THE MICRO TOME

University of Vermont
College of Medicine

HSRF 203 | Voice: (802) 656-0813 | Fax: (802) 656-8892

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

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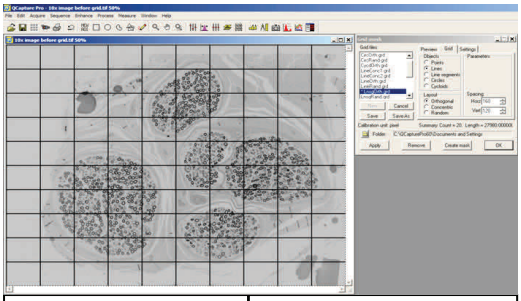
Imaging and Analysis 101: Each issue of the microTOME provides useful tips for a specific imaging application. In this issue we provide a random sampling method for light microscopy images.

Systematic Uniform Random Sampling (SURS) is a method of choosing regions of interest without bias. The sampling can be done at any level (population, tissue, field of view, etc.) and depends on the experimental design. If SURS of tissue regions at the light microscopic level is needed, the QImaging software has some tools to accomplish this.

Begin by capturing a low magnification image of your tissue. Perform a background correction, if needed.
Apply a grid to captured image:
Process menu… Grid Mask.



Grid tab:	Settings tab:
Line orth. grd Layout – Orthogonal Spacing (10X) - Horiz – 160, Vert – 120 Spacing (4X) - Horiz – 48, Vert – 34.4	Margins: *Full size* Draw: Color (choose black) Measure units: *pixels* Click *Apply*, then keep this window open.

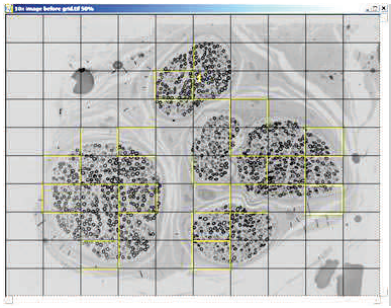
1. Count number of squares falling over tissue. Go to Random.org and generate a random number between 1 and this number. This will be your starting square.

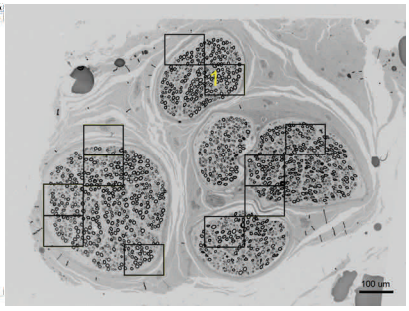
2. To mark the square on the image with the grid, open the *Annotation Tool* (pencil icon on tool bar): Click the rectangle, and then drag out a rectangle the size one grid box on the image. You can also use the text tool to make a '1' in this box.

3. Divide total # of grid boxes falling over tissue by number of fields/images needed. This will be the frequency of sampling (every 2nd, 3rd, or 4th box).

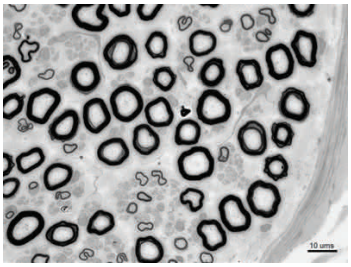
4. Use the *Annotation Tool*, as described above, to place boxes (and numbers, if desired) on all fields to be captured at high magnification. Use the copy and paste tool in this *Annotation Tool* box for efficiency.

5. In *Grid tile* dialog box, click *Remove* to remove the grid and see all the boxes. In *Annotation Tool* dialog box, click the burn tool, to burn boxes onto image. Save image as 'SURS results'.



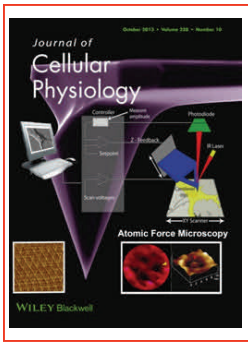


6. Insert the high magnification objective lens needed and capture fields using the SURS results image as a reference.



MIC Outreach Activities

Academic Year 2012-2013 was an active one with many outreach activities both in the MIC lab and off-site. We facilitated 18 sessions of Project MICRO in 9 venues, reaching over 350 students, teachers, and parents. Under the auspices of the Vermont Cancer Center (VCC) and the AGTC, we welcomed over 60 AP Bio students from 3 different high schools for mornings of MIC instrument demonstrations and "hands-on" lung cancer discussions in the Pathology Teaching Lab. The MIC was well represented with CMB and MMG prospective student tours and lectures and hands-on demonstrations with medical students from Japan, FAHC Cytotechnology students, Johnson State College, Middlebury College, Green Mountain College and SUNY-Cobbleskill undergraduates, Pathology residents, and UVM graduate and undergraduate students. We hosted high school students as CVU Grad Challenge mentors, Governor's Institute facilitators, and provided job shadowing experiences for additional middle school and high school students. The year wrapped up with three sessions of MedQuest campers spending the afternoon in the Pathology Teaching Lab learning about the effects of preventable diseases on various organs of the body.



That Devilish Photoshop…… HELP!!!!

MIC is now offering assistance with the creation of image montages and schematic drawings. If you need figures for a manuscript, grant, poster or presentation we can help. Nicole Bouffard has over 10 years of experience with graphic design in Adobe Photoshop and is available for consultation. If you can sketch it, she can digitize it. For more information contact Nicole Bouffard. Nicole.Bouffard@uvm.edu (October 2013 cover - Journal of Cellular Physiology – N.Bouffard & D. Taatjes).

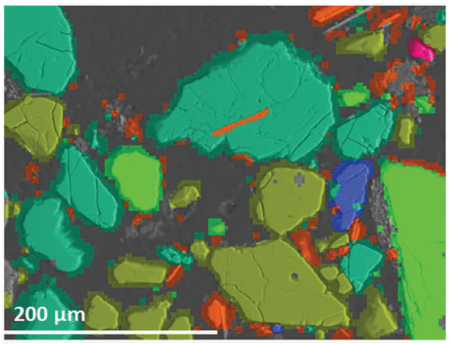
Introducing iLab Solutions

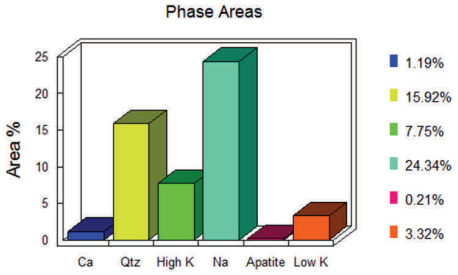
In April 2013 MIC said goodbye to the BioDesktop and hello to iLab Solutions. After weeks of development we have successfully transitioned to the new scheduling software. iLab provides various upgrades to the BioDesktop including the ability to request training and submit work requests. After logging in, take a look at the "About our Core" tab, it contains several manuals explaining how to schedule instrument time, request training and submit work requests. iLab Solutions runs most efficiently with the internet browser Mozilla FireFox. We have found that using other browsers limits access to critical software functionality, therefore we strongly recommend Mozilla FireFox as the browser of choice. Please contact Nicole Bishop or Nicole Bouffard with questions or concerns. To sign up for an iLab account visit https://my.ilabsolutions.com/service_center/show_external/3159

On the Cutting Edge: Highlighting Microscopy in the MIC—

SEM-EDS from Dr. Ross and Rebecca Bourgault, Dept. of Plant and Soil Science

Trees require calcium (Ca) and acquire it from the soil; therefore, if a soil is depleted in Ca, trees cannot thrive. Soils contain different forms of Ca, and these forms vary in their availability for plant uptake. Plants can access Ca only in exchangeable forms, which are soluble or bound to organic matter or soil particle surfaces. However, these exchangeable pools are quickly lost through acid leaching. Loss of exchangeable Ca in northeastern forests has been accelerated in recent years due to acid deposition, and forest productivity in some areas has declined as a result. In the northeast, the parent material of soil is primary minerals derived from glacial drift. As these minerals are weathered, they release Ca (and other nutrients) over time. Both the Ca content and the weathering rate of soil minerals vary considerably. There is not much information available regarding quantitative mineralogy of northeast forest soils. Therefore, researchers from the Department of Plant and Soil Science at UVM, UMass Amherst, and the US Forest Service have been investigating the mineralogy of forest soils in the northeast, and estimating the quantity of Ca in resistant and exchangeable pools. The JEOL 6060 Scanning Electron Microscope with Energy-Dispersive X-ray Spectroscopy (SEM-EDS) at the Microscopy Imaging Center is being used to examine quantitative mineralogy of soil thin sections. Sections of slides have been imaged using INCA software's QuantMap feature, to produce a quantitative mapping of elemental composition. Phase-Map is then being used to identify particles with similar composition and thus mineralogy. Each unique phase is user-defined and represented by a false color (Figure at right). Calcium-rich minerals such as plagioclase feldspars (green in figure), ferrohastingsite (blue in figure), and apatite (magenta in figure) have been identified. Three different sites across NY, VT, and NH with a wide range in total Ca content are being compared for quantities and forms of Ca. Results will help determine the capacity of northeastern soils to replenish available Ca pools.





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
- 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

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