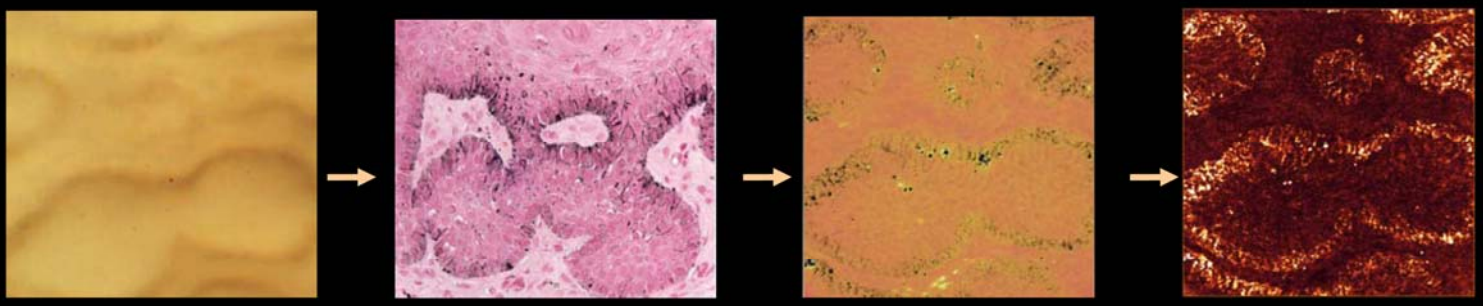
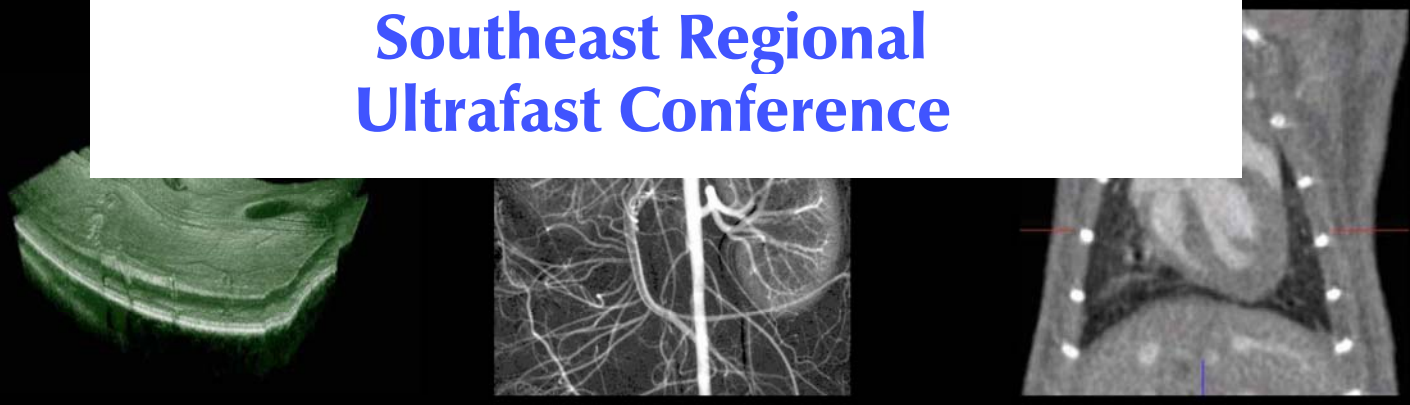


New Directions in Microscopy: Sharper, Deeper, Smarter

Southeast Regional Ultrafast Conference



French Family Science Center
Duke University
December 14-16, 2009

Duke University

Center for Molecular and Biomolecular Imaging

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Welcome to the "New Directions in Microscopy: Sharper, Deeper, Smarter" meeting, which is the annual meeting of the Center for Molecular and Biomolecular Imaging at Duke, and the Southeast Ultrafast Regional meeting, sponsored by Coherent. The meeting takes place in the French Family Science Center (dedicated November 2007), which I think you will agree provides an outstanding venue for research and education.

Optical microscopy has been an essential tool in biology and medical practice for centuries. As imaging technologies evolve from monitors of structure to monitors of function, optical microscopies have also evolved dramatically, driven largely by innovations in laser technology. This symposium will focus on new optical imaging methods which can improve resolution by an order of magnitude or more past the traditional diffraction limit; can overcome scattering and absorption in tissue to produce high resolution images far deeper in tissue than previously possible; and can create entirely new contrast in images, which correlates better with the intrinsic cellular biochemistry (for example, of cancer). Invited presentations will address multiple new methods in superresolution microscopy,nonlinear imaging (two-photon microscopy, with and without fluorescence signatures), and effects of spatial and polarization shaping on the laser to reduce scattering and improve penetration depth. Both *in vivo* and *in vitro* applications will be presented.

CMBI is a Provost-level organization which transcends school boundaries 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. To support this very broad field, our meetings have widely varying themes and foci. The preceding meeting (March 2009), for example, focused on applications of molecular imaging in clinical practice. This meeting returns somewhat more to a basic science focus, but still with significant clinical representation.

This year, our meeting dovetails with the **Southeast Regional Ultrafast Laser** meeting, sponsored by Coherent. CMBI speakers will present on Monday and Tuesday; the Ultrafast meeting will begin Tuesday afternoon and continue on Wednesday, with dinner and an afterdinner presentation on Tuesday night. I think we have attracted an excellent collection of speakers and poster presenters, and I expect that the excitement in both modern optical imaging and ultrafast laser applications in general will be apparent throughout the meeting.

A meeting of this scope does not happen spontaneously. I am very grateful for the outstanding efforts of Mike Conti (CMBI), whose attention to detail was critical to make this work; to Professor Martin Fischer for his help with the program; and for Scott Crane (Coherent) for arranging their support.

New Directions in Microscopy: Sharper, Deeper, Smarter

Monday, December 14, 2009, 2:00-8:00PM

1:30	Registration and Poster Setup, French Foyer
2:00	Welcome and Introduction-Warren S. Warren
2:10	**Michael Levene (Yale)**- Micro-optics and Other Tricks for Extending the Range of Multiphoton Microscopy
2:50	**Martin Fischer (Duke)**- Femtosecond Pulse Shaping for Imaging non-Fluorescent Targets in Tissue
3:20	**Lin Shao (Janelia Farm, HHMI)**- Breaking the Resolution Limit in Widefield Fluorescence Microscopy with Structured Illumination
4:00	Break
4:20	**Kelly Nelson (Duke)**-New Imaging Directions for Dermatology
4:50	**Volker Westphal (MPI Gottingen**)– New Directions in Stimulated Emission Depletion (STED) Microscopy
5:30	**David Smith (Duke)** – Metamaterials and Imaging
6:00	Poster Session and Reception

Tuesday, December 15, 2009, 8:30AM-3:00 PM

8:30	**Warren Zipfel (Cornell)**- Multiphoton Microscopy for High Resolution in Vivo Imaging
9:10	**Joe Izatt (Duke)** – Novel Imaging Techniques for Ophthalmology
9:40	**Jerome Mertz (Boston University)**–Out-of-focus Background Reduction with HiLo Microscopy
10:20	Break
10:50	**Sam Johnson (Duke)**- Current and Desired Capabilities of the Duke Light Microscopy Core Facility
11:20	**Julie Biteen (Michigan)**- Superresolution Imaging of Structural Proteins in Live *Caulobacter crescentus*
12:00	Lunch
1:00	PLENARY SPEAKERS for both meetings **Michael Fayer (Stanford)**- Watching Ultrafast Molecular Motions in Liquids, Molecules, and Proteins **Tuan Vo-Dinh, (Duke)**- Plasmonic Nanoprobes for Cellular Sensing and Imaging

Southeast Regional Ultrafast Laser meeting

3:00	**Thomas Crawford (South Carolina)-** Controlling Nanomagnet Dynamics with Ultrafast Spin Torque Pulses
3:30	**Keith Weninger (North Carolina State)-** Revealing Protein Dynamics with Single Molecule Fluorescence
4:00	**Joel Schwartz (Duke)-** Protein Dynamics of Map Kinase Signaling Revealed by Correlation Spectroscopies
4:30	**Steve Butcher (Director of Scientific Laser Systems, Coherent)-** The Next Generation of Lasers Enabling New Scientific Research
5:00	Dinner, Posters and Laboratory Tours
7:00	**Warren S. Warren (Duke) -** Breasts and Brains, Similarities and Differences: How Lasers fit into Molecular Imaging

Wednesday, December 16, 2009, 8:30AM-4:00 PM

8:45	**Tim Lian (Emory)-** Multi-exciton Dissociation in Quantum Dots
9:15	**Ken Knappenberger (Florida State)-** Surface Plasmon Probing of Electronic Energy Relaxation in Metal Nanostructures
9:45	Break
10:00	**Michael Therien (Duke)-** Nanoscale Agents for in Vivo Optical Imaging and Ultrasensitive in Vitro Diagnostics
10:30	**Kazuo Watanabe (Louisiana State)-** Towards Single Molecule Spectromicroscopy by Combining STM and Ultrafast Lasers
11:00	**Michael Chua (North Carolina, Chapel Hill)-** FRAPing Little Bugs
11:30	**Weiguo (Bill) Yang (Western Carolina)-** Super-resolution Imaging via Optical Gain
12:00	Lunch
1:00	**Anne Vaahtokari (David H. Murdock Research Institute {DHMRI})-** Imaging Technologies at the DHMRI Light Microscopy Core Facility
1:30	**Kenan Gundogdu (North Carolina State)-** Observation of bond-specific oxidation of Si(111)
2:00	**Scott Crane & Steve Butcher (Coherent)-** Laser Round Table

Micro-optics and Other Tricks for Extending the Range of Multiphoton Microscopy

Michael J. Levene
Assistant Professor, Department of Biomedical Engineering
Yale University

The Levene lab applies optical physics, mostly fluorescence, to the development of new approaches to studying biological problems and to address various diseases.

While the bulk of their work uses multiphoton microscopy or fluorescence fluctuation spectroscopy, they have numerous other projects ranging from single molecule biophysics to novel ultrasound imaging approaches for breast cancer screening.

Abstract:

Multiphoton microscopy is often the tool of choice for fluorescence microscopy of thick scattering tissues, including in particular in vivo microscopy. Yet the depth of tissue that is accessible by multiphoton microscopy under routine use is limited to several hundred microns below the surface. I will discuss several approaches using micro-optics, including grandient index lenses and micro-prisms, for extending the range of in vivo multiphoton microscopy. In addition, I will present results from optical clearing of fixed tissues, which allows for multiphoton microscopy through up to 3 mm of tissue, with image stacks encompassing entire mouse organs.

Femtosecond Pulse Shaping for Imaging non-Flourescent Targets in Tissue

Martin C. Fischer
Assistant Research Professor
Duke University

Dr. Fischer's research explores novel nonlinear contrast mechanisms such as two-photon absorption and self-phase modulation for structural and functional imaging in tissue.

Abstract:

Nonlinear optical interactions offer several advantages for molecular imaging in highly scattering tissue. The localized nature of the interaction leads to high spatial resolution, optical sectioning, and larger possible imaging depth than linear methods. However, nonlinear contrast (other than fluorescence) is generally difficult to measure because it is overwhelmed by the large background of detected illumination light. This background can be suppressed by using femtosecond pulse shaping to encode nonlinear interactions in background-free regions of the frequency spectrum. We will discuss two techniques aimed at measuring nonlinear absorptive and nonlinear dispersive contrast, respectively.

Nonlinear absorption offers a dramatically expanded range of molecular contrast, because not all markers that absorb photons release the absorbed energy in the form of light. The difficulty in measuring nonlinear absorption in tissue lies in the fact that, at the typical intensities we would be willing to apply to tissue, nonlinear absorption accounts for at most 10^{-6} of the loss of the main pulse train – the rest of the light is lost to linear absorption or scattering. The challenge is to determine which components of the beam loss come from which mechanism. We will describe a technique that utilizes shaped pulse trains of multiple colors, where an amplitude modulation of the pump beam is transferred onto the probe beam of a different wavelength, thereby generating a new frequency in the probe beam. Using this technique we have been able to detect non-fluorescent metabolic markers in tissue (e.g. the imaging of different types of melanin in pigmented lesions and the mapping of oxygenation content in blood vessels).

While the modulation transfer technique can measure nonlinear absorption processes, it is unable to extract nonlinear dispersive contrast. We have developed a different technique that is able to measure nonlinear phase contrast (e.g. self-phase modulation, SPM) in tissue with very moderate laser power. SPM modulates the index of refraction by an amount proportional to the instantaneous pulse intensity. Its strength is strongly affected by molecular content and on local structure and anisotropy. The key concept our new measurement technique is based on is the fact that nonlinear processes can create new frequency components within a laser pulse, but linear processes (such as scattering or absorption) cannot do this. These spectral changes are typically very small for the low pulse energies allowable in biomedical imaging applications. However, we can efficiently detect the spectral changes by appropriately pre-shaping the pulse spectrum such that the changes show against a small background. Using these pulse shaping techniques we have been able to detect nonlinear optical signatures of neuronal activity in live neurons.

Breaking the Resolution Limit in Widefield Flourescence Microscopy with Structured Illumination

Lin Shao
Research Specialist, Gustafsson Group
Howard Huges Medical Institute, Janelia Farm Research Campus

Dr. Shao works with Dr. Mats Gustafsson on his research in the field of structured illumination.

Abstract:

Structured illumination takes advantage of moiré patterns, which are produced by overlaying one pattern with another. Two rows of chain-link fencing seen from a distance can produce a moiré pattern, as can overlapping layers of gauzy curtains. Moiré patterns can be quite noticeable, even distracting, in digital photographs or on television: They are the reason that TV newscasters are told to avoid jackets with certain designs, such as hounds tooth.

In structured-illumination microscopy, the sample under the lens is observed while it is illuminated by a pattern of light (more like a bar code than the light from a lamp). Several different light patterns are applied, and the resulting moiré patterns are captured each time by a digital camera. Computer software then extracts the information in the moiré images and translates it into a three-dimensional, high-resolution reconstruction.

The principles of resolution enhancement by structured illumination both in 2D TIRF and 3D wide-field microscopy are discussed. In particular, we show that the technique can be applied to live imaging, at least in the case of TIRF imaging, when using a fast pattern-generating device such as a spatial light modulator (SLM). Various imaging results with biological samples are presented to illustrate the potentials of structured illumination.

New Imaging Directions for Dermatology

Kelly Nelson, MD
Assistant Professor, Department of Dermatology
Duke University

Dr. Nelson is the director of the Melanocytic Disease/Pigmented Lesion Clinic at Duke University.

Her current research involves the use of real time confocal microscopy for the diagnosis of skin cancers in order to offer early diagnosis of skin cancers without the use of an invasive skin biopsy.

Abstract:

Within the field of dermatology, technological advances to facilitate early diagnosis of skin cancer are in great demand. While skin lesions at either extreme of the benign to malignant spectrum are easily identified by the well-trained dermatologist, at times subtle malignant lesions may be interpreted as benign, and unusual appearing benign lesions may appear as mimicking skin cancer. Our goal is to avoid unnecessary procedures, while maximizing the "early catch" for skin cancer. To provide clinical context for the role of in-vivo imaging modalities in the dermatologic clinic, we will briefly review the most common cutaneous malignancies (basal cell carcinoma, squamous cell carcinoma, melanoma), and currently used technologies (dermoscopy, optical coherence tomography, high-resolution ultrasound, reflectance confocal microscopy) to enhance diagnostic accuracy for cutaneous malignancy.

New Directions in Stimulated Emission Depletion (STED) Microscopy

Volker Westphal
Biomedical Engineer, Department of NanoBiophotonics
Max-Planck-Institute for Biophysical Chemistry

Dr. Westphal's research at MPI in Gottingen, Germany focuses on STED and other diffraction-unlimited microscopy techniques.

Abstract:

For more than a century, the resolution of a lens-based (far-field) optical microscope has been limited by diffraction, as shown by Abbe. However, in the 1990s it became evident that the limiting role of diffraction can be broken in lens-based fluorescence microscopy if certain fluorophore properties are judiciously integrated into the image formation. The first viable concept of this kind is stimulated emission depletion (STED) microscopy, which, since its experimental validation, has been key to solving a number of problems in biophysics and cell biology.

Since its invention STED microscopy has done many steps forward, up to the point where imaging of living cells and fast dynamic processes with super-resolution has become possible. Recent efforts had gone in the simplification of the STED design, e.g. the employment of simpler and cheaper light sources as well as a reduction of alignment requirements. We will present the latest efforts on these efforts.

Metamaterials and Imaging

David R. Smith
Professor, Department of Electrical Engineering
Duke University

Research in the Smith lab includes photonic crystals, metamaterials and negative index media, plasmon nanophotonics, and self-monitoring composites.

Multiphoton Microscopy for High Resolution in Vivo Imaging

Warren Zipfel
Associate Professor, Department of Biomedical Engineering
Cornell University

Dr. Zipfel is working to optimize the instrumentation for multiphoton microcsopy and produce high-resolution, three-dimensional images of tissues in living tissue inside or outside of the body in order to depict details of cells and cellular processes across the third and fourth dimensions. He also is evaluating more direct clinical roles for multiphoton microcsopy technology.

Novel Imaging Techniques for Opthamology

Joseph Izatt
Professor, Department of Biomedical Engineering
Duke University

Dr. Izatt's research in biophotonics is concerned with the application of optoelectronic technologies to problems in the biomedical sciences. His research centers on the application of optical technologies for non-invasive, high-resolution imaging and sensing in living biological tissues, specifically on the development of technology of Optical Coherence Tomography (OCT), a relatively new medical imaging modality which uses optical radiation to noninvasively image living cells and tissues with resolution approaching the wavelength of light.

Abstract:

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 undergoing clinical trials 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. Functional extensions of OCT for Doppler blood flow imaging, polarization-based tissue birefringence mapping, and molecular imaging using intrinsic and nanoparticle-based contrast media are undergoing rapid development. Recent technology advances have also enabled the design of OCT-based highly phase-stable interference microscopes capable of resolving nanometer-scale structures and motions in living cells in the optical far field 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. This talk will concentrate on applications of these techniques in ophthalmology for noninvasive, micrometer-scale resolution structural and functional imaging of the retina and for quantitative assessment of the optical power and aberrations of the cornea.

Out-of-focus Background Reduction with HiLo Microscopy

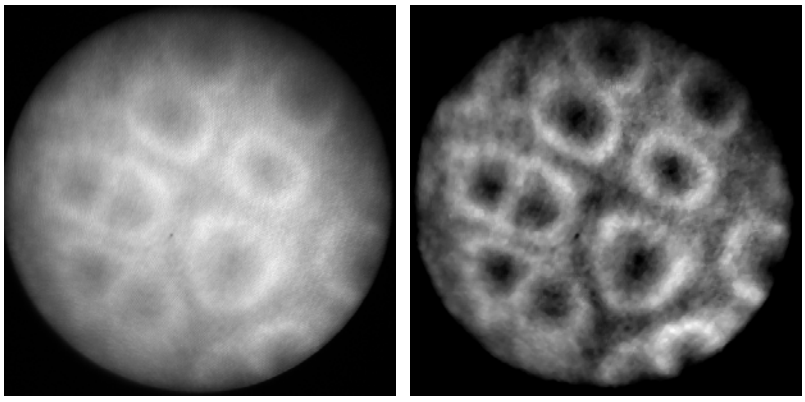
Jerome Mertz
Associate Professor, Department of Biomedical Engineering
Boston University

Dr. Mertz is the director of Boston University's biomicroscopy laboratory. His recent research has included HiLo microscopy, fluorescence endoscopy, dynamic speckle illumination microscopy, differential aberration imaging, nonlinear microscopy, autoconfocal microscopy, and graded field microscopy.

Abstract:

I will describe a new method of obtaining optical sectioning with a standard wide-field fluorescence microscope. The method, called HiLo imaging, involves acquiring two images, one with structured illumination and another with uniform illumination. An evaluation of the local image contrast in the structured-illumination image provides an optically sectioned image with low resolution. This is complemented with high-resolution information obtained from the uniform-illumination image. A fusion of both images leads to a full resolution image that is optically sectioned across all spatial frequencies.

HiLo imaging is fast, robust, and provides a user-defined depth of field. Moreover it works with both fluorescent and non-fluorescent samples, and is generalizable to a variety of illumination and imaging configurations. Demonstrations will be presented of brain tissue imaging with a standard microscope and colon tissue imaging with an endomicroscope.



Conventional endomicroscopy

HiLo endomicroscopy

Current and Desired Capabilities of the Duke Light Microscopy Core Facility

Sam Johnson
Director, Light Microscopy Core Facility
Duke University

Dr. Johnson has been building and running the LMCF since March 2006. During his previous work at the Salk Institute, he became interested in techniques for studying signaling events in living cells. In attempting such techniques he spent a lot of time in rooms with no windows and also learned a fair amount about microscopes, microscopy and image analysis.

Abstract:

The Light Microscopy Core Facility provides a range of optical imaging modalities to a broad array of researchers from across the University and Medical School. This talk will cover several examples of imaging projects being carried out in LMCF using confocal, wide-field, 2-photon and TIRF based imaging of fixed and living samples, and also the visualization, deconvolution and analysis of images. Directions of interest to extend the resources offered will also be mentioned, including a summary of the range of options for implementing super resolution microscopy and how we plan to test these new methods.

Superresolution Imaging of Structural Proteins in Live *Caulobacter crescentus*

Julie Biteen
Assistant Professor, Department of Chemistry
University of Michigan

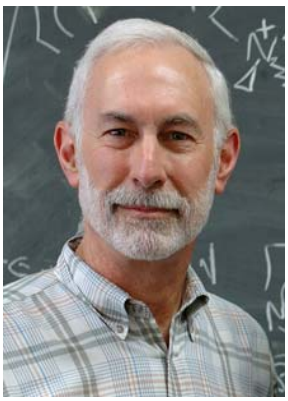
Dr. Biteen's research seeks to maximize the impact of single-molecule fluorescence and nanophotonics by applying them to investigations of live cells. Superresolution techniques based on single-molecule optical microscopy can reach nanometer-scale accuracy. These non-invasive, non-perturbative methods are ideal for investigating biological specimens, and the Biteen group's research is focused on improving these methods and applying them to physiologically relevant problems.

Abstract:

Improving spatial resolution in order to visualize features and phenomena on a sub-diffraction-limited scale is an important challenge for biological imaging. Single-molecule imaging permits nanometer-scale motion to be tracked, and more recently, photoactivation and photoswitching have been used to control single-molecule fluorescent labels and produce images of sub-cellular structures (e.g., PALM, FPALM, and STORM). While previous live-cell studies relied on sophisticated photoactivatable fluorescent proteins, we demonstrate that photoinduced reactivation of the common fluorescent protein EYFP can be used to produce superresolution images of intracellular proteins in living cells.

By imaging only sparse subsets of single fluorophores in each cycle, we localize the emitters beyond the diffraction limit and obtain for the first time sub-40-nm resolution images of the bacterial actin protein, MreB in live *Caulobacter crescentus* cells. To attain true superresolution images, we address limitations arising from physiologically imposed upper boundaries on the fluorophore concentration by employing dark time-lapse periods to allow single-molecule motions to fill in filamentous structures, increasing the effective labeling concentration. We then extend our technique to three dimensions to resolve the midplane ring formed by the prokaryotic cell-division protein, FtsZ. These studies show that EYFP is a useful emitter for *in vivo* superresolution imaging of intracellular structures in bacterial cells.

Watching Ultrafast Molecular Motions in Liquids, Molecules, and Proteins



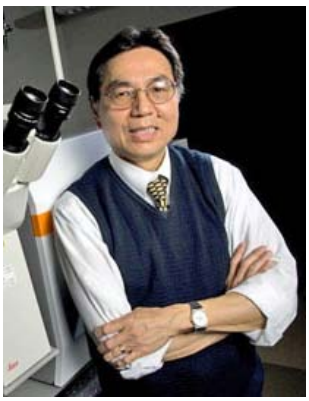
Michael D. Fayer
Professor, Department of Chemistry
Stanford University

Professor Fayer has been a pioneer in the development and application of ultrafast non-linear laser techniques. In large part due to his work, ultrafast nonlinear and coherent spectroscopic techniques such as transient gratings, photon echoes and vibrational echoes have become powerful techniques for studying fast molecular processes, intermolecular interactions, and structure in complex molecular systems. His work has had a profound impact on modern physical chemistry, biophysics, and materials science, and his methods and approaches to the examination of problems involving dynamics and interactions in molecular systems have spread worldwide.

Abstract:

The natural systems we encounter in daily life, from water to the most complex biological structures, are composed of molecular subunits that are inherently small, that is, the nanometer length scale. Because molecules are small, the time scale for molecular motions is very fast, on the order of picoseconds. Constantly evolving inter and intramolecular structures make molecular processes possible. To understand the complex molecular systems that pervade our environment, we need methods that can examine the fast evolution of molecular systems without changing the properties of the systems. Recent technological and theoretical developments have produced a new experimental tool, Ultrafast Two Dimensional Infrared (2D IR) Vibrational Echo Chemical Exchange Spectroscopy, that has achieved this goal. The method makes it possible to study fast molecular motions under thermal equilibrium conditions. The 2D IR chemical exchange experimental methodology is described, and how information is extracted from the 2D IR data is explained. Novel results on liquid, molecular, and protein dynamics are presented.

Plasmonic Nanoprobes for Cellular Sensing and Imaging



Tuan Vo-Dinh
Director, Fitzpatrick Institute of Photonics
Duke University

Dr. Vo-Dinh's research activities have involved biophotonics, laser-excited luminescence spectroscopy, room temperature phosphorimetry, synchronous luminescence spectroscopy, surface-enhanced Raman spectroscopy, field environmental instrumentation, fiberoptics sensors, nanosensors, biosensors and biochips for the protection of the environment and the improvement of human health.

Abstract:

This presentation provides an overview of the development and applications of the plasmonics and surface-enhanced Raman scattering (SERS) nanoprobes and nanostructures for environmental sensing and biomedical diagnostics and ultra-high through put screening. Plasmonics refers to the research area of enhanced electromagnetic properties of metallic nanostructures that produce ultrasensitive and selective detection technologies.

We describe the development of nanoparticle-based SERS technology that has enabled sensitive detection of DNA damage and gene defects. We have developed a novel detection approach that incorporates the "SERS effect modulation" scheme associated with metallic nanoparticles and the DNA hairpin structure. The plasmonics-based MS nanoprobe, referred to as "molecular sentinel" nanoprobe, comprises of a metal nanoparticle and a stem-loop DNA molecule tagged with a Raman label. The nanoprobe utilizes the specificity and selectivity of the DNA hairpin probe sequence to detect a specific target DNA sequence of interest. In the normal configuration and in the absence of target DNA, the stem-loop configuration maintains the Raman label in close proximity to the metal nanoparticle, inducing an intense SERS effect that produces a strong Raman signal upon laser excitation. Upon hybridization of a complementary target DNA sequence to the nanoprobe, the stem-loop configuration is disrupted, causing the Raman label to physically separate from the metal nanoparticle, thus quenching the SERS signal. The SERS nanoprobes have been also applied to hyperspectral bioimaging of cellular systems. Applications in environmental sensing, biomedical diagnostics including the detection of cancer gene probes and infectious diseases are discussed to illustrate the usefulness and potential of plasmonics nanoprobe technology.

Controlling Nanomagnet Dynamics with Ultrafast Spin Torque Pulses

Thomas Crawford
Associate Professor, Department of Physics
University of South Carolina

The Crawford group works to develop metrologies and characterization techniques that enable discovery and innovation in nanomagnetism and nanoelectronics.

Their current projects include reprogrammable parallel nanomanufacturing, ultrafast spin torque switching and magnetodynamics, and novel magnetic materials for biological and radiation sensing.

Abstract:

The last 15 years have seen a dramatic increase in research to understand dynamics in ferromagnetic materials. While largely driven by potential applications in data storage and memory technologies, much of the ongoing research is focused on fundamental questions about how ferromagnets behave at high frequencies, particularly the origin of ferromagnetic damping. Nonetheless, many details remain poorly understood. An important requirement for studying ferromagnetic dynamics is to understand and eliminate extrinsic effects, either by carefully modelling a system, or by experimentally controlling the extrinsic phenomena. Even better would be a ferromagnetic system governed primarily by intrinsic dynamics. The recent discovery of spin momentum transfer, coupled with the giant magnetoresistance (GMR) effect, the subject of the 2007 Nobel prize in Physics, offers the best candidate system yet for studying intrinsic effects: an ellipsoidal nanomagnet. Here spin torque provides a new and exciting handle with which to control nanomagnet dynamics, while GMR provides a method to measure them. In this talk, I will describe a new experimental approach to spin-torque driven magnetodynamics, which was developed at South Carolina. Our approach uses the change in resistance upon nanomagnet switching to map coherent precessional dynamics to either a “0” (unswitched) or a “1” (switched) digital state, allowing precise detection of ultrafast precessional dynamics both at room and low temperatures. Further, we have used this approach to reduce the damping torque in a nanomagnet and observe transient phenomena which occur before a precessing magnetization settles into a zero-damping orbit. Finally, I will discuss extensions to this technique aimed at finding the origins of sub-ps demagnetization in ferromagnets caused by ultrafast laser heating.

Revealing Protein Dynamics with Single Molecule Flourescence

Keith Weninger
Associate Professor, Department of Physics
North Carolina State University

The Weninger group research focuses on revealing the molecular mechanisms at work in complex biological systems with the use of single molecule, optical spectroscopy. They use a variety of optical techniques (including single molecule FRET, single particle tracking, and fluorescence quenching) that are capable of resolving the real-time dynamical motion of individual biological molecules.

Abstract:

Recent technological advances have allowed the motion of individual biological molecules to be observed in real time - an ability that is leading to a highly mechanistic view of biology. In this talk I will describe studies using florescence resonance energy transfer to reveal real-time dynamical motion of biological molecules from several pathways including neurotransmitter release and DNA repair.

Protein Dynamics of Map Kinase Signaling Revealed by Correlation Spectroscopies

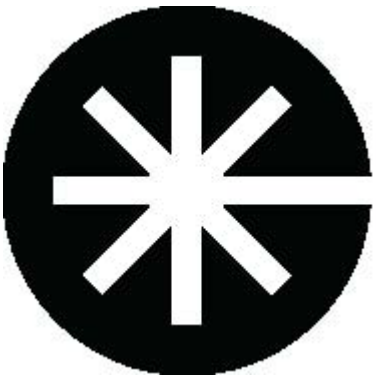
Joel Schwartz
Postdoctoral Associate, Ehlers Lab (Dept. of Neurobiology)
Duke University

The research in the Ehlers Lab is focused at the interface of cell biology and neural circuit plasticity. With a combination of fluorescence and single molecule imaging together with genetic inactivation and mouse genetic models, their work is directed at understanding the organelles and mechanisms for protein trafficking and turnover in dendrites, and the relationship to synapse formation and function.

Abstract:

Correlation spectroscopies, such as fluorescence correlation spectroscopy (FCS,FCCS) and photon counting histograms (PCH), are employed to measure molecular dynamics, interactions, and oligomerization. These strategies were utilized in the model system Saccharomyces cerevisiae to provide insight into the understanding of mitogen activated protein kinase (MAPK) signaling upon activation with mating pheromone. FCS and FCCS data indicate Ste7 (MAPK kinase) interacts with the MAPKs, Fus3 or Kss1 and scaffolding protein, Ste5 associated with Ste7 and Ste11 (MAPK kinase kinase) selectively in the cytosol . PCH analysis revealed cytosolic Ste5s were mostly monomers, and alpha factor treatment increases the dimeric pool of Fus3 and Ste7. Ste5 interacts with Fus3 at the cortex in upon phermone treatment. Two-dimensional PCH provide a measure of absolute stoichometry between Ste50 and the regulatory protein Ste11. The new insight into protein dynamics provided by correlation strategies has prompted the establishment of a novel model for the MAPK kinase signaling in living yeast.

The Next Generation of Lasers Enabling New Scientific Research



Steve Butcher
Director of Scientific Laser Systems
Coherent Inc.

In the highly sophisticated industry of photonics, Coherent, Inc. leads the way, offering reliability, cost, and performance advantages for the widest range of commercial and scientific research applications.

Founded in 1966, Coherent has production and research facilities spanning the world, supplying everything from laser systems and components to laser measurement and control products and precision optics to over 80 countries. Notably, many of its customers are Fortune 500™ leading manufacturers and scientific researchers from numerous universities and institutes across the Americas, Europe, and Pacific Rim.

Markets for Coherent's products include microelectronics (which includes semiconductor test and measurement, flat panel display manufacturing, and advanced packaging), scientific research and government programs, materials processing, and OEM components and instrumentation (which includes such areas as biotechnology, medical imaging and treatment). Since its inception, Coherent has grown through internal expansion and strategic acquisitions of complementary technologies, intellectual property, manufacturing processes, and product offerings. Coherent also consistently invests over 10 percent of its annual revenue into research and development.

The company's strategy is to continue to develop innovative and proprietary products and solutions that meet the needs of customers and that are based on Coherent's core expertise in lasers and optical technologies.

Breasts and Brains, Similarities and Differences: How Lasers fit into Molecular Imaging

Warren S. Warren
James B. Duke Professor and Chair, Department of Chemistry
Professor in Radiology and Biomedical Engineering
Director, Center for Molecular and Biomolecular Imaging, Duke University
2009-2010 Chair, Division of Laser Science, American Physical Society

The Warren group focuses on the design and application of what might best be called novel pulsed techniques, using controlled radiation fields to alter dynamics. The heart of the work is chemical physics, and most of what they do is ultrafast laser spectroscopy or nuclear magnetic resonance.

Abstract:

Recent developments in modern imaging methods (magnetic resonance, PET, CT, optical imaging and others, often in combination) have enabled a critical transition from imaging structure to imaging function, largely by looking for specific molecular signatures (for example, of the changes that occur when cells become malignant). I will try to provide a broad overview of the promise, capabilities, and new directions in these fields, with an emphasis on understanding the connection between what we can measure in tissue and what we would *like* to measure in tissue. I will also highlight the role lasers in general (and ultrafast lasers in particular) play now and will play in the future.

Multi-exciton Dissociation in Quantum Dots

Tim Lian
Professor, Department of Chemistry
Emory University

The Lian group seeks to contribute to the advancement of solar energy conversion science and technology through basic research. Currently, their research efforts are focused on the preparation, characterization and fundamental understanding of photovoltaic and photocatalytic nanomaterials, specifically the fundamental dynamical processes in the materials and their interfaces (such as charge transfer, solvation, energy transfer and relaxation).

Abstract:

Charge transfer to and from quantum dots (QDs) is of intense interest because of its important roles in QD-based devices, such as solar cells and light emitting diodes. Recent reports of multiple exciton generation (MEG) by one absorbed photon in some QDs offer an exciting possibility to improve the efficiency of QD-based solar cells and to design novel multiple-electron/hole catalytic systems. However, the application of the MEG process requires ultrafast exciton dissociation prior to the exciton-exciton annihilation process, which occurs on the 10s to 100s ps time scale. In this presentation we report our recent studies of exciton dissociation dynamics in QDS by electron transfer to adsorbed molecule. We showed that exciton in CdSe can be dissociated on the a few picosecond time scale to various adsorbates. We will discuss the dependence of these rates on the size and the nature of the quantum dots and the dynamics of multiple exciton dissociation.

Surface Plasmon Probing of Electronic Energy Relaxation in Metal Nanostructures

Ken L. Knappenberger
Assistant Professor, Department of Chemistry
Florida State University

Research in the Knappenberger group involves understanding electron- and energy-transfer processes in nanoscale assemblies through development and implementation of novel optical spectroscopy techniques, including single-molecule and time-resolved methods. The resulting detailed chemical information will address critical issues in nanoscale chemical physics and single-molecule analytical chemistry and direct the assembly of carefully designed nanoscale architectures.

Abstract:

Electronic relaxation and interparticle electromagnetic coupling processes in hollow gold nanospheres (HGNs) and HGN aggregates will be described. These plasmon-tunable HGNs exhibit an unexpected, but systematic, blue shift of the surface plasmon resonance spectral position upon nanoparticle aggregation. Femtosecond transient absorption measurements, high-resolution TEM and Finite-Difference Time-Domain calculations will be used to demonstrate that this blue shift results from interparticle coupling, an effect not observed with solid nanospheres. Ultrafast electron-phonon coupling lifetimes for the thin-shelled HGNs increase in aggregates, indicating significant delocalization of the Fermi-gas over multiple particles. A 48-nm HGN with 7-nm shell thickness exhibits ultrafast electron-phonon coupling with a lifetime of 300 ± 100 fs, and upon aggregation, this lifetime increases to 730 ± 100 fs. Interparticle coupling efficiency can be tailored by controlling HGN aspect ratio over a vast range of sizes. Also, it will be shown that time-dependent SPR is a sensitive probe of condensed-phase dynamics.

Nanoscale Agents for in Vivo Optical Imaging and Ultrasensitive in Vitro Diagnostics

Michael J. Therien
Professor, Department of Chemistry
Duke University

The Therien group's research involves the synthesis of compounds, supramolecular assemblies, nano-scale objects, and electronic materials with unusual ground-and excited-state characteristics, and interrogating these structures using state-of-the-art transient optical, spectroscopic, photophysical, and electrochemical methods.

Abstract:

Formed through cooperative self-assembly of amphiphilic diblock copolymers and electronically conjugated porphyrinic near infrared (NIR) fluorophores, NIR-emissive polymersomes (70 nm – 50 mm 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. 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 gives rise to emissive nanoscale vesicles that can not only be selectively targeted, but to nanoscale constructs that detect specific biological markers with exquisite sensitivity.

Towards Single Molecule Spectromicroscopy by Combining STM and Ultrafast Lasers

Kazuo Watanabe
Research Associate, Department of Chemical Engineering
Louisiana State University

Dr. Watanabe works at the Center for Nanophase Material Sciences in Oak Ridge National Laboratory to develop a time-resolved single molecule spectromicroscopy by combining STM and ultrafast lasers with Dr. Minghu Pan and Dr. John Wendelken at ORNL, and Prof. E. Ward Plummer at LSU.

Abstract:

In order to achieve the ultimate spatial, temporal, and energy resolutions for the surface chemical dynamics studies, what is desirable is to combine STM techniques and ultrafast laser spectroscopy. At the present, a couple groups have succeeded in observing STM images under femtosecond laser irradiation. The challenge is to acquire time-resolved STM signal related to photoinduced processes by laser irradiation. Recently tunneling signal in subpicosecond transient time has been detected by a lock-in amplifier referenced to the oscillating delay stage. However, in this scheme, no energy resolution has been obtained so far.

Here we propose a new scheme, the tip-enhanced SFG (sum frequency generation). The STM tip is used to enhance the local electric field near the tunneling junction and the time-resolved SFG signal will be obtained, when combined with a pump pulse. With this scheme the spatial resolution is realized by the STM tip, the time resolution by the ultrafast laser pulses, and the energy resolution by the SFG processes with which molecular vibrations are detected. The time resolution should be vastly superior to the conventional STM scheme where the limitation is the bandwidth of the electronics. In contrast to the latter, the tip-enhanced SFG is a "photon-in-photon-out" method and its time resolution is solely limited by the pulse width of the probe laser pulses.

Recently tip-enhancement has been demonstrated in the TERS (Tip-Enhanced Raman Spectroscopy). Similar tip-enhancement is expected for the tip-enhanced SFG. This method will open up the way to study the single molecule chemical dynamics in the ultimate spatial, temporal, and energy resolutions.

FRAPping Little Bugs

Michael Chua
Director, Michael Hooker Microscopy Facility
University of North Carolina – Chapel Hill

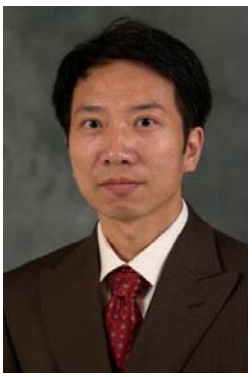
Dr. Chua's research focuses on the development of novel light microscopy and imaging technologies for cell biology and physiology

Abstract:

The microenvironment inside bacteria is not well understood and is difficult to quantify. The high macromolecular content of cytoplasm affects many parameters such as thermodynamic activities, protein aggregation and folding, diffusion, and interpretation of NMR data. With NMR crowding is more significant since proteins need to be over expressed in order to improve NMR sensitivity. In this study protein diffusion in E. Coli was quantified using fluorescence recovery after photobleaching (FRAP) in order to study crowding effects.

E. Coli were either induced to express eGFP or express eGFP with one of α-synuclein, Tau-40, Maltose Binding Protein or Calmodulin. The effect of co-protein expression on eGFP diffusion was measured using raster scanning FRAP on a Zeiss 510 meta confocal microscope optimized for high speed scanning and optimum bleaching with minimum bleaching during readout of eGFP fluorescence. Due to the restricted diffusion space of E.Coli diffusion was analyzed as 1-dimensional diffusion using Fourier analysis. It was found that eGFP diffusion was 7.7 +/- 2.5 um^2s^{-1} in the presence or absence of each of the four over expressed proteins. As a positive control to test the sensitivity of the FRAP technique, eGFP expressing E.Coli were osmotically shocked with 250 mM Sorbitol. This treatment is known to decrease protein mobility and indeed reduced the measured diffusion constant to 2.4 +/- 1.6 um^2s^{-1}. Therefore expression of the four proteins had little effect on eGFP diffusion.

Super-resolution Imaging via Optical Gain



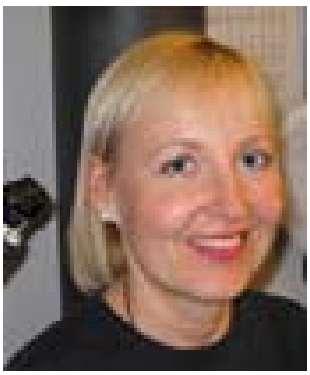
Weiguo (Bill) Yang
Assistant Professor, Department of Engineering
Western Carolina University

Dr. Yang's current research focuses on low loss metamaterials and their applications to photonic and optoelectronic devices, multi-spatial-mode semiconductor laser dynamics, and optical communications systems and subsystems.

Abstract:

In order to get a sharper image than the usual diffraction limit, one only needs to get some part of evanescent spectrum to the imager. Optical gain is found to be able to convert the evanescent waves in air into sustainable propagating waves in the gain media. These sustainable propagating waves carry the high spatial frequency information of a small object in the forms of non-uniform plane waves where the equal phase planes are at an angle with respect to the equal amplitude planes. Pump-probe techniques can then be combined with signal processing approaches to recover the high spatial frequency information at far field and reconstruct the superresolved images. This scheme is fundamentally different from super-lens schemes employing negative index of refraction media (NIM) and hyper-lens schemes employing effective media of indefinite permittivity and/or permeability tensors and does not require or rely on plasmonic or plasmon coupling effects.

Imaging Technologies at the DHMRI Light Microscopy Core Facility



Anne Vaahtokari
Associate Director of Microscopy
David H. Murdock Research Institute

The David H. Murdock Research Institute (DHMRI) is a nonprofit research institute built to provide high quality laboratory services to universities, governments, and businesses. Located at the heart of the North Carolina Research Campus (NCRC) in Kannapolis, DHMRI provides a complete environment containing instrumentation, resident expertise, and well-equipped laboratories that bring together a variety of disciplines (genomics, proteomics, metabolomics, microscopy, and NMR) under one roof.

Abstract:

The Light Microscopy Laboratory at DHMRI is a world-class beta-testing site for Carl Zeiss Microimaging. Besides top-of-the-line instruments, the Light Microscopy Laboratory offers automated image acquisition solutions as well as full range of image analysis services. Today, microscopy is not only used for obtaining images but increasingly for quantative measurements, due to the fact that images are able to carry large amounts of data such as intensities of several signals and simultaneous high-resultion 3D spatial and temporal information for each signal. Remote access capabilities, both for image acquisition and analysis, make the services of the Light Microscopy Laboratory available also for non-local users.

Fully motorized microscopes allow time-saving automated image acquisition such as mosaic (tiling) and multipoint imaging. Automated mosaic imaging is especially useful for tissue sections, and multipoint imaging can be used for multiwell plates. Both are applicable for multichannel and live cell imaging.

Image processing and analysis software includes e.g. deconvolution, 3D reconstruction, tracking, colocalization, ratio imaging, and segmentation-based measurement tools for quantative analysis. The Light Microscopy Laboratory provides expertise both for image acquisition and analysis; from experiment planning to data analysis, the Laboratory staff assists users of the facility.

Observation of Bond-specific Oxidation of Si(111)

Kenan Gundogdu
Assistant Professor, Department of Physics
North Carolina State University

The Gundogdu group's research is aimed at investigation of structural and electronic dynamics in condensed matter systems using ultrafast and nonlinear optical spectroscopy techniques, specifically the dynamics that are relevant to solar energy conversion.

Abstract:

The oxidation of silicon is one of the most technologically important chemical reactions. Because dangling bonds trap and/or scatter carriers, well-organized bonding at Si-SiO_2 interfaces is critical to device performance. Therefore, the dynamics of interface formation is of continuing interest. We use a bond-specific probe, second-harmonic-generation (SHG), and a macroscopic probe, spectroscopic ellipsometry (SE), on two Si orientations to obtain real-time data that reveal in unprecedented detail how H-terminated (111) Si oxidizes.

Laser Round Table

Scott Crane
Senior Sales Engineer, Southeast
Coherent, Inc.

Scott has current responsibility for the Southeast to include, the following states: North and South Carolina, Tennessee, Georgia, Florida, Alabama, Mississippi and Louisiana, and Puerto Rico.

An audience discussion with sales engineer Scott Crane and Director of Scientific Laser Systems Steve Butcher of Coherent, Inc. Among the topics to be discussed:

- The latest in laser technology and upgrades, and what lies at the horizon
- A discussion of what is needed for the next generation of lasers to support future research areas
- How short pulse, higher energy, and extended wavelength range can enable your research
- Solicitation of feedback for ways that service and support can better serve the research community
- What sort of communication channels best serve you?
- Conference feedback… how can we improve to make this a better and more valuable meeting?

A final message from Coherent:

We are always looking for superior talent, and invite qualified students to inquire as to current opportunities for internship program and employment.

We have a very qualified team of support engineers available to help you in any way we can. Please give me a call if I can be of any assistance. Scott.crane@coherent.com or 800-400-3008.