

THE MICRO TOME

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College of Medicine

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MICROSCOPY IMAGING CENTER

HTTP://WWW.UVM.EDU/MICROSCOPYIMAGING

Fall 2012

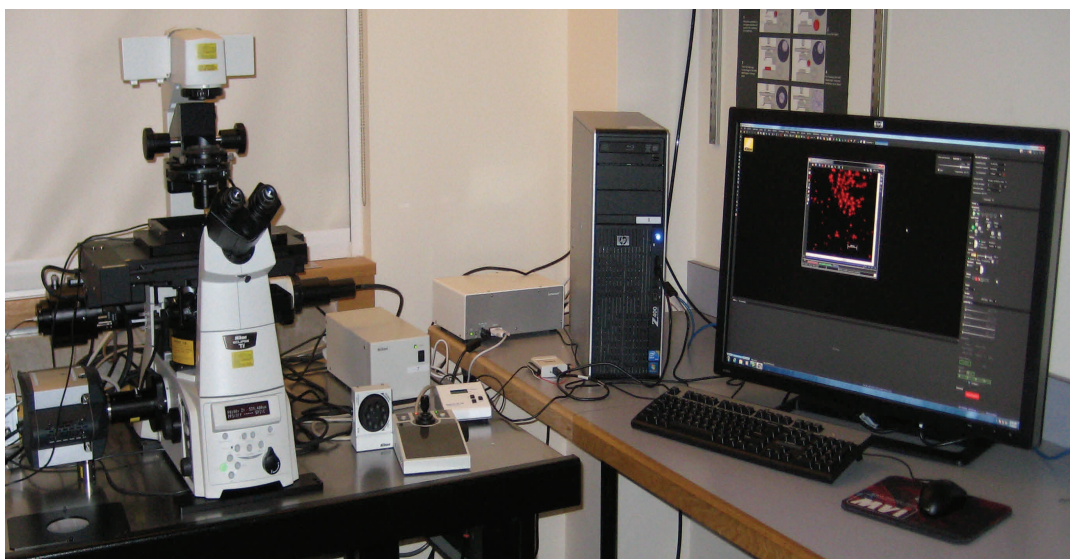
Super Resolution takes UVM by STORM

The Microscopy Imaging Center is pleased to announce that the Nikon STORM super-resolution microscope has been installed in the facility. We have two technicians available to train and assist with all of your STORM imaging needs.

STORM (Stochastic Optical Reconstruction Microscopy) is a super-resolution imaging technique that captures images with a higher resolution than the diffraction limit. Images are created by the sequential activation and localization of photoswitchable fluorophores. A small sub-set of fluorophores are activated and their positions are determined by locating the centroid position of the point spread function of each fluorophore. The fluorophores are then deactivated, and a new subset is activated and imaged. This process is repeated allowing numerous fluorophores to be localized. A super-resolution image is then constructed from all of the localizations.

The Nikon N-STORM super-resolution microscope system consists of a Nikon Ti-E TIRF inverted microscope base with laser modules

delivering excitation at 405 nm, 488 nm, 561 nm, and 647 nm, and a high sensitivity Andor iXON3 DU897 EMCCD camera. This instrument provides resolution in the fluorescence mode of 20 nm lateral and 50 nm axial. Both fixed and live cell preparations can be imaged, as well as imaging in three dimensions. Please contact Doug Taatjes, Nicole Bishop or Nicole Bouffard for further information or to schedule a training session.



MIC Captures New Laser Microdissector

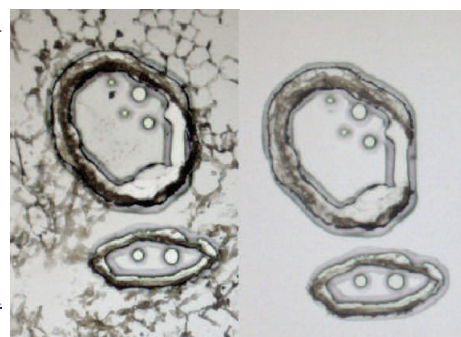
The Microscopy Imaging Center has recently upgraded our Laser Capture Microdissection (LCM) System to the Arcturus^{XT} equipped with a Nikon Eclipse *Ti-E* Microscope. Now you can use both a low-power infrared (IR) laser to melt a thermoplastic film over cells of interest on a plain glass slide, and an ultraviolet (UV) laser to cut around a larger region of interest (ROI), when the tissue is mounted on a polyethylene naphthalate (PEN) membrane slide. http://media.invitrogen.com.edgesuite.net/ab/applications-technologies/LCM/LCM_UV_video.html

The speed involved in microdissection has been greatly increased by the ability to outline all of the ROI or mark individual cells across the entire section, either by drawing directly on the monitor with a stylus or using the drawing tool in the Arcturus^{XT} software. Then, with a click of the keyboard, all of the chosen areas are captured and/or cut by automatic firing of the IR and UV lasers.

You can view your microdissected cells attached to the thermoplastic film before transferring them to a microfuge tube containing the

appropriate buffer for extracting DNA, RNA, or proteins for downstream analysis. The Arcturus^{XT} software allows you to automatically archive images including the cells microdissected, and your tissue before and after microdissection. When using fluorescently-labeled cells, you can also mark cells to microdissect from a static image, allowing you to close the shutter to minimize photo-bleaching.

Please contact Jan Schwarz @ 656-0813 or [ja-net.schwarz@uvm.edu](mailto:jan.schwarz@uvm.edu) to schedule a demo or training on the Arcturus^{XT}.

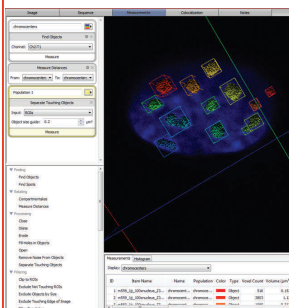


Cells selected for LCM

Microdissected cells on cap



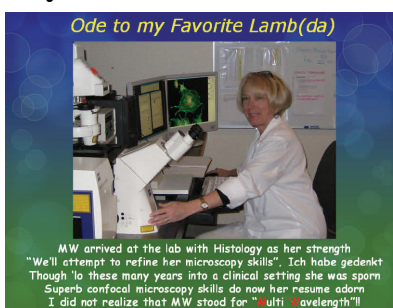
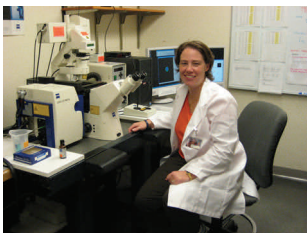
Lost Objects? Find them with Velocity



The 'Find Objects' function in Velocity is a powerful tool which allows the user to identify 2D or 3D areas of interest and quantify them based on staining intensity, size and volume. To start a protocol for finding objects simply drag a finding task such as 'Find Objects' to the protocol pane and select the channel of interest. A histogram of intensity values is shown within the dialog box and can be used to threshold for significant intensities and avoid background. The 'Find Objects' task also allows the exclusion of objects below a size threshold. The default value in this field will exclude most noise in biological images, but can be adjusted. The result of a 'Find Objects' task is then referred to as a 'Population.' A 'Population' can be given a name and can be analyzed for intensity, volume, size as well as many other morphological measurements. Using this tool you can obtain quantitative data on 3D structures within an image that might have previously only been able to be counted as present.

I Don't Know Why You Say Goodbye, I Say Hello...(apologies to The Beatles)

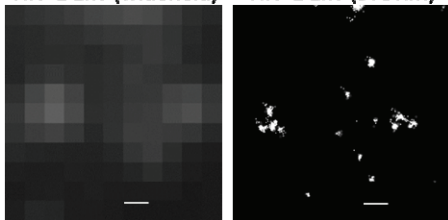
After a long and illustrious career in the MIC, Marilyn Wadsworth retired at the end of March, 2012. Marilyn was a "charter" member of the MIC, having been here when the facility opened in 1993. Marilyn performed many functions in the MIC, including confocal microscopy specialist, image analysis specialist, histotechnologist, Laboratory Safety Office, to name a few. She will always be remembered for her cheerful disposition, infectious smile, and ready laughter. She will be sorely missed, but we are happy for her and Tom, and wish them pleasant voyages and a sunny journey into retirement.



We are also pleased to announce the addition of Nicole Bouffard to the MIC staff. Nicole is a UVM graduate and has worked in the College of Medicine for the past ten years. She brings a wealth of experience in confocal microscopy and image analysis, and is a most welcome addition to our MIC team.

Thali Lab STORMing with HIV Recognition and Fusion

HIV-1 Env (widefield) HIV-1 Env (STORM)



Scale bar = 200nm

Fluorescence microscopy has been used to study the underlying structure and organization of proteins on a subcellular level. Although fluorescence microscopy has provided immense insight into our understanding of the inner workings of cells, this technique is fundamentally constrained by the diffraction limit of the wavelength of light used (Abbe's Law), which puts the limit of resolution at roughly 250nm. Recently, new fluorescence techniques have emerged to overcome the diffraction limit. One such technique,

called stochastic optical reconstruction microscopy (STORM), uses a chemical property of the fluorophore to switch it between a non-fluorescent "off" state and a fluorescent "on" state. By isolating individual fluorophore switching events, the centroid can be determined with high accuracy, generally between 20-30nm (depending on the number of photons gathered), which represents a 10-fold increase in resolution from conventional diffraction limited techniques. Here, we have used STORM to probe the organization of HIV-1 assembly sites. Cells were transfected with a full molecular clone of HIV-1, surface stained for HIV-1 Env, and fixed. HIV-1 Env is a transmembrane protein required for host cell recognition and membrane fusion, and is incorporated into the budding virion at the plasma membrane. Above is a representative image from the STORM instrument. The left panel is the diffraction limited widefield image, and the right panel is the corresponding STORM image. In the STORM image, Env seems to cluster into ~100nm clusters, which correspond nicely to the size of HIV-1 virions revealed by electron microscopy, thus we are likely visualizing individual viral budding sites. This instrument is also capable of 2 and 3 color imaging, which will be highly beneficial in dissecting the sub-diffraction organization of cellular and viral proteins. - Nate Roy

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 Z0
- AR MFP-3D BIO™ Atomic Force Microscope
- Arcturus XT-Ti Laser Microdissector
- CompuCyt Laser Scanning Cytometer
- IVIS Whole Animal Imager
- Olympus BX50 Microscope
- Olympus IX70 Inverted Microscope
- Olympus SZX12 Dissecting Microscope
- Leica MZ16F Fluorescence Dissecting Microscope
- Universal Imaging MetaMorph Workstation
- Velocity 3D Software
- MBF Biosciences Stereo Investigator

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