Australian
Microscopy & Microanalysis
Research Facility

PROFILE

2011

making an impact

Our vision

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

Our mission

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

FOUNDING NODES

SOUTH AUSTRALIAN REGIONAL FACILITY (SARF)

FUNDED BY

contents

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from the minister

World-class research assets are the mark of a nation fit for the twenty-first century. They transform our understanding of the world we confront and, in so doing, transform its challenges into real opportunities. The Australian Microscopy and Microanalysis Research Facility is a testament to our ambition, in research fields of the highest importance to industry and academia alike. It is no less a testament to our phenomenal potential. This is a national facility, of international significance, made possible by many institutions working in partnership. I am proud to support their nation-building mission.

Senator the Hon. Kim Carr

Minister for Innovation, Industry, Science and Research

from the chair

The AMMRF has completed its fourth year of operation. It continues to deliver world-class microscopy and microanalysis capabilities to Australian researchers in universities, government laboratories and industry. In the context of the NCRIS program, the AMMRF could be regarded as being mature and thus there may be little expectation of change to organisational structure and operations. However, it is noteworthy that the organisation continues to evolve in terms of its formal linkages around the world. Additionally, it is striving to be innovative in the key performance areas of research services, research programs and research training. The approach that the AMMRF is taking with the development of a suite of on-line training tools called MyScope is exciting in this regard. MyScope will reduce the amount of instrument time that is booked for training purposes and therefore liberate capacity for research.

As Chair, I was privileged to join the AMMRF contingent that attended the first EU-Australia Workshop on Research Infrastructure, held in Brussels in April 2011. The AMMRF convened one of the thematic streams at the workshop and that provided an incredible opportunity for the facility to meet and interact with comparable European microscopy and microanalysis networks. As a result, views and opinions about possible future technologies and collaborations, which will enable the Grand Societal Challenges, in the European context, or the National Research Priorities in Australia, were discussed. Most importantly, it was clear from the workshop that the AMMRF is a world-class research facility that is admired internationally.

Finally, the AMMRF strongly supported the review of the Strategic Roadmap for Australian Research Infrastructure that was conducted by DIISR during 2011. The AMMRF is satisfied with the position that advanced microscopy and microanalysis has within the Roadmap, which was recently released by Minister Carr. The AMMRF now looks forward to continuing to work towards its implementation.

The Board and I are excited by the performance and impact that the AMMRF is continuing to have, both in Australia and internationally. We trust that you too will be excited by the snapshot of outcomes presented in this Profile.

Dr Gregory R. Smith
Chair of Board

from the ceo

Once again, it is a great pleasure to publish another AMMRF Profile. This document is a record of, and testimony to, the valuable work of our large and steadily growing user community, and is a marvellous credit to all AMMRF staff. I am especially delighted to share with you some of the facility's numbers because they are truly impressive: a user community of 3300, over 200,000 hours of beamtime, numerous prestigious prizes and fellowships awarded to our users, many outstanding research papers and theses, and a healthy smattering of contributions to patents and inventions. And, as our user community continues to grow and diversify, it is clear that the AMMRF staff and facilities are building strong engagement with Australian and international industry.

As we blaze this substantial trail, it is timely to consider the outcomes of the important work undertaken by the Department of Innovation, Industry, Science and Research (DIISR) to reinvigorate the national roadmap for research infrastructure in 2011. This is important work. The AMMRF was pleased to see the formulation of what is clearly the most sophisticated Strategic Roadmap for Australian Research Infrastructure yet. The roadmap's emphasis on national research priorities is a valuable framework, and we welcome the recognition that certain capabilities, such as characterisation, are of special significance because they impact across all research priorities. Pleasingly, the final version of the Roadmap recognises the critical role of microscopy and microanalysis as an enabler of Australia's research frontiers – given the award of the 2011 Nobel Prize for Chemistry to an electron microscopist for the discovery of quasicrystals, it would seem difficult to disagree. The exploration of 'inner space' by the AMMRF's sizable user community is reaping rewards across the disciplines, and continues to demonstrate that advanced facilities coupled with the right blend of technical and scientific staff are the ideal ingredients for a world-class research facility.

Prof. Simon P. Ringer
Executive Director & CEO

governance

Organisational Structure

Established under the Commonwealth Government's National Collaborative Research Infrastructure Strategy (NCRIS), the AMMRF is a national grid of equipment, instrumentation and expertise in microscopy and microanalysis that makes nanostructural characterisation capabilities and services available to the entire Australian research community.

Activities of the AMMRF are overseen by a board composed of an independent chair and the deputy vice-chancellors in charge of research (or their nominees) from the participating universities, plus the AMMRF Executive Director and CEO. The operations team, comprising the node directors and General Manager, meets regularly and reports to the board.

NODES

Nodes are major university-based microscopy and microanalysis centres collaborating to form the core of the AMMRF. They have a wide range of overlapping and complimentary instrument types and expertise, collectively providing infrastructure for high-demand and cutting-edge techniques.

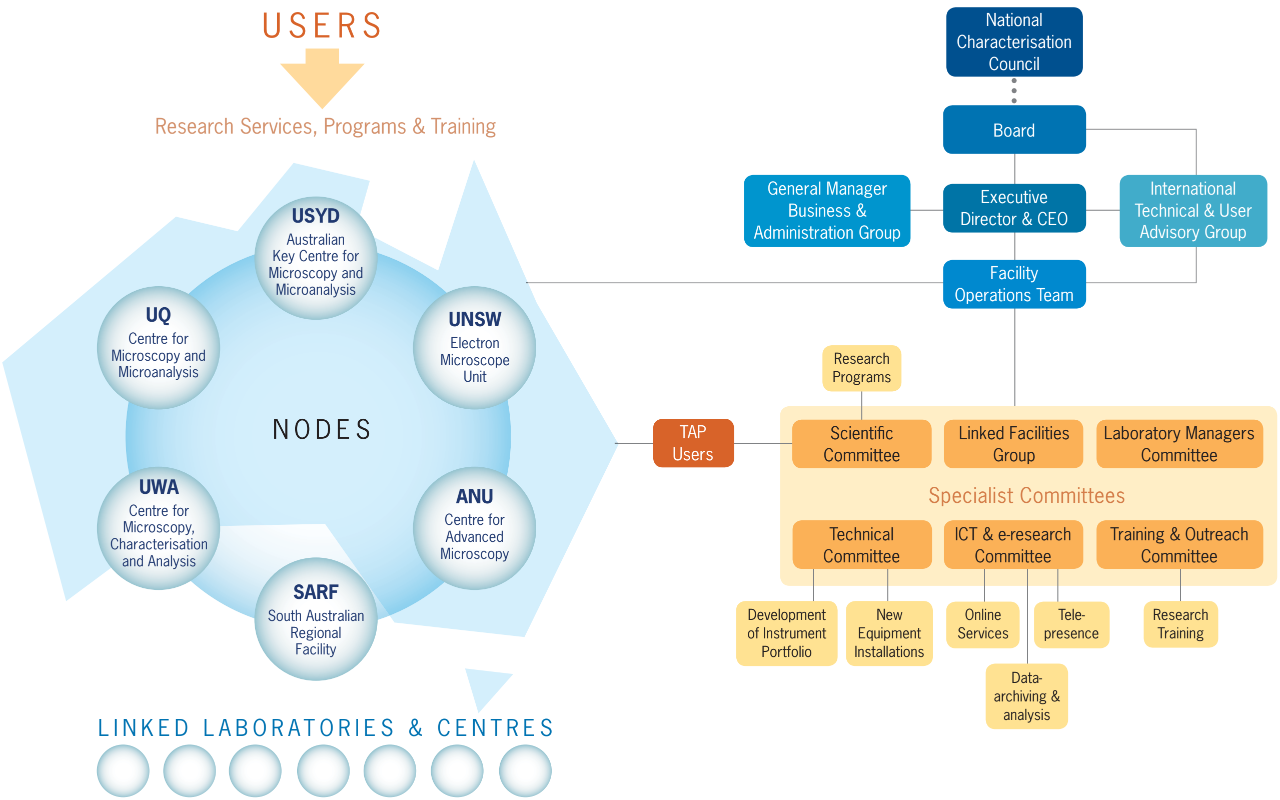
LINKED LABORATORIES

A Linked Lab provides access to specialist instruments within the university sector or within a publicly funded research agency.

LINKED CENTRES

A Linked Centre is established in conjunction with a concentration of specialist users based at a major research centre and each centre has a dedicated microscopist.

For a full list of node, lab and centre contact details please see p.36 or visit our website: ammrf.org.au



BOARD MEMBERS

Dr Gregory R. Smith, Chair

Prof. Alan Lawson
University of Queensland

Prof. Jill Trewhella
University of Sydney

Prof. Margaret Harding
University of New South Wales

Prof. Jim Williams
Australian National University

Prof. Tony O'Donnell
University of Western Australia

Prof. David Day
Flinders University

Prof. Simon Ringer
University of Sydney

International Connections

OPERATIONS TEAM

Executive Director: Prof. Simon Ringer
University of Sydney

Scientific Director: Prof. John Drennan
University of Queensland

Associate Director: Prof. David Sampson
University of Western Australia

Technical Director: Prof. Paul Munroe
University of New South Wales

Associate Director: Prof. Tim White
Australian National University

South Australian Regional Facility (SARF)
Associate Director: Prof. Joe Shapter
Flinders University

Prof. Hans Griesser, Node Director
University of South Australia

Mr John Terlet, Node Director
University of Adelaide

AMMRF General Manager:
Dr Miles Apperley

EUROPEAN UNION – AUSTRALIA BILATERAL WORKSHOP ON RESEARCH INFRASTRUCTURE

Through the work of its International Technical and User Advisory Group (ITUAG) and the development of several strategic overseas connections, the AMMRF is able to obtain a perspective on:

- world's best practice in research facility operation and management
- technology foresighting and emerging microscopy and microanalysis techniques that could impact on Australian research priorities
- potential improvement of the AMMRF user experience
- performance indicators and metrics that enable international benchmarking.

Increasingly, overseas networks and labs are making connections with the AMMRF to understand key operational and organisational features that could be applied to similar infrastructure networks as they are being established in their own regions.

EURO-BIOIMAGING

One such emerging connection is with Euro-BioImaging, a pan-European network of biomedical imaging facilities. The goal of this collaboration is to establish an alliance of mutual benefit in providing national infrastructure to support research in the areas of biological and medical sciences. The collaboration builds on discussions held at the first European Union–Australia Workshop on Research Infrastructure in Brussels on 4–5 April 2011. The meeting enabled Commonwealth Government officials and representatives from the AMMRF, the Australian Synchrotron and the Integrated Marine Observing System to meet with European counterparts and explore opportunities for collaboration around research infrastructure.

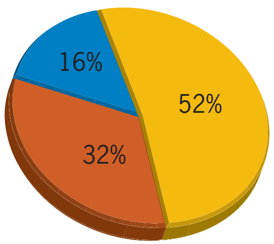
In addition, discussions were held with representatives from other microscopy and microanalysis networks including ESTEEM (Enabling Science and Technology through European Electron Microscopy) and INSTRUCT (Integrated Structural Biology Infrastructure for Europe). AMMRF systems, procedures and policies that enable it to operate as a national facility attracted particular interest due to obvious applications to European programs.

Feedback from the workshop and the progress of our developing international strategic relationships make it clear that the AMMRF is a world-leader in its field.

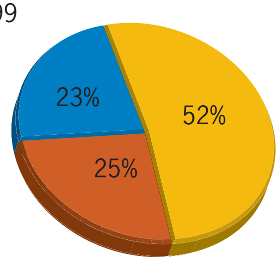
AMMRF 2010–11

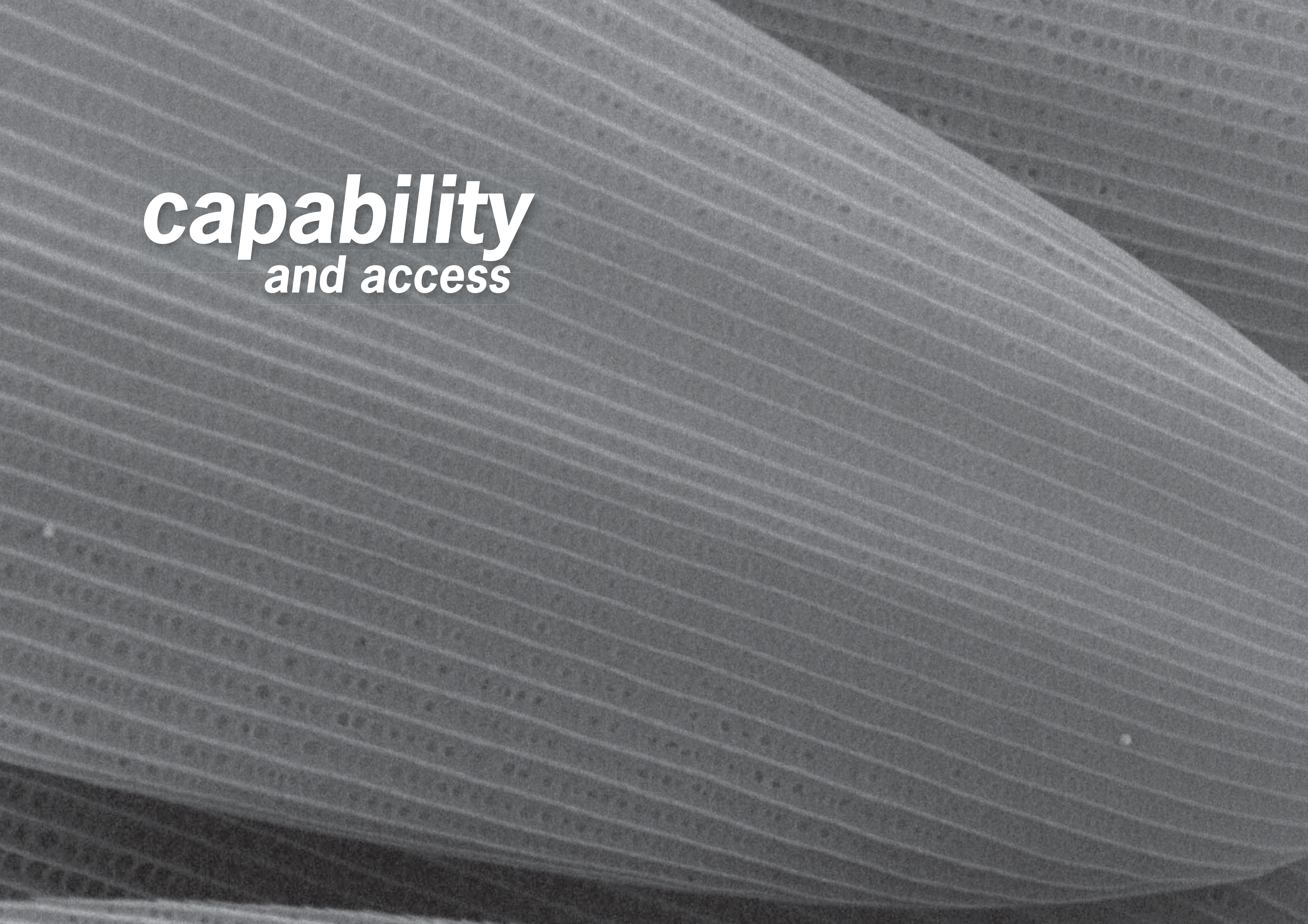
- Physical/Materials Science
- Biological/Medical Science
- Environmental Science/Geoscience

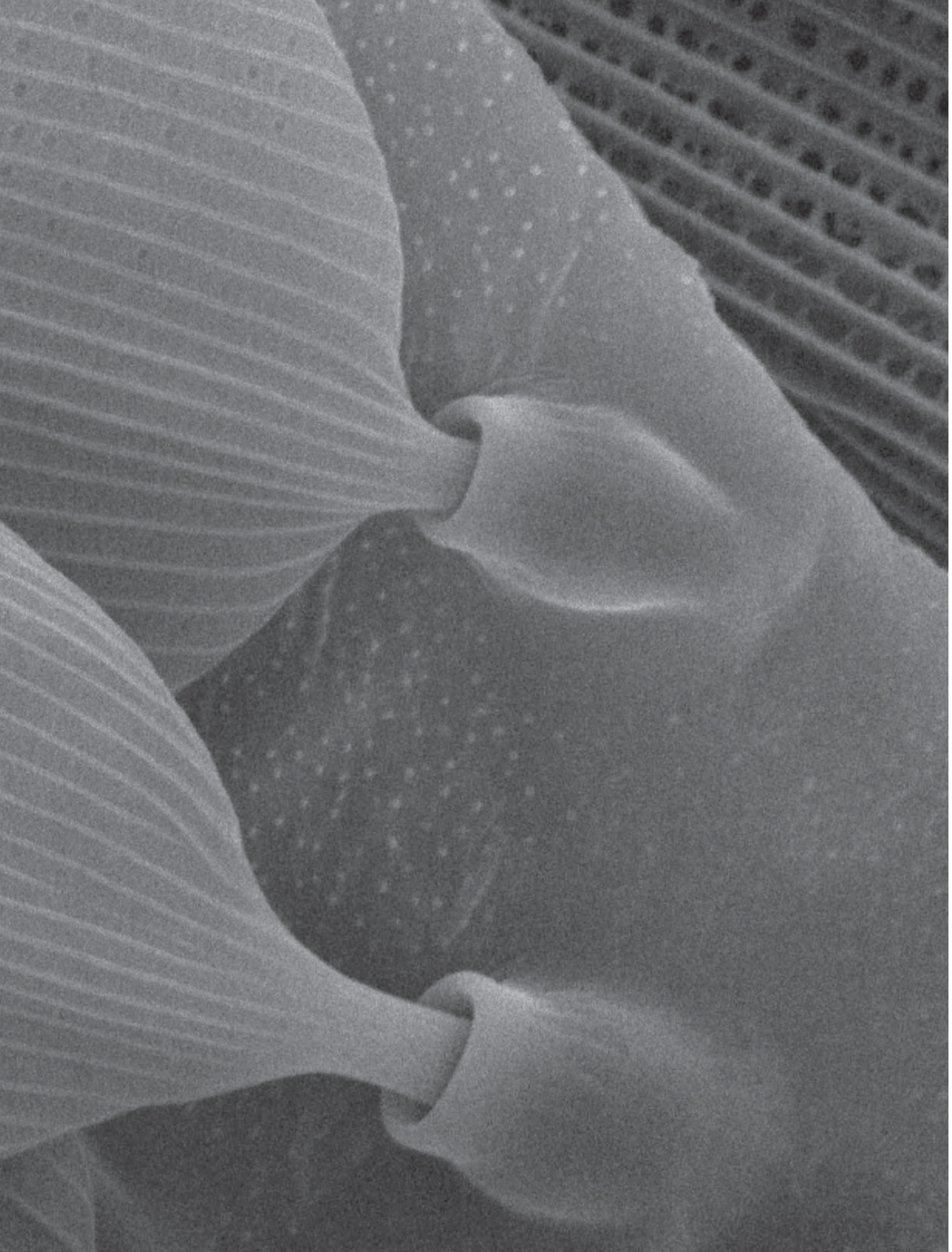
USERS
Total: 3295
By discipline:



INSTRUMENT HOURS
Total: 208,799
By discipline:




capability
and access



ammrf
Australian
Microscopy & Microanalysis
Research Facility

Our capability comprises nearly 200 instruments run by expert staff supporting over 60 different microscopy techniques. They enable finely tailored experimental approaches to diverse research questions.

Our users 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.

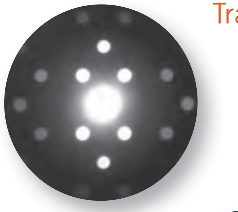
Available to all Australian researchers on the basis of merit, our capability also enables innovation through partnerships with industry and through international collaborations.

capability

WORLD-CLASS RESEARCH INFRASTRUCTURE

Effective research demands advanced infrastructure and AMMRF capabilities don't stand still. They are always being improved through the acquisition of new instrumentation and innovation in the application and adaptation of current technology. For example we develop improved specimen preparation methods and combine techniques for added value. On the opposite page are snapshots of two of these recent innovations.

Major new instruments as well as detectors, lasers and cameras have been installed and become operational around the AMMRF nodes over the past year. Highlights include high-performance focused ion beam (FIB) instruments at the University of Sydney and the University of New South Wales, electron microprobes for materials and geoscience at the University of Adelaide and the University of Western Australia, an X-ray micro- and nanotomography suite at the University of South Australia, and a confocal and multiphoton microscope at the University of Sydney.

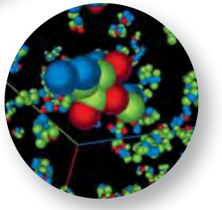


Transmission Electron Microscopy

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

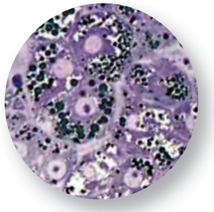
Specimen Preparation

Biological & Materials
Cell Culturing & Molecular Preparation
Thermomechanical Processing



Advanced Ion Platforms

Nanoscale Mass Spectroscopy
Atom Probe Tomography
Ion Milling & Machining
Ion Implantation

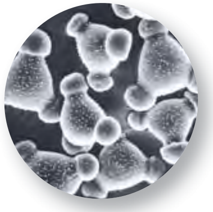


Light and Laser Optics

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

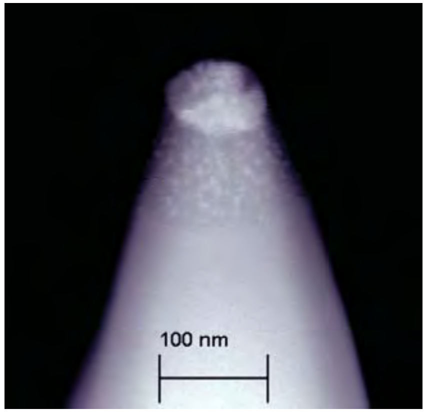
Visualisation and Simulation

Computed Spectroscopy
Computed Diffraction
Image Simulation & Analysis
Data Mining

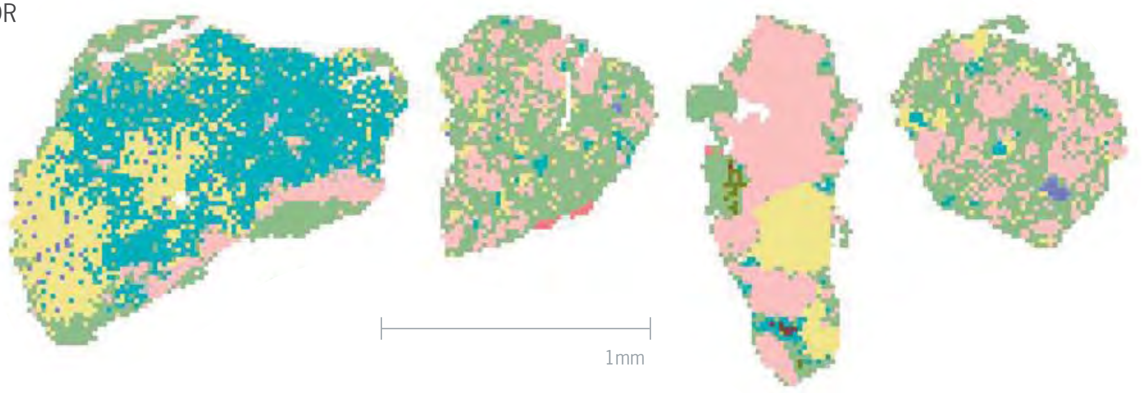
new developments

COMBINING TECHNIQUES FOR BETTER SAMPLE PREPARATION

Focused ion beam (FIB), scanning transmission electron microscopy (STEM) and atom probe tomography are all very powerful techniques. The staff of the AMMRF at the University of Sydney are working to combine FIB and STEM to prepare precisely defined atom probe samples in ways that have not been possible before. The developments are enabled by the advanced features on the new Zeiss Auriga FIB instrument. A custom-made slide-in STEM detector makes it possible for features that are only visible under TEM, such as the nanocrystals shown in this image, to be used to monitor the milling process in real-time. This way, the milling pattern can be controlled as it happens, with very high precision, and the milling can be stopped precisely when the region of interest appears at the desired position (precision <10 nanometres). A secondary-electron image can still be acquired in parallel to easily monitor the overall shape of the tip. This approach is now being successfully applied to various projects, including atom probe tomography of thin-film solar cells that incorporate nano-crystals.



QEMSCAN® FOR CLAYS AND BITUMENOUS SHALES



QEMSCAN® is an automated mineral analysis system that is widely applied in the minerals industry and provides data about the mineralogy, chemistry and texture of a sample by combining energy dispersive microscopy and sophisticated software. Some samples are especially challenging and haven't been analysed by QEMSCAN® before. Staff in the AMMRF at the University of South Australia have developed sample preparation methods that now allow analysis of clays and bitumenous shales, both particularly difficult specimens. Samples containing oils and other organic compounds cannot be prepared by standard block-preparation techniques so modified polishing methods were developed to prevent smearing of the oily components of the bitumenous samples. It was also necessary to select new mounting media to provide effective contrast of organic matter in a mineral matrix. New block-preparation and polishing methods were also developed for the clays, which gave a smooth surface without plucking the flat clay platelets from the samples. The researchers prevented the clays from swelling by using non-aqueous polishing methods. Refined species identification protocol (SIP) files were produced for both the new sample types.

These new developments greatly extend the range of samples that can now be processed for QEMSCAN® analysis.

Image: Clay particles analysed by Qemscan. Different minerals showing as different colors.

access

Your Project

Our website and online tools can help you to find to the right instrument for your project. After you contact the node that houses the instrumentation you require, your project is assessed by the node director and relevant technical staff. Assessment is based on scientific merit and feasibility, along with the availability of the instrument and associated technical expertise.

With our constant focus on improving access through training and efficiency, we are sharing our expertise through online tools.

MyScope

This innovation in training for advanced research provides e-learning modules that help new users progress on our sophisticated instruments.

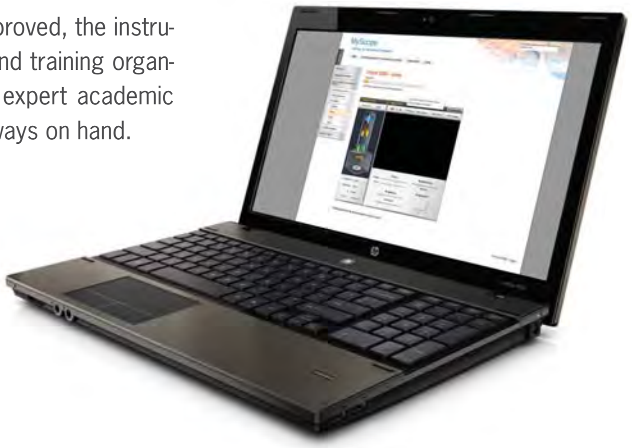
Integrating with traditional learning environments, each module has an interactive virtual instrument, step-by-step instructions and a range of additional resources. This provides a flexible, individual learning path that prepares trainees for intensive one-on-one instruction. Better-prepared trainees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

MyScope is available 24/7 online, free of charge to everyone. It enhances the wide range of training opportunities offered by the AMMRF. Modules are being deployed in stages throughout 2011.

ammrf.org.au/
techniquefinder

ammrf.org.au/
myscope

Once a project is approved, the instruments can be booked and training organised. Support from the expert academic and technical staff is always on hand.



Technique Finder

An online tool to help you identify the appropriate microscopy technique to answer your research questions and find the contact details of the relevant expert staff, who will guide you through the planning, training, data collection and interpretation stages of your experiment.

travel & access program

This highly successful program attracted researchers from across Australia and provided quality access to instruments in the AMMRF nodes. Mainly directed towards the flagship instruments the scheme allowed researchers to access the latest technology in microscopy and microanalysis, which might not have been accessible within their own institutions. Each application was assessed rapidly and successful applicants notified within weeks of submission. Funds were provided for travel, accommodation and for beam-time. On average, researchers were given five days access to any facility, which allowed enough time to obtain useful results and to determine the feasibility of their experiment. In numerous cases, the initial exposure to the instrumentation and laboratory has led to longer-term collaborations.

Due to great demand, the funding for the Travel and Access Program was fully allocated as of January 2011. The TAP received 158 applicants over its lifetime and funded 143 of those. The success of this program clearly demonstrates the need for seed funding to enable pilot studies, providing results that support larger grant applications and strengthen Australian research.

The TAP has contributed crucial elements to significant bodies of work, as illustrated in the adjacent example. Other case studies follow, in which contributions have supported different phases of the researchers' projects.

Scanning electron micrograph of a grain of tranquillityite after SHRIMP analysis and removal of a FIB section.

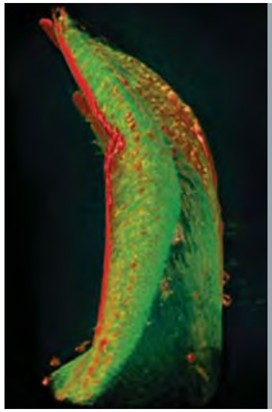
Janet Muhling from the University of Western Australia visited the University of Adelaide and showed that Moon minerals are here on Earth

A collaborative team of researchers from the University of Western Australia (UWA) and Curtin University needed to confirm the identity of some rare-earth and uranium-containing minerals found in small amounts in the Eel Creek Formation in the Pilbara region of Western Australia. Electron microprobe (EPMA) analyses had provided tentative identities for some of the minerals but the fact that several were polymorphous meant that further analysis was required. The aim of A/Prof. Muhling's TAP project was to prepare samples that could be examined by using electron diffraction to identify these enigmatic mineral phases.

The focused ion beam instrument in the AMMRF at University of Adelaide was used by A/Prof. Muhling to prepare extremely thin sections from each of the sample minerals. These were then taken back to the AMMRF at UWA for analysis. The transmission electron microscopy and electron diffraction confirmed the identity of one of the minerals as tranquillityite. This intriguing name hints at the origins of the first examples of this mineral; it was first found in samples of moon rock collected by Apollo 11 astronauts from Mare Tranquillitatis, or the Sea of Tranquility. It was thought to be unique to the Moon until this Western Australian team found it here on Earth. Analysis of the FIB-cut samples revealed that the mineral was only partly crystalline with a structure similar to lunar tranquillityite. Minerals, such as these, that contain radioactive elements like uranium and thorium, are often partly amorphous due to radiation damage to the crystal structure.

Terrestrial tranquillityite seems to be fairly widespread in Western Australian dolerite rocks, although it is only ever present as a minor mineral with small, elongated grains that look fox-red in transmitted light. Geochronological studies were done by sensitive high-resolution ion microprobe (SHRIMP) at the AMMRF Linked Lab at Curtin University and showed that, where the grains are large enough (>10 micrometres), they are excellent geochronometers with potential for dating some hitherto undateable geological formations. This particular Earthly example of tranquillityite was found to be about 1.07 billion years old.

Birger Rasmussen et al., *Geology*, 40 (1), 2012

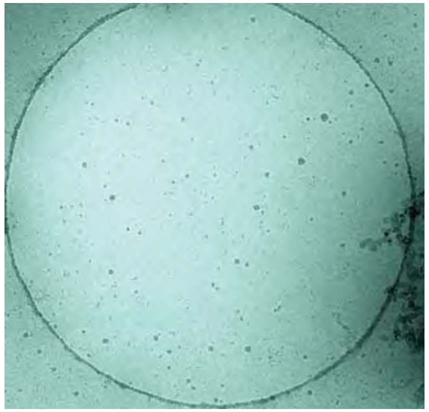


X-ray nano-tomography image of a single chiton tooth. Mineralised areas are in red.

Dr Jeremy Shaw from the University of Western Australia visited the University of Sydney

University of Western Australia researchers are seeking to discover how chitons build the iron-mineralised teeth they need to scrape algae off rocks. This work could inform the development of stronger composite materials.

Recently, Dr Jeremy Shaw used TAP funding to access both the X-ray nano- and microtomography systems in the AMMRF at the University of Sydney to explore the structure of chiton teeth in 3-D at multiple length scales. In the past, studies of tooth structure have often relied on electron microscopy to resolve features at the micro- to nano scale. However, owing to the complex organisation of the tooth's internal structure, it is easy to lose perspective when interpreting 2-D electron micrographs. X-ray nano- and micro-tomography were used to regain this perspective by imaging the entire structure in 3-D. The nanotomography was also able to reveal the 3-D arrangement of the organic fibres within the tooth.



Mr Hadi Khudheyer from Monash University visited the University of Queensland

Postgraduate student, Mr Hadi Khudheyer, is investigating new, non-viral ways to deliver genes into patients for gene therapy. He was producing short pieces of circular DNA (plasmids) complexed with cationic lipopeptides and needed to measure the size of the particles produced. Measurement by dynamic light scattering (DLS) was giving him a wide range of sizes, but it wasn't clear if these were real results or artefacts. By using cryo-TEM in the AMMRF at UQ, he was able to gather reliable information on size and morphology and detect smaller populations of particles that were not detected by DLS. Cryo-TEM also showed that large particles were aggregates of smaller ones. These results made it clear to Mr Khudheyer that homogeneous and stable spherical complexes were being formed.

TAP-FUNDED PROJECTS FROM JULY 2010 – JANUARY 2011

The cranial mechanics of varanid lizards and kingfishers

FIB to prepare wedge-shaped and uniform TEM samples

ToF-SIMS analysis of covalently linked proteins on surfaces

Investigation of ferromagnetic–ferroelectric (multiferrite) interfaces

Au precipitation in bacteria

Nano-CT iron biomineralisation

Evaluation of novel targeted and non-targeted resveratrol conjugates

Biomineralisation in scleractinian reef corals

Investigation of aging behaviour of SSM-HPDC alloys

Investigation of viral inclusion bodies characteristic of reovirus infection in commercially produced *Acheta domesticus* (house cricket)

Damage-tolerant aerospace sandwich composite: a cryo-TEM investigation of the self-assembly interactions of metal binding to oligonucleaotides

Crystallographic study of surface transformations

In-situ localisation of DMSP metabolism by coral-associated bacteria

Internalisation of bacterial vesicles by host cells

Correlative fluroescence and TEM imaging of the malaria parasite *Plasmodium*

Characterisation of the hosts of rare-earth elements in mafic rocks Preparing pillar specimens using FIB for in-situ TEM

Distribution and ecology of zooplankton iridoviruses

Understanding soft–brittle films under ultraprecision machining

Crystallographic structure of the thin-shell skeleton of ash cenospheres

Aluminium-based amorphous alloys

Metal localisation in two tropical marine sponges

Study on the new type of feromagnetic shape memory alloys Ni–Mn–In(Sn)–Co

Plasma-polymerised surface gradients on 3-D scaffolds for tissue-engineering applications

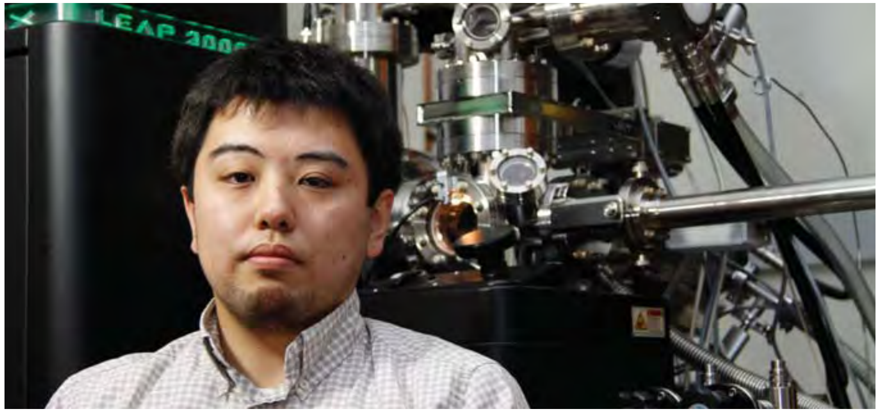
Identification of mucin-utilising organisms in-situ by Nano-SIMS

Diffusion of A1 and PGEs in olivine and spinel

Cryo-tomorgaphy of nuclear pore-like structures in *Gemmata obscuriglobus*

Study of the graphitisation process in diamond using analytical electron microscopy

flagship engineers at your service



DR TAKANORI SATO

IMAGO LOCAL-ELECTRODE ATOM PROBES (SYDNEY)

I run what are the most powerful 'microscopes' in the country. The local-electrode atom probes (LEAP) are a pair of complex instruments that hold a material specimen at an extremely low temperature of –250°C, and an almost outer-space vacuum level of 10^{-9} Pa. We apply precise voltage or laser beam pulses to the specimen to ionise and evaporate individual atoms off the specimen surface. By detecting these ions, we can accurately establish the 3-D positions of the atoms in the material and their elemental identity. Atom probes are the only instruments that can do this.

I work with the researchers, helping them run their samples and reconstruct the 3-D tomography data of structures at the atomic level, often involving hundreds of millions of atoms. The models that result help them understand sub-nanoscale structures and phenomena, beyond what is possible with conventional electron microscopes.

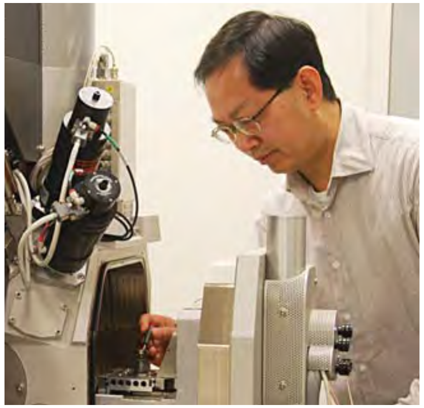
Atom probe tomography is a relatively new technique and more and more people are discovering what it can do. The AMMRF is conducting leading-edge research on advanced light alloys, steels and superconductors, but we are also starting to look into examining some biomaterials and mineral samples. It's very exciting.

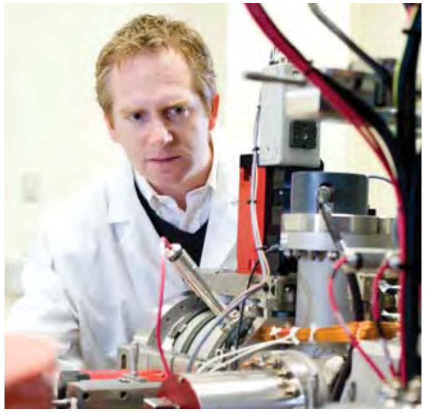
DR KAREN PRIVAT

HIGH-RESOLUTION SEM MICROANALYSIS FACILITY (UNSW)

Having begun work at the University of New South Wales Electron Microscope Unit during the twilight years of our old microprobe, I have been able to really appreciate the improvements in analytical and imaging capabilities that the flagship Hyperprobe has brought to our facility. As I write, I am mapping a specimen with sub-micron features of only subtle compositional variation, using a much higher beam current (100 nanoamps) and lower voltage (5 kilovolts) than I would have used before. This greatly enhances the spatial resolution of the analysis without compromising the sensitivity, which maximises mapping efficiency and the instrument's ability to detect those subtle compositional differences. And while I wouldn't have used our previous microprobe to capture any specimen images, the beam on our Hyperprobe makes it easy to capture great publication-quality images of a sample, even at a few thousand times magnification. Of course, setting up any microprobe analysis can take a lot of time and effort, but once your analysis is running, you can walk out of the room and let the instrument do the work; in the end you're rewarded with quantitative, high-resolution elemental data from your samples, which could be anything from ancient glass or corroded metal artefacts to fuel-cells, commercial coatings, ceramics and micro-circuits.

flagship engineers





DR CHARLIE KONG & MR LEN GREEN

FEI DUALBEAM FIBS: NOVA NANOLAB 200 (UNSW) & HELIOS NANOLAB (ADELAIDE)

I am the flagship engineer for the focused ion beam (FIB) at the University of New South Wales (UNSW) and Len Green runs the other part of the FIB flagship suite, at the University of Adelaide. Both Len and I have a materials science background and find it great to be able to work with such versatile instruments as the NanoLab and Helios. Not only can you perform micro-surgery on any solid material, from soft polymers to hard diamond, revealing substructures at multiple scales, but you can also home in and investigate site-specific submicron-sized features by navigating over large areas with the bird's-eye view of a scanning electron microscope. Sharpen a tip for an atom probe sample within minutes or tell the machine to drill millions of holes for you overnight. You can slice your materials to make sections, just nanometres thick, for transmission electron microscopy (TEM) and you can weld small things together to build your own structure in the micro-world.

It is very satisfying supporting researchers with so many different projects, such as studying quasicrystal structures in alloys, interfaces in nano-composites, multilayer structures for the future generation of solar cells, and all sorts of FIB applications for spintronics and biomaterials.

The researchers come to make use of the FIB's many features for preparing specimens for transmission electron microscopy (TEM) analysis, including high-resolution TEM; rewiring of integrated circuit devices; milling 2-D mechanical patterns; fabrication of 3-D micro-tools and biosensors; 3-D imaging of multiphase materials; 3-D electron back-scatter diffraction and energy dispersive spectroscopy, to name just a few.

A/PROF. MATT KILBURN & ASST/PROF. JOHN CLIFF

CAMECA IMS 1280 & NANOSIMS 50 ION MICROPROBES (UWA)

Matt: I was first introduced to secondary ion mass spectrometry (SIMS) during my PhD; I used the old Cameca IMS 4f in Edinburgh to measure trace-element partitioning in tiny planetary analogues that I made in the lab. Years later, I moved into biomaterials and used SIMS to analyse the surfaces of orthopaedic implants – same technique, very different application. It's this diversity that makes running the NanoSIMS lab at UWA so enjoyable; every week it's a different project, with different materials and different questions being asked.

The NanoSIMS 50 is a highly versatile instrument for earth science, materials science and biomedical applications. The flagship has contributed to publications as diverse as the world's oldest fossils, why stainless steels corrode, and how microbes transport nutrients between organisms within natural ecosystems.

John: I was also introduced to SIMS as a graduate student. I was the first person to use SIMS to study microheterogeneities in soil nutrient cycling. From there, I moved into nuclear forensics and materials analysis. I now look after the Cameca IMS1280, one of the most powerful and versatile SIMS instruments in the world. It can precisely measure, in-situ, the minute isotopic variations in minerals, addressing a wide range of questions from finding ore deposits, detecting signatures of early life, to reconstructing migration patterns of extinct animals. Whether it's depth profiling through thin-films, element mapping in minerals or measuring isotope abundance in bacteria, plant cells or animal tissue, SIMS provides a great combination of spatial resolution and sensitivity.

at your service

DR JOHN DENMAN

PHI TRIFT V NANOTOF TOF-SIMS (UNISA)

My scientific career started off in forensic and analytical chemistry, with my first introduction to time-of-flight secondary ion mass spectroscopy (ToF-SIMS) involving the development of new applications for this technique in the area of trace evidence analysis. The high surface sensitivity of the technique, coupled with the non-destructive mode of operation, means that the integrity of evidence remains intact. Additionally, minimal sample preparation and short analysis times, simultaneous acquisition of organic and inorganic information, and the ability to analyse trace amounts all give ToF-SIMS advantages over other techniques currently used in the forensic field.

I am now working in the more general field of materials science in the AMMRF at the University of South Australia, where I run the PHI TRIFT V nanoToF for a wide range of surface characterisation applications. I really enjoy being able to support researchers in their highly diverse projects in areas such as bio- and polymer interfaces, pharmaceuticals and mineral processing. One day I might be using the sub-micron spatial resolution to image the distribution of coatings on mineral ores, while the next I might be using the state-of-the-art C_{60}-ion source to sputter through an organic matrix to investigate its depth profile.

We get people coming from all over the country to make use of its imaging, spectroscopic and depth-profiling features. They often use the hot/cold stage or the reaction chamber to complete their experiments. Multivariate statistics are also sometimes needed to make the best sense of spectral data, with principal component analysis routinely used to investigate surface modifications or attachments of proteins on a surface.

DR GARRY MORGAN

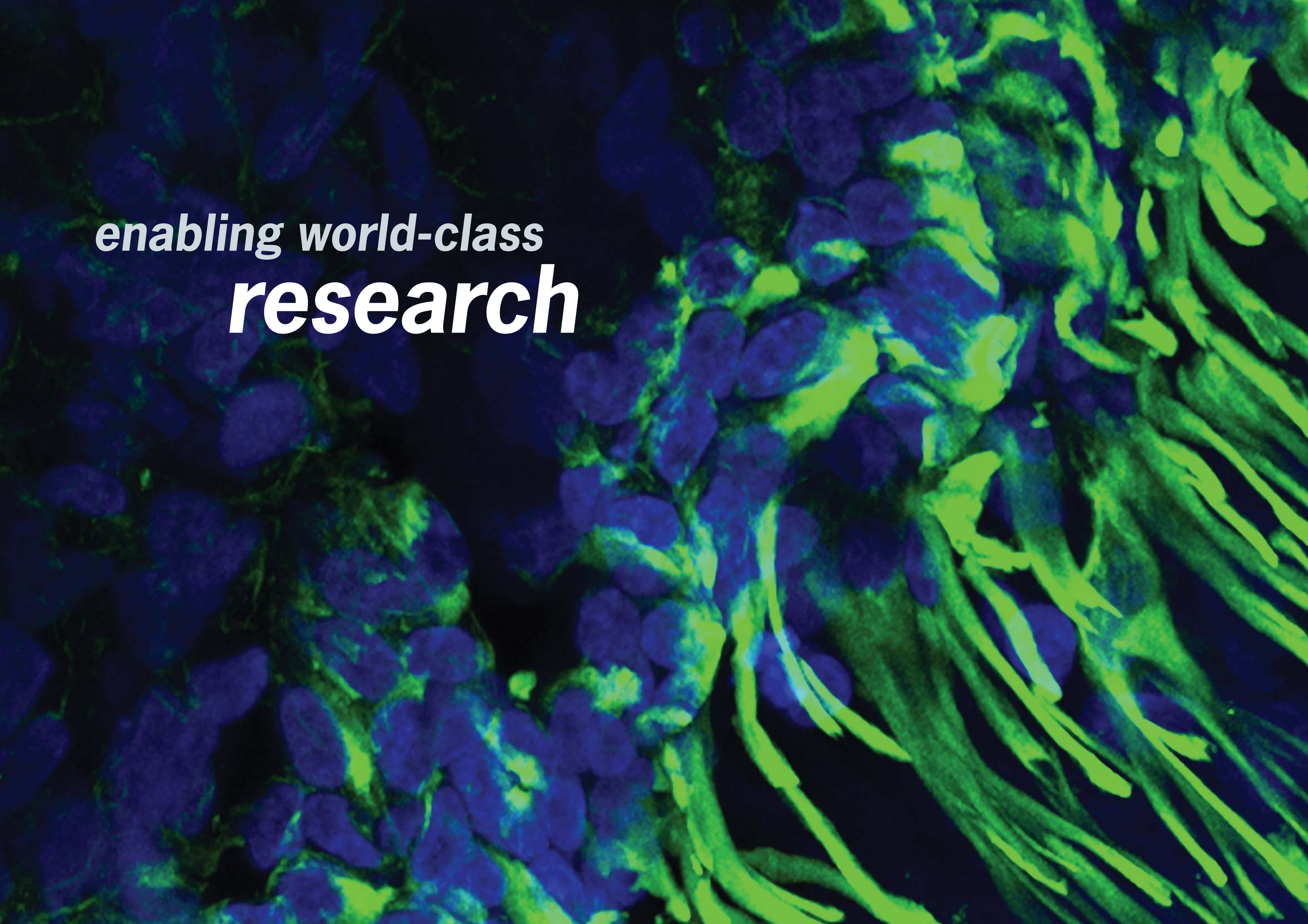
HIGH-THROUGHPUT CRYO-TEM FACILITY (UQ)

The FEI Technai F30 transmission electron microscope (TEM) is the AMMRF flagship instrument of the University of Queensland node. I currently look after this instrument with Dr Kathryn Green. We both have backgrounds in biological sciences, and lots of experience with TEM sample preparation and imaging.

We've got all the necessary equipment for the preparation and imaging of cryo-samples and plastic-embedded specimens. We can freeze samples by using either a high-pressure freezer (HPF) or a plunge-freezer. Plunge-frozen samples can be transferred directly to cryo-holders and put into the TEM while maintaining a temperature of –150°C or lower. If you use HPF you need to cryo-section or chemically fix your samples before imaging in the TEM.

The Technai F30 produces an electron beam powerful enough for imaging relatively thick sections, and can precisely tilt samples to ±70°, making this an ideal instrument for collecting tomographic tilt-series to give you 3-D ultrastructural information. It's also very good for the high-quality, high-resolution imaging of macromolecular assemblies whose structure can be determined by single particle analysis. All of these factors, combined with our high-throughput methods, make this flagship ideal for the collection of cryo-TEM and electron tomography data and we will be happy to help you do it.

Watch out for research stories from our flagship instruments, indicated by the icon: Flagship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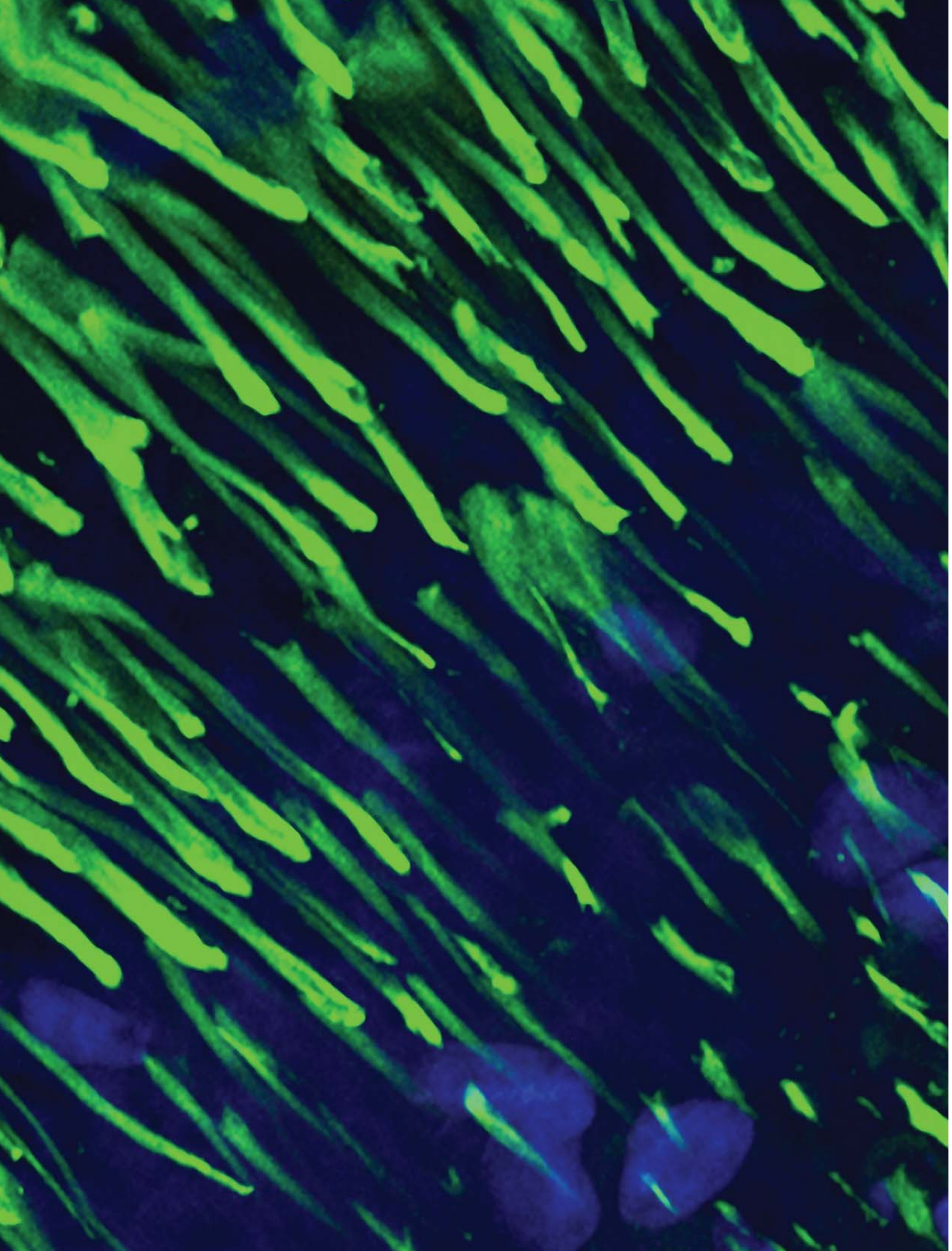
research



ammrf

Australian
Microscopy & Microanalysis
Research Facility

Our primary purpose is to support Australian research, generating new knowledge and feeding innovation in publicly funded 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 National Research Priorities, as indicated by these icons:

Frontier Technologies | Good Health | Sustainable Australia | Safeguarding Australia | Cultures & Communities

BONE-CANCER TREATMENT TRIALS

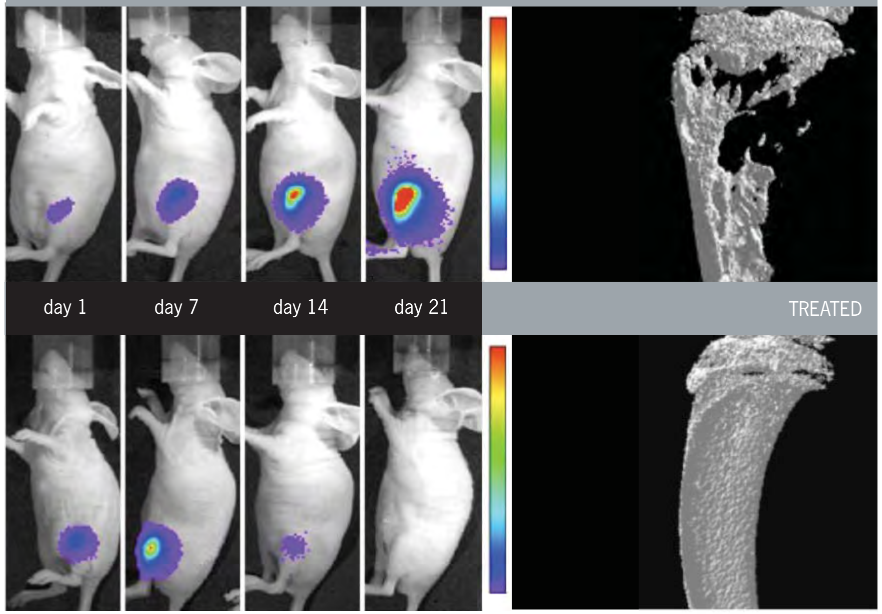
Bone cancer leads to large areas of bone destruction that can be extremely painful and often difficult to treat. Dr Agatha Labrinidis investigated the many types of cancers that affect bone, including metastatic breast and prostate cancer. Her focus was to examine potential new anti-cancer agents and their effectiveness against bone cancer in animal models.

In the AMMRF at the University of Adelaide Dr Labrinidis was able to quantify tumour growth in real-time with bioluminescence imaging, which detects light emitted from cancer cells tagged with the luciferase protein. She also measured corresponding bone destruction with X-ray microtomography on live animals. As a result, she was able to identify extremely small changes in bone micro-architecture. She was then in a position to compare the effects of a number of novel drug treatments on bone tumours, the results of which can be seen in the image.

As a direct result of these evaluations, new anti-cancer agents are now undergoing clinical trials for potential use in human patients. Dr Labrinidis has numerous international collaborations that take advantage of this technology.

Labrinidis et al., *Int. J. Canc.* 127(2), 2010

Bioluminescence imaging (left) of bone tumors in mice and X-ray microtomography (right) of the associated bone healing.



NEW EFFICIENCIES IN MINERAL RECOVERY

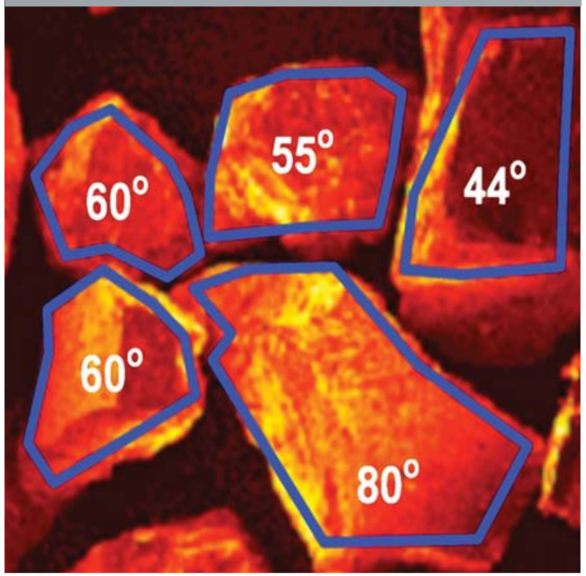
Flotation is the major industrial process used for concentrating valuable base-metal minerals and gold. Ore is ground to a defined range of particle sizes and rising air bubbles attach to the desired particles concentrating them into a froth, which is then collected. The efficiency of the process depends critically on controlling surface-chemistry differences to distinguish between valuable and waste particles. X-ray photoelectron spectroscopy, Auger and time-of-flight secondary ion mass spectroscopy (ToF-SIMS) have been used extensively in the AMMRF at the University of South Australia (UniSA) as a diagnostic tool for particle surface chemistry, directly relating the measurements with flotation behaviour.

The work of Ms Susana Brito e Abreu and Prof. Bill Skinner has produced the first direct coupling of measurements of particle surface chemistry with hydrophobicity and particle behaviour during flotation. The ToF-SIMS enabled the researchers to look at real samples from mines and to separate the determination of surface chemistry from particle-size effects in complex mixtures of mineral particles. This information cannot be measured any other way and is a breakthrough in flotation processing.

When combined with statistical analysis, the chemical data can quickly and accurately predict the best conditions for flotation, saving time and money in mineral liberation. This technique is now offered as a service to industry through the AMMRF at UniSA.

Susana Brito e Abreu and Prof. Bill Skinner, *Minerals Engineering*, 24, 2011

ToF-SIMS image of mineral grains superimposed with the calculated angle between the particle and a droplet of water (contact angle). This is what determines how well a grain clings to a bubble. A higher contact angle means that it will cling better to air bubbles than to the water.



impacthealth

PROBLEM

More than 25% of all deaths in the world are still due to infectious diseases. Vaccines only protect against infections when delivered effectively. Three significant issues are:

- liquid vaccine must be kept cold to retain its activity before it is injected. Only about half the vaccines delivered in developing countries are thought to be effective because of failures in the cold chain
- dealing with used needles is extremely hazardous, especially in HIV-endemic areas where needle-stick injuries are numerous
- injection into muscle is not very efficient in generating an immune response and so requires a large amount of antigen.

SOLUTION

Nanopatch technology for delivering vaccines has been developed at the University of Queensland (UQ) by Prof. Mark Kendall and his team. Small squares of silicon covered with 20,000 tiny projections around 110 micrometres high (see image) are coated with the vaccine, which dries onto the surface. The patches can then be applied to the skin where the projections penetrate just far enough to reach a very particular set of antigen-presenting cells, the most effective cells in establishing an immune response to the vaccine. The adjuvants that currently help boost the immunogenicity of vaccines might not be needed.

The team use scanning electron microscopy in the AMMRF at UQ to monitor the structure and coating of the nanopatches and its effects on the skin.

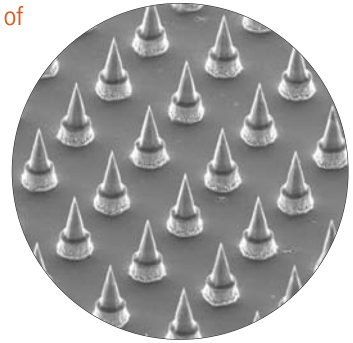
IMPACT

A nanopatch uses less than 1% of the dose of vaccine compared to a syringe.

- Vaccine coating retains full potency for long periods of time without refrigeration.
- The nanopatch is painless and only needs to stay on the skin for a minute or less to be effective.
- Overcomes hazards of needles and is easy to use and dispose of.

The nanopatch will have a major impact on the effectiveness of mass vaccination programs and therefore on the target of eliminating infectious diseases. Vaxxas Pty Ltd has been established to develop this technology (see p.35).

Engineers, mathematicians, material scientists and immunologists working on the nanopatch, won the 2011 Eureka prize for research by an interdisciplinary team.



Germain Fernando et al.
PLoS one 5 (4), 2010.

research

LOOKING INSIDE PARTICLES

The engineering of multi-compartmental nano- and microparticles with precisely controlled shape, size, surface chemistry and mechanical properties is increasingly important in a variety of research areas, such as diagnostics, therapeutics and photonics. Control over internal architecture of particles is gaining importance as an additional design parameter. The characterisation of multi-compartmental polymer particles without the use of fluorescent dyes, is highly desirable in order to confirm specifically introduced chemical functionality.

Confocal Raman spectromicroscopy (CRS) is particularly well suited to studying multi-compartmental particles, allowing the mapping of molecular-vibration signatures of functional groups in different areas of a particle.

Dr Christopher Gibson and Prof. Nico Voelcker from the AMMRF at Flinders University employed CRS to investigate the internal chemical functionality of bi- and tetra-compartmental particles made by their collaborators at the University of Michigan. 50% and 25% of the internal structure respectively had been functionalised with acetylene groups and were visualised by mapping the molecular vibration signature for acetylene. This was overlayed onto an image generated by mapping a typical Raman band for the polymer particles.

Such sophisticated analytical techniques for the characterisation of functional particles are allowing nano- and microstructures to be engineered with a good fundamental understanding of their internal structures.

S.Bhaskar et al. *Small*, 7, 2011.

OLDEST FOSSILISED LIFE ON EARTH

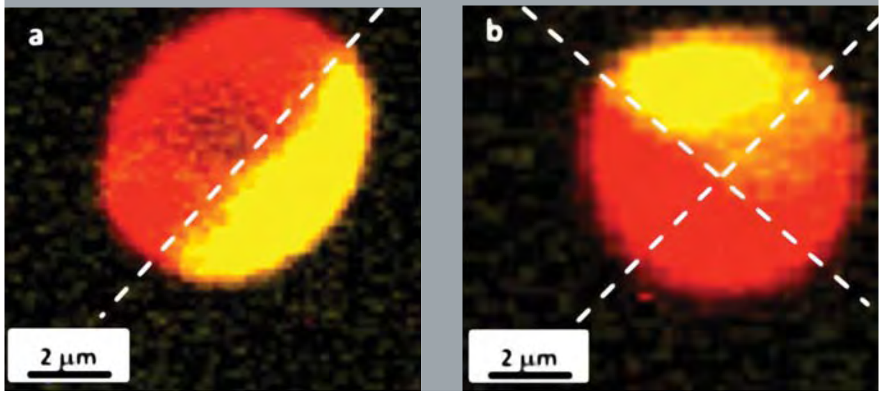
Researchers based in the AMMRF at the University of Western Australia (UWA) have unearthed arguably the best-preserved and oldest pre-three billion year-old microfossils on Earth. Found in the Strelley Pool Formation, in a remote region of the Pilbara, they are at least 3.4 billion years old and are providing insights into the earliest life on Earth. The microfossils consist of remarkably well-preserved carbonaceous cells along with the protective tubes (sheaths) that housed them.

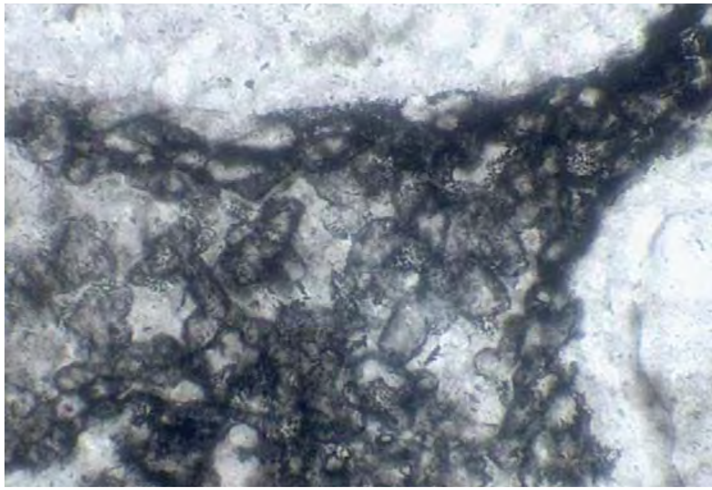
Leader of the team, Dr David Wacey says that direct evidence for early life in the form of microfossils is exceedingly rare and details of the precise nature of the earliest organisms had, until now, proved elusive. The team was able to apply their expertise in electron microscopy and innovative ion-microprobe approaches to convincingly demonstrate that these particular microbes had a metabolism that was based on sulphur. This ability to 'breathe' sulphur compounds has long been thought to be one of the earliest stages in the transition from a non-biological to a biological world. By showing the intimate association of these 3.4-billion-year-old microfossils with the mineral pyrite (FeS_2), the team has provided strong evidence that the earliest microorganisms employed a sulphur-based metabolism.

Evidence from the characterisation of these microfossils extends the fossil record of life on Earth by approximately 300 million years. They also highlight a potential method to be applied to the search for life on Mars.

D. Wacey et al. *Nature Geoscience*, 4, 2011

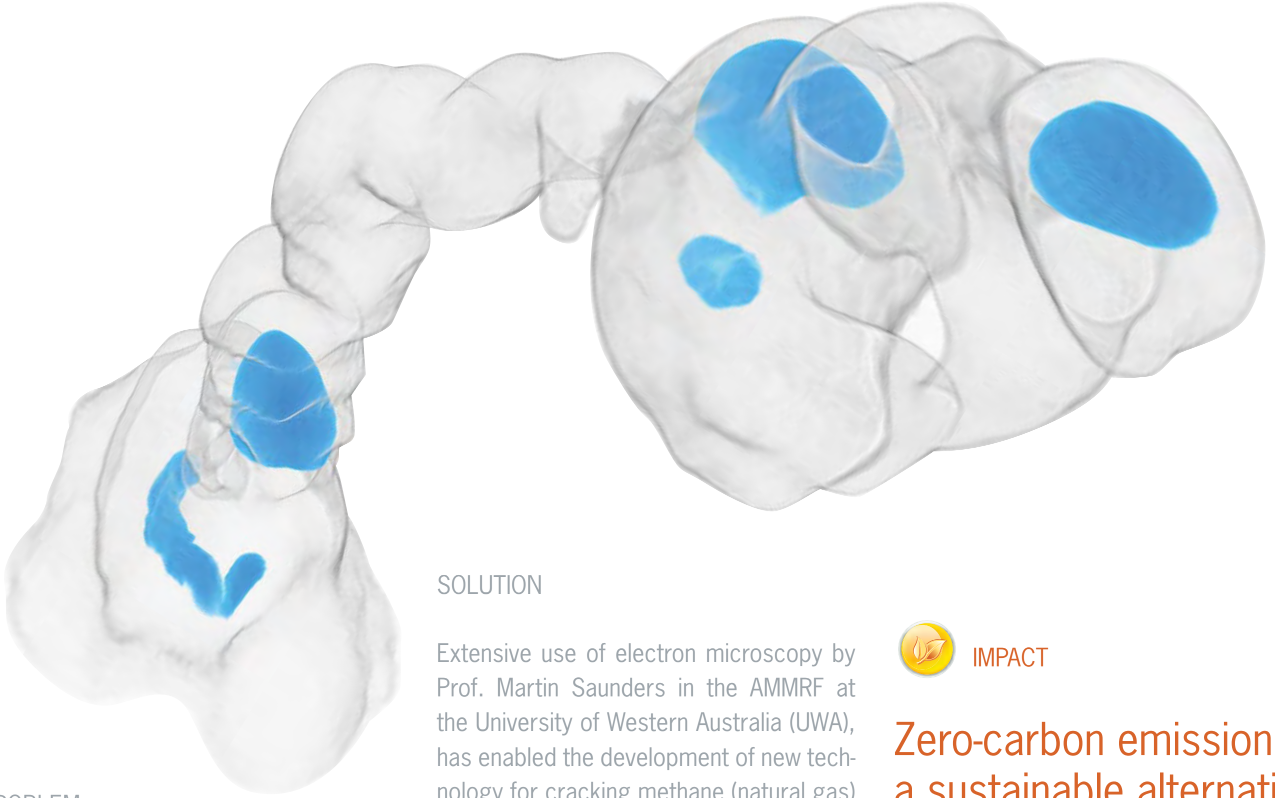
Confocal Raman images of bi-compartmental (a) and tetra-compartmental (b) polymer particles. The yellow regions show the spatial distribution of acetylene groups within these particles. The red regions are generated by mapping the Raman band for an esther group and are present throughout the particles.





Light micrograph showing bacterial and tubular microfossils clustered together around the edges of two quartz grains (top and right). The fossil bacteria are around 20 microns in diameter.

impactenergy



PROBLEM

Methane is the second most significant greenhouse gas after carbon dioxide, with a global warming potential nearly four times that of carbon dioxide.

Hydrogen is a valuable industrial chemical mainly used for the preparation of fertiliser and other applications. Current methods of generating hydrogen create significant emissions of carbon dioxide.

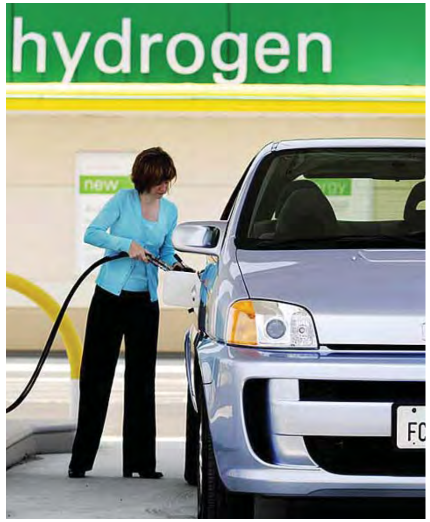
In future, if hydrogen can be made cleanly, it is likely to be a critical component of a reduced-emission energy market.

SOLUTION

Extensive use of electron microscopy by Prof. Martin Saunders in the AMMRF at the University of Western Australia (UWA), has enabled the development of new technology for cracking methane (natural gas) into hydrogen and carbon nanomaterials, with no carbon-dioxide emissions.

As well as hydrogen, self-encapsulating carbon spheres (image above) called carbon 'nano-onions' are produced. These have potential value in nano-electronics, advanced lithium-ion batteries, advanced lubricants, reinforcements in composites and as a storage material in hydrogen fuel-cells.

A spin-off company resulting from this research, Hazer Pty Ltd, has successfully raised capital and is now funding further development work (for more see p37). Patents are in place to cover the cracking process and purification of the carbon nano-onions.



IMPACT

Zero-carbon emission hydrogen fuel production is a sustainable alternative for fuelling vehicles and electricity generation.

Carbon is sequestered as useful nanomaterials.

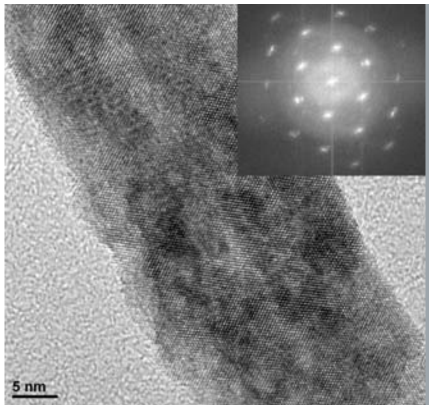
Australian innovation attracting investment and creating jobs for a sustainable future.

IMPROVING EFFICIENCY OF CATALYTIC CONVERTORS

Catalytic convertors in car exhaust systems oxidise residual hydrocarbons and carbon monoxide in the exhaust stream, thus preventing these environmental pollutants from being released into the atmosphere.

Oxides of rare-earth metals are widely used in catalytic convertors to provide oxygen storage capacity under both reducing and oxidising conditions. The stored oxygen is released and reacts with the exhaust gases. Maximising the oxygen storage capacity of these materials is of great interest and can be achieved by controlling the material's morphology to maximise surface area of the most-active crystal faces. Single-crystal nanorods formed using a complex three-step synthesis method, consisting of precipitation, hydrothermal aging, and calcination, were found to be significantly more active for the oxidation of carbon monoxide than conventional nanoparticles.

The high temperatures and pressures used in this method make synthesis complex and difficult to scale up. As a consequence, Dr Aaron Dodd from the University of New South Wales (UNSW) set out to determine whether low-temperature aging could be used as a simpler method for manufacturing high-activity catalytic materials. So far Dr Dodd has demonstrated that low temperature aging can indeed be used to prepare nanoparticles with a controlled size and morphology. Analysis by high-resolution transmission electron microscopy, carried out in the AMMRF at UNSW has also shown that low-temperature aging yields particles with a complex multi-crystalline structure that differ from the single crystals prepared by high-temperature processing. Work is continuing to see if their catalytic activity is also different and if the process is a feasible alternative to current methods.



High-magnification TEM image of a praseodymium-oxide nanorod prepared by low-temperature aging. Inset shows a Fourier transform of the lattice image.

NANOPARTICLES TO TREAT CANCER

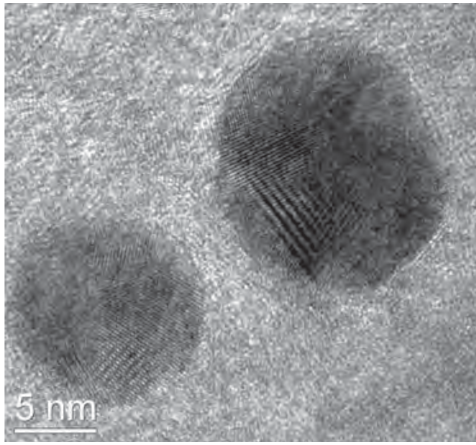
Exciting results are emerging on a potential new approach to treating oral cancers.

Ms Ya-Na Wu is a postgraduate student with A/Prof. Filip Braet in the AMMRF at the University of Sydney and, in collaboration with a team of Taiwanese researchers led by Prof. Dar-Bin Shieh from the National Cheng Kung University, she has found that gold-coated iron nanoparticles selectively reduce cell division in oral cancer cells while having no obvious harmful effects on normal cells. When combined with a commonly used anti-cancer drug, their effect was more pronounced, causing a significant number of tumours to disappear from experimental animals. Ms Wu made extensive use of of fluorescence microscopy and transmission electron microscopy in the AMMRF at the University of Sydney to study the interaction of the particles with cells.

Several properties of the particles are significant in mediating the anti-cancer effects; the thickness of the gold coating, the fact that the coating is gold and critically, the oxidation state of the iron. When the iron oxidises, particles are markedly less effective in killing the cancer cells. Although the precise mechanism by which the iron acts from within the gold coat is still unclear, the iron-in-gold nanoparticles have been shown to cause an irreversible loss of mitochondrial membrane potential. As mitochondria are the power stations of the cell, if they don't work, the cells die.

These particles could be developed into a treatment that, if applied directly onto the affected area in the mouth, could reduce the need for disfiguring facial surgery.

Ya-Na Wu et al. *Biomaterials*, 32, 2011



Transmission electron micrograph of two iron-in-gold nanoparticles showing the crystalline structure of their iron cores.

impact cancer

IMPACT

Better prediction of treatment outcomes and therefore more appropriate interventions.

- Identification of new targets for drug development.
- Ultimately it will be possible to tailor therapy more precisely according to the needs of individual patients resulting in improved outcomes and the possible elimination of recurrent breast cancer.

PROBLEM

Breast cancer is the third most costly cancer in Australia with direct costs to our healthcare system of $331 million in 2005. Around 8,500 new cases occur each year, causing much distress to patients and their families.

Although early detection of breast cancer and advances in therapy have significantly improved survival rates, cancer recurs in approximately 20% of patients. The recurrent cancer is harder to treat and is not well understood. There appears to be a different class of cells that can lead to a localised recurrence of tumours.

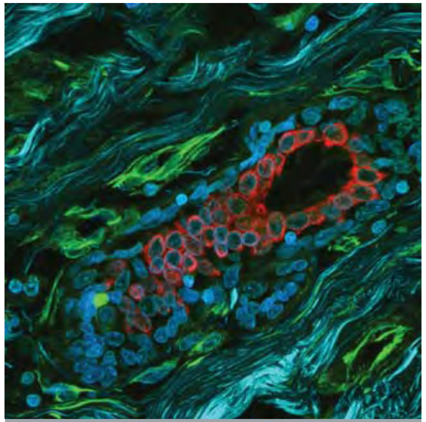
SOLUTION

Dr. Lilian Soon from the AMMRF at the University of Sydney has focused on finding the origins of these cells in the primary tumour and has discovered solitary tumour cells in the breast matrix. She has isolated these solitary, primary tumour cells (SPCs) by using laser-capture microscopy and found that they carry a dormancy (non-proliferative), migratory and stem-cell pattern of gene activity. The SPCs move away from the breast ducts, invading the extracellular matrix where they remain dormant and drug resistant.

Her work, also including scanning electron microscopy and live-cell imaging, demonstrates that:

- genes regulating cell protrusions are over-active in SPCs
- protrusions are important for cell invasion because they interact with the matrix to generate the traction needed for forward movement.
- interfering with these genes can block cell invasion
- knocking down a dormancy gene causes the tumour cells to resume proliferation and therefore become sensitive to cytotoxic drugs.

This leads to a better understanding of different types of breast cancer.



The red area is a milk duct surrounded by connective tissue in a biopsy from a patient with breast cancer. Connective tissue cells are green and collagen fibres, turquoise.
Size: 163 micrometres wide.

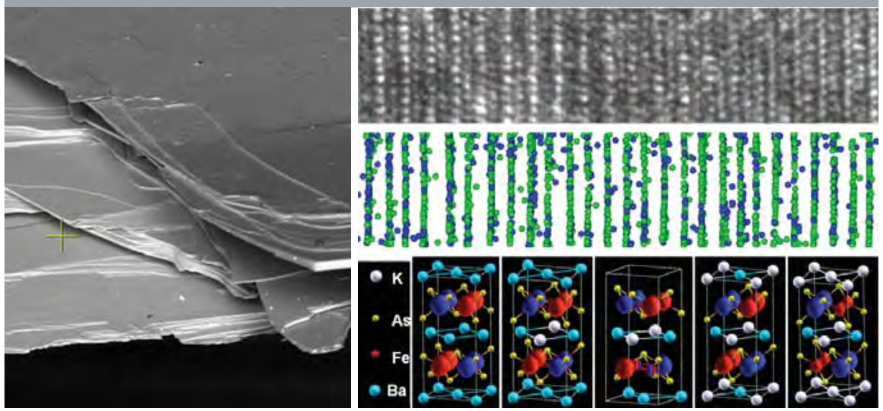
PROBING ATOMS TO UNDERSTAND SUPERCONDUCTIVITY

Superconductors are materials where electrical currents flow without energy loss, but only when they are kept below a critical temperature, usually within about 20 degrees of absolute zero. Some unusual superconductors also appear to exhibit magnetism, although the reason for this is a great mystery.

To investigate these properties, AMMRF scientists from the University of Sydney, led by Drs Rongkun Zheng and Waikong Yeoh, have collaborated with researchers from the University of Wollongong and the Max Plank Institute in Germany. The team used atom probe tomography in the AMMRF at Sydney to look at the precise 3-D arrangement of individual atoms in one of these newly discovered iron-based superconductors, a potassium-doped $BaFe_2As_2$. Their results, and subsequent simulations, showed that the doped potassium atoms are not uniformly distributed within the material and that the way they are arranged in the crystal lattice determines the relative spin density around the iron atoms and therefore the characteristics of the magnetism. It is also the arrangement of the potassium atoms in the lattice that determines the electronic structure of the material, which is critical for the superconductor's performance. These results show how magnetism and superconductivity can survive harmoniously in these new materials. Such advances in superconductor science will have many applications; improving MRI scanners and high-powered motors and potentially in supercomputers, to name a few.

Waikong Yeoh et al. *Phys. Rev. Lett.* 106 (24), 2011

Scanning electron micrograph of the superconductor material (left) with high-resolution transmission electron microscopy (top right) and atom probe tomography (centre). Resulting simulations of possible crystal strctures appear below right.



MICROBAND BOUNDARIES IN THREE DIMENSIONS

Microbands are one of the most commonly found microstructural features in many engineering metals and alloys. The nature of microbands has a strong influence on some essential production-line steps for structural metal, such as shaping and heat treatments. Microbands form after low levels of deformation and are often separated by boundary interfaces, the orientation of which has significant influence on the strength of alloys. Because of the complexities in the microband boundary structures, their crystallographic alignment remains unresolved and a three-dimensional investigation was necessary.

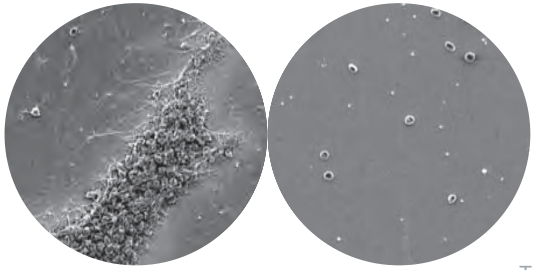
Ms Nasima Afrin Zinnia, a postgraduate student in the School of Material Science and Engineering at the University of New South Wales (UNSW), has made use of the 3-D electron backscatter diffraction (EBSD) facility to investigate microbands in nickel. Ms Afrin located a microband-containing area in a deformed nickel sample and milled into it with a focused ion beam (FIB), mapping the crystal orientations in the exposed surface with EBSD. Sophisticated software enabled the EBSD slices to then be reconstructed to show the microband boundaries in 3-D.

The boundaries contain many irregular curves and bumps instead of being perfectly crystallographically flat. The results demonstrate that the average alignment of the curved microband boundaries nearly coincides with the crystallographic slip planes, which could have consequences for subsequent deformations. This finding helps to understand the hardening behaviour of materials that contain microband structures.

Nasima Afrin et al. *Scripta Materialia*, 64, 2011

The microband boundaries in three-dimensional space.

impact implants



PROBLEM

Medical devices can induce adverse reactions when the body detects the device as foreign, including inflammation, clotting, formation of fibrous scar tissue and, if bacteria find their way to a device, serious infections. For instance, although bare metal stents are used to physically open up occluded blood vessels in the treatment of coronary artery disease, they actually increase inflammation, a cause of the condition in the first place.

In Australia in 2009, there were 22,383 stent insertions at a cost to Medicare of nearly $7 million. Other devices, from pacemakers to artificial hips, can have similar problems. We need a simple way to treat devices that will cloak them from the body's defences.

SOLUTION

Physicists, biochemists and medical researchers have brought their expertise together in a collaboration at the University of Sydney. Led by Prof. Marcela Bilek, Prof. Tony Weiss and Prof. Cris dos Remedios the team have developed patented coating technologies that use plasma induced ion implantation (PIII).

This technique generates a layer rich in free radicals extending about 100 nanometres into the surface, making it highly reactive. Just about any protein or organic molecule can covalently bind to the treated surface and retain its functionality. Variations of the PIII technique can be applied to any material, including polymers and metals. Previously it had been necessary to develop complex and specialised intermediate layers to facilitate attachment. When put into a patient, devices prepared by PIII rapidly become coated with the patients own proteins, effectively hiding the device from the body's defences.

In the AMMRF at the University of Sydney, the team has demonstrated that, when exposed to whole blood, treated metallic surfaces did not initiate clot formation. This is in contrast to untreated metal on which clots can readily form, as can be seen in the micrographs above. Treated materials remain active for at least a year.

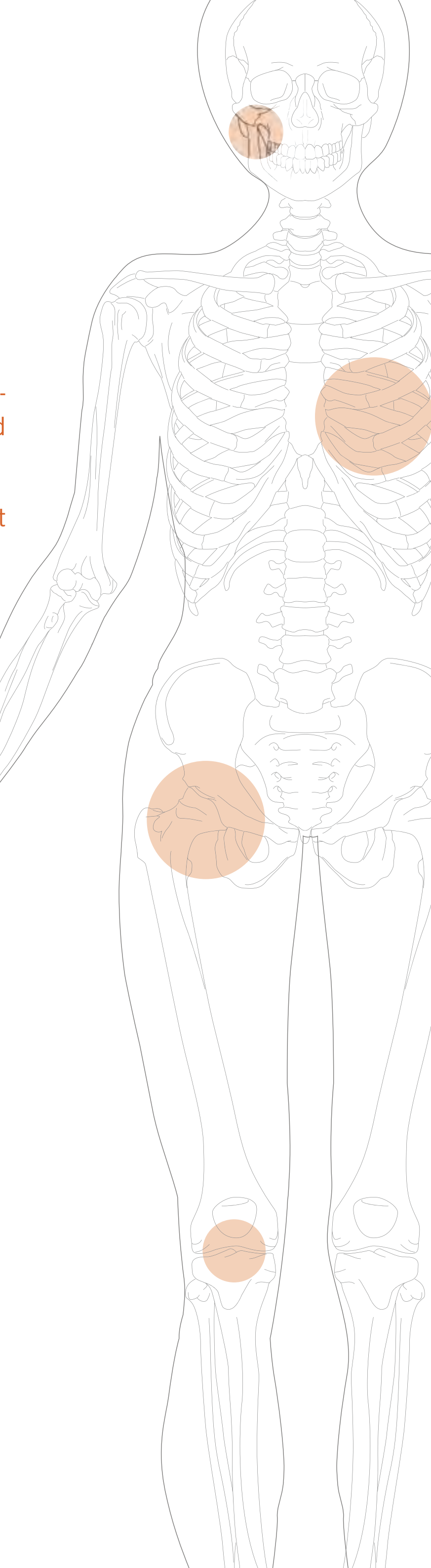
IMPACT

Medical implants with reduced adverse reactions

- Less anti-clotting and anti-inflammatory drug treatment following device insertion – saving money and side effects.
- Less subsequent surgical intervention needed – cost savings for healthcare, faster recovery for patients.
- PIII-treated implants can work with proteins in the patient's own body to prevent adverse reactions;
- alternatively, devices can be pre-coated with organic molecules such as elastin to encourage growth of healthy blood vessel cells, or anti-bacterial compounds to prevent infection.

The anti-bacterial coating will be further developed in a new collaboration with by Prof. Hans Griesser, Director of the AMMRF node at the University of South Australia.

Bilek et al., PNAS, 108 (35), 2011



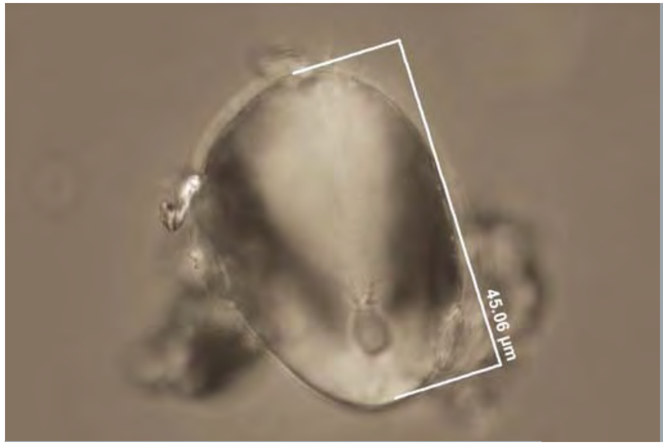
FIRST HUMAN ARRIVALS TO NEW GUINEA

Archaeologists have found new evidence for some of the earliest known settlements in Papua New Guinea. They were occupied during the last ice age when New Guinea and Australia were one landmass, called Sahul.

The study sites are located at 2000 metres in the Owen Stanley Range and are dated from around 44–49,000 years ago. It would have been a cold and difficult environment in which to live. The research team included Dr Judith Field from the AMMRF at the University of Sydney who was responsible for the microscopic analysis of residues on stone tools. She found that there was excellent preservation of starch residues on the tools and this provided direct evidence that starchy plants, largely pandanus nuts and yams, were being processed by those who lived there. Campsites were discovered, buried by volcanic ash, where people made stone tools, hunted small animals, gathered the nuts of the local pandanus trees and processed yams.

Yams only grow at altitudes below those of the Ivane Valley, so the discovery of yam starches on stone tools coupled with the presence of stone from outside the Ivane Valley shows that people were moving between and living in several altitudinal zones. The cold conditions at that altitude would have made life uncomfortable and the Ivane Valley could have been at the very edge of routinely habitable territory. It is no surprise that occupation of the area ceased during the coldest periods of the last ice age.

Glenn R. Summerhayes et al., *Science*, 330, 2010



Light micrograph of a yam starch grain from one of the stone tools.

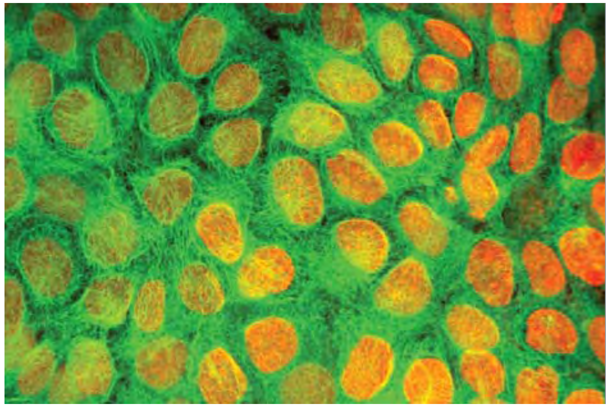
RECREATING THE EAR DRUM

Chronic perforations of the eardrum are a worldwide health problem and a significant issue here in Australia within disadvantaged and indigenous communities. A perforated eardrum can heal without surgical intervention; however, in cases where significant damage to the eardrum has resulted from injury or disease, surgical repair is required in the form of a graft. Current treatments can mend the hole, but do not provide adequate eardrum function to restore good hearing.

Tissue engineering could offer a solution and a team of scientists from the Ear Science Institute Australia (ESIA) and Deakin University, led by Dr Robert Marano, has developed a method of growing donated human eardrum cells on nanosilk fibre membranes. In the AMMRF at the University of Western Australia, they characterised the isolated cells as keratinocytes by using flow cytometry, confocal microscopy and high-resolution slide scanning. The next stages of the project aim to replicate, as closely as possible, the acoustic and bio-mechanical properties of the normal human eardrum in-vitro. Currently, work is evaluating the performance of the artificial eardrums, under conditions that eardrums normally encounter, to see how they measure up against the real thing.

It is envisaged that the successful use of this bio-engineered patch will provide a safe and readily available treatment for chronic ear perforations, allowing natural healing to progress. Once in the ear, the scaffold will slowly dissolve away, leaving only the patient's own regenerated eardrum. To further improve outcomes, the team is identifying growth factors that can be impregnated into the scaffold to enhance cell growth and accelerate healing.

Redmond et al. *J Mol Histol.*, 42 (1), 2011



These cultured tympanic membrane (eardrum)-derived cells are expressing epithelium-specific ets1 indicating that these cells are epithelial. Red fluorescence locates the cell nuclei. Visualised using confocal microscopy.

impact health

PROBLEM

Middle-ear infections (otitis media or OM) and their complications are one of the most common conditions treated in childhood.

It is the most common reason for a child to be prescribed antibiotics and the second-most common reason for pre-school-aged children to undergo surgery.

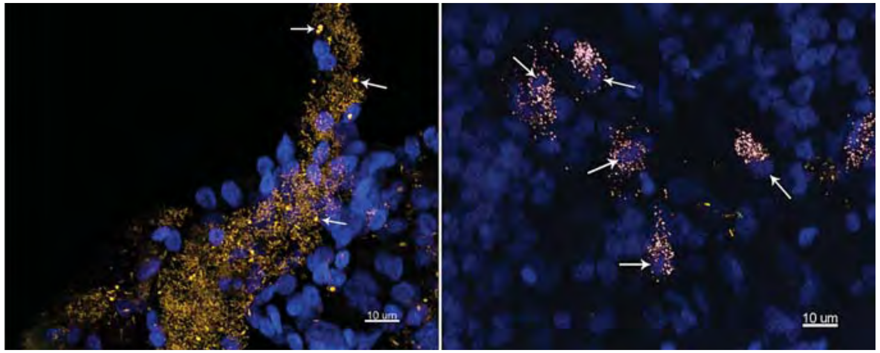
Approximately 659,000 Australians suffered from OM in 2008, 55% of them under fifteen years of age. Treatment cost $391.6 million.

Indigenous Australian children experience much higher rates of OM than their non-indigenous counterparts and this disease is almost universal in many Aboriginal communities.

The main complication is hearing loss with consequential restricted development of speech, language and cognitive abilities. OM is currently treated with antibiotics or surgical intervention to insert grommets. However, recurrent or chronic infections that defy these therapies are rife, often leading to ruptured eardrums.

SOLUTION

A collaborative team based at the University of Western Australia has studied middle-ear biopsies from children undergoing surgery for OM. AMMRF capability at UWA has enabled postgraduate student Ms Ruth Thornton to use confocal microscopy to image fluorescent in-situ hybridisation labelling of the biopsies (images lower left). She has discovered a range of infection-causing bacteria that not only exist in biofilms on the surfaces of cells but that can also invade the cells of the middle ear. Hiding within biofilms and inside cells, the bacteria evade the action of commonly prescribed antibiotics, acting as reservoirs for re-infection. By understanding how these bacteria evade standard treatments, new treatment regimes can be developed. This is likely to require a multi-faceted approach, targeting the non-bacterial components in biofilms and utilising different classes of antibiotics that can get inside the cells and kill the bacteria.



IMPACT

improved health outcomes, less damage to hearing

More effective treatments and prevention strategies will reduce the recurrence or persistence of OM leading to:

- better educational outcomes leading to more able and confident children
- reduced support costs for educational interventions
- based on reduced surgical treatment costs, new treatment regimes could reduce direct annual hospital costs of OM by $2.8 million nationally.

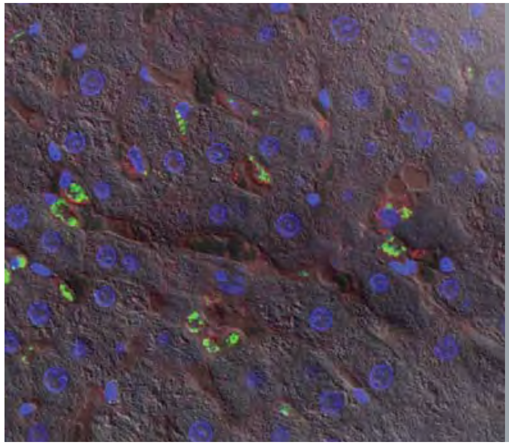
research

NEW STRATEGIES FOR RNAi DELIVERY

Viral diseases tend to be much more difficult to treat than those caused by bacteria, largely due to the fact that viruses use the person's own cellular machinery to propagate themselves. RNA interference (RNAi) technology has the potential to treat various intractable diseases, not only viral ones but also genetic disorders and cancers. It is able to do this by precisely suppressing the production of specific proteins from the host cell or a pathogen.

Led by Dr Thilak Gunatilake, a multidisciplinary group at CSIRO Clayton laboratories is collaborating with Dr Mark Tizard, Dr Tracey Hinton and Dr Paul Monaghan, at the Australian Animal Health Laboratory (AAHL), Geelong to overcome the major challenge of developing effective delivery vehicles for RNAi therapy. By using confocal and live-cell imaging at the AMMRF Linked Lab at the AAHL Biosecurity Microscopy Facility they are able to track fluorescently labelled delivery vehicles inside cells and whole animals. This enables them to establish the behaviour of capsules within the body and their effectiveness in delivering the RNAi 'payload'. This can be seen in the image where a thick section of liver from a rat that had been treated with green fluorescent capsules shows that they end up in a specific subset of cells.

The team also want to understand the role of various ligands (small bioactive molecules attached to the delivery vehicle) in directing RNAi to specific organs, cells or even sub-cellular targets with a view to developing these new reagents into practical therapeutics.



Confocal micrograph of a thick section of rat liver showing that the green capsules travel specifically to Kupffer cells (red). Nuclei are stained blue and tissue morphology is shown by differential interference contrast microscopy.

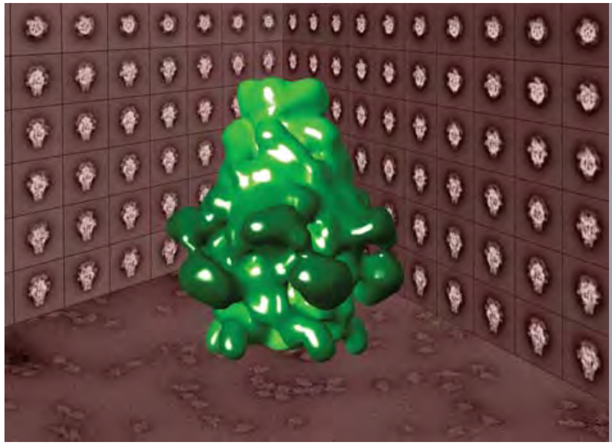
A POTENTIAL NEW BIOINSECTICIDE

The control of harmful insect populations is very important to both the agricultural and horticultural industries. For many years, synthetic insecticides were the most widely used control measures, but, owing to their various side effects, they have now been largely replaced by insecticides of biological origin. These are widely regarded as being more environmentally friendly and safer to humans, wildlife and beneficial insects.

Dr Michael Landsberg and A/Prof. Ben Hankamer are using single-particle analysis on the transmission electron microscope (TEM) in the AMMRF at the University of Queensland to determine the structure of a bacterial protein complex isolated from a native New Zealand grass grub. The protein is currently being investigated for its potential to be deployed as a new bioinsecticide. It is toxic to several insect pests, including a species of moth that has spread worldwide and infests plant crops such as cabbage, broccoli, Brussels sprouts, cauliflower, radish, turnip and mustard seed. It is also effective against the bronze beetle that affects apples and pears. The protein appears to be completely safe and shows no harmful effects towards helpful insects such as honey bees or other beneficial species such as worms.

The protein is an endochitinase and the researchers have identified the areas in the structure responsible for its toxicity. They have developed a hypothesis about how the toxin invades the host and has its effects. This in-depth knowledge could potentially enable the modulation and enhancement of the toxin's actions for maximum effectiveness.

Michael Landsberg et al. *PNAS*, in press



3-D model of the protein structure (green) as determined by single-particle analysis. The 'carpet' shows different views of the protein under the TEM and the 'wallpaper' shows steps in the 3-D reconstruction of the protein's structure from the TEM images.

research

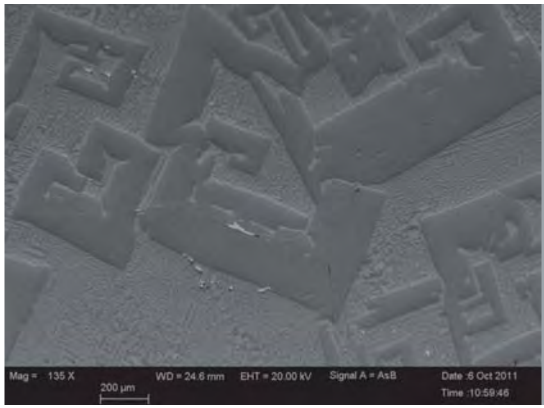
NEW MATERIALS FOR FUEL-CELLS

Solid-oxide fuel-cells produce clean and efficient electricity directly from hydrogen or natural gas, and so are finding applications in portable or emergency power supply and in long-distance electric vehicles. They use an oxide ceramic as the solid electrolyte, which conducts oxygen ions from the cathode to the anode where the fuel is oxidised. Existing technology operates at high temperatures of approximately 800°C to obtain sufficient oxygen conductivity in the solid electrolyte; however, reducing operating temperatures would be more efficient and extend the life cycle of cell components.

One of the promising new oxide ceramics that can operate efficiently at temperatures around 500°C is the family of melilites, composed of calcium, neodymium and gallium. These can accommodate numerous types of metal ions and large excesses of oxygen into their complex crystal structures, but it is difficult to establish a direct causal link between specific crystal chemistries and their performance as solid electrolytes. This challenge arises from their complex microstructures in which hierarchical crystal intergrowth appears at scales from hundreds of micrometres to tens of nanometres. A further complication is that the crystallography is not periodic in three dimensions, but displays long-range distortions. It seems that these distortions might play a role in helping oxygen to move more easily through the material.

AMMRF node director Prof. Tim White at the Australian National University and his team are using microscopy to link the structural complexities of these materials with superior electrolyte performance. Recognising the potential of these intriguing structures is a first step towards optimising and exploiting these materials in next-generation fuel-cells.

Fengxia Wei et al., *J. Am. Chem. Soc.*, 133 (38), 2011



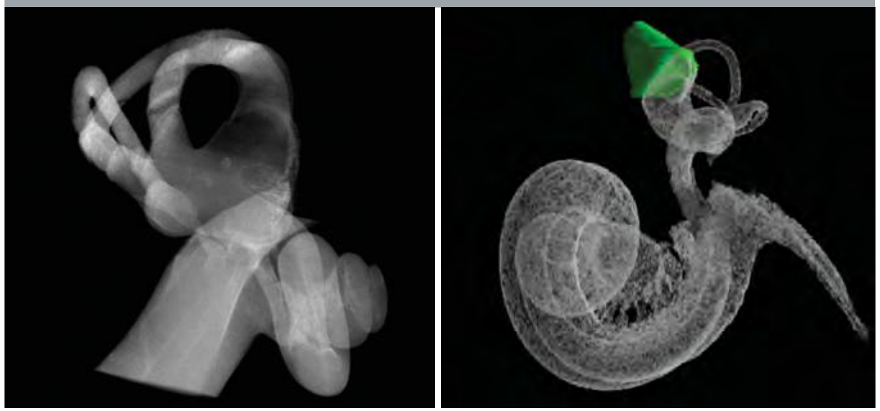
Backscattered electron channelling contrast image that highlights the hierarchical domain structure in melilite.

SECRETS FROM THE LABYRINTH

How do you stay standing? Everybody knows that the ear is what we hear with, but the inner ear, or labyrinth as it is known, is much more than just our hearing organ. It's also our very own gyroscope, accelerometer and altimeter. It's a fluid-filled hydraulic system that monitors our position, orientation and movements, making it possible for us to stand upright. The part of the ear that makes all this possible is known as the vestibule and semi-circular canals – a complex, yet very elegant, arrangement of tubes and sensory membranes in three orthogonal planes that are incredibly responsive to even minute forces.

A/Prof. Allan Jones is using X-ray micro-tomography in the AMMRF at the University of Sydney to give us new insights into these tiny canals. For the first time we can see through the bone with enough resolution to image these membranes and tubes in their exact positions without destroying any of the structure. We can compare our own balance system to that of other animals that have had different evolutionary pressures. Does flight or deep diving require a different labyrinth structure? Does having a range-finding sense, such as bats' sonar, make a difference? These are some of the questions that A/Prof. Jones hopes to answer as he looks at the different labyrinth structures from animals that move in ways we cannot. Some intriguing early observations suggest that the semi-circular canals in their labyrinths tend to be narrower and the sensory membranes different in their orientations and attachments, in animals that encounter stronger forces through either fast movement or deep diving.

X-ray microtomography images of the inner ears from a human (left) and a pilot whale (right) showing the three semi-circular canals and the spiral cochlea.

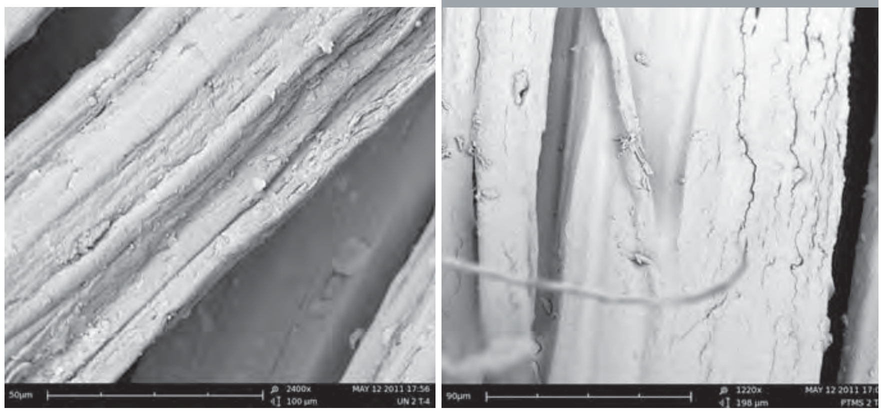


REGENERATING ADELAIDE'S SEAGRASS MEADOWS

Over 5,000 hectares of seagrass meadows have been lost from the Adelaide coastline, probably through past wastewater and effluent disposal. Subsequent seagrass recovery has failed because the denuded sand does not support the small roots of the young plants. The South Australian Department of Environment and Natural Resources (DENR) and the South Australian Research and Development Institute (SARDI) Aquatic Sciences have developed a promising method to enhance natural seagrass recruitment, using sand-filled hessian bags to snag seedlings until adequate root systems develop. However, although the bags successfully trap the seedlings, rapid breakdown of the hessian has led to limited success of the recovery program. A/Prof. Jamie Quinton from Flinders University is working with DENR and SARDI on an ARC Linkage Project to optimise methods of applying environmentally friendly barrier coatings to the hessian to decrease the rate of bacterial breakdown of the bags in the ocean, thus improving their longevity, and increasing retention of seagrass seedlings.

Scanning electron microscopy (SEM) and confocal-Raman microscopy in the AMMRF at Flinders University are revealing the surface morphology and chemistry of natural and modified jute in the hessian. The team is also investigating the mechanical properties of various silane surface-treatments and the reaction conditions required for permanent binding. Sandbags placed either in test marine aquaria or deployed in the field have been recovered and show that the coated hessian is better at resisting bacterial breakdown. Once these factors are more fully analysed, long-lasting coated sandbags can begin to play their fundamental part in restoring Adelaide's seagrass meadows.

Scanning electron micrographs showing untreated (left) and propyltrimethoxysilane-treated (right) hessian.



SCAFFOLDS FOR BONE REPAIR

Bone has a great ability to regenerate, but when large areas are damaged it is often necessary to fill missing areas with replacement materials that will act as porous templates to guide the growth of new bone tissue. The scaffolds are then slowly resorbed by the body leaving only the new bone. Hydroxyapatite was used in the past but is not ideal. More recently, calcium silicate (Ca–Si)-based ceramics have been introduced. The calcium and silicon ions promote new bone formation, but the mechanical properties are quite poor when it is formed into a porous scaffold.

A/Prof. Hala Zreiqat and her group at the University of Sydney have been developing Ca–Si based materials with improved properties and performance, and they have patented a number of these materials.

Trace elements zinc (Zn) and strontium (Sr) were added to give a Sr-enriched hardystonite, which releases bioactive Zn, Ca, Sr and Si ions, all of which encourage bone regeneration. By using scanning electron microscopy in the AMMRF at the University of Sydney, the team has shown that human osteoblasts interact well with the Sr-containing hardystonite.

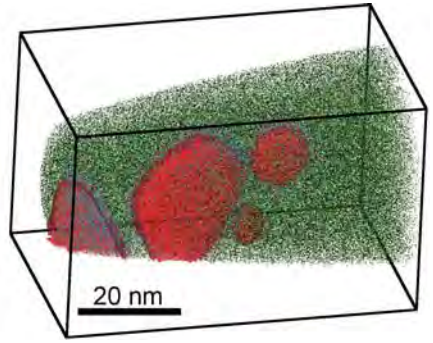
They have also used X-ray microtomography in the AMMRF at the University of Adelaide to evaluate, in animal models, the bone-healing potential of scaffolds made from another Ca–Si ceramic material, baghdadite. Analysis revealed excellent results with more new bone forming on baghdadite than on current commercial products. Sr-enhanced hardystonite and baghdadite both have great potential in orthopaedic applications and tissue engineering. They will next be evaluated in a sheep model, followed by clinical trials.

Hala Zreiqat et al. *Biomaterials*, 31, 2010

Also featured on the ABC TV program Catalyst.

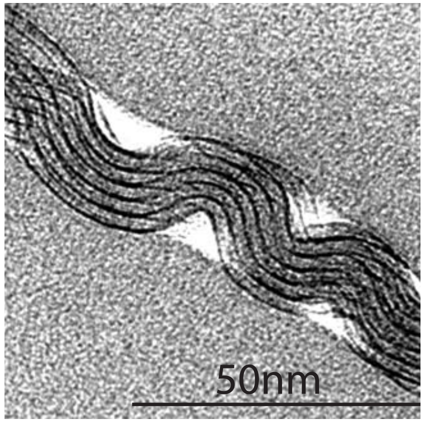
Colour-enhanced scanning electron micrograph of a human bone-forming cell growing on a Sr-enhanced hardystonite surface.

from the linked centres



AUSTRALIAN NUCLEAR SCIENCE AND TECHNOLOGY ORGANISATION (ANSTO)

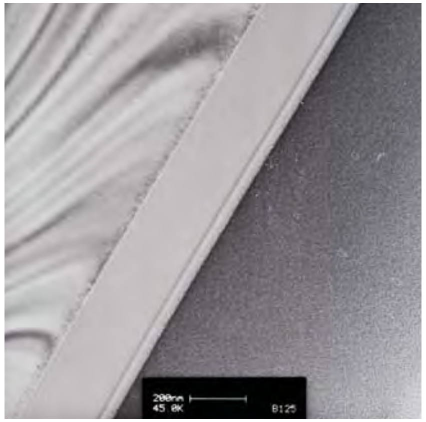
Dr Baptiste Gault is helping ANSTO to investigate how to make metals for nuclear applications that are hard enough to function effectively and yet do not contain commonly used elements such as nickel or copper that convert to long-lived radioisotopes when exposed to radiation. Reduced-activation steels have been developed specifically for the new generations of nuclear reactors. Dr Gault, based in the AMMRF at the University of Sydney, works to understand the properties of these new steels by investigating how radiation affects their microstructure and chemistry at the atomic level. He uses a combination of transmission electron microscopy and atom probe tomography. Initial studies are revealing the structures of the unirradiated material and future work will determine the nature of structural changes that occur once the materials have undergone sustained irradiation.



AUSTRALIAN INSTITUTE FOR BIOENGINEERING & NANOTECHNOLOGY AT THE UNIVERSITY OF QUEENSLAND

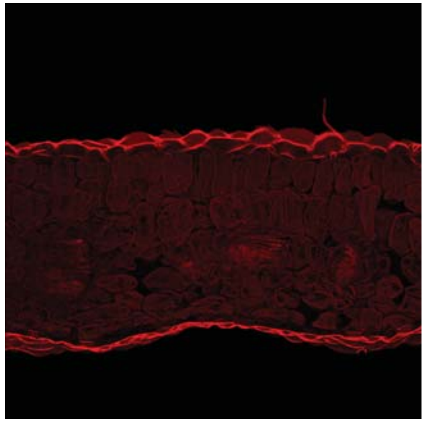
Dr Isabel Morrow is supporting a number of projects including one on a series of new thermostable polyurethane (TPU) nanocomposites. They have been developed in the laboratory of A/Prof. Darren Martin for use in surgical implants, such as heart valves and artificial joints. Dr Morrow is characterising them by cryo-ultramicrotomy and high-resolution transmission electron microscopy (TEM). This allows physical properties to be related back to morphology and results show that adding just 2% (w/w) of engineered clay nanoparticles to polyurethane elastomers dramatically improves their mechanical properties without significantly increasing stiffnes. This is essential for the sustained performance required in a device like a heart valve. Contrary to traditional thinking, the group's low-aspect ratio nanofillers outperformed those with a high-aspect ratio.

Dr Morrow is also investigating the toxicology of nanoparticle exposure through inhalation.



AUSTRALIAN NATIONAL FABRICATION FACILITY AT THE AUSTRALIAN NATIONAL UNIVERSITY

Dr Animesh Basak is based in the AMMRF at ANU, providing scientific expertise to researchers and promoting collaboration between ANFF and the AMMRF. He is supporting ANFF's research in the area of compound semiconductors such as gallium arsenide, indium phosphide, indium gallium arsenide and gallium nitride. These materials are widely used in LEDs, infrared technology, thermo-photovoltaics, fast electronics, high-efficiency solar cells and opto-electronic devices. The main challenge of analysing these materials is their ion-beam sensitivity, which can result in ion-implantation and amorphisation of the crystal structure. By focused ion beam (FIB) techniques, he has successfully developed process parameters to prepare transmission electron microscopy (TEM) samples of these materials. An example, showing indium phosphide with different multilayers appears above.



PLANT BREEDING INSTITUTE AT THE UNIVERSITY OF SYDNEY

Based in the AMMRF at the University of Sydney, Dr Errin Johnson is supporting a wide range of plant-related research through microscopy support and technique innovation. She is currently collaborating on a large-scale project with PBI researchers, including Prof. John Crawford, Dr Tarryn Turnbull and Dr Margaret Barbour, examining the link between leaf form and function by characterising physiological and anatomical changes in spinach leaves grown under different light and CO_2 conditions. By using a range of advanced microscopy techniques, including X-ray microtomography, confocal and focused ion beam SEM, Dr Johnson is applying her expertise to help the researchers construct high-resolution 3-D leaf models.

Other PBI projects include micromorphology studies of native grass, surface area of air spaces in respiring wheat and eucalyptus leaves, and confocal imaging of stripe-rust colonisation in resistant and susceptible wheat cultivars.

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Microscopy & Microanalysis
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Case studies in testing services

PROBLEM

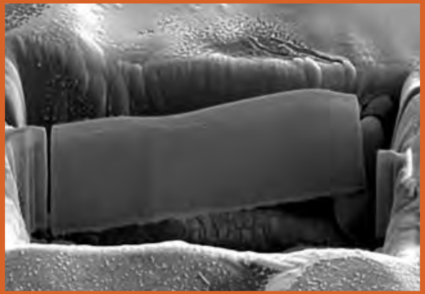
Discolouration on the surface of premium aluminium cladding was occurring when the building product was installed onsite at a particular location. The client, a building materials manufacturer, was interested to know the identity and cause of this discolouration.

SOLUTION

X-ray photoelectron spectroscopy in the AMMRF at the University of South Australia was used to collect surface-specific information from 'good' and 'stained' areas of the product. Survey spectra provided the identity and quantity of all chemical species present, and high-resolution spectra of aluminium provided information about its oxidation state. It was found that an etchant in the local environment was responsible for the removal of a protective aluminium-oxide layer, giving the stained appearance.

PROBLEM

BlueScope Steel wanted to optimise the aluminum–zinc coating on their steel and therefore needed to analyse existing coatings at high magnification.



SOLUTION

Very thin sections were milled from the near-surface region of a 55% Al–Zn-coated steel sample with a focused ion beam instrument in the AMMRF at the University of New South Wales. The specimens were then analysed by transmission electron microscopy in the AMMRF at the University of Sydney. Results enabled BlueScope researchers to modify process design to maximise passivation behaviour, particularly with respect to film thickness, chemical composition and distribution.

PROBLEM

The performance of a reverse osmosis membrane used by SA Water was affected by an unknown foulant. The client wanted to know whether this contamination was of an organic or inorganic nature.

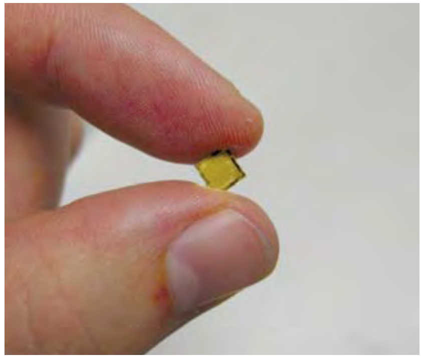
SOLUTION

The surface composition of the membrane's active layer was determined by X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry in the AMMRF at the University of South Australia. These surface-specific analyses allowed for the parallel detection of organic and inorganic species on the surface of the membrane. The comparison of control and fouled membranes showed substantial changes in the surface characteristics of the membrane, which were indicative of the fouling and allowed the material responsible to be identified.

commercialisation

These three Australian companies have recently formed to develop new products arising from research enabled by microscopy and microanalysis at the AMMRF.

VAXXAS PTY LTD



Based in Brisbane this medical device company for vaccine delivery is using a game-changing micro-needle technology (Nanopatch™) developed at the University of Queensland (see p.19).

Prof. Mark Kendall who heads the research team developing the Nanopatch received the Translational Research Excellence Commercialisation Award for the Nanopatch and Vaxxas was a finalist in the Enterprize Business Plan competition in 2010.

Vaxxas has attracted $15 million of investment to continue development of the Nanopatch and take it to clinical trials. Investors, led by OneVentures, include Brandon Capital, the Medical Research Commercialisation Fund (MRCF) and US-based HealthCare Ventures.

www.one-ventures.com/portfolio/vaxxas

HAZER PTY LTD

Current hydrogen-production technologies are significant producers of greenhouse gases, whereas Hazer's technology captures the carbon pollution in the form of graphite, a valuable by-product in its own right.

Work in the AMMRF at the University of Western Australia, was critical to the successful development of this new methane-cracking technology (see p.21) and the subsequent establishment of Hazer Pty Ltd. Their patented technology (see below) delivers cost-effective hydrogen and graphitic carbon through the thermo-catalytic decomposition of methane.

The technology thus gives a clean route to two valuable industrial commodities from an abundant natural resource. The company is based in Perth and has raised equity funding from private investors including Wesfarmers.

ELASTAGEN PTY LTD

Elastagen Pty Ltd is a clinical-stage medical-device company that is pioneering Elastatherapy™, which uses the human protein elastin to naturally repair and augment the skin. The company has arisen out of patented research conducted by Prof. Tony Weiss's group at the University of Sydney and the first clinical trials have demonstrated the biocompatability and safety of their synthetic human elastin in human subjects.

Prof. Weiss's work on elastin for burns treatment was highlighted in the 2010 AMMRF Profile.

Elastagen is a venture-backed private company based in Sydney. Investors include ATP Innovations, Brandon Capital and GBS Ventures.

www.elastagen.com

New technologies enabled by the AMMRF in 2010–11 also include these two patents and other provisional applications.

PATENT# PCT/AU2011/001052

A method of forming a structure in a material

Dr Warren McKenzie has developed a new method of ion beam lithography called Focused Ion Beam Hard Mask (FIBHM). It modifies the surface with gallium ions forming a masked area that encourages deposition, rather than etching, of the modified area when exposed to a plasma.

PATENT# PCT/AU2010/001168

A process for producing hydrogen from hydrocarbons

Prof. Hui Tong Chua and others from the University of Western Australia have developed the process that consists of contacting the hydrocarbon gas with a catalyst at elevated temperature to convert the hydrocarbon gas to hydrogen and solid carbon.

AMMRF 2010–11 USERS FROM INDUSTRY

Total: 148

By sector:

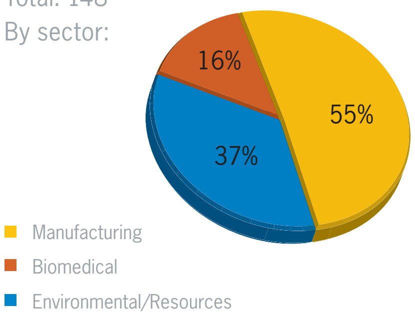


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Image credits for the double-pages and covers:
Cover & Capabilities (pages 6–7) B&W image
Scanning electron micrograph of scales on a moth's wing. Image by Ms Jenny Norman.

Research (pages 16–17)
Confocal micrograph of an intermediate-filament protein, nestin (green), in ondontoblasts; blue shows nuclei. Image by Mr Ramin M. Z. Farahani.

Industry (pages 32–33)
Electron backscatter diffraction image of cast iron from the historic Tharwa bridge in the ACT. Image by Dr Gwénaëlle Proust and Dr Pat Trimby.

Back Cover (colour image)
Autofluorescence and second harmonic generation image of structures in human lung tissue. Image by Mr Gavin Tjin and Ms Ellie Kable.

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