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W.M. KECK LABORATORY FOR BIOLOGICAL IMAGING

Bio-Imaging News

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Leica Introduces the 4pi System

At the Spring 2004 Photonics East meeting in Philadelphia, PA, Leica displayed the first 4Pi microscopy system, which allows scientists better resolution than any other light microscope. The main concept for any 4Pi microscope is to add the wavefronts of two opposite objective lenses. This sharpens the focal point by a factor of 3-7 in the z-direction, allowing for thinner slicing in a z-series. The new system allows imaging at or below 75 nm resolution, smaller than the 250 nm resolution in conventional light microscopes.

The father of 4Pi microscopy and designer of Leica's system, Stefan Hell, is director of the Department of Nanobiophotonics at the Max Planck Institute of Biophysical Chemistry in Gottingen, Germany. He has combined stimulated emission deletion (STED) and confocal microscopy to create his 4Pi system. The STED works by focusing a laser on a 200 nm spot, and then a second ring-shaped laser pushes the excited molecules back down to the ground state before they emit a fluorescent photon. The 4Pi system focuses the two beams onto a single spot, which has allowed Hell to image a 35 nm-wide bacterial membrane.

www.uchsc.edu/ightmicroscopy/sted4pi.htm

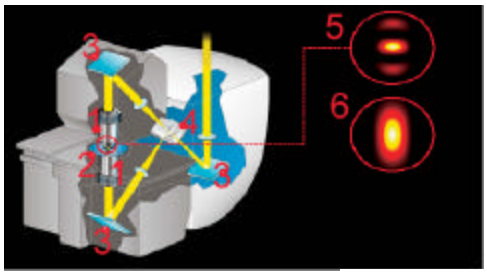
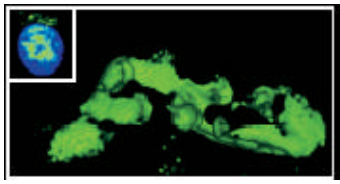
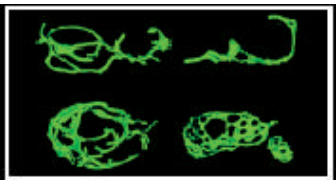


Diagram of Leica's 4Pi Microscope.

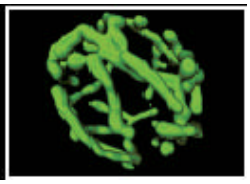
1. Special high NA objectives
2. Sample holder
3. UHQ surface mirrors
4. UHQ non-polarizing beam splitter
5. 4Pi point spread function
6. Two photon point spread function



Golgi apparatus in a live Vero cell at 100 nm spatial resolution. The inset shows and epifluorescence image to correlate the Golgi with the nucleus. Label: EGFP. From Egner et al. (2003) J. Struct. Biol.



Mitochondrial matrix in living yeast for different mutants and growth conditions. Label: EGFP. From Egner et al. (2002) PNAS.



Mitochondrial matrix in living yeast. Label: EGFP. From Gugel, Leica Microsystems, Heidelberg Germany.

In Vivo Imaging with Fiber-Coupled Confocal Microscope

Cell-viZio Technology is a new confocal microscope being sold by Mauna Kea Technology.

This is the first confocal microscope with a custom made fiber optic probe, allowing scientists to image 100 µm under the skin of a living animal. The instrument's design is as follows: light from a 488 nm laser diode is directed into a 30,000 count fiber bundle 2 µm in diameter. On the end of the fiber bundle is a custom made objective with diameter ranging from 350 µm to 1.8 mm. The fluorescence signal is collected trough the same fiber bundle. This microscope presents optical slices of tissue that can be observed at a depth of 80 µm, a lateral resolution of 2.5 µm and axial resolution of 15 µm.

www.maunakeatech.com

Imaging Software

Imaging techniques and image software development are constantly changing and being refined. The relatively innovative techniques of multi-photon and confocal imaging have advanced to a new level. Numerous free and commercial software packages have been developed to deal with images from these types of systems. The following software is recommended for users at the Keck Lab.

- *CircuSoft PFRET*, an analysis program designed to correct spectral bleed-through, was developed at the W.M. Keck Center for Cellular Imaging at the University of Virginia. The program can quantitatively analyze FRET experiments by calculating FRET efficiency and determining the distance between donor and acceptor molecules. This software is optimized for the Radiance 2100 using the 457 nm line with along with GFP and YFP.

www.circusoft.com/pfret.html

- *Confocal Assistant* is a free PC-based program for use with the PIC files from the Bio-Rad MRC-1024 confocal or Radiance 2100 multi-photon microscopes. This software has 3D capabilities.

www.brc.cornell.edu/brcinfo/mif/links.php

- Another software program that is used for confocal/multi-photon imaging is *LaserSharp2000*. This software is available at the Keck Lab and is used specifically on the Bio-Rad Radiance 2100. Contact the Keck Lab for a copy of this software.

- A free program developed at the NIH called *NIH Image* is also useful for image processing and analysis. *NIH Image* is designed for the Mac, but a highly similar PC-based called *Scion Image* is also available.

rsb.info.nih.gov/nih-image/

www.scioncorp.com/frames/fr_scion_products.htm

- *Image J* is free image anaylsis software steaming from based on *NIH Image*, having Mac and PC capabilities. *Image J* aids in analysis of a z-series. This software has been used as an alternative to the *LaserSharp2000* and *Confocal Assistant*.

rsb.info.nih.gov/ij/

- *VisBio* is designed for easy visualization and analysis of multidimensional image data. It was developed by Curtis Rueden at the Laboratory on Computational Imaging (LOCI) at UW-Madison. The program works with in conjunction with *Image J*.

www.loci.wisc.edu/visbio/

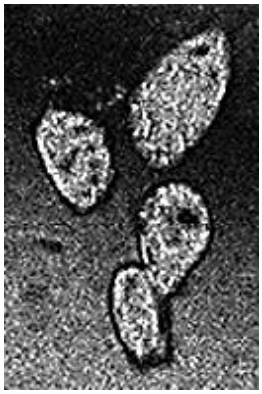
- The Keck Lab has links to more information and other software on its website.

www.keck.bioimaging.wisc.edu/

Confocal Reflection Microscopy

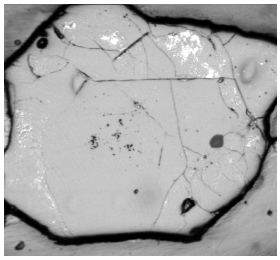
Confocal reflection microscopy can be used to gather additional information from a specimen with relatively little extra effort, since the technique requires minimum specimen preparation and instrument reconfiguration. In addition, information from unstained tissues is readily available with confocal reflection microscopy, as is data from tissues labeled with probes that reflect light. The method also can be used in combination with more common classical fluorescence techniques.

For more information on confocal reflection imaging please contact Lance Rodenkirch at the Keck Lab.



Confocal reflection image showing aggregated 11 nm gold particles attached to an E membrane receptor of A549 carcinoma cell line. Image courtesy of Long Yan, Laboratory of Nimmi Ramanujam, Department of Biomedical Engineering, UW-Madison.

www.engr.wisc.edu/news/ar/2001/bme.html



Confocal reflection image of the oldest known material from the Earth, a single crystal of zircon from the Jack Hills metaconglomerate, Western Australia. Concentric, magmatic growth zoning is shown about the crystal core. The crystallization age has been determined at 4.40Ga. Image courtesy of Lance Rodenkirch and John Valley, Department of Geology and Geophysics, UW-Madison.

www.geology.wisc.edu/zircon/zircon_home.html

Calcium Imaging of Cancer Cells with Chemotherapeutic Treatment

Shigeki Miyamoto's lab in the Department of Pharmacology at UW-Madison has been studying the transcriptional factor NF-κB. Craig Berchtold, in the Miyamoto laboratory, has been studying the relationship between calcium and this transcription factor.

Genomic stress producing double stranded DNA breaks rapidly induces the transcription factor NF-κB. Preliminary studies in the Miyamoto lab suggest calcium mediates this NF-κB cell survival pathway, which can be induced with agents commonly used for cancer treatment. Tumor cells preloaded with the calcium sensitive probe Fluoro-4 elicited a rapid calcium increase with application of the chemotherapeutic agent VP-16 at 10 µM (Figure 1).

Treatment with VP-16 elicited a statistically significant ($P<0.001$) calcium increase at the 2 minute and 2 minute 15 second time points. DMSO application did not produce a statistical significant response within this 5 minute assay (Figure 2).

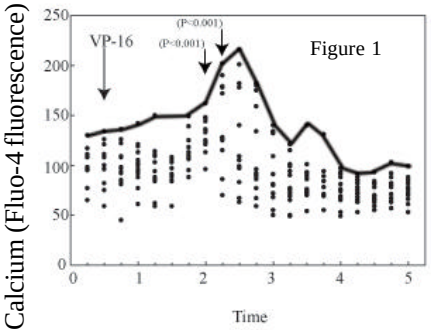
The data represent 4 individual experiments for each treatment with the analysis consisting of at least 3 different regions within the confocal imaging field. Each field measured the response of approximately 8 cells.

The data illustrated in Figure 2 is the most recent data for this experiment.

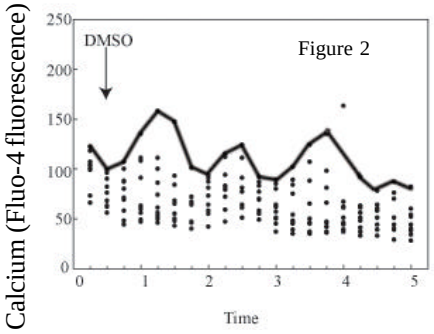
The image clearly represents no increase in calcium activity. An early concern in the experimental design was that DMSO in the culture media could influence membrane permeability in the cells and falsely increase the amount of calcium.

However, DMSO did not appear to effect cytoplasmic calcium levels. Further examination of these studies that used Image J and Sigma Stat 2.0 software are displayed below.

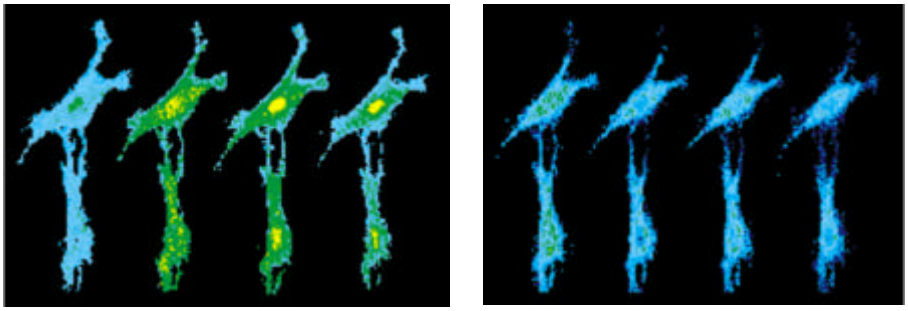
Each newsletter will focus on the research of one laboratory currently involved in biological imaging techniques. If interested in submitting experimental data please contact Lance Rodenkirch.



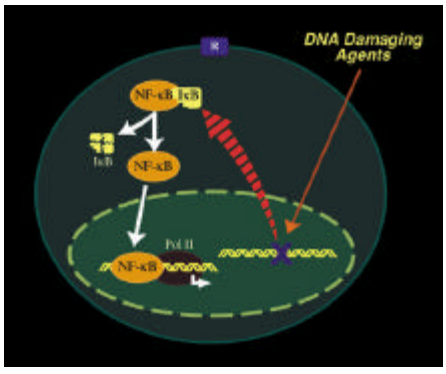
Time



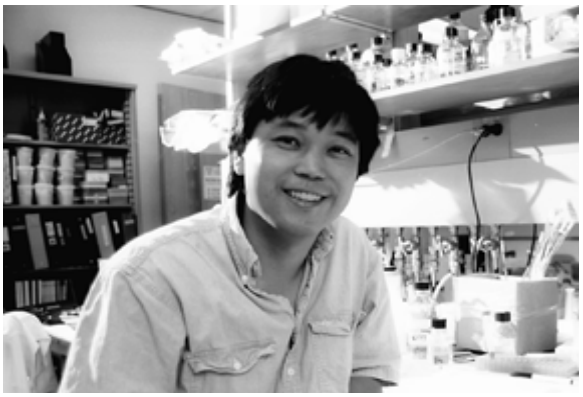
Time



Increase in cytoplasmic calcium during VP-16 treatment. FI (fluorescent intensity) is measured as a span of 0 fluorescent units (black) to a saturated level of 255 fluorescent units (yellow). Calcium measurements were visualized using Fluoro-4 (Molecular Probes) and recorded with a Bio-Rad MRC-1024 laser scanning confocal microscope.



NF-kB activation by DNA damaging agents.



Shigeki Miyamoto, Associate Professor, Department of Pharmacology, UW-Madison.

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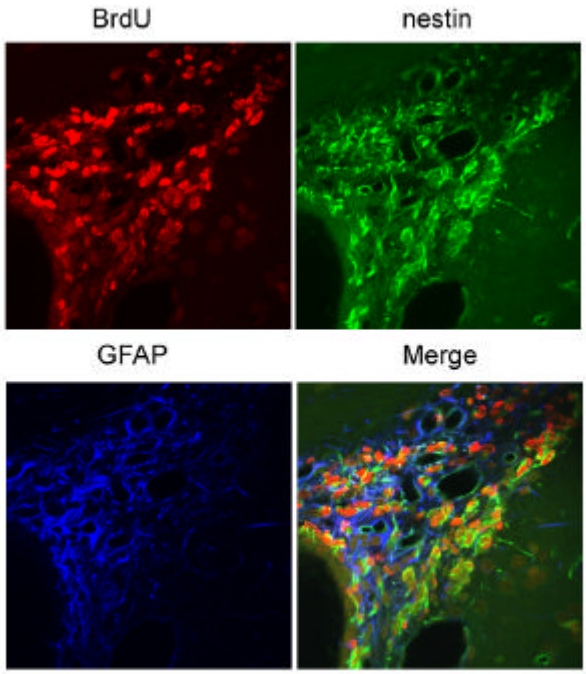
Acknowledged Image



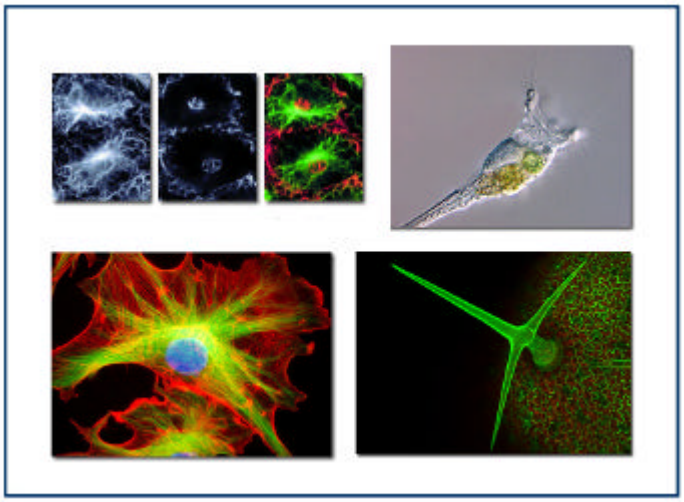
Coronal sections through the subventricular zone, one of two known sites of neural stem cell proliferation in the adult rat brain. This animal received daily injections of bromodeoxyuridine (BrdU), a thymidine-analog incorporated into the DNA of dividing cells, for the last month of its adult life. After sacrifice, sections 40 microns thick were triple immunostained for BrdU (red), a neural stem cell marker nestin (green), and an astrocyte marker GFAP (blue). Images were captured sequentially, using the 60x objective on the Bio-Rad 1024 confocal microscope. Image cortesy of Abigail Rao, Laboratory of Ron Kalil, Department of Opthalmology and Visual Sciences.

The mission of the W.M. Keck Laboratory for Biological Imaging is to provide researchers with access to confocal and multi-photon imaging systems and the expertise to apply these tools effectively. The Keck Lab also is committed to working with users to develop new applications for confocal and multi-photon microscopy in a wide range of research areas.

Fall 2005 Symposium on Biological Imaging

The W.M. Keck Laboratory for Biological Imaging and The Laboratory for Optical and Computational Instrumentation at the University of Wisconsin-Madison along with Promega Corporation are sponsoring the third biannual Symposium on Biological Imaging this fall, tentatively scheduled for September 16th-23rd. The theme of this year's conference is Multidimensional Imaging: Approaches and Innovation. The current list of speakers includes Wolfhard Almers from Oregon Health and Science University, Sanjiv Gambhir from Sanford University, Stefan Hell from the Max Planck Insitute for Biophysical Chemistry, Jennifer Lippincott-Schwartz from the National Institutes of Health, Badrinath Roysam from the Rensselaer Polytechnic Institute, Ted Salmon from the University of North Carolina, and Bruce Tromberg from the University of California at Irvine. Like previous symposia, this year's gathering will include presentations, poster sessions, workshops, product demonstrations, and. The symposium will take place at the Biopharmaceutical Technology Center on the Promega campus in Fitchberg, WI. Additional details and registration information will appear on the Keck Lab's website.

www.keck.bioimaging.wisc.edu



Images courtesy of Nikon.