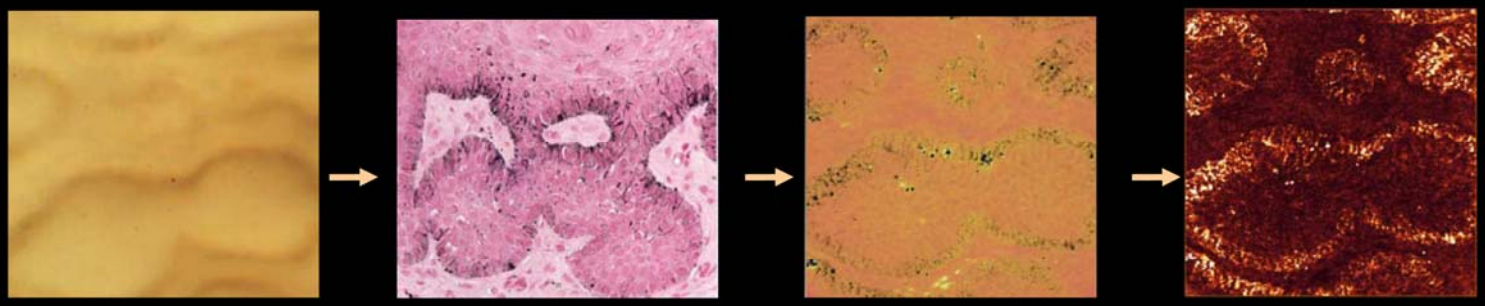

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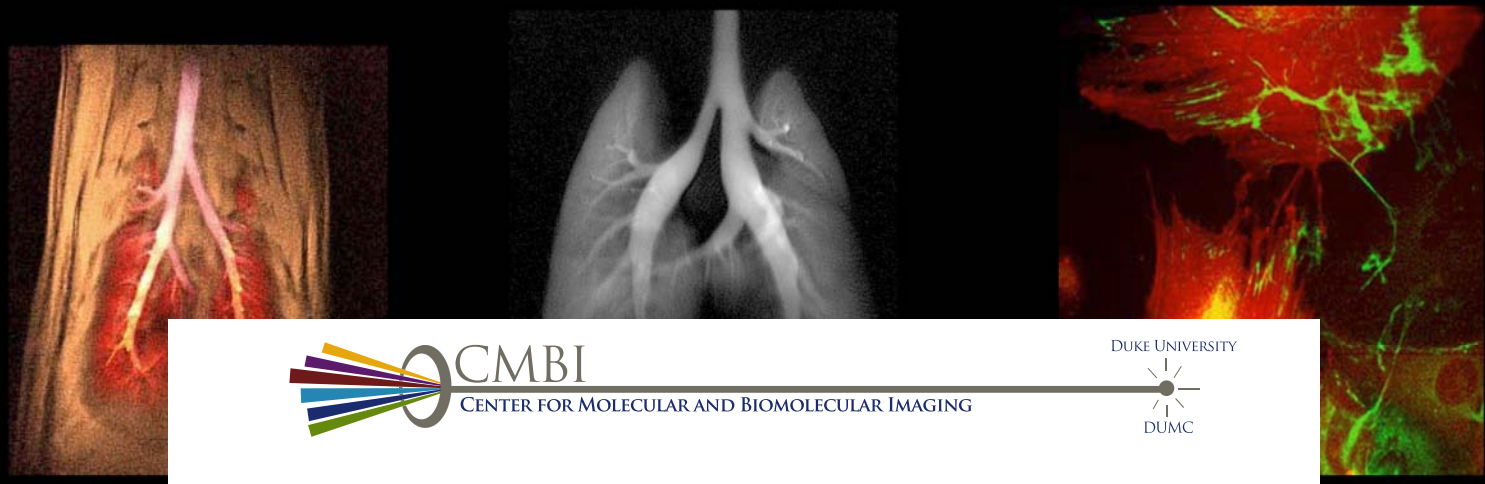
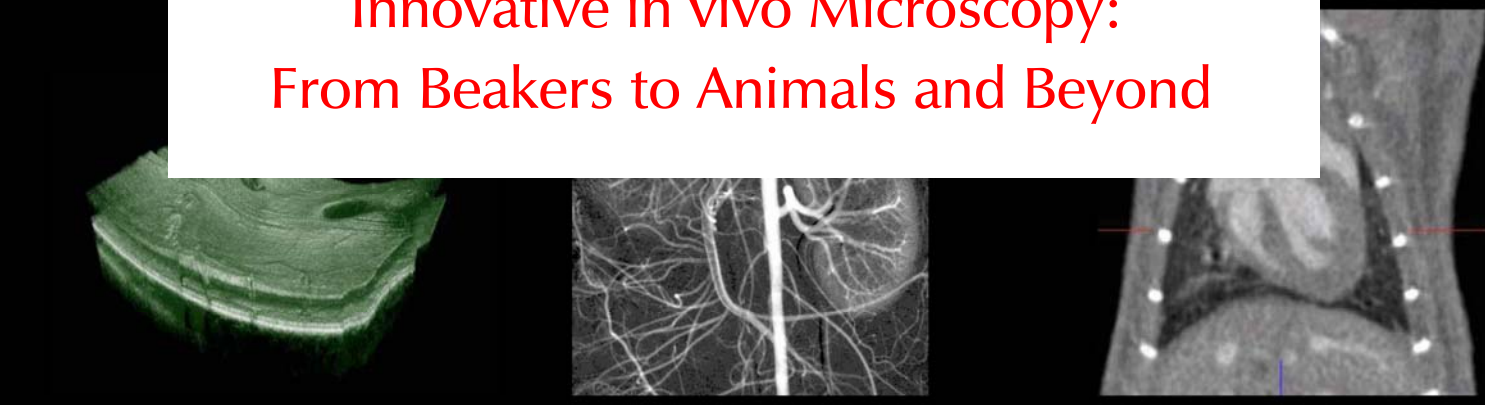
Innovative in vivo Microscopy: From Beakers to Animals and Beyond





French Family Science Center
Duke University
December 18, 2013

Warren S. Warren
James B. Duke Professor of Chemistry, Physics, Radiology, and Biomedical Engineering
Director, Center for Molecular and Biomolecular Imaging

December 18, 2013

Welcome to the Center for Molecular and Biomolecular Imaging 2013 conference, "Innovative in vivo Microscopy: From Beakers to Animals and Beyond." Part of this meeting is a celebration of the renewal of NIH support for the Center for *in vivo* Microscopy. CIVM will enter its 25th year of funding next year under director G. Allan Johnson (our first speaker of the day). CIVM has outstanding capabilities in all core imaging technologies at the preclinical level, with important research programs focusing on understanding animal and human diseases at the microscopic and molecular level. Part of this meeting will also be a celebration of the continued intertwining of disciplines at Duke University. Three new Chemistry professors will be speaking in the afternoon session, and their research has the potential for future collaboration across a wide range of fields including biology, pharmacology, biomedical engineering, radiology, and more.

CMBI is a Provost-level organization which unites several different schools at Duke (interconnecting Trinity College of Arts and Sciences, the Pratt School of Engineering, the Medical School, and the Nicholas School of the Environment) to support the transformative and inherently interdisciplinary nature of modern imaging science. This has a natural connection with one of Duke's greatest strengths, which can best be appreciated on *Google Maps*. If you locate the French Family Science center at 124 Science Drive, and go to the 200 foot scale, you will find all of physics, biology, chemistry, engineering, and computer science, and virtually all of the basic science buildings of the medical school. This extremely unusual proximity can, and does, foster strong connections between departments. It is the aim of this Center to support and strengthen these connections. To this end, our meetings have widely varying themes and foci that we hope will continue to intrigue the best and brightest across the imaging spectrum.

These meetings don't happen spontaneously. I am very grateful for the continuing efforts of Mike Conti (CMBI Manager), for helping to organize the meeting, and to the CMBI steering committee for their thoughts and guidance.

Sincerely,

Warren S. Warren

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

December 18, 2013

French Family Science Center Room 2231

Morning Session

8:00AM	Breakfast – French Family Science Center Atrium	
8:30AM	Al Johnson (Duke University) Director, Center for In Vivo Microscopy	Magnetic Resonance Histology
8:55AM	Chunlei Liu (Duke University) Center for Brain Imaging & Analysis	MRI of Brain and Magnetic Field Interaction
9:20AM	Cristian Badea (Duke University) Center for In Vivo Microscopy	Expanding X-ray CT Towards Functional Imaging
9:45AM	Murali Krishna Cherukuri (NIH) National Cancer Institute	Tumor pO2 and Glycolytic Activity in Pancreatic Cancer Xenografts as Biomarkers to Guide Treatment
10:30AM	Break	
10:55AM	Matthew Merritt (University of Texas, Southwestern) Advanced Imaging Research Center	Hyperpolarized Carbon-13 Studies of Intermediary Metabolism: Gluconeogenesis in the Liver, Compartmentation and Substrate Competition in the Heart
11:40AM	Thomas Theis (Duke University) Center for Molecular and Biomolecular Imaging	Long-lived Hyperpolarized Biomarkers for Molecular Magnetic Resonance Imaging
12:05PM	Bastiaan Driehuys (Duke University) Center for In Vivo Microscopy	Hyperpolarized 129Xe MRI - Introduction, History, and New Developments

12:30PM	Lunch – French Family Science Center Atrium

Afternoon Session

1:50PM	Gang Zheng (University of Toronto) Department of Medical Biophysics	Explore New Frontiers of Molecular Imaging with Porphysome Nanotechnology and Beyond
2:35PM	Steven Malcolmson (Duke University) Department of Chemistry	Identification of Linear Precursors in the Biosynthesis of the Macrocyclic Thiopeptide Antibiotic Berninamycin
3:00PM	Amanda Hargrove (Duke University) Department of Chemistry	Developing Small Molecules to Target Nucleic Acids in Human Disease
3:25PM	Break	
3:45PM	Jennifer Roizen (Duke University) Department of Chemistry	Detecting Fleeting Intermediates in Catalytic C–H Amination Reaction Cycles
4:10PM	Alexandra Badea (Duke University) Center for In Vivo Microscopy	Visual Informatics for Small Animal Imaging – Focus on the Brain!
4:35PM	Martin Fischer (Duke University) Center for Molecular and Biomolecular Imaging	Towards High-Resolution Structural and Chemical Imaging in Entire Mouse Organs

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Magnetic Resonance Histology

G. Allan Johnson, PhD
Director – Center for In Vivo Microscopy
Charles E. Putman Distinguished Professor of Radiology
Professor of Biomedical Engineering and Physics
Duke University

Dr. Johnson is Director of the Center for In Vivo Microscopy, an NIH/NCRR/NIBIB-funded National Biomedical Technology Resource Center (P41 EB0015897), now into its 25th year of funding. His research focuses on magnetic resonance histology, the application of MR microscopy to study tissue architecture.

Abstract:

The closing sentence of Paul Lauterbur's seminal article describing magnetic resonance imaging is " Zeugmatigraphic (imaging) techniques should find many useful applications in studies of the internal structures, states, and compositions of microscopic objects" [1] – an obvious understatement about the impact MRI has had on modern medicine and science. MRI continues to deliver exciting new opportunities for discovery in science and medicine. Magnetic resonance histology, the topic of this presentation is an area in which we have seen dramatic advances over the last few years.

Magnetic resonance histology (MRH), the study of tissue structure- through the use of magnetic resonance imaging was first suggested in 1993 [2]. MRH provides several unique compliments to more traditional optical methods: MRH is nondestructive, is inherently digital and 3 dimensional, and provides particularly fascinating tissue contrast dependent proton sources and their environments in the tissue. This talk will describe the technology that has allowed us to acquire MR images at spatial resolution more than 1 million times that seen in clinical scanners, some of the unique sources of contrast, and some recent applications in genetics and neuroscience.

1. Lauterbur, P.C., *Image formation by induced local interactions - examples employing nuclear magnetic resonance.* Nature, 1973. **242**: p. 190-1.
2. Johnson, G.A., et al., *Histology by magnetic resonance microscopy.* Magnetic Resonance Quarterly, 1993. **9**(1): p. 1-30.

December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Magnetic Resonance Imaging of Brain and Magnetic Field Interaction

Chunlei Liu, PhD
Core Faculty, Duke-UNC Brain Imaging Analysis Center
Assistant Professor of Radiology and Medical Physics
Duke University

Professor Liu is a member of the core faculty at the Duke-UNC Brain Imaging and Analysis Center. His research efforts center on MRI techniques for translational medical applications, particularly *in vivo* imaging of brain white-matter architecture and function.

Abstract:

When the brain is situated in a magnetic field, it creates a small field of its own in response to the presence of the external field. This interaction, though extremely weak, becomes measurable under the strong field provided by MRI scanners. With MRI, this small perturbation field can also be spatially localized and quantified. The strength and direction of the perturbation is influenced by a number of physiologically important factors including molecular composition, cellular organization and neuronal connectivity. By imaging brain magnetic interaction, one may then be able to infer a wealth of information about brain microstructure. Such information include, for example, iron deposit in aging and Parkinson's disease, myelination in brain development, demyelination in multiple sclerosis, and neuronal connectivity. Besides brain, this magnetic interaction is also significant in many other organs including kidney and heart. I will present some recent methodological developments and discuss potential applications.

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Expanding X-ray CT Towards Functional Imaging

Cristian Badea, PhD
Center for In Vivo Micrsocopy
Associate Professor of Radiology
Duke University

Professor Badea's research at CIVM focuses on x-ray-based methods for small animal morphological and functional imaging and 4D tumor imaging using digital subtraction angiography and microCT.

Abstract:

CT imaging is ubiquitous in the clinic. The preclinical equivalent of CT (i.e. micro-CT) is also one of the preferred modalities for small animal imaging. Simple operating procedures, relatively fast scanning protocols, high throughput, plus lower acquisition costs and maintenance, all prove beneficial for micro-CT compared to other imaging modalities. High spatial resolution in micro-CT is a key technical advancement that has allowed researchers to capture increasingly detailed anatomical images in rodents. However, micro-CT does not typically provide functional information, and suffers in general from poor contrast sensitivity.

The overall goal of this talk is to show how to increase the information content delivered by micro-CT beyond anatomy, by developing novel sampling/reconstruction strategies for spectral and dynamic micro-CT. Just as a prism can show that white light consists of a spectrum of colors, since white light is polychromatic, the x-ray beam in a CT system is also polychromatic. Its spectral content can be used to better differentiate between materials. We have demonstrated that by using nanoparticle-based contrast agents and spectral micro-CT methods (via dual or multi-energy scanning), we can increase specificity of detections. On the other hand, dynamic micro-CT imaging methods that we have developed allow us to better characterize functional aspects in cardiac or perfusion studies. And by combining spectral and temporal dimensions in sampling and reconstruction, we demonstrate ways to now further expand the functional information provided by micro-CT to benefit cancer studies.

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Tumor pO2 and Glycolytic Activity in Pancreatic Cancer Xenografts as Biomarkers to Guide Treatment

Murali Krishna Cherukuri, PhD
Head, Biophysical Spectroscopy Section
Center for Cancer Research
National Institutes of Health

Dr. Krishna has been a researcher for the National Cancer Institute for nearly 30 years. He is currently the head of the Radiation Biology Branch's Biophysical Spectroscopy Section. His research interests include studies of free radical processes and developing functional imaging tools to study tumor physiology.

Abstract:

Pancreatic cancer is a malignant neoplasm with an extremely poor prognosis. The 5-year overall survival rate is below 10%. A new drug TH-302 in combination with gemcitabine was approved for Phase 3 clinical trial in locally advanced or metastatic pancreatic adenocarcinoma in December 2012. The TH-302 is a kind of drugs known as hypoxia-activated prodrug (HAP), which activated under hypoxia (low concentration of oxygen) and exhibits anti-tumor effect. A question derived is how clinicians select patients who receive most benefit from treatment with this hypoxia targeting new drug, instead of classical radiation therapy or gemcitabine monotherapy. Electron paramagnetic resonance imaging (EPRI) can non-invasively provide 3D absolute oxygen images. Here, we investigated if the EPR oxygen imaging can predict treatment benefit of oxygen dependent or independent therapies in three different pancreatic cancer xenografts.

Three human pancreatic cancer cells Hs766t, MiaPaca2, and Su8686 were subcutaneously inoculated in hind leg of athymic nude mice. The tumor bearing mice were treated with TH-302 (80mg/kg, ip, 5 days), X-radiation (3Gy, 5 days), or gemcitabine (150mg/kg, ip, twice a week). Tumor oxygen imaging was conducted by a homemade 300 MHz pulsed EPRI scanner using an oxygen sensitive triarylmethyl probe OX063, followed by anatomic MRI scan.

Three pancreatic cancer cell lines showed large difference in tumor oxygenation. Tumor median pO_2 values are 9.1±0.7 mmHg for Hs766t, 11.1±1.0 mmHg for MiaPaca2, and 17.6±1.1 mmHg for Su8686. TH-302 treatment provided survival benefic of 28.6 days in hypoxic Hs766t tumors but only 1.0 days in the most oxygenated Su8686 tumors. In contrast, tumor growth delay by radiotherapy was 10.3 days in Hs766t, 18.6 days in MiaPaca2, and 19.3 days in Su8686 tumors. Gemcitabine treatment was effective in both hypoxic and oxygenated tumors but there seemed to be most effective against the hypoxic Hs766t tumors.

Quantitative oxygen images by EPRI can predict difference in the benefit from oxygen-dependent anti-tumor treatments in individual pancreatic tumor cell lines that may help properly choose the best treatment in patients with pancreatic cancer if EPRI is available in clinic.

December 18, 2013

Hyperpolarized Carbon-13 Studies of Intermediary Metabolism: Gluconeogenesis in the Liver, Compartmentation and Substrate Competition in the Heart

Matthew Merritt, PhD
Advanced Imaging Research Center
Assistant Professor of Radiology
University of Texas Southwestern Medical Center

Professor Merritt's research focuses on measuring metabolism using magnetic resonance and hyperpolarized substrates. His lab is dedicated to developing DNP technology as well as its application for understanding intermediary metabolism in the heart, liver, and in tumors.

Abstract:

Hyperpolarized (HP) carbon-13 is rapidly gaining acceptance as a new metabolic imaging method due to the specificity of the results that it can produce. The chemical shift inherent to the magnetic resonance experiment allows multiple metabolic pathways to be studied with simple endogenously appearing compounds such as pyruvate. However, the apparent simplicity of the experiment belies the complicated nature of the analysis of the data. Here, 3 cases will be presented where a precise understanding of intermediary metabolism is required in order to properly interpret the results obtained with HP pyruvate.

Gluconeogenesis (GNG) in the liver is essential for proper glucose homeostasis in the body. Hepatic GNG depends upon the carboxylation of pyruvate to oxaloacetate and subsequent conversion to phosphoenolpyruvate (PEP), as the direct phosphorylation of pyruvate is energetically unfavorable. Phosphoenolpyruvate carboxykinase (PEPCK) is the enzyme responsible for the first committed step of GNG, PEP formation, and imaging this reaction should give a direct estimate of GNG. HP [1-^{13}C]pyruvate can be used to detect PEPCK flux due to the concomitant release of [^{13}C]bicarbonate, but other competing enzymes such as pyruvate dehydrogenase (PDH) or isocitrate dehydrogenase (IDH) can also release [^{13}C]bicarbonate. In order to more clearly understand the mechanism of hepatic [^{13}C]bicarbonate production, excised mouse livers were perfused with a mixture of HP [1-^{13}C] and [2-^{13}C]pyruvate as well as thermally polarized ^{13}C labeled tracers. The combination of standard ^{13}C isotopomer analysis and the HP studies allows the fraction of [^{13}C]bicarbonate production from PEPCK to be quantitatively determined.

(abstract continues on next page)

Heart failure (HF) remains as one of the primary causes of death in the developed world. Initial results using HP pyruvate to image myocardial infarction have produced some of the most compelling images to date in the HP literature. Previous studies have associated the production of [^{13}C]bicarbonate in the heart with PDH activity alone, but metabolic flux in

the myocardium is subject to substrate competition between ketones, carbohydrates, and fatty acids. PDH flux is regulated by its phosphorylation state as well as the ratios of CoA/Acetyl-CoA and NAD^+/NADH. Propionate is known to dephosphorylate PDH, increasing its specific activity. Mouse hearts were perfused with acetate and glucose as a control, with the experimental group including propionate as a substrate. Propionate increases PDH flux as detected by the production of [^{13}C]bicarbonate from [1-^{13}C]pyruvate, but further measurements indicated that compartmentation in the heart is the basis for a sequestration of pyruvate derived from glucose away from the mitochondria. Interpretation of pyruvate images taken in the heart must account for the phenomenon of compartmentation.

Finally, substrate competition has been measured in the rat heart *in vivo* using a mixture of hyperpolarized [1-^{13}C]pyruvate and [1-^{13}C]butyrate, a 4-carbon fatty acid. Using a fed versus fasted protocol, a variety of downstream metabolites could be detected with high sensitivity and time resolution. Competition between the two substrates leads to significant differences in both cases, and it is suggested that this method could become a clinically relevant paradigm for the diagnosis of heart failure as well as a guide for treatment planning.

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Long-lived Hyperpolarized Biomarkers for Molecular Magnetic Resonance Imaging

Thomas Theis, PhD
Center for Molecular and Biomolecular Imaging
Duke University

Dr. Theis is a postdoctoral associate at Duke University and Visiting Professor at RWTH Aachen University. His research focuses on the dynamics and characterization of molecular targets supporting long-lived singlet states for hyperpolarized MRI.

Abstract:

We present recent breakthroughs creating long-lived hyperpolarization for molecular magnetic resonance imaging (MRI). This promises in-vivo tracking of biomarkers with the unique ability to characterize metabolic transformations in real-time.

Thus far, hyperpolarized MRI has been demonstrated for only a few select molecules in vivo, because it faces a significant challenge - the fast decay of the polarization, which precludes the observation of biochemical processes that take longer than a few tens of seconds. We have recently shown that it is possible to restrict the fast decay by using symmetry-protected nuclear spin states in pairs of chemically equivalent spins, thereby enabling detection of low concentration molecules for minutes.[1]

Our new developments show that, for example, for $^{13}C_2$-diphenyl-acetylene ($^{13}C_2$-DPA) it is possible to convert initial ^{1}H polarization with a T_1 of 6.5s into a disconnected state involving a chemically equivalent ^{13}C pair with a lifetime of 520s thereby extending ^{1}H-polarization lifetime by 80-fold even at large magnetic fields of 8.45T. Furthermore, the polarization stored in the disconnected state can be reconverted into ^{1}H polarization for readout. Starting from ^{1}H hyperpolarization can be advantageous because higher polarization levels can be achieved and the polarization process is faster (a few minutes instead of ~half hour). Moreover, detection of the hyperpolarization on protons results in a sensitivity enhancement of $(\gamma_{1H}/\gamma_{13C})^{7/4}=11.3$. The proposed methodology allows for storage and read-out of the hyperpolarization with proton-irradiation only, making the experiments compatible with clinical scanners.[1]

Finally, a remaining obstacle is that the existing pulse-sequences to access the protected states are limited by excess energy dissipation or high sensitivity to inhomogeneities. We design novel composite and adiabatic pulse excitations which exploit the small *J*-couplings in the molecules to drive the desired transitions. The new pulses work over a wide range of rf amplitudes and offsets, while depositing insignificant amounts of power.[2]

[1] Y. Feng, T. Theis et al. **2013** *"Storage of hydrogen spin polarization in long-lived $^{13}C_2$-singlet order and implications for hyperpolarized magnetic resonance imaging"* ***J. Am. Chem. Soc.***, *135*:9632.

[2] T. Theis, Y. Feng, R. M. Davis, W. S. Warren **2013** *"Spin lock composite and shaped pulses for efficient and robust pumping of dark states in magnetic resonance"* ***arXiv***:1308.5666.
(Just accepted in ***J. Chem. Phys.***)

December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Hyperpolarized 129Xe MRI - Introduction, History, and New Developments

Bastiaan Driehuys, PhD
Faculty, Center for In Vivo Microscopy
Associate Professor of Radiology, Biomedical Engineering, and Medical Physics
Duke University

Professor Driehuys' research focuses on developing and applying hyperpolarized substances in MR imaging. His background is in the atomic physics of producing hyperpolarized noble gases 3He and 129Xe, not only in attacking the basic physics problems of these gases, but in their large-scale development and application to biomedical challenges

Abstract:

Hyperpolarized ^{129}Xe MRI is now emerging as a powerful clinical research tool at Duke and other medical centers around the world. This inhaled agent provides a means to evaluate pulmonary function regionally, with sensitivity to the smallest airways where early disease originates. The technology is now positioned to begin exploiting the most fascinating properties of ^{129}Xe – its solubility in biological tissues and accompanying range of chemical shifts. Hyperpolarized gas MRI has a long and rich development history, much of it directly traceable to the Center for *in vivo* Microscopy. This includes the first *in vivo* ^{3}He MRI, the introduction of diffusion-weighted contrast, and driving the transition from ^{3}He to more sustainable ^{129}Xe. On this 25th anniversary of the CIVM I will take some time to not only introduce the technology and its current state-of-the-art, but also revisit these important historical accomplishments that were enabled by the culture, people and resources of the center.

December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Explore New Frontiers of Molecular Imaging with Porphysome Nanotechnology and Beyond

Gang Zheng, PhD
Professor of Medical Biophysics
University of Toronto
Associate Editor, *Bioconjugate Chemistry*

Professor Zheng's research group focuses on developing translatable technology platforms to combat cancer. Specifically, they have introduced activatable photodynamic therapy agents, developed lipoprotein-like nanoparticles, and recently discovered porphysome nanotechnology that opens a new frontier in cancer imaging and therapy.

Abstract:

Porphyrins are the endogenous chromophores of nature such as hemes in red blood cells and chlorophylls in green plants. Porphyrins and porphyrin-like molecules are well known photosensitizers for photodynamic therapy and fluorescence imaging, and there has been rekindled interest for nuclear medicine given their radioisotope chelating ability. In the course of examining porphyrin self-quenching in liposomes to explore their potential use as activatable photosensitizers, we discovered 'porphysomes', the first all-organic nanoparticles with intrinsic multimodal photonic properties. They are self-assembled from porphyrin-lipid building blocks to form liposome-like bilayer vesicle (~100 nm diameter). The very high porphyrin packing density (>80,000 per particle) results in both 'super'-absorption and structure-dependent 'super'-quenching, which, in turn, converts light energy to heat with extremely high efficiency, giving them ideal photothermal and photoacoustic properties that are unprecedented for organic nanoparticles. Upon porphysome nanostructure dissociation, fluorescence of free porphyrins is restored to enable low background fluorescence imaging. In addition, metal ions (e.g., radioactive copper-64) can be directly incorporated into the porphyrin building blocks of the preformed porphysomes thus unlocking their potential for PET, MRI and radiation therapy. As a result of their organic nature, porphysomes were biodegradable in vivo and induced minimal acute toxicity in mice with high intravenous doses. In a similar manner to liposomes, porphysomes can be easily scaled up via commercial extrusion techniques and the large aqueous core of porphysomes could be passively or actively loaded with drugs, opening up a new avenue for image-guided drug delivery. By changing the way porphyrin-lipid assembles, we developed ultra small porphyrin nanodiscs (<20nm), trimodal (US/photoacoustic/fluorescence) porphyrin shell microbubbles (~2um), and confocal microscopy-controlled porphyrin microreactors (~100um), expanding the purview of porphyrin nanophotonics. Compared with classical "all-in-one" nanoparticles containing many functional modules, the simple yet "one-for-all" nature of porphysomes represents a novel approach to the design of multifunctional nanoparticle and confers high potential for clinical translation.

December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Identification of Linear Precursors in the Biosynthesis of the Macrocyclic Thiopeptide Antibiotic Berninamycin

Steven Malcolmson, PhD
Assistant Professor of Chemistry
Duke University

Research in the Malcolmson group focuses on the discovery of novel methods for the efficient and selective synthesis of small molecule scaffolds. This is primarily achieved through the design and development of new catalysts, thereby enabling new synthetic bond disconnections. The catalysts and methods developed are applied to the synthesis of more complex compounds, consisting of biologically active and other structurally interesting molecules.

Abstract:

Antibiotics constitute one of the most effective classes of therapeutic agents developed over the last century; however, the sustained use of these compounds has led to widespread bacterial resistance, such as in methicillin-resistant *Staphylococcus aureus* (MRSA), necessitating the development of novel antibacterial compounds. Thiopeptides are a class of naturally-produced antibiotics, which display potent antibacterial activity (including against MRSA) but have seen little use clinically.

Of the numerous challenges associated with transforming these compounds into drugs, one that stands out is the enormous structural complexity of thiopeptides, ruling out a pure synthesis approach in generating them. Bacterial fermentation, combined with biological engineering of biosynthesis pathways, is one strategy that may produce sufficient quantities of libraries of related compounds for discovery-phase research. However, for this approach to come to fruition, we first must understand the structural requirements needed for thiopeptides to bind effectively to their biological targets as well as the substrate structural requirements and mode of action of the enzymes involved in the biological assembly of these macrocyclic molecules.

As part of a program directed at examining thiopeptide biosynthesis, we located the gene cluster associated with the assembly of berninamycin, a 35-membered ring thiopeptide produced by *Streptomyces bernensis*, and examined its heterologous expression in several species of *Streptomycetes*. Our studies have revealed that alternate macrocyclic compounds and linear precursors to berninamycin are produced in these heterologous hosts, suggesting an order of events in the late stages of berninamycin assembly and also demonstrating structural requirements for antibiotic efficacy. Such effects may be general to all thiopeptide antibiotics.

December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Developing Small Molecules to Target Nucleic Acids in Human Disease

Amanda Hargrove, PhD
Assistant Professor of Chemistry
Duke University

The Hargrove lab harnesses the unique properties of small organic molecules to study the structure, function and therapeutic potential of long noncoding RNAs (lncRNAs). Working at the interface of chemistry and biology, they employ methods ranging from RNA-targeted small molecule synthesis and array-based pattern recognition to studies of the molecular and cellular biology of nucleic acids.

Abstract:

Therapeutic targeting of nucleic acids has been a long-standing challenge in cancer biology, in part due to the limited chemical space occupied by DNA and RNA relative to proteins that can be exploited for molecular recognition. This challenge has been met for genomic DNA by the development of pyrrole-imidazole polyamide oligomers that recognize the minor groove in a sequence specific manner through highly organized hydrogen bonding interactions. Py-Im polyamides have been found to localize to the nucleus, display transcriptional inhibition of induced pathways and disrupt transcription factor occupancy at chromatin binding sites. In prostate cancer, Py-Im polyamides have been targeted to androgen receptor (AR)-DNA binding sites as well as those of ERG, an ETS transcription factor recently identified as a driver in prostate cancer progression. Key findings include Py-Im polyamide inhibition of ETS-promoter reporter assays, enrichment of genes with known ETS-promoter sequences through gene set enrichment analysis (GSEA) and reduced levels of ERG-driven DNA damage in ERG-positive cell culture. Studies of ERG-positive xenografts show reduced intravasation in chick embryo CAM assays and dose-dependent inhibition of tumor growth in mice.

Specific binding of RNA molecules, however, presents unique challenges. While antisense (silencing) oligonucleotides can be effective against short RNA sequences, the therapeutic applications of siRNA have yet to be realized, and these sequences often fail against longer, more structured targets such as lncRNA. Small molecules have recently been presented as an alternative approach to recognize “undruggable” RNA. Despite significant advancement in the field, selectivity for individual RNA molecules has been particularly difficult to achieve. One proposed solution is the covalent linkage of multiple small molecule recognition units obtained from fragment-based screening. Multivalent interactions, as commonly observed for binding events in nature, will allow specific binding of large, multi-domain RNA molecules. Current screens, however, are generally based on protein-targeted fragments. Large RNA-targeted screening libraries and combinatorial designs offer an auspicious route to single molecule recognition, and resulting inhibition, of lncRNAs.

December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

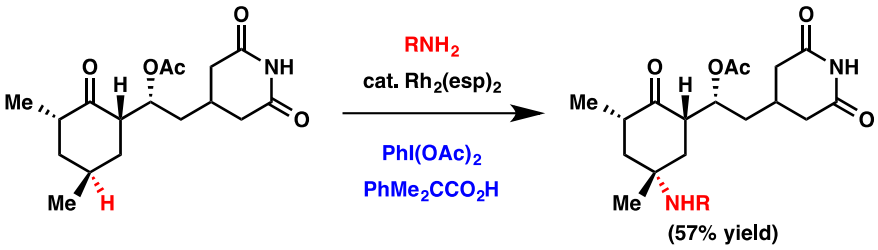
Detecting Fleeting Intermediates in Catalytic C–H Amination Reaction Cycles

Jennifer Roizen, PhD
Assistant Professor of Chemistry
Duke University

The Roizen lab works to access improved antibiotics and selective ion channel inhibitors, with implications for the study and treatment of cancer, heart disease, and neurological disorders. They develop novel reaction methods designed to streamline access to challenging natural products, such as the guaianolide sesquiterpenes.

Abstract:

Over the last 30 years, most new chemical entities that have been approved by the Food and Drug Administration to treat disease have been small organic molecules. The specificity of interactions between small molecules and proteins motivates the Roizen group to develop new methods to access these structures. This talk will highlight efforts toward an intermolecular amination reaction that have resulted in fundamental mechanistic discoveries, which have in turn, facilitated the reaction development process. Both the initial development of the oxidative C–H functionalization method and mechanistic investigations employing desorption electrospray ionization mass spectroscopy (DESI-MS) experiments will be described.



December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Visual Informatics for Small Animal Imaging – Focus on the Brain!

Alexandra Badea, PhD
Center for In Vivo Microscopy
Assistant Professor of Radiology
Duke University

Professor Badea uses MR microscopy to image the rodent brain, including its vasculature. She studies the morphometry and its anatomical variability in normal states, and also in human disease models.

Abstract:

Small animal models provide essential tools to better understand and treat human disease. One of the aims we have set at the Center for In Vivo Microscopy is to advance image-based discovery of anatomical, physiological, and molecular biomarkers in animal models. We aim to synergize the efforts of our colleagues at the CIVM, experts in multiple imaging modalities, to provide an integrated perspective through quantitative and multivariate biomarkers.

The challenges we face come from: 1) the sheer volume of image data acquired from different sources, and with different contrasts such as magnetic resonance microscopy, micro-CT, micro-SPECT, US, multiphoton microscopy; 2) biological variability coming from animal strain, age, or disease phenotype.

But to understand the changes that happen with disease we must first establish the boundaries of normal variability. To this aim we have developed atlases of the brain for some of the most common mouse and rat strains, at adult age and through development period. We share our atlases with the interested community, in an effort to advocate for open science.

Building of top of this foundation, we have implemented pipelines for registration and segmentation, to characterize regional and local structural changes in the brains of rodent models of Alzheimer's disease, autism, or toxic insults.

It is our hope that such pipelines developed for automated, quantitative phenotyping will increase throughput for the analysis of many large data sets, enhance sensitivity through statistical power, while ensuring reproducibility of the analysis.

December 18, 2013

Innovative in vivo Microscopy:
From Beakers to Animals and Beyond

Towards High-Resolution Structural and Chemical Imaging in Entire Mouse Organs

Martin Fischer, PhD
Center for Molecular and Biomolecular Imaging
Center for In Vivo Microscopy
Duke University

Professor Fischer is director of CIVM Core D – Nonlinear Optics. His research efforts focus on mitigating the problem of scattering in three dimensional imaging of everything from human tissue to historical artwork.

Abstract:

Conventional optical microscopes provide high-resolution contrast in thin biological samples such as single cells, but strong scattering reduces their utility in thick samples, limiting studies of cell morphology, metabolism and function in a cell's natural environment. The effects of scattering can be reduced by confocal detection or the localized nature of nonlinear optical microscopy (initially two-photon excited fluorescence, and more recently second-harmonic generation or coherent Anti-Stokes Raman scattering). However, very few intrinsic biological markers give sufficient strength and resolution in tissue, and the use of exogenous (injected or expressed) contrast agents is often not an option. To address this deficiency, we have developed advanced femtosecond pulse shaping and detection technologies to access intrinsic nonlinear signatures that were not previously observable in tissue (for example, because they do not produce fluorescence). The key is to visualize nonlinear interactions in tissue from their effect on the frequency spectrum of an ultrafast laser pulse. The changes are generally small for physiologically acceptable laser powers, but pre-shaping the pulse spectrum can force these changes to occur in uncongested - or even clear - regions of the spectrum. This provides two advantages: the information can be extracted essentially background-free, and it is preserved even in highly scattering tissue (since the spectrum is relatively immune to scattering).

We have extensively explored pulse shaping techniques that can measure a range of nonlinear absorptive processes such as excited state absorption, ground state depletion and stimulated Raman scattering, and nonlinear phase contrast such as cross-phase modulation. Using our shaping techniques we exploited, for example, specific excited state properties of hemoglobin to image microvasculature and differentiate oxygenated versus deoxygenated blood vessels in live mice. We have also been able to differentiate sub-types of melanin in a variety of lesions (from benign nevi to malignant melanoma) in unstained skin at high resolution - a capability that could dramatically improve the diagnosis and understanding of the development of skin cancer. Further we have demonstrated that the nonlinear refractive index can yield contrast in biological cells without the addition of exogenous contrast agents. Here we will describe applications of our microscopy technique and discuss strategies to extend its imaging range both in live tissue and in ex vivo samples.

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