THE MICRO TOME

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

Winter 2005-2006

Welcome to the Winter 2005-2006 edition of the MICROTOME! The Microscopy Imaging Center has had a busy year, with changes in both equipment and personnel. The addition of a new confocal scanning laser microscope, a scanning electron microscope, and new image processing and analysis software will be highlighted below. The other big change in the Center this year was the adoption of a web-based on-line reservation system for all of the equipment, which you can read about on the other side of this newsletter. On the personnel side, we bid a sad farewell to Masha Stern, but wish her much happiness and success in her new position at MicroBrightfield, Inc. in nearby Williston! So, we hope you enjoy this edition of the MICROTOME, and as always we seek your comments and input on the contents of this newsletter, as well as suggestions for improvement of the Microscopy Imaging Center. Wishing you good imaging, happiness and success in the New Year!

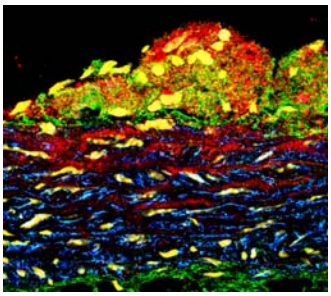
MIC Equipment Tutorials

We are establishing a series of tutorials/seminars by equipment manufacturers to highlight recent advances in the field. The first seminar was presented by Alan Carpino from Arcturus, Inc., on Thursday December 1st. Alan spoke about laser capture microdissection techniques, and the downstream purification and amplification of RNA from captured cells. The seminar was well attended and much useful information was provided. Please watch for upcoming presentations, and let us know if there are specific equipment/ techniques you would like to see presented.

Volocity 3D Rendering Software

Volocity is a high-performance 3D imaging software package providing visualization, exploration and analysis capabilities of multi-channel 3D volumes over time. The software can be used for 2D, 3D, and 4D imaging, and will accept image sequences from both confocal and wide-field microscopes. The Volocity Visualization module provides an extensive array of visualization and publication features. The Volocity 3D view enables the user to interactively explore a 3D rendered object. The viewing point can be placed either inside or outside of the 3D object, and changes of structures over time monitored. Animated movie volumes can be created with the Movie Sequencer module. The Volocity Classification module will identify, measure and track biological structures in 2D, 3D, and 4D. Co-localization analysis can also be performed for two fluorophores in 3D. All in all, this is a very powerful 3D software program, and we encourage you to speak with a staff member for more information related to your specific image analysis needs. This software is available on the Dell computer attached to the Fujix Pictrography 3500 printer in HSRF 206.

Zeiss 510 META Confocal

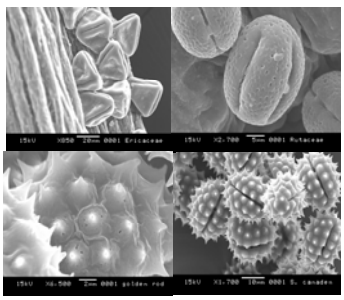


4 channel image projection of an APO E-/- mouse artery cross-section with lesion stained with nuclei -yellow, collagen – green, lipid - red, and actin – blue, captured with a 63X objective

The Zeiss 510 META Confocal Laser Scanning Microscope has been in use for about one year and thanks largely to our diverse user base, we have tested a wide range of its capabilities. From live cell imaging to fixed tissue in paraffin and cryostat preparations, we have been pleased with the performance of the software and optics. The complimentary packages of Metamorph for quantitation and Volocity for 3D quantitation and image enhancement have augmented the confocal images captured. If you would like an introduction to confocal imaging and associated software, please see Marilyn or call 656-0813 to arrange an appointment.

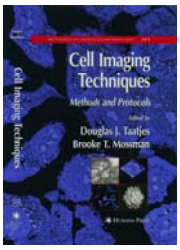
Scanning Electron Microscopy

The new JSM-6060 scanning electron microscope from JEOL Technics, Ltd has been in operation for over one year now and has been used by students and researchers from a variety of departments around campus. Several researchers from the departments of Chemistry and Physics have been trained to operate the microscope and have been very pleased with the ease of capturing digital images of their materials specimens. Students of the Botany Department were able to capture beautiful images of pollen samples this fall and students attending the Governor's Institute for Science and Technology imaged a variety of biological samples collected from different ecosystems in the area. We have also provided imaging services to a variety of local businesses and other researchers in the area using both backscattered electron imaging and secondary electron imaging. The capability of the microscope has excited a lot of students that have visited the facility this year. In addition to providing high resolution (3.5 nm) scanning electron imaging, the system comes with analySIS® image processing and analysis software from Soft Imaging System Corp. and is available for use. One feature we really like is the ability to "stitch" images together very easily. If anyone would like a tutorial on scanning electron microscopy or an introduction to analySIS® software, call Michele von Turkovich at 656-0813 to make an appointment.



Pollen: Botany 109 Images taken on JEOL 6060

MIC Collaborative Publication



Methods in Molecular Biology – **Cell Imaging Techniques - Methods and Protocols** has just been released. Edited by Drs. Doug Taatjes and Brooke Mossman, this book includes several chapters written by UVM authors. It covers protocols using a diverse range of microscopy techniques, from wide-field microscopy, confocal scanning laser microscopy, laser scanning microscopy, laser capture microdissection, atomic force microscopy, and transmission electron microscopy, among others. This book is intended to assist researchers in a variety of techniques and introduce some new techniques for consideration.

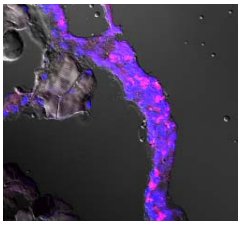
Lab Tours

This year the Microscopy Imaging Center demonstrated the imaging technologies available through the facility to 194 students and researchers. The students ranged from high school level through graduate level and residents. Project MICRO, the Microscopy Imaging Center's middle school outreach program, encompassed 463 students from Charlotte to St. Johnsbury. Recently, Project MICRO was the recipient of two dissecting microscopes and a *Video Flex 7600* microscope camera donated by the Champlain Valley Area Education Center (CVAHEC).

Spotlight on Current MIC Users

One of the projects currently in Dr. Mossman's laboratory in the department of Pathology involves a collaborative effort with Dr. Christopher Landry's group in the Department of Chemistry. This project is investigating the potential use of acid-prepared silica-based mesoporous spheres (APMS), which are approximately 1-2mM in diameter, as vehicles for delivering anti-cancer drugs and other inhibitory constructs to specific cells in the treatment of the asbestos-associated disease, mesothelioma. By coating the APMS with different molecules, such as tetraethylene glycol (TEG), we have shown that the APMS are taken up by mesothelioma cells *in vitro* and into cells local to the injection site when administered intrapleurally to mice *in vivo*. Additionally, by using APMS to deliver the anticancer drug doxorubicin to mesothelioma cells *in vitro*, we dramatically increase the efficacy of the drug compared to incubation of the cells with identical doses of doxorubicin alone.

With the use of confocal scanning laser microscopy in the Microscopy Imaging Center, we have developed techniques that have allowed us to locate fluorescent labeled APMS in cells *in vitro* and in different tissues *in vivo*. By using a combination of Differential Interference Contrast Microscopy and multicolor fluorescence microscopy, we are beginning to determine what types of cells take up the APMS after intrapleural injection in mice. Additionally, using live-cell assays where the cell membranes are stained with a fluorescent lipophilic dye, we are able to track the movement of APMS from the outside of cells through the membrane and can determine the kinetics of uptake of the APMS into cells. This is important to help determine the mechanism of binding of the APMS to the cell and mechanisms of the uptake into the cells. For *in vivo* studies, in order to determine the trafficking of APMS after intrapleural injection in mice, we are also using scanning electron microscopy and energy-dispersive x-ray spectroscopy (EDAX) to locate the silica APMS in different tissues of the APMS-treated mice. These techniques have been critical in allowing us to advance our understanding of the potential use of APMS in therapeutic applications, and will continue to be used to help provide insight into the mechanisms of how the APMS function, both *in vitro* and *in vivo*. *(description provided by S. Blumen)*



APMS in Rib Tissue 72 Hours After Intrapleural Injection ***DIC image*** *taken on Zeiss LSM 510 META Laser Scanning Confocal Microscope* ***APMS*** *are red (Alexa 568)* ***Cell nuclei*** *are blue (TOTO3).*

OCF Online Scheduler

The Microscopy Imaging Center has moved to an online scheduling program to sign-up for equipment use. The scheduler can be found at http://www.uvm.edu/~microimg/OCF_Scheduler . When you schedule time please be aware of the following items: am and pm, entering an event name is a requirement (other people will be able to read this) and deselect the email confirmation (it does schedule your time but it also generates an error message). We do ask if you are unavailable to make your time and can't delete it yourself or you finish early and need your time edited to ask an MIC staff member to perform these activities. This way others will know the instrument is available. The online scheduler also has an information banner located below the instrument name on its calendar that we will use to inform users of maintenance times, updates and upgrades for the instruments.

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
- Dell Image Processing Workstation
- 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 sections 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