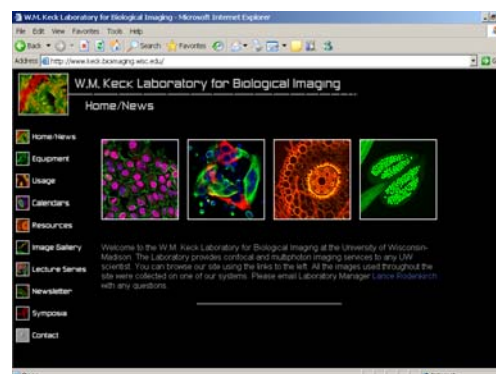




New Website

The W.M. Keck Laboratory for Biological Imaging has launched a new Web site featuring an updated look and additional sections to aid users with imaging. One area especially helpful is linked under the “Resources” tab. This section contains links to other websites providing information for both experienced and novice users. Useful sites for beginners include ones that focus on general microscopy techniques, sample preparation, and the excitation/emission spectra of fluorophores. Other categories of interest to all

users include image processing and analysis freeware, and local microscopy links.



W.M. Keck Lab New Website

www.keck.bioimaging.wisc.edu

Olympus Introduces the IV100

The IV100 Intravital Laser Scanning Microscope is a new instrument from Olympus designed for intravital imaging in small laboratory animals. The instrument utilizes three elongated “stick” lenses, each of which acts as a dipping objective and is inserted directly into tissue. Through the use of these MicroProbe lenses and flexible observation angles, the IV100 allows the observation of organs deep inside specimens.

Live animal imaging is the IV100’s main application in translational research. Other specific imaging uses of the IV100 include: experimental tumor imaging; micro-vascular growth patterns in the brain; cell migration;

stem cell-fate; imaging of low-movement organs such as the kidney, liver, and pancreas; and arthroscopic imaging in rheumatism models.



IV100 Intravital Laser Scanning Microscope

For more information see:

www.olympusamerica.com

Special points of interest:

- Olympus IV100
- Imaging Acronym: FRET
- Featured Lab: Lipton

Quantum Dots

Quantum dots, a new tool for fluorescent labeling, are composed of multiple semiconductor particles that together have properties similar to that of a single atom. A unique feature of quantum dots is that the emission spectrum can be tuned to various wavelength by changing the dot size. As the size is increased, the emission spectrum shifts is shifted into the red. Quantum dots differ from traditional fluorophores such as FITC or Rhodamine in four main respects. First, they have emission peaks that are narrower (full width at half maximum of ~30 nm) and nearly symmetric. Second, their excitation spectra are radically different, increasing exponentially as the illuminating wavelength is decreased. This has the advantage that any quantum dot can be excited efficiently with UV or blue light, but has the disadvantage that excitation selectivity is virtually lost. Third, the excitation efficient is much higher, yielding a significantly brighter signal. Fourth, quantum are much larger than organic dyes, and thus staining protocols may require modification.



Image of fluorescence in various-sized Cadmium Selenide Quantum Dots.

www.evidenttech.com

www.qdots.com

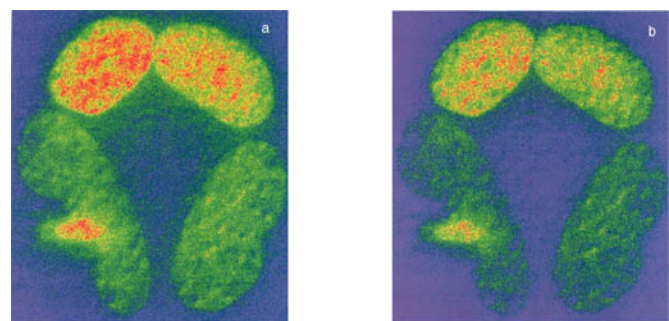
FRET

Fluorescence (or Foerster's) Resonance Energy Transfer, more commonly referred to by the acronym FRET, is a technique used in microscopy to measure the interactions between two biomolecules during both *in vitro* and *in vivo* imaging. FRET is a process involving the transfer of radiationless energy from a donor to an acceptor fluorophore. Normally, an excited fluorophore returns back to the ground state with the emission of a photon. During FRET, however, donor fluorophore transfers that energy to a nearby acceptor fluorophore, which then returns to its ground state by emitting a photon.

The distance over which FRET can occur is limited due to the efficiency of the energy transfer between fluorophores. As the distance increases, the efficiency of energy transfer decreases (according to the inverse sixth power of the distance separating them). This puts the limit over which FRET can occur to about 1-10 nm. However, this is what makes FRET useful in biological macromolecule im-

aging. If FRET occurs, it suggests that the two fluorophores (or whatever they are fused/conjugated to) are very closely associated. By comparison, colocalization by light microscopy is limited in spatial resolution to ~250 nm.

FRET has been used in studies that observe the structure and conformation of proteins and immunoassays, as well as detect nucleic acid hybridization.



DV-FRET bleed-through corrected images for BFP-GFP-Pit-1 protein

www.kcci.virginia.edu/FRET-FLIM/index.php

Featured Research: Peter Lipton



Professor Peter Lipton

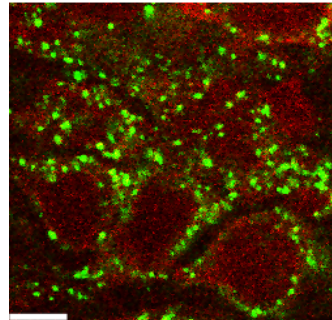
The Peter Lipton Laboratory has examined the permeabilization of lysosomes following *in vitro* ischemia (IVI) and reoxygenation. With the help of student James Windelborn, experiments were performed in the acutely prepared rat hippocampal slice, and lysosome permeabilization was detected immunohistochemically with an antibody raised against the lysosomal protease, cathepsin B.

The images shown have been taken from the CA1 pyramidal cell layer of two slices (scale bar = 10um). Each pseudo-colored image displays Nissl staining in red (Neurotrace 530/615, Molecular Probes) and cathepsin B staining in green (Alexa Fluor 488-conjugated IgG, Molecular Probes). The second panel from each treatment shows only cathepsin B staining. The subcellular distribution of cathepsin B shifts from lysosomal (punctate) to cytosolic (homogenous) when slices are exposed to IVI and reoxygenation (right-hand panels). While this shift can be qualitatively appreciated, a semi-quantitative measurement is necessary to provide statistical rigor to the analysis.

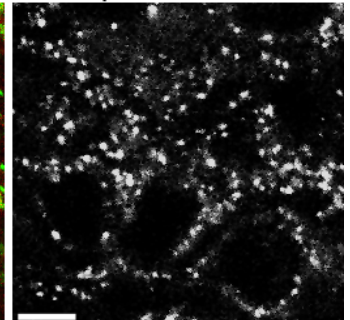
Since an increase in cytosolic cathepsin B represents an increase in lysosome permeability, a method was developed using NIH's ImageJ soft-

Normoxic Control

Nissl + catB

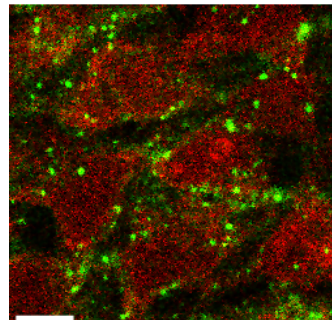


cathepsin B

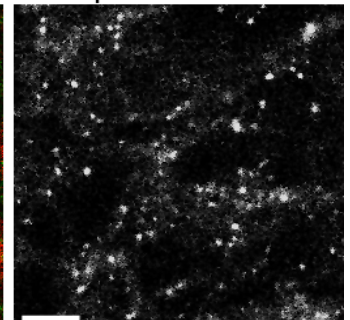


5min IVI + 2hr Reoxygenation

Nissl + catB



cathepsin B



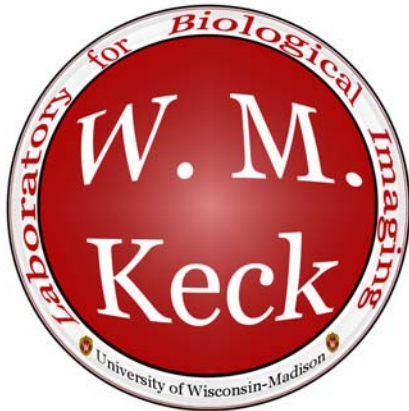
CA1 Pyramidal cell layer of two slices with red staining

ware to eliminate lysosomal and acellular signal while retaining cytosolic signal. The theory underlying the method is that pixels covering acellular areas will be of the lowest relative intensities, while pixels covering punctate areas will be of the highest intensities, since antigen is extremely concentrated in those spots. Eliminating the upper and lower limits with pixel intensity thresholding leaves behind cytosolic signal for analysis. Cytosolic signal within a set of CA1 pyramidal neurons is measured and corrected for the number of cells within the ROI and the total area of the ROI. Using this method, it has been determined that CA1 neurons exposed to 5min IVI and 2hr reoxygenation have approximately 2-times more cytosolic cathepsin B than time-matched normoxic controls. These findings, along with studies revealing that cathepsins are necessary for CA1 neuron damage following IVI, point to an important role for lysosomes in ischemic damage.

**UNIVERSITY OF WISCONSIN -
MADISON**

Primary Business Address:
University of Wisconsin
1300 University Ave
172 Medical Sciences Center
Madison, WI 53706

Phone: 608.265.5651
Fax: 608.265.5536
Email: larodenk@facstaff.wisc.edu



Featured Image

Mouse myocytes, differentiated from embryonic stem cells, were stained for DNA using DAPI (blue), eGFP with Alexa Fluor 488 (green), and smooth muscle actin using Alexa Fluor 568 (red). The DAPI was excited using a multiphoton laser set at 750 nm.

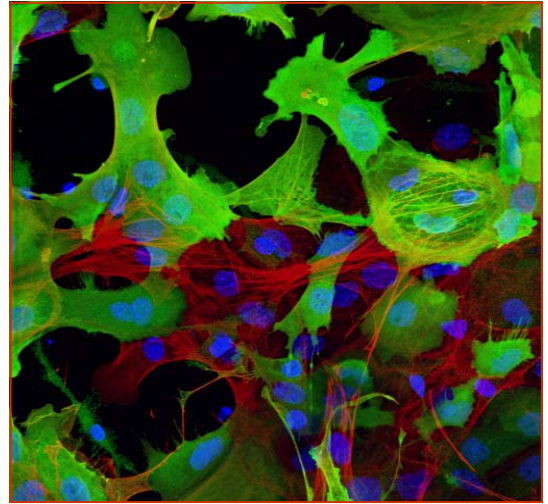


Image Courtesy of Lining Ma (Laboratory of Tim Camp, Department of Medicine).

W.M. Keck Laboratory Completes Five Years of Lecture Series

The W.M. Keck Laboratory concluded its fifth year of the Biological Imaging Lecture Series in May. The Series, which presents a monthly lecture, is sponsored by the UW Department of Anatomy, the UW School of Medicine and Public Health, the Fryer Company, and Promega Corporation. The lecturers discuss biological imaging at a variety of scales and how its application has allowed them to further their research.

The Spring 2006 Series featured:

- Mary Halloran, UW-Madison Department of Zoology
- Paul Ahlquist, UW-Madison McArdle Laboratory for Cancer Research
- Stephen Smith, Stanford University Department of Molecular and Cellular Physiology
- Eugene Myers, Janelia Farm Research Campus, Howard Hughes Medical Institute



Professor Kurt Amann

Kurt Amann, UW-Madison Department of Zoology, kicked off the sixth year of the Biological Imaging Lecture Series on September 12th. He was followed by Nancy Kanwisher (MIT) and Jack Nitschke (UW). The fall portion of the series will conclude with a talk by Erik Dent (UW) titled *Actin Filaments and Fried Eggs: Cytoskeletal Pathways to Neuritogenesis*.

www.keck.bioimaging.wisc.edu