Image Manipulation

A number of fairly recent articles appearing in *Nature* and other journals over the last few years have worried about the possibility of investigators' manipulating images for publication. For example, in a 2006 on-line issue of *Nature* (at www.nature.com/nature/peerreview/debate/nature04996.html) Dale Benos noted that:

> [D]igital image-processing programs make it a simple matter to prettify ugly gels. Unwanted background, smudges and 'non-specific' bands can be easily removed from the final figure. I have always thought that showing only a single band of interest in a figure such as a western blot or immunoprecipitation experiment is a somewhat equivocal practice, although I admit I have done it…If images are manipulated to enhance what we aim to demonstrate, even if our intentions are good, we chip away at the integrity of the scientific enterprise and erase the trust that the public places in our work.

So, are there any rules of thumb that investigators might use to distinguish allowable from unallowable uses of image manipulaton? Or should only raw images be submitted to journal editors?

Expert Opinion: Doctoring the Western Blot (#35)

(Note: Douglas Cromey, Manager of the Cellular Imaging Core of the Southwest Environmental Health Sciences Center at the University of Arizona in Tucson, authored an excellent on-line article in 2007 entitled "Digital Imaging: Ethics." The article is available at http://swehsc.pharmacy.arizona.edu/exppath/micro/digimage_ethics.html. Virtually the entirety of what follows is gratefully taken from that publication, such that it will not be constantly referenced.)

Obviously, digital images are data upon whose accuracy the scientific community depends. Just as the data that appear in the tables of lab reports can be misrepresented or fabricated, so can digital imagery data. A primary but not always realized source for misrepresenting digital imagery data consists in the fact that each individual element of the image, called a pixel, has a numerical value reflecting an RGB (red/green/blue) intensity. Image processing that alters that intensity can improve the visualization of the data. But if that image alteration *mischaracterizes* the data, one has gone too far. "Honest" ethical dilemmas lie in the gray zone between enhancing the image such that its clarity is improved (but its perceived content has not essentially changed) versus enhancing the image such that it now suggests what is, in reality, not there. So, for example, because software image filters change the numerical data in the pixels, they can create image artifacts leading to misinterpretation. As such, they are usually not recommended for biological images.

The resolution of dilemmas over whether or not an investigator has gone too far in manipulating an image is very simple: The investigator should make an unaltered, raw image of the data and retain it, preferably in the original file format. This image is never altered or enhanced; only its copies are. If the investigator honestly believes that an altered version of the original image is preferable for publication, he or she should attach both a copy of the original and the altered image to the manuscript being submitted along with a detailed description of why and how the copied image was altered. The journal editors can then decide which image to publish. If the altered image is chosen, the nature of the alteration should be described in the figure legend and explained in the methodology section of the paper. That way, both the investigator and the journal will maintain transparency so that no accusations of deception or misrepresentation will stand. In a nutshell, that's how to resolve this problem, when it is provoked by honest consternation of whether or not some kind of image manipulation is allowable. The

remainder of this opinion will discuss various technical details and considerations associated with digital image manipulation that Cromey includes in his article.

Cromey begins his remarks with a reminder about the importance of safeguarding and protecting the unaltered, original image because accusations of misconduct will stand or fall based on whether or not the original is available to compare with its copies. Indeed, investigators whose work falls under the FDA's "Final Rule on Electronic Records and Electronic Signatures" (21 CFR part 11, available at www.fda.gov/ora/compliance_ref/part11/frs/background/11cfr-fr.htm) must maintain the integrity of the original image. Similarly, industries whose work products are used in forensic activities or in HIPAA-related aspects of health care might be required to maintain an original image.

Cromey suggests that adjustments to the original image that are usually acceptable are small adjustments in brightness and contrast or reasonable adjustments of the levels and gamma settings. Although cropping an image is usually acceptable, accusations of unethical cropping will occur when the cropping distorts the image, e.g., cropping so as to omit something that contradicts the investigator's hypothesis. If cropping yields a special, "one of a kind" image rather than a representative picture, then the cropping has been performed unethically. Sound guideline language is found in the Journal of Cell Biology's Instructions to Authors (2007) in that image adjustments must not "obscure, eliminate, or misrepresent any information present in the original" and that the original itself must fairly represent the reality of the image such that multiple micrographs can attest to the same empirical reality. (Available at http://www.jcb.org/misc/ifora.shtml). Cromey also makes the following recommendations:

(1) Digital images that are to be compared to one another should be acquired under identical processing considerations. If they are not, the reason should be explained in the publication or in the figure legend.
(2) Enhancing a specific area of an image is extremely questionable. If performed, the selective enhancement(s) must be identified and explained. Similarly, because they can create artifacts in an image, the use of software filters that can lead to misinterpretation are questionable.
(3) Copying objects from one part of an image to another part is extremely problematic while "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."
(4) JPEG compression reduces the file size but it also changes the XY resolution of the image and the intensity value of any given pixel. This kind of compression should be avoided and replaced by a TIF file format.
(5) Care should be exercised when changing the pixel size of a digital image. Decreasing image size will decrease the image's resolution. Increasing image size can cause the software to "guess" at how many pixels need to be created between the existing ones. If the total number of pixels in an image is going to change because of a manipulation, it should be done only once to limit the number of artifacts that might be introduced.

Cromey believes that investigators who overuse image editing such that it becomes morally problematic probably do so from ignorance rather than malevolence. Nevertheless, we might end with Cromey's quoting this stern warning from the Journal of Cell Biology's 2007 Instructions to Authors:

> All digital images in manuscripts accepted for publication will be scrutinized by our production department for any indication of improper manipulation. Questions raised by the production department will be referred to the Editors, who will request the original data from the authors for comparison to the prepared figures. If the original data cannot be produced, the acceptance of the manuscript may be revoked. Cases of deliberate misrepresentation of data will result in

revocation of acceptance, and will be reported to the corresponding author's home institution of funding agency. (Available at http://www.jcb.org/misc/ifora.shtml)

Consequently, if moral arguments that discourage image misrepresentation don't impress or persuade the investigator bent on deception, perhaps the self-interest setbacks that await him or her upon being found out will discourage the act.

Suggested readings:

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Long J. Ethics in the age of digital photography. National Press Photographer's Association, September 1999. Available at http://www.nppa.org/professional_development/self-training_resources/eadp_report/.

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