

THE MICRO TOME

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MICROSCOPY IMAGING CENTER

HTTP://WWW.UVM.EDU/MICROSCOPYIMAGING

The Price is Right... Come on Down!

Summer 2008

Last fiscal year the Microscopy Imaging Center did not increase its rates, and added a special hourly rate for Zeiss Confocal work done after 5pm and on weekends. With the new fiscal year we will be implementing a 2% rate increase for all fees and services and introducing another special hourly rate for the following instruments: the Bio-Rad Confocal, the Laser Scanning Cytometer and the Laser

Capture Microdissector. Each of these instruments will now be charged at a flat rate of \$20/hour regardless of when they are used. So, if you have experiments planned that could benefit from the use of these instruments, this is a great time to take advantage of substantial cost savings. Gas prices may continue to rise, but the MIC is working to stretch your research dollars further!

MIC Named Laboratory Response Network Partner in Bioterrorism Preparedness

Because of the electron microscopy capabilities of the Microscopy Imaging Center, the Vermont Agency of Public Health named the Microscopy Imaging Center their Laboratory Response Network (LRN) partner in bioterrorism preparedness activities. The LRN was established by the Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and became operational in 1999. Its objective is to ensure an effective laboratory response to bioterrorism by helping to improve the nation's public health laboratory infrastructure.

In September 2007, the MIC answered the call for participation by sending a technician to a 3 day training course, "Electron Microscopy for Agents of Bioterrorism" conducted by the Infectious Disease Pathology Branch/Division of Viral and Rickettsial Diseases at the CDC. It was taught by Cynthia S. Goldsmith and Charles Humphrey of the CDC and Sara E. Miller of Duke University Medical Center, Durham, NC. Ten participants from state public health agencies around the country attended.

Because the threat of an intentional release of *Variola* virus exists, the course was developed to provide electron microscopy technicians with the expertise to assist the CDC in rapidly ruling out other infec-

tions that could be confused with smallpox, such as chicken pox which is caused by the *Varicella zoster* virus in the Herpesviridae family. The three day course was structured so that participants attended lectures, participated in discussions, and practiced with virus specimen preparation and evaluation by transmission electron microscopy. Bio-safety and vaccination issues were also discussed.

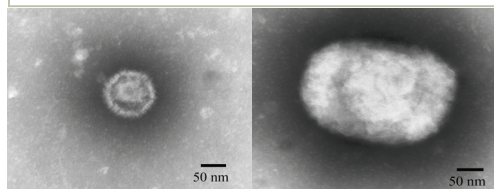
It is the expectation of the CDC that all participants will share the knowledge and skills gained with appropriate network partners in their respective states. Accordingly, an MIC technician has met with the bioterrorism coordinator and other staff at the Vermont State Health Laboratory, given an overview of the course, and has begun training VDH staff in the procedures for collecting and preparing viral specimens for electron microscopic examination, as well as, decontamination procedures. In addition, the MIC is participating in a quality assessment (QA) program for EM viral diagnostics established by and among the CDC course participants. We will also be participating in a world-wide QA program administered by the Robert Koch Institute in Berlin, Germany. For more information on the Laboratory Response Network, go to, <http://www.bt.cdc.gov/lrn/>

Working Safely After Hours

The Microscopy Imaging Center, in compliance with ESF, now has a Working Alone Policy. Our policy states that no laboratory work including sectioning, tissue processing or working with chemicals may be done by non-MIC staff between the hours of 5 pm and 7 am weekdays or on weekends. These hours are considered after normal working hours and the main lab and cutting rooms are closed during this time. The imaging rooms are the only ones available after hours and on weekends. We strongly encourage users doing imaging after hours to take safety precautions. Safety precautions for anyone working alone should include:

1. always notify someone if you are working alone
2. keep a laboratory phone (911 for UVM police services) - or cell phone on you at all times with the UVM police services number - 802-656-3473
3. contact information for MIC staff is located in the facility

Herpesviridae and Poxviridae are two different virus families that cause similar rash illnesses. In the event of a suspected intentional release of variola virus (Poxviridae family), electron microscopy could rapidly rule-out other causes of rash illness such as varicella zoster virus (Herpesviridae). Specimens are negatively stained with 0.5% aqueous uranyl acetate.



Herpesviridae

Poxviridae

Touring Vermont

This year the Microscopy Imaging Center reached over 900 students through facility tours and Project MICRO. Working as a LRN Partner, we also provided a lab tour for the Vermont Army National Guard's 15th Weapons of Mass Destruction Civil Support Team, lead by CPT Cipriano along with 12 other members of the team.



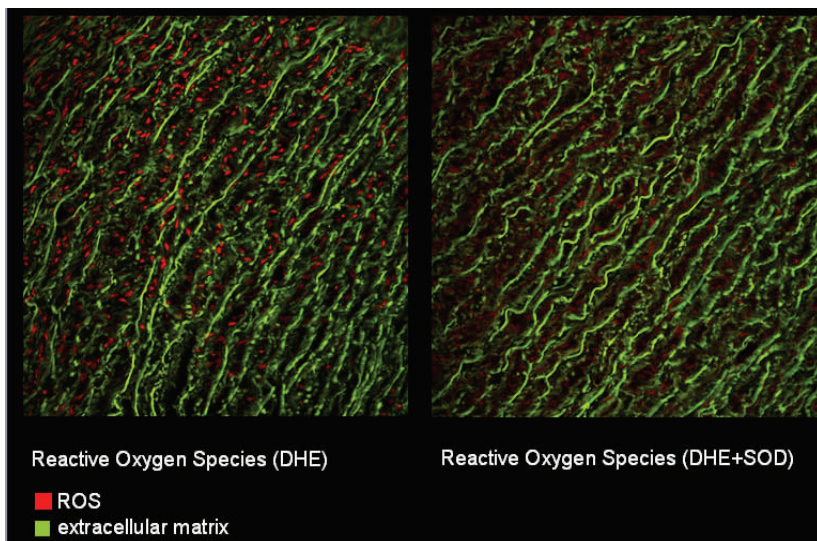
MetaMorph Technical Tip— Total Area or not to Total Area?

When choosing an area measurement in MetaMorph it is often confusing to users which area measurement they need: *total area*, *pixel area* or *area*? *Total area* is the area of the object thresholded including any unthresholded holes present. Think of a doughnut. If the doughnut is thresholded, the total area will be the doughnut plus the hole! *Area* will give you only the thresholded area (doughnut only). This measurement will be expressed in calibrated units such as square microns. The *pixel area* will give you the number of pixels in the object that are thresholded. One note, be sure to look at the Measure Objects Preferences box to see if hole filling is enabled and uncheck it, otherwise any holes will automatically be added into your measurements. Also be sure to check the toolbar at the bottom of your screen to see if your image was calibrated correctly.

All-a-Board at the MIC Depot!

The Microscopy Imaging Center is now using the UVM BioDesktop for all online scheduling of instruments in the facility. This system replaced the OCF Online Scheduler that we used for the past four years. The benefits of using the UVM BioDesktop to the facility are on-campus support for all issues, which are taken care of within 24hrs, tracking usage on instruments and simplification of billing procedures. The MIC Depot can be accessed through the MIC website or <https://biodesktop.uvm.edu/perl/micdepot> whenever you wish to reserve time on a piece of equipment. The imaging equipment is accessible 24 hours a day, 7 days a week (Please see MIC Working Alone Policy—front page). Please sign up only for the hours you will actually need (3-hour maximum without special permission). If you are not able to make your imaging time, please delete your time or call X6-0813 so we can delete your sign up and free up the instrument for another user.

The LSM Measures ROS



Hypertension (high-blood pressure) is a recognized risk factor common to the pathogenesis of all types of aneurysms (e.g. thoracic and abdominal aortic aneurysms) and is known to induce oxidative stress (e.g. reactive oxygen species; superoxide). In studies utilizing a novel human aortic organ culture model with a pressure incubator, we are finding that

simulated pathological pressure (180 mmHg) is associated with a decrease in ROS formation in aneurysmal aortic tissue when compared to control tissue. These findings support our hypothesis that hypertension may contribute to aortic aneurysm expansion by inhibiting ROS associated wall thickening and thereby promote increased wall tension.

ROS formation is measured using the Zeiss LSM 510 META Confocal Laser Scanning Imaging System. Human aortic cryosectioned samples (16 μ m) are incubated with the oxidative fluorescent dye Dihydroethidium (DHE 4 μ M, 37°C, 30min). In the presence of ROS, DHE is oxidized to Ethidium Bromide (EtBr) which is then excited with the 488 nm laser to produce an emission spectrum of 610 nm. Tissues incubated with ROS scavenger Superoxide Dismutase (PEG-SOD 1000U/ml, 37°C, 60min) serve as negative controls. Data are quantized using MetaMorph Imaging Software. (Armin Kiankhooy MD, Lucy Trombley MS, Joseph Schmoker MD – Department of Surgery, Division of Cardiothoracic Surgery)

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