THE MICRO**TOME**

University of Vermont
College of Medicine

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MICROSCOPY IMAGING CENTER

http://www.uvm.edu/medicine/MIC

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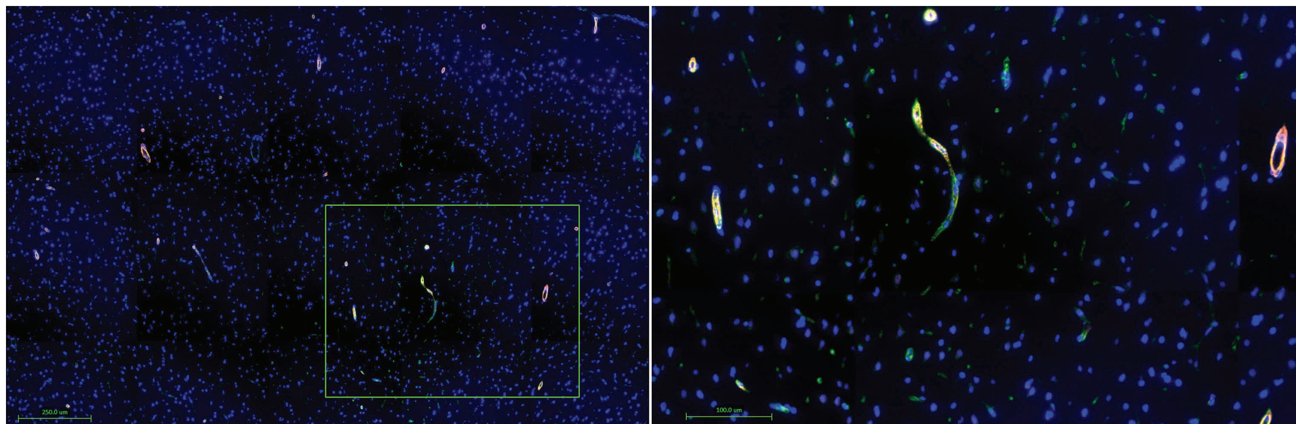
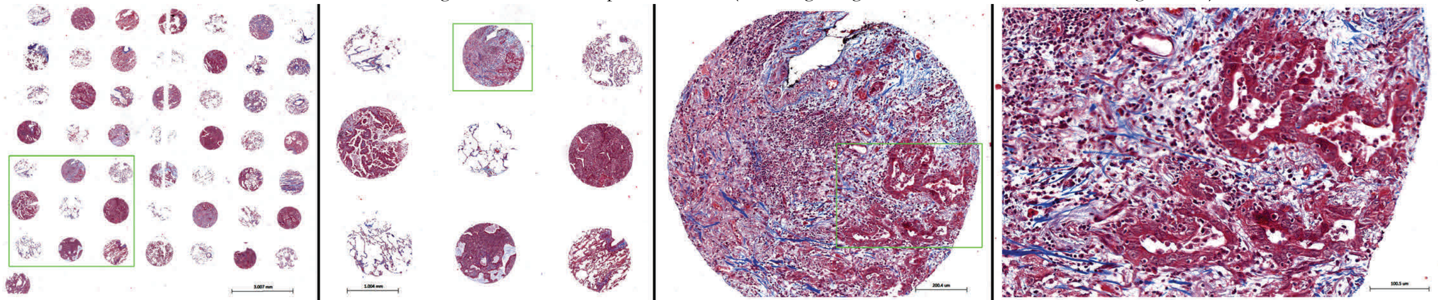
Winter 2016-2017

MIC Becomes more (VERSA)tile

Thanks to a College of Medicine Shared Instrumentation Grant, and matching funds provided by the Department of Pathology and Laboratory Medicine and the Vermont Cancer Center, the MIC acquired in December a Leica VERSA8 whole slide imager. This highly versatile instrument is an automated scanning microscope and image generation scanning system. Some of its features include: integrated brightfield and fluorescence scanner; two high quality CMOS cameras for brightfield or fluorescence image acquisition; batch set-up and automation for unsupervised scanning of up to 8 slides; fluorescent scanning of up to 7 fluorophores/slide; and a wide range of Leica quality objective lenses (1.25, 5, 10, 20, 40, and 63X). Software allows low magnification overview of an entire sample, with high resolution "zoom in" feature to analyze cellular features. Please see Doug or Nicole Bishop to find out more about this new slide scanner and to schedule a demonstration.



Above - 4 color immunofluorescence of rat brain captured at 20x. (left - 7x demagnification tile of original image, right - original full 20x magnification)
Below - Trichrome staining on a TMA slide captured at 20x. (left to right digital zoom of 0.7x, 2x, 10x and original 20x)



The Ethics of Digital Image Manipulation

The popularity in the sciences of image processing software such as Adobe Photoshop, has resulted in a significant up-tick in the number of improperly manipulated images submitted to journals for publication. Most of these instances are the result of a knowledge gap regarding what may be considered improper processing of digital images. Oftentimes images are acquired by students, Post-Doctoral fellows, or laboratory technicians, and the laboratory director may not be aware that images have been manipulated prior to viewing or submission to a journal. Therefore, it is incumbent upon the laboratory director to educate the lab personnel in the proper handling of images. If they do not feel qualified for this task, they should seek guidance from those more knowledgeable in this area. Indeed, many journal "Instructions to Authors" now specifically indicate what is allowable (and not allowable) manipulation of a digital image. For instance, The Journal of Histochemistry and Cytochemistry provides the following instructions concerning images: "Image Adjustments or Enhancements - Images should accurately reflect the original data. Linear adjustments in brightness, contrast, or color balance are acceptable if applied to the entire image. Selective linear adjustments to portions of images are not acceptable. Non-linear adjustments are acceptable only if they are applied to the entire image, are clearly disclosed in the figure legend, and do not alter the interpretation of the original image." Most importantly, always perform any manipulations on a copy of the original figure, retaining the original image in its unprocessed form in the event that any concerns are voiced about improper manipulation of the published image. Our goal in the MIC is to assist you in acquiring optimal images from all of our instruments, and to provide guidelines on the proper use of post-acquisition image processing software. If you have any questions or concerns regarding the manipulation of digital images please contact Doug or any member of the MIC staff for assistance.

How to: MIC Facility and Equipment Acknowledgement

We respectfully request that users acknowledge the Microscopy Imaging Center in any publications resulting from use of the facility or its services. Acknowledgement helps us demonstrate our value to the research community, and it significantly enhances our future efforts to secure funding to bring you even more instruments and services. Anyone who uses the MIC should include the General Acknowledgment (below) in their publication. If you used specific equipment that was purchased with grant funding (AFM or confocal) the corresponding sentence should be added.
General Acknowledgment
Imaging work was performed at the Microscopy Imaging Center at the University of Vermont.

Zeiss 510 META Scanning Confocal
Confocal microscopy was performed on a Zeiss 510 META laser scanning confocal microscope supported by NIH Award Number 1S10RR019246 from the National Center for Research Resources.

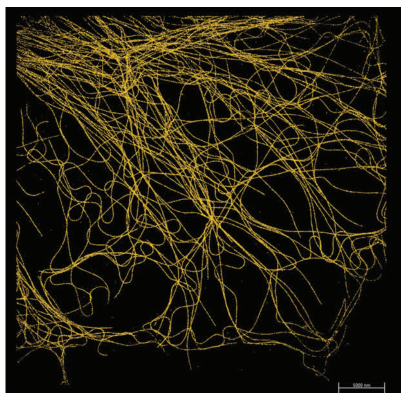
AFM
Atomic force microscopy was performed on an Asylum Research MFP-3 D BIO supported by NIH award number S10RR025498 from the National Center for Research Resources.

For example, an acknowledgement for work done in MIC on the confocal should read:
Imaging work was performed at the Microscopy Imaging Center at the University of Vermont. Confocal microscopy was performed on a Zeiss 510 META laser scanning confocal microscope supported by NIH Award Number 1S10RR019246 from the National Center for Research Resources.
For our records, we would also like to be notified of manuscripts submitted and/or published that acknowledge the facility. Thank you.

Time to Replace Zeiss 510 META Confocal

We are planning to submit an NIH S10 Shared Instrumentation Grant in May to support the purchase of an extended resolution point scanning confocal microscope to replace the existing Zeiss 520 META. We will be hosting a demonstration instrument on-site in the MIC during the week of April 3rd, and we would like to invite anyone with interest in this system to contact us to reserve time on the instrument during the demonstration week to image your own samples, and perhaps to be included on the grant as a "Major" or "Minor" user. Please contact Doug or Nicole with questions or to reserve time.

STORMing with Super Resolution Microscopy



STORM (Stochastic Optical Reconstruction Microscopy) is a super resolution imaging technique that captures images with a higher resolution than the diffraction limit. This technique is designed to provide resolution in fluorescence mode of 20 nm lateral and 50 nm axial. STORM require fluorescent probes whose state can be controlled by reversibly switching between a light and a dark state. The fluorophores must be as bright as possible and require a high contrast ratio between the two states. In the initial configuration, STORM requires pairs of dyes (tandem dye pairs). However, direct STORM (also called continuous or dSTORM) exploits the blinking phenomenon exhibited by certain dyes such as Alexa Fluor 647 and, with the proper imaging protocol, can be used to achieve multi-color STORM imaging. In this constantly developing field it is becoming increasingly clear that careful consideration in sample preparation, fluorophore selection and imaging protocol parameters is vital to STORM imaging success. Recent studies show the importance of imaging buffer choice and how closely linked these choices are to the fluorophores imaged. Buffer chemistry is conducive to specific fluorophores and it is extremely important to investigate appropriate buffer conditions for your imaging needs. The Nikon N-STORM super resolution microscope system has laser modules delivering excitation at 405 nm, 488 nm, 561 nm, and 647 nm, and a high sensitivity Andor iXON3 DU897 EMCCD camera for data collection. Both fixed and live cell preparations can be imaged, as well as imaging in three dimensions. The system is also routinely used for high magnification TIRF imaging. Please contact Doug Taatjes, Nicole Bishop or Nicole Bouffard to discuss your potential super resolution and TIRF imaging project.

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Equipment Available:

- JEOL 1400 TEM
- JEOL JSM 6060 SEM with Oxford INCA EDS system
- Nikon STORM Super Resolution
- Zeiss LSM 510 META Confocal
- Applied BioPhysics ECIS Zθ
- AR MFP-3D BIO™ Atomic Force Microscope
- Arcturus XT-Ti Laser Microdissector
- CompuCyte Laser Scanning Cytometer
- Leica VERSA8 Whole Slide Imager
- IVIS Whole Animal Imager
- Olympus BX50 Microscope
- Olympus IX70 Inverted Microscope
- Olympus SZX12 Dissecting Microscope
- Leica MZ16F Fluorescence Dissecting Microscope
- Universal Imaging MetaMorph Workstation
- Volocity 3D Software
- MBF Biosciences Stereo Investigator
- RNAscope—HybEZ Oven for ISH

MIC Services Provided:

- Morphologic services and consultation at the light and electron microscopy level
- Morphometry
- Light and electron microscopic immunocytochemistry
- Confocal scanning laser microscopy
- Laser scanning cytometry
- Atomic force microscopy
- Scanning and transmission electron microscopy
- Laser capture microdissection
- Super resolution microscopy
- Preparation of paraffin and frozen sections
- Whole animal imaging
- Electric Cell Substrate Impedance Sensing
- Image analysis and processing
- Training for use of the above equipment
- Special histological staining
- Testing of new antibodies and developing new staining techniques