



Proper handling of tissue samples is important for Proteomics, Genomics and Imaging:

A recent article in *BioTechniques* examined the effect of tissue ischemia (*loss of blood supply*) on protein and gene expression (*as measured by microarray and mass spectrometry*). The authors found that initial changes in expression profiles were observed as soon as 5-8 minutes after tissue had been surgically removed. At fifteen minutes they found that 10-15% of all genes were differentially expressed. By thirty minutes the intensity of 30% of the protein peaks (*by SELDI-TOF MS*) had changed more than two-fold. The authors conclude that tissue should be processed rapidly and with identical ischemia time intervals to minimize data variation within sample sets.

See: Spruessel, A, et. al., (2004) Tissue ischemia time affects gene and protein expression patterns within minutes following surgical tumor excision, *BioTechniques* 36:1030-1037.

Ischemia can seriously affect tissue samples submitted for imaging experiments (*e.g., histology, immunofluorescence, immunohistochemistry, electron microscopy*). Fixatives like formaldehyde and glutaraldehyde take time to penetrate into tissue. Thick pieces of tissue can end up being well-fixed on the outside and very poorly-fixed inside. When these large pieces of tissue are sectioned, the staining patterns can be very confusing, since the well-fixed outer areas stain one way and the poorly-fixed inner areas stain with a different pattern.

Here are some important things to remember about fixing tissues:

- Formaldehyde penetrates into tissues at a rate of approximately 0.5mm/hr. Glutaraldehyde penetrates tissues even slower than formaldehyde. Tissue penetration is slower at 4°C than at room temperature.
- Tissue for formaldehyde fixation should have one dimension that is no thicker than 2 US quarters (*approximately 3mm*). Tissue for glutaraldehyde fixation should have one dimension that is no thicker than 1 US quarter (*approximately 1.5mm*).
- The volume of fixative should be at least 20 times that of the tissue. Inverting the fixative container every 30 minutes will ensure proper mixing (*shaking is not necessary*).
- Tissues can be over-fixed and should not be stored long-term in fixatives.
- **Note:** cultured cells (*typically >0.05mm in thickness*) do not need long fixation times.

We encourage users to contact the Core for advice on sample fixation and preparation.

ARL & AZCC Flow Cytometry labs combining:

The Arizona Research Labs Fluorescence Activated Cell Sorting facility and the AZ Cancer Center's flow cytometry core will be combining in late summer or early fall of 2004. The labs will move into newly renovated space in AZCC room 4920. Barb Carolus will manage the combined facility.

The plan is to place a state-of-the-art flow cytometer in the combined facility. Dr. Michael Cusanovich is coordinating the raising of funds from departments and investigators to purchase this instrument. If your lab can support this purchase in any way, please contact Dr. Cusanovich ASAP.

Light Microscopy Tips – new handout available on-line:

This four page handout covers several topics: recommended coverslip thickness, coverslip mounting media, don't mix immersion oils from different vendors, mixing and matching microscope objectives, and cleaning microscope objectives.

The handout is available at: <http://swehsc.pharmacy.arizona.edu/exppath/resources/handouts.html>. Other handouts available at this web address include: [Basic Microscope Alignment](#), [Formaldehyde Fixatives](#), [Digital Imaging Ethics](#), [Contact information for affiliated imaging facilities](#), [Light Microscope Vendors for Southern Arizona](#) and several others.

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August 2004 - FREE Workshops

(Sponsored by the Biotechnology Division, Arizona Research Labs):

Microwave Tissue Processing Workshop	Image J Workshop
<p>When: August 16 - 17, 2004 Where: Marley, Rm 217 Registration: REQUIRED - Limit of 30 participants Cost: FREE Registration: http://imaging.arl.arizona.edu/microwave.php</p>	<p>When: August 20, 2004, from 9:00 - Noon Where: BioSciences West, Rm 243 Registration: REQUIRED - Limit of 21 participants Cost: FREE, Limited seating. Registration: http://bcf.arl.arizona.edu/services/events/</p>
<p>August 16 schedule: 9:00 - 12:00 Understanding the microwave environment 12:00 - 1:00 Lunch Break 1:00 - 4:00 New microwave methods: Fact and Fiction 4:00 - 5:00 Questions and Specific User Techniques</p> <p>August 17 schedule: 9:00 - 12:00 New approaches to old issues: Applications, Clinical/Research. 12:00 - 1:00 Lunch Break 1:00 - 4:00 Immunolabeling and special staining techniques in the microwave: Control Issues. 4:00 - 5:00 Questions and specific user techniques</p>	<p>Description: Hands on session, with basic introduction to image processing, and introduction to image processing routines and plug-ins in ImageJ</p> <p>Other popular image manipulation tools like ImageMagik and Gimp (PC/Mac/LINUX) will also be covered.</p> <p>Users are encouraged to e-mail instructor if they would like specific topics/issues covered</p> <p>Instructor: Nirav Merchant</p>
<p>This workshop is a combination of lecture and demonstration.</p>	<p>Background info (from: http://rsb.info.nih.gov/ij/)</p>
<p>Instructor: Rick Giberson (R&D from Ted Pella, Inc.) has been instrumental in the development of new microwave protocols and equipment.</p>	<p><i>ImageJ is a public domain Java image processing program inspired by NIH Image for the Macintosh. It runs, either as an online applet or as a downloadable application, on any computer with a Java 1.1 or later virtual machine. Downloadable distributions are available for Windows, Mac OS, Mac OS X and Linux.</i></p>
<p>Background info</p> <p><i>Microwave techniques have been used to speed up tissue fixation and processing in histology and EM. The techniques have been used to speed up special stains and improve immunostaining (e.g., antigen retrieval).</i></p>	<p><i>It can display, edit, analyze, process, save and print 8-bit, 16-bit and 32-bit images. It can read many image formats including TIFF, GIF, JPEG, BMP, DICOM, FITS and "raw". It supports "stacks", a series of images that share a single window. It is multithreaded, so time-consuming operations such as image file reading can be performed in parallel with other operations.</i></p> <p><i>It can calculate area and pixel value statistics of user-defined selections. It can measure distances and angles. It can create density histograms and line profile plots. It supports standard image processing functions such as contrast manipulation, sharpening, smoothing, edge detection and median filtering.</i></p>