# PROFILE

Australian
Microscopy & Microanalysis
Research Facility

## 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

THE UNIVERSITY OF SYDNEY

UNSW THE UNIVERSITY OF NEW SOUTH WALES

Australian National University

THE UNIVERSITY OF WESTERN AUSTRALIA *Achieve International Excellence*

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

SOUTH AUSTRALIAN REGIONAL FACILITY (SARF)

THE UNIVERSITY of ADELAIDE

University of South Australia

Flinders UNIVERSITY

FUNDED BY

An Australian Government Initiative

National Collaborative Research Infrastructure Strategy

NSW GOVERNMENT

Queensland Government

Government of South Australia

The Government of Western Australia

# contents



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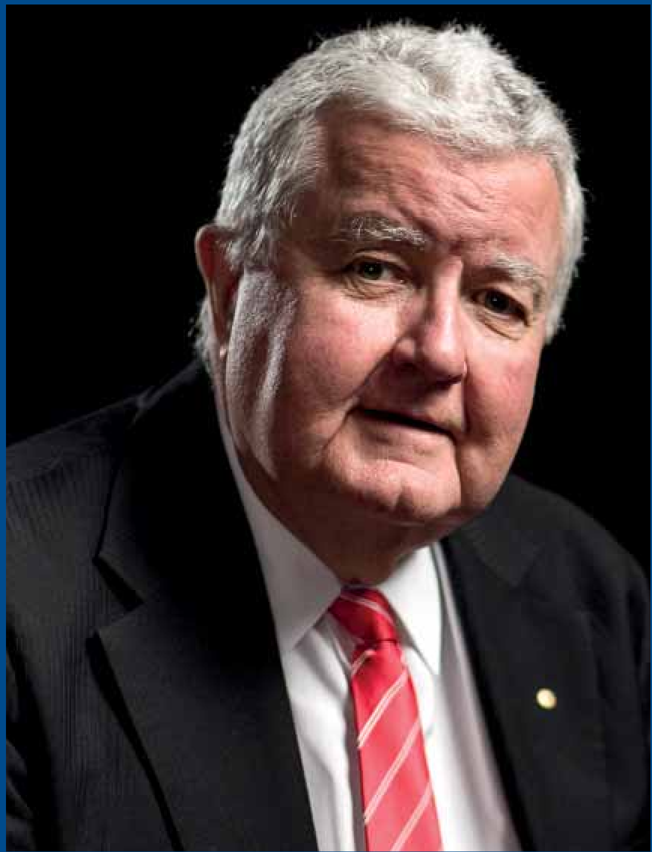
# *from the chief scientist*



Australia's future is underpinned by our ability to develop the science that makes us globally competitive and successful. Scientific developments play an important role in innovation and productivity in laboratories, universities and industry.

That's why world class research assets like the Australian Microscopy & Microanalysis Research Facility are a fundamental part of our national research effort.

The AMMRF has a well-established track record of providing independent expertise to the development of new products and processes by some of Australia's most highly regarded industries.

I commend the work of the AMMRF, as it continues to contribute to our future prosperity.

*Professor Ian Chubb AC*

Australia's Chief Scientist

## from the chair

I am always pleased to see the breadth of outcomes that are generated as a result of researchers accessing the AMMRF's collaborative research infrastructure. From basic research to the most applied industrial applications, the AMMRF has been involved in making a major contribution to Australia's innovation system.

Sometimes, the outcomes of high-quality research can become the first steps towards innovation and commercial impact. Taking these outcomes of research to the next level can lead to new enterprises or to improvements in existing businesses. The AMMRF's research involvement has made impacts across a diverse array of sectors extending from biomedical, to advanced manufacturing, ICT, mining and mineral resources and on to agriculture.

The AMMRF is focused on achieving results on two fronts. It provides world-class research infrastructure that enables scientists to make new discoveries and often, to provide seeds for innovation opportunities. On another front, the AMMRF engages directly with industry. Here, the AMMRF's ability to support complex but highly relevant industry research projects, as well as to support routine testing or analytical services, for a company is paramount. Significant achievements in industrial research integration are testimony to the capabilities of the AMMRF's expert staff and to the existence of world-class instrumentation within its laboratories.

The AMMRF Board is committed to maintaining this capability and is continuing to plan for the future so that the facility can play an ongoing role in meeting the grand research and technology challenges that face Australia.

*Dr Gregory R. Smith*
Chair of Board

## from the ceo

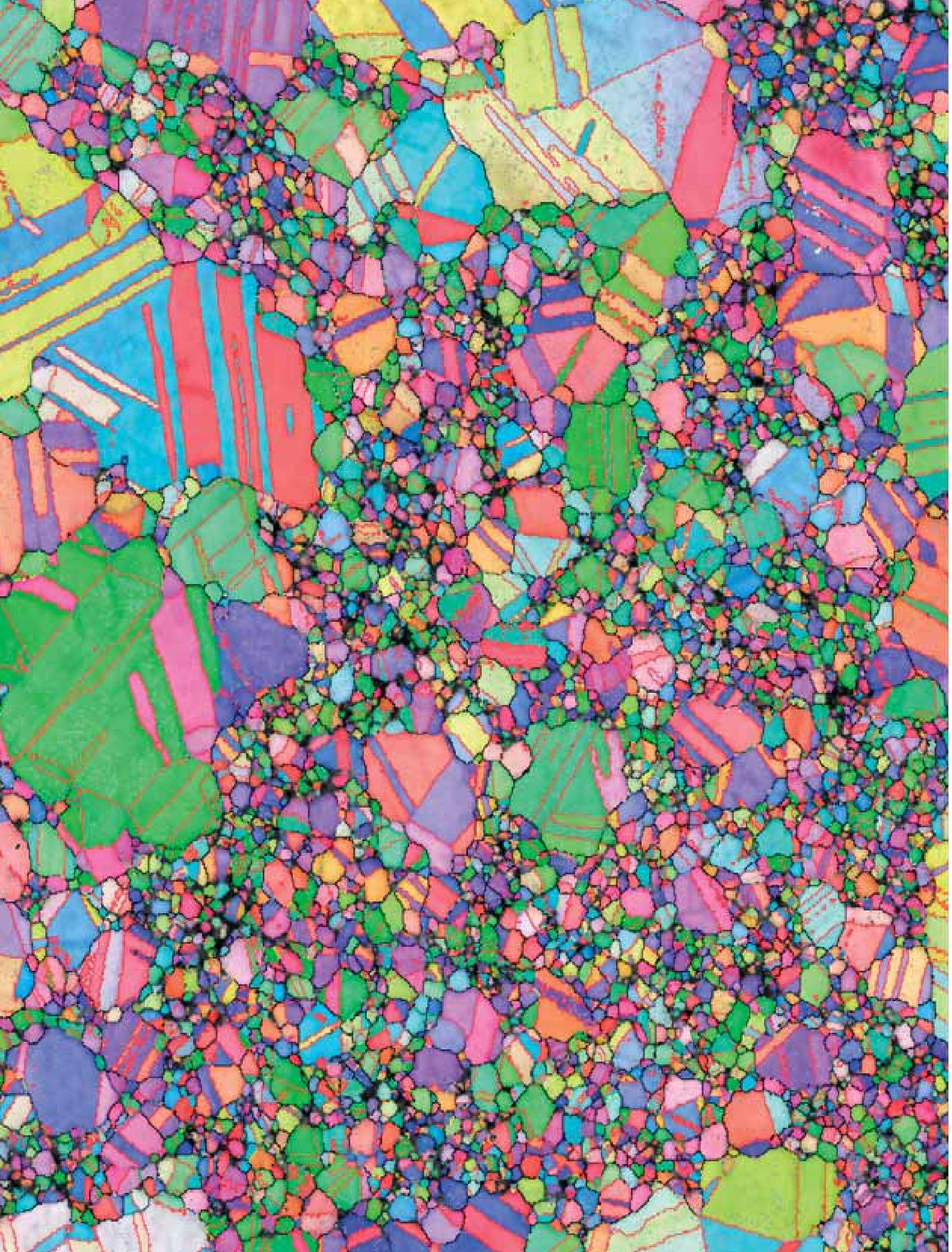
Australia is fortunate to possess a healthy scientific and technological capability. It is clear that we are entering a time where this capability will be put to the test as we address the increasingly acute challenges arising from our national strategic research priorities (SRPs). Broadly, Australia's SRPs relate to the environment, health, food and water, security, and enabling economic growth. Each of these has rich veins of fundamental science and applied technology and each has great potential to enhance the quality of life in Australia.

In this year's Profile the research highlights for me are the new and innovative developments in sensor and diagnostics technology and the new treatment approaches for diseases from glue ear and Hendra virus to cancer and major trauma. All these outcomes have enormous potential to improve the wellbeing of Australians and boost our economy through commercialisation and improved productivity.

Research infrastructure is clearly of crucial importance for the specific investigations that lie at the frontier of our research challenges. Therefore, the strength of our scientific and technological capacity, and its ability to drive innovation, is directly related to the strength of our research infrastructure. After reading this report, I trust that you will agree with me that the AMMRF displays all the hallmarks of a world-class facility, ready for the future as we continue to enable research of the highest quality.

*Prof. Simon P. Ringer*
Executive Director & CEO

# access
## advanced microscopy



ammrf
Australian
Microscopy & Microanalysis
Research Facility

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

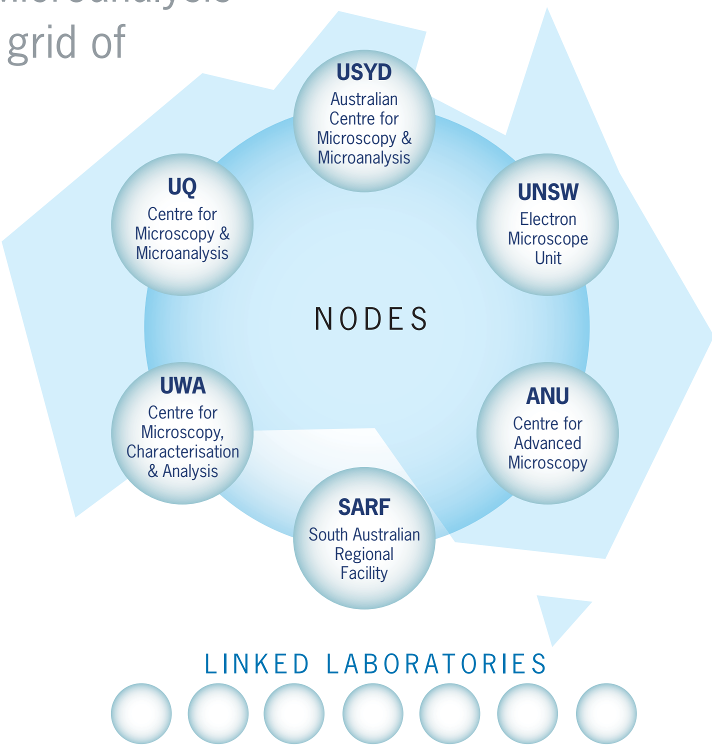
The Australian Microscopy & Microanalysis Research Facility is a national grid of equipment, online tools and expertise dedicated to nano-structural characterisation.

Major university-based centres are the core nodes of the AMMRF. This collaborative network is extended through linkages to specialist laboratories.

Our research infrastructure enables world-class scientific outcomes in fields ranging from engineering to agriculture, archaeology to medicine.

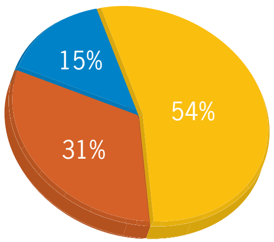
With well-established access policies and processes, the AMMRF supports around 3,000 researchers each year.

To contact us please see p36 or visit our website: ammrf.org.au

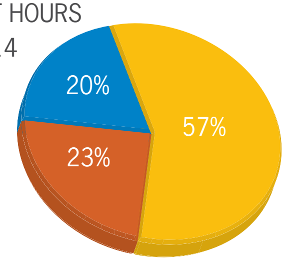



## 2012–13 in figures

USERS
Total: 2,986
By discipline:

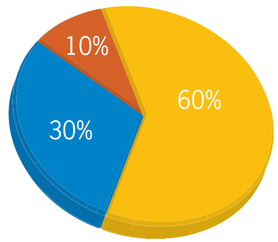



INSTRUMENT HOURS
Total: 218,214
By discipline:




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

USERS FROM INDUSTRY
By sector:




- Manufacturing
- Biomedical
- Environmental/Resources

## International Networks

We maintain and seek to expand a range of strategic international connections. These allow us to understand evolving technical trends and how they can be best used to address emerging global issues in research. Also our International Technical & User Advisory Group (ITUAG), comprises a panel of the world's leading microscopists.

In 2013 we used our formal linkages with EMBL Australia and Euro-BioImaging to host a masterclass in correlative light and electron microscopy (CLEM). This brought European and Australian experts together to provide professional training to Australian researchers and highlighted Australian CLEM capability.

Throughout 2013, the AMMRF at the University of Western Australia continued its important role as a network laboratory of the International Atomic Energy Agency testing samples collected to monitor global nuclear safeguards.

Our international connections enable us to maintain a leading position in world microscopy and collaborative research infrastruture.

# online tools

Our popular range of online tools supports Australian research. They enable researchers to identify appropriate microscopy techniques and expert staff, to learn online, and to analyse and manage their data.

## MYSCOPE

This innovation in training for advanced research provides e-learning modules that help new users learn the fundamentals of microscopy techniques and instrument operation.

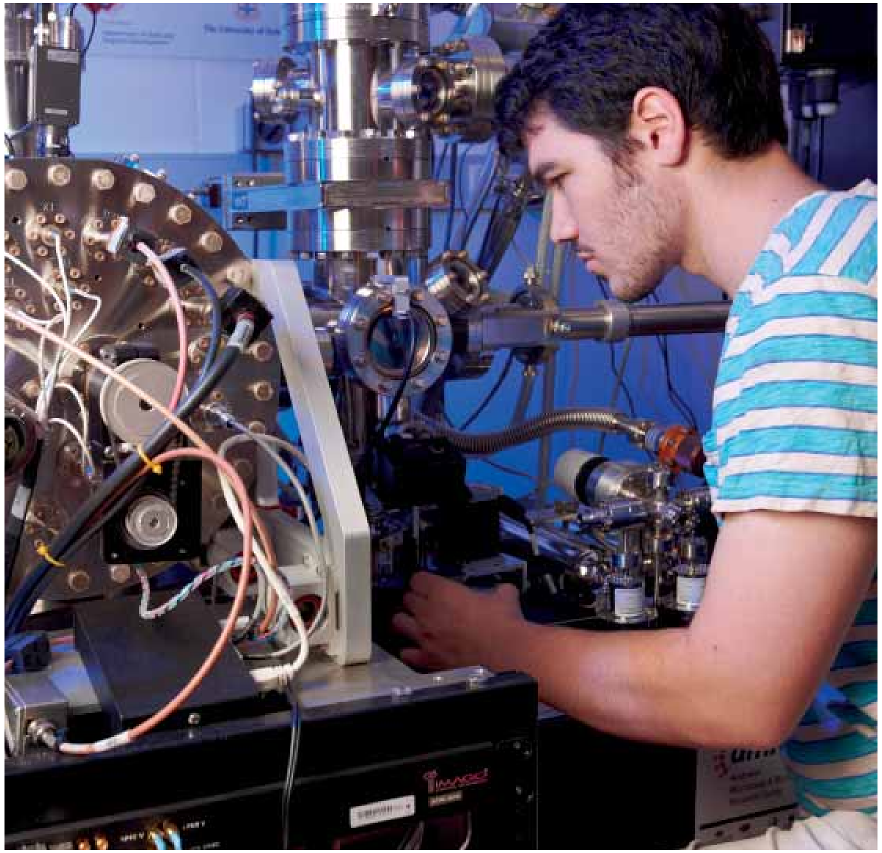
Integrating with traditional learning environments, each module has an interactive virtual instrument, step-by-step instructions and a range of additional resources. This provides a flexible, individual learning path that prepares trainees for intensive one-on-one instruction. Better-prepared trainees improve efficiency and access to the instruments in the AMMRF nodes.

MyScope has taken the AMMRF around the world, with 57,809 visitors in the last year and visitor numbers continue to grow. MyScope supports learning in many more ways than originally foreseen, and is available 24/7 to everyone.

## CVL

The Characterisation Virtual Laboratory (CVL) software infrastructure is being developed to make the benefits of high performance computing accessible to more Australian researchers. It is a cloud application that can be accessed remotely, providing researchers with a ready-made interactive analysis environment for multi-modal and multi-scale data. This allows scientists to focus on using tools and sophisticated analysis techniques, rather than on installing software or copying data.

The AMMRF has developed the atom probe 'workbench', which is on the brink of deployment. Development of the X-ray microtomography 'workbench' by our node at the Australian National University is well underway. These virtual workbenches will allow researchers to store, analyse, visualise and share their data. The CVL is being developed by a consortium including Monash University, the University of Queensland, the Australian National University and the University of Sydney.



## TECHNIQUE FINDER

An online tool to help you to identify the appropriate microscopy techniques, instrument location and staff to answer your research questions. Our experts will guide you through the planning, training, data collection and interpretation stages of your experiment.

To access our online tools visit ammrf.org.au

**57,809**

MyScope visitors in the last year

# advanced microscopy

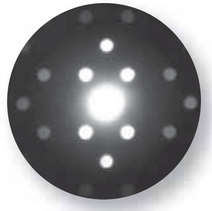
EXPERTISE, EFFICIENCY, EXCELLENCE

Our collaborative facility brings together specialised resources efficiently, making it easier for researchers to access microscopy and microanalysis.

The range of our instruments and techniques develops in response to the demands of the Australian research community and emerging technological trends. Our instruments, research environments and constantly evolving techniques help researchers address pressing issues such as sustainability, health, and agriculture.

Our expert staff support and guide users through planning, training, data collection and analysis to maximise high-quality outcomes.

At the heart of our collaborative infrastructure are flagship instruments. These are world-class platforms, many of which are unique in Australia, and each is supported by a dedicated engineer. These, like the rest of our capability are available to all. Research outcomes from these instruments are flagged.

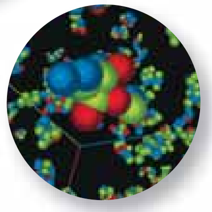


## Transmission Electron Microscopy

Imaging & Analytical Spectroscopy
Cryo-Techniques & Tomography
Phase & Z-contrast Imaging
Electron Diffraction

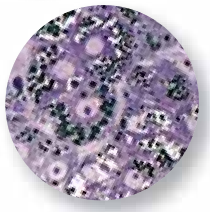
## Specimen Preparation

Biological & Materials
Cell Culturing & Molecular Preparation
Thermomechanical Processing
Ion Milling & Machining
Ion Implantation



## Ion & Spectroscopy Platforms

Nanoscale Mass Spectroscopy
Atom Probe Tomography
Nuclear Magnetic Resonance

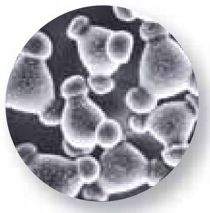


## Light and Laser Optics

Confocal, Fluorescence & Optical Microscopy
Flow Cytometry & Cell Sorting
Live-cell Imaging
Vibrational & Laser Spectroscopy
Laser Microdissection

## Scanned Probe Techniques

Atomic Force Microscopy
Scanning Tunneling Microscopy
Near-field Scanning Optical Microscopy

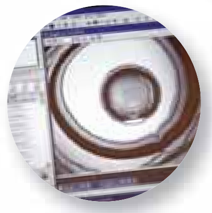


## Scanning Electron Microscopy

Imaging & Analytical Spectroscopy
In-situ Imaging & Testing
Cathodoluminescence
Electron Backscatter Diffraction

## X-ray Technologies

X-ray Diffraction
X-ray Fluorescence
X-ray Micro- and Nanotomography



## Visualisation and Simulation

Computed Spectroscopy
Computed Diffraction
Image Simulation & Analysis
Data Mining

# services to industry

global ADVANTAGE competitive exploration transport ENGINEERING health WATER quality MINING solar PATENTS coatings aviation ENERGY minerals optics fertility innovation nano technology medicine CONSTRUCTION bio fuel implants testing SUSTAINABILITY CONSERVATION food manufacturing climate culture security communications computing brewing DISCOVERY agriculture environment future sensors growth knowledge R&D resources

**100+**

Australian and international companies benefited from our research infrastructure in the past year alone.

## GRANT-AIDED PARTNERSHIPS

This includes Australian Research Council (ARC) 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.

## 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.

# meet the researchers

Dr Lauren Burns recently completed her PhD at RMIT University and is now a research engineer at Boeing Research and Technology – Australia.

## Lauren's highlight

*"Using software to stitch together the 2D slices taken from the micro-CT to create a 3D image that gave me an unprecedented insight into the internal structure of trees."*

Access to the AMMRF was important for my PhD research and the aerospace engineering industry in general because high quality imaging enables us to understand the failure modes of different materials and structures. This helps us design lighter aircraft that are cheaper and more environmentally friendly to operate.

I was investigating bio-inspired design of aerospace parts. I looked into nature, particularly trees and wood, as a shortcut to finding innovative ways to design aerospace carbon fibre composite joints that are stronger and lighter than current designs (full story on p.14).

By using scanning electron microscopy at RMIT University and micro-computed tomography at the University of Sydney I examined the micro-structure of both carbon fibre composite parts and tree branch–trunk joints.

I received a grant from the AMMRF to travel to Sydney to use the micro-CT equipment. I was able to pinpoint the toughening mechanisms that occur within wood and compare the similarities and differences in the way that carbon fibre composite breaks. These images have now been published in several journal papers.

Probably the highlight of working with the AMMRF was my positive experience of the people. They were willing to take the time to show me how to use the equipment and optimise the settings to get the best images for my purposes.

## Iyer's highlight

*"Late nights with Prof. Martin Saunders, capturing fullerene assemblies at the nanoscale, resulting in a "Hot Article" published in the Royal Society of Chemistry journal."*

The facilities at the AMMRF at the University of Western Australia are pivotal for the success of my research and have played a very important role right from the characterisation of nanoparticles following production, to in-vitro and in-vivo analysis in cell lines and animal models.

My research focuses on developing nanoparticle-based therapeutic modalities that mainly aim to overcome traditional approaches for treatment of medical emergencies (full story on p.33).

Microscopy forms an integral part of my research. AMMRF staff have played a pivotal role in enabling me to answer research questions crucial for design of nanoscale drug delivery/imaging agents, right from composition to cellular interactions. The technical and scientific support to interpret data beyond acquisition has been extremely valuable.

I use the AMMRF at UWA for transmission electron microscopy (TEM), scanning electron microscopy (SEM), confocal, NanoSIMS, live cell imaging, flow-cytometry and multispectral imaging. I have received tremendous support from the AMMRF staff: from sample preparation, imaging, analysis, interpretation of data, experimental design and most importantly research output for conferences and publications.

Dr K. Swaminathan Iyer is an ARC Australian Research Fellow in the School of Chemistry and Biochemistry at the University of Western Australia and founder of Eridan Technology Pty Ltd.

Dr Cristi Ciobanu is a Research Associate in the School of Earth and Environmental Sciences at the University of Adelaide.

Cristi's highlight

*"...a paper accepted by the journal* Precambrian Research *in which my group successfully dated the hematite in the giant Olympic Dam deposit – this is the first direct estimation of how old this mineral system actually is. This would not have been possible without the range of instrumentation offered by the AMMRF."*

I specialise in economic mineral deposits and the investigation of ore-forming reactions at the scale at which they take place. This involves microanalysis, at the nano- to micron-scales of the minerals that comprise an ore body (full story on p. 14).

Access to state-of-the-art microanalytical facilities is essential for my research, for publishing the results of this work in leading international peer-reviewed journals, for creating a credible and sustainable scientific profile, and successfully developing new topics of investigation. My track record of 49 publications is due, in no small part, to very good access to AMMRF top-class facilities.

Since the platform was established, I have become a heavy user of the focussed ion beam (FIB) at the University of Adelaide. It has opened up huge possibilities in my research area, specifically, to extract material from the surface of a sample for analysis by TEM. This is supplemented by SEM, electron microprobe and laser-ablation inductively coupled plasma mass spectrometry. I have also used facilities at University of New South Wales and the University of Western Australia.

Instrument usage at subsidised rates is vital to my work and the practical and logistical assistance received from AMMRF staff has been equally important. This assistance has enabled the maximum to be obtained from each session, and has ensured that the results are of the maximum quality achievable.

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Ren's highlight

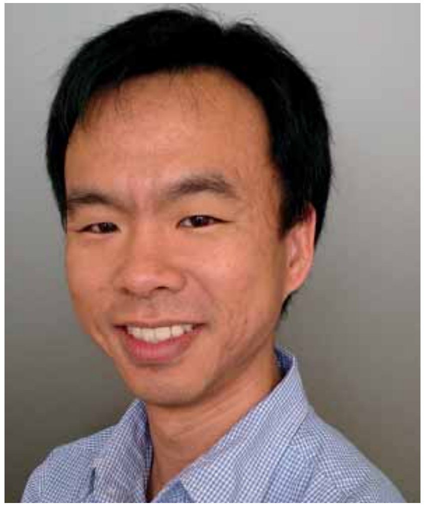
*"As all the ToF-SIMS data was analysed graphically I realised that it all made sense and the results were as good as we hoped they would be."*

I investigate our antimicrobial peptides once they are attached to biomaterials (full story on p. 28). Collaborators in the University of South Australia (UniSA) told us about the AMMRF and it was critical that we had access to ToF-SIMS to elucidate the surface orientation of our novel coatings. It was a fast and relatively straightforward method for our surface characterisation needs.

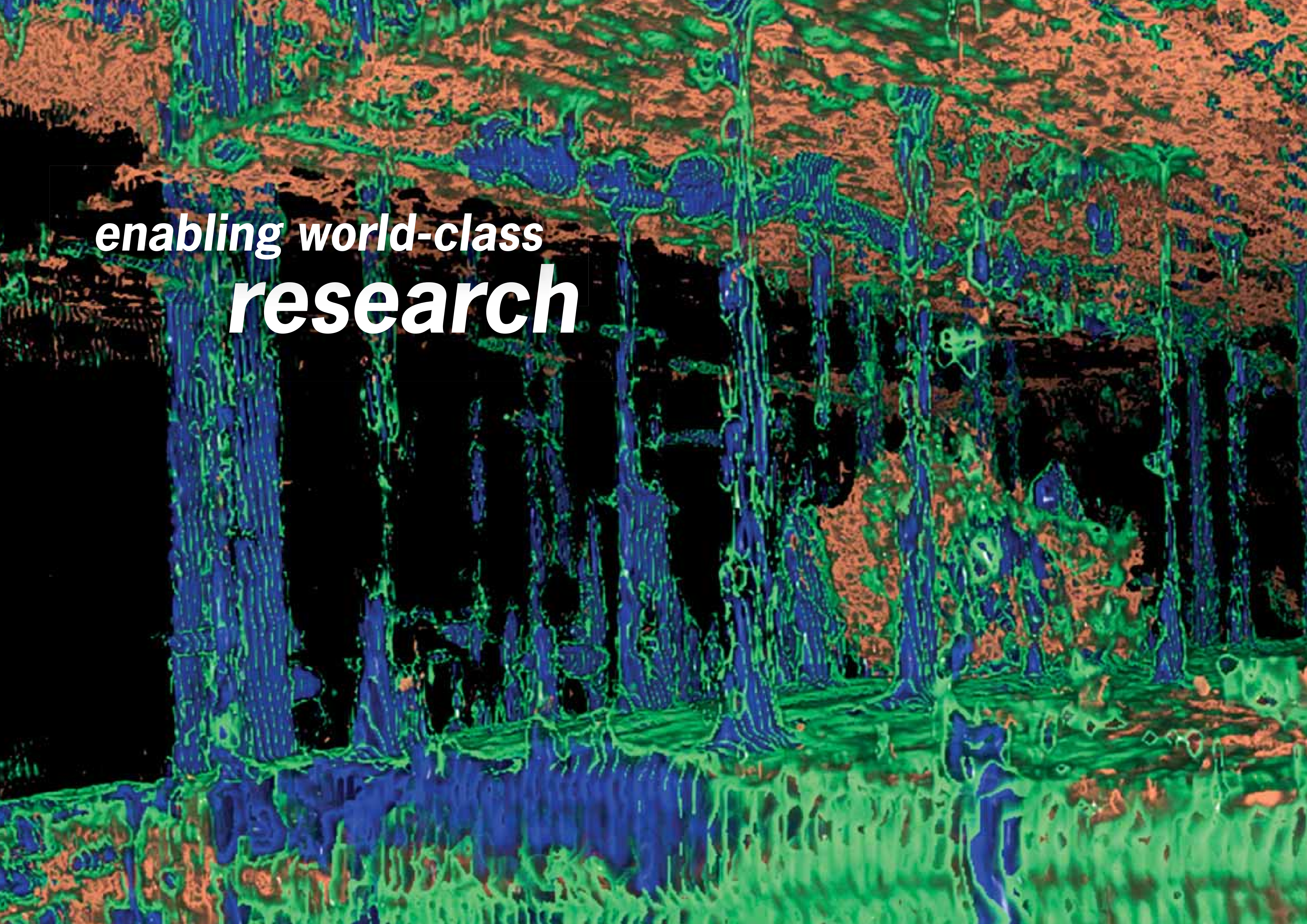
John Denman [the Flagship Engineer] was especially helpful in teaching me the theories behind ToF-SIMS and the methods of analysis. This was essential for us as it was a new technique for our group and the support was critical in our investigations. I also used SEM in the AMMRF at the University of New South Wales node to look at bacterial growth on the surfaces.

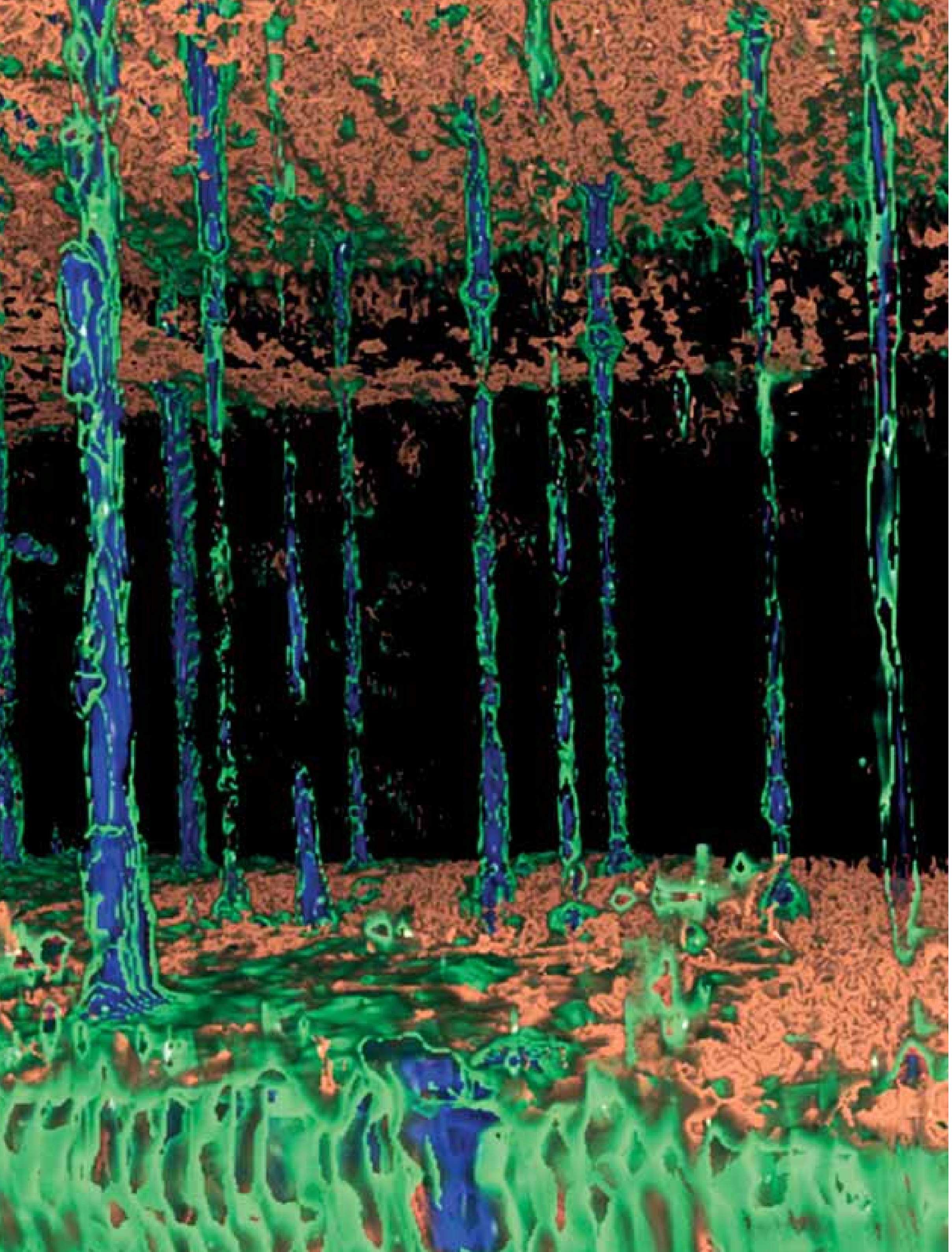
Having the Travel Access Program allowed us to have face-to-face discussions with the technicians that operate the instrument in UniSA. This allowed the experiments to proceed with minimal delays.

Dr Ren Chen recently completed his PhD and is now a Research Associate in the School of Chemistry at the University of New South Wales.



# enabling world-class research





Australian
Microscopy & Microanalysis
Research Facility

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 document some of our contributions to Australia's Strategic Research Priorities, as indicated by these icons:

food & water assets

population health & wellbeing

a changing environment

securing Australia's place in the world

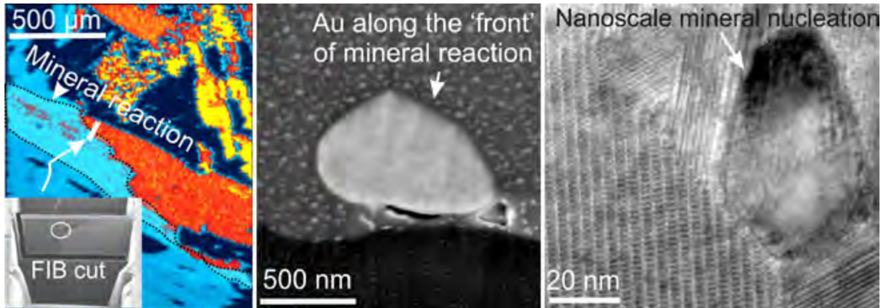
productivity & economic growth

## FINDING INVISIBLE GOLD

An economically significant amount of gold is 'invisible', existing either as gold nanoparticles or bound up in crystal lattices with other elements to form a variety of gold-containing minerals. The concentration of gold in different minerals depends on how the gold first came to the surface of the Earth and the impact of subsequent geological events. These events, including heat, pressure and interaction with infiltrating fluids, can mobilise the gold and cause it to be transported away from its original location. This can lead to it being dissipated or cause it to concentrate within certain bismuth-containing minerals, such as copper–lead–bismuth–sulphides (Cu–Pb–Bi–S). These are particularly good at attracting and trapping gold. Evidence of these movements can be seen at the micro- and nano-scale and can have important implications for gold recovery.

Dr Cristiana Ciobanu and her colleagues from the University of Adelaide are investigating Cu–Pb–Bi–S containing trace amounts of gold by using the complementary techniques of laser-ablation inductively-coupled-plasma mass-spectrometry (LA-ICP-MS), focused ion beam–scanning electron microscopy (FIB-SEM) and transmission electron microscopy (TEM). Sequentially applied, these techniques allowed visualisation of the mineral's chemistry and structure at different scales. They demonstrated that areas with subtle chemical variation, and where gold is present in unexpected places, are also sites of nanoscale mineral nucleation. Gold was seen locked at the 'reaction front' where it has precipitated out of solution as fluid moved through the mineral.

Nigel Cook et al. *Economic Geology* 108, 2013




LA-ICPMS map (left) where the lowest levels of copper are shown in pale blue and highest levels in yellow. The inset shows the FIB section made at the location of the white line in the LA-ICPMS map, with a circle around the gold particle shown in the close-up FIB–SEM image (centre). On the right is a TEM image.

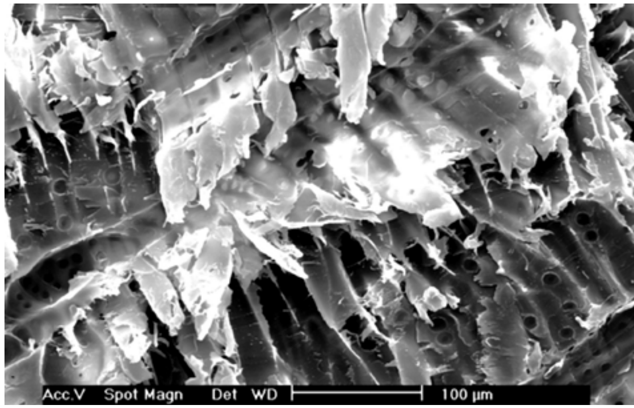
## BIOMIMETIC AVIATION INNOVATION: T-JOINTS

As aviation manufacturing moves to using more polymer composites, new approaches are needed to create high-strength joints. Current methods of joining composite materials tend not to be strong enough to allow full benefit to be gained from the light weight of the composite materials.

PhD student Ms Lauren Burns, an Amelia Earhart Fellow (2010), worked with Dr Stefanie Feih and Prof. Adrian Mouritz at RMIT University (RMITU) on a project with Boeing Research and Technology Australia. Ms Burns set out to develop more damage tolerant T-joints for composite materials used in aviation. Taking tree branches as an evolutionarily successful model of T-joints, Prof. Mouritz's team evaluated the structure, load-bearing and failure properties of tree branches compared to the conventionally engineered T-joints used for composites. They used X-ray microtomography in the AMMRF at the University of Sydney and scanning electron microscopy (SEM) in the Linked Lab at RMITU to understand joint failures across a range of scales. They found three crucial factors contributed to the strength of attachment of the branch to the trunk: an integrated design with the branch embedded into the centre of the trunk; fibres running in the direction of the major stresses; and different fibril densities across the joint to spread out the strain evenly.

The researchers then replicated aspects of tree joints in experimental composite joints. Their modifications achieved improved flexibility and, although the damage onset happened earlier, it progressed more slowly, improving the overall damage tolerance and making it more manageable. Evolutionary success continues to provide design inspiration.

LA Burns et al., *Composite Structures* 94, 2012




SEM showing fibres across a branch fracture surface (left). Scan the QR code to see the X-ray microtomography movie of a branch joint.

# impact energy

## CHALLENGE

The highways of the energy industry are the 30,000km of gas pipelines transporting gas across the country. Their estimated replacement cost exceeds $40 billion. Defects can occur in pipeline welds due to hydrogen-assisted cold cracking (HACC), which can cause welds to fail soon after their construction. The high-strength, low-alloy steels now favoured for pipelines may induce welds more prone to HACC and the fastest pipe welding consumable (new metal melted in during welding) itself introduces hydrogen. With huge costs at stake, the industry needs to find a sweet-spot between the speed and efficiency of pipeline construction, and the risk of cracking.

## SOLUTION

Mr Walter Costin and Dr Animesh Basak at the University of Adelaide, as part of the Energy Pipelines CRC, are investigating the structural basis underlying this problem.

Weld metal differs from the surrounding metal in composition and structure; properties that are affected by the temperature, and chemistry of consumables. The resulting structures are morphologically complex with corresponding variation in local mechanical properties. Conventional mechanical tests of welds at the macro-scale only measure average material properties rather than those of the specific microstructural regions that may have contributed to the failure.

The Adelaide team is using the AMMRF flagship focused ion beam to prepare, manipulate and analyse tiny cantilever-shaped samples from precisely defined regions of welds containing different types of ferrite. They then use electron back-scatter diffraction to examine the resulting cracks. Fine interlocking grains of acicular ferrite were seen to continually deflect the crack path, preventing micro-cracks from reaching the critical length for failure. Ferrite with larger grains in a more parallel alignment put up far less resistance to crack propagation.


## IMPACT

Natural gas provides 20% of Australia's energy. This is expected to double in the next 20 years.

### safer and more manageable pipelines with corresponding cost savings through:

- more reliable welds
- construction efficiency improved by controlling conditions to produce acicular ferrite welds.

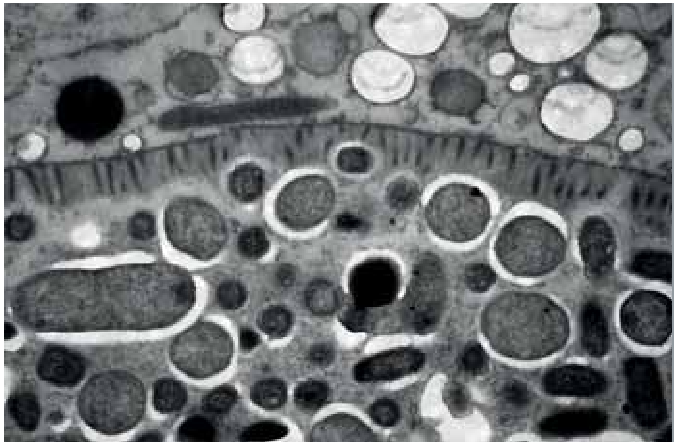

Image courtesy of Dr Ian Brown.

## ADVANCING THE FIGHT AGAINST SHIGELLOSIS

165 million people worldwide each year suffer from Shigellosis, or bacillary dysentery, caused by the very infectious *Shigella* bacteria. Bacteria invade the colonic and rectal cells of their host and cause severe clinical symptoms ranging from watery diarrhea to severe dysentery and fever. *Shigella flexneri* is the major disease-causing *Shigella* species in the developing world but as it only infects humans and other primates there is currently no simple animal model available. This has been one of the major limitations to vaccine development. However, the use of the nematode *C. elegans* as an animal model for several microbial diseases has recently been gaining momentum. This stems from the fact that like the human intestine, the nematode intestine is lined with cells with finger-like microvilli on their surface anchored into a very similar intracellular skeleton.

The feasibility of using *C. elegans* as a model for shigellosis is being investigated by Ms Divya George and A/Prof. Naresh Verma at the Australian National University (ANU). Ms George uses transmission electron microscopy (TEM) in the AMMRF at ANU to explore the interactions between *S. flexneri* and *C. elegans*. She found that before entering the cells the bacteria break down the surface microvilli. Fluid then accumulates within the intestinal cells and body cavity, reminiscent of the watery diarrhea in human infection.

This is the first observation of *S. flexneri* invasion of *C. elegans* intestinal cells and brings us a step closer to confirming *C. elegans* as a viable and valid model to study prevention and treatments for shigellosis.



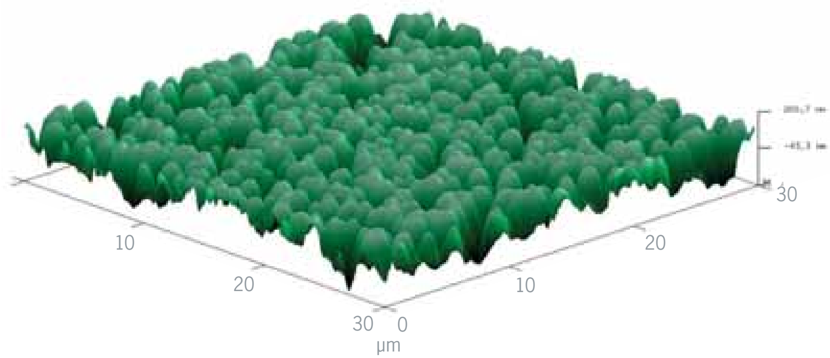
TEM image of a transverse section a nematode showing *S. flexneri* (round mid grey structures) in the intestinal lumen (lower area) and within an epithelial cell (upper area). The horizontal field of view is 10μm.

## SOLAR GETS ROUGH

Thin film polycrystalline silicon solar cells on low-cost glass substrates are now one of the most practical materials for producing commercial units for solar power generation. They combine the high conversion efficiency of crystalline silicon and the low-cost of thin film silicon. PhD student Zamir Pakhuruddin, working with Dr Sergey Varlamov from the University of New South Wales (UNSW), is using liquid phase crystallisation to create high-quality polycrystalline silicon layers on glass substrates for solar cells. This process achieves grains up to millimetres in size making them far superior to those produced by conventional solid phase crystallisation (SPC). The electronic properties of solar cells on glass substrates also show a vast improvement over SPC silicon with significantly greater open-circuit voltages.

To boost the solar cells' efficiency further, the photocurrent needs to be increased.

This can be done by trapping more light. To this end Mr Pakhuruddin is developing rougher silicon films that will trap and absorb more useful photons from the solar spectrum, thereby generating the increased photocurrent. To characterise the surface morphology of his new designs he makes extensive use of atomic force microscopy (AFM) and scanning electron microscopy (SEM) in the AMMRF at UNSW. By optimising light trapping strategies and fabrication processes, Mr Pakhuruddin had already seen increases in optical absorption with his more textured films indicating the likelihood of increased photocurrent and conversion efficiency in the final solar cells.




AFM image of textured silicon film on a glass substrate.

# impact diagnostics

## CHALLENGE

Blood samples are required for diagnostic tests for diseases such as Dengue, HIV and many cancers, as well as testing for banned substances. These must be processed in the lab before detection of the target molecules can be carried out. The collection of blood samples from infected patients with traditional needles and syringes also presents a considerable personal hazard to health workers.

A diagnostic test where no processing and no needles are required would be quicker, cheaper and safer while opening up opportunities for innovative companies in a growing industry currently valued at $6 billion worldwide.

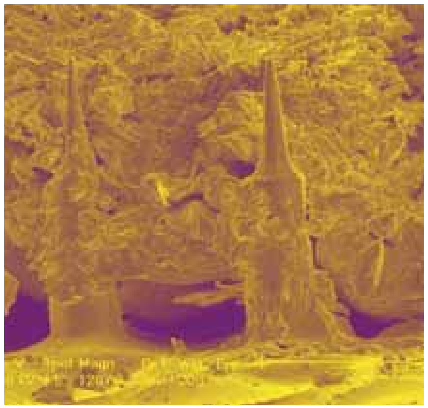
## SOLUTION

Drs David Muller and Simon Corrie, Prof. Mark Kendall and colleagues at the University of Queensland (UQ) are developing microprojection arrays (MPAs) to selectively collect disease-specific molecules directly from the skin of patients. This needle-free biomarker capture system requires no post-collection processing and testing occurs right on the array.

MPAs are structurally similar to the Nanopatch™ being developed for vaccine delivery (described in the 2011 Profile). Arrays of microprojections are coated with antibodies against the target molecule. When the coated MPAs are applied to the skin any target molecule present under the skin's protective surface will bind to the antibodies and be immobilised ready for detection.

The team tested their system on mice injected with a protein diagnostic for Dengue infection. The Dengue protein was detected with a sensitivity similar to current methods. The researchers used scanning electron microscopy in the AMMRF at the University of Queensland to analyse skin penetration of MPAs with different projection lengths (100µm in the image) and shapes, and X-ray photoelectron spectroscopy to analyse the coating process. These techniques inform the optimisation of their technology.

The diagnostics team works closely with Vaxxas, the company commercialising the Nanopatch™, to share knowledge and expertise. Commercialisation of MPAs for diagnostics is also now underway and the work protected under patent PCT/AU2009/000637.



## IMPACT

### Needle-free diagnostic tools

will make crucial diagnostics

### quicker, easier, safer and cheaper

This innovation could bring economic benefits through the

### $6 billion molecular diagnostics global market

**CASE STUDY**: SOLAR ENERGY INNOVATION BY DYESOL

Dyesol Ltd (ASX: DYE) is a publicly listed company with a mission to commercialise dye-sensitised solar cell (DSC) technology. Dyesol has been developing this technology since incorporation in 2004 and is the exponent of considerable IP, including a number of patents and substantial process know-how, for the application of DSC technology onto various substrates. Dyesol produces DSC materials for sale and offers a range of DSC prototyping, assembly, and testing equipment to its customers around the world.

The traditional liquid DSC is fabricated through the application of thin layers of key materials (nano-porous titania, ruthenium dye, electrolyte, platinum catalyst) onto a glass or metal substrate. Technology developments in 2013 have heralded a new era with promising advancements in so-called solid-state dye solar cells. These cells use a related set of key materials, including: nano-porous titania, perovskite light absorber, and solid organic hole transport material. Further R&D work and, in particular industrial scale-up and process development work on this new variation in the technology, is required and ongoing. Laboratory processes such as spin coating or physical vapour deposition for the technology will be replaced by industrially scalable and lower cost processes.

Characterisation of the various materials is critical for an in-depth understanding of the effect of raw materials specifications and process parameters on product performance. Of particular challenge is that while liquid DSC materials utilised very thin (~10 μm) layers of nano-sized constituents, solid-state variations use even thinner layers, with typical film thicknesses of <500 nm. Dyesol has some in-house facilities but makes considerable use of the AMMRF at the Australian National University, for more high-end characterisation. Scanning electron microscopy, transmission electron microscopy and energy dispersive X-ray spectroscopy are examples of critical tools to evaluate variations in device characteristics at the length scales of relevance for this nano-sized set of materials.

DSC, as a value-add technology, enables photovoltaic functionality to be incorporated into the manufacturing process for new building materials such as glass façade and metal sheet roofing. There is a growing global demand for green buildings and green building materials and the Company sees a strong demand for its products when commercialised. The size of the solar photovoltaic market is large and growing. Dyesol's DSC technology is likely to expand the market for solar by adding photovoltaic functionality to products which are currently not solar, in particular building integrated photovoltaics. The work at the AMMRF's ANU node is crucial in maintaining Dyesol's leading position in the solar market place. Achieving success in this area has directly applicable commercial and environmental benefits.

Dr Olivier Bellon, Project Manager – Device R&D, Dyesol (www.dyesol.com)

*"The assistance of the experts and the use of advanced technology housed at the AMMRF has been invaluable to our laboratory in the evaluation of new products and solving complex problems."*

Terry Martyn,
GM Holden Ltd

*"As the SEM is a crucial part of Qantas Engineering's business, this [AMMRF] course allowed our staff to be professionally up-skilled to the high standard that is expected in our industry"*

Wayne Pinder, Engine Fleet Engineering, Qantas Australia

## **CASE STUDY**: PRODUCT INNOVATION BY CALIX

Calix Ltd has developed a low-emissions technology for processing carbonate minerals, with a focus on processing magnesite ($MgCO_3$) to magnesia (MgO). In the Calix process the ground particles lose about half their mass in several seconds. This process has applications in a range of innovative agricultural, building, water and energy products.

To obtain insights into the nature of the magnesia product, and the market opportunities that derive from those characteristics, Calix relied on the facilities at the AMMRF at the University of Sydney.

Mineral analysis of materials from our mine in South Australia showed a magnesite with silica impurities in the form of talc. This material was processed in our pilot plant in Victoria, and the XRD analysis was consistent with the product being a composite of nano-crystalline magnesia. This intriguing result enabled Calix to connect to the academic literature on nano-MgO, which had demonstrated, in the past decade, a broad range of anti-fungal, anti-bacterial and anti-viral actions from these high-surface-area particles. Scanning electron microscopy showed that the particles, of 1–50 micrometres, were characterised by very rough surfaces, which led Calix to the hypothesis that their particles might show similar responses. The high surface area also provides excellent properties for water treatment applications, including heavy metal extraction.

Calix developed techniques to optimise the anti-microbial properties. The results of petri-dish experiments and the first field trials have now validated that hypothesis, first formed from the AMMRF data. The Calix factory at Bacchus Marsh in Melbourne was commissioned in July 2013, with a capacity of 30,000 tonnes. Calix is now bringing a range of anti-microbial and water treatment products to the market.

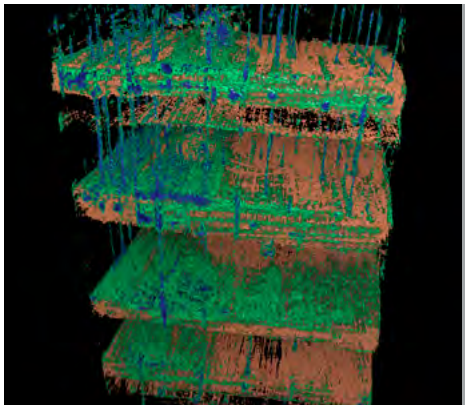
Dr Mark Sceats, Chief Scientist, Calix (www.calix.com.au)

## A NEW APPROACH TO WOOD PRESERVATION

Wood is nature's structural material par excellence and its virtues are obvious. One problem, however, is its longevity when used outdoors. Most woods, except for a handful that are naturally resistant to biological organisms, will quickly rot in the ground. To overcome this problem wood was commonly preserved with a solution of metal salts containing copper, chromium and arsenic. This preservative is still used today, but more environmentally friendly copper-based preservatives, free of chromium and arsenic, are preferred for residential uses. Insoluble copper micro- and nanoparticles are available as an alternative to traditional soluble copper salts. There has been debate over their effectiveness so Prof. Philip Evans from the University of British Columbia and colleagues used helical X-ray microtomography in the AMMRF at the Australian National University to investigate the basis of particulate copper's action at the microscopic level.

The wood's microstructure gives access to destructive fungi through flow paths; rays, resin canals and cut cell lumens. In treated wood, the copper was seen to accumulate in these paths. It was also detected in wood cell walls close to high concentrations of copper particles. The team's results suggest that the copper particles remain in the flow paths where they are needed, providing local concentrations of toxic copper to act against colonising fungi. The team plan to use X-ray microtomography to fully test this hypothesis and link the micro-distribution of copper to the performance of treated wood in the field.

Evans PD, et al. in Nicholas DD, et al., eds *Deterioration and Protection of Sustainable Biomaterials* (in-press bk-2013-00388v)



Reconstructed images of a 19 mm long pine sample showing the particulate copper (green) in the flow channels of the wood. Different bands in the sample have been made transparent to allow visualisation of the copper. Scan the QR code to see on video the power of 3D reconstruction in visualising and understanding data.

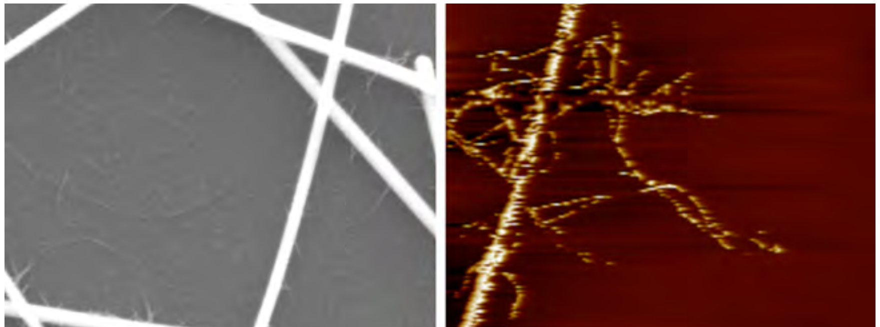
## SEE-THROUGH FLEXIBLE ELECTRODES

Cheap, flexible optoelectronic devices such as organic light-emitting diodes, plastic solar cells and flexible televisions are set to become multibillion dollar industries. They will rely on cheap, easily processed transparent electrodes. The Transparent Electrodes for Plastic Electronics Research Cluster (TEPERC) is looking for ways to produce suitable electrodes.

Indium tin oxide (ITO), the currently available material for transparent electrodes, while transparent, tends to crack when bent and therefore is not suited for high throughput methods such as roll-to-roll manufacturing. Indium is also a very rare and expensive element that is energy intensive to produce.

Silver nanowire networks (SNNs) show promise as ITO replacements. They are flexible and cheap to manufacture. However, to achieve optical transparency, they consist of a network of wires with micrometre-scale gaps between the wires. These gaps make them less conductive than a solid electrode. Dr Andrew Stapleton from Flinders University, as part of TEPERC, is improving these SNNs by bridging the gaps with a secondary conducting network of single-walled carbon nanotubes (SWCNT)s. He found that the silver nanowires form electrical connections with the SWCNTs and allow more charge to be collected and transferred.

Dr Stapelton used scanning electron microscopy and peak force tunnelling atomic force microscopy (PF-TUNA) in the AMMRF at Flinders University to investigate the structure and conduction paths in silver nanowires interwoven with SWCNT. His findings pave the way for commercial development of a new generation of highly efficient nanocomposite electrodes to supply this burgeoning industry.



SEM image of the network (left) and PF-TUNA current map a single silver nanowire with attached SWCNTs (right). Scale: width of SEM is 3.55 micrometres and of TUNA is 4.8 micrometres.

# impact child health

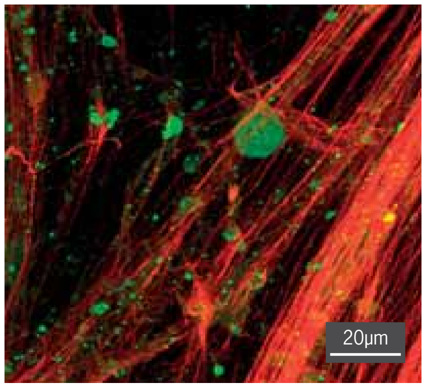
## CHALLENGE

In our 2011 Profile, we highlighted how Dr Ruth Thornton had improved our understanding of middle ear infections, also known as glue ear or otitis media (OM). Approximately 659,000 Australians suffered from OM in 2008, of whom 55% were under 15. Treatment cost $391.6 million. Dr Thornton from the University of Western Australia (UWA) had discovered a range of infection-causing bacteria that exist in the middle ear. Hiding within sticky biofilms and inside cells that line the inner ear, the bacteria evade the action of commonly prescribed antibiotics and act as reservoirs for re-infection.

## SOLUTION

Dr Thornton has continued to use the AMMRF at UWA to progress towards an effective treatment. She noticed that the biofilms are very similar to those found in the lungs of patients suffering from cystic fibrosis (CF). She realised that this similarity might make glue ear succumb to the same drug that is used to break up the biofilms and thin out the lung secretions in CF. Dr Thornton therefore tested the drug, Dornase alfa (a human DNAse), on samples from the ears of children with otitis media. By using confocal microscopy she showed that the drug effectively broke down the biofilms.

Because this drug is already in use for CF it was quick to get to clinical trials for the new application. Consequently clinical trials are now underway in 60 children under the age of five who are each receiving Dornase alfa in one ear during grommet surgery. The other ear will be used as a comparison and receive only surgery. Results will be collected over two years, and a larger national trial is planned. The trial will also extend to indigenous children, who suffer from high rates of severe chronic middle ear infections.




## IMPACT

Dr Thornton says "…more effective treatments will hopefully lead to

**improved hearing, better learning outcomes and a reduced burden on children and their families"**

The new treatment could also:

**reduce healthcare costs by $2.8 million nationally**

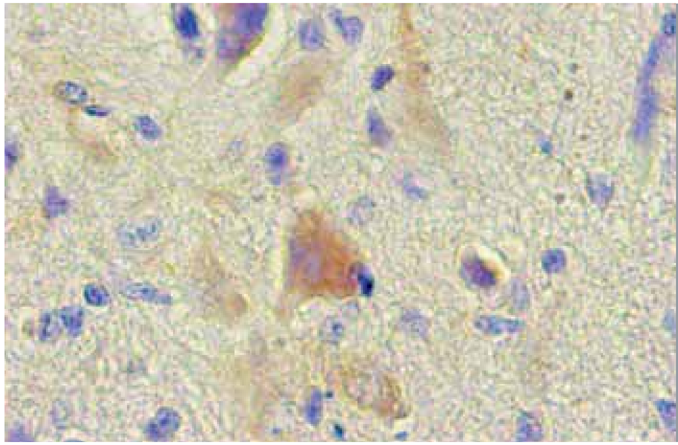
This is the direct annual hospital cost of OM surgical treatment. More effective treatment could also reduce indirect costs of educational interventions.

## UNDERSTANDING SUDDEN INFANT DEATH SYNDROME (SIDS)

SIDS is the main cause of death in young babies, 1–12 months of age, in the developed world. SIDS and obstructive sleep apnoea (OSA) are disorders of arousal and sleep. OSA causes periods of reduced oxygen exposure and both children and adults with OSA are harder to arouse from sleep than healthy people. Mr Nicholas Hunt, Prof. Karen Waters and Dr Rita Machaalani wanted to discover the mechanisms by which environmental conditions known to be predisposing factors for SIDS, like prone sleeping and cigarette smoke, could affect arousal in a similar way to OSA and explain their role in SIDS.

The protein, orexin (also called hypocretin), is a major regulator of sleep and arousal and acts by binding to specific proteins called orexin receptors found in many areas of the brain. The team examined the effects of nicotine and intermittent periods of reduced oxygen on the levels of orexin receptors in piglet brains, particularly in an area involved in arousal and sleep. By using powerful analytical techniques applied to light microscopy in the AMMRF at the University of Sydney they were able to show that these exposures increased the levels of orexin receptors. Functionally, in the piglets, this was associated with decreased arousability. Extrapolating to SIDS and OSA, the results indicate that cigarette smoking and the reduced oxygen experienced by babies if sleeping on their stomach or with their heads covered, can increase orexin receptors, which could be the mechanism by which the decreased arousability occurs.

Nicholas Hunt et al., *Brain Research* 1508, 2013



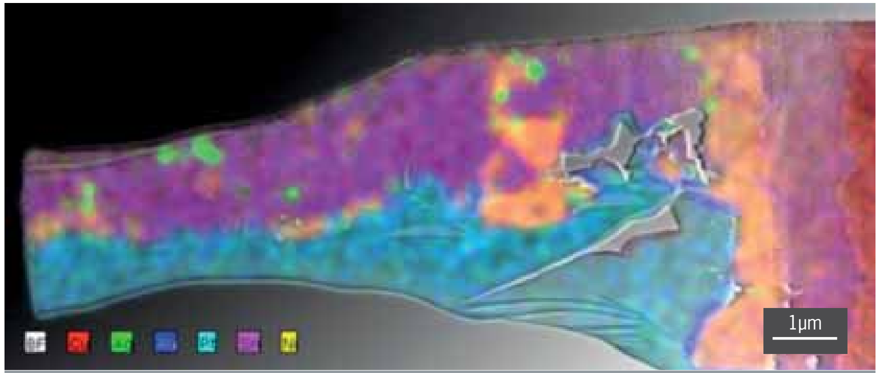
Light microscope image of part of a piglet brain showing orexin receptors in brown. The horizontal field of view is 128μm.

## SOLDERING ON WITH REDUCED TOXICITY: MICROELECTRONICS

Semiconductor industries are seeking alternatives to lead-based solders in order to meet world-wide regulatory requirements restricting their use. Currently, tin-based solders are used, but as devices are getting smaller, the solder spots also need to be smaller. This means that they get hotter, which in turn changes their crystalline structure in such as way as to shorten the solder's lifetime. This adversely affects reliability of microelectronic devices. One solution may be to incorporate nanoscale reinforcement particles to restrict structural changes.

Prof. Paul Munroe and Dr Quadir Zakaria from the University of New South Wales (UNSW) are collaborating with Prof. Haseeb and Ms See Leng Tay, from the University of Malaya to find a solution. Ms Leng visited the AMMRF at UNSW to conduct detailed electron microscopy analysis of commercially available tin–silver–copper (Sn–3.8Ag–0.7Cu) solders to which 20 nanometre-diameter nickel nanoparticles were added. When the nanoparticles were present, nanoscale regions of hard, brittle metal compounds formed in the main body of the solder giving added strength. However, similar compounds also formed in the interfacial layer between the solder and the underlying copper, weakening the connection between the two layers. The nickel appears to have its effect by dissolving into the molten solder.

The researchers are now investigating how to control the alloying conditions and manage the cooling rates to strengthen the solder spot without weakening the interface. This will ensure more reliable, safer and long-lived, miniaturised devices through the effective solder engineering.




Combined elemental map of solder on a copper surface (right) showing tin (pink), copper (red), nickel (yellow) and silver (green).

**impact innovation**

## CHALLENGE

In nature we can observe a spectacular variety of surfaces that can repel or attract water. For example lotus leaves and termite wings have micro-structures that cause water to roll off easily, providing a model for self-cleaning surfaces and stay-dry fabrics. Rose petals are different; their surface holds onto discrete droplets of water rather than causing them to roll straight off. This feature would be useful in humidity control applications, micro-volume diagnostics and micro-scale fluid movement. To find a production method that controls surface structures to give useful properties, and is easy to scale up for industrial applications, is the challenge.

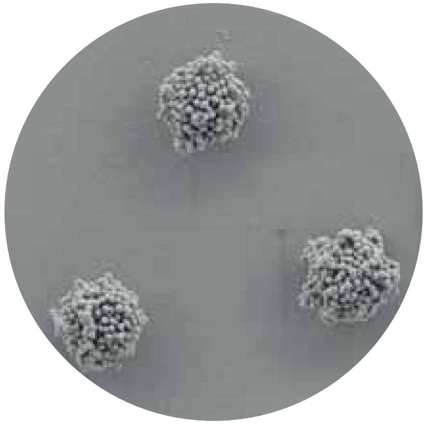
## SOLUTION

Dr Andrew Telford and colleagues at the University of Sydney are designing surfaces with controllable structures in an Australian Research Council Linkage Project with Dulux. They have developed a process to form raspberry-like structures with a two-tier hierarchy of larger (850nm) coated polystyrene core particles chemically bonded through carboxyl and amine groups to smaller (105nm) particles. They used emulsion polymerisation, a technique widely used in the coating industry, to make the basic particles. The subsequent assembly steps use simple chemistry in air and water making the whole process environmentally friendly.

During production of the surfaces, the 'raspberries' are dispersed in water and can readily be spread to form films on glass or other surfaces. When water encounters the final, dry, coated surfaces, discrete droplets form but remain attached to the surface, even when turned upside-down, just like water droplets on a rose petal. The wettability can be customised for defined applications by using different chemicals for the smaller particles.

The team uses scanning electron microscopy in the AMMRF at the University of Sydney to monitor and evaluate the production of the particles and films.

AM Telford et al., *Chem. Mater.* 25, 2013



## IMPACT

### Water-holding surfaces produced using simple chemistry that is easy to scale up for commercial production.

Coated products could generate economic benefits in nano- and biotechnology through:

- container-free micro-scale diagnostics allowing more accurate analysis of small samples without interference from container walls
- micro-tweezers, to pick up and move small liquid droplets with no loss of volume. This would be valuable in the expanding area of microfluidics.

This innovation could also be applied to create drip-free surfaces in condensation-prone areas such as aircraft cabins.

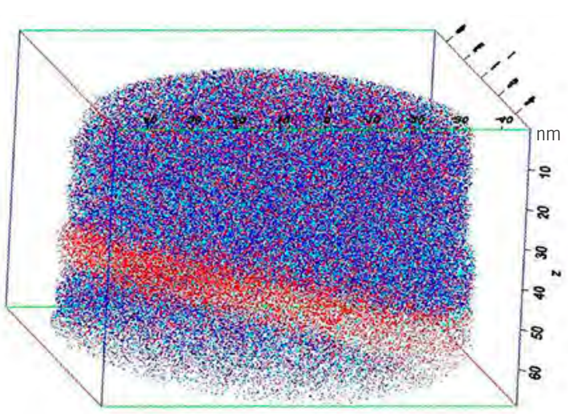
## FUTURE COMPUTING: SEMICONDUCTOR SPINTRONICS

Semiconductor spintronics combine the logic-processing of semiconductors and the information storage of magnetic materials in one material by controlling electrons' spin and charge states. The search is on for materials that allow this to happen efficiently at room temperature, to enable faster, more compact computing. Zinc oxide containing small amounts of cobalt (ZnO:Co) is one of the best candidates, however, it is not clear what underlies its magnetic properties. Speculation abounds, but there is little evidence to back it up. If ZnO:Co is to be used in future computing devices, its magnetic properties must be well understood.

PhD student Ms Li Li and Dr Rongkun Zheng at the University of Sydney (UoS), along with international collaborators, set out to understand the origin of magnetism in ZnO:Co. Ms Li used atom probe tomography, electron and X-ray diffraction, and transmission electron microscopy in the AMMRF at UoS to study the distribution of the cobalt atoms and structural defects. She demonstrated that cobalt is randomly distributed in the material proving that cobalt clustering is not the origin of the magnetism. Crucially, the team did show that the nature of the magnetism not only depends on the concentration of cobalt but also on how hydrogen atoms interact with those cobalt atoms within the crystal. Hydrogen is an unintentional addition to the material so its levels can vary considerably, affecting the magnetism in the process.

These results show the importance of carefully controlling the amount of hydrogen and the cobalt concentration to achieve the desired magnetic properties in manufacturing ZnO:Co for semiconductor spintronics.

Li Li et al., *Phys. Rev. B*, 85, 2012

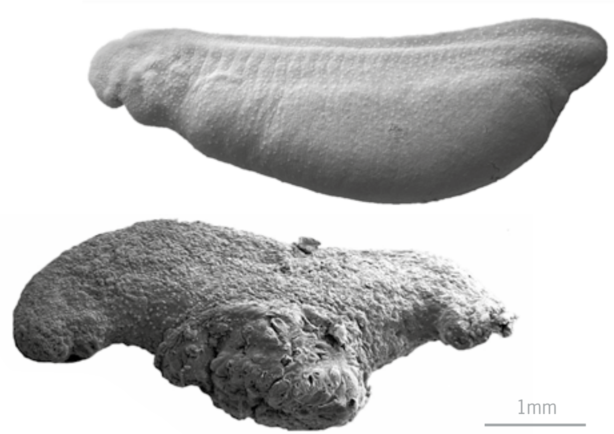



Atom probe dataset of ZnO:Co showing the random distribution of the cobalt atoms (blue) among zinc atoms (red) sitting on a layer of zinc oxide. The accompanying movie shows the dataset rotating – only the cobalt atoms are highlighted.

## LUNGFISH ON THE BRINK

The Australian lungfish, *Neoceratodus forsteri*, is the only surviving species of lungfish in Australia. It can live for up to 80 years and is a protected species, now confined to only three natural environments in southeastern Queensland. It has also been introduced to a few lakes and rivers. The lungfish is currently under threat from human activity, particularly where natural river environments are being converted into large reservoirs for water conservation. These environments rarely have the necessary shallow water and dense cover needed to provide food sources and breeding habitats for adults or for eggs, embryos and hatchling lungfish.

Analysis of embryological development, by scanning electron microscopy (SEM) has been carried out in the AMMRF at the University of Queensland by A/Prof. Anne Kemp from Griffith University. She found that all the eggs from reservoirs gave embryos with abnormalities whereas those from normal rivers gave normal embryos. Her findings reveal far greater damage to lungfish populations than was previously thought from the presence of eggs alone. Lungfish in the Enoggera Reservoir have recently become extinct and this could happen in other reservoirs where suitable spawning sites and food sources are now absent. This absence of viable young lungfish serves to alert us to the need for intervention to modify habitat before the remaining long-lived, mature fish in the reservoirs die out. Reduction in lungfish populations threatens valuable biodiversity and could disrupt the ecology of our watercourses with potentially damaging consequences.




SEMs of a normal lungfish embryo (top) from the Brisbane River at Lowood, and an abnormal one (bottom) of the same age from Lake Samsonvale.

**impact** *innovation*

## CHALLENGE

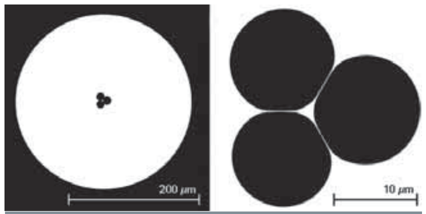
Disease diagnostics and environmental sensing applications would benefit from faster, more sensitive, reusable and cheap metal sensors that are easily deployed. Metal ions are important in diseases such as diabetes and cancer and in many biological processes.

## SOLUTION

Dr Sabrina Heng and her colleagues at the University of Adelaide have combined light-sensitive chemicals with silica optical fibres to develop the first reusable, nanolitre-scale ion sensor that can be regenerated using light.

Their system uses microstructured optical fibres (MOF) of their own design. Each MOF has tiny air holes at its centre. These air holes have been lined with a specialised chemical that has two parts, each with specific functions. One part changes its structure, opening up to become fluorescent in the presence of UV light and closing back to its colourless form when the UV light is switched off. The other part can be tailored to bind different metal ions. When metal ions are bound, they lock the chemical in the open (fluorescent) form. The intensity of the fluorescence when the UV light is switched off depends on the number metal ions present. Treating the complex with white light drives off the metal ions and reverts the sensor chemical to its starting state, ready to be used again. This switching can be done many times without losing reliability or sensitivity.

The sensor platform contains all the components to collect the sample, excite it with a laser and measure the fluorescence with a built in spectrometer. The MOFs have a dual role in this sensor system, the air holes act as nanoscale test tubes and the silica fibre transmits the light to and from the bound chemicals. Dr Heng and her colleagues use scanning electron microscopy (SEM) in the AMMRF at the University of Adelaide to analyse the structure of their MOFs.




SEM image showing the structure of a MOF in cross section.

## IMPACT

Switchable dip sensors for metal ions will bring economic benefits from future commercialisation as they will be:

**faster, more sensitive, reusable, easily deployed and cheaper**

They will enable advances in

**reproductive health, disease diagnostics and environmental monitoring**

The team have demonstrated the concept using lithium ions and have also developed an adaptable zinc-specific sensor suitable for use in biological systems and as a nanoscale dip-sensor that can detect zinc ions down to 100 pM in nanolitre-scale volumes.

Sabrina Heng et al. 2013: *RSC Advances* 3, and *Biomacromolecules* 14 (10).

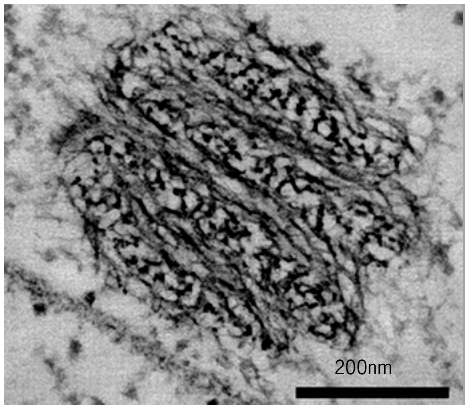
## LIQUID CRYSTALLINE DNA

A major distinction between bacteria and higher organisms (eukaryotes) is that bacteria have no obvious internal membrane-bounded compartments. Thus their DNA is not contained within a nucleus. Planctomycetes are exceptions in that their DNA is highly condensed and packaged in a membrane envelope, appearing very similar to a eukaryotic nucleus. It has been suggested that this arrangement may help these bacteria to survive very high doses of ionising radiation.

Dr Benjamin Yee worked with Prof. John Fuerst and colleagues at the University of Queensland (UQ) to do a detailed multiscale 3D analysis of how DNA is packaged in *Gemmata obscuriglobus*, a species of Planctomycetes. In the AMMRF at UQ they used high-pressure freezing and cryo-substitution to prepare the bacteria, and electron tomography to reveal an orderly hierarchical DNA packing structure. The DNA first appears to assemble, probably with proteins, to form 'beads on a string'. This is wound into bundles that then form into spirals. These then wrap into solenoidal structures running backwards and forwards within the constraining membrane, similar to a ball of wool. These structures are typical of liquid crystals in their periodicity, rotation and the parallel nature of the filaments. The liquid crystal structure is unusual, even for eukaryotes, although a similar arrangement is found in flagellate protists.

This valuable study helps us to understand how different types of organisms have evolved unusual ways of packaging their DNA and how those systems may be able to confer advantages to help them adapt to challenging environmental conditions.

Benjamin Yee et al., *Frontiers in Microbiology* 3, 2012




Cross-sectional image from a tomogram of a *G. obscuriglobus* nucleoid. Scalebar 200nm. Scan the QR code to see a movie of the entire nucleoid.

## TOWARDS IMPROVED REPAIR OF TOOTH DECAY

Tooth decay (dental caries) is the most common infection and can occur at any age. Once decay is established, treatment requires replacement of the lost dentine. The compatibility of the restoration material with the remaining dentine is one of the most important factors affecting the success of treatment. Therefore, understanding how dentine structure is affected by caries is critical to choosing the proper restorative materials.

Dentine is a complex mineralised tissue continually forming throughout life. It is laid down by cells called odontoblasts in response to cues they pick up from their immediate environment. These cells live within the pulp but extend sensory processes through microscopic channels in the dentine, called dentinal tubules.

Ms Nattida Charadram, a PhD student working with Prof. Mike Swain at the University of Sydney (UoS), did a comprehensive study of reactionary dentine, which forms in response to caries. In the AMMRF at UoS, she used focused-ion-beam scanning electron microscopy (FIB-SEM) for imaging & elemental analysis, and X-ray microtomography for 3D modelling. The data showed that the tubules that formed in response to caries are more helical and constricted than those in normal dentine, forming an effective barrier to bacterial penetration. Altered tubular structure and mineral composition combine to increase the elasticity of reactionary dentine.

These findings provide a basis for developing more biocompatible and longer-lasting synthetic materials for repairing tissue lost as a result of tooth decay and for enhancing formation of reactionary dentine to protect dental pulp tissue while attempting to remineralise affected dentine.

Nattida Charadram et al., *Journal of Structural Biology* 181, 2013

X-ray microtomography images of normal tubules (left) and tubules in reactionary dentine (right). Tubules are approximately 2µm in diameter.

# impact skin cancer

## CHALLENGE

Nearly half a million Australians are treated for skin cancers each year and of those, more than 2,000 die. Treatment for these cancers cost the health system half a billion dollars in 2010.

Topical treatments are used for some less aggressive, epidermal skin cancers but treatment times are long and skin reactions can be debilitating.

Tea tree oil (TTO) is a natural cocktail of terpene hydrocarbons with anti-bacterial, antifungal and antiviral actions. It has recently also been reported to quickly kill cancer cells. The basis of this action and how TTO could be developed into a fast-acting effective topical anti-cancer agent needed investigation.

## SOLUTION

Dr Demelza Ireland, Dr Sara Greay and colleagues at UWA examined cell changes in subcutaneous tumours in mice after treatment with TTO. By using flow cytometry and microscopy facilities in the AMMRF at UWA they showed that although TTO stimulated an immune response in the upper layers of the skin, it actually killed the tumour cells by disrupting their internal membrane structures. Interestingly, it only did this when used at a concentration of 10% in dimethylsulphoxide (DMSO) but not in its pure form. DMSO makes the skin permeable so the TTO can reach the tumour cells.

Clinically acceptable formulations are now being developed to optimise TTO delivery before it moves to clinical trials and commercial development. The team is seeking funding to facilitate commercialisation of this work.

Demelza Ireland et al., *Journal of Dermatological Science* 67, 2012

## IMPACT

The tea tree oil formulation is likely to be a clinically

### effective, well-tolerated, topical treatment for some precancerous lesions and skin cancers

- Treatment would be quicker, thus better for patients and cheaper to provide.
- Commercial development would bring economic benefits to Australia.

Tea Tree Image © M.Fagg, Australian National Botanic Gardens

## CULTIVATING AN ACACIA INDUSTRY

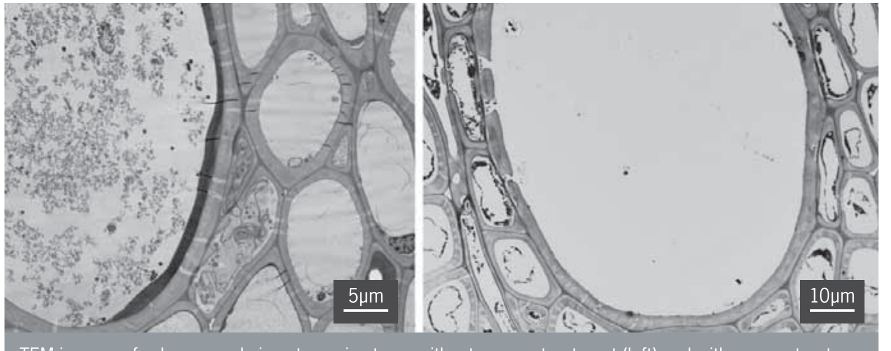
The flowers and foliage of many Australian acacias are very attractive and, although popular, they are under-utilised by florists due to their short vase life. Understanding how to improve this would facilitate a development path for the acacia industry.

Copper is known to extend the life of many cut flowers, but how it does this has remained a mystery. By working with the AMMRF at the University of Queensland (UQ), Dr Kamani Ratnayake from UQ and Prof. Daryl Joyce from UQ and AgriScience Queensland have been investigating how copper acts, and how it could extend the life of cut acacias.

Water must be able to flow unobstructed up the length of the cut stems. Dr Ratnayake has used light microscopy and scanning & transmission electron microscopy to investigate why this doesn't happen well in acacias. She showed that the problem originates a day or two after cutting by the natural wound healing response. A gel oozes out of cells around the cut surface and, over time, completely blocks the water-transport tubes in the stems. Copper was shown to stop gel formation by disrupting metabolic function in the cells around and above the cut. Water could then flow easily into the stems, more than doubling vase life.

She also showed that a single pulse of copper was more effective than its continued presence in the vase water. This would be a practical bonus to the industry as the grower could do the treatment at harvest and nothing more should be necessary down the line.

Kamani Ratnayake et al., *Physiologia Plantarum* 148, 2013




TEM images of xylem vessels in cut acacia stems without copper treatment (left) and with copper treatment (right). Gel can be seen only in the vessel without copper treatment.

## PEPTIDES BATTLE BACTERIA

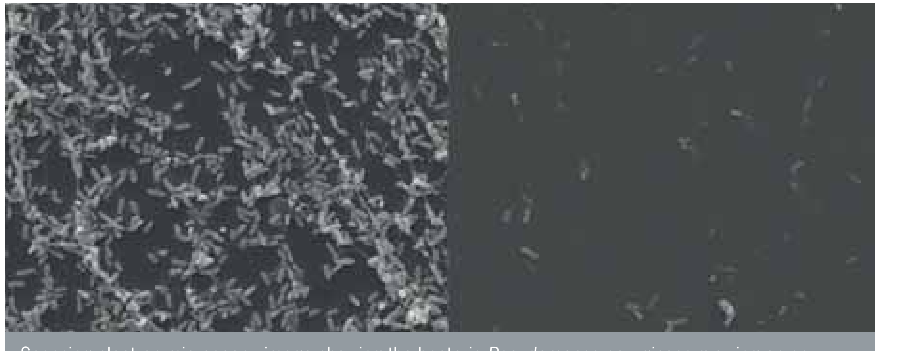
The continuing fight against bacterial infection has an increasing scope. The growing range of implanted and inserted medical devices from contact lenses to cochlear implants and joint replacements bring the concomitant threat of associated infections. New antimicrobials that remain active when attached to devices would bring benefits to patients and help win this ongoing battle.

Profs Mark Willcox and Naresh Kumar from the University of New South Wales (UNSW) have designed a clever new antimicrobial peptide by combining the active regions of two known, but less than optimal, antimicrobial peptides, protamine and mellitin. Known as melimine, this peptide is active against a much broader range of bacteria than either starting peptide and remains active when attached to surfaces, unlike the starting peptides.

Their PhD student Renxun Chen investigated whether the orientation of peptide attachment to surfaces affects its antimicrobial activity. He used the flagship ToF-SIMS in the AMMRF at the University of South Australia to characterise surfaces with peptides attached by either end or near their centre. He then tested the differently coated surfaces for bacterial growth. By using scanning electron microscopy in the AMMRF at UNSW he found that melimine killed bacteria most effectively when attached by its amino-terminal end, exposing the positively charged portion to interact with the bacteria.

This work has led to the team, along with industry partners Cochlear, being awarded an Australian Research Council Linkage grant to further test melimine-coated devices and surfaces. This could significantly reduce the economic burden and personal suffering caused by implant-associated infections.

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



Scanning electron microscopy image showing the bacteria *Pseudomonas aeruginosa* growing on an uncoated surface (left) and a surface coated with peptide (right). Each bacterium is about 2µm in length.

# impact *innovation*

## CHALLENGE

To develop a super-sensitive, fast and widely applicable biosensor with good biological specificity for small biological molecules.

## SOLUTION

Dr Leo Lai, Scientia Prof. Justin Gooding and colleagues from the University of New South Wales (UNSW) innovatively combined antibody–antigen interactions with magnetic nanoparticles to generate an entirely new kind of biosensor. They took gold-coated iron nanoparticles and coated these with the small molecule they want to detect (antigen). They then let antibodies to this target molecule bind completely to the antigen-coated surface of the particles.

These biosensor particles are added to the solution to be tested. Any target molecules present in the solution compete with the particle-bound target molecules and pull some of the antibodies off the nanoparticles. As antibodies are very large molecules, when they are pulled off the particles, the coating becomes thinner and the particles can get closer together. A magnetic field can be used to assemble the iron nanoparticles into a dense array. The closer they are to each other the better an electric current will flow through the assembly. The resistance to that electric current therefore serves as a measure of how close together the particles are, which in turn reveals how many target molecules are present in the test solution.

Photo ©The Coffee Collective

## IMPACT

A patent was filed and the work has attracted considerable commercial interest from the

**$16 billion global biosensor market**

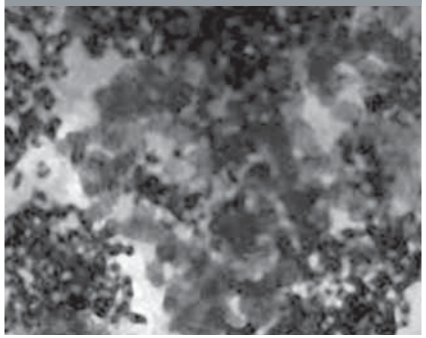
Increased sensitivity will allow:

- earlier detection of diseases and therefore earlier intervention
- detection of lower levels of bioactive chemical contaminants

No sample preparation means

- saving on time, money and personnel
- easier deployment in the field, increasing the range of applications in many industries. These include environmental and agricultural monitoring, food and water quality assurance, and drug testing.

TEM image of the 100 nanometre diameter gold-coated magnetic nanoparticles.



The team found that using this system they could detect target molecules in solution at concentrations between 0.28 femtomolar and 2.8 micromolar in only 40 minutes. This is orders of magnitude more sensitive than existing techniques with the added bonus that the test samples need no preparation.

The system is now being adapted into a generalised biosensing system to also detect microRNA cancer biomarkers with large complementary pieces of DNA instead of antibodies. This can, in principle, be applied to any type of cancer in a liquid biopsy simply by changing the microRNA sequences bound to the biosensor particles.

The researchers used transmission electron microscopy in the AMMRF at the University of Sydney and at UNSW to monitor development of their sensor systems.

Leo M. H. Lai et al., *Angew. Chem. Int.* Ed. 51, 2012.

## 380-MILLION-YEAR-OLD FISH MUSCLES REVEALED

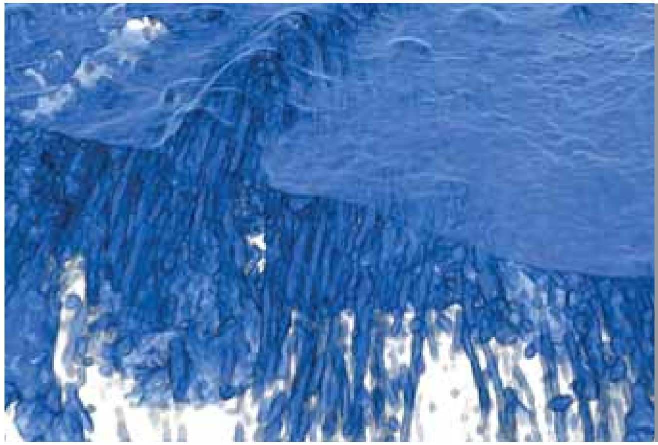
Unlike the vast majority of fossils, the fish found in the Gogo Formation in the Kimberley area of Western Australia (placoderms) have preserved muscles, tendons and skin as well as bone. A/Prof. Kate Trinajstic from Curtin University has worked with Australian and international colleagues to study these unique fossils and their results were published in the journal *Science*.

Piecing together musculature of extinct species generally involves trying to infer the size and position of muscles from attachment marks on the fossilised bones. However, in these fossils of early, jawed fishes (placoderms), the soft tissue is also preserved. X-ray microtomography in the AMMRF at the Australian National University complemented synchrotron studies to look through the rock to see the fossilised tissue. It was able to reveal the placoderms' delicate preserved musculature and tendons. Complete sets of multiple muscles were found, allowing the full restoration of the major neck and trunk musculature.

The most unexpected discovery was the transverse-fibre muscles found in the ventral body wall. These are not seen in higher fish and were previously unknown in the early jawed fish. Elsewhere, these transverse-fibre muscles only appear in four-legged animals.

The researchers also showed for the first time that the important cucullaris muscle, which depresses the head, was present in the most primitive jawed fishes. It has been retained throughout vertebrate evolution, still being present in humans today. The complexity of placoderm musculature was never predicted from skeletons alone and highlights the value of soft tissue fossils in evolutionary studies.

Kate Trinajstic et al., *Science* 341, 2013



X-ray microtomogram of a 1.6mm wide region showing fossil muscle fibres beneath the posterior ventrolateral plate of a *Compagpopiscis croucheri*.

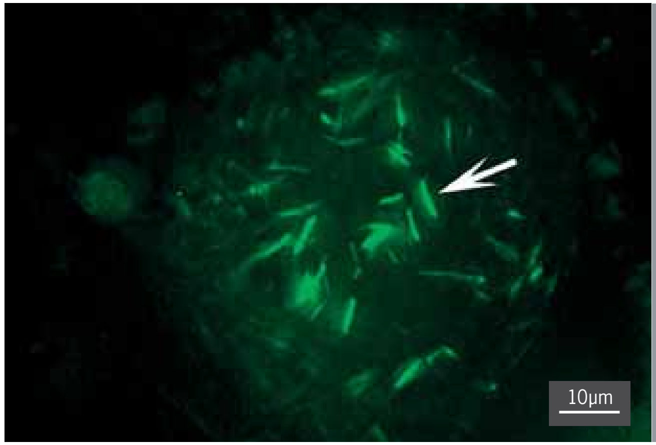
## FIGHTING AGRICULTURAL PATHOGENS

The plant pathogen, *Phytophthora nicotianae*, infects more than 200 species of economically important plants including citrus, tomato, pineapple and passionfruit crops. It spreads by releasing large numbers of motile zoospores that can swim significant distances to reach a potential host. Each zoospore has two morphologically different flagella, one of which has tubular hairs called mastigonemes that are required for forward movement of the zoospore. If the mastigonemes are absent the zoospores can only swim round and round in circles. Mastigonemes are synthesised during asexual sporulation and are assembled onto the flagella during formation of the zoospores.

Research by PhD student Mr Wei Yih Hee, Dr Leila Blackman and Prof. Adrienne Hardham at the Australian National University, shows that two related proteins called PnMas1 and PnMas2 are found in the shaft of the mastigoneme. They also found that a third related protein, PnMas3 is associated with synthesis of the mastigonemes in specialised compartments of the *P. nicotianae* cell.

If mastigonemes are disrupted, the zoospores are unable to swim to suitable infection sites. Therefore understanding the formation and functioning of mastigonemes lays the groundwork for identifying targets for future chemical intervention in the infection cycle.

By using fluorescently labelled PnMas3-containing cells Mr Hee is now investigating the assembly and organisation of mastigonemes onto the zoospore anterior flagellum in living *P. nicotianae* cells.



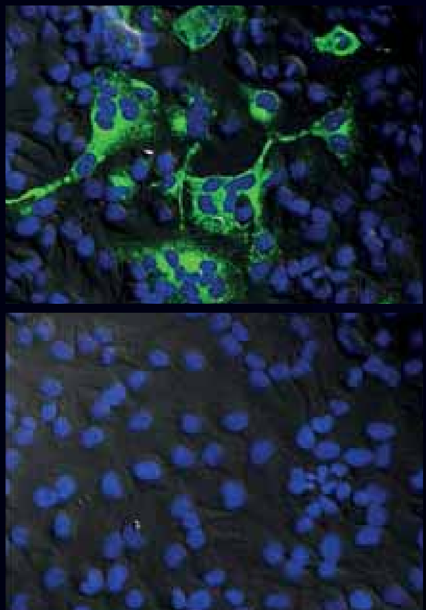

Deconvoluted epifluorescence image of a *P. nicotianae* cell showing fluorescent PnMas3 associated with mastigonemes in the endoplasmic reticulum (arrow).

# impact hendra

## CHALLENGE

Infections with Hendra virus, and the closely related Nipah virus are frequently fatal. Horses infected with Hendra virus can transmit the disease to humans and must be put down. This is an important issue, especially for the equine industry.

- Both viruses emerged from bat reservoirs in the last 15 years and are increasingly prevalent in Australia and South-East Asia.
- More than half of infected people die. There is currently no human vaccine and no effective treatment.



Confocal images showing Hendra virus proteins in green in infected cells (above) and infected cells treated with one of the siRNAs and polyI:C (below). Nuclei are in blue.

## SOLUTION

Interfering RNAs (RNAi) are a natural cellular defence mechanism against viruses. Through base pairing they bind specifically to the viral RNAs and cause them to be broken down.

Small interfering RNAs (siRNA) are beginning to be developed for therapeutic use. They have the advantage of being specific to the viral genes and therefore do not affect healthy cells.

Ms Jana McCaskill and A/Prof. Nigel McMillan from the University of Queensland have designed a range of siRNAs against Hendra virus gene sequences. To evaluate the effectiveness of their siRNAs in suppressing production of viral proteins in infected cells, the team worked with Drs Glenn Marsh and Paul Monaghan at CSIRO’s Animal Health Laboratory (AAHL). The PC4 Zoonosis Suite and Bioimaging Facility at AAHL is an AMMRF Linked Laboratory and provided confocal microscopy images to support the research.

The most effective siRNAs reduced the number of new virus particles produced by up to 98% and when combined with the immunostimulant polyI:C, they achieved a 99.95% reduction.

McCaskill JL, et al., *PLoS ONE* 8(5), 2013

## IMPACT

Combined with innovative delivery methods being developed by A/Prof. McMillan's group and others, these siRNAs could be commercialised into the first

effective therapy to save the lives of people infected with Hendra and Nipah viruses

Photo: Bob Barker / Newspix

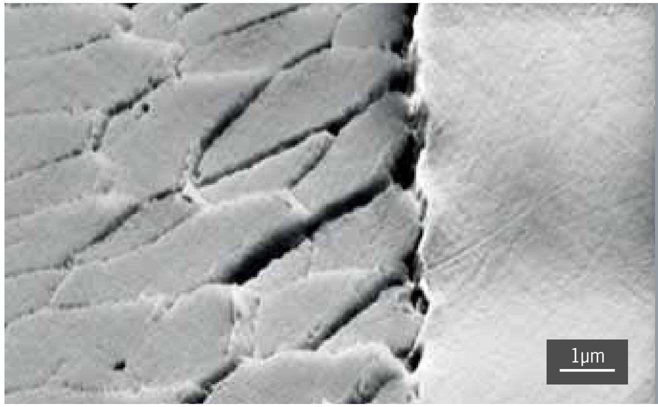
## PAPER NAUTILUS – A FRAGILE FUTURE

The female octopus *Argonauta nodosa*, or paper nautilus, makes one of the thinnest shells in the sea as a floating brood chamber for her developing embryos. It is secreted from specialised glands on the female's arms. Unlike most other shells it doesn't have a protective organic layer, making it particularly vulnerable to climate change as oceans warm and acidify. Honours student Mr Kennedy Wolfe, working with Prof. Maria Byrne at the University of Sydney, investigated the effects of ocean warming and acidification on this organism.

Mr Wolfe used scanning electron microscopy (SEM) and electron backscatter diffraction (EBSD) in the AMMRF at the University of Sydney to examine changes in the structure of the magnesium–calcite shell after exposure to predicted near-future ocean change conditions. After just two weeks the shells were beginning to dissolve. Conditions predicted for the end of the century (pH 7.6 and 24˚C), dissolved shells by approximately 2%. In the most extreme conditions tested (pH 7.2 and 24°C for two weeks), 5% of the shell dissolved away. The complementary imaging techniques showed that dissolution penetrated into the shell along structural boundaries, forming gaps that compromise the integrity and strength of the shell.

In living animals these effects might be mitigated by additional shell secretion, but this would demand greater energy and resources from the female. Ocean acidification is likely to have deleterious effects on paper nautilus reproduction, upsetting fragile food webs, including fish and marine mammals, and reducing valuable biodiversity. This research helps us to understand the limitations of organisms to adapt to our changing environment.

Kennedy Wolfe et al., *Marine Biology* 160, 2013




SEM image of a nautilus shell showing gaps between the individual crystals after treatment in acidic conditions. The surface of the shell is to the right and is adjacent to the resin in which it has been embedded.

## THE WORLD'S THINNEST MATERIAL

Graphene is a material that promises to transform the future. With a single layer of carbon atoms arranged in a honeycomb structure, it is the world's thinnest, strongest and most conductive material. It promises a range of diverse applications including smartphones, ultrafast broadband, computer chips and drug delivery.

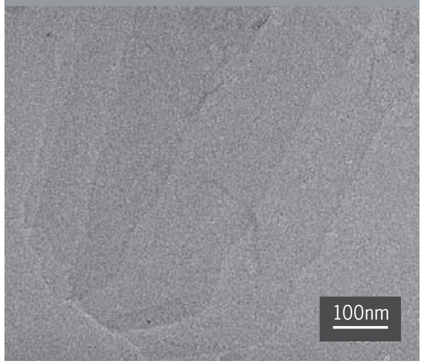
The researchers who won the 2010 Nobel Prize in physics for graphene discovery had used sticky tape to peel one-atomic-layer-thick graphene sheets from a graphite block. This is clearly not feasible for practical graphene production. Current synthetic production methods involve such high energy that the resulting graphene sheets are often damaged during production. This therefore drove researchers to look back to exfoliation methods to find more efficient and practical ways of production.

Prof. Colin Raston, formerly at the University of Western Australia (UWA), now at Flinders University, has worked with his team to develop an easy and completely new method for producing graphene sheets. Their method reduces the chance of defect formation and chemical degradation of the graphene. It makes use of fluid dynamics to establish shear processes generated from the interplay of centrifugal and gravitational forces. This gently lifts and separates the layers from a piece of graphite.

The process is carried out in N-methyl-pyrrolidone (NMP), which keeps the layers separated after lifting. The team used transmission electron microscopy, selected area diffraction and atomic force microscopy in the AMMRF at UWA to reveal the size, shape and crystal structure of the separated graphene sheets and demonstrate the success of their new method.

Xianjue Chen et al. *Chem. Commun.* 48, 2012

TEM image of separated graphene sheets.



# impact targeted drugs

## CHALLENGE

Successful multifunctional drug delivery systems to treat cancer and other diseases are in high demand. A variety of nanoparticles are being developed as drug delivery vehicles but the challenge is to make a single particle system to encapsulate a wide range of drugs, target them to where they are needed and provide a built-in imaging agent to track the location and long-term efficacy of treatment.

## SOLUTION

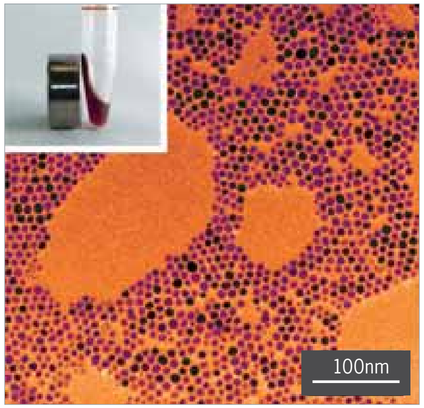
Eridan Technology Pty Ltd has a patented polymer formulation with a highly reactive core made of poly(glycidal methacrylate) (PGMA). This makes it easy to attach virtually any targeting molecule, via an epoxy functional group, making a universally applicable, targeted therapy for cancer and various medical emergencies.

Dr Swaminathan Iyer from the University of Western Australia (UWA) established Eridan Technolgy to commercialise his work. He uses transmission and scanning electron microscopy, live cell and confocal imaging, in-vivo fluorescence imaging and the Flagship NanoSIMS in the AMMRF at UWA. These techniques allow him toevaluate the structure and behaviour of the particles with different drugs in different cells.

The drug is encapsulated within the particle core along with nanoscale iron oxide that gives contrast for magnetic resonance imaging and fluorescent molecules that enable confocal & bioluminescent imaging of the particles within cells. This combination of therapeutic drug and diagnostic imaging actions has led to the term 'theranostic' agents.

The Eridan system gives very efficient drug delivery, even with highly insoluble molecules. Recent pre-clinical trials successfully delivered much lower overall doses of the test drug targeted to prostate cancer in rats, effectively killing the cancer without deleterious effects on the rest of the body.

The nanoparticle formulation is also being tested and marketed as a laboratory reagent to introduce DNA and other molecules into cultured cells. The nanoparticles have already demonstrated superior efficiencies than the currently available commercial gold standards. This is an extremely lucrative additional market.




## IMPACT

There is a multi-billion dollar market for

**targeted delivery systems**

for drugs, DNA and other molecules.

**cancer**

accounted for 19% of Australia's disease burden in 2013 and the suffering of around 10 million people globally. Dr Iyer's system could improve their outcomes.

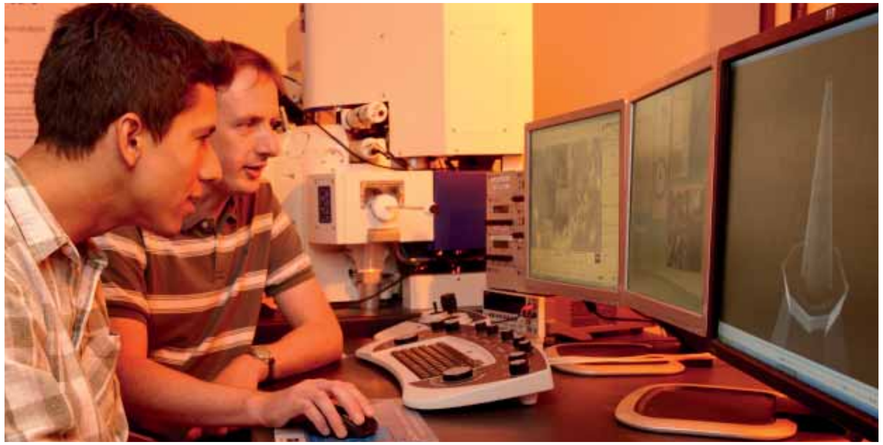
His system could also help treat:

- trauma in the central nervous system
- cardiovascular diseases
- burns, by stimulating skin regeneration
- foetal and maternal disorders in pregnancy

# looking to the *future*

## The Australian Government's Strategic Research Priorities set the challenges for us as a nation and guide our research directions. Since its formation, the AMMRF has consistently demonstrated outcomes that directly address these challenges and enable effective solutions.

With over ten years of experience in operating collaborative, distributed research infrastructure, we are a well-established and efficient organisation. The AMMRF is ready for these challenges and remains committed to providing the best research experience and enabling world-class outcomes for Australian scientists.



Strategic planning is important factor in our preparedness for the future. The next generation of microscopy and microanalysis instrumentation that will tackle Australia's research priorities has been identified and our laboratories are growing and evolving.

The National Collaborative Research Infrastructure Strategy (NCRIS) provided a firm foundation on which to build national capability in advanced microscopy. The AMMRF has all the hallmarks of a successful, high quality collaborative facility, confident it will continue to deliver world-class research outcomes.

## The hallmarks of a world-class research facility

1. Visionary leadership with a focus on national priorities, effective national collaboration and international trends.
2. Growing world-class capability as new instruments are commissioned and expert staff develop innovative techniques and online tools to push the boundaries of characterisation.
3. Laboratories that are evolving as the range of capabilities expand and member institutions increasingly recognise the value of core research infrastructure.
4. Active industry engagement: in analytical services, routine quality assurance analysis or long-term research and development.
5. Demonstrated economic impact to the nation through enabling innovation and consequent new commercialisation opportunities.
6. Track record of world-class research outcomes enabling researchers to publish in higher-ranking journals.
7. High utilisation of facilities with strong demand for the capabilities.
8. Connected internationally to other networks to develop capability and employ best practice in facility operation and management.
9. Effective communications and engagement strategy targeting the research community, stakeholders and the public.
10. Efficient access policies and processes that streamline researcher engagement, training, instrument use, data analysis, management and presentation.
11. Partner laboratories with common values that are financially sustainable.
12. A strategic plan for next-generation capabilities required to meet Australia's Strategic Research Priorities.

ready to meet
***australia's challenges***

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## FEATURED IMAGE CREDITS

Covers, front and inside back: transmission electron micrograph of rough endoplasmic reticulum inside a cell. Part of the nucleus and a mitochondrion are also shown. Ribosomes (the small dots arranged in rows) are 25–30 nm in diameter. Anne Simpson, Univeristy of Sydney.

Back cover: electron microprobe X-ray map showing the levels of magnesium in a rock sample. The highest levels are shown in white then yellow through to the lowest levels in indigo. The horizontal field width is 3.25 mm. Alexander Prohoroff, University of Adelaide.

Contents: photograph by Mike Gal, Univeristy of New South Wales

Access: transmission Kikuchi diffraction in the SEM (SEM-TDK) showing nanocrystalline structures in copper films. To glean crystal orientation information from grains less than 20 nm is unprecedented and this image is an outstanding example of the technique. The horizontal field width is 6.6 μm. Saritha Samudrala, Univeristy of Sydney.

Research: X-ray microtomogram of a piece of pine wood impregnated with copper nanoparticles (green). The horizontal length of the wood is 11 mm. Philip Evans, University of British Columbia.

Centre-spread: cells washed from mouse lungs and imaged on an oil immersion scanner. Samples courtesy of Prof. Peter Henry and Ms Tracy Mann from the University of Western Australia and images by Prof. Paul Rigby. The small red cells (red blood cells) are approximately 5 μm in diameter.

## VIEWING VIDEO VIA QR CODES

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Open the app and hold the phone steady with the QR code centered on the screen (as if to photograph it). When the scan is done you may get a prompt showing you the information in the QR code. In this case it's a web link that plays a video. Just respond to the prompt to visit the URL and enjoy the video. You may find the URL is stored in your browser history. QR codes: p. 14, 20, 24, 26.