THE MICRO TOME

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MICROSCOPY IMAGING CENTER

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

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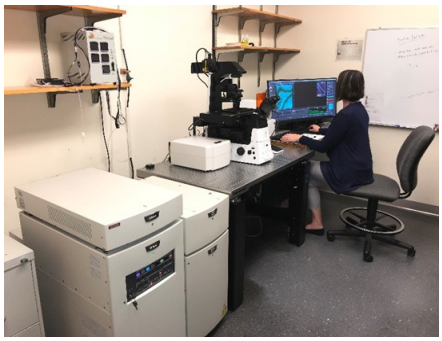
Winter 2018-19

Introducing the State-of-the-Art Nikon A1R HD Laser Scanning Confocal Microscope



Thanks to a successful NIH S10 Shared Instrumentation grant proposal (and a big "shout-out" to all of the Major and Minor Users who provided project descriptions) this past summer the MIC replaced the outdated Zeiss 510 META confocal microscope with the state-of-the-art Nikon A1R HD (high definition) confocal microscope system. The A1R comes equipped with a dual scanning system consisting of a high resolution galvanometer scanner and a high speed resonance scanner. The galvanometer scanner can operate at 8 frames/sec over a 512 x 512 pixel field and can be used to acquire high spatial resolution images at up to 4096 x 4096 pixels. The resonance scanner is able to image at high temporal rates (up to 30 frames/sec at 1024 x 1024 pixels resolution or 420 frames/sec in band scan acquisition mode at 1024 x 32 pixels), making it an ideal choice for live-cell imaging and imaging of photosensitive fluorophores. The dramatically shortened laser exposure time significantly limits photobleaching and the effects of phototoxicity on live specimens. Moreover, the unique combination of resonance and galvanometer scanners on one imaging platform provides the ability to deliver visible light for fluorophore photoactivation, while high-resolution images are simultaneously acquired at up to 30 frames/sec using the resonance scanner. The A1R HD contains a detector unit with 2 ultrasensitive GaAsP and 2 high sensitivity PMT's for visible lasers, and fluorescence filter emission sets for 450/50, 525/50, 600/50 (UV/GFP/RFP/Cy5). Combining these features with laser excitation lines at 405 nm, 445 nm, 488 nm, 514 nm, 561 nm & 640 nm, high quality objective lenses of 10, 20, 40, and 60X, and a live cell imaging chamber should provide an instrument capable of meeting all your confocal imaging needs. Incidentally, the "HD" designation implies that this confocal instrument is capable of providing images at sub-diffraction-limited resolution (approximately 140 nm resolution) surpassing the resolution afforded by prior confocal microscopes, thereby enhancing the information provided in the images.

Utilizing the flexible and easy-to-use NIS-Elements software platform familiar to many Nikon users, customization of settings and configurations tailored for specific imaging experiments can be readily achieved. The A1R HD confocal is equipped for all types of imaging applications possible on a point-scanning confocal, so please drop by and inquire about the new imaging possibilities provided by this instrument!

Training is FREE of charge. Please contact Nicole Bishop Nicole.bishop@uvm.edu or Nicole Bouffard Nicole.bouffard@uvm.edu to schedule your training session.

Changing of the Guard

After 2 decades of meritorious service to the MIC, Jan Schwarz retired at the end of May 2018. Jan was a key member of the MIC, serving as the main diagnostic electron microscopy technologist. In addition, Jan was responsible for training clients in the use of, and performing research with laser capture microdissection and laser scanning cytometry. She served as outreach coordinator for the MIC, and conducted numerous annual "Microscopy Festivals" at local schools and libraries. Jan's presence and team spirit will be sorely missed by us all, and we wish her and her husband Jerry great travels and adventures in their well-deserved retirement.

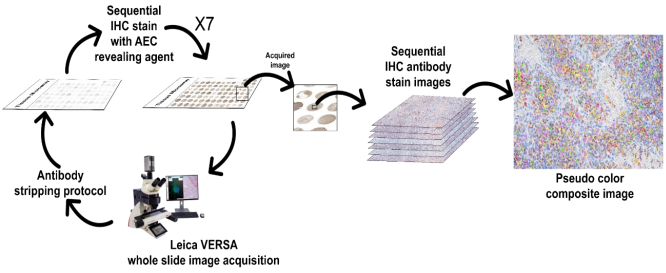
We are also excited to take this opportunity to introduce Jan's replacement Tim Connolly as the newest member of the MIC. Tim recently relocated from Western Massachusetts to UVM in May of 2108. Graduating from the University of Iowa, Tim's career has included working in pharmaceutical, research, and clinical pathology settings. The move to Vermont has increased his opportunities to spend time with family and friends, bike and hike, and spend his evenings with his telescope and camera. Please join us in wishing Tim a warm welcome to the MIC!

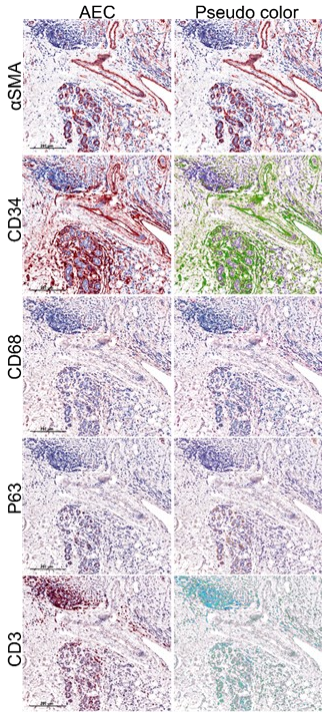
High-Throughput Imaging Query

The MIC is considering options for adding high-throughput imaging technology. If you are interested in this application for your research, please contact Doug Taatjes regarding your specific requirements. Thanks.

MIC Introduces Multiplexed IHC Method for Tissue Sections

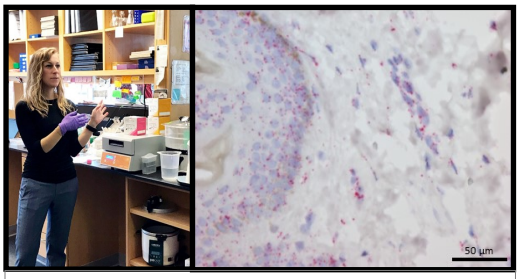
In a collaborative project with Drs. Janet Stein and Mark Evans, the Microscopy Imaging Center is utilizing a method of multiplexed immunohistochemistry to investigate the tumor microenvironment signature of breast cancer. The study is being performed on archived tumor samples of aggressive breast cancer identified via the VBCSS. Using the multiplexed immunohistochemistry (IHC) method we are evaluating and classifying early-stage, screen-detected breast cancers based on whether their stroma exhibit the signature hallmarks of the aggressive tumor microenvironment. The cellular and molecular analyses of the stroma focuses on specific cell types proposed to be involved in disease progression. IHC labeling of well characterized cell surface antigens is being used to define specific populations of cells associated with the tumor microenvironment. To date, we have developed a panel of target antigens (αSMA, CD3, CD58, p63, CD34, Vimentin, Thy1) which, along with morphological indicators, provide insight into the cellular composition of breast tissue both in the tumor and the surrounding regions. TMA (tissue micro-array) slides have been constructed from breast cancer patient tissue. TMA slides contain 3 cores per patient: (1) a sample originating from within the tumor, (2) 1 mm from the first core, and (3) a sample 1 mm from the second core. TMA slides containing ~43 cores (~14 patients per slide) were created for testing with the multiplexed IHC method. Samples were serially stained for the 5-7 targets mentioned above and the presence of the antigen was revealed with AEC. After each stain a 40X image was acquired on the Leica-Aperio VERSA whole slide imager (obtained with an instrumentation grant awarded by the Dean's Office, Larner College of Medicine). Following acquisition of the image, the slide was stripped of antibody and chromagen and the same slide was stained for another antigen. After 5-7 rounds of serial staining and imaging, resulting images were overlayed in Photoshop (adjacent figure) to reveal which cells stained with more than one antibody. This information was then used to evaluate cell types present in breast tumor microenvironment. The immunostaining multiplex technique can be used with either conventional chromogenic dyes, or with fluorescent dyes. Applications of the technique extend beyond tumor samples, and can be used for any study requiring tissue (or cell) staining with multiple antibodies. Please speak with Nicole Bishop, Nicole Bouffard, or Doug Taatjes to learn if multiplex immunostaining may be appropriate for your studies!





RNAscope Bootcamp

In November 2018 The Microscopy Imaging Center hosted a two day seminar/Bootcamp introducing RNAscope technology to our research community. RNAscope is a relatively new technology designed to detect low number RNA copies in various samples via RNA in situ hybridization. It is an excellent alternative to traditional IHC/IF methods when antibody specificity is questionable. Emily Martersteck from Advanced Cell Diagnostics (ACD) provided an introductory lecture (attended by approximately 30 people at UVM, and an additional 20 at Maine Medical Research Institute via video link) and took eight participants through the RNAscope protocol with their particular sample of interest. Sample types tested during the Bootcamp varied widely, and included everything from human cells on coverslips to rat cerebral arteries, and bovine mammary gland tissue. The Bootcamp was a great success and we would like to thank Emily and Jane Clarke from ACD for allowing us to host this two day event.



Emily Martersteck from ACD leading RNAscope Workshop at MIC.

Red dots on paraffin section indicating localization of RNA probes using RNAscope technology.

RNAscope technology is available as a service through the MIC, so please contact Doug Taatjes, Nicole Bishop or Nicole Bouffard for more information and a consultation.

Equipment Available:

- JEOL 1400 TEM
- JEOL JSM 6060 SEM with Oxford INCA EDS system
- Nikon STORM Super Resolution
- Nikon A1R HD Confocal
- 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
- Image analysis and processing
- Training for use of the above equipment
- Multiplex Immunostaining
- Special histological staining
- Testing of new antibodies and developing new staining techniques