, 2016

**15.30-17.00 I-11 Teaching Microscopy: On-line, Hands-on & Remote**

**Chair: Supapan Seraphin**

**Co-chair: Makoto Shiojiri**

**(Orchid Ballroom)**

- 15.30-16.00** Teaching microscopy: sharing wondrous journey into the micro- and nano-world  
**Supapan Seraphin**  
*University of Arizona, USA*
- 16.00-16.15** **OI0101**  
Probing structure and electronic structure of battery materials at atomic scale by scanning transmission electron microscopy  
**Lin Gu**
- 16.15-16.30** **OI0102**  
3D Fourier transform analysis to evaluate a high-performance TEM  
**Kazuo Ishizuka** and Koji Kimoto
- 16.30-16.45** **OI0103**  
Quantitative experimental determination of site-specific magnetic structures by transmitted electrons  
**Xiaoyan Zhong**, Ziqiang Wang, Rong Yu, Jing Zhu
- 16.45-17.00** **OI1101**  
Digital transformation in Microscopy Teaching  
**Silvia Zenner-Gellrich** and Peter Kraemer

Tuesday, May 24<sup>th</sup>, 2016

**15.30-17.30 M-3 Surface, Interfaces, Grain Boundaries, and Coatings**

**Chair: Yuttanant Boonyongmaneerat**

**Co-chair: Sukanda Jiansirisomboon**

**(Dalah-1 Room)**

- 15.30-16.00** Enhancing corrosion resistance of galvanized coating via microstructural engineering and microscopy analysis  
**Yuttanant Boonyongmaneerat**  
*Chulalongkorn University, Thailand*
- 16.00-16.15** **OM0301**  
Challenges in HR-SEM: Direct observation of Micro-, Meso- and Macro-pore openings and fine structure details of nanostructured materials  
**Shunsuke Asahina**, Yusuke SAKUDA, Shunai CHE, Osamu TERASAKI
- 16.15-16.30** **OM0302**  
Metallography Techniques for Microscopy of Electrogalvanized Coatings: Effectiveness of Mechanical, Electrochemical, and Ion polishing  
**Narin Jantaping**, Chaityasit Banjongprasert, Torranin Chairuangstri, Christopher A. Schuh, Yuttanant Boonyongmaneerat
- 16.30-16.45** **OM0303**  
Characterization of the Interface between Metal and Glass Produced by Cold Spray  
**Minghui Song**, Hiroshi Araki, Seiji Kuroda, Kazuhiko Sakaki

- 16.45-17.00 OM0304**  
In-situ Observation of Mutual Reduction between ceramics in TEM  
**N.Ishikawa**, T.Kimura, M.Takeguchi, T Aizawa, T.Inam
- 17.00-17.15 OM0305**  
Real time observation of the reconstructing TiO<sub>2</sub> (001) surface via an Environmental TEM  
Wentao Yuan, Hengbo Li, Hanglong Wu, Ze Zhang, Annabella Selloni, Chenghua Sun, **Yong Wang**
- 17.15-17.30 OM0306**  
In-situ TEM study of oxidation in a nickel-based single crystal superalloy  
**Qingqing Ding**, Tian He, Jixue Li, Ze Zhang

**Tuesday, May 24<sup>th</sup>, 2016**

<b>15.30-17.00 L-3 Cell and Tissues Structures I</b> <b>Chair: Pakorn Kanchanawong</b> <b>Co-chair: Kanokpan Wongprasert</b> <b>(Bu-nga Room)</b>
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- 15.30-16.00** Mapping the nanoscale architecture of cadherin-based adhesions by superresolution microscopy  
**Pakorn Kanchanawong**  
*National University of Singapore, Singapore*
- 16.00-16.30** Adhesion formation and transformation at the cell-matrix interface  
**Cheng-han Yu**  
*University of Hong Kong, China*
- 16.30-16.45 OL0301**  
Histological study of larval development of the blow fly, *Chrysomya megacephala* (Diptera: Calliphoridae)  
**Chutharat Samerjai**, Sangob Sanit, Kwankamol Limsopatham, Suttida Suwannayod, Kom Sukontason, Tarinee Chaiwong, Ratana Leksomboon, Kabkaew L. Sukontason
- 16.45-17.00 OL0302**  
Ultrastructure of Immature Stages of *Lucillia sinensis* Aubertin, 1933 (Diptera: Calliphoridae) for Identification  
**Sangob Sanit**, Chutharat Samerjai, Narin Sontigun, Tunwadee Klong-klaew, Kwankamol Limsopatham, Hiromu Kurahashi, Kom Sukontason, Kabkaew L. Sukontason

**Tuesday, May 24<sup>th</sup>, 2016**

<b>15.30-17.00 L-5 Advanced Techniques in Biological Sample Preparation and Observation</b> <b>Chair: Georg Ramm</b> <b>Co-chair: Keisuke Ohta</b> <b>(Dalah-2 Room)</b>
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- 15.30-16.00** Correlative light and electron microscopy techniques for live-cell imaging and for identifying rare structures in small organisms  
**Georg Ramm**  
*Monash University, Australia*

- 16.00-16.30** Three-dimensional organization of mitochondria-associated membrane revealed by FIB-SEM combined with live cell imaging  
**Keisuke Ohta**  
*Kurume University School of Medicine, Japan*
- 16.30-17.00** Recent developments in preparation of biological samples for electron microscopy  
**Wen An Chiou**  
*University of Maryland, USA*

**Tuesday, May 24<sup>th</sup>, 2016**

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| <b>15.30-17.00</b> | <b>A-7 Aging Science</b><br><b>Chair: Thamthiwat Nararatwanchai</b><br><b>Co-chair: Amornpun Sereemasapun</b><br><b>(Dalah-3 Room)</b> |
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- 15.30-16.00** Scientific method for antiaging research by using bioengineering measurement  
**Thamthiwat Nararatwanchai**  
*Mae Fah Luang University, Thailand*
- 16.00-16.30** Current biomarkers of cellular aging: from morphology to molecular evidences  
**Amornpun Sereemasapun**  
*Chulalongkorn University, Thailand*
- 16.30-16.45** **OA0701**  
 Cerebellar cortical layer atrophy in the aging human-A Post-mortem observation  
**Tahamida yesmin**, Shazia Afrin, Lubna Sharmin, Chandan Kumar Roy,  
 Mohammad Kabir Ahme
- 16.45-17.00** **OA0702**  
 Degenerative Change in Lumbar Facet Joint Surface related with Age in A Thai Population  
**Chanatporn Inthasan**, Sithee Praneatpolgrang, Sukon Prasitwattanaseree,  
 Pasuk Mahakkanukrauh

**Tuesday, May 24<sup>th</sup>, 2016**

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| <b>17.00-18.00</b> | <b>AAT Business Meeting</b><br><b>(Dalah-3 Room)</b> |
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**Wednesday, May 25<sup>th</sup>, 2016**

- 08.00-17.00** **Registration (Graceland Lobby, First Floor)**
- 08.30-09.30** **Keynote Presentation II (Orchid Ballroom)**  
**Innovating New Materials through Aberration-corrected Microscopy**  
**Professor Stephen John Pennycook**  
*National University of Singapore, Singapore*  
 Chair: Prasert Sobhon  
 Co-chair: Kazuo Furuya



**09.30-10.30**      **Keynote Presentation III (Orchid Ballroom)**  
**Visualizing Lipid Regulation of Gene Expression Using New and Classical Imaging Tools**  
**Professor Pavel Hozak**  
*Institute of Molecular genetics ASCR, v.v.i., Czech Republic*  
 Chair: Prasert Sobhon  
 Co-chair: Kazuo Furuya

**10.30-11.00**      **Coffee Break**

**Wednesday, May 25<sup>th</sup>, 2016**

<b>11.00-12.30</b> <b>M-4 Metals, Alloys and Steels</b> <b>Chair: Jer-Ren Yang</b> <b>Co-chair: Torranin Chairuang Sri</b> <b>(Orchid Ballroom)</b>
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**11.00-11.30**      Microstructure observation of Al-Mg-Ge alloy aged at 423K and 473K using TEM  
**Kenji Matsuda**  
*University of Toyama, Japan*

**11.30-11.45**      **OM0401**  
 Aging Strengthening of Interphase-Precipitated Strengthened Copper-Bearing Dual-Phase Steels  
**Cheng-Han Li**, Shao-Pu Tsai, Jer-Ren Yang

**11.45-12.00**      **OM0402**  
 Multi-dimensional analysis of bainite in Cu-Al-Mn alloy by advanced electron microscopy techniques  
 S. Motomura, T. Omori, R. Kainuma, M. Nishida, **T. Hara**

**12.00-12.15**      **OM0403**  
 Spinodal decomposition in CoCuFeMnNi and CuFeMnNiZn high-entropy alloys observed by electron microscopy  
**Ruangdaj Tongsri**, Ussadawut Patakham, Nattaya Tosangthum, Pennapa Muthitamongkol, Plaichumpon Paha, Panida Surawongsakul, Piyanuch Nakpong, Panita Choeychom, Pinai Mungsantisuk

**12.15-12.30**      **OM0404**  
 Analytical investigation of rapidly solidified braze ribbons for transient liquid phase bonding  
**Riza Iskandar**, Ludwig Pongratz, Stefanie Wiesner, Memet Öte, Alexander Schwedt, Kirsten Bobzin, Joachim Mayer

**Wednesday, May 25<sup>th</sup>, 2016**

<b>11.00-12.30</b> <b>I-13 Advances and Applications of Electron Energy loss Spectroscopy</b> <b>I-4 Advances in Electron and X-ray Spectroscopy</b> <b>Chair: Hiroki Kurata</b> <b>Co-chair: Chris Boothroyd</b> <b>(Dalah-1 Room)</b>
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**11.00-11.30**      High-resolution EELS study of organic crystals  
**Hiroki Kurata**  
*Kyoto University, Japan*

- 11.30-12.00** Atomically-Resolved Electron Spectroscopy for Emergent Phenomena at Oxide Interfaces  
**Ming-Wen Chu**  
*National Taiwan University, Taiwan*
- 12.00-12.30** Determining the structure of carbon-based materials using electron energy-loss spectroscopy  
**Chris Boothroyd**  
*Nanyang Technological University, Singapore*

**Wednesday, May 25<sup>th</sup>, 2016**

<b>11.00-12.30</b>	<b>A-4 Cancer Biology and Viruses</b> <b>Chair: George Sai-Wah Tsao</b> <b>Co-chair: Christopher Beng Ti</b> <b>(Bu-nga Room)</b>
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- 11.00-11.30** Live cell imaging of visible Epstein-Barr virus in human epithelial cells  
**George Sai-Wah Tsao**  
*University of Hong Kong, China*
- 11.30-12.00** Precision Medicine in Neuro-Oncology  
**Christopher Beng Ti**  
*Duke-NUS Graduate Medical School, Singapore*
- 12.00-12.30** Detection of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in *Penaeus monodon* by *In-situ* Hybridization at Transmission Electron Microscopic Level  
**Kanokporn Chayaburakul**  
*Rangsit University, Thailand*

**Wednesday, May 25<sup>th</sup>, 2016**

<b>11.00-12.30</b>	<b>L-4 Cell and Tissues Structures II</b> <b>Chair: Steeve Boulant</b> <b>Co-chair: Worawit Suphamungmee</b> <b>(Dalah-2 Room)</b>
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- 11.00-11.30** Dynamics and ultrastructural characterization of the membranous flat clathrin coated arrays  
**Steeve Boulant**  
*German Cancer Research Center (DKFZ), Germany*
- 11.30-12.00** Focused Ion Beam Scanning Electron Microscopy Revealed Three-Dimensional Fine Structure of the Cells  
**Depicha Jindatip**  
*Chulalongkorn University, Thailand*
- 12.00-12.15** **OL0401**  
The combination of atorvastatin and cyanidin-3-glucoside attenuated oxidative stress in vascular smooth muscle cells  
**Rungusa Pantan**, Jiraporn Tocharus, Manussabhorn Phassara, Apichart Suksamrarn, Chainarong Tocharus

**12.15-12.30 OL0402**

Study of the interaction of *Macrobrachium rosenbergii* Nodavirus virus-like particles (MrNV-VLPs) in Sf9 insect cells: the hijacking of caveolin-mediated endocytosis and the possible existence of a protruding VLP capsid ligand domain

**Monsicha Somrit**, Atthaboon Watthammawut, Charoonroj Chotwiwatthanakun, Puey Ounjai, Wattana Weerachatanukul

**Wednesday, May 25<sup>th</sup>, 2016**

**11.00-12.30 A-1 Neuroscience I**

**Chair: Wei Yi Ong**

**Co-Chair: Guo-Fang Tseng**  
(Dalah-3 Room)

**11.00-11.30** Distribution and Function of Phospholipases A2 in the brain

**Wei Yi Ong**

*National University of Singapore, Singapore*

**11.30-12.00** Epigenetic regulation of microglia in the ischemic mouse brain

**S Thameem Dheen**

*National University of Singapore, Singapore*

**12.00-12.30** Cortical, hippocampal and striatal changes accompanied the behavioral alterations in a rat model of infantile hydrocephalus

**Guo-Fang Tseng**

*Tzu-Chi University, Taiwan*

**Wednesday, May 25<sup>th</sup>, 2016**

**12.30-13.30 Lunch Talk**

**Wednesday, May 25<sup>th</sup>, 2016**

**13.30-15.15 M-4 Metals, Alloys and Steels**

**I-13 Advances and Applications of Electron Energy Loss Spectroscopy**

**Chair: Kenji Matsuda**

**Co-chair: Waraporn Piyawit**  
(Orchid Ballroom)

**13.30-14.00** Substructures of lenticular martensite in high-carbon Hig-chromium stainless steel

**Jer-Ren Yang**

*National Taiwan University, Taiwan*

**14.00-14.15 OM0405**

Heat-treated microstructure and property of sintered of Fe-0.85Mo + 4 wt. % SiC + 0.4 wt.% C materials

**Dhriti Tanprayoon** Kittikhun Ruangchai, Amporn Wiengmoon, Rungtip Krataitong, Thanyaporn Yotkaew, Ussadawut Patakham, Ruangdaj Tongsri

**14.15-14.30 OM0406**

Sintered Ductile Iron/Compacted Graphite Iron-like Fe-Mo-Si-C alloy

**Kittikhun Ruangchai**, Amporn Wiengmoon, Monnapas Morakotjinda, Rungtip Krataitong, Dhriti Tanprayoon, Thanyaporn Yotkaew, Nattaya Tosangthum, Ussadawut Patakham, Ruangdaj Tongsri

- 14.30-14.45 OM0407**  
Evidences of Sympathetic Nucleation and Ledgewise Growth Revealed by Scanning Electron Microscopy  
**Wananurat Srijampa**, Amporn Wiengmoon, Ruangdaj Tongsri
- 14.45-15.00 OM0408**  
Characterization of CoCuFeMnNi alloy  
**Panita Choeychom**, Pinai Mungsantisuk, Nattaya Tosangthum, Rungtip Krataitong, Thanyaporn Yotkaew, Ussadawut Patakham, Ruangdaj Tongsri
- 15.00-15.15 OI1301**  
Effects of nickel oxide underlayer on the catalytic properties of manganese oxide nanoparticles  
**Sangmoon Yoon**, Kyoungsuk Jin, Won Woo Chung, Ki Tae Nam and Miyoung Kim

**Wednesday, May 25<sup>th</sup>, 2016**

**13.30-15.30 I-3 New Methods in Diffraction and Imaging**

**Chair: Kenji Tsuda**

**Co-chair: Fu-Rong Chen**  
**(Dalah-1 Room)**

- 13.30-14.00** Study of nanoscale local structures of perovskite-type ferroelectrics using STEM-CBED  
**Kenji Tsuda**  
*Tohoku University, Japan*
- 14.00-14.30** Atomic Resolution Tomography of Nanoparticles Reconstructed from Exit Wave  
**Fu-Rong Chen**  
*National Tsing Hua University, Taiwan*
- 14.30-14.45 OI0301**  
Electron diffraction analysis of microcrystalline of organic molecules  
**Tetsuo Oikawa**, Anna Liew, Yusuke Nishiyama
- 14.45-15.00 OI0302**  
Quantification of crystallinity using energy filtered electron diffraction  
**Byeong-Seon An**, Tae-Hoon Kim, Cheol-Woong Yang
- 15.00-15.15 OI0303**  
A Relativistic-energy femtosecond-pulse electron microscopy  
**Jinfeng Yang**, **Yoichi Yoshida**, **Katsumi Tanimura**
- 15.15-15.30 OI0304**  
MEMS Waveguide Sensor for Photoacoustic Detection  
**Supanee Learkthanakhachon**, Suejit Pechprasarn, Mike Somekh

**Wednesday, May 25<sup>th</sup>, 2016**

**13.30-15.00 I-7 Advanced Light-Optical Microscopy**

**Chair: Suejit Pechprasarn**

**Co-chair: Michael Somekh**

**(Bu-nga Room)**

- 13.30-14.00** Surface wave attenuation coefficient measurement using confocal surface plasmon microscopy  
**Suejit Pechprasarn**  
*Hong Kong Polytechnic University, China*
- 14.00-14.30** Evanescent wave and confocal microscopy  
**Michael Somekh**  
*Hong Kong Polytechnic University, China*
- 14.30-14.45 OI0701**  
Ultra-sensitive biosensor using double-metallic-layer-waveguide structure  
**Mengqi Shen**, Jingkai Meng, Supannee Learkthanakhachon, Suejit Pechprasarn, Michael G. Somekh, Yaping Zhang, Chung W. See
- 14.45-15.00 OI0702**  
Embedded interferometry with dynamic reference beam  
**Wai-Kin Chow**, Suejit Pechprasarn, Michael G. Somekh

**Wednesday, May 25<sup>th</sup>, 2016**

**13.30-15.15 L-6 Confocal and other Microscopies in Biomedical Sciences**

**Chair: David Becker**

**Co-chair: Wattana Weerachatanukul**

**(Dalah-2 Room)**

- 13.30-14.00** Targeting connexins to improve chronic wound healing  
**David Becker**  
*Nanyang Technological University, Singapore*
- 14.00-14.30** C-terminal domain on the outer surface of the *Macrobrachium rosenbergii* Nodavirus capsid is required for Sf9 cell binding and internalization”  
**Wattana Weerachatanukul**  
*Mahidol University, Thailand*
- 14.30-14.45 OL0601**  
Active Targeted Delivery of PLGA-Liposome Nanoparticles Specific for Cervical Cancer Therapy  
**Suphawadee Bunthot**, Somsak Saesoo, Katawut Namdee, Teerapong Yata, Suwatchai Jarussophon, Pawinee Pongwan, Uracha Raktanonchai, Kunat Suktham, Suvimol Surassmo, Nattika Sangkrit
- 14.45-15.00 OL0602**  
The Airyscan Detector from Zeiss: Confocal imaging with improved signal-to-noise ratio, faster speed and super-resolution  
**Xianke Shi**

- 15.00-15.15      OL0603**  
 Nanoscale approaches to characterize the dynamic and biophysical determinants of receptor-mediated virus internalization  
Megan Stanifer, Marta Fratini, Tina Wiegand, Ada Cavalcanti-Adam, Steeve Boulant

**Wednesday, May 25<sup>th</sup>, 2016**

<b>13.30-15.30      A-9 Surgical and Clinical Anatomy</b> <b>Chair: Meechai Srisai</b> <b>Co-chair: Pasuk Mahakkanukrauh</b> <b>(Dalah-3 Room)</b>
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- 13.30-14.00**      A continuing study model from preclinical, clinical, through postgraduate medical education  
**Meechai Srisai**  
*Chulalongkorn University, Thailand*
- 14.00-14.30**      Surgical and Clinical Anatomy  
**Pasuk Mahakkanukrauh**  
*Chiang Mai University, Thailand*
- 14.30-15.00**      Training and Education Center for Clinical Skills  
**Rossarin Rattanalekha**  
*Mahidol University, Thailand*
- 15.00-15.15      OA0901**  
 Stature Estimation from Skull using Craniometric Method in A Thai Population  
Chirapat Inchai, Phuwadon Duangto, Sukon Prasitwattanaseree  
 Pasuk Mahakkanukrauh
- 15.15-15.30      OA0902**  
 Extensor tendons and variations of the medial four digits of hand: a cadaveric study  
Athikhun Suwannakhan, Tulyapruet Tawonsawatruk, Krai Meemon

<b>15.30-16.00      Coffee Break</b>
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**Wednesday, May 25<sup>th</sup>, 2016**

<b>15.30-17.30      Poster Presentation</b>  <p style="text-align: center;"><b>“All presenters should be present at their poster board.”</b></p>
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**Wednesday, May 25<sup>th</sup>, 2016**

<b>17.00-18.00      MST Meeting</b> <b>(Dalah-1 Room)</b>
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**Wednesday, May 25<sup>th</sup>, 2016**

<b>18.30-21.30      Conference Banquet</b> <b>(Orchid Ballroom)</b>
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Thursday, May 26<sup>th</sup>, 2016

08.30-17.00 Registration (Graceland Lobby, First Floor)

Thursday, May 26<sup>th</sup>, 2016

09.00-10.30 **M-6 Amorphous Materials and Quasicrystals**  
**Chair: Eiji Abe**  
**Co-chair: Nattapol Laorodphan**  
**(Orchid Ballroom)**

09.00-09.30 Structure determination of quasicrystals: STEM imaging and hyperspace x-ray crystallography

**Eiji Abe**

*University of Tokyo, Japan*

09.30-10.00 Real time observation of the reconstructing TiO<sub>2</sub> (001) surface via ETEM

**Ze Zhang**

*Zhejiang University, China*

10.00-10.30 Structural Characterization of Amorphous Materials via Electron Beam Radial Distribution Function Analysis

**Manabu Ishimaru**

*Kyushu Institute of Technology, Japan*

Thursday, May 26<sup>th</sup>, 2016

09.00-11.00 **I-8 Scanning Probe Microscopy**  
**I-9 Atom Probe Microscopy**  
**Chair: Michael Hietschold**  
**Co-chair: Tongjai Choolajorm**  
**(Dalah-1 Room)**

09.00-09.30 Scanning Tunneling Microscopy Investigations of Pattern Control in Molecular Self-Assembly

**Michael Hietschold**

*Technische Universität Chemnitz, Germany*

09.30-10.00 Development of nanomanipulator based on scanning probe microscopes for biological applications

**Futoshi Iwata**

*Graduate School of Engineering, Shizuoka University, Japan*

10.00-10.30 Analysis of Geological Materials by Atom Probe: Understanding the Mechano-chemical Behaviour of Zircon

**Julie Cairney**

*University of Sydney, Australia*

10.30-11.00 Revealing the internal structures of nanostructured alloys using atom probe tomography

**Tongjai Choolajorm**

*National Metal and Materials Technology Center, Thailand*



Thursday, May 26<sup>th</sup>, 2016

**09.00-10.30 I-5 In Situ Dynamics in TEM and SEM, Environmental Microscopy**

**Chair: Yoshifumi Oshima**

**Co-chair: Masanori Koshino**

**(Bu-nga Room)**

- 09.00-09.30** The Morphological Study of Wet Biopolymer Capsules with Living Cells by Environmental Scanning Electron Microscope AQUASEM II  
**Vilem Nedela**  
*Institute of Scientific Instruments of the CAS, v. v. i., Czech Republic*
- 09.30-10.00** A novel approach for the scanning electron microscopy at ambient atmospheric pressures  
**Yusuke Ominami**  
*Hitachi High-Technologies Corporation, Japan*
- 10.00-10.15 OI0501**  
In situ TEM Study of Vapor-Solid Nanotube Growth  
**Zhengfei Zhang**, Yong Wang, Hengbo Li, Wentao Yuan, Ze Zhang
- 10.15-10.30 OI0502**  
In situ electron holography of electric potentials inside a solid-state electrolyte: Effect of 3D electric-field leakage  
**Tsukasa Hirayama**, Yuka Aizawa, Kazuo Yamamoto, Takeshi Sato, Hidekazu Murata, Ryuji Yoshida, Craig A. J. Fisher, Takehisa Kato, Yasutoshi Iriyama

Thursday, May 26<sup>th</sup>, 2016

**09.00-10.30 L-7 Microscopy Applications in Pathology and Laboratory Medicine**

**Chair: Suchin Worawichawong**

**Co-Chair: Hing Hiang Lian**

**(Dalah-2 Room)**

- 09.00-09.30** Role of microscopy in diagnosis of transplant kidney  
**Suchin Worawichawong**  
*Mahidol University, Thailand*
- 9.30-10.00** Studying Virus Trafficking in Cells using Immune Fluorescence Microscopy"  
**Mark Douglas**  
*University of Sydney at Westmead Hospital, Australia*
- 10.00-10.30** Evaluation of cells integrity using different fixation time using scanning electron microscopy  
**Hing Hiang Lian**  
*Universiti Kebangsaan Malaysia, Malaysia*

Thursday, May 26<sup>th</sup>, 2016

**09.00-10.30 L-9 Reproductive Biology and Glycobiology**

**Chair: Ken Kitajima**

**Co-Chair: Krai Meemon**

**(Dalah-3 Room)**

- 09.00-09.30** Effects of polyunsaturated fatty acids and prostaglandins on crustacean reproduction  
**Prasert Sobhon**  
*Mahidol University, Thailand*

**09.30-10.00** Glycan-mediated interactions on pig sperm lipid rafts during fertilization  
**Ken Kitajima**  
*Nagoya University, Japan*

**10.00-10.30** Chemical tools to image glycans by metabolic labeling  
**Yann Gueraedel**  
*Univ. Lille, France*

**10.30-11.00 Coffee Break**

**Thursday, May 26<sup>th</sup>, 2016**

**11.00-12.30 M-5 Ceramics and Inorganic Materials**

**Chair: Naoya Shibata**

**Co-chair: Bralee Chayasombat**  
**(Orchid Ballroom)**

**11.00-11.30** Development of atomic-resolution scanning transmission electron microscopy for ceramic interfaces  
**Naoya Shibata**  
*University of Tokyo, Japan*

**11.30-12.00** Assessment of Strain-generated Oxygen Vacancies Using SrTiO<sub>3</sub>Bicrystals  
**Si-Young Choi**  
*Korea institute of materials science, South Korea*

**12.00-12.15** TEM and STEM Investigations of Oxide Thermoelectrics  
**Miran Čeh**  
*Jožef Stefan Institute, Slovenia*

**12.15-12.30 OM0501**  
In situ Studies of Heat Induced Precipitation of Yttria Precursor using Liquid-Cell Transmission Electron Microscopy  
**Sašo Šturm**, Bojan Ambrožič, Nina Kostevšek, Marjan Bele, Kristina Ž. Rožman

**Thursday, May 26<sup>th</sup>, 2016**

**11.00-12.30 I-9 Atom Probe Microscopy**

**I-10 Theoretical Modeling and Analysis in Electron Microscopy**

**Chair: Fu-Rong Chen**

**Co-chair: Raynald Gauvin**  
**(Dalah-1 Room)**

**11.00-11.30** Atomic Scale Mechanical Microscopy  
**Xiao Dong Han**  
*Beijing University of Technology, China*

**11.30-12.00** Quantitative X-Ray Microanalysis and High Resolution Imaging With Monte Carlo Simulations  
**Raynald Gauvin**  
*McGill University, Canada*

- 12.00-12.15 OI0901**  
Improvement of mass resolution in atom probe tomography for oxide materials with surface modification  
**Chang Min Kwak**, Jae Bok Seol, Chan Gyung Park
- 12.15-12.30 OI1001**  
Quality enhancement and strain measurement in HAADF images using Super-Resolution techniques  
G. Bárcena-González, M. P. Guerrero-Lebrero, E. Guerrero, D. F. Reyes, D. González, A. Mayoral, A. D. Utrilla, J. M. Ulloa, **P. L. Galindo**

**Thursday, May 26<sup>th</sup>, 2016**

<b>11.00-12.30 I-5 In Situ Dynamics in TEM and SEM, Environmental Microscopy</b> <b>Chair: Vilem Nedela</b> <b>Co-chair: Yusuke Ominami</b> <b>(Bu-nga Room)</b>
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- 11.00-11.30** *Operand* Transmission Electron Microscope Observation of Lithium Ion Battery–What happened during the Operation  
**Yoshifumi Oshima**  
*Japan Advanced Institute of Science and Technology, Japan*
- 11.30-12.00** Application of Low-voltage S/TEM With EELS and EDS for Soft Materials Analysis  
**Masanori Koshino**  
*National Institute of Advanced Industrial Science and Technology (AIST), Japan*
- 12.00-12.15 OI0503**  
Improved heating stage for EBSD *In-situ* work  
**Seiichi SUZUKI** and Tatsuya FUKINO
- 12.15-12.30 OI0504**  
In-situ TEM study of nano-scale conductive filament used for threshold selector device  
**Byeong Gyu Chae**, Kyung Joon Baek, Jeong Hwan Song, Jae Bok Seol, Hyun Sang Hwang, Sang Ho Oh, Chan Gyung Park

**Thursday, May 26<sup>th</sup>, 2016**

<b>11.00-12.15 L-8 Mass Spectrometry</b> <b>Chair: Mitsutoshi Setou</b> <b>Co-chair: Scott F. Cummins</b> <b>(Dalah-2 Room)</b>
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- 11.00-11.30** Platform of functional metabolic imaging  
**Mitsutoshi Setou**  
*Hamamatsu University School of Medicine, Japan*
- 11.30-12.00** Mass spectral identification of aquatic 'excretomes' that dictate key ecological interactions  
**Scott F. Cummins**  
*University of the Sunshine Coast, Australia*

- 12.00-12.15      OL0801**  
Multimodal approach to improving *Macrobrachium rosenbergii* aquaculture through in-depth studies into MrNV infection and sperm-egg interaction processes  
**Atthaboon Watthammawut**, Monsicha Somrit, Somluk Asuvapongpatana, Charoonroj Chotwiwatthanakun, Wattana Weerachatanukul

**Thursday, May 26<sup>th</sup>, 2016**

<b>11.00-12.30      A-2 Neuroscience II</b> <b>Chair: Sutisa Thanoi</b> <b>Co-chair: Sukumal Chongthammakun</b> <b>(Dalah-3 Room)</b>
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- 11.00-11.30      Deficits in the GABAergic System after Exposure to Abused Drugs**  
**Sutisa Thanoi**  
*Naresuan University, Thailand*
- 11.30-11.45      OA0201**  
Methamphetamine impairs the P-gp transporter in primary rat brain microvascular endothelial cells  
**Pichaya Jumnonprakhon**, Piyarat Govitrapong, Chainarong Tocharus, Jiraporn Tocharus
- 11.45-12.00      OA0202**  
Characterization of olfactory ionotropic glutamate receptors 25a in the giant freshwater prawn, *Macrobrachium rosenbergii*  
**Natwalee Jaikunta**, Thanapong Kruangkum, Charoonroj Chotwiwatthanakun, Prasert Sobhon, Rapeepun Vanichviriyakit
- 12.00-12.15      OA0203**  
Role of Fluoride ingestion in the pathogenesis of Alzheimer's Disease  
**Raghu Jetti**, Raghuveer CV, Mallikarjuna Rao C
- 12.15-12.30      OA0204**  
Entrapment of the Martin-Gruber anastomotic nerve in the forearm: a case report  
**Anu V Ranade**, Rajalakshmi Rai, Muralimanju BV

**Thursday, May 26<sup>th</sup>, 2016**

<b>12.30-13.30      Lunch Talk</b>
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**Thursday, May 26<sup>th</sup>, 2016**

<b>13.30-15.30      M-11 Microscopy and Microanalysis in Industry</b> <b>M-5 Ceramics and Inorganic Materials</b> <b>Chair: Lynne Gignac</b> <b>Co-chair: Paul E. Fishchione</b> <b>(Orchid Ballroom)</b>
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- 13.30-14.00      Imaging 8  $\mu$ m thick semiconductor interconnects using 300-400 keVSE, BSE, STEM and 3D FIB-SEM**  
**Lynne Gignac**  
*IBM T. J. Watson Research Center, USA*

- 14.00-14.30** Unlocking structure and chemistry at the atomic level through advanced TEM specimen preparation  
**Paul E. Fishchione**  
*E.A. Fischione Instruments, Inc., USA*
- 14.30-15.00** Failure Analysis of Semiconductor Devices by FIB-SEM and TEM-EEL  
**Yasufumi Yabuuchi**  
*Panasonic Corporation, Japan*
- 15.00-15.15** **OM0502**  
Synthesis and characterization of mixed phase TiO<sub>2</sub>(B)/anatase TiO<sub>2</sub> thin films on a number of different substrates by a modified low pressure chemical vapour deposition (LPCVD)  
**Yothin Chimupala**, Robert Simpson, Patcharanan Junploy, Rik Brydson
- 15.15-15.30** **OM0503**  
Microstructural evolution of hydroxy/fluoroapatite in the different precursor solution under hydrothermal process  
Wisarat Ngoenthong and **Upsorn Boonyang**

Thursday, May 26<sup>th</sup>, 2016

<b>13.30-15.30</b>	<b>M-7 X-ray Analysis of Geological / Mineralogical Materials</b> <b>M-8 Novel Applications of Material Science and Engineering</b> Chair: Nirawat Thammajak Co-chair: Syo Matsumura (Dalah-1 Room)
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- 13.30-14.00** X-ray Absorption Spectroscopy for Gemology and Mineralogy Applications: A Case Study of Fresh-Water Cultured Pearls"  
**Nirawat Thammajak**  
*Synchrotron Light Research Institute, Thailand*
- 14.00-14.30** Structural Investigation of Some Nanocomposite Powders for Li-ion Battery Anode Applications  
**Makoto Shiojiri**  
*Kyoto Institute of Technology, Japan*
- 14.30-15.00** Atomic Resolution Quantification of Elemental Ratio  
**Mitsutaka Haruta**  
*Kyoto University, Japan*
- 15.00-15.15** **OM0801**  
Preparation of Electrospun Polystyrene Fibrous Membranes Containing Silver Nanoparticles  
**Chutima Srisitthiratkul**, Nuttaporn Pimpha and Wiyong Kangwansupamonkon
- 15.15-15.30** **OM0802**  
Quick Evaluation of Electric Field Surrounding a Charged Particle  
**Katsuhiro Sasaki**, Tomoharu Tokunaga, Takahisa Yamamoto

Thursday, May 26<sup>th</sup>, 2016

**13.30-15.30** I-5 In Situ Dynamics in TEM and SEM, Environmental Microscopy  
I-12 Surface Microscopy with LEEM, PEEM, UHV-SEM, REM and SREM  
**Chair: Vilem Nedela**  
**Co-chair: Ing Eva Tihlaříková**  
(Bu-nga Room)

- 13.30-14.00** Observation of dynamic phenomena using Atmospheric Scanning Electron Microscope (ASEM)  
**Mitsuo Suga**  
*JEOL Ltd., Japan*
- 14.00-14.30** *In-situ* preparation method for repetitive ESEM and SEM observation of plant samples  
**Ing Eva Tihlaříková**  
*Institute of Scientific Instruments of the CAS, v. v. i., Czech Republic*
- 14.30-15.00** Strong perpendicular magnetization of ferromagnetic multi-layers with high brightness and highly spin-polarized LEEM  
**Takanori Koshikawa**  
*Osaka Electro-Communication University, Japan*
- 15.00-15.30** Imaging and tailoring chiral magnetism  
**Andreas Schmid**  
*Lawrence Berkeley National Lab, USA*

Thursday, May 26<sup>th</sup>, 2016

**13.30-14.45** A-11 Reproductive Biology  
**Chair: Samur Thanoi**  
**Co-chair: Somluk Asuvapongpattana**  
(Dalah-2 Room)

- 13.30-14.00** Methamphetamine and Changes of Sperm Quality  
**Samur Thanoi**  
*Naresuan University, Thailand*
- 14.00-14.15** **OA1101**  
Can Parallels be drawn among microscopic features, hysteroscopy and endometrial thickness in patients with Abnormal Uterine Bleeding?  
**Shruti Saralaya**, Supriya K, Suchitra Thunga, Nina A Mahale, Vimala Ramachandran
- 14.15-14.30** **OA1102**  
Alpha-2 macroglobulin is involved in sperm activation and decapacitation factor of the blue swimming crab, *Portunus pelagicus*  
**Thanyaporn Senarai**, Shinji Miyata, Chihiro Sato, Rapeepun Vanichviriyakit, Wattana Weerachatanukul, Prasert Sobhon, Ken Kitajima, Prepee Sretarugsa
- 14.30-14.45** **OA1103**  
Alteration of GABA Concentrations in Rat Testis after Methamphetamine Exposure  
**Paweena Kaewman**, Sutisa Nudmamud-Thanoi, Samur Thanoi

Thursday, May 26<sup>th</sup>, 2016

**13.30-15.30 A-5 Applied and Clinical Anatomical Sciences**

**Chair: Wojciech Pawlina**

**Co-chair: Kittikun Vivapinyo**

**(Dalah-3 Room)**

- 13.30-14.00** Teaching Microscopic Anatomy in Medical Curriculum: Changes, Trends, and Competencies  
**Wojciech Pawlina**  
*Mayo Clinic College of Medicine, USA*
- 14.00-14.15** **OA0501**  
Association between age and distal femoral articular surface of knee joint morphological changes in dry bone in a Thai population  
**Sirimas Prathum**, Sirada Sukhsawasdi Naayuthaya, Robert Mann, Pasuk Mahakkanukrauh
- 14.15-14.30** **OA0502**  
Association between age and tibial articular surface of knee joint morphological changes in dry bone in a Thai population  
**Kan Wangpiriyapanich**, Rattaphon Inphai, Kachitphong Boosuan, Robert Mann, Pasuk Mahakkanukrauh
- 14.30-14.45** **OA0503**  
Association between age and distal patellar articular surface of knee joint morphological changes in dry bone in a Thai population  
**Suphawita Pliannuom**, Suphawit Boondiskulchok, Robert Mann, Pasuk Mahakkanukrauh
- 14.45-15.00** **OA0504**  
Association between age and acetabular morphological changes in dry bones in a Thai population  
**Jirath Suriyasathaporn**, Worachot Chotecharnont, Pandaree Khomkham, Pawika Srinuan, Robert Mann, Pasuk Mahakkanukrauh
- 15.00-15.15** **OA0505**  
Age Estimation from Pubic Symphysis in a Thai Population by Using Method of Boldsen *et al.*  
**Pornhatai Komutrattananont**, Sittiporn Ruengdit, Sukon Prasitwattanaseree, Pasuk Mahakkanukrauh
- 15.15-15.30** **OA0506**  
Age Estimation from the Auricular Surface of the Ilium in a Thai Population by Using Method of Boldsen *et al.*  
**Treerat Gumpangseth**, Phruksachat Singsuwan, Sukon Prasitwattanaseree, Pasuk Mahakkanukrauh

**15.30-16.00 Coffee Break**



Thursday, May 26<sup>th</sup>, 2016

**16.00-17.15 M-11 Microscopy and Microanalysis in Industry**

**Chair: Lynne Gignac**

**Co-chair: Paul E. Fishchione**

**(Orchid Ballroom)**

- 16.00-16.30** Industrial-user-oriented Functionality of Transmission Electron Microscopy Application Software  
**Hiromitsu Furukawa**  
*SYSTEM IN FRONTIER INC., Japan*
- 16.30-16.45 OM1101**  
Scanning electron microscopes with integrated Raman spectrometer revealing new complementary information  
Stefanie Freitag and **Fang Zhou**
- 16.45-17.00 OM1102**  
Surface sensitive imaging using energy filtered SEM at low kV  
**Fang Zhou** and Markus Bose
- 17.00-17.15 OM1103**  
Origin of dramatic oxygen solute strengthening effect in Titanium  
**Qian Yu**

Thursday, May 26<sup>th</sup>, 2016

**16.00-16.45 M-8 Novel Applications of Material Science and Engineering**

**Chair: Nirawat Thammajak**

**Co-chair: Thapanee Sarakonsri**

**(Dalah-1 Room)**

- 16.00-16.30** La ordering in epitaxial  $\text{Li}_{3x}\text{La}_{2/3-x}\text{TiO}_3$  films and its effects on Li-ion conduction  
**Kazutaka Mitsuishi**  
*National Institute for Materials Science, Japan*
- 16.30-16.45 OM0803**  
Inclusion Complex between water-soluble quaternized  $\beta$ -cyclodextrin grafted chitosan and  $\alpha$ -mangostin: Influence of degree of  $\beta$ -CD substituted on chitosan  
**Sarunya Phunpee**, Somsak Saesoo, Anchalee jintapattanakit, Uracha Rungsardthong Ruktanonchai

Thursday, May 26<sup>th</sup>, 2016

**16.00-17.15 I-5 In situ Dynamics in TEM and SEM, Environmental Microscopy**

**I-12 Surface Microscopy with LEEM, PEEM, UHV-SEM, REM and SREM**

**Chair: Takanori Koshikawa**

**Co-chair: Sumet Sakulsermsuk**

**(Bu-nga Room)**

- 16.00-16.30** Structure/Dynamics of Two-Dimensional Layers Studied by Low Energy Electron Microscopy and nano-Low Energy Electron Diffraction  
**Michael S. Altman**  
*Hong Kong University of Science and Technology, China*

- 16.30-16.45**      **OI0505**  
*In-situ* TEM study of deformation behavior in a dual-phase high-entropy alloy  
 AlCoCrFeNi  
Qiannan Wang and Qian Yu
- 16.45-17.00**      **OI0506**  
*In-situ* nanoengineering based on TEM  
 Tao Xu, Hengchang Bi, Shu Wan, Jun Sun, Longbing He, Kuibo Yin, Litao Sun
- 17.00-17.15**      **OI0507**  
 Ultra-high resolution SEM for 3D analysis in biology  
Jarosav Jiruse, Jaroslav Jiruse, Miloslav Havelka, Jan Polster

**Thursday, May 26<sup>th</sup>, 2016**

**16.00-17.00**      **A-6 Teaching Anatomy: On-line, Hands-on & Remote**  
**Chair: Kem A. Rogers**  
**Co-chair: Kulathida Chaithirayanon**  
**(Dalah-3 Room)**

- 16.00-16.30**      Design and Implementation of Online Courses in Anatomy and Histology and the Perception of Both Students and the Faculty  
**Kem A. Rogers**  
*Western University, Canada*
- 16.30-17.00**      Cognitive load and commercial Software Design for Teaching Anatomical Sciences  
**Sonya Van Nuland**  
*Western University, Canada*

**Friday, May 27<sup>th</sup>, 2016**

**08.30-12.30**      **Registration (Graceland Lobby, First Floor)**

**Friday, May 27<sup>th</sup>, 2016**

**09.00-10.00**      **M-10 Mineral Geochronology & Isotope Characteristics by X-ray & Ions**  
**Chair: Boontarika Srithai**  
**Co-chair: Richard Wirth**  
**(Orchid Ballroom)**

- 09.00-09.30**      Reviews of geochronology of igneous rocks: implications for tectonic evolution of Thailand  
**Boontarika Srithai**  
*Chiang Mai University, Thailand*
- 09.30-10.00**      TEM and FIB: significance of nanoinclusions and microstructure for geochronology  
**Richard Wirth**  
*German Research Centre for Geosciences GFZ, Germany*

Friday, May 27<sup>th</sup>, 2016

**09.00-10.00 I-6 EBSD of Synthetic and Natural Materials**

**Chair: Chaiyasit Banjongprasert**

**Co-chair: Yothin Chimupala**

**(Dalah-1 Room)**

**09.00-09.30** Applications of EBSD on Microstructural Investigation of Aluminum and Its Alloys  
**Chaiyasit Banjongprasert**  
*Chiang Mai University, Thailand*

**09.30-09.45 OI0601**  
Study of pseudo-symmetric misindexing in EBSD analysis of  $\gamma$ -TiAl alloys with refined accuracy band detection  
**Wu Jiang**, Niels-Henrik Schmidt, Alberto Palomares-García, Rocío Muñoz-Moreno, Jenny Goulden

**09.45-10.00 OI0602**  
Direct observation of low angle boundary migration during recrystallization using electron channeling contrast imaging and electron backscatter diffraction  
**Jin-Su Oh**, Seung-Moon Baek, Tae-Hoon Kim, Jee-Hwan Bae, Cheol-Woong Yang

**10.00-10.30 Coffee Break**

Friday, May 27<sup>th</sup>, 2016

**10.30-12.00 Closing Ceremony and Award Presentation**

**APMC12 Announcement**

**(Orchid Ballroom)**

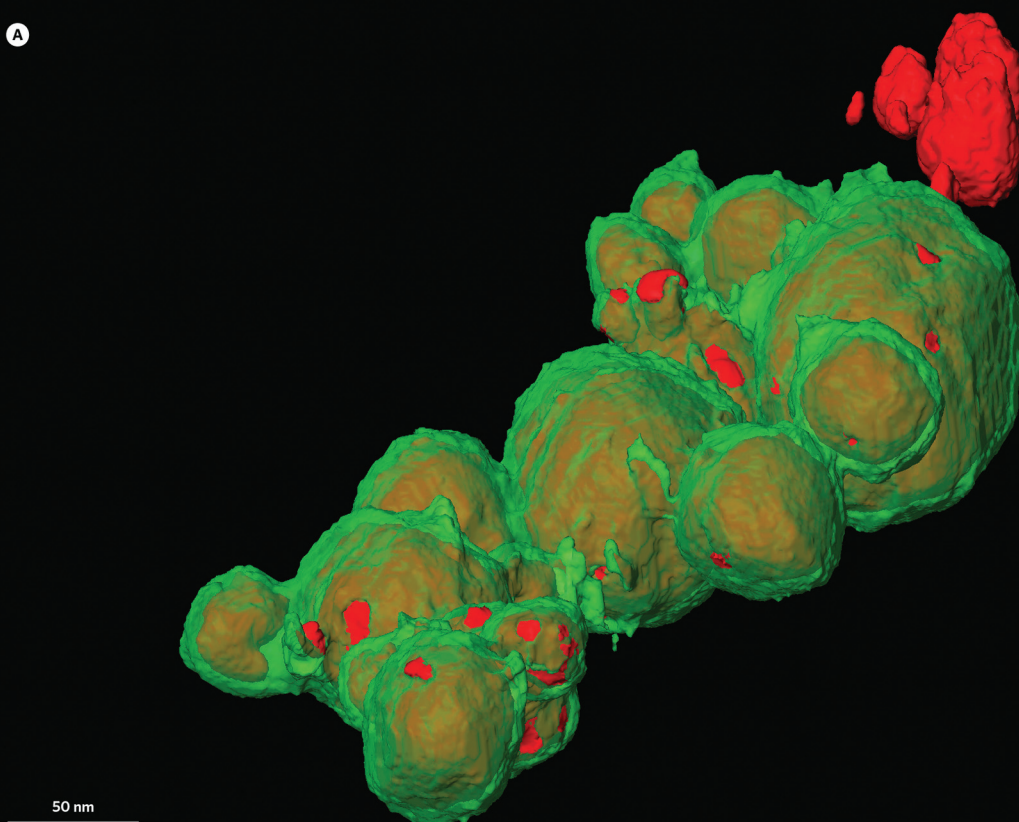
Friday, May 27<sup>th</sup>, 2016

**12.00-13.30 Lunch**

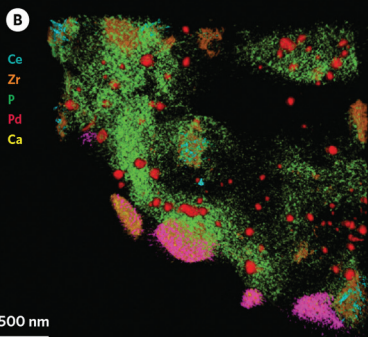
**(Bua Luang Restaurant)**

# **Keynote Presentation**

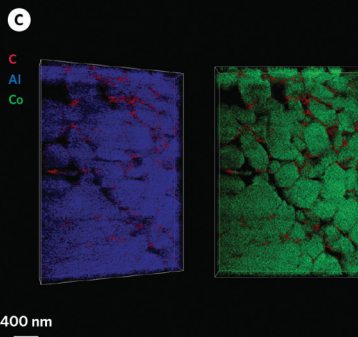
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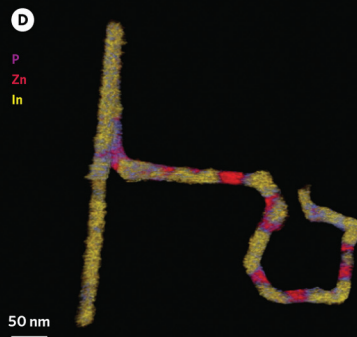
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C



D



**A:** EDS tomogram of Ag-Pt core-shell nanoparticles. Ag cores are shown in the false color of red, covered by green-colored Pt shells, only a few nanometers in thickness. Sample courtesy Prof. Yi Ding and Prof. Jun Luo, Center for Electron Microscopy, Tianjin University of Technology. **B:** Vehicle-aged automotive catalyst. EDS tomogram showing the distribution of Palladium particles (red) relative to other elements. **C:** Battery anode material. EDS tomograms of Carbon-Cobalt and Carbon-Aluminum. **D:** EDS tomogram of P-Zn-In nanotubes. Sample Courtesy of Dr. Reza Shahbazian Yassar, Michigan Tech University.

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**Professor Joachim Mayer**  
Central Facility for Electron Microscopy  
RWTH Aachen University  
52074 Aachen, GERMANY.  
And Ernst Ruska-Centre  
Forschungszentrum Jülich  
52425 Jülich, GERMANY  
E-mail: mayer@gfe.rwth-aachen.de

## **From Nano to PICO – the Next Generation of Aberration Corrected TEMs**

The introduction of aberration correctors has revolutionized the development of TEM and STEM instrumentation. In order to provide a platform for these novel developments and based on the experience with the first aberration corrected TEM, Research Centre Jülich and RWTH Aachen University have jointly founded the Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons (ER-C). Recently, the PICO instrument has been installed at the ER-C, which is the second high resolution TEM in the world with a corrector for the chromatic aberration.

In the presentation, the electron optical background and the initial results obtained with the PICO instrument will be presented. With PICO, atomically resolved images can be obtained with simultaneous correction of the spherical and the chromatic aberration. The benefits of chromatic aberration corrected imaging are particularly large for HRTEM imaging at low accelerating voltages and for energy filtered (EFTEM) imaging with large energy window width. In the present contribution, I will focus on these two applications and will present results from our recent work.

*Joachim Mayer has been professor and head of the Central Facility for Electron Microscopy at RWTH Aachen University since 1999. In 2004, he received a co-appointment as one of the two directors of the newly founded Ernst Ruska-Centre, a German national user facility jointly founded by the Research Centre Jülich and RWTH Aachen University. He studied Physics at the University of Stuttgart, and received his Ph. D. in Physics at the Max-Planck-Institut für Metallforschung, Stuttgart, in 1988. After completing his Ph. D., he joined the Materials Department at the University of California, Santa Barbara, as a postdoctoral researcher for two years and then moved back to the Max-Planck-Institut für Metallforschung, Stuttgart. His research focuses on the application of new methods in electron microscopy in the areas of materials science, nanoelectronics and energy systems.*



**Professor Stephen Pennycook**  
National University of Singapore,  
Department of Materials Science and Engineering,  
9 Engineering Drive 1,  
Block EA, 07-14  
117575 Singapore.  
E-mail: [steve.pennycook@nus.edu.sg](mailto:steve.pennycook@nus.edu.sg)

## Innovating New Materials through Aberration-corrected Microscopy

The aberration-corrected scanning transmission electron microscope (STEM) can provide real space imaging and spectroscopy at atomic resolution with a new level of sensitivity to structure, bonding, elemental valence and even spin state [1,2]. Coupled with first-principles theory, this represents an unprecedented opportunity to probe the functionality of complex nanoscale systems, which in turn can lead to new materials innovations. Examples will be shown of the direct imaging of atomic diffusion within a solid [3], the identification of the active site in a catalyst [4], the role of interface termination on ferroelectricity in BiFeO<sub>3</sub> (BFO) films grown on La<sub>0.5</sub>Sr<sub>0.5</sub>MnO<sub>3-x</sub> (LSMO) [5], the origin of white light emission in ultra-small CdSe nanocrystals [6] and some surprising insights into the role of defects in CdTe solar cells [7]. Finally, some thoughts on future directions for aberration-corrected microscopy will be presented [8].

- [1] W. Zhou, et al., "Direct determination of the chemical bonding of individual impurities in graphene," *Phys Rev Lett*, **109**, 206803 (2012).
- [2] J. Gazquez, et al., "Atomic-Resolution Imaging of Spin-State Superlattices in Nanopockets within Cobaltite Thin Films," *Nano Lett*, **11**, 973–976 (2011).
- [3] R. Ishikawa, et al., "Direct Observation of Dopant Atom Diffusion in a Bulk Semiconductor Crystal Enhanced by a Large Size Mismatch," *Phys Rev Lett*, **113**, 155501 (2014).
- [4] X. Zhang, et al., "Catalytically active single-atom niobium in graphitic layers," *Nature Communications*, **4**, 1924–7 (2013).
- [5] Y.-M. Kim, et al., "Interplay of Octahedral Tilts and Polar Order in BiFeO<sub>3</sub> Films," *Adv Mater*, **25**, 2497–2504 (2013).
- [6] T. J. Pennycook, et al., "Dynamic Fluctuations in Ultrasmall Nanocrystals Induce White Light Emission," *Nano Lett*, **12**, 3038–3042 (2012).
- [7] C. Li, et al., "Grain-Boundary-Enhanced Carrier Collection in CdTe Solar Cells," *Phys Rev Lett*, **112**, 156103 (2014).
- [8] S. J. Pennycook, "Fulfilling Feynman's dream: "Make the electron microscope 100 times better"—Are we there yet?" *MRS Bull*, **40**, 71–78 (2015).

*Stephen J. Pennycook is a Professor in the Materials Science and Engineering Dept., National University of Singapore, an Adjunct Professor in the University of Tennessee and Adjoint Professor in Vanderbilt University, USA. Previously, he was Corporate Fellow in the Materials Science and Technology Division of Oak Ridge National Laboratory and leader of the Scanning Transmission Electron Microscopy Group. Pennycook is a Fellow of the American Physical Society, the American Association for the Advancement of Science, the Microscopy Society of America, the Institute of Physics and the Materials Research Society. He has received the Microbeam Analysis Society Heinrich Award, the Materials Research Society Medal, the Institute of Physics Thomas J. Young Medal and Award, the Hsun Lee Award of the Chinese Academy of Sciences and the Materials Research Society Innovation in Characterization Award. His latest book is "Scanning Transmission Electron Microscopy."*





**Professor Pavel Hozak**

Department of Biology of the Cell Nucleus,  
Institute of Molecular genetics ASCR,v.v.i.,  
Prague, Czech Republic  
E-mail: hozak@img.cas.cz

## **Visualizing Lipid Regulation of Gene Expression Using New and Classical Imaging Tools**

While fluorescent microscopy allows for simultaneous detection of multiple antigens, the electron microscopy (EM) sensitive immunodetection is limited to only two antigens. I will summarize the current possibilities of single molecule visualization inside of cells and tissues, and discuss future needs of researches in biomedicine. In order to overcome the current limitations of immunodetection, we prepared a set of novel nanoparticles (NPs) which fulfil several criteria: size in the frame of 5-12 nm, small size distribution, good contrast and stability in the electron microscope, stability of colloidal solution during conjugation, and surface properties allowing for conjugation with antibodies. With the use of novel NPs, various combinations with commercial gold NPs can be made to obtain a set for simultaneous labelling. For the first time in ultrastructural histochemistry, up to five molecular targets can be identified simultaneously. Using our previously developed tools of spatial statistics one could then map the regions of distribution of multiple molecular targets within the cell, as well as to analyse a high number of individual molecular interactions. Also, we characterized some of the critical steps during (cryo)sample preparation in order to achieve the best preservation of both ultrastructure and antigen in cells.

These methods allowed us to progress with understanding some novel molecular interactions in the cell nucleus. The nucleus is a highly organized cell compartment, where controlled gene expression, DNA replication, and RNA processing occur. These processes are spatially ordered via the nucleoskeleton, which is involved in nuclear compartmentalization and critical for nuclear functioning. In spite of the growing interest and extensive research concerned to the nuclear organization, so far mostly protein complexes have been found as important for spatial nuclear ordering. We describe here novel structures containing phosphatidylinositol 4,5-bisphosphate (PIP2) which contribute as well. Based on scarce literature data relating to PIP2 presence in interchromatin granule clusters and in the nucleolus, we carried out ultrastructural mapping of PIP2-containing structures using pre-embedding immunolabelling and 3D electron tomography, EELS and super-resolution fluorescence microscopy together with molecular biology experiments. We identified previously undescribed nucleoplasmic structures decorated by PIP2 molecules, and demonstrated that these structures, referred to as PIP2 islets, are evolutionary conserved. They are surrounded by nucleic acids and protein-containing compounds. At the islet periphery, PIP2 co-localizes with nascent RNA transcripts and the proteins engaged in Pol II transcription and organization of chromatin. PIP2 islets are sensitive to RNase treatment, and their enzymatic disruption affects the level of transcription. Based on our findings, we suggest that PIP2 islets play an important role in the organization of nuclear architecture providing multiple stable surfaces for the formation of the RNA polymerase I/II transcription complexes.

Taken together, this data allowed us to suggest for the first time that PIP2 as a lipid representative plays an important role in the organization of chromatin architecture and in regulation of gene transcription. The results will be discussed in the frame of the current nucleolar model and lipid functions in the cell nucleus.

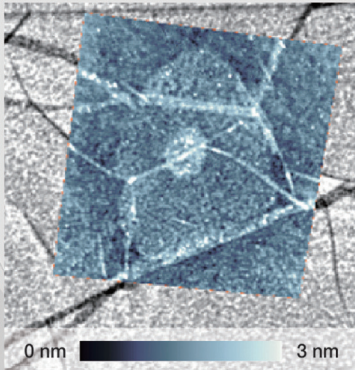
Acknowledgements. We would like to thank for the financial support GACR (15-08738S and 16-03346S), TACR (TE01020118), project, BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University (CZ.1.05/1.1.00/02.0109) from the European Regional Development Fund, and IMG (RVO:68378050). The microscopy work was performed at the Microscopy Centre, Institute of Molecular Genetics AS CR.

*Prof. Pavel Hozak is heading jointly the Department of Biology of the Cell Nucleus and the Microscopy Centre at the Institute of Molecular Genetics in Prague. Since 2007 he is also a full professor in medical biology, Charles University, Prague. His research topics include the definition of higher-order structures in the cell nucleus, mechanisms forming nuclear compartmentalization, identification of nuclear structures active in epigenetic regulation of gene expression, and development of new methods in microscopy. Publications: 145 peer reviewed publications in scientific journals including Cell, Science, Nature Cell Biology. Other involvements: past President, Czechoslovak Microscopy Society, Member of the executive board, European Microscopy Society & International Federation of Societies for Microscopy, Chairman, IMC2014 in Prague ([www.imc2014.com](http://www.imc2014.com)). Further information: <http://nucleus.img.cas.cz>.*

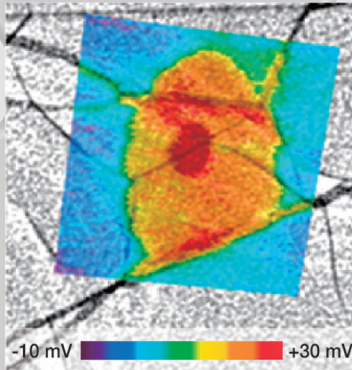
# Symposia in Materials Science

- M1** - Low-Dimensional Nanomaterials: Dots, Wires, and Sheets
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- M7** - X-ray Analysis of Geological/ Mineralogical Materials
- M8** - Novel Applications to Materials Science and Engineering
- M9** - Biomass Materials
- M10** - Mineral Geochronology & Isotope Characteristics by X-ray & Ions
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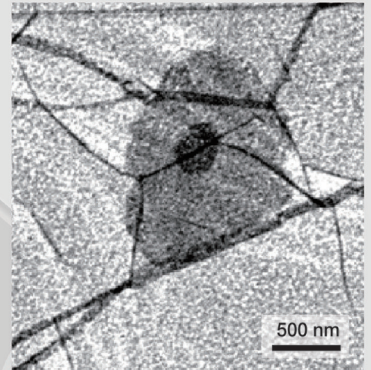
## AFM/SEM Comparative Measurements of Graphene



AFM/SEM overlay \*



KFM/SEM overlay \*



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+1 800 253 3053 microscopy@hitachi-hita.com  
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**Dr. Annop Klamchuen**

Nanocharacterization Laboratory, Nanostructure and Nanometrology  
Research unit, National Nanotechnology Center, NSTDA, 111  
Thailand Science Park, Khlong Nueng, Khlong Luang, Pathum Thani  
12120, THAILAND.  
E-mail: annop@nanotec.or.th

## **Heterostructure of ZnO Nanowire Arrays on Ag Thin Film Prepared by Gas-timing RF Magnetron Sputtering and Its Photocatalytic Activity**

1-D nanostructures of ZnO have been attractive for photocatalytic activity due to its nontoxic nature, low cost and high photochemical reactivity. Since the excitons in ZnO have a short lifetime due to fast charge recombination, utilizing heterostructure between ZnO and Ag has been proposed to separate electrons from excitons through the unidirectional charge transfer across the contact between ZnO and Ag. Although several research groups have successfully fabricated Ag-ZnO nanostructure, the practical large-scale area/forest of Ag-ZnO heterostructure is difficult to fabricate. The used Ag is mostly based on nanoparticle and microplate. Here we demonstrate the fabrication of a photocatalytic heterogeneous structure of high density of ZnO nanowires grown on large-scale Ag thin films. First, the large-scale Ag thin films were fabricated by gas-timing (GT) RF magnetron sputtering technique. A dense structure and a high ratio of (111)/(200) of Ag films could be obtained without any applying additional energy sources. We found that the GT technique not only provides the ability to adjust the number of sputter species from the target, but also generates the self-energy assisted deposition, which related to the atomic peening effect. The optimization of (111)/(200) peak intensity ratio of XRD could be provide for seed-assisted of ZnO nanowires hydrothermal growth. The heterostructure of ZnO nanowire arrays on Ag thin films were then synthesized via hydrothermal process. Density, length and diameter of nanowires as a function of (111)/(200) peak intensity ratio were observed. We found that the density of ZnO nanowires strongly related to (111)/(200) peak intensity ratio of Ag thin film, confirming that facet-selective epitaxial growth between ZnO (002) and Ag (111) occurs. Finally, the photodegradable experiment employed the methylene blue aqueous solution to reveal photocatalytic activity was investigated. The photocatalytic effect of ZnO NWs could be improved through ZnO NWs/Ag heterostructure, which obtained the reaction rate larger than that of ZnO seed/ZnO nanowires 3 orders.

*Dr. Annop Klamchuen was graduated in Chemistry at Department of Chemistry, Faculty of Science, Osaka University and the present position is a leader of Nanocharacterization Laboratory, Nanostructure and Nanometrology Research unit, National Nanotechnology Center, NSTDA.*





**Professor Jin Zou**

Materials Engineering and Centre for Microscopy and  
Microanalysis  
The University of Queensland  
St Lucia, QLD 4072  
Australia.  
E-mail: j.zou@uq.edu.au

## **Impact of Catalysts in Epitaxial Growth of III-V Semiconductor Nanowires**

Epitaxial III-V semiconductor nanowires and their heterostructures have been paid extraordinary attention in recent years due to their unique structural and chemical characteristics and in turn potential properties in optoelectronic, nanoelectronic, and sensing developments. In general, semiconductor nanowires are induced by catalysts, in which the catalysts mediate the one-dimensional growth. However, since the complications of catalysts in inducing the nanowire growth, many nanowires induced by the catalysts have their own structural characteristics. Through using various electron microscopic techniques, many extraordinary physical phenomena were uncovered. In this presentation, I shall summarize our discoveries of impact of catalysts in the growth of epitaxial III-V semiconductor nanowires for the past decade.

*Dr. Jin Zou is a Chair Professor in Nanoscience at the University of Queensland, Australia. In his postgraduate studies, he has been trained as an electron microscopist - Professors K H Kuo and F H. Li (both academia of Chinese Academy of Sciences) supervised his Masters thesis and Professor D. J. H. Cockayne (FRS) supervised his PhD thesis. Since his PhD study (from mid 1989), his research has been focused on the understanding of the evolution of semiconductor nanostructures and the building of their structure-property links through closely correlating the structural characteristics of semiconductor nanostructures determined by electron microscopy and their growth/demonstrated properties. Over the years, Professor Zou published over 550 SCI journal articles, which have attracted over 12,000 citations and led to a H-index of 53.*



**Professor Takeshi Yanagida**  
Institute of Material Chemistry and Engineering,  
Kyushu University,  
6-1 Kasuga-Koen, Kasuga, Fukuoka 816-8580  
JAPAN.  
E-mail: yanagida@cm.kyushu-u.ac.jp

## **Material Design of Single Crystalline Metal Oxide Nanowires and Their Promises for Green-innovation and Life-innovation**

Self-assembled one-dimensional “Single Crystalline Nanowires” have attracted much attention due to not only the fundamental interests in nanoscale-confined physical properties but also novel nano-device applications, where existing nanomaterials have not been applicable. Although nanowires composed of Group VI and III-V compounds have demonstrated their great promises, the feasibility of functional oxide nanowires, whose physical properties are hardly attainable to other materials, has been strongly limited. Here I demonstrate i) a fundamental design concept for creating single crystalline oxide nanowires via vapor-liquid-solid (VLS) pathway, and ii) a development to measure the physical properties of a single nanowire, including electrical and thermal transport properties. By comparing experimental VLS nanowire growth to MD simulations. We found that the difference between LS interface and VS interface on the critical nucleation size essentially allows us to perform VLS nanowire growth. This knowledge can be expanded to discover novel metal oxide nanowires via VLS mechanisms [1] We have constructed highly stable memristive device comprised of single nanowire. [2] The information as to the memristive switching including the carrier type for memristive switching and the spatial switching location, have been extracted by the present nanowire memristor, which had been buried in conventional memristors. [3-4] In addition, we have shown the intrinsic transport properties and applications of various oxide nanowires. [5-6] We believe that the presented approaches by utilizing metal oxide nanowires offers an important platform for investigating not only nanoscale properties of transition metal oxides but also exploring novel nano-devices with other materials, which had not been possible to be integrated onto Si and/or plastic substrate.

### **Related Recent Publications**

- [1] *Nano Lett.*, 15, 6406 (2015), *Phys. Rev. E*, 82, 011605 (2010), *Phys. Rev. E*, 83, 061606 (2011)
- [2] *JACS*, 132, 6634 (2010), *JACS*, 133, 12482 (2011), *Nano Lett.*, 10, 1359 (2010)
- [3] *Nano Lett.*, 11, 2114 (2011), *Nano Lett.*, 12, 5684 (2012), *JACS*, 134, 134567 (2012)
- [4] *Sci. Rep.* 3, 1657 (2013)
- [5] *JACS*, 135, 7033 (2013), *JACS*, 136, 14100 (2014), *Sci. Rep.* 4, 5532 (2014)
- [6] *ACS Nano*, 7, 3029 (2013), *Adv. Mater.*, 25, 5893 (2013), *Sci. Rep.* 4, 5252 (2014), *Sci. Rep.* 5, 10584 (2015)

*Dr. Takeshi Yanagida was an Associate Professor in Institute of Scientific and Industrial Research, Osaka University, and the current position is a Professor in Institute of Material Chemistry and Engineering, Kyushu University. In 2012, he received Young Research Award of Ministry of Education, Culture, Sports, Science & Technology in Japan.*



**Assoc. Prof. Luu Tien Hung**

Department of Electronics and Telecommunications,  
Vinh University, 182 - Le Duan Str., Vinh City,  
Nghe An, VIETNAM.  
E-mail: [hunglt@vinhuni.edu.vn](mailto:hunglt@vinhuni.edu.vn)

## **Synthesis, Characterization, Photocatalytic and Bactericidal Activities of (Co, Ag) - Doped ZnO Nanoparticles**

Among the functional oxides, ZnO has a wide range of applications in optics, optoelectronics, sensors, actuators, energy and biomedical sciences, and spintronics [1]. The photocatalytic degradation of organic pollutants in water and air, using semiconductors such as TiO<sub>2</sub>, ZnO and some other oxides has been in focus of research recently due to their prospects in the environmental detoxification [2,3]. The quantum efficiency of ZnO is in general larger than that of TiO<sub>2</sub> and some times it has revealed better activity than TiO<sub>2</sub>. Besides, ZnO is available at low cost and it absorbs over larger fraction of the solar spectrum than TiO<sub>2</sub>, furthermore it is considered to be a more suitable material for photocatalytic degradation of organic pollutants [4]. Its high chemical stability and low toxicity make it suitable for UV screening applications. Moreover, it is a bactericide and is effective to inhibit both Gram-positive and Gram-negative bacteria [5,6].

In recent years, combustion synthesis has become an effective, low-cost method for production of various industrially useful materials. It has now become a very popular approach for preparation of nanomaterials also. The combustion method has the advantages of easy control of the process, solution concentration, homogeneity and low equipment cost [7].

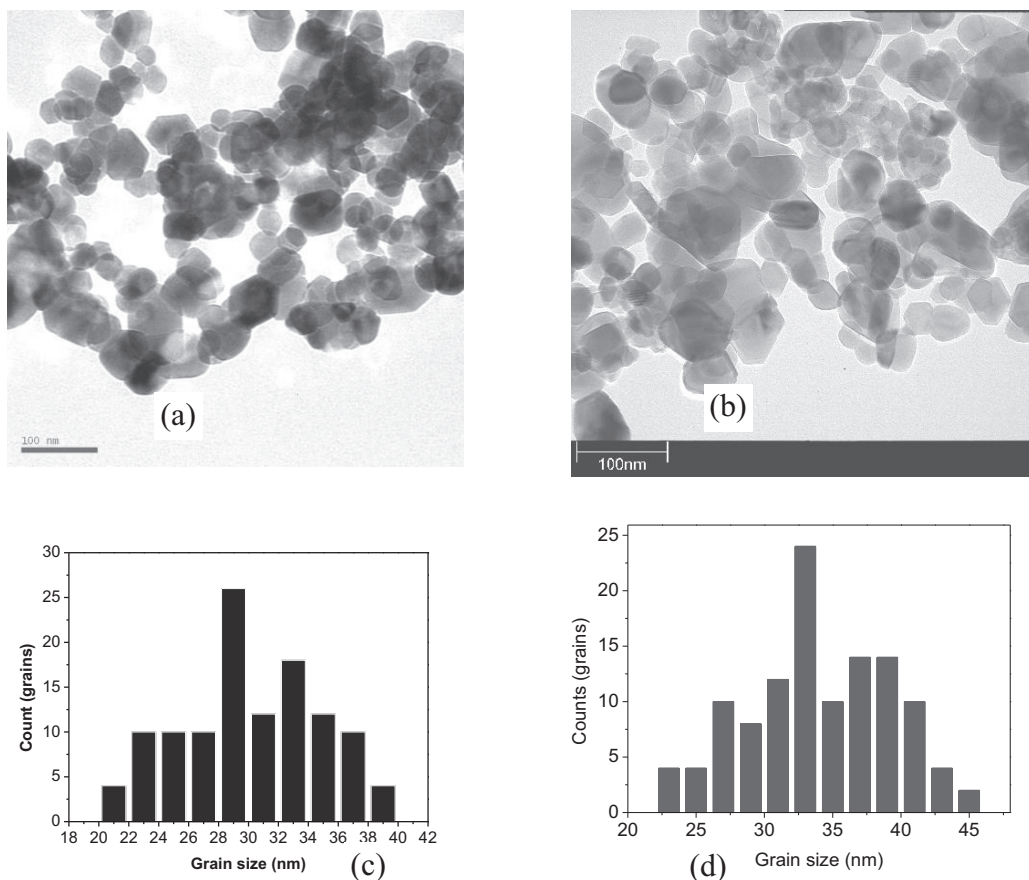
In this work, we present the results of a study on synthesis, characterization, photocatalytic activity and bactericidal property of (Co, Ag) - doped ZnO nanoparticles, which were synthesized by combustion method using polyvinyl alcohol (PVA) agent.

The nanoparticles were characterized by powder X-ray diffraction, energy dispersive X-ray spectroscopy and high resolution transmission electron microscopy. Both of X-ray diffraction (XRD) and selected area electron diffraction (SAED) analyses showed the single phase of hexagonal structure in all the samples. Transmission electron microscopy (TEM) and HRTEM images show that most of the particles have spherical shape and grain size of nanoparticles is ranging from 10 to 60 nm in diameter. Both of shape and grain size of nanoparticles depended on both of Co or Ag ratio and annealing temperatures. Energy-dispersive X-ray (EDX) spectral analysis showed the proportion of elements in the nanoparticles in accordance with the original composition.

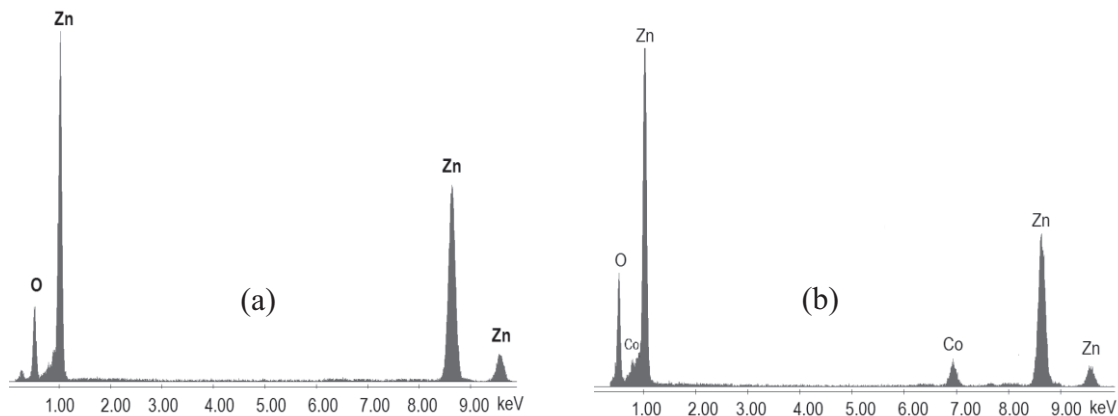
The photocatalytic activity of Co-doped ZnO samples was studied by measuring the decomposition of methylene blue in aqueous solution using UV-VIS spectroscopy. The results showed that photocatalytic ability to decompose methylene blue achieved 70 % with ZnO and 98 % with Zn<sub>0.97</sub>Co<sub>0.03</sub>O samples after 150 minutes of irradiation.

The antibacterial property of Ag-doped ZnO samples was tested bioactive with Escherichia coli ATCC (American Type Culture Collection) 25922 strain. The results showed that the Ag-doped ZnO nanoparticles have the antibacterial properties and are better than pure ZnO nanoparticles.

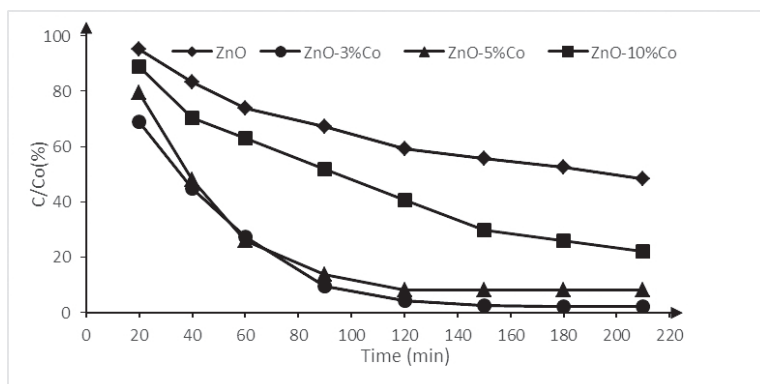




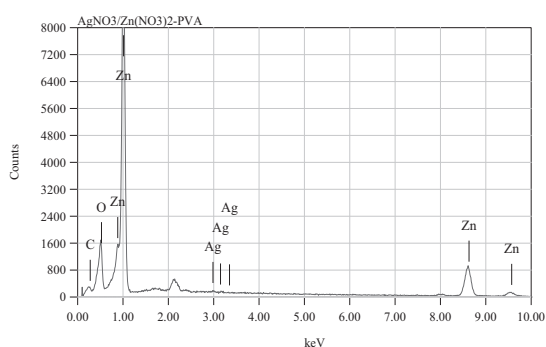
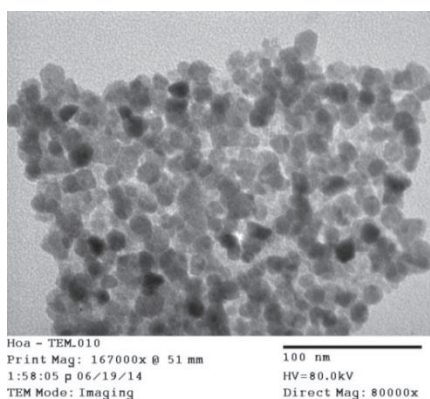
**Figure 1.** BF-TEM micrographs of undoped ZnO (a) and 10% Co-doped ZnO (b) nanoparticles synthesized under conditions at: pH=3 , PVA/metal mole ratio = 2:1 and annealing at 500°C for 60 minutes and (c) and (d) are corresponding grain size distributions, respectively.



**Figure 2.** Energy dispersive X-ray (EDX) spectra of undoped (a) and 10% Co-doped ZnO (b).



**Figure 3.** Photocatalytic degradation curves of cleaning methylene blue under visible light irradiation.



(a)

(b)

**Figure 4.** BF-TEM micrographs of 1% Ag-doped ZnO (a) and corresponding energy dispersive X-ray (EDX) spectrum (b) of nanoparticles synthesized under conditions at: pH=4, PVA/metal mole ratio = 2:1 and annealing at 500°C for 60 minutes.

**Table 1.** Test results the antibacterial activity of the E. coli strain ATCC 25922.

Samples	Results					
	$10^{-3}$		$10^{-5}$		$10^{-7}$	
		S		S		S
Standard sample	283.67	21.73	170.33	37.58	155.33	8.33
T <sub>0</sub> sample	9.00	7.94	3.33	3.51	1.67	1.53
T <sub>1</sub> sample	3.67	3.51	1.67	0.58	0	-

S: Experimental adjusted deviation.

: Average number of colonies.

$10^{-3}$ ,  $10^{-5}$  and  $10^{-7}$  are the diluted concentrations of bacteria

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*Dr. Luu Tien Hung was born in 1974. He is working at Vinh University, Nghean, Vietnam. He received his bachelor's degree in physics from Vinh University, Vietnam in 1996. In 1999, he received his Master's Degree in Physics from the Institute of Physics in Hanoi, Vietnam. In June 2008, he received his PhD in Materials Science from the Institute of Materials Science, VAST. At present, he is associate professor in Physics at Vinh University from 2012. He is the author/co-author of more than 15 international scientific papers. His current research interests include nanostructure magnetic materials and nanomaterials for environments.*

**Dr. Takao Yasui**

Department of Applied Chemistry, Graduate School of Engineering,  
Nagoya University, Nagoya, Japan  
ImPACT Research Center for Advanced Nanobiodevices, Nagoya  
University, Nagoya, Japan  
JST, PRESTO, Graduate School of Engineering, Nagoya  
University, Nagoya, Japan  
E-mail: [yasui@apchem.nagoya-u.ac.jp](mailto:yasui@apchem.nagoya-u.ac.jp)

## Nanowires for Extracellular Vesicles Analysis

Takao Yasui<sup>1,2,3\*</sup>, Takeshi Yanagida<sup>4,5</sup>, Noritada Kaji<sup>1,2</sup>, Sakon Rahong<sup>1,2</sup>, Kazuki Nagashima<sup>4</sup>, Tomoji Kawai<sup>5</sup>, and  
Yoshinobu Baba<sup>1,2,6</sup>

<sup>1</sup>Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Nagoya, Japan

<sup>2</sup>ImPACT Research Center for Advanced Nanobiodevices, Nagoya University, Nagoya, Japan

<sup>3</sup>JST, PRESTO, Graduate School of Engineering, Nagoya University, Nagoya, Japan

<sup>4</sup>Institute of Materials Chemistry and Engineering, Kyushu University, Kasuga, Japan

<sup>5</sup>Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Japan

<sup>6</sup>Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Takamatsu, Japan

Recently, extracellular vesicles have received researchers attention since the extracellular vesicles contain important cellular components: microRNA (miRNA). The miRNA analysis in the extracellular vesicles provides of great clinical importance as biomarkers for a variety of disease states. Several methods have been developed to isolate the extracellular vesicles from body fluid; however, these methods take long time or need large volume to get sufficient volume of the vesicles. In this study, we fabricated nanowires to isolate the extracellular vesicles from 1 mL of body fluid. We compared isolation efficiency of the vesicles and extraction efficiency of the vesicle miRNA using the nanowire devices with those using conventional methods. Nanowires realized over 90 % isolation rate within 10 min, and higher miRNA extraction efficiency. The developed nanowires should allow researchers to develop an analytical method not only for early stage diagnosis but also for discovering new biomarkers.



**Dr. Mati Horprathum**

Optical Thin-Film Laboratory,  
Intelligent Devices and Systems Research Unit,  
National Electronics and Computer Technology Center,  
Pathum Thani 12120, THAILAND.  
E-mail: mati.horprathum@nectec.or.th

## **Controlled Nanostructures by Physical Vapor Deposition with Glancing Angle Deposition Technique for Sensor Applications**

Nanostructural thin-film coatings have attracted enormous attention for the developments of sensor devices. With physical vapor deposition (PVD) and glancing angle deposition (GLAD) technique, a development of well-controlled multi-dimensional morphologies has gained drastic attention because of facile, cost-effective method, high throughput, and its applicability to various materials. In this work, we present the emergence of controlled nanostructure growth by PVD with GLAD, developed at Optical Thin-Film Technology Laboratory, NECTEC, Thailand, utilized for the preparations of the nanostructural for the sensor devices. The different PVD techniques based on DC magnetron sputtering and electron-beam evaporation, both with the integrated GLAD component, were discussed. Based on the technique, the effects of the deposition parameters on the morphologies, physical structures, and sensing properties were investigated according to the nanostructured materials, i.e. silver, tungsten oxide, tin oxide and zinc oxide. In addition, we will also discuss the potentials of the fabrications to improvements towards recent applications, i.e. surface-enhanced Raman scattering (SERS) substrate, ultra-sensitive nanostructural gas sensors and their future developments.

*Mati Horprathum received Ph.D. degree in Physics from King Mongkut's University of Technology Thonburi (KMUTT), Thailand in 2009. He did his 1-year postdoctoral study at Laboratory of Atomic Scale Materials Processing, Institute of Scientific and Industrial Research (ISIR), Osaka University, Japan. He is now a researcher at Optical Thin-Film Laboratory, National Electronic and Computer Technology Center (NECTEC), Thailand. His research interests are glancing-angle deposition, nano-microelectronic mechanic device, surface enhance Raman spectroscopy (SERS), fabrication and characterization of nanostructures, electrochromic thin film, vacuum design and thin film characterization. In 2016, he was awarded the ICO/ICTP Gallieno Denardo Award for "his valuable contributions in the development of optical thin film technology for innovative surface functionality as well as for his commitment in diffusion of optical thin film research in Thailand" from the International Commission for Optics (ICO), USA and the Abdus Salam International Center for Theoretical Physics (ICTP), Italy.*



**Assistant Professor Dr. Sakuntam Sanorpim**

Department of Physics, Faculty of Science

Chulalongkorn University,

Phayathai Rd., Phatumwan,

Bangkok 10330, THAILAND.

E-mail: sakuntam.s@chula.ac.th/ sakuntam@gmail.com

## **TEM Analysis of Cubic GaN Films on GaAs (311) Oriented Substrates Grown by MOVPE**

Cubic GaN (c-GaN) films grown by metalorganic vapor phase epitaxy (MOVPE) were investigated using transmission electron microscopy (TEM) to verify effects of substrate surface orientation on micro-structures in the grown films. To reduce a generation of hexagonal phase, growth temperature of a GaN buffer layer is optimized, since it typically effects on the formation of stacking faults (SFs) in the c-GaN films. The best quality of c-GaN film grown on the GaAs (001) oriented substrate with high cubic-phase purity was achieved by the growth with optimum growth temperature of GaN main layer of 900 oC using a buffer layer grown at 575 oC. SFs were found to localized at the c-GaN/GaAs interface. On the other hand, the film on the GaAs (311) oriented substrate with an identical growth conditions exhibits only the presence of SFs, but the hexagonal single crystal is invisible. These results demonstrate that c-GaN on GaAs (311) exhibits a better film purity with lower density of SFs compared to that in c-GaN on GaAs (001). This might be due to a difficulty of a generation of the (111) step on the GaAs (311) grown surface. However, some dislocations were observed for the c-GaN/GaAs (311).

*Dr. Sakuntam Sanorpim is currently Assistant Professor in Physics at Department of Physics, Chulalongkorn University. His research is focused on the metalorganic vapor phase epitaxy (MOVPE) growth and characterization of III-Nitride semiconductors and dilute Nitride semiconductors. He received a B.Sc. from Chulalongkorn University in 1997, and an M. Eng. from the University of Tokyo, Japan in 2000. He received his Ph.D. in Applied Physics Engineering from the University of Tokyo, Japan in 2003.*



**Dr Hideyuki Takahashi**  
Surface Analysis Business Unit,  
JEOL Ltd.,  
1-2 3chome, Musashino, Akishima,  
Tokyo 1968558, JAPAN.  
E-mail: takahash@jeol.co.jp

## **Depth Quantitative and Chemical State Analysis from Few nm to Several nm using Soft X-ray Emission Spectroscopy**

Terauchi et al. reported a development of wavelength dispersive soft X-ray emission spectrometer (WD-SXES) attached to TEM and high energy resolution of 0.2 eV at Al-L emission. For commercial use, we have modified and developed the WD-SXES consisting of X-ray reflection mirrors, newly developed diffraction gratings; JS50XL and JS200N, and a detector of a back-thinned layered CCD, and attached to electron probe X-ray microanalysers (EPMA, JEOL JXA-8530F or JXA-8230). It covers nominally the energy range from 50 to 210 eV and approximately to 700 eV using higher order X-ray spectra. By using useful characteristic CCD parallel detection for the X-rays, it makes it possible to operate just like conventional energy dispersive spectrometers for EPMA.

“Slow” electrons by using very low accelerating voltages provides the very surface sensitive secondary electron image. Recent models of SEM can use low voltage, down to 10 V, using a deceleration function by stage bias and specially designed electron optics. Using this function of low voltage from 100 V to several kV, WDSXES was applied to analyze depth quantification of trace boron in a silicon device. As a result, it was demonstrated that higher contents of boron, ~4% (40,000 ppm), could be found at a 2 nm depth from the top surface of device compared to just 10 ppm at 500 nm depth. Moreover, depth chemical state analysis of metals were demonstrated from few nm to several nm using alternative low voltages. Using soft X-ray, it is very convenient for stable spectra than electron signals of Auger, XPS or EELS with charging due to X-ray signals not being sensitive to charging. I will report these several features in recent work of soft X-ray spectroscopy.

*PhD was given in Applied Physics in Osaka University, (1997). Now a senior adviser at Surface Analysis Business Unit of JEOL Ltd. He was a manager at application division of Electron Probe Microanalyzer and Transmission Electron Microscope and EDS and WDS development division. He was a co-convenor of ISO202/TC2 from 2006 to 2012 to publish ISO11938 (EPMA mapping method). He is now a guest researcher at Osaka peripheral University. In 2015, he received Birks Award in M & M in USA and Yamazaki Teiichi Award in Japanese MST.*





**Dr Kuttanellore Muraleedharan**

Director

Central Glass & Ceramic Research Institute

Council of Scientific and Industrial Research

196, Raja SC Mullick Road, Jadavpur University PO

Kolkata, India

E-mail: muralee@cgcricri.res.in

## **Transmission Electron Microscopy Characterization of the Structural Evolution of GaN grown by MOCVD on C-Sapphire**

III-Nitrides (AlN, GaN, InN) are promising materials, which find usage in the high power high frequency applications. Bulk GaN substrates are difficult to grow, therefore the GaN based structures have to be grown on foreign substrates.

In this presentation, microstructural evolution of Gallium Nitride grown by Metal Organic Chemical Vapour Deposition (MOCVD) process on c-plane sapphire substrate has been systematically studied using transmission electron microscopy (TEM) and related spectroscopic techniques. The role of nitridation temperature, its effect on GaN film for the subsequent growth processes has been analyzed.

The first step in the growth process is the nitridation of the sapphire layer. It has been observed, that under low temperature (at 530°C) nitridation, Al-O-N complex having a cubic structure is formed and at higher nitridation temperatures (> 800°C), AlN with a cubic structure is formed. The crystallography and orientation of the nitrides are defined.

The low temperature GaN nucleation layer grown on these nitrided layer templates have also been studied and it has been observed that  $\beta$ -GaN is the majority phase for low temperature nitridation and Wurtzite-GaN is the phase for the higher temperature nitridation. The continuity, mosaicity, and crystallography of these low temperature GaN films have been characterized in the as-grown and thermally annealed conditions.

Subsequent high temperature GaN films grown on these templates are found to contain Ga-polar GaN and N-polar GaN as the majority phases for the low and high temperature nitridation cases respectively. Dislocations, stacking faults and inversion domains present in these high temperature GaN films affect the properties of the device as a whole, and have been studied. The differences in the defect structures in Ga-polar GaN and N-polar GaN are described.

*Dr. Kuttanellore Muraleedharan obtained B. Tech (1983) and Ph. D (1994) from Banaras Hindu University-Institute of Technology (BHU-IT), Varanasi, India. He joined Defence Metallurgical Research Laboratory (DMRL), Hyderabad as a Scientist in the year 1984 and led the electron microscopy group during 1989-2010. He has also been the member of Defence Research and Development Organisation (DRDO, India) Think Tank, Director of Materials, DRDO, New Delhi during 2011-2015 and provided advice and insights into materials related activities of DRDO before joining CSIR-CGCRI, Kolkata, India as its 8th Director. He also holds additional charge as Director, CSIR-NML, Jamshedpur, India.*

*Research interests of Dr. Muraleedharan include the Science and Design of Advanced Materials, and Multi-scale Microstructural Characterization using techniques such as Transmission Electron Microscopy and 3D Atom Probe Field Ion Microscopy, as applied to the study of electronic materials and solid-state phase transformations. His research concentrated on the Process-Structure-Property relationships in a variety of materials systems such as alloys based on Ti and its intermetallics Ti3Al and TiAl; specialty steels; Ni base superalloys; high energy rare-earth permanent magnets; and ceramic matrix composites. Dr. Muraleedharan is a member of many professional bodies and has been the Vice President (2011-2013) and President (2013-2015) of the Electron Microscopy Society of India (EMSI), and is currently National Council member of Indian Institute of Metals and Indian Ceramic Society and EMSI.*



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**Associate Professor Dr. Yuttanant Boonyongmaneerat**  
Metallurgy and Materials Science Research Institute,  
Chulalongkorn University,  
Bangkok 10330, THAILAND.  
E-mail: yuttanant.b@chula.ac.th

## **Enhancing Corrosion Resistance of Galvanized Coating via Microstructural Engineering and Microscopy Analysis**

Galvanized coatings of steels are used widely in several applications including automotive, construction, and electronics, owing to their decent corrosion protection at reasonable costs. In many regions including offshore and areas near coastlines, however, corrosion attacks are rather severe and the conventional galvanized coatings cannot well-withstand such harsh saline environments. Further improvements of galvanized coatings are therefore desirable in many industries, and also fundamentally interesting from the academics standpoints.

This presentation provides latest trends of the subject and presents a review of research studies being performed at the Surface Coating Technology for Metals and Materials Research Center, Metallurgy and Materials Science Research Institute (MMRI), Chulalongkorn University, to improve the performance of galvanized coatings. Specifically, by analyzing and engineering the microstructure of the coating materials, using a concert of techniques including electrochemical analysis, finite element simulations, kinetics modeling and crystallographic studies, understanding of the approaches to fabricate galvanized coatings with superior corrosion resistance and structural integrity is realized. The presentation will also discuss the importance of metallography and microscopy techniques to analyze the easily-oxidized galvanized surfaces.

*Dr. Yuttanant Boonyongmaneerat is currently an associate professor at Metallurgy and Materials Science Research Institute, Chulalongkorn University and the head of the Surface Coating Technology for Metals and Materials Research Center. He was the recipients of the Young Scientist Award (2011) and the Yong Technologist Award (2015) of the Foundation for the Promotion of Science and Technology under the Patronage of H.M. the King, and was named top young innovator by TR-35 Singapore, MIT Technology Review (2014).*



**Professor Kenji Matsuda**

Graduate School of Science & Engineering for Research,  
University of Toyama  
3190, Gofuku, Toyama-shi, Toyama, 930-8555, Japan  
E-mail: matsuda@eng.u-toyama.ac.jp

## Precipitation on Al-Mg-Si and Al-Mg-Ge alloys by HRTEM

The precipitation sequence of Al-Mg-Ge alloy has been compared with that of Al-Mg-Si alloy to understand their nucleation and growth mechanism. According to our previous result, Al-Mg<sub>2</sub>Si alloys are as follows; S.S.S.S.→ G.P. zones→ random type precipitate→ parallelogram type precipitate, (order/disordered β'' phase)→ β' phase, (type B, A and C precipitates)→ equilibrium phase β-Mg<sub>2</sub>Si or Si phase. The Random type precipitate is the aggregates of GP zones with vacancies and disordered phase, and the parallelogram type precipitate is a disordered β'' phase. The β'' phase, type A, B and C precipitates are typical precipitates in excess Si type Al-Mg<sub>2</sub>Si alloys which include Si and Mg as the ratio of Mg/Si<1.

Al -0.43 at.% Mg -0.2at.% Ge (Al -1.0 mass% Mg<sub>2</sub>Ge) alloy was fabricated using casting, and homogenized, solution heat treated, quenched in ice water, and then aged at 423K or 473K. Microstructure structure of Al-Mg-Ge alloys was observed by a transmission electron microscopy (TEM) / high resolution transmission electron microscopy (HRTEM) with difference aging times. G.P. zones, random type precipitates, parallelogram type precipitate and β'' phase were confirmed in samples of under-aged and peak-aged conditions. The β' phase, type-A precipitate and equilibrium β-Mg<sub>2</sub>Ge were observed in over-aged. It is quite interested that β'' phase and type-A precipitate exist in this alloy, even this is a pseudo binary Al-Mg<sub>2</sub>Ge alloy. The precipitation sequence in Al-Mg-Ge alloy can be described as: S.S.S.S.→ G.P. zones→ random type precipitate→ parallelogram type precipitate, β'' phase→ β' phase, type-A precipitate→ equilibrium phase β-Mg<sub>2</sub>Ge.

*Dr. Kenji Matsuda is the Professor, Graduate School of Science & Engineering for Research, University of Toyama, Japan, and also the Director, Center for Research and Development in Natural Sciences, and unit leader for Common instrument for research, University of Toyama, and also the cluster manager of the project of creation of new functional and high performance materials at Hokuriku area, Ministry of Economy, Trade and Industry, Japan (2014-2016). He has been a project leader for several projects over the past ten years, funded mainly by Grants-in-Aid for Scientific Research by JSPS and Japan Science and Technology Agency (JST) about research projects of light metals. In 2014, he received the Academic Distinguished Service Award for Senior Researchers in Japan Institute of Light Metals.*



**Professor Jer-Ren Yang**

Department of Materials Science and Engineering,  
National Taiwan University, Taipei, Taiwan  
E-mail: jryang@ntu.edu.tw

## Substructures of Lenticular Martensite in High-carbon High-chromium Stainless Steel

An Fe-1.0C-17Cr (wt.%) stainless steel was subjected to subzero treatment to investigate the substructures of the lenticular martensite. During the course of the isothermal holding in liquid nitrogen (-196 C), the thin-plate martensite formed first, and lenticular martensite later. The substructures of thin-plate martensites and lenticular martensite were examined using transmission electron microscopy, focusing on the details of the midrib [1]. The results provide strong evidence to suggest that thin-plate martensite can be transformed into lenticular martensite. Through electron backscatter diffraction (EBSD) coupled with transmission electron microscopy, comprehensive orientation image microscopy (OIM) of lenticular martensite has been achieved [2]. For a given coarse austenite grain with considerable variants of lenticular martensite, the pole figures indicate that the lenticular martensite in this steel approximately adopts the Kurdjumov-Sachs orientation relationship with respect to the austenite matrix. Through electron backscatter diffraction (EBSD) and convergent beam electron diffraction (CBED), it has been clarified that the spread in diffracted intensity within pole figures is related to the misorientation gradient within the lenticular martensite plate. On the other hand, a given coarse austenite grain with a few variants of lenticular martensite was employed to analyze the orientation relationships of the variant pairs of lenticular martensite plate. The results indicated that zigzag couplings (including spear couplings), the major product of plate martensite in this study, had an absolute dominance of a specific variant pair.

**Keywords** Lenticular martensite; Transmission electron microscopy; Electron backscatter diffraction (EBSD); Convergent beam electron diffraction (CBED); Orientation relationship.

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*Professor Jer-Ren Yang received Ph.D. degree (Materials Science and Metallurgy) from University of Cambridge, England, UK in 1987. In February 1988, he joined the faculty of Department of Materials Science and Engineering, National Taiwan University (NTU), where he has been a full Professor since August 1993. During the period of August 2007 - July 2010, he was the head of the Department. Professor Jer-Ren Yang's research interests include phase transformation of steels, crystal geometry and crystal defects. Professor Yang is a Distinguished Engineering Professor in Taiwan University and has published some 170 international journal papers in the areas of materials science and engineering. Since Nov. 2011, Professor Jer-Ren Yang has been appointed as Director of Advanced Steel Microstructure Control – Engineering Research Center at National Taiwan University, which was founded by CBMM, China Steel Corporation and National Taiwan University. He also has been appointed as President of Microscopy Society of Taiwan since 2012, and elected as Fellow of Institute of Materials, Minerals and Mining, UK since 2014*



**Associate Professor Naoya Shibata**

Institute of Engineering Innovation, The University of Tokyo  
Yayoi 2-11-16, Bunkyo, Tokyo 113-8656, Japan  
E-mail: shibata@sigma.t.u-tokyo.ac.jp

## **Development of Atomic-resolution Scanning Transmission Electron Microscopy for Ceramic Interfaces**

Understanding the structures of interfaces even down to atomistic scales is essential to control the functional properties of ceramic materials. Scanning transmission electron microscopy (STEM) boosted by aberration-correction technology has made possible the direct characterization of localized atomic and electronic structures of many ceramic materials and devices, especially at interface regions. In STEM, a finely focused electron probe is scanned across the specimen and the transmitted and/or scattered electrons at each raster position are detected by the post-specimen detector(s) to form images. Thus, we gain flexibility in determining the contrast characteristics of the STEM images by controlling the detector geometry, and can not only image atomic-scale structures of the materials (even all elements using HAADF and ABF) but can also image local electromagnetic fields inside materials using segmented-type detectors through differential phase contrast (DPC) imaging techniques.

In recent years, we have developed an area detector which we refer to as the "Segmented Annular All Field (SAAF)" detector and which is capable of atomic-resolution STEM imaging. This area detector can obtain 16 simultaneous atomic-resolution STEM images which are sensitive to the spatial distribution of scattered electrons on the detector plane. Using this detector, we can even map atomic electric field inside ceramic materials. In this talk, I briefly introduce our SAAF detector, and also discuss our recent results on the electromagnetic field structure imaging of ceramic materials and devices using the detector, especially at interface regions where structural and compositional changes crucially affects the field structures.

*Naoya Shibata received a PhD in Materials Science in 2003 at University of Tokyo. He was a JSPS Research Fellow at Oak Ridge National Laboratory (2003-2004). He was a Research Associate in Institute of Engineering Innovation at the University of Tokyo (2004-2007) and was an Assistant Professor (2007-2011) and now an Associate Professor (2011-). His research focuses on the grain-boundary and interface structures in oxide materials using atomic-resolution scanning transmission electron microscopy. He received several awards such as Nagase Prize (2015), Seto Award (2015), Sir Martin Wood Award (2013), Kazato Award (2013).*



**Dr. Si-Young Choi**

Department of Materials Modeling & Characterization,  
Korea Institute of Materials Science,  
Sangnam-dong 66, Seongsan-gu,  
Changwon 642-831, SOUTH KOREA.  
E-mail: youngchoi@kims.re.kr

## **Assessment of Strain-generated Oxygen Vacancies Using SrTiO<sub>3</sub> Bicrystals**

Control of atomic-scale defects has attracted attentions in materials community due to the amphoteric characteristic of defects. Defects deteriorate the desired properties of materials but also often improve them, and their effects are even more pronounced in the localized dimensions. Here we directly demonstrate that strain triggers the formation of oxygen vacancies in complex oxides by examining the tilt boundary of SrTiO<sub>3</sub> bicrystals by combining experiments and calculations. Through transmission electron microscopy and electron energy loss spectroscopy, we identify the strains along the tilt boundary and oxygen vacancies in the strain-imposed regions between dislocation cores. First-principles calculations support that strains, irrespective of its type and sign, lower the formation energy of oxygen vacancies, thereby enhancing the vacancy formation. Finally, current-voltage measurement confirms that such oxygen vacancies at the strained boundary result in the decrease of non-linearity of I-V curve as well as resistivity. Our results strongly point that oxygen vacancies are preferentially formed, segregated at the region where strains accumulate, such as heterogeneous interfaces as well as at grain boundaries. Our results confirm that atomic-scale defects can impact on the performance through strain-mediated control and confinement of defects.

*Dr. Si-Young Choi had got his PhD degree in Korea Advanced Institute of Science and Technology in 2004. Since then, he was a fellow researcher in the field of electron microscopy in Oxford University and the University of Tokyo. In 2008, he joined Korea Institute of Materials Science (KIMS) and his present position is Head of Department of Materials Modeling & Characterization in KIMS. His specialty is the atomic scale analysis via aberration-corrected STEM in the variety of functional oxides, such as ferroelectric/piezoelectric perovskite oxides, Li-ion battery cathode oxides, and multiferroic oxides.*



**Professor Miran Čeh**

Department for Nanostructured Materials,  
Center for Electron Microscopy and Microanalysis,  
Jožef Stefan Institute, Ljubljana, Slovenia  
E-mail: miran.ceph@ijs.si

## TEM and STEM Investigations of Oxide Thermoelectrics

It is known that thermoelectric (TE) properties, i.e. figure of merit (ZT), of oxide-based TE materials can be improved by introducing planar faults into the microstructure. It is assumed that in-grown planar faults will reduce thermal conductivity, which would consequently increase the ZT value. In order to successfully tailor thermoelectric properties of chosen thermoelectric materials, it is prerequisite to know the structure and chemical composition of introduced planar faults.

In our investigation, HRTEM and HRSTEM imaging (HAADF, ABF) with EDXS were used to characterize Ruddlesden-Popper-type (RP) planar faults in SrO and CaO-doped  $\text{Sr}(\text{Ti},\text{Nb})\text{O}_{3-\delta}$  (STNO) perovskite and inversion boundaries (IBs) in the  $\text{ZnO}_k(\text{In}_2\text{O}_3, \text{Al}_2\text{O}_3)$  system. All results were obtained in a Jeol ARM-200CF with Cs probe corrector. Sr and/or Ca-rich RP faults in the STNO form either more or less ordered polytypoidic sequences and/or three dimensional networks of isolated RP-faults along low index zone axis in the STNO. HR HAADF STEM showed that Nb content on B sites in the STNO perovskite matrix varies between single atom columns. Within  $\text{In}_2\text{O}_3$ -doped ZnO grains pure indium monolayers are readily observed by the HAADF. These basal inversion boundaries (IB's) are parallel to the  $\{0001\}$  ZnO lattice planes and separate domains with different orientation. Basal IB's are much more clearly resolved by HAADF as opposed to pyramidal IB's. In case of  $\text{Al}_2\text{O}_3$  addition, aluminium is substituting for indium in the basal IB's as well as in the pyramidal IB's.

*Miran Čeh is scientific advisor at the Department for Nanostructured Materials and head of Center for Electron Microscopy and Microanalysis at the Jožef Stefan Institute. He is professor at the Chemistry and Chemical Technology Department at the University Maribor and at the Jožef Stefan Postgraduate School. Miran Čeh is member of the EMAS board, past president of the Slovenian Society for Microscopy, Slovenian delegate to the ESFRI and member of the ESTEEM2 project. Main scope of his work includes structural and chemical characterization of nanostructures in oxide materials using various electron microscopy techniques.*





**Professor Eiji Abe**

Department of Materials Science & Engineering,  
University of Tokyo, 113-8656, Japan  
E-mail: [abe@material.t.u-tokyo.ac.jp](mailto:abe@material.t.u-tokyo.ac.jp)

## Structure Determination of Quasicrystals: STEM Imaging and Hyperspace X-ray Crystallography

T. Seki<sup>1</sup>, H. Takakura<sup>2</sup>, E. Abe<sup>1\*</sup>

<sup>1</sup>Department of Materials Science & Engineering, University of Tokyo, 113-8656, Japan

<sup>2</sup>Division of Applied Physics, Hokkaido University, 060-8628, Japan

Quasicrystals represent aperiodically ordered form of solids with symmetries that are incompatible with a translational order in normal crystals. The structure of quasicrystals can be basically described according to a similar manner of periodic crystals; i.e., a fundamental lattice and its atomic decoration, where the aperiodic ‘quasilattice’ (e.g., Penrose tiling) is defined instead of the Bravais lattices. Atomic decorations around each lattice point are often manifested by the atomic clusters along with a relevant symmetry motif, and the decagonal columnar clusters are commonly defined as a fundamental cluster for decagonal quasicrystals (d-QC) of a two-dimensional quasiperiodic order. For the last decade, the atomic-resolution scanning transmission electron microscopy (STEM) has provided remarkable progresses in determining d-QC structures [1], particularly by unveiling the local cluster symmetry as well as the relevant atomic configurations through the element-sensitive Z-contrast imaging. Recent ultrahigh-resolution imaging with aberration-corrected STEM has provided further insights of local cluster symmetry; atomic configurations within the cluster are not strictly identical but slightly different due to disorder even in the best-ordered d-QCs specimen, breaking weakly the underlying ideal symmetry for each of the local clusters. This is reasonably attributed to the phason-related atomic disorder characteristics of quasicrystals, and their quantitative evaluations in terms of the relevant cluster symmetry has been an important key issue to understand the phason dynamics playing a critical role for a thermodynamic stability. During the x-ray diffraction analysis, such weak disorder may be averaged over the entire specimen without preliminary knowledge about cluster symmetry, and hence the true symmetry of the cluster may possibly be misled.

In the present paper, we have attempted to determine the structure of several d-QCs at the highest reliability ever made, based on a combination between ultrahigh-resolution STEM and hyperspace x-ray crystallography [2]. Through a multivariate analysis of the STEM images [3], we first determine the fundamental cluster and evaluate the relevant local degree of order. Then, the initial model is further tuned by x-ray diffraction refinements, during which the convergence is judged based on not the R-factor alone but comprehensive evaluations, including direct comparison of the Fourier map with the relevant STEM images. The present work establishes how we approach to the structural solution of complex quasicrystals; it is not any longer an never-ending story, which was raised at around a decade ago [4].

**Keywords** quasicrystals, STEM, atomic imaging, structure analysis

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**Professor Ze Zhang**

Center of Electron Microscopy, School of Materials Science and Engineering,  
Zhejiang University,  
Xihu District, Hangzhou, 310027, CHINA.  
E-mail: zezhang@zju.edu.cn

## Real Time Observation of the Reconstructing $\text{TiO}_2$ (001) Surface via ETEM

When a crystal is cleaved to create a surface, a substantial rearrangement of surface atoms (reconstruction) often occurs in order to achieve higher stability. After reconstruction, surfaces may show remarkably different physical and chemical properties with respect to the bulk-truncated ones. Therefore, correctly determining reconstructed geometries and understanding how they form are of paramount importance in surface science [1, 2]. Over the last decades, scanning tunneling microscopy (STM) has been successfully employed to characterize various reconstructions from top view with atomic resolution. Even so, however, STM cannot ‘see’ the sub-surface layers; more importantly, it remains a great challenge to secure a real-time observation of how the reconstruction forms by STM. Here we demonstrate the capability of in-situ environmental transmission electron microscopy (ETEM) to study the dynamics of surface reconstruction [3]. Using ETEM, we have for the first time obtained real-time images of the reconstructing anatase  $\text{TiO}_2$  (001) with atomic scale resolution from both side and top views, and clarified how the most favorable  $(1 \times 4)$  reconstruction is formed. With the support of first principles calculations, we found that such surface evolution is driven by both lowly coordinated atoms and surface stress. This work provides a complete picture of the reconstruction of  $\text{TiO}_2(001)$  and paves the way for future studies of the reconstruction dynamics of other solid surfaces.

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- [4] This work was supported by NSFC and the authors acknowledge Dr Sun at Monash University and Dr Selloni at Princeton University for their contribution to the calculations

*Ze Zhang is a professor in the School of Materials Science and Engineering, Zhejiang University, China. His current research interests focus on electron microscopy study of advanced materials. He was one of the earlier researchers worked on quasicrystals in 1980s. In recent years, Prof. Zhang together with his research team has developed an advanced microscopy technique, with which in-situ external field can be introduced into the microscope retaining the atomic resolution. They successfully apply this advanced technique on in-situ electron microstructure study of the size effects of structural materials, especially on abnormal elasticity and strength of Ni-, Fe-, Cu-, Au-, Zr-, and some alloys. Prof. Dr. Zhang got his Ph. D on 1987 from the Institute of Metal Research, Chinese Academy of Sciences and worked as a research professor from 1991- 2003 in an Electron Microscopy Center of Chinese Academy of Science. He was a professor of Beijing University of Technology on 2003-2010, and is a professor of Zhejiang University from 2010. Prof. Dr. Zhang was elected as a member of Chinese Academy of Sciences on 2001. Now he serves as the chair of Academic Committee board of Zhejiang University since 2012. He is the author of more than 260 papers published on international peer reviewed journals. He is a chief scientist of national basic research program of China, the president of China Association for instrumental Analysis, Chinese Electron Microscopy Society, and the president of Asia Pacific Electron Microscopy Association since 2012.*





**Professor Manabu Ishimaru**

Department of Materials Science and Engineering,  
Kyushu Institute of Technology,  
Sensui, Tobata, Kitakyushu  
Fukuoka 804-8550, JAPAN.  
E-mail: ishimaru@post.atssc.kyutech.ac.jp

## **Structural Characterization of Amorphous Materials via Electron Beam Radial Distribution Function Analysis**

Amorphous materials are often utilized not only as structural and functional materials but also for the synthesis of nanocrystalline and non-equilibrium materials. To control materials properties and processing, it is necessary to obtain information on amorphous structures. Amorphous structures are not completely disordered but exhibit short-range order. The short-range ordered states can be evaluated by a variety of experimental techniques. Radial distribution function analysis is one of the useful ways for understanding atomic correlations and the correlation distances in amorphous networks. In this function, amorphous structures are characterized by the probability of finding another atom at a distance between  $r$  and  $r+dr$  from a specific atom. Radial distribution functions can be obtained by x-ray, neutron, and electron diffraction techniques. Among the diffraction techniques, electron diffraction has the following merits: (1) strong interaction between the material and electrons has the advantage of detecting light atoms; (2) thanks to short wavelength of high-energy electrons, an intensity profile up to high scattering angles is easily obtained; (3) local structures on the nano-scale can be obtained by a combination of other techniques, such as high-resolution electron microscopy and nano-beam spectroscopy analysis.

We have examined a variety of amorphous materials using radial distribution function analyses based on electron diffraction techniques. In the present talk, I'll show some examples of radial distribution functions analyses of covalent amorphous materials.

*Manabu Ishimaru is a professor in the Department of Materials Science and Engineering, Kyushu Institute of Technology. His current research interests focus on the structural characterizations of functional amorphous and/or nano-sized materials. He is experienced in transmission electron microscopy techniques, such as high-resolution electron microscopy and nanobeam electron diffraction, and computer simulations based on molecular-dynamics and Monte Carlo methods. Ishimaru received his BS (1989) and MS (1991) degrees in metallurgy from Tokyo Institute of Technology and PhD degree (1994) in materials science and technology from Kyushu University. He worked in Kyushu University as a research associate (1994-2000) and in Osaka University as an associate professor (2000-2013). He was a visiting researcher in the Materials Science and Technology Division at Los Alamos National Laboratory, USA from 1998 to 1999. He moved to the current position in 2013.*



**Dr. Nirawat Thammajak**  
SLRI-GeM Consortium, Research Facility Division.  
Synchrotron Light Research Institute,  
111 University Avenue, T. Suranaree, A. Muang,  
Nakhon Ratchasima 30000, THAILAND.  
E-mail: n.thammajak@gmail.com

## **X-ray Absorption Spectroscopy for Gemology and Mineralogy Applications: A Case Study of Fresh-Water Cultured Pearls**

X-ray Absorption Spectroscopy (XAS) is a powerful technique for studying local structure in materials using an energy-tuneable source of x-rays from a synchrotron. The absorption of x-rays by an atom can provide a meaningful spectrum which qualitatively and quantitatively related to its chemical compositions, forms, and bonding structure. XAS is therefore sensitive to the oxidation state, coordination geometry, and the distances, coordination number and species of the atoms immediately surrounding the selected element. Hence, the technique is capable to provide an effective analysis of trace elements and dilute compositions, which commonly affect the color of gemstones. A study of gamma irradiated Freshwater Cultured Pearls (FWCPs) by the XAS technique led to a fundamental knowledge of coloring mechanism caused by a trace color-forming composition combined with the calcareous base material of the pearl. Different irradiation doses resulted in a shift of XAS spectra, including a development of a peak-shape feature near the absorption edge. This gamma dose dependence observed in the XAS spectra can provide a substantial evidence for irradiated pearl's identification. Besides, the revealed coloring mechanism can potentially be applied for FWCPs quality improvement. In a feasibility study, an intense x-ray white beam generated by a synchrotron was employed to alter the color of FWCPs. It was observed that an original white pearl was enhanced to a metallic-golden pearls, contrary to the metallic grey pearls obtained from gamma irradiation. This metallic gold color is very unique, and surprisingly; the natural iridescence of the irradiated pearl has also been slightly improved. It should be noted that the intense x-ray beam from a synchrotron stimulates the color change, but does not activate the pearls through nuclear reactions, as in the case of cobalt-60 gamma irradiation. It also has an extra advantage of the synchrotron's specific beam direction that can beneficially be applied with a photolithography technique for imprinting an irradiated pattern on pearls. This invention has already established a new technique of golden pattern imprinting process on FWCPs by means of the irradiation of intense x-rays through a designed mask that partially obscured the beam. The partly through x-ray beam resulted in a high-definition golden pattern with highly detailed line, as fine as micrometer level. ©All rights reserved.

*Dr. Nirawat Thammajak is a scientist at Synchrotron Light Research Institute and a project leader for "SLRI-GeM Consortium", Synchrotron Technology for Gemology and Mineralogy Applications. He has accumulated experiences in chemistry research and experiments with leading synchrotron and neutron facilities around the world during his doctoral degree from University of Oxford. In 2016, he received the Very Good Invention Award in Physical Sciences from National Research Council of Thailand.*



**Professor Makoto Shiojiri**  
Kyoto Institute of Technology,  
Kyoto 606-8585, JAPAN.  
E-mail: shiojiri@pc4.so-net.ne.jp

## Structural Investigation of Some Nanocomposite Powders for Li-ion Battery Anode Applications

Masahiro Kawasaki,<sup>a</sup> Kanganit Sompetch,<sup>b</sup> Viratchara Laokawee,<sup>b</sup> Thapanee Sarakonsri,<sup>b</sup> Takashi Hashizume,<sup>c</sup> and  
Makoto Shiojiri<sup>c,d,\*</sup>

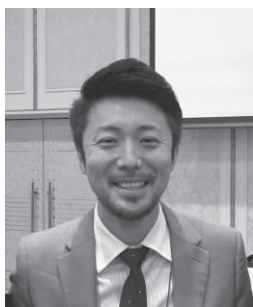
<sup>a</sup>JEOL USA Inc., MA 01960, USA. <sup>b</sup>Depart. of Chem., Chiang Mai Univ., Chiang Mai 50200, Thailand. <sup>c</sup>School of Sci. & Engin., Univ. of Toyama, Toyama 930-8555, Japan. <sup>d</sup>Kyoto Inst. of Tech., Kyoto 606-8585, Japan. \*Present address: 1-297 Wakiyama, Kyoto 618-0091, Japan.

Nanocomposite materials have been widely applied in optical and electrical devices such as thin film transistors, electrodes for solar cells and solid ion batteries, and light emitting diodes as well as in various coatings and catalysts. The structural analysis in a sub-nano scale is indispensable to understand their physical properties. Here, we review our recent high-resolution analytical electron microscopy studies of some nanocomposite powders for Li-ion battery anode applications.

1) **Ge(O)/(graphitic carbon nitride)**: The specimen particles were synthesized by chemical reaction of GeO<sub>2</sub> with NaBH<sub>4</sub> in the basic solution including graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>) powders. The g-C<sub>3</sub>N<sub>4</sub> was arranged by recrystallization of melamine at 600°C under N<sub>2</sub> gas atmosphere. The samples were dried at 60°C or 180°C for 4 h. The g-C<sub>3</sub>N<sub>4</sub> was observed as lamellae of several ten nm or less in size and had an amorphous-like structure with a distorted lattice in an area as small as a few hundred pm in size. The reaction product was Ge(O) particles as fine as several nm in size and composed of Ge and O atoms. Most of the particles might be of GeO<sub>2-x</sub> with the amorphous-like structure that had also a distorted lattice in an area of a few hundred pm in size. In the sample dried at 60°C, the particles were found to be dispersed in a wide area on the g-C<sub>3</sub>N<sub>4</sub> lamella. It was hard to recognize those particles in TEM images but STEM HAADF technique helped us find them instead. The particles in the sample dried at 180°C became larger and were easily observed as isolated lumps. Hence, these powders can be regarded as GeO<sub>2</sub>/g-C<sub>3</sub>N<sub>4</sub> or Ge/GeO<sub>2</sub>/g-C<sub>3</sub>N<sub>4</sub> nanocomposites, and expected to be applicable to anode materials for high energy Li-ion batteries due to Ge catalysis effect, accordingly.

2) **SiSn/(reduced graphene oxide)**: The reduced graphene oxide (rGO) was prepared by a modified Hummers method. A solution of SnCl<sub>2</sub>•2H<sub>2</sub>O dissolved in N<sub>2</sub> bubbled ethylene glycol, added with Si and rGO powders, was reacted with NaBH<sub>4</sub>, followed by rinsing and drying. The nominal composition of the product was C/15 mol.% [Si<sub>0.8</sub>Sn<sub>0.2</sub>]. The powder product consisted of crystalline particles of Sn, Si, and SiO (a mixture phase of Si and a certain SiO<sub>2</sub>) deposited on thin rGO lamellae in the SiSn/rGO powder. The sizes of the Sn, Si and SiO particles were less than 10 nm, although cohered Si and Sn particles became to several hundred nm and several nm in size, respectively. High-resolution transmission electron microscopy revealed that the rGO was amorphous-like graphite with distorted lattices in areas of a few nm or less in size. An electrochemical test was performed for this material, which suggests that SiSn/rGO powders are promising anode materials for lithium-ion batteries with high capacity.

*Dr. Makoto Shiojiri was born in Kyoto, Japan, in Jan. 1936. He is a Professor Emeritus of Kyoto Institute of Technology (1999~) and a Fellow of Faculty of Engineering, University of Toyama, Japan (2014~). He was the President of CAPSEM in 2000-2004 and the vice President of CAPSM in 2005-2012. He is Honorary Members of the Polish Society for Microscopy (2004~), the Japanese Society of Microscopy (2006~), and the Microscopy of Thailand (2008~).*



**Assistant Professor Mitsutaka Haruta**

Institute for Chemical Research,  
Kyoto University,  
Uji, Kyoto, 611-0011, JAPAN  
E-mail: [haruta@eels.kuicr.kyoto-u.ac.jp](mailto:haruta@eels.kuicr.kyoto-u.ac.jp)

## Atomic Resolution Quantification of Elemental Ratio

Elemental mapping by electron energy-loss spectroscopy combined with scanning transmission electron microscopy is particularly useful for non-periodic regions. However, the spatial resolution of an EELS signal is constrained by the delocalization of inelastic scattering and electron channeling process. Then the elemental signals do not necessarily localize at atomic-column positions because the elemental mapping only reflects the two-dimensional distribution of excitation-probabilities at a specific energy loss. These complexities make it difficult to perform quantitative analysis with atomic resolution.

In this study, atomic-resolution quantification of the elemental ratio of Fe to Mn at octahedral and tetrahedral sites in brownmillerite  $\text{Ca}_2\text{FeMnO}_5$  (Fig. 1) is demonstrated using STEM-EELS. It is known that Fe and Mn ions are nearly all ordered but not fully ordered, i.e., a small number of Fe and Mn ions reside in octahedral and tetrahedral sites, respectively.

It was found that a considerable oversampling of the spectral imaging data yields a spatially resolved area that very nearly reflects atomic resolution ( $\sim 1.2 \text{ \AA}$  in radius) for Fe and Mn  $L_{2,3}$ -edge. And the average relative compositions of Fe to Mn within the region were 17.7 to  $82.3 \pm 13.1$  in octahedral sites and 80.7 to  $19.3 \pm 9.9$  in tetrahedral sites.

The true atomic ratio was extracted by estimating the mixing of signals from nearest-neighbor columns using simple simulation based on multislice technique. It was concluded that the ratio of Fe to Mn was 0.13 to 0.87 at octahedral sites and 0.87 to 0.13 at tetrahedral sites. The results correspond reasonably well with the previous neutron diffraction experiment which can correctly decide such information for bulk sample [1]. This means that an experimental oversampling SI data of Fe and Mn  $L_{2,3}$ -edge in perovskite-like structure is probably interpreted with an uncertainty of 10% without simulation.

[1] Hosaka, Y.; Ichikawa, N.; Saito, T.; Haruta, M.; Kimoto, K.; Kurata, H.; Shimakawa, Y. *Bull. Chem. Soc. Jpn.* **2015**, 88, 657-661.

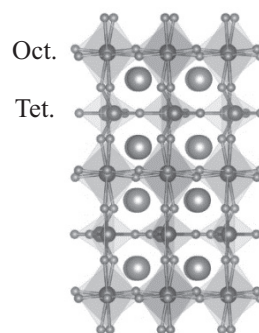


Fig1. Crystal structure of  $\text{Ca}_2\text{FeMnO}_5$

*Dr. Mitsutaka Haruta was a JSPS Post Doctor in Electron Microscopy Group, National Institute for Materials Science and the present position is an Assistant Professor at Institute for Chemical Research, Kyoto University.*



**Dr. Kazutaka Mitsuishi**

All Solid State Battery Specially Promoted Research Team,  
Global Research Center for Environment and Energy based on  
Nanomaterials Science (GREEN),  
National Institute for Materials Science.  
1-2-1 Sengen, Tsukuba, Ibaraki, 305-0047 JAPAN.  
E-mail: Mitsuishi.Kazutaka@nims.go.jp

## **La Ordering in Epitaxial $\text{Li}_{3x}\text{La}_{2/3-x}\text{TiO}_3$ Films and Its Effects on Li-ion Conduction**

Lithium-ion batteries are widely used in everyday life, yet much higher performance and reliability are required for their future applications. At the Global Research Center for Environment and Energy based on Nanomaterials Science (GREEN), which is part of the National Institute for Materials Science (NIMS), we are studying solid state batteries, to achieve high performance by overcoming the limitations imposed by liquid electrolytes. This collaborative research involves both experimentalists and theoreticians in materials science.

Instead of sintered powder materials having been usually used in the studies on solid-state batteries, we grow well-defined epitaxial thin-film devices by pulsed laser deposition. This helps us to relate specific macroscopic properties, such as ionic conductivity, with the orientation and structure of the involved materials. Such devices are also easier to model in first-principles or dynamical simulations.

In this study we grew  $\text{Li}_{3x}\text{La}_{2/3-x}\text{TiO}_3$  (LLTO) thin films on  $\text{NdGaO}_3$ . To control the c-axis orientation direction of LLTO relative to  $\text{NdGaO}_3$ , we used  $\text{NdGaO}_3$  substrates with a small deviation from the 110 axis. The resultant epitaxial thin films were mostly c-axis oriented and contained a small amount of anti-phase boundaries of stacking order of La rich and La poor layers. The density and distribution of those anti-phase boundaries were characterized by scanning transmission electron microscopy. Li-ion conduction was measured for films with different thicknesses to observe the effects of the anti-phase boundary. The relationship between the domain boundary structure and Li-ion conductivity suggests that the a-axis domains and the anti-phase boundaries present in the films are not the principal limiting factors of ionic conductivity [1].

[1] T. Ohnishi, K. Mitsuishi, K. Nishio, and K. Takada, Chem. Mater. 27, 1233–1241 (2015), DOI: 10.1021/cm504033r

*Dr. Kazutaka Mitsuishi works at the In situ Interface Analysis Group Global Research Center for Environment and Energy based on Nanomaterials Science (GREEN), NIMS, Tsukuba, Japan. He is part of the All Solid State Battery Specially Promoted Research Team of GREEN, which is a collaboration among computational and characterization scientists headed by Dr. Kazunori Takada.*





**Professor Takayuki Takarada**  
Division of Environmental Engineering Science,  
Graduate School of Science and Technology,  
Gunma University, Japan

## Low Temperature Catalytic Pyrolysis/Gasification of Biomass Using Natural Products as Catalysts

*Takayuki Takarada, Boodsakorn Kongsomart, Tsedenbal Battsetseg and Naokatsu Kannari  
Division of Environmental Engineering Science, Graduate School of Science and Technology, Gunma University, Japan*

Biomass is quite important energy resources in a near future. The low temperature pyrolysis and gasification of biomass were investigated in a two-stage quartz fixed-bed reactor and an Internally Circulating Fluidized-bed Gasifier (1kg/h) at the temperature range of 450 - 650 °C. Teak sawdust from Thailand, cypress sawdust and swine compost from Japan were used as biomass samples. As natural products for the preparation of catalysts, Loy Yang brown coal from Australia, chicken compost, EFB, black liquor, limestone and limonite were selected. Loy Yang brown coal was used as a catalyst support. Since a low rank coal has a large amount of oxygen-containing functional groups such as carboxyl groups that act as ion-exchange site for metal species, metal cations such as Ni, Ca, Na and K can be incorporated into coals by ion-exchanging with free functional groups. It is well known that finely dispersed metal compounds have a quite large catalytic effect for char gasification in O<sub>2</sub>, CO<sub>2</sub> and steam. The metal-loaded brown coals were prepared by impregnation method. Metal loadings (Ni, K, Na) were about 9wt%, respectively. A commercial nickel catalyst (Ni/Al<sub>2</sub>O<sub>3</sub>, 0.5~1.0 mm, Ni loading 20±2 wt%) and silica sand (0.5~1.0 mm) were used as reference materials.

When swine compost was gasified at 650 °C using silica sand, the gas yield was quite low, that is, 12mmol/g-sample and much tarry materials (about 30wt%) were produced. On the other hand, when Ni-loaded brown coal char and limonite were used in the gasification of swine compost, the gas yield enormously increased by about 4 times compared with that obtained using silica sand and almost no tarry material was produced. Similar results were observed in the gasification experiments using ICFG. The experiments in the fluidized bed demonstrate that ICFG can operate well by using nickel catalysts and the combination of the high performance catalyst with the fluidized bed reactor provide a suitable system for hydrogen, high calorific fuel gas and syngas productions from biomass. The gasification of biomass char was carried out in steam or CO<sub>2</sub> atmosphere. The gasification rates of fixed-carbon in chars physically mixed with catalysts from natural product (black liquor, chicken compost ash and EFB ash) were enormously enhanced compared with that obtained using raw chars.

It was found that natural products examined in this study could be used as quite active and inexpensive catalysts for low temperature pyrolysis and gasification of biomass.

*Professor Takayuki Takarada graduated from Gunma University in March 1974, with the degree of Bachelor of Chemical Engineering and finished his master in March 1976. He joined Mitsubishi Kakoki Kaisha Ltd. for about five years. Then he moved to Tohoku University in 1981. He was awarded Ph. D. in March 1984 from Tohoku University for a thesis entitled: "Study on the catalytic effect for coal gasification". Since 1987 to date, he has served in Gunma University, as an associate professor (1987-1994), as a professor (1994-date) and as a Dean (2005-2008). His research activities covered gasification, catalytic gasification, catalytic pyrolysis, combustion, desulfurization, fluidized bed technology and diamond synthesis by CVD. He was President of The Japan Institute of energy and Vice President of The Society of Chemical Engineers, Japan.*



**Dr.-Ing. Pudji Untoro**

Center for Innovation and Certification,  
Surya Research Center, The Surya Institute.  
Jl. Scientia Boulevard Blok U/7, Summarecon, Gading Serpong  
Tangerang 15810, BANTEN – INDONESIA.  
E-mail: pudji.untoro@surya.ac.id

## **Plasma Arc Technology Processing of Destroying Biomass Materials**

Plasma arc technology is a relatively new technology for processing of destroying biomass materials. A large number of research groups, the world over, are developing plasma gasification systems for treating trash of various kinds and generating useful byproducts, while minimizing the environmental pollution using proprietary processes. This technology has an edge over the other processes involving combustion as it can turn biomass materials into a clean, green, renewable fuel in the form of a synthesis gas (SynGas) with almost no residues that require further disposal. However, major drawbacks for this technique are capacity of the biomass materials depend on the dimension of the plasma beam generated. Recently, an arc plasma prototype was successfully developed for destroying biomass materials in the form of organic and also an-organic materials. The results showed that the biomass is burned with plasma arc largely destroyed and turned into gas within less than 1 minute. With a very short time of destruction will have an impact on the process cost. This plasma can be used also for producing of iron nuggets from iron sand. As result can be obtained with high content of iron oxide without content of elements from organics material, so that it can be used directly as raw material for metal manufacturing industry. Examples of applications will be showed in the presentation and the possibility of potential future implementation. These preliminary results will be followed by various types of biomass and power needed to destroy it. Further works on development of the industrial prototype for destroying of hospital waste and are under development for production of raw materials for metal industry.

*Dr.-Ing Pudji Untoro was a Senior Scientist in Advanced Material at National Nuclear Energy Agency and the present position is a Director at Surya Research Center, the Surya Institute and a member of "Kuark" Magazine Advisory Board. He became a member of national journals editor, "Jurnal Sains Materi", "Jurnal Tekno Fisika" and Jurnal Sains dan Teknologi "Telaah" and elected as a assessors board member of indonesian science institute for national magazines. In 2015, he received the Excellent National Award in Renewable Energy from Indonesian Minister Research and High Education and Award in 107 Indonesian Most Prospective Innovation. He has published more than 60 papers and some books in Indonesian.*



**Dr. Arbi Dimyati**

Center for Science and Technology of Advance Materials  
National Nuclear Energy Agency (BATAN),  
Kawasan Puspiptek Building 71,  
Serpong 15314, INDONESIA.  
e-mail: arbi.dimyati@gmail.com, arbi.dimyati@batan.go.id

## **Arc Plasma Sintering: New Challenge for Low Emission Bio-Waste Treatment**

Arc Plasma Sintering (APS) is a new technique originally designed for consolidation of metal alloys prepared by powder metallurgy at high speed. This technique uses plasma as the heat source which is generated by ionisation of part of Argon gas using low voltage electric DC arc. The main part of Argon gas is used for oxidation protection during the process. The central plasma plume can easily reach heat higher than 2000 °C which is useful for many high temperature processes. The superior advantage of this technique compared to other technique such as the well-known Spark Plasma Sintering (SPS) technology is its quite independent to material conductivity which opens the opportunity for processing of non-metal object such as special waste treatment. In this work it will be shown that the APS can be applied to treat small scale bio and organic waste without producing toxic gases. The production of environmentally harmful gases in general caused by the low temperature combustion can therefore be significantly minimize.

*Dr.-Ing. Arbi Dimyati is a material scientist at the Center for Science and Technology of Advance Materials of Nuclear Energy Agency (BATAN), Indonesia. His research field is especially in high temperature material and electron microscopy. He is now the acting director of Indonesian Society for Microscopy.*





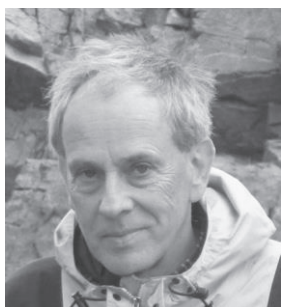
**Dr. Boontarika Srithai**

Department of Geological Sciences,  
Faculty of Science, Chiang Mai University,  
Chiang Mai 50200, THAILAND.  
E-mail: boontarika.s@cmu.ac.th

## **Reviews of Geochronology of Igneous Rocks: Implications for Tectonic Evolution of Thailand**

The lack of absolute age determination has been a problem for geologic works in Thailand, particularly, for geologic maps complying and stratigraphic correlation, understanding the tectonic evolution thus leads to the exploration of natural resources such as ore and petroleum. Not until the last few decades that the advancement in geochronology and sophisticated analytical techniques that yield reliability, accuracy and precision results have shed the light into the new era of research in geochronology. Like in Thailand, the better information about the absolute age of events in geologic timelines has been a useful tool in helping geologists to better understand the tectonics processes that took place in this vicinity and regional scale. Focus on geochronology data obtained from igneous rocks that distributed in northern part of the country and formed four volcanic belts with nearly north-south alignment, the tectonic evolution of Thailand can be depicted. It is generally believed that the collision of Shan-Thai (Sibumasu) and Indochina blocks resulted in the present mainland Southeast Asia, of which the NS-oriented suture zone located along Thailand. The initial stage began with the arc forming by subduction of Paleo-Tethyst underneath Indochina plate, also known as Sukhothai arc, in the late Carboniferous to early Permian. The ongoing process triggered the opening of back-arc basin, to the east of the Sukhothai terrane and the spreading ceased in late Permian resulted in the Nan-Sra Kaeo suture zone. The complete collision of Shan-Thai and Indochina plates occurred in late Triassic, also regarding as Indosinian Orogeny. The main mountain range coupled with S-type granitoid intrusions marked a distinctive feature that extends northward to southwest of China and southward end to the peninsular Malaysia, which is acknowledged for a tin-bearing granite belt. The Post-collision phase took place in late Triassic to early Jurassic. The Cenozoic volcanism was governed by the extensional regime resulted from the India-Eurasian collision that also triggered the opening of Tertiary basins both onshore and offshore in the Gulf of Thailand, where most of petroleum has been produced. However, not always that results from geochronology and biochronology of the same terrane are in a good agreement and this leads to the diversity of interpretations. There are many more challenge issues for geologists and geoscientists to solve regarding the complex tectonic evolution in this region with the assist of geochronology technique.

*Dr.Boontarika Srithai is a lecturer in Geology at Department of Geological Sciences, Chiang Mai University. Her field of interests are mineralogy-gemmology, igneous petrology, geochemistry and geochronology. She and her colleagues at the Igneous Rocks and Related Ore Deposits Research Unit have been working on igneous rocks for tectonic evolutions and ore deposits in Thailand.*



**Dr. Richard Wirth**

German Research Centre for Geosciences GFZ  
Helmholtz Centre Potsdam  
4.3 Telegrafenberg  
Potsdam 14473, GERMANY.  
E-mail: wirth@gfz-potsdam.de

## **TEM and FIB: Significance of Nanoinclusions and Microstructure for Geochronology**

Nanoinclusions and microstructural features of minerals such as zircon or monazite that are relevant for geochronology are of fundamental importance for geological dating. A closed system is the basic requirement for age determination of minerals. The absence or presence of microstructural features such as internal twinning, high dislocation density, fluid inclusions nanoporosity and nanoinclusions can testify for or reject a closed system. Focused ion beam (FIB) sample preparation of electron transparent foils (< 150 nm thick) and subsequent study of such foils in a transmission electron microscope (TEM) reveals the microstructure of minerals and nanoinclusions. TEM is an ideal tool to study the microstructure and inclusions in solid-state materials because it provides chemical composition and structural details simultaneously. In a case study of zircon from Antarctica the importance of the knowledge about the internal structure of zircon for the evaluation of geochronology data is demonstrated. Lead nanospheres (5 – 30 nm in diameter) have been detected in ancient zircon (> 3.4 Ga) from an Archean (2.5 Ga) high-grade metamorphic terrain in East Antarctica. The Pb-nanospheres are commonly associated with an amorphous silica-rich phase, together with titanium and aluminium-bearing phases that could not be determined. These nanoinclusions have been generated during recrystallization of radiation-damaged zircon under high-grade metamorphic conditions. Once formed, the entrapment of Pb-nanospheres in annealed zircon effectively prevents Pb loss, thus explaining why zircon that has experienced such extreme conditions is not completely reset to its metamorphic age. The heterogeneous distribution of Pb can, however, affect isotopic measurement by microbeam techniques, leading to spurious age estimates<sup>(1)</sup>.

<sup>(1)</sup>M. A. Kusiak, D. J. Dunkley, R. Wirth, M. J. Whitehouse, S. A. Wilde, K. Marquardt (2015) Metallic lead nanospheres discovered in ancient zircons. PNAS, 112, 4958–4963.

*Richard Wirth is supervisor of the FIB/TEM laboratory at GFZ German Research Centre for Geosciences, Potsdam, Germany. In 1994 he established the TEM laboratory at GFZ Potsdam, which he has continued to develop by incorporating modern technologies such as the focused ion beam (FIB). His main research areas are micro- and nanoinclusions in minerals, structure and behavior of grain boundaries and amorphous intergranular layers along interfaces in mantle xenoliths.*

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**Dr. Lynne M. Gignac**  
IBM T. J. Watson Research Center  
1101 Kitchawan Rd  
Yorktown Heights, NY 12508 USA  
E-mail: gignac@us.ibm.com

## **Imaging 8 um Thick Semiconductor Interconnects Using 300-400 keV SE, BSE, STEM and 3D FIB-SEM**

Advanced semiconductor chips can have 8-12 levels of interconnects. For reverse engineering and failure analysis purposes, there is a need to image the metal wiring levels without having to delayer the chip. Previously, it has been shown that very high energy (>100 keV) backscattered electrons (BSE) can be used to image multiple, sub-surface Cu interconnect levels. To understand the depth and resolution that Cu or W interconnects in SiO<sub>2</sub> dielectric can be detected by BSE, a 90 nm technology node semiconductor chip with 9 levels of interconnects was characterized by BSE, secondary electron (SE) and dark field (DF)/ bright field (BF) scanning transmission electron microscope (STEM) imaging in transmission electron microscope (TEM)/ STEM using incident beam energies of 300-400 keV.

In the 90 nm node chip, the total interconnect stack thickness was 8 um and consisted of one W contact via level to Si, 8 Cu wiring levels and 1 Al level. A specific region on the chip was imaged by BSE, SE and STEM from the front and back sides. The SE images provided distinct surface details but only had information from shallow depths. The BSE images showed metal levels at varying depths but the location of the level below the surface could not be determined. Though the sample was 8 um thick, good quality BF and DF STEM images could be obtained and, due to severe beam scattering, both BF and DF STEM images had similar contrast. The STEM images had similar information as the BSE images but with inverted contrast.

The region of the chip that was thoroughly characterized by BSE, SE and STEM was then serially sectioned in a dual beam focused ion beam (DB-FIB) system and a 3D volume was created from the SEM image series. The FIB-SEM tomogram correlation with BSE/STEM images determined the depths of the metal levels that were seen in the BSE/STEM images. Cu interconnects in SiO<sub>2</sub> dielectric could be imaged by 400 keV BSE/STEM from depths > 5 um below the surface. A physical model of the interconnect structure was created by 3D printing the FIB-SEM tomogram.

This research was developed with funding from the Defense Advanced Research Projects Agency (DARPA) under SSC Pacific contract HR0011-0060. The views, opinions, and/or findings contained in this presentation are those of the presenters and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S Government.

*Dr. Gignac has B.S. and M.S. degrees from University of Illinois and a Ph.D. from University of Arizona. She is a Research Staff Member at IBM T. J. Watson Research Center and studies nanostructure devices, processing, and materials using electron microscopy. In 2009, she was awarded the Microscopy Society of America Chuck Fiori Outstanding Technologist Award.*



**Dr. Paul Fischione**  
E.A. Fischione Instruments, Inc.  
Export, PA 15632 USA  
E-mail: [pe\\_fischione@fischione.com](mailto:pe_fischione@fischione.com)

## **Unlocking Structure and Chemistry at The Atomic Level through Advanced TEM Specimen Preparation**

Aberration-corrected transmission electron microscopy (TEM) can produce images with sub-Ångström resolution and, therefore, requires pristine specimens for imaging and analysis. Surface damage must be removed to ensure the highest specimen quality. Focused ion beam (FIB) is an indispensable tool for preparing site-specific cross-sectional TEM specimens. However, specimens prepared by FIB typically suffer from amorphous damage, gallium ion implantation, and other artifacts. Low-energy (< 1 kV) argon ion milling has been shown to be an effective tool for post-FIB sample processing to improve specimen quality. Argon ion milling of TEM specimens can enhance specimen quality by reducing specimen thickness, removing gallium implantation, and eliminating amorphous damage.

Ion milling can be performed using a concentrated beam (~ 1 µm diameter) of argon ions. This beam size allows for targeting of the TEM specimen, which reduces the risk of milling the supporting grid material and the subsequent possibility of redeposition of grid material onto the TEM specimen surface.

TEM specimens were prepared using inert gas ion milling with a concentrated beam diameter at low energy. After ion milling, the TEM specimens were analyzed using high-resolution TEM (HR-TEM), high-angle annular dark-field scanning TEM (HAADF STEM), and energy-dispersive X-ray spectroscopy (EDS). The analysis results show the advantages of low-energy, small spot, inert gas ion beam milling.

*Paul Fischione is the chief executive officer of E.A. Fischione Instruments, Inc., which has designed, manufactured, and distributed innovative microscopy devices to the global scientific community for 50 years. Products include specimen preparation devices, imaging detectors, and specimen holders. Fischione also currently serves as the treasurer of the International Federation of Societies for Microscopy (IFSM).*



**Dr. Yasufumi Yabuuchi**

Material Structure Analysis Section,  
Material Solution Department,  
Analysis center, Panasonic Corporation,  
3-1-1 Yagumo-nakamachi, Moriguchi city, Osaka 570-8501, Japan.  
E-mail: [yabuuchi.yasufumi@jp.panasonic.com](mailto:yabuuchi.yasufumi@jp.panasonic.com)

## **Failure Analysis of Semiconductor Devices by FIB-SEM and TEM-EELS**

Failure analysis of damaged SiC power MOSFET samples during TDDB tests can be utilized to investigate the breakdown mechanism as well as to improve the TDDB reliability. The TDDB test sample which deteriorated in this study contained at least one leakage path through the gate oxide. The OBIRCH (Optical Beam Induced Resistance Change) method was used to detect the location of this leakage path. Sample block containing the leakage spot with 20 $\mu$ m width x 20 $\mu$ m long x 10 $\mu$ m height was picked up from the test device and fixed on a Cu half mesh by the micro-sampling method using Hitachi High-Technologies, Co., Ltd. FIB equipment (FB-2200).

The three-dimensional (3D) SEM observation of the leakage spot was carried out using orthogonally arranged type FIB-SEM (Hitachi High-Tech Science Co., Ltd. MI-4000L). The FIB milling at an accelerating voltage of 30kV with 10nm pitch and SEM image capture at an acceleration voltage of 3kV or 5kV were performed alternately with so-called Cut & See mode. From SEM images obtained using FIB-SEM, we constructed 3D image using the software Avizo Fire (Visualization Sciences Group) for the construction and analysis of 3D image. SiO<sub>2</sub> void volumes were then estimated. It is found that there is the influence of SiO<sub>2</sub> void volume on 4H-SiC TDDB failure by the difference in the TDDB lifetime (Tbd) or the gate-to-body resistance (Rbd), and 4H-SiC substrate vendors. The small SiO<sub>2</sub> void plays very important role in the degradation of TDDB reliability.

Using FIB-SEM equipment, another sample blocks were thinned to a thickness of 2 $\mu$ m including the leakage spot from both sides. The UHVEM (Hitachi H-3000) cross-sectional observation at an accelerating voltage of 2.0MV revealed a bright contrast region within the SiO<sub>2</sub> layer. The bright contrast is understood to be caused by the reduction in SiO<sub>2</sub> density with respect to darker contrast regions. Moreover, Si epitaxial growth and penetration into the SiO<sub>2</sub> layer was also confirmed. However, the expected crystalline defects including TSD were not observed in our cross-sectional images. Furthermore thinning the sample from both sides to a thickness of 0.2 $\mu$ m, UHVEM-EELS analysis of the leakage spot was carried out using JEM-1000KRS.

In TDDB failure mechanism analyzed by the present FIB-SEM and UHVEM-EELS techniques, it is considered that SiO<sub>2</sub> voids and Si epitaxial growth are formed at the same time and the gate electrode (poly-Si) and 4H-SiC substrate are connected at the time of breakdown.

*In 1990, Dr. Yasufumi Yabuuchi started to study and develop semiconductor devices such as laser and LED, as an engineer in Matsushita Technoresearch (now, Panasonic Corporation) by structure analysis techniques such as SEM, TEM and FIB. He is a senior engineer in Material Structure Analysis Section, Material Solution Department, Analysis center, Panasonic Corporation.*





**Dr. Hiromitsu Furukawa**

Executive Vice President of SYSTEM IN FRONTIER INC.  
Visiting Professor of Hyogo University  
2-8-3 Shinsuzuharu Bldg.4F, Akebono-cyo, Tachikawa-shi  
Tokyo 190-0012, JAPAN  
e-mail: furukawa@ieee.org

## **Industrial-user-oriented Functionality of Transmission Electron Microscopy Application Software**

Of course, there should not be any difference between academic and industrial use regarding the functionality of transmission electron microscope and its application software. However, from the prerequisites for application software standpoint, there are clear differences, I feel. Especially, the errand inexpensiveness, low introducing and maintenance costs, reliability, robustness, efficiency, and user-friendliness are critical keywords to be accomplished for designing application software for the industrial use. So far, we have been producing various software products that are poised to respond to these popular demand. At this opportunity, I would like to overview several desired functionality by taking few representative software, which seem to be much-needed and welcomed by industrial users, as an example.

### **• TEMOGRAPHY**

A software suit for electron tomography technique comprising a fully automated image acquisition tool, a patented image alignment tool, and a powerful 3D visualization tool.

It was originally developed toward ultimate usability and has been improved over 16 years continuously. Today, more than 350 customers are utilizing this suit in the world.

### **• COLORIST**

‘Segmentation’ is the final step of three dimensional reconstruction process and applicable for various scale, including electron tomography, serial sectioning (optical, SEM, and dual beam FIB), etc. It had to depend on user’s subjectivity and therefore a time-consuming/labor-intense process in many cases. We have developed COLORIST to resolve this problem in response to industrial user’s requests, for more objective, more quantitative, and less difficulty.

### **• STEM MEISTER**

Spherical Aberration Corrector has given rise to very high spatial resolution in STEM imaging. However, extreme installation standards such as vibration, environmental magnetic/AC fields, and operator’s patience came to be required at the same time. ‘Anti Drifting System’, one of the STEM Meister’s unique features could lower such hurdles.

### **• SHOT MEISTER and AUTO PILOT**

One of the primary concerns of industrial user is the time efficiency for data acquisition. Automated data acquisition and reserved recording functions minimize human labor and maximize the service time for electron microscopes simultaneously.

### **• ATTEM**

It is an important to reduce the instrument downtime for improving the time and utilization efficiencies of the instruments. ATTEM records all the hardware status of TEM and watch them in real time. It will notify the instrument supervisor as soon as abnormality occurs, allows to manage troubles beforehand, and enables to monitor the usage situation of the instrument exactly.

*Hiromitsu Furukawa is a Vice President of SYSTEM IN FRONTIER, Tokyo Japan and Visiting professor of Hyogo University, Japan.*

# Symposia in Instrumentation and Development

- I1** - Advances and Applications of Atomic Resolution  
Electron Microscopy
- I3** - New Methods in Diffraction and Imaging
- I4** - Advances in Electron and X-ray Spectroscopy
- I5** - In Situ Dynamics in TEM and SEM, Environmental  
Microscopy
- I6** - EBSD of Synthetic and Natural Materials
- I7** - Advanced Light-Optical Microscopy
- I8** - Scanning Probe Microscopy
- I9** - Atom probe Microscopy
- I10** - Theoretical modeling and Analysis in Electron  
Microscopy
- I11** - Teaching Microscopy: On-line, Hands-on & Remote
- I12** - Surface Microscopy with LEEM, PEEM, UHV-SEM,  
REM and SREM
- I13** - Advances and Applications of Electron Energy Loss  
Spectroscopy



# Materials Characterization

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Colorized EELS elemental map of Ti L<sub>2,3</sub> edges at 456 eV in green, Mn L<sub>2,3</sub> edges at 640 eV in blue, La M<sub>4,5</sub> edges at 802 eV in purple and Sr L<sub>2,3</sub> edges at 1040 eV in red. This work was performed in collaboration with Dr. Phil Rice and Dr. Teva Topura of IBM Almaden Research Center in San Jose, CA who provided expert assistance as well as the TEM specimen and access to their microscope facilities.





**Professor Nobuo Tanaka**

Advanced Measurement Technology Center (AMTC)  
Institute of Materials and Systems for Sustainability (IMaSS),  
Nagoya University, Chikusa-ku, Nagoya, 464-8603, JAPAN.  
e-mail: a41263a@nucc.cc.nagoya-u.ac.jp

## **Research Direction of Environmental Transmission Electron Microscopy for Nano-Materials**

Environmental transmission electron microscopy (E-TEM) has recently attracted a great interest in materials science, chemistry and physics. Current research in this area is focusing on dynamic observation of small catalytic particles with atomic resolution under gaseous atmospheres and in liquids, which is closely related to the development of photo-catalysts, electrodes of fuel-cells and the environmental pollution. In this talk, the author would like to review the development and history briefly since the 1960's, and discuss the advantages and problems. Particularly, since 2007, Nagoya University has been developing a new 1-MV high-voltage environmental (scanning) transmission electron microscope for "reaction science" (RSHVEM), which can be used to observe nano-materials under various gases of more than  $10^4$  Pa pressure, and with heating/cooling and illuminating lights[1,2,3]. The instrument is also used to perform in-situ observation of nano-mechanical operations by using piezo-devices[4] as well as scanning transmission electron microscopy (STEM), convergent beam electron diffraction, electron tomography and elemental analysis by electron energy loss spectroscopy[5]. The new electron microscope has been used to image and analyze various samples including biological ones[6]. The TEM resolution is less than 0.1 nm and STEM one is less than 1 nm, and EELS energy resolution is less than 1 eV at 1MV[5]. In the present talk, various kinds of environmental electron microscopy including those at medium voltages[7] are compared with the present E-HVEM by using data obtained by ours and others. Finally, the future prospects for E-TEM including pulsed TEM[8] are delivered to the audience.

Drs. J. Usukura, S. Arai, S. Muto, M. Kusunoki, K. Yoshida and M. Kuwahara of Nagoya University, T. Fujita of Tohoku University and Y. Takahashi of Kansai University are acknowledged for the present collaboration studies.

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*Dr. Nobuo Tanaka was a professor of Department of Crystalline Materials Science and the director of Ecotopia Science Institute, Nagoya University previously. He has been engaging in studies of nano-science by using high-resolution electron microscopy and nano-diffraction. He was the committee member of IUCr and the program member of IMC-16 and 17(Sapporo and Rio de Janeiro). He was awarded various kinds of prizes, particularly the Minister Prize for Scientific Development in 2014. He is now the president of Japanese Microscopy Society(JSM) and an adjunct senior researcher of Japan Fine Ceramic Center in Nagoya.*



**Dr. Masahiro Kawasaki**  
JEOL USA Inc.,  
11 Dearborn Road, Peabody,  
MA 01960, USA  
E-mail: Kawasaki@jeol.com

## Recent Studies for Materials Characterization with High Resolution Analytical TEM/STEM

Masahiro Kawasaki<sup>1</sup>, Chien-Nan Hsiao<sup>2</sup>, Jer-Ren Yang<sup>3</sup>, Thanapat Autthawong<sup>4</sup>  
Thapanee Sarakonsri<sup>4</sup>, Takashi Hashizume<sup>5</sup>, Takahiro Namiki<sup>5</sup>, and Makoto Shiojiri<sup>5,6\*</sup>

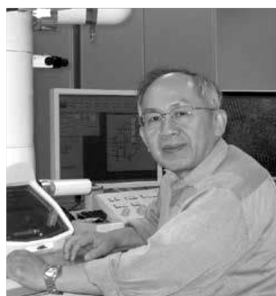
<sup>1</sup>JEOL USA Inc., MA 01960, USA, <sup>2</sup>National Appl. Res. Lab., Instrument Techn. Res. Center, Hsinchu 30076, Taiwan,  
<sup>3</sup>Depart. of Mater. Sci. and Engin., National Taiwan Univ., Taipei 10617, Taiwan, <sup>4</sup>Depart. of Chem., Chiang Mai Univ.,  
Chiang Mai 50200, Thailand, <sup>5</sup>School of Sci. & Engin., Univ. of Toyama, Toyama 930-8555, Japan, <sup>6</sup>Kyoto Inst. of Tech.,  
Kyoto 606-8585, Japan \*Present address: 1-297 Wakiyama, Kyoto 618-0091, Japan

Some of our recent materials characterization studies using high-resolution analytical transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) will be reviewed.

1) **Ru-Pt nanocomposite:** We have fabricated Ru and Pt nanocomposite films using plasma-enhanced atomic layer deposition (PE-ALD), and characterized their structure by means of analytical electron microscopy including the aberration correction technique. Pt and Ru were deposited in the Ar/O<sub>2</sub> plasma ambiance using trimethyl (methylcyclopentadienyl) platinum (IV) and bis (cyclopentadienyl) Ru(II) or bis (ethylcyclopentadienyl) Ru (II) as precursors, respectively. The resistivity of a Pt film deposited on a Si substrate at 300°C was 16.2  $\mu\Omega$  cm, and that of a Ru film was as low as 11  $\mu\Omega$  cm, showing the film to be metallic and not oxidized. It was revealed that the film prepared by successive PE-ALDs of Pt and Ru on a thin amorphous carbon substrate for electron microscopy analysis is a nanocomposite of Ru ribbons and PtRu (7:3) alloy ribbons with 2 ~ 3 nm in width. The Ru ribbon comprised small particles with poor crystallinity of the hcp A3 structure and the PtRu ribbon comprised crystallites with good crystallinity of the fcc A1 structure. The atomic layer deposition would be one of potential techniques to produce Ru/Pt nanocomposites for fuel cell catalysts.

2) ***n*-type CdS<sub>0.9</sub>Se<sub>0.1</sub> powder:** The sample was produced by mixing and annealing of CdS and Se powders that were prepared by a reflux method using Cd(CH<sub>3</sub>CO)<sub>2</sub> 2H<sub>2</sub>O, thiourea and ethylene glycol, and by a solution method using SeO<sub>2</sub>, ethylene glycol and NaBH<sub>4</sub>, respectively. The product was supposed to be crystalline CdS<sub>0.9</sub>Se<sub>0.1</sub> powder from simulation of X-ray diffraction pattern using CaRine 3.1 program and JCPDS card of CdS. Analytical electron microscopy revealed that Cd, S and Se atoms were homogeneously distributed in the powder, which was composed of particles as fine as a few ten nm and in a single crystalline phase, supporting the formation of CdS<sub>0.9</sub>Se<sub>0.1</sub>. This CdS<sub>0.9</sub>Se<sub>0.1</sub> powder is expected for *n*-type thermoelectric materials.

*Dr. Masahiro Kawasaki is the director of TEM applications and business solutions for the Americas, JEOL USA, Inc. Masa is a 1985 graduate of Kyoto Institute of Technology, Japan and was awarded a doctor of engineering degree in 1997. He developed the first S/TEM application for the JEOL TEM in the early 1990s, holds two patents in TEM technology, and is active in technical improvement of TEM applications. He is the 2015 winner of the Microscopy Society of America Chuck Fiori Outstanding Technologist Award for Physical Sciences.*



**Professor Wen-An Chiou**

AIM Lab (Advanced Imaging and Microscopy Laboratory)  
NanoCenter University of Maryland College Park,  
MD 20742-2831  
E-mail: wachiou@umd.edu

## **Ultrafast *In-Situ* and *Ex-Situ* Heating of Al and Ta Nanoparticles**

Nano-Aluminum (Al) has traditionally been used as a fuel due to its high energy density and low cost. Thus, there has been interest in studying oxidizers and optimizing its ignition mechanisms, such as utilization of nanoparticles allows for more homogeneous mixing and interfacial contact that results in increased combustion characteristics. The interaction between the Al core (MP 660 oC) and the Al<sub>2</sub>O<sub>3</sub> shell (MP 2072 oC) of nano-Al is critical to understanding the initiation mechanism for oxidation. This research capitalizes on the unique capabilities of environmental TEM (ETEM) to examine oxidation processes of Nano-Al, Nano-Ta and nano-Al/WO<sub>3</sub> and Ta/Fe<sub>2</sub>O<sub>3</sub> thermites in a super rapid heating environment in and out of an electron microscope.

Al and Tantalum (Ta) nanoparticles of < 50 nm, Fe<sub>2</sub>O<sub>3</sub> and WO<sub>3</sub> nanopowders of < 100 nm were purchased commercially. Ta was chosen due to its high melting point in comparison to Al (3017 oC vs. 660 oC). The thermite was prepared by weighing stoichiometric amounts of nano-Al and WO<sub>3</sub> powders, nano-Ta and Fe<sub>2</sub>O<sub>3</sub>, and homogeneously mixed by sonication in ethanol for 30 minutes prior to deposit onto the TEM grids. A semiconductor-based heating grid/stage (Protochips, Inc.) enabled heating of a sample from room temperature up to a maximum of 1575 K at a rate of 106 K/s. Selected areas of the same particles were examined before and after heating using a Hitachi SU-70 SEM and JEOL 2100 FEG TEM/STEM, equipped with Oxford EDS and Gatan Tridiem EELS/GIF systems.

Morphology of nano-Al and Ta oxide layers before and after various heating treatments was investigated. Morphology of the majority of these particles changed instantly after 1 ms of heating to 1573 oK. Most noticeably, Al particles appeared flattened and coated with a thin layer of W and/or WO<sub>3</sub>. Drastic surface changes of the oxide shell were observed when Ta nanoparticles were heated to and held at 850 oC for 1 ms in air. The result is consistent with a cracking/diffusion initiation mechanism for nano-Al. BSE images and EDS mapping of the untreated thermite sample reveal random distribution of Al and WO<sub>3</sub> particles. Possible evidence of elemental interdiffusion was observed in the HRSEM/EDS images and EDS data. This finding implicates heterogeneous condensed-phase reactions as part of the initiation mechanism in the nano-Al/WO<sub>3</sub> thermite. The Ta/Fe<sub>2</sub>O<sub>3</sub> nanothermite was heated in the TEM to 1200 oC and held for 1 ms. The Fe<sub>2</sub>O<sub>3</sub> appears to wet the surface of the Ta, allowing oxygen to diffuse through the Ta<sub>2</sub>O<sub>5</sub> shell to oxidize the Ta. Unlike Al, Ta does not sinter at areas that are not in contact with the Fe<sub>2</sub>O<sub>3</sub>, as shown by the nanostructure of the unreacted parts of the Ta aggregate.

*Dr. Wen-An Chiou is currently the Director of Advanced Imaging and Microscopy Laboratory (AIM Lab) at the Maryland NanoCenter, University of Maryland. He was a Research Professor at Northwestern University, Evanston, Illinois and also Director at the Materials Characterization Center at University of California, Irvine, California before joining University of Maryland. Dr. Chiou has a broad spectrum of research ranging from materials science to earth science, to biomaterials. He has nearly 40 years of expertise in the use of conventional/high resolution as well as environmental EM, and has received numerous awards in electron microscopy.*



**Associate Professor Kenji Tsuda**

Institute of Multidisciplinary Research for Advanced Materials,  
Tohoku University, Sendai, 980-8577, JAPAN.

E-mail: [k\\_tsuda@m.tohoku.ac.jp](mailto:k_tsuda@m.tohoku.ac.jp)

## **Study of nanoscale local structures of perovskite-type ferroelectrics using STEM-CBED**

The mechanisms of the structural phase transformations in perovskite-type ferroelectrics like  $\text{BaTiO}_3$  have been a matter of discussion for many decades. Coexistence of the displacive and order-disorder characters in the phase transformations was pointed out from many experiments and theories.<sup>1)</sup> In order to solve this problem, it is crucial to examine their nanometer-scale local structures. We have applied the convergent-beam electron diffraction (CBED) method, which is the most powerful technique for determining crystal symmetries of nanoscale local specimen areas.<sup>2)</sup>

We discovered that rhombohedral nanostructures exist in the orthorhombic and tetragonal phases of  $\text{BaTiO}_3$ , while no such nanostructures were found in the rhombohedral phase.<sup>3)</sup> This means that the orthorhombic and tetragonal phases are partially-disordered phases but the lowest-temperature rhombohedral phase is the only ordered phase, indicating the order-disorder character of their phase transformations. Similar rhombohedral nanostructures were observed in the ferroelectric orthorhombic phase of  $\text{KNbO}_3$ ,<sup>4)</sup> while the local structure of the ferroelectric tetragonal phase of  $\text{PbTiO}_3$  was revealed to have the tetragonal symmetry.<sup>5)</sup>

To examine distributions of the nanostructures and local polarizations, we proposed a combined use of STEM and CBED methods (STEM-CBED).<sup>6)</sup> CBED patterns are acquired by scanning the electron probe with a sub-nanometer step. The STEM-CBED method enables picometer-scale sensitivity to atom-displacements and nanometer-scale spatial resolution. Using the STEM-CBED method, two-dimensional distributions of nanoscale fluctuations of the polarization clusters were visualized in the tetragonal phase of  $\text{BaTiO}_3$ <sup>6)</sup> and the orthorhombic phase of  $\text{KNbO}_3$ .<sup>7)</sup>

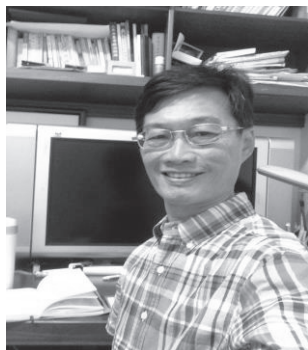
Recently, symmetry breaking in the local structure of the paraelectric cubic phase of  $\text{BaTiO}_3$  has been observed using the STEM-CBED method. The result will be presented in the talk.

These studies were performed in collaboration with Emer. Prof. M. Tanaka and Mr. R. Sano (IMRAM, Tohoku University), and Mr. A. Yasuhara (JEOL Ltd.).

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*Dr. Kenji Tsuda is an Associate Professor in Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, JAPAN. He received the Seto Prize of the Japanese Society of Microscopy in 2004 and the Research Award of the Crystallographic Society of Japan in 2010.*



**Professor Fu-Rong Chen**

Dept. of engineering and System Science,  
National Tsing Hua University,  
Sin Chu 300, Taiwan  
E-mail: [fchen1@me.com](mailto:fchen1@me.com)

## **Atomic Resolution Tomography of Nanoparticles Reconstructed from Exit Wave**

Resolution and sensitivity of the latest generation aberration-corrected Transmission Electron Microscopes allow imaging the vast majority of single atoms from the Periodic Table of Elements with sub-Ångstrom resolution and determining their locations in an image plane with a precision that exceeds the 1.9 pm wavelength of 300 kV electrons. Further progress that exploits this ability requires addressing at leading-edge: (1) there is the need to recover structural information in three dimensions from the two dimensional projection (2), a most noticeable bottleneck to reconstruct 3D structural information are electron beam-induced sample alterations, since a large accumulated electron dose in the range of  $10^4 - 10^5 \text{ e}/\text{\AA}^2\text{s}^{-1}$  is typically required to achieve a needed resolution around one Ångstrom and single atom sensitivity. We present, in the talk, a general tomographic method to recover the 3D shape of a crystalline particle from high-resolution images of a single projection without need for sample rotation. The method is compatible with low dose rate electron microscopy, which improves on signal quality while minimizing electron beam-induced structure modifications even for small particles or surfaces. We apply it to  $\text{MoS}_2$ ,  $\text{NiO}$ ,  $\text{CeO}_2$ ,  $\text{Ge}$ ,  $\text{Au}$  and  $\text{MgO}$  particles and achieve a depth resolution of 1-2 Å, which is smaller than inter-atomic distances.

*Dr. Fu-Rong Chen is a Distinguish Professor in Materials Science and Physics at Department of Engineering and System Science, National Tsing Hua University. In 2012, He received Excellent Research award and Excellent Start-Up Company Award from Ministry of Science and Technology, also outstanding paper from European Microscopy Society.*





**Dr. Chris Boothroyd**

School of Materials Science and Engineering  
Nanyang Technological University  
Block N4.1  
50 Nanyang Avenue  
Singapore 639798  
E-mail: [ChrisBoothroyd@cantab.net](mailto:ChrisBoothroyd@cantab.net)

## **Determining the Structure of Carbon-Based Materials Using Electron Energy-Loss Spectroscopy**

Carbon-based materials come in many different forms such as graphite, diamond and layer based structures like graphene and carbon nanotubes. These can be further modified to alter their properties by for example the addition of functional groups to the surface, as in graphene oxide or by preparation in the form of nanoparticles. High-resolution transmission electron microscopy and scanning transmission electron microscopy can be used determine the morphology of such materials and in many cases the arrangement of the individual atoms. Electron energy-loss spectroscopy provides in addition both chemical information on the atomic species present and information on the type of bonding of the carbon atoms. Here a number of examples of the use of energy-loss spectroscopy for determining the structure of carbon-based materials will be presented.

Graphene oxide is a form of graphene that has been modified by the addition of functional groups to its surface. The structure and distribution of these functional groups, which affect its chemical, electrical and mechanical properties depends on the synthesis method used. Of particular interest is the chemical composition and spatial distribution of these functional groups. We analysed Ba-doped graphene oxide at 80kV using a Cs and Cc corrected scanning transmission electron microscope (STEM) and were able to identify the positions of single Ba atoms along with the location of oxygen and other elements.

The shape of the carbon-K energy loss edge depends not only on the type of bonding of the carbon atoms ( $sp^2$  or  $sp^3$ ) but also on the location of the surrounding atoms. We have analysed a number of different forms of carbon using monochromatic energy-loss spectroscopy and determined the shapes of their carbon edges with high energy resolution. These spectra can provide a way of understanding the bonding in unknown carbon materials. In addition, the low-loss part of the energy-loss spectrum, ie energies below about 20eV, can contain valuable information. We will show how this part of the spectrum can help with understanding the effect of metal dopant atoms on graphene.

*Chris Boothroyd is a Senior Scientist at the Jülich Research Centre, Germany. In the past he worked in the Department of Materials Science and Metallurgy, University of Cambridge, at IMRE, Singapore and at the Center for Electron Nanoscopy, Technical University of Denmark. His recent research interests cover a wide variety of topics related to electron microscopy including aberration-corrected microscopy, energy-loss spectroscopy and electron holography.*





**Dr. Vilem Nedela**

Environmental Electron Microscopy Group,  
Institute of Scientific Instruments of the CAS, v. v. i.,  
Královopolská 147,  
612 64 Brno, Czech Republic.  
E-mail: [vilem@isibrno.cz](mailto:vilem@isibrno.cz)

## **The Morphological Study of WetBiopolymer Capsules with Living Cells by Environmental Scanning Electron Microscope AQUASEM II**

*Vilem Nedela, Eva Tihlarikova & Peter Gemainer  
Environmental Electron Microscopy Group, Institute of Scientific Instruments of the CAS, v. v. i.,  
Královopolská 147, 612 64 Brno, Czech Republic.*

The characterization of the microstructure and study of polyelectrolyte complex microcapsule (PEC) properties represent important challenge for electron microscopy. Development of new PEC uses for biotechnological applications such as an immunoisolation of Langerhans islets for the treatment of diabetes and the stabilization and reuse of enzymes and bacterial cells as biocatalysts is very important. These very beam-sensitive bio-polymer capsules used for cells immobilization/encapsulation are laboratory-produced as a uniform with a controlled shape and size, as well as membrane thickness, permeability and mechanical resistance. Owing to importance of study of above mentioned parameters, samples must be inspected in their fully native and functional state (free of freezing, chemical contamination or preparation resulting in shape distortion). It means observation in thermodynamically stable and fully wet state, precisely reached after very slow changing of conditions in the specimen chamber of ESEM. Violation of these conditions leads to deformation and burst of a thin semipermeable membrane surrounding liquid core, containing live bacterial cells, for example *E. coli*. Destruction is relatively fast and nonreversible. Our results provide evidence that our non-commercial ESEM AQUASEM II allows to study surface morphology of fully wet membrane free PEC microcapsules in their native state, without distortion and shape modification and in addition, while maintaining the conditions for the survival of the live bacteria inside the capsules. Dependence of the PEC microcapsule surface beam sensitivity on chemical composition of the polymer is demonstrated on two types of PEC microcapsules prepared from modified materials. Due to the relatively big size of samples (800  $\mu\text{m}$  in diameter) and their very high beam sensitivity, a combination of our method of gentle and slow specimen chamber pumping procedure and our ionization detector of SEs (beam current up to 40 pA) enhanced for larger field of view (850  $\mu\text{m}$ ) are used. The extent of sample damages due to ESEM observation is evaluated by the juxtaposition of images from the optical microscope and ESEM. Long-term effect of primary electron beam impact on the sample is demonstrated by the effect of sample shrinkage throughout the volume, or deformation of the outer spherical segment.

*Dr. Vilém Neděla is a founder and leader of the Environmental Electron Microscopy Group in ISI CAS and former student of professor Autrata. He is author of two patents, many scientific papers and methods for study of soft and susceptible samples in ESEM as well as scintillation BSE, SE and ionization detectors for SEM and ESEM. He is author of design improvements of the ESEM AQUASEM II. In 2009, he received the Award for the best doctoral thesis from Czechoslovak Society of Microscopy.*



**Dr. Yusuke Ominami**  
Electron Microscope Systems Design 2<sup>nd</sup> Dept.  
Science Systems Design Div.  
Hitachi High-Technologies Corporation,  
Hitachinaka-shi, Ibaraki-ken, Japan  
E-mail: yusuke.ominami.ay@hitachi-hightech.com

## **A Novel Approach for the Scanning Electron Microscopy at Ambient Atmospheric Pressures**

The scanning electron microscope (SEM) has been utilized as a powerful tool for obtaining micro and nanostructures of observed sample. Samples are observed under a vacuum condition so that wet samples require drying before SEM observation. To overcome this inconvenience, Many efforts has been devoted to designing environmental and low vacuum SEMs, which enable imaging samples in gaseous and vapor conditions with the pressure range from  $10^0$  Pa to  $10^3$  Pa. In recent years, SEM methods for observing wet samples under atmospheric pressure have been reported by some investigators. With these methods, the sample space is separated by a thin transparent membrane from vacuum environment where electron beam is propagated. Also, samples attaching to the membrane are observed by SEM.

In the present study, we developed a table-top atmospheric SEM (ASEM) for observing samples which are kept in ambient air conditions but are separated from the membrane. Our ASEM has an inner chamber inside a regular specimen chamber. This inner chamber is equipped with an attachable thin membrane. When a sample should be exchanged, the specimen stage is extracted from the inner chamber. In this configuration, the environment around the sample can be kept in ambient air conditions and changed by evaporation with an additional vacuum pump. Moreover, the higher vacuum observation can be also performed after removing the membrane from the inner chamber. This means that our ASEM enables observation of samples under not only atmospheric but also various-ranged vacuum pressures.

Our ASEM can observes various wet samples. We observed wet regenerated cellulose fibers at atmospheric pressure. After obtaining an image of wet fibers, the specimen chamber was evacuated using the additional vacuum pump and the same fibers were observed in different pressure conditions. Measurement from the SEM images shows that the fibers shrunk approximately 25 % in diameter after evaporation, indicating that the fibers swell with water. We also succeeded in observing wet biological samples including mildew fungus, blood cells and renal glomerulus at atmospheric pressure using light microscope and our ASEM. Since no additional preparation of samples are required between light microscope and our ASEM observation, the technique allows to be widely used for evaluation of various materials. We show some examples of these SEM images and discuss the future of this technique for applying to studies on three-dimensional surface structure of samples by SEM in ambient atmospheric conditions.

*Dr. Yusuke Ominami is a Senior Engineer at Science & Medical Systems Business Group, Hitachi High-Technologies Corporation. In 2015, he received the vacuum technology Award from the Vacuum Society of Japan*



**Professor Yoshifumi Oshima**

School of Materials Science,  
Japan Advanced Institute of Science and Technology,  
1-1 Asahidai, Nomi, 923-1292, JAPAN.  
E-mail: oshima@jaist.ac.jp

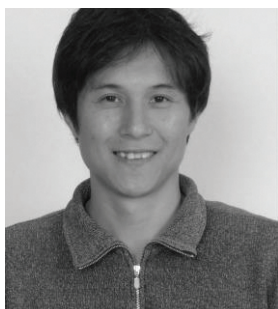
## ***Operand* Transmission Electron Microscope Observation of Lithium Ion Battery – What Happened during the Operation**

Lithium ions have been pointed out not to be homogeneously distributed in the electrode during the battery operation. Structure evolution in the cathode or anode materials during the battery operation has been suggested not to be explained by the results of *ex-situ* measurements. In this study, we developed the nanobattery consisting of  $\text{LiMn}_2\text{O}_4$  nanowires cathode of about 100 nm in diameter, liquid ion electrolyte and  $\text{Li}_4\text{Ti}_5\text{O}_{12}$  crystalline anode. Since the nanowires were bridged between Pt current collector and  $\text{Li}_4\text{Ti}_5\text{O}_{12}$  crystalline anode, their structural evolution could be observed by a transmission electron microscope (TEM) simultaneously with monitoring the cyclic voltammogram [1] (*operand* TEM observation). We observed the nanowire cathode including the interface with ionic liquid during the battery cycles of charge and discharge processes in order to investigate both the structural evolution of the cathode [1,2] and lithium distribution in the cathode [3]. Cyclic voltammetry (CV) was performed by scanning voltage from 3.50 to 5.50 V vs Li/Li<sup>+</sup> at 0.55 mV/s, which was relatively high rate. For structural analysis, the transmission electron diffraction (TED) patterns were taken at the vicinity of the interface with the electrolyte at several voltages during the cycles. The nanowire showed the cubic phase without lithium ions at 4.7 V. However, it changed to the mix of the orthorhombic and cubic phases at 4.25V, suggesting that that large amount of lithium ions must be inserted into the cathode even at the initial stage of the discharge process. And it perfectly became the tetragonal phase at 3.9V, suggesting that the vicinity of the interface has the  $\text{Li}_2\text{Mn}_2\text{O}_4$  crystal. Taking into account that the lithium concentration was about 1 ( $\text{LiMn}_2\text{O}_4$ ) on average at 3.9V, the lithium concentration around the interface was found to be much higher than the average, indicating inhomogeneous lithium concentration. We also investigate the lithium distribution by electron energy loss spectroscopy (EELS) during the battery operation and proposed a new model to explain the lithium diffusion in the nanowire.

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*Dr. Yoshifumi Oshima is an Associated Professor at School of Materials Science, Advanced Institute of Science and Technology, Japan. In 2011, he received the society award (Seto prize) of the Japanese Society of Microscopy.*



**Dr. Masanori Koshino**

Electron Microscopy Group, Nano-Materials Research Institute,  
National Institute of Advanced Industrial Science and Technology  
(AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, JAPAN  
E-mail: [m-koshino@aist.go.jp](mailto:m-koshino@aist.go.jp)

## Application of Low-voltage S/TEM with EELS and EDS for Soft Materials Analysis

Recent advances in electron microscopy has been achieved by the development of aberration correctors and high sensitive detectors in addition to the numbers of stabilized functions of microscope itself. Our methodology of single molecular analysis is unique in a way that we utilize nano carbon materials, such as carbon nanotubes and nanohorns, as a substrate of organic molecules not only for static imaging but also imaging in motion by high-resolution transmission electron microscopy (HRTEM)<sup>1-3</sup>. More advanced technique with aberration correctors, a low voltage electron beam, and a bright and atomic size electron probe is achieved by the combination of scanning transmission electron microscopy and electron energy-loss spectroscopy (STEM-EELS)<sup>4</sup>. The combination of atomic level imaging and spectroscopy has paved the way to characterize low-dimensional materials such as edge structures, defect, and dopants of graphene or h-BN in more details<sup>5-10</sup>. I will discuss the basic concept of these techniques and share the ideas of its application not only for material sciences but also basic science of soft materials.

*A recent work of Dr. M. Koshino demonstrates the atomic level analysis of nano-carbon materials both by imaging and spectroscopy. The analysis covers the interpretation of molecular behavior during the chemical reaction. He has learnt transmission electron microscopy at Kyoto University, Japan, that has conducted a systematic study of organic crystals and the theory of microscopy. He joined several national projects promoted by Japan Science and Technology Agency (JST) and Ministry of Education, Culture, Sports, Science and Technology (MEXT).*

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**Dr. Mitsuo Suga**

Application Management Department, JEOL Ltd.  
3-1-2, Musashino, Akishima, Tokyo 196-8558, Japan.  
E-mail: msuga@jeol.co.jp

## **Observation of Dynamic Phenomena Using Atmospheric Scanning Electron Microscope (ASEM)**

A new Atmospheric Scanning Electron Microscope (ASEM) is able to observe samples directly in liquid or gas under open atmosphere [1, 2]. In this system, an electron-permeable window made of pressure-resistant 100nm-thick silicon nitride (SiN) film, set into the bottom of the ASEM dish, allows an electron beam to be projected from underneath the sample. The electrons backscattered from the samples are captured by a detector positioned below the dish. Above the dish, an optical microscope (OM) realizes quasi-simultaneous observation.

ASEM allows dynamic observations of various chemical and physical phenomena. For example, random movement of silica particles in liquid was observed using ASEM. Electron beam induced self-organization and dispersion of the particles were also observed [2].

Since the ASEM dish is open to the atmosphere, it is possible to add reagents during ASEM observation. Water was added to gypsum, and formations of needle-like structures were observed. Change of the movement of silica particles was also observed by addition of salt solution [3]. In addition, phenomena accompanying rapid volume change such as evaporation can be observed because of the open ASEM dish. Formation of salt crystals and re-crystallization of copper sulfate by evaporation of solvent was also observed [2].

In addition, custom ASEM dishes allow further dynamic observations. The electrochemical ASEM dish has two gold/titanium bilayer electrodes 100  $\mu\text{m}$  apart. Since the sample stage of the ASEM is open to the air, phenomena accompanying volume change can be observed, including electrochemical gas generation. These have not been easily observed with the closed environmental cell. Using saturated NaCl solution as an electrolyte with 2.1 V applied between the electrodes, we observed electrochemical deposition near the cathode [2]. The heated ASEM dish allows observation of temperature-dependent samples, using a heater and a thermocouple around the sample space. A piece of solder (Sn: 42 wt%, Bi: 58 wt%) was placed on the SiN film and observed by ASEM at temperatures between 110 °C and 150 °C. Using the ASEM, we observed that the contrast from the segregated solder changed to be uniform during melting and the uniform contrast had various morphologies during solidification [2]. Some trials to observe and process polymers will be also presented [4].

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*Dr. Mitsuo Suga was a project leader of Atmospheric Scanning Electron Microscope (ASEM) development team, and is assistant general manager of application management department of JEOL Ltd. He is also a guest researcher of Riken. In 2010, he received R&D 100 Award (USA) and MT-10 award (Microscopy Today).*





**Dr. Eva Tihlarikova**

Environmental scanning electron microscopy group,  
Institute of Scientific Instruments of the CAS, v. v. i.,  
Kralovopolska 147,  
612 64 Brno, Czech Republic.  
E-mail: [tihlarik@isibrno.cz](mailto:tihlarik@isibrno.cz)

## **In-situ Preparation Method for Repetitive ESEM and SEM Observation of Plant Samples**

*Eva Tihlarikova, Vilem Nedela*

*Environmental scanning electron microscopy group, Institute of Scientific Instruments of the CAS, v. v. i.,  
Kralovopolska 147, 612 64 Brno, Czech Republic.*

Environmental scanning electron microscope (ESEM) is well known for its ability to observe nonconductive moist or wet samples in optional conditions including in-situ study of their dynamical changes. Nevertheless, observation of susceptible wet biological samples is burdened with the low possibility for repetitive imaging due to sample collapse and relatively low resolution in comparison with scanning electron microscope (SEM).

Methodological improvement using a low-temperature method (LTM) on study of plant samples was firstly introduced in 2015 and was supported by the grant No. GA 14-22777S and LO1212 together with the European Commission (ALISI No. CZ.1.05/2.1.00/01.0017). Results pointed out high sample stability and resistance to beam damage in nonstandard conditions (temperature -20 °C, 400 Pa of air) instead conventionally used conditions (temperature between 0 - 5 °C, water vapor). The LTM allows sample observation in reduced relative humidity, hence with higher resolution.

Now, suitability of the LTM for different not only biological samples and its utilization as an alternative in-situ preparation method for repetitive observation of plant samples is presented. The LTM allows study of the same plant surface microstructure in natural, freeze-dried or sputter coated state and making a compromise between adequate pros and cons (wet structure and in-situ study of dynamical changes vs. low resolution, the highest beam sensitivity and free radicals impact in ESEM; capability for repetitive observation of freeze-dried sample in SEM/ESEM with higher resolution and lower beam sensitivity vs. skills in the LTM and necessity of precise control of the environment in the ESEM specimen chamber; expensive and time-consuming sample preparation for SEM, risk of modification or damage of susceptible microstructures vs. the highest resolution, the lowest beam sensitivity and repetitive observation). Our results show that the possibility for the accurate setting of the working environment in ESEM can be utilized not only for the study of highly susceptible biological samples and polymers, but also for fast and cheap sample preparation. The quality of results is strongly dependent on the working conditions; however high variability of ESEM parameters, such as temperature, freezing velocity and humidity, can be found and set. Despite the fact that this method has many limitations, the surface microstructure is well preserved with minimum artifacts and without expensive and time-consuming chemical treatments.

*Eva Tihlarikova is a researcher at the Environmental Electron Microscopy Group, ISI CAS. In 2013 she presented a new method for in situ study of live specimens in the ESEM. She was also co-author of the new low temperature method for ESEM and new method for study of stem cells in SEM and ESEM. She is currently focused on MC simulations of electron-solid interactions and development of new high sensitive low energy scintillation detectors for SEM and ESEM.*



**Dr. Chaiyasit Banjongprasert**

Department of Physics and Materials Science,  
Faculty of Science, Chiang Mai University,  
Chiang Mai 50200, THAILAND.  
E-mail: chaiyasit.b@cmu.ac.th

## **Applications of EBSD on Microstructural Investigation of Aluminum and Its Alloys**

Electron Backscattered Diffraction (EBSD) has now become a well-established technique among other materials characterization methods. For aluminum alloys, EBSD has now been using as a routine technique to investigate the relationships between microstructures and crystallography which are related to prior processing. The applications of EBSD on aluminum and its alloys will be discussed; focusing on the use of EBSD to realize the relationships between microstructures and processing. EBSD of a variety of processed aluminum alloys will be presented i.e. (1) conventional chill-cast, (2) spray-cast, and (3) severely plastic deformed aluminum alloys. The conventional cast 6xxx aluminum alloys with different Fe contents were characterized by EBSD. An ex-situ EBSD shows that the abnormal grain growth occurred on the chill cast 6xxx with a low Fe content. In addition, the discontinuous recrystallization and grain coarsening was detected.

For spray cast aluminum alloy extended alloying elements and uniform distribution of Zr was achieved. Effective inhibited dynamic recrystallization and grain growth during extrusion. The remained non-recrystallized grains and retained texture were investigated by EBSD.

Equal Channel Angular Pressing (ECAP) is one of the Severe Plastic Deformation (SPD) techniques. Zr and Sc were added in high purity aluminum to promote  $Al_3(Zr, Sc)$  particles and impeded boundary movements during post-heat treatments of aluminum alloys subjected to ECAP. Different ECAP routes: route Bc and route C led to different distribution of  $Al_3(Zr, Sc)$  and thus different degree of retarded boundary movements. The in-depth EBSD analysis can be used to reveal the microstructural evolution after heat treatment.

*Dr. Chaiyasit Banjongprasert is a Lecturer in Metallurgy at Department of Physics and Materials Science, Faculty of Science, Chiang Mai University. In 2015, he received the Thailand Nanotechnology Innovation Award (1<sup>st</sup> prize), awarded under Her Royal Highness Princess Sirindhorn Debaratanasuda.*





**Dr. Suejit Pechprasarn**

Department of Electronic and Information Engineering,  
The Hong Kong Polytechnic University,  
Hung Hom, Kowloon,  
Hong Kong SAR, China.  
E-mail: suejit.pechprasarn@polyu.edu.hk

## Surface Wave Attenuation Coefficient Measurement Using Confocal Surface Plasmon Microscopy

*Suejit Pechprasarn, Wai-Kin Chow, and Michael G. Somekh  
Department of Electronic and Information Engineering, The Hong Kong Polytechnic University,  
Hung Hom, Kowloon, Hong Kong SAR, China.*

Attenuation measurement is crucial in the characterization of Surface Plasmon (SP) sensors and waveguide structures. It is well established that the SP dissipates its energy through two major loss mechanisms [1] (i) coupling loss due to reradiation as the SP propagates along the surface and (ii) ohmic loss occurs due to the resistance in the metal. These two mechanisms can be expressed by Eq (1).

(1)

Where  $I_0$  is the magnitude of surface plasmon in field.  $I$  is an arbitrary constant representing the light intensity of the incident beam.  $L$  is propagation distance.  $\alpha$  and  $\beta$  are coupling attenuation coefficients and ohmic attenuation coefficient respectively.

Theoretical predictions and experimental measurements [2] on the effects of the surface plasmons attenuation have been carried out by a number of researchers. The challenge is to separate the two loss mechanisms. Another attempt to distinguish the two attenuation coefficients involved curve fitting back focal plane SP dip with reflection coefficient model [3]. These back focal measurement methods do have some drawbacks including (1) these measurements rely very much on simplified SP reflection coefficient model (2) for some cases, where the total loss are the same but the ratio between the two losses are inverted, the model can give a very similar solution leading to ambiguity and (3) the back focal plane measurement is less accurate and will not work with lossless surface waves. In other words, this method does not work for dielectric waveguide structures, since there is no dip in the reflectance curve and the model relies on curve fitting the reflectance curve.

In this talk, we will demonstrate the feasibility of measuring the surface plasmon attenuation and quantifying loss mechanisms in surface plasmons (SP) using our recently developed phase spatial light modulator (SLM) based confocal surface plasmon microscope. We can solve Eq(1) and measure  $\alpha$  and  $\beta$  (1) without relying on any model, (2) without ambiguity in the measurement and (3) for a wide range of ratios between  $\alpha$  and  $\beta$  measurement method also works for dielectric waveguides.

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- Dr. Suejit Pechprasarn received his BEng (1<sup>st</sup> class) and PhD from School of Electrical and Electronic Engineering at the University of Nottingham (UK). He then worked as Research Fellow at Institute of Biophysics imaging and Optical Science (IBIOS), the University of Nottingham (UK) and the present position is a Research Fellow at Department of Electronic and Information Engineering, the Hong Kong Polytechnic University (Hong Kong SAR).



**Professor Michael G. Somekh**

Department of Electronic and Information Engineering,  
The Hong Kong Polytechnic University,  
Hung Hom, Kowloon,  
Hong Kong SAR, China.  
E-mail: mike.somekh@polyu.edu.hk

## **Evanescent Wave and Confocal Microscopy**

*Michael G. Somekh, Suejit Pechprasarn and Wai-Kin Chow  
Department of Electronic and Information Engineering, The Hong Kong Polytechnic University,  
Hung Hom, Kowloon, Hong Kong SAR, China.*

Evanescent waves produced by either total internal reflection or by gratings offer scope to confine radiation in at least one dimension into regions far smaller than the optical wavelength. This is very important for the detection of small biomolecules either in solution or within cells.

For imaging we show that evanescent wave microscopy where the scattering of the evanescent waves into propagating waves provides a powerful way to monitor activities in the cell membrane. We use this method to demonstrate different mechanisms of endocytosis which vary with the size of the particle. Combining fluorescence with label free markers allows correlation of physical and chemical responses which gives a very strong indication of the predominant biological mechanism. Sometimes the aim is to make a localised measurement so that one can monitor binding of proteins to a substrate for instance, to follow antibody antigen reactions. Such measurements are of great importance in disease diagnosis. For these type of measurements surface plasmons are especially effective as the surface plasmon propagation properties are highly sensitive to the ambient medium. The problem with surface plasmons when translated to an imaging environment is that their long propagation length degrades the spatial resolution. We have demonstrated that by using a modified confocal microscope the path length of the surface plasmons can be very well defined which both improves measurement precision as well as localization. By placing a spatial light modulator in the back focal plane it is possible to create a highly stable interferometer with a great number of different functions depending on the application. The spatial light modulator allows these different operating modes to be produced within a single instrument and the different configurations are simply controlled by the pattern imposed on the spatial light modulator. We give several examples to demonstrate this including common path operation, single shot interferometry. Attenuation measurements with this instrument are presented in a companion paper.

Finally, we discuss further developments of the modified confocal system in particular we consider how the method may be adapted for wide field operation.

*Chair Professor of Biophotonics and Head Department of Electronic and Information Engineering Professor Somekh received an MA degree in Metallurgy and Materials Science from the University of Oxford in 1976 and a Doctor of Philosophy degree in Microwave Electronics from the University of Lancaster in 1981. He started his academic career in 1981 as Research Associate and later as SERC (now EPSRC) Advanced Research Fellow in the University of Oxford. From 1985 to 1989, he joined the University College London as Lecturer and was the Director of Wolfson Unit for micro-NDE. Professor Somekh joined the University of Nottingham in 1989 and was their Professor of Optical Engineering, Head of Applied Optics Group and Director of Institute of Biophysics Imaging and Optical Science before joining PolyU.*



**Professor Michael Hietschold**

Dr. Nguyen Thi Ngoc Ha, Lars Smykalla, Nguyen Doan Chau Yen, Dr. Marius Toader, Dr. Pavel Shukryna  
Solid Surfaces Analysis Group, Institute of Physics,  
Technische Universität Chemnitz, D-09107 Chemnitz, GERMANY.  
E-mail: [hietschold@physik.tu-chemnitz.de](mailto:hietschold@physik.tu-chemnitz.de)

## **Scanning Tunneling Microscopy Investigations of Pattern Control in Molecular Self-Assembly**

The investigation of self-assembled periodic adsorbate structures on crystalline substrate surfaces is a classical topic of surface physics which has been dominated for a long time by diffraction techniques. The appearance of scanning probe microscopies – especially scanning tunneling microscopy (STM) – has opened the fascinating opportunity of direct real-space imaging with atomic or submolecular resolution.

At the interface between a solution and a crystalline solid, solute (and sometimes also solvent) molecules may deposit in an ordered manner at the solid substrate surface. In-situ studies of the adsorption pattern created this way are possible by ambient STM with the tip immersed in a deposited solution droplet. As an example, trimesic acid (TMA) molecules solved in alkanolic acids may arrange in open adsorption patterns (chicken wire and flower structures) due to H bonding via carboxylic functional groups. At the liquid-solid interface, such type of polymorphism may be controlled by the nature of the solvent (especially its polarity) as well as the concentration of the solutions which opens access to further novel structures. By a controlled increase of molecular packing density of solutions of TMA in alcohols, even a surface-reaction of TMA with coadsorbed solvent molecules (monoester formation with undecanol) has been observed [1-3].

Recent investigations concerning substrate temperature during deposition [4] (Figure 1) and replacement of trimesic acid by the non-planar benzene-triphosphonic acid will be discussed also.

Another approach is based on the self-assembly of molecules at the crystalline surface in ultra-high vacuum (UHV). Under such “ideal conditions” the local electronic structure at the adsorbate-substrate interface can be studied in detail by scanning tunneling spectroscopy (STS) offering insight e.g. into highly localized donation-backdonation charge transfer processes [5]. We demonstrate some examples for the adsorption of phthalocyanines and porphyrines on metal surfaces [6, 7]. As an example, Figure 2 shows a temperature-induced polymerization in a monolayer of brominated Cu-Tetraphenylporphyrin on a Au(111) substrate.

Such kind of investigations may open a way to better understanding the conditions of structure formation and control which is permanently encountered in the biotic world and which might become extremely fruitful for future engineering of molecular architectures and devices.

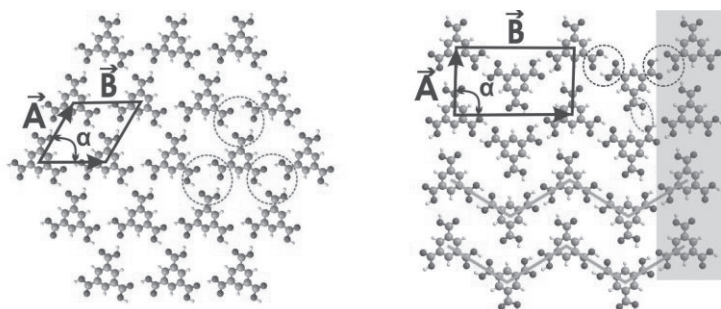


Figure 1. Molecular models of periodic adsorption patterns of trimesic acid molecules deposited from a solution in phenyloctan at 40°C and 60°C, respectively, at the solution-graphite interface [4].

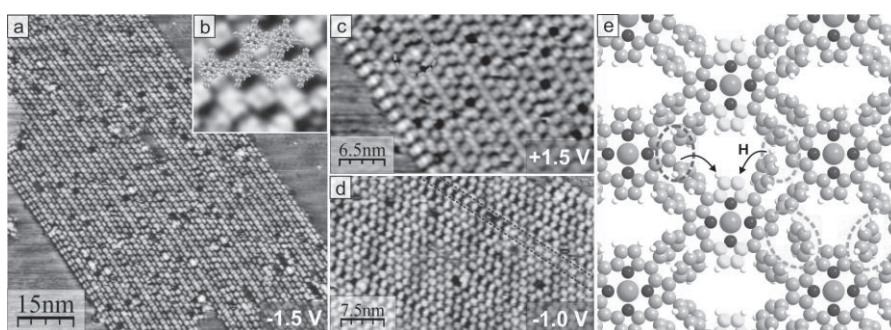


Figure 2. Polymerized monolayer observed after annealing to 350 °C of a OMBE deposited ultrathin film of brominated CuTTP on Au(111). UHV STM images and molecular model visualizing the formation of the network [6].

**Keywords:** Scanning tunneling microscopy (STM), adsorption, solid-liquid interface, ultra-high vacuum, charge transfer, trimesic acid, phthalocyanin, porphyrin.

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*Prof. Dr. Michael Hietschold studied physics and completed Ph.D. 1976 in theoretical solid state physics at Technical University Dresden, Germany. He was a postdoc at Quantum Theory Group of Moscow State Lomonosov University, Soviet Union. Since 1993, he is a professor for Solid Surfaces Analysis and head of the Electron Microscopy Laboratory at the Institute of Physics, Technische Universität Chemnitz, Germany. His research interests are surface physics, nanophysics and ultramicroscopy. He was a guest professor at the National University Ho Chi Minh City, Vietnam, and also lecturing at Portland State University, Oregon, USA. Since 2008 he is advisor for the National Metals and Materials Technology Center (MTEC), Pathumthani, Thailand. Michael Hietschold is a referee for many international scientific journals and funding organizations and has published about 250 scientific papers.*



**Professor Futoshi Iwata**

Department of Mechanical Engineering, Graduate School of  
Engineering, Shizuoka University, Johoku, Naka-ku, Hamamatsu,  
432-8561, JAPAN

E-mail: [iwata.futoshi@shizuoka.ac.jp](mailto:iwata.futoshi@shizuoka.ac.jp)

## **Development Of Nanomanipulator Based on Scanning Probe Microscopes for Biological Applications**

Atomic force microscope (AFM) is well known as surface imaging tool with nanometer scale resolution. AFM can be used not only for surface observation but also for local surface manipulation. Recently, by coupling with a haptic device, the operator can feel the response from the surface during manipulation. However, as for manipulation based on AFM, the probe is used to manipulate the surface; thus, it is impossible to observe the modified surface simultaneously during manipulation. Therefore, real time monitoring systems have been desired to improve the operability.

In this presentation, we describe a novel nano manipulator based on an atomic force microscope (AFM). The body of the manipulator is enough compact to be operated inside the sample chamber of a scanning electron microscope (SEM). It is possible to observe the manipulation situation in the real time observation by using the SEM. The AFM manipulator is coupled with a haptic device for human interface. Thus, the operator can move the AFM probe at any position on the surface with feeling the interaction force detected by the cantilever on the sample surface according to the cantilever deflection. As a performance of the system, biological samples were controllably manipulated under the SEM observation. In order to deal with biological samples in liquid condition, the manipulator can be coupled with an inverted optical microscope. By using the system, we successfully demonstrated manipulation of biological samples in liquid condition. Furthermore, we developed a nanomanipulation technique using a high-speed atomic force microscope (HS-AFM). During manipulation using the AFM probe, the operation is periodically interrupted for a fraction of a second for high-speed imaging that allows the topographical image of the manipulated surface to be periodically updated. With the use of high-speed imaging, the interrupting time for imaging can be greatly reduced, and as a result, the operator almost does not notice the blink time of the interruption for imaging during the manipulation. This creates a more intuitive interface with greater feedback and finesse to the operator. Nanofabrication under real-time monitoring was performed to demonstrate the utility of this arrangement for real-time nanomanipulation of sample surfaces.

*Dr. Futoshi Iwata is a Professor in Micro/ nano mechatronics at Department of Mechanical Engineering, Shizuoka University and in Biomedical Photonics Research Division at Research Institute of Electronics, Shizuoka University. His research interests are development of scanning probe microscopes for nanofabrication and micro/nano manipulations.*





**Professor Julie Cairney**

Australian Centre for Microscopy & Microanalysis,  
The University of Sydney,  
Sydney 2006, AUSTRALIA.  
E-mail: julie.cairney@sydney.edu.au

## **Analysis of Geological Materials by Atom Probe: Understanding the Mechano-chemical Behaviour of Zircon**

The mineral zircon ( $\text{ZrSiO}_4$ ) is ideally suited for radiogenic dating of rocks. Not only does it contain trace amounts of uranium and thorium, enabling dating using radioactive decay in the U-Pb system, but it is also a very robust mineral. It is generally believed to survive a range of geological processes such as erosion, deformation and high-grade metamorphism (up to 900 °C). A fundamental assumption when using the mineral zircon ( $\text{ZrSiO}_4$ ) as a U-Th-Pb geochronometer is that trace elements diffuse negligible distances through the pristine crystal lattice (1). However, recent studies have shown that deformation can modify trace element distributions and Pb/U ratios within zircon (2)

Here, using atom probe tomography, we document the effects of crystal-plastic deformation on atomic-scale elemental distributions in zircon revealing sub-micron scale mechanisms of trace element mobility (3). Target zircon grains were identified from a ~2.5 Ga old gneiss from the Napier Complex, Antarctica. A focused ion beam (FIB) SEM was used to prepare atom probe tips from deformed regions in candidate grains; these tips were characterised using transmission Kikuchi diffraction (TKD) in the SEM and then evaporated in the atom probe. Dislocations that move through the lattice accumulate U and other trace elements. Pipe diffusion along dislocation arrays connected to a chemical or structural sink results in continuous removal of selected elements (e.g. Pb), even after deformation has ceased. However, in disconnected dislocations trace elements remain locked. These findings may have profound implications on the interpretation of trace element concentrations in zircons as well as in other minerals and demonstrate the necessity for a thorough characterisation of the deformation structures within individual grains. They also highlight the importance of deformation on trace element redistribution in minerals and engineering materials.

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*Prof. Julie Cairney is a Professor of Engineering at the University of Sydney and serves as the Director of the Australian Centre for Microscopy and Microanalysis. She is also the Director of the University of Sydney Node of the Australian Microscopy and Microanalysis Research Facility. She leads a research group that focuses on the relationship between microstructure and properties of materials, with particular emphasis on the application and development of new microscopy techniques, and has authored over 150 papers in this field.*





**Dr. Tongjai Chookajorn**  
National Metal and Materials Technology Center  
114 Thailand Science Park, Paholyothin Road,  
Klong Luang, Pathum Thani 12120, Thailand  
E-mail: tongjai.cho@mtec.or.th

## **Revealing the Internal Structures of Nanostructured Alloys using Atom Probe Tomography**

The use of atom probe tomography (APT) technique to investigate the distribution of different atomic species in a specimen has uncovered many important insights on the internal structure of novel materials and its influence on material behavior. In particular, a comparison between atom probe tomography and electron microscopy results often leads to understanding the relationship between chemical, phase, and topographical structures of materials.

For nanostructured alloys, the ability of the APT technique to quantitatively resolve both the spatial distribution and chemical information of atoms on the nanoscale has been a valuable tool for understanding the connection between the intertwined grain structure and atomic distribution, and designing materials with favorable grain boundary segregation of alloying additions. Grain boundary segregation of solute occurs on a very fine scale over an interface and has been captured mostly qualitatively using electron microscopy. The quantitative nature of APT allows more precise measurement of the solute enrichment degree in intergranular regions, which is beneficial not only for engineering purposes such as structural stabilization and improving interfacial cohesion, but also for understanding material's equilibrium structure through thermodynamic calculations. This talk will discuss recent efforts on APT investigation of nanostructured metals such as nickel-, tungsten-, and aluminum-based alloys, as well as advanced high strength steels.

*Tongjai Chookajorn is a researcher at Thailand's National Metal and Materials Technology Center (MTEC), National Science and Technology Development Agency (NSTDA). Dr. Chookajorn completed her Bachelor's degree in Materials Science and Engineering at Carnegie Mellon University and her PhD at the Massachusetts Institute of Technology (MIT), USA with expertise in nanostructured alloys, thermodynamic modeling, and structural characterization using focused ion beam, scanning electron microscopy, transmission electron microscopy, and atom probe tomography.*



**Prof. Xiaodong Han**

Institute of Microstructure and Property of Advanced Materials  
Beijing University of Technology,  
PingLeYuan 100#, Chaoyang District, Beijing, 100124, China  
E-mail: xdhan@bjut.edu.cn

## Atomic Scale Mechanical Microscopy

We developed *in situ atomic scale mechanical microscopy* (ASM) for investigation of the deformation dynamics of materials with simultaneous atomic scale imaging. The *In situ* TEM mechanical testing techniques are generally applicable to regular TEM samples by tensile and compression experiments with simultaneous double tilt, and thus the ability of obtaining atomic scale imaging about the deformation dynamics of the deformed materials. We show examples of the atomic scale deformation dynamics of Ni, Pt, Au and Cu as well as Cu-Zr metallic glass. It was revealed that these materials show unusual large strain elasticity, hyper-elasticity and liquid-like pseudo-elasticity, size-dependent elasto-plastic transitions and the unusual plasticity behaviors at nano-scale. For example, the cross-over plasticity mechanisms from partial dislocation to full dislocation and twins were discovered in deformation of nano single crystalline Cu samples. A long standing puzzle and uncertainties of grain rotation in ultra-small nano-sized polycrystalline materials (grain size less than 8nm) was demonstrated through this *in situ* ASM. It was uncovered that the dislocation climb takes care of the grain rotation behaviors in small grain systems. These results shed lights in understanding the interesting mechanical behaviors and the related dislocation activities of the metallic materials at small scale and useful for designing new materials with strength and ductility as well as those applications in micro- and nano- electronics and mechanics.

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*Xiaodong Han currently works as a professor at Institute of Microstructure and Property of Advanced Materials of Beijing University of Technology. His research interest mainly focuses on developing novel electron microscopy tools and instruments for atomic scale and multi-scale in situ experiments on structural and functional materials. His interests include high strength yet ductile structural materials, energy related materials and physics and catalysis materials, atomic scale understanding solid-solid, liquid-solid and gas-solid reactions etc. He published more 150 papers at international recognized peer-reviewed journals such as Science, Nature Communications, Nano Letters, Physical Review Letters and Acta Mater..*



**Professor Raynald Gauvin**  
Department of Materials Engineering,  
McGill University,  
3610 University,  
Montréal, Québec  
Canada, H3A 0C5.  
E-mail: raynald.gauvin@mcgill.ca

## **Quantitative X-Ray Microanalysis and High Resolution Imaging With Monte Carlo Simulations**

This paper will present how Monte Carlo simulations can be used to perform quantitative x-ray microanalysis in the scanning electron microscope (SEM) and in the transmission electron microscope (TEM) as well as to simulate BSE images of bulk materials as well as bright field and dark field images of transparent foils. Two Monte Carlo free commercial software's, Casino and Mc X-Ray are used in this work. These two Monte Carlo programs can be downloaded at memrg.com. The necessity of using Monte Carlo simulations to perform quantitative x-ray microanalysis non homogeneous materials in the electron microscope will be covered. Contrast reversal in STEM imaging in the SEM will be covered. Quantitative information obtained at the nanoscale with state of the art field emission scanning electron microscopy with an Hitachi SU-8230 coupled with a Bruker FlatQuad SDD EDS system will presented. This paper will demonstrate how the coupling of state of the art FE-SEM and Monte Carlo simulations will lead to a revolution in electron microscopy for the quantitative characterization of real materials at the nanoscale.

*Pr. Gauvin's research interest are related in developing new methods to characterize the microstructure of materials using high resolution scanning electron microscopy with x-ray microanalysis and Monte Carlo simulations. He is the founder of the Monte Carlo program CASINO that has more than 10 000 users. He also developed the Monte Carlo program Win X-Ray and Mc X-Ray that allows performing quantitative x-ray microanalysis. The recent results obtained by his research group in STEM in the SEM have shown that the gap between TEM and SEM is getting narrower. He has more than 300 papers in scientific journals and conference proceedings. He was Invited Speaker in more than 100 international scientific conferences. He won several scientific prizes, most notably the 31st Canadian Materials Physics Medal in 2007 by the Metallurgical Society of the Canadian Institute of Mining, the Heinrich Award in 1997 from the Microbeam Analysis Society of America and the Prix d'excellence du président de l'École for the best Doctorate Thesis defended in 1990 at École Polytechnique de Montréal. Pr. Gauvin was the President of the Inter American Societies of Electron Microscopy (CIASEM) from 2009 to 2011. He was President of the Microbeam Analysis Society of America (MAS) from 2005 to 2006, President of the Microscopical Society of Canada (SMC) from 2001 to 2003 and President of the International Union of the Microbeam Analysis Societies (IUMAS) from 2000 to 2005.*



**Professor Supapan Seraphin**

Department of Materials Science and Engineering,  
Department of Agricultural and Biosystem Engineering,  
and College of Optical Sciences  
University of Arizona, Tucson, Arizona 85721, U.S.A.  
E-mail: seraphin@email.arizona.edu, supapan2000@yahoo.com

## **Teaching Microscopy: Sharing Wondrous Journey into the Micro- and Nano-World**

Microscopy is an eye for science, especially electron microscopy, which can reveal external as well as internal structures down to sub-nanometer scale. “Seeing is believing” is another statement that validates the importance of microscopy to the advancement of science and technology. Improving capabilities and resolution of optical microscopes and electron microscopes is a continuing quest and goes hand-in-hand with scientific discoveries and technological innovations. Effective education of new generations of students and the general public is a requirement for the field in order to pass on the excitement of “seeing and sensing” the incredible world of invisible features. Teaching microscopy has been done in various modes of delivery including face-to-face classrooms, small group demonstrations in front of the instruments, hands-on workshops, on-line tutorials, animation and simulation, and remote-access real-time teaching. This talk will present detail of each mode, compare pros/cons of relevant methods, and give examples of using combinations of various modes to maximize the effectiveness of teaching microscopy.

*Supapan Seraphin is a University of Arizona (UA) Distinguished Outreach Professor, a Faculty Fellow, and a Professor in Materials Science and Engineering Department. She has two joint appointments in College of Optical Sciences and in Department of Agricultural and Biosystem Engineering in College of Agriculture and Life Sciences at UA. She was a 2012 Fulbright scholar teaching and research in Thailand. In 2015, she was a visiting professor at National Materials and Metals Technology Center of Thailand (MTEC).*



**Professor Takanori Koshikawa**  
Osaka Electro-Communication University,  
18-8 Hatsu-cho, Neyagawa, Osaka, 572-8530 Japan  
E-mail: kosikawa@isc.osakac.ac.jp

## Strong Perpendicular Magnetization of Ferromagnetic Multi-Layers with High Brightness and Highly Spin-Polarized LEEM

T. Koshikawa<sup>1)</sup>, M. Suzuki<sup>1)</sup>, K. Kudo<sup>2)</sup>, K. Kojima<sup>3)</sup>, T. Yasue<sup>1)</sup>, N. Akutsu<sup>1)</sup>, A. Dino<sup>3)</sup>, H. Kasai<sup>3)</sup>, E. Bauer<sup>4)</sup>, T. Nakanishi<sup>5)</sup>, X.G. Jin<sup>6)</sup> and Y. Takeda<sup>7)</sup>

1) Osaka Electro-Communication University and Osaka Univ. Osaka, Japan, 2) Ochanomizu University, Tokyo, Japan, 3) Osaka University, Osaka, Japan, 4) Arizona State University, Tempe, USA, 5) School of Science, Nagoya University, Nagoya, Japan. 6) KEK, Tsukuba, Japan. 7) Aichi Synchrotron Light center, Aichi, Japan

We have developed a novel very high brightness and high spin-polarized low energy electron microscope (SPLEEM) [1-3] with a new compact spin-polarized electron gun which enables 3-dimensional spin operation with one device [4]. Current induced domain wall motion is a key phenomenon to realize novel spintronics devices. It has been indicated that domain walls in nanowires with perpendicular magnetic anisotropy can move with lower current density than those with in-plane magnetic anisotropy. Multilayer [Co/Ni] multi-layer is expected as the strong perpendicular magnetic anisotropy. We investigated magnetic property during growth of the [Co/Ni]<sub>y</sub> multi-layer with the above SPLEEM and numerical simulations based on the Landau-Lifshitz-Gilbert (LLG) equation. Fig. 1 shows experimental and simulation results of magnetic domain images of multilayers of pairs of [Co/Ni<sub>2</sub>] on W(110) [5,6]. The numerical simulations well reproduce the magnetic domain patterns observed in the experiments.

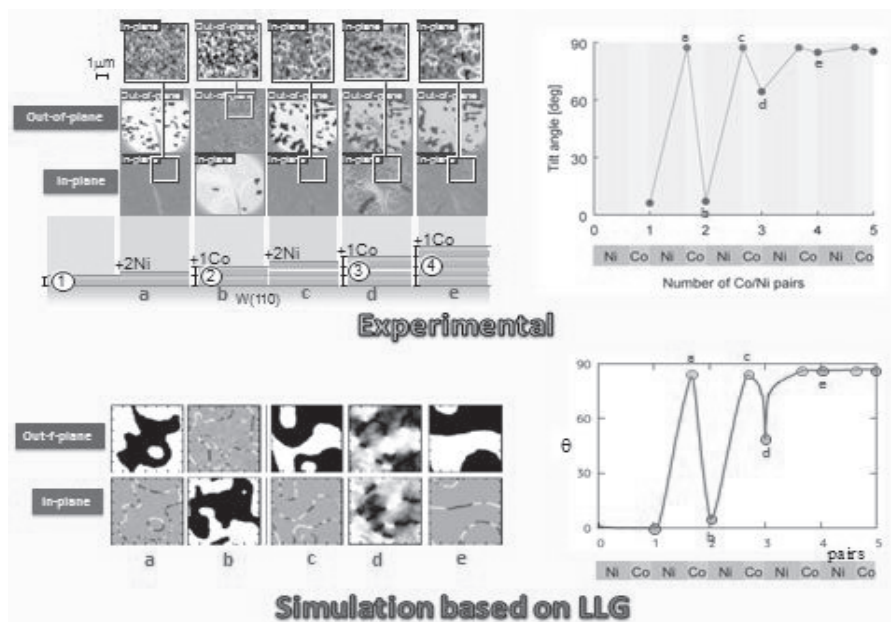


Fig.1 Magnetic domains of Co/Ni multi-layers with experiments and simulations

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*Koshikawa is in the field of Surface Science and has developed very high performed spin polarized low energy electron microscopy (SPLEEM) which has very high brightness and high spin polarization. He received 10 honorable prizes, awards and fellows including a central governmental award (Award of the minister of MEXT).*



**Dr. Andreas Schmid**

Molecular Foundry / National Center for Electron Microscopy  
Lawrence Berkeley National Lab  
One, Cyclotron Rd.  
Berkeley, CA 94720, USA  
Phone: +1 510 486 4564  
Fax: +1 510 486 5888  
E-mail: akschmid@lbl.gov

## Imaging and Tailoring Chiral Magnetism

Chirality in magnetic materials is fundamentally interesting and holds potential for logic and memory applications [1,2,3]. Using spin-polarized low-energy electron microscopy at the National Center for Electron Microscopy of Lawrence Berkeley National Lab, we recently observed chiral domain walls in thin films [4,5]. We developed ways to tailor the Dzyaloshinskii-Moriya interaction, which drives the chirality, by interface engineering [6] and by forming ternary superlattices [7]. We find that spin-textures can be switched between left-handed, right-handed, cycloidal, helical and mixed domain wall structures by controlling uniaxial strain in magnetic films [8]. We also demonstrate an experimental approach to stabilize skyrmions in magnetic multilayers without external magnetic field [9]. These results exemplify the rich physics of chirality associated with interfaces of magnetic materials.

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*Andreas Schmid studies growth and structure of surfaces and thin films. Using tools including scanning tunnelling microscopy and low energy electron microscopy, he is interested in developing and applying in-situ approaches to investigate materials properties under the influence of environmental factors such as sample temperature, deposition flux, applied fields, etc. Since joining the Materials Sciences Division of Lawrence Berkeley Lab in 2001, Andreas has been developing and applying spin-polarized low-energy electron microscopy as a tool to gain insights in diverse research topics related to magnetic phenomena at surfaces, films, and interfaces.*





**Professor Michael S. Altman**  
Department of Physics,  
Hong Kong University of Science and Technology,  
Clear Water Bay,  
Kowloon, Hong Kong SAR, CHINA.  
E-mail: phaltman@ust.hk

## **Structure/Dynamics of Two-Dimensional Layers Studied by Low Energy Electron Microscopy and Nano-Low Energy Electron Diffraction**

Low energy electron microscopy and nano-low energy electron diffraction offer unique and complementary capabilities for studying the structure and dynamics of inhomogeneous surfaces, thin films and two-dimensional layers with high spatial/temporal resolution. These diverse capabilities will be demonstrated by studies of the defect structure of graphene(g)/metals and anomalous diffusive dynamics of the Pb wetting layer on the Si(111) surface. The prevalence of defects in large-area graphene fabricated on metal substrates by chemical vapour deposition is of interest because it may undermine the unique physical and electronic properties that are vital to its use in technological applications or may be exploited to enhance the functionality of graphene. Our investigations reveal the proliferation of lattice orientational disorder and small angle grain boundaries in g/Ru(0001). On the contrary, incommensurability and polymorphism are observed in graphene that exhibits greater orientational uniformity. The flexibility of grapheme on metals is attributed to the coupling of in-plane strain and out-of-plane buckling. Two-dimensional strain mapping in g/Ir(111) reveals inhomogeneous strain relaxation by wrinkles that form due to lattice mismatch with the substrate (Fig. 1). This suggests that it may be possible to strain engineer the properties of graphene if wrinkling can be controlled to form desirable wrinkle networks. Diffusion dynamics in Pb/Si(111) was also investigated by monitoring the relaxation of non-uniform coverage profiles prepared by laser induced thermal desorption. The sensitivity of diffraction satellite peaks produced by the wetting layer to its density is exploited to determine local Pb coverage with 0.001 monolayer precision using nano-LEED. Profile evolution observed in real-space and detailed coverage profiles extracted from reciprocal-space features contradict expectations of classical gradient-driven mass flow. Instead, they suggest an exceptional collective super-diffusive mechanism, whereby the Pb layer slides cohesively over the substrate surface.

*Prof. Michael Altman is Professor and Head of the Department of Physics at the Hong Kong University of Science and Technology. He has served on the Editorial Boards of Ultramicroscopy (2015-present), Surface Science (2008-2014) and Surface Review and Letters (1994-present). Prof. Altman is Fellow of the American Physical Society in the Division of Condensed Matter Physics, cited for his development and use of spin polarized low energy electron microscopy to understand surface processes of solids.*



**Professor Hiroki Kurata**  
Institute for Chemical Research,  
Kyoto University,  
Uji, Kyoto, 611-0011, JAPAN  
E-mail: kurata@eels.kuicr.kyoto-u.ac.jp

## High-Resolution EELS Study of Organic Crystals

Recent developments of a monochromator for transmission electron microscopy provide a new tool for chemical analysis in local area of materials. Owing to the improved energy resolution, the energy range probed by electron energy-loss spectroscopy (EELS) has been extended to visible or infrared region, allowing vibrational spectroscopy in the electron microscope [1, 2]. Moreover, one can also measure the fine energy-loss near-edge structure (ELNES) by exciting electrons in relative shallow inner-shells like a carbon K-edge, being an advantage in the analysis of organic compounds. In the present work, we demonstrate the high energy resolution EELS study of organic crystals performed by a JEM-ARM200F equipped with a double Wien filter [3] and spherical aberration correctors. Figure shows the carbon K-edge ELNES obtained from the crystals of copper phthalocyanine (CuPc) and chlorinated one ( $\text{Cl}_{16}\text{CuPc}$ ). Since the radiation damage is serious for the CuPc crystal compared to the  $\text{Cl}_{16}\text{CuPc}$  one, its ELNES is rather noisy due to the limited electron dosage ( $0.5 \text{ C/cm}^2$ ). However, the chlorination effects are clearly observed in the change of ELNES, which is attributed to the chemical shift of 1s level of carbon atoms because the molecule has three independent carbon atoms with a different 1s binding energy. From the orientation dependence of ELNES, the peaks (a) to (d) can be assigned to the transitions from 1s to  $\sigma^*$  unoccupied molecular orbitals. The peak (a) is related to the excitation of the carbon bonding to hydrogen or chlorine atoms. The intensity of peak (a) in the spectrum of CuPc rapidly decreased with the increase of electron dosage. This means that the primary damage process is C-H bond scission, which is also confirmed by the C-H stretch excitation observed in the infrared region. The low-loss spectra obtained from these crystals will also be presented and compared to the optical measurements.

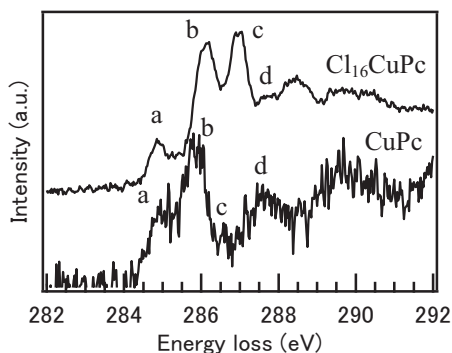


Figure 1. Carbon K-ELNES

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*Dr. Hiroki Kurata is a Professor in Advanced Research Center for Beam Science at the Institute for Chemical Research, Kyoto University.*



**Dr. Ming-Wen Chu**  
Center for Condensed Matter Sciences,  
National Taiwan University,  
Taipei 106, TAIWAN.  
E-mail: chumingwen@ntu.edu.tw

## Atomically-Resolved Electron Spectroscopy for Emergent Phenomena at Oxide Interfaces

With the assistance of modern thin-film growth techniques, perovskite oxides with a three-dimensional crystal structure can now be grown in a layer-by-layer manner at atomic-level precision, opening up vast opportunities for unprecedented phenomena at the two-dimensional (2D) heterostructural interface. The emergence of a conductive interface between the two band insulators,  $\text{LaAlO}_3$  (LAO) and  $\text{SrTiO}_3$  (STO), represents the most celebrated exemplification in this framework. Up to the date, a plethora of oxide interfaces have been reported. It is, however, found that some of the interfaces remain insulating, whereas the same heterostructure-design concept as that of LAO/STO was exploited. To disentangle this puzzle, an atomic-scale spectroscopic characterization across the interfaces is indispensable and the quantitative chemical, electronic mapping by electron energy-loss spectroscopy (EELS) in conjunction with scanning transmission electron microscopy (STEM) represents a powerful technique to this end. In this talk, I will explain the principles of atomically-resolved STEM-EELS and the unveiling of intriguing oxide-interfacial phenomena, ranging from the unexpected existence of a localized 2D electron density at the insulating  $(\text{Nd,Sr})\text{MnO}_3/\text{STO}$  interface to the condensation of the 2D interfacial charges into one-dimensional electron chains by the misfit-dislocation strain field. The origin of interfacial conductivity in the model oxide heterostructure of LAO/STO will be also retackled quantitatively with atomic accuracy.

*Dr. Ming-Wen Chu is a Research Fellow at Center for Condensed Matter Sciences, National Taiwan University. His research interests focus on materials physics by electron spectroscopy at atomic-level precision. So far, he has demonstrated the excitations of dipole-forbidden plasmons in nanoparticles by a fast electron beam, the emergent chemical mapping at atomic resolution by energy-dispersive X-ray spectroscopy, and the tackling of oxide-interfacial phenomena by quantitative STEM-EELS mapping.*

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Sir Michael Stoker Building  
Garscube Campus  
464 Bearsden Road  
Glasgow G61 1QH Scotland (UK).  
E-mail: david.bhella@glasgow.ac.uk

## **Towards *in situ* Structure Analysis of Virus-Host Interactions**

Cryogenic electron microscopy has undergone a revolution in recent years. Technological advances have allowed researchers to solve structures of macromolecular assemblies at close to atomic resolution. Historically virus structure determination has been a reductionist process, requiring us to purify intact virions or viral proteins and solve their structures in isolation. Virus biology is however intimately linked to that of the host cell. Virus particles are dynamic structures exquisitely evolved to function in the cellular environment. Thus to truly understand virus behavior at the structural level will require analysis of virus structures within the cell. Several methods have been developed to prepare cells in a frozen-hydrated state for analysis by cryo-electron tomography. These offer us the tantalizing possibility of three-dimensional structure analysis of the virus-infected cell at macromolecular resolution and cellular scale. I will present an overview of some approaches we have taken to investigating virus-host interactions by cryogenic electron microscopy of the infected cell.

*David Bhella is a programme-leader in the MRC – University of Glasgow Centre for Virus Research (CVR). Research in his laboratory focusses on the characterisation of viruses and virus-host interactions at the structural and molecular level. This is achieved primarily through the technique of cryogenic electron microscopy (cryoEM) and computational three-dimensional image reconstruction.*

*In 2013 David was awarded the Peter Wildy prize for Microbiology Education by the Society for General Microbiology (now the Microbiology Society).*





**Dr. Paula da Fonseca**

MRC Laboratory of Molecular Biology  
Cambridge Biomedical Campus  
Francis Crick Avenue Cambridge CB2 0QH, UK.  
E-mail: pauladf@mrc-lmb.cam.ac.uk

## High Resolution Cryo-EM of Proteasome Complexes as a New Tool for Therapeutic Drug Development

Recent progresses in the areas of electron cryo-microscopy (cryo-EM) and single particle analysis have made these methodologies suitable to determine the structure of proteins to high resolution. This structural information can in principle be used to infer into the detailed molecular mechanisms of proteins and protein complexes. We explored their application to study protein/ligand interactions using the human 20S proteasome core. The proteasome is a highly regulated protease complex fundamental for cell homeostasis and controlled cell cycle progression. The proteolytic active sites of the proteasome are enclosed within its 20S core. In eukaryotes, the 20S core is a 750 kDa complex formed by 7 individual  $\alpha$  and 7 individual  $\beta$  subunits, arranged in a barrel shaped two-fold symmetric  $\alpha\beta\beta\beta\alpha\beta$  assembly. While the 20S proteasome core is a well-established target for cancer therapy, its inhibition is being explored for an increasing range of further therapeutic usages. We used cryo-EM and single particle analysis to determine the structure of the human 20S proteasome core bound to a substrate analogue inhibitor molecule, at a resolution of around 3.5Å. The resulting map allowed the building of protein coordinates as well as defining the location and conformation of the inhibitor at the different active sites. These results serve as proof of principle that cryo-EM is emerging as a realistic approach for more general structural studies of protein/ligand interactions. This has the potential benefits of extending such studies to complexes unsuitable for other methods of structure determination and allowing closer to physiological conditions to be used. Within this context, we extended our studies to assist in the development of new highly specific inhibitors targeting the *Plasmodium falciparum* proteasome. *Plasmodium falciparum* is the parasite responsible for the most severe form of malaria, against which artemisinin is currently the forefront medication. The spreading of artemisinin resistant parasites, first identified in the Southeast Asia, represents therefore a major threat to human health and to the current programs aiming at controlling and eventually eradicating malaria. We determined the structure of the *Plasmodium falciparum* 20S proteasome core bound to a new specific inhibitor, at a resolution of around 3.6Å. This inhibitor was developed by our collaborator Matt Bogyo, Stanford University, based on the profiling of substrate cleavage sites specific to the parasite proteasome. Our structure, and its comparison with that of the human 20S proteasome core, revealed the molecular basis for the inhibitor specificity for the parasite complex. The structure obtained has further guided the improvement of this ligand into a more effective anti-malaria drug prototype, with demonstrated low toxicity to *in vivo* model hosts.

*Dr. Paula da Fonseca graduated in Biochemistry at the Universidade de Lisboa, Portugal, and obtained her PhD in Biochemistry at Imperial College London, UK. She was at the Imperial College School of Medicine and at the Institute of Cancer Research, London, before she started her own research group at the MRC Laboratory of Molecular Biology, Cambridge, UK. Her work focuses on studying the structure and function of cell regulatory protein complexes primarily by electron microscope based methods.*





**Dr. Ming Pan**  
Gatan, Inc.  
5794 W. Las Positas Blvd.  
Pleasanton, CA 94588, USA  
E-mail: mpan@gatan.com

## **Direct Detection and Electron Counting - A Beginning of a New Era for Electron Microscopy**

In the last decade great progress has been made in the instrumentation of transmission electron microscopy (TEM) with the introduction of aberration correctors, electron energy monochromator, and a wide variety of TEM specimen holders designed for in-situ applications. Now a rich wealth of experimental data can be readily generated by the advanced TEM systems. However, the development of suitable image capture device has fallen behind that makes the image detectors become a serious bottleneck to achieving the full potential in performance of an advanced TEM.

Traditional image detectors use scintillator and optical transfer path (fiber-coupling or lens) to convert high energy electrons to photons that are subsequently transferred to the imaging sensor to form an image. One of the disadvantages of this indirect detection approach is the loss of image resolution and sensitivity during the electron-photon conversion and the photon transfer.

Very recently direct detection imaging devices have been successfully developed based on technology advancement made in CMOS (complementary metal oxide semiconductor) design and manufacturing, high speed data architectures, vastly increased memory densities and speed, etc. The elimination of scintillator and the subsequent optical transfer path has significantly improved the detective quantum efficiency (DQE) – a critical measure for resolution and sensitivity of an imaging device. The state-of-the-art direct detection imaging device further boosts the DQE and the image quality under extremely low beam conditions by electron counting at high speed (e.g. 400 fps @ 4k x 4k resolution) to eliminate the sensor readout noise and minimize the electron scattering noise.

This new generation of direct detection imaging device has revolutionized the field of cryo-electron microscopy (cryoEM) in structural biology and now starts to make its impact on many application fields of electron microscopy in materials science, for example, *in-situ* microscopy, 4D STEM, imaging beam sensitive materials, quantitative measurement of radiation damage or quantitative electron microscopy, etc. Direct detection and electron counting are poised to advance electron microscopy to a new era.

*Dr. Ming Pan is the Senior Vice President of Business Development at Gatan, Inc.*



**Professor Jose L. Carrascosa**  
Centro Nacional de Biotecnología (CNB-CSIC)  
Cantoblanco  
28049 Madrid, Spain.  
E-mail: jlcarras@cnb.csic.es

## **Correlation of Cryo-Soft X-Ray Tomography and Electron Microscopy to Study Nanoparticle-Whole Cell Interaction**

Cryo-Soft X-ray Tomography is an emerging technique that benefits from high X-ray penetration into the biological material to image close-to-native vitrified cells at nanometric resolution without the need of chemical fixation or staining agents (1, 2). We have combined this technique with cryo-epifluorescence microscopy and electron microscopy to get 3D information from cancer cells incubated with superparamagnetic iron nanoparticles at a whole cell level (3). In this way we have obtained quantitative statistical analysis of intracellular nanoparticles distribution and accumulation, showing the transfer from the plasma membrane by endosomes towards a region near the nucleus.

Iron identification and quantitation was done by near-edge absorption soft X-ray nanotomography, which combines whole-cell three-dimensional structure determination at 50 nm resolution, with 3D elemental mapping and high throughput.

The results obtained by combination of these tomographic methods are instrumental to provide a basis for selection of the nanoparticles best suited for hyperthermia treatment, drug delivery or image diagnosis in nanobiomedicine.

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*Professor Jose L. Carrascosa is a Research Professor at the Department of Structure of Macromolecules in the Centro Nacional de Biotecnología, CSIC, Madrid, Spain. He is also heading the Nanobiosystems Program at the Imdea Nanosciences Institute at Madrid. He has published more than 227 peer-reviewed publications. He is presently President of the Spanish Society of Microscopy (2013 to date), Member of the Executive Committee of the International Federation of Microscopy Societies (IFSM, 2010 to date), Member of the Executive Committee of the European Biophysics Societies Association (EBSA, 2007 to date), and he was President of the European Microscopy Society (EMS, 2000-2004).*



**Dr. Puey Ounjai**

Department of Biology & Center of Nano-Imaging,  
Faculty of Science, Mahidol University,  
272 Rama VI Rd, Ratchadevi,  
Bangkok 10400, THAILAND.  
E-mail: Puey.Oun@mahidol.edu

## **Structural Insights into Substrate Recognition and Surface Adhesion in *Chlamydomonas reinhardtii***

Surface interactions of planktonic cells govern various microbiological phenomena, particularly in the early establishment of biofilm formation. Flurry of researches has been focused on prokaryotic biofilm formation. While the mechanism governing the interaction of eukaryotic cells with solid surface remains unknown. In unicellular algae, flagellar surface plays an important role in cell-surface adhesion and gliding through several flagellar membrane proteins, in particular, the glycocalyx layer and the flagellar membrane glycoproteins (FMG-1B). Using electron microscopy and tomography, we report a direct evidence that the glycocalyx layer of *Chlamydomonas reinhardtii* flagella is forming s-layer like organization. Immuno-EM suggested that the layer constitutes arrays of flagellar membrane glycoproteins (FMG-1B)—the surface protein that has been shown to be important for making surface contact and gliding motility. This observation provides insights not only on the eukaryotic cell-surface adhesion that may lead to a better understanding of biofilm formation but also offers a fresh perspective on convergent evolution of s-layer proteins.

*Dr. Puey Ounjai is a lecturer at the Department of Biology and head of Center of Nano-Imaging and Mahidol-Olympus Bioimaging Center, Faculty of Science, Mahidol University in Bangkok, Thailand. His lab is focusing on characterization of molecular principles behind various cellular behaviors with particular interest in cell motility, differentiation and biofilm formation.*



**Assistant Professor Pakorn Kanchanawong**

Department of Biomedical Engineering, & Principal Investigator,  
Mechanobiology Institute, National University of Singapore,  
5A Engineering Drive 1, Republic of Singapore 117411  
E-mail: biekp@nus.edu.sg

## **Mapping the Nanoscale Architecture of Cadherin-based Adhesions by Superresolution Microscopy**

Mechanical forces serve as both physical constraints and information cues in cell and tissue biology. Multi-protein assemblies such as the integrin-based focal adhesions and the cadherin-mediated cell-cell junctions are known to sustain, sense, and respond to force in highly complex and adaptive manners. However, the relevant length scale for protein organization within these assemblies is in the sub-diffraction-limit regime, and much has remained unexplored, both in terms of their molecular architecture, and their nanoscale structure-function relationship. We have recently applied superresolution microscopy for molecule-specific interrogation of protein localization and orientation within integrin-based focal adhesions [Kanchanawong et al., Nature 2010; Liu et al., PNAS 2015], yielding insights into their mechanobiological functions. I will discuss our recent studies whereby this strategy was employed to elucidate the nanoscale architecture of the cadherin-based cell-cell adhesions and to probe conformational transitions of the mechanotransducer protein vinculin, revealing how they are regulated by mechanical tension together with specific tyrosine kinase and phosphatase.

*Dr. Pakorn Kanchanawong is an Assistant Professor in the Department of Biomedical Engineering, National University of Singapore, and Principal Investigator at the Mechanobiology Institute, Singapore. He is the recipient of the Singapore National Research Foundation Fellowship in 2011. Dr. Kanchanawong received his Ph.D. in Biophysics from Stanford in 2007 (Advisor: Dr. Steven G. Boxer) and was subsequently trained at the National Institutes of Health as a postdoctoral fellow with Dr. Clare M. Waterman.*



**Assistant Professor Cheng-han Yu**  
School of Biomedical Sciences  
Li Ka Shing Faculty of Medicine, The University of Hong Kong  
21 Sassoon Road, L1-59  
Hong Kong, Hong Kong  
E-mail: chyul@hku.hk

## **Adhesion formation and Transformation at the Cell-Matrix Interface**

Matrix-activated integrins form different cell-matrix adhesion structures. Extracellular microenvironment and traction forces play an important role in adhesion assembly, including focal adhesion, podosome, and cancer invadopodia. We have explored the role of force in podosome development using matrix ligands bound to fluid supported membranes that do not support traction forces. With fluid RGD-membranes, fibroblasts that normally do not form podosomes on rigid matrices will form podosomes within 45 minutes. Podosomes are defined by doughnut-shaped RGD rings and characteristic core components, including F-actin, cortactin, and Arp2/3. Here, we employ RGD-membranes with nano-partitions and demonstrate that dense partitions (lines spaced by 1 $\mu$ m) in RGD-membranes suppress podosome formation. Dense nano-partitioned RGD-membranes locally facilitate contractile traction force development, and cells can pull integrin clusters to the lines and generate force on them. On the other hand, when cells are unable to generate forces on clusters (e.g. with wider separations of partitions (4 $\mu$ m) or myosin inhibition), cells can assemble podosomes. In addition, within a single cell, clusters over continuous RGD-membranes will form podosomes whereas cluster pulled to barriers will not. Inhibition of formin or actomyosin activity does not suppress podosome formation on RGD-membrane. However, inhibition of PI3K activity or activation of RhoA-mediated cellular contractility block podosomes. The local increase of phosphatidylinositol (3,4,5)-trisphosphate level and sequential recruitment of PI3K and PTEN are associated with podosome formation process. Thus, we suggest that force on integrin clusters will stimulate regular adhesion formation, whereas PI3K activation at an integrin cluster in the absence of force will stimulate podosome formation.

*Dr. Cheng-han Yu was an Assistant Professor in the School of Biomedical Sciences, Li Ka Shing Faculty of Medicine at The University of Hong Kong. His research focuses on direct visualization of molecular reorganization and signal transduction by advanced fluorescence microscopy and super-resolution microscopy. He utilizes functionalized supported lipid bilayer membranes to decipher mechano-sensitive signal pathways in cell-matrix adhesion formation and cytoskeletal reorganization. Dr. Yu served as the discussion leader in Gordon Research Seminar: Adhesion Receptor Signaling (2014), as well as the keynote speaker in Gordon Research Seminar: Signaling by Adhesion Receptors (2012). Currently, he focuses on the cross-talk between integrin, Rho GTPase, and kinase in adhesion transformation and cancer invasion. Dr. Yu joined the University of Hong Kong since August 2014. He is a member of Centre for Cancer Research and Strategic Research Theme of Cancer in the University of Hong Kong.*



**Dr. Steeve Boulant**

Department of Infectious Disease, Virology University Heidelberg  
Schaller research group at CellNetworks  
German Cancer Research Center (DKFZ)  
Im Neuenheimer Feld, 581  
69120 Heidelberg, Germany  
E-mail: s.boulant@dkfz.de

## **Dynamics and Ultrastructural Characterization of the Membranous Flat Clathrin-Coated Arrays**

Clathrin-mediated endocytosis (CME) is the most fundamental cellular uptake pathway. Cargo molecules localized outside the cell, after binding to their receptor, are internalized through formation of small transient clathrin structures, referred as clathrin-coated pits. During formation of these endocytic structures, clathrin molecules, adaptors, and regulatory proteins assemble in a coordinate manner to invaginate the cellular membrane resulting in the engulfment of the membrane-bound cargo in cytosolic clathrin-coated vesicle. In this process, membrane curvature is tightly linked to the assembly of the clathrin coat. Beside these clathrin-coated pits, clathrin molecules can also assemble into large, flat, long-lived arrays called clathrin-coated plaques. To date, how these flat clathrin arrays are internalized within the cell and their functions remain unknown.

In this talk, I will focus on a novel microscopy workflow that we have established to characterize the dynamics and ultra-structural organization of clathrin structures (coated-pits and coated plaques). Using this approach, we have revealed unexpected dynamic features of clathrin structures that shed light on the functions of clathrin plaques.

Using micro-contact printing of extracellular matrix (ECM) proteins we were able to spatially and temporally control formation of clathrin-plaques. We found that clathrin plaque formation is not only limited to the basal membrane of cells but they are only found on membranes in direct contact with the substrate. Performing correlative analysis of migrating cells, we unveiled the potential function of these structures acting as “focal adhesions-like” and directly participating in cell attachment to the ECM. Using fluorescence live-cell, optical sectioning microscopy, transmission electron microscopy (TEM) of metal replicas, and correlative light and electron microscopy (CLEM), we thoroughly studied, the dynamics, size, and ultrastructural organization of the clathrin coat in multiple cell lines. We found a great diversity in the dynamics, size and structural organization of the clathrin coats in a cell-type specific manner. Surprisingly, complete correlative analysis revealed that for a large part of the transient clathrin structures, increasing of size does not systematically correlate with an increase in the curvature of the clathrin coat. These clathrin structures display a flat lattice although they have reached their mature size.

We propose that flat clathrin structures, like clathrin-coated pits, contribute to CME and can rearrange into curved scaffolds prior endocytosis. This model challenges the current view of clathrin-coated pit formation where curvature is acquired during coat assembly.

*Dr. Steeve Boulant is a research group leader at the university Heidelberg Germany since 2012.*





**Dr. Depicha Jindatip**

Department of Anatomy, Faculty of Medicine,  
Chulalongkorn University,  
Bangkok 10330, THAILAND.  
E-mail: depicha.j@chula.ac.th

## **Focused Ion Beam Scanning Electron Microscopy Revealed Three-Dimensional Fine Structure of the Cells**

During the study of perivascular cells in rat anterior pituitary glands, considerable data on pituitary pericytes were collected by the use of desmin (pericyte marker) immunohistochemistry, transmission and scanning electron microscopy. These pericytes presented the characteristics as typical pericytes found in other organs. Unexpectedly, desmin immunoelectron microscopy allowed the identification of another type of the perivascular cells in the gland. This novel type did not attach tightly to the vessel. Conventional transmission electron microscopy showed the differences of fine structure between pericytes and novel cells in two-dimensional images. To confirm the results, we applied focused ion beam scanning electron microscope (FIB-SEM), the combination of ion beam milling and scanning electron microscopy to this study. Using this technique, three-dimensional reconstruction and movies of fine structure were created from stacks of two-dimensional images with the helping of software tool. FIB-SEM revealed novel desmin-immunopositive perivascular cells had expanded cytoplasm containing dilated rough endoplasmic reticulum, which formed a circular shape, and had no basement membrane. However, this novel cell type also extended its cytoplasmic processes to the capillary wall. From these findings, our research group now believes that there are two types of perivascular cells—pericytes and desmin-immunopositive perivascular cells—in rat anterior pituitary gland. Further study is needed to clarify more characteristics and functions of these two perivascular cells by introducing the molecular techniques.

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*Dr. Depicha Jindatip is a lecturer in Histology and Anatomy at Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Thailand and a visiting researcher at Division of Histology and Cell Biology, Department of Anatomy, Jichi Medical University School of Medicine, Japan.*



**Dr. Georg Ramm**

Department of Biochemistry & Molecular Biology  
15 Innovation Walk | Room G90  
Monash University | Clayton VIC 3800 | AUSTRALIA  
E-mail: Georg.ramm@monash.edu

## **Correlative Light and Electron Microscopy Techniques for Live-Cell Imaging and for Identifying Rare Structures in Small Organism**

Correlative light and electron microscopy (CLEM) techniques synergistically combine the advantages of optical and electron microscopy, while compensating for the deficiencies of each technique. Major advantages of optical microscopy techniques are that they allow to identify rare events on a large scale and allow for dynamical and functional studies, while electron microscopy achieves high resolution and can provide morphological context. Two CLEM techniques will be discussed, addressing scale using immuno CLEM on Tokuyasu cryo sections and addressing dynamical and functional imaging using live-cell CLEM.

Immuno CLEM on Tokuyasu sections allows finding a rare structure by electron microscopy equivalent to finding a "needle in a haystack." Both optical and electron microscopy are performed on the same section simplifying the correlation between the two techniques. Fluorescently labeled structures of interest are first identified in an overview image by light microscopy and subsequently traced in electron microscopy. For example, we have used immuno CLEM to identify specific cell types in the developing zebrafish brain and actin aggregates in a zebrafish myopathy model.

Live-cell CLEM offers unique insights into the ultrastructural dynamics of cells. However, the correlation of subcellular cell structures observed by live cell imaging with the EM ultrastructure is technically challenging as the same region needs to be tracked in 3D after sample embedding and processing. We have developed a workflow for live-cell CLEM that provides easy guidance for 3D relocalisation. We have used this CLEM-strategy to investigate the fate of mitochondria during induced mitochondrial degradation by mitophagy. In another example, we have manipulated single mitochondria during live cell imaging within a cell and have traced back the same region of interest using electron microscopy.

*Dr Georg Ramm is the head of the Bio EM platform at Monash University in Melbourne, Australia and leads the advanced cellular imaging lab in the Department of Biochemistry. Dr Ramm trained as a Biochemist and obtained a PhD in Cell Biology at Utrecht University in the Netherlands. The focus of his lab is to develop and apply cellular imaging techniques to solve fundamental biological questions regarding the dynamics of cellular organelle traffic and its regulation by metabolic and other factors.*

*Since his recruitment to Monash University in 2009, Dr Ramm has led the development of the electron microscopy platform for the Life Sciences at Monash University. The Monash facility now hosts expertise in both structural and cellular EM and is home to Australia's first Titan Krios cryo TEM.*



**Associate Professor Keisuke Ohta**

Division of Microscopic Anatomy, Department of Anatomy  
Kurume University School of Medicine,  
Asahi-machi 67 Kurume, Japan.  
E-mail: kohta@med.kurume-u.ac.jp

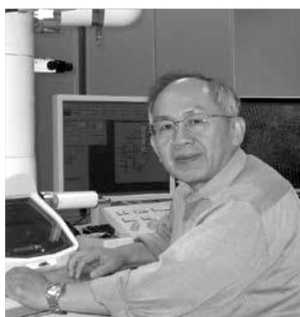
## **Three-Dimensional Organization of Mitochondria-Associated Membrane Revealed by FIB-SEM Combined with Live Cell Imaging**

Correlative light and electron microscopy (CLEM) observation combined with light-microscopic live imaging and focused ion-beam scanning electron microscopy (FIB-SEM) 3D reconstruction is a powerful tool to visualize the electron microscopic structure of the temporospatial specific site. Using this method, we attempted to visualize 3D architecture of mitochondria-associated membranes (MAMs) at a moment of the mitochondrial fission in this study to clarify morphological participation of MAM in the mitochondrial division.

Mitochondria are biological power plants that control various cellular metabolism, cell survival and cell death. Thus, mitochondrial quality control and their homeostasis are critical for cellular homeostasis. Mitochondria homeostasis is now considered to be regulated by mitochondrial dynamics including their continuous fission and fusion and opportune removal of damaged mitochondria. Mitochondrial fission in mammalian cell is mediated by dynamin-related protein 1 (Drp1) that is recruited on the outer membrane of the mitochondria to promote a constriction of the fission site resulting in the eventual division of a mitochondrion into two offspring mitochondria. Additionally, Drp1 protein is known to localize to the endoplasmic reticulum (ER) contact sites on mitochondria frequently and such contact site may play a role in the mitochondrial fission process. However, the ultrastructural detail of this process has not been established because their 3D structure is quite difficult to visualize by using conventional method, even in the MAMs themselves are able to observe under conventional transmission electron microscopy easily. The MAM complex is considered to extend over few  $\mu\text{m}$  of 3D space, and the FIB-SEM tomography is a unique tool to analyze the complete architecture of such scale object with electron-microscopic resolution.

This method we used has enough resolution to evaluate the entire membrane organization of the cell, and the resultant data have shown an organization of MAMs. In the case of the hepatocyte, MAMs occupied 10-20% of the total mitochondrial surface area and the most of MAM on the mitochondrion were connected each other by ERs around the mitochondria and then they form MAM complex around the mitochondrion. A similar organization was also found around the mitochondrial constriction site, but it was difficult to decide whether this constriction was a step of the mitochondrial fission process. To specify the mitochondrial fission site, we observed Drp1-RFP expressing cells. As the FIB-SEM has a convenient characteristic for CLEM observation, we can observe the ultrastructure of the exact site of Drp1-RFP punctuate. In some case, there was a typical mitochondrial constriction in the FIB-SEM reconstruction data; however, we did not observe any ringlike ER tubule around the construction site, which tubule had been reported in a case of yeast. On the other hand, we also observed a small contact of ER tubule just on a mitochondrion, which showed weak Drp1 accumulation, but there was no typical constriction. Such weak constriction may be interpreted as an early phase of mitochondrial fission. These results suggested that the difference in the role of MAMs in the mitochondrial fission process between in mammals and in yeast.

*Dr. Keisuke Ohta is an Associate Professor at the department of anatomy, Kurume University School of Medicine. Dr. Ohta is generally interested in understanding the actual 3D architecture of the cell using FIB-SEM and electron tomography methods.*



**Professor Wen-An Chiou**

AIM Lab (Advanced Imaging and Microscopy Laboratory)

NanoCenter University of Maryland College Park,

MD 20742-2831

E-mail: wachiou@umd.edu

## **Recent Developments in Preparation of Biological Samples for Electron Microscopy**

Electron microscopy (EM) has become a powerful and indispensable tool in scientific and engineering research at the nano- and subnano-meter scale. To accurately understand and deduce the microstructure, composition, and behavior of materials, preparation of a thin, representative, artifact-free sample is crucial. One long-standing challenge for electron microscopists is specimen preparation for interfacial investigation and examination of wet specimen in situ EM. This paper presents unique applications of focused ion beam (FIB) in sample preparation and materials characterization with an emphasis in bio-related materials, as well as an introduction to the application of ionic liquid for the microstructural examination of wet specimens in SEM.

Several materials with different interfaces (e.g., biomimetic mineral and polymer, metal and bio- tissue/minerals), are selected for demonstration and discussion in this paper. Conventional preparation (grinding/polishing/ion milling and ultramicrotoming) of these special interfaces has been difficult due to property differences. Using combined FIB/SEM (dualbeam), specimen cutting and imaging can be performed simultaneously in a vacuum environment. Microstructures and microchemistry can thus be reconstructed and presented in 3D using modern computer software. Examples of FIB cross-sectional TEM results, as well as high resolution TEM and SAD pattern of the tissue layer will be presented. These results demonstrate the unique application of FIB technique to sample preparation and investigation.

An ionic liquid, also known as liquid electrolyte is a salt in the liquid state. Ionic liquid has high conductivity, high affinity to biological organism solid in ambient temperature, high osmotic pressure and zero vapor pressure. These properties allow for investigation of wet samples in a regular high vacuum SEM. Examples of the application of ionic solution to study wet specimen in SEM will be presented.

*Dr. Wen-An Chiou is currently the Director of Advanced Imaging and Microscopy Laboratory (AIM Lab) at the Maryland NanoCenter, University of Maryland. He was a Research Professor at Northwestern University, Evanston, Illinois and also Director at the Materials Characterization Center at University of California, Irvine, California before joining University of Maryland. Dr. Chiou has a broad spectrum of research ranging from materials science to earth science, to biomaterials. He has nearly 40 years of expertise in the use of conventional/high resolution as well as environmental EM, and has received numerous awards in electron microscopy.*



**Professor David Becker**  
Lee Kong Chian School of Medicine,  
Nanyang Technological University,  
Singapore  
E-mail: David.Becker@ntu.edu.sg

## Targeting Connexins to Improve Chronic Wound Healing

Chronic wounds are defined as wounds that do not heal in 3 months but they can often persist for years. These wounds are very debilitating for the sufferer and all too often result in amputation of the lower limb. Life expectancy for patients is reduced to around five years, a mortality rate which is worse than many common cancers. These chronic wounds are a growing problem in society as they have no effective treatments and affect the elderly and diabetics who are rapidly increasing in numbers. In the USA they cost over \$25B a year to treat and are a substantial drain on health care services as well as having a negative impact on the lives and morbidity of sufferers.

We have found that gap junctions play a central role in the wound healing process and are dynamically regulated at the wound site. We find that in acute wounds connexin 43 (Cx43) down regulates in keratinocytes and fibroblasts as they become migratory but Cx43 upregulates in blood vessels as they become leaky and inflamed. In diabetic rodents, where healing is perturbed, Cx43 turns on at the wound edge, preventing cell migration and healing, which can be rescued by application of a Cx43 antisense gel to the wound. In vitro studies show that high levels of glucose can stimulate upregulation of Cx43 and retard migration, which can be rescued by repressing Cx43 protein production. Elevated Cx43 also results in elevated levels of N-Cadherin (N-Cad) and Zonula Occludin -1 (ZO-1), which is associated with elevated cell adhesion. Preventing the Cx43 upregulation also prevents the upregulation of N-Cad and ZO-1. At the same time knocking down Cx43 protein levels results in dynamic changes to the cytoskeleton, enhancing filopodial production and is associated with activation of small GTPases Rac and Rho.

Biopsies from a variety of human chronic wounds, venus leg ulcers, diabetic foot ulcers and pressure ulcers all show a massive overexpression of Cx43 in wound edge keratinocytes and fibroblasts. Second harmonic and multiphoton imaging of these biopsies show that the extracellular matrix of these wounds is highly degraded for a considerable distance from the wound edge and is filled with inflammatory cells. Clinical trials of the Cx43 antisense are currently being conducted on venus leg ulcers and diabetic foot ulcers.

*Dr. David Becker was a Professor in Cellular Imaging at the Department of Cell and Developmental Biology at University College London and is currently Professor of Tissue Repair and Regeneration at the Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.*





**Associate Professor Wattana Weerachatanukul**

Department of Anatomy, Faculty of Science,  
Mahidol University, Bangkok  
Thailand  
E-mail: wattana.wee@mahidol.ac.th

### **C-Terminal Domain on the Outer Surface of the *Macrobrachium Rosenbergii* Nodavirus Capsid Is Required for Sf9 Cell Binding and Internalization**

We have shown that *Macrobrachium rosenbergii* nodavirus (MrNV) was able to infect Sf9 cells and that MrNV virus-like particles (MrNV-VLPs) were capable nanocontainers for the delivery of nucleic acid-based materials. Here, we demonstrated that chymotryptic removal of a C-terminal peptide and its truncated variant (T345-MrNV-VLPs) exhibited a reduced ability to interact and internalize into Sf9 cells. Electron microscopic observations revealed that the loss of C-terminal domain did not affect MrNV-VLP icosahedral conformation, but did noticeably alter the spike structures on the capsid surface. Homology-based modelling of the MrNV capsid with other icosahedral capsid models revealed that this chymotrypsin-sensitive C-terminal domain was not only exposed on the capsid surface, but also constitute the core of the viral capsid protrusion. These results therefore suggest the importance of the C-terminal domain as part of the capsid protrusion and as a ligand for target cell interaction. This work thus offered structural and functional insights into the role of the MrNV C-terminal domain in viral entry into Sf9 cells, which would be useful for development of agents against MrNV infection and for the augmentation in the delivery efficiency of MrNV-VLPs.

#### **Related Publications:**

- 1) Jariyapong P, Chotwiwatthanakun C\*, Somrit M, Jitrapakdee S, Xing L, Cheng HR, **Weerachatanukul W**. Encapsulation and delivery of plasmid DNA by virus-like nanoparticles engineered from *Macrobrachium rosenbergii* nodavirus. *Virus Res.* 179: 140-146. 2014.
- 2) Jariyapong, P, Xing L, van Houten NE, Li TC, **Weerachatanukul W**, Hsieh B, Moscoso CG, Chen CC, Niikura M, Cheng RHR\*. Chimeric hepatitis E virus-like particle as a carrier for oral-delivery Vaccine. 31(2): 417-424. 2013.

*Associate Professor Wattana Weerachatanukul is an academic staff at the Department of Anatomy, Faculty of Science, Mahidol University since 1995. He is heading 4 multidisciplinary research groups including Drug delivery & nano-containers; Biomaterials Science; Shrimp Reproductive Biology; and Three dimension image analyses. He is also the Supervisory Board of the Nanoimaging, Faculty of Science, Mahidol University in collaboration with Olympus Thailand. Currently, he is members of the Microscopy Society of Thailand (MST) and Anatomy Society of Thailand and has actively published more than 53 peer-reviewed publications.*





**Assistant Professor Suchin Worawichawong**  
Renal and urological Pathology, Department of Pathology,  
Faculty of Medicine Ramathibodi Hospital,  
Mahidol University,  
Bangkok 10400, THAILAND  
E-mail: suchin.wor@mahidol.ac.th

## **Role of Microscopy in Diagnosis of Transplant Kidney**

Renal transplantation is the most common solid organ transplant worldwide. The success rate of outcome or graft survival is increased due to improved surgical techniques and immunosuppressive treatment as well as early and accurate diagnosis of the dysfunctional graft, leading to proper and effective management. Histopathological study of the graft biopsy makes a major role in understanding the histological alterations by various pathologic mechanisms such as graft rejection, infection, drug toxicity, recurrent/de novo glomerulonephritis, etc. More than 50 years that we use microscopic examinations of the graft biopsy including light microscopy, immunohistochemistry and ultrastructural study to diagnosis graft dysfunctions as the gold standard. The additional with immunological test and the novel biomarkers in blood and urine increase the sensitivity and specificity for early diagnosis the dysfunctional graft. The renal biopsy is invasive and has more complications, while biomarker is less invasive, but until now there is no specific useful biomarkers to replace the kidney biopsy. Developing of new potential biomarkers for clinical use is promising, but need time and effort. According to the latest Banff classification for renal allograft rejection, histopathology is still the important key in the diagnosis of allograft rejection.

*Dr. Suchin Worawichawong is the Assistant Professor in Pathology and chief of Electron Microscopy Laboratory at Department of Pathology, Ramathibodi Hospital, Mahidol University. He is the executive committee of the Royal College of Pathologists of Thailand and the Councilor of The International Academy of Pathology, Thailand Division.*



**Associate Professor Mark W Douglas**

Storr Liver Centre, The Westmead Institute for Medical Research,  
Marie Bashir Institute for Infectious Diseases and Biosecurity,  
The University of Sydney at Westmead Hospital,  
Sydney, AUSTRALIA.  
E-mail: mark.douglas@sydney.edu.au

## **Studying Virus Trafficking in Cells using Immune Fluorescence Microscopy**

Viruses depend on the infected host to complete their replication cycle, so replication involves complex interactions between viral and cellular proteins. Virus entry, virus protein translation, RNA/DNA replication, virus assembly and virus export each occur in different cellular compartments, so intracellular trafficking is a key part of the replication cycle for most viruses. Long distance transport within cells typically involves the microtubule network, with cellular cargo being carried by microtubule-associated motors. Anterograde (plus-end directed) transport is performed by a family of molecular motors called kinesins, while retrograde (minus-end directed) transport usually involves cytoplasmic dynein.

Herpes simplex virus type 1 (HSV-1) infects sensory neurons, so needs to travel long distances along neuronal axons to establish latent infection (retrograde transport) and following reactivation to cause recurrent infections (anterograde transport). Using a yeast two-hybrid system and *in vitro* pull down assays we identified direct interactions between one of the HSV-1 capsid proteins (VP26) and cytoplasmic dynein light chains (DYNLT1/tctex-1 and DYNLT3/RP3). To confirm a role for these interactions in retrograde transport of HSV-1, we constructed recombinant HSV capsids, either with (VP26+) or without (VP26-) VP26. We injected the recombinant capsids into individual cells in culture and used immune fluorescence microscopy to measure their intracellular trafficking. We confirmed that VP26+ capsids containing VP26 moved towards the microtubule organizing centre (MTOC), but VP26- capsids did not.

Hepatitis C virus (HCV) utilises microtubule-associated transport during viral entry, establishment of replication complexes, redistribution of cellular lipid droplets for virus assembly and during virus egress. In a proteomics screen from HCV-infected cells we identified the kinesin adapter protein calsynenin-1 as a protein likely involved in the HCV replication cycle. Using immune fluorescence microscopy and live cell imaging, we confirmed an important role for calsynenin-1 at various stages of the HCV life cycle, including trafficking of early endosomes after virus entry, establishment of virus replication and export of virus-containing exosomes.

*Dr Mark Douglas is an Associate Professor at the University of Sydney. He is an Infectious Diseases physician and molecular virologist, with a strong research interest in viral hepatitis. After completing a PhD in virology he undertook a NHMRC CJ Martin Postdoctoral Fellowship on hepatitis C virus (HCV) at the prestigious MRC Virology Unit in the UK, working with Dr John McLauchlan. He now heads the Viral Hepatitis Pathogenesis group in the Storr Liver Centre, at The Westmead Institute for Medical Research. His projects are diverse, ranging from basic molecular pathogenesis to clinical research. He runs a hepatitis clinic at Blacktown Hospital, treating patients with Chronic Hepatitis B and Hepatitis C, and also treats patients at Westmead Hospital with a range of Infectious Diseases.*



**Associate Professor Hing Hiang Lian**

Environmental Health Programme, Faculty Health Sciences,  
Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, MALAYSIA  
E-mail: hing61@yahoo.com

## Evaluation of Cells Integrity Using Different Fixation Time Using Scanning Electron Microscopy

<sup>1,5</sup>Ida Muryany, M.Y., <sup>1</sup>NorFadilah, R., <sup>3</sup>Ina Salwany, M.Y., <sup>1</sup>Ghazali, A.R., <sup>4</sup>Zamri-Saad, M., <sup>6</sup>Kaswandi, M.A.  
<sup>1</sup>Sahalan, A.Z. & <sup>7</sup>Muranaka Y.

<sup>1</sup>Biomedical Science Programme, Faculty Health Sciences, <sup>2</sup>Environmental Health Programme, Faculty Health Sciences, <sup>3</sup>Aquaculture Department, Faculty of Agriculture, UPM, <sup>4</sup>Veterinary Diagnostic Department, Faculty Veterinar Medicine, UPM, <sup>5</sup>School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, <sup>6</sup>Institute of Medical Science technology, Universiti Kuala Lumpur, <sup>7</sup>Research Center for UHVEM, Osaka University

The study aims to evaluate the integrity and structural changes of two intestinal cells adhered with *Lactobacillus plantarum* after exposure to different fixation time. HT-29 human colon adenocarcinoma (ATCC® HTB38™) and CCD-18Co human colon fibroblast (ATCC® CRL-1459™) respectively with bacteria cells were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer for two, four, six and 12 hours at room temperature. Our study revealed that the different time of fixation with 2% glutaraldehyde in 0.1 M phosphate buffer can affect and influenced the integrity, viability and durability of cell. HT-29 cells structure remain intact even after long fixation time of 12 hr. However, at 12 hour fixation, the cilia structures on the surface of HT-29 cells were not distinct. The result differ with CCD-18Co cells where this cell was intact at two and four hour fixation time whereas at six hour cells remain intact but the cilia structure was not clearly seen on the surface of cell. However, at 12 hour fixation, the structure of CCD-18Co cells were completely broken and degraded. The cell shrinked, epithelium was lost and the surface of the cell became smooth and all cilia structures were totally damaged. *Lactobacillus plantarum* cells remains stable on both cells, showing that the bacteria cells were unaffected with various fixation time. The present study suggested different cell has different endurance and integrity depending on it's passive forces, electrostatic interaction, hydrophobicity and specific cellular surface components which act as defense system and control the movement of substances in and out of the cells thus leading to the usage of different fixation time for fragile cells.

*Dr. H.L. Hing was an Associate Professor in the School of Diagnostic & Applied Health Science, Universiti Kebangsaan Malaysia and Assoc. Senior Fellow of FUELCELL Institute, UKM. He is currently the General Secretary of Committee of Asia Pacific Committee Societies of Microscopy (CAPSM)*



**Professor Mitsutoshi Setou**

Department of Cell Biology and Anatomy,  
Hamamatsu University School of Medicine  
1-20-1 Handayama Higashi-ku,  
Hamamatsu 431-3192, Japan  
E-mail: setou@hama-med.ac.jp

## Platform of Functional Metabolic Imaging

For the human health care, it is important to know the functional metabolomic status in vivo. After I was trained as human physiologist at the University of Tokyo Hospital, I started molecular and cellular imaging with light microscope at the University of Tokyo, Graduate School of Medicine supported by Special Grant-in-Aid for Center of Excellence from the Japan Ministry of Education, Science, Sports and Culture (MEXT) (Science, 288, 1796-1802, 2000; Nature, 2002, 417, 83-87), and received the AAAS Science Award for Young Scientists (Science, 2002).

Then I started my laboratory, based on imaging research combined with mass spectrometry analysis supported by a Grants-in-aid for Young Scientist A from Japan Society for the Promotion of Science (Cell, 130, 943-957, 2007 and others), and started project to develop imaging mass spectrometry (IMS) for biological and clinical application supported by a Grants-in-aid for Young Scientist S from Japan Society for the Promotion of Science, collaboration with Shimadzu Corporation. The project turned out to be successful and I was received the Promotion Prize from Japan Society for Medical Molecular Morphology (2009), the Promotion Award from the Mass Spectrometry Society of Japan (2012), and the Setou Award from the Japanese Society of Microscopy (2015).

During those efforts, I noticed the importance of cultivate the lab where people with various backgrounds, i.e., medicine, chemistry, physics, computer sciences, biology, optics, collaborate for new technological challenge. We call it “platform” and were supported by the Project for Creation of Research Platforms and Sharing of Advanced Research Infrastructure from MEXT. I think my lab has been successful in this term, more than 20 members including over 10 foreign students were studied and graduated from my lab, it is to say, our lab is human resource platform. Now, our lab is elected and named as an International Imaging Mass Center of Japan. This center is strongly supported by a German global company Bruker Daltonics Inc also. In this talk, I will present our new techniques for the manipulation of functional metabolomics in vivo.

*Dr. Mitsutoshi Setou is a professor and chairman at Department of Molecular Anatomy, Hamamatsu University School of Medicine. He is also an adjunct Professor at National Institute for Physiological Science, a professor and chairman at Department of Cell Biology and Anatomy, Hamamatsu University School of Medicine, ISO committee member, Graduate School of Technology of the University of Tokyo, an honorary professor at the University of Hong Kong and a president of international mass imaging center of Japan. His research interest is to cure aging and age-related diseases. To do so, He developed imaging techniques and visualized various functional metabolites to understand molecular anatomy of aging by biological, clinical and analytical approaches. With those techniques, he found distinct lipid distributions unique to molecular species in age-related diseases. He is now developing techniques to manipulate in vivo distribution of lipid species to cure age-related disorders.*



**Associate Professor Scott Cummins**  
Molecular Communication Research Group,  
University of the Sunshine Coast, Building I,  
Maroochydore 4558, AUSTRALIA.  
E-mail: scummins@usc.edu.au

## **Mass Spectral Identification of Aquatic 'Excretomes' that Dictate Key Ecological Interactions**

Aquatic animals must perceive an extraordinary variety of water-soluble chemicals. To gain insight into the mechanisms underlying olfactory communication in aquatic animals, we have analyzed using mass spectrometry approaches the excretory-secretory biomolecules (excretome) from the freshwater snail *Biomphalaria glabrata*, the marine giant triton snail *Charonia tritonis* and the marine crown-of-thorns starfish *Acanthaster planci*. *B. glabrata* is the key intermediate host for the schistosoma parasite. We have found that this snail releases several abundant molecules, one of which is detected by a parasite, enabling efficient tracking and infection. The *C. tritonis* is a key predator of the crown-of-thorns starfish. Diminished numbers of this snail on the Great Barrier Reef and thought to be one reason for excessive starfish populations. We have also found that *C. tritonis* has several abundant molecules which are detected by the starfish, eliciting an aversion response. In turn, the *A. planci* releases its own bouquet of biomolecules in response to detection of predator odours. The function of the peptide components can be predicted in the context of their similarity with other peptides of known function, as well as their requirement during predator alarm. In summary, we have identified putative pheromones released by a freshwater and marine snail, and a marine starfish, demonstrating their importance in key ecological interactions.

*Dr. Scott Cummins has completed research positions at The University of Texas and University of Queensland before undertaking an ARC Future Fellowship at the University of the Sunshine Coast. He has established the Molecular Communication research group which utilizes multi-omics approaches to discover the molecular toolkit that underlies various chemical communication systems.*



**Professor Prasert Sobhon**

Department of Anatomy, Faculty of Science,  
Mahidol University, Rama VI Road,  
Bangkok 10400, Thailand  
E-mail: prasertso@buu.ac.th, prasert.sob@mahidol.ac.th

## **The Essential Roles of Polyunsaturated Fatty Acids and Highly Unsaturated Fatty Acids (Pufas And Hufas) in Reproduction and Growth of Crustaceans**

Prasert Sobhon<sup>1,2,\*</sup>, Attakorn Engsusophon<sup>2</sup>, Chanudporn Sumpoawnon<sup>2</sup>, Tanapan Siangjam<sup>1,2</sup>, Tipsuda Thongbuakaew<sup>2</sup>, Narin Changklungmoa<sup>1</sup>, Pornanan Kueakhai<sup>1</sup>, Prasert Meeratana<sup>1</sup>, and Chaitip Wanichanon<sup>2</sup>  
<sup>1</sup> Faculty of Allied Health Sciences, Burapha University, Chonburi 20131, Thailand <sup>2</sup> Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand  
\*Corresponding author

Dietary polyunsaturated fatty acids and highly unsaturated fatty acids (PUFAs and HUFAs) are crucial for reproduction and growth of crustaceans. Besides their important roles as membrane components in differentiating and mature germ cells, HUFAs (C20s) serve as precursors for eicosanoids, i.e., prostaglandins, a hormone-like mediators of reproduction in crustaceans as well as other invertebrates. The beneficial effects of PUFA and HUFA-rich food on reproduction were demonstrated by an acceleration of gonadal maturation, germ cell proliferation, and an increase of vitellogenin expression in ovaries as well as hepatopancreas. Additionally, genes involved in eicosanoid signaling were notably influenced by high dietary PUFA and HUFA contents. For examples, the cyclooxygenase gene (Cox), and prostaglandin E synthase (PGES) coding for a central as well as a terminal enzymes in the eicosanoid pathway, are highly responsive to the feed containing high PUFAs and HUFAs. Direct injection of PGE and PGF in crustaceans also induce early gonadal maturation, germ cell proliferation and development. Together these evidence suggest that dietary PUFAs and HUFAs are essential for germ cell differentiation and homeostasis of their cellular physiology. They are also substrates for eicosanoid syntheses, and these molecules in turn can act as endocrine as well as paracrine signalings for gonadal maturation and germ cell proliferation.

### **Acknowledgments**

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*Dr. Prasert Sobhon was a Professor of Anatomy and Cell Biology at Department of Anatomy, Faculty of Science, Mahidol University, and presently is also a Professor at Faculty of Allied Health Sciences, Burapha University. In 1995 he received Thailand Outstanding Scientist Award, from the Foundation for the Promotion of Science and Technology under the Patronage of His Majesty the King of Thailand, and in 2011 Outstanding Researcher Award in Agricultural and Biological Sciences, National Research Council of Thailand.*





**Professor Ken Kitajima**  
Laboratory of Animal Cell Function,  
Bioscience and Biotechnology Center,  
Nagoya University,  
Nagoya 4648601, JAPAN.  
E-mail: kitajima@agr.nagoya-u.ac.jp

## **Glycan-Mediated Interactions on Pig Sperm Lipid Rafts during Fertilization**

Membrane lipid rafts on the cell surface are recognized as a hot spot for cellular interactions and signal transductions. They are unique biomembrane areas enriched in sphingolipids, cholesterol, and glycosylphosphatidylinositol (GPI)-anchored proteins. Although not so well recognized, a unique feature of lipid rafts is an enrichment of particular glycan chains attached on glycoproteins and glycolipids. Few studies, however, have focused on those lipid rafts-localized glycan chains. In 1999, we first reported biochemical characterization of sperm lipid rafts using sea urchin (1), and have since demonstrated the importance of lipid rafts-localized glycans during fertilization (1-4). To extend the significance of lipid rafts-localized glycans to mammalian sperm, we have searched for a highly glycosylated protein in pig sperm lipid rafts (5). Here we report one of unique glycoproteins of uncapacitated sperm, which was named WGA16 after its strong reactivity with wheat germ agglutinin (WGA) lectin (6). WGA16 was a prostate-derived seminal plasma protein, which was deposited initially on the sperm surface, then removed during capacitation. WGA16 is the Jacalin-like family lectin, and can strongly bind to heparin. Unlike other seminal plasma glycoproteins, WGA16 *N*-glycans are terminated by GalNAc or GlcNAc residues. The GlcNAc/GalNAc residues can work as binding ligands for a sperm surface galactosyltransferase, which actually galactosylates WGA16 *in situ* in the presence of UDP-Gal. Interestingly, surface removal of WGA16 is induced by either UDP-Gal or heparin. In the crystal structure, *N*-glycosylated sites and the heparin-binding site are situated opposite sides of the molecule.

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1987, PhD thesis in Biochemistry at Univ. of Tokyo; 1987-1989, Postdoctoral fellowship, Japan Soc. Prom. Sci., Dept. Biophys. Biochem., Univ. of Tokyo; 1989-1996, Assistant Professor, DBB, Grad. Sch. Sci., Univ of Tokyo; 1996-2004, Associate Professor, Grad. Sch. Bioagr. Sci. & Biosci. Biotechnol. Center, Nagoya Univ.; 2004-, Professor, Lab Animal Cell Func., BBC & GSBS, Nagoya University.



**Dr. Yann Guerardel**

Univ. Lille, UMR 8576 – UGSF – Unité de Glycobiologie  
Structurale et Fonctionnelle, F-59000 Lille, France  
E-mail [yann.guerardel@univ-lille1.fr](mailto:yann.guerardel@univ-lille1.fr)

## Chemical Tools to Image Glycans by Metabolic Labeling

The recent surge of interest in sugars and their impact on health and disease stems from the fact that glycosylation has, finally, gained recognition for the pivotal role that it plays in all aspects of biological systems; from embryogenesis to pathogenesis. This comes as no surprise, since the surfaces of animal entire cellular networks, as well as those of bacterial and viral pathogens, and the backbone of most proteins and lipids, are decorated with a dense complement of complex sugar structures. The biosynthesis of glycoconjugates requires the coordinated activity of a cohort of enzymes (including glycosyltransferases, epimerases, sugar transporters...), which work without a genetic template, and can create the myriad of structures that fulfill a variety of crucial functions. However, the functions that glycans play in the physiology and pathophysiology of eukaryotic cells and bacteria are difficult to study by traditional methods that generally require laborious radiolabeling techniques and purification procedures that are incompatible with the *in vivo* experiments. So, despite the obvious relevance of glycosylation to many biologists, glycobiology is still seen as a confidential area. Thus, the development and dissemination of methods and tools for analysis of glycosylation that are transposable to non-specialized laboratories appear to be the most coherent approach to promote new scientific topics at the interface of biology and sugar chemistry.

Recently, the advent of chemical biology has opened new links between biology and organic chemistry, allowing tremendous advances in glycobiology like the development of metabolic oligosaccharide engineering (MOE)<sup>1</sup>. This method consists in the use of a slightly modified monosaccharide which can enter the cell and follow the metabolic pathway. Indeed, if these structural modifications are discrete enough, glycosyltransferases do not discriminate analogues from natural monosaccharides and these are being used for the labeling of glycoconjugates by metabolic incorporations to cell cultures, bacteria or total organisms. Labeled glycans can then be traced using a wealth of methods including fluorescence microscopy after conjugation of alkyl/azide groups to fluorophores or biotin groups by click chemistry, HPLC, western blotting or FACS. Here, we will expose the different methodologies based on metabolic labeling and bio-orthogonal ligation that can be used to track glycosylation and propose applications in different fields of biology. We will also present the novel methodologies developed in our laboratory to shed light on trafficking and cellular uptake mechanisms of sialic acids.

[1] Reutter et al, J. Biol. Chem. 1992, 267, 16934; Bertozzi et al, Science. 1997, 276, 1125.

# Symposia in Anatomical Sciences

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**Associate Professor Wei-Yi Ong**  
Department of Anatomy and  
Neurobiology and Ageing Research Programme,  
National University of Singapore  
Singapore 119260  
E-mail: wei\_yi\_ong@nuhs.edu.sg

## Differential Distribution and Function of Brain Phospholipase A2 Isoforms

The phospholipase A2 family includes cytosolic and secretory isoforms, that are expressed in many tissues of the body, and are also found in snake and bee venom. *Cytosolic phospholipase A2* (cPLA2) is expressed at low levels in the normal forebrain, but is increased during neurodegeneration. cPLA2 releases arachidonic acid from glycerophospholipids on the cell membrane to generate eicosanoids and platelet activating factor. These mediators are involved in neurological disorders such as ischemia, traumatic brain and spinal cord injury, Alzheimer's disease, multiple sclerosis and chronic pain. The expression of cPLA2 is epigenetically regulated by histone proteins and certain naturally occurring compounds / nutraceuticals.

In contrast to cPLA2, *calcium independent phospholipase A2* (iPLA2) is expressed at high levels in the normal brain, but is decreased during neurodegeneration. Mutations in this isoform are found in some cases of Parkinson's disease. iPLA2 catalyzes the release of docosahexaenoic acid (DHA) from cellular membranes. It plays an important physiological role in long-term potentiation and spatial working memory, mediates the antidepressant and antinociceptive effects of the certain antidepressants, and could have a role in the beneficial effect of exercise on spatial working memory.

iPLA2 likely work in tandem with downstream enzymes, the lipoxygenases, which metabolize DHA to achieve its effects. Together, results indicate an important role of fatty acids and their metabolites, in the physiology and pathophysiology of the CNS.

### Representative publications

- 1: Tan CS, Ng YK, Ong WY. Epigenetic Regulation of Cytosolic Phospholipase A(2) in SH-SY5Y Human Neuroblastoma Cells. *Mol Neurobiol*. 2016 (In Press).
- 2: Chew WS, Ong WY. Regulation of Calcium-Independent Phospholipase A(2) Expression by Adrenoceptors and Sterol Regulatory Element Binding Protein-Potential Crosstalk Between Sterol and Glycerophospholipid Mediators. *Mol Neurobiol*. 2016 (In Press).
- 3: Ong WY, Farooqui T, Kokotos G, Farooqui AA. Synthetic and natural inhibitors of phospholipases A2: their importance for understanding and treatment of neurological disorders. *ACS Chem Neurosci*. 2015 Jun 17;6(6):814-31.
- 4: Lee LH, Tan CH, Shui G, Wenk MR, Ong WY. Role of prefrontal cortical calcium independent phospholipase A? in antidepressant-like effect of maprotiline. *Int J Neuropsychopharmacol*. 2012 Sep;15(8):1087-98.

*Dr. Wei-Yi Ong is an Associate Professor in Department of Anatomy and Director of the Electron Microscopy Unit, Yong Loo Lin School of Medicine; and Principal Investigator at the Neurobiology and Ageing Research Programme, Life Sciences Institute, National University of Singapore.*





**Associate Professor S. Thameem Dheen**  
Department of Anatomy,  
Yong Loo Lin School of Medicine,  
National University of Singapore, Singapore  
E-mail: antstd@nus.edu.sg

## Epigenetic Regulation of Microglia in the Ischemic Mouse Brain

Patnala R<sup>1</sup>, Arumugam TV<sup>2</sup>, Dheen ST<sup>1</sup>  
*Department of Anatomy<sup>1</sup>, Department of Physiology<sup>2</sup>, Yong Loo Lin School of Medicine  
National University of Singapore, Singapore 117594.*

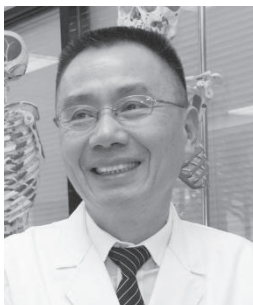
Ischemic stroke induces activation of microglia, the resident immune cells of the brain. While, healthy microglia play multivariate roles ranging from surveillance to protection of the brain parenchyma, a chronic activation as seen in stroke, worsens disease progression. Hence, understanding the mechanism of microglial activation will aid in development of therapeutic strategies that mitigate microglia-mediated neurotoxicity in neuropathologies including ischemic stroke. To address this, we investigated the epigenetic regulation of microglial activation in ischemic stroke by studying histone modification-acetylation, on Lysine 9 of Histone 3 (H3K9ac) seen to be selectively enriched at promoter regions of active or inducible genes. We have shown that modulation of H3K9ac is involved in microglial activation.

H3K9ac has been found to be upregulated in microglia in the peri-infarct zones after ischemic stroke. The H3K9ac upregulation in microglia in response to 1 hr ischemia with 24hr reperfusion was observed in different regions of the brain. A similar upregulation was observed in lipopolysaccharide (LPS)-activated microglia in the adult rat brain, indicating that the H3K9ac upregulation is consistently associated with microglial activation.

It is well established that Histone Deacetylases (HDAC) regulate H3K9ac. In this study, HDAC inhibition altered H3K9ac enrichment and transcription at pro-inflammatory (TNF-alpha, iNOS, STAT 1, IL6) and anti-inflammatory (STAT3 and IL-10) gene promoters in activated microglia in vitro. In the ischemic stroke animal model, HDAC inhibitors downregulated TNF-alpha and iNOS expression in activated microglia and improved the neuronal survival, indicating the neuroprotective ability of HDAC inhibitors *via* microglia.

*Associate Professor S. Thameem DHEEN, the Assistant Dean (Graduate Studies) at the Yong Loo Lin School of Medicine, and Assistant Head, Department of Anatomy, National University of Singapore, obtained his PhD in Neuroscience from the Faculty of Medicine, NUS, and completed his postdoctoral training at the University of Manitoba, Canada. He has been actively involved in teaching gross anatomy to medical and pharmacy students and training graduate students pursuing their MSc and PhD degrees. His research interest is centred on the molecular and epigenetic mechanisms of microglial activation in neurodegenerative diseases. He is currently the President of the Singapore Neuroscience Association and a Governing Council member of International Brain Research Organization (IBRO).*





**Professor Guo-Fang Tseng**

Department of Anatomy, College of Medicine,  
Tzu-Chi University, Hualien, Taiwan  
E-mail: guofang@mail.tcu.edu.tw

## **Cortical, Hippocampal and Striatal Changes Accompanied the Behavioral Alterations in A Rat Model of Infantile Hydrocephalus**

Hydrocephalus is a common neurological disorder in children characterized by abnormal dilation of cerebral ventricles due to impairment of cerebrospinal fluid flow or absorption. Clinical presentation of hydrocephalus varies with chronicity and often shows cognitive dysfunction and physical disabilities. Using the kaolin-induced hydrocephalus juvenile rat model, we found hydrocephalus impaired rats' performance in Morris water maze task and on constant speed or accelerating rotating cylinder tasks. Serial 3-dimensional reconstruction of coronal sections of the whole brain freshly froze in situ with skull shows that hydrocephalus reduced the volumes of both cerebral cortex and hippocampus, the two regions highly related to cognition. Morphologically, pyramidal neurons of the somatosensory cortex and hippocampus appear to be distorted. Intracellular dye injection and subsequent 3-dimensional reconstruction and analyses showed that the dendritic arbors and ends of layer III and layer V cortical pyramid neurons were reduced in acute hydrocephalus and maintained at the same level into chronic hydrocephalus. The total dendritic length of CA1, but not CA3, pyramidal neurons was also reduced. The reduction of dendritic spines on primary somatosensory cortical pyramidal neurons however exacerbated with chronicity, while those on CA1 and CA3 hippocampal pyramidal neurons remained at the same level throughout the acute and chronic hydrocephalus. At the same time, expressions of both the presynaptic terminal marker- synaptophysin and glutamatergic postsynaptic marker- postsynaptic density protein 95 (PSD95) were also reduced. The coincidence of these changes suggests that the reductions of excitatory connectivity in the somatosensory cortex and hippocampus could have underlain the learning and memory deficits associated with hydrocephalus.

In the striatum, hydrocephalus reduced the level of striatal presynaptic markers synaptophysin and vesicular Glutamate Transporter 1 and postsynaptic glutamatergic marker PSD95. The expressions of GluR2/3 were also specifically reduced. These suggest that while reducing cortical drive to the striatum, hydrocephalus increased calcium-permeable glutamate receptor channels in striatal neurons. In addition, hydrocephalus reduced striatal dopamine receptor D1 but increased D2 expression. These suggest that hydrocephalus also altered the balance of the direct and indirect striatal pathways to compromise motor control. (Supported by grants from the Ministry of Science and Technology and Tzu Chi University)

*Dr. Guo-Fang Tseng is a professor of Anatomy in the College of Medicine, Tzu Chi University in Hualien, Taiwan. He leads the renowned humanistic-based Silent Mentor Program that won praises around the world and has received front page coverage in the Wall Street Journal in 2009, 04, 22 by Ian Johnson, a Pulitzer-prize honored journalist. He was the professor and head of Anatomy of National Taiwan University before 2006. He had received many teaching excellence awards from students and institutions over the years.*



**Associate Professor Dr Sutisa Thanoi**

Department of Anatomy, Faculty of Medical Science,  
Naresuan University, Phitsanulok, 65000, THAILAND.  
E-mail: sutisat@nu.ac.th

## **Deficits in the GABAergic System after Exposure to Abused Drugs**

Recently, drugs of abuse are serious public health and social problems worldwide, including Thailand. It has been documented that abused drugs can induce neurotransmitter alterations related to psychotic behavior including cognitive impairment. GABAergic neurotransmission has been suggested to be implicated with psychosis and cognitive impairment. Therefore, our study aimed to investigate the effect of abused drugs such as methamphetamine (METH) and dextromethorphan (DXM) on the neuronal mechanisms underlying the GABAergic system in animal models. Behavioral stereotypy, hyperlocomotion, cognitive impairment and neophobia were observed following METH administration. METH can induce neuronal cell loss in the frontal cortex (FC) and the hippocampus. The reduction of neuronal cell density in these brain areas may lead to alteration in the neuronal transmission between brain regions and may contribute to psychotic behaviors and cognitive deficits. This study also showed an increase in GABA<sub>B</sub>1-immunoreactive (IR) neurons in the hippocampus and up-regulation in GABA<sub>A</sub>  $\alpha$ 1-IR neurons in both the FC and the hippocampus. Moreover, decreased glutamic acid decarboxylase (GAD)<sub>65</sub>- and GAD<sub>67</sub>-IR neurons were observed in the FC and the hippocampus. Up-regulation of GABA receptors may be a regulatory response to the reduction of GABA concentration resulting from decrease in GAD protein expression. Moreover, down-regulation in GABA<sub>B</sub>1-IR neurons in the FC may be a response to the neurotoxic effect of METH. In addition, decreased parvalbumin- and calbindin-IR neurons in the FC and the hippocampus and decreased calretinin-IR neurons in the FC were also observed after METH administration. These results suggest abnormalities of GABAergic function after exposure to METH. We also observed the effect of DXM on GABAergic system. The hyperlocomotion and anxiety-like behaviors were found in animals after exposure to DXM. Furthermore, reduction in GAD<sub>67</sub> protein expression was found in the FC. GABA concentration was also decreased in the FC after DXM withdrawal. These results revealed a transient effect of DXM on the GABAergic system. The results of our study demonstrate that abused drugs can induce psychotic behavior, cognitive impairment, neuronal damage and GABAergic deficits. Moreover, these findings also provide evidence to support an important role of the GABAergic system in drug dependence.

*Dr. Sutisa Thanoi is an Associate Professor in Anatomy at Department of Anatomy, Faculty of Medical Science, Naresuan University and the present position is the director of Centre of Excellence in Medical Biotechnology at Naresuan University.*



**Associate Professor George W. Yip**

Department of Anatomy,  
National University of Singapore,  
4 Medical Drive, Block MD 10,  
Singapore 117594, SINGAPORE.  
E-mail: georgeyip@nus.edu.sg

## **Heparan Sulfate 6-O-Sulfotransferase 2 in Breast Cancer**

Breast cancer is the most frequently diagnosed cancer in women worldwide, accounting for one quarter of all female malignancies. Despite improvements in healthcare over the years, it remains a leading cause of cancer-related deaths. Heparan sulfate is a long, linear and highly negatively charged glycosaminoglycan that is ubiquitously distributed in the body. By modifications in the number and positions of sulfate groups on its backbone, heparan sulfate can bind to various growth factors and their receptors, thereby regulating a myriad of cellular activities. Heparan sulfate 6-O-sulfotransferase 2 (HS6ST2) is an enzyme that catalyses the transfer of sulfate groups to position 6 of the *N*-sulfoglucosamine residues of heparan sulfate. To determine if HS6ST2 has biological roles in breast cancer, we knocked down its expression in breast cancer cells using siRNA and found significant increases in cell migration and invasion. The findings were corroborated by complementary observations of breast cancer cells that overexpress the HS6ST2 enzyme. Furthermore, node-positive breast cancer tissues showed lower levels of HS6ST2. Through transcriptome analysis using high density oligonucleotide microarrays, we have identified novel molecules that are regulated by HS6ST2. In this presentation, recent data on the functional roles and expression patterns of these molecules will be examined, and their possible application in predicting clinical outcomes will be discussed.

*Dr. George W. Yip is an Associate Professor in the Department of Anatomy and an Assistant Dean of Research in the Yong Loo Lin School of Medicine, National University of Singapore. He is also the Head of Grants Management in the Research Office, National University Health System. He is a current recipient of the Clinician Scientist Award from the National Medical Research Council of Singapore.*



**Associate Professor Samuel Tay Sam Wah**  
Department of Anatomy,  
Yong Loo Lin School of Medicine  
National University of Singapore, SINGAPORE.  
E-mail: anttaysw@nus.edu.sg

## **Mesenchymal Stem Cells Secretome Alters Microglial Proliferation via The Expression of the ECT2 Gene**

There is increasing evidence that activated microglia are associated with the pathophysiology of various neurodegenerative diseases. In recent years, mesenchymal stem cells (MSC) have been proposed to have specific immunosuppressive properties with a potential to moderate neuroinflammation, but little is known about the role of the MSC secretome on microglial proliferation via the activation of the ECT2 gene, which is related to the cell cycle. Thus, the present study aimed to elucidate the effects of the MSC secretome (containing extracellular vesicles called exosomes) in altering microglial proliferation via the ECT2 gene.

Mouse MSC were cultured from male C57BL/6J mouse tibia and fibula. The immortalised microglia cell line, BV2 cells, and primary microglia from C57BL/6J mouse pup brains were used in the experiment. ECT2 expression was studied using RT-PCR, Western blot and immunofluorescent staining. Proliferation analyses were performed using alamarBlue® and BrdU assays. Immunofluorescent analysis showed that the ECT2 protein expression was increased in activated microglia. The increased expression of ECT2 was further confirmed by Western blot. Both immunofluorescent analysis and Western blot showed an increase in ECT2 protein expression after culturing in MSC complete medium (containing the secretome). Proliferation of BV2 cells was increased after being induced by LPS at either 6 or 24 hours, and the effect was slightly enhanced in the presence of the MSC secretome. The present study showed that the MSC secretome increases microglial proliferation and protein expression of ECT2, and it is well established that an increase in microglial proliferation in the central nervous system exacerbates neurodegenerative diseases. Thus, further studies have to be carried out to elucidate the role of the ECT2 gene in microglial proliferation, and by targeting the suppression of this gene may lead to a better therapy. Although MSC have been widely used as a cellular therapy, further investigations are mandatory to identify, characterize and modify their secretome before they can be safely employed as a therapeutic agent for the amelioration of neurodegenerative diseases.

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**Key words:** Mesenchymal stem cells (MSC), secretome, microglia, proliferation, neurodegenerative diseases.

*Dr. Samuel Tay Sam Wah is an Associate Professor at the Department of Anatomy, Yong Loo Lin School of Medicine, NUS. He teaches Gross Anatomy and Histology to Medical Year 1 students, as well as Year 1 Residency students at NUHS.*



**Professor Yindee Kitiyanant**

Department of Anatomy,  
Faculty of Science, Bangkok 10330, and  
Institute of Molecular Biosciences, Nakhon Pathom 73170,  
Mahidol University, THAILAND.  
E-mail: yindee.kit@mahidol.ac.th

## **Modeling B–Thalassemia By Using Induced Pleuripotent Stem Cells (Ipscs)**

Human stem cells are important tools of choice for disease modeling, since it is possible to produce patient-specific pluripotent stem cells (iPSCs). Autologous stem cell transplantation is a potential alternative treatment for personalized cell-based therapies both in regenerative medicine and for individuals with hereditary anemia including  $\beta$ -thalassemia. Beta-thalassemia is an inherited blood disease caused by genetic variations in  $\beta$  hemoglobin gene. Disturbance of pre-mRNA splicing process resulting in decreased or absent production of  $\beta$  globin chain in The most two common  $\beta$ -thalassemic mutations affecting pre-mRNA splicing process are HemoglobinE (HbE) and IVS2-654 which are prevalent in Thailand and China. The well-known management for thalassemia patients is routine blood transfusion together with iron chelation treatment. Although hematopoietic stem cell transplantation is an only accepted permanent effective cure for  $\beta$ -thalassemia, HLA matched healthy donor are limited and insufficient. In present study human iPSCs carrying IVS2-654/ $\beta^E$  genotype was successfully established. A reduced production of correctly spliced  $\beta$ -globin Mrna was demonstrated in erythroid cells derived from established patient-specific iPSCs. The undifferentiated pluripotency gene markers were detected in the patient-reprogramming iPSCs. The embryoid bodies and three germ layer differentiation capacity were observed. The modified U7 small nuclear RNA containing antisense against IVS2-654 mutation can be used to correct aberrant splicing in erythroblasts differentiated from IVS2-654  $\beta$ -thalassemia/HbE iPSCs. The data collectively suggest that patient-specific iPSCs can be used for disease modeling and the potential way of antisense-based therapy serve as a foundation in gene therapy for correction of abnormal  $\beta$ -globin pre-transcripts.

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*Dr. Yindee Kitiyanant is a Professor Emeritus of Department of Anatomy, Faculty of Science, Mahidol University. Her research interest focuses on reproductive biology in farm animal as well as stem cell biology. By bridging assisted reproductive biotechnology and stem cell research, she moves the research works toward the area of regenerative medicine. The team of researchers from Faculty of Science, Institute of Molecular Biosciences and Faculty of Medicine, Ramathibodi Hospital are working together on iPSCs-derived blood cells for disease modeling.*



**Professor George Sai Wah Tsao**  
School of Biomedical Science  
Li Ka Shing Faculty of Medicine  
University of Hong Kong  
Hong Kong SAR CHINA  
E-mail: gswtsao@hku.hk

## **Live Cell Imaging of Visible Epstein-Barr Virus in Human Epithelial Cells**

Life-long latent infection of Epstein-Barr virus (EBV) is established in memory B cells after primary infection. EBV infection is also associated with specific types of carcinoma with lymphoepithelioma properties including the undifferentiated nasopharyngeal carcinoma and EBV-associated gastric adenocarcinoma. How the EBV establishes latency and persistence in infected epithelial host cells are poorly understood. The replication of EBV genomes in each cell cycle and their segregation to daughter cells are crucial for the persistence of EBV in continuously proliferating cancer cells. Previous studies using EBV-derived plasmids (containing a portion of EBV genomes, oriP) revealed the imperfect replication and non-random segregation of these plasmids in infected cells. However, the dynamics of whole EBV episomes in live epithelial cells during type II latency have not been previously examined. In present study, latent infection of a newly engineered visible EBV in human epithelial cells was established. The visible EBV genomes could be visualized as fluorescence foci in infected live epithelial cells, hence providing a platform to image the replication and segregation of EBV genomes automatically and reproducibly throughout cell cycle. The dynamics of replication, segregation and cellular localization of visible EBV genomes in live epithelial cells could be resolved at single cell level. Live cell imaging of visible EBV revealed that the viral genomes replicated during mid-late S phase and asynchronously among the viral genomes within the same infected epithelial cell. Surprisingly, amplification of EBV genomes to 3-4 folds was observed in 9.5% of the infected cells. On average, EBV genomes replicated to 1.55 fold per cell cycle per infected cell. The preferred localization of EBV episomes is localized at the perichromatic regions of host nucleus during interphase. Lastly, EBV episomes segregated relatively equally in an average of 68% of cells, but failed to do so in the remaining 32% of cells. The EBV amplification and unequal segregation may compensate for the imperfect replication, giving rise to a wide range of distribution of EBV copy per cell within a clonal population. Thus, EBV episomes are retained in cells receiving higher copy of EBV genomes during cell division but consistently lost in cells receiving lower copy of EBV

*Professor George Tsao graduated from the Department of Biology, the Chinese University of Hong Kong, Hong Kong. After graduation, he was awarded a Shell Postgraduate Scholarship to pursue postgraduate study at the Institute of Cancer Research, University of London. Professor Tsao received postdoctoral training at the Dana Farber Cancer Institute, Harvard Medical School. Before joining the University of Hong Kong, he was Assistant Professor at the Brigham and Woman's Hospital, Harvard Medical School. Professor George Tsao is currently the Research Cluster Convenor of Cell Signaling and Integrative Biology, School of Biomedical Science, Li Ka Shing Faculty of Medicine, University of Hong Kong. He is also the Director of the Faculty Core Imaging Facility and Deputy Director of the Center for Cancer Research, Li Ka Shing Faculty of Medicine, University of Hong Kong. Professor Tsao has research interests in defining the biology and pathogenic role of Epstein-Bar virus (EBV) infection in nasopharyngeal carcinoma. He was an elected officer of the Governing Board of the International Association for Epstein-Barr Virus and Associated Diseases (2010-2012).*





**Associate Professor Christopher Beng-Ti Ang**  
Senior Consultant & Head, Department of Neurosurgery, NNI,  
Duke-NUS Graduate Medical School, Singapore  
E-mail: ang.beng.ti@singhealth.com.sg

## Precision Medicine in Neuro-Oncology

The diffuse gliomas are a commonly encountered primary central nervous system neoplasm, accounting for up to 80% of primary malignant brain tumours. Tumor recurrence following therapy is inevitable and tumour progression is ultimately fatal. At present, international standard of care is composed of maximal surgical resection where feasible followed by radiation therapy and chemotherapy using temozolomide. Based on histological appearance, diffuse gliomas are separated into three categories: astrocytomas, oligodendrogliomas and a poorly defined group of mixed oligoastrocytomas. The grading of malignancy in these tumors is also histological, using the World Health Organization scheme from II to IV based on histomorphology, proliferation and the presence of microvascular proliferation or necrosis. Our understanding of the molecular basis of glioma initiation, tumor progression and treatment failure is however evolving and a molecular, rather than a histomorphological profile of diffuse gliomas is emerging.

Our Neuro-Onocology Program has been evolving in tandem and has been able to establish a NNI brain tumour resource comprising patient-derived glioma cells, their matching xenografts and original tumors - this tissue/ cellular resource has the capability to reform xenografted tumors that recapitulate the molecular heterogeneity of the tumors. In addition, we developed a core systems biology capability that is able to establish transcriptomic programs from our patient-derived tumor cells, which link to cellular phenotype and characteristics and which correlate with survival information in large public clinical glioma databases. With these capabilities, we have thus entered the new phase of Precision Medicine where in place of administering a standard chemotherapy for all patients with malignant glioma, we may stratify patients into treatment cohorts that can benefit from targeting of specific molecular pathways. To date, the studies which have evaluated DNA methylation profiles/ CpG island hypermethylator phenotypes, isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) mutations and 1p19q co-deletion status clearly identify specific subsets with improved prognosis. However, a reliable paradigm to define useful biomarkers to guide therapeutics has yet to be established. We will show the efforts in our Neuro-Oncology Research Program to uncover molecular determinants of glioma phenotype and recurrence and in so doing, achieve functional validation of diffuse gliomas into specific treatment groups so as to optimize patient survival outcomes.

*Dr. Ang trained both at the National Neuroscience Institute (NNI) in Singapore and in Vancouver, Canada at the University of British Columbia Division of Neurosurgery. His research during his residency was directed at unraveling the molecular mechanisms regulating oligodendrocyte maturation and myelination. His clinical interests are in endonasal skull base and pituitary tumour surgery and adult surgical neuro-oncology. His lab studies precision medicine for glioblastoma. He contributes to medical education in the Brain & Behaviour Programme of the Duke-NUS Graduate Medical School.*



**Assistant Professor Kanokporn Chayaburakul**

Anatomy Unit, Faculty of Science  
Faculty Building (#4), Rangsit University,  
Paholyothin Rd., Maung-Ake, Luekhok  
Patum Thani 12000, THAILAND.  
E-mail: kanokporn.c@rsu.ac.th

**Detection of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in *Penaeus Monodon* by *In-Situ* Hybridization at Transmission Electron Microscopic Level**

*In situ*-hybridization procedure was developed to detect infectious hypodermal and hematopoietic necrosis virus (IHHNV) of infected juvenile *Penaeus monodon* at the ultrastructural level. The tissues were fixed, dehydrated, and embedded in Unicryl<sup>TM</sup> hydrophilic resin. A 515 bp IHHNV-specific DNA probe, labeled with DIG-11dUTP, was tested on both semi-thin section and ultrathin sections, and the sections were examined under light and transmission-electron microscopes, respectively. The hybridized probe was detected by means of an anti-DIG antibody conjugate to 10 nm gold particles, cytoplasm, nuclear penetration, through replication in the nucleus, and release from the cell by exocytosis. This report is the first description of *in-situ* hybridization to detect IHHNV at the ultrastructural level. The result showed positive *in-situ* hybridization of IHHNV-infected shrimp tissue at TEM in nucleolus and cytoplasm.

*Assistant Professor Dr. Kanokporn Chayaburakul had been a head of International Medical Program (India) for 5 years (2005-2009). At present position is the head of anatomy unit, Faculty of Science, Rangsit University, Patum Thani.*



**Professor Wojciech Pawlina, M.D.**  
Department of Anatomy,  
Mayo Clinic College of Medicine, Mayo Clinic  
Stabile Bld 9-38C,  
Rochester, MN, 55902, USA.  
E-mail: pawlina.wojciech@mayo.edu

## **Teaching Microscopic Anatomy in Medical Curriculum: Changes, Trends, and Competencies**

Medical education is changing in response to the ongoing transformation of the health care system in the United States. Medical curricula are being redesigned to prepare the current students to meet society's healthcare needs. Teaching basic sciences, including microscopic anatomy, is influenced by new trends in curricular design such as: decreased allocated time for basic sciences, new student-centered pedagogical approaches, and increasing use of educational technologies (virtual microscopy, audience response systems, electronic multimedia resources, mobile learning technologies). Traditional format of microscopic anatomy course that utilized only light microscopes and glass slides is slowly disappearing being transformed to multi-subject integrated student-centered activities utilizing virtual microscopy. The memorization of microscopic details has been replaced by learning clinical reasoning based on histological findings in healthy and diseased tissues and organs from real patients. Instead of traditional lecture-based presentations current trend favors interactive approaches, as team-based learning, small group interactive sessions, and modified problem-based or case-based learning. Collaboration with pathologists who can emphasize differences between normal and pathologically changed tissues using clinically specific descriptions and terms is beneficial for students when transition into clinical environment. Formative feedback is being given more frequently (often using audience response system), so students can improve and adjust their learning style preferences. Assessment of histology knowledge is moving towards competencies which are expressed in skills and behavior that each student should demonstrate at the end of the course. Thus the modern microscopic anatomy course should be viewed not only as a basic science course teaching students static descriptive morphology of cells and tissues, but also as an early opportunity to teach and evaluate new skills and competencies for tomorrow's physicians.

*Dr. Wojciech Pawlina is a Professor of Anatomy and Medical Education and Chair of the Department of Anatomy at Mayo Clinic College of Medicine in Rochester, Minnesota. He is also Director of the Procedural Skills Laboratory and teaches anatomy and histology to first year medical students. He is the author of widely used histology textbook entitled "Histology, A Text and Atlas with Correlated Cell and Molecular Biology" and several other books on the subject of anatomy, histology and medical education. He is a recipient of many education and teaching awards from medical students and recently he received the Distinguish Mayo Educator Award for his work with students, residents and fellows.*



**Professor Kem A. Rogers, PhD**  
Professor and Chair  
Department of Anatomy and Cell Biology  
Schulich School of Medicine and Dentistry  
Western University  
London, Ontario, Canada N6A 5C1  
E-mail: Kem.Rogers@Schulich.uwo.ca

## **Student and Instructor Evaluation of An Online Systemic Human Anatomy Course With Laboratory: Flexibility of Online Learning is Valued While Student-Teacher Interaction Must Improve**

An online section of an undergraduate face-to-face (F2F) systemic human anatomy course with a prosection laboratory commenced in 2012-13. Lectures for F2F students were broadcast in both live and archived format to online students using Blackboard Collaborate virtual classroom software. Laboratories were delivered online by a teaching assistant (TA) who manipulated 3-dimensional computer models (from Netter's 3D Interactive Anatomy) in the virtual classroom. The course was studied for two years to assess the impact of delivery format on anatomy grades and reveal student and instructor perceptions of learning/teaching online to formulate future course modifications. Student performance measures (multiple choice term tests and short answer laboratory tests) were statistically identical between the sections (Mann-Whitney U,  $p < 0.001$ ) and there were strong, positive correlations ( $r > 0.63$ ,  $p < 0.01$ ) between incoming grade average and final anatomy grade in both sections. These data suggest that prior academic performance, and not delivery format, predicted anatomy grades. A mixed-methods approach was taken to assess the perception of the online learning environment through qualitative individual interviews (20 online, 20 F2F; 9 instructors) and quantitative surveys (101 online, 543 F2F). While students valued pace control, schedule and location flexibility of archived lessons, they preferred the unique-hands on experience of the prosections. Instructors and teachers concluded that the F2F environment was more conducive to student-teacher communication and engagement. A smaller student:teacher ratio and frequent formative student feedback to instructors may improve communication in future years.

*Dr. Kem Rogers is a professor and Chair of the Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry at Western University, London, Canada. He is the coordinator and instructor for the undergraduate histology course and his research interests range from models of cardiovascular disease to educational scholarship. Co-authors: Stefanie M. Attardi, Ph.D., Suwhan Choi, B.M.Sc. (Hons), Noah M. Mintz, M.Sc., John Barnett, Ph.D.*



**Sonya Van Nuland**

Supervisor: Dr. Kem A. Rogers  
Medical Sciences Building Room 422  
Department of Anatomy and Cell Biology  
Schulich School of Medicine and Dentistry  
Western University, London, Canada  
N6A 5C1  
E-mail: svannula@uwo.ca

## **Are E-Learning Tools The Answer? A Comparison Of E-Learning Tools With Traditional Kinesthetic Tactile Learning Approaches in the Anatomical Sciences**

Increasing class sizes and a reduction in laboratory hours have increased the popularity of commercial anatomy e-learning tools. Our previous research (n=70) compared a simple 2-dimensional e-learning tool (A.D.A.M. Interactive Anatomy) to a more complex tool that allows for a more 3-dimensional perspective (Netter's 3D Interactive Anatomy). Despite the differences in how these e-learning tools present information, student ability to learn anatomical material, and their mental effort while doing so, known as cognitive load, were identical between e-learning tools. However, when students with low spatial ability studied anatomical content with the more complex tool (Netter's 3D Interactive Anatomy), their performance scores were significantly lower than those students with high spatial ability ( $p=0.007$ ,  $R^2=0.103$ ). These results indicate that e-learning tool software design can differentially influence students based on their spatial ability, but questions remain regarding how these e-learning tools compare to more traditional learning processes, such as physically manipulating a skeleton. Studies are ongoing to determine how performance scores are impacted when students study a bony joint using a physical skeleton compared to a simple commercial software program (A.D.A.M. Interactive Anatomy). Student performance on anatomical post-tests will be compared to their mental rotation test (MRT) score (a measure of spatial ability) in an effort to determine the relationship between spatial ability and the effectiveness of models versus software. Using a novel dual-task methodology, undergraduate anatomy students from Western University, Canada (n=75) are being assessed using a baseline knowledge test, Stroop observation task response times (a measure of cognitive load), MRT scores and an anatomy post-test (a measure of learning). We hypothesize that the acquisition of anatomical knowledge by students, regardless of their spatial ability, will be superior when learning is associated with a real model, rather than currently available e-learning tools. Results of this study will determine if currently available e-learning tools are effective in delivering anatomical education; alternatively, if this is not the case, we will have identified a major weakness in the strategy to move traditional education online.

*SONYA E. VAN NULAND, M.Sc., is a Ph.D. student (Clinical Anatomy Program) in the Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry at Western University, London, Canada. She is a teaching assistant in the undergraduate histology laboratory and her research interest is anatomical e-learning tool effectiveness.*

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**Professor Thamthiwat Nararatwanchai**  
School of Anti Aging and Regenerative Medicine  
Mae Fah Luang University,  
Bangkok 10110, Thailand  
E-mail: pitipalungwachira@hotmail.com

## **Bioengineering Measurement and Application in Anti Aging Research**

Bioengineering Measurement is the investigative method which use multi-probe adapter and connect to different probes. Their functions are operated on the display by the turning knob and MPA software. All data are automatically stored as a database in the software. The settings in the program are very flexible and can be selected by the user according to different applications. This modern, high quality electronics of the system make it economical, extremely easy to handle, precise and quick measurement. It is used for research and development of the cosmetics, biologic age, raw materials, development of household products, the pharmaceutical industry and for the treatment of many dermatologic diseases etc..

*Professor Dr. Thamthiwat Nararatwanchai graduated MD from Faculty of medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. He's got PhD in Dermatology (Electron Microscope and Immunohistochemistry) from Juntendo University, School of Medicine Tokyo, Japan. He has been the Director Mae Fah Luang University Hospital, Asoke (2008-2010) and the President of Microscopy society of Thailand (2008-2010). His present positions are as follows: 1) Director of Cosmetic Health Innovation Center (CHIC) and Mae Fah Luang University Hospital, Panjabhum, Sathorn 5 (2014-Present), 2) Advisor to the Dean, School of Anti-Aging and Regenerative Medicine (2010-Present), 3) Advisor to the Director Mae Fah Luang University Hospital (2010-Present), 4) President of Thai Association of Anti-Aging and Regenerative Medicine (2007-Present), 5) President of Thai Society of Anti-Aging Music Therapy (2014-Present), 6) Chairman of Thai Aesthetic Physicians (TAP) (2014-Present).*



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**Assistant Professor Amornpun Sereemaspun**  
Division of Histology and Cell Biology,  
Department of Anatomy, Faculty of Medicine,  
Chulalongkorn University, 1873 Rama 4 road, Patumwan district  
Bangkok 10330, THAILAND.  
E-mail: amornpun.s@chula.ac.th

## **Current Biomarkers of Cellular Aging: From Morphology to Molecular Evidences**

The growing of aged population is concerned as one of the major health and socioeconomic problems worldwide. Understanding the biology of cellular aging is critical to health promotion as well as diseases treatment. Since 2013, the hallmarks of aging has just published, this made a great changes in aging science knowledge. The paradigm shift in the cellular senescence shed light in the diagnostics, therapeutics and prevention of accelerated aging of the cell. Currently, numerous types of biomarker of aging have been developed currently, but the validity and the real implementation for biomedical applications have not yet been clearly elucidated. In this talk, various biomarkers of cellular aging were evaluated in many cell types. Cells were induced to be senescence by reactive oxygen species. Biomarkers of aging; including cell morphology, biochemical, genetic, and epigenetic markers, were evaluated both in vitro and ex vivo. Additionally, results of aging parameters of cellular aging from blood samples of healthy people with various chronological ages of Thai population would be discussed in this talk. Our results suggest the insight for appropriate selection of biomarker of aging in the further biomedical implementation.

*Asst. Prof. Dr. Amornpun Sereemaspun received M.D. degree from Faculty of Medicine at Siriraj hospital, Mahidol University. He continued studying Ph.D. (Human molecular biology) in Jichi Medical University, Japan. From 2006-present, He has been working in Nanobiomedicine Laboratory, Division of Histology and Cell Biology. Department of Anatomy, Faculty of Medicine, Chulalongkorn University. His present position is Assistant Professor, Head of Division of Histology and Cell Biology, and Head of Nanobiomedicine Laboratory. His recent research of interest are Nannotechnology application in medicine, Nano-cell interaction in cellular senescence, and Nanotoxicology.*



**Professor Ingrid Y Liu**  
Institute of Medical Sciences,  
Tzu Chi University,  
Hualien 970, TAIWAN  
E-mail: ycliu@gms.tcu.edu.tw

## **Using Behavioral Training Paradigms and Mouse Models to Delineate Memory Formation Pathways and Select Novel Drug Targets**

Our laboratory has been focusing on two research themes: 1) investigation of molecular mechanism underlying memory formation, and 2) identification of genes or chromosomal abnormalities associated with congenital brain disorders. We have used mouse models, behavioral paradigms, systems biology, and advanced genomic and cell biology techniques to achieve our research goals. In this presentation, our recent research findings will be reported. We previously discovered that Brain-Derived Neurotrophic Factor (BDNF) is critical for formation of contextual fear memory. This discovery set the foundation for elucidating the detailed molecular basis of contextual fear memory. Thus our laboratory extended the study to investigate the significance of Erk1/2 pathway, downstream of BDNF signaling, in contextual and trace fear conditioning. We demonstrated that Erk1/2 expressed in the hippocampal CA1 neurons is also critical for the two types of hippocampus-dependent memory. Activation of BDNF signaling requires calcium influx to the neurons, we thus further investigated the novel role of the  $Ca_v3.2$  T-type calcium channel in memory formation. We have demonstrated that the  $Ca_v3.2$  gene is important in contextual-associated memory. Nuclear calcium activating the cAMP response element binding protein (CREB) plays a key role in learning/memory acquired by synaptic activity. In mouse hippocampal neurons, the activating transcription factor 3 (*Atf3*) is a direct target of CREB. The *Atf3* gene is also found critical in repressing fear memory formation. Besides, we are making effort to investigate the effect of coral drugs on treating memory impairment by targeting to the identified important molecules.

*Dr. Ingrid Y Liu is a Professor in Institute of Medical Sciences, and the present provost at Tzu Chi University, Taiwan. She has received numerous TCU awards with excellent research articles and excellent teaching.*



**Dr. Akihito Ishigami**

Team Leader, Head,  
Research Team for Functional Biogerontology,  
Molecular Regulation of Aging  
Tokyo Metropolitan Institute of Gerontology (TMIG)  
35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015 JAPAN  
E-mail: ishigami@tmig.or.jp

## **Abnormal Protein Citrullination in Alzheimer's Disease**

Citrullinated proteins are the products of a post-translational process in which arginine residues undergo modification into citrulline residues when catalyzed by peptidylarginine deiminases (PADs) in a calcium ion-dependent manner. Enzymatic citrullination abolishes positive charges of native protein molecules, inevitably causing significant alterations in their structure and functions. To elucidate the involvement of protein citrullination in human neuronal degeneration, we examined whether citrullinated proteins are produced during Alzheimer's disease (AD). By Western blot analysis using anti-modified citrulline antibody, citrullinated proteins of varied molecular weights were detected in hippocampal tissues from patients with AD but not normal humans. Two of the citrullinated proteins were identified as vimentin and glial fibrillary acidic protein (GFAP) by using two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry. Interestingly, PAD2 was detected in hippocampal extracts from AD and normal brains, but the amount of PAD2 in the AD tissue was markedly greater. Histochemical analysis revealed citrullinated proteins throughout the hippocampus, especially in the dentate gyrus and stratum radiatum of CA1 and CA2 areas. However, no citrullinated proteins were detected in the normal hippocampus. PAD2 immunoreactivity was also ubiquitous throughout both the AD and normal hippocampal areas. PAD2-enrichment coincided well with citrullinated protein-positivity. Double immunofluorescence staining revealed that citrullinated protein- and PAD2-positive cells also coincided with GFAP-positive cells, but not all GFAP-positive cells were positive for PAD2. Like GFAP, which is an astrocyte-specific marker protein, PAD2 is distributed mainly in astrocytes. These collective results, the abnormal accumulation of citrullinated proteins and abnormal activation of PAD2 in hippocampi of patients with AD, strongly suggest that PAD has an important role in the onset and progression of AD and that citrullinated proteins may become a useful marker for human neurodegenerative diseases.

*Dr. Akihito Ishigami is a Team Leader of Research Team for Functional Biogerontology and a Head of Molecular Regulation of Aging in Tokyo Metropolitan Institute of Gerontology which institute is a main basically gerontological research center in Japan. He has been studying about aging research for more than 30 years and published one hundred twenty or more papers until now.*



**Professor Gavin P Reynolds**  
Biomolecular Research Centre  
Sheffield Hallam University  
Sheffield U.K.  
E-mail: gavin.reynolds@hotmail.com

## **Parvalbumin Deficits in Psychotic Illness - from Pathology to Epigenetics**

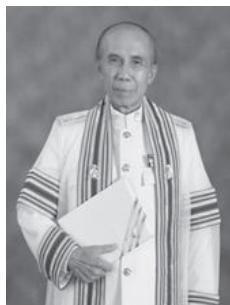
The hippocampal deficit in the calcium binding protein (CBP) parvalbumin (PV) is one of the most robust findings in the brain in schizophrenia. This diminished expression of PV, which also extends to the frontal cortex, indicates a dysfunction or deficit in a subgroup of GABAergic cells important in the inhibitory control of glutamatergic neurons. Thus PV immunoreactivity (IR) provides a neuropathological marker for GABAergic dysfunction in schizophrenia which may also relate to disturbances of glutamate neurotransmission in the disease.

This PV deficit is also found in several animal models of schizophrenia including the sub-chronic administration of phencyclidine (PCP), which results in enduring cognitive and other behavioural abnormalities in rodents. Both isolation rearing and neonatal lipopolysaccharide administration, mimicking developmental insult, also result in cognitive deficits reminiscent of schizophrenia symptoms and in losses of hippocampal PV-IR. We have also found that a drug regime in rats mimicking methamphetamine dependence also results in profound deficits of PV and, to a lesser extent, the other CBPs found in cortical GABAergic neurons, calbindin and calretinin. This may underlie the psychosis associated with methamphetamine abuse.

In trying to understand the mechanism underlying the development of these PV deficits, we have looked to epigenetic influences. There is a report demonstrating an effect of a neurotoxic challenge on DNA methylation of the PV promoter sequence in mice; we determined whether this site might also be affected in schizophrenia and the sub-chronic PCP model. We have identified equivalent sequences in rat and human and determined methylation at CpG sites in these sequences. We find hypermethylation of the PV promoter in hippocampal brain tissue both in schizophrenia and in the PCP model, indicating a mechanism for reduced PV expression. Interestingly this was also observed in DNA extracted from blood samples from subjects with schizophrenia compared to unaffected controls. We also observed the same effect in people with methamphetamine dependence, particularly those with drug-induced psychosis.

These findings indicate that PV hypermethylation is another feature in addition to PV deficits in both psychotic illness - whether schizophrenia or drug-induced - and its animal models. As yet we have not demonstrated a direct correlation between PV promoter hypermethylation and gene expression, although such a link seems highly likely.

*Gavin Reynolds is Honorary Professor at Sheffield Hallam University and Professor Emeritus, previously Chair of Neuroscience, at Queen's University Belfast. His research interests lie in two directions: one is understanding the neurotransmitter pathology of schizophrenia and other psychiatric disorders, while the other is investigating the mechanisms underlying both symptom relief and the adverse effects of psychiatric drugs. He has international collaborations with Visiting Professor appointments in Malaysia, Thailand and China, as well as at the Institute of Psychiatry in London. He is Past-President of the British Association for Psychopharmacology.*



**Professor Meechai Srisai**  
Department of Anatomy  
Faculty of Medicine,  
Chulalongkorn University  
Bangkok 10330 Thailand  
E-mail : m.srisai@hotmail.com

## **A Continuing Study Model From Preclinical, Clinical, Through Postgraduate Medical Education**

Basic procedures required by Thai Medical Council are essential for undergraduate curriculum to acquire skill. Medical students need to practice in a suitable environment and the best one should be similar to the real situation in clinical practices.

The Chula Soft Cadaver Surgical Training Center has been established since 1998. This center provides soft cadavers for training undergraduate medical students and also residents and staffs at Chulalongkorn Medical School for 19 years.

The training modules are regularly set up in medical curriculum at the faculty of medicine for 5th and 6th year medical students in 2016 and 2017, respectively. The program includes endotracheal intubation, lumbar puncture etc. in Department of Anesthesiology. In orthopedic surgery, student may get experience in joint aspiration, carpal tunnel decompression etc. In Department of Trauma and Emergency, medical students may be able to practice on wound sutures, wound dressing, venipuncture, central venous access and intercostal drainage insertion etc.

In Thailand, most of people are Buddhists who are willing to donate their bodies for the study of anatomy and surgical practices. It is very important to conclude that the donated bodies or cadavers are of greatest benefits to medical education and in fact, the whole mankind.

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**Professor Pasuk Mahakkanukrauh**

Department of Anatomy, Excellence center of osteology research and training, Cadaveric surgical and training center  
Faculty of Medicine, Chiang Mai University,  
Chiang Mai 50200, THAILAND.  
E-mail: pasuk034@gmail.com

## **Surgical and Clinical Anatomy**

Surgical and Clinical Anatomy implies something you do in clinic and in surgery, why anatomy is a body of knowledge of the normal human body. As Hippocrates used to say 500 years before Christ that the natural form of the human body is the beginning of the medical science. So Surgical and Clinical Anatomy is the medical science you practice in clinical and surgical setting.

As for the Surgical and Clinical Anatomy we use in our medical school in Chiang Mai: we have evolved from a whole year dissection of the whole body in the beginning to a block system in which the medical students are exposed to periodical dissection and alternating clinical anatomy lectures selectively and to a day of on hand practice on performing cut-down, tracheostomy, intercostal drainage and subcutaneous nodule excision.

For residency training: we provide fresh cadaver for surgical, orthopedic, emergency-room, and ear-nose-throat residence to practice appropriate skills to protect the safety of their patients.

So these are the Surgical and Clinical Anatomy in medical education that we have been using up to now.

*Dr. Pasuk Mahakkanukrauh was a professor in Anatomy at Department of Anatomy, Faculty of Medicine, Chiang Mai University. and the present position is a professor and director of the Excellence center of osteology research and training and the vice director of Cadaveric surgical and training center, Faculty of Medicine, Chiang Mai University. In 2013, she received the Outstanding Preclinical Teacher Award in Faculty of Medicine and National Excellent Teacher Award (Council of the University Faculty Senates of Thailand).*





**Assistant Professor Rosarin Ratanalekha**  
Department of Anatomy,  
Faculty of medicine Siriraj Hospital,  
Prannok, Bangkoknoi, Mahidol University,  
Bangkok 10700, THAILAND.  
E-mail: rosarin.rat@mahidol.ac.th

## **Training and Education Center for Clinical Skills**

The main objectives of Siriraj Training and Education Center for Clinical Skills (SiTEC) are; to recruit essential procedures, train experiences in simulated environment, and provide surgical skill training in fresh and soft cadavers before managing real patients,. We are dedicated to providing quality instructional programs to medical students, residents, fellows and other health care professionals. This center is on the 4th floor of Srisavarindhira Building consisting of 2 operation theatres for training (8 beds), 2 operation theatres for training (2 beds), and a lecture room. All rooms include visual aids and International video tele-conference equipments. Furthermore, the area includes microsurgery operation theatre (10 seats), surgical and operational equipments and simulators. Department of Anatomy supports the fresh cadavers and provides a special formula of chemical agent in preparing soft cadavers for training. Therefore, SITEC has been established to make our hospitals and medical safer from human errors, using robotic simulators allow medical students to practice on robots before they ultimately work on real humans and also trained to respond to stressful situations.

*Dr. Rosarin Ratalekha was Assistant Professor in Anatomy, at Department of Anatomy, Faculty of Medicine Siriraj Hospital. She graduated MD. from Faculty of Medicine Siriraj Hospital, Mahidol University. The present position is a Vice Dean of Student Affairs, Faculty of Medicine Siriraj Hospital. She is one of the committee of Siriraj Training and Education Center for Clinical Skills (SITEC) who is taking care about the fresh cadavers for the surgical skill training.*

A10\_1



**Professor Takashi Yashiro**

Division of Histology and Cell Biology, Department of Anatomy,  
Jichi Medical University School of Medicine, Tochigi, Japan  
E-mail: tyashiro@jichi.ac.jp

## **Analysis of Cell to Cell and Cell to Extracellular Matrix Interactions in Anterior Pituitary Gland**

Anterior pituitary gland is a master endocrine organ that controls growth, reproduction, metabolism, and stress response. It is commonly accepted that hormone secretion from the gland is regulated by hypothalamic-pituitary-target organ axis. In addition, cell to cell and cell to extracellular matrix local interactions has been recognized as another mediators that maintain anterior pituitary cell functions effectively and appropriately. In anterior pituitary gland, there are 5 types of endocrine cells, non-endocrine cells (folliculostellate cells, vascular endothelial cells, and pericytes), and various extracellular matrix (collagens, laminins, proteoglycans). Our research group has been investigating significance of their local interactions more than decades and has identified several important paracrine/autocrine, juxtacrine (via cell adhesion), and matricrine (via extracellular matrix) factors that influence cell/gland functions. Recently, we developed 3 dimensional (3D) cell culture using rat anterior pituitary cells. The resulting cell aggregate possesses in vivo-like cell and extracellular matrix architecture of anterior pituitary gland, which allows us to study cell to cell and cell to extracellular matrix interactions. In combination with this cell culture system, we utilized S100b-GFP transgenic rats, which express GFP in folliculostellate cells, to study novel local interactions mediated by folliculostellate cells. In this symposium, I am going to introduce our recent progress in anterior pituitary research in relation to local cell to cell and cell to extracellular matrix interactions.

*Dr. Takashi Yashiro is a Professor at Division of Histology and Cell Biology, Department of Anatomy, Jichi Medical University School of Medicine. In 2010, he received the Yoshimura Prize from Japan Association of Pituitary Research for his outstanding achievements in the field of pituitary research. He was also awarded Honorary Professor of Mongolian National University of Medical Sciences for the contribution in the development of Biomedical Sciences in Mongolia in 2015.*



**Professor Apiwat Mutirangura**

Center of Excellence in Molecular Genetic of Cancer and Human Disease,  
Department of Anatomy, Faculty of Medicine, Chulalongkorn University,  
King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand  
E-mail: mapiwat@chula.ac.th

## **The Role of Epigenetic Regulation on Repetitive Sequences in Cancer Development**

The functions of epigenetic modifications at repetitive DNA sequences involve in gene expression regulation and genome integrity. Changes in these epigenetic modifications play significant roles in cancer development. There are two types of repetitive sequences, interspersed repeat sequences and tandem repeat sequences. Interspersed repeat sequences such as Long Interspersed Element-1s (LINE-1s) are heavily methylated in human genome. However, in cancer several factors including oxidative stress and smoking genome widely reduces LINE-1 methylation. LINE-1 hypomethylation causes at least two consequences in cancer. Genome methylation maintains physiologic replication independent endogenous DNA double strand breaks (RIND-EDSBs). This newly described type of EDSBs possesses physiologic function in reduction of genomic stress. Consequently, genome wide hypomethylation can promote genomic instability. Another crucial function of intragenic LINE-1 methylation is to regulate gene expression. When LINE-1 is demethylated, LINE-1 transcription occurs. In cancer, intragenic LINE-1 hypomethylation promote LINE-1 RNA, pre-mRNA and argonaute 2 complexes and then limit mRNA production. In conclusion, global hypomethylation in cancer not only promotes genomic instability but also intragenic LINE-1 hypomethylation down regulated many genes.

Recently, we reported cis-regulatory roles of mononucleotide A-T repeats. In mammal, whereas A-repeats can be found more frequently up-stream to transcriptional start sites, T-repeats are found more down-stream. While long A-T repeats are found more in house-keeping genes, shorter repeat sequences are frequently present in tissue specific genes. The transacting factors of long A-T repeats are argonaute protein binding to A-repeats. In many cancer long A-repeats are frequently found in up-regulated genes. Here we inhibited argonaute protein binding to A repeat by peptide nucleic acid A15. Significant reduction in cell growth was discovered.

*Dr. Apiwat Mutirangura is a Professor in Human and Molecular Genetics at Center of Excellence in Molecular Genetic of Cancer and Human Disease, Department of Biology, Department of Anatomy, Faculty of Medicine, Chulalongkorn University. He received Outstanding Researcher Award in 2006 and Outstanding Scientist Award in 2008, THAILAND.*



**Professor Boon Huat Bay**  
Department of Anatomy,  
Yong Loo Lin School of Medicine,  
National University of Singapore  
S 117 594, SINGAPORE.  
E-mail: antbaybh@nus.edu.sg

## **Morphology of Breast Cancer Cells Overexpressing The Y-Box Binding Protein 1 As Observed by Immunofluorescence Confocal Microscopy**

Jia Pei Lim<sup>1</sup>, Sukanya Shyamasundar<sup>1</sup>, Jayantha Gunaratne<sup>1,2</sup> and Boon Huat Bay<sup>1</sup>

<sup>1</sup>Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, 4 Medical Drive, Block MD10, S117594, Singapore.

<sup>2</sup>Quantitative Proteomics Group, Institute of Molecular and Cell Biology, Agency for Science Technology and Research, 61 Biopolis Drive, S138673, Singapore

The Y-box binding protein-1 (YB-1) is an evolutionary conserved DNA and RNA binding protein that is known to have pleiotropic functions, including regulating fundamental cellular processes. Elevated YB-1 protein levels have been observed in a variety of cancers and associated with poorer prognosis, chemoresistance and metastatic spread in breast cancer. In this study, non-invasive MCF-7 breast cancer cells were transfected with a YB-1 expression plasmid and processed for confocal microscopy. Paraformaldehyde fixed cells were blocked with 0.1% BSA, followed by incubation with anti-vinculin, anti-paxillin and anti-YB-1 antibodies at 4°C overnight, before incubation with the relevant secondary antibodies. A cell migration assay was also performed using polycarbonate membrane transwell inserts. YB-1 overexpressing MCF breast cancer cells (MCF7-YB-1 cells) were observed to be more elongated and had loose cell-to-cell contact as compared to the vector only control cells. Immunofluorescence staining showed cytoplasmic localization of the YB-1 protein, with MCF-YB-1 cells displaying disruption in intercellular junctions and increased cellular protrusions. There was also a significant increase in cell migration in MCF7-YB-1 cells. It would appear that YB-1 has the propensity to induce cytoskeletal changes and enhance the migratory properties of breast cancer cells *in vitro*

*Dr. Boon Huat Bay's research interest is in Cancer Biology, in particular the utility of biological markers of malignancy and drug design based on tumour biology and molecular-targeted cancer therapy. He also has an interest in nanotoxicology, especially, investigating the cytotoxic and genotoxic effects of gold, silver and zinc oxide nanoparticles, using various microscopy and technological platforms in different model systems.*



**Associate Professor Samur Thanoi**  
Centre of Excellence in Medical Biotechnology  
And Department of Anatomy  
Faculty of Medical Science  
Naresuan University  
Phitsanulok 65000, THAILAND.  
E-mail: samurt@nu.ac.th

## **Methamphetamine and Changes of Sperm Quality**

Methamphetamine (METH) is generally known to damage neurons and induce psychosis. Recently, it has also been reported its effects on reproductive system. The aims of our studies was to investigate the effects of METH administration on sperm quality and structures related to sperm production. Our initial work demonstrated that the percentage of normal sperm morphology was decreased in acute group (one dose of a dose of 8 mg/kg) when compared with control. Total numbers of sperm count were significantly decreased in acute and sub-acute (4 mg/kg for 14 days) groups. Apoptotic activities were most abundant in the seminiferous tubules of acute treated animals with a highly significant increase in the number of apoptotic cells per tubule. Then, we designed our experiments to imitate METH addiction in human. The results showed that the percentages of normal sperm motility and normal sperm morphology were significantly decreased in animals receiving METH, especially in escalating dose (ED METH) and escalating dose-binge (ED-binge METH) groups when compared with control. In addition, sperm concentrations in ED METH and ED-binge METH groups were numerically decreased. Immunohistochemistry demonstrated that PR, ER $\alpha$  and ER $\beta$  immunoreactive cells were significantly decreased in spermatogenic cells and especially in Sertoli cells in all METH-treated groups. Furthermore, mRNA expression of PR, ER $\alpha$  and ER $\beta$  were also significantly decreased in all METH-treated animals. These results indicate that METH can induce abnormal sperm quality and structures related to sperm production. These changes of sperm quality may relate to increased apoptotic activity inside the testis and the reduction of PR, ER $\alpha$  and ER $\beta$  expressions in male germ cells and Sertoli cells which are essential for sperm production and development of sperm.

*Associate Professor Dr. Samur Thanoi is currently a lecturer in Department of Anatomy, Faculty of Medical Science, Naresuan University and a member of Centre of Excellence in Medical Technology, Naresuan University. He is now also acting as a Dean of Faculty of Medical Science.*



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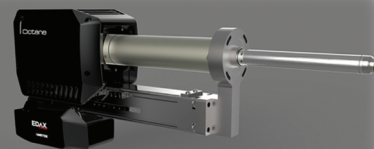
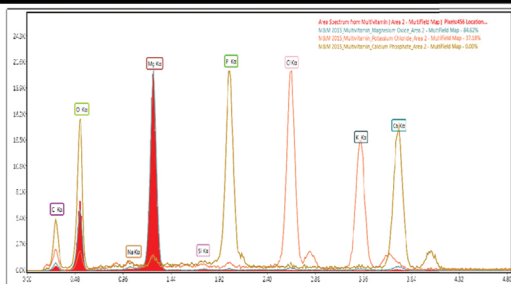
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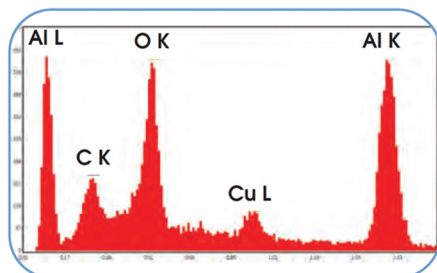
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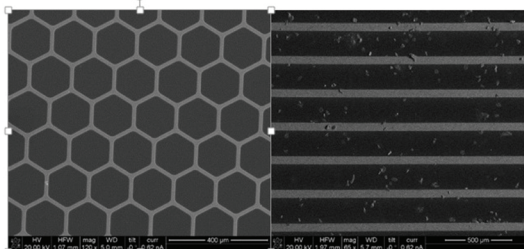
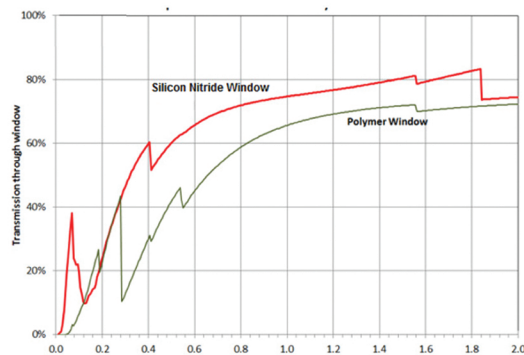


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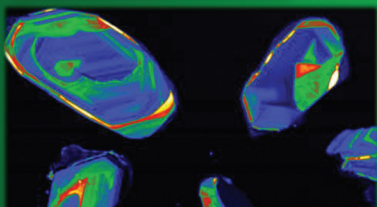
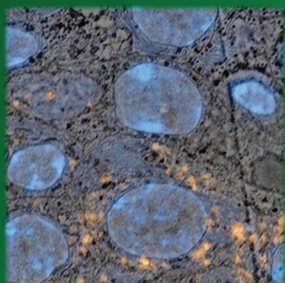
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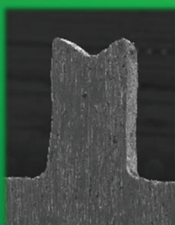
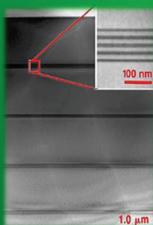
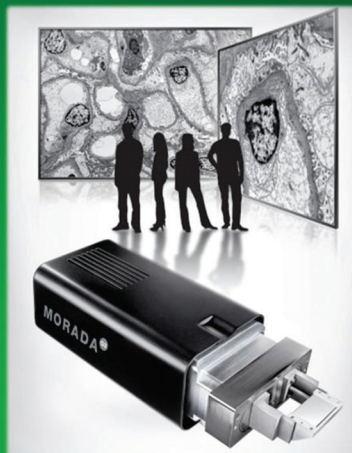


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## Exploring the Configuration of Atomic Defects and the Multi-path Migration of Mo Adatoms in Atomically Thin MoS<sub>2</sub> by Statistic ADF-STEM

Jinhua Hong<sup>1</sup>, Zhixin Hu<sup>2</sup>, Yuhao Pan<sup>1,2</sup>, Wei Ji<sup>2</sup>, Chuanhong Jin<sup>1\*</sup>, Jun Yuan<sup>3</sup>, Ze Zhang<sup>1\*</sup>

<sup>1</sup>State Key Laboratory of Silicon Materials and School of Materials Science and Engineering, Zhejiang University, Hangzhou, Zhejiang 310027, P. R. China

<sup>2</sup>Beijing Key Laboratory of Optoelectronic Functional Materials & Micro-Nano Devices, Department of Physics, Renmin University of China, Beijing 100872, China

<sup>3</sup>Department of Physics, University of York, Heslington, York, YO10 5DD, United Kingdom

\*Corresponding author, e-mail: chhjin@zju.edu.cn

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### Abstract

Defects in two-dimensional monolayer molybdenum disulphide (and other transition metal dichalcogenide) may be responsible for large variation of electric and optical properties. In this talk, I will firstly present a joint experiment-theory investigation of point defects in monolayer MoS<sub>2</sub> prepared by mechanical exfoliation, physical and chemical vapor deposition. Defect species are systematically identified and their concentrations determined by aberration-corrected scanning transmission electron microscopy, and also studied by *ab-initio* calculation. Secondly, I will also talk about the experimental determination of kinetic pathway of Mo adatoms in MoS<sub>2</sub> by statistic ADF-STEM imaging.

**Keywords** atomic defect, migration pathway, ADF-STEM, *ab-initio* calculations

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## Real-time Study of the Growth Behavior of InAs Nanorods Using *in situ* RHEED Transmission mode and TEM

Janghyun Jo<sup>1</sup>, Youngbin Tchoe<sup>2</sup>, Hwangsun Kim<sup>2</sup>, Gyu-Chul Yi<sup>2</sup>, Miyoung Kim<sup>1\*</sup>

<sup>1</sup>Department of Materials Science and Engineering, Seoul National University, 1 Gwanak-ro, Seoul, Korea

<sup>2</sup>Department of Physics and Astronomy, Seoul National University, 1 Gwanak-ro, Seoul, Korea

\*Corresponding author, e-mail: mkim@snu.ac.kr

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### Abstract

Assessing *in situ* structural information during growth processes is essential for comprehensive understanding of growth behavior.<sup>1,2</sup> Reflection high-energy electron diffraction is one of the time-resolved observation techniques, but its application has only been limited to the study on the surface structure of thin films.<sup>3</sup> We employ reflection high-energy electron diffraction in a transmission mode to investigate the growth behavior of In<sub>x</sub>Ga<sub>1-x</sub>As(GaAs)/InAs coaxial nanorods on a Si(111) substrate in real time. Electrons propagating parallel to a substrate are scattered by nanostructures and form diffraction patterns at the detector, thereby enabling acquisition of structural information during growth of various nanostructures. Such information would be complementary to that obtained from transmission electron microscopy, leading to better understanding on structural evolution. We found structural transformation of InAs nanorods having thick zincblende and wurtzite phases into their alternating structures at the very early growth stage, which results from local transition of the growth condition from In-rich to As-rich. Additionally, strain relaxation process via lattice constant accommodation was clearly revealed during epitaxial coating of GaAs shell layers. We believe that this unique approach can advance our understanding of nanostructure growth mechanisms and stimulate a new field of research concerning the *in situ* observation of nanostructure growth.

**Keywords** reflection high-energy electron diffraction, transmission electron microscopy, growth mechanism, indium arsenide

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## Exploring the Composition-related Stability for Layered Cathode Material for Lithium Ion Battery by Analytical STEM

Wei Huang<sup>1</sup>, Chunyang Wu<sup>1</sup>, Yewu Zeng<sup>1</sup>, Chuanhong Jin<sup>1,\*</sup>, Ze Zhang<sup>1</sup>

<sup>1</sup>State Key Laboratory of Silicon Materials, Zhejiang University, Hangzhou, Zhejiang 310027, P. R. China

\*Corresponding author, e-mail: chhjin@zju.edu.cn

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### Abstract

In recent years, series of high capacity lithium-transition metal (TM) oxides with layered structure have been reported as cathode materials in lithium ion batteries (LIBs). So far, the practical application of these materials in LIB are severely restricted by fast capacity loss and voltage fading during the electrochemistry cycling, and they could be further attributed to the structural and chemical instabilities upon cycling. In this work, we try to understand the component-related stability of the lithium rich layered material in a Mn-Ni-Co system by using advanced analytical scanning transmission electron microscopy (STEM). We found that the proper increase of the content of nickel can suppress the layered to spinel transition in an aging process, while the excess of cobalt will harm the structure stability. These results will be suggestive for the composition design of high performance layered cathode material.

**Keywords** lithium ion battery, lithium rich cathode material, scanning transmission electron microscopy, composition design

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## Interacting Magnetic Nanoparticles of Three Dimensional Magnetic Vortex in Different Configuration Assemblies

Min-Kwan Kim<sup>1</sup>, Prasanta Dhak<sup>1</sup>, Ha-Youn Lee<sup>1</sup>, Jae-Hyeok Lee<sup>1</sup>, Myoung-Woo Yoo<sup>1</sup>, Jehyun Lee<sup>1</sup>, Kyoungsuk Jin<sup>2</sup>, Arim Chu<sup>2</sup>, Ki Tae Nam<sup>2</sup>, Hyun Soon Park<sup>3</sup>, Shinji Aizawa<sup>4</sup>, Toshiaki Tanigaki<sup>4,5</sup>, Daisuke Shindo<sup>4,6</sup>, Miyoung Kim<sup>2</sup>, and Sang-Koog Kim<sup>1\*</sup>

<sup>1</sup> National Creative Research Initiative Center for Spin Dynamics and Spin-Wave Devices, Nanospinics Laboratory, Research Institute of Advanced Materials, Department of Materials Science and Engineering, Seoul National University, Seoul 151-744, South Korea

<sup>2</sup> Department of Materials Science and Engineering, Seoul National University, Seoul 151-744, South Korea

<sup>3</sup> Department of Materials Science and Engineering, Dong-A University, Busan 604-714, South Korea

<sup>4</sup> Center for Emergent Matter Science (CEMS), RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan

<sup>5</sup> Hitachi, Ltd., Central Research Laboratory, Hatoyama, Saitama 350-0395, Japan

<sup>6</sup> Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Sendai 980-8577, Japan

\*Corresponding author, e-mail: sangkoog@snu.ac.kr

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### Abstract

Assembly of magnetic nanostructures are promising building blocks for bio applications, information storage and recording media<sup>1-5</sup>. Achieving the self-assembled magnetic nanostructure requires controlled assembly from magnetic nanoparticles. However, despite recent insight into the magnetic nanoparticles, interaction between coupled nanoparticles of three dimensional magnetic vortex remain elusive. Here, we have explored how interactions between different configuration assemblies are modified by magnetic field induced by magnetic vortices. By means of scanning electron microscope, we observed self-assembled configurations as secondary particles that consist of 1 to 4 primary nanoparticles. With the help of micromagnetic simulations and off-axis electron holography, we investigate the experimentally observed assemblies including spin structures and energy. The results reveal that particular configurations are enormously stable and unchangeable, while the others are unstable and readily transformed to stable configurations. The underlying physics are well understood in terms of the giant magnetic binding energy induced by spin-exchange energy of magnetic vortex. Accordingly, manipulating intermediate assemblies could play a key role in controlling the dynamic self-assembly of complex nanostructures. This work constitutes a milestone towards the diverse novel approaches for the advanced application of self-assembled nanostructures from magnetic nanoparticles.

**Keywords** nanoparticle, self-assembly, magnetic interaction, electron holography

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## Investigation on Defect Related Luminescence in Semi-polar InGaN/GaN Quantum Well by Using TEM-CL

Mi-Hyang Sheen<sup>1</sup>, Sung-Dae Kim<sup>1</sup>, Jongjin Jang<sup>2</sup>, Okhyun Nam<sup>2</sup>, Young-Woon Kim<sup>1\*</sup>

<sup>1</sup> Research institute of Advanced Materials, Department of Materials Science and Engineering, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-744, Republic of Korea

<sup>2</sup> LED Technology Center, Department of Nano-Optical Engineering, Korea Polytechnic University, Siheung 429-793, Republic of Korea

\*Corresponding author, e-mail: [youngwk@snu.ac.kr](mailto:youngwk@snu.ac.kr)

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### Abstract

InGaN/GaN quantum wells (QWs) grown on a c-plane sapphire has strong spontaneous and piezoelectric polarization field<sup>1</sup>, which induces a spatial separation of electrons and holes in the quantum wells and hinder a recombination of carriers, known as a quantum-confined stark effect (QCSE)<sup>2</sup>. Since the QCSE is one of the factors to deteriorate the emission efficiency of light-emitting diode, InGaN/GaN was grown on m-plane sapphire substrates to reduce the QCSE, taking advantage of the semi-polar nature of the plane.<sup>3</sup> The InGaN/GaN quantum wells formed on the semi-polar substrate, however, resulted in complex microstructures like basal plane stacking faults (BSFs), prismatic stacking faults (PSFs), partial dislocations (PDs), and threading dislocations (TDs). Understandings the luminescence characteristics from defects in semi-polar InGaN/GaN QWs are critical steps to improve the efficiency of semi-polar LED. We report microstructural and optical analysis results on (11-22) semi-polar InGaN/GaN QWs by using home-built cathodoluminescence stage for transmission electron microscopy. The semi-polar GaN epilayers were grown on the hemispherical patterned sapphire substrates (HPSS) and planar m-plane sapphire substrates by metal organic chemical vapor deposition. The CL mappings overlaid microstructures clearly reveal uneven distribution of luminescence and the wavelength shift in the vicinity of BSFs and dislocations, originated from different substrates.

**Keywords** GaN, semi-polar, defect, cathodoluminescence, transmission electron microscopy

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## Strain Analysis by STEM Moiré Method

Yukihito Kondo<sup>1\*</sup> and Noriaki Endo<sup>1\*</sup>

<sup>1</sup> JEOL Ltd. 3-1-2, Musashino Akishima 196-8558 Tokyo Japan

\*Corresponding author, e-mail: kondo@jeol.co.jp

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### Abstract

Strain is measurable using a moiré fringe arisen from interference of gratings of STEM raster and crystalline lattice. Accuracy of measured strain is improved with two techniques: one is averaging and the other is compensation of specimen drift during acquisition of STEM moiré pattern using a non strained region. The resulted typical accuracy reached to be 0.1 % in standard deviation, which is agree with industrial request. The data on typical high k metal gate sample, whose stress was made by Si-Ge mixed crystal, are shown as strain maps. The resulted strain map showed the compressive strain along [110] direction. The time to acquire data is 2 min. And analysis time to obtain a final resulted strain map is less than 3 min.

**Keywords:** moiré, STEM, strain analysis

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## Challenges in HR-SEM: Direct Observation of Micro-, Meso- and Macro- pore Openings and Fine Structure Details of Nanostructured Materials

Shunsuke ASAHINA<sup>1\*</sup>, Yusuke SAKUDA<sup>1</sup>, Shunai CHE<sup>2</sup>, Osamu TERASAKI<sup>3\*</sup>

<sup>1</sup> JEOL Ltd., 3-1-2 Musashino, Akishima, Tokyo, 196-8558, Japan

<sup>2</sup> School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P. R. China

<sup>3</sup> Dept of Materials & Environmental Chemistry, EXSELENT, Stockholm Univ, Stockholm, Sweden & Graduate School of EEWS, KAIST, Daejeon, Republic of Korea

\*Corresponding author, e-mail: sasahina@jeol.co.jp, terasaki@mmk.su.se

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### Abstract

New technical developments in LVHRSEM have made it possible to acquire SEM images at extremely low impact energy even below 100 eV on single nm spatial resolution. This method is becoming popular for observing fine details of surface structures in nanoporous materials. This is because that the materials such as Zeolites, MOFs and mesoporous silica are electron sensitive and non-conductive. In this report, we focused on showing advantages of LVHRSEM especially fine structures on external surfaces of nanostructured materials through examples using electrons with ultra-low impact energy.

**Keywords** Low voltage SEM, Meso porous silica, Zeolites,

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## Metallography Techniques for Microscopy of Electrogalvanized Coatings: Effectiveness of Mechanical, Electrochemical, and Ion polishing

Narin Jantaping<sup>1</sup>, Chaiyasit Banjongprasert<sup>2</sup>, Torranin Chairuangsr<sup>3</sup>, Christopher A. Schuh<sup>4</sup>,  
Yuttanant Boonyongmaneerat<sup>5\*</sup>

<sup>1</sup> Nanoscience and Technology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand

<sup>2</sup> Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>3</sup> Department of Industrial Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>4</sup> Department of Materials Science and Engineering, Massachusetts Institute of Technology, Massachusetts, USA

<sup>5</sup> Metallurgy and Materials Science Research Institute, Chulalongkorn University, Bangkok 10330, Thailand

\*Corresponding author, e-mail: yuttanant@gmail.com

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### Abstract

Electrogalvanized coatings have been employed widely in various applications, owing to their excellent corrosion protection for steel components. However, to date, there presents only minimal microscopy studies on electrogalvanized coatings. This is partly due to the challenges in preparation of their surfaces, which are easily oxidized and exhibit refined nano-scaled crystallite size. If microscopy studies of the coating materials could be carried out effectively, further improvements of materials properties through analysis of the coatings' micro- and nano-structures would be enabled.

In the present study, mechanical polishing, electrochemical polishing, and ion polishing for preparations of electrogalvanized surfaces were systematically and comparatively examined. The investigations, which were performed in relation to scanning electron microscopy (SEM), indicated that the electrochemical polishing of some specific formulations provided more decent results with improved visibility of microstructural features, as compared to the more conventional mechanical polishing routes that utilize oil-based diamond suspension or colloidal silica. Such superior results were obtained as the surfaces were etched in a controlled manner without rapid attack on the zinc's grains, which were prone to metal dissolution. Furthermore, the transmission electron microscopy (TEM) study suggests that electropolishing also provides a more appreciable surface characteristic than the conventional metallography method. Finally, the focused ion beam (FIB) investigated in this work was determined to be the most suitable technique to prepare a surface of galvanized coatings for TEM examination, despite its intricate and long protocols, as it provides exceptionally thin sample without inducing surface defects.

**Keywords** Electrodeposited zinc; Sample preparation; Metallography; Electron microscopy techniques

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## Characterization of the Interface between Metal and Glass Produced by Cold Spray

Minghui Song<sup>1\*</sup>, Hiroshi Araki<sup>1</sup>, Seiji Kuroda<sup>1</sup>, Kazuhiko Sakaki<sup>2\*</sup>

<sup>1</sup> National Institute for Materials Science, Sengen 1-2-1, Tsukuba, 3050047 Japan

<sup>2</sup> Shinshu University, 4-17-1 Wakasato, Nagano, 3808553 Japan

\*Corresponding author, e-mail: Minghui.SONG@nims.go.jp

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### Abstract

Cold spray uses a high pressure gas to accelerate powder particles onto a substrate to form a coating at temperatures much lower than the melting point of the sprayed particles. It can also be sprayed onto non-metallic substrates such as ceramics and glass. Among the several reported metals such as Al, Cu, Ti, and Sn, Al exhibits excellent adhesion to alumina, lead zirconate titanate (PZT), or glass. However, the mechanism of its adhesion has not been well understood yet. Recently, we reported that an interface layer is formed at the interface between cold sprayed Al particles and a glass substrate. This layer may contribute to the good adhesion of Al onto a glass substrate. In the present work, the interface layer was further analyzed with EELS and other methods to reveal the chemical and structure nature of the interface. The chemical condition of the layer was revealed to be close to that of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>. The formation of the interface layer and the resulted interface structure was considered to result in the stabilization of Al-glass interface and a relatively good adhesion.

**Keywords** EELS, TEM, cold spray, interface, glass

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## In-situ Observation of Mutual Reduction between ceramics in TEM

N.Ishikawa<sup>1\*</sup>, T.Kimura<sup>1</sup>, M.Takeguchi<sup>1</sup>, T Aizawa<sup>2</sup> and T.Inami<sup>2\*</sup>

<sup>1</sup>National Institute for Materials Science (NIMS), 1-2-1, Sengen, Tsukuba, Ibaraki 305-0047 Japan

<sup>2</sup>Faculty of Engineering, Ibaraki University, 4-12-1, Nakanarusawa-cho, Hitachi, Ibaraki 316-8511 Japan

\*Corresponding author, e-mail: ISHIKAWA.Nobuhiro@nims.go.jp

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### Abstract

The carbon dioxide in the iron and steel industries accounts for about 5% of the total world emission because of the use of coal as reductant, heat source, carburization and so on. Most of research projects for reduction of carbon dioxide are still depend on the carbon as reductant and it is difficult to reduce much amount of emission. Recently we found that several kinds of ceramics which do not include carbon could reduce iron-oxide with in-situ electron microscopy. Some of reactions were promoted by high energy electron irradiation. The observation of these reactions enabled by the development of the way of specimen preparation. These reaction systems do not generate carbon dioxide and these results are expected to apply for low carbon dioxide emission iron-making process.

**Keywords:** in-situ observation, reduction, hematite, composite oxide

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## Real Time Observation of the Reconstructing TiO<sub>2</sub> (001) Surface via an Environmental TEM

Wentao Yuan<sup>1</sup>, Hengbo Li<sup>1</sup>, Hanglong Wu<sup>1</sup>, Ze Zhang<sup>1</sup>, Annabella Selloni<sup>2</sup>, Chenghua Sun<sup>3</sup>, Yong Wang<sup>1\*</sup>

<sup>1</sup> Center of Electron Microscopy and State Key Laboratory of Silicon Materials, School of Materials Science and Engineering, Zhejiang University, Hangzhou 310027, China

<sup>2</sup> Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States

<sup>3</sup> ARC Centre for Electromaterials Science, School of Chemistry, Monash University, Clayton, Victoria 3800, Australia

\*Corresponding author, e-mail: yongwang@zju.edu.cn

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### Abstract

As the oxide surfaces have a remarkable impact on their physical and chemical properties, research on oxide surfaces attracts considerable attention in the past decades. Tremendous advances have been made in determining the surface structures of the micro-sized crystals by scanning tunneling microscopy from top-view, however STM cannot 'see' the sub-surface layers to acquire crucial information of surface stress; and more importantly, it remains a great challenge to secure a real-time observation of surface dynamics of oxides in a gaseous environment at the atomic scale. Here, using an environmental transmission electron microscopy, we directly observe the reconstruction dynamics on the (001) surface of anatase TiO<sub>2</sub>. We have for the first time obtained real-time images of the reconstructing anatase TiO<sub>2</sub> (001) with atomic-level resolution from both side and top views, and clarified how the most favorable (1x4) reconstruction is formed. With the support of first principles calculations, we found that such surface evolution is driven by both lowly coordinated atoms and surface stress. This work provides a complete picture of the reconstruction of TiO<sub>2</sub> (001) and we anticipate this in situ real-time technique can be readily applied to study the dynamic formation and evolution of surface reconstructions on other systems.

**Keywords** oxide surface, surface reconstruction, surface dynamics, environmental transmission electron microscopy (ETEM), in situ TEM

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## In-situ TEM Study of Oxidation in a Nickel-based Single Crystal Superalloy

Qingqing Ding, Tian He\*, Jixue Li\*, Ze Zhang\*

Center of Electron Microscopy and State Key Laboratory of Silicon Materials, Department of Materials Science and Engineering, Zhejiang University, Hangzhou 310027, China

\*Corresponding author, e-mail: hetian@zju.edu.cn and jx\_li@zju.edu.cn

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### Abstract

Oxidation of a nickel-based single crystal superalloy was studied by in-situ environmental transmission electron microscopy, energy dispersive spectrometer and electron energy loss spectroscopy, energy dispersive spectrometer and electron energy loss spectroscopy in detail. The oxidation product of  $\gamma$  and  $\gamma'$  phase was respectively consisted of porous NiO nanoparticles and  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> crystallites with a strong crystallographic texture. In addition, oxygen concentration of interface is found to be much higher than  $\gamma'$  and  $\gamma$  phase in elemental mapping obtained by electron energy loss spectroscopy and the closer near sample edge, the higher oxygen concentration at the interface. Hence we deem that oxygen diffusion path in nickel based single crystal superalloy is more inclined to be the interface rather than the previously thought matrix channel. Moreover, phase transformation from ordered  $\gamma'$  phase (L1<sub>2</sub>) to disordered  $\gamma$  phase (fcc) was also observed and was a slow process with the generation of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> crystallites. This directly indicates that aluminum oxidation resulting in aluminum depletion caused the  $\gamma'$ - $\gamma$  phase transformation, which can severely degrade creep properties of thinned superalloys.

**Keywords:** Oxidation; In-situ TEM; Electron energy loss spectroscopy; Nickel-based superalloy; Oxygen diffusion path

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## Aging Strengthening of Interphase-Precipitated Strengthened Copper-Bearing Dual-Phase Steels

Cheng-Han Li, Shao-Pu Tsai, Jer-Ren Yang\*

Department of Materials Science and Engineering, National Taiwan University, Taiwan

\*Corresponding author, e-mail: jryang@ntu.edu.tw

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### Abstract

In order to decrease the carbon emission and energy saving, light-weight design for high-strength low-alloy (HSLA) steels plays an important role in automotive industries in recently years. Dual-phase (DP) steels (composed of soft ferrite and hard martensite) as one type of HSLA steels were commercialized by the end of 20<sup>th</sup> century. DP steels demonstrate special mechanical properties with combination of good strength and formability; therefore, DP steels have been widely implemented in automotive industries. The study we present in the paper is an attempt to design an unprecedented high-strength dual-phase steel. In this research, carbide precipitation during austenite ( $\gamma$ ) to ferrite ( $\alpha$ ) transformation called interphase precipitation is utilized to dramatically enhance of strength of ferrite. A two-step isothermal heat treatment followed by quench-tempering process for low-carbon copper-bearing titanium-added steel is conduct. Co-precipitation effect of interphase-precipitated carbide and copper particle and microstructure evolution are identified with the aid of transmission electron microscopy. Mechanisms for secondary hardening in both phases are elucidated. This new-generation material provides great interest both for industrial application and scientific research.

**Keywords** Dual-phase steel; Interphase precipitation; Copper-bearing Steel; Aging hardening

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## Multi-dimensional Analysis of Bainite in Cu-Al-Mn Alloy by Advanced Electron Microscopy Techniques

S. Motomura<sup>1</sup>, T. Omori<sup>2</sup>, R. Kainuma<sup>2</sup>, M. Nishida<sup>3</sup>, T. Hara<sup>3,4\*</sup>

<sup>1</sup> Department of Applied Science for Electronics and Materials, Kyushu University, Kasuga, Fukuoka, Japan

<sup>2</sup> Department of Materials Science, Graduate School of Engineering, Tohoku University, Sendai, Miyagi, Japan

<sup>3</sup> Department of Engineering Science for Electronics and Materials, Faculty of Engineering Sciences, Kyushu University, Kasuga, Fukuoka, Japan.

<sup>4</sup> Research Center for Structural Materials, National Institute for Materials Science, Tsukuba, Ibaraki, Japan

\*Corresponding author, e-mail: HARA.Toru@nims.go.jp

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### Abstract

Bainitic transformation has both characteristics of diffusionless and diffusional transformations. This type of transformation has been found in various alloy systems, from steel to noble metal based alloys. Despite many researches have been carried out, the mechanism of bainitic transformation has still been unclear. In order to understand bainitic transformation in Cu-Al-Mn alloy, 'multi-dimensional' analysis using recently developed electron microscopy techniques are applied. 'Multi-dimensional' means that a parameter, such as time, temperature, crystal orientation, chemical composition etc., is simultaneously varied in addition to two-dimensional microscopy image. Techniques utilized in this study are: (i) heating in-situ SEM observation to reveal growth manner of bainite, (ii) three dimensional morphological analysis of bainite using orthogonally-arranged FIB-SEM, which is specially designed to realize precise three-dimensional analysis, and (iii) high-efficiency EDS-STEM compositional analysis with large detecting solid-angle (~2str.) with double detectors to clarify the contribution of diffusion on bainite forming. From these observations and analyses, we obtained detailed morphological, crystallographic, and compositional information which is indispensable to discuss the mechanism of bainitic transformation.

**Keywords** : scanning electron microscope, in-situ observation, serial-sectioning, energy-dispersive X-ray spectroscopy, bainitic transformation

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## Spinodal Decomposition in CoCuFeMnNi and CuFeMnNiZn High-entropy Alloys Observed by Electron Microscopy

Ruangdaj Tongsri <sup>1\*</sup>, Ussadawut Patakham <sup>1</sup>, Nattaya Tosangthum <sup>1</sup>, Pennapa Muthitamongkol <sup>1</sup>, Plaichumpon Paha <sup>2</sup>, Panida Surawongsakul <sup>2</sup>, Piyanuch Nakpong <sup>2</sup>, Panita Choeychom <sup>3</sup> and Pinai Mungsantisuk <sup>3\*</sup>

<sup>1</sup> Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, PathumThani 12120 Thailand

<sup>2</sup> Department of Chemistry, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand

<sup>3</sup> The Sirindhorn International Thai-German Graduate School of Engineering (TGGs), King Mongkut's University of Technology North Bangkok (KMUTNB), 1518 Pracharajsai 1 Road, Wongsawang, Bangsue, Bangkok 10800, Thailand

\*Corresponding author: ruangdt@mtec.or.th

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### Abstract

Two equiatomic multicomponent alloys with compositions of CoCuFeMnNi and CuFeMnNiZn were designed using several empirical criteria of high entropy alloys. Theoretically, both high entropy alloys ought to have disordered face-centered cubic solid solutions. Experimental processing of these two alloys was performed by using mechanical alloying followed both sintering and melting. Microstructures of sintered and cast alloys were characterized by optical and scanning electron microscopy. In sintered alloys, oxidation of manganese could not be avoided so *in situ* manganese oxide-reinforced high entropy alloy matrix composites were obtained. The composite matrices were identified as two Fe-based and Cu-based disordered face-centered cubic solid solutions existing in the forms of modulated spinodal structure. In cast alloys, solidified dendritic structures were commonly observed in both alloys. Although the dendritic structures could be refined by high a cooling rate and a grain refiner addition but the spinodal decomposition in both dendrites and interdendrites still occurred. In some cases, egg type or core-shell particles were observed in interdendrites of cast CuFeMnNiZn high entropy alloy. Within the core, spinodal decomposition was also observed. It may be implied that immiscibility of Fe and Cu still exists in Fe- and Cu-containing high entropy alloys.

**Keywords:** High entropy alloys, mechanical alloying, sintering, melting, microscopy, spinodal decomposition

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## Analytical Investigation of Rapidly Solidified Braze Ribbons for Transient Liquid Phase Bonding

Riza Iskandar<sup>1\*</sup>, Ludwig Pongratz<sup>2</sup>, Stefanie Wiesner<sup>2</sup>, Memet Öte<sup>2</sup>, Alexander Schwedt<sup>1</sup>, Kirsten Bobzin<sup>2</sup>, Joachim Mayer<sup>1\*</sup>

<sup>1</sup> Central Facility for Electron Microscopy (GFE), RWTH Aachen University, AhornStr. 55, Aachen 52074, Germany

<sup>2</sup> Surface Engineering Institute (IOT), RWTH Aachen University, KackertStr. 15, Aachen 52072, Germany

\*Corresponding author, e-mail: iskandar@gfe.rwth-aachen.de

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### Abstract

Transient liquid phase (TLP) bonding is a diffusion based joining process that offers great potential for different metallic systems. TLP bonding makes it possible to produce bonds where brazed joint and base materials are almost chemically identical [1]. In this work, flexible Sn78Cu22 and Sn75Cu20Ge5 braze ribbons with a homogenous element distribution were manufactured by a melt spinning process [2]. The aim of this work is to analyse the microstructure and to understand the melting behaviour as well as the solidification process of the braze ribbons. A JEOL field emission SEM JSM-7000F and ZEISS in-column corrected omega filter Libra 200 FE were used to analyse both braze ribbons and final joints.

EBSD and TEM investigations on both types of braze ribbons revealed grain morphology evolutions and formation of metastable  $\text{Cu}_x\text{Sn}_y$  and Ge rich phases due to rapid solidification. Furthermore, analysis of brazed joints with AlSi7Mg0.3 base material showed that all phases formed in the joint are embedded in a Sn matrix. In the case of Sn78Cu22, intermetallic  $\text{Cu}_x\text{Al}_y$  phases and a small number of Si precipitates were found. In the Sn75Cu20Ge5 joint, intermetallic  $\text{Cu}_x\text{Al}_y$ ,  $\text{Ge}_x\text{Al}_y$  and  $\text{Ge}_x\text{Si}_y$  phases were found [3]. The results suggest that germanium addition induces a change of the intermetallic  $\text{Cu}_x\text{Al}_y$  phases.

**Keywords** transient liquid phase bonding, rapid solidification, EBSD, AEM

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## Heat-treated Microstructure and Property of Sintered of Fe-0.85Mo + 4 wt. % SiC + 0.4 wt.% C Materials

Dhriti Tanprayoon<sup>1</sup>, Kittikhun Ruangchai<sup>2</sup>, Amporn Wiengmoon<sup>2</sup>, Rungtip Krataitong<sup>1</sup>, Thanyaporn Yotkaew<sup>1</sup>, Ussadawut Patakhram<sup>1</sup>, Ruangdaj Tongsri<sup>1\*</sup>

<sup>1</sup> Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120 Thailand

<sup>2</sup> Department of Physics, Faculty of Science, Naresuan University, Pitsanulok-Nakhon Sawan Road, Tha Pho, Muang, Pitsanulok 65000 Thailand

\* Corresponding author: ruangdt@mtec.or.th

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### Abstract

The sintered Fe-0.85Mo + 4 wt. % SiC + 0.4 wt.% C alloy exhibiting ductile iron/compacted graphite iron-like microstructure, in which vermicular graphite particles were enveloped by ferrite grains, which were surrounded by pearlitic structure, was austenitized at 850 °C for 1h. After that, the alloy was cooled with different cooling rates. Microstructural characterization by optical microscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy revealed that the heat-treated alloys exhibited different microstructural features depending on the cooling rate after homogenization. With the slowest cooling rate, the ductile iron/compacted graphite iron-like microstructure transformed to that consisting of vermicular graphite particles surrounded with polygonal ferrite (PF) grains and coarse pearlite islands distributed in ferrite matrix, respectively. With increased cooling rate, both the PF grains and pearlite islands became refined. With further increase of cooling rate, the matrix around the vermicular graphite particles to be upper bainite and/or martensite. The microstructural features had strong influences on mechanical properties of the heat-treated alloys.

**Keywords:** Sintered Fe-Mo-Si-C alloy, heat-treated alloy, microstructural characterization, microscopy, mechanical property

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## Sintered Ductile Iron/Compacted Graphite Iron-like Fe-Mo-Si-C alloy

Kittikhun Ruangchai<sup>1</sup>, Amporn Wiengmoon<sup>1</sup>, Monnapas Morakotjinda<sup>2</sup>, Rungtip Krataitong<sup>2</sup>, Dhriti Tanprayoon<sup>2</sup>, Thanyaporn Yotkaew<sup>2</sup>, Nattaya Tosangthum<sup>2</sup>, Ussadawut Patakham<sup>2</sup>, Ruangdaj Tongsri<sup>2\*</sup>

<sup>1</sup> Department of Physics, Faculty of Science, Naresuan University, Phitsanulok-Nakhon Sawan Road, Tha Pho, Muang, Phitsanulok 65000, Thailand

<sup>2</sup> Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, PathumThani 12120, Thailand

\*Corresponding author, e-mail: ruangdt@mtec.or.th

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### Abstract

Sintered Fe-Mo-Si-C alloys were prepared from pre-alloyed Fe-Mo powder added with fixed 4 wt.% silicon carbide powder and varied graphite powder contents. Microstructural investigation was performed by using optical microscopy and scanning electron microscopy in association with energy dispersive X-ray spectroscopy chemical analysis. The microstructural characterization showed that the sintered alloys had microstructural features similar to that of a ductile iron and/or compacted graphite iron. Black nodular and/or vermicular particles were enveloped by ferrite grains, which were surrounded by pearlitic structure. The counts of nodular/vermicular particle, ferrite and pearlite fraction ratios were performed by using optical micrographs. Addition of graphite powder to Fe-Mo + 4 wt.%SiC powder mixture resulted in decrease of black nodular/vermicular particle fraction, increase of pearlite fraction and slight change of ferrite fraction. The increase of added graphite powder content also caused morphological change from black nodular to black vermicular particles. Characterization using scanning electron microscopy and energy dispersive X-ray spectroscopy chemical analysis revealed that black nodular particles were either graphite or Fe-Mo-Si-C/graphite core-shell particles whereas vermicular particles were totally composed of carbon. The microstructural features of showed influence on mechanical property of the sintered Fe-Mo-Si-C alloys.

**Keywords** Fe-Mo-Si-C alloy, Sintering, Microstructural Characterization, Microscopy, Mechanical Property

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## Evidences of Sympathetic Nucleation and Ledgewise Growth Revealed by Scanning Electron Microscopy

Wananurat Srijampan<sup>1</sup>, Amporn Wiengmoon<sup>1</sup>, Ruangdaj Tongsri<sup>2\*</sup>

<sup>1</sup> Department of Physics, Faculty of Science, Naresuan University, Phitsanulok-Nakhon Sawan Road, Tha Pho, Muang, Phitsanulok 65000, Thailand

<sup>2</sup> Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, PathumThani 12120, Thailand

\*Corresponding author, e-mail: ruangdt@mtec.or.th

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### Abstract

Sintered ultralow carbon (0.0055 wt.%) and low carbon (0.1 wt.%) Fe-Cr-Mo steels were characterized using optical microscopy and scanning electron microscopy. Different microstructures were found to be due to the influences of carbon content and cooling rate. With the slow cooling rate of 0.1 °C/s, the sintered ultralow carbon steels exhibited coarse polygonal ferrite grains and pores. No micrometer-sized sub-structures were revealed by scanning electron microscopy. With higher cooling rates of 4.0 and 5.4 °C/s, the sintered ultralow carbon steels exhibited evidences of sympathetic nucleation and ledgewise growth of ferrite plates, whose dimensions were 500 nm wide, 100 nm thick and varied lengths. In the sintered low carbon steels, thick bainite layers were observed along PF grain boundaries when the material was cooled at 0.1 °C/s. Increases of cooling rates to 4.0 and 5.4 °C/s, the sintered low carbon steels also showed ledgewise-grown ferrite plates. However, the ferrite plates in the sintered low carbon steels were thinner but longer than those in the sintered ultralow carbon steels. These fine ferrite plates were thought to be one of basic units of Widmanstätten and bainite structures. The presence of sub-structures of ferrite plates strengthened the sintered steels.

**Keywords** Sympathetic nucleation, Ledgewise growth, Widmanstätten, Bainite, Mechanical property

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## Characterization of CoCuFeMnNi Alloy

Panita Choeychom<sup>1</sup>, Pinai Mungsantisuk<sup>1</sup>, Nattaya Tosangthum<sup>2</sup>, Rungtip Krataitong<sup>2</sup>, Thanyaporn Yotkaew<sup>2</sup>, Ussadawut Patakham<sup>2</sup>, Ruangdaj Tongsri<sup>2\*</sup>

<sup>1</sup> The Sirindhorn International Thai-German Graduate School of Engineering (TGGS), King Mongkut's University of Technology North Bangkok (KMUTNB), 1518 Pracharajsai 1 Road, Wongsawang, Bangsue, Bangkok 10800, Thailand

<sup>2</sup> Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, PathumThani 12120, Thailand

\*Corresponding author, e-mail:[ruangdt@mtec.or.th](mailto:ruangdt@mtec.or.th)

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### Abstract

High-entropy alloy with nominal composition of CoCuFeMnNi was designed using empirical criteria for solid solution formation. According to the design, the alloy was predicted to have face-centered cubic solid solution. Experimental validation was conducted by using two approaches, e.g., mechanical alloying followed by sintering and mechanical alloying followed by melting. Characterizations using X-ray diffraction technique, scanning electron microscopy and energy dispersive X-ray spectroscopy revealed different alloy microstructures produced by two approaches. Using the first materials processing approach, an in-situ composite of CoCuFeNi high-entropy alloy matrix reinforced with MnO particles was obtained. The CoCuFeNi high-entropy alloy matrix, observed by using scanning electron microscopy and energy dispersive X-ray spectroscopy, showed microstructure consisting of spinodally decomposed Fe-based and Cu-based Widmanstätten plates oriented perpendicular to each other. The microstructure of the as-cast CoCuFeMnNi high-entropy, produced by the second approach, indicated that the melt solidified with dendritic regime. The dendrites were Fe-rich whereas the interdendrites were Cu-rich materials. However, in both dendrites and interdendrites there were clear evidences of spinodal decomposition to form Fe-based and Cu-based Widmanstätten plates.

**Keywords** Mechanical alloying, Sintering, Melting, High-entropy alloy, Spinodal decomposition

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## In situ Studies of Heat Induced Precipitation of Yttria Precursor using Liquid-Cell Transmission Electron Microscopy

Sašo Šturm<sup>1\*</sup>, Bojan Ambrožič<sup>1,2</sup>, Nina Kostevšek<sup>1,2</sup>, Marjan Bele<sup>3</sup>, Kristina Ž. Rožman<sup>1\*</sup>

<sup>1</sup>Department for Nanostructured Materials, Jožef Stefan Institute, Ljubljana, Slovenia

<sup>2</sup>Jožef Stefan International Postgraduate School, Ljubljana, Slovenia

<sup>3</sup>Laboratory for Materials Chemistry, National Institute of Chemistry, Ljubljana, Slovenia

\*Corresponding author, e-mail: saso.sturm@ijs.si

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### Abstract

Yttria nanoparticles (NPs) have shown to be an efficient up-conversion phosphor material with great potential ranging from theranostics to photovoltaics. Their efficient fabrication is bound to the understanding of the colloidal chemistry during their formation. In contrast to various analytical methods, where the kinetic data are deduced from large sampled volumes, in situ transmission electron microscopy (TEM) combined with liquid cell offers the unique possibility of studying spatial and temporal evolution of individual NPs, allowing a comprehensive reconstruction of early stage events that are vital for the formation of final NPs.

Temperature controlled nucleation of yttria precursors by urea precipitation method was facilitated inside the Jeol JEM 2100 TEM equipped with Protochips liquid flow cell with heating capabilities. The abrupt nucleation of NPs was observed when the temperatures in the cell was raised above 90°C. The resulting products were either faceted particles or dendritic nanostructures. While the faceted nanoparticles did not experience significant morphological changes during the observation, dendritic structures experienced rapid growth, fragmentation and dissolution. This phenomenon was explained by changes in the cell environment due to a combined effect of water and urea decomposition, destabilizing the dendritic structure and promoting the formation of more stable crystalline yttria precursors.

**Keywords** In situ TEM, Liquid TEM, Nucleation, Nanoparticles, Yttria

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## Synthesis and Characterization of Mixed Phase TiO<sub>2</sub>(B)/anatase TiO<sub>2</sub> Thin Films on a Number of Different Substrates by a Modified Low Pressure Chemical Vapour Deposition (LPCVD)

Yothin Chimupala,<sup>1,2\*</sup> Robert Simpson,<sup>2</sup> Patcharanan Junploy<sup>3</sup> and Rik Brydson<sup>2\*</sup>

<sup>1</sup> Department of Industrial Chemistry, Faculty of Science, Chiang Mai University, Huaykaew, Chiang Mai, 50200, Thailand

<sup>2</sup> School of Chemical and Process Engineering, Faculty of Engineering, University of Leeds, LS2 9AJ, UK

<sup>3</sup> Department of Chemistry, Faculty of Science, Chiang Mai University, Huaykaew, Chiang Mai, 50200, Thailand

\* Corresponding author, e-mail: yothin.chimupala@cmu.ac.th

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### Abstract

TiO<sub>2</sub>(B) or “bronze” is a TiO<sub>2</sub> polymorph which is difficult to synthesise in a pure form and does not commonly exist in minerals. TiO<sub>2</sub>(B) potentially plays an important role in applications both as a photocatalytic component alongside anatase for degradation reactions and as an anode material in lithium ion batteries due to its distinctive crystal structure which exhibits large channels and voids. In this research, titania thin films of a mixed phase of TiO<sub>2</sub>(B) and anatase were successfully synthesised by a Low Pressure Chemical Vapour Deposition (LPCVD) process onto a number of different substrates including a soda lime glass, silicon wafer, fused quartz, highly ordered pyrolytic graphite (HOPG) and pressed graphite flake (grafol). The optimal LPCVD condition for preparing this mixed phase of TiO<sub>2</sub> was 550°C (actual temperature) with a 1 mL/s N<sub>2</sub> flow rate. Thin films samples were mainly characterised using scanning and transmission electron microscopy which exhibited a columnar morphology together with smaller equi-axed particles. Additionally, X-ray diffraction, Raman spectroscopy, X-ray Photoelectron spectroscopy were used to investigate the thin films. Phase formation mechanisms during LPCVD process have been proposed. Methylene blue degradation tests indicated an increase of photocatalytic activity for the mixed phase anatase/TiO<sub>2</sub>(B) thin films.

**Keywords** TiO<sub>2</sub>(B), LPCVD, Thin films, TEM, SEM

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## Microstructural Evolution of Hydroxy/fluoroapatite in the Different Precursor Solution under Hydrothermal Process

Wisarat Ngoenthong, Upsorn Boonyang<sup>\*</sup>

Molecular Technology Research Unit, School of Science, Walailak University, Thailand

<sup>\*</sup>Corresponding author, e-mail: upsorn.bo@wu.ac.th

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### Abstract

This work is focused on the potential role of pH and starting precursor for the morphology of hydroxy/fluoroapatite (HFAP) particles during their synthesis by hydrothermal process. Phase composition, crystal structure and morphology of hydroxyl/fluoroapatite were studied by powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) combined with energy dispersive X-ray spectroscopy (EDS). Under hydrothermal process, 150 °C for 12 h, precursor with different phosphate and fluorine source shows different final product morphology. The effect of starting precursor, phosphorous source, in ammonium hydrogen phosphate condition showed the prism-like microstructure. Besides, hexagonal crystallites bundle of flowerlike-HFAP nanorods were present in phosphoric acid solution. While the results showed that different of fluorine source; sodium fluoride and ammonium fluoride did not affect the morphology of HFAP.

**Keywords** hydroxy/fluoroapatite; hydrothermal, morphology

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## Preparation of Electrospun Polystyrene Fibrous Membranes Containing Silver Nanoparticles

Chutima Srisitthiratkul, Nuttaporn Pimpha and Wiyong Kangwansupamonkon\*

National Nanotechnology Center, National Science and Technology Development Agency 111  
Thailand Science Park, Phahonyothin Road, Pathum Thani 12120, Thailand.

\*Corresponding author, e-mail: wiyong@nanotec.or.th

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### Abstract

Fibrous membranes with antibacterial activity were prepared from 10%w/v polystyrene (PS) solution containing silver nitrate ( $\text{AgNO}_3$ ) in the amounts of 1 –5 % by weight of PS by electrospinning. N, N-Dimethylformamide (DMF) was used as both the solvent for PS and reducing agent for  $\text{Ag}^+$  ions. The enhancement in the reduction process was achieved with UV irradiation. The structure and surface elemental composition of the nanofibers were characterized by SEM, TEM and EDX spectra. Electrospinning of both the base and the  $\text{AgNO}_3$ -containing PS solutions resulted in the formation of smooth fibers with average diameters of 500 and 280-350 nm, respectively. A larger number of silver nanoparticles with diameter ranging between 5 to 10 nm were observed adjacent to and at the fiber surface after the UV irradiation. Increasing the initial concentration of  $\text{AgNO}_3$  resulted in increase the amount of as-formed silver nanoparticles. The PS fibrous membranes containing silver nanoparticles exhibited excellent antibacterial activity against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* microorganisms. Thus, these membranes are promising materials for biomedical applications.

**Keywords:** PS nanofibers, Electrospinning and Antibacterial activity

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## Quick Evaluation of Electric Field Surrounding a Charged Particle

Katsuhiro Sasaki\*, Tomoharu Tokunaga, Takahisa Yamamoto\*

Department of Quantum Engineering, Faculty of Engineering, Nagoya University, Furo-cho, Chikusa, Nagoya, Japan

\*Corresponding author, e-mail: khsasaki@nagoya-u.jp

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### Abstract

We have been developing a simple method to measure the electric/magnetic field in a transmission electron microscope, named Shadow Image Distortion method<sup>1)</sup>. The method can be realized with a minor modification, in which the selected area diffraction aperture is replaced to a dedicated aperture (Shadow Image Aperture) for this method. The method was tested both for the dielectric and metallic particles with sub-micron size in diameter. The diffuse bright contrast of the distorted shadow image aperture was obtained only for the dielectric particle made of latex. The detailed analysis of the electric field surrounding the latex particle, which is due to the charging under electron beam irradiation, demonstrates the measurement of the electric field distribution with a few nanometers of resolution. The amount of the charge of the particle was quantitatively evaluated to be  $4.28 \times 10^{-18}\text{C}$ . The results were compared to the reported electron holography studies<sup>2,3)</sup> and showed that the comparable measurement could be available. The theoretical limit of spatial resolution and compatibility to electron holography will be discussed.

**Keywords:** Shadow image distortion, electron holography, electric field, charged particle.

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## Inclusion Complex between Water-soluble Quaternized $\beta$ -cyclodextrin Grafted Chitosan and $\alpha$ -mangostin: Influence of Degree of $\beta$ -CD Substituted on Chitosan

Sarunya Phunpee<sup>1</sup>, Somsak Saesoo<sup>1</sup>, Anchalee jintapattanakit<sup>2</sup>, Uracha Rungsardthong Ruktanonchai<sup>1\*</sup>

<sup>1</sup> National Nanotechnology Center, National Science and Technology Development Agency, Thailand Science Park, Pathumthani, Thailand

<sup>2</sup> Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

\*Corresponding author, e-mail: uracha@nanotec.or.th

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### Abstract

The compound  $\alpha$ -mangostin was isolated from the pericarp of the mangosteen (*Garcinia mangostana* Linn) fruit.  $\alpha$ -mangostin has recently been used for medicinal purposes such as anti-inflammatory and anti-microbial as well, meanwhile, its poor aqueous solubility and low oral bioavailability resulting in limited those therapeutic applications. Water-soluble quaternized  $\beta$ -CD grafted chitosan (QCD-g-CS) as a new carrier for hydrophobic drugs has been previously developed by our group. This study aimed to investigate the influence of QCD-g-CS with several of the degree  $\beta$ -CD substituted on chitosan (DS) ranging from 5 to 22% on the physicochemical and biological properties of  $\alpha$ -mangostin and QCD-g-CS complexes. The QCD22-g-CS demonstrated the highest entrapment efficiency and the strongest mucoadhesive response than that of QCD5-g-CS and QCD11-g-CS. It was due to its higher amount of  $\beta$ -CD grafted on chitosan backbone, which could be more loaded  $\alpha$ -mangostin in  $\beta$ -CD cavity and also hydrophobic core of QCD-g-CS self-aggregation. The *ex-vivo* mucoadhesion of those derivative was performed using porcine buccal mucosa. The high DS provided that larger amount of  $\beta$ -CD resulting in stronger hydrogen bonding and the electrostatic interaction. Moreover, internalized particles into buccal mucosa cell were observed by flow cytometry and confocal laser scanning microscopy. The exposed charge on the surface of the particles had a significant effect on the rate of endocytosis in tested cell. The morphologies of those derivatives were investigated using TEM as well.

**Keywords:**  $\alpha$ -mangostin,  $\beta$ -CD, chitosan, inclusion complexes, mucoadhesive

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## Comparison of Bio-oil and Extracted Residue from Acid and Enzyme Hydrolysis Processes of Microalgae

Nuapon Duongbia<sup>1</sup>, Suraphon Chaiwongsar<sup>2</sup>, Chatchawan Chaichana<sup>3</sup>, Suparin Chaiklangmuang<sup>1,\*</sup>

<sup>1</sup> Department of Industrial Chemistry, Faculty of Science, Chiang Mai University,  
Chiang Mai 50200, Thailand

<sup>2</sup> Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna,  
Chiang Mai 50200, Thailand

<sup>3</sup> Department of Mechanical Engineering, Faculty of Engineering, Chiang Mai University,  
Chiang Mai 50200, Thailand

\*Corresponding author, e-mail: suparin.c@cmu.ac.th

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### Abstract

Bio-oil production from wet microalgae using hydrolysis process is the worthy technique to reduce the processes and energy of the production. This research has studied the acidic and enzymatic hydrolysis processes of *Spirulina platensis* contained high moisture for bio-oil production. The lipid or crude bio-oil yields from the research were in range of 3.5-13 wt.%. The properties of the extracted algae residues including, higher heating value, total sulfur content, surface morphology using SEM and chemical composition analysis by EDS were investigated.

**Keywords:** Microalgae, *Spirulina platensis*, Hydrolysis, Wet extracted, SE

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## Scanning Electron Microscopes with Integrated Raman Spectrometer Revealing New Complementary Information

Stefanie Freitag and Fang Zhou\*

Carl Zeiss Microscopy GmbH, Market Segment Material Sciences, Germany

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### Abstract

Scanning electron microscope (SEM) delivers surface sensitive topographical and compositional images with high resolution. Whereas Raman spectroscopy allows for the identification and quantification of solid state materials and molecules through their unique vibrational and rotational energy level structure. The integration of Raman spectrometer into an SEM allows exact sample navigation as well as correlation of SEM image contrasts and chemical information obtained using Raman spectroscopy which enables the differentiation between oxides and organic as well as inorganic material.

SEM images of graphene sample revealed information about topography features like wrinkles and residues from the production process like adhesives or particles. Subsequently the sample is transferred to the Raman system within one chamber in vacuum or analyzed with a retractable Raman accessory. Point measurement and a full mapping including confocal imaging may be achieved, followed by the correlation of SEM images and Raman maps. Thus, the information whether pristine graphene or graphene oxide or reduced oxide or graphene with further functional groups was produced is delivered using this SEM and Raman combination.



## Surface Sensitive Imaging using Energy Filtered SEM at Low kV

Fang Zhou and Markus Böse\*

Carl Zeiss Microscopy GmbH, Carl Zeiss Str. 22, Oberkochen, Germany

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### Abstract

The SEM with an adapted electron spectrometer offers the analysis capability to detect and image the energy filtered secondary electron (SE) and backscattered electron (BSE) signals with high spatial resolution. Due to the different SE as well as BSE spectra of different elements or composites high material contrast can be imaged to reveal the sample surface compositional information. Furthermore, the surface charging or surface potential causes SE spectra shifts which can be detected and imaged to reveal the potential distribution on the sample surface. In particular, higher surface sensitivity and higher resolution of SEM images are expected at low-kV primary beam energy because of the notably reduced scattering volume. Series of images are taken using the advanced energy selective backscattered (EsB) detector which allows quantitative analysis and improved accuracy of mapping where more than one element or composite is involved. By taking a series of images, it is also possible to extract the SE and BSE spectra profile from particular features.

## Origin of Dramatic Oxygen Solute Strengthening Effect in Titanium

Qian Yu\*

Department of Materials Science and Engineering & Center for Electron Microscopy, Zhejiang University, Hangzhou, China

\*Corresponding author, email: yu\_qian@zju.edu.cn

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### Abstract

Structural alloys are often strengthened through the addition of solute atoms. However, given that solute atoms interact weakly with the elastic fields of screw dislocations, it has long been accepted that solution hardening is only marginally effective in materials with mobile screw dislocations. By using transmission electron microscopy and nanomechanical characterization we report that the intense hardening effect of dilute oxygen solutes in pure  $\alpha$ -Ti is due to the interaction between oxygen and the core of screw dislocations that mainly glide on prismatic planes. First-principles calculations reveal that distortion of the interstitial sites at the screw dislocation core creates a very strong but short-range repulsion for oxygen consistent with experimental observations. These results establish a highly effective mechanism for strengthening by interstitial solutes.

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## Probing Structure and Electronic Structure of Battery Materials at Atomic Scale by Scanning Transmission Electron Microscopy

Lin Gu\*

Institute of Physics, Chinese Academy of Sciences/Beijing National Laboratory for Condensed, Matter Physics, Beijing, 100190, P.R.China

\*Corresponding author, e-mail: l.gu@iphy.ac.cn

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### Abstract

Lithium ion batteries (LIBs) and sodium ion batteries (SIBs) are two kinds of the promising energy storage devices due to their high energy density and power density, low cost and high safety, respectively. Performance optimization of them necessitates information about structural evolution of electrode materials at an atomic scale. However, limited understanding for the structural evolution of electrode materials at atomic resolution, especially the Li ions and Na ions distribution and arrangement at the atomic level, underlying electrochemical process substantially hinders our exploration of reaction mechanism and further performance optimization. The annular-bright-field (ABF) imaging in aberration-corrected scanning transmission electron microscopy (STEM) allows simultaneous imaging of light and heavy elements, providing an unprecedented opportunity to probe the nearly equilibrated local structure of electrode materials after electrochemical cycling at atomic resolution. In this report, we will present our recent efforts on revealing the atomic-scale structure of selected electrode materials with different charge and/or discharge state, e.g., the lattice distortion, phase interface structure, transition metal migration, surface reconstruction with (partial) intercalation and de-intercalation (1-6); information of electronic structure was also be obtained by atomic resolved electron energy loss spectroscopy (7), extending the current understanding of electrochemical reaction mechanism.

### Keywords

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## 3D Fourier Transform Analysis to Evaluate a High-Performance TEM

Kazuo Ishizuka<sup>1,2,\*</sup> and Koji Kimoto<sup>2</sup>

<sup>1</sup> HREM Research Inc., 14-48 Matsukazedai, Higashimatsuyama, Saitama, Japan

<sup>2</sup> National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki, Japan

\*Corresponding author, e-mail: ishizuka@hremresearch.com

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### Abstract

The resolution of HRTEM has been improved down to sub-angstrom by correcting the spherical aberration of the objective lens, and the information limit is thus determined mainly by partial temporal coherence. Thus, a method to measure the partial temporal coherence becomes important more than ever. We have compared the 3D FT analysis with the diffractogram analysis (2D FT analysis) to evaluate a high-performance TEM. The significant difference of the 3D FT analysis from the diffractogram analysis is the capability to extract linear image information from the image intensity, and further to separate two linear image contributions on the Ewald sphere. Therefore, we can use a thick sample or a sample made from strong scattering elements. Furthermore, we can apply the 3D FT analysis for the case of a small defocus spread that is expected for a Cc-corrected microscope or a microscope with a monochromator.

**Keywords** HRTEM, information limit, 3D FT analysis, diffractogram analysis

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## Quantitative Experimental Determination of Site-specific Magnetic Structures by Transmitted Electrons

Xiaoyan Zhong<sup>1\*</sup>, Ziqiang Wang<sup>1</sup>, Rong Yu<sup>1</sup>, Jing Zhu<sup>1</sup>

<sup>1</sup> National Center for Electron Microscopy in Beijing, Key Laboratory of Advanced Materials (MOE), State Key Laboratory of New Ceramics and Fine Processing, School of Materials Science and Engineering, Tsinghua University, Beijing, China.

\*Corresponding author, e-mail: xyzhong@mail.tsinghua.edu.cn

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### Abstract

Electron energy-loss magnetic chiral dichroism technique is invented as a new route for detecting magnetic signals in a transmission electron microscope with a high spatial resolution. Using this technique, we first experimentally demonstrate that the use of transmitted electrons allows us to quantitatively determine atomic site-specific magnetic structure information on a nanometre scale. From one NiFe<sub>2</sub>O<sub>4</sub> nanograin in composite films, we extract its atomic site-specific magnetic circular dichroism spectra and achieve the quantitative magnetic structure information by constructively selecting the specific dynamical diffraction conditions in the experiments. Our work opens a door to meet the challenge of exploring the magnetic structures of magnetic materials at the nanoscale using transmitted electrons.

**Keywords** EMCD; TEM; magnetic structure; site-specific; NiFe<sub>2</sub>O<sub>4</sub>

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## Electron Diffraction Analysis of Microcrystalline of Organic Molecules

Tetsuo Oikawa<sup>1\*</sup>, Anna Liew<sup>1</sup>, Yusuke Nishiyama<sup>2</sup>

<sup>1</sup> JEOL Asia Pte. Ltd., Corporation Place, 2 Corporation Road 618494, Singapore

<sup>2</sup> JEOL Resonance Inc., 1-2, Musashino 3-chome, Akishima, Tokyo 196-8558, Japan

RIKEN CLST-JEOL Collaboration Center, Tsurumi, Yokohama, Kanagawa 230-0045, Japan

\*Corresponding author, e-mail: [oikawa@jeolasia.com.sg](mailto:oikawa@jeolasia.com.sg)

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### Abstract

Transmission electron microscopy (TEM) imaging and electron diffraction (ED) were applied to small sized single crystal of *L-histidine.HCl.H<sub>2</sub>O* as one of the organic material. In order to reduce the electron irradiation damage, MDS (Minimum Dose System) was used in both imaging and ED observation. A critical dose of the *L-histidine* was measured as 10 - 20 e<sup>-</sup>/nm<sup>2</sup> at room temperature, and the ED was obtained at dose condition of 10 e<sup>-</sup>/nm<sup>2</sup>. The experimental diffraction pattern is in good agreement with simulated pattern.

**Keywords** TEM, electron diffraction, MDS, critical dose, L-histidine

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## Quantification of Crystallinity Using Energy Filtered Electron Diffraction

Byeong-Seon An<sup>1</sup>, Tae-Hoon Kim<sup>1</sup>, Cheol-Woong Yang<sup>1\*</sup>

<sup>1</sup>School of Advanced Material Science and Engineering, Sungkyunkwan University, Suwon, 16419, Korea

\*Corresponding author, e-mail: cwyang@skku.edu

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### Abstract

The amount of crystalline phase presented in the nanomaterials is an important parameter to control correlation between the properties and the microstructure, which occurred in the process. The distribution of crystalline phase in materials depends on the synthesis and switching process such as phase change materials<sup>1</sup>, flexible-OLEDs<sup>2</sup>, cellulose polymorphs<sup>3</sup> and glass-ceramics<sup>4</sup>. However, although crystallinity in nanomaterials play a key role in characteristics, these conventional quantification methods are hard to apply to current applications at a nanoscale level due to the spatial limitation of sampling<sup>5-7</sup>. Therefore, it is essential to develop a technique for the quantification of crystallinity at the nanoscale.

Thus, in this study, quantification technique using energy filtering of electron diffraction in transmission electron microscopy (TEM) is applied to quantitative analysis of standard sample deposited amorphous film on single-crystalline Si. The samples were investigated by energy filtered TEM to minimize inelastic scattering signal. Crystallinity was calculated by separation of crystalline and amorphous intensities from histogram of total intensity using azimuthal average of electron diffraction. To estimate reliable crystallinity quantification, accurate crystallinity was measured by taking the selected area aperture image, and the reliability of crystallinity quantification is investigated by comparing experimentally measured and calculated results.

**Keywords** energy filtering, transmission electron microscopy, crystallinity, electron diffraction

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## A Relativistic-energy Femtosecond-pulse Electron Microscopy

Jinfeng Yang<sup>1</sup>, Yoichi Yoshida<sup>1</sup>, Katsumi Tanimura<sup>2</sup>

<sup>1</sup> The Institute of Scientific and Industrial Research, Osaka University, Japan

<sup>2</sup> Research Center for Ultra-high Voltage Electron Microscopy, Osaka University, Japan

\*Corresponding author, e-mail: yang@sanken.osaka-u.ac.jp

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### Abstract

A relativistic-energy ultrafast electron microscopy using a radio-frequency (RF) femtosecond-pulse electron gun based has being developed at Osaka University. We succeeded to generate a 100-fs-pulse electron beam with energy of 3.1 MeV using the RF gun driven by a femtosecond laser. Using such electron pulses, we constructed the first prototype of relativistic-energy femtosecond-pulse electron microscopy. In the demonstrations of ultrafast electron diffraction measurement, we succeeded to observe excellent-quality electron diffraction patterns under the single-shot observation. In transmission electron microscopy (TEM) demonstrations, the observation of TEM imaging of polystyrene microparticles with the diameter of <1 micron was succeeded by averaging 10,000 electron pulses with the electron energy of 3.1 MeV. The relativistic-energy single-pulse electron imaging is also available under the low-magnification observation, i.e. 300 times. In the next step, we propose the improvement of the electron generation and injection system to furthermore reduce the beam emittance. We will focus the electron beam at the sample and increase the magnification of the imaging up to 10,000 times.

This work is supported by a Basic Research (A) (Grant No. 26246026) of Grant-in-Aid for Scientific Research from MEXT, Japan.

**Keywords** ultrafast electron microscopy, TEM, femtosecond electron pulse

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## MEMS Waveguide Sensor for Photoacoustic Detection

Supanee Larkthanakhachon<sup>1\*</sup>, Suejit Pechprasarn<sup>1,2</sup>, Mike Somekh<sup>1</sup>

<sup>1</sup>Department of Electrical and Electronics Engineering, Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

<sup>2</sup>Biomedical Engineering program, Department of Physics, Faculty of Science, Rangsit University, Pathum Thani, Thailand 12000

\*Corresponding author, e-mail: supanee.larkthanakhachon@polyu.edu.hk

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### Abstract

In this work, we present a numerical analysis of a Microelectromechanical system (MEMS) waveguide ultrasound detection sensor. The sensor made of 2 gold thin films, act as mirrors, are separated by 416.67 nm creating an optical waveguide. The top and bottom gold films have the thickness of 1  $\mu\text{m}$  and 40 nm, respectively. The top mirror is suspended in the air fixing on left and right side allowing the beam to deflect upon an acoustic pressure incident. The detection of the deflected mirror was performed optically at 633 nm wavelength laser incidents on the bottom mirror. As the air gap spacing between 2 mirrors change due to the pressure, the angle of reflectance minimum also change. We discuss the sensitivity and dynamic range of the system and compare with competing technologies. The configuration allows a sensitive measurement with the sensor size of 50 x 50  $\mu\text{m}^2$  which open up the possibility of arranging the sensor into arrays and performing novel ultrasound imaging technique.

**Keywords** photoacoustic, ultrasound, MEMS, waveguide, sensor

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## In situ TEM Study of Vapor-Solid Nanotube Growth

Zhengfei Zhang<sup>1</sup>, Yong Wang<sup>1\*</sup>, Hengbo Li<sup>1</sup>, Wentao Yuan<sup>1</sup>, Ze Zhang<sup>1</sup>.

<sup>1</sup> Center of Electron Microscopy and State Key Laboratory of Silicon Materials, School of Materials Science and Engineering, Zhejiang University, Hangzhou 310027, China

\*Corresponding author, e-mail: yongwang@zju.edu.cn

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### Abstract

The growth of metal oxide nanotubes has been widely investigated, however, the mechanism regarding how nanotube form remains elusive due to the lack of real time growth information. Here we report a direct observation of  $W_{18}O_{49}$  nanotube growth through thermally oxidizing a tungsten filament in an environmental TEM. We first reveal that the nanotube growth is realized via the sidewall epitaxy on the leader nanowire, which is different from the nanowires coalescence mechanism proposed previously. Furthermore, our in situ results demonstrate that higher oxygen pressure leads to the growth of nanotubes, but low oxygen pressure results in the growth of nanowires. Such nanotube growth is presumably ascribed to the maximization of heat dissipation during fast growth. These findings may enrich our present understanding of the growth dynamics of metal oxide nanotubes and provide insight for fabricating metal oxide nanotubes.

**Keywords** In situ TEM, metal oxide, nanotube growth, vapor-solid, sidewall epitaxy

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## ***In Situ* Electron Holography of Electric Potentials Inside a Solid-State Electrolyte: Effect of 3D Electric-Field Leakage**

Tsukasa Hirayama<sup>1\*</sup>, Yuka Aizawa<sup>1</sup>, Kazuo Yamamoto<sup>1</sup>, Takeshi Sato<sup>1</sup>, Hidekazu Murata<sup>2</sup>, Ryuji Yoshida<sup>1</sup>, Craig A. J. Fisher<sup>1</sup>, Takehisa Kato<sup>3</sup>, Yasutoshi Iriyama<sup>3</sup>

<sup>1</sup> Nanostructures Research Laboratory, Japan Fine Ceramics Center, 2-4-1 Mutsuno, Atsuta-ku, Nagoya, Japan.

<sup>2</sup> Faculty of Science and Technology, Meijo University, 1-501 Shiogamaguchi, Tempaku-ku, Nagoya, Japan

<sup>3</sup> Department of Materials, Physics and Energy Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan

\*Corresponding author, e-mail: t-hirayama@jfcc.or.jp

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### **Abstract**

*In situ* electron holography can be used to measure electric-potential distributions in all-solid-state lithium-ion batteries during the charge-discharge cycle [1,2]. When a voltage is applied to the electrodes of a transmission electron microscopy (TEM) specimen, electric fields generally spread three-dimensionally around the specimen. Since the phase shift of an electron wave is proportional to the integral of electric potential along the electron trajectory, the influence of the three-dimensional (3D) electric field on the reconstructed phase image needs to be considered in order to accurately measure the potential distributions inside the specimen. In this study, the phase distributions in the observation plane were numerically simulated taking into account the 3D electric potential distributions around a lithium phosphorus oxynitride (LiPON) electrolyte specimen [3]. The simulated profiles were compared with experimental profiles to obtain the precise potential distributions inside the electrolyte. Based on the precise potential distributions, a model of lithium-ion and lithium-vacancy distributions inside LiPON is proposed. Part of this work was supported by the RISING project (NEDO).

**Keywords** electron holography, 3D electric potential, solid electrolyte, electric-potential distribution

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## Improved Heating Stage for EBSD In-Situ Work

Seiichi SUZUKI<sup>1\*</sup>, Tatsuya FUKINO<sup>1</sup>

<sup>1</sup> TSL Solutions KK

\*Corresponding author, e-mail: info@tsl-japan.com

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### Abstract

Dynamic observation of grain growth and phase transformation by EBSD with In-situ heating stage is very helpful to understand the problems. We have developed a heating stage which can heat up the specimen over 1273K and reported at REX&GG meeting in 2013. But its reachable temperature was very much depending on the contact condition between the specimen and the heater. The improved specimen holding method with high temperature carbon past improved the contact. As a result, the maximum specimen temperature over 1273K was achieved stably. Re-crystallization and phase transformation process of rolled IF steel and recrystallization of heavily deformed SUS304 stainless steel were observed by EBSD using this heating stage.

We have also tested a new method to make back scattered electron (BSE) images by using EBSD pattern signal at higher temperature. BSE detector which generally uses Si diode doesn't work at higher temperature. But EBSD patterns can be acquired even over 1273K of the specimen temperature. And we confirmed BSE images can be acquired by using EBSD pattern at 1273K.

It is confirmed that In-situ heating experiment at higher with EBSD analysis will be a very helpful technique to understand re-crystallization and phase transformation. .

**Keywords:** EBSD, In-Situ heating, re-crystallization, phase transformation, BSE image

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## In-Situ TEM Study of Nano-Scale Conductive Filament Used For Threshold Selector Device

Byeong Gyu Chae<sup>1</sup>, Kyung Joon Baek<sup>1</sup>, Jeong Hwan Song<sup>1</sup>, Jae Bok Seol<sup>2</sup>, Hyun Sang Hwang<sup>1</sup>, Sang Ho Oh<sup>3</sup>, Chan Gyung Park<sup>1,2\*</sup>

<sup>1</sup> Department of Materials Science and Engineering, POSTECH, Pohang, Republic of Korea

<sup>2</sup> National Institute for Nanomaterials Technology, Pohang, Republic of Korea

<sup>3</sup> Department of Energy Science, Sungkyunkwan University, Suwon, Republic of Korea

\*Corresponding author, e-mail: cgpark@postech.ac.kr

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### Abstract

As one of the most promising candidates for future non-volatile memory, resistive random access memory (ReRAM) has received a great deal of attention. The switching mechanism is based on the abrupt change in the electrical resistance due to the formation and the rupture of the conductive filament in oxide layer. Recently, the threshold selector device with high performance has been investigated to suppress the sneak current problem, which can lead to a device failure. However, the understanding of the threshold switching mechanism has not been clear in the actual device scale.

In the present study, the microstructural evolution in the Ag/TiO<sub>x</sub>-based threshold selector device has been investigated by using in-situ electrical probing in TEM. This technique can directly exhibit both the evolution of the nano-scale conductive filaments and the corresponding I-V characteristics. The detailed morphology of the conductive filament was directly observed by using high-resolution TEM. Based on the observed morphology and the characteristics of the conductive filament, we suggested that the threshold switching characteristics was induced by the spontaneous rupture of the nano-scale conductive filament. In addition, it was also demonstrated that the growth of the large conductive filament during repetitive cycles could lead to the device degradation.

**Keywords** ReRAM, selector device, threshold switching, conductive filament, in-situ TEM

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## In-Situ TEM Study of Deformation Behavior in A Dual-Phase High-Entropy Alloy AlCoCrFeNi

Qiannan Wang<sup>1\*</sup>, Qian Yu<sup>1</sup>

<sup>1</sup> Center of Electron Microscopy and State Key Laboratory of Silicon Materials, School of Materials Science and Engineering, Zhejiang University, Hangzhou 310027, China

\*Corresponding author, e-mail: wangqn@zju.edu.cn

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### Abstract

Certain high-entropy alloys (HEAs), emerging as a new frontier for research, can have either high strength or high ductility. Single-phased fcc structured HEAs are ductile but not strong enough; while single-phased bcc structured HEAs, on the other hand, can be very strong but at the price of brittleness. Therefore, it is still difficult for a single-phased HEA to achieve a reasonable balance between strength and ductility. Recently, a new type of eutectic HEA (AlCoCrFeNi) was designed, possessing a fine lamellar fcc/bcc microstructure, which showed an unprecedented combination of high fracture strength and considerable tensile ductility at room temperature. By using in situ high-resolution transmission electron microscopy, here we show some unique deformation behaviors of this dual-phase HEAs, rarely achieved in single-phase HEAs, including the transformation from an ordered solid solution into a disordered solid solution in fcc-structured phase and the characteristic dislocation behaviors.

### Keywords

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## In-situ Nanoengineering Based on TEM

Tao Xu<sup>1</sup>, Hengchang Bi<sup>1,2</sup>, Shu Wan<sup>1,2</sup>, Jun Sun<sup>1</sup>, Longbing He<sup>1,3</sup>, Kuibo Yin<sup>1</sup>, Litao Sun<sup>1,2,3\*</sup>

<sup>1</sup>SEU-FEI Nano-Pico Center, Key Laboratory of MEMS of Ministry of Education, Collaborative Innovation Center for Micro/Nano Fabrication, Device and System, Southeast University, Nanjing 210096, P. R. China

<sup>2</sup>Joint Center for Advanced Carbon Materials of Southeast University and Jiangnan Graphene Research Institute, Changzhou 213100, P. R. China

<sup>3</sup>Center for Advanced Materials and Manufacture, Joint Research Institute of Southeast University and Monash University, Suzhou 215123, P. R. China

\*Corresponding author, e-mail: E-mail: slt@seu.edu.cn

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### Abstract

Based on the idea of "setting up a nanolab inside a TEM", we present our recent progress in nanoengineering including in situ growth, nanofabrication with atomic resolution, nanodevice construction and possible applications (e.g. a 5nm-diameter hole on graphene for third-generation gene sequencing, the spongy graphene as an ultra-efficient sorbent for oils and organic solvents, etc.). The electron beam can be used as a tool to induce nanofabrication on the atomic scale. Additional probes from a special-designed holder provide the possibility to further manipulate and measure the electric/mechanical properties of the nanostructures in the small specimen chamber of a TEM (the space between the pole pieces in the object lens of a commercial TEM is usually less than 5 mm). Recently, the optical signal also was introduced into the electron microscope to enrich the coverage of investigation inside the "multifunctional nanolab". All phenomena from the in-situ experiments can be recorded in real time with atomic resolution.

**Keywords** In situ Microscopy, Nanoengineering, TEM, Nanomaterials

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## Ultra-High Resolution SEM for 3D Analysis in Biology

Jaroslav Jiruse<sup>1\*</sup>, Miloslav Havelka<sup>1</sup>, Jan Polster<sup>1</sup>

<sup>1</sup> TESCAN Brno, s.r.o., Libusina trida 1, 62300 Brno, Czech Republic

\*Corresponding author, e-mail: jaroslav.jiruse@tescan.cz

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### Abstract

3D tomography is a popular and powerful technique that reliably provides crucial insights into biological tissues. The combination of processing using a focused ion beam and imaging using scanning electron microscopy is especially useful. Here, we describe a new scanning electron microscope column providing ultra-high resolution and enhanced analytical capabilities enabling long-time processing. It overcomes the problems associated with ion beam splitting and thermal instabilities and delivers a new detection system suitable for biological applications.

**Keywords:** SEM, FIB, energy-filtered BSE, 3D reconstruction, tomography

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## Study of Pseudo-Symmetric Misindexing in EBSD Analysis of $\gamma$ -TiAl Alloys with Refined Accuracy Band Detection

Wu Jiang<sup>1\*</sup>, Niels-Henrik Schmidt<sup>2</sup>, Alberto Palomares-García<sup>3</sup>, Rocío Muñoz-Moreno<sup>4</sup>, and Jenny Goulden<sup>2</sup>

<sup>1</sup> Oxford Instruments NanoAnalysis Singapore Office, 10 Ubi Crescent, Singapore

<sup>2</sup> Oxford Instruments NanoAnalysis, Halifax Road, High Wycombe, UK

<sup>3</sup> IMDEA Materials Institute, Eric Kandel Street, Madrid, Spain

<sup>4</sup> Department of Material Science and Metallurgy, University of Cambridge, Cambridge, UK

\*Corresponding author, e-mail: wu.jiang@oxinst.com

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### Abstract

Cubic pseudosymmetry is commonly observed when analyzing EBSD patterns of  $\gamma$ -TiAl alloys, which arises from its close tetragonal c/a ratio of about 1.018. Pseudosymmetry is a known limitation in EBSD and occurs in many phases where the Kikuchi patterns are extremely similar for different crystal orientations, such that indexing may not clearly identify the correct orientation. Very slight differences in inter-band angle can separate candidate solutions, and then accurate band detection is essential to identify the correct one among solutions. It has been confirmed that the band detection based on high-accuracy Hough transform settings improves band detection accuracy, and is useful in mitigating pseudosymmetric misindexing. However, these settings result in greater image transform time and reduce data acquisition speeds.

In this study, a new band detection method with refined accuracy is introduced to solve the classic pseudosymmetric issue. For  $\gamma$ -TiAl alloys, this method offers significant improvements of orientation accuracy, and can run at reasonable data acquisition speeds. It is sufficiently sensitive to resolve fine differences in inter-band angle to nearly eliminate the pseudosymmetric misindexing. The new method can further improve the phase discrimination, identifying more 5% tetragonal  $\gamma$ -TiAl phase than the conventional band detection.

**Keywords** EBSD, Pseudosymmetry, Indexing, TiAl alloy

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## Direct Observation of Low Angle Boundary Migration During Recrystallization Using Electron Channeling Contrast Imaging and Electron Backscatter Diffraction

Jin-Su Oh, Seung-Moon Baek, Tae-Hoon Kim, Jee-Hwan Bae, and Cheol-Woong Yang\*  
School of Advanced Material Science & Engineering, Sungkyunkwan University, Suwon 16419, Korea

\*Corresponding author, e-mail: cwyang@skku.edu

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### Abstract

Study on recrystallization behavior is important for practical industrial application because metals or alloys may lose its characteristics by recrystallization under external heat. It is known that recrystallization behavior of metal is influenced by misorientation across a boundary. In the present research, effect of low angle boundary between recrystallized grain and deformed matrix on boundary migration is directly observed using state-of-the-art scanning electron microscopy techniques which are comparable and, at the same time, complementary: electron channeling contrast imaging and electron backscatter diffraction.<sup>1</sup> Pure nickel deformed by shot peening was investigated due to the characteristics which induces plastic deformation gradient at surface.<sup>2</sup> Despite electron channeling contrast imaging allows of measuring dislocation density in a low level of deformation<sup>3</sup>, it is difficult to quantify degree of deformation induced by shot peening. Also, electron backscattering diffraction cannot give the exact value solely. However, combination of both techniques is able to correct value of average dislocation density so that stored energy near the boundary can be estimated quantitatively. Through this process, threshold stored energy for boundary migration during recrystallization is evaluated as function of misorientation angle.

**Keywords** Electron channeling contrast imaging, electron backscatter diffraction, low angle boundary, stored energy, boundary migration

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## Ultra-Sensitive Biosensor Using Double-Metallic-Layer-Waveguide Structure

Mengqi Shen<sup>1,2\*</sup>, Jingkai Meng<sup>2</sup>, Supanee Larkthanakhachon<sup>2</sup>, Suejit Pechprasarn<sup>2,3</sup>, Michael G. Somekh<sup>2</sup>, Yaping Zhang<sup>1</sup>, Chung W. See<sup>4</sup>.

<sup>1</sup> International Doctoral Innovation Centre, The University of Nottingham Ningbo China, 199 Taikang East Road, Ningbo, China.

<sup>2</sup> Department of Electronic and Information Engineering, the Hong Kong Polytechnic University, Hong Kong SAR, China.

<sup>3</sup> Biomedical Engineering program, Department of Physics, Faculty of Science, Rangsit University, Pathum Thani, 12000, Thailand.

<sup>4</sup> School of Electrical and Electronic Engineering, The University of Nottingham, University Park, Nottingham, NG7 2RD, UK.

\*Corresponding author, e-mail: zx15211@nottingham.edu.cn

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### Abstract

Surface plasmon resonance (SPR) has been developed as a leading technology in bio-sensing because of its high sensitivity to the change at the surface and its characteristics of label-free and real-time detection when observing bio-molecular interactions[1]. In traditional confocal SPR measurement[2], high numerical aperture (NA) is required which places a heavy burden on the system alignment and the processing of the system output. The aim of this study is to obtain better sensitivity in a low NA system. Our design is a new waveguide structure which combined Kretschmann configuration[3] and Otto configuration[4]. By carefully control the gap-distance (e.g. 200nm gap-distance) between metal layers, the conventional SPR mode is split into symmetric mode and anti-symmetric modes[5]. Its dispersion relation is further decided by the gap between metal layers, which not only makes the required NA for SPR excitation smaller, but also gives a better sensitivity. Besides, waveguide modes also exist due to the ‘sandwiched’ structure and it has been proved better bulk sensitivity can be achieved as we demonstrate that 10 times compared to conventional SPR. Thus in this novel waveguide structure, both waveguide modes and SPR modes can be employed in bio-sensing with promising performance.

**Keywords:** Surface plasmon resonance; symmetric mode; anti-symmetric mode; waveguide; sensitivity.

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## Embedded Interferometry with Dynamic Reference Beam

Wai-Kin Chow<sup>1\*</sup>, Suejit Pechprasarn<sup>1,2</sup>, Michael G. Somekh<sup>1</sup>

<sup>1</sup> Department of Electronic and Information Engineering, The Hong Kong Polytechnic University, Hong Kong

<sup>2</sup> Biomedical Engineering program, Department of Physics, Faculty of Science, Rangsit University, Pathum Thani, Thailand 12000

\*Corresponding author, e-mail: terry.waikin@connect.polyu.hk

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### Abstract

We have previously demonstrated how a defocused confocal microscope can operate as an embedded interferometer to measure the k-vector of the surface plasmon on a localized scale. This is essentially an interference between surface plasmon signal and reference signal from normal incidence as a function of defocus  $z$  so called  $V(z)$ . Since the system is a defocused system, the signal amplitude decays very quickly as it goes out of focus and the inference signal amplitude becomes very small at far defocus distance. In this talk, we discuss some recent improvements in this system demonstrating that the k-vector of surface plasmon can be recovered in low light input power environment. This can be achieved by phase modulation on the reference beam to delay the amplitude decay. Here we discuss two methods to enhance the reference signal amplitude by (1) providing Axicon reference beam illumination and (2) making the reference beam stationary at pinhole. As a result, the interference signal can be greatly enhanced by a stronger reference beam. We also show that the methods described here are more reliable and robust than surface plasmon dip position measurement and improve the signal to noise ratio of the recovered k-vector.

**Keywords** surface plasmon, microscope and interferometry

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## Improvement of Mass Resolution in Atom Probe Tomography for Oxide Materials with Surface Modification

Chang Min Kwak<sup>1</sup>, Jae Bok Seol<sup>2</sup>, Chan Gyung Park<sup>1,2\*</sup>

<sup>1</sup> Department of Materials Science and Engineering, POSTECH, Pohang, Republic of Korea

<sup>2</sup> National Institute for Nanomaterials Technology, Pohang, Republic of Korea

\*Corresponding author, e-mail: [cgpark@postech.ac.kr](mailto:cgpark@postech.ac.kr)

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### Abstract

Understanding the field evaporation mechanism in non-conductive materials is essential in understanding the mass resolution in laser-pulsed atom probe tomography (APT). We have studied the influence of metallic capping layers, such as Co, Ni and Ag, for MgO tips on the mass resolving power in APT. Among the metallic capping layers, we found that the Ag capping layer could lead to a profound improvement in mass resolving power due to the homogeneous heat distribution at the tip surface between the laser side and the other side. It was revealed that the Ag capping with high thermal diffusivity could promote the compositional uniformity as well as the reduced fraction of multiple events for oxygen ions. Thus, we have demonstrated that sample preparation using metallic capping onto APT tips is very much useful in improving mass resolving power, reducing both multiple events and surface radius anisotropy. In addition, we proposed the systematic experimental sequences for the correlative analysis using atom probe tomography and transmission electron microscopy in order to directly observe the change of the field evaporation sequences occurring at tip surface.

**Keywords** Atom probe tomography, Transmission electron microscopy, Metallic capping

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## Quality Enhancement and Strain Measurement in HAADF Images Using Super-Resolution Techniques

G. Bárcena-González<sup>1</sup>, M. P. Guerrero-Lebrero<sup>1</sup>, E. Guerrero<sup>1</sup>, D. F. Reyes<sup>2</sup>, D. González<sup>2</sup>, A. Mayoral<sup>3</sup>, A. D. Utrilla<sup>4</sup>, J. M. Ulloa<sup>4</sup>, P. L. Galindo<sup>1</sup>

<sup>1</sup>Department of Computer Science and Engineering, Faculty of Engineering, University of Cádiz, Avenida de la Universidad 10, Puerto Real, Spain

<sup>2</sup>Department of Material Science and Metallurgy Engineering and Inorganic Chemistry, Faculty of Sciences, University of Cádiz, Avenida República Saharaui, Puerto Real, Spain

<sup>3</sup>Institute of Nanoscience of Aragon and Laboratory of Advanced Microscopies, University of Zaragoza, C/ Mariano Esquillor, Zaragoza, Spain

<sup>4</sup>Institute for Systems based on Optoelectronics and Microtechnology, Polytechnic University of Madrid, Ciudad Universitaria, Madrid, Spain

\*Corresponding author, e-mail: pedro.galindo@uca.es

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### Abstract

The Super-Resolution technique allows the reconstruction of a high resolution image by combining several low-resolution images from the same sample. In this work, we consider an experimental High Resolution High Angle Annular Dark Field (HRHAADF) image with an exposition time of 8 seconds and a Super-Resolution image generated from a series of 8 frames taken every second of an InAs/GaAs quantum dot. The images have been aligned using Vandewalle's algorithm and normalized cross-correlation. For the reconstruction, the Non-Local Means denoising algorithm has been adapted for Super-Resolution purposes.

The Super-Resolution reconstructed image shows higher Signal-Noise Ratio values and a better atomic column roundness. Strain analyses for both images have been calculated and compared using Geometrical Phase Analysis and Peak Pairs Analysis. In order to compare the accuracy, we have analyzed an unstrained area in the substrate where the theoretical strain value is expected to be zero. Although mean strain values are closely around zero, its standard deviation is significantly reduced using the Super-Resolution image. This precision enhancement can be considered a key factor in the strain mapping in high resolution electron microscopy images and will permit to make more accurate measurements in the analysis of materials.

**Keywords** Super-Resolution, high-angle annular dark field, signal-to-noise ratio, roundness, strain analysis

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## Digital Transformation in Microscopy Teaching

Silvia Zenner-Gellrich<sup>1</sup>, Peter Kraemer<sup>1</sup>

<sup>1</sup> Carl Zeiss Microscopy GmbH, Königsallee 9-21, 37085 Göttingen, Germany

\*Corresponding author, e-mail: [silvia.zenner-gellrich@zeiss.com](mailto:silvia.zenner-gellrich@zeiss.com)

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### Abstract

In education and teaching, digital transformation is found in the relevant tools: From blackboards and exercise books to smart boards, tablets and apps. Digital technologies has changed the way of communication and the way of learning and processing information too. Challenge for nowadays teachers is to understand and address language and style of their students.<sup>1</sup>

Microscopy training courses are an essential part in the curricula of Life- and Earth-Sciences. Apps have the potential to revolutionize student training courses in microscopy teaching too.<sup>2</sup> To monitor the learning success in a student lab, the teacher is forced to view into each and every microscope. Because this is time consuming, only limited time stays for focusing on the lessons content. Digital classrooms, as wifi-enabled networks, support teachers in spending more time for content. Monitoring becomes efficient by seeing into all microscopes at one glance, with ZEISS Imaging app Labscope. By having all microscope images in view, sharing selected images e.g. via beamer opens new possibilities for an interactive training course. Now students can explain their images to the whole group. In addition, the app shows students who need individual support, so that the training course benefits from a personalized learning atmosphere.

**Keywords:** digital transformation, digital classroom, ZEISS Imaging App Labscope

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## Effects of Nickel Oxide Underlayer on the Catalytic Properties of Manganese Oxide Nanoparticles

Sangmoon Yoon<sup>1</sup>, Kyoungsuk Jin<sup>1</sup>, Won Woo Chung<sup>1</sup>, Ki Tae Nam<sup>1</sup> and Miyoung Kim<sup>1\*</sup>

<sup>1</sup> Department of Materials Science and Engineering, Seoul National University, Seoul 151-744, Korea

\*Corresponding author, e-mail: mkim@snu.ac.kr

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### Abstract

Developing an efficient electro-catalyst for oxygen evolution reaction (OER) has drawn strong attention in the field of chemistry and materials science because it is the major bottleneck in a hydrogen economy through water splitting. As a result, various attempts have been made to reach beyond the volcano limitations that the OER catalysts have<sup>1</sup>. We newly developed a manganese oxide/nickel oxide heterostructure catalyst to enable for OER to occur at a much lower overpotential than other manganese oxide catalysts. (490mV of overpotential @5mA/cm<sup>2</sup> under neutral condition and 350mV of overpotential @10mA/cm<sup>2</sup> under basic condition) Moreover, this catalyst showed great stability such that nearly constant current was observed during the 40,000 sec of electrolysis. These experimental results present that synergetic effects are formed by constructing the heterostructure. Here, we investigated the electronic structure of the manganese oxide/nickel oxide catalyst using scanning transmission electron microscopy and electron energy loss spectroscopy to understand the roles of the nickel oxide underlayer. We found that nickel ions are diffused over the manganese oxide nanoparticles and hybridized with the surface manganese atoms. Also, the nickel ions on the surface of the nanoparticles affect the stoichiometry of manganese oxides at the surface, which may be closely related to the enhancement of catalytic reactions. .

**Keywords** Oxide nanoparticles, Oxygen evolution reaction, Electron energy loss spectroscopy

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## **Making the Practically Impossible ‘Merely Difficult’ – Cryogenic FIB Lift-Out for ‘Damage Free’ Soft Matter Imaging**

Christopher Parmenter<sup>\*1</sup>, Michael Fay<sup>1</sup>, Cheryl Hartfield<sup>2</sup>, Hoda Eltaher<sup>3</sup>

<sup>1</sup> Nottingham Nanoscale and Microscale Research Centre, University of Nottingham, UK

<sup>2</sup> Oxford Instruments Nanoanalysis, Omniprobe Products, Concord USA

<sup>3</sup> Wolfson Centre for Stem Cells, Tissue Engineering and Modelling (STEM), School of Pharmacy, University of Nottingham, UK.

\*Corresponding author, e-mail: Christopher.Parmenter@nottingham.ac.uk

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### **Abstract**

The preparation of thinned lamellae from bulk samples for Transmission Electron Microscopy (TEM) analysis has been possible in the Focused Ion Beam Scanning Electron Microscope (FIB-SEM) for over 20 years via the in situ lift-out method. Lift-out offers a fast and site specific preparation method for TEM analysis, typically in the field of materials science. This work presents the successful lift-out of high-water content lamellae, under cryogenic conditions (cryo-FIB lift-out). Strategies are explored for maintaining cryogenic conditions, grid attachment using cryo-condensation of water and protection of the lamella when transferring to the TEM.

**Keywords** Cryo-FIB, Cryo-Lift-out, Cryo-TEM preparation, soft-matter, biological-EM

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## Actin-Bundling Formation of Dimeric Human Fascin-1 was Determined by IHRSR and Tomography Methods

Jeong Min Chung, Sangmin Lee and Jung, Hyun Suk\*.

Department of Biochemistry, College of Natural Sciences, Kangwon National University, 1, Kangwondeahak-gil, Chuncheon-si, Gangwon-do, 200-701, Korea.

\*Corresponding author, e-mail: [hsjung@kangwon.ac.kr](mailto:hsjung@kangwon.ac.kr)

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### Abstract

Fascin-1, a major actin cross-linking protein, is expressed in most vertebrate tissues. It organizes actin filaments into well-ordered bundles that are responsible for the extension of dynamic membrane protrusions like microspikes, filopodia and invadopodia from cell surfaces and for the functionality of these protrusions in cell migration. Therefore, studying their regulatory mechanism at molecular level is important to understand cell motility. Recent studies showed that fascin-1 contains two actin-binding sites, actin binding site 1 and actin binding site 2, thereby facilitating efficient bundling between adjacent actin filaments. However, it is not clear how fascin-1 induces actin bundling and the location of fascin-1 along the actin filaments. Here we have investigated this question structurally, by determining the dimeric structure of fascin-1 and the complex structure of actin/fascin-1. Biochemical experiments and electron microscopic analyses of fascin-1 shows that N-terminal region containing the actin binding site 1 and actin binding site 2 is responsible for bundling actin filaments and the C-terminal region is important for forming of dimeric structure of fascin-1. Three dimensional structures of actin/fascin-1 complex was obtained by using electron microscopy and cryo-electron microscopy and determined by Iterative helical real space reconstruction (IHRSR) and tomography method. In conclusion, dimer formation of fascin-1 through intermolecular interactions of C-terminal region is essential for actin filaments bundling and these findings provide insightful information into actin-bundles formation by actin-bundling proteins.

**Keywords** Fascin-1, Actin-bundling protein, Cryo-Electron microscopy, Iterative helical real space reconstruction.

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## Histological Study of Larval Development of the Blow Fly, *Chrysomya megacephala* (Diptera: Calliphoridae)

Chutharat Samerjai<sup>1\*</sup>, Sangob Sanit<sup>1</sup>, Kwankamol Limsopatham<sup>1</sup>, Suttida Suwannayod<sup>1</sup>,  
Kom Sukontason<sup>1</sup>, Tarinee Chaiwong<sup>2</sup>, Ratana Leksomboon<sup>2</sup> and Kabkaew L. Sukontason<sup>1</sup>

<sup>1</sup> Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

<sup>2</sup> College of Medicine and Public Health, Ubon Ratchathani University, 85 Sathollmark Rd., Warinchamrap, Ubon Ratchathani 34190, Thailand

\*Corresponding author, e-mail: chutharat.smj@gmail.com

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### Abstract

*Chrysomya megacephala* is a forensically important blow fly species, and its larvae are associated commonly with human corpses. In order to determine the postmortem interval (PMI) in forensic investigation, the age of the oldest maggot collected from the corpses must be assessed. Therefore, this study aimed to investigate the morphological age estimation of *C. megacephala* larvae. The larval morphological and histological changes were observed every 6 hours from newly hatched larvae until the third instar by using the histological method, hematoxylin and eosin staining. The results displayed longitudinal section of larval organs. The newly hatched larvae demonstrated a small mouth part and dense proliferated cells of the hypodermis and alimentary canal cells. Then, the anterior end of 6 and 12 hours-old larvae showed dorsal organs and a prominent pharynx and pharyngeal muscle which were observed easily and enlarged during larval development. After that the skeleton muscle was observed clearly in the larvae from when they were 18 hours-old and it continued to enlarge until they were 60 hours-old. Moreover, the fat cells were spherical or elliptical shape and scattered around the body cavity, and had highly vacuolated cells in the late instar larva. Therefore, this information would be useful for roughly estimate of the age of the larvae, and thereby, could be applied in estimating the PMI of a corpse.

**Keywords** *Chrysomya megacephala*, larval development, histology, forensic entomology

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## Ultrastructure of the Immature Blow Fly, *Lucillia sinensis* Aubertin, 1933 (Diptera: Calliphoridae)

Sangob Sanit<sup>1\*</sup>, Chutharat Samerjai<sup>1</sup>, Narin Sontigun<sup>1</sup>, Tunwadee Klong-klaew<sup>1</sup>, Kwankamol Limsopatham<sup>1</sup>, Hiromu Kurahashi<sup>2</sup>, Kom Sukontason<sup>1</sup> and Kabkaew L. Sukontason<sup>1</sup>

<sup>1</sup> Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

<sup>2</sup> Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan.

\*Corresponding author, e-mail: [sang.ngeab@gmail.com](mailto:sang.ngeab@gmail.com)

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### Abstract

Blow flies of the genus *Lucilia* are medically and forensically important worldwide. In Thailand, four species of *Lucilia* are listed, i.e., *L. cuprina*, *L. porphyrina*, *L. papuensis* and *L. sinensis*, of which information on *L. sinensis* is limited to its morphology. Therefore, the aim of this study was to describe the ultrastructure of its immature stages (egg, larva and puparia) by using a light microscope (LM) and Scanning Electron Microscope (SEM). The egg of *L. sinensis* is similar morphologically to those of other *Lucilia* species in having a relatively wide and upright median area. Of all larval stages, the obvious characteristic of *L. sinensis* is the caudal segment, which differs from that of other *Lucilia* species. It has three prominent pairs of dorsal tubercles (inner, median and outer), of which the outer dorsal tubercles are the longest. The anterior spiracle of the second and third instars bear 8-10 papillae that are arranged in a single row. The intersegmental spines between the prothorax and mesothorax of the third instar show an arrangement of spines in continuous individual rows, with each spine having a single sharp tip. Each posterior spiracular disc displays a complete peritreme encircling three straight spiracular slits, and a prominent button. The puparium is coarctate and displays prominent outer dorsal tubercles. The bubble membrane in young puparia is positioned dorsolaterally on each side of the first abdominal segment, and comprises ≈58 mammillate structures. Such information provides more detailed and important morphological features that can distinguish *L. sinensis* accurately from other *Lucilia* species.

**Keywords** *Lucillia sinensis*, Blow fly, larvae, maggot, pupa

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## The Combination of Atorvastatin and Cyanidin-3-Glucoside Attenuated Angiotensin II Induced Oxidative Stress in Vascular Smooth Muscle Cells

Rungsa Pantan<sup>1</sup>, Jiraporn Tocharus<sup>2</sup>, Manussabhorn Phassara<sup>1</sup>, Apichart Suksamran<sup>3</sup>, Chainarong Tocharus<sup>1,\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>2</sup>Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>3</sup>Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

\*Corresponding author, e-mail: chainarongt@hotmail.com

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### Abstract

Reactive oxygen species play the crucial role in various signaling pathways that underline vascular inflammation and pathogenesis of atherosclerosis. Although statins are commonly used for moderate atherosclerosis treatment, many studies have reported their adverse effects. In this study the cyanidin-3-glucoside, a known strong anti-oxidant from fruits has been used in combination with statins to lowering the concentration of statins used in atherosclerosis. Human aortic smooth muscle cells (HASMCs) were induced by angiotensin II (Ang II), a potent activator of NADPH-oxidase and reactive oxygen species enhancer. Pretreatment cells with atorvastatin and cyanidin-3-glucoside showed the synergistic effect in the diminishing of reactive oxygen species which was mediated by the suppression of NOX-1. Furthermore, the combination of atorvastatin and cyanidin-3-glucoside inhibited NF-κB signaling. This present study suggests that the combination of atorvastatin and cyanidin-3-glucoside has a potential effect against Ang II induced oxidative stress which related to anti-inflammation effect in atherosclerosis treatment.

**Keywords:** Cyanidin-3-glucoside, Atorvastatin, Atherosclerosis, Vascular smooth muscle cell

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## **Study of the Interaction of *Macrobrachium rosenbergii* Nodavirus Virus-Like Particles (MrNV-VLPs) in Sf9 Insect Cells: the Hijacking of Caveolin-Mediated Endocytosis and the Possible Existence of a Protruding VLP Capsid Ligand Domain**

Monsicha Somrit<sup>1</sup>, Atthaboon Watthammawut<sup>1,3</sup>, Charoonroj Chotwiwatthanakun<sup>2</sup>, Puey Ounjai<sup>4,5</sup>, Wattana Weerachatanukul<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Mahidol University, 272 Rama VI Rd., Bangkok, Thailand

<sup>2</sup> Nakhonsawan campus, Mahidol University, Nakhonsawan, Thailand

<sup>3</sup> Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Bangkok, Thailand

<sup>4</sup> Department of Biology, Faculty of Science, Mahidol University, 272 Rama VI Rd., Bangkok, Thailand

<sup>5</sup> Center of Excellence on Environmental Health and Toxicology, Office of Higher Education Commission, Ministry of Education, Bangkok, Thailand. 10400

\*Corresponding author, e-mail: wattana.wee@mahidol.ac.th

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### **Abstract**

*Macrobrachium rosenbergii* nodavirus (MrNV) is the etiological agent of white tail disease (WTD) in the giant freshwater prawn *M. rosenbergii*. In our past studies, we generated *Macrobrachium rosenbergii* nodavirus virus-like particles (MrNV-VLPs) that were capable of being “nanocontainers” through the uptake of foreign plasmids after viral capsid assembly. Nevertheless, the studies into the binding and internalization of this particular virus into susceptible host cells have been lacking. To this end, we have shown for the first time that Sf9 cells are susceptible to MrNV infections, and serve as cells that can propagate and amplify this virus for further studies. Furthermore, our team has demonstrated through correlative microscopic techniques such as confocal, electron microscopy and flow cytometry the capability of using MrNV-VLPs as a tool to examine viral entry mechanisms in insect cells. Moreover, homology-based capsid protein subunit modeling and icosahedral constructions demonstrated the existence of protruding C-terminal domain on the outer surface of capsid. Enzyme digestion of C-terminal peptides and recombinant modifications were performed for revealing the existence, function and role of viral protrusions/ligands in Sf9 cell binding. Through the use of inhibitors/agonists and fluorescent markers, viral entry was observed to utilize primarily a caveolin-dependent mechanism.

**Keywords** *Macrobrachium rosenbergii*, nodavirus, Sf9, C-terminal domain

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## Active Targeted Delivery of PLGA-Liposome Nanoparticles Specific for Cervical Cancer Therapy

Suphawadee Bunthot<sup>1</sup>, Somsak Saesoo<sup>1</sup>, Katawut Namdee<sup>1</sup>, Teerapong Yata<sup>1</sup>, Suwatchai Jarussophon<sup>1</sup>, Pawinee Pongwan<sup>1</sup>, Uracha Raktanonchai<sup>1</sup>, Kunat Suktham<sup>1</sup>, Suvimol Surassmo<sup>1</sup>, Nattika Sangkrit<sup>1\*</sup>

<sup>1</sup> National nanotechnology Center (NANOTEC), National Science and Technology Development Agent, Thailand Science Park, Pathumthani 12120, Thailand

\*Corresponding author, e-mail: nattika@nanotec.or.th

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### Abstract

At the present, targeted drug delivery systems have become one of the most distinguished aspects in the cancer therapeutics. The development of a novel and effective targeted nanocarriers is still a major challenge in the research of nanomedicine and in pharmaceutical industry. In this study, novel PLGA-liposome nanocarrier (PLGA-Lip) was prepared. The PLGA was a core and phospholipid bilayer was a coated membrane facilitating cellular internalization. The particle size diameter was  $320 \pm 20$  nm with a zeta potential of  $-4.83 \pm 0.8$  mV. The PLGA-Lip were conjugated with cancer targeted monoclonal antibody (bevacizumab) generating an active targeted PLGA-Lip. Therefore, this designed nanoparticle is able to specifically bind to the VEGF protein resulting in angiogenesis inhibition of tumor. The binding efficiency of PLGA-Lip was performed by using parallel plate flow chamber as mimicking blood circulatory system. The targeted PLGA-Lip presented four times higher binding than non-targeted in cervical cancer cells. In addition, confocal imaging technique was employed to observe the cellular internalization. The result confirmed that targeted PLGA-Lip provided a higher ability in tumor cell entry more than the non-targeted one. Finally, the *in vivo* study was demonstrated under *in vivo* imaging system to verify the specific delivery effect. The VEGF expression cell line, SiHa, was injected subcutaneously in a nude mice, producing the mouse model xenograft of cervical cancer. Fluorescence-labeled active targeted and non-targeted PLGA-Lip were administrated via tail vein injection. Imaging fluorescence indicated that the active targeted nanoparticle showed higher fluorescence intensity. This result confirmed that active-targeted nanocarriers were specific localization around cervical cancer. In conclusion, this study proposed the novel active targeted drug carrier for a great potency in cancer diagnostic and therapeutic.

**Keywords** PLGA, liposome, active targeted-nanoparticles, bevacizumab, *in vivo* imaging

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## **The Airyscan Detector from Zeiss: Confocal Imaging with Improved Signal-To-Noise Ratio, Faster Speed and Super-Resolution**

Xianke SHI<sup>1</sup>

<sup>1</sup>Regional Application Specialist, APAC, Carl Zeiss Microscopy

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### **Abstract**

With Airyscan, ZEISS introduced a new detector concept for confocal laser-scanning microscopy (LSM). Whereas traditional LSM designs use a combination of pinhole and single-point detectors, Airyscan is a 32-channel gallium arsenide phosphide photomultiplier tube (GaAsP-PMT) area detector that collects a pinhole-plane image at every scan position. Each detector element functions as a single, very small pinhole. Knowledge about the beam path and the spatial distribution of each detector channel enables very light-efficient imaging with improved resolution, speed and signal-to-noise ratio.

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## Nanoscale Approaches to Characterize the Dynamic and Biophysical Determinants of Receptor-Mediated Virus Internalization

Megan Stanifer<sup>1</sup>, Marta Fratini<sup>1,2</sup>, Tina Wiegand<sup>2</sup>, Ada Cavalcanti-Adam<sup>2</sup>, Steeve Boulant<sup>1\*</sup>

<sup>1</sup> Department of Infectious Disease, Schaller research group at CellNetworks and DKFZ, University of Heidelberg, Germany.

<sup>2</sup> Institute of Physical Chemistry, University of Heidelberg, Germany.

\*Corresponding author

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### Abstract

Viruses are strict intracellular parasites; they need to hijack the host endocytic machinery to gain access to the intracellular environment in order to replicate. Although a lot of information is available on the mechanisms regulating endocytosis, very little is known concerning the general principles by which viruses induce their uptake by the target cell. Additionally, since viruses strongly interact with the extracellular environment, cells have to generate sufficient forces to disengage this interaction in order to uptake the viral particles. The biophysical determinants regulating this process remain unknown.

This project aims at developing novel surface chemistry and FRET-based force sensor tension microscopy approaches to study early steps of virus-induced endocytosis and to measure the force generated by a cell during uptake of a single virus particle.

We use live-cell fluorescent microscopy to monitor virus uptake. We observed that when “free” viruses are provided to cells, the clathrin dependent endocytic machinery is efficiently recruited to single viral particles and mediates their internalization. On the contrary, after immobilization on surfaces using “click-chemistry”, virus internalization failed. The clathrin machinery was recurrently recruited to the same particles demonstrating that viruses actively signal to promote their internalization. Additionally, by coupling viruses to molecular FRET-based force sensor, we demonstrated that cells were able to generate forces >100pN per virus particles.

Our novel surface-chemistry/tension microscopy approaches represent a new powerful methodology to dissect virus/receptor mediated signaling, early stages of virus entry, and to characterize the biophysical determinants that lead to virus uptake.

**Keywords** Virus entry, force sensor tension microscopy, endocytosis

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## Multimodal Approach to Improving *Macrobrachium rosenbergii* Aquaculture through In-Depth Studies into MrNV Infection and Sperm-Egg Interaction Processes

Atthaboon Watthammawut<sup>1,3</sup>, Monsicha Somrit<sup>1</sup>, Somluk Asuvapongpatana<sup>1</sup>, Charoonroj Chotwiwatthanakun<sup>2</sup>, Wattana Weerachayanukul<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Mahidol University, 272 Rama VI Rd., Bangkok, Thailand

<sup>2</sup> Nakhonsawan campus, Mahidol University, Nakhonsawan, Thailand

<sup>3</sup> Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Bangkok, Thailand

\*Corresponding author, e-mail: wattana.wee@mahidol.ac.th

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### Abstract

The giant freshwater prawn *Macrobrachium rosenbergii* is an important economic species; but aquacultural yields has been greatly affected by diseases such as white-tail disease (WTD) caused by *Macrobrachium rosenbergii* nodavirus (MrNV), and by the loss of quality broodstock/larvae. Our group focused on host-pathogen interactions of MrNV by examining the binding/internalization processes of both live MrNV and recombinantly-generated MrNV virus-like particles (MrNV-VLPs) in virally-susceptible Sf9 insect cell cultures. Correlative techniques including fluorescent/electron microscopy, PCR, biochemical inhibition and structural modelling revealed that the MrNV/VLPs required C-terminal domain for Sf9 binding/internalization, while depending on the caveolin-mediated endocytotic pathway for infection. Furthermore, our group focused on producing pathogenic-free, genetically-advantaged offspring through the development of techniques towards successful *in vitro* fertilization (IVF). Successful induction and high-fidelity gauging of increased sperm activity have enabled augmented ability of sperm to penetrate/fertilize spawned eggs in *in vitro* settings. Along with findings of the crucial role of carbohydrate moieties in the sperm/egg interaction, we have been able to better understand the intricacies of the fertilization process. In all, our group's studies have set a firm foundation for the development of highly-specific antiviral agents for MrNV infection, and for the advancement for successful IVF in this valuable species of prawn.

**Keywords** *Macrobrachium rosenbergii*, nodavirus, Sf9, sperm induction, carbohydrates

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## Methamphetamine Impairs the P-gp Transporter in Primary Rat Brain Microvascular Endothelial Cells

Pichaya Jumnonprakon<sup>1</sup>, Piyarat Govitrapong<sup>2</sup>, Chainarong Tocharus<sup>1</sup>, Jiraporn Tocharus<sup>3,\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>2</sup> Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Bangkok, Thailand

<sup>3</sup> Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

\*Corresponding author, e-mail: [jtocharus@gmail.com](mailto:jtocharus@gmail.com)

### Abstract

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Methamphetamine (METH) is an addictive drug and has been reported that caused blood-brain barrier (BBB) impairment which is the primary event of neurodegeneration. Previous studies found that METH-impaired BBB by mediated the oxidative stress, apoptosis, and paracellular permeability with the METH accumulation in brain endothelium. However, the exact mechanisms of BBB impairment are still unclear. Therefore, we hypothesized that METH impairs the P-gp transporter, an important structure of endothelial efflux, that caused BBB impairment by mediated the negative responses in brain endothelium. Our results showed that METH directly impairs the P-gp transporter by decreased the efflux of Rho123, P-gp ATPase activity, P-gp protein expression, and increased Rho123 accumulation. Moreover, the impairment of P-gp with METH treatment was closely associated with the increasing of reactive oxygen species (ROS), apoptotic cells, paracellular permeability and the reduction of transendothelial electric resistance (TEER). Thus, our data indicated that the P-gp transporter was the important target in METH administration prior mediated the BBB impairment by inducing the negative responses.

**Keywords** Blood-brain barrier, Cell stress, Methamphetamine, P-gp transporter, Tight junction

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## Characterization of Olfactory Ionotropic Glutamate Receptors 25a in the Giant Freshwater Prawn, *Macrobrachium rosenbergii*

Natwalee Jaikunta<sup>1,2</sup>, Thanapong Kruangkum<sup>1,2</sup>, Charoonroj Chotwiwatthanakun<sup>2,3</sup>, Prasert Sobhon<sup>1</sup>, Rapeepun Vanichviriyakit<sup>1,2\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

<sup>2</sup>Center of Excellence for Shrimp Molecular Biology and Biotechnology (CENTEX Shrimp), Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

<sup>3</sup>Mahidol University, Nakhonsawan campus, Nakhonsawan 60130, Thailand

\*Corresponding author, e-mail: rapeepun.van@mahidol.ac.th

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### Abstract

Pheromones are chemical complexes that play role in communication and sex or mating interactions in animals. Previously, our group has reported that the short lateral antennules (slAn) of the male giant freshwater prawn, *Macrobrachium rosenbergii* possess odor receptor setae playing role in sex pheromone perception (Kruangkum et al., 2013). Moreover, the transcriptome analysis of slAn reveals partial sequences of ionotropic glutamate receptors (iGluRs) gene family which reported as olfactory ionotropic receptors (IRs) in other arthropods. Thus, this study aims to characterize the full cDNA sequences of IR25a and other variant IR genes expressing in the slAn of *M. rosenbergii*. We also performed *in situ* hybridization to localize the gene expression in the olfactory receptor neurons of the slAn.

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## Role of Fluoride Ingestion in the Pathogenesis of Alzheimer's Disease

Raghu Jeti<sup>1\*</sup>, Raghuveer CV<sup>2</sup>, Mallikarjuana Rao C<sup>3</sup>

<sup>1</sup> Department of Anatomy, Melaka Manipal Medical College, Manipal University, Manipal, India

<sup>2</sup> Department of Pathology, Yenepoya Medical College, Yenepoya University, Mangalore, India

<sup>3</sup> Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India

\*Corresponding author, e-mail: raghujetti@yahoo.co.in

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### Abstract

Fluoride is present in water, toothpaste, mouth washes and other dental products. Its excessive ingestion leads to fluorosis. Fluorosis is associated with decrease in learning, memory and intelligence quotient (IQ). Several studies have reported that IQ of children living in endemic fluorosis areas is significantly less than the children living in non-endemic areas. The fluoride exposure is found to be associated with rise in neurodegenerative diseases. Alzheimer's disease is found to be common among people living in endemic fluorosis regions. This experiment was aimed to explore the relationship between fluorosis and Alzheimer's disease. Adult male Wistar rats were divided in to control and fluoride groups; Control animals received 0.5 ppm fluoridated water; fluoride group animals received 100ppm fluoridated water for 30 days. Animals were sacrificed after 30 days and brain tissue was collected, processed and sectioned; sections were stained with Congo red; slides were examined with polarized microscope. Compared with known amyloid positive tissue, control and fluoride group animal brain sections did not show apple green birefringence. Hence they are free from amyloid deposits. Short term fluoride exposure seem to have no effect on pathogenesis of Alzheimer's disease. However chronic fluoride exposure may play role in development of Alzheimer's disease.

**Keywords** Fluorosis, Alzheimer's disease, Amyloid deposits

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## Entrapment of The Martin-Gruber Anastomotic Nerve in The Forearm: A Case Report

Anu V Ranade\*, Rajalakshmi Rai\*\*, Muralimanju BV\*\*

\*Assistant Professor, Department of Basic Medical Sciences, College of Medicine, University of Sharjah, Sharjah, PO BOX 27272. UAE

\*\*Associate Professor, Department of Anatomy, KMC Mangalore, Manipal University, Karnataka, INDIA.

Email: aranade@sharjah.ac.ae

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### Abstract

**Background:** Martin Gruber anastomosis (MGA) is the known abnormal communication between median and ulnar nerves in the forearm. It serves as alternative motor pathway in case of damage to median or ulnar nerve.

**Objective:** To report entrapment of MGA between vena comitans and ulnar artery in the forearm of a formalin fixed cadaver and relate it to compression neuropathies and altered innervation of the intrinsic muscles of the hand.

**Results:** An inch below the elbow joint, a communicating branch between median nerve and ulnar nerve i.e Martin Gruber anastomosis was observed forking between the vena comitans and ulnar artery.

**Conclusion:** The MG anastomosis and its related neuro-vascular variations could be of great clinical importance in traumatic or nerve entrapment lesions. Because, even with complete lesion of the median nerve some intrinsic hand muscles supplied by it may escape being paralyzed which might lead to the misdiagnosis of nerve related injury of upper limb.

**Key words** Marin- Gruber anastomosis, median nerve, ulnar nerve, communication

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## Association between Age and Distal Femoral Articular Surface of Knee Joint Morphological Changes in Dry Bone in a Thai Population

Sirimas Prathum<sup>1</sup>, Sirada Sukhsawasdi na ayuthaya<sup>1</sup>, Robert Mann<sup>2</sup>, Pasuk Mahakkanukrauh<sup>3,4,5\*</sup>

<sup>1</sup>Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>2</sup>Professor in Anatomy and Pathology, John A. Burns School of Medicine, University of Hawaii.

<sup>3</sup>Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>4</sup>Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand

<sup>5</sup>Excellence Center in Osteology Research and Training Center )ORTC(, Chiang Mai University, Chiang Mai, 50200, Thailand

\*Corresponding author, e-mail: pasuk034@gmail.com

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### Abstract

**Background:** Age estimation from skeleton is the core of identification process in Forensic Science. Forensic scientist can estimate the age by observing the morphological changes in joints, especially knee joint. The morphological changes with age in knee joint are suitable age estimators because knee joints change as people age. Moreover, studying knee joints also help in estimating the tendency of Osteoarthritis in Thailand. Therefore, the researchers would like to study knee joints of Thai cadavers to observe the relationship between age and morphological changes. This study will be done in femur.

**Objective:** To indicate the association between age and femoral articular surface of knee joint morphological changes in dry bone in a Thai population

**Result:** There was a significant relation between age and distal femoral articular surface. The highest association in left femur is marginal osteophytes on anteromedial surface of medial part (0.637). The highest association in right femur is marginal osteophytes on posteromedial surface of medial part (0.437). The association is calculated by correlation methods. The correlation indicates that age and the deformities have the significant relationship.

**Conclusion:** Medial surface area is the most associated area for age and the degeneration of distal femoral articular surface.

**Keyword** knee joint, distal femoral articular surface of knee, knee joint morphological changes

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## Association between Age and Tibial Articular Surface of Knee Joint Morphological Changes in Dry Bone in a Thai Population

Kan Wangpiriyapanich<sup>1</sup>, Rattaphon Inphai<sup>1</sup>, Kachitphong Boosuan<sup>1</sup>, Robert Mann, PhD<sup>2</sup>, Pasuk Mahakkanukrauh<sup>3,4,5\*</sup>

<sup>1</sup>Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>2</sup>Professor in Anatomy and Pathology, John A. Burns School of Medicine, University of Hawaii.

<sup>3</sup>Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>4</sup>Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand

<sup>5</sup>Excellence Center in Osteology Research and Training Center (ORTC), Chiang Mai University, Chiang Mai, 50200, Thailand

\*Corresponding author, e-mail: [pasuk034@gmail.com](mailto:pasuk034@gmail.com)

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### Abstract

**Background:** Age estimation from skeleton is the core of identification process in Forensic Science. Forensic scientist can estimate the age by observing the morphological changes in joints, especially knee joint. The morphological changes with age in knee joint are suitable age estimators because knee joints change as people age. Moreover, studying knee joints also help in estimating the tendency of Osteoarthritis in Thailand. Therefore, the researchers would like to study knee joints of Thai cadavers to observe the relationship between age and morphological changes. This study will be done in tibia.

**Objective:** To indicate the association between age and tibial articular surface of knee joint morphological changes in dry bone in a Thai population

**Result:** There was a significant relation between age and articular surface of tibia. The highest association in left tibia is marginal osteophytes on posterolateral surface of medial part (0.507). The highest association in right tibia is marginal osteophytes on anteromedial surface of lateral part (0.566).

**Conclusion:** Besides age, the morphology of tibial articular surface can change by how people weigh down on knee joint hence it is different in each person.

**Keywords** tibial articular surface, tibia, knee joint

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## Association between Age and Distal Patellar Articular Surface of Knee Joint Morphological Changes in Dry Bone in a Thai Population

Suphawita Pliannuom<sup>1</sup>, Suphawit Boondiskulchok<sup>1</sup>, Robert Mann, PhD<sup>2</sup>, Pasuk Mahakkanukrauh<sup>3,4,5\*</sup>

<sup>1</sup>Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>2</sup>Professor in Anatomy and Pathology, John A. Burns School of Medicine, University of Hawaii.

<sup>3</sup>Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>4</sup>Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand

<sup>5</sup>Excellence Center in Osteology Research and Training Center )ORTC(, Chiang Mai University, Chiang Mai, 50200, Thailand

\*Corresponding author, e-mail: pasuk034@gmail.com

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### Abstract

**Background:** Age estimation from skeleton is the core of identification process in Forensic Science. Forensic scientist can estimate the age by observing the morphological changes in joints, especially knee joint. The morphological changes with age in knee joint are suitable age estimators because knee joints change as people age. Moreover, studying knee joints also help in estimating the tendency of Osteoarthritis in Thailand. Therefore, the researchers would like to study knee joints of Thai cadavers to observe the relationship between age and morphological changes. This study will be done in patella.

**Objective:** To indicate the association between age and patellar articular surface of knee joint morphological changes in dry bone in a Thai population

**Result:** There was a significant relation between age and distal patellar articular surface. The highest association in left patella is surface osteophytes of superomedial part (0.479). The highest association in right patella is surface osteophytes of inferomedial part (0.268).

**Conclusion:** Medial surface area is the most associated area

**Keyword** knee joint, distal patellar articular surface of knee, knee joint morphological changes

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## Association between Age and Acetabular Morphological Changes in Dry Bones in a Thai Population

Jirath Suriyasathaporn<sup>1</sup>, Worachot Chotecharnont<sup>1</sup>, Pandaree Khomkham<sup>1</sup>, Pawika Srinuan<sup>1</sup>, Robert Mann, PhD<sup>2</sup>, Pasuk Mahakkanukrauh, MD<sup>3,4,5\*</sup>

<sup>1</sup>Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>2</sup>Professor in Anatomy and Pathology, John A. Burns School of Medicine, University of Hawaii.

<sup>3</sup>Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>4</sup>Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand

<sup>5</sup>Excellence Center in Osteology Research and Training Center (ORTC), Chiang Mai University, Chiang Mai, 50200, Thailand

\*Corresponding author, e-mail: [pasuk034@gmail.com](mailto:pasuk034@gmail.com)

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### Abstract

Estimation of age at death from human skeletal remains is useful in Forensic Anthropology. Several studies show that morphological changes in the acetabular region which is well-preserved are associated with age. So, there is a possibility to use this region as an effective age predictor for adult specimens. The purpose of this study is to determine the applicability of seven morphological variables by evaluating the correlation between variable score and age of Thai specimens, using 35 males and 15 females from Forensic Osteology Research Center, FORC.

The correlation analysis between the evaluated scores and age at death shows that variable 1 (Acetabular groove) is the most associated with age at death with correlation coefficient of 0.753 in left male specimens. Moreover, the correlation analysis among seven variables shows the relationship between variable 6 (activity of the acetabular fossa) and variable 7 (porosities of the acetabular fossa) with correlation coefficient of 0.598 in right male specimens. In addition, there is no difference between male and female as well as left and right.

These results indicate an association between acetabular morphological changes and age. Therefore, the acetabulum is one of the capable age predictors for both male and female adult Thai specimens.

**Keywords** forensic anthropology, morphological change, human aging process

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## Age Estimation from Pubic Symphysis in a Thai Population by Using Method of Boldsen et al.

Pornhatai Komutrattananont<sup>1</sup>, Sittiporn Ruengdit<sup>2</sup>, SukonPrasitwattanaseree<sup>3</sup>, Pasuk Mahakkanukrauh<sup>1,2,4\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

<sup>2</sup> Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

<sup>3</sup> Department of Statistics, Faculty of Science, Chiang Mai University, 50200, Thailand.

<sup>4</sup> Excellence in Osteology Research and Training Center (ORTC), Chiang Mai University, 50200, Thailand.

\*Corresponding author, email: pasuk034@gmail.com

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### Abstract

Estimating ages of skeletons is major parameters to construct biological profiles. Pubic symphysis is accepted as morphological indicator, which provides precision of adult age estimation for identification of human skeletal remains. The objective was to apply method of Boldsen et al. for evaluating age from pubic symphysis in a Thai population and to determine correlation between age, all component and overall score. This study applies 5 components of method of Boldsen et al. on a modern Thai skeleton from Department of Anatomy at Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University (N = 260, range 20-90). A statistical analysis of correlation was calculated using the Pearson correlation test. Results suggested that age estimation was significant correlation between overall score and age that is similar to correlation of ventral symphyseal margin so, no necessary to assess all components. This method was considered as a valid age indicator for application to unknown age Thai skeleton in forensic practice.

**Keywords** forensic anthropology, skeletal age estimation, pubic symphysis, identification, Thai population

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## Age Estimation from the Morphological Change of the Auricular Surface of the Ilium in Thai Population

Treerat Gumpangseth<sup>1</sup>, Phruksachat Singsowan<sup>2</sup>, Sukon Prasitwattanaseree<sup>3</sup>, Pasuk Mahakkanukrauh<sup>1,2,4\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

<sup>2</sup> Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

<sup>3</sup> Department of Statistics, Faculty of Science, Chiang Mai University, 50200, Thailand.

<sup>4</sup> Excellence in Osteology Research and Training Center (ORTC), Chiang Mai University, 50200, Thailand.

\*Corresponding author, e-mail: [pasuk034@gmail.com](mailto:pasuk034@gmail.com)

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### Abstract

Age estimation is one of biological profile for primary identification of unknown individuals. The auricular surface of the ilium is the commonly skeletal remains in age estimation because this is a part of the pelvis which is well-preserved after death. The objectives were to study the relationship between age, features and composite score and develop age estimating method from auricular surface by applying method of Blodsen et al. in a Thai population. The study samples consist of 220 Thai pelvises of known sex and age at death from the Department of Anatomy at Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University. There were 9 components of auricular surface for assessment. The present study showed that the composite score was significantly correlated with actual age. The composite score could be used in age estimation and it is possible to apply this method in creating the equation which is useful for age estimation in forensic context.

**Keywords** forensic anthropology, age estimation, auricular surface, pelvis

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## Cerebellar Cortical Layer Atrophy in the Aging Human- A Post-Mortem Observation

Tahamida yesmin<sup>1\*</sup>, Shazia Afrin<sup>2</sup>, Lubna Sharmin<sup>3</sup>, Chandan Kumar Roy<sup>4</sup>, Mohammad Kabir Ahme<sup>4</sup><sup>5</sup>,

<sup>1</sup> Department of Anatomy, Faculty of medicine, University Kuala Lumpur, Royal College of medicine Perak, Malaysia.

<sup>2</sup> Department of Dental Public Health, Saphena Women's Dental College, 111 DIT Road, Malibagh, Dhaka 1217, Bangladesh

<sup>3</sup> Department of Anatomy, Qwest University. Perak, Malaysia

<sup>4</sup> Department of microbiology, Faculty of medicine, University Kuala Lumpur, Royal College of medicine Perak, Malaysia.

<sup>5</sup> Department of biochemistry Faculty of medicine, University Kuala Lumpur, Royal College of medicine Perak, Malaysia

\*Corresponding author, e-mail:tahamida.yesmin@yahoo.com

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### Abstract

**Aim and Objective:** The change in the thickness of cerebellar cortex layers with increasing age is evident change of number and morphology of cells of the cerebellar cortex that causes disorders of fine movement, hypotonia and postural changes. The present study was done to see the histological changes of cerebellum in different age groups of Bangladeshi people. **Methodology:** This cross sectional descriptive type of study was designed and done in the Department of Anatomy, Dhaka Medical College, , which was performed on the cerebellum of 28 Bangladeshi people, collected during autopsy examination of unclaimed dead bodies, from Department of Forensic Medicine. Paraffin blocks of cerebellum were cut at 5mm thickness and stained with routine Harris' Haematoxyline and Eosin (H & E) stain. **Result:** Histological studies revealed that gray matter was more prone to shrieked with age that the white matter. The age related change of molecular layer and granular layer start earlier than Purkinje cell layer but Purkinje cell layer more prone to change with age than other two layers of cerebellar cortex. **Conclusion:** The thickness of cerebellar cortex was decreased with age, were statistically highly significance. Further study on morphological changes of Purkinje cell and granular cell with electron microscope was recommended.

**Keywords** white matter, Cerebellar cortex, age

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## Degenerative Change in Lumbar Facet Joint Surface Related with Age in a Thai Population

Chanatporn Inthasan<sup>1</sup>, Sithee Praneatpolgrang<sup>2</sup>, Sukon Prasitwattanaseree<sup>3</sup>,  
Pasuk Mahakkanukrauh<sup>1,2,4\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

<sup>2</sup>Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

<sup>3</sup>Department of Statistics, Faculty of Science, Chiang Mai University, 50200, Thailand.

<sup>4</sup>Excellence in Osteology Research and Training Center (ORTC), Chiang Mai University, 50200, Thailand.

\*Corresponding author, e-mail :pasuk034@gmail.com

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### Abstract

The lumbar facet joints play important roles in loading transmission, stabilizing the motion segment in flexion and extension movements and kinematic rotation. Age is one of the important roles of degenerative changes of the vertebral column, the osteophyte. Several studies found that the degenerative change of vertebrae significantly related to age and vertebral column which was still in the burn situation. The purpose of this study was to examine correlation between degenerative changes of lumbar facet joints (maximum osteoarthritis score for each vertebra and mean lumbar osteoarthritis score) and age in a Thai population. The result showed that mean lumbar osteoarthritis score was the highest correlation with age, suggested that this method was possible to apply in other vertebral regions in order to calculate effective equations for age estimation for forensic anthropology in a Thai population.

**Keywords** facet joints, osteophyte, age. forensic anthropology

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## Stature Estimation from Skull using Craniometric Method in A Thai Population

Chirapat Inchai<sup>1\*</sup>, Phuwadon Duangto<sup>2</sup>, Sukon Prasitwattanaseree<sup>3</sup>  
Pasuk Mahakkanukrauh<sup>1,2,4\*</sup>

1Department of Anatomy, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

2Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, 50200, Thailand.

3Department of Statistics, Faculty of Science, Chiang Mai University, 50200, Thailand.

4Excellence in Osteology Research and Training Center (ORTC), Chiang Mai University, 50200, Thailand.

\*Corresponding author. E-mail: pasuk034@gmail.com

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### Abstract

Stature estimation is one of the important components in biological identification of human skeletal remains. Several authors have reported the various parts of the body which can estimate the stature. In many cases, only skull is available for medico-legal examination. If the stature can be estimated from the skull, it is useful for forensic anthropology. The aim of the present study was to establish correlation between stature and cranial measurements. The study consisted of 250 dry skulls (158 male and 92 female skulls) obtained from The Forensic Osteology Research Center, Department of anatomy, Faculty of medicine, Chiang Mai University. Seven cranial measurements in vertical dimension included Basion-Bregma Height (ba-b), Cranial Base Length (ba-n), Nasal Height (n-n), Frontal Chord (n-b), Occipital Chord (l-o), Orbital Height and Mastoid Length were also made on each skull. The correlations between stature and each of the cranial measurements were assessed with Pearson correlation coefficients. The result indicated that there are five cranial measurements with high significant correlation coefficient of stature. Their correlation coefficient (r) values ranged from 0.366 to 0.597. In conclusion, cranial measurements may be useful for stature estimation, when the skull is available for forensic examination.

**Keywords** Stature estimation, Skull, Cranial, Craniometric analysis.

## Extensor Tendons and Variations of the Medial Four Digits of Hand: A Cadaveric Study

Athikhun Suwannakhan<sup>1,3</sup>, Tulyapruet Tawonsawatruk<sup>2</sup>, Krai Meemon<sup>3\*</sup>

<sup>1</sup> Mahidol University International College, 999 Phutthamonthon 4s Road, Salaya, Nakhonpathom, 73170, Thailand

<sup>2</sup> Department of Orthopaedics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama VI Road, Ratchathewi, Bangkok, 10400, Thailand

<sup>3</sup> Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Ratchathewi, Bangkok, 10400, Thailand

\*Corresponding author, e-mail: krai.mee@mahidol.ac.th

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### Abstract

A total of 100 cadaveric limbs were dissected to study the anatomy of the forearm and hand extensor musculature. Four types of contributions to the index finger were found for the extensor indicis proprius, including one new type where the double tendons of the extensor indicis proprius inserts volar and radial to the extensor digitorum communis of the index finger. Four anomalous muscles were identified including the extensor medii proprius (in five cadavers), extensor digitorum brevis manus (in one cadaver), extensor indicis et medii communis (in four cadavers) and extensor pollicis et indicis (in four cadavers). The absence of the extensor indicis proprius in four cases was substituted by either the extensor indicis et medii communis or the extensor pollicis et indicis. Two previously unrecognized cases were found. In one hand, the anomalous extensor indicis et medii communis was present along with the extensor pollicis et indicis. In another hand, both the extensor medii proprius and the extensor digitorum brevis manus were present, and the extensor medii proprius tendon inserted to the tendon of the extensor digitorum brevis manus. Awareness of the variations on the dorsum of the hand is essential for diagnosis, surgical planning and treatment of diseased hands.

**Keywords** extensor tendons, anatomical variations, extensor medii proprius, extensor digitorum brevis manus, extensor indicis et medii communis, extensor pollicis et indicis

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## Can Parallels be Drawn Among Microscopic Features, Hysteroscopy and Endometrial Thickness in Patients with Abnormal Uterine Bleeding?

Shruti Saralaya<sup>1\*</sup>, Supriya K<sup>1</sup>, Suchitra Thunga<sup>1</sup>, Nina A Mahale<sup>1</sup>, Vimala Ramachandran<sup>1</sup>  
Department of Obstetrics and Gynaecology, Faculty of Medicine, Manipal University, Kasturba Medical College Mangalore,

\*Corresponding author, e-mail: shrutisaralaya@gmail.com

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### Abstract

**Background:** Abnormal Uterine Bleeding (AUB) generally describes all patterns of bleeding that result from a wide variety of causes including anovulation, uterine pathology and coagulopathies. AUB is the most common complaint that a woman in reproductive age group presents with.

**Objectives:** The aim of the present study is to see if we can find any similarity between microscopic features, hysteroscopy findings and endometrial thickness of patients with AUB.

**Methods:** Women presenting with AUB, who underwent Hysteroscopy followed by endometrial curettage between January 2015 to January 2016 were included in the study. The Endometrial thickness, hysteroscopic findings and Histopathological reports were tabulated and results obtained.

**Results:** In the total of 112 patients included in the study, non secretory endometrium was the commonest microscopic finding in 27(24%), followed by Simple endometrial hyperplasia without atypia in 24(21.42%), Normal endometrial tissue was seen in 20(17.85%), endometrial polyps in 17(15.17%). Endometrial malignancy was seen in 4 cases. 5 cases of atrophic and pill endometrium were seen. Complex endometrial hyperplasia with atypia and without atypia was seen in 1 case each.

**Conclusion:** We found that in patients with endometrial polyps and endometrial cancers, the endometrial thickness and hysteroscopic findings are comparable. In hyperplasias and normal histology of endometrium, the endometrial thickness and hysteroscopic findings vary considerably.

**Keywords** endometrial thickness, hysteroscopy, microscopy.

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## Alpha-2 Macroglobulin is Involved in Sperm Activation and Decapacitation Factor of the Blue Swimming Crab, *Portunus pelagicus*

Thanyaporn Senarai<sup>1\*</sup>, Shinji Miyata<sup>2</sup>, Chihiro Sato<sup>2</sup>, Rapeepun Vanichviriyakit<sup>1,3</sup>, Wattana Weerachatanukul<sup>1</sup>, Prasert Sobhon<sup>1,4</sup>, Ken Kitajima<sup>2</sup>, Prepee Sretarugsa<sup>1</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

<sup>2</sup> Graduate School of Bioagricultural Sciences and Bioscience and Biotechnology Center, Nagoya University, Nagoya 464-8601, Japan

<sup>3</sup> Center of Excellence for Shrimp Molecular Biology and Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

<sup>4</sup> Faculty of Allied Health Sciences, Burapha University, Chonburi 20131, Thailand

\*Corresponding author, e-mail: tsenarai@gmail.com

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### Abstract

In mammals, the specific-secreted epididymal proteins have function in sperm maturation and seminal plasma can prevent sperm capacitation. In *Portunus pelagicus*, sperm maturation is occurred in the middle spermatic duct (MSD) which contained the specific-protein band, 250 kDa. In this study, we aim to characterize this protein in MSD fluid and examine its biological component and expression.

The 250 kDa of MSD fluid was analyzed by MALDI-TOF MS/MS and its amino acid sequencing was confirmed using N-terminal amino acid sequencing and results showed that 250 kDa protein was alpha-2 macroglobulin ( $\alpha 2M$ ). Then, DNA of 250 kDa protein had been cloned by RT-PCR technique. For *in situ* hybridization,  $\alpha 2M$  mRNA was expressed in spermatic duct (SD), hepatopancreas and hemocyte. In the expression of  $\alpha 2M$  on sperm, it gradually decreased from SD until to spermatheca. The carbohydrate moieties of  $\alpha 2M$  (250 kDa) revealed that several kinds of lectins were detected, especially ConA.

The present study demonstrated for the first time that in the MSD fluid of male blue swimming crab contains 250 kDa protein, a  $\alpha 2M$ . This protein was synthesized from spermatic duct, hepatopancreas and hemocyte and expresses mainly in MSD and MSD-sperm. We suggest that  $\alpha 2M$  may be involved in sperm activation and also acts as decapacitation factor in the male blue swimming crab.

**Keywords** alpha-2 macroglobulin, blue swimming crab, decapacitation

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## Alteration of GABA Concentrations in Rat Testis after Methamphetamine Exposure

Paweena Kaewman, Sutisa Nudmamud-Thanoi and Samur Thanoi \*<sup>1</sup>

<sup>1</sup> Department of Anatomy and Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

\*Corresponding author, e-mail: samurt@nu.ac.th

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### Abstract

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter which it does not only play a role in the central nervous system, but it also plays a role in the peripheral tissue including in the reproductive system. In male reproductive system, a GABAergic system was found in the testis which regulated Leydig cell proliferation and androgen production. Moreover, the inhibitory effect of GABA to spermatogenesis has been reported in *in vitro* study by inhibiting proliferation of spermatogonia stem cells. Methamphetamine (METH) has been reported the adverse effects on male reproductive system, leading to the reduction of sperm quality including sperm concentration, sperm motility and normal sperm morphology. Nevertheless, the main mechanisms of these effects remained unclear. The hypothesis of this study is that METH administration can change the GABA concentrations in testis. Thus, this study aims to determine the alteration of GABA concentrations in testis after METH exposure. Male Sprague–Dawley rats were used in the model of METH abuse. They were divided into control, acute binge (AB-METH), escalating dose (ED METH) and escalating dose-binge (ED-binge METH) groups. After sacrifice, the concentrations of GABA in rat testis were investigated by using high-performance liquid chromatographic (HPLC) method. The results showed that the GABA concentrations of all METH-administrated groups were significantly increased when compared to the control group. These results suggested that the increase of GABA concentrations in testis might be the cause of poor sperm quality or the homeostasis responding after METH exposure.

**Keywords** Methamphetamine, GABA concentrations, Rat testis

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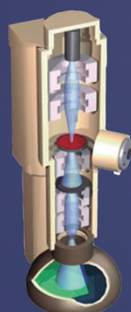


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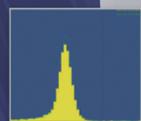
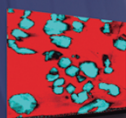
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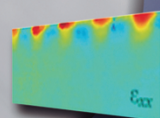
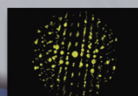


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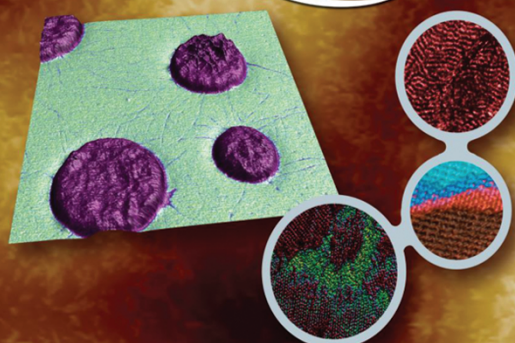


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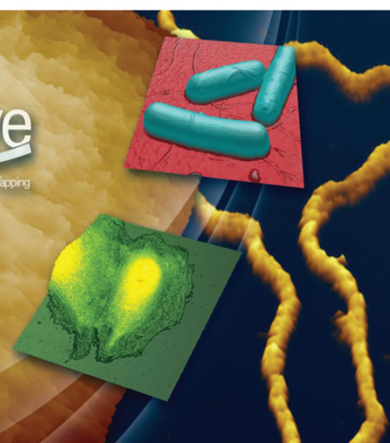


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## Bamboo Charcoal Powder Made from Culms of *Bambusa multiplex*

Visittapong Yordsri<sup>1,\*</sup>, Chanchana Thanachayanont<sup>1</sup>, Chabaiporn Junin<sup>1</sup>, Masahiro Kawasaki<sup>2</sup>,  
Tetsuo Oikawa<sup>3</sup>, and Makoto Shiojiri<sup>3,†</sup>

<sup>1</sup> National Metal and Materials Technology Center, 114 Thailand Science Park, Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand

<sup>2</sup> JEOL USA Inc., 11 Dearborn Road, Peabody, MA 01960, USA.

<sup>3</sup> JEOL Asia PTE Ltd., Co Regn No: 199500744W, 2 Corporation Road #01-12, Corporation Place 618494, Singapore

<sup>4</sup> Kyoto Institute of Technology, Kyoto 606-8585, Japan.

<sup>†</sup> Present address: 1-297 Wakiyama, Kyoto 618-0091 Japan; shiojiri@pc4.so-net.ne.jp

\* Corresponding author, e-mail: [visittay@mtec.or.th](mailto:visittay@mtec.or.th)

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### Abstract

Bamboo charcoal powder made from culms of *Bambusa multiplex* has been studied by means of analytical scanning transmission electron microscopy, aiming at fabricating and applying to advanced materials.

**Keywords** Charcoal, bamboo, *Bambusa multiplex*, analytical scanning transmission electron microscopy

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## Graphene- and N-doped graphene-based gas sensor for ethanol detection

Sumet Sakulsermsuk<sup>1\*</sup>, Pisith Singjai<sup>1</sup>, Surachet Phadungdhitihada<sup>1</sup> and Supab Choopun<sup>1</sup>

<sup>1</sup> Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

\*Corresponding author, e-mail: sssakulsermsuk@gmail.com

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### Abstract

Ethanol concentration in blood for drunk drivers is important factor for prosecuting. Graphene, two-dimensional carbon material, has high electrical conductivity and high surface area per mass, which is promising for gas sensor applications. We fabricated graphene and N-doped graphene based gas sensors for detecting ethanol gas. Graphene was synthesized by chemical vapor deposition (CVD) on copper foils. Doping nitrogen into graphene was achieved by annealing graphene with NH<sub>3</sub> at 400 °C. Measuring by atomic force microscopy obtained ~ 1.4 nm of thickness of graphene. A few layers and single layer graphene can be observed by transmission electron microscope. Raman spectrum on N-doped graphene showed the effect of doping in G and 2D peaks. Measurement of gas sensors were performed in varying concentrations of ethanol gas and temperatures of the sensors. Responding resistance of both sensors to ethanol gas showed characteristic of n-type semiconductor. Sensitivity of graphene at 25 °C and N-doped graphene at 200 °C was derived close to 1.

**Keywords** 3-5 words: Graphene, CVD, ethanol, gas sensors

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## Controlled Release Urea Fertilizer via Polymer-Layer Silicate Nanocomposite

Kannika Sitthisuwannakul<sup>1</sup>, Wiyong Kangwansupamonkon<sup>1\*</sup>

<sup>1</sup>National Nanotechnology Center, National Science and Technology Development Agency, 111 Thailand Science Park, Phahonyothin Road, Khlong1, Khlong Luang, Pathum Thani, 12120, Thailand

\*Corresponding author, e-mail: wiyong@nanotec.or.th

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### Abstract

In order to minimize the loss of nutrient to the environment, many types of the controlled release fertilizer have been developed consistently, especially, a polymer coated fertilizer (PCF). Nanoclay or layer-silicate nanoparticle is one of the famous natural materials for polymer composites and plays an important role for controlling the release rate of nutrients due to its advantages especially to improve the water barrier and increase mechanical properties. In this research, we interested in using the nanoclay including an alkyd resin as the polymer-layer silicate nanocomposite layer on urea granules and investigated the role of this nanocomposite layer. Herein, the nanocomposite films at different nanoclay contents (0%, 3%, 5% and 10%wt) were created as the demonstrated urea coated layer and characterized *via* IR, XRD and universal testing machine. Moreover, the coated urea were characterized by FESEM and EDXs. The releasing test performed in the water at 60 °C and the nitrogen quantitative measurement performed using elemental analyzer. The results show that the increasing of nanoclay contents do not affect to the thickness of all the nanocomposite coated urea layers, the thickness is approximately 52  $\mu\text{m}$ . The sigmoidal kinetic format was used as releasing model with high correlation coefficients and the diffusion indices exhibit inversely to the yield's strength of the nanocomposite layer.

**Keywords** Controlled release fertilizer, Polymer-layer silicate, Sigmoidal

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## Microstructural Characterization of Gold Nanoparticles Impregnated on Mesocellular Silica Foam

Nithi Thananukool<sup>1</sup>, Bralee Chayasombat<sup>2,\*</sup>, Chanchana Thanachayanont<sup>2</sup>, Seeroong Prichanont<sup>1</sup>

<sup>1</sup> Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Phayathai Road, Bangkok 10330, Thailand

<sup>2</sup> National Metal and Materials Technology Center, Thailand Science Park, Paholyothin Road, Pathumthani 12120, Thailand

\*Corresponding author, e-mail: [braleec@mtec.or.th](mailto:braleec@mtec.or.th)

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### Abstract

Mesocellular silica foam (MCF) is a type of mesoporous silica with a structure that is composed of uniform spherical cells interconnected by windows [1]. This structure gives a 3D pore network and is expected to have better substance diffusion compared to the 2D pore structure of conventional mesoporous silica such as SBA-15 or MCM-41 [2]. For this advantage, MCF is suitable for various applications such as a catalyst support in an advance catalysis system or immobilizing enzyme in biosensors. In this study, spherical MCF was synthesized by a modified MCF synthesis method [3]. After template removal, Au particles were impregnated on the MCF by the chemical reduction method. The MCFs before and after gold impregnation were characterized using x-ray diffraction, scanning electron microscopy and transmission electron microscopy (TEM). Figures 1 (a) and (b) show TEM images of MCF before and after gold impregnation. It shows that the reduced gold nanoparticles were well distributed into the internal structure of the MCF. Concentration of the gold precursor and reduction time was found to effect the size and distribution of the gold nanoparticles. By optimizing of these factors, gold nanoparticle dispersion on MCF can be controlled to create a gold modified MCF material that is matched with the required applications.

**Keywords** Mesocellular Silica Foam, Gold Nanoparticle, Transmission Electron Microscopy

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## Estimation of Edge Structure of Triangular Graphene on SiC (0001) Using *Moiré Pattern on STM Image*

Akkawat Ruammaitree\*

Department of Physics, Faculty of Science and Technology, Thammasat University,  
Paholyothin Road, Prathumthani, Thailand

\*Corresponding author, e-mail: u4605070@hotmail.com

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### Abstract

Graphene is a two dimensional carbon material which has many exotic properties, especially electronic properties. In case of small graphene islands, the electronic properties depend on their edges. Nevertheless, direct determination of graphene edge structure using *scanning tunneling microscope* is difficult since the carbon-carbon bond length is very small ( $\sim 1.4\text{\AA}$ ) and the level between graphene and non-graphene regions is much different. In this report, the estimation of edge structure of triangular graphene on SiC (0001) is demonstrated by analyzing orientation of graphene lattice and *moiré pattern*.

**Keywords:** graphene, silicon carbide, *moiré pattern*, scanning tunneling microscopy, *reflection high-energy electron diffraction*

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## Non-vacuum Locally Patterned N<sup>+</sup> and P<sup>+</sup> Doped Layers for Interdigitated Back Contact Solar Cells by Ultrasonic Spray Technique

Sung-Yen Wei<sup>1\*</sup>, Jian-Wei Liao<sup>2</sup>, Shen-Ming Yu<sup>1</sup>, Wen-Chin Sun<sup>1</sup>, Shen-Chuan Lo<sup>1</sup>

<sup>1</sup> Material and Chemical Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan

<sup>2</sup> Engineering and System Science department, National Tsing Hua University, Hsinchu, Taiwan

\*Corresponding author, e-mail: wei@itri.org.tw

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### Abstract

The emitter and back surface field doping layer of Interdigitated-Back-Contact solar cells were commonly acquired by POCl<sub>3</sub> diffusion or BBr<sub>3</sub>, BCl<sub>3</sub> diffusion<sup>1-3</sup>. However, this kind of diffusion method was expensive, complicated and the toxic doping sources are harm for the environment. Phosphoric acid and boric acid were used as low-cost, nontoxic n-type and p-type doping sources to make emitter and BSF for silicon solar cell<sup>4</sup>. In this research, a non-vacuum, low cost process of emitter and back surface field fabrication for Interdigitated-Back-Contact Solar cells was established. The dilute phosphoric acid and boric acid were coated on Si wafer by ultrasonic spray technique and used as nontoxic and low cost p/n doping sources. The patterned diffusion area can be defined by using an easily removed mask and Al<sub>2</sub>O<sub>3</sub> as a protective layer. The sheet resistance of the p-type and n-type doping area can be well controlled by varying spraying time and annealing temperature. The dopant distribution was revealed by electron energy loss spectrum technique in high resolution transmission electron microscope. In phosphorus doping, the sheet resistance ranging from 28 to 125Ω/□ were achieved. In boron doping, sheet resistance from 30 to 125Ω/□ could be acquired.

**Keywords** patterned-diffusion, back-contact, solar cell, boric acid, phosphoric acid

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## The Surface Morphology Analysis of Rolled Ag-Sheath Bi,Pb-Sr-Ca-Cu-O Superconducting Wire

Hendrik<sup>1,a\*)</sup>, Pius Sebleku<sup>1,b)</sup>, Sigit Dwi Yudanto<sup>1,c)</sup>, Bintoro Siswayanti<sup>1,d)</sup>, Andika Widya Pramono<sup>1,e)</sup> and Agung Imaduddin<sup>1,f)</sup>

<sup>1</sup> Research Center for Metallurgy and Materials-Indonesian Institute of Sciences (P2MM-LIPI) Kawasan Puspiptek, Serpong, Tangerang 15314, Indonesia

\*E-mail of the corresponding author:<sup>a\*)</sup> hend027@lipi.go.id

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### Abstract

The critical temperatures of BPSCCO wire superconductor can be achieved in Ag sheath-monofilament BPSCCO (round wire) when the sample Ø is reduced to 4 mm during wire drawing. This study was aimed to analyze the affect of sintering time and sintering temperature in Ag-sheath wire superconductor. Starting materials were inserted into Ag tube and rolled untill it has diameter of 4mm. And then, heated at 860°C. We have two samples with heating time of 8 hours and 24 hours. We analyzed surface morphology with SEM and temperature dependence resistivity with four point probe. SEM images showed cavities along cross sectional surface of the samples. The sample with the heating time of 24 hours has more cavities compared to the 6 hours one. We also confirmed the critical temperature of superconducting at around 70 K..

**Keywords:** BPSCCO, wire superconductor, Tc, surface morphology, SEM

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## The effect of aging treatment on the in-situ transformation of precipitates in AA7050 aluminum alloys

Tsai-Fu Chung<sup>1\*</sup>, Bo-Ming Huang<sup>1</sup>, Jer-Ren Yang<sup>1</sup> and T. Ohmura<sup>2</sup>.

<sup>1</sup> Department of Materials Science and Engineering, National Taiwan University, Taipei 106, Taiwan.

<sup>2</sup> National Institute for Materials Science, 1-2-1 Sengen, Tsukuba, Ibaraki 305-0047, Japan.

Corresponding author, e-mail: [f03527003@ntu.edu.tw](mailto:f03527003@ntu.edu.tw)

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### Abstract

The strengthening of AA7050 aluminum alloys is based on the aging treatment. An evidence to indicate that not only  $\eta'$  phases but also GP zones during aging treatments contribute hardness is indecisive. Analyzing the microstructure and **orientation relationships** of  $\eta'$  has improved to elucidate its evolution, but the interaction between dislocations and  $\eta'$  still remains to be solved. In this work, we find the **in-situ transformation** when  $\eta'$  gradually transforms as a whole into  $\eta$  in a single precipitate during **two-step aging treatment** and find special **plateau-effect** in the load-depth curve with using the nano-indentation. Studying the nanometer-sized precipitates in two-step aging treatment make us clearly what kinds of precipitates dominate the strength of AA7050 aluminum alloys.

**Keywords** orientation relationships, in-situ transformation, two-step aging treatment, and plateau-effect.

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## Comparative Nanostructure Analysis of Lithium Titanate Composite Material Synthesized by Solid State Reaction for Lithium Ion Battery Application

Fadli Rohman<sup>1\*</sup>, Achmad Subhan<sup>1</sup>, Riza Iskandar<sup>2</sup>, Arbi Dimyati<sup>3</sup>, Bambang Prihandoko<sup>1</sup>

<sup>1</sup> Research Center for Physics, Indonesian Institute of Sciences, Kawasan Puspiptek Serpong, Tangerang Selatan, Indonesia

<sup>2</sup> Central Facility for Electron Microscopy (GFE), RWTH Aachen University, Ahornstr. 55, Aachen 52074, Germany

<sup>3</sup> National of Nuclear Agency, Kawasan Puspiptek Serpong, Tangerang Selatan, Indonesia

\*Corresponding author, e-mail: fadli.rohman@lipi.go.id

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### Abstract

Lithium Titanate (LTO/Li<sub>4</sub>Ti<sub>5</sub>O<sub>12</sub>)-based spinel has been widely used as the anode material in the lithium ion battery due to its excellent properties such as safety, splendid Li-ion insertion/extraction reversibility and ultra-long life time. In this work, we carried out structure and morphology characterization of spinel LTO nanoparticles composite synthesized by solid state reaction method. This method has advantages since it was easy to do through stoichiometric reaction. This method was used because of the easiness of the process compared to hydrothermal and other method. Characterizations were performed by means of X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and High Resolution Transmission Electron Microscopy (HRTEM). X-ray diffraction analysis obviously identified peaks of anatase phase TiO<sub>2</sub> (accordance with JCPDS Card no. 84-1285) and Li<sub>4</sub>Ti<sub>5</sub>O<sub>12</sub> (accordance with JCPDS Card no. 26-1198). The SEM images show the particle morphology which is exhibited agglomeration with average particles size is about 100 - 300 nm. Further analysis by TEM measurements revealed well-define nano particles and confirmed sizes between 100 – 300 nm. Some small nano-rods particles about 10-30 nm in size could be also observed. The phase identification analysis using SAED ring patterns are in agreement with XRD observations which revealed the presence of both anatase phase TiO<sub>2</sub> (JCPDS Card no. 84-1285) and spinel Li<sub>4</sub>Ti<sub>5</sub>O<sub>12</sub> (JCPDS Card no. 26-1198). Moreover, HRTEM analysis cannot found and observed any pore which indicates that well-crystalline particles were successfully synthesized.

**Keywords** LTO/TiO<sub>2</sub> composite material, electron diffraction, HRTEM, LIBs

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## Microstructural Characterization of $Y_xGd_{1-x}Ba_2Cu_3O_y$ Coated Conductors with Different Film Thicknesses of One Coating in Multiple Coating TFA-MOD Process

Ryuji Yoshida<sup>1\*</sup>, Takeharu Kato<sup>1</sup>, Ko-ichi Nakaoka<sup>2,3</sup>, Kazunari Kimura<sup>2,3</sup>, Takato Machi<sup>2,3</sup>, Teruo Izumi<sup>2,3</sup>, and Yuh Shiohara<sup>2,3</sup>

<sup>1</sup> Nanostructures Research Laboratory, Japan Fine Ceramics Center, Nagoya, Japan

<sup>2</sup> Industrial Superconductivity Technology Research Association, iSTERA

<sup>3</sup> Superconductivity Research Laboratory, ISTEK, Kawasaki, Japan

\*Corresponding author, e-mail: [r\\_yoshida@jfcc.or.jp](mailto:r_yoshida@jfcc.or.jp)

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### Abstract

We achieved high in-field critical current densities of  $BaZrO_3$  (BZO) doped  $Y_xGd_{1-x}Ba_2Cu_3O_y$  (YGdBCO) superconducting coated conductor fabricated by improved trifluoroacetate-metal organic deposition (TFA-MOD) process. In the TFA-MOD process, coating, drying, and calcination processes were repeated to form a thick YGdBCO film. Features of this improved TFA-MOD process include the repetition of the coating / calcination of an extremely thin film with the two-step crystallization process. We observed the BZO doped YGdBCO layers fabricated by the improved TFA-MOD with different thicknesses ( $d_{\text{once}}$ ) of one coating in multiple coatings, using transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS), to investigate the relationship between the  $d_{\text{once}}$  and the BZO particle size for enhancement of the in-field properties. In the finally crystallized films using the  $d_{\text{once}}$  of 170 nm, 100nm, 30 nm, and 18 nm, the smallest average size of the BZO particles was accomplished by the sample of  $d_{\text{once}}$  of 30 nm. From the EDS line analyses of calcined films with different thicknesses of  $d_{\text{once}}$ , we found the distinct difference in the compositional characteristic segregation length corresponding to the thickness of  $d_{\text{once}}$ . These results strongly suggest that the crystal growth and coarsening kinetics of the BZO particles are limited by the diffusion within the constrained distance of the thickness of  $d_{\text{once}}$ .

This research is supported by the Development of Medical Devices and Systems for Advanced Medical Services from the Japan Agency for Medical Research and development, AMED.

**Keywords:** Coated conductor, TFA-MOD, TEM, EDS

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## FIB/SEM/TEM Study of $\text{EuBa}_2\text{Cu}_3\text{O}_y$ Coated Conductor with $\text{BaHfO}_3$ Nanorods

Takeharu Kato<sup>1\*</sup>, Daisaku Yokoe<sup>1</sup>, Ryuji Yoshida<sup>1</sup>, Akira Ibi<sup>2,3</sup>, Tomo Yoshida<sup>2,3</sup>,  
Teruo Izumi<sup>2,3</sup>, Tsukasa Hirayama<sup>1</sup>, and Yuh Shiohara<sup>2,3</sup>

<sup>1</sup> Nanostructures Research Laboratory, Japan Fine Ceramics Center, Atsuta-ku, Nagoya, Japan

<sup>2</sup> Industrial Superconductivity Technology Research Association (iSTERA),

<sup>3</sup> Superconductivity Research Laboratory, International Superconductivity Technology Center (ISTEC), Takatsu-ku, Kawasaki, Japan

\*Corresponding author, e-mail: tkato@jfcc.or.jp

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### Abstract

We characterized the nanostructures of  $\text{EuBa}_2\text{Cu}_3\text{O}_y$  (EuBCO) coated conductors containing  $\text{BaHfO}_3$  (BHO) nanorods fabricated by PLD on Hastelloy<sup>TM</sup> tapes with textured buffer layers [1] using FIB, SEM and TEM. The minimum in-field  $I_c$  value in the measurements of  $I_c$ - $B$ - $\theta$  of the EuBCO layer with 3.6  $\mu\text{m}$  in thickness was 141.2 A/cm-w at 77 K in 3 T [1]. The EuBCO layers were mainly composed of  $c$ -axis oriented EuBCO grains containing BHO nanorods with an average diameter of 4.5 nm. Some outer growth EuBCO grains, which had different orientations compared with those of the matrix of  $c$ -axis oriented grains and obstructed supercurrents, were formed on Cu-oxide grains. In addition, Ba-Cu-oxides, which were considered to be a liquid phase during the PLD process and solidified in the cooking after the process, were distributed on the surface of the film composed of the  $c$ -axis oriented EuBCO grains. 3D reconstruction of the outer growth EuBCO grains, Cu-oxides and Ba-Cu-oxides in the EuBCO layer was performed using a series of cross-sectional SEM images of the EuBCO layer acquired in an FIB-SEM system [2]. From these results, we found the important (V-L-S) crystal growth mode of the PLD process for enabling high deposition speed of thick EuBCO layer containing BHO nanorods as well as having high in-field current ( $I_c$ - $B$ ) properties.

This research is supported by the Development of Medical Devices and Systems for Advanced Medical Services from the Japan Agency for Medical Research and development, AMED.

**Keywords**  $\text{BaHfO}_3$ , nanorod,  $\text{EuBa}_2\text{Cu}_3\text{O}_y$ , FIB-SEM, 3D reconstruction

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## Sample Preparation Techniques for Studying Wheat Gluten and Poly (Butylene Succinate) Blended Morphology by the Scanning Electron Microscope

Warunee Borwornkiatkaew<sup>1</sup>, Onuma Santawitee<sup>1\*</sup>, Sudsiri Hemsri<sup>2</sup>, Chanchai Thongpin<sup>2</sup>

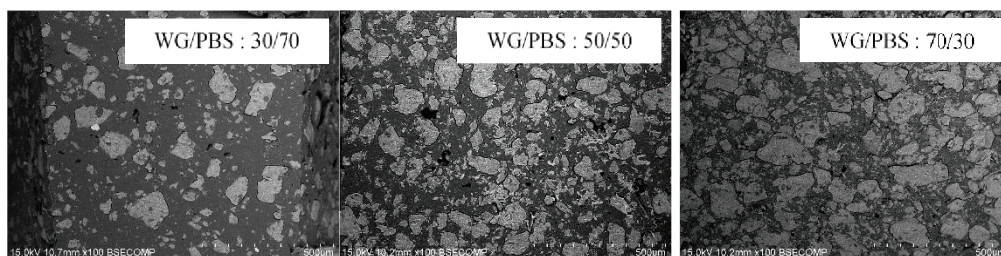
<sup>1</sup>National Metal and Materials Technology Center, Phahonyothin Road, Pathumthani, Thailand

<sup>2</sup>Department of Materials Science and Technology, Faculty of Engineering and Industrial Technology, Silpakorn University, Rachamankana Road, Nakornpathom, Thailand

\*Corresponding author, e-mail: [onumas@mtec.or.th](mailto:onumas@mtec.or.th)

### Abstract

Wheat gluten/Poly(butylene succinate) blended, WG/PBS blended, was prepared for using as inexpensive, opaque and biodegradable containers such as pots, trays and boxes. Morphological analysis by scanning electron microscope (SEM) was studied in order to explain mechanical properties of blended. However, SEM micrographs of WG/PBS blended could not clearly identify each phase because the contrast between WG and PBS phase was not different. Consequently, in this research, two sample preparation methods: etching with chloroform vapor and staining with osmium tetroxide techniques were compared. According to the results, staining WG/PBS blends with osmium tetroxide for 45 minutes was the best sample preparation technique in increasing contrast of these blended. WG/PBS blended was immiscible, and its tensile strength and elongation at break in 70/30 composition were low due to aggregations of WG phase and weak interfacial adhesion between WG and PBS phase. On the other hand, elongation of WG/PBS blends were improved by increasing PBS contents in blended.



SEM micrographs at X100 magnification of WG/PBS blended after staining with osmium tetroxide for 45 minutes.

**Keywords** wheat gluten, poly(butylene succinate), morphology, contrast

## Microstructure and Electrochemical Corrosion Behavior of Electroplating Trivalent Chromium Coating on Stainless Steel 304

Surassawadee Paliphot<sup>1</sup>, Sankum Nusen<sup>1</sup>, Torranin Chairuangsi<sup>1\*</sup>, Thapanee Sarakonsri<sup>2</sup>,  
Siriporn Daopiset<sup>3</sup>

<sup>1</sup> Department of Industrial Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai  
50200, Thailand

<sup>2</sup> Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200,  
Thailand

<sup>3</sup> Thai-French Innovation Institute, King Mongkut's University of Technology North Bangkok,  
Bangkok 10500, Thailand

\*Corresponding author, e-mail: tchairuangsi@gmail.com

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### Abstract

Stainless steel 304 (SS304) is a potential material as the bipolar plate for proton exchange membrane fuel cell. However, its corrosion resistance is too low to survive in the hostile environment. In the present study, chromium-film coatings on 304 stainless steel plate were electrodeposited from chromium chloride solution containing controlled concentration of formic acid, ammonium sulphate and potassium bromide, was studied. Factors affecting coating including concentration of formic acid up to 2.0 M, concentration of potassium bromide up to 0.05 M, current density up to 10 A/dm<sup>2</sup>, coating temperature up to 25°C and coating time up to 30 min were investigated by controlling the concentration of ammonium sulphate at 0.7 M. Characterisation of coating film was performed by scanning-electron microscopy and X-ray diffraction. Corrosion resistance to sulfuric acid solution of coated and bare steel plates was determined by potentiodynamic technique. Comparative discussion on effectiveness of both electrolytic solutions will be given.

**Keywords** trivalent chromium plating, surface morphology, corrosion resistance

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## Visualization of Active Reaction Sites on Gold Nanoparticulate Catalysts

Tadahiro Kawasaki<sup>1,2,3\*</sup>, Kaname Yoshida<sup>1</sup>, Takafumi Ishida<sup>2,3</sup> and Takayoshi Tanji<sup>2,3</sup>

<sup>1</sup> Nanostructures Research Laboratory, Japan Fine Ceramics Center,  
2-4-1 Mutsuno, Atsuta-ku, Nagoya, 456-8587, Japan

<sup>2</sup> Institute of Materials and Systems for Sustainability, Nagoya University, 1 Furo-cho, Chikusa-ku,  
Nagoya, 464-8603, Japan

<sup>3</sup> GREEN, 1-1, Namiki, Tsukuba, 305-0044, Japan

\*Corresponding author, e-mail: t\_kawasaki@jfcc.or.jp

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### Abstract

A gold nanoparticulate supported catalyst [1,2] has been investigated in order to identify its active sites, where catalytic reactions occur, by means of visualizing reaction products with an environmental TEM (ETEM). One of the catalytic reactions gold can perform is selective oxidation of propene ( $C_3H_6$ ) to propene oxide ( $C_3H_6O$ ), which is a very important process in the chemical industry. Since propene oxide (PO) has a low vapor pressure ( $5 \times 10^{-4}$  Pa @RT), the PO remains in liquid form if the surrounding pressure is controlled to be more than this value. Here, the PO catalytic product might be observed in liquid form but it is almost impossible to visualize it in gas form. In this study, we have observed *in-situ* catalytic propene epoxidation on the gold nanoparticles supported on anatase  $TiO_2$  using a windowed-type environmental TEM [3], and have clarified the active sites of the gold catalysts by revealing the distribution of the PO molecules. According to our experimental results, it is directly proved that the active sites are the perimeter of Au/ $TiO_2$  interface, which is consistent with a model previously proposed [2] and experimental results in chemistry [4].

**Keywords** Windowed-type environmental TEM, Gold catalyst, active site

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## Characterization of Thermal Sprayed Nicko-Shield 200 Coating

Phuangphaga Daram<sup>1</sup>, Suphitcha Moonngam<sup>1</sup>, Chaiyasit Banjongprasert<sup>1,2\*</sup>

<sup>1</sup> Department of Physics and Materials science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>2</sup> Materials Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

\*Corresponding author, e-mail: chaiyasit.b@cmu.ac.th

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### Abstract

The microstructure and chemical composition of Ni-based alloy coating; Nicko-shield 200 (12-25wt%Cr, 8-15wt%Mo, 10wt%other element) were prepared by arc spray method onto mild steel substrate. The effects of heat treatments on the microstructure of Nicko-shield 200 coatings were also investigated at 1100°C for 60 min. The microstructure of as-sprayed and heat treated coatings was analyzed by scanning electron microscopy )SEM(, X-ray diffraction )XRD( and optical emission spectroscopy )OES(. The results show that as-sprayed and heat-treated coatings were dense with a low porosity of ~ 2-3 vol%. EDS and OES confirmed that the coatings contained Ni, Cr, Mo, Al, O and Si elements. EDS shows that the matrix phases contained Ni, Cr and Mo element. The coatings consisted of Ni(Cr,Mo) matrix phase and oxide layers. After heat treatment, it was found that high Mo phase appeared in the matrix and a fraction of oxide layers increased inside intersplat. The oxides were mostly  $\text{Cr}_x\text{O}_y$ . XRD indicated that both coatings consisted predominantly of  $\gamma$ -Ni phase, but heat-treated coating consisted of small peaks of  $\text{Ni}_3\text{Al}$ ,  $\text{Ni}_3\text{Mo}$ ,  $\text{NiO}$ ,  $\text{Cr}_2\text{O}_3$  and  $\text{NiCr}_2\text{O}_4$ . The formation of oxides in the coatings after heat treatment is also thought to be beneficial to the corrosion resistance and mechanical properties.

**Keywords** Ni-based alloys, Thermal sprayed coating, Microstructure, Heat treatment

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## Microstructural Investigation of CuAgZr Alloy Processed by Severe Plastic Deformation

Waraporn Piyawit<sup>1\*</sup> and Panya Bauhombura<sup>1</sup>

<sup>1</sup> School of Metallurgical Engineering, Institute of Engineering,  
Suranaree University of Technology, 111 University Avenue, Nakhon Ratchasima, Thailand

\*Corresponding author, e-mail: wpiyawit@sut.ac.th

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### Abstract

High pressure torsion is one of the “top down” severe plastic deformation procedures that has been successfully used to produce ultrafine grains and/or nanostructured grains in various kind of metallic materials. CuAgZr alloy is a variant of CuAg alloy that has been remarkably known as a high strength and high electrical conductivity alloy. Strengthening mechanisms for CuAgZr alloy processed by high pressure torsion can be achieved through precipitation hardening, solid solution hardening, work hardening and grain boundary strengthening. Appropriate age hardening process can increase strength of the alloy in conjunction with increasing electrical conductivity. The microstructures of heavily deformed and aged CuAgZr samples are examined by using conventional and high resolution transmission electron microscope. After low temperature aging treatment, the X-ray diffraction analysis and selected area diffraction pattern indicate that Ag-precipitate presents in Cu matrix. Microstructure of the low temperature annealed sample shows no significant grain growth and the average grain diameter is well below 100 nm with less amount of dislocation tangles observed. This can be suggested that the low temperature aging treatment helps improve the electrical conductivity of severely deformed CuAgZr alloy by lowering the amount of dislocation without eliminating the effect of grain boundary strengthening from high pressure torsion process.

**Keywords** high pressure torsion, copper alloy, ultrafine grained materials, precipitation strengthening

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## Effects of Austenite Reversion on Impact Toughness of Martensitic Steels in High Temperature Tempering

Hung-Wei Yen<sup>1</sup>, Yu-Chen Lin<sup>1</sup>, Meng-Hsuan Chiang<sup>1</sup>, Delphic Chen<sup>2</sup>, Yuan-Tsung Wang<sup>2</sup>, Ching-Yuan Huang<sup>2</sup>, Hsin-Chih Lin<sup>1</sup>

<sup>1</sup> Department of Materials Science and Engineering, National Taiwan University, Roosevelt Road, Taipei, Republic of China

<sup>2</sup> Iron and Steel R&D Department, China Steel Corporation, Chung Kang Road, Kaohsiung, Republic of China

\* Hsin-Chih Lin, e-mail: hclinntu@ntu.edu.tw

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### Abstract

Advanced offshore steels have microstructure of tempered martensite in order to reach both high strength and excellent impact toughness. However, in the conventional S690Q offshore steel, tempering at 720 °C leads to detrimental effects on the toughness due to the coalescence of martensite laths. The current work, *via* X-Ray energy-dispersion spectrum (EDS) mapping in scanning transmission electron microscope (STEM), discovered nickel- and copper-enriched martensite laths in an offshore steel with modified composition. The phenomenon is attributed to austenite reversion, which occurs between original martensite laths at 720 °C. These reversed austenite laths transformed into martensite in cooling and their effects on prevention of lath coalescence greatly improved the impact toughness. This discovery, along with the principle of alloy design, can be applied to prevent from the embrittlement when martensitic steels are tempered at higher temperature.

**Keywords** Offshore steel, tempered martensite, reversed austenite, X-ray energy-dispersion spectrum, scanning transmission electron microscopy

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## In-depth Study of the Deformation Microstructure in an Austenitic Stainless Steel upon Shot peening

Yinsheng He<sup>1</sup>, and Keesam Shin<sup>1\*</sup>

<sup>1</sup> School of Nano & Advanced Materials Engineering, Changwon National University, Changwon 51140, Korea

\*Corresponding author, e-mail: keesam@changwon.ac.kr

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### Abstract

Shot peening has been used for long time as a means of surface finish and introduction of compressive stress on the surface which retards crack initiation and propagation. It is being applied industrially to parts such as boiler tubes to reduce corrosion rate. While shot peening is useful in many aspects of machine part usage, it is getting a new attention as a means of introducing a gradient structured surface layer on metals and alloys. The shot peened gradient surface layer is often found to have a nanocrystalline, ultra-fine grain and various deformation microstructures. Earlier reports indicated that the grain refinement mechanism in an austenitic ( $\gamma$ ) stainless steel 304 after ultrasonic nanocrystalline surface modification peening involved dislocation intersection, deformation twin and the martensitic transformation. This work focuses on the development and evolution of the martensite natures by combined using of EBSD and TEM. The results indicate that the formation of single direction  $\epsilon$ -martensite (hcp) plate at the low strain region, which is believed as the gliding of the Shockley partial dislocations on every other  $\{111\}\gamma$  plane. With the increase of the strain, the  $\alpha'$ -martensite (bcc) inclusions were observed at the intersectional of the two  $\epsilon$ -martensite plate with the COR of Kurdjumov-Sachs. As the increase of strain, the  $\alpha'$ -martensite islands were found near the deformation twin with the COR of Nishiyama-Wassermann. The formation of  $\alpha'$ -martensite was suggested as the two shear mode involved the first shear of  $\frac{a_\gamma}{18}[\bar{1}\bar{1}2]$  on every  $(11\bar{1})_\gamma$  planes and the second shear of  $\frac{a_\gamma}{16}[\bar{1}2\bar{1}]$  on every  $(111)_\gamma$  planes.

**Keywords:** Austenitic Stainless Steel, Deformation Microstructures, Martensite Transformation

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## Ex-situ EBSD Study on Abnormal Grains in 6063 Aluminium Billets

Kanokwan Uttarasak<sup>1</sup>, Wanchai Chongchitnan<sup>2</sup>, Julathep Kajornchaiyakul<sup>3</sup>, Chaiyasit Banjongprasert<sup>1\*</sup>

<sup>1</sup> Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Huay Kaew Road, Chiang Mai, Thailand

<sup>2</sup> CAP C E L CO.,LTD, Klong 6 Road, Pathumtani, Thailand

<sup>3</sup> National Metal and Materials Technology Center (MTEC), Pahonyothin Road, Pathumthani, Thailand

\*Corresponding author, e-mail: chaiyasit.b@cmu.ac.th

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### Abstract

The 6063 aluminium billet alloy is one of the most common raw materials for extruded aluminium products. Homogenization is used to improve extrudability and surface finish of extruded products by reducing the non-uniformity. Fe content of 6063 aluminium billets plays an important role on abnormal grain growth during homogenization. This work aims to study the effect of Fe content on the grain growth behaviors of homogenized 6063 aluminium billets. The 6063 billets were homogenized at 580 °C with an increment of holding time. The as cast and as homogenized samples were characterized using ex-situ electron backscatter diffraction (EBSD) under a JSM-IT 300 LV with 20 kV and 2.5 - 4.0 micron step sizes. It was found that discontinuous recrystallization and grain boundaries migrations were main mechanisms for abnormal grain growth during homogenization. In addition, a significant change of misorientation angles was observed on the 6063 billet with a lower Fe content whereas minimal change in the higher Fe-containing billets. More details on the grain growth mechanisms related to EBSD investigations will be discussed throughout.

**Keywords** Ex-situ EBSD, Abnormal Grains, Homogenization, 6063 Aluminium Billets

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## Microstructure and Hardness of Ag Alloys for Invisible-Setting Jewelry

Natthaphol Chomsaeng<sup>1\*</sup>, Titaree Chumrod<sup>1</sup>, Suttawan Imurai<sup>2</sup>, Pornkiat Churnjitapirom<sup>3</sup>, Chutimun Chanmuang<sup>4</sup>

<sup>1</sup> Jewelry Materials Research and Development Center, Faculty of Gems, Burapha University, Chanthaburi Campus, Chanthaburi, Thailand

<sup>2</sup> Division of Gemological Science, Faculty of Gemological Sciences and Applied Arts, Rambhai Barni Rajabhat University, Chanthaburi, Thailand

<sup>3</sup> Faculty of Dentistry, Mahidol University, Bangkok, Thailand

<sup>4</sup> Institute of Mineralogy and Crystallography, University of Vienna, Wien, Austria

\*Corresponding author, e-mail: natthaphol@bua.ac.th

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### Abstract

Invisible setting is an attractive setting in gold jewelry where, normally, high-hardness 18 K gold is used. However, due to increasing gold price, jewelers prefer working on silver to gold. Usually, silver is alloyed with 7.5 wt%Cu, “sterling silver”, to increase its hardness (60-70 HV) [1-2]. But the setting requires higher hardness of sterling silver. This work focused on improving hardness of the sterling silver by adding 0.5-1.5 wt.%Al along with age hardening. Molten alloys were casted at 1000 °C into 650°C investment mold. The samples were solution-treated at 750 °C for one hour and then aged at 300°C varying from 1 to 9 hours. Optical microscope (OM) and scanning electron microscope (SEM) equipped with EDS were used for microstructure investigation. Hardness of the as-cast and aged samples was measured by Vickers microhardness tester. Dendritic- $\alpha$ -Ag rich phase was obviously seen in every as-cast alloys and gave the maximum hardness to the alloys at 80.58 HV. The alloy obtained its maximum hardness at 135.16 HV after aging for 1 hour. Microstructure and hardness of the aged samples depended on the aging temperatures and times. The maximum hardness of the alloy was due to precipitation in Ag matrix.

**Keywords** Silver Alloy, Aged Hardening, Invisible Setting Jewelry

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## Microtexture of Cu-based Shape Memory Alloy

Chutimun Chanmuang<sup>1\*</sup>, Bamika Kumnerdphonpittaya<sup>2</sup>, Oumaporn Juntakool<sup>2</sup>  
and Saisamorn Niyomsoan<sup>2</sup>

<sup>1</sup> Institut für Mineralogie und Kristallographie, Universität Wien, 1090, Austria

<sup>2</sup> Faculty of Gems, Burapha University, Chanthaburi Campus, Chanthaburi, 22170, Thailand.

\*Corresponding author, e-mail: chutimun.chanmuang@univie.ac.at

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### Abstract

Shape Memory Alloys (SMAs) is one among smart materials that can remember its original shape and able to return to a pre-deformed shape after being heated. Shape memory effect derives from a martensitic transformation induced by stress and/or temperature changes (1). Cu-based alloys provide a more economical alternative compared with Ni-Ti due to their low cost, variety of interesting mechanical and thermal properties (2, 3). In this work, the Cu-Zn-Al shape memory alloys were performed by lost-wax casting technique with melting temperature at 1100 °C and mold temperature at 650 °C. Shape memory effect, annealing behavior and microstructure were studied. Optical microscope, Scanning Electron Microscope (SEM) and Energy X-ray Diffraction Spectroscopy (EDS) were used for microstructural characterization. It can be concluded that the microstructure of as-cast alloy contained  $\alpha$ -phase texture surrounded by matrix phase. EDS revealed that the  $\alpha$ -phase was enriched with Cu, while Al was more soluble in the matrix phase. The needle-like martensitic texture was found only in the matrix. This texture provided hardness of the matrix phase (181 HV). However, to study the microstructure and texture of these phases in higher magnification, the result from Transmission Electron Microscope (TEM) will be discussed.

**Keywords** Shape Memory alloy, Cu-based Alloys, Microtexture

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## Scanning Electron Microscopy of ZSM-5 Zeolite from Hydrothermal Synthesis for Use as a Reforming Catalyst

Nongkhran Chaiwong<sup>1\*</sup> and Nongnuch Rueangjitt<sup>1</sup>

<sup>1</sup>Department of Industrial Chemistry, Faculty of Science, Chiang Mai University,  
Huaykaew Road, Chiang Mai 50200, Thailand

\*Corresponding author, e-mail: nongkhran.ch@cmu.ac.th

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### Abstract

ZSM-5 zeolites synthesized from hydrothermal method were characterized by Scanning Electron Microscopy (SEM). ZSM-5 zeolite is widely used as a catalyst and plays a very important role for reforming process of hydrocarbons in petroleum and petrochemical industries in Thailand. This research aims to design and fabricate a pressurized vessel reactor for synthesizing ZSM-5 zeolite catalyst by using TPABr as a direct-structuring template. In addition, a preliminary study of hydrothermal synthesis was conducted to examine the key factors affecting the crystallization of ZSM-5 zeolite as follows: molar ratio of silica-to-alumina, pH and hydrothermal temperature. From the results of SEM and XRD analysis, ZSM-5 zeolite crystals can be formed in the case of adding TPABr. The surface morphology of ZSM-5 zeolite was shown by Secondary Electron Images (SEIs). The optimum condition of this preliminary study was a silica-to-alumina molar ratio of 140, a pH of 8 and a hydrothermal temperature of 220°C, which corresponds to the highest percentage of Relative Crystallinity (%RC). The SEM results exhibited the shape of microscopic ZSM-5 spheres at the optimum condition. Crystal size of synthesized ZSM-5 zeolite was in the range of 5 to 30 micron.

**Keywords:** ZSM-5, Zeolite, Hydrothermal

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## Preparation and Characterization of $\text{Bi}_2\text{VO}_{5.5}$ Nanopowder

Prakasit Intaphong<sup>1</sup>, Jitrephan Phanmalee<sup>1</sup>, Suchanya Kojinok<sup>2</sup>, Sukon Phanichphant<sup>3</sup>,  
Wiyong Kangwansupamonkon<sup>4</sup>, and Pusit Pookmanee<sup>1,5\*</sup>

<sup>1</sup> Department of Chemistry, Faculty of Science, Maejo University, Chiang Mai, 50290, Thailand

<sup>2</sup> Institute of Product Quality and Standardization (IQS), Maejo University, Chiang Mai, 50290, Thailand

<sup>3</sup> Materials Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>4</sup> National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency, Pathumthani, 12120, Thailand

<sup>5</sup> Nanoscience and Nanotechnology Research Laboratory (NNRL), Faculty of Science, Maejo University, Chiang Mai, 50290, Thailand

\*Corresponding author, e-mail: pusit@mju.ac.th

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### Abstract

Bismuth vanadate ( $\text{Bi}_2\text{VO}_{5.5}$ ) nanopowder is a ferroelectric, non-centrosymmetric and polar orthorhombic class. The preparation methods of  $\text{Bi}_2\text{VO}_{5.5}$  nanopowder were solid state reaction, sol-gel, co-precipitation, hydrothermal, solvothermal and microwave method. Microwave method has been developed to be a facile synthetic method for inorganic nano-scale materials. In this research,  $\text{Bi}_2\text{VO}_{5.5}$  nanopowder was prepared by microwave method at 300-500 Watt for 6 min. Bismuth nitrate pentahydrate ( $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ ) and ammonium vanadate ( $\text{NH}_4\text{VO}_3$ ) were used as the starting precursors with mole ratio of 2:1 in 2-propanol medium. The phase and structure of  $\text{Bi}_2\text{VO}_{5.5}$  nanopowder were characterized by X-ray diffraction (XRD). Orthorhombic structure was obtained without calcinations step. The morphology of  $\text{Bi}_2\text{VO}_{5.5}$  nanopowder was investigated by scanning electron microscopy (SEM). The particle was irregular shape and agglomerate of 300-400 nm. The chemical composition of  $\text{Bi}_2\text{VO}_{5.5}$  nanopowder was determined by energy dispersive X-ray spectroscopy (EDXS). It found the chemical compositions are bismuth, vanadium and oxygen with the ratio of Bi:V of 2:1. The functional groups of  $\text{Bi}_2\text{VO}_{5.5}$  nanopowder was determined by fourier transform infrared spectroscopy (FTIR). The stretching vibration of  $\text{VO}_{5.5}^{6-}$  and bending vibration of Bi-O showed the wavenumber at 765 and 500  $\text{cm}^{-1}$ , respectively.

**Keywords** Bismuth vanadate, microwave method, X-ray diffraction, scanning electron microscopy, energy dispersive X-ray spectroscopy

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## Electrochemical Fabrication of Bismuth Ferric Oxide (BFO) Thin Film for High Photoelectrocatalytic Activity of Water Oxidation under Visible Light Irradiation

Chotiwid Wiriyaichalerd, Chatchai Ponchio\*

Department of Chemistry, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathumthani, 12110 Thailand

\*e-mail: [chatchai@rmutt.ac.th](mailto:chatchai@rmutt.ac.th)

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### Abstract

Bismuth ferric oxide thin film was successfully fabricated on fluorine doped tin oxide by a galvanostat technique with a condition of  $6 \text{ mA/cm}^2$  for 15 min at room temperature. The thin film was characterized by scanning electron microscopy, X-ray diffraction and X-ray photoelectron spectroscopy techniques. UV-Visible spectrophotometry was used to study band gap energy and photo responding. The bismuth ferric oxide thin film was applied as photo anode for study photoelectrocatalytic activity of water oxidation under visible light irradiation. Charge transfer resistance and capacitance of the electrode were confirmed by electrochemical impedance spectroscopy. The photocurrent from water oxidation was depended on temperature and current density of bismuth ferric oxide thin film fabrication. There are presents the simple and high efficiency bismuth ferric oxide electrode fabrication technique for water oxidation.

**Keywords:** Bismuth ferric oxide, Water oxidation, Electrochemical deposition, Photoelectrocatalytic activity

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## Enhanced Photoelectrocatalytic Activity of CuO Electrode by Coupling with TiO<sub>2</sub> for Water Reduction under Visible Light

Wannasiri Srevarit<sup>1</sup>, Sanchai Kuboon<sup>2</sup>, Annop Klamchuen<sup>2</sup>, Chatchai Ponchio<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Faculty of Science and Technology,  
Rajamangala University of Technology Thanyaburi, Pathumthani, 12110 Thailand

<sup>2</sup>National Nanotechnology Center (NANOTEC), National Science and Technology  
Development Agency (NSTDA), Phathumthani 12120, Thailand

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\*e-mail: Chatchai@rmutt.ac.th

### Abstract

CuO photocathode electrode was coupled with TiO<sub>2</sub> for enhancing photoelectrocatalytic activity of water reduction under visible light irradiation. The CuO thin film was deposited at the first layer on fluorine doped tin oxide by electrodeposition and following with annealed process. TiO<sub>2</sub> thin film was fabricated by radio frequency sputtering at the second layer on CuO electrode. The coupled CuO/TiO<sub>2</sub> electrode surface was characterized by X-ray diffraction, scanning electron microscopy, X-ray photoelectron spectroscopy and UV-vis spectroscopy. The photo current from water reduction was studied to confirm the function of TiO<sub>2</sub> deposited on an CuO electrode. The cooperated TiO<sub>2</sub> at the outer side with CuO could be enhanced the photo current more than that pure CuO for three times. There are a successful development of photocathode with high efficiency of water reduction under visible light irradiation.

**Keywords:** Photoelectrocatalytic, water reduction, radio frequency sputtering, CuO/TiO<sub>2</sub> photocathode

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## Photoelectrocatalytic Performance of FTO/Cu<sub>2</sub>O for Nitrite Degradation under Visible Light

Chatchai Ponchio\*, Pattaranit Thongthep, Suporn wongchatee, Pattaraporn Temchuen.  
Department of Chemistry, Faculty of Science and Technology, Rajamangala University of  
Technology Thanyaburi, Pathumthani, 12110 Thailand  
\*e-mail: [chatchai@rmutt.ac.th](mailto:chatchai@rmutt.ac.th)

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### Abstract

Cuprous oxide (Cu<sub>2</sub>O) thin film was prepared on fluorine doped tin oxide by an electrodeposition technique. Scan electron microscopy, X-ray diffraction and UV/Vis spectroscopy were used to study the film characterization. The fabricated Cu<sub>2</sub>O film was applied as photocathode for nitrite (NO<sub>2</sub><sup>-</sup>) degradation by photoelectrocatalytic technique under visible light irradiation. The optimum condition was investigated with high efficiency nitrite degradation up to 100% for 60 min. Moreover, the incorporated solar cell system as bias potential unit was presented the % nitrite degradation up to 100% for 70 min. This developed technique shows high efficiency, simple and low cost could be an excellent alternative method for nitrite degradation in water.

**Keywords** Photoelectrocatalytic, Nitrite degradation, Cuprous oxide

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## Effect of Carbon and Cooling Rate on Phase Transformation and Hardness in Sintered FeCrMo Steels

Amporn Wiengmoon<sup>1\*</sup>, Wananurat Srijampan<sup>1</sup>, Ruangdaj Tongsri<sup>2</sup>

<sup>1</sup> Department of Physics, Faculty of Science, Naresuan University, Pitsanulok-Nakhon Sawan Road, Tha Pho, Muang, Pitsanulok 65000 Thailand

<sup>2</sup> Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120 Thailand

\*Corresponding author, e-mail: ampornw@nu.ac.th

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### Abstract

In this paper, the effect of carbon content and cooling rate on the microstructure and hardness of Fe-3.0wt.%Cr-0.5wt.%Mo-0.01wt.%C steel was investigated. The pre-alloyed powder was mixed with 0.2 and 0.3wt.%C. The powder mixtures were compacted with hydraulic compactor at room temperature to green density of 6.5 g/cm<sup>3</sup>. The green compact were sintered at 1280 °C in vacuum furnace and cooled by nitrogen gas with cooling rate of 0.1 and 4.0 °C/s. From XRD, OM and SEM results revealed that the microstructure of 0.2wt.%C sintered steel with the slow cooling rate of 0.1°C/s consisted mainly of polygonal ferrite (PF). While the steel containing 0.3wt.%C, consisted of PF and bainite. With higher cooling rate of 4.0 °C/s, bainite, martensite ( $\alpha'$ ) and some retained austenite ( $\gamma$ ) were found. The Vickers micro-hardness of the steels containing 0.2 and 0.3wt.%C with the cooling rate of 0.1 °C/s were 106.7 HV0.1 and 127.3 HV0.1, respectively. With higher cooling rate of 4.0 °C/s, Vickers micro-hardness was increased up to 304.5 HV0.1 and 436.1 HV0.1 respectively. These steels exhibited increases of hardness with increasing carbon content and cooling rate due to the transformations from austenite that formed during sintering, to hard bainite and martensite structures [1-3].

**Keywords:** Sintered FeCrMo steels, Microstructure, Phase transitions, Scanning electron microscopy, Hardness

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## Physical and Electrical Properties of Ternary System $\text{Bi}_{0.5}(\text{Na}_{0.80}\text{K}_{0.20})_{0.5}\text{TiO}_3\text{-}0.005\text{LiNbO}_3\text{-Ba}(\text{Ti}_{0.90}\text{Sn}_{0.10})\text{O}_3$ Lead-Free Piezoelectric Ceramics

Pimpilai Wannasut<sup>1</sup>, Pharatree Jaita<sup>1</sup>, Anucha Watcharapasorn<sup>1,2</sup>, Sukanda Jiansirisomboon<sup>1,3\*</sup>

<sup>1</sup> Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>2</sup> Materials Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>3</sup> School of Ceramic Engineering, Institute of Engineering, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand

\*Corresponding author, e-mail: Sukanda.jian@sut.ac.th

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### Abstract

The ternary system of lead-free piezoelectric ceramics with the formula of  $(1-x-y)\text{Bi}_{0.5}(\text{Na}_{0.80}\text{K}_{0.20})_{0.5}\text{TiO}_3\text{-}x\text{LiNbO}_3\text{-}y\text{Ba}(\text{Ti}_{0.90}\text{Sn}_{0.10})\text{O}_3$  or  $(1-x-y)\text{BNKT-}x\text{LN-}y\text{BTS}$  ( $x = 0.005$  and  $y = 0, 0.01, 0.02, 0.03, 0.04$  and  $0.05$  mol fraction) were successful prepared by solid-state and mixed oxide method. All sample were sintered at the temperature of  $1125^\circ\text{C}$  for 2 h. The sample investigated on phase, microstructure and dielectric properties. The highest density of  $5.89\text{ g/cm}^3$ . X-ray diffraction pattern showed pure perovskite structure without any detection of a secondary phase. Scanning electron micrographs of all sample presented a cubic-like grain shape with side length of  $0.44 - 0.87\text{ }\mu\text{m}$ . The highest dielectric ( $\epsilon_r = 1775$ ,  $\tan\delta = 0.0534$ ) were obtained at  $0.995\text{BNKT}0.005\text{LN-}0.04\text{BTS}$  sample.

**Keywords** Ternary system, Lead-free piezoelectrics, X-ray diffraction, Piezoelectrics.

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## Electron Diffraction Study of Bismuth Borate Glasses

Nattapol Laorodphan<sup>1\*</sup>, Torranin Chairuangsr<sup>2</sup>, Kenji Tsuda<sup>3</sup>

<sup>1</sup> Department of Industrial Chemistry and Textile Technology, Faculty of Science,  
Maejo University, 63 Moo 4, Sansai, Chiang Mai, Thailand

<sup>2</sup> Department of Industrial Chemistry, Faculty of Science, Chiang Mai University, 239, Huay Kaew  
Road, Muang, Muang, Chiang Mai, Thailand

<sup>3</sup> Frontier Research Institute for Interdisciplinary Sciences, Tohoku University,  
Aramaki aza Aoba 6-3, Aoba-ku, Sendai, Japan

\*Corresponding author, e-mail: Nattapol.Lao@gmail.com

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### Abstract

Bismuth borate glasses have been widely used in many electronic devices, for instance, photonic switches and an optical fibre for communications, due to the optical non-linearity properties of these glasses. In this study, the changes of the structure of binary bismuth borate glasses with a nominal composition of  $x\text{Bi}_2\text{O}_3-(1-x)\text{B}_2\text{O}_3$ , where  $x$  is varied from 0.25–0.65, was investigated using electron microscopy in transmission electron microscopy and Raman spectroscopy. Physical properties of glasses including density and molar volume were also examined. The results showed that the density and the molar volume of glasses increase with increasing bismuth oxide content. One-dimensional electron density profile was converted from halo pattern using “ProcessDiffraction” software. The different position of the first bright ring was observed. These differences correspond to the difference in bond distances between B-O found in these bismuth borate glasses. The electron density profiles of glasses were also compared with an amorphous carbon. Raman spectra also reveal the changes of the coordination number of boron indicating the formation of tetragonal boron;  $[\text{BO}_4]^-$  from trigonal boron;  $[\text{BO}_3]$ .

**Keywords** Bismuth Borate Glasses, Electron Diffraction, Raman Spectroscopy

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## Raman study of ion-irradiated FIB foils of zircon (ZrSiO<sub>4</sub>)

Lutz Nasdala<sup>1\*</sup>, Shavkat Akhmadaliev<sup>2</sup>, Andreas Artach<sup>1,3</sup>, Chutimun Chanmuang<sup>1</sup>, Gerlinde Habler<sup>4</sup>, Christoph Lenz<sup>1,5</sup>

<sup>1</sup> Institut für Mineralogie und Kristallographie, Universität Wien, 1090 Wien, Austria

<sup>2</sup> Institut für Ionenstrahlphysik und Materialforschung, Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany

<sup>3</sup> present address: Sandoz GmbH, 6250 Kundl, Austria

<sup>4</sup> Department für Lithosphärenforschung, Universität Wien, 1090 Wien, Austria

<sup>5</sup> present address: Institute of Materials Engineering, Australian Nuclear Science and Technology Organisation, Lucas Heights, NSW 2234, Australia

\*Corresponding author, e-mail: lutz.nasdala@univie.ac.at

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### Abstract

The mineral zircon (tetragonal ZrSiO<sub>4</sub>) incorporates trace amounts of the actinides U and Th. Due to corpuscular self-irradiation (mainly alpha-decay events), zircon accumulates structural damage over geologic periods of time. The quantification of radiation effects is however difficult, because of the mostly uncertain thermal (and hence annealing) history of natural samples.

Here we report Raman spectroscopic results obtained from zircon samples whose damage was produced by irradiation with 1–10 MeV Au ions in a Tandem accelerator. The samples irradiated were ca. 1.5 µm thick lamellae produced using a FIB (focused ion beam) system. The sample thickness corresponds to the penetration depth of MeV Au ions. The lamellae hence contain the vast majority of the damage created, but no un-irradiated volumes (whose presence would bias analytical results).

Total fluences up to  $4.25 \times 10^{13}$  Au/cm<sup>2</sup> (corresponding to  $\leq 0.079$  dpa) resulted in notable broadening of the  $\nu_3(\text{SiO}_4)$  Raman band of zircon. Irradiation with  $1.2 \times 10^{14}$  Au/cm<sup>2</sup> (0.224 dpa) was sufficient to amorphize the target, as evident from the complete loss of Raman bands of crystalline ZrSiO<sub>4</sub>.

**Acknowledgments:** Funding for this research was provided by the Austrian Science Fund (FWF) through grant P24448–N19, and University of Vienna Doctorate School IK052.

**Keywords** Zircon, Heavy-ion irradiation, Focused ion beam, Raman spectroscopy

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## Crystal-to-Amorphous-to-Crystal (C-A-C) transition in $\text{Cr}_2\text{M}$ (M = Hf or Zr) Laves phase

Takeshi Nagase<sup>1,2 \*</sup>

<sup>1</sup> Research center for ultra-high voltage electron microscopy, Osaka University, 7-1 Mihogaoka, Osaka 567-0047, Japan

<sup>2</sup> Division of Materials and Manufacturing Science, Graduate School of Engineering, Osaka University, 2-1, Yamada-Oka, Suita, Osaka 565-0871, Japan

\*Corresponding author, e-mail: [t-nagase@uhvem.osaka-u.ac.jp](mailto:t-nagase@uhvem.osaka-u.ac.jp)

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### Abstract

The  $\text{Cr}_2\text{Ti}$  Laves phase was reported to show the typical Crystal-to-Amorphous-to-Crystal (C-A-C) transition which exhibits an amorphous single phase formation after the C-A transition and disappearance of an amorphous phase after the subsequent A-C transition [1]. In this study, electron-irradiation-induced phase transition in  $\text{Cr}_2\text{M}$  (M = Hf and Zr) Laves phase was investigated by high voltage electron microscopy (HVEM), focusing on the difference in the C-A-C transition behavior among the  $\text{Cr}_2\text{X}$  (X = Group 4 elements Ti, Zr, and Hf) Laves phases. Laves phase with C14 structure was obtained in rapidly solidified melt-spun ribbon in  $\text{Cr}_{66.67}\text{M}_{33.33}$  (M=Hf or Zr) (at.%) alloys. Under MeV electron irradiation, the  $\text{Cr}_2\text{Hf}$  and  $\text{Cr}_2\text{Zr}$  Laves phases with C14 structure transformed into an amorphous phase, which was followed by the crystallization of an amorphous phase, indicating the occurrence of the crystalline-to-amorphous-to-crystalline (C-A-C) transition.

A part of work was supported by JSPS KAKENHI Grant Number 15K06484, and scientific research grant from Kansai Genshiryoku Kondankai.

**Keywords** High Voltage Electron Microscopy, In situ observation, Solid state amorphization, Crystallization, Laves phase

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## Microanalysis Study on Thai Historical Glasses

Pisutti Dararutana\*

Royal Thai Army Chemical Department, Phaholyothin Road, Chatuchak, Bangkok, Thailand

\*Corresponding author, e-mail: pisutti@hotmail.com

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### Abstract

Cultural heritage objects are highly heterogeneous. Due to the complex nature of materials and objects, extremely sensitive, spatially resolved, multi-elemental and versatile analytical instruments are needed. The techniques employed should be as non invasive as possible and able to give complementary information from macroscopic to nanometer scales. In this work, SEM-EDS, PIXE, EPMA and SRXRF were used to analyze chemical composition and microstructure of historical glass samples collected from Thailand including ancient glass beads, ancient decorative glasses and old-styled gold mosaic glass. It can be concluded that these techniques in combination are powerful for the investigation of heterogeneous glassy materials.

**Keywords** *Thai historical glass, SEM-EDS, PIXE, EPMA, SRXRF*

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## Environment-friendly Disposable Nonvolatile Resistive Switching Memory Composed of Biodegradable Nanocellulose

Kazuki Nagashima<sup>1\*</sup>, Umberto Celano<sup>2</sup>, Hirotaka Koga<sup>3</sup>, Masaya Nogi<sup>3</sup>, Fuwei Zhuge<sup>1</sup>, Gang Meng<sup>1</sup>, Yong He<sup>1</sup>, Jo De Boeck<sup>2</sup>, Malgorzata Jurczak<sup>2</sup>, Wilfried Vandervorst<sup>2</sup> and Takeshi Yanagida<sup>1</sup>

<sup>1</sup> Institute for Materials Chemistry and Engineering, Kyushu University, 6-1 Kasuga-koen, Kasuga, Fukuoka, 816-8580, Japan

<sup>2</sup> imec, Kapeldreef 75, B-3001 Heverlee (Leuven), Belgium

<sup>3</sup> The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka Ibaraki, Osaka, 567-0047, Japan

\*Corresponding author, e-mail: kazu-n@cm.kyushu-u.ac.jp

### Abstract

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Single-use, disposable nonvolatile memory devices hold a promise for the novel applications on internet of everything (IoE) technology by storing the health status of individual human in daily life. However, conventional memory devices are not disposable since they are mostly composed of non-renewable, non-biodegradable, and some toxic materials, causing a serious damage for ecological system when they are emitted to the environment. Here we demonstrate an environment-friendly, disposable nonvolatile memory device composed of 99.3 vol.% nanocellulose. Our memory device consists of a nanocellulose-based resistive-switching layer and nanopaper substrate. The device exhibited the nonvolatile resistive switching with the capability of multilevel storage and the potential scalability down to single nanofiber level (ca. 15 nm). Furthermore, the biodegradability of our memory device was confirmed by burying it into the natural soil for 26 days.

**Keywords** Cellulose nanofibers, nanopaper, resistive switching memory, biodegradation, disposable

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## Momentum-Resolved Electron Energy-Loss Spectroscopy of MoS<sub>2</sub>

Chien-Ting Wu<sup>1\*</sup>, Ming-Wen Chu<sup>2</sup>

<sup>1</sup> National Nano Device Laboratories, National Applied Research Laboratories, Hsinchu City, Taiwan

<sup>2</sup> Center for Condensed Matter Sciences, National Taiwan University, Taipei 106, Taiwan

\*Corresponding author, e-mail: ctwu@narlabs.org.tw

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### Abstract

Momentum( $q$ )-resolved electron energy-loss spectroscopy (EELS) is a classical, while nowadays largely unexploited, method to study electronic properties of materials. The grand advantage of this technique is that the electronic essence of given excitations, ranging from single-particle interband transitions to collective plasmon oscillations, can be unambiguously identified through the corresponding dispersion characteristics in reciprocal,  $q$ -resolved space.

In this study, we report  $q$ -dependent EELS studies in free-standing MoS<sub>2</sub>. With the assistance of optimal  $q$  resolution and simultaneous extended  $q$ -resolving range, we were able to explore the plasmon dispersion characteristic of 2D collective excitations along the two principal in-plane directions,  $\Gamma M$  and  $\Gamma K$ . In addition, a further tilting intending to tackle the out-of-plane electronic contribution was also performed.

We also conducted the impact-parameter dependent EELS investigations of the layered MoS<sub>2</sub> in nonetheless spatially-resolved approach in scanning transmission electron microscopy (STEM). These STEM-EELS studies show that the predominant excitations at  $\sim 23$  eV arise from the resonances of  $\pi+\sigma$  plasmons. Moreover, we observed several peaks with the characteristics of surface excitations, showing intriguing decays in peak intensities as a function of impact parameters (0–10 nm) from the material surface. Discussions corroborating the  $q$ - and spatially-resolved EELS investigations are also elucidated.

**Keywords:** electron energy-loss spectroscopy, MoS<sub>2</sub>, plasmons

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## Direct Observation of Inhomogeneous Growth and Dissolution of Silver Nanoparticles Using In Situ Liquid TEM

Tae-Young Ahn, Seung-Pyo Hong, Seong-Il Kim, and Young-Woon Kim\*

Department of Materials Science and Engineering, Seoul National University, 599 Gwanak-ro  
Gwanak-gu Seoul, Korea

\*Corresponding author, e-mail: [youngwk@snu.ac.kr](mailto:youngwk@snu.ac.kr)

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### Abstract

The properties of metal nanoparticles are significantly affected by their size distribution and aspect ratio, as well as faceting of the crystallographic surfaces. The aim of this presentation is to gain insight into the inhomogeneous growth and dissolution of silver nanoparticles using real-time liquid transmission electron microscopy. The solution used to fill the liquid cell was prepared by dissolving silver nitrate ( $\text{AgNO}_3$ ) in deionized (D.I) water.

Small equiaxed silver nanocrystals were observed for the first 5 seconds with electron beam irradiation. Inhomogeneous, preferential growth was observed started from one corner of the frame after 35 seconds. Growth of the silver nanoparticles from seeds was anisotropic, with unidirectional growth. Subsequently, growth of nano-particles located at the center of the screen occurred after 55 seconds. In the same region of unidirectional growth, dissolution of nanoparticles were also observed. After 75 seconds of exposure, reactions of particle growth were occurred in the region of the unchanged. Finally, after 95 seconds, growth saturated, and silver nanoparticles covered the entire observation area. The interpretation of the inhomogeneous growth and dissolution of silver nanoparticles were to be discussed using the preferential reaction by the radiolysis.

**Keywords** In-situ TEM, Liquid cell, Radiolysis, crystal growth

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## Effect of Ti Diffusion on the Microstructure of $\text{Ge}_2\text{Sb}_2\text{Te}_5$ in Phase-Change Memory Cell

Jucheol Park<sup>1\*</sup>, JunSoo Bae<sup>2</sup>, Sang-Hoon Lee<sup>1</sup>

<sup>1</sup> Future Strategy Research Institute, Gumi Electronics & Information Technology Research Institute, Gumi 730-853, South Korea

<sup>2</sup> Advanced Technology Development Team, Memory Division, Samsung Electronics Co., Ltd, Nongseo-Dong Mt.24, Giheung-Gu, Yongin-si, Gyeonggi-do, 449-900, Korea

\*Corresponding author, e-mail: jcpark13@geri.re.kr

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### Abstract

The dependence of the microstructure of  $\text{Ge}_2\text{Sb}_2\text{Te}_5$  (GST) on Ti diffusion into GST by annealing in  $\text{Ge}_2\text{Sb}_2\text{Te}_5/\text{Ti}/\text{TiN}$  PRAM stack was studied by various TEM techniques. The microstructure and crystal structure of GST was identified with high resolution electron microscopy (HREM) and image simulation technique, and the Ti diffusion into GST was revealed by STEM-EDS analysis. It was observed that Ti atoms of Ti/TiN thin layers were incorporated into GST cell through several thermal annealing steps and they could retard the phase transition from FCC into HCP phase partially and restrain the increase of grain size. Thus, it is concluded that Ti diffusion can affect the microstructure of GST including the type of the crystal phase and grain size of GST. It was shown that the insertion of diffusion barrier between TiN and GST could block Ti diffusion into GST and make it possible for FCC phase to completely transform into HCP phase.

**Keywords** 3 PRAM,  $\text{Ge}_2\text{Sb}_2\text{Te}_5$ , microstructure, FCC, Ti diffusion

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## Synthesis of Black Phosphorus Nano-Powder and TEM Studies of Structural Evolution

Jonghan Won<sup>1\*</sup>, Chang Geun Song<sup>1</sup>, Hyung Joong Yoon<sup>1</sup>, Cheul Jong Choi<sup>2</sup>, Jucheol Park<sup>3</sup>

<sup>1</sup> The Advanced Nano Surface Research Group, Korea Basic Science Institute, 169-148 Gwahak-ro, Daejeon, Korea

<sup>2</sup> Gumi Electronics & Information Technology Research Institute, 17 Chomdangieop, Gumi, Korea

<sup>3</sup> School of Semiconductor and Chemical Engineering, Semiconductor Physics Research Center, Chonbuk National University, Jeonju, Korea

*\*Corresponding author, e-mail: [jhwon@kbsi.re.kr](mailto:jhwon@kbsi.re.kr)*

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### Abstract

Physical properties of black phosphorus can be tremendously different from bulk when it exists as a single layered structure. Recently, a single layered structure black phosphorus has attracted great attention due to great potentials in optical and electronic application. To achieve single atomic layered BP structure, high purity and large amount of BP source is indispensable. Up to now, several methods such as high pressure (up to 12 kbar) sintering or transport reaction with mineralization additives have been used to synthesize black phosphorus structure. However, those methods still have problem such as difficulty in synthesis process or remaining of additives, respectively. To overcome those weaknesses, we synthesized black phosphorus nano-powder from red phosphorus by means of high speed attrition milling method. The microstructure of synthesized black phosphorus nano-powder was studied using x-ray diffractometer, (s) transmission electron microscopy with EELS in detail.

**Keyword:** Black phosphorus, attrition milling, transmission electron microscopy, nano-powder

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## Backscattered Electron Technique for Carbon Nanotube Characterization

Visittapong Yordsri<sup>1,2\*</sup>, Winadda Wongwiriyan<sup>2,3,4</sup>, Chanchana Thanachayanont<sup>1</sup>

<sup>1</sup> National Metal and Materials Technology Center, 114 Thailand Science Park, Paholyothin Rd., Klong 1, Klong Luang, Pathumthani, Thailand

<sup>2</sup> College of Nanotechnology, King Mongkut's Institute of Technology Ladkrabang, Chalokkrung Rd., Ladkrabang, Bangkok, Thailand

<sup>3</sup> Nanotec-KMITL Center of Excellence on Nanoelectronic Device, Chalokkrung Rd., Ladkrabang, Bangkok, Thailand

<sup>4</sup> Thailand Center of Excellence in Physics, CHE, Si Ayutthaya Rd., Bangkok, Thailand

\*Corresponding author, e-mail: visittay@mtec.or.th

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### Abstract

Carbon nanotubes (CNTs) are one of the most promising nanocarbon materials that have been widely used in many research fields and applications. A high-resolution technique such as transmission electron microscopy (TEM) is generally required to characterize the internal structure of CNTs. However, because of the high cost and high degrees of expertise in operation and data analysis of TEM, there is a challenge to find a new technique with simple and quick analysis. In this study, the backscattered electron (BSE) is utilized for characterizing different structures of CNTs. The BSEs are primary electrons that have been scattered by sample atoms. As the intensity of the BSEs is proportional to the mean atomic number of the atoms, they provide information in sample composition by an image with different contrast. Elements of greater atomic mass or high density appear brighter than lighter element or lower density in BSE image. Using BSE technique, CNTs with similar diameter but difference in their internal structures can be directly identified. Fig. 1 shows BSE images obtained from multiwalled CNTs and bamboo-like CNTs. Differences in arrangement of carbon atoms at tubular wall structure can be clearly observed.

**Keywords:** backscattered electron, carbon nanotubes, bamboo-like carbon nanotubes, internal structure

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## Three Dimensional Shape Analysis of Palladium Nanoparticles by DART

Tomokazu Yamamoto<sup>1, 2\*</sup>, Koji Shigematsu<sup>1</sup>, Syo Matsumura<sup>1, 2, 3</sup>

<sup>1</sup> Department of Applied Quantum Physics and Nuclear Engineering, Kyushu University,  
744 Motoooka, Nishi-ku, Fukuoka 819-0395, Japan

<sup>2</sup> JST ACCEL, 7 Gobancho, Chiyoda-ku, Tokyo 102-0076, Japan

<sup>3</sup> Ultramicroscopy Research Center, Kyushu University  
744 Motoooka, Nishi-ku, Fukuoka, 819-0395, Japan

\*Corresponding author, e-mail: yamamoto@hvem.kyushu-u.ac.jp

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### Abstract

Palladium nanoparticles are important catalysts in organic chemistry. The nanoparticle size, shapes and surface information are important factors for their catalytic properties. The electron tomography is useful tool to determine three dimensional (3D) structure of nanomaterials. Filtered back projection (FBP) and simultaneous iterative reconstruction technique (SIRT) are often used for electron tomography reconstruction. However these conventional algorithm cannot reconstruct accurate shape under limitation of image acquisition angle in TEM. In addition, these conventional algorithm need has a many images. In resent years several advanced reconstruction algorithms, such as discrete algebraic reconstruction technique (DART) and compressed sensing base algorithm were developed, which make it possible to reconstruct more accurate 3D structure from a small number image set. In this study, shapes of palladium nanoparticles were measured by electron tomography with SIRT and DART algorithms for a small number set of HAADF-STEM images and comparison of reconstructed images was performed. The cuboidal shape were successfully reconstructed by both algorithm. While SIRT reconstructions show severe missing wedge artifacts for the projection angle range smaller than  $\pm 60^\circ$ , DART reconstructions show more accurate shape even for a projection range of  $\pm 50^\circ$  with angle step of  $10^\circ$ .

**Keywords** palladium nanoparticle, electron tomography, DART

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## Substructure of Nanometer-Sized Unit of Lath Martensite in Low Carbon Steel by Ausforming

Ya-Ling Chang<sup>1</sup>, Shao-Pu Tsai<sup>1</sup>, Po-Yu Chen<sup>1</sup> and Jer-Ren Yang<sup>1</sup>

<sup>1</sup>Department of Materials Science and Engineering, National Taiwan University, Taipei, Taiwan

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### Abstract

This research mainly focuses on the microstructure of lath unit in lath martensite structure after ausforming process. The specimen was deformed at 900°C with 50% compressive strain followed by quenching to room temperature to obtain nanometer-sized lath martensite structure. In a single prior austenite grain, the dislocation slip and strain orientation map are investigated by transmission electron backscatter diffraction (t-EBSD) and scanning transmission electron microscopy (STEM). It is believed that these two high resolution characterization methods improve the understanding of deformation substructure in nanoscale.

**Keywords** transmission electron backscatter diffraction (t-EBSD), scanning transmission electron microscopy (STEM), lath martensite.

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## Mechanism of Nodule Particle Formation in Sintered Fe-Mo + SiC Alloys Revealed by SEM and SXES

Ussadawut Patakham<sup>1</sup>, Bralee Chayasombat<sup>1</sup>, Dhriti Tanprayoon<sup>1</sup>, Kittikhun Ruangchai<sup>2</sup>, Amporn Wiengmoon<sup>2</sup>, Ruangdaj Tongsri<sup>1\*</sup>

<sup>1</sup>Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120 Thailand

<sup>2</sup>Department of Physics, Faculty of Science, Naresuan University, Pitsanulok-Nakhon Sawan Road, Tha Pho, Muang, Pitsanulok 65000 Thailand

\*Corresponding author: ruangdt@mtec.or.th

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### Abstract

Ductile iron-like microstructure was observed in the Fe-Mo +  $x$  wt.% SiC ( $x = 3$  and  $4$ ) material sintered at high temperatures. In this type of microstructure, black nodular particles were enveloped by ferrite grains, which were surrounded by pearlitic structure. Since the materials processing route employed for production of the Fe-Mo +  $x$  wt.% SiC material is sintering, which is an uncommon process for producing a ductile iron, characterization of black nodular particles is necessary to understand their formation mechanism. Characterization of several large black nodular particles using scanning electron microscopy and soft X-ray emission spectroscopy revealed interactions between SiC particle and Fe-Mo matrix. Due to the characterization results, large black nodular particles generally showed core-shell structures. The cores and shells were different from particle to particle. The melt created around the cores of SiC and/or Fe-Si-C was the cause of core-shell structure formation. In some cases, during cooling the sintered materials, graphite was found to nucleate and grow on the surface of Fe-Si-C core, formed within peripheries of the prior SiC particle sites. Characterization of fine black nodules revealed that these particles were graphite particles, which recrystallized from the melt, similar to the case of conventional cast ductile-iron.

**Keywords:** Fe-Mo-Si-C alloy, sintering, ductile iron-like microstructure, core-shell particle, graphite crystallization

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## ***In Situ* Observation of Electrochemical Reactions in All-Solid-State Li-Ion Batteries by Electron Holography and Spatially-Resolved TEM EELS**

Kazuo Yamamoto<sup>1\*</sup>, Atsushi Shimoyamada<sup>1</sup>, Yasutoshi Iriyama<sup>2</sup>, Tsukasa Hirayama<sup>1</sup>

<sup>1</sup> Japan Fine Ceramics Center, 2-4-1 Mutsuno, Atsuta, Nagoya, Japan.

<sup>2</sup> Dep. of Materials, Physics and Energy Engineering, Nagoya University, Nagoya, Japan.

\*Corresponding author, e-mail: k-yamamoto@jfcc.or.jp

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### **Abstract**

All-solid-state Li-ion batteries having incombustible solid electrolytes are promising candidates of next-generation batteries because they have some advantages of safety, long lifetime, low cost and high energy density. However, they have a serious problem that an interfacial resistance of Li-ion transfer is very large at the electrode/solid-electrolyte interfaces. To evaluate the battery reactions around the interfaces, we used electron holography and spatially-resolved TEM EELS during the charge-discharge cycle in a TEM. We prepared a TEM sample of Au/LiCoO<sub>2</sub>/Li<sub>1-x-y</sub>Al<sub>y</sub>Ti<sub>2-y</sub>Si<sub>x</sub>P<sub>3-x</sub>O<sub>12</sub>-solid-electrolyte/Pt by FIB and applied a voltage between Au and Pt. We successfully observed local electric-potential changes due to the Li insertion reaction around the solid-electrolyte/Pt interfaces by electron holography [1]. We also detected the Li concentration distribution around the interfaces by TEM EELS [2,3]. The Li profiles were similar to the local potential profiles. We show the details of the above methods and results in the conference.

One part using electron holography in this work was financially supported by Chubu Electric Power Co., Inc., and the other part using the EELS was supported by the RIGING project of the New Energy and Industrial Technology Development Organization (NEDO).

**Keywords:** Li-ion battery, electron holography, EELS

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## Electron Microscopy Study of Pure Zn and Zn-(0.5-3)wt.%Mg Alloys Related to Their Electrochemical Behavior in KOH Solution

Sankum Nusen<sup>1\*</sup>, Manut Jaimasit<sup>2</sup>, Torranin Chairuangsr<sup>1</sup>

<sup>1</sup> Department of Industrial Chemistry, Faculty of Science, Chiang Mai University,  
Chiang Mai 50200, Thailand

<sup>2</sup> Department of Materials Science, Faculty of Science, Phayao University,  
Phayao 56000, Thailand

\*Corresponding author, e-mail: nusen.san@gmail.com

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### Abstract

Zinc alloys containing 0.5 to 3 wt% of magnesium have been prepared by casting. These zinc alloys are expected to have improved electrochemical behavior in alkaline solution, so that they may be used as the anode in zinc-air batteries instead of pure zinc which has a drawback during recharging. Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy-dispersive X-ray spectrometry (EDS) were used to characterize the as-cast microstructure of pure zinc and the zinc alloys. It was found that the as-cast microstructure consists of dendrites of zinc-rich solid solution and the eutectic structure of zinc-rich solid solution and a magnesium-rich phase, expected to be  $Mg_2Zn_{11}$ . The volume fraction of the eutectic structure increased as the magnesium content in the alloy increased. Electrochemical behavior of the zinc alloys was studied by a self-corrosion testing and a potentiodynamic testing at room temperature using KOH solution as the electrolyte. Corroded surfaces of the zinc alloys after the self-corrosion testing and the potentiodynamic testing in the anodic range were characterized by SEM-EDS in comparison to those of the pure zinc. During self-corrosion testing, hydrogen evolution increased as the magnesium content and the volume fraction of the eutectic structure in the alloys increased. Self-corrosion occurred preferentially at the eutectic zinc-rich solid solution. This indicates that magnesium addition in the experimental range enhances self-corrosion of the zinc alloys in the potassium hydroxide solution. During the anodic range in the potentiodynamic testing, corrosion occurred preferentially on pro-eutectic dendrites of the zinc-rich solid solution, whereas the corrosion rate of both eutectic phases are comparable. From polarization curves, corrosion current of the Zn-3wt.%Mg alloy is slightly higher than that of pure zinc, suggesting their alternative potential. Finally, advantages and disadvantages of the Zn-Mg alloys and further study on recharging experiment will be discussed.

**Keywords** Zn-Mg Alloy, Zn Anode, SEM,

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## Preparation of Silicon-Germanium-Tin Nanocomposites on Nitrogen Doped Graphene for Use as Anode Materials in Lithium-ion Batteries

Thanapat Autthawong<sup>1\*</sup>, Thapanee Sarakonsri<sup>1,2</sup>

<sup>1</sup> Department of Chemistry, Faculty of Science, Chiang Mai University, 239 Huay Kaew Rd, Suthep, Muang, Chiang Mai 50200 Thailand.

<sup>2</sup> Materials Science Research Center, Faculty of Science, Chiang Mai University, Suthep, Muang, Chiang Mai 50200, Thailand.

\*Corresponding author, e-mail: Maman\_Chem@hotmail.co.th

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### Abstract

Lithium ion Batteries are widely used as energy source in electronic devices. The current commercial lithium ion battery is based on the use of graphitic carbon anode which provides low theoretical capacity. Therefore, new materials that have higher theoretical capacity and energy density are attracted especially Silicon, Germanium and Tin. Nonetheless, using of Silicon, Germanium and Tin as bulk electrode materials has high volumetric changing that lead to occur electrode cracking during cycling. Nitrogen doped graphene (NrGO) is proper to use as supporting materials to solve these problems. Because it has high surface area and can act as excellent supporting material to obstruct the volumetric changing. In addition, nitrogen-doping level could help improve the conductivity, rate capability and reversible capacity of graphene sheet. Silicon-Germanium-Tin nanocomposites on n-doped graphene are synthesized in this research by solution method. Two step processes are applied. First, Silicon-Tin nanocomposites was prepared on n-doped graphene. Second, Silicon-Tin/NrGO are dispersed in Germanium solution which was reduced by NaBH<sub>4</sub> solution to get Silicon-Germanium-Tin nanocomposites on n-doped graphene. The products are primary characterized by x-ray diffraction and scanning electron microscopy and transmission electron microscopy techniques. The obtained silicon-germanium-tin product is well distributed on n-doped graphene and appeared as small particle size. It is therefore suitable for the further use as the next generation anode materials.

**Keywords** Anode materials, Lithium ion battery, Nanocomposites

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## Preparation of Coral-like Magnesium Aluminium Layered Double Hydroxides on Activated Carbon for Waste Water Treatment Application

Patsaya Songkhum<sup>1</sup>, Kritapas Laohhasurayotin<sup>1\*</sup>

<sup>1</sup> National Nanotechnology Center, National Science and Technology Development Agency,  
Phaholyothin Rd., Pathumthani, Thailand,

\*Corresponding author, e-mail: [kritapas@nanotec.or.th](mailto:kritapas@nanotec.or.th)

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### Abstract

Human activities have affected the quality of our water environment – lakes, canals, rivers and etc. There are many approaches to deal with water pollution, including membrane separation, oxidation reaction, adsorption and ion-exchange. To our knowledge, activated carbon (AC) adsorption has been demonstrated as a facile and low-cost technique for water remediation; nevertheless, AC is not a universal material for all kinds of contaminants. Layer double hydroxides (LDHs), stable and safe materials, have widely used for toxic elimination by ion-exchange process. Recently, cooperative effects of Magnesium Aluminium (MgAl) -LDHs and AC for waste water treatment have been discovered. The preparation of coral-like MgAl-LDHs on AC was carried out by co-precipitation method. The diffraction peak of MgAl-LDHs shows major phase in amorphous carbon, and surface functional groups were examined using fourier-transform infrared (FT-IR) spectroscopy. The morphologies of MgAl-LDHs on AC were observed in form of coral-like MgAl-LDHs structure presented on AC surface by scanning electron microscope (SEM) equipped with energy dispersive x-ray (EDX) spectroscopy. Anion exchange property of MgAl-LDHs and adsorption behavior of AC are studied as promising effects to remove harmful contaminants (i.e. inorganic and organic chemical waste) from water resource. Cr(VI) and NO<sub>3</sub><sup>-</sup> ion were selected as waste models for waste remediation.

**Keywords** MgAl; LDHs; coral; adsorption; ion; exchange

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## Texture Orientation of Ag Thin films grown via Gas-Timing RF Magnetron Sputtering and their SERS Activity

Pongbordin Ukahapunyakul<sup>2</sup>, Annop Klamchuen<sup>1\*</sup>, Chaweewan Sapcharoenkun<sup>1</sup>, Alongkot Tritong<sup>1</sup>, Panita Kasamechonchung<sup>1</sup>, Mati Horprathum<sup>3</sup>, Supanit Porntheeraphat<sup>3</sup>, Jiti Nukeaw<sup>4</sup> and Nurak Gridsadanurak<sup>2\*</sup>

<sup>1</sup> National Nanotechnology Center (NANOTEC), NSTDA, 111 Thailand Science Park, Paholyothin Rd., Klong Nueng, Klong Luang, Thailand,

<sup>2</sup> Department of Chemical Engineering, Faculty of Engineering, Thammasat University, 99 Moo1, Paholyothin Rd., Klong Nueng, Klong Luang, Thailand,

<sup>3</sup> National Electronics and Computer Technology Center (NECTEC), NSTDA, 111 Thailand Science Park, Paholyothin Rd., Klong Nueng, Klong Luang, Thailand

<sup>4</sup> College of Nanotechnology, King Mongkut's Institute of Technology Ladkrabang, Chalongsak Rd., Ladkrabang, Bangkok 10520, Thailand

\*Corresponding author, e-mail: annop@nanotec.or.th

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### Abstract

Sputtered silver (Ag) thin films have been attractive for several advanced technologies, especially, an application for surface enhanced Raman scattering (SERS) substrate. It has been known that crucial factors of highly-sensitive SERS substrate based on Ag thin film are depended texture orientation and morphology of thin film. Although several research groups have demonstrated the controllability in the texture and surface morphology of sputtered Ag thin films, the external energy sources such as substrate bias voltage, heating the substrate during growth or post annealing and ionized magnetron sputter deposition (IMSD) are necessary to include. Such external added techniques make a complex and high cost operation and are not available to fabricate sputtered Ag film at room temperature. Here we demonstrate a special technique to control a texture orientation of Ag thin films via using gas-timing (GT) rf magnetron sputtering. We found that the GT technique as to the on-off sequence switching can be attributed to the alternate sequence between deposition mode and energy-assisted mode during thin film growth. Such switching modes through the GT technique not only provide the ability to adjust the number of sputter species from the target but also generate self-energy assisted, which related to the atomic peening effect. In addition, we exhibit that the high (111)/(200) ratio of Ag films strongly affects on the SERS activity of Ag films due to the small grain size and the gap between grain edges (i.e. hot spot effect). Our results highlight that utilizing the GT technique enables us to perform the texture engineering of metal thin films, which can apply for various research fields.

**Keywords** Silver thin films, Gas-timing rf magnetron sputtering, Atomic peening effect, Surface enhanced Raman spectroscopy (SERS)

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## Alumina Thin Film Coating by DC Magnetron Sputtering

Saisamorn Niyomsoan<sup>1\*</sup>, Paramet Laphasampan<sup>1</sup>, Thanchanok Sricum<sup>1</sup>, Kodchaporn Chotikaphukkana<sup>1</sup>, Nirun Witit-anun<sup>2</sup>

<sup>1</sup> JMAT, Faculty of Gems, Burapha University, Chanthaburi campus, Chanthaburi 22170, Thailand

<sup>2</sup> Department of physics, Faculty of Science, Burapha University, Chon Buri, 20130 Thailand

\*Corresponding author, e-mail: sniyomsoan@gmail.com; [saisamor@buu.ac.th](mailto:saisamor@buu.ac.th)

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### Abstract

Alumina ( $\text{Al}_2\text{O}_3$ ) thin film has widely been under investigation with several coating techniques. They are transparent. However, their hardness depends on the deposition methods. This study focused on coating alumina thin film on semi-precious gemstones by DC Magnetron sputtering method. Aluminium metal and oxygen gas were used as a target and reagent gas, respectively. Three coating parameters were varied; deposition distance at 5 -10 cm, oxygen flow rate at 4-8 sccm, and deposition time at 60 - 120 minutes. Influence on color of the gemstone substrate was investigated by using UV-vis NIR Spectrophotometer ranging from 300 to 800 nm and reported in CIE  $L^*a^*b^*$  color space. Microstructure of the films at all conditions was observed by Field Emission Scanning Electron Microscope (FE-SEM) equipped with EDS. The semi-precious gemstones after coated with aluminium oxide thin film at all conditions had their color obviously changed. Coated fluorite and apatite changed to different hues while other coated stones still maintained the same hue. Increasing deposition distance decreased the film thickness but rather provided smoother film surface without altering Al:O atomic ratio. The film thickness was in a range of 100 nm. Scratch resistance of the coated semi-precious gemstones was also investigated.

**Keywords**  $\text{Al}_2\text{O}_3$  thin film, DC magnetron sputtering, microstructure

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## Modification of Sintered Fe-Mo-Si-C Alloy by Cu and Ni Additions

Monnapas Morakotjinda, Rungtip Krataitong, Thanyaporn Yotkaew, Nattaya Tosangthum, Bhanu Vetayanugul, Ussadawut Patakham, Ruangdaj Tongsri \*

Powder Metallurgy Research and Development Unit (PM\_RDU), Thailand National Metal and Materials Technology Center, 114 Paholyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120 Thailand \*Corresponding author : ruangdt@mtec.or.th

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### Abstract

Sintered Cu- and Ni-containing Fe-Mo-Si-C alloys were prepared from pre-alloyed Fe-Mo, plus fixed 4 wt.% silicon carbide, plus fixed 2 wt.% Cu or Ni and varied graphite powder contents. Microstructural investigation was performed by using optical microscopy and scanning electron microscopy in association with energy dispersive x-ray spectroscopy chemical analysis. The microstructural characterization showed that the sintered alloys had microstructural features similar to that of a ductile iron and/or compacted graphite iron, depending on added graphite content. Without graphite addition, black nodular particles were enveloped by ferrite grains, which were surrounded by bainitic structure. With increasing added graphite content, the black nodular particles were changed to compacted graphite-like particles and the bainite fraction was increased. Tensile strengths and hardness of the sintered materials also increased with increasing added graphite content (up to 0.3 wt.%) whereas ductility of the sintered materials were governed by type and content of alloying Cu or Ni elements.

**Keywords:** Fe-Mo-Si-C alloy, Cu and Ni additions, sintering, microstructural characterization, microscopy, mechanical property

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## Cs-corrected STEM Study on Sr-doped Lanthanum Cobaltite Thin Films

Yoonkoo Kim<sup>1</sup>, Miyoung Kim<sup>1\*</sup>

<sup>1</sup> Department of Material Science and Engineering, Seoul National University, A Road, Seoul, South Korea

\*Corresponding author, e-mail: mkim@snu.ac.kr

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### Abstract

Perovskite cobaltite thin films attract considerable interests in terms of their remarkable magnetic and relevant transport properties which are highly dependent on subtle structural distortions generated by strain from substrates. As a result, fundamental understanding of the strain relaxation mechanisms of the cobaltite thin films is essential for manipulating and tuning their magnetic properties.

Particularly, the presence of dark stripes in Lanthanum cobaltite thin films is known to be responsible for their magnetic properties and to be related to the strain relaxation.[1][2]. So far, various strain relaxation mechanisms, including the spin-state ordering of Co cations[1] and the ordering of oxygen vacancies in the film[2], have been proposed to account for the origin of dark stripes. Nevertheless, the exact process of strain relaxation in La/Sr cobaltite thin films is still under debate.

Here, we study the relation between the dark stripes and oxygen vacancies in the cobaltite thin films. We prepared Sr-doped lanthanum cobaltite films with different Sr compositions and under different annealing conditions. We compare the density of dark stripes in these films, and there were no difference in the dark stripes in the samples with the Sr composition larger than 0.3. Oxygen deficiencies and electronic structures in the dark strips are further examined using electron energy loss spectroscopy to understand the nature of the structural modulation.

**Keywords** Aberration-corrected STEM, Lanthanum Cobaltite, Electron Energy Loss Spectroscopy

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## Study of Low Voltage Contrast Imaging of Graphene by the Linkage with SEM and SPM

Shuichi Takeuchi<sup>1\*</sup>, Yoichiro Hashimoto<sup>1</sup>, Kazunori Ando<sup>2</sup>, Takeshi Sunaoshi<sup>1</sup>, Atsushi Miyaki<sup>1</sup>, Masahiro Sasajima<sup>3</sup>, Takehiro Yamaoka<sup>2</sup>

<sup>1</sup> Application Development Dept., Hitachi High Technologies Corp., Hitachinaka, Japan

<sup>2</sup> Analytical Application Engineering Section Tokyo 2, Hitachi High-Tech Science Corp., Kawasaki, Japan

<sup>3</sup> Electron Microscope Systems Design 1<sup>st</sup> Dept., Hitachi High-Technologies Corp., Hitachinaka, Japan

\*Corresponding author, e-mail: shuichi.takeuchi.vf@hitachi-hightech.com

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### Abstract

To clarify the origin of image contrasts of graphene observed with the Scanning Electron Microscope (SEM), the relationship between the contrasts, the thickness which means the number of layers and the difference of surface potential was studied with the linkage of SEM and Scanning Probe Microscope (SPM). The linkage of stage coordinates between SEM and SPM was achieved by registering three alignment positions in the both systems. Darker patch structures which have a few levels of darkness and some darker line structures were observed by SEM on graphene surface which was grown by Chemical Vapor Deposition (CVD) technique. About 0.3 nm difference of height which is almost same as one layer thickness of graphene was observed between the patch structure and surrounding area with Atomic Force Microscope (AFM). Up to 40 mV difference of surface potential between the patch structure and the surrounding area was confirmed by AFM-based technique Kelvin Force Microscope (KFM). These results imply that the SEM contrasts might be provided by the difference of surface potential which is caused by the difference of its thickness.

**Keywords** Scanning Electron Microscope (SEM), Kelvin Force Microscope (KFM), Atomic Force Microscope (AFM), graphene, surface potential

## A Sample Extend Abstract Title Design and Application of In-situ TEM Holder with Electrochemical Function at Operating Condition

Mu Tung Chang<sup>1\*</sup>, Shen-Chuan Lo<sup>1</sup>, C.Y.Hsieh<sup>1</sup>, Sung-Yen Wei<sup>1</sup>, Ren-Fong Cai<sup>1</sup>

<sup>1</sup>Dept. of Electron Microscopy Development and Application, Material and Chemical Research Laboratories, Industrial Technology Research Institute (ITRI), No. 195, Chung Hsing Road, Sec. 4, Hsinchu 31040, TAIWAN

\*Corresponding author, e-mail: mtchang@itri.org.tw

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### Abstract

Solid state Li-ion battery has the potential for the next generation battery with high energy density and safe performance. In-situ Transmission Electron Microscope (TEM) with electrochemical platform is a powerful technique for driven-reaction mechanism study of solid state Li-ion battery with spatial resolution down to atomic scale. In this study, we develop the design and application of in-situ TEM holder with electrochemical function at operating condition. The retractable kit which is compatible both in the focus ion beam (FIB) and TEM is fabricated in this design. It is very similar to real environment and scale in Li-based battery. In addition, the combination of in-situ TEM/STEM image and EDS/EELS analysis represents a helpful tool to link the physical and chemical structure of observed target to their macroscopic properties. The achievements of this study will be adopted by ITRI as the technical base for future analysis works which are related to in-situ TEM observations. The design and application of in-situ TEM holder with battery platform at operating scale is to establish advanced electron microscopy analysis techniques for unknown mechanism study.

**Keywords** in-situ TEM, Li-ion battery, electrochemistry, solid state Li-ion battery

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## First Results of Prototype EELS System for Low Voltage Cold FE-SEM

Satoshi Okada<sup>1\*</sup>, Yu Yamazawa<sup>1</sup>, Zulihuma Yassenjiang<sup>1</sup>, Takeshi Sunaoshi<sup>1</sup>, Kazutoshi Kaji<sup>1</sup>

<sup>1</sup> Nanotechnology Products Business Group, Hitachi High-Technologies Corp., Hitachinaka-shi, Japan

\*Corresponding author, e-mail: satoshi.okada.rj@hitachi-hightech.com

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### Abstract

For various fields of research and development, a role of an electron microscope is becoming more important for not only imaging but also chemical characteristics analysis. Since inorganic materials such as carbon nano-tube and graphene sheet are not strong against electrons accelerated to higher than 100 kV, to lower the accelerating voltage is one of effective approaches for reducing damage. Field-emission scanning electron microscope (FE-SEM), Hitachi SU9000, gives not only secondary electron (SE) images but also scanning transmission electron microscope (STEM) images at 30 kV or lower voltages. In present study, we have developed a prototype of an electron energy loss spectrometer (EELS) for SU9000. Energy resolution is attainable to less than 0.4 eV near energy width of a Cold-Field Emission electron gun. SU9000 that performs high spatial resolution imaging can also provide elemental and chemical information with high energy resolution by using the prototype of EELS system.

**Keywords** SEM, EELS, low-voltage

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## Quantitative Microanalysis at Low Voltage with a WDS Electron Microprobe Equipped with a Fe Column

Pierre-Yves Corre<sup>1</sup>, Chrystel Hombourger<sup>2</sup> and Michel Outrequin<sup>2</sup>

<sup>1</sup> Ametek, CAMECA Business Unit, 19/36 Mckee St, Maroubra, 2035 NSW Australia

<sup>2</sup> CAMECA, 29 Quai des Grésillons, 92622 Gennevilliers Cedex, France

\*Corresponding author, e-mail:

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### Abstract

Thanks to its precision, its reproducibility and its stability, Electron Microprobe is a well suited technique for accurately analyzing nearly all chemical elements at concentration levels down to few 10's ppm with a spatial resolution of about 1  $\mu\text{m}$ , which is relevant to microstructures in a wide variety of materials and mineral specimens.

With the development of the Schottky emitter and its implementation as electron source in Electron Microprobe, small features are commonly analyzed down to sub-micrometer scale. Thanks to the high brightness of the Schottky emitter, a fine focussed electron beam can be achieved with both high and stable beam currents even at low accelerating voltages ( $\leq 10$  keV).

Since X-rays are generated from a much larger diameter than the diameter of the incident electron beam, it is necessary to optimize the two interdependent parameters, accelerating voltage and beam diameter, in order to take full advantage of the FEG electron source for X-ray analysis. The electron beam diameter increases when decreasing the electron beam energy. The interaction volume - within which scattered electrons generate X-rays - decreases with the electron energy. Thus a small beam diameter is not always synonym of a small interaction volume and optimized conditions are obtained when the analytical spatial resolution is primarily limited to the diameter of the X-ray emission volume in a specific material.

The ability to accurately quantify precipitate phases on the micrometer and sub-micrometer scale when working at low beam energy with high spatial resolution will be illustrated in examples such as Dunite, igneous rock locally enriched with Platinum. The analytical resolution determined from X-ray maps will be presented. All samples were analyzed with the CAMECA SXFiveFE.

[1] The authors acknowledge Ph. de Parseval and B. Abily from GET for sharing samples.

**Keywords** fe-epma, quantitative analysis, x ray mapping

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## Scanning Transmission Electron Microscope –Transition Edge Sensor (STEM-TES)

Keiichi Tanaka<sup>1\*</sup>, Toru Hara<sup>2</sup>, Kazuhisa Mitsuda<sup>3</sup>, Keisuke Maehata<sup>4</sup>

<sup>1</sup> Hitachi High-tech Science Corp., 36-1 Takenoshita, Oyama-cho, Shizuoka, Japan

<sup>2</sup> National Institute for Materials Science, 1-2-1 Sengen, Tsukuba, Ibaraki, Japan

<sup>3</sup> Japan Aerospace Exploration Agency, 3-1-1 Yoshinodai, Chuo-ku, Sagami-hara, Kanagawa 252-5210, Japan

<sup>4</sup> Kyushu university, 944 Motooka, Nishi-ku, Fukuoka, Japan

\*Corresponding author, e-mail: keiichi.tanaka.sf@hitachi-hightech.com

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### Abstract

Scanning transmission electron microscope- transition edge sensor (STEM-TES) system has been developed to detect the small X-ray peaks caused by the minor elements which are buried for x-ray peaks of the major elements for a small area (typically  $< 10$  nm). This energy resolution is ten-fold higher than the Si(Li) type EDS. The TES is a kind of energy dispersive x-ray detector with high energy resolution ( $< 10$  eV). The TES system needs to cool the detector around 0.1 K to achieve the high energy resolution. Our system adopted the separate type dilution refrigerator to operate the system continuously. The Si lattice image was observed without being affected by the vibration of mechanical cooler used in this system. The peak center positions of X-ray spectrum ( $\text{Si-K}_\alpha$ ) was stable within 1 eV between 1<sup>st</sup> spectrum and 2<sup>nd</sup> spectrum. The detection time was 600 s for 1<sup>st</sup> and 2<sup>nd</sup> spectrum. The 2<sup>nd</sup> spectrum was obtained after the 600 s the 1<sup>st</sup> spectrum. The present system is very stable. Judging from two separate measurements, energy deviation is less than 1 eV and the resolution achieved between  $8 \pm 0.3$  eV.

**Keywords** 3-5 words (*STEM, TES, EDS, Spectrum*)

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## Valance and Coordination Mapping Using Monochromated STEM EELS

He Tian\*

State Key Laboratory of Silicon Materials and School of Materials Science & Engineering, Zhejiang University, China

\*Corresponding author, e-mail: hetian@zju.edu.cn

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### Abstract

STEM EELS technique, especially combined with Monochromator, has been proved with great ability to map valence and coordination at nano, even atomic scale. Here, I will report some applications of this method, by taking advantage of high-angle annular dark field STEM in combination with monochromated EELS, with high spatial and energy resolution:

(i) Understanding the alteration processes of the pigments is of key importance both to optimize the conditions in which works of art are stored and to allow selection of appropriate restoration treatments. We will demonstrate how insights into the alteration mechanism of pigments can be gained at the nano-scale level. 100 years old paint was investigated using state of the art transmission electron microscopy and spectroscopy, revealing the mechanism why some bright yellow colors in Vincent Van Gogh's paintings (for example in *Les Alyscamps*, 1888) are turning brown over time.

(ii) By combining atomic resolution HAADF STEM, GPA, ABF STEM, and monochromated STEM EELS, the coordination and oxidation state of near interface cations has been determined in great detail. Here, we will demonstrate the feasibility of oxidation state control by strain modification and show direct evidence of how a local strain field change the atomic coordination and introduces atomic displacements leading to a ferroelectric like polarization near an interface.

**Keywords** *Valance mapping, coordination mapping, STEM EELS*

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## Measurement of Fogging Electron Current in Scanning Electron Microscope

Yoshifumi Hagiwara<sup>1,2</sup>, Taku Noda<sup>1,2</sup>, Masatoshi Kotera<sup>1</sup>, Raynald Gauvin<sup>2</sup>

<sup>1</sup>Major in Electrical and Electronic Engineering,  
Osaka Institute of Technology, 5-16-1, Omiya, Asahi-ku, Osaka, Japan

<sup>2</sup>Department of Materials Engineering,  
McGill University, 845 Rue Sherbrooke, Montreal, Canada  
Yoshifumi Hagiwara, E-mail: yoshi\_oit120@yahoo.co.jp

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### Abstract

Electron beam is used in various nanotechnologies. However, if an insulator is irradiated by the beam, electrons are accumulated in the specimen, and negative charging occurs. Not only beam electrons, but also fogging electrons, which are produced by multiple backscattering events occurred between the specimen and the optical components in the vacuum. It comes to be recognized that the fogging electrons give a wide-range contribution in charging. The contribution on the energy deposited is negligible, but if the electron stays at the position provided for a long time, the accumulated charge continues to deliver the influence.

We have measured the current to understand the influence of fogging electrons at the specimen surface in a scanning electron microscope. We made a printed circuit board to measure the current, as a function of electron beam acceleration voltage. When electron beam is irradiated on the central annular electrode, fogging electrons flow out to the ground from each electrode, fogging electron current is measured at the outer electrode. We found that the number of fogging electrons with an irradiated electron shows a clear linearity as a function of acceleration voltage, and the electron energy to make the same number of fogging electrons is 6keV.

**Keywords** scanning electron microscope, measurement of fogging electron current distribution

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## Technical Development of Cryo-SEM Workflow for Direct Observation of a Hydrated Emulsion Adhesive

Yuri Nishino<sup>1,2\*</sup>, Ayumi Ishihara<sup>1,3</sup>, Yoshiko Ito<sup>1,3</sup>, Atsuo Miyazawa<sup>1,2\*</sup>

<sup>1</sup> Graduate School of Life Science, University of Hyogo, 3-2-1, Kouto, Kamigori-cho, Ako-gun, Hyogo, Japan

<sup>2</sup> RSC-University of Hyogo Leading Program Center, RIKEN SPring-8 Center, 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo, Japan

<sup>3</sup> Leica Microsystems K. K., 1-29-9 Takadanobaba, Shinjuku-ku, Tokyo, Japan

\*Corresponding author, e-mail: atsuo@sci.u-hyogo.ac.jp

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### Abstract

To analyzing the internal structures of multi-component fluid materials, such as emulsions including inter-particle spacing by the techniques of cryo-electron microscopy, it is necessary to physically fix the material and make wide areas with their smooth cross-sectional surface. We have developed a system that involves the following steps: preserving the structure of an emulsion adhesive using cryo-fixation in its moisture state and during the drying process after being coated, preparing the cross-sections of the internal structure using a cryo-ultramicrotome, and transferring the cross-sections into a cryo-scanning electron microscope for the observation via a cryo-transfer system. This system could allow the direct observation of the structural changes occurring in hydrated emulsion adhesives during drying<sup>1</sup>.

**Keywords** cryo-scanning electron microscopy, cryo-ultramicrotome, cryo-transfer system

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## Development of Trajectory Simulation of Fogging Electrons in a Vacuum Specimen Chamber

Kazumasa Terada<sup>1\*</sup>, Taiki Nishino<sup>2</sup>, Taku Noda<sup>2</sup> and Masatoshi Kotera<sup>1,2</sup>

<sup>1</sup> Department of Electronics, Information and Communication Engineering,  
Osaka Institute of Technology, 5-16-1, Omiya, Asahi-ku, Osaka, Japan

<sup>2</sup> Major in Electrical and Electronic Engineering,  
Osaka Institute of Technology, 5-16-1, Omiya, Asahi-ku, Osaka, Japan

\*Corresponding author, e-mail: Kazumasa\_pyon@yahoo.co.jp

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### Abstract

Electron beam can be focused in nanometer scale, and it is an indispensable tool in nanotechnologies. However, recently it is known that fogging electrons made by multiple re-backscattering events between specimen surface and optical components of the vacuum chamber can be a cause of degradation of the spatial resolution. The fogging electrons spread several 10 mm, and they may be a cause of a global charging in the optical system. Therefore, we develop a simulation in order to gain the quantitative knowledge of fogging electrons. Primary electron trajectory is expressed based on the continuous slowing down approximation, and cascade multiplication of secondary electrons is considered using a Monte Carlo simulation.[1-4] To check the validity, we measured a radial distribution of fogging electron current at the specimen surface in an ordinary scanning electron microscope. The current is measured by concentric annular electrodes. Here, the beam current is 1nA and the electron beam acceleration voltage is 0.8kV. If electrons stop in the specimen, they are assumed to flow as the current. The agreement is good, but if the radius is large, the difference becomes appreciable, especially at larger working distances, and we are trying to find the reason of this phenomenon.[5]

**Keywords** Monte-Carlo simulation, multiple-backscattered-fogging-electrons

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## Structure Retrieval Using Segmented Detectors in Scanning Transmission Electron Microscopy

H. G. Brown<sup>1\*</sup>, A. J. D'Alfonso<sup>1</sup>, Z. Chen<sup>2</sup>, A. J. Morgan<sup>3</sup>, M. Weyland<sup>4,5</sup>, C. Zheng<sup>2,6</sup>, M. S. Fuhrer<sup>2</sup>, S. D. Findlay<sup>2</sup> and L. J. Allen<sup>1</sup>

<sup>1</sup>School of Physics, University of Melbourne, Parkville, Victoria 3010, Australia

<sup>2</sup>School of Physics and Astronomy, Monash University, Clayton, Victoria 3800, Australia

<sup>3</sup>Centre for Free-Electron Laser Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany

<sup>4</sup>Monash Centre for Electron Microscopy, Monash University, Clayton, Victoria 3800, Australia

<sup>5</sup>Department of Materials Science and Engineering, Monash University, Clayton, Victoria 3800, Australia

<sup>6</sup>Department of Civil Engineering, Monash University, Clayton, Victoria 3800, Australia

\*Corresponding author, e-mail: hbrown@student.unimelb.edu.au

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### Abstract

Segmented detectors are often used in scanning transmission electron microscopy because they allow measurement of magnetic and electric fields and are sensitive to light atoms [1]. Here a method for the retrieval of the sample transmission function using segmented detector data is presented.

For a thin sample, scattering of the illumination may be represented by multiplication with a transmission function. A linearized set of equations are constructed that relate the signal measured by segmented detectors to the transmission function for each probe position in a scanning transmission electron microscopy set up. The resulting matrix equation is solved using the conjugate gradient least squares [2] method.

Application to a simulated dataset confirms the validity of the retrieval method. Quantitative agreement between the retrieved transmission function for an experimental dataset from a MoS<sub>2</sub> monolayer and that simulated using parametrized atomic scattering factors is achieved if an appropriate amount of sulfur sputtering by fast electrons is assumed. Two samples of SrTiO<sub>3</sub>, 35 Å and 78 Å thick, provide an important test for cases where the sample is not as thin: quantitative retrieval is not possible but the retrieved transmission function is more sensitive to light atoms than most conventional imaging techniques.

**Keywords** Atomic resolution electron microscopy, Segmented detectors, Phase retrieval

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## Single-shot Electron Diffraction Using Relativistic-energy Femtosecond Electron Pulse

Ryo Asakawa<sup>1\*</sup>, Jinfeng Yang<sup>2</sup>, Takafumi Kondoh<sup>2</sup>, Koichi Kan<sup>2</sup>, Masao Gohdo<sup>2</sup>, Yoichi Yoshida<sup>2</sup>, Katsumi Tanimura<sup>3</sup>

<sup>1</sup> Graduate School of Engineering Osaka University, Japan

<sup>2</sup> The Institute of Scientific and Industrial Research, Osaka University, Japan

<sup>3</sup> Research Center for Ultra-high Voltage Electron Microscopy, Osaka University, Japan

\*Corresponding author, e-mail: [asakawa81@sanken.osaka-u.ac.jp](mailto:asakawa81@sanken.osaka-u.ac.jp)

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### Abstract

A single-shot ultrafast electron diffraction (UEM) based on a photocathode radio-frequency (RF) electron gun has been constructed in Osaka University for the study of ultrafast phenomena in materials. The RF gun driven by high-power RF generates a strong electric field of 100 MV/m and accelerates the electron pulse in the relativistic energy region to reduce the space-charge force in the pulse. We have succeeded to generate a 100 fs electron pulse with the energy of 3 MeV and the electron number of  $10^7$  in the pulse using the RF gun, a copper cathode and a 90 fs UV laser. The beam emittance (convergence angle) is essential in many aspects of electron diffraction. We improved the beam emittance down to 0.14 mm-mrad using a 0.5-mm-diameter condenser aperture downstream the RF gun. The energy spread is about  $10^{-4}$ . Using the 100 fs electron pulse, we measured the high-quality single-shot relativistic-energy electron diffractions from the single crystal materials, such as Au and Mica. The single-shot UED has been also succeeded to observe and understand the photo-induced phase transition in the single crystal gold.

**Keywords** Ultrafast electron diffraction, Femtosecond electron pulse, Photocathode RF gun

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## **An History of EBSD ... A One Thousand Factor!**

Francois Brisset

ICMMO, University of Paris-Sud / Orsay, CNRS

\*Corresponding author, e-mail: francois.brisset@u-psud.fr

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### **Abstract**

Electron BackScatter Diffraction (EBSD) is becoming a very interesting technic in material science as soon as materials are crystalized. It really started in 1992 when it became fully automatic. However, the first record is as far as 1928 and it is very passionate to observe the evolution of this powerful analysis technic for scratch to the new developments. During this talk, we will travel through time and describe the evolution of the EBSD technique from the observation of the first Kikuchi lines, the introduction of the scanning electron microscope to the most recent advances. In fact, we will discover a story time!

**Keyword:** EBSD, History, Kikuchi patterns, SEM

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## Application of Non-Tiling Extra-Large Field Observation and Analysis Using SEM

Yuji Hasebe<sup>1\*</sup>, Seiichi Suzuki<sup>2</sup>, Shunsuke Asahina<sup>1</sup>, Takeshi Nokuo<sup>1</sup>

<sup>1</sup> JEOL Ltd. 3-1-2 Musashino, Akisima, Tokyo 196-8558 Japan

<sup>2</sup> TSL Solutions K. K. SIC2-401, 5-4-30, Nishihashimoto, Midori-ku, Sagamihara, Kanagawa, 253-0131 Japan

\*Corresponding author, e-mail: yhasebe@jeol.co.jp

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### Abstract

Usually, the extra-large field observation and analysis using Scanning Electron Microscopy (SEM) are difficult to avoid image distortion due to non-uniformed magnetic field of usual objective lens. Recently, we have developed LDF (Large Depth of Focus) mode that enables to acquire extra-large field of images with minimized image distortion in both observation and analysis such as EDS and EBSD even larger than 4 x 4 mm field. Actually, the LDF mode is using a magnetic lens located above the objective lens in order to focus an incident beam with very long working distance. So, that makes it possible to realize low distortion image and EDS-MAP at large field without using conventional method like a tiling function. Now, it is able to obtain much less distortion image and perform EDS, EBSD analysis at extra-large field by using LDF function in a short acquisition time.

**Keywords** SEM, Extra Low magnification, EDS, EBSD

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## Grating Structured Light Illumination Microscopy

Alexandr Melnikov<sup>1\*</sup>, Michael G. Somekh<sup>1</sup>

<sup>1</sup> Department of Electronic and Information Engineering, Biophotonics Group, Faculty of Engineering, The Hong Kong Polytechnic University, 11 Yuk Choi Road, Hung Hom, Hong Kong SAR, PRC

\*Corresponding author, e-mail: alex.melnikov@connect.polyu.hk

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### Abstract

Structured illumination is employed in fluorescence microscopy to extend resolution in all three dimensions. It has been demonstrated that a factor of 2 improvement can be achieved.<sup>1</sup> In this paper we introduce another approach to both lateral and axial resolution enhancement by using a combination of a fine transmission grating and tilted illumination to create a synthetic aperture of higher numerical aperture than the actual objective lens has. A grating produces mutually coherent light beams, which interfere in the sample to create a fringe pattern varying both laterally and axially. Change of the tilt angle of the light illuminating the grating, results in fringe pattern variations “in time-domain”, assigning unique intensity signature to every object as a function of its spatial position. Fourier transform-based signal analysis allows reconstruction of the three-dimensional image.

The finest optical sectioning is achieved by driving transmission angle for specific diffraction orders from  $-\pi$  to  $+\pi$ . In this case the depth of a single section will be  $\lambda/(2n)$ , where  $\lambda$  is the wavelength and  $n$  is the refractive index of the medium.

Lateral resolution is proportional to  $d\delta/(1.8\lambda)$ , where  $\delta$  is resolution according to Rayleigh criterion<sup>2</sup> and  $d$  is a grating period in units of wavelength.

**Keywords** Grating structure, Tilted illumination, Fringe pattern, Intensity

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## Dependence of the Working Distance and the Applied Bias on the Surface Potential Distribution of Insulating Specimen Irradiated by Electron Beam

Takuya Kawamoto\*, Masashi Tokai, Masatoshi Kotera  
Major in Electrical and Electronic Engineering,  
Osaka Institute of Technology, 5-16-1, Omiya, Asahi-ku, Osaka, Japan  
\*Corresponding author, e-mail: pe3d778b72lrd5q@i.softbank.jp

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### Abstract

Scanning Electron Microscopy is used in various purposes of technologies. But we observe anything that has less conductivity, the specimen charges up positively or negatively. To explain the reason, we have constructed an electrostatic force microscope system in our scanning electron microscope specimen chamber. In previous research, we know that the charging up is built by Fogging electrons, which are produced by multiple backscattered electrons between specimen surface and structure of the sample chamber, especially the objective lens plate. In the present study we measure surface potential distribution in a 300 nm-thick resist film on 70 nm-thick Cr layer on a bulk glass substrate. If the electron beam energy is 30keV and the current is 1nA, irradiation area is 100um×100um, the potential distribution obtained by fogging electrons distribute in a large lateral distance. Results are obtained at the bias applied to the specimen are 0 and +200V in the present experiment. Fogging electrons exist over the specimen surface are attracted toward the specimen surface, and they produce a negative surface potential. If working distance is large, the absolute value of the distribution is small. The distribution reaches a large range of around 1.5mm for any working distances.

**Keywords** scanning electron microscope, fogging electron

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## **A Handy Method for High Quality Specimen Preparation for Chip-based in-situ Transmission Electron Microscopy**

Ya Wang<sup>1\*</sup>, Jun Liu<sup>1</sup>, Lu Chen<sup>1</sup>, Yong Wang<sup>1</sup>

<sup>1</sup> Center of Electron Microscopy and State Key Laboratory of Silicon Materials, School of Materials Science and Engineering, Zhejiang University, Hangzhou 310027, China

\*Corresponding author, e-mail: yawang@zju.edu.cn

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### **Abstract**

The process to prepare conventional cross section TEM specimen using focused ion beam has been well established. However, it is still difficult to prepare high quality specimen on micro-size chips for in situ experiments from bulk materials. By using a common 45° sample stage and a standard 52° tilted FIB, we report a high quality specimen process for chip-based in-situ TEM experiments.

**Keywords** FIB, in-situ TEM, thermal chips, cross-section sample

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## Low Energy X-ray Analysis for Light Element and High Spatial Resolution SEM-EDS

James Sagar<sup>1\*</sup>, Wu Jiang<sup>2</sup>, James Holland<sup>1</sup>, Simon Burgess<sup>1</sup>

<sup>1</sup> Oxford Instruments NanoAnalysis, Halifax Road, High Wycombe, UK

<sup>2</sup> Oxford Instruments NanoAnalysis Singapore, Ubi Crescent, Singapore

\*Corresponding author, e-mail: james.sagar@oxinst.com

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### Abstract

A new type of EDS detector, X-Max Extreme, has been developed for SEM-based elemental characterization at low accelerating voltage and short working distance. This detector uses windowless operation with high sensitivity electronics to improve the effective solid angle for the detection of low energy X-rays by 10-100x. In combination with ultra-high resolution FEG-SEM chemical analysis is now possible for lighter elements and smaller structures.

Enhanced detectability for light elements, provides new information for borides, carbides, nitrides and oxides in a wide range of nano-materials, nano-structures and defects. Light elements can also be detected when present in lower concentrations such as boron in alloys and dopants in Si and other electronics. Lithium has also been detected from materials including Li-ion battery anodes with this this new technology [1]. Further developments in Li analysis are actively being pursued. The capability to detect very low energy X-ray lines means much lower accelerating voltage can be used for elemental analysis. Characteristic lines for all elements can be excited at 2kV. Even at 1 kV most important elements can be detected, meaning sub 10nm resolution and surface sensitive analysis in the SEM can be achieved for many materials including semiconductors.

**Keywords** 10nm, SEM-EDS, Lithium, Semi-conductor

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## Optimization of the FIB Induced Damage in TEM Diamond Samples

Sergey Rubanov \*

Advanced Microscopy Facility, Bio21 Institute, University of Melbourne, Australia

\*Corresponding author, e-mail: [sergey@unimelb.edu.au](mailto:sergey@unimelb.edu.au)

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### Abstract

It is very hard to prepare a TEM diamond samples using traditional methods of preparation. However, diamond can be easily micro-machined using FIB technique and cross-sectional TEM sample of diamond can be prepared in a few hours. However, Ga FIB milling results in formation of the damage layers which aggravates the quality of high-resolution imaging. In the present work the damage after FIB milling in diamond was studied using high-resolution and analytical electron microscopy. The way of the optimization of the sample preparation was demonstrated.

**Keywords** diamond, damage, amorphisation, FIB

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## Scanning Confocal Microscope Using Digital Micromirror Device (DMD)

Sani Boonyagul<sup>1</sup>, Kodchakorn Ittipornnusun<sup>1</sup> and Suejit Pechprasarn<sup>1,2\*</sup>

<sup>1</sup> Biomedical Engineering Programme, Department of Physics, Faculty of Science, Rangsit University, Pathumthani, Thailand, 12000

<sup>2</sup> Department of Electronic and Information Engineering, the Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China

\*Corresponding author, e-mail: suejit.p@rsu.ac.th, suejit.pechprasarn@polyu.edu.hk

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### Abstract

Scanning confocal microscope has played an important role in biological and biomedical imaging [1], because its higher resolution compared to conventional widefield microscope and its capability in optical sectioning and 3D imaging [2]. Of course, these features come with very expensive cost. There are 3 conventional methods to construct a confocal system, which are (1) sample scanning using 3D motorized sample stage [2] (2) beam scanning using high speed galvo mirrors and a motorized stage to control the z axis [3] and (3) lens scanning using Nipkow disk [4].

In this poster, we will demonstrate that system by employing a digital micromirror device (DMD) [5]; a parallel confocal scanning can be realized. The DMD confocal system has been aligned so that the DMD pattern is projected on the image plane of the objective lens. The sample image and the DMD image are then imaged using a CCD camera. By ensuring, that the projected DMD pixel image is smaller than the diffraction limited spot size of the objective lens. In order to avoid crosstalk between adjacent pixels, we can set the confocal spots to be separated by distance more than  $1.22\lambda/NA$  or a series of orthogonal code can be employed.

**Keywords** Scanning Confocal Microscope, Digital Micromirror Device, Parallel Confocal Scanning

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## Effect of Methionine on Growth Rates of the White Shrimp, *Litopenaeus vannamei*

Benjamart Pratoomthai<sup>1\*</sup>, Addison L. Lawrence<sup>2</sup>, Susmita Patnaik<sup>2</sup>

<sup>1</sup> Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Samsen road, Dusit, Bangkok, Thailand

<sup>2</sup> Texas AgriLife Research Mariculture Laboratory at Port Aransas, Texas A&M System, 1300 Port Street, Port Aransas, Texas 78373, USA

\*Corresponding author, e-mail: maew\_199@hotmail.com

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### Abstract

A 35 days feeding trial was conducted to evaluate the potential of using methionine as feed ingredient on Pacific White shrimp, *Litopenaeus vannamei* performance. Experimental tanks were stocked (size:  $0.32 \pm 0.12$  g) with *L. vannamei* at a density of 60/m<sup>2</sup> (6 shrimp/tank). Experimental diets consisted of control diet, and methionine-supplemented diet. A standard reference research diet was also included in the trial to verify the experimental system, water quality, and shrimp health conditions. Control diet was limited levels of methionine-containing protein sources, and no fish meal. Methionine hydroxyl analogue calcium (MHAC) was gradually supplemented to the control diet (0.134, 0.260, 0.402, 0.536, 0.670 % of diet corresponding to 0.465, 0.577, 0.690, 0.802, 0.915% of methionine). At the end of the trial, all surviving shrimp were counted and weighed. The results showed no significant differences in shrimp survival (range: 91.7% to 100%) between the control diet and methionine-supplemented diets ( $P > 0.05$ ). The final weight and growth rate of shrimp fed the control diet were significantly lower than those of shrimp fed the methionine-supplemented diets. Treatment fed the control diet showed significantly higher feed conversion ratio (FCR) (2.57) compared to FCRs of methionine-supplemented diets (FCR range 1.91 to 2.49). Results indicated that *L. vannamei* has a requirement for dietary methionine.

**Keywords** Methionine, *Litopenaeus vannamei*, growth rate

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## Human Dermal Fibroblast Cells are Unchangeable to Cellular Morphological Aging with Gold Nanoparticles Exposure

Chalerm Sri Chayatsatid<sup>1,2</sup> and Amornpun Sereemasun<sup>2,3\*</sup>

<sup>1</sup> Graduate Program in Medical Science, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Bangkok,

<sup>2</sup> Nanobiomedicine Laboratory, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Bangkok, Thailand

<sup>3</sup> Division of Histology and Cell Biology, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Bangkok, Thailand

\*Corresponding author, e-mail: amornpun.s@chula.ac.th

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### Abstract

Gold nanoparticles (AuNPs) have been widely used in biomedical applications, cosmetic and aesthetic due to safe for living organisms and anti-aging. However, there has never really studied the cellular morphological aging with gold nanoparticles exposure on human dermal fibroblast cells (HDFs). The recent study has been designed to investigate the effect of gold nanoparticles on cellular senescence in HDFs. The cellular senescence was determined by cytochemical staining of senescence-associated- $\beta$ -galactosidase (SA- $\beta$ -gal) and formation of senescence-associated heterochromatin foci (SAHF). Compared to control group, we found that treatment with 5 ppm and 50 ppm of AuNPs size 20 nm demonstrated an increase in SA- $\beta$ -gal positive cells but unalterable SAHF within the nuclei. These findings suggest that AuNPs is not resulted in cellular morphological aging.

**Keyword** gold nanoparticles, cellular senescence, SA- $\beta$ -gal, SAHF

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## The Ethanol Extract from Red Seaweed *Gracilaria fisheri* Inhibits Biofilm Formation of *Vibrio harveyi*

Kulwadee Karnjana<sup>1</sup>, Chumporn Soowannayan<sup>2</sup>, Kanokpan Wongprasert<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

<sup>2</sup> National Center for Genetic Engineering and Biotechnology, and Centex Shrimp Chalermprakiat Building, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

\*Corresponding author, e-mail: kanokpan.won@mahidol.ac.th

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### Abstract

*Vibrio harveyi* (*V. harveyi*) is a pathogenic bacteria of vibriosis, a disease that causes massive losses of shrimp culture in hatchery. Previous study demonstrated that *V. harveyi* survives in hatchery environment within biofilm. This study aims at evaluating an anti-biofilm effect of the ethanol extract from red seaweed *Gracilaria fisheri* (*G. fisheri*). The virulent strain of *V. harveyi* were treated with the ethanol extract (5 and 10 µg/ml) and biofilm was grown for 72 h. Biofilm biomass determined using crystal violet assay showed that bacteria treated with the ethanol extract decreased biofilm biomass to 48.16% and 50.86% of control, respectively. Biofilm imaged by confocal laser scanning microscopy (CLSM) revealed that the ethanol extract treated bacteria showed thinner biofilm and higher density of dead bacteria in the biofilm than control. Moreover the treated bacteria demonstrated decreased expression of *LuxR* gene, a master regulator gene for biofilm development and bacterial virulence. The overall results suggests that the ethanol extract from *G. fisheri* has an ability to eradicate biofilm formation of the virulent strain *V. harveyi*.

**Keywords** *Vibrio harveyi*, *Gracilaria fisheri*, ethanol extract, biofilm formation

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## The Ethanol Extract from the Red Seaweed *Gracilaria fisheri* Inhibits Biofilm Formation by *Staphylococcus epidermidis*

Sakun Thala<sup>1,2</sup>, Kulwadee Kanjana<sup>1</sup>, Kanokpan Wongprasert<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of science, Mahidol University, Bangkok, Thailand.

<sup>2</sup> Department of Anatomy, University of medicine (2), Yangon, Myanmar.

\*Corresponding author, e-mail: kanokpan.won@mahidol.ac.th

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### Abstract

Previously, the ethanol extract from the red seaweed *Gracilaria fisheri* (*G. fisheri*) was found to inhibit bacterial growth. However, the effect of the ethanol extract on biofilm formation which is an important process for microbial virulence and adaptive resistance to conventional antibiotics has not been evaluated. Biofilm formation by *Staphylococcus epidermidis* (*S. epidermidis*), an opportunistic bacteria, is the major cause of device-related nosocomial infections such as valvular and device-associated infective endocarditis, osteomyelitis, pneumonia. Therefore, the present study aimed to examine the effect of the ethanol extract from *G. fisheri* on biofilm formation of *S. epidermidis*. The antimicrobial activity against *S. epidermidis* was evaluated and minimum inhibitory concentration (MIC) was determined. The MIC<sub>50</sub> against *S. epidermidis* of the ethanol extract was 1.43 mg/ml. *S. epidermidis* biofilm was grown with the ethanol extract at concentrations that did not affect the bacterial planktonic growth (0, 10, 50, 100, 200 µg/ml) for 24 h. Biofilm biomass was determined using crystal violet assay and biofilm feature was evaluated by confocal laser scanning microscopy. The results showed that treatment with the ethanol extract at concentration 100 µg/mL prevented biofilm formation and eradicated development of mature biofilm in *S. epidermidis*. In conclusion, the present study suggests that the ethanol extract from *G. fisheri* targets *S. epidermidis* biofilm formation and is a potential agent for the prevention of biofilm-related microbial resistance.

**Keywords** Ethanol extract, *Gracilaria fisheri*, *Staphylococcus epidermidis*, biofilm

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## Plumbagin, a Compound Extracted From *Plumbago indica*, Inhibits Proliferation and Induces Apoptosis of Stromal Cells Derived From Endometriotic Endometrium'

Sineenart Songkoomkrong<sup>1</sup>, Piyachat Chansela<sup>2</sup>, Morakot Sroyraya<sup>1,3</sup>, Narin Changklungmoa<sup>4</sup>, Jaruwan Poljaroen<sup>1,3</sup>, Sawaek Weerakiet<sup>5</sup>, Yada Tingthanatikul<sup>5</sup>, Areepan Sophonsritsuk<sup>5</sup>, Anna Wongkularb<sup>5</sup>, Srithean Lertvikool<sup>5</sup>, Prasert Sobhon<sup>1,4\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of science, Mahidol University, Bangkok, 10400, Thailand

<sup>2</sup> Department of Anatomy, Phramongkutklao College of Medicine, Bangkok, 10400, Thailand

<sup>3</sup> Mahidol University, Nakhonsawan Campus, Nakhonsawan, 60130, Thailand

<sup>4</sup> Faculty of Allied Health Sciences, Burapha University, Chonburi, 20131, Thailand

<sup>5</sup> Department of Obstetrics and Gynecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, 10400, Thailand

\*Corresponding author, e-mail: prasert.sob@mahidol.ac.th

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### Abstract

Endometriosis occurs from abnormal implant of endometrial cells at locations outside the uterine cavity. Endometriosis causes chronic pelvic pain and subfertility in affected woman. The exact cause of endometriosis is uncertain and the ectopic cellular growth is defined as benign even though the disease is highly debilitating. Endometriosis can be abated by drug and hormonal treatments, such as progestin and GnRH analog. It can also be reduced or prevented by use of natural products as a traditional medicine or nutraceutical supplement. Plumbagin (PB) is a natural compound extracted from a plant, *Plumbago indica*, whose chemical structure is 5-hydroxy-2-methyl-1, 4-naphthoquinone. PB exhibits highly potent biological activities, including anti-cancer, anti-oxidant and anti-inflammation. In this study, we investigated the effect of PB on the proliferation of endometriotic stromal cells that were isolated and purified from ovarian endometrioma. Ectopic stromal cells treated with varying concentrations of PB showed decreased cell viability as measured by MTT assay in a dose-dependent manner. Furthermore, PB dose-dependently induced apoptosis clonogenicity of the stromal cells from ectopic endometrium. These findings suggest that PB has a strong inhibition of cell growth by acting through the apoptosis pathway, and that it may be used as an alternative naturally-derived drug which less side effect to hormonal cycle in endometriosis patients.

**Keyword** Plumbagin, endometriosis, stromal cells, apoptotic cell death

## **Acute Toxicity Screen of Aqueous Leaf Extract from *Nerium oleander* Against *Pomacea canaliculata* Lamarck**

Passapan Sriwichai \*

Faculty of Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Sanai, Chiangmai, 52090, Thailand

\*Corresponding author, e-mail: passapani@hotmail.com

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### **Abstract**

Aqueous extracts from leaves of *Nerium indicum* were evaluated for its acute toxicity activity against *Pomacea canaliculata* (golden apple snail) under laboratory conditions. Efficiency of *N. indicum* extract on the snails was studied for 24 and 48 h after treatment; 3 replicated experiments were performed. Each treatment contained 10 snails in the tank. The results showed that all tested concentrations (250, 500, 1,000, 1500 and 2000 mg/L) of the crude extracts caused a 100 % of the mortality rate of *P. Canaliculation* at 24 h and 48 h. These results indicated that the extract could be an effective molluscicide against *P. Canaliculation*. The toxicity of the water extract of *N. indicum* on golden apple snail was also evaluated through changes of histological structures of the tissues/organ. At the lowest sub lethal concentrations exposure, the golden apple snail showed the damages in the heart and hepatic tissues. These results suggested that water extracts from fresh leaves of *N. indicum* were lethal to *P. canaliculata*.

**Keywords** *Nerium indicum*, *pomacea canaliculata*, Toxicity

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## Effect of Estrogen on Angiogenesis in Bovine Follicular Cells

Bongkoch Turathum<sup>1\*</sup>, Yindee Kitiyanant<sup>2</sup>, David Bates<sup>3</sup>

<sup>1</sup> Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Samsen Road, Dusit, Bangkok, Thailand

<sup>2</sup> Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, Thailand

<sup>3</sup> Department of Oncology, Faculty of Medicine and Health Science, Queen Medical Centre, Nottingham, United Kingdom

\*Corresponding author, e-mail: bongkochei@gmail.com

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### Abstract

The ovarian follicular development and ovulatory cycle are depended on angiogenesis and vascular regression. In the ovary, angiogenesis occurs under the control of the vascular endothelial growth factor-A (VEGFA) family of proteins, which are generated as both pro-(VEGF165) and anti (VEGF165b)-angiogenic isoforms by alternative splicing. Estrogen is a necessary hormone to modulate angiogenesis via effects on endothelial cells. To determine the effect of estrogen on the VEGF165, VEGF165b and its receptor (VEGFR<sub>2</sub>) during ovarian follicular development, we cultured follicular cells that were collected from small, medium and large size of bovine follicles and measured VEGF165b, VEGF165 expression by western blot and immunofluorescence. The results showed the expression of VEGF165 was decreased and the expression of VEGF165b was increased in follicular cells of large follicle after culture in medium with estradiol. The expression of VEGFR2 was increased in all size of follicle. In conclusion, estrogen is necessary for angiogenesis during development of follicles in bovine.

**Keywords** *VEGF165, VEGF165b, VEGFR2, Follicular cells, Estradiol*

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## The Sesamin Encouraging Effects on Chondrogenic Differentiation of Human Amniotic Fluid-Derived Mesenchymal Stem Cells

Suteera Narakornsak<sup>1</sup>, Runchana Markmee<sup>1</sup>, Waleephan Tancharoen<sup>1</sup>, Naree Poovachiranon<sup>1</sup>, Paisan Thaojamnong<sup>1</sup>, Lamaiporn Peerapapong<sup>1</sup>, Junjira Keawdee<sup>1</sup>, Nonglak Boonma<sup>1</sup>, Witoon Tasuya<sup>1</sup>, Peeraphan Pothacharoen<sup>2</sup>, Sirinda Aungsuchawan<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Chiang Mai University, Intawarorose Road, Chiang Mai, Thailand

<sup>2</sup> Thailand Excellence Center for Tissue Engineering and Stem Cells, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Intawarorose Road, Chiang Mai, Thailand

\*Corresponding author, e-mail: sirinda001@hotmail.com

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### Abstract

The most important musculoskeletal degenerative disease in worldwide is osteoarthritis (OA). Sesamin, is a major abundant lignan compound consisting in *Sesamun Indicum Linn.*, which has been described in various pharmacological effects and health benefits. However, the promotion effect of sesamin on chondrogenic differentiation has not been observed. Aim of this study was to investigate the effects of sesamin on cell cytotoxicity and differentiation potential of human amniotic fluid-derived mesenchymal stem cells (hAF-MSCs) into chondrocyte-like cells. The results manifested that sesamin did not toxic to hAF-MSCs in all treatment time periods (24, 48, and 72 hours). Furthermore, this study found that the sesamin combined into culture medium could be promoted the chondrogenic specific genes, which related to ground substance protein synthesis including *COL2A1*, *COL11A1*, and *COMP* genes. Besides, *SOX9* and *AGC* genes were observed in the early stage of cell cultivation. Additionally, histological analysis revealed the higher positive staining in the sesamin treated group when compared with control group. In conclusion, this study suggested that the sesamin might be used as a chondrogenic inducing factor.

**Keywords** Sesamin, Chondrogenic differentiation, Amniotic fluid, Mesenchymal stem cells

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## Characterization of Multiple Contact Synapse in Cerebellum of Tottering Mice

Hyun-wook Kim<sup>1</sup>, Eun-Soo Park<sup>1</sup>, Kea Joo Lee<sup>2</sup>, Im Joo Rhyu<sup>1,\*</sup>

<sup>1</sup> Department of Anatomy, College of Medicine, Korea University, Seoul 136-705, Korea.

<sup>2</sup> Research Division, Korea Brain Research Institute, Daegu 701-300, Korea

\*Corresponding author, e-mail: irhyu@korea.ac.kr

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### Abstract

Tottering (tg/tg) mouse is an ataxic model that carries an autosomal recessive mutation in the gene coding for the P/Q-type calcium channel. Ultrastructural investigation has also found that individual PF boutons in tottering mice often make multiple contacts with dendritic spines of Purkinje cells, which is called multiple synaptic boutons (MSB). Interestingly, MSB was also observed in the same locus of the mouse cerebellum housed in environmental enrichment condition (EE). We investigated whether MSBs observed from different situation are some nature or not.

The MSBs from each animal model were observed using serial sectioning transmission electron microscopy (ssTEM) and analyzed for the dendritic spine origin. The MSBs were generally observed in EE showed the PCs were originated from the same dendrites (sdMSBs), however MSB observed in the tottering mouse showed increased proportion of the PCs were originated from different dendrites (ddMSBs). Both groups were increased total proportion of MSB significantly than normal condition, but the character of synapse was different. These results imply that synaptic connectivity is closely related to its functional status. These conditions differences in dendritic spine origination may offer insight into the morphological event indicate neuronal plasticity of the cerebellum.

**Keywords** Multiple synaptic bouton, Ataxia, Environmental enrichment, Plasticity, ssTEM

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## Characterization of Oocyte, Follicle Cells and Follicular Fluid Proteins from Porcine Follicles

Mayuva Areekijserree\*, Hatairuk Tungkasen, Waramanee Pongsawat, Somrudee Phetchrid, Suwapat Jaidee, Supinya Yoomak

Department of Biology, Faculty of Science, Silpakorn University, Sanamchandra Palace Campus, Muang, Nakhon Pathom 73000, Thailand

\*Corresponding author, e-mail address: maijackee@yahoo.com

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### Abstract

Our project was studied characterization of oocyte, follicle cells and follicular fluid proteins from porcine follicles of pig (Large White X Landrace X Duroc) ovaries received from local slaughter house. The porcine follicular fluid (pFF) of selected healthy follicles with diameter ranging between 2-8 mm was aspirated. The porcine oocyte and the follicle cells (pFC) were separated from the fluid. Characterization of porcine oocyte showed 5 types, they were intact-, multi-, partial cumulus cell-layer oocytes, completely denuded oocyte, and degenerative oocyte. It showed high percentage (more than 56 %) of intact- and multi- cumulus cell-layer oocyte complexes which have high potential to develop and mature in vitro. We also studied a method for in vitro culture conditions of pFC, which optimized with regard to ultrastructural- characterized-differentiation. They were cultured in M199 with Earle's salts (Sigma Company, St Louis MO) supplemented with 10% heat-treated fetal calf serum (HTFCS), 2.2 mg/mL NaHCO<sub>3</sub>, 1M Hepes, 0.25 mM pyruvate, and 50 µg/mL gentamycin sulfate, and hormones (15 µg/mL pFSH, 1 µg/mL LH, 1µg/mL estradiol in ethanol) at 37°C , 5% CO<sub>2</sub> in 95% air atmosphere, and high humidity for 24-44 h. The ultrastructure was investigated by using transmission electron microscopy (TEM). The results showed that, pFC recapitulated in vivo-like morphology and ultrastructure on 24 h. They were normal nucleus as well as organelles in the cytoplasm. The pFC changed their character from round shape to tear drop (or columnar) shape after 44 h. Furthermore, pFF, which contains several proteins (secretion by granulosa and theca cells) were detected on gel electrophoresis and follow analyzed by LC/MS/MS. The pFF showed several proteins which play important roles on stimulation and regulation on growth and development of oocyte and gamete fertilization. This finding could be used for a model in a standardized culture system as a candidate tool for in vitro toxicity testing and biotechnology research. Acknowledgements: The project was funded by a grant from Silpakorn University Research & Development Institute (SURDI) and Faculty of Science, Silpakorn University, Thailand

**Keywords** porcine follicular fluid proteins, porcine follicle cells, porcine oocyte, TEM

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## Effects of Combined Chondroitinase ABC and 17beta-Estradiol on Astrocyte Aggregation and Glia Scar Degradation

Bamrungsuk K<sup>1</sup>, Chompoopong S<sup>2\*</sup>

<sup>1</sup>Department of Physiology, <sup>2</sup>Department of Anatomy, Faculty of Medicine Siriraj hospital, Mahidol University, Bangkok 10700, Thailand.

\*Corresponding author. E-mail: supin.cho@mahidol.ac.th

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### Abstract

Spinal cord injury (SCI) results in more chondroitin sulfate proteoglycan (CSPG) expression from accumulated reactive astrocytes at the lesion site which can cause glia scarring and block the axonal regeneration. To determine the effects of combined chondroitinase ABC (ChABC) and 17beta-estradiol (E2) on astrocyte aggregation and glia scar degradation, the C5 hemiconfusion injury was performed in rats. Forty adult male Sprague Dawley (SD) rats were divided into four groups; sham laminectomy (SH), moderate SCI injury (SCIM), SCIM treated with ChABC (SCIM-Ch), and SCIM treated with combined ChABC and E2 (SCIM-Ch-E). The 0.1 U of ChABC was applied immediately, day 3, day 7, and day 11 after injury. The 300 µg/kg of E2 was intraperitoneally injected at 30 min after injury, daily for two weeks and then weekly until sacrificed. The GFAP immunoperoxidase and C-4-S immunofluorescence stained section were evaluated the number of astrocytes and expression of degraded CSPG, respectively by using computer image analysis. The results was that the combined ChABC and E2 treatment could significantly decrease the number of astrocytes (SCIM, 12.35±0.51, SCIM-Ch-E, 7.30±0.27) and showed more degraded CSPG activity (SCIM, 3.90±0.67, SCIM-Ch-E, 20.30±3.75) after injury. The GFAP staining showed hypertrophic astrocytes with extended processes aggregated at lesion in SCIM. The survival neurons in SCIM-Ch-E were surrounded by activated astrocytes with short processes Therefore, SCI causes the increase of inflammatory cytokines related to GFAP expression. Accumulated reactive astrocytes at lesion increase CSPG. The CS-GAG side chains of CSPG interact with CSPG transmembrane receptor protein tyrosine phosphate rho (PTP rho) on neuronal membrane. Rho activation can cause the actin depolymerization which results in collapse of growth cones and block axonal regeneration. The E2 treatment reduced proinflammatory cytokines and decreased astrocytes aggregation. ChABC treatment could abolish the binding to CSPG receptor results in glial scar degradation which provides physical and biochemical characteristics of architecture in glia scar tissue promoting for neuronal growth. Otherwise, E2 increases proteoglycan for cell proliferation, adhesion and migration. This proteoglycan is also the substrate of ChABC. Therefore, it may suggest that the immediately short course of ChABC treatment after injury can prevent the late CSPG degradation which may support axonal growth under the E2 effect. These findings could be potentially useful to further manipulate the treatment of SCI in patients.

**Keywords** Chondroitinase ABC, Estrogen, Glial scar, CSPG Spinal cord injury, Chondroitinase ABC, Estrogen, Glial scar, CS

## Neural Differentiation of Human Dental Pulp Stem Cells Using Conditioned Medium of Embryonic Chick Dorsal Root Ganglion

Cho Zin Linn<sup>1\*</sup>, Thanasup Gonmanee<sup>1</sup>, Sujittra Jueatin<sup>3</sup>, Hathaitip Sritanaudomchai<sup>2</sup>, Sittipon Intarapat<sup>1</sup>, Rattanavijit Vijitruth<sup>3</sup>, Yindee Kitiyanant<sup>1</sup>, Charoensri Thonabulsombat<sup>1</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand

<sup>2</sup> Department of Oral Biology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

<sup>3</sup> Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

\*Corresponding author, e-mail: charoensri.tho@mahidol.ac.th

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### Abstract

Progressive degeneration of spiral ganglion neurons in the inner ear, which lead to sensorineural hearing loss, could be compensated by cell-based therapy. Human dental pulp stem cells (hDPSCs) are one of the multipotent adult mesenchymal stem cells because of having properties of differentiating into chondrocytes, osteocytes and adipocytes as well as neurons. Chick dorsal root ganglion conditioned medium (DRG-CM) is the medium collected from culturing the DRG of day 8 chick embryo having glia derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF) which are important factors in neuronal differentiation of stem cells. In this study, we investigated whether neural differentiation can be induced for hDPSCs cultured with the DRG-CM. The result indicated that DRG-CM supplemented in the dental pulp stem cell media can induce differentiation of hDPSCs into mature neurons.

**Keywords** Human dental pulp stem cells, conditioned medium, neural differentiation

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## **YB-1 Overexpression Increases Migration and Invasion of MDA-MB-231 Breast Cancer Cells**

Sukanya Shyama Sundar, Lim Jia Pei, Yu Yingnan and Bay Boon Huat  
Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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### **Abstract**

Breast cancer is the most common cancer in women and the second leading cause for cancer death. Despite advances in treatment, the highly metastatic nature of tumors has given rise to the urgent need for developing novel therapeutic and prognostic markers. Y box binding protein-1 (YB-1) is a DNA/RNA binding that regulates diverse cellular processes and is critical for malignant transformation and metastasis. YB-1 is known to be overexpressed in several cancers. To understand the role of YB-1 in breast tumors, YB-1 was stably overexpressed in MDA-MB-231 breast cancer cells. Stable overexpression of the YB-1 gene in MDA-MB-231 cells was confirmed by RT-PCR and the YB-1 protein by Western blot analysis. Overexpressing YB-1 were observed to decrease cell proliferation, but resulted in increased migratory and invasive ability of cells as compared to the control cells. Immunofluorescence by confocal microscopy revealed no obvious changes in the actin cytoskeleton (F-actin) or focal adhesion-associated molecules (Paxillin) between control and YB-1 overexpressed cells. Taken together, YB-1 regulates the migratory and invasive capability of MDA-MB-231 breast cancer cells and thus, promotes metastasis. Identifying key genes that mediate YB-1 function would help develop novel prognostic markers and therapeutic targets to combat breast cancer metastasis.

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## Hemolytic Uremic Syndrome: A Case Report

Sakol Suwalak \*, Winyou Mitarnun

Electron Microscopy Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

\*Corresponding author, e-mail: sakon.s@psu.ac.th

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### Abstract

Hemolytic uremic syndrome (HUS) is characterized by generalized thrombotic microangiopathy in the small sized by vascular insufficiency vessels and causes acute renal failure [1]. The etiologies of HUS include infection of Shiga toxin-producing bacteria, viral infections, mycoplasma infection, chemicals, post-partum, malignancies and mutation of genes encoding proteins involved in the properdin pathway of complement system [2,3]. In this study, we compared the kidney pathology in a patient with HUS and a mouse intragastrically infected with Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7. The patient was a six-year-old boy presented with acute renal failure from mycoplasma infection. He denied history of acute diarrhea and fever. The histopathology of the HUS kidney shows microangiopathy in the small blood vessels combined with multiple microthrombi in the capillary lumens of the glomerulus [Figure 1]. The mouse kidney shows only moderate expansion of the glomerulus mesangium and endothelial cell swelling causing partial occlusion the capillary lumen. There is no microthrombus and microangiopathy [Figure 2]. The ultrastructure of the HUS kidney shows mark thickening of the glomerular basement membrane and endothelial cell swelling [Figure 3]. The ultrastructure of the infected mouse kidney shows mesangial cells and matrix proliferation and expansion with mark endothelial cell swelling [Figure 4]. The primary pathology of HUS and endotoxin of the Shiga-toxin producing bacteria is the damage to the capillary endothelium thereby causing renal vascular insufficiency and initiating the thromboembolic process.

**Keywords** Hemolytic uremic syndrome, Thrombotic microangiopathy, Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, Shiga-toxin producing bacteria

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## Dysmorphic Mitochondria and Endoplasmic Reticulum in Type II Diabetic Rat

Sumate Ampawong<sup>1</sup>, Duangnate Isarangkul<sup>2</sup>, Supattra Suwanmanee<sup>1</sup>, Pornanong Aramwit<sup>3\*</sup>

<sup>1</sup> Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, 420/6, Ratchawithi Road, Ratchathewi, Bangkok, 10400, Thailand

<sup>2</sup> Department of Microbiology, Faculty of Science, Mahidol University, 272, Rama VI Road, Ratchathewi, Bangkok, 10400, Thailand

<sup>3</sup> Bioactive Resources for Innovative Clinical Applications Research Unit and Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, PhayaThai Road, Phatumwan, Bangkok 10330 Thailand

\*Corresponding author, e-mail: aramwit@gmail.com

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### Abstract

**Background:** Mitochondria and endoplasmic reticulum are very important organelles in eukaryotic cell which have been described as “the powerhouse of cell” and “protein factory”, respectively. Their abnormalities cause several diseases, e.g. cardiovascular and neurological diseases, especially type II diabetes mellitus. However, most of the studies rather focus on mitochondrial and endoplasmic reticulum function than their cellular architecture.

**Objectives:** Our study aimed to identify microscopic and ultrastructural changing in pancreas and the extracted mitochondria from heart and kidney in streptozotocin-induced type II diabetic rat compare to control group.

**Results:** Unlike normal rat, the degeneration of islet of Langerhans (pyknotic nuclei, vacuolated cells, and cellular hypertrophy) and pancreatic exocrine gland (single cyst formation and acinar cell hypertrophy) were obviously found in diabetic rat with scarce inflammation ( $p < 0.05$ ). In diabetic rat, mitochondrial extraction from both liver and kidney exhibited higher number of dysmorphic architectures (swelling, ghost, and vacuolated mitochondria) than normal rat ( $p < 0.05$ ). Moreover peculiar dense and dark endoplasmic reticulum, deposited throughout of the degenerative exocrine gland's cytoplasm, was only found in diabetic rat.

**Conclusion:** Hyperglycemic state induced by streptozotocin can lead to dysmorphic mitochondria and endoplasmic reticulum. These are crucial factors involving in the severity and pathogenesis of type II diabetes mellitus.

**Keywords** Diabetes mellitus, Endoplasmic reticulum, Mitochondria, Rat

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## Correlation between Histological Parameters in Decalcified Femoral Cortex and Human Age in Adult Thai Cadavers Using Computerized Imaging Technique

Hathaichanok Chompoopuen<sup>1</sup>, Jongkolnee Settakorn<sup>2</sup>, Karnda Mekjaidee<sup>3</sup>, Wichan Thumthong<sup>4</sup>, Pasuk Mahakkanukrauh<sup>5,6\*</sup>

<sup>1</sup>Forensic Osteology Research Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>2</sup>Department of Pathology Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>3</sup>Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>4</sup>Department of Computer Science and Information Technology, Rambhai Barni Rajabhat University, Chanthaburi, 22000, Thailand

<sup>5</sup>Excellence in Osteology Research and Training Center (ORTC), Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>6</sup>Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

\*Corresponding author, e-mail: pasuk.m@cmu.ac.th

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### Abstract

Age estimation is an important step in biological identification of the skeletal remains. In adult human, histological age estimation is often based on the evidence of bone remodeling. The purpose of this study is to assess the applicability of using the advanced computerized imaging technique for identifying the histological parameters that are able to predict the human age in decalcified femoral cortex of adult Thai cadavers. The pilot bone samples were from seven male and one female cadavers with the age range of 41 to 86 years. All cadavers were sent for routine autopsy at the department of Forensic Medicine, Chiang Mai University. One small pieces of anterior mid-shaft femoral cortical bone were histologically prepared and stained with Sirius red. The images were captured at 100x magnification using Olympus DP73. The digitalized images were analyzed by Olympus CellSens Standard Program. The studied parameters included fragment osteon density, intact osteon density, percentage of lamella bone, perimeter of haversian canal, and area of haversian canal and osteon. Three best correlated parameters with age were perimeter of haversian canal ( $r=0.970$ ), intact osteon density ( $r=0.877$ ), and area of haversian canal ( $r=0.864$ ). Therefore, further development of this technique is promising for the better age estimation.

**Keywords** histology, forensic science, femur cortex, age estimation

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## Anti-Oxidant and Anti-Aging Effects of Natural Flavonoid and Diarylheptanoid

Sirinad Tankruad<sup>1</sup>, Pawanrat Chalorak<sup>1</sup>, Prapaporn Jattujan<sup>1</sup>, Patoomratana Tuchinda<sup>2</sup>, Prasert Sobhon<sup>1</sup>, Krai Meemon<sup>1,\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand 10400

<sup>2</sup>Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand 10400

\*Corresponding author, e-mail: [krai.mee@mahidol.ac.th](mailto:krai.mee@mahidol.ac.th)

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### Abstract

Flavonoid and diarylheptanoid have exhibited several properties for health benefit including anti-oxidant, anti-aging, anti-inflammatory, anticancer, antibacterial, and antiviral activities. In this study, we investigated on antioxidant and anti-aging effects of natural flavonoid and diarylheptanoid. Antioxidant activity was examined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The result showed that flavonoid had high antioxidant activity with the median effective concentrations (EC<sub>50</sub>) of 19.66 µg/ml, whereas diarylheptanoid showed low antioxidant activity with EC<sub>50</sub> of more than 2000 µg/ml. The study of anti-aging activity was performed by using *Caenorhabditis elegans* (*C. elegans*) as an animal model. Flavonoid and diarylheptanoid at concentration of 100 µg/ml significantly increased longevity of *C. elegans* with mean lifespan increased for 7.36 and 5.86 %, respectively ( $p < 0.05$ ). Stress resistance activity of flavonoid and diarylheptanoid was then determined by heat shock and oxidative stress assays. The results indicate that the flavonoid and diarylheptanoid had anti-oxidant and anti-aging properties that are independently related.

**Keywords** flavonoid, diarylheptanoid, antioxidant, anti-aging

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## Human Sex-Identification Using Loop-Mediated Isothermal Amplification (LAMP) Technique of *TTY* gene in Urine Samples

Gun Anantasomboon<sup>1\*</sup>, Anucha Wimonchat<sup>1</sup>, Kanokporn Chayaburakul<sup>1</sup>, Sirinda Angsachawan<sup>2</sup>, Tadsanee Punjanon<sup>1</sup>

<sup>1</sup>Anatomy Unit, Department of Medical Science, Faculty of Science, Rangsit University, Patumthani 12000,

<sup>2</sup>Department of Anatomy, Faculty of Medicine, Chaing-Mai University, Chaing-Mai, 50200, Thailand

\*Corresponding author, e-mail: ananta\_rsu@yahoo.com

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### Abstract

Sex determination of the sources of forensic DNA evidence is an importance step in crime investigation. In this study, we applied Loop-mediated isothermal amplification (LAMP) for human sex-identification with urine specimens. LAMP is a novel molecular technique for DNA or RNA amplification, which is simplicity of single-tube procedure, rapidity, and low cost. The target sequence is amplified at a constant temperature of 60-65°C with either two or three sets of specific primers, and a *Bst* DNA polymerase. Two primer sets for *TTY* (Testis specific transcript Y-linked) gene upon the long arm of Y-chromosome was specifically designed and examined with minimal DNAs (10 ng of total DNAs) extracted from suspended cells in urine samples of the males and the females. Results revealed positive LAMP-*TTY* reactions as smear bands, specifically with all of the male DNA samples, but gave negative results with female DNAs. The sensitivity test of LAMP-*TTY* was found to be higher than the conventional PCR and it may be equivalent to the nested-PCR amplification. We have planned to combine a condition of visible bio-reporter for direct notification when the positive LAMP-*TTY* reaction is occurred. The authors are also confident that this finding will lead to a new practical application in the fields of medical science, athlete gender, forensic and crime investigations.

**Keywords** sex identification, Y-chromosome, *TTY* gene, urine sample, LAMP

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## Association between LINE-1s Methylation with Methamphetamine Use

Korakot Kraijak<sup>1</sup>, Apiwat Mutirangura<sup>2</sup>, Rasmon Kalayasiri<sup>3\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Program Medical Science, Bangkok, Thailand

<sup>2</sup> Department of Anatomy, Faculty of Medicine, Center of excellence in Molecular Genetics of Cancer and Human Diseases, Chulalongkorn University, Bangkok, Thailand

<sup>3</sup> Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

\*Corresponding author, e-mail: rasmon.k@chula.ac.th

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### Abstract

Recent studies demonstrated that epigenetic alteration of gene expression induced by substance such as methamphetamine (MA) regulate neuronal plasticity and lead to substance dependence. Long interspersed nuclear element 1s (LINE-1s) has been used as surrogate of overall global methylation levels. The hypomethylation of LINE-1s can promote genomic instability of genome. The aim of this study was to measure the methylation level of LINE-1s that affected by MA. Our result revealed that MA induced increases hypomethylation of LINE-1s. And genomic instability might be a new targets for the treatment of addictive disorders.

**Keywords** LINE-1s, methamphetamine and hypomethylation

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## Selective Localization of Cannabinoid Receptor Type1 (CB1) in the Kidney of the Restraint-Stress Mouse

Waraporn Sakaew, Surang Chomphoo, Sawetree Pakkarato, Yada Polsan, Wiphawi Hipkaeo  
Department of Anatomy, Faculty of Medicine, Khon kaen University, Mittaparp Rd., Khon Kaen,  
Thailand

\*Corresponding author, e-mail: warapsa@kku.ac.th

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### Abstract

The cannabinoid receptor type1 (CB1) is a G protein-coupled cannabinoid receptor. It is activated by the endocannabinoid system that plays a role in the regulation of energy homeostasis, immunity and stress response. Considering that the kidney is an organ that regulates the electrolyte homeostasis of the body, the immuno-localization of this molecule was examined in mouse kidneys under normal and restraint-stress conditions. In the experiment group the restraint-stress was given by overnight enclosure in a wire-cage tightly fitted to their body, whereas the control animals were treated under normal condition. The kidneys were collected and subjected for immunohistochemistry with anti-mouse CB1 antibody.

CB1 was selectively positive in the cytoplasm of the epithelial cells of S2 and S3 segments of the proximal tubules and the collecting duct. Different from the normal group, immunoreactivity was additionally detected in the epithelial cytoplasm of the parietal layers of Bowman's capsules, S1 segment of the proximal tubules and the distal tubule of the stress-animal.

These findings suggest that the stress-induced increase in CB1 expression in a variety of kidney tissues could represent an adaptive response to acute stress, which might help maintain the homeostatic regulation of the body under a restraint-stress condition.

**Keywords** The cannabinoid receptor type1, immunohistochemistry, proximal tubule\

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## Overexpression of AQP4 and GFAP Related to Clinical Sign of Cerebral Malaria Mice Model

Lanaprai Kwathai<sup>1</sup>, Supin Chompoopong<sup>2</sup> Thanaporn Rungruang<sup>2\*</sup>

<sup>1</sup> Faculty of Science, Mahidol University, Bangkok, Thailand

<sup>2</sup> Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

\*Corresponding author, e-mail: thanaporn.run@mahidol.ac.th

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### Abstract

Cerebral malaria (CM) is the most severe neurological complication of infection with *Plasmodium falciparum*. Without any treatments often causes coma and death within 24 hours. Deficits in cognitive function are often observed after anti-malaria drug treatment. The aquaporin-4 (AQP4) is abundant in brain and plays roles regulating water homeostasis. AQP-4 highly expressed in ependymal cells and astrocytes end-feet membranes facing cerebral capillaries and pia mater. Although, AQP4 related to brain edema in several neurological disease but the pattern of AQP4 expression in CM is still unclear. Therefore, this study was aimed to evaluate the expression of AQP4 at astrocyte end feet in CM mice model, Mice were inoculated intraperitoneally with *Plasmodium yoelii* lethal (PyXL). Then observed sign of CM and recorded % of parasitaemia daily. After mice were sacrificed, brains were collected and processed for paraffin section. Immuno-peroxidase staining for AQP-4 and GFAP were performed in 3 µm thickness of coronal sections. The results showed that infected CM mice without any treatments died within 8 days after inoculation and they showed clinical signs of neurological involvement such as convulsion and ataxia before death. Immuno-peroxidase staining of brain showed that the expression of AQP4 at astrocyte (indicated by GFAP) foot process was higher in CM group than non-infected group. Average number of reactive astrocyte of hippocampus in CM group was shown two times more expression than sham group (mean 44.5 and 21 cells/area respectively) and in cerebral cortex (mean 106.5 and 38.5 cells/area in CM and sham groups, respectively). Moreover, vessel congestion and enlargement of perivascular space were also found in CM mice. This experiment demonstrated AQP4 overexpression related to clinical sign of cerebral malaria mice. Thus, this finding may be new biochemical molecule for early detect severity of CM and early treatments. This study may be clearly mechanism of AQP4 for further researchs in novel therapeutic treatments of CM and might be a target for therapeutic intervention to prevent CM.

**Keywords** Cerebral malaria, AQP4, Brain edema, Astrocyte, Blood-brain barrier

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## **Proliferative Myositis of the Vastus Medialis: Benign Lesion Mimicking Sarcoma on Imaging**

Chinnawut Suriyonplengsaeng<sup>1</sup>, Supamit Leemasawat<sup>2</sup>, Jariya Waisayarat<sup>3,\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand

<sup>2</sup> Department of Surgery, Sunpasittiprasong Hospital, Ubon Ratchathani, 34000, Thailand

<sup>3</sup> Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400, Thailand

\*Corresponding author, e-mail: jariya.wai@mahidol.ac.th

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### **Abstract**

Proliferative myositis is a rare benign intramuscular fibroblastic proliferation mimicking sarcoma clinically, radiologically and sometimes histologically. We presented a case of proliferative myositis mimicking sarcoma radiologically, but confirmed its benignity by histopathology. A 47-year-old woman with diabetes mellitus type 2 and essential hypertension presented with a 3-month history of a mild painful slow-growing mass at her right medial thigh without history of trauma. Physical examination revealed afebrile, BP 159/95 mmHg and fixed firm mass at the right medial thigh with mild tenderness. Laboratory investigations were unremarkable. Magnetic resonance imaging demonstrated an ill-defined patchy heterogeneous lesion (9.0x3.1x3.3 cm) at the inferior portion of the right vastus medialis muscle. Right femur and femoral vessels were normal. The differential diagnoses included intramuscular abscess or soft tissue sarcoma with intratumoral hemorrhage. Surgical excision was then performed. Tissue sections unveiled an ill-defined intramuscular lesion composed of proliferation of stellate fibroblasts/myofibroblasts in collagenous stroma and giant cells with abundant basophilic cytoplasm, called ganglion-like cells. These lesional cells infiltrated between myofibers displaying checkerboard pattern. The tissue findings combined with transmission electron microscopic study were compatible with proliferative myositis. Pathological examination is very crucial for definite diagnosis in order to exclude sarcoma and avoid unnecessary radical surgical excision.

**Keywords** Proliferative myositis, pseudosarcoma, intramuscular fibroblastic proliferation, ganglion-like cells

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## A Novel Mutant Affecting Rice Leaf Morphology during Developmental Process

Punyavee Dechkrong<sup>1</sup>, Jun-Ichi Itoh<sup>2\*</sup>

<sup>1</sup>Central Laboratory and Greenhouse Complex, Research and Service Center, Faculty of Agriculture at KamphaengSaen, Kasetsart UniversityKamphaengSaen Campus, MalaimanRoad, KamphaengSaendistrict,NakhonPathom, Thailand.

<sup>2</sup>Laboratory of Plant Breeding and Genetics, Department of Agricultural and Environmental biology, Graduate School of Agricultural and Life Sciences, the University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo, Japan.

\*Corresponding author, e-mail: ajunito@mail.ecc.u-tokyo.ac.jp

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### Abstract

High photosynthesis of plants is considered as a key factor for enhancing yield. Leaf is a crucial organ affecting the capability of light capture. Thus, the proper size and shape of leaves can enhance the photosynthetic efficiency, which lead to yield increase. The mutation and natural variations of plants can be genetic resources for high yield in rice breeding. For instance, the increased-width of flag leaf indicates a positive correlation to the yield per plant [1], and the modified-expression level of *OsBAK1* gene can improve agronomic traits [2]. In this research, to find a new genetic factor that regulates leaf development in rice, a morphogenetic mutant (12T-S-221 line) was characterized and analyzed. The 12T-S-221 line was a recessive mutant showing the abnormal leaf blade and sheath boundary, auricleless, sterile panicles, and irregular shape of spikelets. In addition, to identify the causal gene of this mutant phenotype, genetic analysis was performed. Total 56 mutant plants among F<sub>2</sub> population crossed with cv. Kasalath were isolated and analyzed for rough mapping and fine mapping. As the genotyping result, the candidate region of the causal gene of 12T-S-221 mutant was refined between 100 to 115 cM and located nearby a marker OSJNBb0065L20-K-3 on chromosome 3.

**Keywords** leaf blade and sheath boundary, auricleless, sterile panicles

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## Pollen Types of the Subtribe Andrographinae, Tribe Ruellieae, Family Acanthaceae in Thailand

Winai Somprasong<sup>1\*</sup> Yuppadi Paopan<sup>2</sup> Piyanan Thanomchat <sup>2</sup>

<sup>1</sup>Department of Agriculture, 50 Phahon Yothin Rd., Bangkok 10900 THAILAND

<sup>2</sup>Kasetsart University Research and Development Institute, Kasetsart University, 50 Phahonyothin Rd., Bangkok THAILAND

\*corresponding author, e-mail:winny.th@gmail.com

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### Abstract

The Andrographinae is one of the famous subtribes in the Tribe Ruellieae (Acanthaceae). The most famous genera in this subtribe are *Andrographis* Wall ex Nees, *Graphandra* Imlay, *Gymnostachyum* Nees and *Phlogacanthus* Nees. A large number of them diverse in the tropical countries, especially Thailand, but they have no revision before. The study aims to clarify the pollen types among them. The result showed that there are types, namely tricolporate with distinct aperture margin (*Andrographis laxiflora* and *Phlogacanthus curviflorus*, *P. pauciflorus*, *P. pedunculatus*, *P. pulcherrimus*, *P. rectiflorus*, *P. vitellinus*); tricolporate, open reticulate tectum with scattered granules (*Gymnostachyum leptostachyum*); tricolporate, open reticulate tectum (*G. signatum*, *G. trilobum*, *G. venustum*)

**Keywords :** Subtribe Andrographinae, Acanthaceae, Pollen type

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## The Discrepancies of Wing Hamuli Numbers in Indo-Malayan Stingless Bees Observed under Scanning Electron Microscope (SEM)

Zubaidah A.H<sup>1\*</sup>, A.R Shamsul Bahri<sup>2</sup>

<sup>1</sup> Electron Microscope Unit, Faculty of Medicine, University of Malaya, 50503 Kuala Lumpur

<sup>2</sup> School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu

\*Correspondent author, e-mail: zubae@um.edu.my

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### Abstract

Wing hamuli in stingless bee are considered as one of the unique characteristics and could be used as the features in taxonomy and identification of species. The number of wing hamuli differs from species to species. Wing hamuli can be found on the hind wing, which link the fore wing of the stingless bee for a distant foraging. In this experiment, nine Indo-Malayan stingless bee species were collected from a specialized collection center in Sekayu, Terengganu, Malaysia (Indo-Malayan Meliponine Respository Sekayu) and stingless bee farm in Jalan Kebun, Selangor. Uncoated fresh samples were introduced into the low-vacuum chamber of the SEM and observed for their wing hamuli on both the right and left wing. Observation under the SEM shows clearer hamuli morphology as compared to stereomicroscopy. Result shows the number of wing hamuli on left and right wing of certain stingless bees are not similar. Samples of *Geniotrigona thoracica* exhibits variation wing hamuli. Some has 8 hamuli on the left wing and 9 on the right wing or 10 hamuli on left and 9 hamuli on the right or both wings have 8 hamuli. There are also variation in *Homotrigona aliceae* wing hamuli. Some has 7 or 8 hamuli on both wings or 8 hamuli on right and 7 on the left. On the other hand, *Homotrigona fimbriata* shows both with either 8 or 7 hamuli. Most of *Tetrigona apicalis* shows 7 hamuli on both wings except, one sample with 8 hamuli on the left and 7 on the right. *Heterotrigona erythrogastra* (7 hamuli), *Heterotrigona itama* (7 hamuli) *Tetragonilla collina* (6 hamuli), *Tetrigona melanoleuca* (6 hamuli) and *Tetrigona vidua* (7) exhibit a similar number of hamuli on both wings.

**Keywords** Stingless bee, wing hamuli, meliponine

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## ESEM and EDX Observations on Fresh Longan Fruit after Sulphur Dioxide Fumigation

Suchanya Kojinok<sup>1\*</sup>, Anuchit Ruangvittayanon<sup>2</sup>, Jakraphong Phimphimol<sup>3</sup>

<sup>1</sup> Institute of Product Quality and Standardization (IQS), Maejo University, Chiang Mai 50290, Thailand

<sup>2</sup> Synchrotron Light Research Institute, Nakhon Ratchasima 30000, Thailand

<sup>3</sup> Department of Postharvest Technology, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai 50290, Thailand

\* Corresponding author, e-mail: suchanyakoji@gmail.com

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### Abstract

Sulphur dioxide (SO<sub>2</sub>) fumigation is widely used to control browning and antimicrobial, especially in the production of fresh longan fruit (*Dimocarpus longan* Lour.). However, the toxic of SO<sub>2</sub> residues in peel and pulp is one of the most important problem for export markets [1-3]. In this study, the environmental scanning electron microscopy (ESEM) with the gaseous secondary electron detector (GSED) equipped with an energy dispersive X-ray microanalysis (EDX) was applied to observation and elemental analysis on fresh longan fruit after the vertical forced-air circulation system of SO<sub>2</sub> fumigation, which the SO<sub>2</sub> residues immediately after fumigation was range of 1,500-2,000 mg·kg<sup>-1</sup> on peel and 5 mg·kg<sup>-1</sup> on pulp, respectively. X-ray mapping showed the main chemical element and SO<sub>2</sub> residues in peel tissue and aril surfaces. The result indicated that SO<sub>2</sub> residues were highly accumulated in both outer and inner surfaces of peel tissue after the storage durations for 10 days. However, SO<sub>2</sub> residues in the aril surface decreased significantly, and were not detected after 6 days of storage. This study reveals that ESEM and EDX analysis can be used to support on the observation of SO<sub>2</sub> residues on fresh longan fruit, which will be developed suitable packages for future.

**Keywords** Longan, SO<sub>2</sub> fumigation, ESEM-EDX, GSED

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## Effect of Kaffir Lime (*Citrus hystrix*) Oil on the Blow Fly Larvae: *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae)

Suttida Suwannayod<sup>1</sup>, Kabkaew L. Sukontason<sup>1</sup>, Pradya Somboon<sup>1</sup>, Anuluck Junkum<sup>1</sup>, Benjawan Pitasawat<sup>1</sup>, Ratana Leksomboon<sup>2</sup>, Tarinee Chaiwong<sup>2</sup>, Kwankamol Limsopatham<sup>1</sup>, Sangob Sanit<sup>1</sup>, Narin Sontigun<sup>1</sup>, Chutarat Samerjai<sup>1</sup>, Tunwadee Klongklaew<sup>1</sup>, Kom Sukontason<sup>1\*</sup>

<sup>1</sup> Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

<sup>2</sup> College of Medicine and Public Health, Ubon Ratchathani University, Ubon Ratchathani, Thailand

\*Corresponding author, e-mail: kom.s@cmu.ac.th

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### Abstract

The blow flies, *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) are medically important species worldwide, yet control of their populations is needed. This study determined the efficacy of Kaffir lime (*Citrus hystrix*) oil on both species. These flies were reared under ambient temperature in a laboratory at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, northern part of Thailand. Analysis using gas chromatography–mass spectrometry (GC–MS) revealed that the major constituents of *C. hystrix* were  $\beta$ -pinen (24.62%), sabinene (22.06%) and limonene (19.30%), consecutively. The bio-efficacy of this oil was performed on the 3<sup>rd</sup> instar as a larvicidal bioassay using the dipping method which control larvae were dipped in 80% ethanol. Mortality was assessed at 24 and 48 h post exposure, while alteration was analyzed by scanning electron microscope (SEM) and histopathology. The results showed a median lethal concentration (LC<sub>50</sub>) value of 71.44 and 67.21 ml/L at 24 and 48 h, respectively, for *C. rufifacies*; and 82.56 and 65.06 ml/L at 24 and 48 h, respectively, for *C. megacephala*. Treated larvae were determined under SEM by displaying shrunken, swollen and bleb formations on the integument. Histological investigation showed alteration of the alimentary cells by vacuolization of the gut, fat body and Malpighian tubule cells. No alteration was observed in the control larvae.

**Keywords** *Citrus hystrix*, blow fly, histopathological, ultrastructural study

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## Visualization of Biomedical Protein Complex with Single Particle Analysis

Sangmin Lee, Jeong Min Chung and Jung, Hyun Suk\*.

Department of Biochemistry, College of Natural Sciences, Kangwon National University,  
1, Kangwondeahak-gil, Chuncheon-si, Gangwon-do, 200-701, Korea.

\*Corresponding author, e-mail: [hsjung@kangwon.ac.kr](mailto:hsjung@kangwon.ac.kr)

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### Abstract

Electron microscopy is a successful application to structural studies in biological and material sciences. In biology, the goal of this approach is to visualize general appearances of target macromolecules. To achieve additional structural details with greatly improved signal-to-noise ratio, following computational analysis can be used. One of the analytical methods is single particle analysis which is carried out to combine the data from many individual molecular images randomly distributed and oriented in single forms. Last decades, single particle analysis followed by cryo-electron microscopy has been solved the structures of many macromolecules at high resolution. Nevertheless, it is still regarded as challenging approaches with a low success rate and time-consuming process. One of main reason can be heterogeneous structure of target objects, not amenable to the high resolution studies. In this study, we introduce relatively quick techniques to produce medium resolution structure of macromolecules, therefore, it can be subjected to evaluation steps to choose best macromolecular candidate adopting homologous structure for further processing in cryo-electron microscopy, so termed pre-screening techniques. This report offer informative technical advances of the pre-screening techniques, single particle analysis, to those who are interested in structural biology on a basis of nano-, and bio-sciences.

**Keywords** Transmission electron microscopy, Cryo-Electron microscopy, Single particle analysis.

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## Microscopy of Internal Structures Related to the Morphogenesis and Gravitropism of Yam Tubers

Michio Kawasaki<sup>1\*</sup>, Satoko Koizumi<sup>1</sup>, Shun Sasaki<sup>1</sup>

<sup>1</sup> Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

\*Corresponding author, e-mail: kawasaki@hirosaki-u.ac.jp

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### Abstract

There are many unclear points related to the mechanisms of morphogenesis and gravitropism of yam tubers, although it is important to control the tuber shapes for agriculture. For understanding the mechanisms, we investigated the role of actin filaments and sedimentary amyloplasts in morphogenesis and gravitropism of tubers in the lines and varieties of yam. The pattern of spatial distribution was specific in tuber tips and resembled the pattern in columella of yam roots under confocal laser scanning microscopy. Connections between actin filaments and sedimentary amyloplasts were observed in tuber tips. Tip parts of tuber treated by cytochalasin D, which is an actin polymerization inhibitor, remarkably enlarged. Inverted tubers did not bend downward after the inhibitor treatment. The number of sedimented amyloplasts in tuber tips was greater in elongated tubers than in spherical tubers under light and electron microscopies. Distribution area of sedimented amyloplasts in tuber tips was wider in tubers having a wide tip than in elongated tubers. The results indicated that sedimentary amyloplasts and actin filaments would be involved in morphogenesis and gravitropism of the tubers.

**Keywords** actin filament, amyloplast, gravitropism, morphogenesis, yam

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## 3D Approaches Visualizing Helically Ordered Nano-Bio Materials

Hyung Joong Yun<sup>1</sup>, Chel-Jong Choi<sup>2</sup> and Hyun Suk Jung<sup>3\*</sup>

<sup>1</sup>Advanced Nano Surface Research Group, Korea Basic Science Institute, 169-148

Gwahak-ro, Daejeon, 305-333, Republic of Korea

<sup>2</sup>School of Semiconductor and Chemical Engineering, Chonbuk National University, Jeonju, 561-756, Republic of Korea

<sup>3</sup>Department of Biochemistry, Kangwon National University, 1 Kangwondaehak-gil, Chuncheon-si, Gangwon-do, 24341, Republic of Korea

\*Corresponding author, e-mail: [hsjung@kangwon.ac.kr](mailto:hsjung@kangwon.ac.kr)

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### Abstract

Projection-matching single particle three dimensional reconstruction following to electron microscopy is the one of common methods to visualize three dimensional structures of nano-bio materials and its interaction at near-atomic resolution. In this report, we demonstrated the potentiality of using artificial structure as an initial reference model for its computational processing by describing an example experiment in which we obtained ~ 2000 single particle images from cryo-electron microscopy. The reconstruction using the model provided correct matching parameters of single particle experimental images with projection images of the model, ultimately resulting in generating informative three dimensional volume of helically ordered nano-bio materials. Comparative analysis confirmed that the resultant volume has a remarkably similar structure to the proposed atomic model. These results provided suggestive evidence for using this technical approach as a useful analytical tool for determining macromolecular structure in the model independence way. In addition, the finding goal can be used for evaluating high resolution structure processed from the reference model of atomic structure since it is a ideal method to eliminate any possibilities of model-bias.

**Keywords** Single particle three dimensional reconstruction, Cryo-Electron microscopy, Nano-bio materials.

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## Prismatic and Needle-Like Crystals of Calcium Oxalate in Thai Herbs

Yupadee Paopun<sup>1\*</sup>, Piyanan Thanomchat<sup>1</sup>, Supapan Seraphin<sup>2</sup>

<sup>1</sup> Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, 50 Ngamwongwan Rd., Ladyao, Chatujak, Bangkok 10900, THAILAND

<sup>2</sup> Department of Materials Science and Engineering, University of Arizona, Tucson, Arizona 85721, U.S.A.

\*Corresponding author, e-mail: rdiydp@ku.ac.th

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### Abstract

Many plants store crystalline calcium oxalate (CaOx) in response to excess calcium in the cytoplasm. This is beneficial in multiple phases of a plant's life cycle. CaOx is an insoluble and metabolically inactive salt. Plant may accumulate this salt at an average of 6.3% of plant dry weight and up to 80% in some species. The crystals may form in various parts of the plants and may exist in either monohydrate or dehydrate forms with diverse morphologies. A combination of genetic and environmental factors plays roles in regulating the crystal amount, shape, size, and functions. In Thailand, a number of reports have investigated the presence of calcium oxalate in certain indigenous vegetables. However, there is no report on any detailed microstructure and elemental analysis of the crystals in the following Thai herbs, (1) krawan (Siam Cardamom (*Amomum krevanh* Pierre.)); (2) realyai (Buatard Cardamom (*Amomum xanthioides* Wall.)); (3) krasang (*Peperomia pellucida* Korth.); and (4) purple allamanda (*Thunbergia laurifolia* Lindl.). In this study, we used scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) to characterize the size, shape, and elemental composition of the crystals in leaves of these Thai herbs.

**Keywords** calcium oxalate in Thai herbs, prismatic and needle-like calcium oxalate

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## A Comparison between the Two Techniques for Preparing Lemon Grass Leaf for Scanning Electron Microscopy

Piyanan Thanomchat<sup>1\*</sup>, Yupadee Paopun<sup>1</sup>

<sup>1</sup> Scientific Equipment and Research Division, Kasetsart University and Development Institute, Kasetsart University, 50 Ngamwongwan Road, Bangkok, 10900, Thailand

\*Corresponding author, e-mail: rdipnt@ku.ac.th

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### Abstract

A preparation technique of biological sample is an important step for scanning electron microscopic to preserve cell structure. Therefore, this study aimed to compare *Cymbopogon citratus* Stapf. (Lemon grass) leaf sample preparing by chemical fixation (using 2.5% glutaraldehyde followed by 1% osmium tetroxide and drying with critical point dryer) and a cryotechnique (using freeze dryer for 18 hours). In general, chemical fixation is used for structural preservation of samples but double fixation, using glutaraldehyde and osmium tetroxide is highly toxic. Freeze drying technique was carried out by deeping the samples into liquid nitrogen and drying us by freeze dryer under vacuum at -70 °C. The results showed that chemical fixation caused low shrinkage of epidermal cells similar to freeze drying method. Although leaf sample preparation by freeze drying method takes longer time than chemical fixation, osmium tetroxide which are harmful to user and environment is eliminated. Thus sample preparation by freeze drying method is probably an advantage for scanning electron microscopy.

**Keywords** freeze drying, chemical fixation, lemon grass, SEM

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## The Application of Cryogenic Focused Ion Beam Scanning Electron Microscopy to Hydrogel Characterization

Christopher Parmenter<sup>1\*</sup>, Abdulraman Baki<sup>2</sup>, Kevin Shakesheff<sup>2</sup>

<sup>1</sup> Nottingham Nanoscale and Microscale Research Centre, University of Nottingham, Nottingham, UK

<sup>2</sup> Wolfson Centre for Stem Cells, Tissue Engineering and Modelling (STEM), School of Pharmacy, University of Nottingham, Nottingham, UK

\*Corresponding author, e-mail: Christopher.parmenter@nottingham.ac.uk

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### Abstract

Hydrogels are typically prepared for electron microscopy by the removal of water, which can lead to deformation and structural change. Cryo-techniques present an opportunity to image samples in a more native state and Cryogenic Focused Ion beam scanning electron microscopy can be used to provide cross-sectional and three dimensional images, which will aid understanding of the native structure. We present the application of cryo-FIB-SEM to a hydrogel and explore the effects of controlled dehydration. Subsequent image analysis yields improved understanding of the system and this is contrasted with a fully hydrated imaging approach. The potential application of this technique is briefly explored in three dimensions and more widely its possible impact on the hydrogel community.

**Keywords** cryo-FIB-SEM, Hydrogel, Pore-size, Characterization, Hydrated

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## Macroscopic Anatomy of the Vascular Foramina of Human Hamate Bone

B.V. Murlimanju\*, P.J. Jiji, Ashwin Krishnamurthy, Vandana Blossom

Department of Anatomy, Kasturba Medical College, Manipal University, Bejai, Mangalore-575004, Manipal University, Manipal, Karnataka, India.

\*Corresponding author, e-mail: flutemist@gmail.com

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### Abstract

**Background:** The aim of the present study was to study the topography and number of vascular foramina in the hamate bones of South Indian population.

**Methods:** The present study included 47 human hamate bones, among them 20 belonged to left side and 27 were right sided.

**Results:** The number ranged between 11 and 40 in each hamate bone. They were ranged between 1 and 11 in number at the palmar surface, lateral side. The foramina were between 0 and 4 at the medial side of the palmar surface, which is very close to the base of the hook. There were foramina observed at the apex of the hook, medial side which ranged between 0 and 6. The foramina at the distal surface ranged between 0 and 4 in each hamate bone. The foramina were highest at the dorsal surface, which ranged between 5 and 16 in each hamate bone. The number ranged between 0-3 at the proximal surface and 0-8 at the lateral surface.

**Conclusion:** We believe that the present study has provided additional information about the morphology and topography of vascular foramina of the hamate bones in South Indian population.

**Keywords:** avascular necrosis, non-union, vascular foramen

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## Anatomical Study of the Retaining Ligaments and Its Relationship to the Facial Soft Tissue of the Midface

Sukanya Uruwan <sup>1\*</sup>, Tanvaa Tansatit <sup>1</sup>, Benrita Jit-aree <sup>1</sup>, Thirawass Phumyoo <sup>2</sup>, Chalermquan Rungsawang <sup>1</sup>

<sup>1</sup> The Chula Soft Cadaver Surgical Training Center and Department of Anatomy, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Rama 4 Road, Pathumwan, Bangkok, Thailand

<sup>2</sup> Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Samsen road, Dusit, Bangkok, Thailand

\*Corresponding author, e-mail: bbss\_pid@hotmail.com

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### Abstract

The anatomical knowledge of retaining ligaments is essential to prevent the complications during face lift procedure. The purpose of this study was to investigate detailed anatomic morphology of retaining ligaments and the relationship relate to the facial soft tissue landmarks. The dissection was performed on the midfaces in 28 hemi-faces of Thai soft embalmed cadavers. The major zygomatic retaining ligament closely related to the facial nerve branch in the distance of  $7.31 \pm 7.26$  mm. The upper masseteric retaining ligament located posterosuperior (region 3) of the masseter muscle. The nerve branch was beyond from the ligament  $0.98 \pm 2.04$  mm, releasing ligament should perform more than 2 mm from the ligament to prevent the injury. The most important masseteric retaining ligaments located on middle (region 5) and posteromiddle (region 6) of the muscle. There consist of the numerous and firmly ligaments, releasing both ligaments might simplify moving of the skin and soft tissue to achieve face lift technique.

**Keywords** Face lift, masseteric retaining ligament, zygomatic retaining ligament, facial nerve

## Equations to Determine Sex from the Hip and the Sacral Bones in Thais

Jureeporn U-pathi<sup>1</sup>, Supap Saenphet<sup>2</sup>, Sompoch Thanachaikun<sup>3</sup>, Karnchana Harnsiriwattanakit<sup>3</sup>, Paiwan Sudwan<sup>3\*</sup>

1 Department of General Education, Kanchanabhisek Institute of Medical and Public Health Technology, Nonthaburi 11150, Thailand

2 Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

3 Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

\*Corresponding author, e-mail: paiwan.sudwan@cmu.ac.th

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### Abstract

Equations to determine sex using osteometric analysis of the pelvic girdle in Thais was made on 3 parameters of sacrum, namely anterior length (antl), anterior – superior breadth (asb) and maximum transverse diameter of base (mtb) and 8 parameters of hip bones, namely maximum depth of greater sciatic notch (mdg), ischial length (isl), total height (th), acetabulum diameter (acd), iliac breadth (ilib), obturator foramen width (obfw), obturator foramen height (obfh) and width of greater sciatic notch (wgn). The pelvic girdle consists of 115 paired hip bones and 115 sacra, of 83 males and 32 females. The result shows that the mtb and antl of sacrum and mdg, isl, th, acd, ilib, and wgn of the pelvis were significantly different between sex. By logistic regression analysis, the equation for the right, the left and both sides together of hip bones were created with the high accuracy of sex determination. The conclusion suggests that the results from osteometric pelvic girdle data in Thais can be used in the fields of human anatomy, forensic science, and anthropologists for determining sex dimorphism.

**Keywords** Sex determination; Hip bone; Sacrum; Osteometric Analysis; Thais

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## Music Innovation Enhance Student Learning in 21<sup>st</sup> Century Education: Anatomy and Physiology in Bali, Indonesia

Pisit Poltana<sup>1,2\*</sup>, I Made Sundayana<sup>3</sup>, Paradorn Yingyoud<sup>1,2</sup>, I Dewa Ayu Rismayanti<sup>3</sup>, I Dewa Putu Agus Wahyu Erawan<sup>3</sup>, Ni Nyoman Ari Ratnadi<sup>3</sup>, Napadon Leaudnakrob<sup>2,4</sup>

<sup>1</sup> Boromarajonani College of Nursing, Changwat Nonthaburi, Tiwanon Road, Muang, Nonthaburi, 11000, Thailand

<sup>2</sup> Center of Excellence for Innovative Learning and Teaching in Anatomy & Physiology  
Praboromarajchanok Institute (CEILTAP), 47/99 Moo 4, Tiwanon Road, Muang, Nonthaburi, 11000, Thailand

<sup>3</sup> Sekolah Tinggi Ilmu Kesehatan Buleleng (STIKES Buleleng), Jalan Raya Air Sanih Km. 11 Bungkulan, Singaraja-Bali, Indonesia

<sup>4</sup> Boromarajonani College of Nursing, Uttaradit, Muang, Uttaradit, 53000, Thailand

\*Corresponding author, e-mail: ppoltana@bcnnon.ac.th, ppoltana@hotmail.com

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### Abstract

The cross-sectional and descriptive research design was conducted. It aimed to 1) explore satisfaction of nursing and midwifery students on learning by utilizing the Anatomy and Physiology's music entitled the "Cranial Nerve", 2) compare satisfaction of the music between nursing and midwifery students, and 3) in-depth interview based on students' self report for using of the music. The music entitled "Cranial Nerve", as an innovation for learning and teaching in the 21<sup>st</sup> century, was created in Bahasa Indonesia. Subjects were the second year nursing students (n= 50) and also midwifery students (n= 50) in Bali, Indonesia. Simple random sampling was applied in collecting data from STIKES Buleleng. Quantitative findings revealed that overall of satisfaction of nursing and midwifery students on learning by utilizing on the Anatomy and Physiology's music entitled "Cranial Nerve" was placed on a high level. The nursing students gave higher satisfaction than midwifery students. Qualitative findings revealed the music made nursing and midwifery students easily memorized on the content, more understanding on the content, being happy on learning, and creative capacities for lifelong learning.

**Keywords** Music of Anatomy and Physiology, Innovation, Nursing and Midwifery Students' Satisfaction, Learning and Teaching in 21<sup>st</sup> Century

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## Dimensions of the Lateral Orbital Part of the Orbicularis Oculi Muscle Implication for Botulinum Toxin Injection in Embalmed Cadavers

Thirawass Phumyoo <sup>1\*</sup>, Tanvaa Tansatit <sup>2</sup>, Benrita Jit-aree <sup>2</sup>, Sukanya Uruwan <sup>2</sup>, Chalermquan Rungsawang <sup>2</sup>

<sup>1</sup> Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Samsen road, Dusit, Bangkok, Thailand

<sup>2</sup> The Chula Soft Cadaver Surgical Training Center and Department of Anatomy, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Rama 4 Road, Pathumwan, Bangkok, Thailand

\*Corresponding author, e-mail: oncestep@hotmail.com

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### Abstract

This study aimed to determine the dimensions of the lateral orbital part of orbicularis oculi muscle (LOOOM) and measure the thicknesses of overlying subcutaneous cheek and lateral orbital fat (SCLOF) and underlying suborbicularis oculi fat (SOOF) which are essential for effective correction of crow's feet and lateral brow ptosis using botulinum toxin injection. This study was investigated on 40 orbits of 20 Thai embalmed cadavers. Five incisions were performed by radiating at the lateral orbital rim to equally divide the LOOOM into five levels. There were two upper oblique (UO1 and UO2 lines), lateral (L line), and two lower oblique (LO1 and LO2 lines) All directions were measured the thicknesses of SCLOF and SOOF at 0, 10 and 20 mm distances from the lateral orbital rim. The results of this study found that the SCLOF thickness over the LOOOM was between 2.7 to 6.3 mm and the SOOF ranged between 1.8 to 3.6 mm. The closed points to malar eminence were the thickest in both the SCLOF and the SOOF. Although the LOOOM extended more than 1.7 cm in all measured directions, the allowing effective injection of botulinum toxin should be done at approximately 5 mm distant apart from the orbital rim.

**Keywords** Orbicularis oculi muscle, botulinum toxin injection, subcutaneous cheek and lateral orbital fat, underlying suborbicularis oculi fat

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## Types of Rhomboid Fossa on Dried Clavicles in Northeastern Thais

Ailadda Kaewma<sup>1\*</sup>, Apichakan Sampannang<sup>1</sup>, Panya Tuamsuk<sup>1</sup>, Jaturat Kanpittaya<sup>2</sup>, Sitthichai Iamsaard<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mitraparp Road, Khon Kaen, Thailand

<sup>2</sup> Department of Radiology, Faculty of Medicine, Khon Kaen University, Mitraparp Road, Khon Kaen, Thailand

\* Corresponding authors, e-mail: ailadda.kaewma@hotmail.com and sittia@kku.ac.th

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### Abstract

The rhomboid fossa of clavicular bone is used to determine the age and sex in anthropology and forensic sciences. The variant types of rhomboid fossa on inferior surface have been reported in many races except in Thais. Therefore, this study was aimed to classify the types of the clavicular rhomboid fossa in Northeastern Thais. The identified 470 Northeastern Thais dried clavicles (264 males and 206 females) were observed and recorded for the types of rhomboid fossa. The results showed that Thai-rhomboid fossa could be classified into 4 types: Type 1 (depressed) Type 2 (flat), Type 3 (elevated) and Type 4 (smooth), respectively. The incidences of rhomboid fossa were Type 1 (3.62%), Type 2 (19.57%), Type 3 (76.60%), and Type 4 (0.21%), respectively. Additionally, it was found that the percentage of type 1 in males (5.69%) was much greater than that of female (0.97%) compared to other types. This incidence of rhomboid fossa types may be a basic knowledge using in sex identification of Northeastern-Thai clavicular remains.

**Keywords** Rhomboid fossa, clavicle, Northeastern Thais

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## Double Superior Vena Cava in Thai Cadaver

Pornsawan Duangsuwan<sup>1\*</sup>, Kosol Puriwattanakul<sup>1</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand

\*Corresponding author, e-mail: pornsawan.d@psu.ac.th

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### Abstract

Double superior vena cava is one of the most common thoracic venous anomalies, due to the embryogenical failure of regression of the left anterior cardinal vein. During routine dissection of the thorax region, we found a case of double superior vena cava in a 54-year-old Thai female cadaver. Both the vena cavae were formed as continuations of brachiocephalic veins of the corresponding sides. The right superior vena cava was normal and opened into the right atrium. The left superior vena cava opened into the dilated coronary sinus that drained into the right atrium between the opening of inferior vena cava and right atrioventricular orifice. No communication was observed between the two vena cavae. The hemiazygos and accessory hemiazygos veins drained normally into the azygos vein and opened into the right superior vena cava. No other associated variations were observed. Double superior vena cava can lead to serious complications during catheterization via subclavian or internal jugular vein. Awareness of this anomaly may therefore reduce confusions and thus would help to avoid further complications.

**Keywords:** double superior vena cava, coronary sinus

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## Ligamentous Band of Obliterated Left Common Iliac Vein Coexisting with Aortic Aneurysm: an Uncommon Variation

Malivalaya Namking\*, Worawut woraputtaporn, Wunnee Chaijaroonkhanarak, Nawaporn  
Techataweewan, Wilaiwan Mothong)

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mitraphap Road, Khon  
Kaen, Thailand

\*Corresponding author, e-mail: malnam@kku.ac.th

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### Abstract

This uncommon variation was found in an 82 year-old-female body during pelvis dissection. The left common iliac, external iliac and internal iliac veins were completely obliterated and turned to be ligamentous bands. There were two large veins in the left pelvis, one is the left ovarian vein, another is the communicating vein connecting between obturator and inferior epigastric veins. These two veins served as collateral venous circulation for obliterated common iliac vein. On the sagittal section cutting through pelvic organs, numerous dilated blood vessels were observed along the walls of uterus, urinary bladder, rectum and anal canal. This could be the formation of transpelvis collaterals, connecting both internal iliac veins, thus creating outflow to the right common iliac vein. A large abdominal aorta aneurysm was found just above the bifurcation. This may due to a long sustained resistance in the vein then causing chronic pulsatile compressive force on the arterial wall. This variation is reasonable to be a congenital anomaly since the venous circulation was able to be compensated by collateral circulation until 82 years of life. Unlike the iliac vein compression in May-Thurner syndrome that causes many suffering symptoms needed for treatment.

**Keywords** Obliterated vein, ligamentous band, common iliac vein

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## Absence of the Musculocutaneous Nerve and Associated Compensation by the Median Nerve – A Case Report

Prasad A.M.

Department of Anatomy, Melaka Manipal Medical College (Manipal Campus), Manipal University, Madhav Nagar, Manipal, Karnataka State, India. 576104

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### Abstract:

The musculocutaneous and median nerves frequently show variations from their normal course. During a routine dissection class for first year medical students, an adult male cadaver aged approximately 60 years, was found to have unique variations in the median and musculocutaneous nerves. Those variations were observed unilaterally in the right upper limb and included the complete absence of the musculocutaneous nerve. The median nerve was found to supply the coracobrachialis, biceps, and brachialis muscles in the arm. In addition, the lateral cutaneous nerve of the forearm was found to originate from the median nerve in the upper part of the arm and continued downward and laterally between the biceps and brachialis muscles until the elbow. At the elbow, the nerve emerged on the lateral border of the biceps and brachialis muscles and entered the forearm. In cases of bone fractures in the upper limb, such as fracture of humerus, the resulting injuries to the median nerve can be mistaken for injuries of the musculocutaneous nerve. Therefore, it can be concluded that knowledge of such variations is of clinical significance to orthopedic surgeons, general surgeons, sport medicine experts, physiotherapists and anesthesiologists.

**Keywords:** Musculocutaneous nerve, median nerve, lateral cutaneous nerve of the forearm, variation

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## A Rare Case of Duplication of the Tendon of Palmaris Longus Muscle

Deepthinath R, Sudarshan S

Department of Anatomy Melaka Manipal Medical College, Manipal University, Manipal, Karnataka, India-576104.

\*Corresponding author, e-mail: rdnath@gmail.com

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### Abstract

Palmaris longus is one of the muscles of the superficial flexors of the anterior compartment of the forearm. It takes origin as a slender belly from the anterior surface of the medial epicondyle of the humerus. Its belly runs downwards and converts into tendon approximately at the junction of the proximal one-third and distal two-third of the forearm. The present case was observed during routine cadaveric dissection for the first year medical students. We found the duplication of palmaris longus muscle in an approximately 50 year old male. The origin of the muscle belly of palmaris longus was seen to be normal, however the insertion of the tendons were different. The tendons inserted into the flexor retinaculum and palmar aponeurosis respectively. In clinical studies, a check on the presence of palmaris longus is done as a preoperative evaluation for use in grafts. Such rare cases of interest provide vital information for clinicians and surgeons during tendon graft.

**Keywords** Palmaris longus, duplication, flexor retinaculum, palmar aponeurosis

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## Macroscopic Anatomy of the Vascular Foramina of Human Triquetrum Bone

B.V. Murlimanju, P.J. Jiji, Ashwin Krishnamurthy\*, Y. Lakshmisha Rao  
Department of Anatomy, Kasturba Medical College, Manipal University, Bejai, Mangalore-575004,  
Manipal University, Manipal, Karnataka, India.  
\*Corresponding author, e-mail: anataashwin@gmail.com

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### Abstract

**Background:** Triquetral bone is the second commonly fractured carpal bone. Its fracture can cause injury to the arteries and can lead to avascular necrosis. There is no data available about the vascular foramina of the triquetrum. The aim was to study the topography and number of vascular foramina in the triquetra of South Indian population. **Methods:** The present study included 18 human triquetral bones, among them 11 belonged to left side and 7 were right sided. The triquetra were macroscopically observed for the location and number of the vascular foramina at each surface. **Results:** The vascular foramina were observed in all the triquetral bones (100%). The number ranged between 8 and 20 in each triquetrum bone. They were ranged between 1 and 5 in number, over the palmar and 3-10 over the dorsal surfaces. The number ranged between 2-8 at the proximal surface and 0-4 at the medial surface. **Conclusion:** The morphological knowledge of the vascular foramina, their location and number are essential to understand the concepts of non-union and avascular necrosis of the triquetrum bone. The data is enlightening to the plastic surgery and the operating hand surgeon.

**Keywords** Avascular necrosis, non-union, triquetrum, vascular foramen

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## Relation Between Latissimus Dorsi and Teres Major and Its Surgical Importance

Rajalakshmi Rai

Department of Anatomy, KMC Mangalore, Manipal University, Mangalore, Karnataka, INDIA

e-mail: rajalakshmi.raai@manipal.edu

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### Abstract

**Background:** Latissimus dorsi (LD) and teres major (TM) are sharing similar function. Both are muscles of the back of the trunk with different origin but adjacent insertion. Both LD and TM, individually, are preferred for tendon transfer surgeries in rotator cuff repair.

**Objectives:** Therefore the present study is carried on 24 embalmed male cadavers to observe any modification in the attachment of LD and TM and examine the relation between two.

**Result:** An accessory slip of muscle was found between the above two muscles in 16% of the cadavers studied. Fascial connection between latissimus dorsi and teres major are well known, but such muscular link between LD & TM is comparatively unusual.

**Conclusion:** Such attachment between two muscles may hamper the mobility of the muscle during any transfer procedure. Therefore the results of present study suggests that during transfer surgeries the TM need to be transferred along with the LD as a single unit.

**Keywords** Latissimus dorsi, teres major, transfer surgeries, shoulder injury repair

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## Morphology of Caudate Lobe of Liver in South Indian Population

Dr. Prameela.M.D

Associate Professor, Department of Anatomy, Kasturba Medical College, Mangalore, Manipal University e-mail: prameela.md@manipal.edu

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### Abstract

**Background:** The caudate lobe is situated on the posterior surface of the right lobe of the liver. A thorough knowledge of normal and variant anatomy of caudate lobe is essential for diagnostic imaging, minimal invasive procedure and for the better surgical outcome. **Objective:** This study was undertaken to study the morphology and variations of caudate lobe. Twenty four formalin fixed liver were examined for variations in the shape, presence of fissures and accessory processes. **Result:** Common type of variation found was the presence of a vertical fissures and prominent papillary process. **Conclusion:** Knowledge about these types of anomalies helps the radiologists to avoid possible errors in interpretations and subsequent misdiagnosis, and the surgeons for planning appropriate surgical approaches.

**Keywords** Caudate lobe, Papillary process, Fissures

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## Incidence of Double Left Suprarenal Vein and Its Clinical Implication

Kimaporn Khamanarong<sup>1\*</sup>, Tarinee Sawatpanich<sup>1</sup>, Somsiri Ratanasuwan<sup>1</sup>, Anucha Ahooja<sup>2</sup>, Kittisak Sawanyawisuth<sup>3</sup>

Department of <sup>1</sup>Anatomy, <sup>2</sup>Radiology, and <sup>3</sup>Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand

\* Corresponding author, E-mail: kkimap@kku.ac.th

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### Abstract

Venous drainage of each adrenal gland usually drains by a single suprarenal vein. The right suprarenal vein is usually very short and courses transversely and directly drains to the inferior vena cava. Whereas, the left suprarenal vein is longer than the right suprarenal vein and generally receives the drainage from the left inferior phrenic vein before entering the superior border of the left renal vein. The left suprarenal vein may have several anatomical variants. The objective of this study was to examine the incidence of double left suprarenal veins in Thai population. We studied 175 embalmed cadavers with age between 19 and 90 years. Of those, 4 cadavers (2.29%) had double left suprarenal veins. Even though the incidence of double left suprarenal veins is low, interventionists and surgeons should be acknowledged of this variation. Performing adrenal blood sampling and laparoscopic adrenalectomy may increase risk of intraoperative hemorrhage in these population.

**Key words:** double left suprarenal veins, adrenal sampling, laparoscopic adrenalectomy

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## Morphological Study of the Human Spleen in South Indian Population

Tanuj Kanchan<sup>1</sup>, B.V. Murlimanju<sup>2</sup>, Vasudha V. Saralaya<sup>2\*</sup>

<sup>1</sup>Department of Forensic Medicine and Toxicology, Kasturba Medical College, Manipal University, Mangalore-575001, Manipal University, Manipal, Karnataka, India.

<sup>2</sup>Department of Anatomy, Kasturba Medical College, Manipal University, Mangalore-575004, Manipal University, Manipal, Karnataka, India.

\*Corresponding author, e-mail: vasudha.vs@manipal.edu

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### Abstract

**Purpose:** Spleen is the largest lymphoid organ of the study. It is purple in color and tetrahedral in shape. It is located in the right hypochondrium and sometimes gets ruptured during the road traffic accidents. It is also enlarged in diseases like typhoid and leukemia. The morphology of the spleen is important to diagnose the splenomegaly. The aim of the present study was to study the morphology of spleen in South Indian population.

**Methods:** The present study included 48 human spleens, among them 36 belonged to male and 12 belonged to female population. The morphology of the spleen was studied. It was just a macroscopic observational study.

**Results:** It was observed that, 27.1% of the spleens were wedge shaped. The notches were more commonly seen over the superior border. It was found that 12.8% of the spleens had fissure over the diaphragmatic surface. The accessory spleens were not observed in the present study.

**Conclusions:** The morphological data of spleen is important to the clinicians to avoid the misinterpretations. We believe that the present investigation has given important data about the morphology of spleen from the South Indian population.

**Keywords** Morphology, Morphometry, Spleen

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## A Central Database of the Cadavers for Missions of the Anatomy Department

Ranida Quiggins<sup>1,\*</sup>, Phatcharanant Thongsuk<sup>1</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Chiang Mai University, Inthawarorot Road, Chiang Mai, Thailand

\*Corresponding author, e-mail:ranida.quiggins@cmu.ac.th

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### Abstract

Receiving cadavers is one of the responsibilities of the Department of Anatom. In the past, data regarding cadavers has been recorded into log books. The retrograde study of the cadaveric data for year 2014 took several days to analyze. The difficult experiences in satisfying protocols led to the idea of creating a data base for the cadavers using the Excel program as a central database, starting on February, 2015. The hypothesis of this study is that the digital database of the cadavers and service requests for using the cadavers probably reduces the time needed for of data analysis each year, and improves management of the system. The secretary regularly records the cadaveric data in the Excel program after the head of the department recognizes that the cadavers have been received. The results of the recorded database over the past year shows the total number of the cadavers received, the current cadaveric data of different categories, and any trends revealed in the cadaveric data. This digital data analysis for 2015 took less time than that done in 2014. This data could be used to construct an effective management plan for the following year.

**Keywords** Body donation, cadaver, database

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## Intraosseus Perfusion : Optional Practise on Cortical Bone of Proximal Tibia in Thai Population

Manita Saythanprom<sup>1</sup>, Arun Sawasdipong<sup>1</sup>, Niti Matheesiriwat<sup>1</sup>, Porncharn Saitongdee<sup>2\*</sup>

<sup>1</sup>Department of Trauma and Emergency Medicine, Phramongkutklao Hospital, Thailand

<sup>2</sup>Department of Anatomy, Faculty of Science, Mahidol University, Thailand

\*Corresponding author, e-mail:porncharn.sai@mahidol.ac.th

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### Abstract

Intraosseous access in critically ill patients after failure to access peripheral vein, is an essential procedure. The intraosseous route is a safe optional practise , rapid application, and minimal interruption in PCR. For intraosseus puncture , a needle tip must placed in medullary space. Therefore, an average of the proximal tibia cortical thickness measurements required to minimize errors and complications. We aim to determine the thickness of proximal tibia cortical bone in Thai cadaver by gross sectional cadavers base study. Fifty cadavers were included in this study. Average left and right proximal tibia cortical bone thickness in male and female were  $3.63\pm0.62$  mm,  $3.66\pm0.65$  mm,  $3.20\pm0.63$  mm and  $3.19\pm0.61$  mm respectively. Among age less than 60 years, average cortical bone thickness at left and right proximal tibia were  $4.00\pm0.71$  mm and  $3.75\pm0.71$  mm respectively. In conclusion, the average of cortical thickness of proximal tibia in male and female were  $3.64\pm0.64$  mm and  $3.20\pm0.062$  mm respectively. The thickness of cortical bone was decreased according to the age. There is no significant difference between the mean value of cortical thickness on left and right proximal tibia.

**Keywords** Intraosseous infusion, Proximal tibia, Cortical bone

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## Flipping the Anatomy Classroom at Chiang Mai University

Nutcharin Pakdeewong<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

\*Corresponding author, e-mail: nutcharin.o@cmu.ac.th

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### Abstract

To improve the effectiveness of anatomy pedagogy and promote the 21<sup>st</sup> century skills of health science students, the author developed a flipped classroom educational model and conducted a pilot study in anatomy course for undergraduates of various allied health science professions. 213 second-year students of Faculty of Associated Medical Sciences at Chiang Mai University, who registered for Basic Anatomy 301233 in the Academic Year 2015, were assigned to learn by the flipped classroom model. The students had to view online materials along with lecture handout prior to class; after that, the in-class session concentrated on knowledge application of learning content by discussions and doing exercises based on learning objectives under the guidance of a facilitator. To evaluate the knowledge achievement, the quiz was sequentially used; before self-learning activities, before beginning the class, and after completing the class. The statistical analysis revealed that the average score significantly and progressively increased regardless of student major ( $p < 0.05$ ). Overall student feedback through in-class reaction, online response and informal queries after the experience was positive concerning the self-directed and interactive aspects of such structure of integrative education. The results highly recommend to implement this model in teaching anatomy in Thai undergraduates.

**Keywords:** flipped classroom, student-centered learning, anatomy, health science education

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## The vertebral Levels of the Supracristal Line

Pattama Amarttayakong<sup>1</sup>, Worawut Woraputtaporn<sup>1</sup>, Wanassanun Pannangrong<sup>1</sup>, Nichapa Phunchago<sup>1</sup>, Kimaporn Khamanarong<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mittraphap Road, Khon Kaen, Thailand

\*Corresponding author, e-mail: kkimap@kku.ac.th

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### Abstract

The supracristal line, the line joining the highest point of the iliac crest on each side, is a clinical landmark for lumbar puncture. It is commonly stated to cross the midline at the L4 or L4-5 spinal level on imaging. The purpose of this study is to assess the vertebral level of the supracristal line in adults from plain film radiography of abdomen. The subjects were 196 adult patients (101 males and 95 females) with the average age of 60.3 years (25-92) undergoing plain abdominal radiography at Srinagarind Hospital during 2015. Spinal deformities such as scoliosis, osteoporosis, sacralization and lumbarization were excluded. The vertebral levels crossed by the line joining the superior margin of the iliac crests were assessed. The results showed that the supracristal lines were located at L4 in 27%, L4-5 in 37% and L5 in 36%. The most vertebral levels transected by the supracristal lines were L4-5 in males (40%) and L5 in females (48%). The present study should help the physicians to identify accurate vertebral level in the performance of lumbar puncture.

**Keywords** supracristal line, iliac crest, lumbar puncture

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## The Structure of *Pandanus amaryllifolius* Roxb. by Optical Microscopy and Transmission Electron Microscopy

Patcharee Umrung

Scientific Equipment and Research Division

Kasetsart University Research and Development Institute, Kasetsart University, Bangkok, 10900.

rdipru@ku.ac.th

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### Abstract

*Pandanus amaryllifolius* Roxb. is the only species belonging to the family pandanaceae that has fragrant. The food industry used various herbals as condiments to improve flavor and aroma. *P. amaryllifolius* Roxb. Is one such herb. It is used in folk medicine as an anti-inflammatory properties, against joint pains and strained muscles, bladder diseases, diabetes. The samples of were collected from gardens in Bangkok, Thailand during January 2016. The present study was to investigate of the leaves of *P. amaryllifolius* Roxb. Was studied by optical microscopy (OM) and transmission electron microscopy (TEM). These leave preparation and chemical structure replaced with Spurr's Rasin and cut into thick sections (1µm thick) and thin section (100 nm.). The results of the study with the optical microscopy (OM). The structure of the normal leaves consisted of a continuous layer of, mesophyll cell, parenchyma, phloem, xylem. In study with transmission electron microscope .TEM can see the details of the internal structure of the cell, cell wall, Vacuole, chloroplast and mitochondrion .This result is used for basic research of plant.

**Keywords** Ultrastructure, *Pandanus amaryllifolius* Roxb., Optical microscope(OM), Transmission electron microscopic (TEM)

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## Effects of *Pueraria mirifica* Extract on Depression-Like Behavior and Density of Dopaminergic Neurons in Ovariectomized Mouse Brain

Kirati Niyomrat<sup>1</sup>, Wandee Udomuksorn<sup>2</sup>, Ekkasit Kumarnsit<sup>3</sup>, Surapong Vongvatcharanon<sup>4</sup>,  
Uraporn Vongvatcharanon<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand

<sup>2</sup> Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand

<sup>3</sup> Department of Physiology, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand

<sup>4</sup> Department of Oral Surgery (Anesthesiology), Faculty of Dentistry, Prince of Songkla University, Hatyai, Songkhla, Thailand

\*Corresponding author, e-mail: uraporn.v@psu.ac.th

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### Abstract

Estrogen has modulatory roles on dopaminergic neurotransmitter associated in the process of depression. *Pueraria mirifica* (PM) is phytoestrogen rich plant. This study investigated effects of PM extract on depression-like behavior and density of dopaminergic neurons in ovariectomized mouse brain. Female ICR mice were divided into SHAM and ovariectomized groups (OVX) treated with either vehicle (PM0), estradiol benzoate (40 µg/kg, E40) or PM at 25 (PM25), 50 (PM50) and 100 (PM100) mg/kg or treated with Bupropion (25 mg/kg) for 90 days. Depression-like behavior was investigated using the dark/light box test and open field test. The density of tyrosine hydroxylase immunoreactivity neurons in substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) was also measured. The results demonstrated no significant difference in locomotor activity among groups. One-way ANOVA revealed significant difference among groups for dark/light box test [ $F(6,41)=2.910$ ,  $p=0.021$ ]. Multiple comparisons indicated significant increase in time spent in dark box of PM0 group. However, PM replacement was found to restore this parameter to baseline levels seen in SH group. Moreover, the density of dopaminergic neurons appeared to be reduced in PM0 and restored by PM treated groups. This study suggested possible beneficial property of PM replacement for depression.

**Keywords** *Pueraria mirifica*, depression, tyrosine hydroxylase, bupropion

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## Histological Study of the Gastrointestinal Tract of *Pomacea Canaliculata*

Piyachat Songvijit<sup>1</sup>, Apichart Ngernsoungnern<sup>2</sup>, Piyada Ngernsoungnern<sup>2\*</sup>

<sup>1</sup> School of Pharmacology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

<sup>2</sup> School of Anatomy, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

\*Corresponding author, e-mail: npiyada@sut.ac.th

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### Abstract

In the present study, histology of epithelial cells lining the gastrointestinal tract of the *Pomacea canaliculata* was studied. It was observed that the snail's gastrointestinal tract consisted of the esophagus, stomach, intestine, and rectum. The esophageal crop was not apparently seen. Using conventional hematoxylin and eosin staining, the result showed that morphology of the epithelial cells was similar in all parts of the gastrointestinal tract. Each part of the gastrointestinal tract was covered by simple columnar epithelium with microvilli presented at the apical region of the cells. Moreover, both neutral and acidic mucin-secreting cells were observed in parts of the snail's gastrointestinal tract.

**Keywords** Gastrointestinal tract, golden apple snail, *Pomacea canaliculata*

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## The Effects of Maqui Berry (*Aristotelia chilensis*) on Liver Morphology of Parasitized Mice.

Tanwarat Thanwisate<sup>1</sup>, Thanaporn Rungruang<sup>1\*</sup>, Supin Chompoopong<sup>1</sup>, Aikkarach Kettawan<sup>2</sup>

<sup>1</sup> Department of Anatomy, Faculty of Siriraj Medicine Hospital, Mahidol University, Prannok Road, Bangkok, Thailand.

<sup>2</sup> Institute of Nutrition, Mahidol University, Salaya, Nakorn Pathom, Thailand.

\*Corresponding author, e-mail: thanaporn.run@mahidol.ac.th

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### Abstract

Malaria is a mosquito-borne infectious disease of humans. Liver is the first target organ of malarial infection and malarial parasite induced free radical on erythrocytes that lead to the cell burst. *Aristotelia chilensis* or maqui berry is a purple berry that grows wild throughout parts of southern Chile. It has high antioxidant compound that may help to scavenge free radicals and produced by malarial parasite. The objective of this study was to investigate the effects of *A.chilensis* on liver morphology of *Plasmodium yoelii* 17XL infected mice. In this study, the extract of maqui berry was injected to parasite-infected mice. We investigated the morphology of the liver by observing liver color, weights, and sizes. For liver size, the treatment group showed no significant difference when compare with parasite group, whereas the parasite group showed a significant difference ( $p = 0.028$ ) when compared with the control group. For liver weight, there was also no significant difference when the treatment groups were compared with infected group. In conclusion, maqui berry did not potentially helps their morphology of infected-livers return to normal appearance.

**Keywords** *Malaria, Hepatoprotective, Maqui berry, Antioxidant, and Morphology*

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## GRIA3 Gene Polymorphism (rs502434) is Associated with Methamphetamine Dependence in Male Thai Population

Sri-arun Iamjan<sup>1,2</sup>, Samur Thanoi<sup>1,2</sup>, Paritat Watiktinkorn<sup>3</sup>, Gavin P Reynolds<sup>4</sup>, Sutisa Nudmamud-Thanoi<sup>1,2\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

<sup>2</sup> Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

<sup>3</sup> Synphaet Hospital, Bangkok, Thailand

<sup>4</sup> Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK

\*Corresponding author, e-mail: sutisat@nu.ac.th

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### Abstract

Methamphetamine (METH) is an addictive drug common used in Thailand. Chronic METH abuse leads to psychosis which resembles positive symptoms of schizophrenia. These are involved in the disturbance of glutamatergic neurotransmission.  $\alpha$ -amino-3 hydroxy-5 methyl-4 isoxazole propionic acid glutamate receptor subunit 3 is a component in glutamatergic system which encoded from GRIA3 gene. It plays critical roles in processes of synaptic plasticity and included in etiology of schizophrenia. Moreover, the variant of GRIA3 gene has been reported in association with schizophrenia susceptibility. However, it has not been observed in association with METH dependence. Therefore, the objective of this study is to investigate the association of GRIA3 gene polymorphism (rs502434) with METH dependence and METH-dependent psychosis in the Thai population. Rs502434 SNP genotyping was performed in 102 male controls and 100 METH-dependent subjects (53 METH-dependent psychosis) using real time PCR high-resolution-melting and sequencing techniques. The results found the significant difference in GRIA3 allele frequency in controls compared to METH dependence and METH-dependent psychosis ( $p=0.049$  and  $0.023$ , respectively), which G allele was significant greater in both of METH-dependent groups. These results indicated that G allele of rs502434 is associated with vulnerability to METH dependence and METH-dependent psychosis in the Thai population.

**Keywords** methamphetamine, gene polymorphism, rs502434, GRIA3, Thai population

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## The Effects of GnRH on Ovarian Cell Proliferation and Estrogen Release during the Reproductive Cycle of the Tropical Abalone *Haliotis asinina*

Parinyaporn Nuurai<sup>1\*</sup> and Prasert Sobhon<sup>1,2</sup>

<sup>1</sup> Faculty of Allied Health Sciences, Burapha University, Chonburi 20131, Thailand

<sup>2</sup> Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

\*Corresponding author, e-mail: parinyaporn@bua.ac.th

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### Abstract

Gonadotropin releasing hormone is an important regulator of reproductive function in vertebrates and invertebrates including abalone. However, the correlations between its bioactivity and cell proliferation rates as well as estrogen synthesis and release in a tropical abalone, *Haliotis asinina*, during the reproductive cycle has not yet been studied. In this report we have investigated the effects of *H. asinina* GnRH (HasGnRH) on the ovarian cell proliferation and estrogen release into the hemolymph during the reproductive cycle which were determined by *in vitro* BrdU assay and ELISA assay, respectively. Groups of one-year-old female abalone at proliferative, mature and spawning phases were injected with HasGnRH at doses of 250 and 500 ng/gBW exhibited increased proliferation of oogonia and early oocytes compared to the control group ( $P < 0.05$ ). Moreover, the numbers of BrdU-labelled gonial cells were increased to a significantly highest level at the mature phase and slightly decreased thereafter during the spawning phase. In addition, injections of HasGnRH at doses of 250 and 500 ng/gBW stimulated increased estrogen level in the hemolymph ( $P < 0.05$ ) which showed initial increase during the proliferative phase and reached the highest values at the mature phase, and then significantly decreased at the spawning phase. These findings provide important evidence indicating the biological functions of GnRH in the reproduction which could be applied in abalone aquaculture by using the hormone to stimulate the ovarian maturation and oocyte production.

**Keywords** Gonadotropin releasing hormone, *Haliotis asinina*, ovarian cell proliferation, estrogen, 5-bromo-2'-deoxyuridine assay

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## Effects of Germinated Brown Rice on Liver Morphology of *P.yoelii* 17XL Infected Mice.

Poonyawee Jirattanawan<sup>1</sup>, Thanaporn Rungruang<sup>1\*</sup>, Aikkarach Kettawan<sup>2</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

<sup>2</sup> Institute of Nutrition, Mahidol University, Nakorn Pathom, Thailand

\*Corresponding author, e-mail: thanaporn.run@mahidol.ac.th

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### Abstract

Malaria is a lethal infected disease with evidence of multiple drug resistance. During liver stage infection, plasmodium parasite causes more oxidative stress in hepatocyte resulting in hepatocyte rupture and liver injury, respectively. Germinated brown rice (GBR) contains antioxidant components which can reduce oxidative stress and prevent liver injury. In this study, Germinated brown rice extract was injected intraperitoneally into *Plasmodium yoelii* infected mice. Liver morphology of mice was observed in term of color, size, and weight. The results showed that there was no significantly difference among positive control group, parasite-high dose group and parasite-low dose group in size, weight and color. Compared with negative control, positive control and parasite-high dose showed significant difference in weight. Liver histopathology need to be further study to confirm about the cause of higher weight in infected groups. In conclusion, germinated brown rice extract is not able to improve liver morphology of *P.yoelii* infected mice.

**Key word** :Germinated brown rice, malaria, liver morphology

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## Anatomical Study of the Midface Perforator Artery: Implication for Entry Site Determination of Filler Injection

Chalermquan Rungsawang <sup>1\*</sup>, Tanvaa Tansatit <sup>1</sup>, Thirawass Phumyoo <sup>2</sup>, Benrita Jit-aree <sup>1</sup>, Sukanya Uruwan <sup>1</sup>

<sup>1</sup> The Chula Soft Cadaver Surgical Training Center and Department of Anatomy, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Rama 4 Road, Pathumwan, Bangkok, Thailand

<sup>2</sup> Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Samsen road, Dusit, Bangkok, Thailand

\*Corresponding author, e-mail: chalermquan@hotmail.com

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### Abstract

The use of filler injection is often associated with vascular complications, such as bruising and ecchymosis. The aim of this study was to investigate the course and location of the midface perforator artery. This would enable the safest entry sites for filler injection to be determined. Twenty eight hemi-face specimens of Thai soft embalmed cadavers were studied at the Faculty of Medicine, Chulalongkorn University. In this study, the midface perforator artery was classified into three groups according to its origin. The perforator artery originated from the buccal branch of facial artery (57.1%, Type I), the parotid artery (25%, Type II) and directly from the facial artery (17.9%, Type III). The distance of buccal perforator (Type I) at the level of upper alar crease, to lateral canthal line (axis X) and Frankfort's horizontal line (axis Y) was  $2.56 \pm 5.98$  mm and  $-16.98 \pm 6.06$  mm. The distance of parotid perforator (Type II) was  $4.19 \pm 10.75$  mm (axis X) and  $-13.87 \pm 3.42$  mm (axis Y). Finally, the distance of facial perforator (Type III) was  $11.19 \pm 10.75$  (axis X) and  $-15.98 \pm 5.27$  mm (axis Y). In conclusion, lateral port of Beut techniques at 1.5 cm inferolateral to lateral canthus is safe injection.

**Keywords** Facial artery perforator, filler injection technique, midface volumization, injectable filler complications

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## Ultrastructural and Phenotypic Studies of the *Dystrobrevin*-Deficient *C. elegans* Reveals Severe Muscle Degeneration

Prapaporn Jattujan, Krai Meemon, Worawit Suphamungmee\*

Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Ratchathevi, Bangkok 10400, Thailand.

\*Corresponding author, e-mail: worawit.sup@mahidol.ac.th

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### Abstract

A congenital disorder of muscle degeneration, called Duchene's muscular dystrophy (DMD), is an inherited in an X-linked recessive manner. The symptoms are displayed as a slow progressive weakness of muscles beginning from limbs to a whole body, including cardiac muscle. Lacking of the key protein, dystrophin, causes progressive muscle degeneration, as dystrophin structurally connects to the actin filaments at one terminus while the other interacts with an integral domain of dystroglycan complex. Among involving molecules, dystrobrevin acts as a modulator protein exerting a powerful function of dystrophin for strengthening the cell stability. Previous study showed delayed progression of muscle degeneration following an overexpressed *dystrobrevin* (*dyb*) gene in DMD worms indicated that dystrobrevin protein may regulate muscle stability via a myofilament contraction and perhaps with other modulator molecules. Our study aims to investigate muscle degeneration as a consequence of *dyb* gene deficiency in *C. elegans* in comparison with *dystrophin* (*dys*) gene deficiency. In comparison to the *wild-type* worms, *dyb*-lacking worms exhibited a slow, undirectional locomotion and some degrees of deformed muscular wall and internal organs. Similar result was observed from the *dys*-deficient worms. Ultrastructure of the *dyb*-lacking and *dys*-lacking worms showed a severe degeneration of the muscle bundles, swollen and deteriorated mitochondria, and replacement of fibrotic tissues. However, *dys*-deficient worms revealed more severe in both phenotypic appearance and ultrastructural investigation than *dyb*-deficient worms. Life span data of *dyb*-deficient worms also showed a significant decrease, compared to the *wild-type*. Results of this study indicated an influential function of dystrobrevin, although still unknown, in regard to indirectly stabilize the muscle cell integrity during muscular dystrophy.

**Keywords:** Muscular dystrophy, DMD, Dystrobrevin, Dystrophin, *C. elegans*

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## Piperine Attenuates Thermal Hyperalgesia in Diabetic Peripheral Neuropathy in Streptozotocin-induced Diabetic Rats

Sureeporn Nak-ung<sup>1</sup>, Waree Tiyafoonchai<sup>3</sup>, Samur Thanoi<sup>1,2</sup> and Sutisa Nudmamud Thanoi<sup>1,2</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medical Sciences, Naresuan University, Phitsanulok, Thailand

<sup>2</sup> Centre of Excellence in Medical Biotechnology, Faculty of Medical Sciences, Naresuan University, Phitsanulok, Thailand

<sup>3</sup> Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand

\*Corresponding author, e-mail: sutisat@nu.ac.th

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### Abstract

Diabetic mellitus (DM) is a metabolic disorder resulting from the deficits in insulin secretion and insulin function that lead to chronic hyperglycemia. Chronic hyperglycemia can cause several serious complications including diabetic peripheral neuropathy (DPN). Piperine is a hot pungent extracted from long pepper (*pepper longum*) and black pepper (*pepper nigrum*) that it has been reported anti-inflammation and anti-arthritis effects in rats. However, the effects of piperine on DPN have not been described. Therefore, the purpose of this study was to investigate the potential therapeutic effect of topical cream containing either long pepper extract or piperine on DPN. Male Sprague-Dawley rats were induced to DPN by a single dose of streptozotocin (60 mg/kg i.p). Diabetic rats were treated with topical cream containing long pepper extract, piperine and capsaicin. Thermal hyperalgesia, a symptom of DPN, was evaluated in all animal groups. Although, there was no effect of long pepper extract cream on thermal hyperalgesia, improvement of thermal hyperalgesia in DPN was observed in piperine treatment group when compared with vehicle group. The result indicates that piperine is an active component that can reduce painful in DPN rats. Therefore, piperine might be a potential therapeutic agent for treatment of peripheral neuropathy.

**Keywords** Piperine, *Pepper longum*, Diabetic peripheral neuropathy

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## **Immuno-Electron Microscopic Localization of Arf (ADP-Ribosylation Factor) Type 6 in Submandibular Ductal Epithelial Cells of Mice**

Somsiri Ratanasuwan\*, Apussara Tachow, Atsara Rawangwong, Surang Chomphoo, Suthankamon Krongyut, Wilaiwan Mothong, Nongnut Aubandit, Wiphawi Hipkaeo  
Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mittraparp Road, Muang Khon Kaen, Thailand

\*Corresponding author, e-mail: [somsiri@kku.ac.th](mailto:somsiri@kku.ac.th)

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### **Abstract**

Arf6 (type 6 of ADP ribosylation factors, a group of GTP-binding proteins) has recently been implicated in the endocytotic and exocytotic pathways at the plasma membrane. Its intracellular localization was examined in the mouse submandibular gland which represents the exocrine glands exerting the regulated secretion of saliva stored in the secretory granules via exo- and endo-cytosis. The immunoreactivity was localized on the basolateral plasmalemma of the ductal epithelial cells, especially the striated and granulated convoluted cells. The immunoreactivity was neither detected in the apical plasmalemma of the ductal cells nor in the acinar epithelial cells. In immuno-electron microscopy, gold-labeling representing Arf6-immunoreactivity was found to be in association with the plasma membranes of the ductal epithelial basal foldings, and of vesicles of various sizes subjacent to the plasma membranes. No gold-labeling was seen on Golgi apparatus, rough ER or mitochondria. The present finding suggests that the membrane dynamics is exerted in the basolateral plasma membranes of the ductal epithelium, in contrast to the expected finding that the apical membranes are active in the membrane dynamics related to the exocrine secretion.

**Keywords:** Arf6, submandibular gland, mice

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## Development of Oocytes in the Golden Apple Snail, *Pomacea Canaliculata*

Anucha Chomnonlao<sup>1</sup>, Piyada Ngernsoungnern<sup>2</sup>, Apichart Ngernsoungnern<sup>2\*</sup>

<sup>1</sup> School of Pharmacology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

<sup>2</sup> School of Anatomy, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

\*Corresponding author, e-mail: apichart@sut.ac.th

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### Abstract

The golden apple snail is an invasive mollusk. The snail has a short reproductive cycle and it can lay eggs throughout the year. In the present study, development of oocytes of the snail was observed. The oocytes were classified based on their diameters and morphologies into five stages; oogonia, previtellogenic oocyte (Oc1), early vitellogenic oocyte (Oc2), late vitellogenic oocyte (Oc3), and mature oocyte (Oc4). This study could be useful for understanding the reproductive biology of this snail species.

**Keywords** Golden apple snail, *Pomacea canaliculata*, oocyte, oogenesis

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## Anatomical, Light and Scanning Electron Microscopical Study of Ostrich (*Struthio camelus*) Integument

Doaa, M. Zaghloul<sup>1</sup> and Samir, El-Gendy<sup>2\*</sup>

Histology & Cytology<sup>1</sup> and Anatomy<sup>2</sup> departments Faculty of Veterinary Medicine, Alexandria University, Egypt

\*Corresponding author

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### Abstract

The current study dealt with the gross and microscopic anatomy of the integument of male ostrich in addition to the histological features of different areas of skin by light and SEM. The ostrich skin is characterized by prominent feather follicles and bristles. The number of feather follicles was determined per cm<sup>2</sup> in different regions. The integument of ostrich had many modifications which appeared as callosities and scales, nail and toe pads. They were sternal, pubic and Achilles tendon callosities. The vacuolated epidermal cells were seen mainly in the skin of legs and to a lesser extent in the skin of back and Achilles areas. Higher lipogenic potential was expressed by epidermis from glabrous areas of ostrich skin. The dermal papillae were found in the skin of feathered area of neck and back and this was not a common finding in bird's skin which may give resistance against shearing forces in these regions of ostrich skin. The thickness of the keratin layer of ostrich varied, being thick and characteristically loose in the skin at legs, very thin and wavy at neck, while at Achilles skin area, scale and toe pad were thick and more compact, with the thickest very dense and wavy keratin layer at the nail. The dermis consisted of superficial layer of dense irregular connective tissue characterized by presence of many vacuoles of different sizes just under the basal lamina of the epithelium of epidermis and deep layer of dense regular connective tissue. This result suggested presence of fat droplets in this layer which may be to overcome the lack of good barrier of cutaneous water loss in epidermis.

**Keywords** Ostrich, Anatomy. Light microscopy, integument, Skin modifications, Scanning electron microscopy

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## Effect of androgenic gland-specific insulin-like hormone on spermatogenesis in juvenile *Penaeus monodon*

Prawporn Thaijongrak<sup>1,2</sup>, Wanida Santimanawong<sup>2</sup>, Charoonroj Chotwiwatthanakun<sup>2,3</sup>, Waratit Keereerak<sup>4</sup>, Somjai Wongtripop<sup>4</sup>, Boonsirm Withyachumnarnkul<sup>1,2,5</sup>, Rapeepun Vanichviriyakit<sup>1,2\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand

<sup>2</sup>Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok, Thailand

<sup>3</sup>Mahidol University, Nakhonsawan campus, Nakhonsawan, Thailand

<sup>4</sup>Shrimp Genetic Improvement Center, Surat Thani, Thailand

<sup>5</sup>Aquatic Animal Biotechnology Research Center, Prince of Songkla University Surat Thani Campus, Surat Thani, Thailand

\*Corresponding author, e-mail: [rapeepun.van@mahidol.ac.th](mailto:rapeepun.van@mahidol.ac.th)

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### Abstract

In crustaceans, sex differentiation and development of male sexual characteristics have been shown to be regulated by an insulin-like androgenic gland hormone. This hormone is released from a male unique androgenic gland located at the distal part of spermatic duct. In this study, we aimed to investigate role of *Penaeus monodon* insulin-like androgenic gland hormone (PmIAG) in juvenile shrimp using RNA interference technique. Male shrimp (6-7 g body weight, 50 shrimp) were intramuscularly injected with double stranded RNA specific to *PmIAG* gene at concentration of 5 µg/g body weight, every 3 days for 40 days. In control group, another 50 male shrimp were injected with double stranded RNA specific to *EGFP* gene at concentration of 5 µg/g body weight, at the same time interval of the treatment group. RT-PCR result showed significant down regulation of *PmIAG* gene in the *PmIAG* knockdown group. Moreover, histological study showed small seminiferous tubules with delayed spermatogenesis in the *PmIAG* knockdown shrimp, suggesting the role of PmIAG in regulation of spermatogenesis especially in juvenile shrimp. Underlying mechanism of the delayed spermatogenesis is being studied. In addition, it is interesting to examine whether male to female sex reversal would be accomplished in long-term knockdown of *PmIAG* expression.

**Keywords** Insulin-like androgenic gland hormone, *Penaeus monodon*, spermatogenesis

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## Effect of *Syzygium Aromaticum* Merr. et Perry (Clove) Extract on Liver Morphology of *P.yoelii* 17XL-Infected Mice.

Apinya Sayinta<sup>1</sup>, Thanaporn Rungruang<sup>1</sup>, Aikkarach Kaettawan<sup>2</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

<sup>2</sup> Institute of Nutrition, Mahidol University, Nakorn Pathom, Thailand

\*Corresponding author, e-mail: thanaporn.run@mahidol.ac.th

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### Abstract

The objective of this study was to investigate clove use to the morphology changes of the liver caused by *P.yoelii* 17 XL infection on ICR mice. Clove is the major sources of phenolic compounds to prevent free radical and inhibits oxidative stress in RBC infected parasite. The mice were divided into 4 groups: NC group served as non parasite infection, PC group were infected with parasite, P+LD group was served as low dose treatment, and P+HD group was served as high dose treatment. After mice died, livers were removed, and colors, weight and size of liver were measured. The results revealed that color of the livers were similar among PC, P+LD and P+HD group and the weight of liver in all groups were not significant different ( $p < 0.05$ ) from. However, the relative weight of P+LD and P+HD significant reduced compared to PC group. Additionally, the width of P+LD liver was significantly shorter than PC group. In conclusion, the results suggested that mice treatment with low dose of cloves reduced the size and relative weight of the liver than treatment with high dose. However, low dose of clove treatment could not completely reduce the liver damage from *P.yoelii* 17XL infection in the liver.

**Keywords** malaria, clove, liver, morphology

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## Effects of *Pueraria Mirifica* Replacement on Estrogen Receptor Levels in Hippocampus of Ovariectomized Rats

Uraporn Vongvatcharanon<sup>1\*</sup>, Wasan Hayeeyahya<sup>2</sup>, Wandee Udomuksorn<sup>3</sup>, Ekkasit Kumarnsit<sup>4</sup>, Surapong Vongvatcharanon<sup>5</sup>,

<sup>1</sup> Department of Anatomy, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

<sup>2</sup> Department of Traditional Thai Medicine, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand

<sup>3</sup> Department of Pharmacology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

<sup>4</sup> Department of Physiology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

<sup>5</sup> Department of Oral Surgery (Anesthesiology), Faculty of Dentistry, Prince of Songkla University, Songkhla, Thailand

\*Corresponding author, e-mail: uraporn.v@psu.ac.th

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### Abstract

Estrogen plays an important role in learning and memory. The action of estrogen is mediated via the estrogen receptors:  $\alpha$  (ER $\alpha$ ) and  $\beta$  (ER $\beta$ ). Thus alteration of estrogen levels may affect estrogen receptor level. *Pueraria mirifica* (PM) is phytoestrogen rich and this can bind to both estrogen receptors. Therefore, this study aimed to investigate the effect of PM replacement on estrogen receptor level in the hippocampus of ovariectomized rats. Female Wistar rats were divided into Sham and ovariectomized groups (OVX) (treated with either vehicle) (PM0), estradiol benzoate (0.04 mg/kg, E) or PM at 50 (PM50), 500 (PM500) and 1000 (PM1000) mg/kg for 90 days. The brain sections containing hippocampus were stained for ER $\alpha$  and ER $\beta$  using immunohistochemistry technique. The intensity of immunoreactivity was evaluated using image pro-Plus software. In the hippocampus, weak intensity of ER $\alpha$  immunoreactivity and strong intensity of ER $\beta$  immunoreactivity were observed. In the PM0 group, a significant decrease of ER $\beta$  intensity was found compared to that of the Sham ( $p < 0.05$ ). An increase intensity of ER  $\beta$  was found in the E, PM50, PM500 and PM1000 groups. Our data suggested that PM has estrogenic activity similar to estrogen. The increase of ER $\beta$  level in hippocampus may help to improve learning and memory in ovariectomized rats.

**Keywords** *Pueraria mirifica*, estrogen receptor, learning and memory, ovariectomized rats, hippocampus

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## The Expression and Localization of Cannabinoid Receptor 1 (CB1) in the Submandibular Gland of Mice

Tansita Arnanteerakul<sup>1\*</sup>, Wipawee Thoungseabyoun<sup>1</sup>, Laorrat Phuapittayalert<sup>2</sup>, Kanoktip Petcharat<sup>3</sup>, Sawetree Pakkarato<sup>1</sup>, Wiphawi Hipkhaeo

<sup>1</sup>Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mittraparp Road, Muang Khon Kaen, Thailand

<sup>2</sup>Division of Anatomy and <sup>3</sup>Division of Biochemistry and Nutrition, Faculty of Medical Science, University of Phayao, Phayao, Thailand

\*Corresponding author, e-mail: [suptee@kku.ac.th](mailto:suptee@kku.ac.th)

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### Abstract

In view of the fact, the human marijuana users often show the dry mouth symptom. The present was attempted to examine the expression and localization of cannabinoid receptor 1 (CB1) in mouse submandibular gland (SMG), which was originally identified in brain, using western blotting and immunohistochemistry techniques. The immunoblotting of 40 kDa CB1 band in mouse SMG was detected. The immunoreactivity was confined to ductal cells, but not acinar cells, in normal glands, and it was distinct on the basolateral plasmalemma of the granular convoluted ductal (GCD) cells. In the glands after secretory stimulation by isoproterenol, a sympathomimetic drug, translocation of the immunoreactivity from the basolateral plasmalemma of the GCD to the apical surface areas of the striated ductal cells was obviously observed. The immunoreaction was additionally detected some, but not all, serous demilune in which it was seen in forms of thin canaliculi. This finding suggests that CB1 may play an important role in submandibular gland under the sympathetic control.

**Keywords:** CB1, submandibular gland, mouse

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## Activated Microglia Influence Neuroblastoma Cell Proliferation in a Co-culture Model

Rubina Shakya<sup>1</sup>, Tanapol Limbunrueng<sup>1</sup> and Sukumal Chongthammakun<sup>1,\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Science, Mahidol University, Payathai Road, Bangkok, Thailand

\*Correspondence: [sukumal.chong@mahidol.ac.th](mailto:sukumal.chong@mahidol.ac.th)

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### Abstract

Inflammation is a common factor in many neurodegenerative diseases which also partly regulates neurogenesis. Neurogenesis is modulated in response to brain injury and neurodegeneration. Microglia are the main inflammatory cells in the CNS, their exact role in regulating neurogenesis remains largely unknown in health and even more elusive in disease. We investigated the interaction between activated microglia and neuroblastoma cells in cell co-culture to further explore the effect of estrogen as a possible therapeutic strategy for neurodegeneration. Neuroblastoma cells showed reduced proliferation in the presence of conditioned medium from activated microglia. Pretreatment and co-treatment with estradiol significantly improved the neuroblastoma cell proliferation. The findings suggest that the influence of activated microglia on neuroblastoma cell proliferation could be modulated by estrogen which provided a novel treatment strategy of neurodegenerative diseases.

**Keywords** Neuroinflammation, Proliferation, Estradiol

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## Effects of Young Coconut Juice on the Numbers of Goblet Cells in the Stomach of Ovariectomized Rats

Pichnaree Kraokaew<sup>1</sup>, Chutchawan Muenpo<sup>1</sup>, Piyakorn Boonyoung<sup>2</sup>, Kanyapak Chunchula<sup>2</sup>, Nisaudah Radenahmad<sup>2\*</sup>

<sup>1</sup>Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

<sup>2</sup>Department of Anatomy, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

\*Corresponding author: nisaudah.r@psu.ac.th

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### Abstract

Estrogen deficiency in postmenopausal women is associated with bone resorption and bone formation, probably because it is involved with transcellular  $\text{Ca}^{2+}$  absorption. Hormone replacement therapy (HRT) has been proposed to prevent bone loss in postmenopausal women. However, HRT can also induce varieties of cancers and other side effects. Young coconut juice (Y CJ) containing the phytoestrogen,  $\beta$ -sitosterol, was investigated for its beneficial effects on delaying osteoporosis using an ovariectomized rat model. In this study, we used the periodic acid Schiff (PAS) staining to quantify the goblet cells. Goblet cells prevent any precipitation between  $\text{Ca}^{2+}$  and negative charges by binding  $\text{Ca}^{2+}$  and their secretion of mucin possibly enhances transcellular  $\text{Ca}^{2+}$  absorption. We found that the number of goblet cells of the fundic and pyloric epithelium was highest in the ovx receiving Y CJ at 10 mL/kg BW group (OY10), compared with the control groups (sham, ovariectomized (OW) and ovariectomized rat that received estradiol benzoate (OB) groups). However, the number of goblet cells of those glands of the OY10 group was significantly higher than the OW group but no significant differences were detected when compared with the OB group. This results that Y CJ may have beneficial effects on delaying osteoporosis in postmenopausal women, as detected by the numbers of goblet cells.

**Keywords** Young coconut juice, goblet cells, osteoporosis, stomach, calcium

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## Effects of Young Coconut Juice on the Numbers of Argyrophil Endocrine Cells in the Stomach of Ovariectomized Rats

Thunwa Binlath<sup>1</sup>, Chutchawan Muenpo<sup>1</sup>, Piyakorn Boonyoung<sup>2</sup>, Kanyapak Chunchula<sup>2</sup>, Nisaudah Radenahmad<sup>2\*</sup>

<sup>1</sup> Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

<sup>2</sup> Department of Anatomy, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

\*Corresponding author, e-mail:nisaudah.r@psu.ac.th

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### Abstract

Estrogen is an important hormone that plays a key role in bone formation, and is possibly involved with  $\text{Ca}^{2+}$  absorption, particularly in the transcellular  $\text{Ca}^{2+}$  absorption. In women, age-related estrogen depletion is an increasing risk factor on the rate of bone loss. Hormone replacement therapy has been proposed to prevent bone loss in postmenopausal women, however, it has been considered to have many negative side effects. Young coconut juice (Y CJ) containing the phytoestrogen,  $\beta$ -sitosterol, was investigated in this study for its beneficial effects on delaying osteoporosis using an ovariectomized (ovx) rat model. In the present study we used Grimelius staining to quantify the argyrophil endocrine cells, that are most likely relating to  $\text{Ca}^{2+}$  absorption, and might have an influence on osteoporosis. The numbers of argyrophil endocrine cells in the fundus and pylorus of the stomach of the ovx group (OW) was the lowest. Among the three doses of Y CJ (10, 20, and 40 mL/kgBW) ovx rats receiving Y CJ at 10 mL/kgBW (OY10) was shown to be the best dose to preserve the argyrophil endocrine cells. There were significantly higher numbers than for the OW group but were not significantly different from the sham group. This study has indicated that Y CJ has beneficial effects on preventing osteoporosis by preserving  $\text{Ca}^{2+}$  absorption as detected by the numbers of argyrophil endocrine cells in postmenopausal women, using an ovx rat model.

**Keywords** Young coconut juice, Osteoporosis, Argyrophil endocrine cell, Stomach, Calcium

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## Retinal and White Matter Damage Related to Memory Deficits in Voice-Cued Fear Test attenuated by Sea Cucumber Extracts (*Holothuria Scabra*)

Thitipong Naowasiri<sup>1</sup>, Thanaporn Rungruang<sup>1</sup>, Narawut Pakaprot<sup>2</sup>, Thongchai Taechowisan<sup>3</sup>, Prasert Sobhon<sup>4</sup>, Supin Chompoopong<sup>1\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, <sup>2</sup>Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, <sup>3</sup>Department of Microbiology, Faculty of Science, Slipakorn University, Nakorn Pathom 73000, Thailand. <sup>4</sup>Department of Anatomy, Faculty of Sciences, Mahidol University, Phayathai, Bangkok 10700, Thailand,

\*Corresponding author, e-mail: [supin.cho@mahidol.ac.th](mailto:supin.cho@mahidol.ac.th)

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### Abstract

Vascular dementia (VD) is the second most prevalent type of dementia with involved in white matter (WM) damage and related to memory deficits. Sea Cucumber Extracts (*Holothuria Scabra* or HsE), one of marine source, used as supplementary food and transitional medicine in Asia. HsE composed of nutritional benefits, triterpenoid saponins which may act as a natural antioxidant and help in damage pathway. This study was aimed to determine the effects of HsE on VD in the mice model of chronic cerebral hypoperfusion using voice-cued fear (VCF) conditioning test, non-vision dominant. The chronic cerebral hypoperfusion was induced by permanent bilateral common carotid artery occlusion (BCCAO) in forty ICR mice. After the occlusion, HsE was injected intraperitoneally for ten days, and VCF conditioning test was then performed after ten days of HsE treatment. At the end of test, all mice were sacrificed, and the eyes and brain were removed for histopathological determination of retinal layer and WM in corpus callosum and optic tract. The result was that BCCAO induced cerebral hypoperfusion, reduced the thickness of outer plexiform layer to  $4.33 \pm 1.02\%$  of retinal thickness, showed WM damage with myelin index,  $25.76 \pm 11.72$  and lost in learning and memory in VCF with the percent freezing,  $23.26 \pm 15.32\%$  at the testing day 4. HsE improved the percent thickness of outer plexiform layer to  $5.57 \pm 2.21\%$  of retinal thickness significantly at  $p < 0.05$ . WM damage with myelin index to  $50.96 \pm 24.05$  and memory retention with increased freezing behavior,  $35.80 \pm 23.51\%$ , at  $p = 0.798$ . The conditioned fear is often measured with freezing (a period of watchful immobility). Mice learned to stop moving as fear response, when the learning and memory process was interrupted with damaged white matter such as corpus callosum, the freezing behavior was decreased. In conclusion, HsE attenuated the memory deficits, showed more freezing in VCF, and improved retina and WM damages, Therefore, HsE may be suggested for using as drug in the alternative medicine

**Keyword** Bilateral Common Carotid Arteries Occlusion (BCCAO), Cognitive impairment, *Holothuria Scabra* extracts, white matter damage, retina

## Immunohistochemical Study of PIP5kinase in Brown Adipose Cells of Adult Mice Under Normal and Cold Stressed conditions

Tarinee Sawatpanich\*, Wipawee Toungeabyoun, Apussara Tachow, Waraporn Sakaew, Kowit Chaisiwamongkol, Pattama Amarttayakong, Wiphawi Hipkaeo  
Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mittraparp Road, Muang Khon Kaen, Thailand

\*Corresponding author, e-mail: tarinee@kku.ac.th

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### Abstract

Phosphatidylinositol 4-phosphate 5-kinase (PIP5kinase) synthesizes phosphatidylinositol 4,5-bisphosphate (PI<sub>4,5</sub>P<sub>2</sub>) through phosphorylation of PI<sub>4</sub>P. PI<sub>4,5</sub>P<sub>2</sub> is a remarkably versatile membranous phospholipid and plays crucial roles in the phosphoinositide signal transduction, actin cytoskeleton reorganization, clathrin-dependent endocytosis, and regulation of membrane morphology. The present study was attempted to examine the changeability of this kinase molecule of heat generating brown adipocytes in response to cold stress. In immunoblotting, a distinct immunoreaction band of 80 kDa for PIP5kinase, the same size as that of the brain, was detected in normal brown adipose tissue and it slightly decreased in intensity in 3 day-cold stressed mice. In immuno-light microscopy, intense immunoreaction was detected as forms delineating individual lipid droplets of various sized in almost all brown adipocytes of normal specimens. The immunoreactivity decreased in intensity in almost all brown adipocytes of the stressed mice and, together with the remarkable decrease in sizes of lipid droplets, the brown adipose tissue appeared entirely as finely textured nets with weakly immunostained and thin trabeculae. This finding suggests that PIP5kinase may play an important role in relation to heat generation of brown fat.

**Keywords:** PIP5K, brown fat, mice

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## Expression and Localization of Phospholipase C (PLC) $\beta$ 3 in Sublingual Gland Epithelial Cells at Several Postnatal Developmental Stages of Mice

Wiphawi Hipkaeo<sup>1\*</sup>, Atsara Rawangwong<sup>1</sup>, Suthankamon Krongyut<sup>1</sup>, Kanoktip Petcharat<sup>2</sup> Laorrat Phuapittayalert<sup>3</sup>, Damrong Hipkaeo<sup>1</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mittraparp Road, Muang Khon Kaen, Thailand

<sup>2</sup>Division of Biochemistry and Nutrition and <sup>3</sup>Division of Anatomy, Faculty of Medical Science, University of Phayao, Phayao, Thailand

\*Corresponding author, e-mail: wiphawi@kku.ac.th

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### Abstract

The salivary gland is important for maintenance of human body physiology via its saliva secretion, and it exhibits the rapid metabolic response and signal transduction of phosphoinositide (PI) after stimulation of certain cell surface receptors. We examined the expression and localization of phospholipase C (PLC) $\beta$ 3, a key enzyme of PI signaling through the generation of two second messenger: inositol triphosphate (IP3) and diacylglycerol (DAG), in the sublingual gland of mice. In immunoblotting, a distinct immunoreaction band of 150 kDa was detected. The immunoreactivity for PLC $\beta$ 3 was distinctly detected in the serous demilune in almost all acini under light microscopic (LM) observation. The immunopositive serous cells appeared in various forms such as polygonal, lunar and triangular shapes with their wider sides facing the acinar basis and their slender tips extending toward the acinar lumen. In immuno-DAB-electron microscopy (EM), immuno-positive portions revealed mainly adjacent to the serous secretory granules while immunonegative mucous cells were seen to be apposed to adjacent immunopositive cells in one and the same acinus. This finding suggests that the PLC $\beta$ 3 may play an important role of salivary secretion of the serous cells in sublingual gland.

**Keywords:** PLC $\beta$ 3, sublingual gland, mouse

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## The Proliferative Effect of Sea Cucumber Extract (*Holothuria Scabra*) on Human Placenta-Derived Mesenchymal Stem Cells

Jutarat Saengsuwan<sup>1</sup>, Napamane Kornthong<sup>1\*</sup>, Supawadee Duangprom<sup>1</sup>,  
Wittawas Srikawnawan<sup>1</sup>, Sirikul Manochantr<sup>2</sup>, Prasert Sobhon<sup>3,4</sup>

<sup>1</sup>Chulabhorn International College of Medicine, Thammasat University,  
Pathum-thani 12121, Thailand

<sup>2</sup>Department of Preclinical Sciences, Faculty of Medicine, Thammasat University,  
Pathum-thani 12121, Thailand

<sup>3</sup>Department of Anatomy, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

<sup>4</sup>Faculty of Allied Health Sciences, Burapha University, Chonburi 20131, Thailand

\*Corresponding author, e-mail: napamaneenatt@gmail.com

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### Abstract

Mesenchymal stem cells (MSCs) are multipotent stem cells with the ability of self-renewal and differentiation into many different types of cells, including osteocyte, adipocyte and chondrocyte. Currently MSCs therapy is expected to be a promising tool for the treatment of degenerative and inflammatory disease. Sea cucumbers (*Holothuria scabra*) are able to regenerate most of their internal organs after a typical evisceration process. The various types of regulatory molecules have been reported as candidates for growth-promoting factors that can be found in echinoderms. The sea cucumbers were separated into 2 parts, body wall and viscera. They were extracted by using 0.1M acetic acid comparing to 0.1M phosphate buffer saline. SDS-PAGE was used for the protein profile analysis of body wall and viscera. We found that protein extraction from 0.1M PBS represents the protein profile more than protein extraction from 0.1M acetic acid in both body wall and viscera. In this experiment, MSCs were isolated from placenta and treated with the protein extracts in different concentration to test the proliferative effect and cell viability by using MTT assay and growth curve. We found that the protein extraction from sea cucumbers may stimulate MSCs proliferation. This study is the first report on the proliferation of *H.scabra* on the human MSCs and need to be explored.

**Keywords** Sea cucumber, Mesenchymal stem cells, *Holothuria scabra*.

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## Pathology of the Toxicity Rat Liver Induced by Dextromethorphan and Pre-Germinated Brown Rice Consumption on Liver Tissue Recovery

Kusuma Suphakhong<sup>1</sup>, Siriluk Veerasakul<sup>1</sup>, Sutisa Nudmamud-Thanoi<sup>1</sup>, Samur Thanoi<sup>1</sup>, Ittipon Phoungpetchara<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

\*Corresponding author, e-mail: ittiponnu47@gmail.com

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### Abstract

Rice is the staple food in Asia including Thailand. Pre-germinated brown rice (PGBR) is unpolished rice with high benefits for human health as it contains  $\gamma$ -oryzanol, vitamin E and  $\gamma$ -aminobutyric acid (GABA). The purpose of this research is to investigate the effects of PGBR on hepatotoxicity which induced by a high dose of dextromethorphan. A total of 70 male Sprague Dawley rats were divided into fourteen groups: control, dextromethorphan 30 mg/kg BW (DXM), DXM withdrawal (15, 30, 60 days), PGBR 5 g/kg BW (15, 30, 60 days), DXM with GABA supplement 0.8 mg/kg BW (15, 30, 60 days) and DXM with PGBR (15, 30, 60 days); five animals per each group. All rats were treated once a day orally. After euthanasia, livers were collected for histopathological investigation. The results showed that significant increases of normal hepatocytes were found in all groups of DXM treated with PGBR for 15, 30 and 60 days. Moreover, reductions of hepatic vascular degeneration and sinusoidal congestion were also observed in all groups of DXM treated with PGBR compared with DXM treated group.. These results suggest that PGBR may have a beneficial effect on hepatocyte regeneration after liver toxicity. It also promotes liver tissue recovery from hepatic vascular degeneration and sinusoid expansion.

**Keywords** pre-germinated brown rice, hepatocyte recovery, hepatotoxicity, dextromethorphan

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## Improvement of Cognitive Impairment in Morris Water Maze by Sea Cucumber Extracts (*Holothuria Scabra*)

Farhana Padungraksart<sup>1</sup>, Thanaporn Rungruang<sup>1</sup>, Narawut Pakaprot<sup>2</sup>, Thongchai Taechowisan<sup>3</sup>, Prasert Sobhon<sup>4</sup>, Supin Chompoopong<sup>1\*</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

<sup>2</sup> Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

<sup>3</sup> Department of Microbiology, Faculty of Science, Slipakorn University, Nakorn Pathom 73000, Thailand

<sup>4</sup> Department of Anatomy, Faculty of Sciences, Mahidol University, Phayathai, Bangkok 10700, Thailand

\*Corresponding author, e-mail: [supin.cho@mahidol.ac.th](mailto:supin.cho@mahidol.ac.th)

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### Abstracts

Sea Cucumber Extracts (*Holothuria Scabra* or HsE) is a valuable source of several kinds of substances that can serve as natural health products, contain saponins or triterpene glycosides. Pharmacological studies indicate anti-inflammatory and anticancer properties of the sea cucumber saponins. It may enhance learning and memory and promote brain functions. To determine the therapeutic potential of HsE as an alternative treatment for cerebral ischemia in the mice, the chronic cerebral hypoperfusion was induced by permanent bilateral common carotid artery occlusion (BCCAO). In forty ICR mice, after the occlusion, HsE was injected intraperitoneally for ten days. The Morris Water Maze (MWM) was then performed after ten days of HsE treatment. At the end of experiment, all mice were sacrificed, and the brains were removed for histopathological determination and counting of the hippocampal CA1 neurons. The results showed that BCCAO induced cerebral hypoperfusion, increased the pyramidal cell death in the hippocampal CA1 area and impaired the spatial learning and memory. HsE improved memory retention in MWM, The CO+HsE group swam up to the platform with escape latency time ( $15.91 \pm 2.46$  sec,  $p < 0.05$ ) compared with injury group ( $18.17 \pm 0.49$  sec,  $p < 0.05$ ). Therefore, HsE could attenuate the memory deficits and the pyramidal cell death in hippocampal CA1 area. Role of HsE to decrease neuronal cell death is still needed to be further investigated. It could be concluded that HsE improved the cognitive impairment induced by BCCAO, suggesting the therapeutic effects of HsE against the cerebral hypoperfusion.

**Keyword** Bilateral Common Carotid Arteries Occlusion, Cognitive impairment, *Holothuria Scabra* extracts, hippocampal CA1 neurons

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## CD34+ Cells Reduce Neuronal Apoptosis Induced by Okadaic Acid

Chanchai Songthaveesin<sup>1\*</sup>, Wanna Sa-nongdej<sup>2</sup>, Sukumal Chongthammakun<sup>3</sup>

<sup>1</sup>Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakhon Pathom, 731702, Thailand. <sup>2</sup>School of Nursing Ramathibodi Hospital, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400, Thailand. <sup>3</sup>Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand.

\*Corresponding author

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### Abstract

This study aimed to determine the neuroprotective effect of CD34+ cells in okadaic acid (OKA) induced neurodegenerative disorder by using the *in vitro* co-culture model. The primary neuronal cell cultures were prepared from fetal bovine cerebral cortex then exposed to 50 nM OKA for 24 h followed by co-culturing with CD34+ cells. *Ninety-five percent of the* CD34+ cells used in our study were positive for brain derived neurotrophic factor (BDNF) determined by flow cytometric analysis. Primary neuronal cells exposed to 50 nM OKA and co-culturing with CD34+ cells were examined for apoptotic cell death using the primary antibody against Annexin V, immunofluorescence staining, and transmission electron microscopy (TEM) studies. The results of flow cytometric analysis showed that OKA induced 44.3% and 40.4% of early and late apoptosis, respectively. Co-culture with CD34+ cells for 24 h significantly ( $p < 0.05$ ) reduced both early (15.4%) and late (4.6%) apoptotic neuronal cell death induced by OKA. A number of vacuoles and condensed nuclei also significantly decreased in neurons treated with CD34+ cells. The present findings suggested that CD34+ cells reduce both early and late apoptotic neuronal cell death by affecting the functional cytoplasmic and nuclear organelles. The BDNF expression in CD34+ cells might play an important role in reduction of the neuronal apoptosis. CD34+ cells had the potential to be used as a therapy for neurodegenerative diseases. However, further studies are need to define the exact role of CD34+ cells.

**Keywords:** Apoptosis, CD34+ cells, Neurotrophic factors, Okadaic acid

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## The Effect of *Sauropus androgynus* Water Extract on Liver Morphology of *Plasmodium*-Infected Mice

Chawin Korsirikoon<sup>1</sup>, Thanaporn Rungruang<sup>1\*</sup>, Supin Chompoopong<sup>1</sup>, Aikkarach Kettawan<sup>2</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok, Bangkok Noi, Bangkok 10700, Thailand

<sup>2</sup>Institute of Nutrition, Mahidol University, Salaya, Nakon Pathom 73000, Thailand

\*Corresponding author, e-mail: thanaporn.run@mahidol.ac.th

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### Abstract

Our aim of this study was to investigate the morphological changes of the liver caused by *Plasmodium* infection and treated with *Sauropus androgynus* water extract (SE), a Thai traditional herbal plant due to parasitic infection in female ICR mice. The mice were divided into 4 groups: non-malaria infected mice served as control (NC), malaria infected mice only (PC), and other two groups; mice were inoculated with *Plasmodium yoelii* 17XL prior to injected with SE at the doses of 27 mg/kg and 80 mg/kg (P+SEL and P+SEH), respectively. All mice were sacrificed at the end of the experiment on day 25 and liver tissues were removed and fixed immediately. We also observed color and measured size and weight. The differences in liver color between the groups PC, PSEL and PSEH were found similar. Compared to NC there are significant differences in liver weight and size of PC, P+SEH and P+SEL. However, our current reports are not sufficient to determine the efficacy of SE to normalize the liver morphology on *Plasmodium*-infected mice. Thus, we suggested that the biochemical analysis, mechanism of action and histopathological examination need to be investigated further. In conclusion, *Sauropus androgynus* water extract is unable to normalize the liver morphology on *Plasmodium*-infected mice.

**Keywords** *Sauropus androgynus*, *Plasmodium yoelii* 17XL, Antimalarial effect, Malaria

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## Morphological Alterations of Gastric and Duodenal Mucosa in Functional Dyspepsia Patients

Kanmanee Kaewklin<sup>1,2</sup>, Wiroj Chonsungnoen<sup>3</sup>, Sutisa Nudmamud-Thanoi<sup>1,2</sup> and Samur Thanoi<sup>1,2\*</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medical science, Naresuan University, Thailand

<sup>2</sup>Centre of Excellence in Medical Biotechnology

<sup>3</sup>Fort Somdet Phranaresuan Maharat Hospital, Phitsanulok, Thailand

\*Corresponding author, email: [samurt@nu.ac.th](mailto:samurt@nu.ac.th)

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### Abstract

Functional dyspepsia (FD) is a clinical syndrome defined by chronic or recurrent pain, or discomfort of upper gastrointestinal (GI) tract. However, the pathophysiology of FD is still unknown. Although, several factors have been suggested to be causes of FD, morphological changes of upper GI tract may be one of the causes of FD symptoms. Thus, the purpose of this study was to investigate the morphological changes of gastric and duodenal mucosa in the FD patients. Twelve female patients were biopsied and small pieces of tissues were collected from fundus, body, antrum of stomach and duodenum. The tissues were analysed by light microscopy with Masson's Trichrome staining and transmission electron microscopy. The results demonstrated morphological abnormalities including vascular congestion and intestinal metaplasia in fundus, body and antrum of stomach. Collagen storage in goblet cells was found in duodenum. Moreover, lymphoid follicle formation was observed in both stomach and duodenum. Apparently, red blood cell deformities were found either in the vessels or inside interstitial spaces and numerous secretory granules were observed in mucous neck cell using TEM analysis. These results suggest morphological abnormalities of tissues in upper GI tract which may affect on cell functions and lead to FD symptoms.

**Keywords** Functional dyspepsia, Gastrointestinal tract, Masson's Trichrome, Transmission electron microscope

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## Effects of Methamphetamine on Alpha-1 Adrenergic Receptor in Rat Testis

Siriporn Janphet<sup>1</sup>, Sutisa Nudmamud-Thanoi<sup>1</sup>, Samur Thanoi<sup>1\*</sup>

<sup>1</sup> Department of Anatomy and Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

\*Corresponding author, e-mail: samurt@nu.ac.th

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### Abstract

Methamphetamine (METH) activates central and sympathetic nervous system by increasing central and peripheral norepinephrine (NE). There is a previous study reported the alteration of NE concentration after METH exposure in rat testis which may also affect on adrenergic receptor changes. This study, therefore, aimed to investigate the alteration of alpha 1 adrenergic receptor (A1AR) expression in rat testis after METH administration. Sixteen male rats were divided in to four groups consisting of a control and three METH-treated groups. The expression of A1AR was determined by immunohistochemistry. Localization of this receptor was found in sertoli cell and all germ cells especially in spermatocyte and elongated spermatid. Quantitatively, the percentage of total immunoreactive cells in seminiferous tubule was significantly increased in escalating binge dose group (ED-binge) compared to control. Moreover, the changes of A1AR in each cell type were found. The percentage of A1AR positive cells of spermatogonia was significantly decreased in ED-binge group. On the other hand, the significance increase of A1AR expression was found in spermatocyte and elongated spermatid of ED-binge and escalating groups, respectively. In conclusion, this study demonstrated that METH affects the expression of A1AR in rat testis that may result in abnormality of spermatogenesis.

**Keywords** Methamphetamine, Alpha-1 adrenergic receptor, Norepinephrine

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## The Effect of *Cinnamomum Verum J.Presl* Water Extracts Treatment on the Liver of *Plasmodium Yoelii* 17XL Infected Mice.

Peerapa Techaniyom<sup>1</sup>, Thanaporn Rungruang<sup>1</sup>, Aporn Chuncharunee<sup>1</sup>, Aikkarach Kettawan<sup>2</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, <sup>2</sup> Wanglang Road, Bangkok, Thailand,

<sup>2</sup>Institute of Nutrition Mahidol University, Phutthamonthon 4 Rd., Salaya, Phutthamonthon, Nakhon Pathom Thailand

\*Corresponding author, e-mail: thanaporn.run@mahidol.ac.th

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### Abstract

Malaria is transmittable disease in tropical country around the world. Researchers, attempted to explore the new drug due to drug resistance. Cinnamon bark is one of the most medicinal plant has been used in cosmetic, food as spice, pharmaceuticals, and traditional herb for long time. The various constitution of cinnamon has been reported antioxidant and anti-parasitic properties. The aim of this study was to investigate the effect of *Cinnamomum verum J.Presl* water extracts treatment in *Plasmodium yoelii* 17XL infected mice liver. Mice were divided into 4 groups and 5 mice per each groups Negative control (NC) group, Positive control (PC) group ( $1 \times 10^8$  parasite/mouse), Parasite + *Cinnamomum verum J.Presl* low dose (PCiL) 40 mg/kg group and Parasite + *Cinnamomum verum J.Presl* high dose (PCiH) 200 mg/kg group. All mice received intraperitoneal route administration of *Cinnamomum verum J.Presl* water extract for 25 days. After the date of death or day of sacrificed, liver was collected for morphology observation in term of color, size and percent relative organ weight. Our results have shown that in case of length, liver weight and percentage of relative organ weight in PCiH group is not significantly different to those of NC group. In conclusion, *Cinnamomum verum J.Presl* water extracts treatment was not able to promote normally liver morphology in *Plasmodium yoelii* 17XL infected mice.

**Keyword:** antioxidant, anti-parasitic, Cinnamon, Malaria

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## Anti-Cancer Cells Effect of the Extracts from the Sea cucumbers, *Holothuria scabra*, in Breast Cancer Cells (Triple-Negative MDA-MB-231 Cell Line)

Laphatrada Yurasakpong<sup>a</sup>, Somjai Apisawethakarn<sup>b</sup>, Kulathida Chaithirayanon<sup>a\*</sup>, Prasert Sobhon<sup>a</sup>

<sup>a</sup>Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Ratchathewi, Bangkok 10400, Thailand.

<sup>b</sup>Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Wattan, Bangkok 10110, Thailand.

\*Corresponding author, e-mail: Kulathida.cha@mahidol.ac.th

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### Abstract

The most common cancers occurring worldwide are breast cancers that are usually classified according to their expressions of estrogen receptor (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2). The highly aggressive and invasive MDA-MB-231 cell is the triple-negative breast cancer (TNBC) type that is normally resistant to several anti-cancer agents due to a lack of receptor expression. Therefore, the treatment of this cancer is more difficult than other types of breast cancer. Sea cucumber, *Holothuria scabra*, is a marine animal which belongs to the phylum Echinodermata. Its extract is believed to be essential to accelerate wound healing and relieve arthritic joint pain. Thus, it is widely used as traditional Asian medicine. Previous studies have shown an anti- cancer activity of the edible sea cucumber extract in leukemia cells. However, the beneficial contribution of *H. scabra* extracts against breast cancer is limited. The purpose of this study was to investigate the potential anti-cancer properties of *H. scabra* extracts in MDA-MB-231 breast cancer cell line. MDA-MB-231 cell was treated with secondary metabolite obtained from ethyl-acetate extract of body wall *H.scabra* (BWEA). Then, the effect of BWEA on MDA-MB-231 cell cytotoxicity and cell migration were evaluated by using MTT assay and wound scratch assay, respectively. The results revealed that BWEA decreased cell viability and migration of MDA-MB-231 cell. In conclusion, these findings suggested that this extract from *H. scabra* might enhance anti-cancer effects in MDA-MB-231 breast cancer cells. And further experiments need to prove the other potentialities of sea cucumber.

**Keywords** *Holothuria scabra*, Breast Cancer, Anti-cancer activity

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## Improved Method for High Quality of Silicone S-10 Plastinated Specimens

Nutchaya Amornmettakit, Noppadol Piumpook, Pheeravut Tantisuvanitchkul, Ploy Pattanakitsakul, Mathee Oungsiriporn, Supin Chompoopong\*  
Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi, Bangkok 10700, Thailand

\*Corresponding author, e-mail: supin.cho@mahidol.ac.th

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### Abstract

Plastination is the process of preservation of anatomical specimens by impregnation with curable polymers like silicone, epoxy or polyester resins which keep the human anatomic specimens in a dry, odorless, and available specimen for teaching and research. Currently, the development of plastination in Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University has been performed using Silicone S-10 standard plastination technique. The formalin fixed tissues were dehydrated in acetone. Forced impregnation using a vacuum chamber and finally the specimens were hardened in a gas chamber. Before impregnation, protocol was modified by placing the specimens in a mixture of acetone and Silicone S-10, 1:1 for 1 week to increase the infiltration ability of the silicone into tissue. In addition, the remodeling process must be taken to keep a more natural looking and interesting specimen. Therefore, this study resulted in more complete impregnation, and showed more deep details of specimens.

**Keywords** Plastination, Silicone S-10, impregnation

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## Differentiation of Executive Function in Age Range and Sex in Healthy Thai Subjects

Bupachad Khanthiyong<sup>1</sup>, Samur Thanoi<sup>1,2</sup>, Sutisa Thanoi<sup>\*1,2</sup>

<sup>1</sup> Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

<sup>2</sup> Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

\*Corresponding author, e-mail: sutisat@nu.ac.th

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### Abstract

Executive function is a higher cognitive ability that associated with goal-directed behavior. The performance of executive function is depended on development of stage. Recently, the evident reports have shown controversial information. Therefore, the aim of this research is to provide evidence of the differentiation of executive functions in age range and sex by using the Wisconsin Card Sorting Test (WCST). WCST was performed for assessing the executive function in early adulthood (n=63), middle-aged (n=38) and aging (n=6) healthy volunteers. The results of this research were revealed by examining the score differences from WCST between age range and sex. The WCST could be categorized into 5 groups: The score on total errors indicating nonspecific cognitive impairment, total correct indicating formal operational thinking, total card indicating serving as task termination and data normalization, categories completed resulted in cognitive set shifting and perseverative error reflecting cognitive flexibility. The results showed that scores on total errors, total cards and categories completed of early adulthood were significantly different when compared with the middle-age groups ( $P<0.002$ ,  $P<0.003$ ,  $P<0.005$  respectively). Moreover, the differences were also observed in aging group but these did not reach significance. These results can be interpreted that early adulthood is good performance executive function when compared with middle age and aging. The results also showed that there were significant differences between male and female subjects in total error and total cards ( $P<0.029$ ,  $P<0.022$  respectively). Additionally, the results found that there were significant difference between male and female subjects in total cards and categories completed scores in early adulthood ( $P<0.005$ ,  $P<0.020$  respectively). It is indicated that sex difference related to executive function on the cognitive set shifting. Moreover, significant differences between the early adulthood and middle-aged groups in the scores of total errors, total cards and categories completed were observed in female subjects ( $P<0.007$ ,  $P<0.013$ ,  $P<0.006$  respectively). These results indicate that the performance of executive function is related to age range differences on nonspecific cognitive impairment and cognitive set shifting. Moreover, the scores on total errors, total correct, total cards and perseverative error were found significantly differences between the male early adulthood and the middle-aged subjects ( $P<0.008$ ,  $P<0.023$ ,  $P<0.003$ ,  $P<0.001$  respectively). The results indicate that the performance of executive function is related to age range differences on nonspecific cognitive impairment and reflecting cognitive.

**Keywords** Executive function Healthy subjects Wisconsin Card Sorting Test

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## Effects of Attire Herbal Compresses on Reducing Low Back Pain at Sakon nakhon Thai Traditional Medicine Hospital (Luang Pu Fab Supatto)

Ronnachai Poowanna,<sup>1\*</sup> Jongkol Poonsawat,<sup>1</sup> Wiwat Sriwicha,<sup>1,2</sup> and Thanawat Nuangsri<sup>1</sup>

<sup>1</sup>Department of Thai Traditional Medicine, Faculty of Natural Resources, Rajamangala University of Technology ISAN Sakon nakhon Campus, Sakon nakhon 47160.

<sup>2</sup>Director of Sakon nakhon Thai traditional medicine hospital (Luang Pu Fab Supatto), Sakon nakhon, 47160 Thailand.

\*Corresponding author, e-mail: Ronnachai\_p1@outlook.com

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### Abstract

**Background** The herbal compresses or “Luk Prakob” in Thai folk medicine have been used in Thailand for a long time. But Luk Prakob have the limitation of usage because the treatment with Luk Prakob would takes a long time, proportion doctor to patient 1 of 1 and therefore insufficient in requiments. Provenance to Attire Herbal Compress or “Attire of Luk Prakob” (ALPK) **Objectives** the quasi experimental research was to study effect of attire herbal compression reduction for back pain at Sakon nakhon Thai traditional medicine hospital (Luang Pu Fab Supatto). **Methods** The participants comprised of the patients with low back pain comparison at before and after the ALPK in 30 persons. **Results** most sample group aged more than 60 years old. The most diagnosis was low back pain in period 3 days causes of bent neck, perk and heavy lifting. The pain scores after treated with the attire herbal compression were significantly lower than that of before treatment at  $p < 0.05$ . **Conclusion** This study showed that the attire herbal compression dramatically decreases back pain and could be applied to other patients.

**Keywords** Attire of Luk Prakob” (ALPK), Herbal compression, Low back pain

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**Email:** [alan@nanomegas.com](mailto:alan@nanomegas.com)

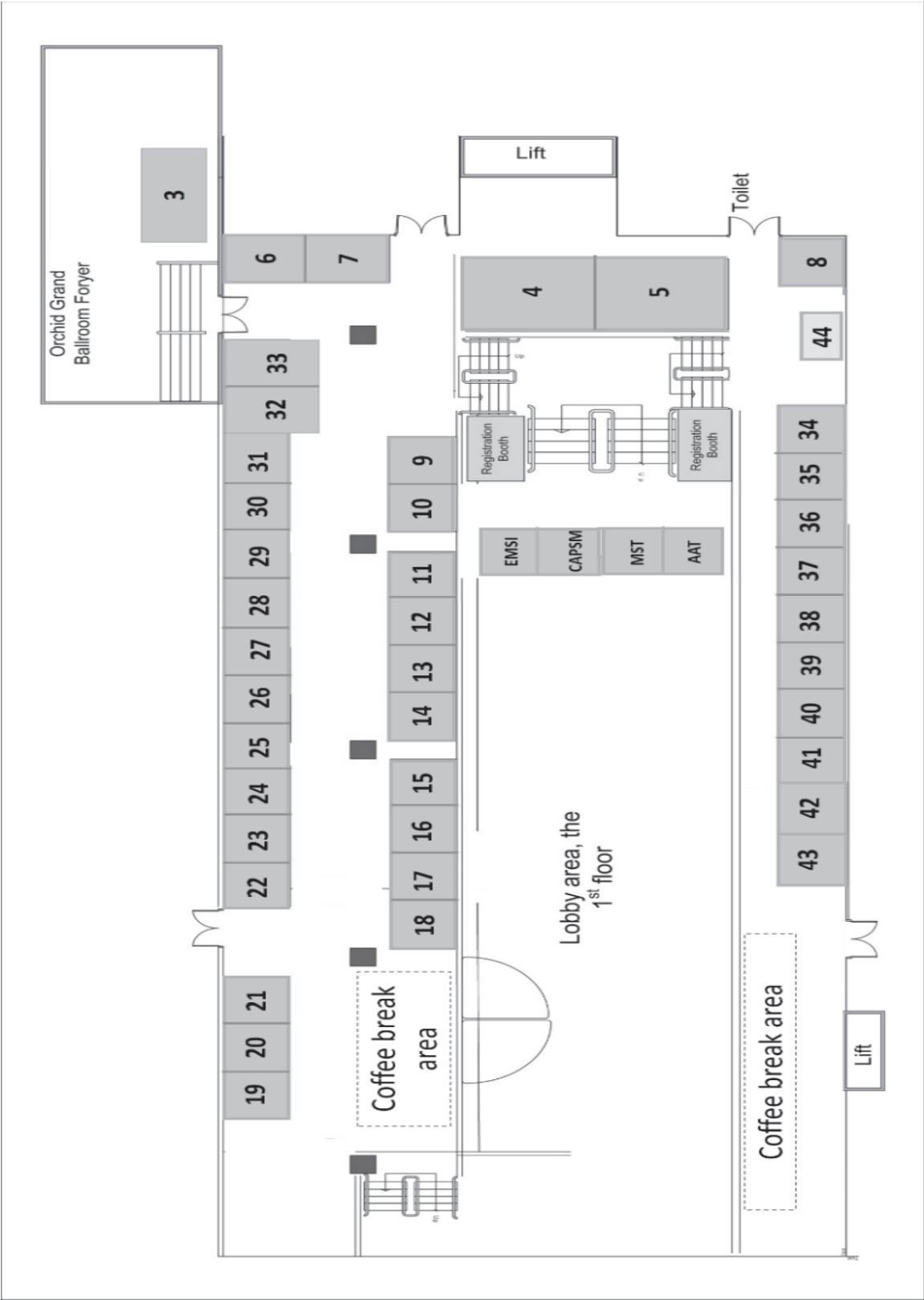
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**Email:** [sales@raithasia.com](mailto:sales@raithasia.com)  
**Contact Persons:** Evan Toh or Dr. Andrew Yu  
**Email:** [evan@tlg.sg](mailto:evan@tlg.sg) or [yu@raithasia.com.hk](mailto:yu@raithasia.com.hk)

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**Tel:** +65 6396 6386 / **Fax:** +65 6298 8043  
**Website:** [www.springer.com](http://www.springer.com)  
**Contact Person:** Loyola DSilva, PhD  
**Email:** [loyola.dsilva@springer.com](mailto:loyola.dsilva@springer.com)

APMC11 Exhibition Floor Plan



Booth Number	Exhibitors		Booth Number	Exhibitors	
3	HORIBA (Thailand) Limited		24,25	Leica Microsystems (SEA) Pte Ltd	
4, 5	Jeol Asia Pte Ltd		26,27	Carl Zeiss Co Ltd	
6, 7	HREM Research Inc		28,29	Gatan Inc	
8	EMSIS ASIA Pte Ltd		30,31	Bruker Singapore Pte Ltd	
9,10	Becthai Bangkok Equipment & Chemical Co Ltd		32,33	Hitachi High-Technologies Corporation	
11	Electron Microscopy Sciences		34,35	Absotec Co Ltd	
12	Diatome Ltd		36	E.A. Fischione Instruments Inc	
13,14	Oxford Instruments Pte Ltd		37	LMS Instruments Co Ltd	
15,16	FEI Company		38	Hollywood International Co Ltd	
17,18	Crest Nanosolution (Thailand) Co Ltd		39	Microscopy (official journal of The Japanese Society of Microscopy)	
19	Olympus Singapore Pte Ltd		40	Microscopy & Analysis	
20,21	Hi-Tech Imaging Co Ltd		41	Extrad Instruments (Thailand) Co Ltd	
22	EDAX Inc		42	NanoMEGAS SPRL	
23	CAMECA		43	Raith Nanofabrication	
			44	Springer	

## Lunch Talk Program APMC11

<b>Date</b>	<b>24th May 2016 (12.30 – 13.20)</b>				
<b>Room</b>	Orchid Grand Ballroom		Dalah 1.2		
<b>Company</b>	Jeol Asia Pte Ltd	Bunga Bruker Singapore Pte Ltd	Hitachi High-Technologies Corporation	FEI Company	Dalah 3 Gatan Inc
<b>Title</b>	1) In-situ experiments using 300kV Cs corrected TEM (JEM-ARM300F) with gas-cell type specimen holder 2) Introduction of multi-purpose electron microscope (JEM-F200)	Advanced applications using an annular four-channel silicon drift detector	Hitachi SEM introduction utilizing cutting-edge interface, usability, and application	New detection techniques and data management, the secrets behind the FEI workflow solutions for life sciences and material science	Spectral and compositional analysis with Gatan Microscopy Suite 3
<b>Speaker</b>	1) Dr. Ichiro Onishi 2) Mr. Akira Yasuhara	Max Patzschke	Ms. Mari Sakaue	Dr. Eric Van Cappellen	Dr. Ray Twisten
<b>Date</b>	<b>25th May 2016 (12.30 – 13.20)</b>				
<b>Room</b>	Orchid Grand Ballroom		Dalah 1.2		
<b>Company</b>	Jeol Asia Pte Ltd	Bunga Bruker Singapore Pte Ltd	Oxford Instruments Pte Ltd	Hi-tech Imaging Co Ltd	Dalah 3 HREM Research Inc
<b>Title</b>	Introduction of Latest Line-up of JEOL scanning electron microscopes	Tools for advanced electron backscatter diffraction in the SEM	1) Low Energy X-ray Analysis for Light Element and Ultra High Spatial Resolution SEM-EDS 2) New Developments in Confocal Instrumentation	Latest developments in Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) - From static surface characterisation to three-dimensional organic and inorganic micro area analysis	Quantitative electron microscopy using DM plug-ins
<b>Speaker</b>	Mr. Tan Teck Siang	Aimo Winkelmann	1) Dr. Wu Jiang 2) Dr. Arsene Chang	Sven Kayser	Dr. Pedro Galindo and Dr. Kazuo Ishizuka
<b>Date</b>	<b>26th May 2016 (12.30 – 13.20)</b>				
<b>Room</b>	Orchid Grand Ballroom		Dalah 1.2		
<b>Company</b>	Carl Zeiss Co Ltd	Bunga Bruker Singapore Pte Ltd	Crest Nanosolution (Thailand) Co Ltd	Leica Microsystems (SEA) Pte Ltd	Dalah 3 EDAX Inc
<b>Title</b>	Latest developments on multi-ion beam technology - making the transition from nanofabrication to nanomanufacturing	Micro-XRF on SEM for trace element analysis	To be confirmed	Cryo preparation-from high pressure freezing to (cryo) EM	EBSD application for material characterization with power of OIM analysis
<b>Speaker</b>	H. Chris Park	Wang Rui	To be confirmed	To be confirmed	To be confirmed



## 1) In-situ Experiments using JEM-ARM300F with Gas-Cell Type Specimen Holder

Ichiro Ohnishi  
EM Business Unit,  
JEOL Ltd.,  
3-1-2 Musashino, Akishima, Tokyo 196-8558, Japan  
email: ionishi@jeol.co.jp

Recently, we developed the 300 kV atomic resolution electron microscope - JEM-ARM300F (GRAND ARM). The JEM-ARM300F has two types of objective lens pole-piece: FHP (Full High resolution Pole-piece) and WGP (Wide Gap Pole-piece). One of these pole-pieces, the WGP is useful for the multi-purpose microscopy. In particular, its “wide gap” achieves the high efficiency in EDS analysis, because the design is optimized for multi-silicon-drift-detectors with large sensor size of 100 mm<sup>2</sup>, which can provide not only a high solid angle (~1.63 sr), but also a high take-off angle (>25 degrees). Also, the WGP can accommodate the special specimen holders such as heating, cooling, liquid etc. Generally speaking, the atomic resolution electron microscope are not able to use these holders because it is too thick to accommodate. Various in-situ experiments are available by the combination of JEM-ARM300F, WGP and special holders.

We conducted in-situ experiments under the gaseous environment, utilizing JEM-ARM300F with WGP and a gas-cell-type holder (“Atmosphere 200”, Protochips). The JEM-ARM300F with Atmosphere 200 could provide highly stable performance in in-situ experiments at a high temperature (up to 1000 degrees) under high gas pressure (up to 10<sup>5</sup> Pa). Moreover, in-situ chemical analysis - EDS and EELS - were also available under the high gas pressure (up to 10<sup>5</sup> Pa). In this Luncheon talk, I will present several results of in-situ observation and analysis, utilizing JEM-ARM300F and Atmosphere 200.

## 2) Introduction of Multi-purpose electron microscope / JEM-F200

Akira Yasuhara  
EM Business Unit,  
JEOL Ltd.,  
3-1-2 Musashino, Akishima, Tokyo 196-8558, Japan  
email: ayasuhar@jeol.co.jp

We just developed the newest Field Emission Transmission Electron Microscope “JEM-F200” (nickname “F2”). Though it is very easy-to-use, JEM-F200 can provide the higher performance in resolution, throughput and stability than any other non-aberration corrected FE-TEM.

Its stable Electron Optics and firm Mechanical Design are guaranteed by JEOL experience and knowledge about the development of Cs-corrected Electron Microscope. On the other hand, “Improved Cold FEG” and “Dual SDD system” provide the high resolution imaging, but also the high efficiency analysis.

JEM-F200 is the great tool to help your studies and researches in life science as well as material science. I will introduce JEM-F200 in this luncheon talk.



## Advanced Applications Using an Annular Four-Channel Silicon Drift Detector

Max Patzschke\*, Andi Käppel, Igor Nemeth  
Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany

\*Corresponding author, e-mail: max.patzschke@bruker.com

### Abstract

Special configuration has been recently developed for certain applications in order to improve certain limitations:

A special multi element concept is the XFlash® 5060FQ, an annular detector which can be placed between the pole piece and the sample in a standard SEM. The four SDD elements have an active area of 15 mm<sup>2</sup> each resulting in a total of 60 mm<sup>2</sup>. This large active area and the annular geometry, where the detector elements are very close to the x-ray source, lead to an extremely large solid angle of more than 1 sr. This is a value which is typically 100 times larger than a 10 mm<sup>2</sup> detectors in a conventional setup. Therefore extremely high count rates can be achieved easily even with low probe currents, and can be processed with four separate electronic channels in parallel, leading to maximum output count rate of more than 1,100,000 cps [1,2].

These properties make the detector an ideal device for high speed mappings, sensitive samples and his relatively high take-off angle lead to a significant reduction of shadowing effects on rough surfaces.

Examples benefiting from these latest developments will be presented.

**Keywords:** EDS, FlatQuad, high speed mapping, no shadowing effects, high input count rate

### References:

- [1]H. Soltau et al., Microsc. Microanal. 15 (Suppl.2) (2009), 204
- [2]R. Terborg, M. Rohde, Microsc. Microanal. 17 (Suppl.2) (2011), 892

APMC11 Lunch Talk  
24<sup>th</sup> May 2016  
Venue: Dalah 2



## **New Detection Techniques and Data Management, the Secrets behind the FEI Workflow Solutions for Life Sciences and Material Science**

Dr Eric Van Cappellen  
FEI Company

Modern laboratories often implement complex workflows including specimen preparation, imaging on various imaging tools such as Light microscope, Scanning Electron Microscopes and Transmission microscopes. Each of those techniques involves multiple detection schemes resulting in large amounts of data that need interpretation.

This seminar will describe some of the novel imaging methods and techniques such as the new FEI SEM in-column and post-column detectors on SEM's as well as the new compound SEM lens system.

FEI Company's latest TEM and STEM technology involves new STEM imaging forming techniques, such as iDPC. Also new imaging strategies allowing observation of beam sensitive samples and 2-D materials will be highlighted.

The seminar will also provide more information about FEI's data management and processing software tools, such as our multiscale workflow software and various imaging and rendering software tools.

APMC11 Lunch Talk  
24<sup>th</sup> May 2016  
Venue: Dalah 3



## **Spectral and Compositional Analysis with Gatan Microscopy Suite 3**

Dr. Ray Twesten  
Gatan, Inc.  
5794 W. Las Positas Blvd.  
Pleasanton, CA 94588 USA  
Tel +1.925.463.0200  
Email: [info@gatan.com](mailto:info@gatan.com)

Gatan Microscopy Suite® (GMS) 3 is the latest version of the industry leading Gatan Microscopy Suite software package. GMS 3 includes fundamentally redesigned methods for spectral and composition analysis. These model-based EELS and EDS methods allow simple, consistent extraction of composition and enhanced separation of overlapping signals. New features such as workflow support, color mapping and data layout management help make GMS 3 the next generation analysis platform for next generation electron microscopists.



APMC11 Lunch Talk  
25<sup>th</sup> May 2016  
Venue: Orchid Grand Ballroom



## **1) Introduction of Latest Line-up of JEOL Scanning Electron Microscopes**

Tan Teck Siong  
Sales Director / Application Group Leader,  
JEOL ASIA Pte Ltd.,  
2 Corporation Road #01-12 Corporation Place Singapore 618494  
email: [tstan@jeolasia.com.sg](mailto:tstan@jeolasia.com.sg)

JEOL Ltd has recently introduced a few new models of Field Emission SEM and Tungsten SEM with latest state of the art technology. The features of individual models are highlighted and explained. JSM-7800F Prime, one of the world's highest resolution FESEM has been well accepted by researchers worldwide. Latest experimental data will be presented.



## Tools for Advanced Electron Backscatter Diffraction in the Scanning Electron Microscope

Aimo Winkelmann\*

Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany

\*Corresponding author, e-mail: [aimo.winkelmann@bruker.com](mailto:aimo.winkelmann@bruker.com)

### Abstract

In the scanning electron microscope (SEM), important local structural information on materials can be provided by electron backscatter diffraction (EBSD). This method is based on the measurement of Kikuchi diffraction patterns formed during the backscattering process of electrons from a crystalline region sampled by the SEM beam. Firstly, the basic mechanism of Kikuchi pattern formation is reviewed, noting the resulting local resolution of orientation and phase data on the order of and below 0.1  $\mu\text{m}$ . Based on the various types of information contained in the Kikuchi patterns, an overview will be given on the general EBSD data analysis workflow for the main application areas, including orientation determination and phase identification of polycrystalline materials. New advanced developments will be mentioned, including configurable detection setups, the application of Kikuchi pattern simulations, and the extended analysis of the complete Kikuchi pattern signal.

**Keywords:** Scanning Electron Microscopy, Electron Backscatter Diffraction, Orientation Mapping, Phase Identification

### References:

- [1] A Winkelmann, C Trager-Cowan, F Sweeney, A P Day, P Parbrook, Ultramicroscopy 107, 414 (2007)
- [2] A Winkelmann, G Nolze, Appl. Phys. Lett. 106, 072101 (2015)
- [3] G Nolze, C Grosse, A Winkelmann, J. Appl. Cryst. 48, 1405 (2015)
- [4] A Winkelmann, G Nolze, Ultramicroscopy 149, 58 (2015)

## 1) Low Energy X-ray Analysis for Light Element and Ultra High Spatial Resolution SEM-EDS

James Sagar<sup>1\*</sup>, Wu Jiang<sup>2</sup>, James Holland<sup>1</sup>, Simon Burgess<sup>1</sup>

<sup>1</sup> Oxford Instruments NanoAnalysis, Halifax Road, High Wycombe, UK

<sup>2</sup> Oxford Instruments NanoAnalysis Singapore, Ubi Crescent, Singapore

\*Corresponding author, e-mail: [james.sagar@oxinst.com](mailto:james.sagar@oxinst.com)

A new type of EDS detector, X-Max Extreme, has been developed for SEM-based elemental characterization at low accelerating voltage and short working distance. This detector uses windowless operation with high sensitivity electronics to improve the effective solid angle for the detection of low energy X-rays by 10-100x. In combination with ultra-high resolution FEG-SEM chemical analysis is now possible for lighter elements and smaller structures.

Enhanced detectability for light elements, provides new information for borides, carbides, nitrides and oxides in a wide range of nano-materials, nano-structures and defects. Light elements can also be detected when present in lower concentrations such as boron in alloys and dopants in Si and other electronics. Lithium has also been detected from materials including Li-ion battery anodes with this new technology [1]. Further developments in Li analysis are actively being pursued. The capability to detect very low energy X-ray lines means much lower accelerating voltage can be used for elemental analysis. Characteristic lines for all elements can be excited at 2kV. Even at 1 kV most important elements can be detected, meaning sub 10nm resolution and surface sensitive analysis in the SEM can be achieved for many materials including semiconductors.

**Keywords:** 10nm special resolution, SEM-EDS, Lithium, Semi-conductor

### References:

1. Hovington P, Timoshevskii V, Burgess S, et al. Can we detect Li K X-ray in lithium compounds using energy dispersive spectroscopy? Scanning. 999 (2016) 1-8.

## 2) New Developments in Confocal Instrumentation

Arsene Chang\*

Andor Technology, Oxford Instruments, 7 Millennium Way, Springvale Business Park, Belfast, BT12 7AL, UK

\*Corresponding author, e-mail: [a.chang@andor.com](mailto:a.chang@andor.com)

For many years, confocal microscopy has been one of the most popular instruments to researchers who wants to get high quality fluorescence images. There are different ways of doing confocal imaging, the most common technique is called point-scanning confocal. But following the development of genetically engineered fluorescent proteins, the rise of studies of the dynamics of fluorescently tagged molecules in living cell have emphasized the need not only for high-resolution imaging systems like point-scanning confocal, but also capable of collecting images rapidly and with illumination levels low enough to avoid damaging light-sensitive fluorophores and cells.

Other than point-scanning confocal, recently spinning disk confocal has been widely used in live cell imaging applications, due to its fast speed, high resolution and low photo-bleaching rate. Here we present Borealis Perfect Illumination Technology which significantly improves the performance of spinning disk confocal, we can now deliver a solution which not only excels in live cell imaging, but should now be considered for fixed tissue volumetric imaging, bringing speed and image quality benefits over the typically used point scanning confocal microscope.

Borealis addresses the traditional limitations of spinning disk confocal such as background noise from scattered light, the need for high power lasers to overcome low throughput, and poor uniformity which impacts image quality and analysis. We offer a solution to outperform traditional point scanning technology; extending the confocal spectral range into the near infra-red, so enabling a wider choice of fluorophores and associated deeper imaging; increasing image capture rate, so improving productivity by an order of magnitude; and most importantly higher quality images with a large dynamic range through using cameras for detection.

**Keywords:** Point scanning confocal, Borealis Perfect Illumination Technology

APMC11 Lunch Talk  
25<sup>th</sup> May 2016  
Venue: Dalah 2



## **Latest developments in Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) - From static surface characterisation to three-dimensional organic and inorganic micro area analysis**

Sven Kayser  
ION-TOF GmbH, Heisenbergstr.  
15, 48149 Muenster, Germany

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a very sensitive and powerful surface analytical technique. It provides detailed elemental and molecular information about surfaces, thin layers, interfaces and full three-dimensional analysis of the sample. Since the original conception, the contribution of Münster by its academics and entrepreneurs to the development and spread of the TOF-SIMS technique has been significant and unceasing.

Today, TOF-SIMS has become a standard requirement for a surface analysis laboratory and has overtaken other, longer established surface techniques, both in the performance and the number of units now being sold. In this presentation we will provide an overview of the latest technical developments and discuss the application progresses in the field of nanotechnology and life science.

APMC11 Lunch Talk  
25<sup>th</sup> May 2016  
Venue: Dalah 3



## Quantitative Electron Microscopy using DM plug-ins

Dr. Pedro Galindo<sup>1</sup> and Dr. Kazuo Ishizuka<sup>2,\*</sup>

<sup>1</sup> Universidad de Cádiz, Avda. Universidad de Cádiz, 10, 11519 Puerto Real (Cádiz) Spain

<sup>2</sup> HREM Research Inc., 14-48 Matsukazedai, Higashimatsuyama, Saitama, 355-0055 Japan

\*Corresponding author, e-mail: [ishizuka@hremresearch.com](mailto:ishizuka@hremresearch.com)

### Abstract

During this Lunch Talk we will introduce our suite of the DigitalMicrograph (DM) plug-ins for Quantitative Electron Microscopy, including Exit Wave Reconstruction, STEM and EELS Deconvolution, Strain Mapping, HR(S)TEM Noise Filters, Scan Noise corrector, Multivariate Analysis for SI data, Rocking Beam Electron Diffraction etc.



## Latest Developments on Multi-Ion Beam Technology - Making the Transition from Nanofabrication to Nanomanufacturing

H. Chris Park\*

ZEISS Microscopy, Carl Zeiss Pte Ltd., 50 Kaki Bukit Road, 415926 Singapore

\*Corresponding author, e-mail: [chris.park@zeiss.com](mailto:chris.park@zeiss.com)

### Abstract

Combining a high brightness Gas Field Ion Source (GFIS) with unique sample interaction dynamics, helium and neon ion microscopy provide nanomachining capabilities in the sub-10 nm regime and offer new and complementary insights into the structure and function of nanomaterials [1, 2]. Besides imaging, the helium ion beam can be used for fabricating nanostructures at the sub-10nm length scale. The neon probe size is greater than helium and is measured between 1-2nm. The sputter yield of Ne is about 30X higher than He, and the Ne beam has a shallower penetration depth resulting in lower sub-surface damage. Inert by nature and with a moderate sputtering rate, the neon ion beam presents an attractive alternative to Ga<sup>+</sup> FIB for use in applications sensitive to Ga<sup>+</sup> implantation and structural damage. In addition to Helium and Neon beams, the Orion NanoFab multi-ion beam platform comes with the option of a state-of-the-art Ga FIB for a versatile nanomachining workstation.

In this talk, we will introduce a new automation software module that offers the capability to create complex automated workflows. Sophisticated programming is facilitated by recipe building templates designed for easy modification and layering of multiple recipes. Examples will be presented of how this module is implemented for a variety of applications from multi-site array patterning to multi-process device nanofabrication.

**Keywords:** Helium ion microscopy; nanofabrication; lithography

### References:

1. Smentkowski, V.S., Denault, L., Wark, D., Scipioni, L., and Ferranti, D, Microscopy and Microanalysis 16(Suppl.2), (2010) p.434
2. Wu, H., Sijbrandij, S., McVey, S., Notte, J., Microscopy and Microanalysis 21 (Suppl 3), (2015) p. 701-702



## Micro-XRF on SEM for trace element analysis

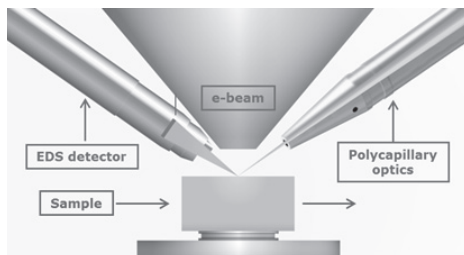
R. Wang\*, A. Wong  
Bruker Singapore  
\*rui.wang@bruker.com

### 1 INTRODUCTION

Micro-XRF on SEM or XTrace is a micro-spot X-ray source for attachment to any SEM with a free inclined port on the specimen chamber. It adds the capabilities of a complete micro-XRF spectrometer to the electron microscope. Limits of detection are improved 20 to 50-fold in the mid to heavy element range. Additionally, larger sample volumes become accessible as X-rays have higher information depth.

The use of polycapillary X-ray optics allows the generation of high fluorescence intensities on very small sample areas. The X-ray optics collects tube radiation from a large solid angle and concentrates the X-rays on spot down to 35 $\mu$ m. The generated X-ray fluorescence spectrum is measured with the XFlash<sup>®</sup> silicon drift detector (SDD), belonging to the QUANTAX EDS system. The schematic drawing is as shown in Figure 1. The use of an XFlash<sup>®</sup> SDD gives excellent energy resolution up to 121eV. Count rates of about 40kcps can be achieved in the analysis of metals, using a 30 mm<sup>2</sup> active area detector. The high intensity produced by the polycapillary optics and the reduced spectral background of X-ray excitation leads to an enhanced sensitivity for trace elements. The improvement is approximately 20 to 50 times compared to electron excitation. Also, because X-ray excitation is more efficient for higher-Z elements, detection limits down to 10 ppm can be achieved. This makes XTrace most interesting for the trace analysis of heavy elements in an electron microscope.

Both EDS and XTrace systems are controlled by the ESPRIT 2.0 software under a single interface. Results of both methods can be combined for optimum accuracy, making perfect use of the light element performance of electron induced EDS and the sensitivity for heavier elements of XRF. The software also supports measurement of objects, line scans and maps using the SEM specimen stage.



**Figure 1.** Schematic drawing of Micro-XRF and EDS in a SEM chamber.

### 2 CONCLUSION

Micro-XRF on SEM extends the analytical limit of your EDS to as low as 10ppm. Applications include failure analysis, determination of trace elements in solids of all kinds, qualitative and quantitative compositional analysis, including combined quantification of Micro-XRF and EDS spectra and finally, element distribution analysis in form of stage line scan and map.

## **EBSD Application for Material characterization with power of OIM analysis**

EDAX Inc.

91, McKee Drive, Mahwah, NJ, 07430, USA

Tel: +1-201-529-4880 / Fax: +1-201-529-3156

[www.edax.com](http://www.edax.com)

1. Single software application providing full capability for comprehensive microstructural investigation and analysis of EBSD mapping data. There are no issues with multiple applications trying to communicate with each other, and it is easy to correlate different types of analysis with each other (orientation maps with pole figures for example).
2. Over 40 different map options, 16 point-to-point boundary options, and 52 chart options provide the widest range of data outputs to provide the data analysis needed for the wide range of requirements in materials research.
3. OIM Templates allow users to select, customize, and save specific maps, charts, and plots as a group to facilitate easy repeated analysis.
4. OIM Project Tree organizes EBSD data by project, sample, and partition, and allows easy viewing and selection of the different maps, charts, and plots available within the project tree.
5. Partitioning functionality allows users to define a subset of data based on point properties (including confidence index, phase, EDS, and crystal orientation) and grain properties (including grain size, grain aspect ratio and grain orientation spread). Can define a partition, or define a neighbor partition, only neighbors (either points or grains) satisfying the defined partition. Partitions can be created using user-specified quantitative ranges, or in a manual point-and-click mode. Boolean logic operators can be applied to combine multiple partition definitions.
6. Processor allows for automated analysis of multiple datasets using pre-defined templates. Data cleanup, cropping, and rotation can be applied to each dataset. Batch parameters settings can be saved for subsequent runs. Batch processor can also be used for 3D dataset alignment based on orientation data.
7. Interactive tools allow users to manually interrogate datasets to extract information on points, grains, orientations, grain boundaries, triple junctions, defined vectors on sample, local unit cell overlay, and local defined plane traces. Output of interactive analysis can be customized, saved, and exported.
8. Highlighting tools allow users to select regions in maps, charts, and plot, and see these areas displayed on all other maps, and charts to allow easy correlation of multiple measurements outputs (to determine if small grains have a different texture than large grains for example).
9. Full export capabilities of including orientation data, misorientation data, grain files, partition data, reconstructed grain boundaries, and triplet points to allow access to data for external processing and analysis. Outputs include text files and HDF5 data formats.
10. OIM Analysis allows analysis of hexagonal measurement grids, which provides 6 equal-distance neighbors compared to a square grid which provides 4 neighbors of one distance and 4 neighbors of another distance. A hexagonal grid provides a higher density of measurement points per area and allows better determination of triplet points and reconstructed boundaries.



**5-in-1 fantasy: Giralope.**



**5-on-1 reality: Bruker's  
range of analytical  
tools for SEM.**



● **EDS, WDS, EBSD, Micro-XRF and Micro-CT - five  
analysis methods for SEM from a single source.**

- QUANTAX EDS: provides highest energy resolution and maximum throughput
- QUANTAX WDS: offers maximum sensitivity in the low energy range
- QUANTAX EBSD: sets new standards in combined EBSD/EDS
- XTrace: adds Micro-XRF analysis capabilities to the SEM
- Micro-CT for SEM: reveals the internal microstructure of samples

**Someone has to be first.**



Prince Mahidol Hall, Mahidol University

### Organized by:

- Department of Anatomy, Faculty of Science, Mahidol University
- Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University
- Microscopy Society of Thailand (MST)
- Anatomy Association of Thailand (AAT)