Volume 1, Issue 1

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W. M. KECK LABORATORY FOR BIOLOGICAL IMAGING

Bio-Imaging News

Bio-Rad Radiance Rainbow

Soon, The W. M. Keck Laboratory for Biological Imaging will be adding a new multiphoton microscope to complement the current Bio-Rad 1024 confocal microscope.

With funding awarded to The Keck Laboratory through a NIH Shared Instrumentation grant, a Bio-Rad Radiance 2100 Rainbow hybrid confocal/multiphoton imaging system has been purchased.

The Radiance 2100 Rainbow system will include a 4-line Argon, and green HeNe and red diode lasers. These six lasers emit at 457, 477, 488, 514, 543 and 633nm. The 457nm line will excite CFP allowing for CFP/YFP FRET pairs.

The Radiance 2100 system also will be equipped with a tunable Ti: Sapphire ultra-fast laser from Coherent. The tuning range for this multiphoton laser is rated at 720nm to 930nm, but may extend to 1um in practice. The Radiance 2100 will be mounted on a Nikon TE2000 inverted microscope to take advantage of the improved live-cell imaging capabilities of multiphoton excitation.

This new imaging system also will include an advanced software suite that will allow for full sensitivity spectral detection and un-mixing. The Rainbow Spectral Detection System will provide 3nm spectral resolution in 10nm steps from 450-670nm. It is the first confocal imaging system to accomplish this without the use of dispersive elements such as gratings or prisms.

The Rainbow uses sets of long-pass and short-pass filters to establish 'spectral windows' that can be defined to optimally detect any overlapping fluorophores. One unique aspect of

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BioRad Radiance Rainbow system equipped with inverted microscope configuration

Biological Imaging Lecture Series Spring 2004

The Biological Imaging Lecture Series, in its third year of existence, provides students, staff, and faculty the opportunity to hear lectures on new and established imaging techniques. The Series is held on the first Tuesday of every month in 341 Bardeen from 4 to 5pm. Refreshments are served. Hope to see you all there!

February 3 --- Patricia Keely, The Department of Pharmacology and Biomedical Engineering, UW–Madison. " Regulation of Breast Cell Behavior by Collagen Matrices: Molecular and Imaging Approaches"

March 2 --- Jeff Hardin, The Department of Zoology, UW-Madison. "Imaging Embryos in the Fourth Dimension"

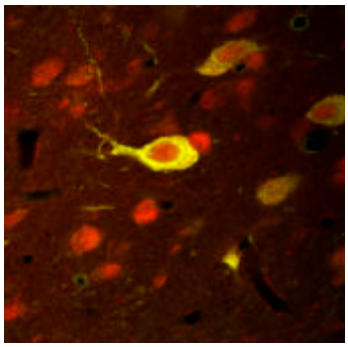
April 6 --- Ammasi Periasamy, The Departments of Biology and Biomedical Engineering and Director of the W. M. Keck Center for Cell Imaging, University of Virginia. "Dynamic Imaging of Protein Molecules: FLIM-FRET Microscopy"

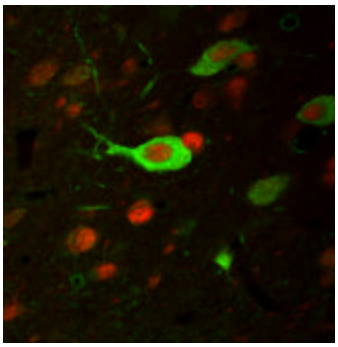
May 4--- Jeff Lichtman, The Department of Anatomy and Neurobiology, Washington University. "Monitoring Synapse Elimination in Fluorescent Neonatal Mice"

Inside this issue:

Why use Sequential Confocal Scanning?

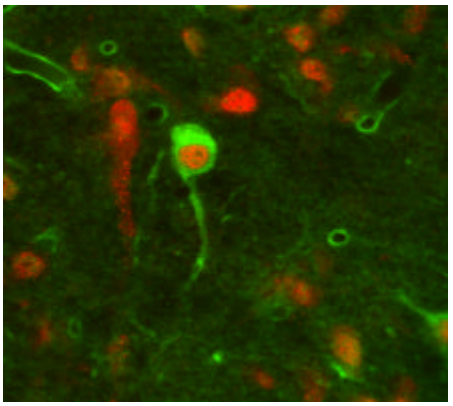
Sequential confocal imaging is a technique in which known fluorochromes are excited sequentially with individual laser lines. The collected images are then merged during post-imaging processing. When this technique is employed, possible crosstalk, aka "bleedthrough", is eliminated. This is an important consideration because when simultaneous imaging of multiple fluorochromes is done using multiple laser lines for excitation, false positives may be recorded by shared emission among the fluorochromes. FITC or fluorescein is the most common culprit in this situation. The maximum absorption of FITC is 488nm, but it can be excited out to nearly 650nm. When simultaneously imaging double-labeled samples with FITC and a red dye, such as Texas Red, long wavelength fluorescence from FITC can appear in the red PMT. Such cross-talk can be minimized by substituting an alternative fluorophore for FITC. For Example, Oregon Green or Alexa 488 are green long wavelength fluorophores that have been designed to have a much shorter emission tail. The images below show a cell double-stained for two antigens, one found exclusively in the cell cytoplasm and the other in the cell nucleus. A red fluorophore was used to detect the nuclear antigen and FITC was used to detect the cytoplasmic antigen. However, while FITC emits optimally at 520nm, it can emit out to 650nm. With simultaneous imaging using the green and red PMTs, both channels will collect emission from FITC. This will give the false impression that the two antigens are co-localized in the cell cytoplasm (left image, yellow). Sequential imaging avoids false positives by collecting fluorescence emission first with one channel and then with the other (right image). The difference is very pronounced and the results show two completely different images. The sequential method should be employed when co-localization results are critical.

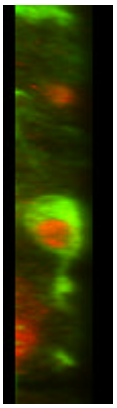




Vertical Section– A Powerful Confocal Method

An underutilized feature of the Bio-Rad 1024 confocal system is the vertical section command. A vertical section (or XZ section) allows you to choose a line through a feature of interest from your normal XY image. That line is scanned repeatedly as the focus motor is moving thus producing a cross-section profile through the feature. A vertical section can be accomplished by setting Z start to focal plane above the feature of interest and setting Z stop to below the feature of interest. This ensures that the entire feature is sectioned. Kahlman averaging can be set in exactly the same way as for a Z-series. The left image represents a full XY scan, while the right image is a full Z scan along a line centered over a feature of interest. Using the vertical section function allows spatial information to be collected while minimizing photobleaching.





New Products in Light Microscopy

Vector Laboratories has developed VECTASHIELD hard-set mounting medium. This product has anti-fade and anti-photo bleaching properties, but unlike conventional VECTASHIELD mounting medium this new product hardens when coverslipped. The hardening decreases movement of the slide and coverslip which lessens specimen damage. VECTASHIELD hard-set also comes with DAPI. For additional information go to Vector Laboratories website; www.vectorlabs.com.

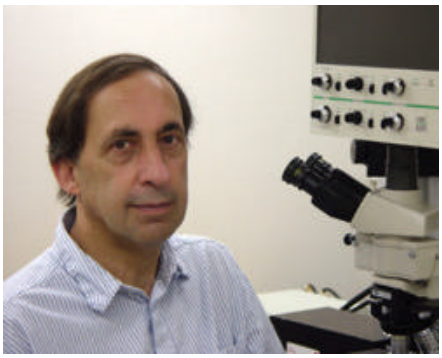
BD Biosciences has a new product on the market, a 35 mm BD Biocoat coverslip-bottom dish (Cat. #354077). This dish has low background fluorescence and is useful when working with inverted microscopy, fluorescence imaging, and confocal microscopy. For additional information go to BD Biosciences website; www.bdbiosciences.com.

Molecular Probes is now offering the Slow Fade Light Antifade Kit. This new antifade kit prolongs the fluorescence emission of fluorescein and helps maintain the initial fluorescence intensity of fluorescein. If used in photomicroscopy, Slow Fade Light Antifade Kit should help to increase signal-to-noise ratio. This new formula is an improvement upon the original, because it quenches the fluorescence of AMCA and Cascade Blue fluorophores only slightly.

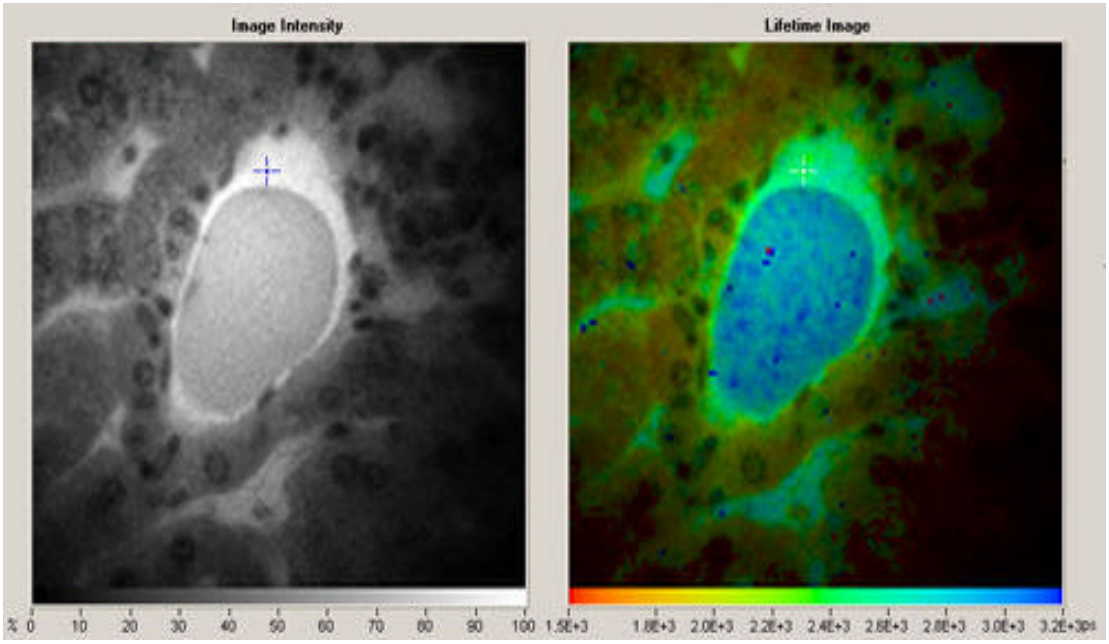
Featured Research

In every issue of the Biological Imaging Newsletter there will be an article featuring imaging research applications here at UW-Madison. This research will involve confocal or multiphoton microscopy in collaboration with The W. M Keck Laboratory for Biological Imaging.

Collectively, the techniques of photon-based experimental manipulation and advanced optical imaging of live tissues define the field of *Biophotonics*. A leader in *Biophotonics* research at the UW-Madison is Professor John White of the Departments of Anatomy and Molecular Biology. Dr. White's NIH funded Instrumentation group the "Laboratory for Optical and Computational Instrumentation" (LOCI) is committed to developing advanced instrumentation and software for live cell imaging. LOCI aims to catalyze interdisciplinary collaborations between diverse departments in projects involving the use of advanced biophotonics techniques. Collaborations are currently being pursued in LOCI with the Departments of Biomedical Engineering, Computer Science, Pharmacology, Physics, Space Science and Zoology. Current research activities include the development of new multiphoton imaging systems for studies of living specimens, the development of spectral/lifetime imaging instrumentation (figure one), the development of software for the analysis of dynamic three-dimensional images of cells and tissues and the development of educational outreach projects.



Professor John White



Fluorescence Lifetime image of a tranverse methyl green-stained section though a liver lobule of a Cynomolgus monkey

In addition to its research activities LOCI is a Bio-Imaging research partner with the Keck Laboratory assisting with the recent acquisition of the Radiance Multiphoton system and is a co-organizer with the Keck Laboratory of the semiannual UW-Madison/Promega Biological Imaging Symposium.

For more information about LOCI see http://www.loci.wisc.edu or contact Assistant Director Kevin Eliceiri (eliceiri@wisc.edu).

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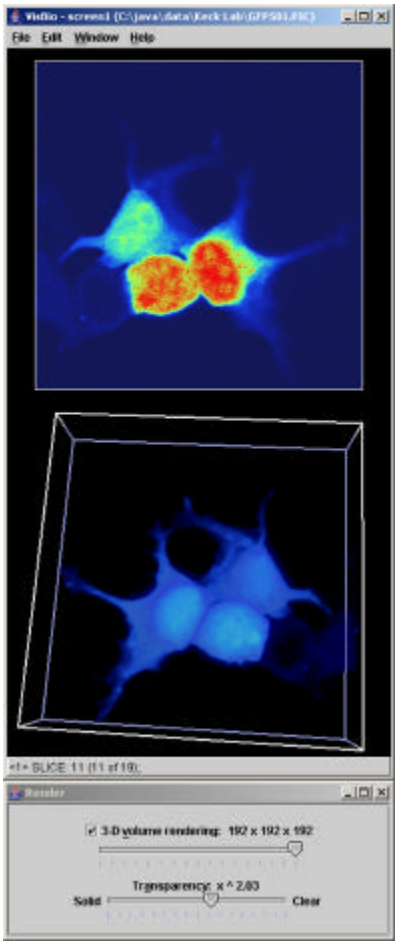
The mission of the W.M. Keck Laboratory for Biological Imaging is to provide researchers with access to confocal and multiphoton imaging, and the expertise to apply these tools effectively. The Laboratory also is committed to working with users to develop new applications for confocal and multiphoton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



Left panel illistrates VisBio, a freeware program developed at The University of Wisconsin Madison. For more information please visit the following web address http://www.loci.wisc.edu/visbio/

Image represents three dimensional reconstruction of human embryonic kidney cells that were transfected with a plasmid construct expressing GFP.

Courtesy of Carrie Gravel, Lance Rodenkirch, Kevin Eliceiri, and Peggy Farnham-University of Wisconsin.

New Addition to The W.M. Keck Laboratory for Biological Imaging

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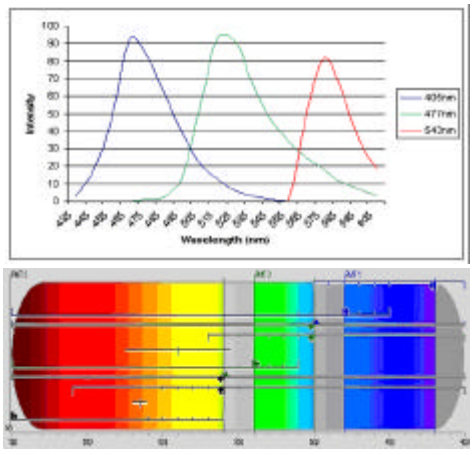
this microscope is that the spectral resolution is constant across the entire detection range and is not adversely affected when the confocal aperture is opened beyond its 'optimal' size. This allows for the ability to open the confocal aperture in order to image and resolve faintly expressing fluorescent proteins.

Deep tissue imaging makes scattered light difficult to resolve. However, the Radiance 2100 system has an optimized design for multiphoton collection in deep tissue. Bio-Rad has designed a system with 2 levels of enhanced detection of scattered light. First, the Signal Enhancement Lens system enables detection of photons scattered outside the cone of light that would normally pass through fully open confocal apertures. Second, an external or Direct Detector System (DDS) collects the emitted light from immediately behind the objective lens (at the position of the epi-fluorescence dichroic) rather than allowing the light to pass back through the scanning system.

Currently, LaserSharp2000 software has the capability to plot intensities from within user-defined regions of interest and to blank the beam outside of the regions. In other words, one can set the intensity to different specified values.

With the addition of the Time Course optional software module, LaserSharp2000 also allows the user to design and conduct multi-phase time course experiments. For example, one can first, scan at high power in order to photobleach selected regions, and then scan the entire region at a lower power to collect data from the photobleached area.

Additional features of the LaserSharp2000 suite will include 4D visualization, a FRET package developed at the Keck Center for Cellular Imaging (KCCI), UV-Charlottesville, under Ammasi Periasamy, and a Z-motor control that will move the stage in increments



as small as 50nm. The images above represent fluorescent emission wavelengths that can be separated into 3nm blocks using Bio-Rad's LaserSharp2000 software package. Fluorochromes, e.g. Fluorescein and GFP, which have overlapping emissions, can be viewed independently of one another.