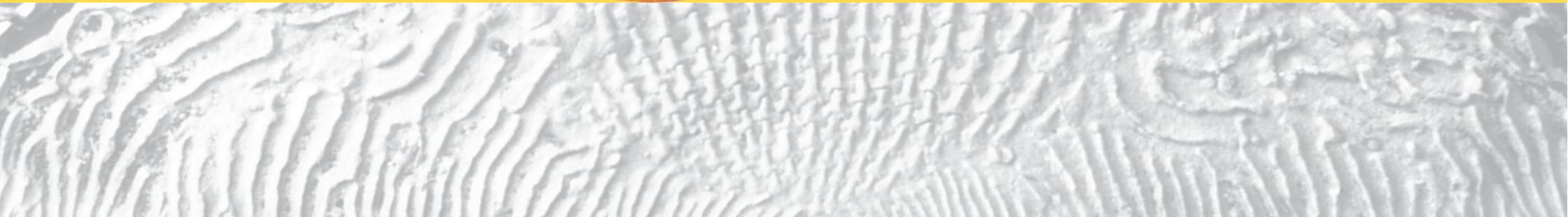


PROFILE



2014



Our vision

The AMMRF is Australia's peak research facility for the characterisation of materials through macro, meso, nano and atomic length scales by means of advanced microscopy and microanalysis.

Our mission

The AMMRF is a user-focused, interdisciplinary organisation that employs microscopy and microanalysis to explore structure–function relationships of materials in the physical, chemical and biological sciences and their technologies. Accessible to all Australian researchers, the facility provides a quality user experience enabled through the provision of world-class research services, research training and research programs.

FOUNDING NODES

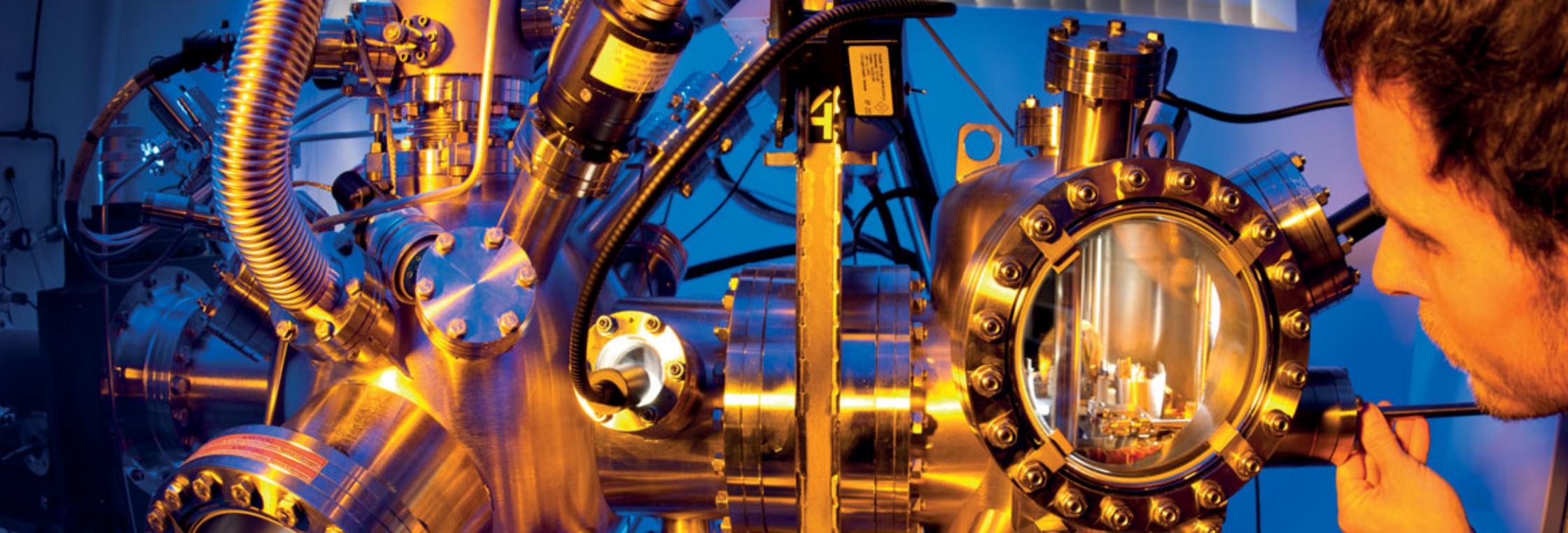


SOUTH AUSTRALIAN REGIONAL FACILITY (SARF)



FUNDED BY





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from the minister



Keeping Australia at the forefront of world-class scientific research is key to our national prosperity. Our ability to address global issues – such as health and ageing, sustainable energy and water – through world-leading research, puts Australia in a position to respond to both immediate and future challenges.

That is why the Australian Government supports the National Collaborative Research Infrastructure Strategy (NCRIS). NCRIS provides the equipment and expertise to enable Australian researchers to compete with the world's best, and Australia's industries to increase their productivity through innovation.

The Australian Microscopy & Microanalysis Research Facility (AMMRF) is leading the way in research that is helping us find the answers to big questions in fields as diverse as health, agriculture, engineering and archaeology.

World-class research assets like the AMMRF make important contributions to Australia's prosperity and the Australian Government is pleased to support this important facility through NCRIS.

The Hon. Christopher Pyne MP

Minister for Education

from the chair



Economic growth and increased productivity are dependent on the translation of research outcomes to successful innovation and technology. These critical steps are occurring with the integral support of Australia's ongoing collaborative national research infrastructure.

The Australian Government's recent announcement of the Industry Growth Centres Initiative highlights areas of significance to Australia's future prosperity. Microscopy and microanalysis at the AMMRF underpins research and development in the key areas of:

- food and agribusiness
- mining equipment, technology and services
- medical technologies and pharmaceuticals
- advanced manufacturing
- oil, gas and energy resources.

I am continually pleased to see that the AMMRF is able to demonstrate the breadth of its contribution to the nation's research and innovation as it supports new and established industries and more effective healthcare technologies. The outcomes from AMMRF-enabled research contribute strongly to the body of new knowledge that is being widely applied for national benefit.

Dr Gregory R. Smith
Chair of Board

from the ceo



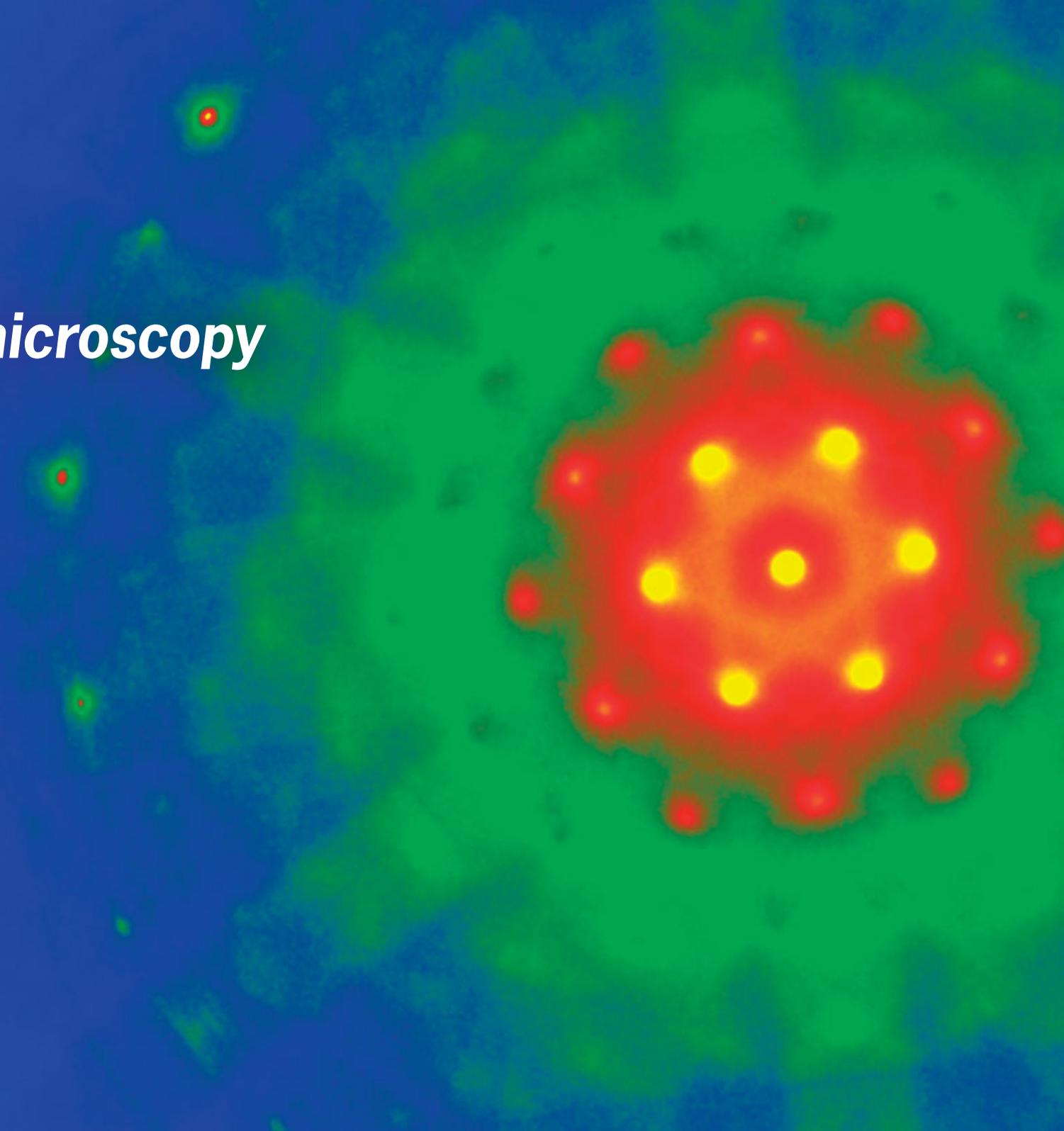
Looking over the past year gives me a very positive outlook for our future. We are going from strength to strength enabling exciting research projects, patents and industry outcomes with expanding capability and expertise.

Our Strategic Planning Workshop held earlier this year contributed to this positive outlook because we captured great ideas and enthusiasm from our staff and users on how to further improve our practices and streamline our services in the future. There was also an excellent session where we were able to engage with our colleagues from the Department of Education. They emphasised the importance of reporting and compliance, things they recognise that we do consistently well.

The availability of world-class research infrastructure is something emphasised by Nobel laureate, Prof. Brian Schmidt, who was a special guest at the workshop. He was clear and passionate in his support of our collaborative research infrastructure, saying that it is the biggest single issue for enabling excellent science.

Prof. Simon P. Ringer
Executive Director & CEO

access
advanced microscopy





Our facility comprises nearly 300 instruments run by expert staff supporting over 60 different microscopy techniques. Together we enable finely tailored experimental approaches to diverse research questions.

Researchers are supported through all aspects of their project, from the original idea to planning, training, data collection and analysis, through to writing papers and grant applications.

Available to all Australian researchers on the basis of merit, our facility also enables innovation through partnerships with industry and through international collaborations.

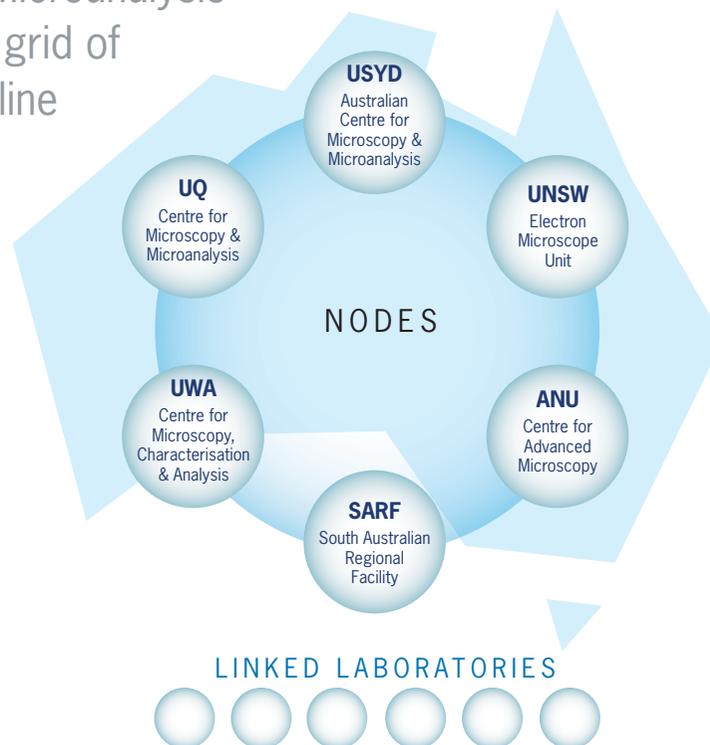
collaboration: efficiency

The Australian Microscopy & Microanalysis Research Facility is a national grid of instruments, expertise and online tools dedicated to nano-structural characterisation.

Major university-based centres are the core nodes of the network, collaborating to make efficient use of sophisticated, distributed capability.

We enable discovery and innovation in fields such as engineering, agriculture, healthcare and geoscience. Our facility complements other national research infrastructure to contribute significantly to Australia's wellbeing and economic growth.

The AMMRF supports around 3,000 researchers each year.

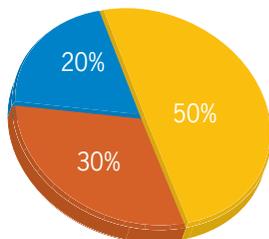


AMMRF capability continues to foster scientific engagement and discovery across the globe. International research collaborations bring scientists to Australia to use our microscopes and expertise, while our staff regularly travel to labs in other countries to share knowledge and experience.

International staff interaction and knowledge sharing continued to be a focus of the collaboration between AMMRF and Euro-BioImaging. A proposal was prepared for a global bioimaging project to support exchanges of staff between Australian and European facilities as well as joint training courses and the development of online tools.

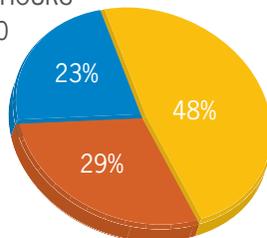
2013–14 in figures

USERS
Total: 3,035
By discipline:

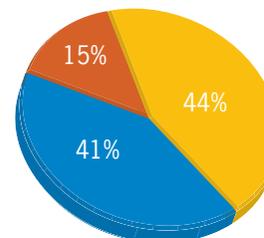


- Physical/Materials Science
- Biological/Medical Science
- Environmental Science/Geoscience

INSTRUMENT HOURS
Total: 220,820
By discipline:



USERS FROM INDUSTRY
By sector:



- Manufacturing
- Biomedical
- Environmental/Resources



AMMRF strategy is informed by strategic connections with peer organisations overseas.



The AMMRF Chairman, CEO, COO and two node directors with experts from our International Technical & User Advisory Group.



The international profile of the AMMRF was enhanced at the 2014 International Conference on Research Infrastructure (ICRI 2014) held in Athens, Greece. The AMMRF was selected to join the ICRI2014 exhibition alongside a range of pan-European research infrastructure

facilities. This provided an outstanding opportunity to showcase the AMMRF amongst key international peers.

The International Technical & User Advisory Group (ITUAG) informs the strategic outlook of the facility through advice on emerging technologies, technique application and trends in research infrastructure development and operations in other countries. A meeting of the ITUAG

was convened during the 18th International Microscopy Congress held in Prague, Czech Republic, in September 2014. AMMRF staff actively supported the successful bid by Australia to host the next international congress in Sydney in 2018. The CEO & Executive Director of the AMMRF, Prof. Simon Ringer, presented the winning bid with Prof. Paul Munro of UNSW. Together they will chair the 19th

International Microscopy Congress in Sydney in 2018.

The outcome is a recognition by leading international peers that Australia has world-class research infrastructure in microscopy and microanalysis and demonstrated leadership in the development and application of this capability.

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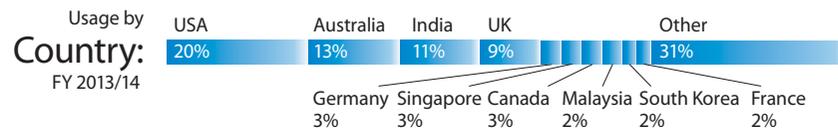
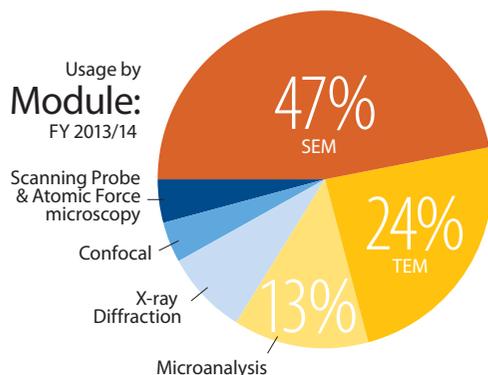
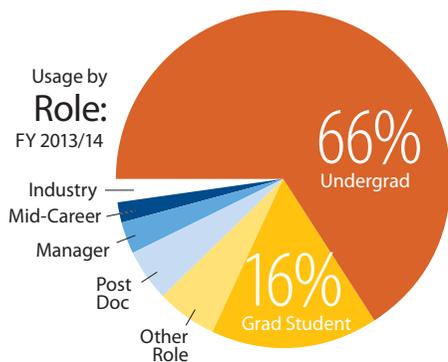
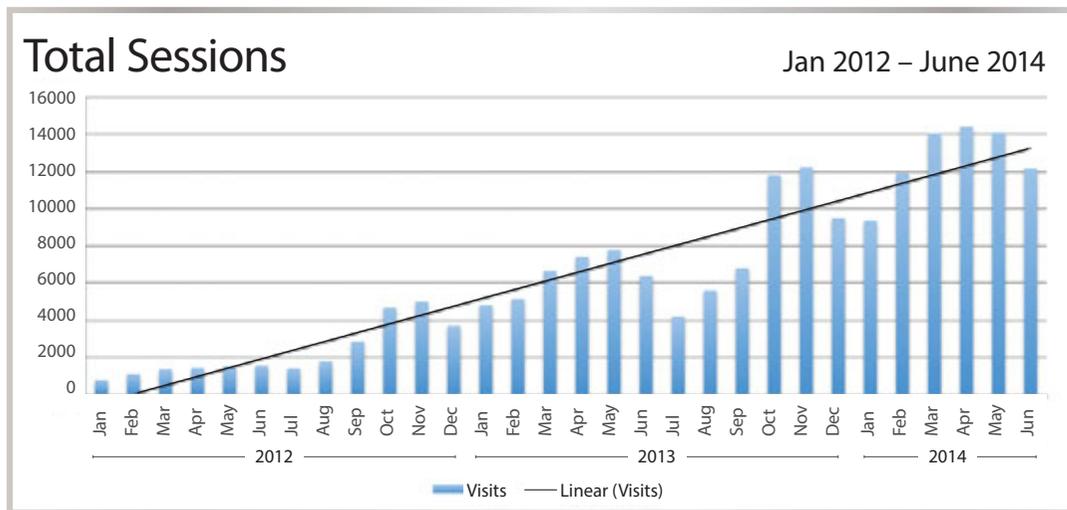


online tools: productivity

Ongoing collaborative work has produced a suite of online tools that supports Australian research. Figures on this page show the popularity and global influence of our online training tool: **MyScope**.

100,000+

MyScope visitors in the last year



MyScope

Significant gains in efficiency and productivity have been documented as MyScope is used to prepare researchers for on-instrument training.

Because on-instrument training is reduced with MyScope, instrument and staff availability for research is improved. MyScope engenders positive attitudes towards online learning and enhances knowledge.

Technique Finder

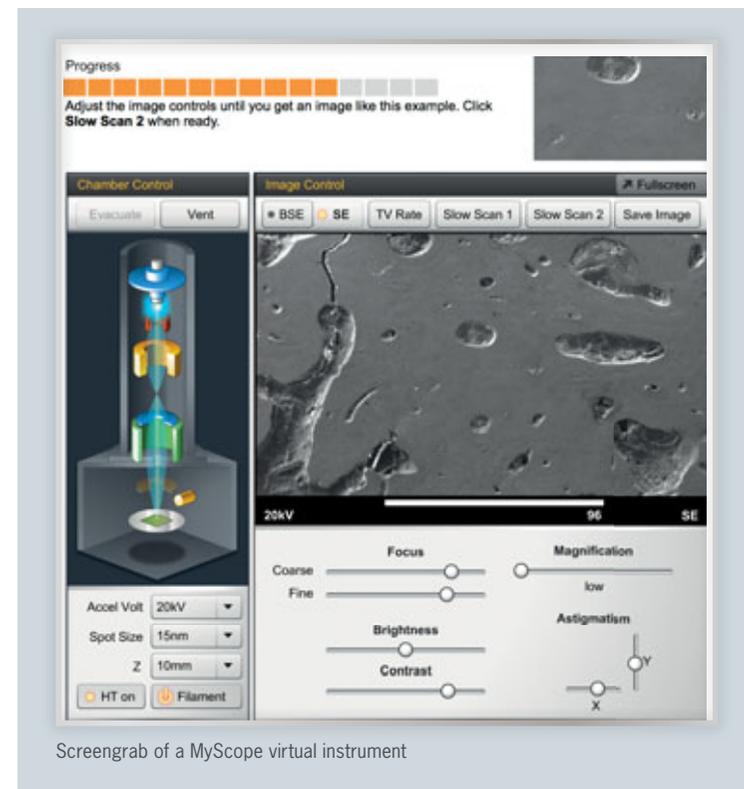
This tool enables researchers to identify the relevant microscopy techniques to enable scientific discovery.

The user-friendly interface presents options based on choices from a list of biological or physical experimental types. It matches researchers with the location of relevant instruments and expertise they need to get started. This feeds neatly into MyScope where they can begin their training. Technique Finder is also being used for a wide range of teaching applications.

CVL is a cloud-based analysis platform for multimodal and multiscale data.

The AMMRF has developed the atom probe and X-ray microtomography workbenches, which are now adding value to research around the world. They continue to build the AMMRF's reputation as world leaders in these advanced techniques.

The CVL is being developed as part of a NeCTAR project by a consortium including Monash University, the University of Queensland, the Australian National University and the University of Sydney.

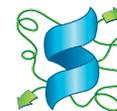


Screengrab of a MyScope virtual instrument



CVL

Characterisation
Virtual Laboratory



structural
biology



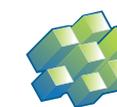
neuro
imaging



X-Ray



atom
probe



imaging
tools

EFFICIENCY, PRODUCTIVITY AND INNOVATION

 At the heart of our collaborative infrastructure are the flagship instruments. These are world-class platforms, many of which are unique in Australia. The productivity of each one is maximised by a dedicated expert. Outcomes from these flagships are highlighted in full-page features throughout these pages.

Our collaborative facility makes it easy for all researchers to access specialised microscopy and micro-analysis. The range of our instruments and techniques develops in response to the demands of the Australian research community and emerging technological trends. It now includes helium ion microscopy and the super-resolution technique of ground-state depletion microscopy.

Two of our nodes now also have the capability to cut sections inside a scanning electron microscope and serially image the exposed block face. This provides more scope for three-dimensional imaging, particularly of biological samples.



industry access



"Cochlear needs advanced microscopy and analysis equipment to support aspects of our device evaluations."

Cochlear's ongoing relationship with the AMMRF at the University of Sydney has supported an efficient and thorough evaluation pathway for our devices. This has helped Cochlear achieve important objectives in continuously improving hearing for our recipients around the world."

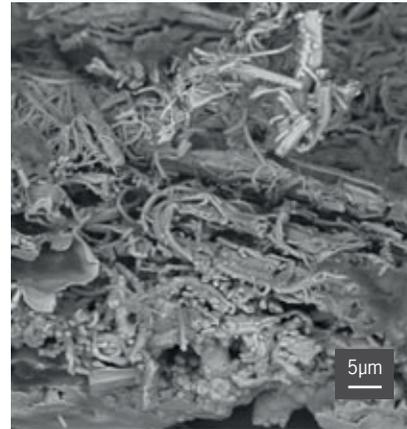
Dennis Balacano, Cochlear

"We have been using both scanning and transmission electron microscopy in the AMMRF at the University of Queensland for the last five years.

The work we do with the AMMRF has been essential in developing optimised structures for our electrode materials, allowing us to partner with major international companies

to bring our patented materials to market for use in personal electronics and electric vehicles."

Stephanie Moroz, Nano-Nouvelle



"AMMRF's continued provision of microscopy to analyse the element concentration in metal particles of the helicopters drive train provides us with superior information to reduce turnaround time of aircraft in maintenance and *ensures the safety of our fleet, which is provided to the Ambulance Service of New South Wales for patient transport and retrieval.*"

Jeff Bahls, CHC Helicopters

CONTRACT R&D

These relationships occur where an industry partner funds the costs of research, including instrument fees, consumables and salaries for research staff or student scholarships.

TESTING SERVICES

Testing and consultancy services such as failure analysis, identification of contaminants, quality assurance and forensic testing are available at commercial rates.

TRAINING & ACCESS TO INSTRUMENTS

Industry employees can be trained and provided with access privileges in-line with their level of competency. Access to AMMRF instruments is charged at commercial rates.

Participation in AMMRF training and specialist courses builds competency for companies that have microscopy capability in-house.

GRANT-AIDED PARTNERSHIPS

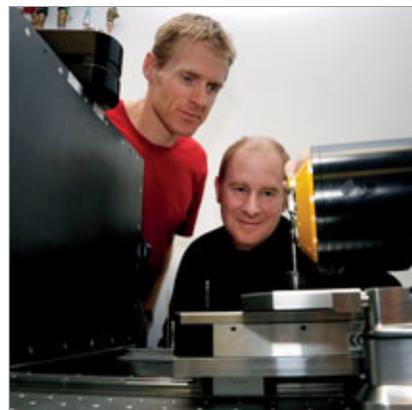
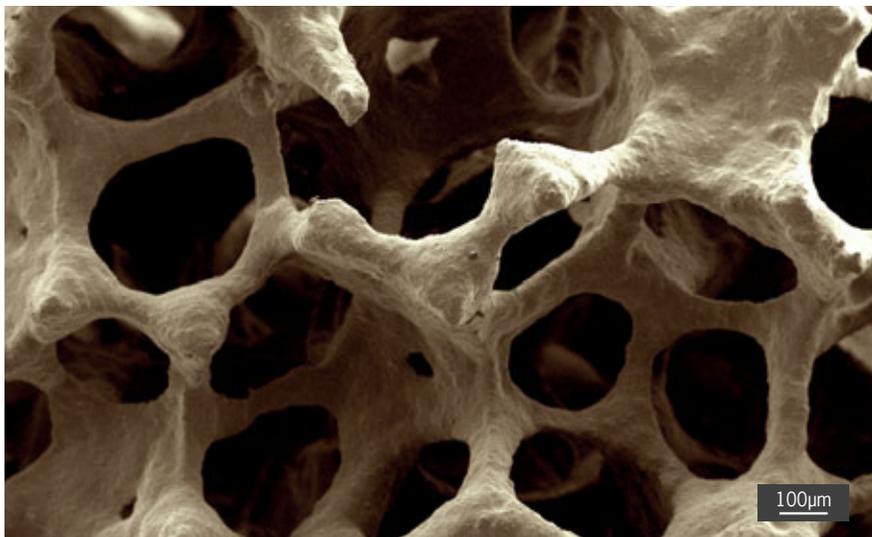
These include Australian Research Council Linkage Projects, which are an ideal way for industry partners to access the full range of academic and technical expertise that exists within the AMMRF. They provide long-term alliances to solve major research questions for industry and extend the research profile of the academics.

BONE SCAFFOLDS BUILD AUSTRALIAN BUSINESS

The University of Sydney has granted an exclusive licence to Australia's largest manufacturer of orthopaedic devices, Advanced Surgical Design & Manufacture Limited (ASDM) (ASX:AMT), to bring a new material called strontium-hardystonite-gahnite (Sr-HT-Gahnite) to market. It is a biocompatible ceramic that can treat bone loss, even in load-bearing parts of the skeleton. In preliminary studies, Sr-HT-Gahnite has duplicated the mechanical strength, elasticity and bioactivity of bone. Importantly, it is 100 times mechanically stronger than the synthetic bone substitutes in clinical use today. This will greatly extend the applicability of bone substitute materials into areas where currently only natural bone grafts, with their limited availability, or metal implants, can provide the necessary strength.

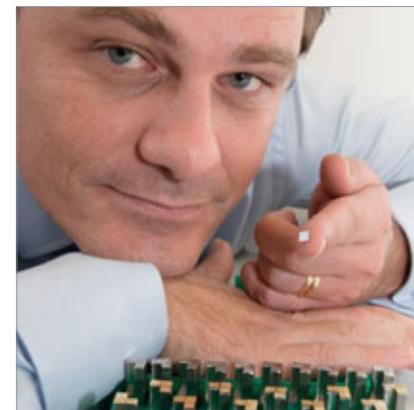
Prof. Hala Zreiqat and her research group have been working for more than 20 years on developing this technology. Scanning electron microscopy and in-vivo X-ray microtomography in the AMMRF at the University of Sydney and the University of Adelaide has enabled their research.

ASDM CEO Tom Milicevic said, "This is an exciting project for ASDM and fits our strategy of developing a portfolio of products in orthopaedics. This material is a novel breakthrough technology with huge global potential. According to the 2010 Global Data report, the bone graft market was valued at US\$1.9 billion and is forecast to grow by a 8.3% compound annual growth rate, more than doubling the market by 2020."



\$76 MILLION FOR ANU SPIN-OFF

Technology developed by researchers in the AMMRF at the Australia National University (ANU) and the University of New South Wales (UNSW) is at the heart of the sale of Lithicon AS to FEI for \$76 million. Applications of ANU's patented helical X-ray microtomography instrumentation, combined with 3D image analysis software (ANU and UNSW), spawned spin-off company Digitalcore – formed in 2009 to provide porosity solutions to the oil and gas industry. Its success led to the formation of Lithicon when Digitalcore merged with Norway's Numerical Rocks in 2013. In conjunction with the acquisition of Lithicon, FEI has obtained the helical X-ray microtomography instrument and associated software from ANU, through a licensing and development agreement. This will lead FEI's expansion into X-ray microtomography instrumentation and form an ongoing relationship with ANU.



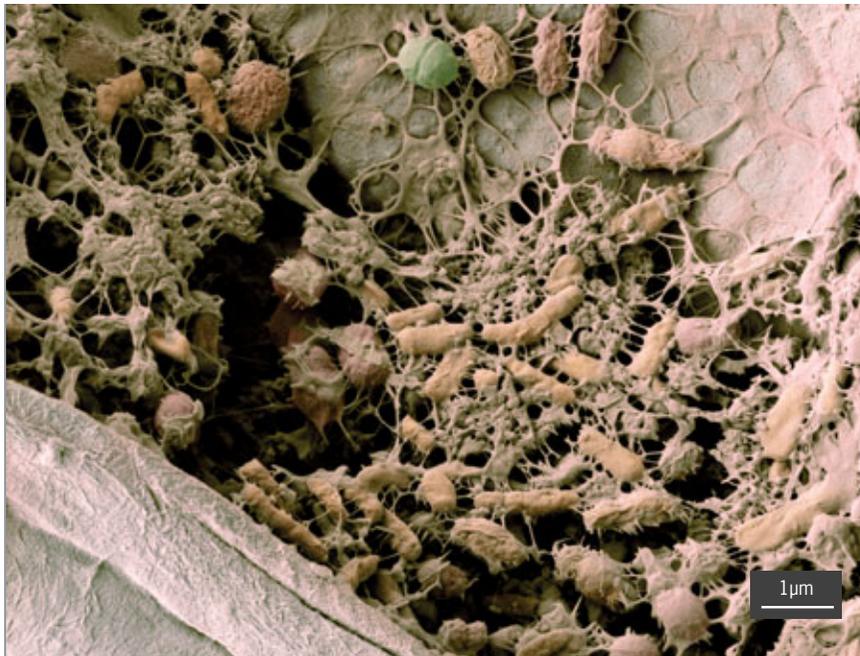
NANOPATCH™ FOR POLIO VACCINATION

The World Health Organization (WHO) is to provide funding to spin-off company, Vaxxas, so it can develop the Nanopatch™ for polio vaccination. WHO will fund pre-clinical studies and the development of good manufacturing practices. Successful completion of this research, and all necessary clinical development and regulatory approvals, would open the opportunity to Vaxxas to supply Nanopatches to help secure a lasting polio-free world. Development of the Nanopatch™ has depended on microscopy in the AMMRF at the University of Queensland.

As recently as 1988, more than 350,000 polio cases occurred annually in more than 125 endemic countries. Concerted efforts to eradicate the disease have reduced incidence by more than 99%, and efforts are being intensified to finally eradicate the strains that are still being transmitted.

patents

At least 35 patents have been filed or granted in the last year as a result of research enabled by the AMMRF. Some are highlighted in the research section, others are below.



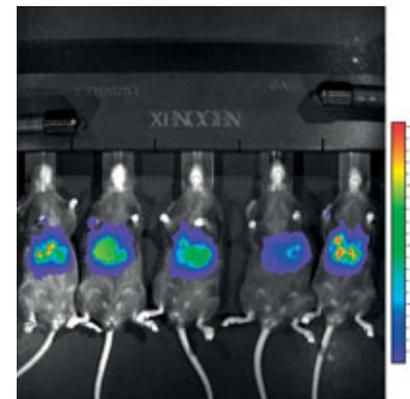
BATTLING BIOFILMS

2014900998 was filed to cover the synthesis of polymeric nanoparticles for the dispersion of biofilm. Biofilm is one of the main causes of death in hospital. The work behind this patent describes the design of small nano-objects using living radical polymerisation to encapsulate therapeutic molecules to disperse biofilm.

A/Prof. Cyrille Boyer and his group were able to have sustained release for several days, preventing the build up of biofilm. They used transmission electron microscopy in the AMMRF at the University of New South Wales to characterise these nano-objects.

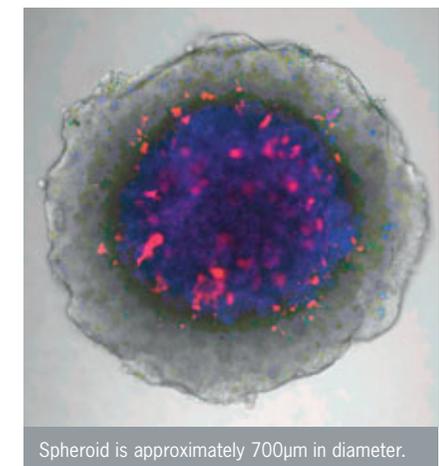
HIV AND HEPATITIS C VACCINES

PCT/AU2013/000509 describes a new DNA-based vaccine system for HIV and hepatitis C. Prof. Eric Gowans, from the University of Adelaide (UoA), has developed a cellular vaccine and method of inducing an immune response in a subject. The new system causes a small amount of tissue inflammation at the injection site in the skin, which stimulates the abundant antigen presenting cells (dendritic cells) in the area to become active. This generates an immune response robust enough for the vaccination to be successful. Bioluminescent imaging in the AMMRF at UoA helped validate the processes.

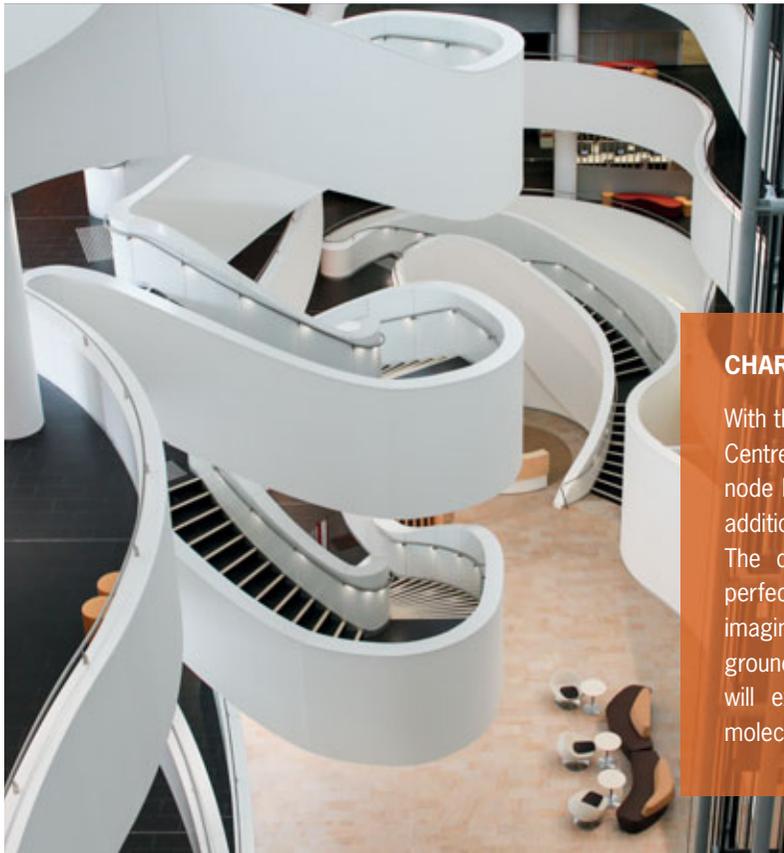


CLEVER CHEMISTRY TARGETS TUMOURS

Patents have been granted in territories around the world for a novel method to deliver drugs to the centre of hard-to-treat solid tumours. Prof. Brian Hawkett and Dr Nicole Bryce have developed the method that relies on the distinctive chemistry in these tumours to specifically control the release of anti-cancer drugs to the most difficult-to-reach cancer cells. The work used imaging of living spheres of cultured cells called spheroids that mimic the environment inside a tumour. This was done in the AMMRF at the University of Sydney.



Infrastructure developments are improving our provision of top-end microscopy through new, purpose-built spaces. They facilitate internationally recognised research that benefits the health and welfare of the community, advances scientific understanding and informs tertiary curriculums.



CHARLES PERKINS CENTRE

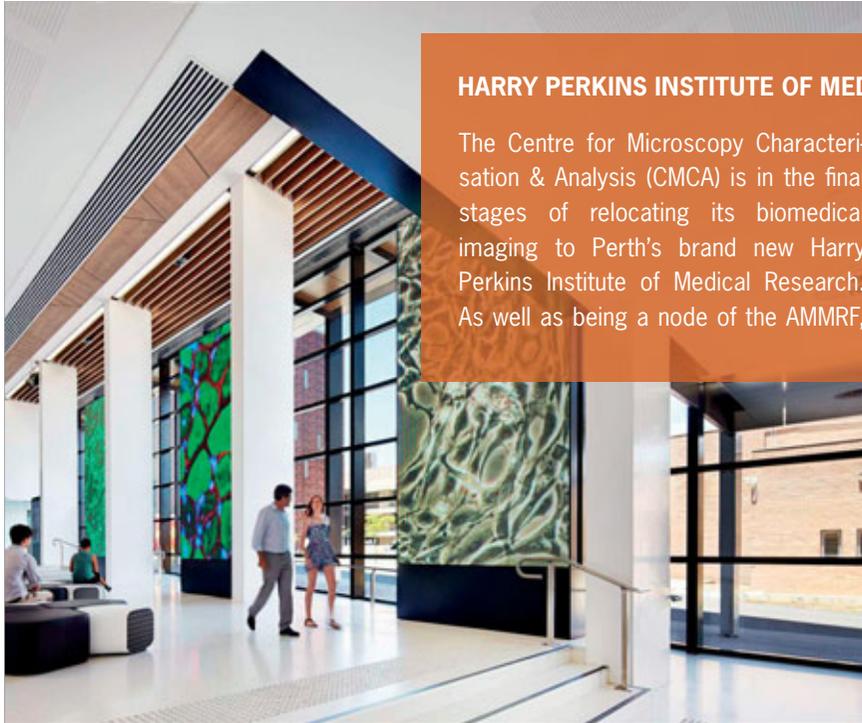
With the opening of the Charles Perkins Centre (CPC), our University of Sydney node has taken on the management of additional imaging capability in the CPC. The custom-built space provides the perfect environment for light and laser imaging, including the super-resolution ground-state depletion instrument. This will extend our capacity to support molecular-imaging focused research.

NEW HELIUM ION MICROSCOPE

The AMMRF Linked Laboratory at the Queensland University of Technology has commissioned the country's first helium ion microscope, pioneering an exciting technology for Australian researchers.



The Zeiss Orion Nanofab operates in a similar fashion to a gallium-ion focussed ion beam (FIB) instrument. Researchers will be able to exploit the system's unique combination of high resolution, surface sensitivity and depth of field in a broad range of disciplines from nanomaterials to biological ultrastructure studies.



HARRY PERKINS INSTITUTE OF MEDICAL RESEARCH

The Centre for Microscopy Characterisation & Analysis (CMCA) is in the final stages of relocating its biomedical imaging to Perth's brand new Harry Perkins Institute of Medical Research. As well as being a node of the AMMRF,

the CMCA is also a node of the National Imaging Facility (NIF).

From their new location, CMCA's biomedical research applications team is led by Drs Matt Linden and Kirk Fiendel, and A/Prof. Paul Rigby. They will enable

extensive optical and confocal microscopy; flow cytometry and cell sorting platforms alongside small animal in-vivo X-ray microtomography; multispectral imaging and the recently installed NIF flagship Bruker 9.4T MRI.

NEW MICROIMAGING AND ANALYSIS PRECINCT AT ANU

Our Australian National University node relocated this year to a purpose-built premises at the John Curtin School of Medical Research. The new precinct, including the Centre for Advanced Microscopy (CAM) and the Computer Tomography Lab, includes state-of-the-art instrument rooms where close attention was given to minimising the impact of noise, vibration, electromagnetic fields and also to maintaining temperature stability. This has resulted in significant improvements to instrument

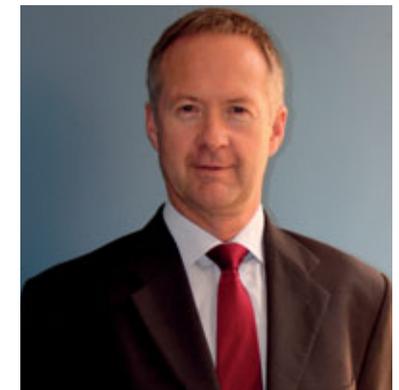
performance and therefore better results for researchers. The X-ray micro- and nano-tomography labs, 3D printing area and brighter, more spacious offices and teaching spaces further contribute to a vibrant new facility.



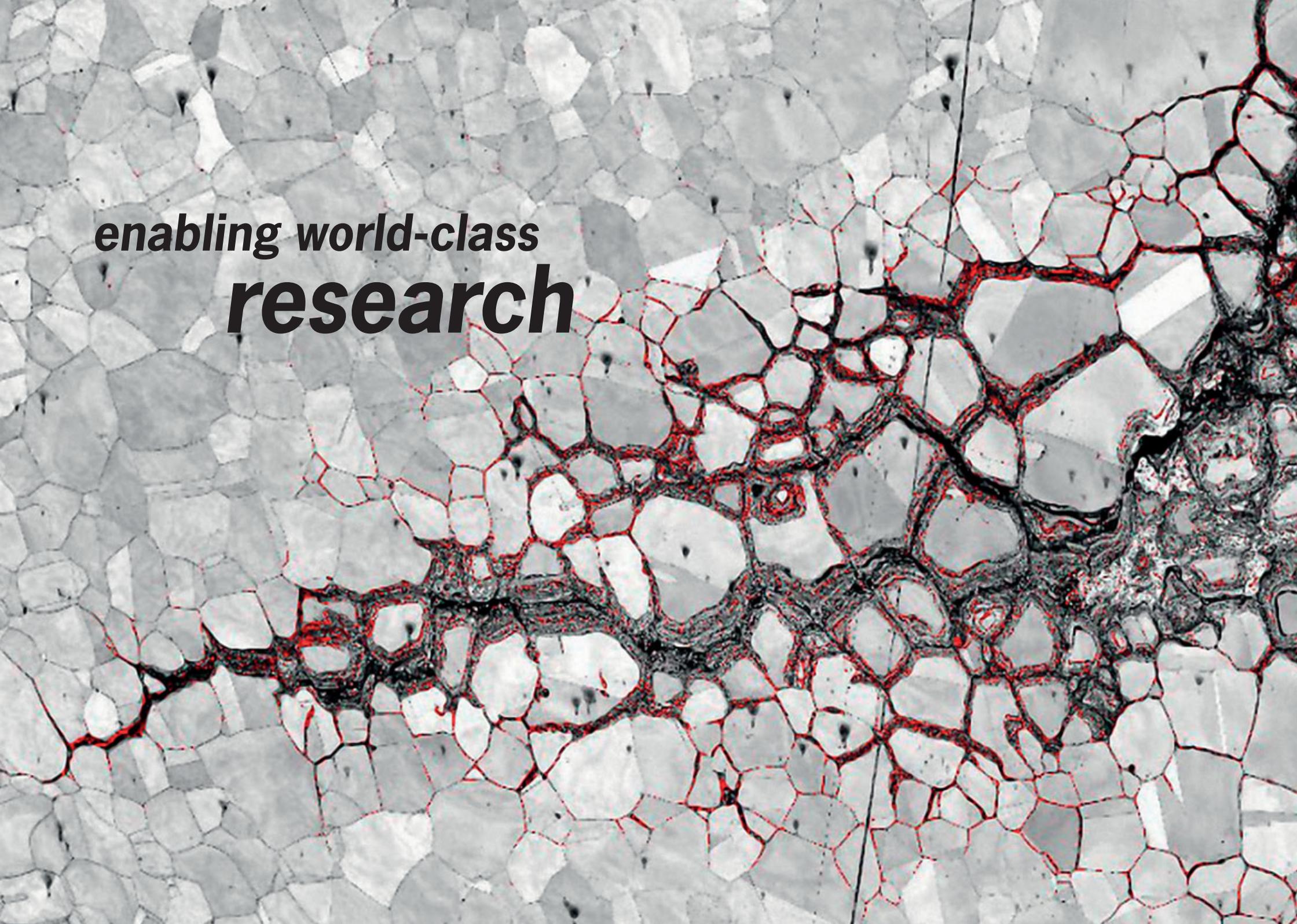
IEEE DISTINGUISHED LECTURER: DAVID SAMPSON

Prof. David Sampson, Director of the AMMRF at the University of Western Australia, was a 2013 IEEE Distinguished Lecturer in Photonics. This award recognises his work in photonics to enable development of a microscope in a needle for deep tissue imaging. This has led to his worldwide lecture tour that included:

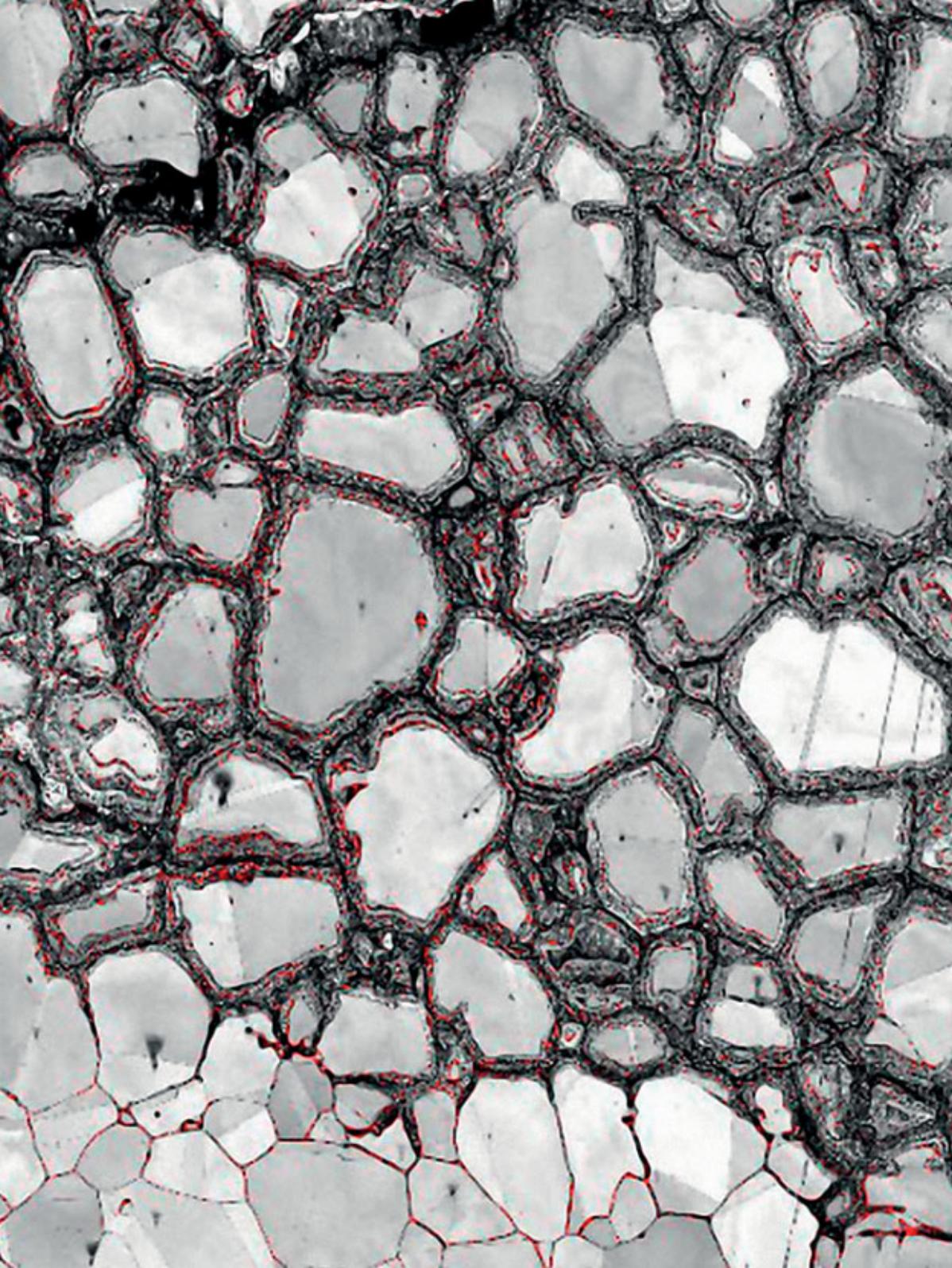
- College of Optics & Photonics, University of Central Florida, USA
- Department of Electrical & Computer Engineering, University of California, USA
- School of Physics & Astronomy, University of St Andrews, Scotland



- National Research Council, Ottawa, Canada
- University of Toronto, Canada
- University of Hong Kong, China
- University of Sydney, Australia.

A microscopic image of plant tissue, likely a cross-section of a stem or root. The image shows a network of cells with distinct cell walls. A prominent feature is a vascular bundle, which is stained in red and black, contrasting with the surrounding greyish tissue. The red staining highlights the cell walls, while the black staining appears to be concentrated in the vascular bundle's structure.

enabling world-class
research



Our primary purpose is to support Australian research, generating new knowledge and feeding innovation in the academic and industrial sectors. Our instrumentation and expertise extend the range of inspirational and world-class research outcomes from Australian science.

The reports on the following pages demonstrate that the research we support is already contributing to the areas of importance identified in the Industry Growth Centres Initiative. Their alignment is indicated by these icons:



food & agribusiness



medical technology & pharmaceuticals



mining technology & services



advanced manufacturing



oil, gas & energy resources



MICRO-TECTONICS ENABLE STRETCHY ELECTRONICS

Stretchable electronics are set to become a multimillion-dollar industry, with wearable devices such as fitness trackers and smart watches appearing recently in the mainstream market. Currently, such devices use rigid conventional electronic elements embedded in elastomeric materials to allow the device to stretch.

Next-generation, fully stretchable transparent devices will use thin layers of oxide materials completely integrated with elastomeric materials. However, oxide materials are brittle and have processing temperatures often in excess of 300°C. This has prevented their incorporation into stretchable electronic devices.

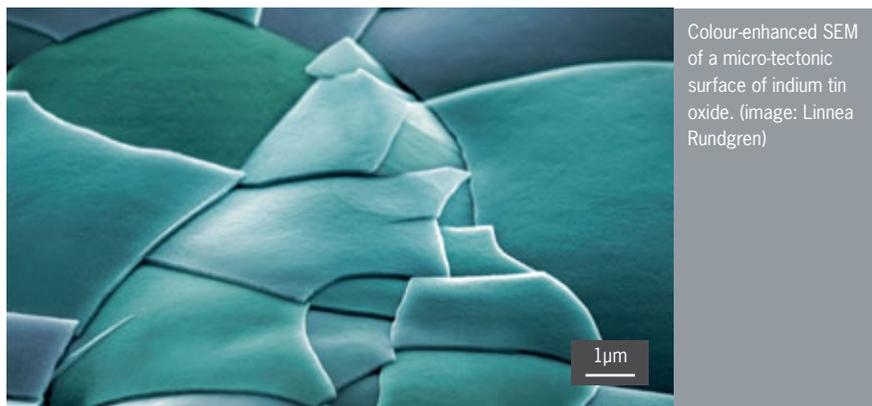
Mr Philipp Gutruf, working with Dr Sharath Sriram and Dr Madhu Bhaskaran at RMIT University (RMITU), has developed a new method in which the electronics are made on rigid silicon (to withstand the high temperatures) and then transferred in a series of steps onto a flexible

substrate. He used scanning electron microscopy (SEM) with a custom-made stretching apparatus at the AMMRF Linked Laboratory at RMITU to investigate device surfaces and image them under stress.

This led to Mr Gutruf discovering the micro-tectonic effect, where microscale plates of the oxide materials are generated through cracking introduced during the fabrication process. The plates slide over each other, very much like geological plates, relieving stress while retaining electrical functionality during stretching. This fortuitous discovery helps to overcome the brittleness of the oxides.

Mr Gutruf found that indium tin oxide, the industry standard for transparent conductors, has a stretchability of up to 15%, making it suitable for wearable devices. This research is a leap forward in realising stretchable transparent electronics.

P. Gutruf et al., *NPG Asia Materials* 5, 2013



Colour-enhanced SEM of a micro-tectonic surface of indium tin oxide. (image: Linnea Rundgren)



3D VISUALISATION OF ASTHMA TREATMENTS

Respiratory diseases affect a significant number of people – asthma affects approximately one in twelve people worldwide and this number continues to increase. Australia has an unusually high prevalence by international standards: our ratio of asthma sufferers approaches one in ten.

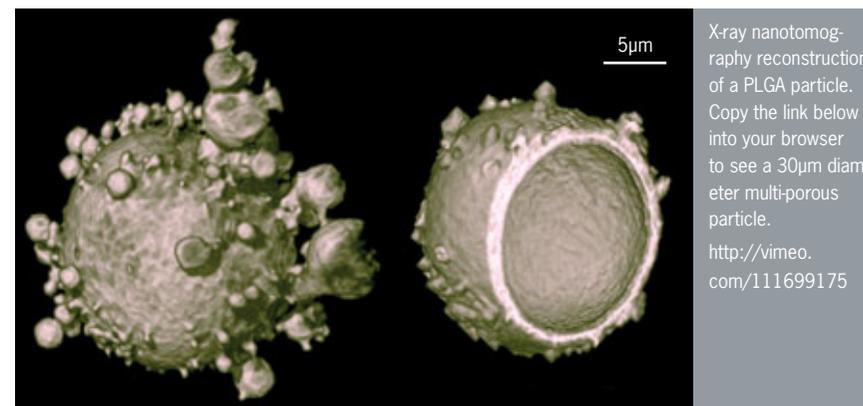
Management of respiratory diseases requires efficient drug delivery methods. One of the keys to successful treatment is carrier particles that efficiently deliver the drugs deep into the lungs.

Using X-ray nanotomography in the AMMRF at the University of Sydney, researchers led by Prof. Kim Chan have been able to visualise the internal structures of porous pharmaceutical particles in a non-destructive manner at resolutions approaching 50 nanometres. Previous studies have required sectioning of the particles through microtomy or focused ion beam microscopy. These methods

have left researchers unsure as to whether the delicate internal features had been impacted by the pre-imaging treatment.

Prof. Kim's team has imaged a variety of different drug carrier particles, including sugars, polymers, proteins and lipids. The technique allows visualisation and quantification of the internal porosities with confidence that the data obtained is representative of the true nature of the particles.

Overall, X-ray nanotomography enables characterisation of drug delivery particles in greater detail. The team continues to use the quantitative capabilities of the X-ray nanotomography system to explain and predict the aerodynamic behaviour of their particles. This will assist them to develop more efficient drug delivery products to improve the quality of life for patients suffering from respiratory diseases.



X-ray nanotomography reconstruction of a PLGA particle. Copy the link below into your browser to see a 30µm diameter multi-porous particle. <http://vimeo.com/111699175>



flagship atom probe

ATOM PROBE MICROSCOPY is used to develop:

- improved alloys for power generation, transport and construction
- superconductors, semiconductors and nanoparticles

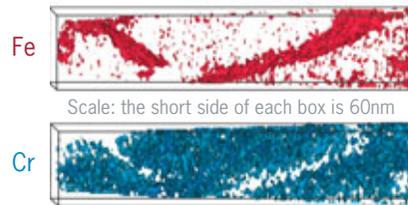
Flagship atom probe instruments at the University of Sydney (UoS) are run by the Flagship Engineer Dr Takanori Sato. Learn how they are improving the efficiency of renewable energy technology.

CONCENTRATED SOLAR POWER (CSP) is a growing renewable energy technology, with some plants already in operation. It has the potential to provide energy on the scale of coal-fired power stations. An array of mirrors concentrates the sun's energy onto a receiver where it heats a heat transfer fluid (HTF). This can be either stored until thermal energy is required or used to generate steam to drive a turbine, producing electricity in the traditional way.

The effective use of CSP requires cost-effective and corrosion-resistant materials for the tubes that receive the concentrated sun rays and carry the HTF. Such components must operate for extended periods at high temperatures and withstand thermal cycling between around 900°C in the day and ambient temperature at night. Nickel-based alloys can withstand these extremes but are prohibitively expensive. The most practical compromise materials are high-temperature austenitic stainless steels (ASSs), but



these can only operate at considerably lower temperatures than required for the next-generation plants. Development of cost-effective, heat-resistant steels will enable solar thermal plants to reach their



<http://vimeo.com/111699174>

maximum potential, bringing greater efficiencies and cheaper electricity.

FAILURE MECHANISMS of current steels in practical service in CSIRO's pilot CSP plant in Newcastle are the focus of Mr Alex La Fontaine, a PhD student with A/Prof. Julie Cairney at UoS. Pipes cycled between 970°C and ambient temperature for four days and nights were examined using complementary microscopy techniques, including the flagship atom probe.

Large intergranular corrosion cracks extended deep into the tube wall, indicating a dramatic and rapid structural failure. Microscopy showed that the extreme heat causes chromium within the steel to move away from the grain boundaries, allowing corrosion-causing oxygen to penetrate from the surface and oxidise the surrounding steel. When the temperature drops, the crystal structure of the oxidised steel changes, opening up cracks along the grain boundaries allowing more oxygen in and creating a vicious circle of corrosion.

The atom probe was used to look closely at these corroded surfaces revealing that iron-rich oxides are present, rather than the protective chromium oxides (see image), weakening the overall structure. A silica layer, intended to improve corrosion resistance, also cracked during thermal cycling, weakening the metal still further.

The design of more corrosion-resistant steel should result from the identification of these high-temperature failure mechanisms. Compositional changes to include aluminium as a more effective corrosion-proof layer will be created, tested in service, then it too will be evaluated.

Researchers in the field recognise that this kind of analysis is particularly challenging without the high-resolution and 3D capabilities offered by the atom probe.

A. La Fontaine et al., *Corrosion Science* 85 (1), 2014
 A. La Fontaine et al., *Scripta Materialia* DOI: 10.1016/j.scriptamat.2014.09.028

⊕ KNEE MICROARCHITECTURE TO MONITOR OSTEOARTHRITIS

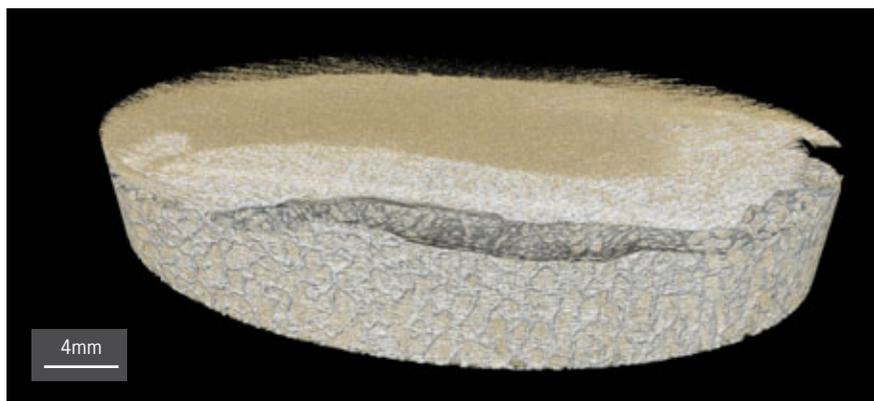
Knee osteoarthritis is a musculoskeletal disease currently affecting about 660,000 Australians. It is a major health cost, which includes knee replacement surgery as there are no early diagnostic tools or adequate preventative therapies.

Research has shown that bone marrow lesions (BMLs) directly beneath the knee cartilage (subchondral bone) correlate with symptoms and disease progression but can only be seen using magnetic resonance imaging (MRI). University of Adelaide (UoA) PhD student Ms Dzenita Muratovic and her supervisors, Dr Julia Kuliwaba and Prof. David Findlay, wanted to characterise BMLs at the tissue level and investigate if BMLs seen in different MRI imaging modes are characterised by different tissue morphology.

Ms Muratovic used MRI to localise BMLs, then characterised the subchondral

bone microarchitecture in 50 knee specimens from patients undergoing total knee replacement for osteoarthritis. Assisted by AMMRF staff at UoA, Ms Muratovic developed a specific X-ray microtomography imaging and analysis protocol for large, complex specimens. Her team were the first to use this protocol to analyse the 3D microarchitecture of BMLs. Their images showed different degrees of change in the subchondral bone. One BML type represents an earlier stage of osteoarthritic disease progression, whereas another is likely to represent a later stage and is, therefore, harder to treat.

The team proposes MRI of BMLs as a specific biomarker to identify individuals at risk of progressive osteoarthritis, and as a way to monitor therapy. New ways of monitoring this common condition will be clinically important.



3D reconstruction of X-ray tomography data of a whole tibial plateau showing loss of cartilage.

FROM DUSTBALLS TO SPACE ROCKS

One of planetary science's major questions is 'how do we form solid rocks from gas and dust?' Prof. Phil Bland at Curtin University has proposed a mechanism based on modelling rock formations from the disc of dense gases and dust (the protoplanetary disc) that orbits a new star. The model suggests that the first pre-planets were highly porous dustballs, resembling giant balls of fairyfloss. Slow impacts between dustballs would generate shock waves and high temperatures – sufficient to increase the density and fuse dust grains together to create solid rock.

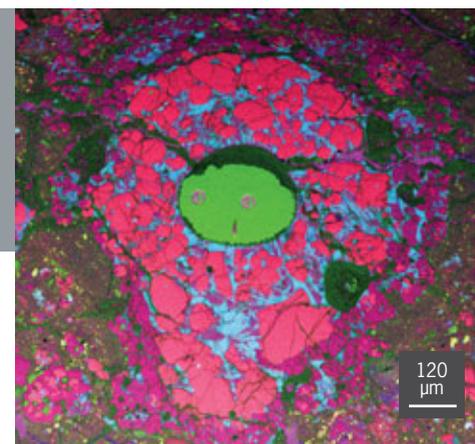
In the protoplanetary disc some millimetre-sized dust balls were melted, possibly by localised shock waves caused by turbulence in the disc. They resolidify, forming spherical droplets called chondrules. Chondrules are often

found within meteorites, surrounded by a fine-grained matrix, thought to be the fused dust grains. If slow impacts have occurred, some deformation and alignment of matrix grains around the solid chondrules should be seen in the rocks.

Prof. Bland's PhD students, Ms Lucy Forman and Mr Luke Daly, are analysing meteorites to find supporting physical evidence. Mr Daly used complementary microscopy techniques in the AMMRF at the University of Sydney to analyse the composition and orientation of individual crystals and found them to be aligned around the chondrule. This suggests compression along one axis, consistent with a slow impact immediately before the grains were fused.

These results are the first tangible evidence of the earliest rock-forming process in our developing solar system.

Elemental map of part of a meteorite with a chondrule in the centre (green and pink). Aligned magenta grains can be seen around the chondrule.



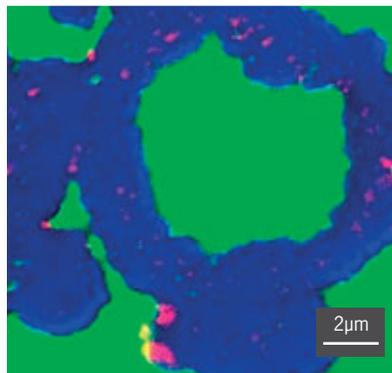
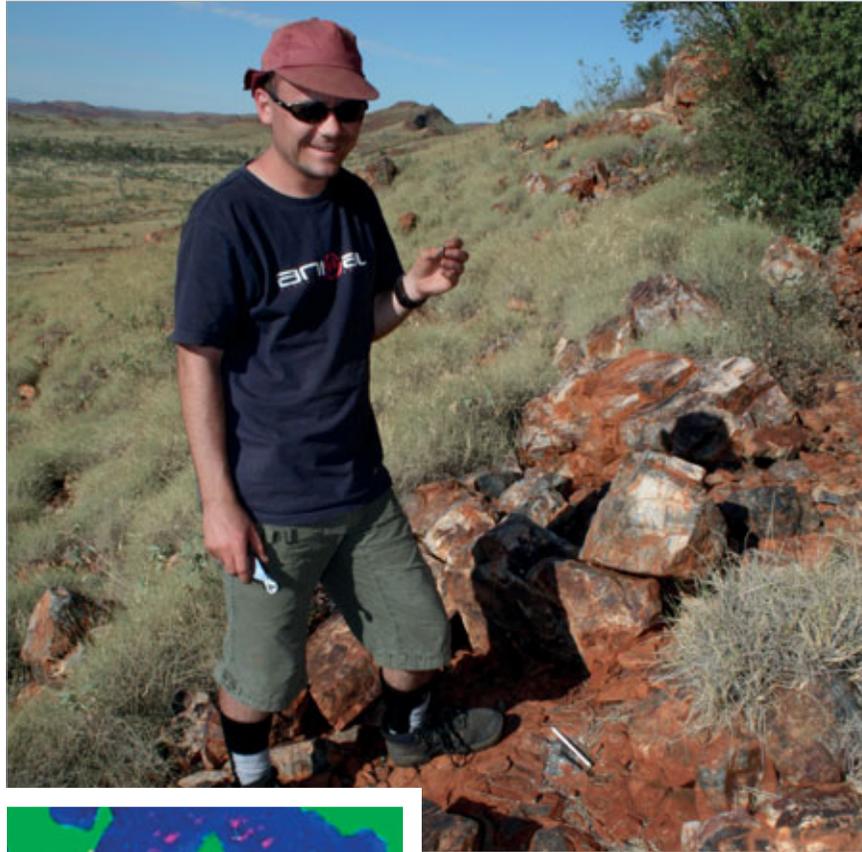
flagship ion probes

STUDIES INTO AGRICULTURE, health and minerals are enabled by the flagship ion probe suite (NanoSIMS 50 and IMS 1280) and its dedicated engineer, Dr Matt Kilburn at the University of Western Australia (UWA). 2014 Future Fellow, Dr David Wacey, has used the ion probes extensively over many years to amass a significant body of work revealing the nature of some of the earliest life on Earth.

WHEN DID LIFE FIRST APPEAR on Earth, what form did it take and how can we recognise life on other planets? These are the big questions that interest Dr Wacey. The earliest life on Earth would have been very small, morphologically simple and probably only subtly different from co-occurring non-biological organic material. Fortunately, AMMRF instrumentation gives researchers a fighting chance to determine what is and what is not ancient fossilised life.

The UWA flagship has enabled Dr Wacey to collect unique in-situ elemental and isotopic data from rock samples up to 3.5 billion years old. This allows the precise ageing of the rocks and lets him associate the position of elements associated with life, such as carbon and nitrogen, with sulfur and structural features of potential fossils.

ULTRATHIN SLICES from potential microfossils were prepared so cell-wall microstructure and nanoscale chemistry could be studied by transmission



NanoSIMS ion image of a 1900 million-year-old pyritic *Huroniospora* microfossil: sulfur is blue; oxygen, representing quartz, is green; and nitrogen, pink. The discontinuous ring of nitrogen is interpreted as a chemical ghost of the original organic microfossil wall.

electron microscopy, complementing the ion probe results. The flagship focused-ion beam instruments at the University of Adelaide and the University of New South Wales were used for this precision work. Accurate 3D models of the microbial fossils were also constructed using serial sectioning.

DR WACEY'S RESEARCH HIGHLIGHTS

- He identified the earliest undisputed fossil life found so far on Earth (3.4 billion-year-old) and found that it had a sulfur-based metabolism, thought to be one of the earliest stages in the development of life.
- He found bacteria called *Gunflintia* caught in the act of being consumed by other bacteria. Their association with specks of the iron sulfide mineral, pyrite, strongly suggests that the pyritic sulfur originates from bacterial metabolism.
- He was part of a team that linked microbial activity with the formation of small iron oxide spheres on Earth. These spheres are very like structures found on Mars.
- He discovered that clay minerals are excellent at preserving cell structures, allowing him to see one billion-year-old cell membranes.
- He helped demonstrate that viruses can mediate the mineralisation of microbial matter and that many nanospheres in the geological record are likely to be fossilised viruses.

Dr Wacey also wrote the first textbook on the subject: *Early Life on Earth – A Practical Guide*. As well as shedding light on the origins of life on Earth, these discoveries will help scientists to recognise what is and what is not extraterrestrial life.

⊕ PLATELETS IN HEALTH AND DISEASE

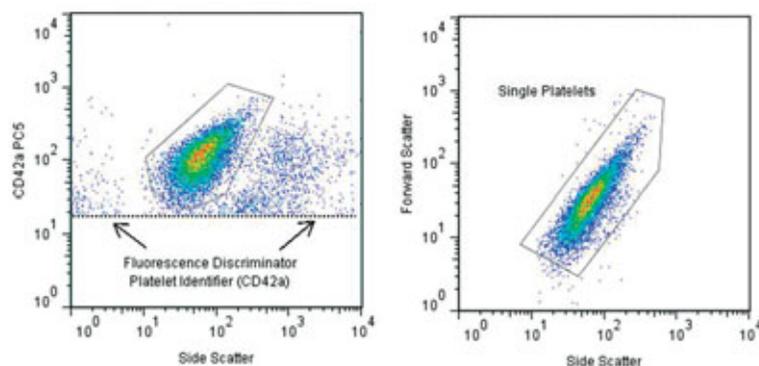
Platelets are blood elements that control blood clotting. While their activation is important in limiting bleeding following an injury, excessive platelet activation increases the risk of heart attack and stroke. Dr Matthew Linden at the University of Western Australia (UWA), with colleagues from RMIT University, investigated the combined effects of brief bouts of high-intensity exercise and caffeine on platelet function.

Flow cytometry in the AMMRF at UWA revealed that a single bout of intense exercise resulted in a 60 to 90 minute spike in platelet activation. This corresponds with the time during which there is increased risk of heart attack and stroke during and briefly after intense exercise. However, while caffeine is often anecdotally associated with sudden stroke, especially in the context of energy drinks, the researchers found no

exacerbation of the exercise effect with caffeine use.

Dr Linden's team also investigated flavonols for their potential to inhibit platelet activation. Flavonols are molecules widely found in fruits and vegetables and are reported to improve the outcomes of heart disease. Mice that were injected with flavonols before a simulated heart attack or stroke showed significantly reduced narrowing of blood vessels. Furthermore, by inducing diabetes in mice, the researchers found that flavonols were able to reverse the platelet hyperactivation that is associated with diabetes. Flavonols are therefore a promising preventative for heart attack and stroke, with the potential to avoid negative effects associated with other antiplatelet therapies.

S. Mosawy et al., *Platelets* 24, 2013



A cytogram showing how platelets with different properties are identified by flow cytometry.

🔍 NANOPARTICLES – THROUGH THE ATOM PROBE LOOKING GLASS

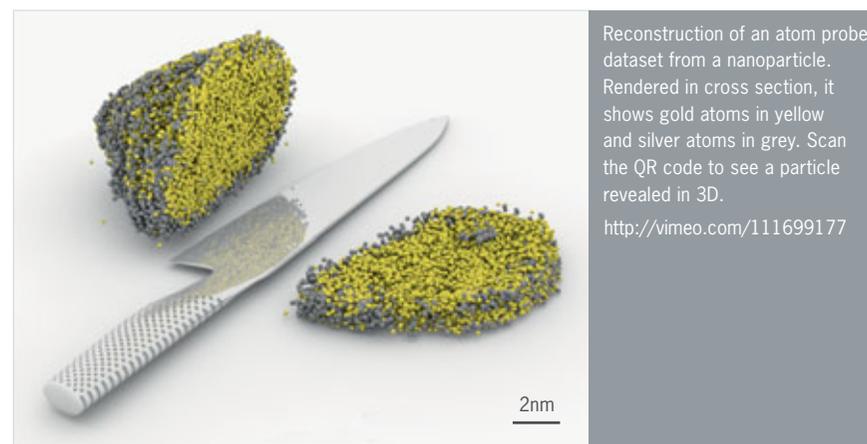
Silver-coated gold nanoparticles play an important role in fuel cells, plasmonics and certain glucose biosensors, and have the potential for use in a wide range of emerging technologies. Until now it hasn't been possible to thoroughly check the three-dimensional distribution of the different types of atoms in the nanoparticles after they've been made. This limits the information available to the researchers who need to be able to correlate the nanoparticles' performance with the detailed atomic structure.

Dr Peter Felfer, working with A/Prof. Julie Cairney and Prof. Thomas Maschmeyer at the University of Sydney (UoS) has used atom probe microscopy in the AMMRF at UoS to manipulate the 15 nanometre-wide nanoparticles so that they can be analysed in the atom probe, something not successfully done before.

The results showed that the particles did consist of a gold core inside a silver shell but there were many gaps in the coating and even some unexpected chemical traces left behind from the manufacturing process. These residual atoms were not evenly distributed and seem to have prevented the particles from being properly coated with silver. A good coating of the reactive silver on the particle surface is crucial for its performance, so gaps would be having a significant impact on the final function.

This atom probe evidence is now enabling the researchers to fine-tune the synthesis of their nanoparticles, which should lead to optimised performance. This, in turn, will lead to greater efficiencies in a whole host of emerging applications.

P. Felfer et al. *Angewandte Chemie*, 126 (42), 2014

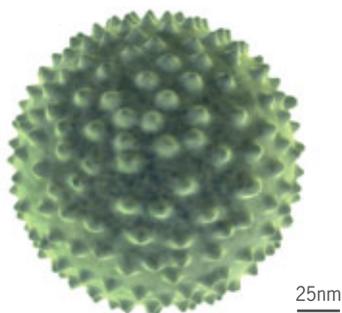


Reconstruction of an atom probe dataset from a nanoparticle. Rendered in cross section, it shows gold atoms in yellow and silver atoms in grey. Scan the QR code to see a particle revealed in 3D.

<http://vimeo.com/111699177>

flagship cryo-tem +

STRUCTURAL STUDIES OF CELLS and molecules are enabled by the flagship cryo-transmission electron microscope (TEM) and its dedicated engineers, Mr Garry Morgan and Dr Kathryn Green, at the University of Queensland (UQ). 2009 Future Fellow, Prof. Chengzhong Yu, uses electron tomography on the cryo-TEM to unravel 3D structures in many of his projects on novel nanomaterials for biomedical, agricultural and environmental applications.

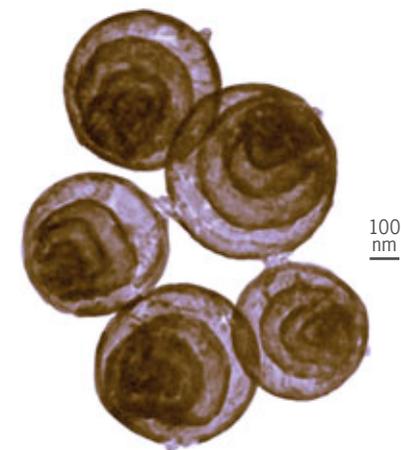


MULTISCALE SILICA NANOPARTICLES inspired by the surface roughness of viruses have been developed by Prof. Yu and his team at UQ. Silica is biocompatible and easy to work with at a chemical level, making it easy to modify. In generating controlled, quantifiable roughness, Prof. Yu used electron tomography on the Flagship TEM so he could visualise and measure his particles in three dimensions.

Getting molecules into cells is important for therapeutic and research applications such as drug delivery and the

experimental genetic modification of cells. Prof. Yu's rough particles bound and delivered proteins and genetic molecules into cells more efficiently than a standard commercial product. It is a significant step forward and opens up an opportunity for development and commercialisation of these rough particles as a high-efficiency cellular delivery product. A patent (2013904973) has been filed to cover these particles.

REMOVAL OF ARSENIC contamination from groundwater and drinking water is also being tackled by Prof. Yu's group through the development of iron oxide nanostructures. Naturally occurring arsenic contamination affects more than 100 million people worldwide and causes severe health problems. By using electron tomography, he has demonstrated the need to keep the nanostructures non-aggregated to achieve a high capacity for arsenic removal.



He has achieved this by utilising simple and scalable spray-drying technology from the food industry, followed by sequential calcination, to produce porous, multi-shelled hollow nanoparticles with discrete, well-separated 20 nanometre-thick layers. These nanoparticles therefore have a large, accessible surface area for the efficient adsorption of arsenic ions.

A patent has been filed for this invention (PCT/AU2014/050038) and Prof. Yu is currently working with industry partners in an ARC Linkage Project to scale up production and develop millimetre-scale porous supports to immobilise the nanoparticles (movie link in image). This will lead to practical products for high-throughput wastewater treatment and for a household drinking-water treatment device.

This project will bring economic benefits through Australian industry and improve the quality of life for people all over the world.

L. Zhou et al., *Chem. Commun.*, 49, 2013.



ANCIENT COLLISIONS THAT BUILT MOUNTAINS

An ancient collision between the Indian and Asian continental plates created the Himalayas. However, the exact time of the collision is controversial. PhD student Adrianna Rajkumar and her supervisor Prof. Geoffrey Clarke, at the University of Sydney (UoS), are attempting to clarify this mystery using Indian rocks.

Previously, researchers dated these rocks by crushing them to produce isolated zircons. The zircons were then dated, but without context to where in the rock they came from, which gave ages that were unexpectedly old (e.g. 60 million years).

Ms Rajkumar and Prof. Clarke recognised that the rocks record multiple stages of mineral growth. Dating the zircon grains while they were still in the whole rock – and, therefore, still associated with specific minerals – would help

to date the mineral growth stages. This would inform a more accurate rock age.

Ms Rajkumar and Dr Pat Trimby at the AMMRF at the UoS scanned whole sections of rock, picking out zircons in backscatter electron images and confirming their composition using energy dispersive X-ray spectrometry. This method can correlate the age of the zircons with the rock's history. Their results explain some of the anomalous dates previously reported, which are likely to be related to zircons from the original parent rocks formed before the start of the India–Asia collision.

Refining the date of collision and the ages of the parent rocks, and determining the depth of the rocks when the zircons grew, will shed light on the complex geological history of the India–Asia collision.



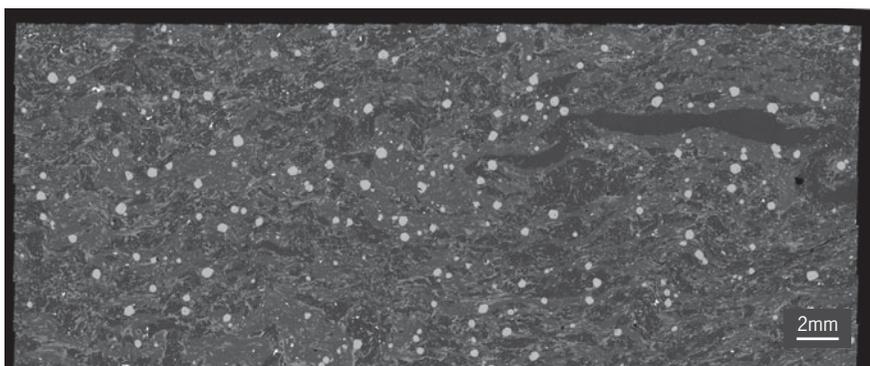
ALUMINIUM SOIL TOXICITY EXPOSED

Worldwide, 40% of all arable soils are acidic. Although all soils contain aluminium, it becomes soluble – and toxic to plants – only in acidic soils. Aluminium toxicity, therefore, has a negative impact on food production in these areas. In Australia, for example, soil acidity costs \$1.5 billion per year in forgone agricultural production. With the global population exploding, it is imperative that more food be produced from these soils.

For the past 110 years, it has been known that aluminium decreases root growth, although the mechanisms for its toxicity have never been identified until now. In the AMMRF at the University of Queensland, ARC Future Fellow Dr Peter Kopittke, with the expert help of Mr Rick Webb and Dr Kathryn Green, carefully prepared root samples to elucidate how aluminium affects root growth.

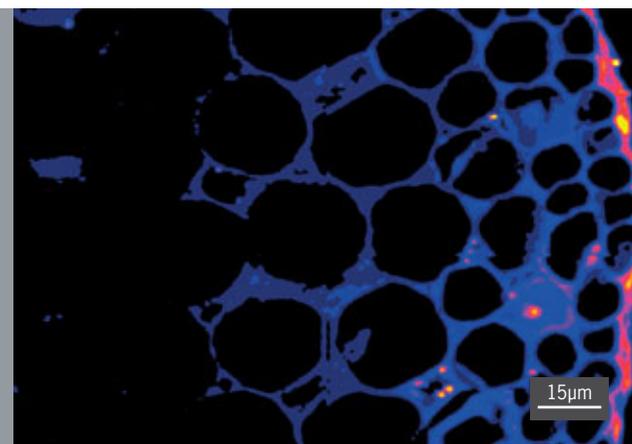
Dr Kopittke used sensitive characterisation approaches, including a beamline on the Italian Synchrotron, accessed through the Australian Synchrotron via the International Synchrotron Access Program. This allowed him to demonstrate that aluminium accumulates in roots within only minutes of exposure to the heavy metal. Combined with high-resolution analyses over time, molecular biology and flow studies, Dr Kopittke and his colleagues have been able to show, conclusively, that aluminium binds very strongly to the cell walls and, within five minutes, stops them expanding, blocking root growth.

This is the first step towards finding a solution to aluminium accumulation and increasing global food production from nearly half of the world's arable soils.



A typical whole, thin section of rock from India. The automated EDS analysis detected 1982 zircons (too small to be seen in this image).

A microscopic map collected using the Italian Synchrotron (ELETTRA), showing the levels of toxic aluminium in roots of soybean. Highest levels are shown in yellow.



flagship tof-sims +



SURFACE ANALYSIS of anything from pharmaceuticals to coated glass and ore samples is enabled by the flagship time-of-flight secondary ion mass spectrometer (ToF-SIMS) at the University of South Australia (UniSA). Flagship Engineer, Dr John Denman manages this powerful instrument.

Learn how A/Prof. David Morton from Monash University and his team are using the ToF-SIMS to improve pharmaceutical production.

EACH TABLET YOU SWALLOW is the result of complex research and technology. Efficient tablet production depends on the ability of powdered drugs to flow and compress into tablets that remain intact. The drug's interaction with the manufacturing machinery at optimal production rates is also important. Various chemicals lubricate and aid the flow of the powdered drug to the tablet presses but these can interfere with tablet formation.

Magnesium stearate (MgSt) is an important lubricant and A/Prof. Morton's group has shown that dry coating drug particles with MgSt via their process of mechanofusion improves powder flow. They have relied on the flagship ToF-SIMS throughout their project to evaluate the effectiveness of the coating methods.

MECHANOFUSION IS SAFER, simpler, more environmentally friendly and potentially more cost-effective than the solvent-based coating processes. However, because of the intense forces generated during coating, the team wanted to discover if this method is suitable for drugs such as ibuprofen, that have a low melting point.

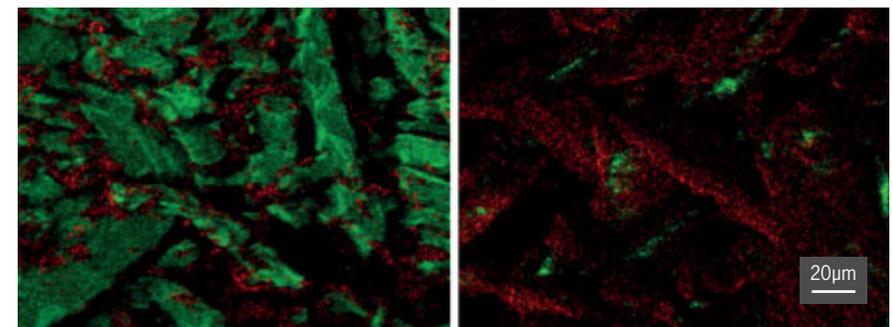
Ibuprofen is used at high doses so there isn't much spare space in the tablets for lubricants and binders. It also melts easily during high-speed tablet making, sticking to the machinery. It requires a slower production speed, reducing productivity.

By using the flagship ToF-SIMS and X-ray photoelectron spectroscopy to examine the coverage of MgSt on the micrometre-scale ibuprofen particles, the researchers demonstrated that mechanofusion gave a much thinner yet highly effective coating compared with traditional methods.

As well as much improved powder flow, the real surprise came when PhD student Ms Li Qu tested the coated drug's ability to be compacted into tablets. She found that despite the lubricant's presence, tablet formation was not substantially diminished and neither was the release rate of drug from the tablets.

THIS SINGLE-STEP, INNOVATIVE process for dry coating a drug powder with MgSt demonstrates for the first time improved flow, tabletability and maintenance of dissolution rate. This success with ibuprofen bodes well for other drugs and therefore for the wider pharmaceutical industry.

L. Qu et al., *Drug Development and Industrial Pharmacy*, DOI:10.3109/03639045.2014.908901, 2014



ToF-SIMS images showing coverage of ibuprofen powder (green) with 1% MgSt (red) using the industry standard method (left) and mechanofusion (right).



MAKING RED GAC SUITABLE FOR CULTIVATION

Momordica cochinchinensis or, as it is commonly called, red gac, is native to north-eastern Australia and South-East Asia. Its fruit has the highest levels of beta-carotene and lycopene of all known fruits, including carrots and tomatoes. This feature makes it appealing to develop as a commercial food crop. The plant is scarce in the wild, perhaps partly because male and female flowers are produced on separate plants and the male flowers tend to mature before the female flowers. This discrepancy adversely affects fruit production and would be a problem for commercial cultivation.

In an attempt to overcome this limitation, Dr Tien Huynh from RMIT University (RMITU) and Dr Sophie Parks from NSW Primary Industries are collaborating with Orga Corporation (Thailand) and Southern Seed Corporation (Vietnam). The team are investigating ways to preserve pollen for

controlled fertilisation. Successful pollen storage would improve the rate of fruit production to a level where commercial cultivation is viable.

Scanning electron microscopy (SEM) in the AMMRF Linked Laboratory at RMITU confirmed that the structure of frozen and refrigerated pollen was unchanged during long-term storage. Field experiments confirmed that stored pollen was suitable for producing fruit in the field. Fertilisation using stored pollen is now used on farms across Asia to ensure fruit production. In Australia, field trials are currently under way with the University of Newcastle for improved fruit quality. The aim is to produce fruit for the Asian market.



SEM image of red gac pollen after long-term storage.



METAL NANOPARTICLES FOR NANOSENSORS

Templates for metal nanoparticle composites are important for fabricating 2D materials for nanosensors. Nanosensors can be used as catalysts, to control chemical reactions, and as sensor systems, to detect small molecules in environmental applications. The manufacture of these nanoparticles focuses on maximising their active surface area, and controlling their size and morphology.

Manufacturing noble metal nanoparticles often involves the presence of stabilisers. Stabilisers control the nanoparticle's size and morphology, but they can cover the surface, blocking the binding molecule required for sensing. This is overcome by dispersing noble metal nanoparticles that are 'naked' (devoid of surfactants) on the surface of inorganic 2D materials.

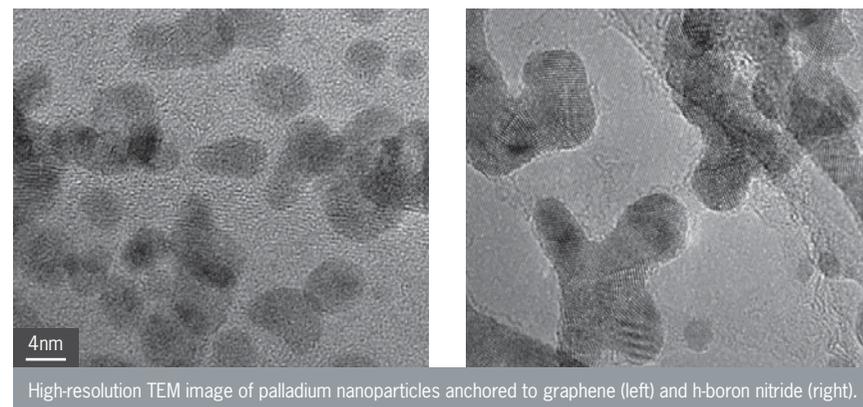
Prof. Colin Raston and his team covered graphene and hexagonal boron nitride (h-BN) nanosheets with 'naked' ultrafine nanoparticles of two noble metals

– palladium and platinum. Graphene and h-BN offer a high specific surface area, flexible structure, excellent mechanical strength and overall chemical stability, which suit nanosensor production.

Prof. Raston used transmission electron microscopy (TEM) in the AMMRF at the University of Western Australia to characterise graphene/h-BN–noble metal hybrids which were around five nanometres in diameter – ideal for catalytic reactions. High-resolution TEM and fast Fourier transform identified the characteristic lattice fingerprint for palladium and platinum nanoparticles, confirming the metals' presence.

These materials can now be used to fabricate devices using a drop-casting technique, also developed by Prof. Raston's research team. They may also have a role in organic transformations for the pharmaceutical industry.

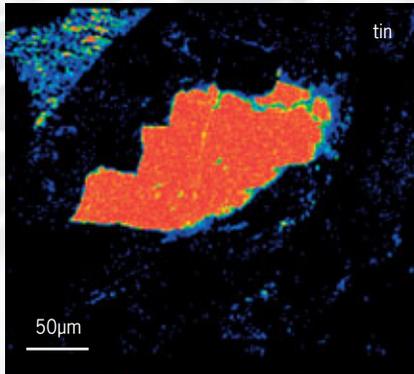
Chen et al., *Chemical Communications* 49(1160), 2013



High-resolution TEM image of palladium nanoparticles anchored to graphene (left) and h-boron nitride (right).



flagship epma

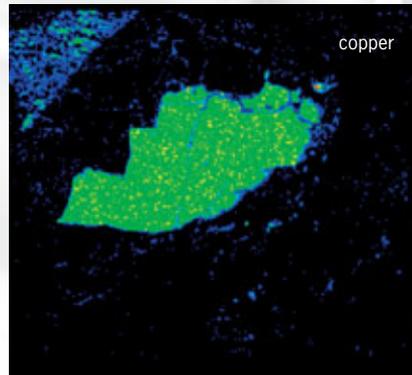


ELEMENTAL ANALYSIS of samples such as metals, geological materials, coal, ceramics, glass and plastics are enabled by the flagship electron probe microanalyser (EPMA) at the University of New South Wales (UNSW). Flagship Engineer, Dr Karen Privat manages this versatile instrument. Learn how the EPMA supports industry by enabling accurate analysis of potential silver deposits.

THE APPEALING LUSTRE OF SILVER (Ag) has seen it used in jewellery and ornaments for thousands of years. Today, silver consumption is dominated by industrial applications (44%), followed by jewellery and coins (31%). Australia has the second largest of the world's economic reserves of silver and ranks fourth in production after Mexico, China and Peru. Silver production in Australia was worth \$1.678 billion in 2012, continuing to

make silver minerals desirable targets for exploration.

In mineralised hydrothermal systems, heated fluids generated beneath the Earth's surface circulate and mix, getting trapped in cracks and faults where minerals can precipitate and form vein deposits. Hydrothermal mineral deposits are a significant source of precious and base metals such as gold, silver, copper, tin, lead, zinc, bismuth, antimony and tungsten.



INDUSTRY PARTNER, SILVER MINES Ltd, initiated a project to investigate the economic potential for exploiting silver-lead-zinc mineralisation as part of the Mole River Project, located in the New England region of northern NSW. A number of locations in the region are considered prospective for small, high-grade hydrothermal silver-lead-zinc deposits. The project aimed to understand the origin

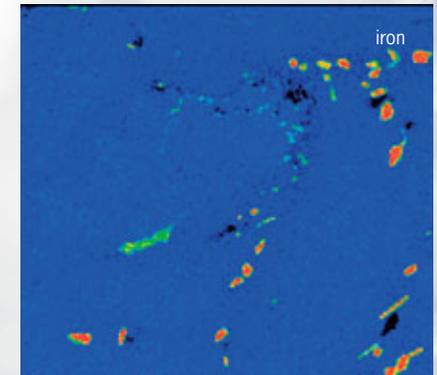


of the mineralisation and more about the geology of the region. It was also important to identify the sources of silver in the mineral system. Mineralisation in the area occurs as veins of polymetallic sulfides, dominated by sphalerite (zinc), arsenopyrite (arsenic) and galena (lead).

Ms Claire Orlov, an honours student working with Dr Ian Graham and A/Prof. David Cohen, used the flagship EPMA at UNSW to conduct microchemical analysis of individual sulfide minerals to determine the sources of silver in the deposits. Using EPMA data, Ms Orlov was able to determine that silver was present in small quantities of the minerals acanthite, pyragryrite, freibergite and stephanite, which contain 40–70% Ag by weight. Minor amounts of pure silver were also identified, and low levels were found in galena. Silver concentrations were found to be generally low across the samples used in the project,

which had been collected from a number of locations throughout the area.

SOME MISIDENTIFIED MINERALS were revealed by EPMA, most notably a common sulfide mineral that was originally labelled tetrahedrite, a Ag-bearing mineral, which was considered to be a primary source of high-grade silver in the area. This was actually found to be an unusual tin-iron-copper sulfide that is



as yet unidentified, and which contains no silver at all, as can be seen in the images.

THANKS TO THIS STUDY, the model of the Mole River silver-rich deposits has had to be rethought, affecting targets for future exploration. It may also help to improve silver prospecting on a regional scale.



EFFICIENCIES IN COPPER EXTRACTION

Sulfide and oxide minerals can be extracted from ore by a process called froth flotation, where a collector chemical is added to a mixture of water and finely ground ore. One end of the collector molecule attaches preferentially to the exposed sulfide or oxide minerals, while the other end repels water, carrying the mineral-containing particles to the surface on rising air bubbles, where they are recovered in the froth. Although this important industrial process has been used for more than a century, how particular collector molecules interact with mineral surfaces is not always known.

In a Linkage Project with Australian Metallurgical Services (AMS), Prof. Alan Buckley from the University of New South Wales set out to understand how a collector called hydroxamate binds to copper and iron minerals. Hydroxamate has been used to a limited extent to concentrate copper

minerals from some weathered sulfide ores. However, since good copper sulfide ore is running out, efficient ways are needed to extract copper from the more difficult oxidised ores.

The mechanisms by which hydroxamate adsorbs onto copper and iron minerals had been proposed based on X-ray photoelectron spectroscopy. Additional corroborating information was gathered by Prof. Buckley, working with Dr John Denman on the time-of-flight secondary ion mass spectrometry in the AMMRF at the University of South Australia. They discovered that on copper oxide, a collector multilayer is created that imparts very good floatability. This demonstrates how hydroxamate can recover copper from oxidised copper ores, and therefore render previously uneconomic mines potentially viable.

Buckley et al., *Minerals Engineering*, 64, 2014



Froth flotation of chalcopyrite, a copper-iron-sulfide mineral. Photograph courtesy of Sam Noonan.



NANOFIBRES FOR NERVOUS SYSTEM REPAIR

Nanofibrous scaffolds can be used in tissue engineering to provide structural support to growing cells in damaged tissue. For example, the scaffold can be wrapped around damaged nerves of people with acute trauma or neurodegenerative disorders. In many cases, nerve damage is permanent. This is changing, however, because this 'internal bandage' can provide support and growth factors to the tissue – allowing it to heal.

Ms Kiara Bruggeman at the Australian National University (ANU), under Dr David Nisbet's supervision, produced a polylactic acid nanofibre scaffold using electrospinning. The fibre was then made functional by attaching proteins to it. To optimise the effectiveness of the scaffold, it is important to determine the fibre dimensions, orientation, consistency, surface characteristics and any

undesirable artefacts. Ms Bruggeman used scanning electron microscopy (SEM) in the AMMRF at ANU to do this.

The team used protein immobilisation techniques to trap various proteins on the scaffold: proteins such as interleukin 10, which guides the immune response to nerve injury, and brain-derived neurotrophic factor, which promotes neuronal differentiation and proliferation. This allows more healing and recovery because the trapped proteins remain active for longer than would proteins in solution, which degrade or diffuse rapidly.

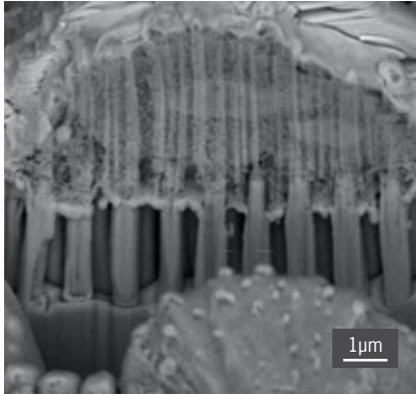
Since neural injuries can lead to irreversible damage and disability, these findings could transform treatment – and therefore the future quality of life – for those with nervous system conditions.

R. Chen et al., *Acta Biomaterialia* 8, 2012

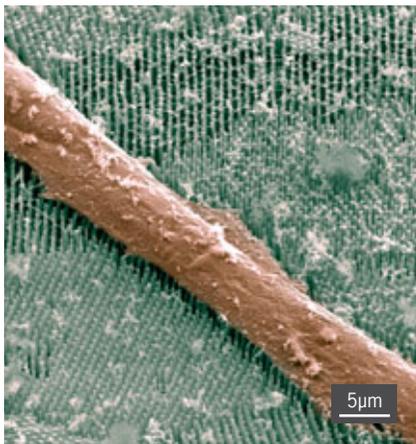


Colour-enhanced SEM image of the nanofibre scaffold.

flagship fib +



OUR FLAGSHIP FOCUSED ION BEAM (FIB) instruments are managed by Dr Animesh Basak at the University of Adelaide and by Dr Charlie Kong at the University of New South Wales. They are in huge demand for direct analysis of samples and preparing samples of hard materials for other forms of microscopy. Learn how the FIB at Adelaide is enabling Dr Roey Elnathan to study the interface between cells and silicon.



GENE THERAPY introduces new genetic material into targeted cells to counter disease-causing mutations. It is now recognised as a viable strategy for treating a number of disorders.

To develop new gene therapy approaches, researchers grow cells in the laboratory (cell culture). Introducing DNA and RNA into cultured cells is called transfection and helps researchers understand how genes work.

Dr Roey Elnathan and Dr Bahman Delalat, working with Prof. Nico Voelcker at the University of South Australia have developed arrays of vertically aligned silicon nanowires (VA-SiNW) to transfer DNA into cultured cells. The arrays, constructed from a silicon chip, resemble a tiny bed of nails. The construction method, called metal-assisted chemical etching, is easy to implement, inexpensive and not hazardous. It enables fine control over the length, diameter, shape, porosity and spacing of the nanowires. Dr Elnathan used scanning electron microscopy (SEM) in the AMMRF at the University of South Australia and the University of Adelaide to monitor this process. He aims to identify the optimal VA-SiNW architectures to promote molecular transport across the cell membrane.

CELLS WERE GROWN on the nanowire arrays (left), which had been coated with the DNA for transfer. Dr Elnathan compared a wide variety of nanowire architectures for their transfer efficiency



A cell culture flask in the lab.

and found that nanowire height was critical. Many cell types were tested successfully and even cells that are normally hard to transfect took up the DNA.

The combination of SEM and focused FIB milling allow Dr Elnathan to probe intricate interactions between VA-SiNW and cells – preserving the cell structure while revealing the nanowires inside (image top left).

These rapid advances in the fabrication of VA-SiNW arrays and their

subsequent interactions with diverse biocomponents are an interesting emerging platform, applicable in diverse life science contexts. The cost-effective manufacturing technique has the potential to make this efficient transfection technology widely accessible.

R. Elnathan, et al., *Nano Today* 9 (2), 2014

➕ EFFECT OF MICROGRAVITY ON TUMOUR CELLS

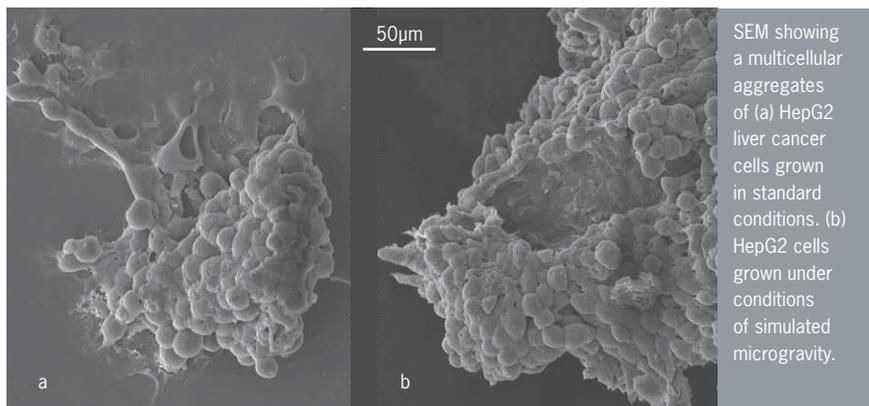
Astronauts have a high probability of being exposed to ionising cosmic and solar radiation, and have an increased risk of developing cancer. Tumourigenesis may be compounded by the relative lack of gravity in space, known as microgravity.

PhD student Mr Theo Orfanos, working with A/Prof. John Foster at the University of New South Wales (UNSW), is studying the effects of microgravity on tumourigenesis by comparing the morphology of cancerous cell aggregates grown under standard mammalian cell culture conditions with aggregates grown in simulated microgravity. Here, the cells are grown in a rotating wall vessel bioreactor, which forces the cells into a constant state of free-fall and, therefore, simulated microgravity.

Using scanning electron microscopy and analytical software in the AMMRF at UNSW, the researchers compared cancer cells grown in simulated microgravity with

those grown in standard culture conditions. They observed that multicellular aggregates of many types of cancer cells were much larger in simulated microgravity, but that the individual cells were almost half the size, a significant decrease. They also saw large smooth areas on the surface of these aggregates that were not present in standard culture conditions. By using a protein-based approach they demonstrated that exposure to microgravity also removes a molecular signalling pathway present in many of the cell types. These effects have not been seen before, making this a new frontier in cell biology.

With more space travel on the horizon, such as Mars missions and space tourism, the group hopes to expose any associated risks so that measures can be employed to protect the travellers.



SEM showing a multicellular aggregates of (a) HepG2 liver cancer cells grown in standard conditions. (b) HepG2 cells grown under conditions of simulated microgravity.

➕ 3D IMAGING OF HYDROGELS

Water-based gels (hydrogels) based on complex sugars (polysaccharides) have applications in protein separation and purification in biotechnology, and for drug delivery in medicine.

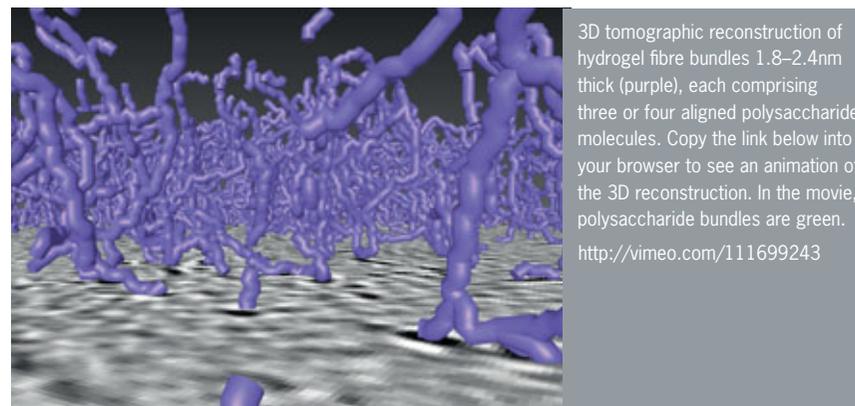
The chemistry of the component molecules and the solution in which they are formed control both how the hydrogels assemble and their macroscopic properties. To determine the basis of the macroscopic properties of hydrogels, Dr Andrew Leis and his colleagues from the CSIRO visualised the gels at the nanoscale in 3D.

Many techniques suitable for visualising ordered crystalline materials do not cope with heterogeneous materials such as hydrogels. Therefore, Dr Leis and his colleagues used electron tomography in the AMMRF Linked Laboratory at the CSIRO Australian Animal Health Laboratory to observe the gels. Visualising the actual gels is more informative than assuming gel characteristics based on models.

They saw that the nanoscale structure of the gels had different degrees of disorder, but still had defining characteristics. When hydrogels form at different pHs or in the presence of different ions, their macroscopic properties, such as stiffness and opacity, reconciled perfectly with what they expected to see at the nanoscale.

The team can now refine the dynamic simulations of molecular structure and function, and test the assumptions upon which these simulations are based. Because heterogeneities at the nanoscale can now be studied, fundamental questions can be answered about how the hydrogels self-assemble and the structural hierarchies that exist within them. In potential applications of hydrogels, perfecting their structure is likely to result in improved industrial efficiencies.

Leis et al., *Proceedings of the International Symposium on Computational Models for the Life Sciences*, pp. 206–213, 2013



3D tomographic reconstruction of hydrogel fibre bundles 1.8–2.4nm thick (purple), each comprising three or four aligned polysaccharide molecules. Copy the link below into your browser to see an animation of the 3D reconstruction. In the movie, polysaccharide bundles are green.
<http://vimeo.com/111699243>



REDUCING DIESEL POLLUTION

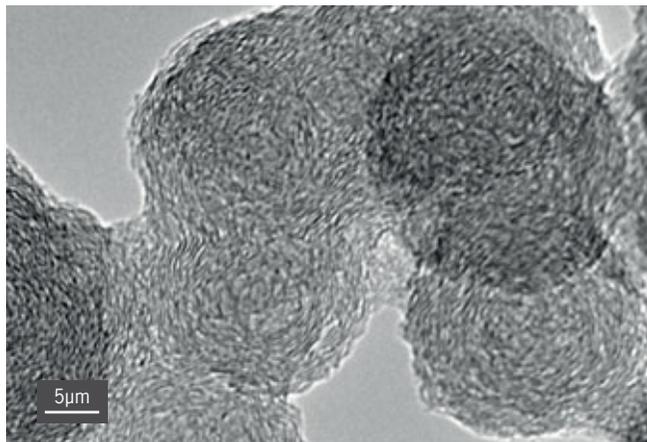
Diesel-powered vehicles and equipment are widely used due to their high fuel efficiency and durability. The downside is that they emit soot and particulate matter. These include sulfates, elemental carbon, organic and carcinogenic compounds, and heavy metals such as arsenic, selenium, cadmium and zinc. The ultrafine particulates, less than 100 nanometres in size, make up 90% of diesel soot pollution and are small enough to penetrate the lung cells. The public-health problems associated with diesel emissions have intensified scientific efforts to develop solutions for reducing these emissions.

Soot is formed by the conversion of the hydrocarbon gases to solid carbon-containing particles, a process that is far from being fully understood. Prof. Dongke Zhang and his research team at the University of Western Australia (UWA) are testing a new catalyst, called ferrous

picrate, to improve fuel efficiency and reduce soot emissions. The research is part of an ARC Linkage Project with partner organisations Fuel Technology Pty Ltd and BHP Billiton Iron Ore Pty Ltd.

The team used high-resolution microanalysis in the scanning and transmission electron microscopes in the AMMRF at UWA to study diesel soot from fuels with and without the catalyst treatments. The catalyst substantially improved combustion in diesel engines, leading to less fuel consumption and a reduction in the overall soot emission. The structure of the soot was the same with and without the catalyst, suggesting that the catalyst promotes efficient fuel combustion and leaves less hydrocarbon available to form soot.

D. Zhang, et al., *Proceedings of the of Combustion Institute* 34 (1), 2013



TEM image of soot from the catalyst-treated diesel.



NEW VIRUS COULD HOLD THE KEY

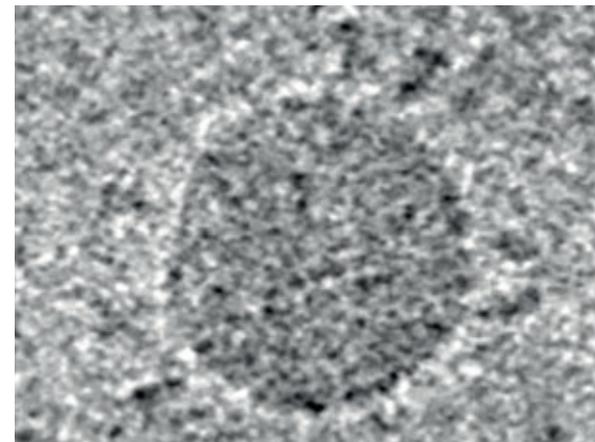
Mosquitoes transmit a range of diseases to humans and other animals. These include malaria and viral diseases such as dengue fever and Ross River fever. Understanding how mosquitoes handle viral infections could reveal new routes to preventing the spread of mosquito-borne viral diseases.

Dr David Warrilow from the Queensland Department of Health and Dr Dan Waterson from the University of Queensland (UQ) worked with researchers from around Australia to investigate a new species of mosquito virus discovered in Darwin. Casuarina virus, as it has been named, is a member of the family Mesoniviridae. This is the first report of a mesonivirus in Australia.

Cryo-electron microscopy in the AMMRF at UQ showed that the viruses are spherical particles 65 nanometres

in diameter with 15-nanometre-long club-shaped projections on their surface. The team also sequenced the RNA genome of this virus, which, along with the microscopic structural information builds a detailed picture of this new virus. It suggested that the projections consist of three copies of the protein arranged together and set into the virus's lipid coat. Surface proteins make a virus specific to its host by only recognising and sticking to complementary proteins on a particular cell type in a particular range of organisms.

Casuarina virus doesn't seem able to infect vertebrate cells or to be associated with any disease. However, it may be able to prevent mosquitoes being infected by other viruses, which makes it a potentially valuable tool in the fight against mosquito-borne viral diseases.



Cryo-electron micrograph of a casuarina virus particle showing surface projections. Copy the link below into your browser to see sequential views of a virus particle. <http://vimeo.com/111699178>



SUPERCAPACITORS TO GIVE E-CARS A BOOST

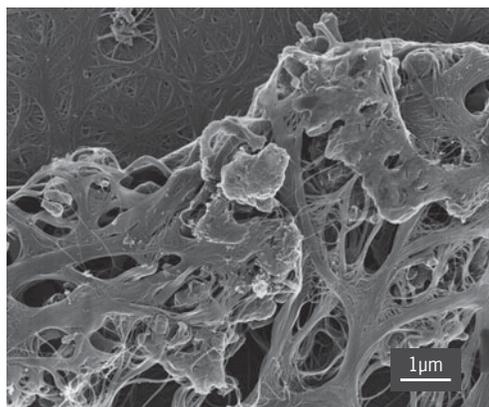
The amount of energy and the speed with which it can be delivered are the key attributes of a mobile energy source. The results of research into batteries with increased energy density are visible everywhere – from mobile phones to cordless power tools to the Tesla electric sports car. Vehicles in particular need an extra energy spurt for acceleration, and this is where supercapacitors come in. They hold limited charge, but can deliver it very quickly – the perfect complement to mass-storage batteries.

A team at Queensland University of Technology (QUT) led by Dr Jinzhang Liu and Prof. Nunzio Motta is developing new-generation supercapacitors using advanced carbon materials. They will be smaller, lighter, cooler and chemically stable. An electrochemical process exfoliates graphite to produce graphene sheets in a liquid suspension. Filtering the sheets out of this suspension results in

a thin ‘paper’ of graphene with extraordinary electrode properties. Double-walled carbon nanotubes were used to form a high-current collector sheet, and to form composite materials with graphene. A suite of microscopy techniques, including helium ion microscopy in the AMMRF Linked Laboratory at QUT, was used to characterise the graphene and carbon nanotubes.

The completed devices generated a power density a thousand times higher than current lithium batteries. This power density provides the oomph needed for starting and hill climbing. These carbon-based devices are both strong and light, making it possible that vehicles of the future could be powered by their own body panels.

M. Notarianni et al., *Nanotechnology* 25 (43) 2014



He ion microscope image of carbon nanotube-graphene composite.



PROTEIN STRUCTURES FOR MEMBRANE CAVES

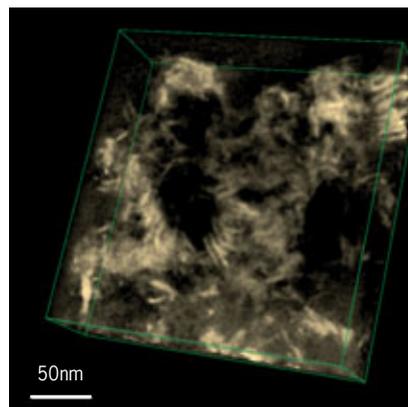
Caveolae are abundant nanoscale cave-shaped structures in the membrane of many types of cells, including those in muscles, fat tissue and blood vessels. They are thought to provide stretch resilience to muscles and blood vessels and manage a turnover of lipids in the membrane. Reduction or lack of the caveolar proteins leads to problems ranging from tumours and neuronal diseases to life-threatening abnormalities in skeletal and heart muscles, lipodystrophy and metabolic imbalance. These clinical symptoms are linked to abnormalities in caveolae formation but the cause is not understood at the molecular level.

Dr Oleksiy Kovtun at the University of Queensland (UQ) is attempting to decipher the molecular architecture and cooperative action of proteins with lipid membranes in caveolae formation. He isolated a set of caveolar proteins called cavins. Biophysical analysis revealed that

they formed a large hierarchical protein assembly. To understand the structure of this assembly, Dr Kovtun used X-ray crystallographic techniques. This revealed the nature of the first product in the hierarchy, but the form of larger cavin assemblies remained elusive.

By using electron microscopy (EM) in the AMMRF at UQ, he was able to make the breakthrough that revealed that the principal building block of cavin complexes are rod-shaped assemblies and that they have a specific arrangement in the final coat. EM imaging also suggested that cavin alone is sufficient for the characteristic membrane curvature of caveolae. These pioneering structural studies will help us to understand how caveola are formed and the causes of caveolae-linked pathologies.

O. Kovtun, et al. *Developmental Cell* 31, 2014



3D electron tomography image showing rod-shaped cavin assemblies. Copy the link below into your browser to see a tilt series of cavin rods.
<http://vimeo.com/111699242>



NANOWIRE COMPOSITION FOR OPTOELECTRONICS

Optoelectronics uses the combination of light and electronics to make more sensitive and improved devices, Semiconductor nanowires have the potential to be important building blocks in this area and are therefore attracting much attention from researchers. The appeal of this technology lies in the ability to fine-tune the wavelength. For example, the nanowire indium gallium arsenide (InGaAs) can be adjusted from the near infrared to the infrared region, making it suitable for applications in infrared emission lasers and photovoltaics.

The main challenge when producing semiconductor nanowires from materials such as InGaAs is controlling the nanowire composition, and achieving homogeneity along and across the nanowires. This is essential if the nanowires are to have any practical or commercial use.

PhD student Ms Amira Ameruddin, under the supervision of Prof. Chennupati Jagadish at the Australian National University (ANU), is studying the chemical composition of InGaAs nanowires. She used energy dispersive X-ray mapping with the scanning transmission electron microscope (STEM) in the AMMRF at the Australian National University to observe cross-sections of the nanowires. These techniques revealed that the nanowires have high-quality crystallinity, and uniform morphology and composition when grown under certain controllable conditions.

Her STEM results helped Ms Ameruddin to identify the optimum conditions for producing homogeneous nanowires. This is a significant step towards realising the full potential of InGaAs nanowires in future applications – including solar energy.



USING GEOLOGICAL TIME TO FIND GOLD

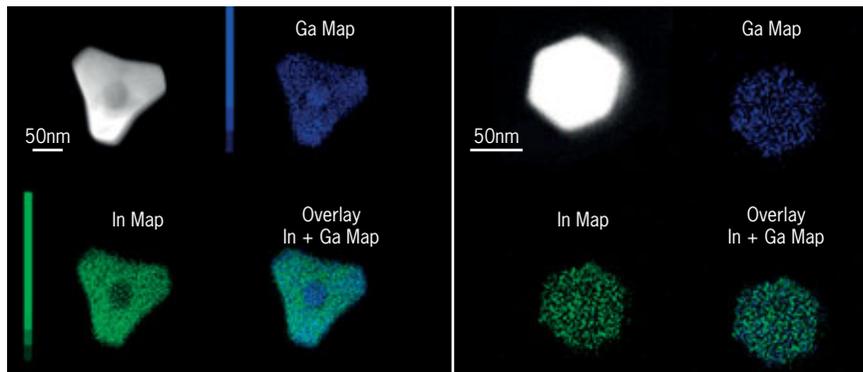
Time is often referred to as the fourth dimension (4D), and measuring geological time can be used to determine the evolution of any given terrane (a geologically consistent area of rock). In turn, geologists can use this 4D evolution to aid mineral exploration.

Working with colleagues from the National Geophysical Research Institute in Hyderabad, India, Prof. Neal McNaughton and his Curtin University (CU) colleagues measured the age of igneous titanite found in southern India. Titanite is a common mineral formed when granite magmas cool and crystallise. A sensitive, high-resolution ion microprobe (SHRIMP) was used to produce backscattered electron images in the AMMRF Linked Laboratory at CU – one of which is shown in the associated image. The brighter areas represent remnants

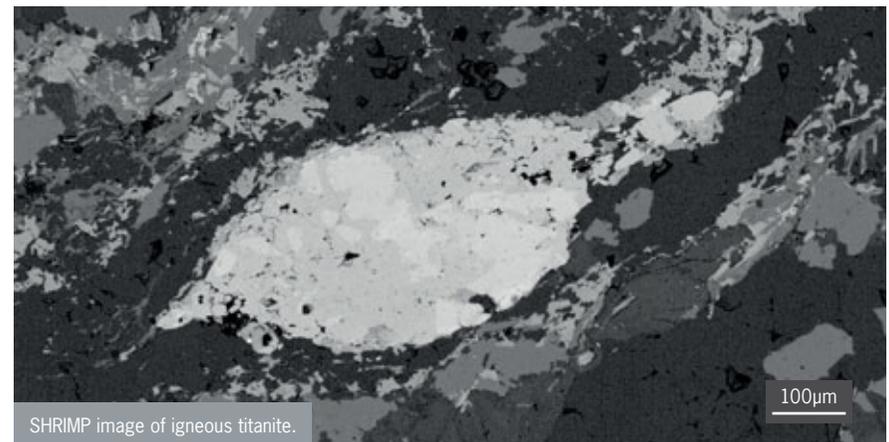
of igneous titanite formed 2,570 million years ago. The darker areas are recrystallised and altered titanite, formed when the original igneous grain was flattened during a subsequent deformation event 2,516 million years ago.

The biggest advantage of using SHRIMP is its capability to date both generations of titanite in situ on the same grain. Importantly, nearby gold mineralisation – which accompanied the titanite deformation – was independently dated with SHRIMP and yielded the same age. These data allowed the 4D evolution of the gold deposit to be quantified and integrated with similar data from other gold deposits. This led to a 4D picture of the mineralisation process in the terrane, providing new gold exploration concepts that are applicable worldwide.

Mohan et al., *Precambrian Research* 243, 2014



STEM images of cross-sections of InGaAs nanowires showing non-uniform (left) and uniform (right) composition.



SHRIMP image of igneous titanite.

Australian Research Council Future Fellows are leading young researchers forging a dynamic research path for a healthy and productive Australia. The AMMRF already provides microscopy to many of these researchers, enabling them to achieve the excellent results on which their awards are based. It is expected that over half of this year's science, technology, engineering and maths award winners will use microscopy to achieve their goals. Here are just some of the Fellows we support.



Australian
National
University

DR VANESSA ROBINS works with the X-ray microtomography group at the Australian National University as a mathematician developing new algorithms for extracting topological and geometric measures from 3D digital images. The Fellowship will enable her to develop new quantitative relationships between these measures of shape and the physical properties of microstructured materials. This will improve scientific understanding of natural materials and engineering design of advanced manufactured materials.



THE UNIVERSITY OF
SYDNEY

DR YANBO WANG'S primary research focus is structural characterisation of advanced structural and functional materials using transmission electron microscopy techniques, and the relationship between structure and mechanical properties. He is part of the Centre for Advanced Materials Technology at the University of Sydney.



THE UNIVERSITY OF
WESTERN AUSTRALIA

DR PETER MUNRO is currently developing theoretical models of optical microscopes, focusing on biomedical applications. He plans to use these models during his Future Fellowship to solve inverse problems in optical microscopy, with a view to seeing deeper into biological tissue. He is based in the Centre for Microscopy, Characterisation & Analysis at the University of Western Australia.



Australian
National
University

DR AJAY NARENDRA is interested in the navigational abilities of animals in different ecological and temporal niches. His research spans across fields of behavioural ecology, neuroethology, sensory ecology, cognition and community ecology. Dr Narendra is a researcher in the College of Medicine, Biology & Environment at the Australian National University.



THE UNIVERSITY
OF QUEENSLAND

DR CHUN-XIA ZHAO is an Associate Group leader at the Australian Institute for Bioengineering & Nanotechnology at the University of Queensland. She has been focusing on the development of soft and hard materials through designing new biomolecules (peptides and proteins), and developing new green platform technologies for drug delivery, vaccine delivery and controlled release.



A/PROF. VIPUL BANSAL, is the Director of the Ian Potter NanoBioSensing Facility, and leads the NanoBiotechnology Research Laboratory at RMIT University. His multidisciplinary team works on the interface of materials chemistry, molecular biology and bioelectrochemistry to develop a wide variety of multifunctional nanomaterials. Being able to control surface properties of these materials allows A/Prof. Bansal's team to use them for advanced biomimetics, biosensing, bioimaging, drug delivery, antibacterial, wound healing, photocatalysis and flexible electronics applications.



UNSW
AUSTRALIA

DR DEWEI CHU'S research focuses on novel nanoelectronics from solution-processed oxide thin films. He aims to theoretically and experimentally develop advanced ionic conductive oxide superlattices for new and completely different transistors to surmount the fundamental limit of current silicon semiconductor technologies. He is based at the University of New South Wales, in the School of Materials Science & Engineering.

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FEATURED IMAGE CREDITS

Covers, front & back: scanning electron micrograph of a scale from the fish, *Girella tricuspidata*
Dr Dianne Hughes, University of Sydney.

Back cover circle: colour-enhanced scanning electron micrograph of zinc oxide nanonails grown radially with a nanoparticle in the centre. The average diameter of the nanonail head is approximately 1.5µm, and the shank length is approximately 3µm.
Dr Jinzhang Liu, Queensland University of Technology.

Inside back cover: transmission electron micrograph of a section of optic nerve from an embryonic rabbit. Horizontal field of view is 27µm. Image courtesy of Dr Ian Kaplin.

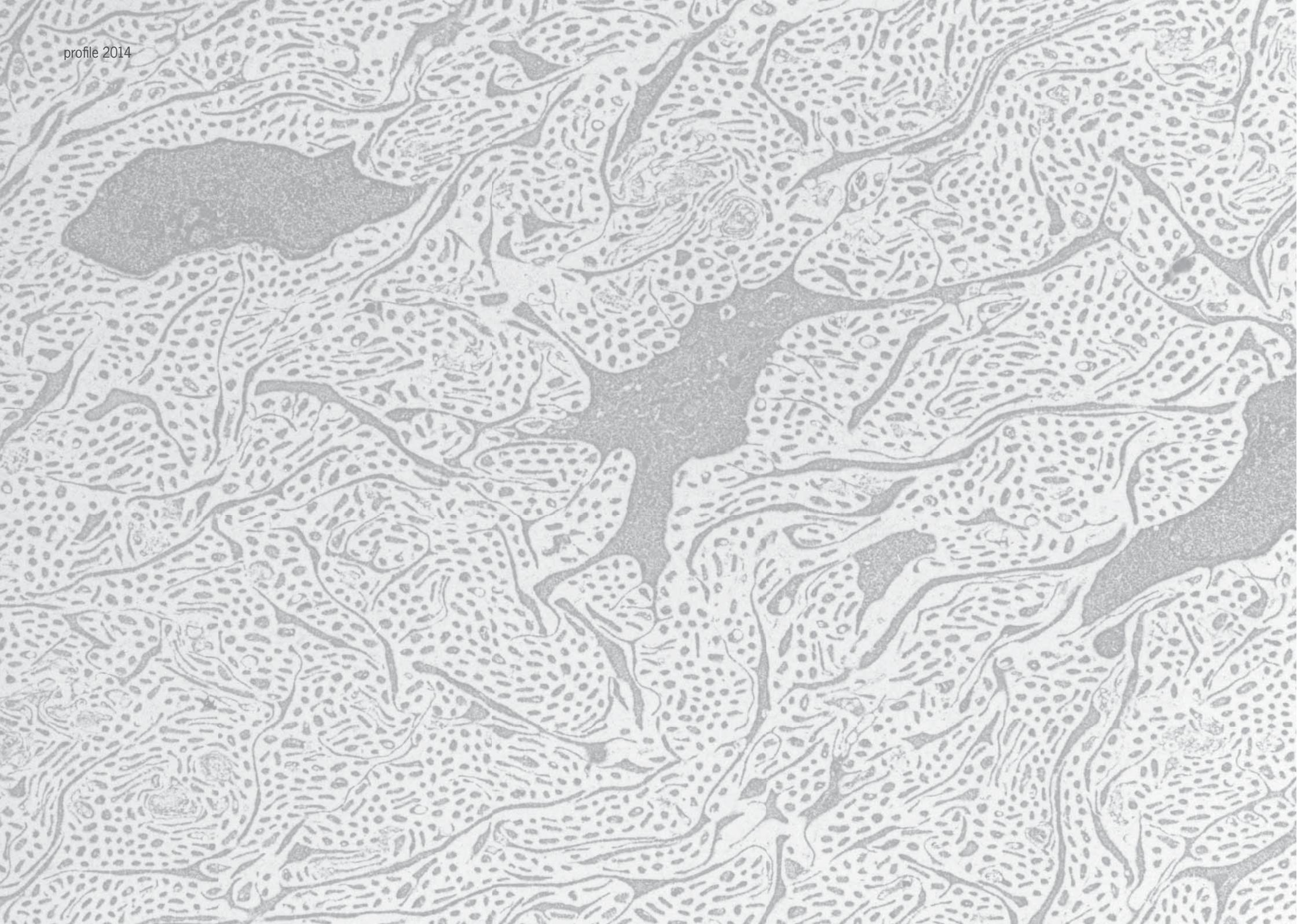
Contents: the Omicron Multiscan Lab Ultra-high Vacuum Scanning Tunneling Microscope (UHV-STM) at the Central Analytical Research Facility, Queensland University of Technology (QUT) – an AMMRF Linked Laboratory. Image courtesy of QUT.

Access: three-fold symmetric diffraction pattern of a pure silicon crystal with pseudo-colour rendering. Dr Hongwei Liu, University of Sydney

Research: electron backscatter diffraction image of a corrosion crack from a high-temperature austenitic steel. The red areas show the altered crystal structure around the grain boundaries that contribute to further cracking. The width of the area shown in the image is 1.4mm. See the full story on page 19. Mr Alex La Fontaine, University of Sydney

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