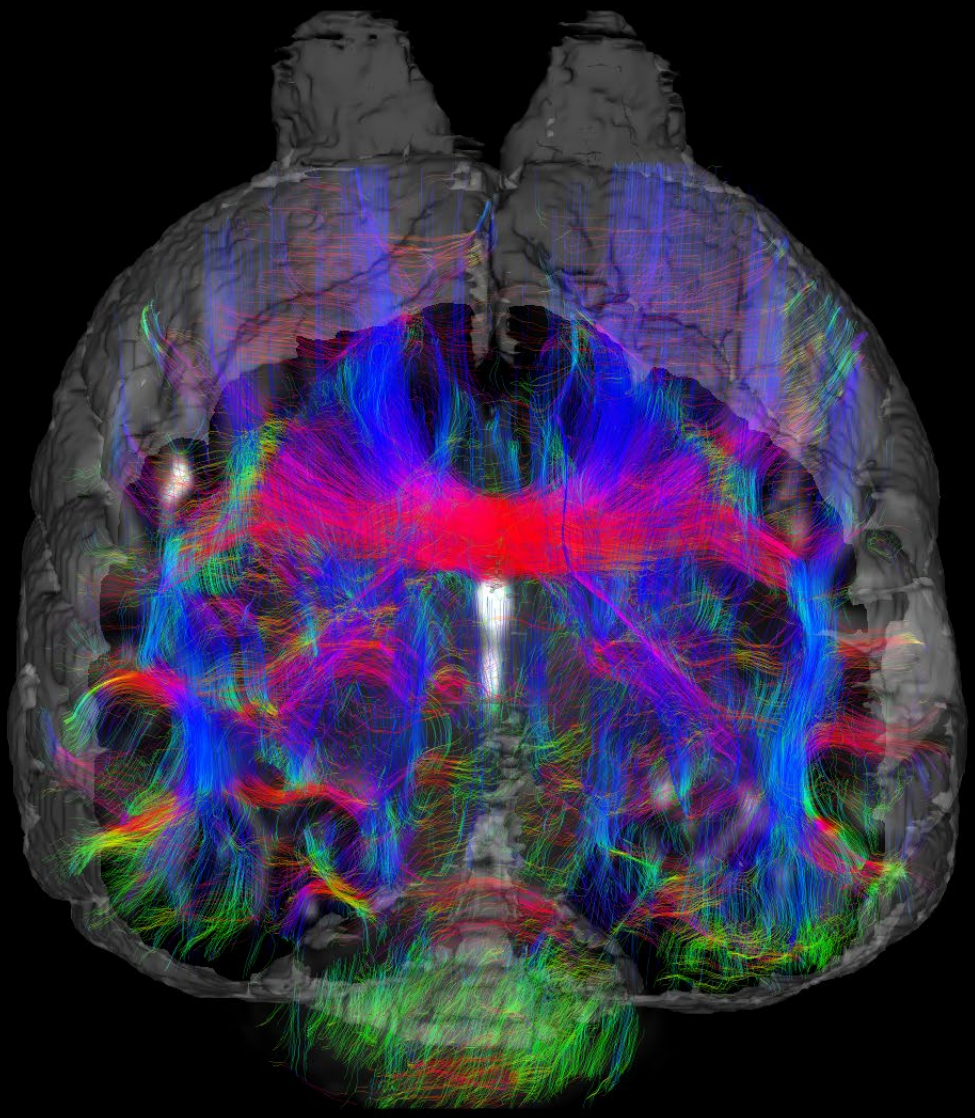
National Imaging Facility

National Imaging Facility Quarterly Newsletter Issue One 2017



Whole brain neuronal connectivity pattern (connectome) of the short-beaked echidna (spiny anteater) as revealed by Diffusion Tensor Imaging and fibre tracking

Image courtesy of Dr. Andre Bongers - Mark Wainwright Analytical Centre, Biological Resourcas Imaging Laboratory, The University of New South Wales

Director's Message

In This Issue

We are privileged to be able to share with you great research stories in every issue of our newsletter. This issue is no exception. Stories that describe research that use imaging to reveal new knowledge for which there is no alternative. Whether it be conservation or preservation, developing new therapies, or guiding new treatments, imaging has a role.

The Tasmanian Tiger, almost brought back to life. Who would have thought the latest in imaging technology could be used to learn about brain morphology and connectivity in an extinct species. The curators at the Australian Museum, were not about to allow a valuable sample to be dissected for conventional histology. So the team at the University of New South Wales node of NIF, non-invasively looked inside the brain.

Equally valuable is the brain of individuals and the varied functions. I am sure that all of us would appreciate that the good parts of the brain are preserved, during surgery to remove lesions that are interfering with proper brain function. That is what is being done at The Florey node of NIF. Preserving language, while treating epilepsy. Talking of epilepsy, the Queensland node of

NIF has been imaging the zebra-fish, building probabilistic models of the brain, bound to make important contributions to the development precision medicine to treat conditions affected by brain connectivity.

If you are not into brains, how about what is happening at the NIF node at the Western Sydney University. They are using brain imaging techniques, to image colon cancer, and provide important information for use in radiotherapy.

The use of imaging in research is only limited by your imagination. So, let your imagination go wild, think of the most wayout idea of how imaging can help your research, and then come and talk to the experts across the nation. Or just read about the great things that are being done to advance knowledge in many disciplines.

The use of imaging in research is only limited by your imagination.

Professor Graham Galloway
Director of Operations

Elucidating brain structure and connectivity in extinct and endangered Australian animals

Australasia has a unique population of native mammals and birds. The monotremes (platypus and echidna) are found nowhere else in the world and our variety of marsupials reflects a remarkable evolutionary radiation that has produced many exceptional forms. Studying the way that brain structure has evolved in Australian mammals can teach many valuable lessons about the way that genes and developmental mechanisms act in concert to meet the requirements of an ecological niche.

At the current stage, knowledge about brain connectivity in many of these interesting Australian endemic species is very limited as they are often rare and endangered or even extinct. Ethical and technical constraints severely limit the options for brain researchers to access live animals or to use classical (usually destructive) anatomical methods on precious brain samples.

A very promising approach to non-invasively collect missing information about the connectome of these evolutionary significant mammals is MRI and Diffusion Tensor Imaging (DTI) in preserved brain samples. Structural MRI yields strong contrast in soft tissues and is able to deliver high resolution information about brain regions. DTI measures the anisotropy of water diffusion in the brain and uses this a probe to quantify brain fibre structure. These datasets can be used as the basis for fibre tracking algorithms to reconstruct the structure of the white matter brain fibres and thus non-destructively elucidate connectivity between significant brain regions.

Brain tissues of rare and endangered animals held in museum collections can provide a very rich source of information when modern imaging methods such as DTI and fibre tracking methods are used to map connections between the cerebral cortex and deeper brain structures. This gives us valuable data on patterns of connectivity within the brain and allows direct comparisons with the brains of placental mammals that are regularly studied by neuroscientists.

This exciting new approach to studying brain evolution in rare or extinct animals is being carried out by an international team of collaborators at the University of New South Wales node of National Imaging Facility, using the preserved brain collection at the Australian Museum in Sydney.

Using the high field pre-clinical MRI system at UNSW's Biological Resources and Imaging Laboratory (Mark Wainwright Analytical Centre) the group studies and analyses the brain structure and connectome of a great diversity of Australian animals. Among them are the endangered endemic monotremes (echidna and platypus), rare marsupials such as the bilby or numbat as well as animals that are already extinct such as the thylacine or "Tasmanian Tiger". The project will provide a unique database of brain structure for Australia's exceptional wildlife that will be invaluable to scientists from around the world.

For more information on this project, contact Dr. Andre Bongers (andre.bongers@unsw.edu.au) or Prof. Ken Ashwell (k.ashwell@unsw.edu.au).

Collaborators

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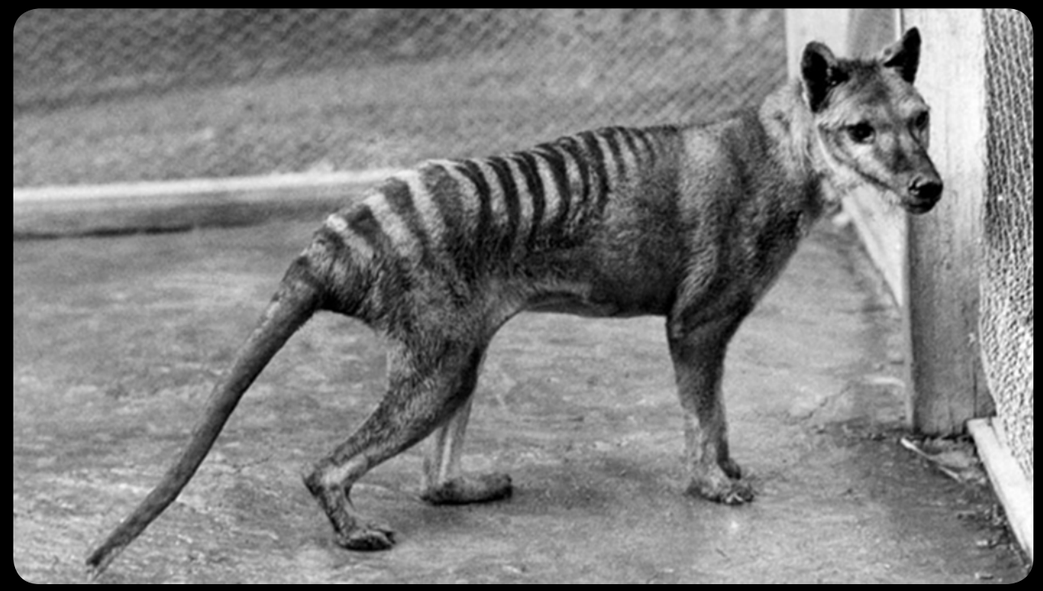
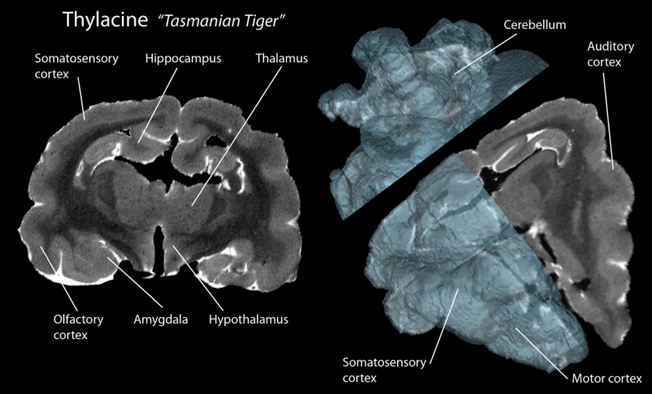
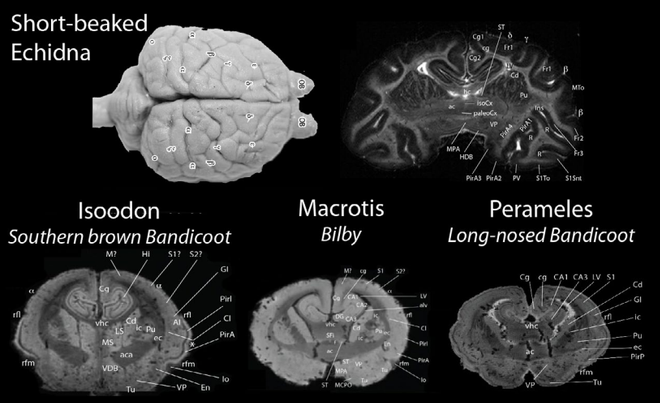




Coronal T2w MRI slice and 3D volume representation of a brain of the extinct the Thylacine (Tasmanian "Tiger").



Cortical mappings on representative MRI slices of long-term preserved brain samples of rare or endangered Australian animals. Upper Row: Brain sample of a short-beaked echidna (Spiny anteater). Lower Row: Three evolutionary related marsupials. Left: Southern brown bandicoot (Isoodon), Middle: Bilby (Macrotis), Right: Long-nosed bandicoot (Perameles)

Case Study: Brain MRI in the Extinct "Tasmanian Tiger" (Thylacine)

The thylacine "Tasmanian Tiger" was once common across Australia. It vanished from the mainland several thousand years ago, but persisted in Tasmania until the early 20th century. A government bounty scheme for hunters from 1830–1914 finally drove it extinct there. Most reports say the last thylacine died in captivity in Hobart Zoo in 1936, but it may have survived in the wild until the 1940s.

With such few data from living animals, scientists have turned to the anatomy of museum specimens to make educated guesses about their behaviour. Though the thylacine had a stronger bite force than the dingo, the anatomy of its head and neck may have meant it was less suited to taking large prey. Its elbow joint suggests it was more of an ambush than pursuit predator, and an analysis of its teeth hints it was a 'pounce-pursuit' predator that hunted preys in 1kg–5kg range. In an attempt to better understand what thylacine was capable of, the team of collaborators scanned two century-old brains. Although MR Imaging of museum samples is a challenging endeavour (due to long preservation times), the team of researchers at the UNSW node of National Imaging Facility could successfully collect precious brain imaging data of this extinct species. The high resolution MRI and DTI studies revealed the relative complexity of the thylacine's brain regions devoted to planning and decision-making, which would be consistent with a predatory ecological niche.

Berns GS, Ashwell KW. Reconstruction of the cortical maps of the Tasmanian Tiger and comparison to the Tasmanian devil. PLoS One. 2017 Jan 18;12(1):e0168993.
Cherupalli S, Hardman, CD, Bongers A, Ashwell KW. Magnetic resonance imaging of the brain of a monotreme, the short-beaked echidna (Tachyglossus aculeatus). Brain, Behavior and Evolution, (Submitted)

Assessing regional lateralisation of language function in the human brain

Research Project

When human brain surgery is necessary to remove a lesion, it is critical to preserve language function. But how do we know which regions of your brain are responsible for language? NIF Informatics fellow A/Prof. David Abbott has recently summarised the complexity of the human language system, and how we can map it, in an article for The Conversation[1]. Working together with others from the Florey node of National Imaging Facility including Dr. Chris Tailby and Prof. Graeme Jackson, David has developed improved neuroimaging methods for quantitative assessment of language laterality. In a study just published in the journal NeuroImage: Clinical[2], they reveal the potential variability in lateralisation across different brain regions within an individual. Here, we look at the critical importance of advancing quantitative analysis methods for functional magnetic resonance imaging (fMRI).

Most of the brain activity involved in your language function is likely occurring in the left side of your brain, however some people use a mix of both sides, and, rarely, some have right dominance for language. How do we know this? Before the era of advanced medical imaging, most of our knowledge came from observation of unfortunate patients with injuries to particular parts of their brain. One could relate the approximate region of damage to their particular symptoms. Language function was mapped to several brain regions by studies undertaken by anatomists Paul Broca, Carl Wernicke and others in the second half of the 19th century and Norman Geschwind and others in the mid-20th century.

Weak electrical stimulation of the brain while a patient is awake (undertaken for example in some patients undergoing surgery for epilepsy) can also be used to cause temporary deficits. In the mid- 20th century this helped neurosurgeons including Wilder Penfield to determine functions disturbed by stimulation to particular brain regions. Some of Penfield's observations shed more light on which side of the brain is most involved in language function. Broca's work had suggested language function arose from the left of the brain, and indeed Penfield observed this in most people he studied (including left and right-handers). However in some he observed that language function could be largely on the right side of the brain.

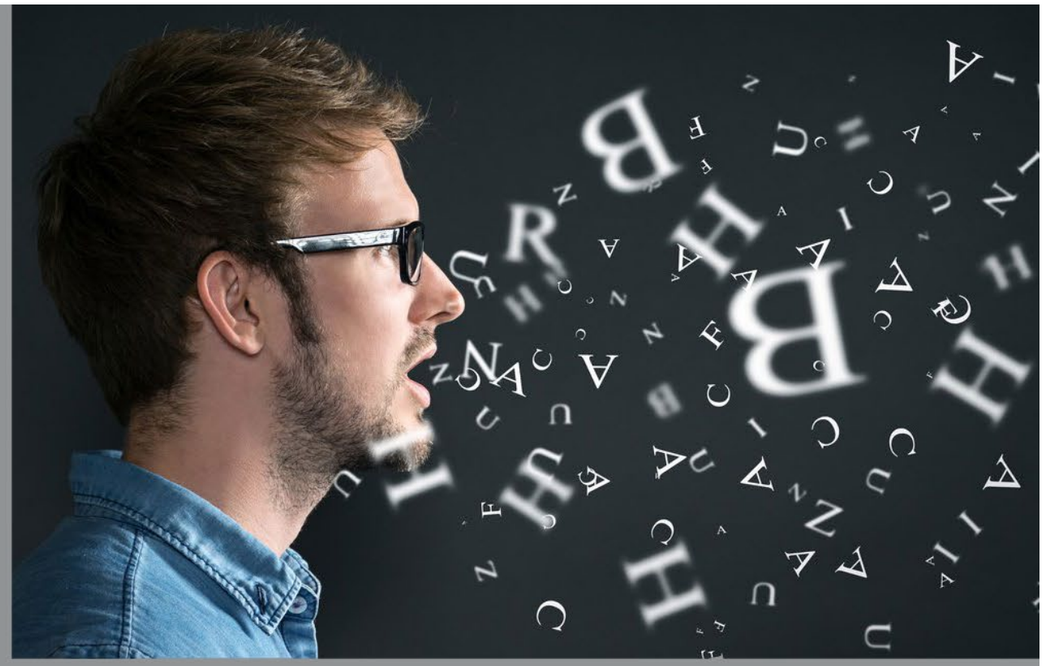
Presurgical language mapping can also be undertaken with a technique that involves anaesthetising one side of your brain. Invented in the mid- 20th century, this test is known as the Wada test, named after Juhn Wada who first described it. The anaesthetic is administered through a catheter inserted into one of the main arteries leading to one side of the brain.

Nowadays we can obtain a much better view of brain function by using harmless brain imaging techniques, especially functional magnetic resonance imaging (fMRI). The fMRI signal changes depending upon whether blood is carrying oxygen (oxygenated haemoglobin, which is diamagnetic so slightly reduces any applied magnetic field) or has delivered up its oxygen (deoxygenated haemoglobin, which is paramagnetic so slightly increases any applied magnetic field). Changes in this signal closely follow local neuronal activity (i.e. brain function).

Neuroimaging has revealed that much more of our brain is involved than previously thought. We now know that there are numerous regions in every major lobe of the brain (frontal, parietal, occipital and temporal lobes, and the cerebellum) that are involved in our ability to produce and comprehend language.

Despite this knowledge, "Which is the dominant hemisphere?" is a question that arises frequently in patients considered for neurosurgery. The concept of the dominant hemisphere implies uniformity of language lateralisation throughout the brain. However, it is increasingly recognised that this is not necessarily the case in a healthy brain, and it is especially not so in neurological diseases such as epilepsy.

Therefore a method was developed by David and his collaborators to objectively quantitate laterality of language. The method permits measurement of the laterality of function in various sub-lobar cortical, subcortical and cerebellar regions of interest. Robust (reliable & reproducible) quantitative determination of language laterality is non-trivial due to the inherently low signal to noise ratio of fMRI, and confounding signals that can arise from subject motion and other physiological noise sources.



Variance in subject performance can also influence activation on fMRI. The method improves robustness by evaluating laterality over a wide range of statistical thresholds. The method was utilised to investigate regional lateralisation of language activation in 30 subjects: 12 healthy controls and 18 focal epilepsy patients. Three different block design language fMRI paradigms were studied in each subject, to tap different aspects of language processing. This was done to determine which of the three tasks was most sensitive to laterality in each region, and how the quantity of data collected affected the ability to robustly estimate laterality across these regions.

In healthy subjects, it was found that lateralisation was stronger, and the variance across individuals smaller, in cortical regions, particularly in the Inferior Frontal (Broca) region. Lateralisation within temporal regions was dependent on the nature of the language task employed, highlighting the need to carefully consider task selection with respect to the particular aims of a study. Employing more than one task may be advisable.

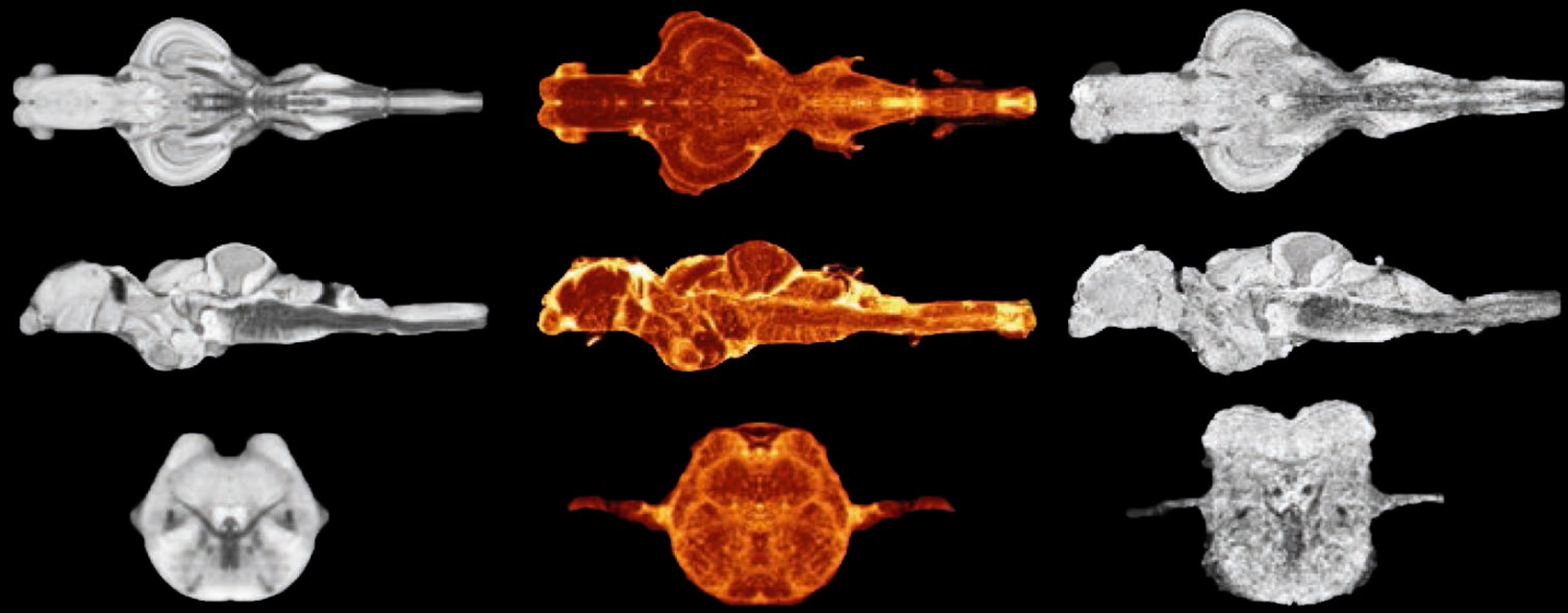
One of the healthy controls was left lateralised anteriorly and right lateralised posteriorly. Departures from normality occurred in ~15-50% of focal epilepsy patients across the different regions, with atypicality most common in the Lateral Temporal (Wernicke) region. Across tasks and regions the absolute magnitude of the laterality estimate increased and its across participant variance decreased as more cycles of task and rest were included, stabilising at ~4 cycles (~4 minutes of data collection).

This work highlights the importance of considering language as a complex task where lateralisation varies at the sub-hemispheric scale. This is especially important for pre-surgical planning of focal resections where the concept of 'hemispheric dominance' may be misleading. The presented method is a precision medicine approach that enables objective evaluation of language dominance within specific brain regions and can reveal surprising or unexpected anomalies that may be clinically important for individual cases.

For more information on this project, contact A/Prof. David Abbott (david.abbott@florey.edu.au).

1. Abbott D. What brain regions control our language? And how do we know this? [Internet]. The Conversation. [cited 2017 Mar 6]. Available from: http://theconversation.com/what-brain-regions-control-our-language-and-how-do-we-know-this-63318
2. Tailby C, Abbott DF, Jackson GD. The diminishing dominance of the dominant hemisphere: Language fMRI in focal epilepsy. NeuroImage: Clinical. 2017;14:141–50.

Neuroimaging Phenotypes in Zebrafish



Comparison of resolution and contrast achieved between a minimum deformation model (a) and a single brain data set (c). The minimum deformation model minimizes individual differences and only exhibits structures present throughout the population. (b) Standard deviation map with areas in yellow highly variable between individual brains and areas in red very consistent.

Zebrafish has become an established model in neuroscience due to the ease with which gene discovery, chemical screening, behaviour, and disease modelling can be performed. More recently, neuroimaging, a crucial pre-clinical technique for probing tissue structure, examining volumetric changes, and studying in vivo brain activity has also been applied to zebrafish. Neuroimaging plays a crucial role in phenotyping research and in studies of neurological diseases. By examining the neuroanatomy of animal models, morphological abnormalities can be identified and correlations made with behaviour. Magnetic Resonance Imaging (MRI) and Diffusion Weighted Imaging (DWI) are frequently used pre-clinically to identify morphological phenotypes in knockout models of neurological diseases. The zebrafish brain is particularly attractive for neuroimaging due to its small size, numerous translucent strains, and distinct forebrain organization.

In a book chapter[1], published by Dr. Jeremy Ullmann and Dr. Andrew Janke, the Informatics Fellow at The University of Queensland node of National Imaging Facility, a range of imaging techniques that have been utilized to examine the zebrafish brain are discussed. Among these are MRI, DWI, Optical projection tomography (OPT), Optical Imaging, and Electron Microscopy. While many of these methods have only begun to be utilized in zebrafish, correlating neuroimaging phenotypes with behaviour in zebrafish has a bright future.

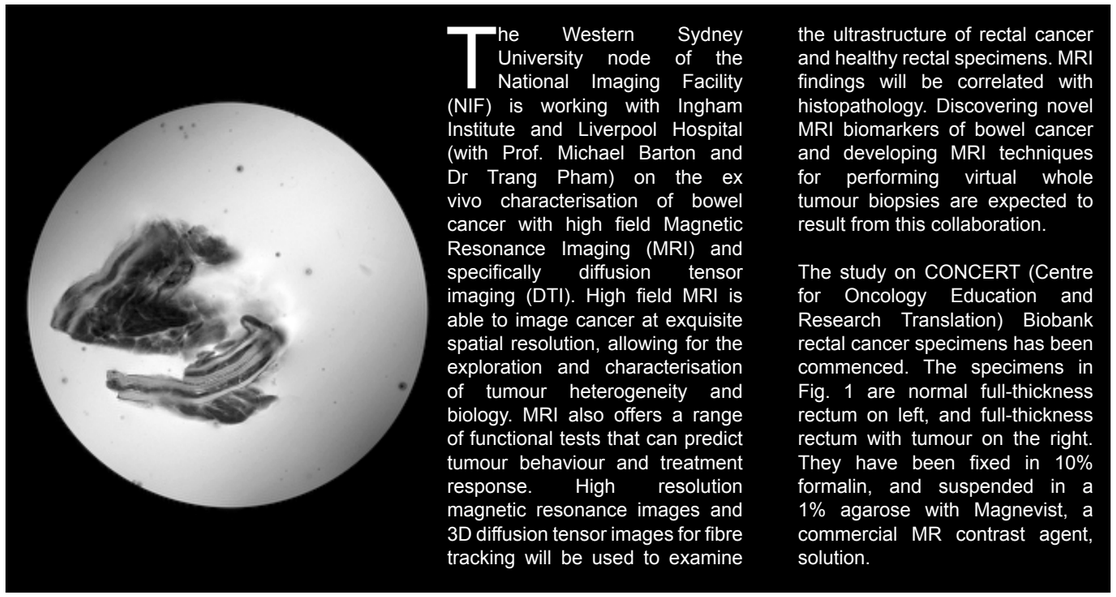
MRI visualizes the anatomy of the brain by exploiting differences in the relaxation values of various microstructures. By altering repetition times and echo times contrast can be optimized and different neuroanatomical structures visualized. MR imaging was initially developed for human brain but subsequent improvements in coil design and magnetic field strength have enabled a range of species including fish to be imaged. MRI permits the acquisition of in-vivo three-dimensional volumes of the whole brain eliminating the need for tedious sectioning. By imaging the whole brain many histological artifacts such as shrinkage, tearing, and variations in labeling are minimized and instead ‘re-slicing’ of the data in any arbitrary orientation is possible. In zebrafish, MRI was first used in to visualize the entire anatomy of the adult zebrafish, where ex vivo MRI was performed on a 9.4 T magnet with in vivo experiments performed using a flow-through chamber, resulting in images with an inplane resolution of 78 µm. Other studies were able to obtain higher resolution and good contrast to noise ratios in an in situ preparation by dissecting the brain out of the skull. The concurrent development of zebrafish-specific fixation and incubation protocols with gadolinium-based contrast agents led to the acquisition of 10 μm^3 images and the creation of a singlebrain high-resolution atlas. Although this singlebrain atlas describes many brain regions in the adult zebrafish brain, a probabilistic atlas (figure above) that minimizes individual differences and instead is based upon a large population provides better resolution. The resultant data set would only exhibit structures present throughout the population and generate mean morphometric measures that represent the population.

In general, neuroimaging modalities for larval zebrafish show great promise for phenotyping. These techniques have primarily been used to understand the fundamental workings of the zebrafish brain such as which neurons are responsible for escape behaviour, swimming speed, and swim posture, however similar imaging techniques could be applied to examining the seizure network in epileptic fish, or functional connectivity in autism models. This would be the first time these networks were examined across the entire brain yet still at the single cell scale! When coupled with microfluidics and automated screening platforms, precision medicine at a whole new level becomes possible.

For more information on this work, contact Dr. Jeremy Ullmann (j.ullmann@uq.edu.au).

1. Ullmann, Jeremy FP, and Andrew L. Janke. “Neuroimaging Phenotypes in Zebrafish.” *The rights and wrongs of zebrafish: Behavioral phenotyping of zebrafish*. Springer International Publishing, 2017. 273-289.

Colon Cancer Characterisation



The Western Sydney University node of the National Imaging Facility (NIF) is working with Ingham Institute and Liverpool Hospital (with Prof. Michael Barton and Dr Trang Pham) on the ex vivo characterisation of bowel cancer with high field Magnetic Resonance Imaging (MRI) and specifically diffusion tensor imaging (DTI). High field MRI is able to image cancer at exquisite spatial resolution, allowing for the exploration and characterisation of tumour heterogeneity and biology. MRI also offers a range of functional tests that can predict tumour behaviour and treatment response. High resolution magnetic resonance images and 3D diffusion tensor images for fibre tracking will be used to examine the ultrastructure of rectal cancer and healthy rectal specimens. MRI findings will be correlated with histopathology. Discovering novel MRI biomarkers of bowel cancer and developing MRI techniques for performing virtual whole tumour biopsies are expected to result from this collaboration.

The study on CONCERT (Centre for Oncology Education and Research Translation) Biobank rectal cancer specimens has been commenced. The specimens in Fig. 1 are normal full-thickness rectum on left, and full-thickness rectum with tumour on the right. They have been fixed in 10% formalin, and suspended in a 1% agarose with Magnevist, a commercial MR contrast agent, solution.

Fig. 1 Specimens of normal full-thickness rectum on left, and full-thickness rectum with tumour and adjacent normal rectum on the right.

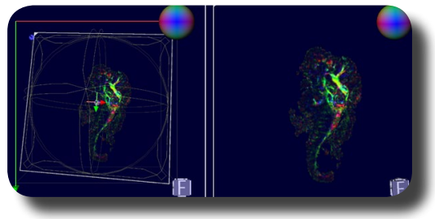


Fig. 2 Slices of DT images of a rectal cancer specimen. The colours correspond to the components of the principle eigenvectors.

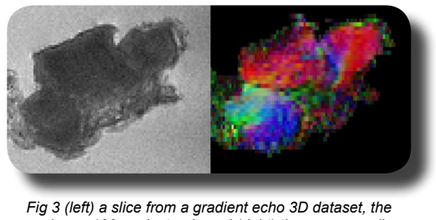


Fig 3 (left) a slice from a gradient echo 3D dataset, the voxels are 100 µm isotropic and (right) the corresponding slice of a diffusion tensor dataset colour coded according to the principle eigenvalue, the voxels are 200 µm isotropic. The colour coding clearly reveals the directions of the bowel muscle fibres.

3D DTI scans with 200 µm isotropic voxels have been obtained on the High Field 11.7 T Bruker Avance MRI located in the Biomedical Magnetic Resonance Facility at the Western Sydney University node of NIF. The rectal cancer sample has a heterogeneous anisotropic structure (Fig. 2 and 3). The different colours correspond to different directions of maximum diffusion as is indicated by the coloured sphere at the top right-hand side of the DT images. Anisotropy of the tissue can be seen very clearly. Diffusion thus provides tissue microstructural information at the cellular level which is not possible on the basis of traditional MRI alone.

The specimens are histologically examined for direct correlation of MR DTI findings with histology. Of the specimens scanned so far MRI-Pathology reveals a correlation between MRI anisotropy, and tumour heterogeneity and fibrosis. The specimens are to be subsequently analysed on the clinical MRI-Simulator (3T), and the Australian MRI-Linac at Liverpool Cancer Therapy Centre to translate high-field findings to low field clinical MRI protocols.

For more information on this project, contact Dr. Tim Stait-Gardner (t.stait-gardner@westernsydney.edu.au).

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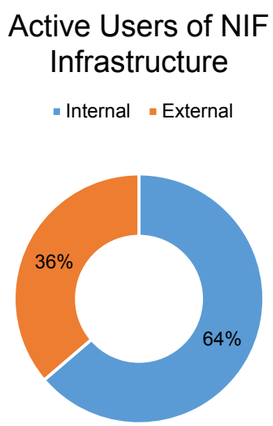
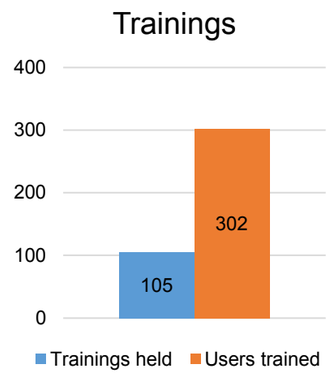
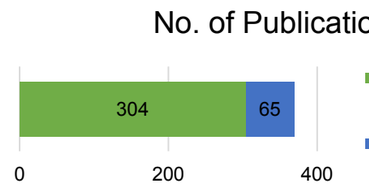
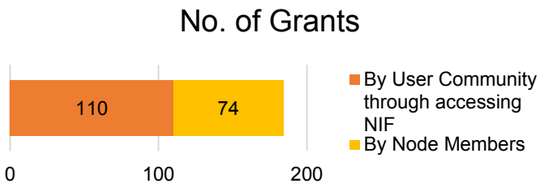
2016 in Review









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