Center for Toxicology, Southwest Environmental Health Sciences Center (an NIEHS Center)

# Cellular Imaging Core Newsletter

**April 2004** **http://swehsc.pharmacy.arizona.edu/exppath/**

## The importance of choosing the correct image file format

"The reason for recording images in scientific studies is not to keep remembrances of familiar objects and scenes, but to record the unfamiliar. If it is not possible to know beforehand what details may turn out to be important, it is not wise to discard them. And if measurement of features is contemplated (to measure size, shape, position or color information), then lossy compression, which alters all of those values, must be avoided." - **John C. Russ, Ph.D., p48, Seeing the Scientific Image**, http://www.drjohnruss.com/archives/000013.html

We agree with Dr. Russ, and urge all of our users to specifically avoid using the JPEG file format for scientific data. JPEG compression uses the discrete cosine function to reduce the file size, however, it also changes the XY resolution of the image and the intensity value of any given pixel. A tutorial on the problems with JPEG compression is available at: http://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/jpegcompression/ .

For most users, the preferred "lossless" file format for scientific images is TIFF *(tagged image file format)*. While it is true that TIFF files can become quite large *(a 4096x4096 pixel, true color 24bit image is approximately 50MB)*, file size is not a suitable reason to use the JPEG format.

If you have questions about digital image file formats, other digital imaging issues, or would like help in learning how to set up figures for publications, contact Doug Cromey.

## Key points from the IHC/IF workshop:

For those of you who were unable to attend the Immunohistochemistry/Immunofluorescence workshop in late February, we've distilled out a few key points.

- Meticulously following a standardized fixation protocol will lead to the most consistent results. *"Fixation is the single most important step that ultimately influences the outcome of IHC."* The protocol should address the type of fixative, the volume of fixative, the maximum time between biopsy/necropsy and immersion in the fixative, the size of the sample, the length of time for fixation and how soon the sample will be processed for paraffin histology.
- There are a number of techniques for antigen unmasking. Some work better than others, but there is not a "universal" antigen unmasking technique. Unmasking techniques are not a panacea; tissue can be over-processed and the antigen can become "lost" as a result. Unmasking can also expose potential sources of background staining.
- Blocking steps are an important way to avoid false-positive or non-specific background staining. Potential sources of non-specific staining include protein interactions, endogenous enzymes, endogenous biotin, cross-reactivity of the primary antibody *(this is why negative controls are so important)* or poorly fixed tissue that allows the antigen to diffuse from its original site.
- Replacing an existing stock of antibody with a new lot number may require an adjustment in dilution factors. This can be a particular problem with polyclonal antibodies.
- Monoclonal antibodies are very specific; however, if the antigenic epitope is slightly different, the monoclonal may not stain anything. Polyclonal antibodies stain a number of epitopes, however, they have a lower specificity and a tendency toward higher levels of background staining.
- Secondary antibodies must target the correct primary antibody. This includes the specific species and the immunoglobin sub-fraction of the primary antibody.

As a reminder, the Cellular Imaging Core has a web page with a great deal of information about Formaldehyde fixatives. We expect to have a PDF version of the information available on-line shortly. See: http://swehsc.pharmacy.arizona.edu/exppath/resources/formaldehyde.html

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