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

# Experimental Pathology Core

***February 2001*** ***http://swehsc.pharmacy.arizona.edu/exppath/***

## *Digital Image do's & don'ts:*

Doug Cromey has added a section on imaging ethics to the Core's Microscopy & Imaging Resources web site. Since he has not been able to locate any specific guidelines for digital imaging on the WWW, he is posting these ten guidelines. If you have questions, comments or feedback, please contact Doug.

1) **Scientific digital images are data.** The data are arranged spatially in an xy matrix *(or grid)* and each individual element *(pixel)* has a numerical value that represents a grayscale or RGB intensity value. These data are a numerical sampling of the sample as presented by the data acquisition system *(e.g., microscope)* to the sensor *(e.g., CCD camera)*. The data acquisition system and sensor are subject to all the limitations and aberrations that physics and instrument design may impose on the two devices. To the observer's eye the image data may appear to accurately represent what can be seen, however, it is the user's responsibility to understand the limitations of the particular instrument.

2) **Intensity measurements of digital images should be performed on raw data and the data should be calibrated to a known standard.**

3) **Manipulation of digital images should always be done on a copy of the raw image data.** The original raw data file is the standard to which the final image can be compared.

4) **Simple adjustments to the entire image are <u>usually</u> acceptable.** This would include techniques that are similar to standard darkroom techniques *(e.g., different contrast grades of paper, changes in development time)*. With digital images this would include performing "reasonable" adjustments of the levels and gamma settings *(adjusting the brightness & contrast is not recommended)*.

5) **Cropping an image is <u>usually</u> acceptable.**

6) **Manipulations that are specific to one area of an image and are not performed on other areas are questionable.** This would include techniques analogous to "dodging" and "burning" in a photographic darkroom. This is a disputed issue. Purists would state that selective enhancement should never be performed, however, there are occasions when it is legitimate to enhance a specific area in an image. Honesty is the best policy, if portions of an image for publication were selectively enhanced, the author should state it clearly in the figure legend.

7) **Use of software "filters" to improve image quality is usually <u>not recommended</u> for biological images.** Commercial software designed for desktop publishing cannot be counted on to appropriately and scientifically manipulate the data in a digital image. Digital image filters are typically mathematical functions *(convolution kernels)* that numerically change the data in the image. If not carefully used, they can create artifacts in an image that can lead to misinterpretation of the data. If filters must be used, they should be noted in the figure legend of published images *(include software version, specific filters & any special settings that were used)*.

8) **Cloning objects into an image, or from other parts of an image, is very questionable.** Users often consider using the technique of cloning sections of an image to "clean up" a dirty preparation. If the image requires this much processing, the best solution is to go back and take another image from the sample or a new sample prepared under the same conditions. Use of cloning techniques to create objects in an image that did not exist there originally *(e.g., "creating" a new gel band)* is completely unethical.

*Continued on next page…*



| ***Contacts***: | *R. Clark Lantz, Ph.D.* | *Core Director* | *626-6716* | *LSN 447* | *clark-lantz@ns.arizona.edu* |
|---|---|---|---|---|---|
| | *Douglas W. Cromey, M.S.* | *Core Manager* | *626-2824* | *AHSC 4212A* | *cromey@arizona.edu* |
| | *Claire M. Payne, Ph.D.* | | *626-2870* | *AHSC 6111* | *claire-payne@ns.arizona.edu* |
| | *Kathleen Kunke, B.S.* | | *626-5514* | *LSN 625* | *kathleek@u.arizona.edu* |

9) **Beware of lossy compression.** There are very few good reasons to use the JPEG file format on scientific digital images *(other than displaying an image on a web page)*. 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. If you must use JPEG, perform the compression as the last thing that is done to an image. With most image manipulation programs, opening & closing a JPEG image multiple times runs the compression algorithm on the image multiple times, further degrading the image each time.

10) **Beware of resampling an image.** Changing the size of an image can introduce resampling artifacts. Decreasing the image size *(downsampling)* can cause the xy resolution in an image to be greatly reduced and if the size reduction is not by a power of two, the software program has to be "creative" in determining the intensity values of each pixel *(guessing)*. Increasing the image size *(upsampling)* causes the software to interpolate *(guessing)* to "create" pixels in between the existing pixels. In either case, insert a scale bar prior to resampling so that you can keep track of the magnification *(its nearly impossible to do afterwards)*.

**PHOTOSHOP TIP:** if you are only changing the dpi of the image for different output devices *(e.g., printers),* uncheck the **resample image** box that's found at the bottom of the window that appears when invoking the **IMAGE|IMAGE SIZE** menu item. By doing this you change the scale of the image *(72 dpi, 300 dpi, etc)* without changing the number of pixels in the width or height boxes.