

CENTRE FOR MICROSCOPY AND MICROANALYSIS THE UNIVERSITY OF QUEENSLAND

Making the invisible visible

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Director's Report

The year 2015 has continued to be a time of rejuvenation and growth for the CMM with the completion of the implementation plan following the very favourable Centre review in 2014.

It has been both a privilege and a pleasure to serve as the Acting Director of the Centre for Microscopy and Microanalysis over the past 11 months. This period falls between the retirement of Prof John Drennan in mid-2015, who over the past two decades continued to develop the CMM into a modern worldclass microscopy and microanalysis facility, and the start of Prof Roger Wepf's tenure as the next Director of the CMM in what promises to be an exciting new chapter for the Centre.

2015 continued to be a time of rejuvenation and growth for the CMM with the completion of the implementation plan following the very favourable Centre review in 2014. Some of the more highly-visible initiatives include programs to enhance the Centre's outreach activities and better showcase its capabilities and achievements.

The collective achievements of the staff of the CMM were recognised in the Vice Chancellors' 2015 UQ Awards for Excellence. The entire CMM team received a commendation for their Excellence in Service. This commendation was a strong recognition of their dedication in providing high-quality microscopy and microanalysis facilities and training.

It is also very pleasing to report that continued strategic investment has been secured, allowing the centre to upgrade key instrumentation and thus remain at the forefront of research capabilities. Much needed funding was provided by UQ to reinvigorate the entry level transmission electron microscopy instruments in the CMM, providing more effective access to research tools for life and materials sciences, as well as enhancing our ability to provide effective research training to students and researchers. Following on from our previous success in national competitive funding schemes we saw the final commissioning of the thin-film and micro X-ray diffraction instrument as well as two state-of-the art electron imaging cameras, all of which have greatly enhanced the capabilities of the Centre.

Moreover, this past year saw a greater degree of certainty on the national scene with the announcement of both continued investment and a long-term strategy for NCRIS funding. Such news is welcome to both our Centre and the national network - Australian Microscopy and Microanalysis Research Facility (AMMRF) – for which the CMM is the QLD node. In addition to the succession of the Director, 2015 has also seen some changes to other key staff within the centre. We welcomed a new Client Liaison Officer, Ms Jenny Brown, following Kay Hodge's promotion to a new challenge within the School of Chemical Engineering at UQ. Dr Matthias Floetenmeyer has been appointed as the manager of the Cryo-EM facility and flagship engineer for the F30 cryo-TEM instrument following the return of Mr Garry Morgan to his alma mater in Boulder, Colorado.

The provision of high quality teaching and training has remained at the forefront of the CMM throughout this year, as in previous years. The entire CMM team continues to work hard in developing and delivering training in advanced characterisation theories and practice. One specific program worth noting is CMM's continued involvement in developing materials for the online training tool - MyScopeTM - in collaboration with the AMMRF. The project is receiving increasing attention, both nationally and internationally, and is also attracting corporate interest. The CMM played a large role in both the inspiration and ongoing development of these tools and it is pleasing to see them grow.

Finally, I truly must thank the CMM team for their dedication and continual and determined hard work throughout the year and for their support during the time of transition.

Associate Professor Kevin Jack Acting Director



ABOUT CMM

The Centre for Microscopy and Microanalysis (CMM) is an interdisciplinary research, training 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. 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.

CMM is also a partner to industry and offers consultation, testing services, access to instruments, contract research and long-term research collaborations tailored to the needs of business.

The CMM is a foundation member and the Queensland Node of the Australian Microscopy and Microanalysis Research Facility (AMMRF). Under AMMRF, major microscopy facilities (Nodes) and linked laboratories unite to form an extensive network. As part of the AMMRF, we can offer our clients access to online teaching and learning resources such as TechFiTM, MyScopeTM and CVL.

FINANCE

2015 OPERATIONAL FUNDING

REVENUE	
FEES AND CHARGES	887,370
OTHER INCOME	4,358
DEFINED CENTRAL FUNDING SCHEME	1,531,406
OPERATING LEVEL ALLOCATIONS	-332,666
EXECUTIVE LEVEL ALLOCATIONS	1,427,798
TOTAL REVENUE	3,518,266
EXPENDITURE	
ACADEMIC SALARIES	750,683
ACADEMIC SALARIES	750,683
GENERAL SALARIES	1,172,141
OTHER EMPLOYMENT COSTS	14,418
GENERAL OPERATING EXPENSES	195,981
PROFESSIONAL & OTHER SERVICES	8,603
EQUIPMENT & MINOR WORKS	1,062,715
TRAVEL	42,632
HOSPITALITY	1,379
OTHER EXPENSES	3,695
TOTAL EXPENDITURE	3,252,247

Operating Result

266 019

REVENUE RESEARCH INCOME 407,835 INVESTMENT 2,124 OTHER INCOME 5,033 DEFINED CENTRAL FUNDING SCHEME 0 OPERATING LEVEL ALLOCATIONS 10,666 EXECUTIVE LEVEL ALLOCATIONS TOTAL REVENUE 425,658 EXPENDITURE ACADEMIC SALARIES 38,597 GENERAL SALARIES 212,879 OTHER EMPLOYMENT COSTS 718 GENERAL OPERATING EXPENSES 3,598 **PROFESSIONAL & OTHER SERVICES** 0 EQUIPMENT & MINOR WORKS 180,490 TRAVEL 16,701 HOSPITALITY 2,676 OTHER EXPENSES 37 TOTAL EXPENDITURE 455,696 **Operating Result**

2015 RESTRICTED FUNDING

INSTRUMENTATION

Transmission Electron Microscopes

12 Scanning Electron Microscopes

4 X-ray Analytical Tools

2 Nano Fabrication Units

28 Sampling Preparation Items

\$22 Million Infrastructure across four laboratories

AIBN LABORATORY

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

The 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 two 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

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 the 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 equiped with 325nm, 514nm and 785nm wavelengths plus stage mapping and confocal depth profiling

X-RAY FACILITY Manager: A/Prof Kevin Jack (k.jack@ug.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

2D silicon drift detector

Rigaku SmartLab thin-film and micro-diffraction XRD High-intensity rotating anode source 5 axis goniometer Parallel, focused and Bragg-Brentano collimation Anton Parr SAXSess SAXS Capillary, gel and powder cells Princeton CCD detector

Kratos Axis Ultra XPS

Angular-resolved and 2D mapping modes Argon-ion bombardment

Anton-Paar heating stage and Oxford cryostream

INSTRUMENTATION

QBP LABORATORY Manager: Mr Garry Morgan (cmm@uq.edu.au)

Located in the Queensland Bioscience Precinct, the Cryo-Transmission Electron Microscopy (TEM) Facility is a laboratory that was purposebuilt for standard and cryo-TEM sample preparation and analysis, as well as electron tomography of both resin-embedded and cryo-samples (for three-dimensional 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-tiltrotate holder, Gatan cryo-α-tilt-rotate tomography holder FEI/Gatan α/β-tilt holder, Gatan high α-tilt cryotransfer system holder

OCCUPATIONAL HEALTH & SAFETY

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ALL UQ OH&S goals were achieved

176 Laboratory workers inducted in 2015

275 Laboratory workers accessed CMM facilities in 2015

116 Active risk assessments from CMM staff group

2015 Report

Excluding contractors, there were 300 workers (WHS Act 2011) who used Centre facilities in 2015, 297 of which were lab workers - reflecting the strong laboratory focus of the CMM.

Workplace Health and Safety is a high priority in all aspects of CMM operations. Our diverse client base, wide range of processes, and multiple sites all present a substantial challenge to our efforts to provide and maintain a safe workplace for all. To achieve this goal, the CMM has two dedicated OH&S staff (total 1.6 full-time staff), an active safety committee which meets quarterly, and staff committed to implementing UQ OH&S policy and reaching the Centre's goals.

In CMM facilities, three incidents were reported, with one resulting in injury. This is similar to previous years. The injury event (laceration) occurred to a contractor engaged in decommissioning of CMM equipment and was not the result of 'standard' CMM activities. The non-injury incidents (falling light fitting, pest infestation) related to infrastructural issues in one of our oldest facilities – the Hawken Engineering facility. These issues have been corrected and it is hoped that funding for a refurbishment will be approved to eliminate similar potential incidents.

Reviews of incidents have resulted in refined operational procedures, intended to reduce this low injury/incident rate even further in future years.

Excluding contractors, there were 300 workers (WHS Act 2011) who used Centre facilities in 2015, 297 of which were lab

workers - reflecting the strong laboratory focus of the CMM. 176 full laboratory safety inductions were conducted for clients/staff. This is a marked increase over the previous year (107 in 2014). An additional 31 after-hours lab inductions were successfully completed in 2015, reflecting high instrument usage and the demand for 24 hour use of the Centre.

Improvements achieved in 2015 include the increased completion rate of local site inductions for contractors (12 new inductions) and the documentation of completion of online modules for all workers.

As in previous years, there was a high number/complexity of risk assessments for new samples/chemicals, indicating the broad range of materials/techniques clients were employing at the Centre. (There were 626 active risk assessments on the UQ database - not authored by CMM staff - which list CMM as the workplace.) Individual assistance was provided to clients and staff in the development of risk assessments for samples and chemicals or processes introduced into the Centre.

As part of the University, the Centre applied the University's current OH&S Policy and strived to achieve the associated goals. (The goals and CMM's performance indicators for 2015 are in Table 1 on the next page.) We maintained a high standard in 2015 and aim to maintain these standards in future years.





EDUCATION & OUTREACH

CMM Staff Involved in nine undergraduate courses in 2015

175 Users trained one-on-one in 2015

100 Users trained in courses in 2015

Training & Education

Training is an ongoing resource for clients who need to expand their technique base over the years of their involvement with the Centre.

Training and education are an integral part of the registration process for new clients using the Centre for Microscopy & 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 every few months, with dates supplied on the Centre's website.

Sessions are augmented by access to MyScopeTM (http://www. ammrf.org.au/myscope/), an on-line education site developed by the Centre in collaboration with five tertiary institutions, the Australian Microscopy & Microanalysis Research Facility and the Australian Government Office for Learning and Teaching. A workshop on X-ray photoelectron spectroscopy is also offered every few months. Training in other techniques is via ad hoc oneon-one sessions. As such, Centre staff also supply training for transmission electron microscopy, light microscopy (excluding confocal), X-ray diffraction, electron backscatter diffraction, electron-beam lithography, focussed ion-beam microscopy, cryo-SEM and TEM, tomography, and sample preparation including chemical fixation, high pressure freezing and freezesubstitution, critical point drying, ultramicrotomy, and sample coating.

Centre staff members also deliver lectures and practicals into undergraduate classes. Subject codes include: ARCS2000, BIOL3006, BIOL3207, CHEM2056, CHEM3013, CHEE4301 and ERTH 3003, ENGG7602, PHYS3900, SCIE2020. For large classes the Centre is able to bring the lab to the classroom through the use of an audio and video linkup to show equipment, lab space and real-time data acquisition. For example, in August 2015 the labclassroom link was used for a workshop for students in CSI UQ: Introduction to Forensic Science (SCIE2020). Here Centre staff undertook imaging and real-time analysis of four famous paintings from the 1400s, 1900s, 1960s and 1980s. Samples of paint from the art works were supplied to us on loan from the Queensland Art Gallery/ Gallery of Modern Art, Centre for Contemporary Art Conservation (CCAC). In addition to benefiting the educational aims of the course and engaging student's interest, the data gathered were previously unknown to CCAC so supported their ongoing scientific and conservation activities.

Centre research and academic staff members have particular areas of expertise and therefore supervise research higher degree candidates. Supervision is facilitated by links with Schools outside the Centre and collaborations with UQ faculty. In addition Centre staff members help to arrange workshops and give talks and presentations in areas of their expertise (listed on the following page). The Centre also hosted industry visitors from China, Korea, Finland, Germany and Canada throughout the year.

Outreach activities are a core part of Centre business, impacting on wider community engagement and the profile of The University of Queensland. Workshops allow prospective students to see the resources available when they undertake degrees at UQ, and showcase current research. Outreach activities in 2015 included two workshops for the National Youth Science Forum, and one for the three-day InspireU Indigenous Science Camp for high school students held at The University of Queensland in July.



Photo of workshop on scanning electron microscopy for the InspireU Science camp, courtesy of Andrew Dillon, Faculty Outreach and Engagement Program Coordinator, UQ.

Developing the MyScope TM Outreach tool

MyScope TM is an online site for training in advanced research tools, developed through a government grant from the Office of Learning and Teaching (led by Bronwen Cribb) and significant support from the Australian Microscopy & Microanalysis Research Facility (AMMRF).

With an average of 15,000 users per month, and including scanning electronmicroscopy, transmission electronmicroscopy, scanning probe microscopy/atomic force microscopy, light microscopy, specifically confocal microscopy, microanalysis, and X-ray diffraction, in 2015 MyScope moved into the next phase. Overseen by an AMMRF teaching committee, MyScope engages corporate partnerships aimed at new developments and sustainability of the site. A venture has been formalised with FEI to develop a MyScope outreach tool. The aim is to make microscopy education accessible to the global community, in a format that encompasses a broad range in age, educational background, and socio-economic background. Supporting the need for engagement with STEM (Science, Technology, Engineering and Maths), it targets school-age (8yrs upwards) children and their science education.

The outreach tool incorporates a simulated SEM as well as a pathway to explore magnification and microscopy. Bronwen Cribb has worked with AMMRF staff to develop the site concept and has provided the range of images for the simulated SEM. The site is due to be launched in April 2016.

MyScope publications and presentations:

M Apperley, J Whiting, B Cribb, A Ceguerra, 2015, The Use of Online Tools in Microscopy and Microanalysis Core Facilities, Microscopy & Microanalysis Meeting, August 2015, Portland, OR, USA.

B Cribb et al, 2013, MyScope: a national approach to education in advanced microscopic characterization through integrated learning tools, Office for Learning and Teaching, Australian Government Department of Education, ISBN: 978-1-925092-03-5



Human head louse

Education & Outreach

Workshops and presentations for 2015

Монтн	PROFESSIONAL ACTIVITIES
February	 Oral presentation: 13th Biennial Australian Microbeam Analysis Symposium, AMASXIII, University of Tasmania, Hobart, Australia Organiser and teacher in Correlative Light Electron Microscopy Workshop associated with 2015 Microscopy New Zealand Conference Invited Oral Presentation: Probing and controlling nanoscale structure in polymers and thin films, at the 3rd Australian Angular Scattering Workshop, Geelong, Victoria, Australia
March	 FEI Correlative Microscopy and CLEM demonstration Invited talk: Symposium of 2D Nanomaterials. 2D layer-structured metal chalcogenides
May	 Workshop: New Methods for Freeze Substitution; Serial Blockface Scanning Electron Microscopy (SBFSEM) Workshop: CMM/AMMRF laboratory workshop on electron microscopy techniques Oral presentation at Adelaide Microscopy: Serial Blockface SEM: Does thi mean the end of TEM as we know it? Invited Talk in Array Tomography Workshop, Centre for Advanced Microscopy, ANU, Canberra.
June	 Workshop: Invited teacher in MRI Bio-Cryo Course for Cellular EM. University of Utah, Salt Lake City, UT, USA Invited talk at the 8th International Conference on Materials for Advanced Technologies of the Materials Research Society of Singapore on Open Education Resources in science and the MyScopeTM experience, Singapore Keynote talk: 3rd International Workshop on Nanostructured Materials - Properties and Characteristics. <i>Ternary III-V nanowires</i>
July	» JEOL EPMA and soft X-ray seminar at UQ
August	 Microscopy & Microanalysis meeting Portland, Oregon, USA Workshop: Cryo-preparation for Biological EM; Workshop: Specimen Preparation for Biological Microscopy Workshop: MyScopeTM
September	» EDAX X-ray Analysis seminar at QUT.
October	» Keynote talk: 16th Beijing Conference and Exhibition on Instrumental Analysis. <i>Defect-free III-V nanowires</i>
November	» Discussion session at School of Biological Sciences: high-impact teaching approaches

RESEARCH



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- » Growing gold nuggets
- » Natural fibres
- » Milk powder enhanicing probiotics
- » Molecular cell biology
- » Growth of epitaxial III-V semiconductor nanowires
- » Development of thermoelectric nanostructures
- » Biomaterials for bone healing
- » Polymer nanostructures, heterogeneity and advanced polymeric materials
- » Design of new processing protocols for biological samples
- » Growth of high-quality epitaxial InAs nanowires

'Growing' gold nuggets

This research highlighted the contribution of bacteria to the formation of gold grains and nuggets and was recently published in *Geology (Jeremiah Shuster)*

The wealth generated by the Australian mineral resource industry makes a significant contribution to the nation's socioeconomic strength. With respect to gold, Australia is within the top five largest gold-producing countries in the world and generates export revenue of approximately \$14 billion dollars per annum. However, innovation in mineral exploration is urgently needed to aid in supplying the increasing global demand for natural resources, such as gold. The research of Prof Gordon Southam and Dr Jeremiah Shuster in The Vale-UQ Geomicrobiology Laboratory, University of Queensland (UQ), focuses on the fundamental understanding of how bacteria control the distribution of gold in the natural environment.

The first millimetre-scale gold grains were 'grown' in the laboratory using an experimental bioreactor that simulated the environmental conditions of a placer deposit. This research highlighted the contribution of bacteria to the formation of gold grains and nuggets and was recently published in *Geology* (Shuster and Southam, 2015). In this study, bacteria and exopolymeric substances transformed soluble gold into nanophase particles, i.e., nanometre-size colloids and crystalline octahedral gold. Biofilms produced in the bioreactor mediated the aggregation of these nanophase particles by acting like a glue, ultimately consolidating the millimetre-scale grains. These findings confirmed the importance of organic cycling in the biogeochemistry of gold and supported the 'radical' field

observations of Friese (1931). Through collaboration with Dr Frank Reith from the University of Adelaide, we have developed field-scale experimental bioreactors to better understand the dispersion of gold in the Australian regolith and how microbial weathering of gold-bearing materials contributes to the occurrence of gold found in natural sediments. Research in the geomicrobiology of gold involves both laboratorybased experiments that we can test specific biogeochemical processes and analysing natural gold grains recovered from complex natural systems, e.g., river sediment. To understand the biogeochemical cycling of gold, the structure and chemistry of gold grains are characterised using a combination of scanning and transmission electron microscopy and qualitative elemental spectroscopy in the QLD AMMRF node at CMM UQ. These highresolution techniques reveal the relationship between bacteria and colloidal gold particles, which are key to understanding the dispersion of gold in nature.

Industrial innovation is often facilitated by step-changes in our fundamental understanding of scientific systems. The advanced multi-disciplinary understanding of the biogeochemistry of gold is creating important opportunities to harbour the use of biotechnology in mineral exploration to keep Australia at the forefront of mining innovation. By understanding the contribution of bacteria to the biogeochemical cycling of gold, novel strategies can be developed to target undiscovered gold in Australia.



Natural fibres

Natural fibres are an attractive alternative to synthetic fibres when used as the reinforcing phase in polymer composite materials. Natural fibres such as hemp and flax offer a good balance between stiffness and toughness, while being both light and environmentally friendly due to their low carbon footprint.

In natural fibre composites, the quality of the final product is often dictated by the interfacial adhesion of the fibre to the matrix. This adhesion is very sensitive to the organic molecules that occur naturally on the fibre surface. Investigating these fibre surfaces is very problematic as most techniques, which are surface sensitive to organic molecules, analyse multiple fibres simultaneously. This gives the average surface composition, rather than microstructural information required for material development.

Traditional SEM is limited to either secondary electron (SE) images that produce good surface topography, but little chemical information, or backscatter electron (BSE) imaging, which is generally a high voltage technique applied to inorganic materials. Both these imaging modes provide minimal material contrast from thin carbon based organic materials on the surfaces of natural fibres.

To address these and other limits of the traditional scanning electron microscope (SEM) techniques, a new SEM technique was developed that utilised the latest generation of in-lens electron detectors. To enable organic insulators to be imaged in their unaltered state without coating, natural fibre specimens were imaged in the SEM at the E2 charge balance point. Low voltage backscattered images that provided useful material contrast of the organic species on the natural fibre surface were obtained using a new 'on-axis' style in-lens detector that filtered out the inelastically scattered electrons with an electrostatic filter grid.

This novel energy filtered in-lens SEM technique has subsequently proven to be very successful in the imaging and analysis of natural fibres. It has led to two publications in the *Journal of Applied Polymer Science*, each with a journal cover image (see *CMM Highlights* in this report).

Milk powder enhancing probiotic performance

Claire Gaiani

This research was conducted as part of a competitive European grant (Marie Curie Action: International Outgoing Fellowships for Career Development

Dr Claire Gaiani from the University of Lorraine (LIBio, France) was a visiting academic at the SAFS (School of Agriculture and Food Science), University of Queensland, with her travel funded under a competitive European grant (Marie Curie Action: International Outgoing Fellowships for Career Development). Dr Gaiani's project is focused on the use of milk powder to enhance probiotic performance. This required an in-depth study of interactions between milk components and probiotics bacteria, based on the development of new methodologies to characterise the bacterial location within the structure.

With the help of the CMM staff and their Field Emission SEMs. Dr Gaiani studied the use of probiotics in infant formula, and was able to identify milk matrices efficiently protecting the bacteria (right images). With classical milk matrices (left images), probiotics were mostly located at the surface and not in the core of the particle, meaning that bacteria are not protected during food processing, shelf-life and gastric transit. With optimised formulations and processes, bacteria are found correctly encapsulated in the matrix and not visible at the surface allowing the release of viable bacteria in the intestine.



Molecular cell biology of the cell surface

Our fundamental cell biology aimed at understanding this enigmatic organelle has led us into areas of immense biomedical interest, including muscular dystrophy, liver regeneration, lipodystrophy (abnormal fat tissue), and cancer.

The cell surface, or plasma membrane, forms a crucial interface 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. Our research proceeds from detailed studies of the molecular mechanisms of microdomain formation in model systems (in vitro reconstitution, formation of caveolae in bacteria) to in vivo studies in fish and mammals.

Our work has particularly focussed on caveolae, abundant features of the plasma membrane of vertebrate cells. Our fundamental cell biology aimed at understanding this enigmatic organelle has led us into areas of immense biomedical interest, including muscular dystrophy, liver regeneration, lipodystrophy (abnormal fat tissue), and cancer. Despite their discovery in the middle of the last century, the cellular roles of these surface pits have only gradually been revealed. Until the 1990s, only electron microscopy could be used to study caveolae but our discovery of caveolins, the major membrane proteins of caveolae, subsequently allowed caveolar function to be dissected using molecular techniques. Recently, we also identified and characterised a new family of cytoplasmic proteins, termed cavins, which have a key role in caveolar dynamics and function. It is now clear that a mechanistic understanding of both caveolins (-1, -2 and -3) and cavins (-1, -2, -3 and -4) is crucial to understanding caveolar structure and function. With mutations in both caveolins and cavins identified in patients, caveolar dysfunction has now been linked to a wide range of human disease conditions including lipodystrophy, muscular dystrophies, cardiac disease, infection, osteoporosis, and cancer.

Electron microscopy is a crucial technique for our cell biological studies of caveolae. We are increasingly using Serial Blockface electron microscopy (Gatan 3View/Zeiss Sigma SEM, with Rick Webb and Robyn Webb) to gain whole cell and entire tissue ultrastructural data. We have now progressed to the study of caveolar function in whole zebrafish embryos by generating high resolution electron microscopic data through the entire depth of the embryo. To complement these 3D electron microscopic approaches we developed a new technique for detection of proteins in cells and in organisms which utilises a peroxidase enzyme, called Apex, attached to a small protein that binds to any green fluorescent protein (GFP)-tagged target (Ariotti et al, Developmental Cell 2015). This method allows rapid high-

throughput electron microscopic localization of entire families of proteins for the first time.

Finally, we have extended our studies aimed at providing a full 3D electron microscopic analysis of cancer cells. 3D datasets obtained by Serial Blockface electron microscopy have been converted into a real-time virtual reality experience (in collaboration with Angus Johnston, Monash University, and John McGhee, UNSW) allowing the user to 'move' through the cell and explore its many features. These approaches are providing a completely new user experience and will hopefully prove a very powerful educational and research tool.



Figure 1. Caveolae in a fibroblast detected using Apex. Note the electron dense staining associated with caveolae at the cell surface (arrows) as shown at higher magnification in the inset.

Growth of epitaxial III-V semiconductor nanowires

This research is part of a collaboration with Professors C. Jagadish at ANU and Wei Lu at Shanghai Institute of Technical Physics.



One-dimensional nanowires made of III– V compound semiconductors have attracted significant attention in the recent decade due to their distinct physical and chemical properties that can potentially lead to a wide range of applications in the nanoelectronics and optoelectronics. Therefore, understanding the growth of epitaxial III-V semiconductor nanowires is essential for the development of future nanowire-based devices. In this multifacet collaborative program, III-V semiconductor nanowires were epitaxially grown along different orientations and their structural and compositional characteristics are investigated.

Development of thermoelectric nanostructures (Jin Zou)



Thermoelectric nanostructures, which can realise the direct conversion from thermal energy to electricity, provide opportunities to harvest useful electricity from waste heat or sunlight. Therefore, thermoelectric energy conversion is considered a promising technique to alleviate global energy demand. In this program, we employed cost-effective techniques, including conventional solvothermal, microwave assisted solvothermal and chemical vapor deposition, to synthesize various chalcogenide-containing semiconductor nanostructures and their thermoelectric performances are evaluated using our newly installed facilities.

Biomaterials for bone healing

In these studies a range of methods including surface spectroscopy, diffraction, imaging and performance testing are used to develop an understanding of the structure-property-performance relations in these and composite materials in general



Mineral deposition after immersion of a composite scaffold in simulated body fluid

Bone is a natural composite comprising of a matrix of collagen fibres and nanometre sized hydroxyapatite (HAP) crystals. The use of synthetic HAP nanoparticles is of much scientific interest in artificial bone replacement materials due to their similarity to bone minerals in composition, size and crystallinity. In developing synthetic bone replacement composites some key aspects to obtaining the desired compressive and tensile properties are a high degree of dispersion of the particles in a polymer matrix or scaffold and strong adhesion between the phases of the composite. In order to enhance this, the chemical nature of the HAP surface can be modified to increase both the adhesion between the inorganic and organic phases to reduce inter-particle attractive forces.

A number of approaches are currently being investigated and these include an understating of the growth of HAP in situ to the modification of synthetic HAP by salinization of the surface of the particles and reactive coupling of selected organic moieties. For example we are preparing functionally modified HAP surfaces by covalently attaching heparin, a glycosaminoglycan which is known to interact favourably with proteins and to promote bone healing. The addition of a stable heparin surface layer can promote the dispersion of HAP particles in solutions necessary for the fabrication of polymer scaffolds and also can be used to modulate the release profile of BMP-2 to ensure a more longlived and favourable profile. The effect of these modifications on the dispersion and presentation of the filler in porous PCL scaffolds and their overall effects on the mechanical properties have also been investigated. In these studies a range of methods including surface spectroscopy, diffraction, imaging and performance testing are used to develop an understanding of the structure-property-performance relations in these and composite materials in general.



XPS and TEM images of functionalised HAP particles

Polymer nanostructures, heterogeneity and advanced polymeric materials

The development of polymeric and hybrid materials which can be used in the production of high-density data storage devices, processors and chemical sensors as well as understating structure-property-performance relationships in these materials are key elements of my research programs. These systems include polymeric materials with high-sensitivities to photon degradation, photo-switchable chemistries that can change their nano-structure upon irradiation with light and advanced polymer architectures which can heal defects in photolithography or which are being used to model and understand the role of chemical heterogeneity in photoresists performance. In all of the projects the nano-scale structure of the materials and their chemical, surface and performance properties are characterised using a range of advanced characterisation methods including small-angle X-ray scattering, grazing incidence SAXS, electron microscopy, X-ray photo electron spectroscopy, NMR spectroscopy, dynamic mechanical analysis and dielectric spectroscopy.



¹³C NMR of a block copolymer used in understanding chemical heterogeneity in photoresists

Design of new processing protocols for biological samples

This research is part of a collaboration with Kent McDonald from UC Berkeley, USA.

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 singlecelled 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. 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.



Left: Single image from a 3D data set of a sponge larva taken using SBF-SEM. The sample was processed by using these new methods. Right: 3D data set of cultured insect cells infected with baculovirus, also processed suing these methods.

Growth of high-quality epitaxial InAs nanowires

Zhi Zhang

This work is part of Zhi Zhang's PhD thesis under the supervision of Professor Jin Zou and collaborating with Professors Wei Lu and Pingping Chen at Shanghai Institute of Technical Physics.

InAs nanowires have attracted special research interest due to their distinct properties such as very high electron mobility and narrow bandgap, which have made them a promising candidate for applications in nanoelectronics such as high-performance transistors. However, in order to achieve these superior properties of InAs nanowires for practical applications, it is critical to control their crystal structures and structural quality. In this project, through detailed morphological, structural, and compositional characterisation using a wide range of electron microscopic techniques, coupled with designated nanowire growth, we have been able to grow defect-free InAs nanowires with different crystal structures (namely zinc-blende or wurtzite) and/or different growth directions with altered side facets. This study led to a 2015 UQ Graduate School's Dean Award.







Model to control the growth of InAs nanowires with different growth directions with different structures, in which ZB=zinc-blende, WZ=wurtzite, V/III=the ratio of As and In the vapour during the growth.

HIGHLIGHTS



Research collaboration with National Institute for Materials Science (NIMS), Center for Green Research on Energy and Environmental Materials

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Fuel cells are a clean and efficient power source for generating electricity using hydrogen and oxygen. To develop the high quality fuel cell device adequate for practical use, the design of the interface between active metal and support material at the atomic scale on the basis of microanalysis is an important challenge. The group of Dr Mori (NIMS, Tsukuba, Japan) has frequently visited at

CMM at UQ to use their facilities and develop an international collaboration.

IN 2015, DR TOSHIYUKI MORI VISITED CMM TO WORK ON THE FOLLOWING TWO JOINT-PROJECTS:

1. Microanalysis of CeOx nanowire loaded with low concentration Pt for development of polymer electrolyte membrane fuel cells

Polymer electrolyte membrane fuel cells (PEMFCs) show high power density below 80°C with both vehicle application and residential scale application being actively developed in Japan. To develop the high quality PEMFCs, the concentration of Pt of the cathode in PEMFCs has to be minimised. For this challenge, Dr Toshiyuki Mori and his postdoctoral research Dr Shipra Chauhan attempted to design the surface and interface of low concentration Pt loaded CeOx nanowire/C cathodes which show better oxygen reduction reaction activity than conventional Pt/C cathodes. To conclude the key nano-structural features of their electro-catalysts, Dr Mori brought the low concentration Pt loaded CeOx nanowire/C electrode and Pt loaded CeOx nanoparticle/C electrode to CMM and observed their microstructures with Dr

Graeme Auchterlonie who has long collaboration history with Dr Mori.

Figure 1(A) and 1(B) show the selected area electron diffraction patterns, high resolution and low magnification TEM images taken from 5wt% Pt loaded CeOx nanowire/C and 5wt% Pt loaded CeOx nanoparticle/C, respectively. To activate the surface of electro-catalysts, electrochemical conditioning process is required. Since Dr Mori assumed that the active surface is created during the electrochemical conditioning process, he observed the electrochemically treated samples at CMM. Figure1(A) clearly indicates that the electrochemically pretreated Pt loaded CeOx nanowire/C consists of well-crystalline CeOx and nano-sized Pt particles. In contrast, the electrochemically pretreated of low crystallinity CeOx and small Pt particles, as shown in Figure1(B).

On the basis of these interesting data, Dr Mori and Dr Shipra Chauhan successfully fixed the best conditioning process and maximize the cathode performance observed for low concentration Pt loaded CeOx nanowire/C. This work has been published in the *ACS* journal.

2. Characterisation of microstructure of high functional brownmillerite compound for solid oxide fuel cell application

The high functional brownmillerite compound Ca2(Al,Mn)O5 has attractive much attention for application of fuel cell technology because of its high oxygen storage property. To maximise its oxygen storage property, the design of defect structure of aforementioned brownmillerite compound is key issue.



Figure 1 High resolution TEM image (i), low magnification TEM image (ii) and SAED pattern (iii) taken from 5wt% Pt loaded CeOx nanowire/C (A, left) and 5wt% Pt loaded CeOx nanoparticle/C (B, right).

Overseas Visitors & Collaborators

Dr Mori is working with Professor Teruki Motohashi (Professor of Kanagawa University) to develop this material in Japan. To conclude the relationship between chemical property and defect structural feature of $Ca_2(AI, Mn)O_5$, Dr Mori observed its microstructural features with Graeme using EELS analysis technique. The defect structure of $Ca_2(AI, Mn)O_5$ was observed at NIMS in advance. In the pre-observation of the aforementioned compound at NIMS, the stacking faults were clearly observed by TEM observation (Figure 2(A)). However, it was hard to understand why the stacking faults were appeared in that material. The EELS analysis at CMM clearly indicated that the electrical structure around dopant AI element shows unusual situation as compared to the conventional brownmillerite

compound which consists of same space group.

Although it is well known that the EELS analysis of Al element in oxide is quite difficult because of low intensity of EELS signal of Al in oxide.

As demonstrated in Figure 2(B), the unique two different EELS peaks of AI were clearly observed by EELS at CMM. This indicates that heterogeneous electrical structure around AI exists in the brownmillerite compound and aforementioned two dimensional defect structure (i.e. stacking fault) is formed by this heterogeneity at nano-scale.

It is expected that the chemical property of brownmillerite compound will be maximized by control of formation of stacking fault in the brownmillerite compound in the near future on the basis of this microanalysis result.



Dr Shelly Arreguin, EAPSI Fellow

In June 2015, Shelly Arreguin, from the University of Washington, was hosted at the Centre for Microscopy and Microanalysis (CMM) as a participant in the East Asia and Pacific Summer Institutes (EAPSI) program at the University of Queensland. The Australian EAPSI program was highly competitive with 30 EAPSI Fellows selected for placement in 2015. The program was co-sponsored by the U.S. National Science Foundation (NSF) and the Australian Academy of Science (AAS) and was held for 8 weeks from 23 June to late August 2015.

During her time at CMM Shelly's main interested was the defect analysis of irradiated silicon carbide (SiC), particularly the grain boundary structure of Boron Carbides (added for strength) incorporated into SiC. Shelley's work at CMM, UQ primarily involved HRTEM, SAED, and STEM/HAADF and the following TEM micrograph shows the complexity within her irradiated structures.



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37 International collaborators across 13 countries





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Applied Polym

CMM on the cover

Images resulting from CMM research have been featured on the cover of the prestigious Wiley publications, *Journal of Applied Polymer Science* and *Traffic.*

Applied Polymer science



Q&A

Meet the new Director Professor Roger Wepf


Professor Wepf, an eminent electron microscopist with an impressive track record of leadership in the European microscopy community, has been appointed the new Director of CMM. Professor Wepf succeeds Emeritus Professor John Drennan, who retired last year.

Professor Roger Wepf, who is the current President of the European Microscopy Society (EMS), joined in may 2016 from the Scientific Centre for Optical and Electron Microscopy (ScopeM) at ETH Zurich, where he was Technical Director.

His scientific background is broad and includes cell biology, biophysics and materials science. His research interests are focused on developing advanced methods for imaging, sample preparation and correlative microscopies.

What do you see as the most exciting opportunities and/or challenges for CMM in the next 12 months?

One of the biggest opportunities for the Centre is atomic *insitu* investigation, which we can dive into by acquiring a stateof-the-art corrected TEM/STEM system. I believe we can also explore new nano fabrication pathways with the procurement of a dedicated Electron Beam Lithography system. We are very fortunate that both instruments have been financed by UQ's DVCR with support from the faculty of Science. We can therefore acquire these instruments relatively soon.

These additions to CMM's capacity will boost chemical, physical, bio-nanomaterial and classical material science. We can be at the forefront in nanostructure design and characterisation at the atomic scale.

A further opportunity lies in CMM's extended collaboration with other UQ platforms, facilitating team networking and therefore effectiveness.

As the new Director of CMM, what will be your key focus areas for 2016/2017?

Establishing an environment including refurbishing existing laboratories to host the new multi-million dollar equipment investments is a key priority. I would also like to enhance the networking capacity within the CMM team by taking advantage of integrative technologies and peer-to-peer training and team exchange opportunities.

Also, I will establish a small instrument and methods development group to close existing gaps where suppliers are either not able or not willing to close, for example, inert gas and cryo connectivities between analytical tools, to be able to characterise very sensitive materials such as lithium base batteries, catalysts, nanomachines and complex bio-organic systems. Regarding your belief that UQ, with the appropriate support, can be at the forefront of connecting excellent imaging tools that exist around the world, what sort of support does the CMM need and where will it come from?

To help bring CMM at the forefront of micro/nanoscopy and analytics we need two large moves here at UQ. Firstly we need to upgrade the old cryo-EM flagship facility to a world leading and national Australian platform for molecular and cellular structural biology. To achieve this we will need co-investment from faculties, institutes, government and even philanthropists to make the invisible nano-machinery, on which nature is based and all life functions, visible.

Secondly, to cope with the future needs of nano and subnanometre analytics with the sensitivity of modern mass spectroscopy, Australia and UQ will need a scientific instrument development program to combine for example, electron and ion optics technologies with mass spectroscopy detection efficiency. Furthermore a new X-ray analytical laboratory based system that is fully integrated into the CMM platform using Australian Synchrotron technology, will allow the transference of samples from one to the other analytical tool with no modification required (instrument integration or connectivity) between CMM instruments and characterising samples before accessing the Synchrotron in Melbourne.

You've noted that Australian researchers work and collaborate well together. Do you hope to extend CMM's networks and partnerships.

We will try to reinforce the AMMRF network by taking an active part in networking, setting up the next 5-10 years road map, investing in large data management and processing, and launching a new master classesand workshops. In addition to this I think we need to join forces with the facilities at Monash University and the Australian Synchrotron Beam lines and the different AMMRF nodes to start a national scientific instrument development program to prepare the future analytical forefront for Australia Research Community.

Additionally, to encourage the next generation of young scientists we plan to establish a Masters Course in nano-Microscopy and analysis as a starting point for 'future' nano characterisation courses.

Executive



Australian Institute of Bioengineering and Nanotechnology (AIBN) Laboratory





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HAWKEN LABORATORY



MR RON RASCH SENIOR SCIENTIFIC OFFICER/ LABORATORY MANAGER HAWKEN







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317 CLIENT PUBLICATIONS

relative .

CMM STAFF PUBLICATIONS

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