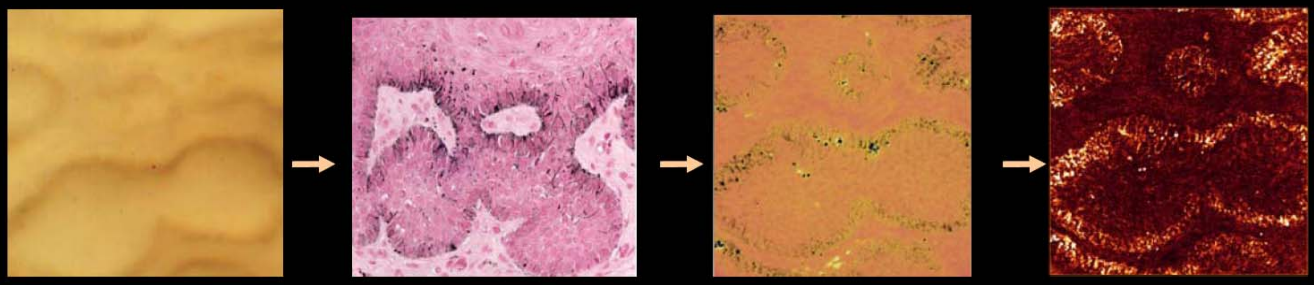
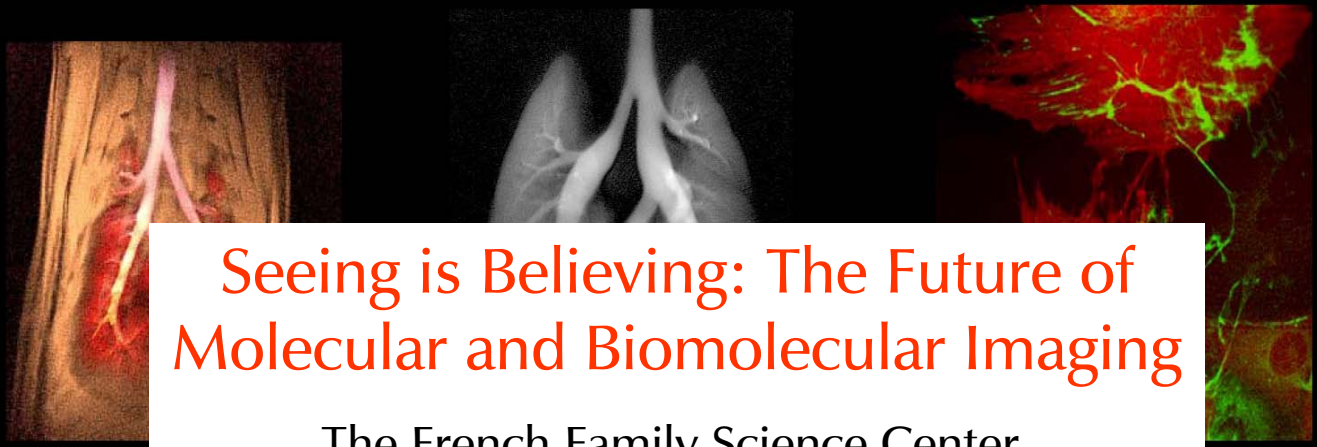


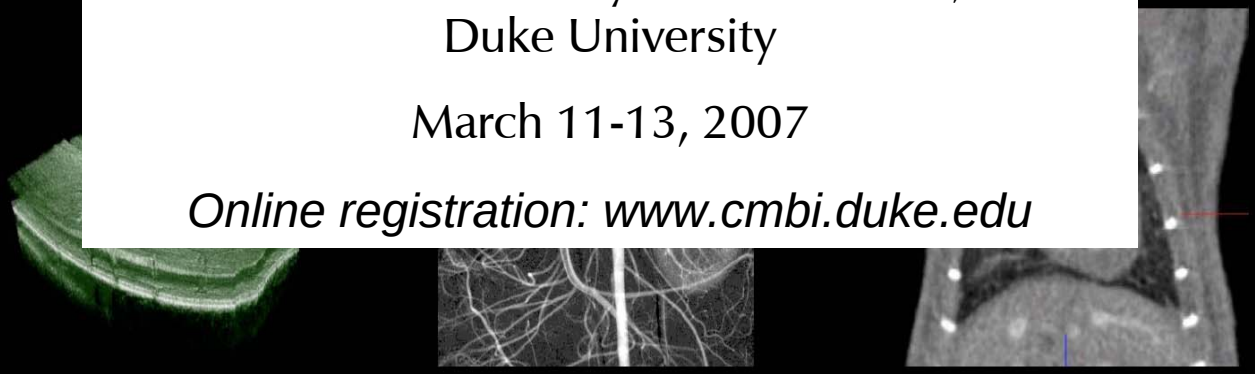


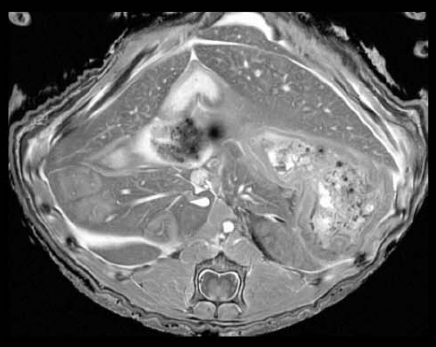
Seeing is Believing: The Future of Molecular and Biomolecular Imaging

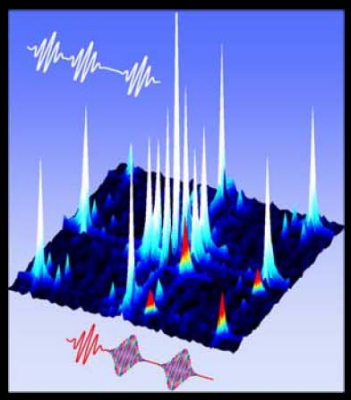
The French Family Science Center,
Duke University

March 11-13, 2007

Online registration: www.cmbi.duke.edu







Duke University
Center for Molecular and Biomolecular Imaging

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Many of the spectacular achievements of twentieth century science followed the same simple paradigm. As new directions in basic atomic or molecular physics matured, they were adopted by chemists and applied physicists. This work in turn enabled applications in biological, clinical, and environmental science, driven both by universities and by innovative companies. Imaging technologies provide many of the best-known examples of this evolution, such that as we complete the first decade of the twenty-first century, it is widely recognized that *development of advanced imaging technologies* holds great promise. This discipline ranges from basic science and engineering through preclinical and clinical applications; it provides the critical underpinning for the development of molecular medicine and advanced environmental monitoring; and it enables the scientific disciplines which attempt to understand complex materials and biomaterials. For example, molecular imaging (the localization of specific molecular signatures in an image) has been identified by the U. S. National Cancer Institute as an "extraordinary opportunity" and scientific priority for cancer research. In fact, recently NIH created an additional institute (NIBIB) dedicated to this field.

In September 2006, Duke University launched a strategic plan that will invest $1.3 billion over the next five to eight years in students, faculty, programming and facilities. One of four initiatives that transcends school boundaries supports **the development of novel imaging technologies and algorithms to extract specific molecular and cellular signatures.** As a kickoff meeting for this initiative, the Center for Molecular and Biomolecular Imaging has assembled a two-day conference to highlight emerging directions and opportunities in this exciting field. This meeting will also celebrate the move of Duke's Chemistry department (and much of Physics and Biology) into the new French Family Science Center. Finally, it will provide an overview of unique or unusual capabilities, both at Duke and in the region. For example, the after-dinner presentation on March 12 will focus on activities being initiated by the North Carolina Research Campus in Kannapolis-roughly $1B (largely private money) is being invested to transform an old cotton mill site into the most technologically advanced biotechnology campus in the country. It is an amazing story, and it provides some unique opportunities for research collaborations.

Please make plans to attend.

Tentative Schedule

Sunday, March 11, 2007

2-4 Registration and Poster Setup, French Foyer

4-4:20 Introduction and Welcome
Warren S. Warren; Peter Lange

4:20-6:10 Finding New Sources of Contrast
Tom Meade (Northwestern), Gary Pielak (UNC), Carola Leuschner/Challa Kumar (LSU); Martin Fischer (Duke)

6:10-8:00 Poster session and reception, French Foyer (even posters manned)

Monday, March 12, 2007

8-9 Continental Breakfast

9-10:40 Advances in Imaging Technologies
Carolyn Larabell (LBL), Ian Anderson (ORNL), Anuj Kapadia (Duke), John Simon (Duke)

10:40-11 Break

11-12:40 Molecular Imaging in Pharmaceutical Development
Susanta Sarkar (GSK), Eric Toone (Duke), Michael Therien (Penn), Michael Zalutsky (Duke)

12:40-2 Lunch and poster session (odd posters manned)

2-3:40 Clinical and Diagnostic Applications
Dan Sullivan (NIH), H. Kim Lyerly (Duke), Bruno Maraviglia (Rome), Gigi Galiana (Princeton)

3:40-4 Break

4-5 Translating Imaging Technologies
Tuan Vo-Dinh (Duke), Nimmi Ramanujam (Duke), Al Johnson (Duke)

5-6 Lab tours: Fitzpatrick, CIVM, French

7-9:30 Reception and Dinner at Washington Duke
After Dinner program: Kannapolis (Andy Conrad)

Tuesday, March 13, 2007

Session organized jointly with the Fitzpatrick Institute for Photonics

8-9:15 Continental Breakfast

9:20-10:40 *Advances in Optical Microscopies*
Mark Schnitzer (Stanford), Tom Brown (St. Andrews), Dan Fu (Princeton)

10:40-11:00 Break

11-12:30 *Commercial Imaging Technologies*
David Brady (Duke), Richard Levenson(CRI), Joe Izatt (Duke)

Afternoon session: the Carolina Photonics Consortium (organized by the Fitzpatrick Institute)

~ Sunday, March 11, 2007 ~

Warren S. Warren
Director, Center for Molecular and Biomolecular Imaging
Duke University

Introduction and Welcome
4:00 - 4:20 pm

Finding New Sources of Contrast
4:20 - 6:10 pm

Thomas J. Meade
Northwestern University

Eliminating the Background in MR Imaging:
The Future Looks Bright
4:20 - 4:50 pm

Fundamental biological and clinical questions have driven technological advances in an area of research known as biological molecular imaging. The direct observation of ongoing developmental events in living embryos and the descendants of individual precursors in an intact embryo can be labeled by microinjection of stable, nontoxic, MR lineage tracers. Since a complete time-series of high-resolution three-dimensional MR images can be analyzed forward or backward in time, it is possible to reconstruct the cell divisions and cell movements responsible for any particular descendant(s). In order to understand the mechanisms of gene expression in whole animals, we have developed a library of multimodal MR probes that are biochemically activated in-vivo. The lanthanide chelates modulate fast water exchange with the paramagnetic center, yielding distinct relaxivity states. The modualtion is triggered by two types of events: *i*. enzymatic processing of the contrast agent and, *ii*. the reversible binding of an intracellular messenger.

Gary J. Pielak
The University of Carolina Chapel Hill

High-resolution NMR of Proteins in Living Cells
4:50 - 5:20 pm

Our goal is to understand the effects of macromolecular crowding on biochemical processes by acquiring atomic-level information on proteins under actual biological conditions. To this end, we have pioneered in-cell NMR. I will talk about our characterization of two intrinsically disordered proteins under the crowded conditions found in living *Escherichia coli*. I will also touch on our next step--using in-cell NMR in eukaryotic cells to study a key protein in neurodegenerative disease, the intrinsically disordered proteins, α-synuclein. This protein is an excellent candidate not only because of its disease relevance, but also because macromolecular crowding has extremely large effects on the properties of disordered proteins.

Our understanding of protein structure and function has grown enormously in the last 100 years. We have progressed from pondering what role, if any, polypeptides play in the cell, to unraveling, at the atomic level, the mechanisms of enzymes and the molecular bases of protein-protein interactions vital to understanding human disease. Our accumulating wealth of knowledge has largely come from *in vitro* studies performed under conditions far different from those found in biology. For example, most biochemical examinations of protein behavior are performed at concentrations in the μg-to-mg-per-mL range, but the insides of cells, where most proteins perform their work, have protein concentrations of >300 mg per mL. Thus, our knowledge comes from data acquired under conditions that are far from physiological relevant, and theory predicts these differences can have extremely large effects on biophysical parameters. Moving beyond the test tube by performing truly *in vivo* studies in living eukaryotic cells by using NMR spectroscopy is the next frontier in protein chemistry.

Carola Leuschner
Pennington Biomedical Research Center

Development of Functionalized Nanoparticles for Early Detection and Treatment of Cancers and Metastatic Disease
5:20 - 5:50 pm

Despite new discoveries of drugs and treatment combinations for cancer the mortality rate did not improve over the last decades. Over 600,000 new cases of breast cancer were reported in the US in 2004. In the year 2000 more than 41,000 deaths occurred from breast cancer in the US alone. There is an urgent need to improve treatment and imaging in patients diagnosed with distant metastatic cancers. Furthermore new drugs and contrast agents need to be developed to monitor the efficacy of response to treatments. Nanotechnology for treatment and diagnostics has

been the focus of developmental research in the past 10 years and continues to grow exponentially. This thriving field of research focuses on materials, which are in the nanometer scale, opening up new avenues that can lead to the development of highly specific compounds for treatment and imaging. Nanoparticles open new opportunities to treat cancers by hyperthermia, to encapsulate drugs like doxorubicin, camptotecin or radiolabeled compounds that can be functionalized for higher specificity to reduce systemic exposure, and to deliver specifically nanoparticles to tumors, metastases peripheral organs and the brain. More than 52 % of human breast, 86 % of prostate cancers overexpress functional receptors for luteinizing hormone releasing hormone (LHRH). In nude mouse models, these xenografts and their metastases can be targeted through receptors for LHRH linked to a membrane disrupting peptide or superparamagnetic iron oxide nanoparticles (SPION) [Leuschner et al J Biomed Nanotech 2005; Leuschner et al Prostate 56, 2003, Leuschner et al Breast Cancer Res Treat 2006]. We have developed nanoparticles that specifically target LHRH receptors for specific delivery, higher efficiency and faster accumulation of nanoparticles within tumor cells. The nanoparticles are iron oxide based particles of less than 30 nm diameter, they are monodisperse, neutral of charge and have a high saturation magnetization. Surface modification allows binding of membrane disrupting peptides on these functionalized nanoparticles creating particles that enhance magnetic resonance imaging sensitivity in vivo and can at the same time destroy tumors and metastases.

Key findings of our studies include: 1. Improved cellular uptake of LHRH-functionalized nanoparticles in target cells through receptor mediated endocytosis, 2. In vivo body distribution of LHRH functionalized nanoparticles specifically target, accumulate in and destroy tumors and metastases expressing LHRH receptors, 3. repeated injection of LHRH-SPION increased the accumulation of nanoparticles in tumors and metastases and were retained over a period of 4 weeks 4. LHRH functionalized nanoparticles have low phagocytotic index, 5. Accumulation of LHRH-SPIONs enhanced resolution in MR images. 6. LHRH functionalized iron oxide based nanoparticles are not toxic and easily incorporated and excreted through iron homeostasis. 7. Magnetic resonance imaging showed increased resolution to 300 micrometer in tumor tissue of LHRH-SPION injected mice.

We have further demonstrated the usefulness of nanoparticles for diagnosis and treatment by linking covalently a ligand molecule and a drug to superparamagnetic iron oxide nanoparticles (SPION). This new nanoparticle construct is currently developed for monitoring and treatment of tumors and metastases. For imaging and monitoring treatment response LHRH and the lytic peptide hecate have been covalently linked to SPIONs, (LHRH-SPION-Hecate). In this study we tested whether LHRH-SPION-Hecate specifically target and accumulate and destroy in metastatic cells from breast cancer xenografts. Key findings of this study include: 1. Highly specific targeted delivery of LHRH-SPION-Hecate in tumors and metastases was observed as accumulation of SPIONs in metastatic cancer cells of peripheral tissues, lymph nodes, brain and bones. 2. LHRH-SPIONs alone were ineffective in reducing tumor volume or tumor weight, nor did it cause destruction of tumors or metastatic cells. 3. LHRH-SPION-Hecate caused significant reduction in tumor volume, tumor weight and metastases. 4. Ironoxide nanoparticles were retained in diseased tissue. 5. Co-injection of LHRH inhibited the accumulation of LHRH-SPION and LHRH-SPION-Hecate in target tissue.

Conclusion: LHRH conjugated SPION or in conjugation to a cytotoxic drug can serve as contrast agent and anticancer drug that specifically increased the sensitivity of detection of metastases and disseminated cells in lymph nodes, bones and peripheral organs in MRI and opens the possibility to monitor a treatment response in a non-invasive manner. The application in nanotechnology targeting the LHRH receptor for treatment and imaging will be outlined and the latest findings and discoveries will be summarized for this highly important receptor. This approach may have promising applications for treatment and imaging and facilitate monitoring of treatment response in patients.

Challa S. S. R. Kumar
Louisiana State University

Influence of size and shape of SPIONs on contrast enhancement in MRI
5:20 - 5:50 pm

We have recently demonstrated that spherical SPIONs of ~15 nm in diameter covalently bound with LHRH specifically target LHRH receptors. The targeting was found to be more efficient and faster, than reported so far with other SPIONs, leading to higher accumulation of nanoparticles within tumor cells [Leuschner et al J Biomed Nanotech 2005; Leuschner et al Breast Cancer Res Treat 2006]. An enhancement in MRI contrast has also been observed in nude mice inoculated with human breast cancer cells [ISMR 2007] Based on these encouraging results, more systematic studies are being planned in order to investigate the influence of size and shape of SPIONs on the MRI contrast through development of appropriate synthetic methods and modeling tools. The presentation will focus on sharing some of these novel ideas.

Martin C. Fischer
Duke University

Novel Structural and Functional Contrast Mechanisms for High-resolution, Nonlinear Optical Tissue Microscopy
5:50 - 6:10 pm

Traditional methods for optical imaging of tissue can be divided into two categories that trade off resolution and imaging depth. The first category is linear optical microscopy, which yields high resolution images of targets as deep as ~100 μm below the surface. The penetration depth is generally limited by strong absorption and scattering of tissue in the wavelength range used for fluorescence excitation of biologically relevant markers. The second category is optical diffusion

tomography. From a large set of transmission data it can reconstruct information from targets several centimeters inside tissue, but with a scattering-limited resolution of several millimeters. Recent developments in nonlinear optical microscopy point the way toward new techniques that combine the advantages of these two categories. Two-photon fluorescence microscopy, for example, can yield high-resolution images at depths of up to ~1 mm, but its applicability is limited to fluorescent targets.

Our research currently focuses on two novel nonlinear signatures: two-photon absorption (TPA) and self-phase modulation (SPM) during ultrafast laser pulse propagation in tissue. Two-photon absorption (TPA) imaging can provide contrast in non-fluorescent molecules while retaining the high resolution and sectioning capabilities of nonlinear imaging modalities [T. Ye *et al*, *Proc. SPIE* **6089** (2006)] In the long-wavelength water window, tissue TPA is dominated by the endogenous molecules melanin and hemoglobin with an almost complete absence of endogenous two-photon fluorescence. A complementary nonlinear contrast mechanism is self-phase modulation (SPM), which can provide intrinsic signatures that can depend on local tissue anisotropy, chemical environment, or other structural properties. One of the difficulties using TPA or SPM in biological systems is that, for physiologically acceptable power levels, these nonlinear effects are extremely small and are typically overshadowed by a large linear background. To overcome this problem, we have developed a unique approach where ultra-fast laser pulses are shaped in such a way that nonlinear interactions in the tissue create new light components [M. C. Fischer *et al*, *Opt. Lett.* **30** (2005)]. In contrast to conventional methods, we can extract these components background-free. With this technique we are able to detect small changes in TPA and SPM with microscopic resolution on a sub-millisecond timescale.

Here we report on a microscopy setup to simultaneously acquire 3D, high-resolution TPA and SPM images [M. C. Fischer *et al Proc. SPIE* **6442** (2007)]. We have acquired data in mounted B16 melanoma cells with very modest laser power levels. We will also discuss the possible application of this measurement technique to neuronal imaging. Since SPM is sensitive to material structure we can expect SPM properties of neurons to change during neuronal firing. Using our hole-refilling technique we have demonstrated strong novel intrinsic nonlinear signatures of neuronal activation in a hippocampal brain slice. The observed changes in nonlinear signal upon collective activation were up to several tens of percent, unlike other intrinsic optical signal changes on the percent level. These results show that TPA and SPM imaging can provide important novel functional contrast in tissue using very modest power levels suitable for *in vivo* applications.

~ Monday, March 12, 2007 ~

Advances in Imaging Technologies
9:00 - 10:40am

John D. Simon
Duke University

Free Electron Laser Photoelectron Emission Microscopy (FEL-PEEM) of Melanosomes
9:00 - 9:20 am

Free-electron laser photoelectron emission microscopy (FEL-PEEM), femtosecond absorption spectroscopy, and EPR measurements of oxygen photoconsumption are used to probe the threshold potential for ionization of eumelanosomes and pheomelanosomes isolated from human hair. FEL-PEEM data show that both pigments are characterized by an ionization threshold at 282 nm. However, pheomelanosomes exhibit a second ionization threshold at 326 nm, which is interpreted to be reflective of the benzothiazine structural motif present in pheomelanin and absent in eumelanin. The lower ionization threshold for pheomelanin is supported by femtosecond transient absorption spectroscopy and ESR oximetry experiments. The lower ionization potential observed for pheomelanin could be an important part of the explanation for the greater incidence rate of UV-induced skin cancers in the group of red-haired individuals.

Carolyn A. Larabell
Lawrence Berkeley National Laboratory

Soft X-ray Nano-tomography of Biological Cells
9:20 - 9:50 am

X-ray microscopy can image whole, hydrated, biological specimens up to 10 microns thick with a spatial resolution better than 50 nm. Soft X-ray microscopy uses photons with energies between the K shell absorption edges of carbon (284 eV, λ=4.4 nm) and oxygen (543 eV, λ=2.3 nm). These photons readily penetrate the aqueous environment while encountering significant absorption from carbon- and nitrogen-containing organic material. In this energy range, referred to as the 'water window,' organic material absorbs approximately an order of magnitude more strongly than water, producing a quantifiable natural contrast and eliminating the need for contrast enhancement procedures to visualize cellular structures.

The experiments presented here were performed at the Advanced Light Source using the full field transmission X-ray microscope, XM-1. This microscope employs a bend magnet X-ray source and zone plate condenser and objective lenses. The condenser zone plate acts as a monochromator and the X-ray images are recorded directly on a cooled, back-thinned 1024x1024 pixel CCD camera. The sample holder was a rotationally symmetric glass tube; the region containing the sample was 10 microns in diameter with a wall thickness of 200 nm. Live cells were loaded into the tube, rapidly frozen by a blast of liquid nitrogen-cooled helium gas, and maintained at –140 degrees C by a steady flow of cold helium gas. The image sequence spanned 180 degrees and consisted of either 45 images spaced by 4 degrees or 90 images spaced by 2 degrees. The images were aligned to a common axis and computed tomographic reconstruction was used to obtain the 3-D X-ray linear absorption coefficient. Volume rendering and animation of reconstructed data was performed using the 3-D program, Amira. The tomographic reconstructions generate 3-D images at approximately 50 nm isotropic resolution and reveal high fidelity views of the internal architecture of cells.

The high penetrating power, coupled with a near absence of reflection at the interface of dissimilar materials, makes X-rays an ideal probe for studying cellular morphology and examining the location of labeled proteins in single cells. We have used this imaging approach to reveal remarkable details of the nuclear and cytoplasmic architecture of fully hydrated whole cells. We have also localized molecules in the nucleus and cytoplasm of whole, hydrated cells using immunogold labeling protocols. We are developing additional labels uniquely suited to x-ray imaging to enable simultaneous localization of multiple proteins. Using the x-ray linear absorption coefficient, quantitative information about cellular structures and molecular distributions can be obtained from the reconstructed data.

No Photo Available

Ian S. Anderson
Oak Ridge National Laboratory

The Spallation Neutron Source: New Directions in Neutron Spectroscopy and Imaging
9:50 - 10:20 am

Anuj J. Kapadia
Duke University

Medical Imaging through Neutron Stimulated Emission Computed Tomography (NSECT)
10:20 - 10:40 am

Neutron spectroscopic techniques are showing great potential in detecting element concentration in biological tissue. Neutron inelastic scatter spectroscopy has the ability to diagnose several disorders in the human body that are characterized by changes in element concentration in the affected tissue, e.g. breast cancer (Al, Br), prostate cancer (Ca, Cu,), and liver iron overload (Fe). The preferred method of diagnosing most of these disorders is invasive biopsy, which is an unpleasant procedure that can have several potential complications. Our neutron imaging technique, called Neutron Stimulated Emission Computed Tomography (NSECT), uses a beam of fast neutrons to non-invasively detect element concentration changes within biological tissue in-vivo and create two-dimensional tomographic images of element distribution through a single non-invasive tomographic scan. The working principle of NSECT is as follows. Neutrons incident on tissue scatter inelastically with an atomic nucleus in the tissue and excite it to a higher energy level that is often unstable. The excited nucleus then rapidly decays to its ground states, emitting gamma photons whose energy is equal to the difference between the two energy states. These energy state differences are generally well-known and are unique to most elements and isotopes. The energy and intensity of the emitted gamma photons is measured by an energy-sensitive detector to identify the emitting nucleus and determine its concentration in tissue.

NSECT experiments are currently performed at the Triangle Universities Nuclear Laboratory using a Van-de-Graaf accelerator as the neutron source. Experimental gamma spectra have been obtained from several samples ranging from solid metal phantoms to bovine liver samples and human breast tissue. Tomographic images have been obtained and reconstructed from a solid metal phantom. Results from these experiments suggest that NSECT has the ability to detect elevated element concentrations in the liver and breast at clinically acceptable dose levels.

NSECT has the potential to develop into a portable screening and diagnostic imaging modality with the use of high-flux portable neutron sources and portable gamma detectors. Efforts are being made to improve the sensitivity of the system to allow trace element measurement through time-of-flight background reduction and attenuation correction. In-depth dose analysis is being performed using Monte-Carlo simulations. Our final goal is to implement a low-dose portable scanning system that allows a non-invasive diagnostic alternative for breast cancer, iron overload and several other disorders.

Molecular Imaging in Pharmaceutical Development
11:00 - 12:50pm

No Photo Available

Susanta Sarkar
Glaxo-Smith-Kline

Molecular Imaging in Drug Discovery
11:00 - 11:30 am

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Eric J. Toone
Duke University

A Stochastic, Cantilever Approach to the Evaluation of Solution Phase Thermodynamic Quantities
11:30 - 11:50 am

The omics revolution in biology is rapidly changing modern medicine, as the diagnosis and treatment of disease becomes driven by the identification of molecular markers, rather than patient reported symptoms and radiological evaluation. Omic approaches facilitate sub-typing and prospective diagnosis of disease from a specific molecular basis, long before symptoms become apparent. Metabolomics – the quantitative evaluation of constellations of metabolites – is an especially powerful approach to diagnosis and treatment since the metabolome represents an end-point snap-shot of the interaction of genetic makeup, protein expression, lifestyle, and environmental factors. The use of metabolomic diagnosis remains hindered by analytical methodology, which today primarily encompasses magnetic resonance and mass spectrometry coupled to chromatographic separation. The greatest need in analytical methodology is throughput rather than sensitivity: most metabolites of interest are present in relatively high concentrations (nM to mM), but the volume of patient samples requires analysis of thousands per day. Building on advances in nanotechnology that facilitate evaluation of single-molecule binding events, we have developed a mechanical approach to the evaluation of solution phase thermodynamic quantities, including concentrations. The approach uses stochastic force-sensed binary evaluation of binding in a competitive configuration that permits quantitative measurement of analyte concentrations. Through the use of magnetically-probed arrays of cantilievers, the approach is readily amenable to formats that facilitate the rapid, quantitative evaluation of virtually any analyte in optically complex milieu.

Michael Therien
University of Pennsylvania

***In Vivo* Optical Imaging Enabled by Soft-Matter Analogues of the Quantum Dots**
11:50 - 12:20 pm

Formed through cooperative self-assembly of amphiphilic diblock copolymers and electronically conjugated porphyrinic near infrared (NIR) fluorophores, NIR-emissive polymersomes (70 nm – 50 μm polymer vesicles) define a family of organic-based, soft matter quantum dot analogues that are ideally suited for *in vivo* optical imaging and high sensitivity *in vitro* diagnostic applications. We show that membrane incorporation of a wide range of related multi-porphyrinic fluorophores enables emission energy modulation over a broad domain of the visible and near infrared spectrum (600-950 nm). Long-wavelength optical excitation of such assemblies generates intense, highly localized emissive signals capable of penetrating through the dense tumor tissue of live animals. New nanoscale polymersomal vesicles in which the component amphiphilic diblock polymers are derived from two previously FDA-approved building blocks have been delineated, providing for fully bioresorbable probes and delivery agents. Excited-state transient dynamical studies provide insights into how NIR-emissive polymersomes can be further optimized for *in vivo* deep-tissue fluorescence-based imaging. Conjugation of cell surface receptor specific peptides or antibodies to the polymersome surface provide emissive nanoscale vesicles that can be selectively targeted.

Timothy DeGrado
Indiana University School of Medicine

Imaging Lipid Metabolism with PET
12:20 - 12:50 pm

PET has unique power as an imaging modality to provide noninvasive measurements of biochemical rates using small molecular substrates. This talk will summarize our experience with developing fatty acid and choline tracers for PET imaging of lipid metabolism. Both anabolic and catabolic pathways may be probed in a variety of applications that include myocardial and hepatic fatty acid oxidation, and phospholipid metabolism in tumors.

Clinical and Diagnostic Applications
2:00 - 3:40pm

H. Kim Lyerly
Duke University

Imaging and the Development of Targeted Cancer Therapies
2:00 - 2:20 pm

No Photo Available

Daniel M. Sullivan
National Institutes of Health

Imaging: The NIH Perspective
2:20 - 2:50 pm

No Photo Available

Bruno Maraviglia
University of Rome "La Sapienza"

Human Brain Function Investigation by Magnetic Resonance: New Multi-Modal Perspectives
2:50 - 3:20 pm

The insufficiency of tools that provide information on the Brain Function , in a real time scale, is limiting the advancement of comprehension of the fundamentals of most of today's open questions. A laboratory (MARBILab) for research on Human Brain Function and Architecture by developing new NMR methods and systems, also in combination with other techniques, was recently created in Rome. Some research lines were established in the last few years with fast growing productivity.

In this talk I will provide a brief description of the main activities in MARBILab, while more attention I will give to the results we obtained, after the success in combining **EEG and fMRI** for **simultaneous measurements*** opened to us the possibility of new targets. Understanding how the human brain works requires knowledge of its functional neuroanatomy : where different

processing areas are, what type of processing is performed, how processing is organized among different areas and which is the temporal dynamics of functional events. fMRI allows fine spatial localization of the metabolic response induced by neural activation but it does not reflect neural activation directly and cannot reveal its temporal sequence. Conversely EEG gives fine temporal resolution of neural activity of sources capable of generating far-field potentials, but has poor spatial resolution. It is then reasonable to expect that the combination of these two techniques may improve localization of neural generators and enhance the temporal resolution of fMRI focuses.

The application of this tool pertains to both cognitive neuroscience and clinical neurology such as epilepsy. Indeed simultaneous Event Related Potentials (ERP) and fMRI are particularly valuable for obtaining perfect correspondence of experimental conditions and cognitive states between two types of recordings. With regard to epilepsy or other spontaneous brain rhythms, the simultaneous multi-modal approach is the only valid strategy to localize unpredictable neuronal events.

Among the results sprouting from the simultaneous combination of EEG and fMRI, I will report further information that can arise from time resolved NMR spectroscopy in areas of the activated brain cortex. Indeed the study of localized metabolic activation, thanks to the combination with EEG, was applied for the first time to spontaneously activated areas of the visual cortex in the FOS (Fixation-Off Sensitivity) Epilepsy.

Further frontiers will probably be reached with the development of multi-modal techniques with increased spatio-temporal resolution. An ultimate goal could be the development of real time applications such as Brain Computer Interface; highly precise spatio-temporal information on brain dynamics is another field to be widely explored. Also a more accurate insight of the neural basis of the measured signals can derive from the multi-modal approach.

No Photo Available

Gigi Galiana
Princeton University

Intermolecular Multiple Quantum MR Thermometry
3:20 - 3:40 pm

Current methods for monitoring temperature with MR are inadequate for guiding hyperthermic cancer treatments because of their precarious accuracy in vivo. We present a physically different method based on the detection of water-fat iZQCs (intermolecular Zero Quantum Coherences) which produce a far more accurate map of absolute temperatures. Novel but simple modifications to the HOMOGENIZED pulse sequence allow us to isolate the relevant signal, and early applications demonstrate an order of magnitude improvement in temperature accuracy, both in vitro and in vivo.

Translating Imaging Technologies
4:00 - 5:00pm

Nimmi Ramanujam
Duke University

Physiologic, Metabolic, and Structural Alterations in Breast Cancer: Assessment via Optical Technologies
4:00 - 4:20 pm

A major focus of our laboratory is the development of optical technologies for enhanced diagnosis of breast cancer. One area that has been intensively studied is the use of optical spectroscopy as an adjunct to percutaneous image-guided core-needle biopsy. Core-needle biopsy is an increasingly used technique for the diagnosis of breast lesions due to attractive advantages over traditional surgical biopsy; however, it may result in a false-negative rate of 1-7% [1] and a repeat biopsy rate of 9-18% [2] due to limited sampling accuracy. Optical probes which could be inserted into the needle and used to survey the surrounding tissue, and which could discriminate malignant tissue from benign with high sensitivity and specificity, would significantly increase the efficiency of this diagnostic procedure. This is made possible by the various chemical and structural changes which occur in cancer which may be probed in a sensitive and specific manner via optical spectroscopy.

Chemical parameters such as hemoglobin content and saturation, lipid and water content, and metabolic rate may be assessed via optical spectroscopy. Likewise, structural changes such as nuclear-to-cytoplasmic ratio and extracellular matrix remodeling may also be observed in this manner. Thus, optical spectroscopy of tissues may be viewed as a direct analog to traditional assessment of tissue structure and function using histological techniques, yet resulting in real-time diagnosis. Further enhancing our approach is the use of fast inverse Monte Carlo-based methods of spectral analysis, which allow extraction of the absorption, fluorescent, and scattering properties of human tissues for a wide range of optical properties and probe geometries [3,4]. This physically-based method allows for the near real-time determination of biochemical and structural composition of the tissue, and is superior to non-parametric or empirically-based methods of spectral analysis. To this end, we are currently conducting clinical trials on patients undergoing core-needle biopsy (mostly benign tissues) or surgical tumor resection (mostly diseased tissues). Optical properties of the tissues are extracted from the measured diffuse reflectance and fluorescence spectra, and are associated with a gold-standard histopathologic diagnosis for proper classification of tissues based on optical spectroscopy. Exposure to tissues with a wide range of clinical diagnoses will allow for the development of a trained classification algorithm which has high predictive accuracy.

The utility of optical spectroscopy for assessment of tissue structure and function is also being exploited for the characterization and therapeutic monitoring of solid tumors in small animal models. Our current efforts in this area are directed at the study of hypoxia in mammary carcinomas grown in the flanks of nude mice. Non-invasive measurements of hemoglobin saturation and content may be made across the skin of nude mice, and are an attractive

alternative to more invasive oxygen monitoring techniques (needle electrodes, for example). The use of optical spectroscopy in the monitoring of therapeutic targets in tumors may be instrumental in the development of effective therapies for human and animal cancers.

Tuan Vo-Dihn
Duke University

Optical Biosensing and Imaging
4:20 - 4:40 pm

This lecture provides an overview of the research activities in our laboratory related to the development and application of advanced biophotonics, molecular spectroscopy, and nanosensors for molecular sensing and imaging. The first research area involves the development of sensors based on metallic nanoprobes that can produce the surface-enhanced Raman scattering (SERS) effect for ultrasensitive biochemical analysis and gene diagnostics. The SERS-based "Molecular Sentinels" nanoprobe technology has the potential for cellular diagnostics and imaging.

Combining the exquisite specificity of biological recognition probes and the excellent sensitivity of laser-based optical detection, biosensors are capable of detecting and differentiating bio/chemical constituents of complex systems in order to provide unambiguous identification and accurate quantitation, and open new horizons for biolomedical sensing. This area of research involves the development of nanosensors and nanoprobes for *in vivo* analysis of a single living cell [molecular markers, apoptosis following photodynamic (PDT) cancer treatment]. Such nanosensors could be used to study *in situ* intracellular signaling processes and to study gene expression and molecular processes within sub-compartments inside individual living cells.

For *in vivo* medical diagnostics, we have developed an optical diagnostic procedure based on laser-induced fluorescence (LIF) and hyperspectral imaging (HSI) for direct *in-vivo* cancer diagnosis without requiring biopsy. LIF measurements were conducted during routine gastrointestinal (GI) endoscopy examinations of patients. The fiberoptic probe was inserted into the biopsy channel of an endoscope and lightly touched the surface of the tissue being monitored. The LIF measurement was completed in approximately 0.6 second for each tissue site. The results of this LIF approach were compared with histopathology results of the biopsy samples and indicated excellent agreement (98%) in the classification of normal tissue and malignant tumors of GI cancer in clinical studies involving over 100 patients.

G. Allan Johnson
Duke University

Advanced Imaging at the Center for *in vivo* Microscopy
4:40 - 5:00 pm

~ Tuesday, March 13, 2007 ~

Advances in Optical Microscopies
9:00 - 10:20am

Mark Schnitzer
Stanford University

Long-term and portable cellular level imaging of the mouse brain using fluorescence microendoscopy
9:00 - 9:30 am

Fluorescence microendoscopy is an emerging optical modality providing cellular level imaging in deep brain tissues that have been inaccessible to *in vivo* microscopy. One- and two-photon fluorescence microendoscopy are based on minimally invasive micro-lenses (350-1000 micron diameter) and offer micron-scale resolution of cells in deep brain areas. For tracking cellular changes over the long-term, we have developed a chronic mouse preparation that has enabled *in vivo* microendoscopy imaging of hippocampal neurons up to a year after an initial surgery. We have also built compact (~2 gram) microendoscopy instrumentation for brain imaging in freely moving mice.

C. T. A. Brown
St. Andrews University

Activating Your Microscope - Optical Transfection of Mammalian Cells
9:30 - 10:00 am

For many, using a microscope is a passive process. In this talk, I will show how we have used the microscope to interact with the samples under study. In particular I will focus on the use of multiphoton microscopy setups to perform optical transfection of mammalian cells. Our studies have shown that such an approach can lead to high transfection efficiencies. We will see further that if the laser's mode of operation can be changed, such techniques can be combined with optical micro-manipulation to enable a wide range of functionality to be achieved using a single platform. We will also demonstrate that the introduction of non-Gaussian light beams can significantly simplify non-linear processes pointing the way to automation of such techniques with relatively simple alterations of conventional microscopes.

No Photo Available

Dan Fu
Princeton University

Nonlinear Absorption Provides New Contrast in Multiphoton Microscopy of Tissue
10:00 - 10:20 am

Multiphoton microscopy has rapidly developed into the world of biological imaging over the last decade. The majority of multiphoton imaging is done with two-photon fluorescence and second harmonic generation. However, there is only a handful of endogenous fluorophores have these properties. Broadening the range of molecular targets that we can image *in vivo* will be tremendously helpful in some particular areas like melanoma imaging or tumor angiogenesis. We have demonstrated a new method of multiphoton imaging with nonlinear absorption measurement. If one of two synchronized mode-locked pulse trains at different colors is intensity modulated, the modulation transfers to the other pulse train when nonlinear absorption takes place in the medium. We can easily measure 10^{-6} absorption changes caused by either instantaneous two-photon absorption or relatively long lived excited state absorption with a RF lock-in amplifier. We demonstrated three dimensional imaging of melanin and hemoglobin in both cells and tissue samples with subcellular resolution. Besides, the two types of melanins (eumelanin and pheomelanin) as well as the two forms of hemoglobin (oxyhemoglobin and deoxyhemoglobin) can be differentiated due to their distinct excited state dynamics. This chemical selectivity in imaging could provide new opportunities in tissue imaging, particularly melanoma imaging and tumor angiogenesis.

Commercial Imaging Technologies
10:50 - 12:00 pm

David Brady
Duke University

Molecular Imaging Using Coded Aperature Spectroscopy
10:50 - 11:10 am

Recent advances in optical design and compressive imaging enable single shot/real time molecular imaging for vibrational and fluorescence spectroscopies. These advances are enabled by system design to integrate sensing and chemometric analysis. In contrast with conventional dispersive or transform spectroscopy, coded multiplex spectroscopy can be designed to emphasize targeted spatial and spectral features for specific applications. This talk reviews code design in spectral sensors and imaging systems and describes strategies for joint optimization of sensor codes and molecular image estimation algorithms. Example experimental results focus on in vivo alcohol chemometrics and on pharmacuetical identification and quality control.

No Photo Available

Richard Levenson
Cambridge Research Instrumentation

From Microscopes to Mice: Multispectral Imaging for Brightfield, Fluorescence, and Small-Animal Imaging
11:10 - 11:40 am

Expression array and flow cytometry methods have led to greater appreciation of the complexity of macromolecular interactions in normal and diseased states. However, it is important not just to measure overall expression of specific molecules, but also their spatial distribution at various scales, while preserving cellular and tissue architectural features. This task is made more difficult in the case of co-localized signals, and in the presence of autofluorescence commonly found, for example, in formalin-fixed specimens. Multispectral imaging (MSI) alleviates these difficulties, enabling the simultaneous imaging and quantitation of four or more chromogenic markers in a tissue section in brightfield (and more for fluorescently labeled specimens), even in the presence of spatial and spectral overlap. A key advantage of the described tunable filter technology is that it removes the requirement for expensive and complex confocal instrumentation. In addition, the same approach can be used for pre-clinical small animal imaging.

Brightfield microscopy and chromogenic immunohistochemical approaches are typically employed in pathology research. Immediate applications of MSI can be seen in the quantitative study of estrogen-receptor (ER) and progesterone (PR)-expression and co-localization in breast cancer as well as in double-immunophenotyping in hematopathology. Fluorescence is typically used to enable the sensitive detection of multiple analytes at once, but autofluorescence and signal cross-talk limits achievable performance. Reagents such as quantum dots help with but do not overcome these problems, which are exacerbated in the case of formalin-fixed tissues. However, multispectral unmixing techniques permit the detection of signals otherwise obscured due to autofluorescence typical of histopathology specimens.

In small animal imaging, autofluorescence is also a major barrier to sensitive detection and quantitation in the intact (skin-on) mouse. MSI plus unmixing results in targets appearing displayed against a black, near-zero background. Compared to conventional imaging systems, improvements in sensitivity of as much as 300-fold have been demonstrated. While moving to the NIR can reduce autofluorescence, this strategy limits the potential for signal multiplexing. However, a multispectral approach allows for the simultaneous detection of as many as 5 different spectrally and spatially overlapping fluorescent signals.

Coordinated use of multispectral imaging techniques in in-vivo and ex-vivo analyses can be valuable, since in some experimental models of cancer, for example, cells need to be characterized before implantation, the resulting tumors imaged, and then subsequently re-examined histologically.

Joseph Izatt
Duke University

Optical Coherence-based Imaging and Sensing in Biomedicine and Biotechnolgy
11:40 - 12:00 pm

Optical coherence-based imaging techniques including optical coherence tomography (OCT), optical coherence microscopy (OCM), and spectral domain phase microscopy (SDPM) use low-coherence spectral interferometry to obtain nanometer to micron-scale measurements of structure, motion, and molecular composition in living cells, tissues, and organisms. OCT has become a standard diagnostic tool in clinical ophthalmology, and is under investigation for other human diagnostic applications including cancer detection and evaluation of cardiovascular disease. Within the past few years, dramatic technology advances have increased the performance of OCT and OCM systems manyfold, and are now capable of micron-scale two and three-dimensional functional and molecular imaging noninvasively in living systems. Applications of these new technologies for noninvasive, quantitative characterization of ophthalmic disease progression, and for high-throughput phenotyping of small animal models of disease and genetic manipulation are particularly compelling. Related technology advances have enabled the design of highly phase-stable interference microscopes capable of resolving nanometer-scale structures and motions in living cells with ms temporal resolution. These new capabilities are being used to probe cellular internal and external surfaces and their responses to chemical and mechanical stimuli. I will review progress in these areas at Duke and at Bioptigen, a local spinoff company which is commercializing OCT technology.