

## **BIOLOGY OF REPRODUCTION'S GUIDELINES FOR DIGITAL IMAGES**

Images submitted as illustrative material supporting a manuscript should be minimally processed to the extent that the final image must correctly represent the original data and conform to current standards for ethical scientific imaging. Authors should retain the unprocessed data and metadata files, as this information may be required for manuscript evaluation. 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 or funding agency. A useful resource for a discussion of guidelines for digital imaging ethics can be found on the WWW at:

[http://swehsc.pharmacy.arizona.edu/exppath/micro/digimage\\_ethics.html](http://swehsc.pharmacy.arizona.edu/exppath/micro/digimage_ethics.html)

### **Microscopy**

Adjustments such as brightness, contrast, or color balance are acceptable if they are uniformly applied to the whole image and as long as they do not obscure, eliminate, or misrepresent any information present in the original, including backgrounds. The same adjustments should be equally applied to representative controls. Threshold manipulation, expansion or contraction of signal ranges and the altering of high signals should be avoided. Should authors determine such changes are necessary, they must be justified to reviewers and disclosed in the figure legend. If 'pseudo-coloring' and nonlinear adjustments such as gamma are used, this must be disclosed. Adjustments of individual color channels are sometimes necessary on 'merged' images, but this should be noted in the figure legend. Immunohistochemistry and immunofluorescence data should include appropriate, representative controls either in the figures or in supplementary data provided for review or publication. Information on the full characterization of antibodies used for microscopy or blots should be provided. A useful discussion regarding the use of antibodies is presented by Saper CB, *J Comp Neurol* 2005; 493:477-478. For microscopy data, inclusion of the following information in "Materials and Methods" can facilitate evaluation/interpretation of images and is encouraged:

- 1) Specify microscopy equipment (make and model of microscope, objective lens information including numerical aperture, filter sets, and wavelength cutoff and bandwidth data used for fluorescence imaging), camera systems, and image processing software.
- 2) Resolution at which images were acquired.
- 3) Any subsequent software used for image processing, with details about types of operations involved (e.g., type of deconvolution, 3D reconstructions, surface or volume rendering, gamma adjustments, etc.).

### **Gels and Blots**

Inclusion of positive and negative controls and molecular size markers should be included on each gel and/or blot in the main figure or in an expanded supplementary figure. Use of cropped gels and blots is permissible if it improves the clarity and conciseness of the presentation. However, cropped gels must retain important bands and cropped blots should retain at least six band widths above and below the band. In such cases, the cropping must be mentioned in the figure legend and the supplementary information for review should include full-length gels and blots wherever possible. Vertically sliced gels that juxtapose lanes that were not contiguous in the experiment must have a clear separation or a black line delineating the boundary between the gels. High-contrast gels and blots are discouraged, as overexposure may mask additional bands. Authors should strive for exposures with gray backgrounds. Multiple exposures should be presented in supplementary information if high contrast is unavoidable. Immunoblots should be surrounded by a black line to indicate the borders of the blot, if the background is faint. For quantitative comparisons, appropriate reagents, controls and imaging methods with linear signal ranges should be used.

*(Approved by the Board of Directors of the Society for the Study of Reproduction by electronic vote, October 20, 2006.)*