

# Bio-Imaging News



SUMMER ISSUE 2005

## 2005 Symposium on Biological Imaging

On Friday, September 9, 2005 the W.M. Keck Laboratory for Biological Imaging and the Laboratory for Optical and Computational Instrumentation at the University of Wisconsin-Madison, in cooperation with Promega Corporation and the BioPharmaceutical Technology Center Institute will be holding "The 3rd Symposium on Biological Imaging," with a main focus on the approaches and innovations to multidimensional biological imaging.

### OVERVIEW

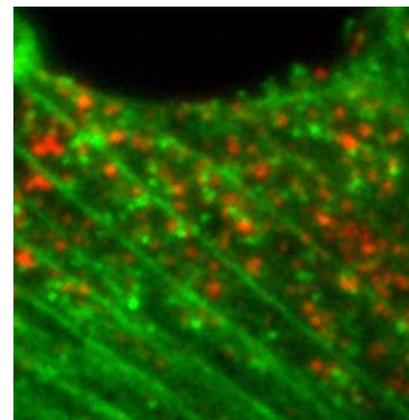
The ability of imaging technology in biology to move beyond the generation of static two-dimensional images of fixed specimens has become increasingly impor-

tant in understanding the real-time dynamics of biological systems, whether they are cells in culture or intact organisms. A shift towards multidimensional in vivo imaging is key not only for the study of normal structure and function, but also for shedding light on the pathophysiology of actual or model diseases, the pharmacology of drugs, as well as the potential toxicity of drugs and environmental contaminants.

The addition of information about volume to image data, typically by the collection of multiple XY image planes, has allowed for the visualization of living structures as they exist in three dimensions. It has also created the demand for increasingly sophisticated imaging software to collect, display, and analyze the large data sets that are generated. Adding the dimension of time

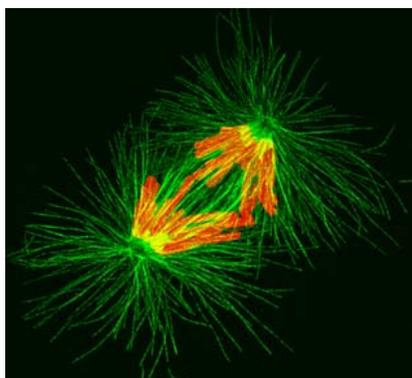
to two- or three-dimensional images introduces similar computational challenges, but has been invaluable in visualizing and quantifying protein interaction and transportation, membrane dynamics, nucleic acid modification, and changes in ion concentration within cells.

Although there has been substantial progress in improving the time scale and spatial extent of imaging data collected, the traditional lateral and axial resolution limits that are imposed when images are collected with wavelengths of visible light have remained largely intact. The Symposium keynote address will be delivered by Stefan Hell. His lecture will describe imaging techniques recently developed in his laboratory at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany that have exceeded the diffraction limit of visible light, allowing a substantial increase in the resolution of fluorescence microscopy to nanometer scale imaging.



The Symposium will also present other recent advances in multidimensional biological imaging. Lectures and poster presentations will highlight how these techniques can be applied in solving a variety of biological problems, ranging from imaging subresolution structures to intact human tissue. Topics covered will include: total internal reflection (TIRF) microscopy, fluorescence resonance energy transfer (FRET), whole animal molecular imaging, protein tracking with GFP, nanoscale fluorescence microscopy, multidimensional image analysis, and near-infrared diffuse optical imaging. In addition, the Symposium will feature interactive workshops on fluorescence lifetime imaging microscopy (FLIM) and automated software-based methods for image acquisition and analysis.

- For additional information on the symposium visit: <http://www.btc1.org/bioimaging/2005/>



## FEATURED LAB: Paul Ahlquist Lab



Professor Paul Ahlquist of the Institute for Molecular Virology at the University of Wisconsin – Madison is currently studying the mechanisms of virus replication, gene expression, evolution, and virus-host interactions.

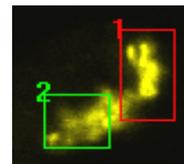
In particular they are exploring the novel, RNA-based pathways and virus-host interactions underlying replication, gene expression and evolution by positive-strand RNA viruses, the largest class of viruses. They are also studying selected replication processes of a reverse-transcribing virus, hepatitis B virus, which is also a major human tumor virus. Their studies integrate molecular genetics, genomics, biochemistry and cell biology to address fundamental questions in virus replication and virus-cell interactions.

Billy Dye, a postdoc in Paul Ahlquist's lab in the Institute for Molecular Virology, is using Fluorescence Loss In Photobleaching (FLIP) to monitor the intracellular movement of a viral replication protein. Protein A, the RNA-dependent RNA polymerase of flock house virus (FHV), is expressed in yeast (*S. cerevisiae*) cells as a fusion to YFP and localizes to outer mitochondrial membranes. (Shown in the top left panel of part A is a fluorescence micrograph of a single yeast cell expressing protein A-YFP.) FLIP is performed by repeatedly photobleaching one area of a live cell (box 1, red) while monitoring the fluorescence of a non-photobleached area (box 2, green). If the fluorescent protein is highly motile, the fluorescence intensity in box 2 will decrease rapidly as protein moves from box 2 into box 1 and is photobleached. Protein A exhibited low motility, seen as a slow decline in the intensity of box

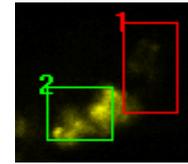
2 (A, graph) and the persistence of fluorescence in box 2 after 400 seconds of photobleaching box 1 (A, bottom left panel). In contrast, when an endogenous yeast mitochondrial protein was expressed as a YFP fusion and assessed by FLIP (B), a rapid loss of fluorescence in box 2 was observed, indicating high motility.

**A**

### FLIP with FHV protein A-YFP



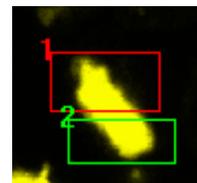
before bleaching



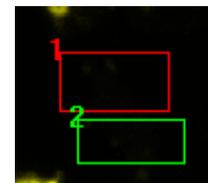
after bleaching

**B**

### FLIP with a motile YFP fusion



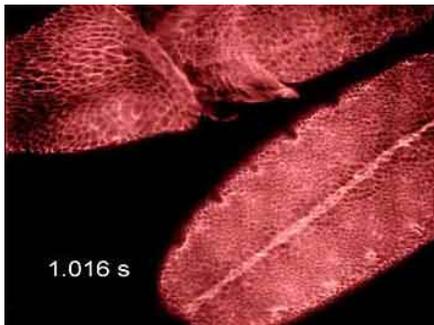
before bleaching



after bleaching

## Zeiss Introduces the LSM 5 Live

Carl Zeiss International recently introduced the exclusive LSM 5 LIVE, a laser scanning microscope designed for live cell imaging. The goal of this microscope is to provide scientists with a closer view of cellular interaction mechanisms



and processes within living samples.

The LSM 5 LIVE not only opens new the doors in confocal microscopy, but it does so at a faster speeding enabling 120 images to be recorded per minute. Also featured in this model is a new optical concept, that provides high resolution imaging and exceptional sensitivity.

The LSM 5 LIVE is an ideal instrument to use in the imaging of the



movement of intracellular molecules in ultra-fast processes such as cell adhesion, cell motility and cell signaling. The combination of the microscope's CCD line detector, zoom optics, and ultra fast Z-drive permit the user to obtain 3D images stacks every second.

## Fluorescence Loss in Photobleaching (FLIP)

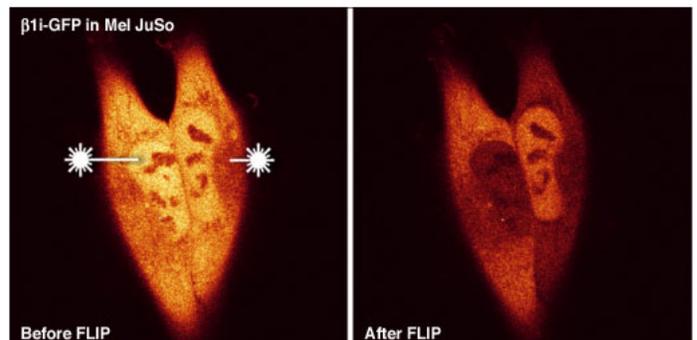
The technique of Fluorescence Loss in Photobleaching (FLIP) has recently gained popularity. FLIP is used today in cellular imaging to investigate the dynamics of molecular mobility in membranes or living cells.

In FLIP an area on a sample experiences repetitive photobleaching, while the disappearance of fluorescence in a region adjacent is monitored. Information obtained from this procedure can reveal the location,

movement, and interactions of proteins.

Other similar techniques that are also gaining popularity include Fluorescence Recovery After Photobleaching, Fluorescence Lifetime Imaging, and Fluorescence Localization After Photobleaching.

A combination of FLIP and the aforementioned techniques are helping to discover new aspects of protein dynamics and the biological processes that they regulate.



## Preview of Fall Lecture Series

The Biological Imaging Series will resume this fall for the fourth consecutive year. This series provides students, staff, and faculty with an opportunity to learn more about a variety of imaging methods.

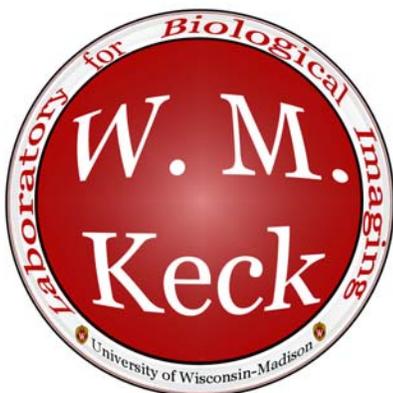
This year the series will be held on the second Tuesday of the month in 341 Bardeen, unless otherwise noted. Refreshments are served.

For the Spring 2005 Semester, the Biological Imaging Lecture Series has a number of interesting lectures lined up, starting with our September 13th Lecture given by Pawan Sinha from the Department of Brain and Cognitive Sciences at the Massachusetts Institute of Technology.

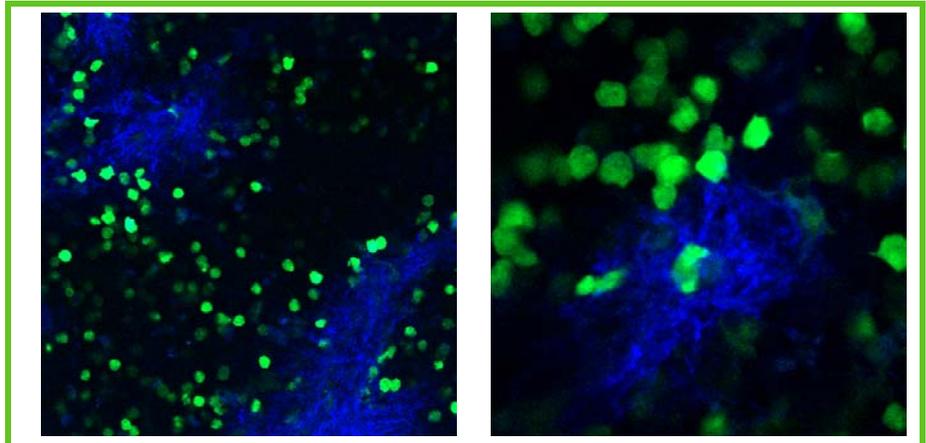
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## ACKNOWLEDGED IMAGE



Whole animal *in vivo* image of mouse spleen acquired using multiphoton excitation at 900nm. Macrophages expressing eGFP ( green ) are seen intermingled with autofluorescent collagen fibers ( blue ). Image courtesy of Lance Rodenkirch ( W.M. Keck Laboratory for Biological Imaging ) and Jacquelin Walisser ( Laboratory of Chris Bradfield ).

## The W.M. Keck Laboratory Upgrades Bio-Rad MRC 1024 Confocal System

Recently The W.M Keck Laboratory for Biological Imaging extensively upgraded it' s Bio-Rad MRC 1024 confocal microscope with the following changes:

The operating system has been upgraded from OS2 Warp to Windows NT.

A new computer has been installed with a much larger hard drive and a CD burner.

An acoustic optical tunable filter ( AOTF ) has also been installed, which will enable users to vary the power of the laser lines independently of each other.

The display has been switched to a dual monitor system, providing for a greater imaging work space.