ammrf NEWS

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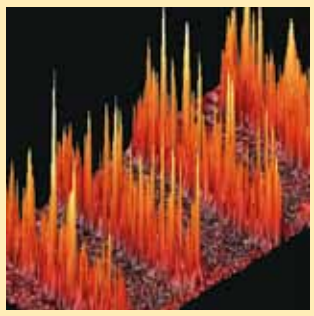
AUSTRALIAN MICROSCOPY & MICROANALYSIS RESEARCH FACILITY



- Anaphylaxis molecules revealed
- LIEF success brings new instruments to a node near you
- Excellent teaching rewarded
- More courses on offer than ever before

RESEARCH

Go with the flow – flow cytometry delivers results

AMMRF @ UWA

Flow cytometry is a technique for counting microscopic particles, such as cells, and is one that has many and diverse applications. Here are two examples from recently published work emerging from the Centre for Microscopy, Characterisation and Analysis (CMCA) at the University of Western Australia (UWA) that demonstrate this diversity. They highlight how the technique is being used to enable significant advances in research.

The first example, published in *The Journal of Allergy and Clinical Immunology*, is the largest ever study of anaphylaxis in humans, and the second, reported in *Cytometry A*, involves the development of a high-throughput technique for the rapid measurement of the number of chromosome sets in plants.

Anaphylaxis is a concern for many of us, especially those parents whose children suffer from serious allergies to things like peanuts and bee stings. Assist/Prof. Shelley Stone, a researcher at UWA, has recently led an ambitious project that set out to discover what biological molecules were mediating the process. However, measuring the mediators of an anaphylactic attack as it is happening is quite a tricky thing to do and consequently had not been done before. In this new study, blood samples were collected for analysis as soon as patients were admitted to emergency departments, usually within one hour of the onset of the attack. Further samples were collected as time progressed. Eight emergency departments around Australia took part in the study and a total of 76 patients were followed. Their attacks had been initiated by various common triggers such as food, drugs, insect bites or stings.

The team wanted to measure the levels of a large number of proteins and other molecules in their often rather small samples. Techniques available for use on the flow cytometer allow many of these proteins to be measured simultaneously, making a comprehensive analysis possible. Assist/Prof. Stone commented, "We were able to investigate serum levels of a number of cytokines by using the Cytometric Bead Arrays and the FACS Canto II flow cytometer. The ability to multiplex in order to investigate a large number of mediators was essential to this project, as often we only had a small amount of serum".

Their investigations have revealed some interesting results, some expected and others less so. Levels of the proteins interleukin-6 (IL-6) and interleukin-10 (IL-10) were raised in patients with acute anaphylaxis. IL-6 is a protein that induces inflammation and plays a number of different roles in the immune system. It may contribute to the severity of the allergic reactions seen in the patients. It could also be perpetuating the whole-body effects of severe anaphylaxis. There are already several drugs under development to block IL-6 activity that could also be investigated in the treatment of acute anaphylaxis.

The fact that IL-10 was also raised was rather unexpected. This is an anti-inflammatory protein whose primary function is to dampen immune responses. The team suggested that the IL-10 is being produced as the immune system tries to turn off the allergic response initiated by the trigger.

Assist/Prof. Stone and her colleagues are now moving ahead to investigate genetic differences in patients who suffer from anaphylaxis in the hope of understanding how the IL-10 gene is controlled in these people.

Continued on page 2

Experimental canola plants.

Assist/Prof. Shelley Stone in her lab at the Western Australian Institute for Medical Research.

LAB NEWS

AMMRF welcomes two new Linked Centres

The constant aim of the AMMRF is to reach out to researchers who can benefit from our facilities. One of the many ways we do this is through the establishment of specific relationships with major research centres in which a concentration of researchers have interests that can only be met through the application of advanced microscopy. These become our Linked Centres. A jointly funded support engineer plays a pivotal role in assisting the centres' researchers perform high-level microscopy and microanalysis.

Two new Linked Centres have recently come into the fold: the Australian National Fabrication Facility (ANFF) node at the Australian National University (ANU) and the Institute of Materials Engineering at the Australian Nuclear Science and Technology Organisation (ANSTO).

The ANU node of the ANFF has expertise and facilities for growing photonic and electronic materials, and processing and fabrication of devices such as micro-electromechanical systems (MEMS). A support engineer will facilitate linkages between the characterisation and fabrication centres and will bring a unique blend of skills in characterisation and fabrication with the experience to enable them to advise researchers from ANU on the most appropriate techniques and facilities to solve the users' research problems. The engineer will assist in the training of researchers from the node in fabrication processes, and microscopy and microanalysis techniques, and will assist in the acquisition of data and data analysis and interpretation.

The new centre at ANSTO will benefit from facilitated access to advanced characterisation capability, in particular, high-resolution transmission electron microscopy and atom probe tomography in the AMMRF at the University of Sydney. A dedicated scientific and technical support officer, will be appointed at the node to provide expertise and advice to ANSTO's researchers and to undertake high-level microscopy and microanalysis for the centre at the University of Sydney and other nodes as appropriate. ANSTO have several projects related to materials used in nuclear engineering to which AMMRF capability will be applied. ■

ANFF

Australian Government

ansto

RESEARCH

LIEF success extends AMMRF capability

There were unassailable smiles fixed to the faces of many AMMRF staff following the last LIEF announcement as the nodes attracted $4.61 million of funding. The new advanced microscopy instrumentation that was supported will extend AMMRF capability in the wake of increasing user demand and exciting new collaborations.

The Australian Key Centre for Microscopy and Microanalysis at the University of Sydney will be able to buy a much-needed additional instrument that combines a focused ion beam and scanning electron microscope for nanoscale characterisation and fabrication, and a similar instrument to reside at the Electron Microscope Unit at the University of New South Wales. It will also be in a position to boast Australia's first dedicated five-dimensional multiphoton-microscopy platform.

The Centre for Advanced Microscopy at the Australian National University will now purchase a new analytical transmission electron microscope for the investigation of functional materials, earth processes and novel condensed matter, as well as a multiphoton confocal microscope for biochemistry and cell biology.

At the University of Western Australia, the Centre for Microscopy, Characterisation and Analysis applied for a micro-CT, one of the most sought-after types of instrument in the AMMRF, and a multispectral imaging system, both for live small animal models. With its LIEF success, the CMCA can now acquire these instruments.

Adelaide Microscopy, the AMMRF facility at the University of Adelaide, will acquire a high-resolution environmental scanning electron microscope, which will have wide application across a range of disciplines from photonics to plant functional genomics.

In addition to these instruments in the core nodes, the AMMRF Linked Lab at the Queensland University of Technology has attracted $0.8 million for an ultra-high-vacuum scanning tunneling microscope to advance the understanding of novel materials. ■

Continued from page 1

Understanding variations could ultimately lead to better treatments for this alarming condition.

The efficiencies provided by flow cytometry are one of the attractions of this technology and are an essential element in the methodology described in the second paper.

In higher organisms, DNA is organised into chromosomes, and humans, like most animals, have two sets. Any deviation from this is lethal. However, unlike us, plants are exceptionally tolerant of changes in the number of chromosome sets (ploidy) and many species can grow reasonably well over a range of ploidies (sugarcane has up to 14 sets of chromosomes) although fertility is generally best in diploids. In plant taxonomy, ploidy series are commonly seen among related species and can be used to help distinguish and classify them. Plant breeders use changes in ploidy to affect the performance of plants as crops. For example, bread wheat is hexaploid, while durum wheat, used for pasta, is tetraploid.

Ploidy is traditionally determined by preparing cells, staining the chromosomes and counting them under a microscope. This is labour intensive and, for many plant species, is technically challenging due to small, indistinct and 'sticky' chromosomes.

For Assist/Prof. Matthew Nelson and his colleagues from UWA, who need to determine the ploidy of about 7000 canola samples each year, a better approach was needed. They have used the CMCA facilities to help them develop a fast, high-throughput method to achieve their goal. Using tiny pieces of baby canola leaves they process all the samples in parallel using 96-well plates. The cells are broken open with metal beads to release the nuclei, which are then stained with fluorescent dyes. A special high-throughput sampler loads the samples into the flow cytometer where the fluorescence is measured. The more fluorescence, the more sets of chromosomes in the samples. Assist/Prof. Nelson explains, "This throughput improvement was made possible by integrating existing parallel-handling approaches and a sample-acquisition plate attached to the flow cytometer into a good workflow system. This new efficient protocol takes only about 15% of the time per sample of previous methods and also allows us to tell which plants are diploid when the plant is still very young, saving a lot of time and plant maintenance resources by allowing us to discard useless sterile plants early on, providing significant savings in time and money." ■

Rapid ploidy determination will greatly assist plant breeders.

Call for workshop plans

At the 2009 AMMRF Strategic Planning Workshop, preliminary plans were made for technique-based workshops in the area of electron probe microanalysis, TEM tomography, SEM, light and laser optical microscopy and spectroscopy techniques. These workshops will enable staff to get together for a dedicated time specifically to discuss, learn about and develop skills around specific techniques. They will strengthen our ability to deliver capability in these areas and assist in the strategic planning for future investments.

The planning of technique workshops in the areas of electron backscattered diffraction, focused ion beam operation and application, and electron probe microanalysis is underway. If AMMRF staff are interested in developing other technique workshops they should submit plans to their node director for consideration. A brief description of the structure, the proposed outcomes and how they will support capability delivery needs to be prepared. Support to bring AMMRF staff together as well a contribution towards workshop costs is available. The specialist technique workshops replace the single large strategic planning workshop this year.

This is a great opportunity for our staff to consolidate and further develop relationships established at the annual strategic workshops. ■

EXECUTIVE DIRECTOR'S COLUMN

While Simon Ringer has been enjoying a well-earned break with his family, I have the pleasure of providing the first column of the new year. I would like, on behalf of the AMMRF team, to wish the readership of this newsletter all the very best for 2010 and hope that the new year brings success in your endeavours.

Around the nodes, instrumentation is running smoothly in the main, and the prospects for further significant, and technologically advanced, outputs are high. The demand for instrument time is constantly increasing across the nodes and the number of applications from institutions outside the AMMRF to use our instruments is steadily growing. The range of universities, industrial organisations and other government bodies that are coming to use the facilities is testament to the fact that the AMMRF is open to supporting quality scientific ideas, wherever they originate, and is willing to provide the advanced characterisation techniques that the research requires.

The need to continually upgrade and replace instrumentation is an important part of developing our infrastructure and the recent ARC LIEF round will see several nodes expand their instrument suites. More details of the various successes can be found in the adjacent article. One of the clear strengths of the AMMRF is that the links we have forged enable us to mount strong collaborative grant applications that have a high likelihood of success. A well-crafted, collaborative and supported application will still get funded, despite the very competitive atmosphere.

2010 will see the Australian Conference on Microscopy and Microanalysis hosted in Brisbane by the Centre for Microscopy and Microanalysis at the University of Queensland. The theme for this meeting is the excellence of the home-grown microscopy and microanalysis community here in Australia. The conference will highlight its research strengths in order to inspire and engage the delegates. The speakers that have accepted our invitations to speak come from a wide range of disciplines and many are strong users of the AMMRF facilities. The quality of research that will be promoted is clearly being underpinned by the access to the investments made in instruments and infrastructure through the AMMRF.

Challenges within nodes to accommodate the changing demands for access to instrumentation and the continuing pressure to perform will ensure that 2010 will be as busy as ever. However, the strong base and goodwill that is the backbone of our organisation will see 2010 be a strong year of discovery and achievement. ■

Regards,
John Drennan
Acting Executive Director & CEO

COMMUNITY

Supporting conferences in 2010

A number of conferences will be sponsored by the AMMRF in 2010, and numerous presentations will feature work carried out using AMMRF capability. These meetings provide an opportunity for us to support the essential process of scientific exchange and increase the awareness of what we offer to the research community. The conferences span topics from nanotechnology at the *International Conference on Nanoscience and Nanotechnology (ICONN 2010)* in Sydney in February to specialised plant structures at *Plasmodesmata 2010*. Latest advances in cell biology will also be supported at the *10th Hunter Cell Biology Meeting* in March.

A major sponsorship opportunity is the *21st Australian Conference on Microscopy and Microanalysis (ACMM-21)* in Brisbane in July where the AMMRF will be sponsoring the internet lounge and the best micrograph award, so make sure you bring along your best images. This biennial conference is a must for many microscopists as it is known to bring together the leading lights in the field of microscopy and microanalysis in Australia. Around the same time are several specialist meetings that will also be supported by the AMMRF, including *IFES 2010*, the *52nd International Field Emission Symposium* in Sydney, and the *Scanning Probe Microscopy Workshop* in Adelaide. Both these meeting are organised by staff from the local AMMRF nodes.

As well as the AMMRF supporting these conferences, the directors of the two NSW nodes, Prof. Simon Ringer and Prof. Paul Munroe, are leading Australia's bid to host the *18th International Microscopy Congress* in Sydney in 2014. If successful, this will bring thousands of international experts in microscopy and microanalysis to Australia, providing important interactions with our international colleagues and the chance to raise our profile further within the international research community. ■

LAB NEWS

RMIT Microscopy and Microanalysis Facility moves up in the world

AMMRF @ RMIT

The RMIT Microscopy and Microanalysis Facility (RMMF), one of the AMMRF's Linked Labs, has upsized, moving into splendid newly refurbished labs, designed specifically to accommodate the wealth of advanced capability that the facility has available. The labs housing the instruments all have glass walls with huge sliding glass doors opening onto a central corridor, giving easy access and generating a unified and open atmosphere in the facility.

Discreet side rooms carry the cooling and pumping systems, so the labs themselves are quieter and far more comfortable to work in. Unlike most facilities with large pieces of sensitive instrumentation, the RMMF is not located in the basement but three floors up in a multi-storey building with views! This enviable location keeps them at a reasonable distance from the multitude of vibrations and electromagnetic fields emanating from the constant stream of Melbourne trams rattling past the door outside on Swanston Street.

Along with the expert staff and postgraduate students who run the facility, these new developments will help to offer a better and more comfortable service to our users. There is a huge amount of experience in materials science in the facility with a strong emphasis on materials engineering and solid-state and surface physics. It provides high-quality electron microscopy and microanalysis capability to academic and industrial researchers involved in the characterisation of surfaces, interfaces and bulk materials and their transformation during thermo-mechanical processing.

The Director of the RMMF, Prof. Dougal McCulloch will be happy to discuss with you how the facility can add extra value to your research with the range of techniques they have on offer. ■

The new labs at the RMIT Microscopy and Microanalysis Facility (RMMF).

TEACHING

Teaching award for Professor Joe Shapter

AMMRF @ SARF

Prof. Joe Shapter, Deputy Director of the South Australian Regional Facility, our South Australian node, has been rewarded for his excellent and effective teaching, receiving one of the Australian Teaching and Learning Council's 2009 Awards for University Teaching. Prof. Shapter is more than just a passionate teacher – he has initiated a new and innovative degree course in nanotechnology, which exposes students to practical, cutting-edge science right from the outset of their studies, allowing them to experience exciting scientific concepts and developments. They also take an active part in shaping their own learning experiences.

Prof. Shapter recognises the importance of engaging students with real-life issues, encouraging them to consider not just scientific facts but also their relation to issues facing society, thereby keeping the course of study relevant. These approaches are among many features that have made this course the model for similar courses that are now run at other universities. ■

TECHNOLOGY

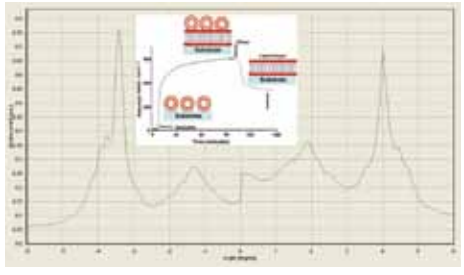
What is optical waveguide lightmode spectroscopy?

Techniques that enable researchers to monitor the dynamic binding of macromolecules, such as proteins, to a surface, have a large range of applications in the life sciences, chemistry and designing biomedical devices. Optical waveguide lightmode spectroscopy (OWLS) is such a technique. It is a very sensitive method that measures alterations in the refractive index of the sensor surface when molecules adsorb to it. As such, it does not need the molecules to be labelled with fluorescent or radioactive tags as is often required in other analytical methods. The technique has a time resolution of seconds, which allows real-time adsorption kinetics to be measured.

The set-up comprises a flow-through cell over the test surface, which sits immediately above a wave-guiding film through which light from a helium-neon laser is diffracted by an optical grating to initiate its propagation along the waveguide by total internal reflection. The test surface is positioned within the resulting evanescent field and it is this field that probes the optical properties of the solution. A solution containing the molecules of interest can be pumped into the cell so that adsorption (and desorption if the solution is replaced by saline) can occur at the active surface. Binding can be detected at levels down to ng/cm^2.

Some of the numerous applications of OWLS include protein–DNA interactions, ligand–receptor binding, investigations of lipid bilayers, behaviour of biocompatible materials, monitoring of environmental pollution, and the interaction of surfaces with blood plasma and serum. It can also be used to measure the attachment of living cells to biomaterial surfaces, making it a valuable tool for the development of medical devices and antibacterial coatings.

This technology is available through the AMMRF at the University of South Australia. If you are interested in using this technique or would like more information, please contact Prof. Hans Griesser (ph. 08 8302 3703, email hans.griesser@unisa.edu.au). ■



OWLS incoupling incident angles showing the two polarisation modes of the incident laser.

TEACHING

AMMRF teaching timetable 2010

The University of Sydney, Australian Key Centre for Microscopy and Microanalysis	
Introductory Microscopy and Microanalysis	Mar 8–18; Jul 26 – Aug 5
Biological Specimen Preparation for TEM & SEM	Mar 22–26; Aug 23–27
Materials Specimen Preparation for TEM & SEM	Apr 13–16; Oct 5–11
TEM of Crystalline Materials	Apr 19–22; Oct 12–15
Research Methodology	Jul 29 – Oct 14
Light Microscopy Workshop	May 10; Aug 30
Fluorescence Microscopy Techniques	May 11–14
Introduction to Confocal Microscopy	May 17–21; Aug 31 – Sep 3
Operation of the Transmission Electron Microscope	May 3–7; Aug 9–13
Operation of the Scanning Electron Microscope	Apr 23–30; Aug 16–20
Stereology	May 24–25; Sep 6–7
Image Analysis	May 26–28; Sep 8–10
Microscopy of Biomolecular Processes, consisting of *Advanced Light and Laser Microscopy* *Advanced X-ray, Probe and Electron Microscopy*	Sep 13–24
Nanostructural Analysis of Materials, consisting of *Advanced Scanning Electron Microscopy and Microanalysis* *Advanced Transmission Electron Microscopy* *Focused Ion Beam Technology* *Atom Probe Tomography*	Oct 18–29
The University of Western Australia, Centre for Microscopy, Characterisation and Analysis	
Scanning Electron Microscopy	Mar 8–12; June 28 – Jul 2; Nov 15–19
Transmission Electron Microscopy	Mar 15–19; Jul 5–9; Nov 22–26
Electron Microprobe Analysis	Mar 22–26; Jul 19–23; Nov 29– Dec 3
Optical and Confocal Microscopy	Mar 1–5; June 21–25; Nov 8–12
Introduction to Flow Cytometry	Mar 9–10; June 29–30; Nov 16–17
Preparing Images for Theses and Publication; Tissue Fixation and Immuno-labelling for Biological Samples; Specimen Preparation for Biological TEM; High-resolution TEM; WDS X-ray Microanalysis; Energy-filtered TEM; Selected Area Electron Diffraction; Electron Energy-loss Spectroscopy; Variable-pressure/Environmental SEM	TBA
Australian National University, Centre for Advanced Microscopy	
Introduction to Electron Crystallography	May 10–21 (by videoconference)
The University of New South Wales, Electron Microscope Unit	
Introduction to Electron Microscopy	Mid-Apr; Mid-Sep
Introduction to Microanalysis; Introduction to Diffraction	TBA
SARF: Ian Wark Research Institute	
Nano-ToF Workshop	TBA
Advanced Electron Microscopy and EDS	Subject to demand
SARF: Adelaide Microscopy	
SEM, TEM, Dualbeam FIB, EPMA, LAICPMS, Solution ICPMS, X-ray MicroCT, Laser Microdissection, Confocal & Optical Microscopy, Sample Preparation	Available on demand
Introduction to Ultramicrotomy	May 19–20
SARF: Flinders Microscopy	
Advanced SPM Techniques	Feb 20
Basic AFM Techniques	End of Apr
The University of Queensland, Centre for Microscopy and Microanalysis	
Cryo-SEM	Jan 18–22; Mar 15–19; May 10–14; Jul 19–23; Sep 13–17; Nov 15–19
Introduction to SEM and TEM	Feb 1–12; Mar 29 – Apr 13; May 31 – Jun 11; Aug 2–13; Oct 4–15; Nov 29 – Dec 10
Upgrade Training (e.g. Advanced SEM and TEM)	Feb 22 – Mar 6; Apr 19–30; Aug 16 – Sep 3; Oct 18 – Nov 5; Dec 13–24
X-ray Theory Course & Microprobe Training	Mar 2–4; Apr 28–30; Jun 29 – Jul 1; Sep 1–3; Nov 2–4
ACMM21 Conference Workshops (details to be announced)	Jul 11–15
Ad hoc training for AFM, XRD, and Cryo-TEM techniques on application.	
Curtin University, John de Laeter Centre	
SHRIMP II Introductory Course	Feb 22 – Mar 1; Sep 27 – Oct 4

OUT OF THE FRAME

The secret life of an atom probe scientist

AMMRF @ USYD

The AMMRF's new Atom Probe Scientist at the Australian Key Centre for Microscopy and Microanalysis, Peter Liddicoat, has been leading a double life. As far as we all knew, he was just a bright young scientist who has recently completed his PhD and is now working hard at passing on his expertise in the technically demanding area of atom probe tomography. However, we have discovered that he is also our very own national sporting hero.

Peter is a member and co-captain of the Australian Ultimate Frisbee team, runners-up last year in the Asian Championships for Beach Ultimate. These were held at the end of October last year on the beach at Boracay in the Philippines. Eleven national teams from around Asia took part. After three days of intense play, scorching weather, a few hospital calls, and the lashing of a small tsunami, both Australian teams, in the open and mixed divisions, made the finals, but both, unfortunately, were defeated by the local teams who, it must be said, had been undefeated at home since 2003! Up next is the World Ultimate Club Championships in Prague in July where the best clubs in the world will battle it out for supremacy. Peter's team, *Colony*, will be there, but will Peter? ■

CMCA enrols its first very own PhD student

AMMRF @ UWA

The Centre for Microscopy, Characterisation and Analysis (CMCA) at the University of Western Australia (UWA) has been granted permission to enrol postgraduate research students directly through the centre, creating exciting new opportunities for students to undertake microscopy-focused research at the AMMRF at UWA. This is a significant development for the centre, and reflects the excellence of its research profile.

The first CMCA-based PhD student, Rahi Varsani, commences in March, with further enrolments anticipated later in the year. Rahi has been awarded a PhD scholarship to continue his honours research on nanomaterial characterisation under the supervision of the node's deputy director, Prof. Martin Saunders. Rahi will present his research at both Australian and US microscopy conferences in 2010. ■

The AMMRF is funded by

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Queensland Government

Government of South Australia

STAFF NEWS

Australian National University

Elizabeth Richter recently joined the Centre for Advanced Microscopy at the Australian National University as Centre Manager. Elizabeth has just recently made Australia her home, having emigrated here from Canada in late 2008. She brings a wide array of skills and experience to the role, having worked in the Australian Government, environmental consulting, tertiary institutions and laboratories. She enjoys travelling and has worked in India, Europe and the Caribbean. Elizabeth studied at Wilfrid Laurier University and Niagara College in Ontario, and is looking forward to growing with the Centre for Advanced Microscopy. ■

The University of Sydney

Dr Patrick Trimby has joined the Australian Key Centre for Microscopy and Microanalysis (AKCMM) at the University of Sydney as SEM manager. Pat has a PhD in geology from the University of Liverpool, with a strong focus on the technique of electron backscattered diffraction (EBSD), a theme continued at Utrecht University. He then spent 10 years in commercial electron microscopy, at HKL Technology in Denmark and then at Oxford Instruments in Sweden. Pat has gained a wealth of experience in many electron microscope techniques and is looking forward to using these skills in his new role at the AKCMM. ■

Queensland University of Technology

Dr Peter Hines, although new to the Analytical Electron Microscopy Facility at Queensland University of Technology (QUT), will be well known to many as senior microscopist in charge of SEM at the Australian Key Centre for Microscopy and Microanalysis (AKCMM) at the University of Sydney. Having migrated north, he is now the AMMRF microscopist at the QUT facility, one of the AMMRF Linked Labs. Peter has a degree in metallurgical engineering and a PhD in materials engineering from the University of Queensland. He has a great deal of experience in all aspects of SEM, training and user support. ■

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