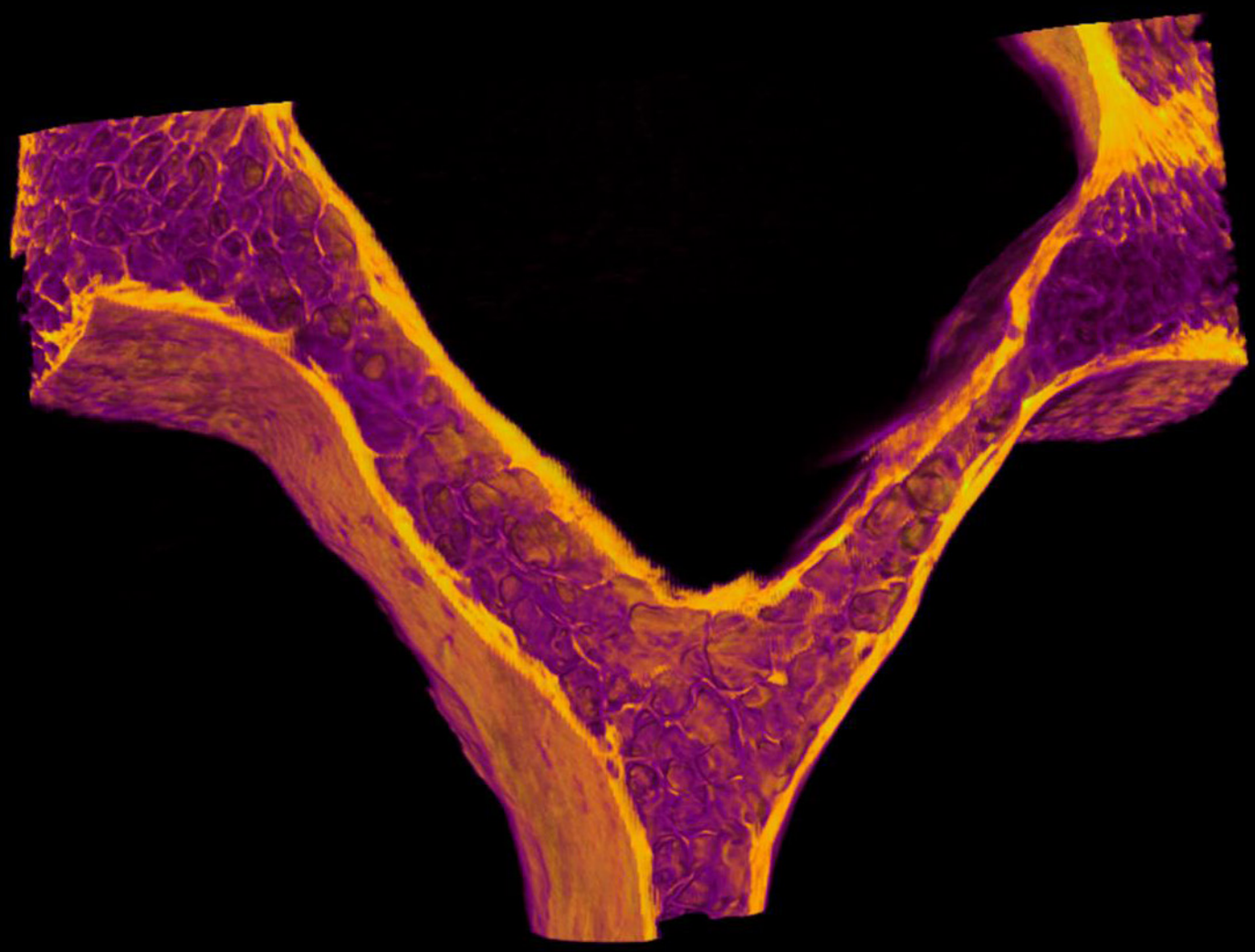
National Imaging Facility

Exploring Inner Space

NIF Quarterly • Q4, 2013

Bee imaging: Single projection through a 15µm isotropic 3D scan of a bee head showing eyes, brain and mouth anatomy. Images acquired on the 16.4T micro imaging MRI system using a 3D gradient echo sequence.
- Dr Jeremy Shaw (CMCA), Dr Michael House (School of Physics), Mr Alastair Boyd (CMCA), University of Western Australia, in collaboration with Dr Gary Cowin, NIF-University of Queensland.



3D image of volume rendered longitudinal section slice of the human vertebrae created using CTAn.
- See NIF Focus Story 1 for more project details.

Director's Message

"This quarter has seen a wide range of opportunities to showcase NIF, and wherever I have presented, there has been a desire to engage with us in leading research."

Communication, communication, communication! Outreach to the scientific research community has to be our mantra. This issue of the NIF Quarterly coincides with the 50th Anniversary of Electron Microscopy at the University of Western Australia, during which they will be celebrating "Excellence in Research", an event at which I look forward to representing NIF. What a milestone, and one for which the team at UWA have every right to be proud. Their success has been the result of excellence in science and reaching out to the needs of their scientific community. I congratulate the CMCA (Centre for Microscopy, Characterisation and Analysis) on this achievement, and trust that in another 50 years, imaging will amongst their list of proud achievements. The two featured articles in this issue would suggest that this should not be a problem.

This quarter has seen a wide range of opportunities to showcase NIF, and wherever I have presented, there has been a desire to engage with us in leading research. From Biological Psychiatry, to Clinical Anatomy, or Computer Scientists to Physical Scientists and Engineers, research, your research, across the breadth of NIF is making an impression. It has been an easy task for me to make an impact. I am but a vehicle for your success. The great images and powerful stories sell themselves. And, importantly, there is a hunger to make use of the great resources that exist within NIF, and it is the responsibility of NIF to share what we have. So, whether you are part of the NIF team, or one of our valued users, we need to communicate with each other, about the scientific problems that you have and the potential solutions that we offer.

I also had the privilege of participating in the 3rd European Union - Australia Workshop on Research Infrastructure, with a theme of Healthy Ageing. Professor Oliver Speck, from EuroBioimaging, joined us for this workshop. Not only was it a chance to share with our European colleagues, but also to partner with other Australian Research Infrastructure. And importantly, as well as being organised by the great team from the Research Infrastructure Branch of the Australian Government and their counterparts from the European Commission, they were enthusiastic in their participation. Be assured, they are working hard to present a strong case for national research infrastructure.

In closing, I wish all our staff and all our users a very joyous Christmas and Happy New Year.

Professor Graham Galloway
Director of Operations

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NIF Focus Story - 1

UWA Node:

Characterisation of the cortical shell and trabecular architecture of the lateral masses of the human cervical spine

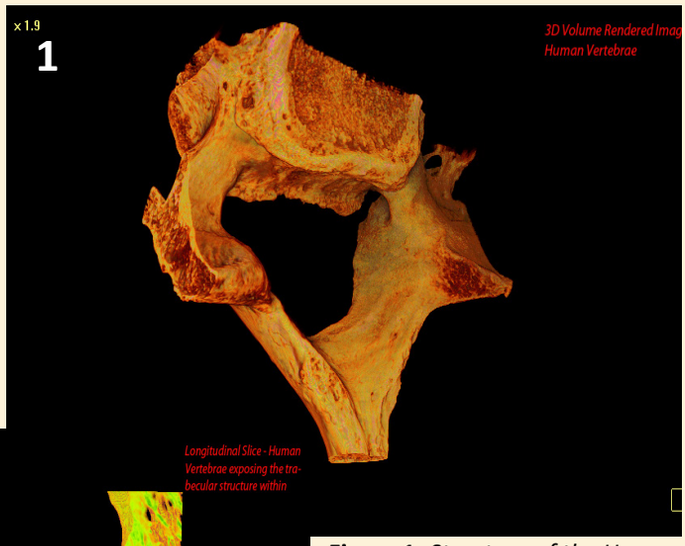


Figure 1: *Structure of the Human vertebrae created using CTAn.*

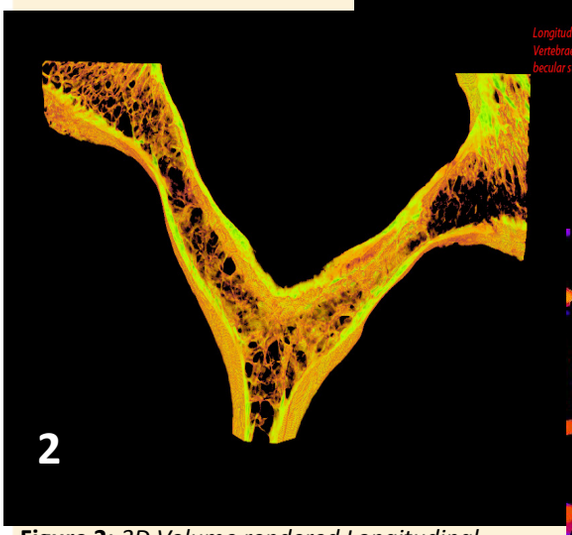


Figure 2: *3D Volume rendered Longitudinal Section Slice of the Human vertebrae created using CTAn.*

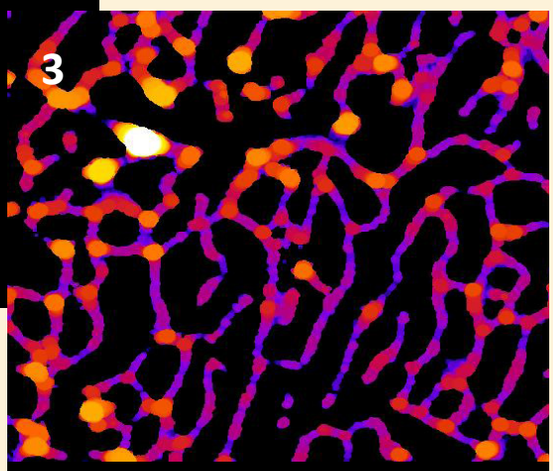
Professor Brett Kirk from the School of Civil and Mechanical Engineering at Curtin University and PhD students Matthew Oldakowski and Intan Oldakowska are developing a novel orthopaedic fastener for use in the lateral mass of the cervical spine during spine stabilisation surgery.

The cortical morphology and trabecular architecture of the lateral mass are critical to the design of the fastener, but is not well characterised. With help from NIF researcher A/Prof Matt Linden and NIF facility fellow Diana Engineer at the Centre for Microscopy, Characterisation and Analysis at The University of Western Australia the team analyses micro computed tomography scans of cadaveric cervical vertebrae for these characteristics and to describe the effect of gender and age. Sheep vertebral bodies are also analysed to determine whether they are an appropriate model for testing prototypes.

Finite element analysis is performed on prospective designs of the novel orthopaedic fastener using geometry extracted from the CT scans. The prototypes are manufactured by Professor Tim Sercombe from the School of Mechanical and Chemical Engineering at UWA using Selective Laser Melting, a 3D printing process, to allow the manufacture of designs which cannot be manufactured by conventional means.

Fresh frozen sheep vertebrae will be scanned before drilling (to determine sample properties), after drilling (to locate the fastener in the finite element model and investigate the effect of drilling and awling on trabecular architecture) and after the fastener has been implanted and expanded (to verify the finite element model.)

Initial scans by the Skyscan 1176 Micro CT were at 9 µm resolution and 90kV and reconstructed using NRecon. However preliminary measurements of volume fraction and trabeculae thickness (see figure 3 & 4) were insensitive to resolution reduction up to 18 µm and so subsequent scans were taken at this resolution to reduce reconstruction time.



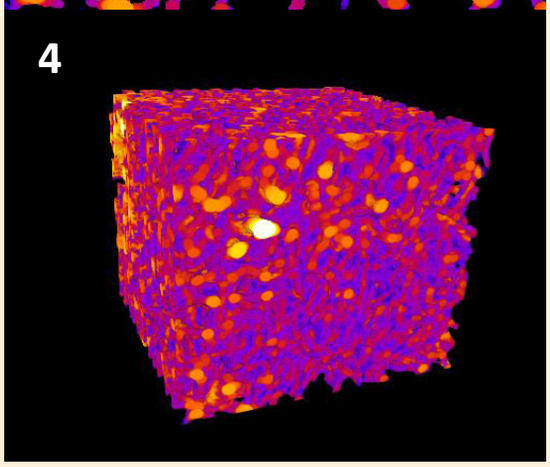


Figure 3: *Trabeculae thickness heat map slice using the BoneJ plugin on ImageJ.*

Figure 4: *Trabecular thickness heat map slice using the BoneJ plugin on ImageJ.*

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NIF Focus Story - 2

UWA Node:

The Role of Carotid Rete Variability in Sheep

Professor Stuart Bunt from the School of Anatomy, Physiology and Human Biology at The University of Western Australia leads a project examining the role of the carotid rete in drought adaptation of different sheep breeds. Sheep use the carotid rete as a counter- current heat exchanger in a process known as selective brain cooling to delay evaporative heat loss and promote water retention, allowing them to survive for longer in dry conditions. Development of a larger carotid rete should allow for more efficient heat exchange and make the animal more suited to drier environments. With his masters student Bradley Kinsella and help from NIF WA acting node director A/Prof Matt Linden and NIF facility fellow Diana Engineer from the Centre for Microscopy, Chatacterisation and Analysis, Prof Bunt analyses the surface area of the rete using micro CT to determine the heat exchange efficiency in the arid-adapted Merino and temperate-adapted Suffolk. This information will help determine if there is any link between rete surface area and heat exchange efficiency.

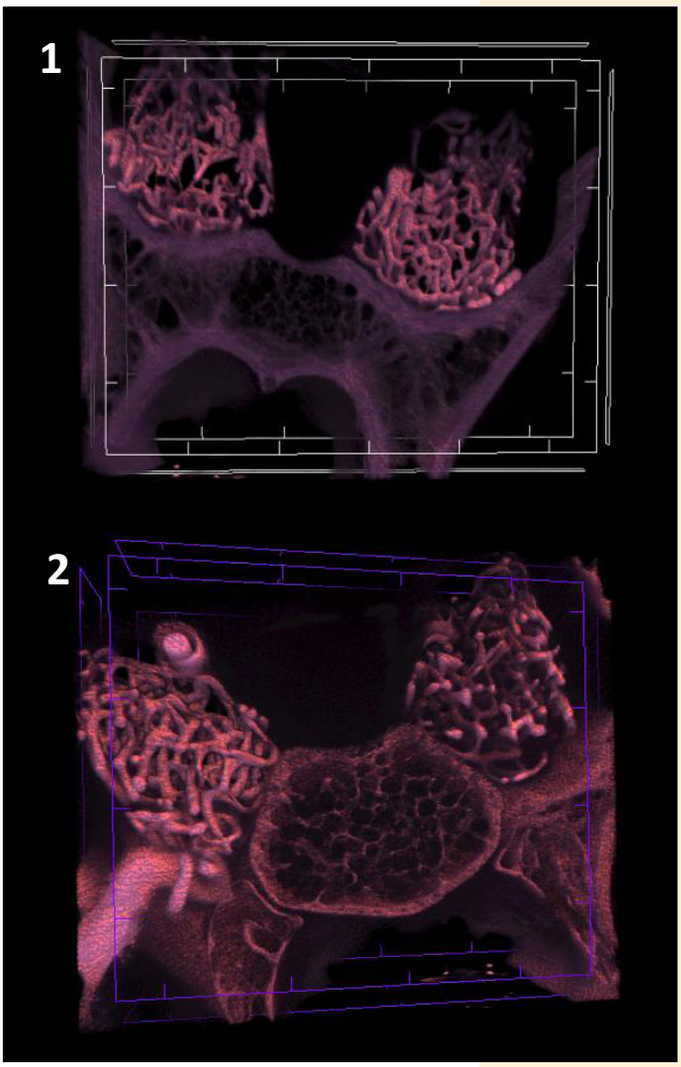

Figure 1: *Vertical Section Slice of the Carotid Rete of Sheep enclosed by bone, created using NRecon and CTVox.*

Figure 2: *3D Volume rendered image of the carotid rete of sheep created using CTVox*

Figure 3: *3D Volume rendered image of a selected Volume of Interest within the carotid rete of sheep created using CTAn*

Samples consist of blocks of bones containing the carotid rete injected with the iodine solution and immediately scanned at 35µm resolution on the Skyscan 1176 Micro-CT. A relatively high voltage and strong filter is required as the rete is completely surrounded by bone (an electron dense material).

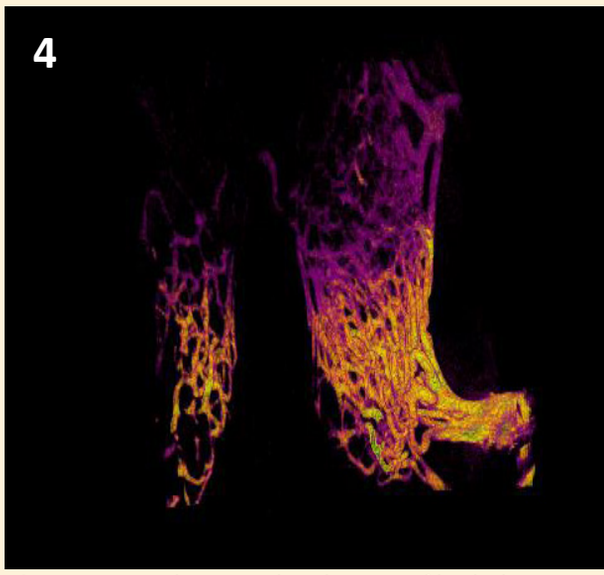

Figure 4: *3D Volume rendered image of both the carotid retes of sheep created using CTAn*

Scanned images were reconstructed using the Feldkamp cone beam reconstruction algorithm with NRecon Software. CTAn was used to calculate surface area and volume. The mean diameter and distance between rete vessels was determined by calculating the structure thickness and structure separation.

For more info about projects, researchers, and facilities available at CMCA, please go to www.cmca.uwa.edu.au, or through National Imaging Facility at www.anif.org.au.

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NIF Focus Story - 3

Florey Node:

Assessing MRI Biomarkers in a Rat Model of Concussion

Concussion, a subset of mild traumatic brain injury (mTBI), is gaining prominence as a serious medical and societal issue worldwide. Of particular concern are individuals in combative arenas, as there is emerging evidence to suggest that recurring mTBIs may result in cumulative and chronic neurological impairments, mental illness and neurodegenerative disease. Locally, there is growing media coverage on the risk to AFL players, from grassroots to elite level, and on-going debate over the management of concussion and return to play decisions. Although the pathophysiological effects of concussion are still not fully understood, is has been proposed that the detrimental effects are in part due to the recurring mTBIs occurring while the brain has not yet recovered from the initial mTBI and is in a period of increased vulnerability.

The current clinical management is based on assessing for the resolution of neurocognitive impairments, however an asymptomatic state may not accurately indicate that the brain has fully recovered. Although more conventional MR methods have failed to identify changes associated with mTBI, advanced methods are potentially sensitive to the subtle changes that occur in the concussed brain.

Meet David Wright:

David Wright is the NIF Facility Fellow at the Florey Node in Parkville, Victoria. David spent 8 years working in neuroimaging at the Research Institute for Brain and Blood Vessels in Akita, Japan, before commencing at the Florey in 2009. Currently a PhD candidate at the University of Melbourne, David is investigating the use of MRI in assessing traumatic brain injury with a focus on concussion and return to play decisions.

A research team lead by Dr Sandy Shults at the University of Melbourne have employed an internationally unique animal model of single and repeated mTBI, in conjunction with MRI and behavioural biomarkers, to investigate: (a) whether MRI can detect injury and assess recovery from concussion; (b) the relationship between MRI and behavioural biomarkers; and (c) to assess the effects of repeated concussion.

MR measures included diffusion-weighted, perfusion and susceptibility-weighted imaging and magnetic resonance spectroscopy. Preliminary results suggest that MRI can detect structural abnormalities long after the resolution of behavioural impairments in animals given a single concussion. In particular, tractography is especially sensitive to the effects of concussion identifying a number of brain regions that are potentially affected.

A second injury, given after the full recovery of behavioural impairments but before the recovery of structural abnormalities identified with MRI, results in exacerbated motor deficits, persisting cognitive deficits and worsened structural damage as shown with MRI.

These results together suggest that the post concussive monitoring of patients with MRI may be a more accurate method of assessing recovery than current clinical methods.

For more details about the project, or access to imaging facilities at Florey, please contact David at david.wright@florey.edu.au, or visit NIF at www.anif.org.au.

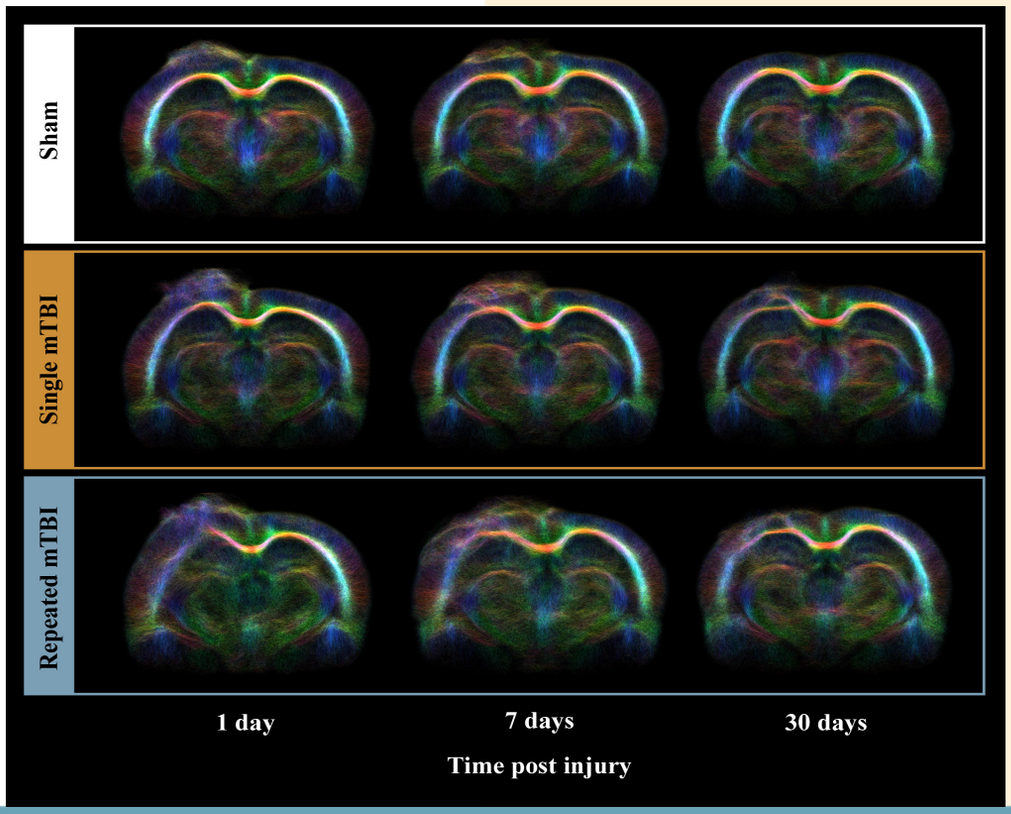

Figure 1: *Tractography images at days 1, 7 and 30 days post injury. Individual tractography images were warped to the groups template image and the total streamlines for each combined.*

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NIF Focus Story - 4

UQ Node:

Tracking of Dendritic Cells using Fluorine-19 label by MRI in mouse model

Biomedical science prepares for another revolution with immunotherapies being widely regarded as the next pillar of medicine. The success of cell based immunotherapies, such as dendritic cell (DC) therapies will require answers to several critical questions including the safety, efficacy, biodistrubution, metabolism kinetics and persistence of the administered cells. Although there is an international effort to address all of these questions, they remain major challenges for the translation of any new cellular therapy. On the regulatory side, these questions relate not only to traditional measures of drug safety and efficacy but also to the fundamentally different and complex characteristics of cellular therapies, such as expansion and persistence following infusion and the therapeutic kinetics of the different cell based therapies. Frequently the function of cellular therapies are characterised and measured by metabolic tests and histological examinations which provides valuable information with regards to the function of transplanted cells but not the complete picture.

At The Centre for Blood Cell Therapies (CBCT) at The Peter MacCallum Cancer Centre (Peter Mac) in Melbourne, clinicians and researchers, A/Prof Simon Harrison, Dr Dominic Wall, Ms Noelene Bergen and Dr Jyoti Arora, engage in cell tracking to try and further understand the fate of cellular therapies following administration. The aim of human in vivo imaging (or tracking) of transplanted cells is to collect non-invasive, real-time, serial, spatial and quantitative measures of cell migration, expansion kinetics and persistence using inert intracellular labels. Although this information is not currently a regulatory requirement for the approval of new cell and tissue therapies, these techniques can provide invaluable in vivo, post infusion data.

About Centre for Blood Cell Therapies (CBCT):

CBCT is located at Peter Mac and has a fully cGMP compliant facility. It has an aim of incorporating imaging into the assessment of cellular therapies. For more information about CBCT and their current projects, please go to http://petermac.org/research/enabling-research/blood-cell-therapies.

In a collaborative effort with imaging scientists at the NIF University of Queensland Node, they share experience using a commercial fluorine-19 (^{19}F) label to track DC by MRI in a mouse model. In their previous studies the feasibility and limitations of tracking using nuclear scintigraphy (^{111}In-oxine) and PET (^{18}F-FDG or ^{64}Cu-PTSM) were shown. In the current study they have demonstrated that labelling with ^{19}F is feasible and can be imaged on a clinically applicable MRI platform. The images below (unpublished data) demonstrate that MRI can detect DC migration to the draining popliteal lymph node at 24 hours post administration. The results of this study form the basis of the plan to develop this technology for use in future clinical trials in cellular therapies. The CBCT group collaborates within various departments at Peter Mac and externally with UQ and various biotechnology groups. This study was supported by Prima Biomed, Siemens Australia and National Imaging Facility (Subsidised Access Program).

Figure 1: *^{19}F DC tracking in mice using 3T clinical scanner. (i) ^{1}H image of mouse with ^{19}F reference on the left and gel capsule marker at the tip of the right foot pad, (ii) ^{19}F image of mouse showing 19F reference and signal from right foot of mouse extending or migrating medially, (iii) composite ^{19}F and ^{1}H image of mouse indicating tracking to the draining lymph node.*

For more info on cell tracking at CBCT or collaborative opportunities please contact Dr Jyoti Arora, Jyoti.arora@petermac.org, or, Dr Dominic Wall, Dominic.wall@petermac.org.

For access to imaging facilities at UQ Node, please contact Dr Gary Cowin at gary.cowin@cai.uq.edu.au.

NIF Focus Story - 5

UWS Node:

Brain Structure and Volume Variation of Australian Agamid Lizards under Constrating Natural and Sexual Selection

Australian National University PhD student Daniel Hoops is accessing the University of Western Sydney (UWS) NIF node to investigate how sociality, sexual selection and natural selection act on brain structure using Australian agamid lizards. Daniel is supervised by Prof. Scott Keogh at the ANU and Prof. Martin Whiting at Macquarie University. Using the 11.7 T MRI located at UWS Campbelltown Campus, UWS Facility Fellow Tim Stait-Gardner and UWS PhD student Yanurita Dwihapsari are scanning over 280 lizard brains with 100 µm isotropic voxel resolution.

Figure 2. *A typical slice of a 100 um isotropic resolution scan. The sequence used is a 3D gradient echo with TR = 40 ms and TE = 8 ms, FOV = 11 mm × 11 mm × 16 mm and with total scan time just under 12 minutes. Taking brains in and out means about four brains can be scanned an hour.*

Figure 1. *An ochre dragon, one of the fourteen species of Australian agmid lizards used in this study. Photo by Angus Kennedy.*

Daniel, Tim, and Yanurita are also scanning 10 brains at 50 µm isotropic voxel resolution to create the first ever 3D lizard brain atlas. Jeremy Ullmann from the Centre for Advanced Imaging at the University of Queensland (UQ), using software written by UQ NIF Informatics Fellow Andrew Janke, has been able to create an average brain, which, once segmented, will form the Atlas. This project illustrates the strength of NIF, drawing on equipment and expertise across nodes.

Australian agamid lizards were chosen for this study because of their variation in natural and sexual selection and because they have been well characterised leaving ample ecological data to investigate for correlations with the MRI data. This is made relatively straight-forward by the well-resolved Australian agamid phylogeny, enabling phylogenetically controlled comparisons. There is a wealth of ecological data to analyse with the brain data. Fourteen species have been selected for investigation, chosen specifically for contrasts in their behaviour, ecology and mating strategies. The brains of ten males and ten females from each species (total 280 brains) will be compared and differences in overall brain size and a variety of lizard brain regions such as the cortex, limbic region and cerebellum.

Given the number of brains that need to be scanned for this project a high-throughput technique was developed at the UWS node of NIF in collaboration with PhD student Ben Moroney. This technique enabled an average of four brains to be scanned per hour. So far well over two hundred brains have been scanned and the high-resolution scans for the atlas are complete. The average volumes and surface-based morphometries of the brains, telecephalons, and cortical subregions of each species will be used for statistical analysis.

The imaging component of this project is partially funded through NIF's Subsidised Access Program.

For more information about the project please contact Prof. Scott Keogh, scott.keogh@anu.edu.au, and Daniel Hoops, daniel.hoops@anu.edu.au. For access to the imaging facility, please contact Dr Tim Stait-Gardner, t.stait-gardner@uws.edu.au.

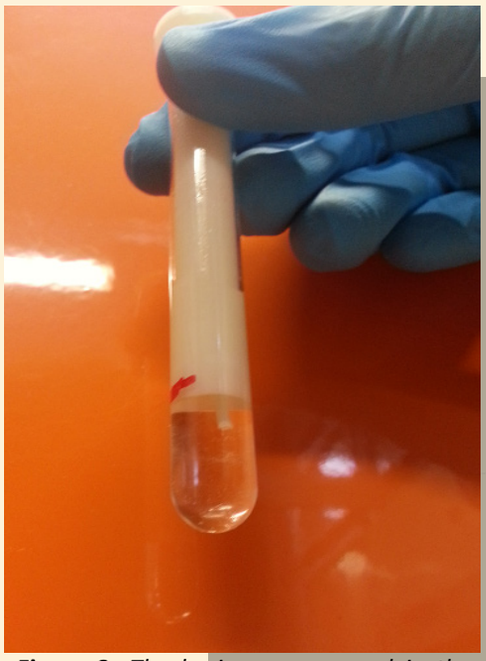

Figure 3. *The brains are scanned in the fluoridated oil Fomblin in which they are buoyant. They are held in place by a sample holder designed by Ben Moroney and printed on a 3-D printer.*

Projects at UWS Node:

The NIF UWS Node houses the 7 T Small Animal scanner and 11.7 T MRI scanner at the Hawkesbury and Campbelltown campuses, respectively. Provisions exist for conducting imaging of small animals under anaesthesia in a specialised live animal imaging probe with ancillary respiratory monitoring equipment and cardiac gating. In addition to ^{1}H, the node is also able to conduct imaging using heteronuclie such as ^{13}C, ^{19}F, ^{23}Na, and ^{31}P.

Most recent projects at the UWS node include: "Mice models of preeclampsia" with Prof. Annemarie Hennessy and Gabriele Bobek; "Starch Gelatinisation in rice kernel during drying in a fluidized bed" with Dr George Srzednicki.

A little friend joining the NIF party at UWS node, posing in front of 11.7 T MRI Bruker Avance II 500 MHz Spectrometer.

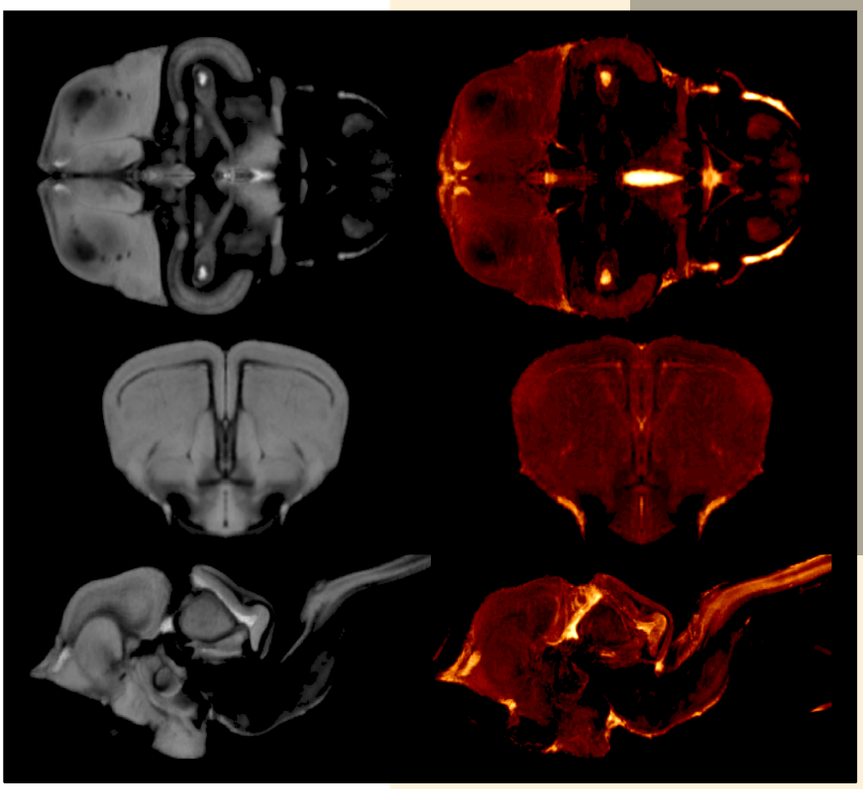

Figure 4. *Three orthogonal slices from the tree dimensional atlas dataset scanned at NIF-UWS node and processed at NIF-UQ node.*

NIF News

Congratulations NIFers! NHMRC and ARC Grant Success

As announced in late October 2013, a number of NIFers have been successful in this year's NHMRC and ARC funding rounds. Congratulations to the following Nodes!

NHMRC 2013:

• 'Improving our Understanding of hearing Voices', Prof. Susan Rossell, Dr Matthew Hughes, Dr William Woods, Emeritus Prof. Patricia Michie, and Dr Neil Thomas ($446,093); combined MEG and fMRI project (Swinburne Node).

• 'Development of folding in the fetal cerebral cortex, identifying fundamental mechanisms and their susceptibility to disruption in normal and abnormal pregnancy', Prof. David Walker, Dr Joanne Britto, A.Prof. Leigh Johnston, Dr Mary Tolcos, Prof. Gary Egan ($588,568; UMelb/Monash Node).

• Practitioner Fellowship – Prof. Graeme Jackson ($542,217; Florey Node).

ARC LIEF 2013:

• 'Ultra-high resolution magnetic resonance imaging (MRI) system for physical applications', Prof. Bill S Price et al; ($1,064,000; UWS Node).

Australia's First 7 T Human MRI

After long long long anticipation, the first in Australia 7 T Human MRI is here, installed at NIF-UQ! Final installation/Commissioning will be early 2014, follow NIF on Twitter for latest updates! So this is basically how it went:

2013 ANZMAG and APNMR Brisbane Covention Centre

This year's combined conference of 5th Asia-Pacific NMR Symposium (APNMR5) and 9th Australian & New Zealand Society for Magnetic Resonance (ANZMAG) was held in Brisbane Convention Centre (27th - 30th October), and the event was great success!

The Imaging Workshop - *New Approaches in MRI*, was one of the greatest features of the conference. Chaired by NIF Director Prof. Graham Galloway, the workshop had attacted 55+ attendees, and was very well represented by NIF members accross all nodes. Emphasising the broad applicability and progress imaging technologies have achieved, the workshop showcased a wide range of projects where imaging techniques can support both research and clinical settings, and also the challenges needed to overcome.

A number of NIF Node Directors are also involved in the organising committee of ANZMAG and APNMR - Thank you to the organisers the event was a great success and we look forward to next ANZMAG/APNMR!

Missed the event? See next page for photos of all actions at the Imaging Workshop. Or http://apnmr2013.org/ for conference abstracts.

CMCA turns 50!

The host of NIF's University of Western Australia Node, Centre for Microscopy, Characterisation and Analysis is hosing their 50th Anniversary Friday, 6th December, 2013.

Although UWA had only joint NIF in 2012, it is one of the most exciting nodes with a new imaging laboratory currently constructing at the QEII campus (due early 2014), which will house a NIF flagship 9.4T MRI scanner, and will be made available to all researchers. For more info about CMCA and facilities available at the NIF-UWA Node, please go to http://www.cmca.uwa.edu.au; or the NIF website.

Big "Thank You!" to all those who have contributed to all issues of NIF Quarterly in 2013. NIF is always 'nosey' about any updates at your node and new research achievements made by our researchers and users community. NIF is proud to broadcast our news - let us know by emailing communications@anif.org.au, or tweet us if you are cool!

Have a well-deserved break and safe holiday over Christmas, look forward to hear from you in 2014. For updates on NIF - FOLLOW US ON TWITTER @NIFAus

NIF Nodes:

- University of Queensland
- University of Western Australia
- University of New South Wales
- University of Sydney / ANSTO
- University of Western Sydney
- University of Melbourne
- Monash University
- Florey Institute of Neuroscience and Mental Health
- Swinburne University of Technology
- Large Animal Research & Imaging Facility

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