From the Director

The Centre for Microscopy, Characterisation and Analysis (CMCA) exists to serve the wider research community – to apply its state-of-the-art expertise to turn good research into great research. To achieve this requires many attributes. Of course, it begins with having the best instruments, and raising the funds to acquire these is a relentless focus. The load is lightened, however, by the increasing number of researchers coming to us to manage and operate their cutting-edge infrastructure. An example initiated in 2010 is Winthrop Professor Shaun Collin’s new multiphoton deep-tissue imaging facility. Working closely with Prof. Collin and his group, CMCA has identified a new location for the facility in the life sciences precinct, one that meets his group’s as well as the broader needs. These are win-win partnerships. CMCA brings to the table its critical mass and increasingly broad expertise in operating science infrastructure and our leading researchers bring their science challenges to be tackled.

Interconnection is another key attribute. The CMCA is at the intersection of an increasingly rich network of structures that help us work together. Locally, we have just renewed the agreement for the Western Australian Centre for Microscopy (WACM) – which links the four WA publicly funded universities. Through the associated Nanoscale Characterisation Centre of Excellence, we have worked closely to support researchers with the ‘last mile’ in processing and visualising their image data. This need is becoming recognised nationally as a key emerging issue – and we are well placed to stay ahead of the curve.

At the European-Australia Workshop on Research Infrastructure I attended this year in Brussels, I was struck by the extent to which Australia is leading the world in the science infrastructure space. The CMCA has many national interconnections via the National Collaborative Infrastructure Strategy (NCRIS), commencing with its founding membership of the Australian Microscopy and Microanalysis Research Facility (AMMRF), a facility that is widely seen as setting the benchmark in infrastructure provision. In 2010, the CMCA became the WA node of the National Imaging Facility (NIF) in founding the Western Australian capability in preclinical bioimaging – see the feature on page 6. CMCA has already partnered with AuScope, the NCRIS geoscience resource, on the IMS 1280 ion probe platform, and as we go to press is commencing its first collaboration with the Australian National Fabrication Facility (ANFF) in X-ray powder diffraction installed at CMCA@Physics. On the data front, we are also working with the Australian National Data Service (ANDS) locally and through the AMMRF.

A final key attribute of the CMCA is its broad interdisciplinary intersection. Like Rome in the ancient world, a visit to CMCA will always bring surprises – in who you meet and what you learn. And we are broadening our horizons through joint appointments in data and imaging with IVEC, and joint fellows with Animal Biology, with Earth and Environment and soon with Electrical Engineering – we are interconnected.

The 1 July 2011 transfer of responsibility for nuclear magnetic resonance spectroscopy, single-crystal X-ray diffraction and biological mass spectrometry infrastructure to CMCA@Bayliss presents further new synergies. Increasingly, interdisciplinary research requires multiple infrastructure capabilities. In 2011, CMCA will be examining this one-stop shop concept in assessing where to from here. So, what can’t you do at CMCA? Come and tell us.
Affiliations

The CMCA is a focus for microscopy and microanalysis activities across Western Australia and, through strong links and collaborations, has both a national and international reputation.

Established in 1964 as a science infrastructure facility within The University of Western Australia to support research activity, the Centre has a long and distinguished record of collaboration with researchers, industry and government agencies in the provision of research expertise and technology.

Its world-class facilities, valued at $40M, comprise an extensive range of microscopy, microanalysis and imaging instruments across five sites.

Nanoscale Characterisation Centre WA (NCC) and the Western Australian Centre for Microscopy (WACM)

CMCA is the lead node of the State Government funded Centre of Excellence, the Nanoscale Characterisation Centre WA (NCC). NCC’s focus is on providing infrastructure and expertise to maximise benefits to the Western Australian community from institutional and industry research and development in WA.

The NCC is founded upon an ongoing collaborative agreement between the four publicly funded universities in Western Australia, which together form the Western Australian Centre for Microscopy (WACM).

The primary objective of the NCC is to provide continuity in major equipment acquisitions to ensure that the State’s researchers and industry have access to state-of-the-art characterisation technology. NCC is funded by the WACM member universities and the State Government.

National Imaging Facility (NIF)

Established under the National Collaborative Research Infrastructure Strategy (NCRIS), the National Imaging Facility focuses on provision of state-of-the-art magnetic resonance, molecular and allied imaging technologies for application to animals, plants and materials for the Australian research community.

A successful 2009 LIEF grant for a preclinical imaging facility has recently seen the CMCA become the Western Australian node of the facility, the first organisation in the country to host two NCRIS capabilities. The facility will feature new capabilities for Western Australia: in-vivo micro-CT and multispectral imaging. To these instruments will be added micro magnetic resonance imaging in 2013.
AuScope
AuScope is a characterisation capability funded through the National Collaborative Research Infrastructure Strategy (NCRIS) with a focus on the structure and evolution of the Australian continent. AuScope’s aim is to establish world class research infrastructure to characterise the structure and evolution of the Australian continent in a global context from surface to core in space and time.

In partnership with the AMMRF, the Government of Western Australia and UWA, AuScope enabled the establishment of the world-class ion microprobe facility at CMCA, for the benefit of all Australian researchers.

Australian Microscopy and Microanalysis Research Facility (AMMRF)
Established under the Commonwealth Government’s National Collaborative Research Infrastructure Strategy (NCRIS), AMMRF is Australia’s peak research facility for the characterisation of materials through advanced microscopy and microanalysis.

The AMMRF facilitates access for all Australian researchers to world-class equipment, instrumentation and expertise through a national grid of nodes with varying microscopy capabilities and an array of flagship instrument platforms at the international cutting edge.

The CMCA is the West Australian node of the AMMRF and features the flagship ion probe capabilities, which consist of the NanoSIMS 50 and IMS 1280. Each of these instruments is unique to the Southern Hemisphere.

The CMCA also collaborates with the John de Laeter Centre, which enjoys linked laboratory status in the AMMRF, in the management and operation of Western Australia’s ion probe facilities.

Australian National Fabrication Facility (ANFF)
Established under NCRIS, the Australian National Fabrication Facility (ANFF) provides researchers and industry with access to state-of-the-art fabrication facilities.

The capability provided by ANFF enables users to process hard materials (metals, composites and ceramics) and soft materials (polymers and polymer-biological moieties) and transform these into structures that have application in sensors, medical devices, nanophotonics and nanoelectronics.

The CMCA houses the newly commissioned X-ray powder diffractometer.
Navigating your way to world-class microanalysis
Researchers have a new tool to help navigate the myriad of microscopy and microanalysis techniques to find which is best for their research, thanks to the introduction of the AMMRF Technique Finder.

Users input what they want to measure and at what scale. The Technique Finder suggests techniques, where they can be found, how they work and who to contact.
NEW
Bayliss characterisation infrastructure

The CMCA’s suite of research infrastructure capabilities available to users will be expanded to include biological mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR) and X-ray diffraction (XRD) techniques. Primarily located at the CMCA@Bayliss site, these additions will further support the world-class research of our users.

NEW
LIEF success boosts scanning electron microscopy

LIEF success in 2010 will also provide an expansion to analytical SEM facilities at CMCA. A new analytical SEM and additional EDS detector will provide enhanced data quality, reduced analysis times and improved productivity for CMCA users across all disciplines. Additionally, a low temperature cryo-system offering new opportunities to the biosciences, will form a unique state-of-the-art microanalytical facility for WA that will support major research initiatives in minerals and mining, energy, engineering, neuro and medical science, and plant and animal biology.

NEW
ANFF powder XRD

A new X-ray diffractometer acquired through the Australian National Fabrication Facility (ANFF) initiative has been installed at CMCA@Physics and will complement the capabilities of the newly acquired crystallography instruments at CMCA@Bayliss.
Bioimaging Facility

The Bioimaging Facility is a landmark in Western Australia. No longer will WA’s researchers need to travel interstate to image live animal models of basic biology and disease.
In June 2010, the CMCA became the first microanalysis facility to become a node of both the AMMRF and its sister NCRIS Characterisation capability, the National Imaging Facility (NIF). This new engagement highlights the synergies and continuities between imaging capabilities and makes it even easier for researchers to access the instruments that they need.

The driving force for the CMCA to instigate an association with the NIF was the identification of a gap in micro-imaging facilities available to Western Australian researchers, who currently have to travel interstate for analysis.

Facilitated by the UWA Bioimaging Initiative, a 2010 CMCA LIEF bid to establish a Western Australian Small Animal Imaging Core Facility, and an invitation to join the University of Queensland’s EIF3 bid for the Centre for Advanced Imaging, led to the proposal that CMCA become the WA node of NIF.

The success of both bids, with a combined value of $7M, will see the development of a state-of-the-art Bioimaging Facility, suitable for in vivo imaging of small animals.

Already installed in M Block at the Queen Elizabeth II Medical Centre, is a suite of instruments including an X-ray micro-computed tomography platform and multi-spectral imaging. This Facility will be further strengthened with the addition of WA’s first high resolution magnetic resonance imaging (MRI) system set for installation in 2013.

**Multispectral imaging**

Multispectral imaging is a versatile and sensitive tool for cellular and molecular imaging.

Used to detect, localise and quantify extremely low levels of light emitted by a bioluminescent or fluorescent gene, molecule or cell, the CMCA’s IVIS Lumina II and CRI Maestro 2 can be used to image small animals, animal tissue, cells and other materials such as plants.

**X-ray micro-computed tomography (Micro-CT)**

Micro-CT imaging is a powerful modality providing non-invasive 3D morphological detail. The Bioimaging Facility features the Skyscan 1176 In Vivo system which provides high resolution 3D imaging. Tissue differences in X-ray absorption enable 3D imaging of bone, adipose and lung tissues. Contrast agents can also be used to provide 3D detail of blood vessels, lymphatics and gastrointestinal spaces.

Able to scan in vivo small animals, variable X-ray voltages and filters also provide scanning flexibility to allow imaging of a wide range of ex vivo samples.

**High-resolution magnetic resonance imaging (MRI)**

MRI of small animals has become a standard tool in many biological and pharmacological applications and is an extremely flexible technique for assessing anatomy and structure, physiological and functional parameters and molecular and cellular processes within live animals. MRI does not rely on the use of ionising agents but instead is based on the intrinsic magnetic properties of hydrogen, which is abundant in animals in the form of water and fat tissues largely. MRI provides excellent soft tissue contrast and high spatial resolution and facilitates fast imaging without restrictions on penetration depth.

Researchers in WA can now follow biological processes in real time, to track tumour growth, disease progression or drug mechanisms in vivo as well as use current instrumentation within the Centre to visualise tissue, cellular or sub-cellular processes with still greater resolution and detail.
The CMCA is a significant training and teaching hub within the university, providing major research training opportunities for postgraduate students and actively contributing to undergraduate teaching.

In 2010, the Centre continued to provide its regular series of training courses for researchers wanting to access the array of advanced microscopy and microanalytical instruments housed in CMCA. The three rounds of core training courses (in March, July and November), attracted more than 350 participants and more than 90 also attended one or more of the advanced workshops in microscopy and image analysis offered by Centre staff during the year.

The number of PhD students accessing Centre facilities for their research continues to rise, with 169 students registered as CMCA users in 2010 compared to 114 in 2009.

A strong cohort of Honours and final year research project students also made use of the CMCA, with more than 67 registered in 2010, predominantly from Engineering, Nanotechnology and Chemistry.

A highlight for the Centre in 2010 was the enrolment of the first PhD student directly into the CMCA, Rahi Varsani. This exciting development required a change to University rules to permit PhD supervision through CMCA, which is intended to provide greater opportunities for microscopy-focused research projects to be supervised by CMCA staff. This move reflects the Centre’s standing within the University as a hub of academic excellence in microscopy and microanalysis. It is anticipated that PhD enrolments in CMCA will build as students begin to recognise the unique opportunities provided by conducting their research within the Centre.

Online courses
A team of experts in research-led teaching from six universities within the AMMRF, including CMCA’s Associate Professor Janet Muhling, was recently successful in a 2010 grant application to the Australian Learning and Teaching Council for funding to develop an integrated range of virtual microscopy tools. The team will support a national blended-learning approach in microscopy that will cater to the increasing numbers and diversity of students who require education and training in microscopy. These tools will be openly accessible to educators around the country.

The first module on scanning electron microscopy (SEM) is now online and accessible via the CMCA website. Teams of experts around the AMMRF are busy developing the additional five modules that will be rolled out in the near future.

First PhD student tastes immediate success
In 2010, Rahi Varsani became the first PhD student to directly enrol through CMCA and he quickly developed a knack for winning prizes. Having decided to continue working with his Honours supervisor Professor Martin Saunders, Rahi was awarded the Trans-Tasman Bursary by the Australian Microscopy and Microanalysis Society for a presentation at the ACMM-21 conference in Brisbane on his Honours research about carbon nanomaterials formed by the catalytic cracking of methane. He quickly followed this up with an award for his talk at the ARCNN/ARNAM 2010 Joint Workshop in Adelaide. Rahi will use the Trans-Tasman Bursary to attend the 2011 NZ Microscopy meeting in Wellington to present a paper on his PhD research into magnetic nanomaterials for biomedical applications.
### 2009 Courses

- **Total**
  - EDS X-ray analysis: 59
  - TEM: 63
  - Flow cytometry: 24
  - SEM: 105
  - Optical/confocal microscopy: 46
  - **Total**: 297

### 2010 Courses

- **Total**
  - EDS X-ray analysis: 56
  - TEM: 52
  - Flow cytometry: 62
  - SEM: 113
  - Optical/confocal microscopy: 70
  - **Total**: 353

*INSTRUMENT DECOMMISSIONED IN 2010*

**Image / Tissue Microarray**

Acquired with CMCA’s Aperio Scanscope.
Industry

In 2010 CMCA has continued to support industry and identify solutions to industry problems across a range of areas. Engagement with a growing number of organisations has ranged from the provision of consulting services to substantial research contracts.

CMCA facilities and staff have played a significant role in assisting the mining industry with exploration. Facilitated by the Centre for Exploration Targeting at UWA, analysis of gold, nickel and iron ore samples is helping to identify and validate new and advanced exploration methods which will enable the WA minerals industry to maintain its competitive edge.

In vitro fertility specialists and PathWest are also frequent users of CMCA facilities, as are the peak medical research institutes in WA, the Lions’ Eye Institute, the Telethon Institute for Child Health Research and the Western Australian Institute for Medical Research.

A 2010 highlight was the opportunity for CMCA to host the Alumina Technical Panel (ATP), comprising research and development managers of the alumina producers with technical capabilities in Australia. Australia is the largest supplier of bauxite worldwide and the panel was very impressed by the Centre’s capabilities and their capacity to contribute to alumina-based R&D projects. Substantial contract consulting has subsequently stemmed from this engagement.

Energy & Minerals
- Alcoa World Alumina
- Atlas Iron
- AusQuest
- BHP Billiton
- Crossland Resources
- CSIRO Earth Science & Resource Engineering
- Geological Survey of WA
- Manhattan Corporation
- Oilfield Production Technologies
- Pathfinder Exploration
- Pterodia
- RioTinto
- Sumatra Copper & Gold

Engineering & Environmental
- Advanced Geomechanics
- Australian Reference Laboratories
- Botanic Gardens and Parks Authority
- Coffey MPL
- CSIRO Land & Water
- Department of Water
- Envirolab Services
- Matrix Composites & Engineering
- National Environmental Resources
- Polymer Inspection Testing & Certification
- Site Environmental & Remediation Services
- SLR Consulting Australia
- TSW Analytical
- Water Corporation

Biomedical & Miscellaneous
- Antaria
- Department of Agriculture and Food, WA
- Fertility Specialists of WA
- National Measurement Institute
- PathWest
- Phylogica
- Pivet
- St John of God Pathology
**Going with the flow speeds up plant research**

Canola Breeders Western Australia Pty Ltd is a private partnership between growers, the Grains Research Development Corporation and researchers in Plant Biology at The University of Western Australia that breeds canola plant varieties. Since 2008, CMCA has helped Canola Breeders to successfully breed new high yielding canola varieties for difficult environments in Australia and elsewhere.

Plant breeders use changes in ploidy (the number of sets of chromosomes) to affect the performance of plants as crops. Traditional methods to determine ploidy are labour intensive and, for many plant species, technically challenging due to small, indistinct and 'sticky' chromosomes.

A high throughput flow cytometry protocol to analyse seedling ploidy, developed in collaboration with CMCA’s Assistant Professor Kathy Heel, takes only 15% of the time per sample and allows ploidy characterisation of very young plants. Useless sterile plants can be discarded much earlier, reducing plant maintenance and speeding up canola variety development which is estimated to save around $250,000 per annum as well as substantial time to market for seedlings. This has a positive knock-on effect for the Australian canola industry and the Australian economy.


**RioTinto diamonds**

The colour of natural pink Argyle diamonds is due to an unidentified crystal-line defect that causes the colour of the diamonds to be affected by certain wavelengths of light. Rio Tinto Diamonds wanted to characterise this change, find the optimal wavelength at which to perform a reversal and to discover the underlying cause of the defect and its behaviour.

Identifying the characteristics of this defect will provide Rio Tinto with methods for verifying authenticity of Argyle pink diamonds. The properties of this crystalline defect could also be of use in research into optical systems and technologies.

www.riotintodiamonds.com
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IMAGE / SYNTHETIC MATRIX SCAFFOLD FOR BONE.
IMAGE BY PETER DUNCAN ACQUIRED AT CMCA.
Ongoing research at The University of Western Australia (UWA) has been developing novel technology for the cracking of methane (natural gas) into hydrogen and graphitic carbon.

The research collaboration, led by Professor Hui Tong Chua of the UWA Centre for Energy, makes extensive use of electron microscopy facilities at the CMCA to understand the cracking process and to characterise the resulting nanomaterials.

Hydrogen is a valuable industrial chemical, at present mainly used for the preparation of ammonia for fertiliser and other applications. However, in future, it is likely to be a critical component of a reduced-emission energy market.

Current methods of generating hydrogen are energy inefficient and directly or indirectly create significant amounts of carbon dioxide emissions. The current research generates hydrogen with no carbon dioxide emissions.

In addition to the clean production of hydrogen, the reaction produces significant quantities of graphitic carbon nanomaterials, predominantly in the form of nano-onions. Consisting of sequentially self-encapsulating carbon shells, the carbon nano-onions have potential application and value in a range of areas, in particular, as high-quality electrodes for use in electrochemical processes and batteries. A tonne of the carbon material has already been requested for trials by a Chinese battery manufacturing company, although the process has to be scaled up considerably first.

The research has had strong support from the private sector since inception. Initially supported by Wesfarmers and the US technology development group XLTG Inc. through an ARC Linkage Project, the research has formed the basis of a new spin-out company, Hazer Pty Ltd, which has successfully raised capital from seed investors and is now funding further development work at the University. Patents are in place to cover the cracking process and purification of the carbon nanomaterials. The company name, Hazer, is an acronym of Hydrogen And Zero Emission Research.

The researchers rely on CMCA’s high-resolution scanning and transmission electron microscopy (SEM and TEM) facilities to investigate the structural and chemical properties of the carbon and the catalyst materials used in the cracking process. CMCA Deputy Director, Professor Martin Saunders, is a core partner in the research team, providing expertise in advanced TEM techniques to correlate the properties of the catalyst with those of the resulting carbon nanomaterials. He has co-supervised several students involved in the project, including Rahi Varsani who recently received an award from the Australian Microscopy and Microanalysis Society for his research on the use of electron tomography to investigate the 3-D morphology of the nanomaterials.

The capital raised by the formation of the spin-out company is being invested into the ongoing research at UWA to further develop and scale-up the methane cracking process. Microscopy will continue to play a crucial role in this future development.

Plasmonics: manipulating the optical properties of materials at the nanoscale

For hundreds of years stained glass windows have exploited the way light interacts with metal nanoparticles in the glass to produce their spectacular colours.

The ability to control how light interacts with matter is fundamental to the development of materials with novel optical properties and end applications. In modern times this has led to the field of plasmonics, where nanoparticles are synthesized to achieve specific properties with potential applications in areas such as biosensors, new computing technologies, solar cells, etc.

At the nanoscale, the shape and size of nanoparticles defines the so-called Localised Surface Plasmon Resonance (LSPR), which controls how light interacts with electromagnetic fields at the surface of the particles. Thus, by changing the shape, size or spatial arrangement of the nanoparticles, systems with specific optical properties can be created.

The design of novel nanomaterials that underpin plasmonic applications has driven the rapid progress in advancing plasmonic materials over the last few years. Control of metal nanoparticle shape, density, and spacing is continually improving with novel synthetic techniques. Nevertheless, to continue advancing plasmonics, new metallic nanostructure systems must be developed that offer unique properties and are superior to Ag, Au, and mixtures thereof – currently the most widely exploited metals and bimetallic systems. In this context, the development of bimetallic alloy nanoparticles is attractive because changing their composition can control their optical properties, whereas the properties of single metal nanoparticles can only be tuned by manipulating their shape or size.

Size-independent tuning by compositional changes is advantageous for applications such as chip-scale plasmonic optical components that must operate in the infrared or near-infrared wavelengths but seek to exploit the sub-wavelength dimensions that are typical of plasmonic nanostructures.

Assistant Professor Alexandra Suvorova and Professor Martin Saunders at CMCA, in collaboration with researchers at the Institute of Inorganic Methodologies and Plasmas, Bari, Italy and Duke University, USA have been studying new plasmonic nanostructures based on bimetallic systems. They have developed a method to tune the properties of GaMg nanostructures by carefully controlling their composition. It was shown that GaMg alloys provide a large energy/wavelength tunability of the surface plasmon resonance, good thermal stability and reduced impact of oxidation on the optical properties. The chemical information has been visualized using advanced analytical TEM techniques such as energy-filtered transmission electron microscopy and high-angle annular dark field scanning transmission electron microscopy. This work has been published in the journal Small.


Australian Academy of Science Fellowship

Dr Alexandra Suvorova, whose main interest lies in electron microscopy of nanomaterials, spent four weeks with Dr Losurdo at the Institute of Inorganic Methodologies and Plasmas, Italy and Dr Werner at the Max Planck Institute, Germany in August 2010. Her fellowship saw her examine the growth and optical properties of plasmonic nanostructures, followed by the structural and compositional analysis of samples using the MPI aberration-corrected analytical TITAN transmission electron microscope. Understanding the nanomaterials growth and properties is essential for their exploitation in a variety of technologies, including chemical sensors, biosensors and optical devices.
Corrosion is an extremely costly failure mechanism, consuming 3–5% of GDP. Stainless steel, despite its name, is not immune to corrosion, called pitting corrosion.

This devastating failure mechanism – which can manifest as anything from rust spots on a kitchen appliance to a large hole in a tank or pipe – appears, apparently at random. Pitting corrosion is the starting point for crevice corrosion, another nasty scourge where the stainless steel rusts rapidly in narrow gaps. Currently, to overcome pitting corrosion, more expensive grades of steel, containing more chromium and also containing molybdenum, need to be used.

Stainless steel is an alloy of iron and chromium and owes its general corrosion resistance to the presence of a very thin layer of chromium oxide that forms on the surface, protecting the underlying steel. When pitting corrosion does occur, it nucleates around unavoidable sulphur impurities in the steel. Manganese is added during production to sequester the sulphur as microscopic inclusions of inert manganese sulphide (MnS).

As a consequence, most inclusions in the steel remain unreactive. However some appear to become especially active and react around their edges. Professor David Williams from the University of Auckland is working to understand this phenomenon by using the NanoSIMS to examine the composition around MnS inclusions.

He found that the inclusions were surrounded by a thin skin (less than 100 nm) of reactive iron sulphide (FeS). This reactive halo is likely to be the reason for the corrosion initiation that occurs around inclusions. FeS is unstable relative to MnS at steel-manufacturing temperatures – indeed the reason for adding manganese is to prevent the formation of FeS. These results point to a possible means of processing the steel to remove the FeS halo around the inclusions and hence mitigate this important form of corrosion. If the steel were cooled more slowly, there would be more time for the manganese to thoroughly sequester the sulphur and maximise the formation of the more energetically favourable MnS, reducing the likelihood that pitting corrosion could initiate in the future life of the final product.

Improved processes will lead to a reduction in the potential for corrosion, in turn leading to longer life of stainless steel and therefore reduced replacement costs and the ability to use less expensive types of stainless steel in certain applications.

Anaphylaxis is a severe allergic reaction triggered in some people by foods, insect stings and drugs. The incidence of anaphylaxis has increased dramatically over the last ten years. The most severe cases are characterised by hypotension, hypoxia and possible unconsciousness.

As anaphylaxis is an unexpected, life-threatening event that requires emergency management, defining the biological mediators that influence the clinical presentation and severity of the reaction has been difficult. A range of immune mediators have been implicated by studies in animal models and cultured cells, but work on acute human anaphylaxis is lacking.

Assistant Professor Shelley Stone and Professor Simon Brown from the Western Australian Institute for Medical Research have identified a number of potential immune mediators involved in anaphylaxis. The Emergency Department Anaphylaxis (EDA) study is a unique collaboration of eight emergency departments in Western Australia and New South Wales that are collecting sequential blood samples from patients with acute anaphylaxis. A total of 432 patients were enrolled in the EDA, including 98 with severe anaphylaxis, making this the largest study of anaphylaxis in the world.

The research team has been utilising flow cytometry to quickly and accurately detect a number of markers in a large number of samples. Serum levels of 15 immune mediators were measured using cytometric bead arrays (CBAs) and analysed using the FACSCanto II flow cytometer equipped with a 96-well plate reader. The technique allows simultaneous detection of multiple analytes in as little as 50µl of serum. The ability to multiplex and investigate a large number of mediators was essential to this project due to the low volume of sample available.

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

However, the elevation of IL-10 was unexpected. This is an anti-inflammatory protein whose primary function is to dampen immune responses. The team suggest that the IL-10 is being produced as the immune system tries to turn off the allergic response initiated by the trigger. This research was published in the Journal of Allergy and Clinical Immunology and was nominated the best paper of 2009 by the Royal Perth Hospital Medical Research Foundation.

Assistant Professor Stone and her colleagues are now moving ahead to investigate additional mediators of anaphylaxis including anaphylatoxins and platelet activating factor. Characterisation of the molecules present during anaphylaxis is a starting point for the development of new treatments for this life threatening condition.

Each year more than 2,000 Australians die from chronic liver diseases, cirrhosis and cancers of the liver, gall bladder and bile ducts. In addition, liver cancer is the most rapidly increasing cancer worldwide with hepatitis B and C significantly increasing the risk of liver cancer.

Liver progenitor cells (LPCs) have an enormous potential for use to treat liver disease. LPCs are induced during chronic liver injury replacing damaged hepatocytes (liver cells) and cholangiocytes (bile duct cells). In a variety of liver diseases including hepatitis B and C, alcoholic and non-alcoholic fatty liver disease, LPC numbers correlate with disease severity. Strikingly, LPCs are almost always associated with inflammatory cells, suggesting the inflammatory cells may provide cytokine signals, such as growth factors, that underpin the regeneration process. Identifying the inflammatory cells and cytokines, which support the LPC response in chronic liver disease, is crucial in designing strategies to augment LPC-mediated liver regeneration and optimise liver repair.

Professor George Yeoh, in collaboration with researchers from the Liver Laboratory, Centenary Institute of Cancer Medicine and Cell Biology and from the Department of Surgery at The University of Regensburg, Bavaria, Germany seeks to understand the relationship of LPCs with inflammatory cells as they co-exist in liver disease.

Fluorescence microscopy was used to analyse the types of cells present and their location in the damaged liver across both diet-induced and immune-mediated liver injury models. This approach identified macrophages and CD8+ T cells, which are actively recruited to the injured liver, as key players (image displayed in the UWA Science Library). While T cells increased in numbers during liver regeneration and contribute to the process, they are not essential. The close association between macrophages and LPCs however suggests a paracrine relationship between these cells.

Subsequently, the research team analysed the types of cytokines and measured their content in the liver of diseased mice by flow cytometry using Cytometric Bead Arrays (CBAs). Invading and liver-resident macrophages were found to be the most important cytokine producers. The common feature between the models is that TNFα is not only induced but its level is sustained during initiation of the LPC response. As a result, Professor Yeoh is now investigating the possibility of regulating LPCs by moderating levels of macrophages in diseased liver.

Knowledge of factors that regulate LPCs will allow us to control their numbers in diseased liver in order to balance the positive outcomes from regenerating the liver and negative outcome of developing liver cancer. This knowledge is also expected to underpin strategies to grow, differentiate and mature LPCs into useful hepatocytes for use in liver assist devices and bioartificial livers.


World first – ancient DNA isolation from fossilised eggshells

In a world first, an international team, led by Dr Michael Bunce of Murdoch University, including researchers from the United Kingdom, Denmark, New Zealand and the United States, has successfully isolated ancient DNA from the fossilised remains of eggshells from extinct birds.

The work, published in the Proceedings of the Royal Society, generated considerable excitement. DNA was extracted from fossilised eggshells up to 19,000 years old. Although used for a variety of dating techniques ancient eggshell was not previously considered to be a source of DNA.

Eggshell fragments excavated from deposits across the globe are widely used in archaeological and paleontological research to infer dates, diets and environmental change, largely on the basis of their excellent biomolecule preservation. DNA is generally found in all biological materials but has not previously been isolated from eggshell. Dr Michael Bunce and PhD student Charlotte Oskam, from Murdoch University’s ancient DNA (aDNA) lab, led a large international collaboration to investigate why this is so.

When attempting to isolate aDNA it is important to understand its location within the sample. CMCA’s Associate Professor Paul Rigby was able to visualise the preserved genetic material within the eggshell matrix by using confocal imaging, together with fluorescent double-stranded DNA-binding dyes. It is most likely that the DNA in eggshell has come from abraded cells incorporated during the formation of the eggshell within the bird. Localising the aDNA in the eggshell was a key step that enabled the researchers to optimise their sampling protocols and develop an extraction technique specifically tailored to eggshell. The researchers were able to recover the aDNA from extinct megafaunal birds such as the giant New Zealand moa and the elephant bird from Madagascar. The DNA preservation seemed to extend back to nineteen thousand years in an emu eggshell from an archaeological site near Margaret River. To date, this is the oldest authenticated aDNA ever retrieved from Australia.

This work is the first description of successful isolation and visualisation of aDNA from fossil avian eggshell and showcases its utility for investigating the genetics of eggshell in fossil and archaeological deposits. The techniques described also have applications in the fields of conservation and forensic genetics. Lastly, genetic profiles, when analysed together with 14C dating and stable-isotope profiles, will significantly enhance the accuracy of understanding both past biodiversity and extinction processes.

Earth sciences

Looking at invisible gold

Gold is one of the world’s most precious metals. In many large gold deposits, the gold itself is invisible – dissolved within the crystal lattice of other minerals.

In a similar way to which tree rings can be used to infer information about past climate change, minerals containing invisible gold, such as pyrite, can be used to derive information about how gold deposits form.

Dr Shaun Barker and colleagues from the University of British Columbia and the University of Nevada, Las Vegas visited the CMCA to examine pyrite grains in samples that contained invisible gold from two gold deposits in northern Nevada, USA.

These deposits are examples of the very important Carlin-type class of gold deposit, responsible for about 10% of current global gold production. Using the NanoSIMS to map gold, other trace elements and sulphur isotopes within selected, small pyrite grains (5–50 µm) from each deposit, they were able to resolve, for the first time, that gold was deposited in at least two discrete episodes in each deposit. Each episode of gold deposition was accompanied by increased concentrations of trace metals that are often associated with magmas.

These results emphasise that gold may be deposited in episodic bursts during the formation of gold deposits, and has important implications for the genesis and controls on Carlin-type gold deposit formation.


Image / Pyrite grain from the ‘Banshee deposit’, northern Carlin Trend, Nevada, USA with NanoSIMS maps of the distribution of gold and sulphur isotopes.
Little is known about the migratory behaviour of extinct Australian mammals, in particular that of the extinct rhinoceros-sized diprotodon, *Diprotodon optatum*.

The diprotodon was one of the most widespread Pleistocene marsupials living in Australia before the last ice age. Teeth of these animals retain information about their environmental and physiological conditions over the time period that is recorded in the growth of the teeth.

The analysis of teeth and bones has established that relationships exist between oxygen isotopes and climate, carbon isotopes and diet, and strontium isotopes and geographical regions. Therefore, by measuring strontium, carbon and oxygen isotopes from the tooth enamel, it is possible to reconstruct the migration patterns of the megafauna. As part of this investigation, an 80,000-year-old fossilised diprotodon incisor, held at the Western Australian Museum, was analysed by Lynette Howearth of Curtin University.

Combining data from the ion probe at the CMCA, where she analysed small spots around 40 µm in diameter from uncontaminated enamel regions, with data obtained using thermal ionisation mass spectrometry (TIMS) at Curtin University of Technology, the signatures of strontium isotopes in the tooth were determined precisely. These results combined with previously obtained carbon and oxygen isotope signatures point to the animal having spent portions of its life in at least two different geological settings. A further comparison of strontium isotope patterns in the diprotodon tooth with those of modern kangaroo teeth from the Pilbara suggests that the animal spent most of its time on the Roebourne Plains and summered at Du Boulay Creek, a distance of 175-200 kilometres away. Migration patterns of extinct fauna are important for studies of palaeobiology, rates of evolution, extinction and speciation, and paleoclimatic reconstructions.
Craters and critters

The end-Permian mass extinction occurred 252 million years ago, and is the largest of the five biotic crises that punctuate the fossil record of the last 540 million years.

The cause of this catastrophe is still unknown, even though its biological effects were more than twice as severe as those unleashed by the 65 million-year old impact that created the crater at Chicxulub in Mexico. The Chicxulub impact is thought to have caused the extinction of the dinosaurs. An ongoing study of several major impact craters around the world is being spearheaded by Assistant Professor Eric Tohver from the School of Earth and Environment and Dr Fred Jourdan from Curtin University. One aim is to provide more accurate age constraints on large impact events to assess their effects on the life on Earth.

Their geochronological investigation of the 40 km wide Araguainha crater in central Brazil is yielding interesting information. The effects of shock metamorphism and recrystallisation are clear in the pictured grain of zircon (right).

Diagonal and horizontal, light-colored striations cutting across the grain (inset) are two sets of deformation features of a type caused only by the compression wave generated by impact events. The smaller discrete crystals growing on the surface of the larger crystal indicate that new zircon growth nucleated on older zircons in the impact melt. The age of this newly crystallised material was investigated with the sensitive high-resolution ion microprobe (SHRIMP) at the John de Laeter Centre for Isotope Research. The results indicate an impact event occurring at approximately 252 million years ago.

Earlier, less comprehensive dating results gave an impact date of 244 million years, whereas these new results clearly place the impact in the same time window as the end-Permian mass extinction. This coincidence of timing means that the impact is a factor that must be considered when searching for causes of this biotic crisis. Taken together with other impacts, this data contributes to the idea that mass extinctions may share a common extraterrestrial cause.
Centre highlights

IMAGE / MICRO-CT OF CHITON TEETH. IMAGE BY JEREMY SHAW, ACQUIRED AT AMMRF NODE AT THE UNIVERSITY OF SYDNEY.
Sten Littman is a geochemist who has joined the CMCA as the SIMS support engineer after working at the Department of Nuclear Safeguards and Security at the Institute of Transuranium Elements (ITU) in Germany. There, he managed the SIMS laboratory that analysed the isotopic composition of uranium particles in environmental swipe samples from around the world.

Dr Tamara Abel joined the CMCA in early 2010 to provide academic support for the Centre’s optical and microscopy instrumentation. Tamara is familiar with UWA, having worked as a research assistant and then completed a PhD in Medical Science at the Centre for Orthopaedic Research, where she then continued as a postdoctoral fellow.

Tamara has a strong background in molecular and cell biology and brings a wide variety of skills to the centre, including experience with X-ray micro-CT technology. This has stood her in good stead as she facilitated the set up of the newly operating CMCA Bioimaging Facility at CMCA@QEII.

Dave Adams graduated from Baylor University, Waco, Texas with a Masters of Science studying igneous petrology and volcanology and a Bachelor of Arts in German, with a minor in Geology. Subsequently he commenced his PhD at Oregon State University where he investigated trace elements in melt inclusion in olivine and plagioclase pheocrysts to determine origin and melt regime. He also worked on diffusion of elements in olivine (olivine speedometry) from Crater Lake, Oregon to determine the time between injection of new mafic magma into a relatively silicic magma chamber and eruption.

Dave became familiar with electron microprobe analysis during his years of study, bringing back to life Baylor University’s old AMRAY AMR-1000 and updating the out-dated film camera with a computer imaging system.

While at Oregon State University, he was the student microprobe technician in charge of their CAMECA SX-100 microprobe working with researchers from the University, around the country, and from industry.

Prior to joining CMCA, Dave worked with the US Geological Survey in Denver, Colorado in the Denver Microbeam Laboratory where he primarily worked with SEM and electron microprobe analysis.

Lecia Khor joins the CMCA as a postdoctoral research fellow from industry, having graduated from UWA in 1998 with a PhD in Geochemistry on the topic of the nature and control of contaminants in altered ilmenites.

On the strength of her PhD research on characterisation and analysis of ilmenite grains, Lecia was employed at Cirrus Logic in the USA where she managed the electron and focused ion beam laboratories responsible for reverse engineering semiconductor devices to elucidate mechanisms of failure. She was also engaged in complex device modification using Focused Ion Beam (FIB) and the development of novel methods for physical deprocessing. Subsequently she worked as a Product Development Scientist for PharmaForm LLC, a contract pharmaceutical manufacturing company.

Lecia has come full circle and returns to UWA with a primary interest in the investigation of solid state and structural chemistry, and a current focus on lime chemistry.

Staff news
Staff list

W/Prof David Sampson
  Director
Prof Martin Saunders
  Deputy Director
Alynka Youngman
  Centre Manager
Steve Parry
  Laboratory Manager
Dr Tamara Abel
David Adams
Liz Albert
Dr Lindsay Byrne
Asst/Prof John Cliff
Assoc/Prof Peta Clode
Dana Crisan
Peter Duncan
Dr Monica Gagliano
  Joint appointment with
UWA School of Animal Biology
Prof Brendan Griffin
Jeanette Hatch
Asst/Prof Kathy Heal
Assoc/Prof Andrew Johnson
Honorary
Asst/Prof Lecia Khor
Assoc/Prof Matt Kilburn
Lyn Kirilak
Prof John Kuo
  Honorary
Tracey Lee-Pullen
Sten Littmann
Dr Janet Muhling
John Murphy
Tracey O’Keefe
Dr Anthony Reeder
Assoc/Prof Paul Rigby
Dr Jeremy Shaw
Dr Brian Skelton
Asst/Prof Alexandra Suvorova
Dr David Wacey
  Joint appointment with
UWA School of Earth and Environment
Botany under the microscope: anthers to Zostera

Professor John Kuo retired in 2010 as the longest serving academic member of the CMCA, joining the then Electron Microscopy Centre at UWA in 1978. Recognised internationally for his use of advanced microscopy techniques in botanical science, Professor Kuo has enabled several generations of Western Australian scientists to better understand plant function through the visualisation of plant anatomy in situ. His contributions to research on crops, Western Australian native plants and seagrasses, have shaped significant improvements for biodiversity and the economic landscape of the Western Australian community.

A symposium, Botany under the microscope: anthers to Zostera, was held to mark John’s retirement and featured guest speakers who highlighted John’s contributions to botanical research, particularly through microscopy. Speakers included John’s PhD advisor, Professor Margaret McCully, world renowned expert on seagrasses, Professor Cornelus den Hartog, former and current colleagues, former students and his daughter Ivana (now a post-doctoral microscopist at Yale).

The kumi-e by Western Australian artist, Mutsuko Bonnardeaux, are created from paper using the traditional Japanese method known as Washi (‘Wa’ meaning Japanese and ‘shi’ meaning paper). The paper was handmade and dyed by the artist from imported Kozo (Mulberry) and Mitsumata (Daphne) bark.

The other is displayed in the Centre, in acknowledgement of Professor Kuo’s contribution to the Centre for more than 30 years and his world leading expertise in seagrass anatomy and taxonomy.

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Electron microscope images of seagrasses

Electron Microscope Images of Seagrasses is derived from black and white microscopic images of Halophila ovalis and Posidonia australis. Framed against an ocean-like background, the artwork features: a Posidonia australis leaf with details of its epidermal cell (left) and vascular cells (middle); three green/blue diatoms found on a Posidonia australis leaf surface; the surface of a Halophila leaf (bottom right) and a spherical orange Halophila seed.

Seagrasses

Mutsuko Bonnardeaux
Seagrasses depicts seagrasses common to the Western Australian coast, featuring the endemic species Posidonia coriacea (left), Posidonia sinuosa (centre), the flowering Posidonia australis (right) and the globally distributed, oval-leafed Halophila ovalis (bottom).
Live cell microscopy at Purdue and Pittsburgh Universities

During 2010 Dr Paul Rigby spent three months at Purdue University mainly concentrated on web-based virtual-reality tools for teaching microscopy. The university’s Envision Center is an advanced virtual-reality centre where Paul was taken on a virtual tour of the only certified virtual pharmacy preparation laboratory world-wide. In conjunction with Professor Robinson (an AMMRF International Technical User Advisory Group (ITUAG) committee member), he also developed a web-based Flash application to assist with basic training and assessment on a Nikon light microscope. It is planned to make this tool available for use in training sessions within CMCA.

The opportunity to work with Professor Simon Watkins (also a member of the ITUAG committee) in Pittsburgh was a highlight as Paul honed his skills on multi-colour TIRF and more live-cell microscopy while seeing how Professor Watkins runs his very successful Centre for Biologic Imaging.

Now back in the Centre users have benefited from Paul’s newfound knowledge and experience.
BioGENEius biotechnology prize
Assistant Professor Kathy Heel from the CMCA recently mentored 15-year-old high-school student, Emily Phillimore from Shenton College in Perth, to success in the sanofi-aventis International BioGENEius Challenge of Western Australia, coordinated by the WA Department of Commerce.

The BioGENEius Challenge provides an opportunity for motivated and talented highschool students to work with a professional scientist who mentors them as they undertake research in the field of biotechnology.

Emily’s project was entitled ‘Fuelling our future – unlocking the potential of an ancient oil crop – Camelina sativa’. Under the supervision of Assistant Professor Heel and Assistant Professor Janine Croser, Emily determined the genome size and variability of the species by using flow cytometry, correlating the results with key biochemical, morphological and agronomic traits. She identified plants with very high levels of omega-3 fatty acid and others with profiles suited to use as a biofuel.

On the basis of project reports, laboratory journals and scientific posters, the WA panel judged Emily the joint winner. She then travelled, with her parents and mentors, to the Bio2010 conference in Chicago where she competed with 13 other international finalists from Canada and the USA, presenting her work to 2000 people and manning her poster to discuss it with all comers. Although Emily didn’t win the event, she was highly commended and had an inspiring trip, hanging out with the other finalists and getting to meet Bill Clinton and George W. Bush.
International honours  
Professor Brendan Griffin has been recognised by the international microscopy community with two very important honours reflecting the important role Brendan plays at a global level.

At the 17th International Microscopy Congress (IMC-17) held in Rio de Janeiro in September 2010, the General Assembly of the Federation of International Societies for Microscopy (IFSM) elected Professor Griffin to the position of General Secretary of IFSM. Professor Griffin is the second Australian to achieve this honour, preceded only by the late Professor David Cockayne FRS.

Professor Griffin has also been elected as a Fellow of the Microscopy Society of America (MSA), an honour reserved for only a small fraction of the MSA membership and intended to recognise members who have made significant contributions to the advancement of the science and practice of microscopy imaging, analysis or diffraction techniques. Professor Griffin was presented with his Fellowship Certificate at the 69th Annual Meeting of the MSA held in Nashville, Tennessee.

The Fellowship Certificate contains the following citation:

“For outstanding original contributions as well as being an internationally recognised leader in the theory and application of variable pressure scanning electron microscopy.”

Professor Griffin is the first Australian to receive this honour, though we are confident he will not be the last.
Science meets Parliament
Director of the CMCA, Winthrop Professor David Sampson attended 2010 Science meets Parliament where he highlighted the importance of microscopy for Australian research and innovation. Discussion of research outcomes with Senator Louise Pratt and Sophie Mirabella, MP, included the identification of nanogold with the CAMECA NanoSIMS 50 and resulted in a subsequent visit to CMCA from Ms Mirabella within the month.

AMMRF Flagship Ion Probe Facility Open House (5iAS)
A combined CMCA and Centre for Exploration Targeting Open House showcased the world-class AMMRF Flagship Ion Probe Facilities, in September 2010.

Thirty of the world’s top archean geologists in Perth for 5iAS visited the CMCA which is conveniently located in close proximity to some of the oldest archean geological features in the world in the West Australian Pilbara. Visitors, who included world-class geology researchers John Valley, Sue O’Reilly and Boswell Wing, were impressed with the quality of the facilities made available to all Australian researchers at nominal cost.
Publications

Image: A cross section through a Deschampsia antarctica root seen with a fluorescence microscope. Image by Peta Clode and Dan Murphy acquired at CMCA.
**2009**

**Books and book chapters**

**Journals**


Total

- Biological/biomedical sciences: 58
- Environmental/geosciences: 16
- Physical sciences: 19
- Total: 101


72 K. Trinajstic and A.D. George, Microvertebrate biostatigraphy of Upper Devonian (Frasnian) carbonate rocks in the Canning and Carnarvon Basins of Western Australia, Palaeontology, 52: 641-659, 2009.


2010 publications

<table>
<thead>
<tr>
<th>Biological/biomedical sciences</th>
<th>Physical sciences</th>
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<tr>
<td>Physical sciences</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

**Journals**


71 R.A. McLaughlin and D.D. Sampson, Clinical applications of fiber-optic probes in optical coherence tomography, Optical Fiber Technology, 16: 467-475, 2010.


Got it covered

Plant, Cell & Environment, Applied and Environmental Microbiology and Parasitology featured images acquired at CMCA by Foteini Hassiotou, Jeremy Shaw and Peta Clode respectively.

The theoretical reconsiderations when estimating the mesophyll conductance to CO₂ diffusion in leaves of C₃ plants by analysis of combined gas exchange and chlorophyll fluorescence measurements

1500 J.-C. Domec, S. Palmroth, E. Ward, C. A. Maier, M. Thérézien & R. Oren

1513 Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted, intact tobacco leaves

R. Zhang, J. A. Cruz, D. M. Kramer, M. E. Magallanes-Lundback, D. Dellapenna & T. D. Sharkey

1525 Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of Miscanthus relative to switchgrass?

F. G. Dohleman, E. A. Heaton, A. D. B. Leakey & S. P. Long

1538 Myzus persicae (green peach aphid) salivary components induce defence responses in Arabidopsis thaliana

M. De Vos & G. Jander

1548 Spatial and temporal analysis of non-steady elongation of rice leaves

B. Parent, G. Conejero & F. Tardieu

1551 Photobiological properties of the inhibition of etiolated Arabidopsis seedling growth by ultraviolet-B irradiation


1561 Leaf hydraulics and drought stress: response, recovery and survivorship in four woody temperate plant species

C. J. Blackman, T. J. Brodribb & G. J. Jordan

1573 Stomatal crypts may facilitate diffusion of CO₂ to adaxial mesophyll cells in thick sclerophylls

F. Hassiotou, J. R. Evans, M. Ludwig & E. J. Veneklaas

1584 Proteomic analysis of β-aminobutyric acid priming and abscisic acid – induction of drought resistance in crabapple (Malus pumila): effect on general metabolism, the phenylpropanoid pathway and cell wall enzymes

D. Macarisin, M. E. Wisniewski, C. Bassett & T. W. Thannhauser
COVER IMAGE / A CROSS SECTION THROUGH A DESCHAMPSIA ANTARCTICA ROOT SEEN WITH A FLUORESCENCE MICROSCOPE. IMAGE BY PETA CLODE AND DAN MURPHY ACQUIRED AT CMCA.

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