

Ontario Consortium for Small Animal Imaging

Research Highlights 2005-2006

Multi-Coil Magnetic Resonance Imaging of Mice

The pioneering efforts of researchers at the Hospital for Sick Children (SickKids) have won the Mouse Imaging Centre (MICe) international recognition as a leader in MRI mouse imaging. MICe is the only laboratory, internationally, with the ability to image 16 mice simultaneously. Three prestigious journals have featured MICe MR images including *NeuroImage* (Volume 29, January 2006), *Physiological Genomics* (Volume 24, April 2006) and the *Journal of Neuroscience* (Volume 26, April 26, 2006). Most notably, Dr. Mark Henkelman has been appointed a Fellow of the Royal Society of Canada. This appointment recognizes the world-class research excellence embodied in Dr. Henkelman. To date, the MR has been used extensively for imaging mouse brains (350 cases have been studied), for imaging live mice with tumours (several hundred cases have been studied), and for imaging the whole anatomy of the mouse. New sequences (software) for mouse cardiac imaging have been developed and implemented for multiple-mouse imaging. As research efforts expand, significant expertise is gained in specific anatomical areas including cancer (tumours), cardiology, and neurosciences (brain). Future efforts will focus on expanding this unique expertise into other anatomical areas.

During this past year, Willem van Heiningen has been recruited from the University of Waterloo to implement the software for the separate transmit/receiver coils for each mouse. In collaboration with our industrial partner, Varian, significant progress has been made towards the development of a prototype scanner. Upgrades to the current scanner provide a separate "transmit/receiver" for each of the nineteen channels. As each channel is capable of imaging one mouse, the upgrade will allow for simultaneous imaging of 19 mice. Varian, a leading manufacturer of pre-clinical imaging devices, has contributed \$660,000 to this project as well as technical advice. Varian's investment has been invaluable to the project and has helped enable the development of a prototype device. Mr. van Heiningen has contributed significantly to the operationalization of the prototype.

Collaborations with other labs have expanded internationally as well as locally. Internationally, collaborations have begun with Dr. Stuart Berr of the University of Virginia Health System (Charlottesville, VA), Dr. Mariealle DeBiasi of the Baylor College of Medicine (Baylor, TX), Dr. John Morrey of Utah State University (Logan, UT), and Dr. Eugene Yu of the Roswell Park Cancer Centre (Buffalo, NY). Nationally, collaborations have begun with Dr. Sean Egan of the Hospital for Sick Children, Dr. Ross Ethier of the University of Toronto, Dr. Rod McInnes of the Canadian Institutes of Health Research, and Dr. Xiao-Yan Wen of the Ontario Cancer Institute. Three peer-reviewed papers were published this past year in prestigious journals including the *Journal of Magnetic Resonance* and *Magnetic Resonance in Medicine*. Finally, a European patent has been granted for "Concurrent MRI of Multiple Objects".

Non-Invasive Magnetic Resonance Assessment of Small Animal Physiology

As discussed in previous reports, research efforts in this area have focused on the application of newly developed technology for use in studying mouse models of disease. Research efforts in this area have continued over the past year, and progress has been made in imaging inflammation, specifically in regards to multiple sclerosis, spinal cord injury and atherosclerosis. Most notably, refinements in MR technology have enabled researchers at the Robarts Research Institute to image a single cell *in vivo* for the first time. Researchers have applied this technology to cancer metastasis with excellent results. One paper has been published and one paper is in press.

ORDCF funding has also enabled the recruitment of two new postdoctoral fellows specializing in cellular MR imaging and the imaging of transplanted islet cells. Dr. Dan Jirak and Dr. Jan Kriz will begin working in Dr. Paula Foster's lab in August. Both were among the first in their field to publish data. Their expertise will prove invaluable to research efforts at Robarts.

Ultrasound Biomicroscopy of Mice: Technology and Applications Development

Research in this project has propelled VisualSonics through a phase of extremely rapid growth such that the company is now the world's leading provider of high-resolution ultrasound microimaging systems. In early 2006, the company delivered its 200th system, reaching this milestone in just over three years and validating the significant demand for microultrasound technology among biotechnology, pharmaceutical and medical research organizations. Starting with revenues of a few hundred thousand dollars per quarter in 2003, VisualSonics has experienced over 100% compounded growth annually, quickly reaching its current \$24 million in annualized sales. Microultrasound is now the world's fastest growing preclinical imaging technology. The Toronto Star had a full-page analysis of VisualSonics in its business section on March 13, 2006.

The next generation of VisualSonics imaging systems will rely on arrays of finely pitched, very high-frequency transducers. In the past year, the first 256 element linear arrays were produced. Researchers at Sunnybrook Research Institute are finetuning the fabrication process and expect to begin production this year. These devices will allow imaging to be performed at very high frame rates, thereby enabling researchers to obtain pictures of moving organs and bring an entire image (as opposed to a specific area) into focus. Two patents, one on the actual transducer, and one on the imaging system including the critical beamformer, have been applied for. Work on contrast agents has focused on angiogenesis and is leading towards the development of targeted contrast molecular imaging. Towards this end, researchers have developed automated methods to acquire and analyze images of microbubble contrast agents that have been targeted to specific angiogenically active molecules such as the VEGFR2 receptor. These results were reported at the meeting of the American Association for Cancer Research in April of 2006 (Conference abstract 6). Targeted imaging will be one of the critical drivers of success going forward. Collaborations with Dr. Robert Kerbel, a Canada Research Chair and distinguished investigator in angiogenesis at Sunnybrook Research Institute, have continued to examine the effect of different dosing schedules and the impact of new therapeutics on experimental tumour growth. A paper testing the vascular normalization hypothesis was published in *Cancer Research* in April 2006 (Ref 6). It showed that antiangiogenic agents targeting the VEGFR2 receptor caused significant and prolonged depression of vascular function following treatment.

A major technological achievement was made through the development of ultra-high frame-rate imaging based on ECG locked retrospective reconstruction of mouse heart images. This patented method allows visualization of the living heart at frame rates exceeding 1,000 per second and opens the door to detailed biomechanical analysis. The first publication on this technology was reported in *Ultrasound in Medicine and Biology* in 2006 (Ref 7). The commercial version of the product is now available and its use is being actively pursued by numerous international groups.

Computed Tomographic Microscopy for Mouse Imaging

A newly established academic collaboration with Dr. Rob Burgess at the Jackson Labs has led to the identification of a new vascular dementia disease from a mouse at the Jackson Labs. The gene responsible for this disease is currently being identified and results will be published shortly. The use of CTM in identifying the gene was instrumental in this discovery. Finally, a major study focusing on placental vascularization has been completed resulting in the characterization of a mouse model of intrauterine growth restriction (IUGR). Two peer-reviewed papers were published this past year in prestigious journals including *Physics and Biology in Medicine* and the *American Journal of Physiology*.

Dynamic Computed Tomographic (CT) Microscopy

As discussed in last year's report, a dynamic CT scanner prototype was installed at Robarts Research Institute in October 2004. Since its installation, the scanner has been relocated to a newly established preclinical imaging facility at the Robarts Research Institute. The new facility provides better control of temperature and humidity as well as better access to relevant animal handling facilities. Research efforts focused on improving the technical features and capacity of dynamic CT microscopy have continued. Specifically, during this past year researchers have optimised the x-ray procedures for perfusion measurement studies affecting the heart, brain, lung and other mouse tissues. The optimization of x-ray procedures is especially important in image quality (optimisation of signal-to-noise ration) and in reducing the animal's exposure to radiation. In addition to enhancing the technical capabilities of the CT scanner, researchers have explored and identified the optimal contrast agent for use in dynamic perfusion measurements performed with 3-D CT. After considering several different contrast agents, researchers determined that an ionic contrast agent (Hypaque) should be used due to its lower viscosity. Researchers have also developed a protocol for use when injecting small animals with an ionic contrast agent. Two papers have been published describing the successes of this aspect of the project.

Researchers at Robarts Research Institute have also developed a test object and a methodology for the quantitative assessment of image quality in micro-CT imaging using a specially designed phantom and custom software. This approach was presented at the annual meeting for the The International Society for Optical Engineering (SPIE), in San Diego, California. This approach was also presented at the Congress of the Canadian Association of Physicists where it received an award as a finalist in the poster competition. Finally, a US provisional patent application has been filed (June 23, 2006). Discussions are underway with private-sector partners to license this technology.

PET Imaging of Small Animals

A primary objective of this project is to be able to apply modern drug discovery methodologies to the development of new tracers/radiopharmaceuticals. Researchers at McMaster University have successfully developed and validated (*in vitro*) two new molecular imaging agents for small animal and translational research. With these agents in hand, ^{18}F and $^{99\text{m}}\text{Tc}$ -labeled insulin analogues, researchers have begun to evaluate their *in vivo* distribution using small animal PET and SPECT, respectively. The results of this work are being written up as a patent application, and one of the products is moving towards human trials. The success of these research efforts has resulted in expanded collaborations with industrial partners Biotage Inc. and Pfizer.

Researchers also published the development of a revolutionary way to radiolabel and purify compounds (*Journal of the American Chemical Society*-2006). This method can be used to generate libraries of novel radiopharmaceuticals in highly effective specific activity. This work was the subject of a patent application and researchers are currently negotiating a licensing agreement with one of our OCSAI partners. In addition to the two patents filed this year, six papers were published in peer-reviewed publications including the *Canadian Journal of Chemistry*, the *Journal of the American Chemistry Society*, and the *Journal of Medical Chemistry*. Additionally, 19 presentations were made at international conferences and other academic centres including The Society of Nuclear Medicine Annual Meeting (June 2005), and the 16th International Symposium on Radiopharmaceutical Chemistry.

The recruitment of highly qualified personnel (HQP) has been very instrumental in research successes. Three postdoctoral fellows (PDFs) were hired last year, Dr. Andrea Armstrong (University of Calgary-Alberta), Dr. Patrick Causey (Queen's University-Ontario), and Dr. Travis Besanger (McMaster University-Ontario). Two of them (Armstrong and Causey) had their first publications accepted recently. Besanger will be submitting two patent applications shortly. Additionally, Causey and Besanger were instrumental in setting up biology labs for screening novel radiopharmaceuticals (at McMaster University). Their expertise gives OCSAI scientists the ability to design, construct, and screen (both *in vitro* and *in vivo*) new molecular imaging agents. McMaster University has become one of only a few places in Canada that has this capacity.

Three-dimensional Imaging of the Mouse

Capitalizing on last year's efforts, researchers at Robarts Research Institute have completed testing and development of segmentation software used in three-dimensional (3-D) ultrasound for the quantification of tumours in mouse models. The segmentation software has been eagerly anticipated as it allows scientists to measure accurately the size of the tumour as well as track its growth through the measurement of variability. Measurement of variability establishes a benchmark that informs the scheduling of imaging sessions and the interpretation of changes in tumour volume. For scientists conducting longitudinal preclinical studies of mouse models of cancer, this feature is invaluable as it enables efficient study management. The software has been developed for use in 3-D ultrasound, which further enhances its utility. A key feature of 3-D ultrasound technology is its low variability, which significantly improves the ability of scientists to monitor the progression of the tumour. This past year, a study was successfully conducted to validate the methodology used to analyze the volume of tumours from 3D micro-ultrasound images. This study laid the groundwork for further development efforts in also providing inter- and intra-observer variability in determining the volume. The results identified the major sources of variability, allowing researchers to improve semi-automated feature of the volume segmentation software. Researchers are also extending their research efforts to encompass that analysis of segmentation in mouse vessels.

In addition to its scientific successes, this project has experienced several other notable outcomes. Most notably, the segmentation software was successfully licensed to the industrial partner for this project, VisualSonics. The unique relationship between the London and Toronto OCSAI laboratories and VisualSonics demonstrates the success of the OCSAI project in linking industry to science. Secondly, the attraction of Dr. Lyubomir Petrov, a postdoctoral fellow from Finland, significantly enhanced research efforts in this area. Collaborations were expanded within London to include Dr. Jim Xuan, Dr. Alex Lucas, Dr. Arthur Brown and Dr. David Hill. Internationally, collaboration with Dr. Norman Greenberg of the Fred Hutchinson Cancer Research Centre in Seattle, Washington was established. In total, five peer-reviewed papers were published and six presentations were made, including three made at two prestigious international conferences, the IEEE (Institute of Electrical & Electronic Engineers) International Symposium on Biomedical Imaging and the SPIE (The International Society for Optical Engineering) International Symposium on Medical Imaging.

Image Analysis and Informatics for Mouse Imaging

The image analysis project focuses primarily on the development of computer software used to register different anatomical areas of the mouse. The ability to register the anatomies of different mice and compare different features is an essential tool in developing anatomical atlases of different aspects of the mouse. To date, registration methods have been developed and successfully tested for the mouse brain and for whole body anatomical images. A key element of the registration software for processing imaging data, pipeline computer algorithms, is in place and has aided researchers in software development efforts. Further, a complete database identifying the mouse with all its relevant imaging procedures has been developed, implemented and is now used to store all the mouse imaging data. This database will significantly aid further efforts in developing atlases of different mouse anatomies. The recruitment of Dr. Jason Lerch from the Montreal Neurological Institute as a post-doctoral fellow, has aided considerably in research efforts in the systematic processing of three-dimensional imaging data.

In the past year, a collaboration with Dr. Xenios Papademetris of Yale University has been developed to address the issue of limb motion in image registration. As many imaging studies involve live mice, software must be developed to account for the movement of limbs without compromising the registration process. Two peer-reviewed papers were published this past year in journals including *Physiological Genomics* (cover illustration) and *Journal of Neurosciences* (cover illustration).

Mouse Models of Human Disease

Research efforts towards the development of a mouse model that corresponds to a specific human disease have progressed. Ongoing research efforts refine the models already in place and document new disease areas. To date, a number of diseases have been studied including schizophrenia, Alzheimer's disease, Huntington's disease, lupus, Intrauterine Growth Restriction, asthma, cancer, cardiac developmental abnormalities, organ transplantation and kidney disease. Results from studies in each of these areas have been published with additional publications in press.

With regards to the field of vascular development, researchers have made significant improvements in the area of optical projection tomography (OPT). A patent application has been filed on reconstruction methods for OPT. Once granted, the patent will be licensed to Bioptonics for commercialization in the UK. Two peer-reviewed papers were published this past year in prestigious journals including *NeuroImage* (cover illustration).