

Lester Wolfe Workshop in Laser Biomedicine

Femtosecond Microscopy and Microsurgery: Make It Fast!

Tuesday, April 18, 2006

4:00-6:00 pm

Thier Conference Room, Thier Research Building
Massachusetts General Hospital
50 Blossom Street, Boston

Refreshments served at 3:30pm

Modern developments in laser technology have provided the ability to deliver very short pulses of light in the femtosecond range. This technology has led to novel applications in tissue imaging such as multiphoton fluorescence microscopy and second harmonic generation microscopy. Ultra short laser pulses also allow unprecedented control of surgery on a sub-cellular scale. This workshop will explore these issues.

Subcellular Surgery and Nanosurgery

Eric Mazur, Harvard University

In Vivo Brain Imaging Using One- and Two-Photon Fluorescence Microendoscopy

Mark Schnitzer, Stanford University

New Techniques in Confocal Microscopy

Jerome Mertz, Boston University

Sponsored by the GR Harrison Spectroscopy Laboratory, MIT, MGH Wellman Center for Photomedicine, the Harvard-MIT Division of Health Sciences and Technology, and the Center for the Integration of Medicine and Innovative Technology (CIMIT)

SUMMARY 4/18/2006

The meeting was devoted to the Lester Wolfe Workshop in Laser Biomedicine and focused on “Femtosecond Microscopy and Microsurgery”. Eric Mazur, PhD, Harvard, spoke on “Subcellular Surgery and Nanosurgery”. This work stems from the lab’s development of femtosecond laser pulses as tools for micromachining, using optical breakdown to controllably remove small (μm^3 scale) volumes of material. Initial studies of the technique used materials such as glass and silicon as targets. The laser source is usually a Ti:sapphire laser producing 50-fs, 1-mJ pulses at 800 nm. Threshold energies for material removal are in the nJ ranges. In more recent work femtosecond laser pulses are used to manipulate sub-cellular structures inside live and fixed cells, to selectively disrupt individual mitochondria in live bovine capillary epithelial cells and to cleave single actin fibers in the cell cytoskeleton network of fixed human fibroblast cells. Using YFP labeling, the actin fibers have been cut at desired locations and their retraction as a function of time observed. The technique has also been used to micromanipulate the neural network of *C. Elegans*, a small nematode, by sniping individual axons in specific neurons and observing the effect on behavior.

Mark Schnitzer, PhD, Stanford University, described “In vivo Brain Imaging Using One- and Two-photon Fluorescence Microendoscopy”. 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 based on minimally-invasive microlenses (350-1000 microns in diameter) offer micron-scale resolution and have enabled visualization of neurons and blood cells in deep areas of the live mammalian brain. One-photon imaging is limited to depths of about 100 microns, while two-photon excitation, typically using 800-nm light, allows deeper (500-micron) penetration. Numerous (> 100) endoscopic microscopes, based on GRIN lenses integrated with optical fibers for focusing and for relaying of light, have been built. Microendoscopy has recently been used to obtain the first-ever images of the auditory hair cells and hair bundles within the live mammalian cochlea. A chronic mouse preparation has been developed and used to enable *in vivo* microendoscopy imaging of fluorescent CA1 hippocampal pyramidal cells over several months after an initial surgery. The lab has also built a compact (3.9 gram) two-photon fluorescence microendoscope that is based on fiber-optics and intended for brain imaging in freely moving mice.

Jerome Mertz, PhD, Boston University, described “New Techniques in Confocal Microscopy”. Several novel 3-dimensional imaging techniques have been developed over the recent years. Some of these, such as two-photon fluorescence or second-harmonic generation microscopy, involve the use of femtosecond lasers. Others, such as structured-illumination microscopy, involve the use of high-contrast illumination patterns. Two new 3-dimensional imaging techniques, both adapted to imaging in relatively thick tissue, were described. Autoconfocal microscopy is a non-fluorescence scanning confocal microscopy technique that makes use nonlinear detection, via a nonlinear crystal, to produce a “virtual pinhole”. Autoconfocal microscopy can readily be combined with 2-photon microscopy, producing simultaneous co-localized fluorescence and phase-gradient images in thick tissue. The second technique is called dynamic speckle illumination microscopy. This simple and robust fluorescence imaging technique leads to out-of-focus blur rejection similar to a confocal microscope, but without laser scanning.