ANNUAL REPORT 2016

CENTRE FOR MICROSCOPY AND MICROANALYSIS THE UNIVERSITY OF QUEENSLAND

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Making the invisible visible

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The Centre for Microscopy and Microanalysis is a research facility dedicated to an understanding of the structure and composition of all natural and man-made materials across different scales. Our challenge is to meet present needs of researchers for microstructural characterisation and to equip The University of Queensland to meet new horizons in analysis research.

Mission

Our service to the university community is provided in three key areas, namely a comprehensive suite of analytical instrumentation, strongly motivated and experienced personnel and high standard of training programs for university researchers. Our highly experienced, specialist staff are committed to providing a supportive and resourceful working environment where clients receive expert advice and training that equips them to achieve their research goals.

Overview

The Centre for Microscopy and Microanalysis (CMM) is an interdisciplinary research, teaching and service centre. We play an integral role within the research programs of The University of Queensland and participate in both undergraduate and postgraduate education. We provide a comprehensive suite of analytical instrumentation and a high standard of training programs for university researchers.

CMM is a foundation member and the Queensland Node of the Australian Microscopy and Microanalysis Research Facility (AMMRF) which was established in July 2007 under the Commonwealth Government's National Collaborative Infrastructure Strategy (NCRIS).

CMM actively supports and initiates microscopy and microanalysis related research and development projects with the aim to maintain future technological competitiveness for UQ. CMM is also in charge of the renewal of instruments to ensure the optimal balance in instruments dedicated to general education, service and research and to further push research projects beyond the current frontiers.

CMM is all about its users, about helping and sharing, making the research better and expanding the knowledge in experimental sciences and engineering.



It is my great pleasure to welcome you to the 2016 annual report of the Centre for Microscopy and Microanalysis. We have once again had a very successful year and together the CMM team has managed several exciting changes. Let me list a few of these stories before I get to the large topic of 2016.

- We received two new client training and advanced research TEM's, which have been fully installed and are up and running, ready for client training from January 2017.
- To ensure that our services remain current and valid to our stakeholders, we conducted two CMM staff team retreats to find missing gaps in our current capabilities. In these retreats we worked mainly to enhance collaborative work between the different CMM labs, which led to more convergent collaborations for the benefit of ongoing research projects of our users. One of the future outcomes will be to establish a scientific advisory board and obtain direct feedback for strategic development of CMM from UQ Cls and researchers.
- As in every year, the CMM team was committed to providing high quality teaching and training for our stakeholders, through workshops, one-on-one training, and our staff involvement in undergraduate teaching and post graduate supervision.
- This year also saw the official launch of the MyScope Outreach in Australia, held in the Maker Space at The Ian Potter Foundation Technology Learning Centre, Questacon, Canberra.

Details of our activities in training and teaching can be found on pp 30-39.

Throughout 2016 we welcomed a number of visitors and collaborators from 60 institutes, including visitors from the USA, Europe and Asia. These international networks, together with the national exchange with NCRIS facilities, are vital for the ongoing expansion towards future frontiers for characterisation capabilities frontiers for the Faculty of Science, UQ, and AMMRF on a national scheme.

It has now been nearly a year for me since I stepped in to the role of Director, to further develop CMM to a world-class characterisation facility. It is a special honor for me to serve a highly motivated team, and supportive Faculty of Science and university. As changes in management often come along with uncertainty and unknowns, new challenges and opportunities, it is not always an easy time for the people involved. Some changes are not always obvious and my aim is to integrate existing knowledge and CMM's high level know-how into all changes to come. I also invite all to be part of the journey, to be open to let new things happen and bring in new views, methods and analytical capabilities, evaluate them and finally integrate the best ones, always aiming towards the future needs of UQ researchers and establishing one of the best world-class characterisation facilities.

In addition, I hope to foster scientific opportunities by bringing scientific methods together to generate new insights and approaches in originally separated fields. This means engaging in all areas of experimental science by better coordinating and linking existing methods and approaches with new capabilities. This kind of convergence of methods – better known in our field as correlative approaches - are driven solely by the needs to solve more and more complex problems that our users face in their more and more holistic research area and towards the "green industrial wave".

I am delighted that our university recognised these activities at CMM from the beginning of my appointment and granted funding for the future expansion of CMM into new application areas and characterisation opportunities:

- the capability to characterise materials at 70 pico-meter with atomic scale spectroscopy and the expansion to run experiments (in-situ) in the spherical corrected Transmission and Scanning Transmission Electron Microscopy (TEM/ STEM) to allow material testing at the nanometer and atomic scale
- the capability to use electron beams to write patterns with 3-10nm resolution (Electron Beam Lithography) into resists to create future structures for quantum physics
- the capability to use the sensitivity of mass-spectroscopy for imaging and hence localise molecules in tissue sections (Imaging Mass Spectroscopy)

See more about further investments and enhancements inside this annual report where you will also find discoveries from different user groups along the convergent use of characterisation methods.

Some of the new instruments have been procured and will arrive in 2017/18, others are under evaluation, and some future tools are being developed and currently built. We will also continue to recruit, which is essential to bring these techniques to a spin and expand UQ characterisation frontiers. This will guarantee access to a world-class facility and expertise across all micro to nano to atomic scale and share your know-how with a team to make your research even better.

CMM is all about its users, about helping and sharing, making the research better and expanding the knowledge in experimental sciences and engineering.

Our success in 2016 is a result of strong support from the Faculty of Science – thanks to Stephen Walker and Ian Gentle - the former Provost (Max Lu) and massive support over the last months and generous support from our DVCR Robyn Ward and her team (Al McEwan and Stephen Love). We owe Robyn, Al, Stephen and Ian and all others involved a great thank you.

Finally I would like to thank all centre staff especially the centre admin and maintenance staff for their excellent support and contribution throughout 2016 and for the on-going changes we are working on in 2017/18.

I hope you enjoy the 2016 Annual Report.

Professor Roger Wepf, Director, CMM



REVENUE	
Fees and Charges	714,413
Other Income	13,320
Defined Central Funding Scheme	7,641,816
Operating Level Allocations	394,396
Executive Level Allocations	1,028,172
TOTAL REVENUE	9,792,117
EXPENDITURE	
Academic Salaries	851,287
General Salaries	1,229,762
Other Employment Costs	22,081
General Operating Expenses	162,696
Professional and Other Services	32,140
Equipment and Minor Works	7,323,425
Travel	77,058
Hospitality	4,681
Other Expenses	4,200
TOTAL EXPENDITURE	9,707,330
Operating Result	84,787



Centre for Microscopy and Microanalysis



REVENUE	
Research Income	441,219
Investment	2,969
Other Income	4,800
Defined Central Funding Scheme	0
Operating Level Allocations	5,604
Executive Level Allocations	
TOTAL REVENUE	454,592
EXPENDITURE	
Academic Salaries	59,278
General Salaries	232,010
Other Employment Costs	414
General Operating Expenses	9,178
Professional and Other Services	0
Equipment and Minor Works	25,210
Travel	11,128
Hospitality	
Other Expenses	5,048
TOTAL EXPENDITURE	342,266
Operating Result	112,326





Executive

PROFESSOR ROGER WEPF



Director

PROFESSOR ROBERT PARTON



Deputy Director

A/PROFESSOR KEVIN JACK



Deputy Director

Australian Institute of Bioengineering and Nanotechnology (AIBN) Laboratory

MR RICHARD WEBB



Senior Laboratory Manager AIBN

DR HUI DIAO



Dual Beam FIB/SEM Engineer

DR GRAEME AUCHTERLONIE



Research Officer

MS MUN TENG (ABBY) SOO



Casual Research Officer

MS ROBYN CHAPMAN



Scientific Officer

MS RACHEL TEMPLIN



Research Assistant

Hawken Laboratory

MR RON RASCH



Senior Scientific officer/Lab Manager, Hawken

MS EUNICE GRINAN



Senior Technical Officer

MS YING YU



DR KIM SEWELL



Scientific Officer

Senior Technical Officer

X-ray Facility

A/PROFESSOR KEVIN JACK



X-ray Suite Manager

DR BARRY WOOD



Scientific Manager

MS ANYA YAGO



Research Officer

Queensland Bioscience Precinct

DR MATTHIAS FLOETENMEYER



Laboratory Manager, QBP

DR KATHRYN GREEN



Scientific Officer

DR ERICA LOVAS



Casual Laboratory Assistant

Affiliate Researchers

PROFESSOR JIN ZOU



Affiliate Professor

DR TOSHIYUKI MORI



Affiliate Researcher

Postdoctoral Fellow

DR KUN ZHENG



Postdoctoral Research Fellow

Administration

MS JILL PRESCOTT



Centre Manager

MR ROBERT GOULD



Senior Scientific Officer, Workplace Health and Safety Coordinator

MS WENDY ARMSTRONG



Senior Scientific Officer & OH&S Support Officer

MR ANDREW STARK



Senior Technical Officer

MS JENNIFER BROWN



Client Liaison Officer

Affiliate Appointments

EMERITUS PROFESSOR JOHN DRENNAN



DR BRONWEN CRIBB



DR JUSTIN KIMPTON



CMM INFRASTRUCTURE













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2016 HIGHLIGHTS

Permission granted to refurbish Hawken Laboratory

Installation of 2 new client training and advanced research TEMs, which offer either high contrast with EDX/STEM, or high resolution with lattice imaging possible. These replace the100kV TEMs and mark the beginning of negotiations for CMM to host a Hitachi Hi-Tech Training Centre.

Ordered Australia's first Cs-corrected STEM/TEM (Hitachi HF5000) allowing 78pm resolution in STEM and fast data acquisition for *in-situ* TEM/STEM experiments.

CMM in conjunction with the ARC Centre EQuS ordered two Lithography systems (Raith), including a top end EBPG5150 and an eLINE Plus with additional 5 GIS additive patterning capabilities.

Mater Hospital donated 6 mass spectrometry instruments including 2 Maldi-TOF instruments allowing CMM to build UQ's imaging mass spectroscopy capacity.



CMM is The University of Queensland's electron and X-ray microscopy and microanalysis core facility and the Queensland Node of the national microscopy and microanalysis network (AMMRF). With strengths in high throughput methods for single particle analysis and cryo-electron microscopy, CMM applies these techniques to the analysis of new materials and living systems.

CMM's platform goals are to facilitate and provide research excellence through a focus on providing world-class facilities matched with an equally high level of expertise, thereby meeting the characterisation requirements of local and national researchers and industry. CMM achieves this via a concept of supporting discovery from user inception of experiments to publication/outcomes through excellence in consultation, training, measurement and finally analysis.

CMM's dedicated academic, research, technical and administrative staff support a diverse range of instrument platforms including electron microscopy and microanalysis, X-ray diffraction and spectroscopy and novel imaging modalities.

2016 saw significant investment in new analytical capacity that has already been delivered or will be operational in 2017.

Looking forward to 2017 and beyond, a number of additional projects are planned:

- CMM seeks to collaborate with UQ's Sustainable Minerals Institute (SMI) on a table top scanning XRF system based on the newest high-flux X-ray sources, using the Australian Synchrotron control and data acquisitions technology, so as to act as a gateway instrument to the Synchrotron with its higher resolution and sensitivity. When completed this will be a 24/7 research tool for screening and *in-situ* experiments.
- CMM plans to increase the X-ray measuring capacity by adding further detectors and increasing the X-ray source flux over time, with an MEI lead by the SMI.

- CMM, teaming up with UQ's Institute of Molecular Bioscience (IMB), is seeking MEI support to modify the FIB/ SEM to a fully RT or Cryo (-180°C) compatible workhorse for processing material at low temperature. This is also of interest for material science, polymer science as well as for life-science and will help to image and process volatile materials such as Indium in III/V semiconductors, mercury or lithium materials, as well as beam sensitive materials like polymer, polymer composites or frozen hydrated samples, for example cells or tissue samples for direct site-specific imaging/analysis or extraction of ultra-thin lamellas for atomic resolution imaging in our future frontiers STEM/TEM.
- CMM has teamed up with UQRFG to support a proposal to establish a GPU/CPU cluster for large scale image processing such as serial section images (3View, Array tomography), single particle cryoTEM, STEM/TEM Tomography and future in-situ data-streams and correlative microscopy data-sets.
- Looking ahead CMM also has significant plans to review and commence the replacement of existing infrastructure, including replacing the existing SAXS system via a LIEF grant application.



AIBN Laboratory Manager: Mr Richard Webb (r.webb@uq.edu.au)

CMM facilities are located on the ground floor of the Australian Institute for Bioengineering and Nanotechnology Building. These purpose built laboratories house four state of the art transmission electron microscopes and three scanning electron microscopes plus a range of sample preparation facilities. In addition the laboratory is equipped with a sophisticated optical microscopy suite with three optical microscopes in various configurations. JEOL JEM-1010A TEM (SIS Veleta CCD camera)

JEOL JEM-1010B TEM (SIS Veleta CCD camera)

FEI Tecnai F20 FEGTEM (Gatan OneView Camera, Oxford EDS, STEM BF/DF/HAADF, FEI Low Background Double Tilt Holder, Gatan Low Background Double Tilt Holder, Gatan High Temperature Double Tilt Holder (1000°), Nanofactory 30997 Double Tilt STM Holder, Gatan GIF EELS/ EFTEM)

JEOL JEM-2100 TEM (LaB6, Oxford EDS, STEM BF/HAADF, Gatan Orius SC1000 digital camera, JEOL Double Tilt Holder, Gatan Low Background Double Tilt Holder)

JEOL JSM 7800F SEM (Super hybrid Objective Lens Retractable BE detector, RAITH Elphy Quantum Lithography System)

Zeiss Sigma SEM (VP, Gatan 3View 2XP)

FEI Scios FIB (Oxford EDS, Oxford EBSD, STEM Detector, Easy Lift micromanipulator, Gas Injection system with platinum, tungsten, silicon and selective carbon etching)



Hawken Laboratory Manager: Mr Ron Rasch (r.rasch@uq.edu.au)

The Hawken laboratory is the scanning electron microscopy laboratory of CMM. There are a series of scanning electron microscopes that can examine a very broad range of sample types. In addition to microscopy, many instruments are capable of microanalysis using a wide variety of techniques. The laboratory also houses a microprobe instrument that provides quantitative elemental analysis from polished specimens. Philips XL30 SEM (FEI BSE detector, EDAX EDS, FEI chamber IR camera, Oxford Cryo-preparation Unit)

JEOL JSM-7100F SEM (Retractable BE detector, JEOL JED EDS system, Gatan ALTO Cryo system)

JEOL JSM-6460LA (JED-2300 UltraMinicup EDS detector, Analysis Station and Phase ID software, Low vacuum SE detector)

JEOL JSM 7001F SEM (Super hybrid Objective Lens, Retractable BE detector, JEOL JED EDS system, Oxford INCA Wave WDS stand alone system)

JEOL JSM-6610 SEM (LaB6 filament, OXFORD Xmax SDD EDS, OXFORD EBSD)

JEOL JCM-5000 Neoscope SEM

Hitachi 3500 SEM (Low vacuum)

JEOL JXA-8200 EPMA (5 x WDS Spectrometers, JEOL EDS Be Window Spectrometer)

Renishaw Raman System (Three laser system equipped with 325nm, 514nm and 785nm wavelengths plus stage mapping and confocal depth profiling)



X-ray Facility

Manager: A/Prof Kevin Jack (k.jack@uq.edu.au)

The X-ray analysis facility at CMM is based on level two of the Chemistry building and provides a range of X-ray techniques for studying chemical composition, nano-scale size and crystalline phases in a range of materials. Bruker D8 Advance powder XRD (Cu and Co X-ray sources, 90 position sample robot, Pixel-array detector)

Rigaku SmartLab thin-film and micro-diffraction XRD (High-intensity rotating anode source, 5 axis goniometer Parallel, focused and Bragg-Brentano collimation, 2D silicon drift detector, Anton-Paar heating stage and Oxford cryostream)

Kratos Axis Ultra XPS (Angular-resolved and 2D mapping modes, Argon-ion bombardment)

Anton Parr SAXSess SAXS (Capillary, gel and powder cells, Princeton CCD detector)



QBP laboratory

Manager: Dr Matthias Floetenmeyer (m.floetenmeyer@uq.edu.au)

Located in the Queensland Bioscience Precinct, the Cryo-Transmission Electron Microscopy (TEM) Facility is a laboratory that was purpose-built for standard and cryo-TEM sample preparation and analysis, as well as electron tomography of both resin-embedded and cryo-samples (for threedimensional analysis). FEI Tecnai 12 TEM (Direct Electron LP Digital Camera)

JEOL JEM-1011 TEM (Olympus SIS-Morada CCD Camera)

FEI Tecnai F30 Cryo-FEGTEM (Gatan K2 Summit Camera)

JEOL JCM-5000 Neoscope SEM

Cryo-holders for the FEI-Tecnai microscopes (3 Gatan cryo transfer systems)

Tomography holders for the FEI-Tecnai microscopes (Fischione 2040 α -tilt-rotate holder, 2 Gatan α -tilt-rotate holder, Gatan cryo- α -tilt-rotate tomography holder FEI/Gatan α/β -tilt holder, Gatan high α -tilt cryo-transfer system holder)

CMM RESEARCH

















Growth of epitaxial III-V semiconductor nanowires

Jin Zou

(Collaborating with Professors C. Jagadish, ANU and Wei Lu, Shanghai Institute of Technical Physics)

III-V semiconductor nanowires have received extraordinary attention in recent years because of their unique physical and chemical properties, which in turn gives them great potential as building-blocks for many advanced devices for nanoelectronic and optoelectronic applications. In general, nanowires are induced by catalysts, and catalysts play a complicated role during the nanowire growth. In this project, III-V semiconductor nanowires were epitaxially grown on different substrates (such as Si) and using different catalysts. Also, their structural and compositional characteristics are investigated.

InAs nanowires epitaxially grown on Si substrates with different orientations using different metallic catalysts

Development of thermoelectric nanostructures

Jin Zou

(Collaborating with Professors Jeff Snyder and Mercouri Kanatzidis, Northwestern University)

Thermoelectric nanostructures directly convert waste heat or sun heat to electricity, provide opportunities to harvest useful electricity, which has been considered a promising candidate to generate clean energy. In this program, we employed low cost and environment friendly approaches, such as solvothermal, microwave assisted solvothermal, to synthesize various chalcogenide-containing semiconductor nanostructures and their thermoelectric performances are evaluated using our newly installed facilities. Modelling the properties are also undertaken to understand the thermoelectric performance of our nanostructures.

EBSD and TEM studies of $Bi_2Te_{2.7}Se_{0.3}$ nanostructures containing a large amount of nanograins and grain boundaries that strengthen the phonon scattering for enhancing the thermoelectric performance.





Two types of planar defects found in ternary $Sn_{i-x}Bi_xTe$ nanoribbons.

Development of topological insulating nanostructures

Jin Zou

(Collaborating with Professor Faxian Xiu, Fudan University and Kyeongjae Cho, The University of Texas at Dallas)

Topological insulators, which feature surface conductivity, are a new type of materials discovered in the last decade. Due to their inherent exotic physical properties, topological insulators have been considered as a promising candidate for next-generation electronic and spintronic devices. In this project, we fabricate a range of topological insulating nanostructures and understand their structural characteristics and properties.

In-situ transmission electron microscopy

Professor Jin Zou

(Collaborating with Professor Xiaodong Han, Beijing University of Technology)

The ultimate goal of materials research is to develop materials with desirable properties for practical applications. It has been well documented that the properties of materials are determined by their structures and/or their structural behaviour under external stimuli, including heat, applied stress and/ or electrical voltage/field. With recent developments, in-situ TEM is playing a key role in understanding the intrinsic properties of individual nanostructures. In this program, we are employing in-situ TEM techniques to measure strain-stress curves and/or current-voltage curves of individual nanostructures.

A schematic diagram of in-situ TEM setup for measuring the current-voltage curve. (a) The probing system. (b) Enlarged schematic diagram of the dashed area in (a). (c) Demonstration of a deformation process using the movable tip. (d) Schematic current-voltage curves under different deformation states.





Fabrication of microscale bridges for advanced mechanical testing

James L. Mead

(This research is part of James L. Mead's PhD thesis under the supervision of Professor Han Huang)

Microscale thin films and multilayers are material systems that are intrinsic to the operation of microelectronics. The emergence of flexible electronics for wearable technology, medical implants, and next-generation solar cells, has placed increased importance on the mechanical performance of such devices. The interfacial adhesive strength of a multilayer is of particular interest as delamination of the layers is a common failure mechanism. To improve our understanding of interfacial failure, it is critical to develop versatile techniques for characterising interfacial adhesion on such a small scale.

In this project, a novel method for inducing stable delamination within multilayers has been established by combining focused ion-beam milling, scanning electron microscopy, and nanoindentation. Micro-bridges containing the interface of interest are milled into the top surface of a layered system using a FIB dualbeam facility. To induce interfacial failure in the micro-bridges, they are placed under load using a nano-indenter in either three-point or four-point bending configurations. Examination of the micro-bridges before and after indentation using scanning electron microscopy allows for the dimensions of the bridge and the nature of failure to be recorded. Mechanical theory is then used to analytically calculate the adhesive strength of the interface of interest.

(a) Focused ion-beam milled microbridge within a Al/SiN/GaAs multilayer system (45° tilt, FEG-SEM), (b) Centre region of microbridge, showing substrate fracture, fracture deflection, and interfacial delamination (45° tilt, FEG-SEM), (c) schematic showing nanoindentation induced delamination of a microbridge

Visualisation of the inner workings of the cell

Robert G. Parton

(Institute for Molecular Bioscience, Centre for Microscopy and Microanalysis)

The cell is the fundamental unit of life. The human body contains approximately 200 different types of cells. These cells have different molecules, structures, and functions that are essential for the organs of the body to function. Our work involves using electron microscopy to explore the cell and to determine what goes wrong in human disease. In particular, we have focussed on the plasma membrane that encloses the cell and forms a crucial barrier between the cell and the outside world. The plasma membrane is a mosaic of specialised regions with specific functions. The overall aim of our research is to dissect the structure, function, and composition of specific plasma membrane microdomains and to understand how microdomain dysfunction leads to disease. These studies have implications for diverse cellular processes and disease conditions including muscular dystrophy, liver regeneration, and cancer.

In 2016 we studied a number of different aspects of cellular function but mainly centred on the plasma membrane and how the plasma membrane responds to the cell's environment. In particular, we gained new insights into endocytosis, the process by which cells engulf part of their surface membrane together with material in the external environment. Endocytosis plays a crucial role in detecting signals for growth or in taking nutrients up into the cell. Membranous vesicles pinch off from the plasma membrane and must then reach their destination in the cell, an organelle called an endosome which is crucial for the cell to function. This process uses a protein called Rab5, a small protein that can switch between active and inactive forms, and a long filamentous protein, called Early Endosomal Antigen 1 (EEA1). We showed using high resolution electron microscopy that EEA1 filaments form a dense layer of filaments that extends out from the surface of endosomes. Vesicles covered in active Rab5 bind to the EEA1 coat triggering the individual EEA1 filaments to change their conformation (Murray et al, Nature 2015). This allows the vesicle to reach the surface of the endosome and fuse with its membrane. This may be a universal mechanism which makes sure that vesicles find the right destination within the cell and that they deliver their contents efficiently to their targets.

We are also interested in less well-understood endocytic processes that may give clues into processes such as metastasis, the means by which cancer cells move from primary tumours to distant sites in the body. We have dissected the structure of the major compartments and analysed the role of specific molecules involved in an enigmatic endocytic process which is crucial for cells to move. New state-of-the-art 3D electron microscopic methods, combined with novel genetic tags, are giving new insights into the structure and molecular make-up of this pathway.

Finally, we have continued our studies aimed at providing a full 3D electron microscopic analysis of cancer cells. Our 3D datasets of a migrating cancer cell generated in collaboration with Robyn Chapman and Rick Webb by Serial Blockface electron microscopy have been segmented by Dr Nick Ariotti (Parton laboratory) and then converted into a realtime virtual reality (VR) experience (by John McGhee, UNSW in collaboration with Angus Johnston, Monash University; funded by our ARC Centre of Excellence in Convergent Bio-Nano Science and Technology). This allows the user to 'move' through the cell and explore its many features in a VR experience we have called Journey to the Centre of the Cell. These approaches are providing a completely new user experience and are a very powerful educational and research tool. Our VR Journey to the Centre of the Cell has been featured on TV (ABC Catalyst and Channel 10 Scope) and in other media.



Figure 1. (Above) Electron tomography of early endosomes Two images from a tilt series of early endosomes in a fibroblast. A network of fine filamentous material surrounds each endosome. Smaller membrane-enclosed structures, possible fusing vesicles, are embedded in the network of filaments.



Figure 2. (Right) Converting 3D electron microscopic data into a Virtual Reality experience The image shows a colour-coded representation of the cell (eg. red mitochondria, blue nucleus) generated from Serial Blockface scanning electron microscopic data. This is then converted into the VR file.

Facile synthesis of large-pore bicontinuous cubic mesoporous silica nanoparticles for intracellular gene delivery Kevin Jack

(In collaboration with Chengzhong (Michael) Yu, UQ)

Mesoporous silica nanoparticles (MSNs) have many promising applications in e.g., molecular separation, energy and catalysis and as biomedicine; however, the synthesis of MSNs with a bicontinuous cubic (Ia3d) mesostructure and large pore size has been a long-standing challenge. Through this research, we have undertaken a systematic study on the synthesis of KIT-6 type MSNs (KIT-6-MSNs) using fluorocarbon-hydrocarbon mixed surfactants as templates and ethanol as a co-solvent.

KIT-6-MSNs have a pore size of 9 nm and a particle size in the range of 200–400 nm. The role of ethanol in the synthesis of KIT-6-MSNs is crucial because ethanol increases the miscibility of

fluorocarbon and hydrocarbon surfactants, thereby increasing the packing parameter and favouring the formation of a bicontinuous structure while the fluorocarbon surfactants aid in the formation of nanosized morphology. After hydrophobic modification, the large-pore KIT-6-MSNs show a high loading capacity towards genetic molecules and successfully deliver the siRNA into a human colorectal carcinoma HCT116 cell line, knocking down the gene expression associated with cancer cell survival and proliferation.



Perturbation of the experimental phase diagram of a diblock copolymer by blending with an ionic liquid Kevin Jack

(In collaboration with Kristofer Thurecht and Idriss Blakey, UQ)

Understanding the phase behaviour of block copolymer/ionic liquid mixtures is an important step toward their implementation in commercial devices.

We have undertaken a program of research using high throughput and systematic small-angle X-ray scattering (SAXS) of the lyotropic phase behaviour of a series of polystyrene-bpoly(methyl methacrylate) (PS-b-PMMA) block copolymers in a range of ionic liquids. The ionic liquid induces disorder-toorder transitions for a number of low molecular weight block copolymers which typically would not phase separate due to their low molecular weight. This allows access to features sizes and nanoscale structures that are significantly smaller (approx. 7 nm) than could be achieved in the native self-assembly of such materials.

The onset points of these transitions are used to calculate the dependence of the effective Flory–Huggins interaction parameter (χ eff) on the ionic liquid concentration. This enabled the construction of an expanded experimental phase diagram, which reveals that after taking volumetric swelling into account, at higher ionic liquid concentrations, the experimental phase boundaries shift significantly when compared to theoretical

calculations for block copolymer melts. Our research is also able to demonstrate that the scaling of the domain spacing with ionic liquid concentration is dependent on the molecular weight for low degrees of polymerization.

These findings represent an important tool in future investigations that target specific self-assembled morphologies to suit a desired application through the development of new understanding and design rules.





Design of New Processing Protocols for Biological Samples

Richard Webb

(In collaboration with Kent McDonald at UC Berkeley)

Standard processing protocols used for preparing biological samples for viewing in an electron microscope entail fixation and dehydration procedures which induce structural artefacts. In recent years low temperature techniques have been introduced in order to prevent these issues. One of the most widely used techniques is freeze substitution, which involves leaving a fast frozen sample in an organic solvent at low temperatures for extended periods. It has always been assumed that it took several days in order for the solvent, usually acetone, to fully substitute the ice within the sample.

In a collaboration with Dr Kent McDonald from UC Berkeley, we have designed new protocols which show that the long protocols are totally unnecessary and that the process can be completed within less than three hours. In fact, we have shown that for single-celled samples, such as bacteria and tissue culture cells this process can take 90 minutes or less. We have now tried this on a very extensive range of samples and been able to prove that the new short protocols work for all biological samples. We believe that for some samples the results may actually be an improvement over the older longer procedures. We have also been able to develop this protocol further so that the full processing of a biological sample for electron microscopy, from live sample to looking at it in the microscope, can be completed within one day, compared to the past where it often took a week or more.

Single image from a 3D data set of a sponge larva taken using SBF-SEM. The sample was processed by using these new methods.

Also we have been working on a process that will allow preservation of a fluorescence signal in the sample. This is particularly important as it allows a region of a sample that has been identified by the fluorescent marker at the light microscope level to be identified again after processing so the same region can again be observed in the electron microscope. We have now used this technique to prepare samples successfully for the synchrotron and the NanoSIMS. A new development is a modification of this technique to process samples for the technique of serial blockface scanning electron microscopy which allows collection of three dimensional data about samples.





Unaffected tissue (left) and under skin browning damaged tissue (right) from 'Honey Gold' mangoes. Using iodine staining it is shown that there is an accumulation of starch in the affected tissues.

Skin Disorders of Mangoes

Richard Webb (In collaboration with Dr Daryl Joyce, DAAF)

Mango is one of the most popular tropical fruits grown in Australia. 38,500 ton of mango were produced for the domestic and export market between 2008 and 2009 (and this amount is increasing annually). There are several disorders that are of major concern to the Australian mango industry due to the resulting downgrading of fruit quality and loss of market confidence.

Skin disorders including lenticel discolouration, underskin browning and resin canal have become prevalent in recent times. Lenticel damage is recognised as a serious problem because of the adverse effect on fruit appearance and economic value. Under-skin browning is a grey-brown 'bruise'-like injury under the epidermis with no damage to the flesh and this defect is usually obvious only at marketplace. Resin canal disorder is characterised by dark grey or brown discolouration in a finely branched pattern which relates to a darkening of the underlying resin canals and vascular bundles. The general objective of the current research project is to characterise the anatomy and biochemistry of the physical and chemical properties of four Australian mango cultivars ('B74', 'Kensington Pride', 'Honey Gold', 'R2E2') in relation to these skin disorders. Through light and electron microscope studies it will be possible to gain a greater understanding of the disorders. This knowledge should contribute to the development of new or optimised postharvest treatments that consistently deliver high quality fruit to consumers.



Crayfish and their worms evolve in unison

Climate change could be affecting Australian spiny mountain crayfish and the animals that live on them, according to an international study that includes University of Queensland research.

UQ Centre for Microscopy and Microanalysis researcher Dr Kim Sewell said the study reconstructed the evolutionary history of 37 species of Euastacus crayfish and 33 species of temnocephalans, which are their flatworm passengers.

"These worms, often mistaken as leeches, have been known to science for more than 100 years," Dr Sewell said.

"Australia is now recognised as the centre of world diversity for the group."

Dr Sewell said fishers often mistook the worms for parasites that could mean the crayfish are unsuitable to eat.

"The worms are actually ectosymbionts, which means they occupy their hosts without causing harm and could even play a role in cleaning the crayfish.

Dr Sewell has studied the worms for almost 30 years, and has collected DNA tissue samples from most of the known species of Australian Euastacus spiny mountain crayfish and their temnocephalan ectosymbionts.

He said the study revealed co-evolutionary patterns and host-shifts during a period of extensive environmental change covering at least 80 million years.

"Euastacus crayfish are cool-climate specialists, and are most distinct in tropical northern Australia where they are now restricted to high mountain streams," he said.

"Protection of these vulnerable cravfish is critical for their survival and the survival of their unique symbionts, which together provide an exceptional insight into the phenomenon of co-evolution through space and time."

The study, published in the Proceedings of the Royal Society B, involved a collaboration of genetics and biology experts from the University of Cambridge and the Natural History Museum in the United Kingdom and from The University of Queensland, the Queensland Museum, James Cook University, and Latrobe University, Wodonga.

A short video showing temnocephalan worms attached to a spiny mountain crayfish (Euastacus spinifer) from Sydney can be viewed online (youtube.com/ watch?v=RlwqRGI0ITY). Credit: Jasper Montana and David Blair from James Cook University

(From CMM news)



Images: Feature image - Dr Kim Sewell with a large spiny Gippsland crayfish Euastacus kershawi in Victoria. Image by Susan Lawler, Latrobe University, Wodonga. Left Light microscope images of a typical five-tentacled temnocephalan, Temnosewellia c.f rouxi, and a pair of two-tentacled temnocephalans, Diceratocephala boschmai, from cultured redclaw crayfish. Both species are about 5mm in length. Images by Katherine Thompson and David Blair

Complex bacterium writes new evolutionary story

A University of Queensland-led international study has discovered a new type of bacterial structure which has previously only been seen in more complex cells.

Research team leader UQ School of Chemistry and Molecular Biosciences microbiologist Emeritus Professor John Fuerst said the study had found pore-like structures in a bacterium called *Gemmata obscuriglobus*. Co-authors include researchers from CMM, Richard Webb, Dr Kathryn Green and previous member of staff, Garry Morgan.

"The pore-like structures appeared embedded into the bacteria's internal membranes, and showed some structural features similar to those in more complex organisms," he said.

"This is a remarkable evolutionary finding, since most bacteria do not possess these structures".

"Finding nuclear pore-like structures in the bacterial species *Gemmata obscuriglobus* is significant for understanding how the cell nucleus and the pores embedded in its membrane envelope could have evolved - a major unsolved problem in evolutionary cell biology."

Professor Fuerst said the bacterium, which was first isolated from Maroon Dam in South-East Queensland in 1984 by UQ researchers Dr Peter Franzmann and Professor Vic Skerman, now constituted one of the most complex bacteria known.

He said the finding suggested that the evolution of complex cell structures may not be unique to eukaryotes, which are organisms containing a nucleus and other structures (organelles) encased in a membrane.

"The research finding is consistent with previous data my lab has published indicating that the *Gemmata obscuriglobus* bacterium contains a nuclear body compartment, which parallels the eukaryote nucleus."

Professor Fuerst said the discovery was important for understanding how the first complex cells may have originated.

"The results are of evolutionary significance, since the origin of eukaryotes is a major event in life's history," he said.

Professor Fuerst said nuclear pore complexes (NPCs) were important in transporting molecules between the nucleus containing the DNA and the rest of the cell contents in eukaryote organisms such as protozoa, fungi, animals and plants.

"They are dotted over the surface of the membranes separating the nucleus from the rest of the cell and enable communication between the nucleus and other parts of the cell," he said.

"Like the membrane-bounded nucleus, NPCs had been thought to be restricted to eukaryotes."

The researchers used a combination of techniques including advanced electron microscopy, a protein analysis method called proteomics, and bioinformatics genome analysis to make the discovery.

The study, published in PLOS ONE, has been supported by Australian Research Council Discovery project grants to Professor Fuerst's laboratory.

Co-authors include researchers from UQ's Centre for Microscopy and Microanalysis; CSIRO; University of Illinois; and University of Canterbury, New Zealand.

(From CMM news)





NORTH AMERICA

CANADA

The Hospital for Sick Children

USA

University of California Berkeley University of California Irvine University of California Los Angeles University of Texas Medical School (Houston) Northwestern University (Evanston) The University of Texas (Dallas) Penn State University, PA Canada

EUROPE

CZECH REPUBLIC

Institute of Scientific Instruments, Czech Academy of Sciences (Brno)

FINLAND University of Turku, Finland

GERMANY

Centre for Scientific Computing, Goethe University (Frankfurt) CEOS GmbH (Heidelberg) Ernst Ruska Centre (Julich) Max Planck Institute of Molecular Cell Biology and Genetics (Dresden) The European Molecular Biology Laboratory (Heidelberg) Saarland University (Saarbrucken)

THE NETHERLANDS

University of Technology (Delft)

NORWAY

University of Oslo

SPAIN

Centro Nacional de Biotecnologia (CNB)/CSIC University of Barcelona

SWITZERLAND

Basel Biozentrum ETH Zurich Paul Scherrer Institute (Villigen)

UK

Francis Crick Institute (London) University of Warwick



27 National collaborators

33

International collaborators across 13 countries



ASIA

CHINA

Beijing University of Technology Fudan University Hebei University of Technology Shanghai Institute of Technical Physics, Chinese Academy of Sciences Tongji University Tsinghua University Zhejiang University

INDIA

National Centre for Biological Sciences, Bangalore

JAPAN

Tohoku University

NATIONAL COLLABORATORS

ANSTO, Lucas Heights Australian National University Australian Synchrotron Baker IDI Heart & Diabetes Institute (St Kilda) Bragg Institute, Australian Nuclear and Science and Technology Organisation (ANSTO) Curtin University Deakin University **Eco Sciences Precinct** Bio21, Melbourne Queensland University of Technology Garvan Institute of Medical Research La Trobe University Monash University Murdoch Children's Research Institute **QIMR** Berghofer Medical Research Institute Queensland Department of Agriculture and Forestry Translational Research Institute University of Melbourne University of New South Wales University of Queensland (multiple Schools, Institutes and Research Centres) University of Southern Queensland University of Sydney University of Western Australia

CMM TEACHING, TRAINING AND OUTREACH















2016 HIGHLIGHTS

CMM Staff contributed to 10 undergraduate courses in 2016

108 users trained in 10 CMM training courses

416 attendees at 7 CMM workshops



Training and education are essential prerequisites to full registration for new clients using the Centre for Microscopy and Microanalysis. Few clients enter the Centre with skills at a level sufficient to undertake independent data-gathering or experimentation on the equipment available. Training is also an ongoing resource for clients who need to expand their technique base over the years of their involvement with the Centre. Formal classes are offered for scanning electron microscopy and microanalysis regularly, with dates supplied on the Centre's website.

Centre users can also access MyScope™ (http:// www.ammrf.org.au/myscope/), an on-line education site developed by CMM in collaboration with five tertiary institutions, the Australian Microscopy and Microanalysis Research Facility and the Australian Government Office for Learning and Teaching. This year MyScope expanded to MyScope Outreach, providing an education tool for school students and other interested learners.

Basic classes in SEM/microanalysis and X-ray photoelectron spectroscopy are offered every few months. This year we also offered a workshop and practical session on CyberSTEM. Training in other techniques is via one-on-one sessions.

Centre staff members also deliver lectures and run practicals for a number of undergraduate classes.

Other training highlights include an intensive two-day course to the Vacuum Society of Australia. CMM gave a JSM 6460LA SEM demonstration to the Australian and New Zealand Association for the Advancement of Science (ANZAAS) students (year 12) and conducted an SEM tour for Indigenous students. Twelve students attended this tour/workshop. And as it is never too early to encourage an interest in microscopy, CMM had two groups of the youngest students visit us from our neighbouring Kindergarten, Campus Kindy.

Centre research and academic staff members were also invited to share their expertise to an international audience, including the Light to 3D Electron Microscopy Workshop in Germany and 3View Users Forum also in Germany. Centre members also attended conferences in France, the USA and China.

Outreach activities are a core part of Centre business, impacting on wider community engagement and the profile of The University of Queensland. In 2016 CMM introduced the 'Frontiers in Microscopy and Microanalysis' seminar series, which included specialists from industry and academia, both local and international.

Another new initiative in 2016 was the 'image of the month' competition. This outreach activity has a dual purpose, promoting affiliation and recognition of CMM and providing us with a platform to share some of the amazing images produced in our facilities.

Contributions to undergraduate teaching by CMM

- □ Kevin Jack: CHEM2056 and CHEM3013
- Bronwen Cribb: BIOL3207 lecturer, ARCS2000 practical,
- Ron Rasch: ENGG7602 practical, SCIE2020 lectures and workshop/practical
- □ Kim Sewell: ARCS2000 workshop/practical, CHEM3013 practical
- Rob Parton: BIOL3006
- Barry Wood: CHEE4301

Training, education, workshop activities by month



Centre member	Title
Kevin Jack	Advanced characterisation of polymers by X-ray and neutron scattering. 17th Australian Polymer Summer School. Brisbane, February 2016
Rick Webb	3View users forum. EMBL Germany, March 2016
Rick Webb	Light to 3D electron microscopy workshop. EMBL Germany, March 2016
Roger Wepf	From 3D light to 3D electron microscopy, 3D CLEM: Past, present and future of correlative microscopy. EMBL Germany, March 2016
Roger Wepf	Past, present and future of correlative microscopy (analytics). ARC Centre of Excellence in Advanced Molecular Imaging, Monash University, June 2016
Roger Wepf	Chairman - <i>Welcome and Introduction for the session: Big Data in Microscopy</i> . European Microscopy Conference 2016. Lyon, France, August 2016
Roger Wepf	Cryo ME. European Microscopy Conference 2016. Lyon, France, August 2016
Roger Wepf	<i>Recent advances in cryomethods for skin studies.</i> European Microscopy Conference 2016. Lyon, France, August 2016
Roger Wepf	<i>Correlative microscopy and microanalysis: Towards nm Tof-SIMS imaging.</i> Imaging COE Meeting/ ARC Centre of Excellence in Advanced Molecular Imaging at IMB/UQ, August 2016
Roger Wepf	Future needs in electron microscopy and material characterisation - short presentation to Chief Scientist Alan Finkel and NCRIS Expert Working Group. UQ, September 2016
Roger Wepf	Material science characterizations with electrons, X-ray and ions: current CMM research enhancement possibilities with an outlook to next frontiers in imaging and microanalysis. School of Mechanical and Mining Engineering Seminar, UQ, October 2016
Roger Wepf	<i>Soft matter (Cryo) electron microscopy and microanalysis.</i> 36APS 2016 – Australian Polymer Symposium in Mantra Lorne (VIC), November 2016





MyScope[™] Outreach launched by AMMRF and corporate partner FEI

Developed in collaboration with science educators, research organisations, CMM and an industry partner FEI, MyScope Outreach went live in April at the USA Science & Engineering Festival in Washington DC. Students were excited to explore the world at the micro and nanometer scale with the Scanning Electron Microscope Simulator. CMM will continue to support this innovative Science, Technology, Engineering and Mathematics (STEM) resource throughout its development bringing the thrill of science and discovery to Australia's future scientists.

MyScope Outreach is a free, online tool designed to make microscopy education and outreach accessible to a global community. It specifically targets schoolage (Kindergarten to year 12) children and their associated science education programs.

"We are very pleased with the outcome of this partnership with FEI. MyScope Outreach brings the excitement and power of microscopy to young people around the world. FEI is the first company to join our MyScope[™] Corporate Partner Program and with more than 60 years of microscopy innovation and leadership, we know they share our passion and commitment to communicating the power of microscopy for discovery and solving global challenges," states Prof. Julie Cairney, CEO, AMMRF.

"MyScope is an easy-to-use platform that teaches young students about electron microscopy and gives them a glimpse of the microscopic world," said John Williams, vice president of corporate marketing, FEI. "We hope that it will help get more young students excited about entering into science, technology, engineering and math (STEM) careers."





Occupational health & safety

OHS Risk Management is a vital component of The University of Queensland's safe system of work. Managing occupational health and safety risks is an ongoing, continuously improving process. At UQ, qualitative techniques for risk assessment, to provide a structured, systematic approach to decision making with respect to the health and safety of our people.

With four laboratory facilities housed within The University of Queensland, CMM places a high priority on all aspects of OH&S. The diversity of CMM's facilities and the wide range of processes undertaken, plus our diverse client base from both internal (within the university), and external provide substantial challenges in CMM's efforts to provide and maintain a safe workplace for all.

To achieve our OH&S Goals, CMM has two dedicated OH&S staff (total 1.6 full-time staff) and an active safety committee which meets quarterly. All our staff are committed to implementing UQ's OH&S policy.

In 2016 CMM had one injury reported (slip/trip on journey to work). The continued low level injuries CMM experienced in 2016 is testament to the diligence of staff and clients.

In 2016 UQ introduced a new incident reporting and risk management database. The new database, UQSafe-Risk, requires all risk assessments to be completed in the new system from the beginning of April 2017. This system allows for the greater integration of industry reporting and management with risk management. CMM currently has 575 active risk assessments (93% approved and 50% audited). This shows the diverse range of materials analyses at CMM.

Excluding CMM staff and contractors, there were approximately 290 clients that accessed CMM facilities in 2016. 111 individuals completed 181 inductions.

ALL - UQ OHS goals were achieved 111 - Clients inducted in 2016 290 – Clients accessed CMM facilities in 2016

Image of the month

In August 2016 CMM introduced "The CMM Image of the Month Competition'. This is where CMM clients enter the competition by submitting an image that they have taken on one of our instruments. CMM awards the best image and the winner receives a prepaid coffee card.

They also have their winning image displayed on the banner of the CMM website and included in our online gallery which is open to the public. This is a great way to exhibit the images taken on CMM instruments and continue to build the connection between our staff and our clients. Here are the winning images submitted from August to December.

View our full gallery on https://www. flickr.com/photos/cmmatuq August

October

This image was taken at CMM by Mr Rohit Gaddam and Ms Xin Fan on the JSM 7001F using 15 kV with spot 6 at 10mm WD. The image represents MoS2 sheet which in the present image looks like a bird (peacock).

> 1µm 21, (7,000 15.0)



10

Image taken by Lisa Walton - It is a bis of Lorenzini of the Brownbanded bamba pup (approx 20 cm long). This is the org the environment and sends them to the part of this species' electro sensory sys JEOL JCM 5000 Neoscope.

10 kV x 150



8/03/2016 0kV SEI SEM



ected ampulla; a part of the ampullae bo shark (*Chiloscyllium punctatum*) gan that receives electric signals from brain. No published images for any tem exist to date. Taken using the

200 μm

<image>

15.0

1µm

X 4,500

2016 CMM seminar series

The Frontiers in Microscopy and Microanalysis seminar series introduces the student and staff to advances in microscopy and nanoscopy with an emphasis on light microscopy, electron microscopy and analysis as well as x-ray microscopy and analytical methods. Both methodological and technological progress as well as applications in various scientific fields are discussed.

Seminar highlights included visits from industry representatives and academics from the USA, Switzerland, Japan, China, France and The Netherlands

JULY

FIB/SEM Scios High resolution and throughput for 2D and 3D analytical characterisation.

Dr Brandon Van Leer FEI, USA

Over the past 20 years, focused ion beam (FIB) and SEM/FIB instrumentation has transformed scientists' ability to investigate materials to develop new sample preparation methods to becoming the industry standard and "work-horse" for site-specific cross-section analysis, S/TEM sample preparation (cross-section or plan view) and nanoscale patterning/prototyping applications.

JULY JULY JULY **High Resolution SEM** The Missing Link: **Thermal Probe Correlative Microscopy & 3D** Lithography SEM for Life Sciences NanoFrazer - Swisslitho AG Mr N. Yamamoto Robert Kirmse, Ruth Redman, JEOL Japan Rene Hessling, Terence Da Silva Dr Philip Paul Zeiss CTO & Founder of SwissLitho AG **NOVEMBER OCTOBER UQ Cryo-EM Symposium Protochips In Situ EM-**Systems: Integrated Adam Frost **Solutions Tailored for the** University of California, San Francisco **Core Laboratory** DOGmas, CAT tails, and a Structural Surprise: New Concepts in Protein Quality Control & Neurodegeneration Steve Shannon Qing Wang Thermo Fisher Scientific, formerly FE/ Company Protochip, USA Advancing Structural Biology with Cryo-EM **NOVEMBER NOVEMBER** DECEMBER The NanoSIMS SOL: Atomic-Level Control of Integrated correlative **Trace Element and Isotope Quantum Materials: From** microscopy - SECOM & analyses at sub-µm scale in Quantized Anomalous Hall HP-Cathodoluminescene -**Environmental.** Effect to High Tc Supercon-**SPARC Geosciences, Materials &** ductivity **Health Sciences** Dr Daan van Oosten Slingeland Delmic, Delft NL Professor Qi-Kun Xue **Dr Philippe Saliot** Tsinghua University China **Cameca France**



Centre provides practical training to CSIRO Melbourne

The CSIRO Manufacturing Group at Clayton, Melbourne Victoria recently installed a new Gatan Alto 2500 cryopreparation chamber on their ultrahigh resolution Zeiss Merlin Scanning Electron Microscope (SEM) and invited Kim Sewell to visit for 2 days to provide practical training on the new equipment. Kim presented an open seminar to the group titled "Cryogenic SEM, Freezing – fast and furious, Preserving structure".

The seminar content, like the subsequent practical component, was targeted towards assisting the group to promptly establish an efficient and effective cryo-SEM capacity. Recognition and reduction of artefacts was a particular focus of the presentation with the concomitant recommendation that the group consider the future use of high pressure freezing for suitable samples. Individual and small group practical training on the cryo-SEM covered routine and specialized sample preparation for biological and physical science samples, risk management, and imaging techniques for various liquids and soft materials. Training samples included plant leaves, cold-sore cream, diatoms, microbeads in exfoliating facial cream, as well as selected research samples from CSIRO clients.

Phillip Karallis from Thomson Scientific Instruments (TSI) generously provided expert help with equipment operation over the two days. At the conclusion of the training, Kim was delighted to pass on to the groups a large volume of video and other cryo-SEM resources he had gathered from relevant courses over the years. CSIRO felt Kim's time and expertise was invaluable and has opened a positive contact for future continued collaboration between CMM and CSIRO in this rare capability field in Australia.



Image from the CSIRO cryo-SEM of a fractured frozen component of commercial cold sore cream (Aciclovir 5% w/w).

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