Digital Imaging Ethics

It is strongly advised that EMS Users follow the MSA Policy on Digital Image Manipulation.

"Ethical digital imaging requires that the original uncompressed image file be stored on archival media (e.g., CD-R) without any image manipulation or processing operation. All parameters of the production and acquisition of this file, as well as any subsequent processing steps, must be documented and reported to ensure reproducibility.

Generally, acceptable (non-reportable) imaging operations include gamma correction, histogram stretching, and brightness and contrast adjustments. All other operations (such as Unsharp-Masking, Gaussian Blur, etc.) must be directly identified by the author as part of the experimental methodology. However, for diffraction data or any other image data that is used for subsequent quantification, all imaging operations must be reported."

This policy was formulated by the Digital Image Processing & Ethics Group of the MSA Education Committee and was adopted as MSA policy at the Summer Council meeting August 2-3, 2003.

Guidelines for the proper acquisition and manipulation of scientific digital images:

(from Douglas W. Cromey, M.S. - Manager, Cellular Imaging Core Southwest Environmental Health Sciences Center, University of Arizona, Tucson, Arizona)

See ***here*** for the original article

These guidelines were written for life science imaging but are relevant to materials science microscopy as well.

1. **Scientific digital images are data that can be compromised by inappropriate manipulations.** Images are data 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 specimen 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.

"The basic message is that humans are not very good observers, that our vision system ignores a lot of information, that having names and labels for recognized features is very important, and that we often think we see what we expect to see." - Dr. John Russ (1)

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

Digital Imaging Ethics

Maintaining a copy of the unaltered original image is the user's only protection against accusations of misconduct. This is also the only way that users can recover from a mistake in image processing. Data should be archived to media that are not easily altered (e.g., CD-R or DVD-R) (2). Maintaining the image in the original file format is highly recommended.

"Individual's and corporations whose research falls under the United States FDA's "Final Rule on Electronic Records and Electronic Signatures" (21 CFR part 11) have mandatory requirements for maintaining the integrity of the original image. This would include labs using "Good Lab Practices". Other industries where maintaining the original image is required would include; forensics (rules of evidence) and health care (liability, HIPAA)."

3. Simple adjustments to the entire image are usually 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. Because changes in gamma are non-linear, many journals are requiring that these types of adjustments be described in the figure legend or the methods section. Small adjustments to the brightness and contrast are usually acceptable, however, large adjustments are not recommended. This is because it is very easy to truncate intensity information in the image using brightness and contrast.

4. Cropping an image is usually acceptable. Avoid acquisition bias. Capturing images that only confirm the lab's "preferred hypothesis" is a form of unethical cropping. Consider the following observation by microscopy core facility director Dr. George McNamara.

"*I suspect that most published micrographs are "exemplary", "best of", or, "the only one we took", or "the only one that fit our hypothesis" (I call the latter two categories, "N=1 experiments"). If you are putting together figures, and you select for publication a micrograph based on any of these categories, at least be honest to the reviewers and editor and say so (hopefully they'll tell you to go back and collect data correctly ... even better, your coauthors should tell you ... best of all, your inner super-ego should tell you).*

What you should be publishing are representative micrographs. That means you need to acquire sufficient images to document/quantify the experiment. Your specimen and images should be good enough that any of the micrographs can be used. In fact, if you can only publish one micrograph per treatment group, use a random number generator to pick which one..." - Dr. George McNamara (3)

After you have selected a specific image to use in a figure, what is your motivation for cropping that image? Is it to improve the "composition" of the image, or is it to hide something that disagrees with the hypothesis?

Remember to leave yourself enough pixels so that the image will reproduce well in a scientific journal. If you have to crop too much out, it's time to re-image your specimen. Don't let Photoshop replace good science.

Digital Imaging Ethics

5. Digital images that will be compared to one another should be acquired under identical conditions, and any post-acquisition image processing should also be identical. Any processing of images that are to be compared should be identical, especially if they will be published as a group of images in a single figure. If there is a compelling reason that the images in a figure were processed differently, this must be explained in the publication or figure legend. Honesty is the best policy. If background subtraction or white-level balancing (to compensate for uneven illumination, etc) was performed, this should be acknowledged in the methods section.

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 very rare 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 not recommended for biological (*or materials*) 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 change the numerical data in the pixels in the image. If the filters are not used carefully, they may 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. The note should include software version, specific filters and any special settings that were used.

Software filters and to some extent “cloning” are sometimes used to clean up the background of an image. Scientists must always remember the possibility that someone will look at their data in a way they hadn’t considered. Perhaps the reader will find that the collagen matrix, support media, interface between two structures, or other “unimportant” features in the image contains information that will spark an idea for their research. If the author changes the “unimportant” things to enhance the “important” things, they have lied to the reader.

8. Cloning or copying objects into a digital image, from other parts of the same image or from a different 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. The 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.

Use of cloning and/or copying is asking for trouble. Most of the falsified image cases that the Office of Research Integrity sees use these techniques. Professional journals that closely examine images (e.g., Journal of Cell Biology) can detect these sorts of things pretty routinely.

Combining images (e.g., two similar gels combined into one figure) is acceptable at most journals only if it is clear to the editors & reviewers that the two images are from separate sources. Often this means a

small gap between the two images or a black line that delineates the two images. Scientifically, it is better to re-run the experiment, rather than paste images together.

9. Intensity measurements should be performed on uniformly processed image data, and the data should be calibrated to a known standard. Be aware that some instruments are subject to a number of known fluctuations over time as well as having other physics/electronics limitations. If you are unaware of, or can't account for, the limitations of the acquisition instrument, you should not be performing intensity measurements.

10. Avoid the use 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 and saving a JPEG image multiple times runs the compression algorithm on the image multiple times, further degrading the image each time.

"*…many aspects of scientific and industrial usage involve subsequent processing of a digital image, for example to enhance features or count items. Using any form of lossy compression for images in this context may create problems - after all the information thrown away during lossy compression is generally that information that is imperceptible to a human eye - not necessarily showing the same characteristics as computer image processing software.*" - Joint Photographic Experts Group (JPEG) (4)

"*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.*" - Dr. John Russ (1)'

"*It is tempting to acquire your image files in JPEG format to save disk space, but doing so compromises your data. Always use TIF format.*" - Journal of Cell Biology (5)

"*Even with large scientific image formats the cost of storage is vanishingly small. It, therefore, makes no sense not to save an original unprocessed and uncompressed image file. The MSA (Microscopy Society of America) format for this storage is the TIFF file format.*" - J.M. MacKenzie, M.G. Burke, T. Carvalho & A. Eades (2)

Important - *Users of the Adobe Acrobat writer software should be aware that the default setting in this program is to apply JPEG compression to any images embedded in the document. These settings can be changed by the user.*

11. Magnification and resolution are important. Digital images of real world objects sample an object in a way such that each pixel in the image has a scale. This scale may be in meters per pixel for satellite images or in tenths of microns per pixel for microscope images. Ideally the scale is the same in both the

X and Y dimensions; however, this is not always the case. The magnification of the image is determined by the difference between the original scale of the pixel and the scale of the pixel in its final form (e.g., paper printout, projected on the wall of a large lecture hall). Since it is often impossible to know in advance what the final magnification will be, a scale bar of known size is the best way to express the magnification. Journals may resize your image, so providing a numerical magnification number in a figure legend may result in errors.

The ability of a microscope to resolve (separate two small, adjacent objects) is limited by the wavelength of light used and the numerical aperture of the objective lens (Rayleigh criterion).

"*In most cases, to ensure adequate sampling for high-resolution imaging, an interval of 2.5 to 3 samples for the smallest resolvable feature is desirable.*" - Spring, K.R., Russ, J.C., Parry-Hill, M.J., Fellers, T.J., Zuckerman, L.D. & Davidson (6)

Note that this statement means 2.5-3 samples (pixels) should be used to capture the smallest resolvable features in each of the three spatial dimensions (XYZ). Other dimensions, such as time and/or wavelengths, should also be correctly sampled to avoid artifacts. Undersampling (using too few pixels to describe a spatial feature in a sample) can lead to artifacts masquerading as real structures. Oversampling is not as problematic, however, it should be noted that oversampling does not yield any additional spatial resolution information from the specimen.

12. Be careful when changing the size (in pixels) of a digital image. Changing the size of an image (the number of pixels in X and Y) can introduce resampling artifacts. Decreasing the image size (downsampling) can cause the XY resolution in an image to be greatly reduced. 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). Using a power of two is slightly better, since this is a form of averaging, and while the resolution is still decreased, it is decreased in a more reproducible manner.

Increasing the image size (upsampling) causes the software to interpolate (guessing) to "create" pixels in between the existing pixels. Upsampling an image does not increase the resolution, in fact it may make it more difficult to resolve features because of aliasing artifacts. In either case, users should insert a magnification scale bar prior to resampling (magnification may be nearly impossible to calculate afterwards).

Users should only change the total number of pixels in an image one time to avoid compounding any artifacts that might be created.

References:

(1) Seeing the Scientific Image (parts 1,2,3), John Russ, Proceedings Royal Microscopy Society 39(2); 39(3); 39(4) (2004) or available on-line at <http://www.drjohnruss.com/downloads/seeing.pdf>

(2) Ethics and Digital Imaging, J.M. MacKenzie, M.G. Burke, T. Carvalho & A. Eades. MicroscopyToday 12:40-41. (2006)

Digital Imaging Ethics

(3) Crusade for Publishing Better Light Micrographs – Light Microscopy publication guidelines, George McNamara, Congressman Julian Dixon Image Core, The Saban Research Institute of Children's Hospital Los Angeles, Los Angeles, CA
<http://home.earthlink.net/~geomcnamara/CrusadeBetterMicrographs.htm>

(4) Scientific and Industrial, Joint Photographic Experts Group,

(5) The JCB will let your data shine in RGB, Mike Rossner and Rob O'Donnell, Journal of Cell Biology 164:11-13. (2004)

(6) Digital Image Sampling Frequency, Spring, K.R., Russ, J.C., Parry-Hill, M.J., Fellers, T.J., Zuckerman, L.D. & Davidson, M.W. (2006)