Fusion image microPET/CT (using TSPO ligand PBR111) showing the TSPO expressing tumor cells in a TSPO knock-out animal and size comparison of a mouse versus human brain, demonstrating the high resolution and contrast of the PET/CT.
Director’s Message

NIF News
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Time-of-flight angiography of the human brain at 7 Tesla
- Dr Zuyao Shan, Centre for Advanced Imaging, NIF - University of Queensland node.
What is happening with NCRIS and what does that mean for NIF?

I am sure that most of you are watching, with interest, the political discussion about Higher Education and research, and will have seen news articles, stating that NCRIS is at risk. However, for NIF, it is business as usual, and we are working hard to ensure that the world-leading imaging infrastructure continues to be available for your research. We are confident that NIF is delivering on the mandate, as highlighted in the December Quarterly:

1. NCRIS delivers world class research facilities so that Australian researchers can solve complex problems both here in Australia and around the globe.
2. NCRIS is the most efficient and strategic way to invest in national scale research infrastructure, driving collaboration to bring economic, environmental, health and social benefits for Australia.

We remain positive about the future, and so have revamped the website, are organising stakeholder meetings with industry, and are celebrating a new installation, demonstrating that NIF continues to grow.

We are working together with other NCRIS capabilities, see the story about a new joint appointment at the University of Western Australia in data management, analysis and visualisation. This is an extremely important theme for NIF, and one in which we are working hard to deliver capability to you, the users of imaging facilities across Australia.

Read about the latest research stories from bees to skin-grafts, multi-modal imaging, from molecule to system.

I trust that you enjoy this NIF Quarterly. It is released to coincide with the Universities Australia Conference “Future Sense: universities shaping the new era.” I strongly believe that NCRIS is part of that new era, and NIF will continue to contribute to research and education, across the whole sector.

Professor Graham Galloway
Director of Operations

“National Imaging Facility is working hard to ensure that the world-leading imaging infrastructure continues to be available for your research.”
New Research Edge for UWS
Official Launch of Bruker 14.1 T MRI & Perkin-Elmer Quantum GX MicroCT

The internationally renowned Biomedical Magnetic Resonance Facility (BMRF) directed by Prof. Price contains state-of-the-art equipment for Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI). The Facility has been undergoing refurbishment in preparation for the installation of a Bruker 600 MHz (i.e. 14.1 T) wide-bore spectrometer. At the time of writing, this installation is in progress. This new instrument was acquired through an Australian Research Council Linkage Infrastructure, Equipment and Facilities (ARC LIEF) grant (LE140100009, Price et al. “Ultra-high resolution magnetic resonance imaging (MRI) system for physical applications”) and will be made available to the wider research community via NIF. The new Bruker 600 MHz MRI will further reinforce BMRF’s world-standing. The facility will be the focal point for more than 100 Australian academics and Higher Degree Research students and will include clinicians, biochemists, chemists and physicists. Internationally, it will attract the best and brightest academics, research partners and HDR students. The new MRI will provide unique insights into a diverse variety of areas. Expected outcomes will range from agricultural advances, higher performing energy storage solutions, to more effective cancer treatments and advance Australia’s fundamental scientific capabilities.

Importantly, the node has amassed a world recognised concentration of expertise from fundamental magnetic resonance development (i.e., quantum mechanics and pulse sequence design) to application support and data modelling. The operation of the instrument requires specialist input and a high degree of training and the node’s expertise can be drawn upon by researchers and collaborators accessing the equipment. Projects already planned for the instrument include: probing GSH interactions in biological systems; probing antibiotic peptide assembly using diffusion NMR (dNMR); probing the chemical stability of peptide structures; probing cellular uptake using dNMR; establishing imaging markers representative of oncology pathology and treatment outcome; measuring grey matter loss in animal models of synaptic dysfunction; understanding diffusion in non-molecular solvents and application to understanding reaction outcomes in these systems; and others.

In addition to the new MRI, the node has also obtained a Perkin-Elmer Quantum GX microCT imaging system for high performance CT imaging. Mice, rats and rabbits can all be imaged with this instrument at resolutions approaching 4.5 μm. The microCT will complement the MRIs and will widen the array of projects possible at the UWS node.

Key facts:
- The Bruker 14.1 T system is 280,000 times stronger than the Earth’s magnetic field.
- The 14.1 T magnet contains kilometres of superconducting wire carrying a current of 234 amps.
- 750 litres of liquid helium and an equal volume of liquid nitrogen are required when the magnet is initially charged.
- To keep the coils in a superconducting state they are immersed in 130 litres of liquid helium. This helium is topped up every couple of months. Every week the magnet is topped up with liquid nitrogen.

To celebrate the installation and commission of the two new instruments at NIF-UWS node, a scientific symposium will also be held on the 31st March, 2015. The symposium will bring together leading domestic and international experts on topics ranging from medical, environmental, mining and industrial applications of the technology.

Invited speakers include Professor Jörg Kärger (Leipzig), Professor Peter Basser (NIH), Dr Kirk Feindel (UWA), Dr Konstantin Momot (QUT).

The symposium is open to all including people from outside academia (e.g., clinicians and industry) and students who aspire to enter this exciting area.
New addition to the NIF family
Dr Andrew Mehnert
CMCA, NIF - UWA node

Andrew joined the Centre for Microscopy, Characterisation and Analysis (CMCA) at the University of Western Australia (UWA) in December as Senior Lecturer in Data Management, Analysis and Visualisation. His position is funded under the National Collaborative Research Infrastructure Strategy (NCRIS) 2013 in part by the National Imaging Facility (NIF) and in part by the Australian Microscopy & Microanalysis Research Facility (AMMRF). His role is to introduce, develop and apply data management, analysis and visualisation expertise in collaboration with researchers using microscopy and imaging techniques across various disciplines at UWA.

Andrew obtained his PhD in electrical engineering from the University of Queensland (UQ) in 2004 – Thesis: “Image analysis for the study of chromatin distribution in cell nuclei with application to cervical cancer screening”. He subsequently held the positions of Research Fellow and then Senior Research Fellow in medical image analysis in the School of Information Technology and Electrical Engineering at UQ. From 2011 until his present appointment at UWA, he held the positions of Assistant Professor and then Associate Professor in medical image analysis jointly with the MedTech West centre (located at Sahlgrenska University Hospital, Gothenburg, Sweden) and the Department of Signals and Systems at Chalmers University of Technology.

Andrew’s research interests focus on the development of medical image analysis methods for clinical imaging applications. He has both academic and commercial research experience in medical image analysis in the areas of optical microscopy (cervical cancer screening), MRI (breast, brain, musculoskeletal) and X-ray CT (forensic identification of human dental remains). This has included research on visualisation, denoising, spatial co-registration, segmentation, parametric modelling of contrast enhancement for dynamic contrast-enhanced MRI and classification (feature extraction, feature selection and classifier design, training and validation).

Andrew is based at the Harry Perkins Institute of Medical Research at the QEII Medical Centre (CMCA@Perkins) but also spends a couple of days a week on the UWA Crawley campus (CMCA@Physics). His contact details are as follows:

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It’s here! Welcome to the new NIF website

After months of anticipation and hard work, the National Imaging Facility is excited to announce the launch of a new website – a make over! The new site www.anif.org.au has extensive information about the NIF’s imaging capabilities, expertise, scientific leadership, image gallery, and much more!

A built-in scientific forum is the perfect virtual ground for all NIF’s scientists to bounce ideas off each other, ensuring the knowledge flow amongst all nodes, so that most efficient and appropriate solutions can be shared amongst our users.

The new website is also about engaging with the wider Australian research community. The website has live twitter feed, LinkedIn, YouTube and Google+ features so NIF can better connect with the users.

Special THANKS! to the web developers and Dr Michelle McCleary for months of discussions back and forth to make this new website happen. The new website is a live project, thank you to the NIF team for feeding through new updates and info!
An overwhelming body of behavioural evidence exists demonstrating that many animals can detect and utilise the Earth’s magnetic field for navigation and orientation.

NCRIS Collaboration:
The Search for Magnetoreceptive Cells in the Honeybee Apis mellifera

Animals are known to detect and utilise the Earth’s weak magnetic field for orientation and navigation, with evidence collected from a broad range of species, including insects, birds, reptiles and mammals. Despite a wealth of data demonstrating magnetoreceptive behaviour, little progress has been made in understanding the mechanistic basis of the sense. Unravelling the cellular basis of the magnetic sense remains one of the great unsolved problems in biology. Such research is fundamental to understanding animal behaviour and ecology and will provide important insights into neuronal function and cognition. A leading explanation for how animals might transduce a magnetic stimulus into a neuronal response is the magnetite hypothesis, which is based on an interaction between magnetite nanoparticles and specialised nerve cells.

Magnetotactic bacteria are the “smoking gun” for the existence of a magnetically sensitive cell. These organisms possess chains of 100 nm sized single crystals of magnetite, which are used to orient the bacteria towards regions more favourable for growth (Fig 1A). However, finding similar structures in animals has frustrated scientists for almost 50 years. The reasons for this are many, but can be summarised as the classic “needle-in-a-haystack” problem, with these potentially rare cells not being limited to a specific anatomical location making the task of discovery highly problematic. A further complication is the fact that iron is a common biological and environmental element, which creates difficulties associated with misidentification and contamination.

Clearly, new approaches must be developed and applied in order to progress our understanding of the cellular mechanisms that underlie magnetoreception. Dr Jeremy Shaw at The University of Western Australia’s Centre for Microscopy, Characterisation and Analysis (CMCA) recognises that a multi-modal and multidisciplinary approach will be needed to find magnetosensory cells. Dr Shaw draws upon the expertise, advanced imaging and microanalytical capabilities available at networks such as the National Imaging Facility (NIF) and the Australian Microscopy and Microanalysis Research Facility (AMMRF), to address the magnetoreception problem.

Although electron microscopy will be a key tool for finally characterising the ultrastructure of magnetoreceptor cells, no single technique will be sufficient for the search. Using honey bees as a model system, Dr Shaw and colleagues are exploring the applicability of MRI, X-ray micro-CT, optical and electron based imaging platforms for probing the structure and composition of the bees various body parts across a range of length scales. Ultimately, techniques are needed that will be able to pin point target regions in whole parts (head, thorax and abdomen) before characterising them at high resolution in situ.

APPROACH and FINDINGS

- Magnetic nanoparticles, similar to those found in magnetotactic bacteria, are theorised to form the basis of a magnetoreceptor cell in animals (Figure 1A).
- Proof of concept – If such particles exist in the honeybee, can they be extracted successfully (Figure 1B and C)?
- Bees are separated into body parts and subjected to the digestion process to extract particles, which can be characterised using a range of imaging and analytical techniques (Figure 2A and B).
- The final aim is to reveal where the extracted material originates from and study it in situ, as shown for iron particles in the honeybee abdomen (Figure 3A-C).
The successful recovery of particles from bacteria and honeybees demonstrates that similar material associated with a magnetic particle based magnetoreceptor could be collected and examined.

High resolution MRI, using the 16.4T instrument at the University of Queensland’s NIF node, offers a means to investigate intact honey bee specimens in three dimensions. In addition to obtaining detailed anatomical information, it is hoped that strongly magnetic features, such as magnetite bearing cells, may influence the relaxation rates of surrounding tissue and give rise to anomalies that give away the location of the sense. However, imaging of the honey bee head reveals a complex structural landscape and MRI will not be a silver bullet. Correlative imaging techniques and novel experimental approaches will be needed to compliment these data in order to finally resolve the mystery of animal magnetoreception.

This work has been supported by the Australian Research Council (DE130101660) and by two grants from the University of Western Australia (Research Development Award and UWA-UQ Bilateral Research Collaboration Award).

The authors acknowledge the facilities, and the scientific and technical assistance of the National Imaging Facility and the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, a facility funded by the University, State and Commonwealth Governments.

For more details about the project, please contact Dr Jeremy Shaw at jeremy.shaw@uwa.edu.au.

For info on MRI imaging, please contact NIF Facility Fellow Dr Gary Cowin at gary.cowin@uq.edu.au. Or please visit NIF website http://www.anif.org.au.
The 18 kDa translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor (PBR), is expressed in the injured brain and has become known as an imaging marker of “neuroinflammation” - an indication of active disease. TSPO expression is best interpreted as a non-diagnostic biomarker and disease staging tool that refers to histopathology rather than disease etiology. The therapeutic potential of TSPO as a drug target is mostly based on the current understanding that it is an outer mitochondrial membrane protein required for the translocation of cholesterol and thus regulates the rate of steroid synthesis. This pivotal role together with the evolutionary conservation of TSPO has underpinned the belief that any loss or mutation of the TSPO gene should be associated with significant physiological deficits or be outright incompatible with life. However, against prediction, full TSPO knockout mice are viable and across the animals’ lifespan do not show the phenotype expected if cholesterol transport and steroid synthesis were significantly impaired. Thus, the “translocation” function of TSPO remains to be better substantiated.

Following five years of intensive research, scientists from the Australian Nuclear Science and Technology Organisation (ANSTO), The University of Sydney and University of Wollongong have confirmed the existence of generations of apparently healthy mice living without the mitochondrial translocator protein 18 kDa (TSPO).

The research, having been recently published in Nature Communications, is gaining significant attention as it breaks the previously held belief that TSPO was essential to life. The TSPO knockout (C57BL/6-Tspo<sup>tm1GuWu</sup>) mouse model has significant implications for both the medical professional and the pharmaceutical industry as it enables better targeted drug design. TSPO, is a mitochondrial outer membrane protein that is highly conserved across species from bacteria to mammals. The TSPO is associated with crucial cellular functions: steroid hormone production, transport of cholesterol into the mitochondria, as well as energy metabolism and the regulation of inflammatory immune responses.

TSPO expression levels have been shown to be highly increased in a variety of disease states and to correlate with the severity of illness and rate of illness progression. Over the last fifteen years, research into the TSPO as a widely applicable diagnostic and therapeutic target has intensified significantly. As of 2014, there have been well over 30 clinical trials involving the measurements or the therapeutic targeting of TSPO in disease conditions ranging from inflammation to neurodegeneration and behavioural illnesses.

The use of the TSPO knockout mouse model will allow for the molecular function of TSPO in disease to be understood in greater detail, and be tested in vivo under realistic and controlled conditions. This is a crucial step towards the rational and accelerated development of next generation therapeutics targeting TSPO related functions in disease.

“...The paper of R.B. Banati and co-workers is definitely a breakthrough... (it) rejuvenates basic science around TSPO and the potential design and development of drugs interacting with this target.”

Gerard Le Fur, Academie des Sciences, France

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The radiotracers available from ANSTO are synthesised and validated, and have either been characterised using a number of LifeSciences technology platforms and capabilities including Chemistry (synthesis, radiosynthesis, automated radiosynthesis, process optimisation & validation, lipophilicity and in vitro stability); in vitro Biological characterisation (radioreceptor binding in membrane fractions or cells, cell uptake, autoradiography, immunocytochemistry); in vivo Biological characterisation (pharmacology, metabolism, in vivo PET/SPECT quantification in relevant animal models) or advanced studies in pre-clinical or clinical trials.

More detailed information about the full range of radiotracers available at ANSTO, focusing on neuroscience and other systems can be found at: www.ansto.gov.au/LifeSciences.

*In recognition of the birth place and the fact the mitochondrial energy production in microglia of TSPO knock out animals may be altered, the animals were named Guwiyang Wurra ('Fire mouse') in the local Dharwal language.

### Partnering for Success:

The mitochondrial translocator protein (TSPO) knockout (C57BL/6-Tspo<sup>tm1GuWu</sup>) is a mouse model developed at ANSTO, Australia’s leading nuclear science and research organisation, in collaboration with the Brain & Mind Research Institute, The University of Sydney.

The characterisation of the animals was carried out in partnership with the joint ANSTO-University of Sydney NIF node. The work is ongoing and included other partners and NIF nodes, such as the UNSW NIF node, to integrate and leverage from each node unique imaging competencies. The research using the TSPO knockout mouse model also involves the NCRIS Deuteration Facility at ANSTO and encompasses a unique range of nuclear technologies. It extends from the structural characterisation of the protein in membranes employing neutron and x-ray scattering, to pre-clinical experiments and clinical trials, using the specific radiotracer [18F] PBR111 to understand, monitor and treat the acute and chronic illnesses in which the TSPO is regulated.

Information about the history of the joint ANSTO-University of Sydney NIF node can be found under: http://royalsoc.org.au/generator/assets/Journal/J_Proc_RSNSW_Vol_146_1_Nos_447_448_Buttner.pdf

Herma Buttner et al., 2013; Nested partnerships and interdisciplinary science: from the National Medical Cyclotron to the research cyclotron of the National Imaging Facility; Journal and Proceedings of the Royal Society of New South Wales, vol. 146, nos. 447 & 448, pp. 25-43. ISSN 0035-9173/13/010025-1925
Industry:

Replacement of the Skin Graft in Full-Thickness Wounds

Studies conducted by A/Prof. John Greenwood AM, Director Adult Burn Service, Royal Adelaide Hospital, on behalf of PolyNovo Biomaterials Pty Ltd, Port Melbourne, Victoria.

Challenge

In major burn injuries (>50% total body surface area), burn size exceeds the available area for the harvesting of skin grafts and result in the patient becoming profoundly immunosuppressed. The surgical excision of a large (albeit compromised) portion of the mechanical barrier to bacterial invasion results in a high risk of mortality or severe morbidity from sepsis in those who survive the initial insult. Over the past 10 years, we have been assessing a bio-degradable polyurethane with the aim of producing a cheap, any size, easy to store and use dermal scaffold. The desire to make the material easy to mass-produce, reaching a global market and making the materials cheaper to the end user, drove us to develop a foam technology. With the matrices less dense, they became lighter, cheaper and degraded yielding with a smaller volume of degradation products. It was compared in a porcine model against spun mats and found to be superior in terms of wound contraction despite being less dense.

How did the facility help?

The animal trial studies would not have been possible if they could not be performed in high quality surgical suites, and with the level of post-operative care that the pigs received in the National Imaging Facility (NIF) – Large Animal Research & Imaging Facility (LARIF) node.

Outcome

Further studies were conducted to assess the effect of time of skin graft on wound healing. The biodegradable temporizing matrix (BTM) resulted in much better outcomes in the immediately grafted group, rather than the delayed graft group. We realized that the biodegradable temporizing matrix would have to be sealed to prevent water loss and the rapid wound contraction that occurs between days 4 and 11 post-injury. When the matrices were ‘sealed’ with an impermeable polymer sheet on their superficial surfaces, they were far more effective at preventing wound contraction than those that remained ‘unsealed’. We noted a thick layer of scar tissue forming superficial to the integrated unsealed matrix, where no mechanical or chemical barrier to continued proliferation and collagen deposition was being offered. Where the matrices were sealed, dermal proliferation and collagen deposition stopped at the seal.

Following optimising refinements of the BTM, the matrix was tested with autologous porcine composite skin. Split skin grafts were harvested from three pigs and used to create autologous Composite Cultured Skins in vitro over the next 28 days. The skin graft donor sites were then deepened to the panniculus adiposus to create deep wounds with no residual dermal or epidermal elements. BTMs were introduced into these porcine wounds. All this occurred at Day 0. The BTMs were allowed to integrate and the CCSs were returned to their host at Day 28. By day 7, most of the wounds demonstrated a stratified squamous epithelium over the ‘neo-dermis’.

Further optimizations of the BTM resulted in absence of wound contraction by day 21 and the presence of a stratified squamous epithelium by day 10 post-CCS application. A novel method of bond application to enable the product to be made more easily and therefore more cheaply was evaluated in October 2013. This product gave the same results as described immediately above, and has progressed to human trials.

Utilising the facilities available at the NIF-LARIF node, the PolyNovo research team was able to generate eight publications from these data. More importantly though, the product is now in human clinical trials, and could vastly improve the outcome for patients that have endured serious burn injuries that require debriding to the level of “full-thickness”, by reducing the need for skin grafts, and increasing the time to formation of “new skin”, whilst reducing wound contraction. This would result in shorter hospital stays, less infection from donor sites and less formation of unsightly scar tissue.

In addition to the human gains, if the product is successful in human trials, approved by Regulatory Bodies and marketed, and used by only 10% of Plastic Surgeons for wound repair, it would still generate $100 million US annually.
REFERENCE

For more information on the projects, please contact NIF-LARIF Node Director Dr Tim Kuchel at tim.kuchel@sahmri.com.

For the more information on accessing the LARIF, PIRL, and SAHMRI capabilities, please go to https://www.sahmri.com/research-support/research-facilities-and-equipment/research-services/sahmri-pirl.

ABOUT LARGE ANIMAL RESEARCH & IMAGING FACILITY (LARIF):
The NIF-LARIF Node is hosted by the Preclinical, Imaging and Research Laboratories (PIRL), which is part of the South Australian Health and Medical Research Institute (SAHMRI).

Officially opened on the 22nd May 2013, LARIF provides cutting edge research services by utilising MR, CT and other imaging modalities for research using large animal models of human disease. Many current neurodegenerative disease and neurotrauma models use sheep. Sheep have gyrenencephalic brains, which are similar to humans in structure and size and therefore are a stepping stone in neurological and pharmacological research between the lissencephalic brain of rodents and the human brain.

The NIF-LARIF Node has a 1.5 T clinical MR scanner with ample coils and software, a 16 slice CT scanner, flat detector digital C-ARM, mobile X-ray equipment as well as two dual-energy X-ray Absorptiometry (DXA) scanners and anaesthesia equipment. Image files can be downloaded remotely via a password access system. Access to fully staffed operating surgical suites and support operations, animal accommodation and office accommodation for external researchers is also provided. In addition, off-site researchers have access to a large range of animal models of human disease. The imaging modalities, surgical suites and supporting services are linked to a large animal holding facility with CCTV monitoring equipment with remote access capability. LARIF can also provide experimental rooms, tissue collection facility, a range of on-site options for temporary storage of samples, animal holding rooms for groups housing or individual pens, and rooms to hold sheep in expandable metabolism crates.

Dr Tim Kuchel, LARIF Node Director
Dr Tim Kuchel, is the director of the Preclinical, Imaging and Research Laboratories (PIRL) of the South Australian Health and Medical Research Institute (SAHMRI). It is PIRL which hosts the NIF funded Large Animal Research and Imaging Facility (LARIF). Dr. Kuchel also is the Node director of the Australian Phenomics network (APN).

Dr. Kuchel has more than 30 years’ experience in animal research with a special interest in imaging modalities, experimental surgery and comparative anaesthesia. The capacity to provide or help develop Large Animal Models of human disease is the core business of LARIF and PIRL, and interactions with industry to add to existing translational medicine capacity are being actively pursued by Dr. Kuchel and his team.
NIF Nodes:
University of Queensland
University of New South Wales
University of Sydney / ANSTO
University of Western Sydney
University of Melbourne
Florey Institute of Neuroscience and Mental Health
Monash University
Swinburne University of Technology
Large Animal Research & Imaging Facility
University of Western Australia

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