THE MICRO TOME

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MICROSCOPY IMAGING CENTER
HTTP://WWW.UVM.EDU/MICROSCOPYIMAGING

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# Digital Imaging and the Ethics Thereof...

The issue of the manipulation of digital images has come to the forefront recently due to several publicized cases. Two members of the MIC (D.T. and M.W.) attended a brief workshop on the ethical manipulation of digital issues held at the annual Spring meeting of the New England Society for Microscopy in May, 2006. The consensus of the meeting was that there really is no strong universal consensus on how this issue should be handled. The Microscopy Society of America has issued a policy on this issue. We in the MIC have considered this issue in depth and how it may impact our clients. Based upon these considerations, we have adopted a modified statement from the Journal of Cell Biology as the MIC Policy on the Manipulation of Digital Images:

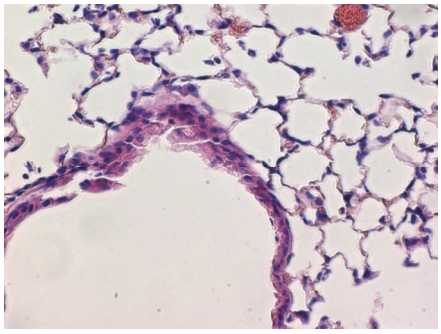
**"No specific feature within an image may be enhanced, obscured, moved, removed or introduced. The grouping of images from different fields of view or exposures must be made explicit by the arrangement of the figure (e.g., using dividing lines) and in the text of the figure legend. Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the whole image (and to corresponding control images as well), as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g., changes to gamma settings) must be disclosed in the figure legend."**

It is our policy in the MIC to encourage clients to optimize their cell/tissue processing and image capture parameters initially so that post-image capture manipulation is unnecessary. It is also good policy to relate any image manipulation performed in the figure legend, even if deemed to be minor. *However, remember that the "raw" image must be maintained, so that any manipulations are saved to a "copy" of the image. Journals may request to see the original raw, unaltered image .*

If you have any questions regarding this policy, please see a member of the MIC staff. A good discussion describing issues related to digital image manipulation can be found in the references on our website (http://www.med.uvm.edu/microscopyimaging/).

# Olympus IX70 Inverted Microscope gets an Upgrade and a New Camera

Last May, the facility upgraded the computer attached to the Olympus IX70 Inverted Microscope and purchased a new camera and software package. The microscope is now outfitted with a Retiga 2000R digital CCD camera from QImaging. The camera comes with an RGB color (liquid-crystal) filter which allows the monochrome camera to produce color images. The recommended applications include brightfield, fluorescence, histology and cytology, FISH and more. The new software (QCapture Pro) provides imaging and analysis capability for acquiring, enhancing and analyzing images. Real time image preview and capture, color management, time lapse sequences and full control over all camera features are some of the benefits offered with the QCapture Pro software.



H&E of mouse lung. Slide provided by the Suratt Lab.

# MIC Fee Update

The Microscopy Imaging Center has reviewed the fees of our facility and similar facilities and has decided to hold its fees for fiscal year 2008. There will not be an increase in price for instrument use and services provided. Along this same line, we have initiated a discounted fee for after hours (instrument time must start after 5pm otherwise current prices are in effect) and weekend use for the Zeiss LSM 510 META Confocal. This new fee is $25 per hour for both College of Medicine and UVM users. This service is for users who are proficient with the instrument to free up time during the day for those users who need assistance.

# Seminars, Presentations and Demonstrations, Oh My!

In our continuing series of tutorials and seminars we have now heard from Molecular Probes, CRi and QImaging. From Molecular Probes, Kelly Lundsten spoke about advances in fluorescent probe technology. Luke Brennan from CRi demonstrated both a multispectral and an in-vivo imaging system. Lastly, David Hitrys from QImaging presented on CCD cameras, what to look for when purchasing a new camera and how to decode all the terminology associated with cameras. The seminars were well attended and a wealth of useful information was presented. Please watch for upcoming presentations, including Qdots and Molecular Machines—Laser Cutting. We welcome feedback on presentations that we have had and suggestions for future presentations.

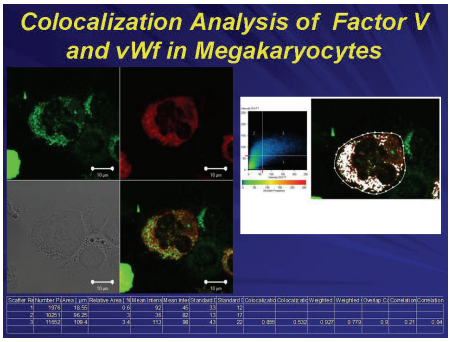
## Cryostat for Unfixed Human Tissue

The facility (together with Dr. David Krag) has purchased a Minotome Cryostat (International Equipment Company) for cutting unfixed human tissue. The cryostat is located in the microtomy room (203B HSRF) of the Microscopy Imaging Center. Requirements for the use of this cryostat are documented completion of blood borne pathogen training through ESF and facility training by Jan Schwarz.

## Colocalization

Many requests are brought to the MIC for colocalization analysis of confocal images. In a biological sense, colocalization is defined by the presence of two (or more) different molecules in the same spatial location in a specimen. In the context of a digital image (for instance, a confocal image), colocalization refers to colors emitted by different fluorescent dyes sharing the same pixel (or voxel) in an image. Sophisticated software is currently available for performing quantitative colocalization analysis of digital images. In the MIC, colocalization analysis can be performed with Molecular Devices MetaMorph software, Improvision Volocity software, MIPAV (Medical Image Processing, Analysis & Visualization; NIH Shareware), and Zeiss colocalization software (packaged with the confocal software). All of the software packages provide a variety of "colocalization statistics", derived from algorithms developed by E. Manders. While a variety of software analysis packages are available, the key to accurate colocalization is in specimen preparation and imaging. On the specimen labeling side, choose specific, high quality antibodies, fluorophores with widely separated emission spectra, and perform multiple controls. On the instrumentation side, image with high quality apochromatic objective lenses, image the fluorophores in sequential mode, and sample according to the Nyquist theorem. Also, of paramount importance is to subtract background fluorescence from the images prior to analysis. With all of these issues kept in mind, accurate quantitative colocalization can be performed on confocal images. Please see an MIC staff member with any questions or to discuss potential applications.

## Spotlight on MIC User




Dr. Beth Bouchard in the Department of Biochemistry is identifying the cellular events that regulate endocytosis of the critical coagulation protein factor V by megakaryocytes, platelet progenitor cells, using $CD34^+$ *ex vivo*-derived megakaryocytes as a model cell system. Confocal microscopy and colocalization analyses are being used to define its intracellular trafficking pathway, and to test the hypothesis that it undergoes retrograde transport through the *cis*-Golgi network prior to its trafficking to and storage in a-granules. Colocalization of endocytosed, fluorescently-labeled factor V with various, fluorescently-labeled, anti-organelle antibodies (e.g. clathrin-coated pits, early and late endosomes, and the Golgi apparatus) and fluorescently-labeled antibodies against megakaryocyte/platelet a-granule markers (e.g. fibrinogen, vWF and P-selectin) is being defined. The images are being analyzed by the Zeiss colocalization software to determine the time-dependent colocalization of factor V with each of these various markers. To date, these studies have demonstrated that subsequent to its clathrin-dependent, receptor-mediated endocytosis, factor V is trafficked first to early endosomes, followed by late endosomes, the Golgi apparatus, and a-granules. These techniques will also be useful in future studies as we continue to identify the mechanisms involved in factor V uptake.

## MIC Lab Tours and Project MICRO

This school year the Microscopy Imaging Center toured and demonstrated our facility for 259 students and others. Laboratory personnel demonstrated the imaging technologies available and discussed some of their current applications. The students ranged from grade school through graduate level and included FAHC residents along with prospective faculty. MIC's student outreach program, Project MICRO, encompassed 932 elementary students from Middlebury to Missisquoi.

### Equipment Available:

- JEOL 1210 STEM
- JEOL JSM 6060 SEM
- BioRad MRC 1024 Confocal LSM
- Zeiss LSM 510 META Confocal
- Olympus IX 70 Inverted Microscope for fluorescence and phase contrast
- Eppendorf Microinjector System
- DI Atomic Force Microscope
- Arcturus PixCell II LCM
- Zeiss Axioplan 2 Microscope
- CompuCyte Laser Scanning Cytometer
- Olympus BX50 Microscope
- Universal Imaging MetaMorph Workstation
- Volocity 3D Software
- Multiple Dell Image Processing Workstations
- Fujix PictroGraphy Printer

### MIC Services Provided:

- Morphologic services and consultation at the light and electron microscopy level
- Morphometry (semi-quantitative morphology)
- Light and electron microscopic immunocytochemistry
- Confocal scanning laser microscopy
- Laser scanning cytometry
- Atomic force microscopy
- Scanning and transmission electron microscopy
- Laser capture microdissection
- Preparation of paraffin and frozen sections
- Training for use of the above equipment
- Special histological stainings
- Testing of new antibodies and developing new staining techniques
- Photo quality printing for publications and posters, computer-assisted digital imaging and analysis