

## Guidelines for Image Processing

Image manipulation can be a form of research misconduct. It is critical that none of the adjustments made change the actual data in the image. In general, simple linear adjustments to an entire image are acceptable (ie, *reasonable* adjustments of brightness, contrast, or levels), whereas nonlinear adjustments that alter the shape of the histogram or create oversaturated or undersaturated pixels should be avoided. Manipulations that obscure, erase, or misrepresent image elements are almost always unacceptable.

Adjustments that are acceptable under most circumstances:

- Cropping
- Downsampling (decreasing the size of an image)
- Small brightness or contrast adjustments to an entire image
- Applying a false color to a grayscale fluorescence image
- White balancing a color image to more closely represent the actual colors of the sample (ideally, this should be done at the time of acquisition)
- Histogram stretching of the entire image to remap the values of the brightest and darkest pixels to the minimum and maximum intensities available (typically done with the 'levels' tool in Photoshop). This is a linear adjustment and does not alter the shape of the histogram. A histogram stretch can be performed for each channel of an RGB image.
- Inversion (black to white or white to black). This is commonly done with images of gels stained with ethidium bromide.
- Minor gamma adjustments to the histogram to improve contrast. This can be done as long as the channel is not to be used for intensity quantification.

Adjustments that are acceptable under some circumstances:

(These adjustments and the software used to make them should be specified)

- Upsampling (increasing the size of an image)
- Histogram equalization (nonlinear redistribution of the histogram)
- Correcting background shading or removing blemishes outside the perimeter of the sample

Adjustments that are not acceptable:

- Cloning image elements to remove defects (eg, using the Photoshop 'clone stamp' or 'healing brush' tools to clean up a small area of an image)
- Using software filters (eg, Photoshop tools like 'liquify,' 'blur,' 'add noise,' 'reduce noise,' 'despeckle,' or 'sharpen')

- Color adjustments
- Removing (erasing) large portions of an image, even if outside the area of interest
- Histogram stretches or brightness or contrast adjustments that overprocess the image by reshaping the histogram (creating oversaturated pixels or artificially clean backgrounds)
- Using the 'auto levels,' 'auto contrast,' or 'auto color' tools in Photoshop
- Major gamma adjustments to the histogram
- Lossy compression (eg, conversion to JPEG file format)
  - Note: JPEG copies are fine (and encouraged) for creating presentations
- Adding or removing image elements
- Adjustments that obscure image elements
- Splicing of multiple images (most often occurs with images of gels)
  - Note: If splicing is needed, all spliced images must be separated by a white or black line
- Copying a portion of one image into another
  - Note: Insets are acceptable but must be clearly defined by a border

Other points and advice:

- Images that are to be compared with one another (especially when intensity values are to be quantified) require identical acquisition parameters and postacquisition processing.
- Describe the methods used for image acquisition and postacquisition processing in the Methods section or the appropriate figure legend. Include the type of microscope, name of the software program, tools within that program, and important settings.
- Keep the original data file unchanged from the time of acquisition. Do image processing on a copy of the original.
- Capture color images in TIFF format or in the proprietary format of the acquisition software.
- Capture multichannel/multidimensional grayscale images (eg, those from fluorescence microscopy) in the proprietary format of the acquisition software. Then, export multichannel 8-bit RGB images in TIFF format.
- The size of a TIFF file may be reduced with LZW compression (lossless compression).
- The JPEG file format is not appropriate for scientific digital images. JPEG employs lossy compression, resulting in the loss of data that cannot be recovered.
- Assemble figures in graphics programs like Adobe Photoshop or Illustrator to avoid accidental reduction in resolution or lossy compression.
- Consult the *Instructions to Authors* document of your target journal to familiarize yourself with their guidelines for image manipulation.