

Research Integrity Guidance Note

Use and misuse of Imagery software May 2015

The manipulations of imagery has become easy and new electronic tools can provide a valuable means of showing imagery data more clearly. Adobe Photoshop is perhaps the most widely used of the packages available which include Paint Shop Pro, Corel Photopaint, Pixelmator, Paint.NET, or GIMP and others.

However, when using such manipulations, it is essential that the manipulation carried out is **described when presenting the data**. This enables the reader to fully understand the process(es) being used and be able to reproduce the data herself/himself. Many journals now require such image manipulations to be described and for the 'raw' pre-enhanced data to be available.

However, the ease of using Photoshop and similar packages brings its own temptations. Many such manipulations are inappropriate and may be considered as research misconduct or, at the very least, irresponsible conduct of research **and lead to allegations of breaches of research integrity and, if proved, to disciplinary sanctions and the retraction of papers.**

The *Journal of Cell Biology* states that "No specific feature within an image may be enhanced, obscured, moved, removed or introduced." Again, adjustments such as major contrast changes may not be acceptable. There is always a need to properly record and retain research imagery which may need to be produced for reviewers of papers or by examiners of theses.

A number of papers on the subject of imagery manipulations has been produced, especially in the biomedical and life sciences of which the most instructive and clear is by Rossner and Yamada in the *Journal of Cell Biology*. Although specifically addressing techniques in life sciences research, the messages contained within it may be applied to other disciplines.

Detailed guidelines from *Nature* are reproduced below together with the *Science* statement as to what is not allowed

References:

Cromey, D.W. Avoiding Twisted Pixels: Ethical Guidelines for the Appropriate Use and Manipulation of Scientific Digital Images
Sci Eng Ethics (2010) 16:639–667

Cromey, D.W., Digital Images Are Data: And Should Be Treated as Such
Methods Mol Biol. 2013; 931: 1–27. NIH Public Access Author Manuscript Author manuscript

Rossner, M. and Yamada, K.M. What's in a picture? The temptation of image manipulation
Journal of Cell Biology Volume 166, Number 1, 2004, 11-15

Lan, T.A., Talerico, C., Siontis, G.C.M. Documenting Clinical and Laboratory Images in Publications - The CLIP Principles
CHEST / 141 / 6 / June, 2012 1626 - 1632

Digital Image Ethics: Introduction to Image Editing Ethics
University of Arizona, South West Environmental Sciences Center

Guidelines produced by *Nature* are given below:

Nature Imagery Policy

The policy outlined on this page applies to the *Nature* journals (those with the word "*Nature*" in their title). Nature Publishing Group (NPG) publishes many other journals, each of which has separate publication policies explained on its website.

Image integrity and standards

Images submitted with a manuscript for review should be minimally processed (for instance, to add arrows to a micrograph). Authors should retain their unprocessed data and metadata files, as editors may request them to aid in manuscript evaluation. If unprocessed data are unavailable, manuscript evaluation may be stalled until the issue is resolved. All digitized images submitted with the final revision of the manuscript must be of high quality and have resolutions of at least 300 d.p.i. for colour, 600 d.p.i. for greyscale and 1,200 d.p.i. for line art.

A certain degree of image processing is acceptable for publication (and for some experiments, fields and techniques is unavoidable), but the final image must correctly represent the original data and conform to community standards. The guidelines below will aid in accurate data presentation at the image processing level; authors must also take care to exercise prudence during data acquisition, where misrepresentation must equally be avoided.

- Authors should list all image acquisition tools and image processing software packages used. Authors should document key image-gathering settings and processing manipulations in the Methods.
- Images gathered at different times or from different locations should not be combined into a single image, unless it is stated that the resultant image is a product of time-averaged data or a time-lapse sequence. If juxtaposing images is essential, the borders should be clearly demarcated in the figure and described in the legend.
- The use of touch-up tools, such as cloning and healing tools in Photoshop, or any feature that deliberately obscures manipulations, is to be avoided.
- Processing (such as changing brightness and contrast) is appropriate only when it is applied equally across the entire image and is applied equally to controls. Contrast should not be adjusted so that data disappear. Excessive manipulations, such as processing to emphasize one region in the image at the expense of others (for example, through the use of a biased choice of threshold settings), is inappropriate, as is emphasizing experimental data relative to the control.

When submitting revised final figures upon conditional acceptance, authors may be asked to submit original, unprocessed images.

Electrophoretic gels and blots

Positive and negative controls, as well as molecular size markers, should be included on each gel and blot – either in the main figure or an expanded data supplementary figure. For previously characterized antibodies, a citation must be provided. For antibodies less well characterized in the system under study, a detailed characterization that demonstrates not only the specificity of the antibody, but also the range of reactivity of the reagent in the assay, should be published as Supplementary Information or in an antibody profile database (e.g., [Antibodypedia](#), [1DegreeBio](#)). The display of cropped gels and blots in the main paper is encouraged if it improves the clarity and conciseness of the presentation. In such cases, the cropping must be mentioned in the figure legend. (Some journals require full-length gels and blots in supplementary information wherever possible.)

- Quantitative comparisons between samples on different gels/blots are discouraged; if this is unavoidable, the figure legend must state that the samples derive from the same experiment and that gels/blots were processed in parallel. Vertically sliced images that juxtapose lanes that were non-adjacent in the gel must have a clear separation or a black line delineating the boundary between the gels. Loading controls (e.g., GAPDH, actin) must be run on the same blot. Sample processing controls run on different gels must be identified as such, and distinctly from loading controls.
- Cropped gels in the paper must retain important bands.
- Cropped blots in the body of the paper should retain at least six band widths above and below the band.
- High-contrast gels and blots are discouraged, as overexposure may mask additional bands. Authors should strive for exposures with grey backgrounds. Multiple exposures should be presented in supplementary information if high contrast is unavoidable.
- For quantitative comparisons, appropriate reagents, controls and imaging methods with linear signal ranges should be used.

Microscopy

Authors should be prepared to supply the editors with original data on request, at the resolution collected, from which their images were generated. Cells from multiple fields should not be juxtaposed in a single field; instead multiple supporting fields of cells should be shown as Supplementary Information.

Specific guidelines: Adjustments should be applied to the entire image. Threshold manipulation, expansion or contraction of signal ranges and the altering of high signals should be avoided. If 'Pseudo-colouring' and nonlinear adjustment (for example 'gamma changes') are used, this must be disclosed. Adjustments of individual colour channels are sometimes necessary on 'merged' images, but this should be noted in the figure legend.

We encourage inclusion of the following with the final revised version of the manuscript for publication:

- In the Methods, specify the type of equipment (microscopes/objective lenses, cameras, detectors, filter model and batch number) and acquisition software used. Although we appreciate that there is some variation between instruments, equipment settings for critical measurements should also be listed.
- A single Supplementary Methods file (or part of a larger Methods section) titled 'equipment and settings' should list for each image: acquisition information, including time and space resolution data (xyzt and pixel dimensions); image bit depth; experimental conditions such as temperature and imaging medium; and fluorochromes (excitation and emission wavelengths or ranges, filters, dichroic beamsplitters, if any).
- The display lookup table (LUT) and the quantitative map between the LUT and the bitmap should be provided, especially when rainbow pseudocolour is used. If the LUT is linear and covers the full range of the data, that should be stated.
- Processing software should be named and manipulations indicated (such as type of deconvolution, three-dimensional reconstructions, surface and volume rendering, 'gamma changes', filtering, thresholding and projection).
- Authors should state the measured resolution at which an image was acquired and any downstream processing or averaging that enhances the resolution of the image.

Nature Editorials providing more detail for these policies:

Nature Cell Biology: Gel slicing and dicing: a recipe for disaster

<http://www.nature.com/ncb/journal/v6/n4/pdf/ncb0404-275.pdf>

Nature Cell Biology: Beautification and fraud

<http://www.nature.com/ncb/journal/v8/n2/pdf/ncb0206-101.pdf>

Nature Cell Biology: Appreciating data: warts, wrinkles and all

<http://www.nature.com/ncb/journal/v8/n3/pdf/ncb0306-203a.pdf>

Nature: Not picture perfect

<http://www.nature.com/nature/journal/v439/n7079/full/439891b.html>

Nature Methods: A picture worth a thousand words (of explanation)

<http://www.nature.com/nmeth/journal/v3/n4/full/nmeth0406-237.html>

Nature Immunology: Spot checks

<http://www.nature.com/ni/journal/v8/n3/full/ni0307-215.html>

Nature Nanotechnology: Image rights and wrongs

<http://www.nature.com/nnano/journal/v5/n9/full/nnano.2010.184.html>

Science (AAAS) states:

About modification of figures:

Science **does not allow** certain electronic enhancements or manipulations of micrographs, gels, or other digital images. Figures assembled from multiple photographs or images, or non-concurrent portions of the same image, must indicate the separate parts with lines between them. Linear adjustment of contrast, brightness, or color must be applied to an entire image or plate equally. Nonlinear adjustments must be specified in the figure legend. Selective enhancement or alteration of one part of an image is not acceptable. In addition, *Science* may ask authors of papers returned for revision to provide additional documentation of their primary data.