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From: Scientific Editing and Research Communication Core
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Scientific Editing and Research Communication Core

Image Integrity: Guidelines for trainees and faculty

Approximately 20%–35% of biomedical research manuscripts are flagged for problems caused by editing of raw images. Although the intent of authors who make these edits is not always to deceive or hide information, such changes to images can be interpreted as inappropriate manipulation.

To assess the extent of image editing as well as the extent of inappropriate image manipulation, the American Association for Cancer Research (AACR) used an artificial intelligence (AI)-based image-checking program to retrospectively screen provisionally accepted manuscripts in 10 AACR journals over 16 months. The AI-based screening software flagged 208 out of 1367 submissions for image integrity issues. Notably, only 5 out of the 208 (2.4%) flagged manuscripts were found to involve inappropriate image manipulation. In the remaining cases (97.5%), image edits were done in good faith and were honest errors not intended to falsify data. Nonetheless, these mistakes unnecessarily exposed the authors to suspicion. Here we describe expectations for image processing, and present basic principles for maintaining image integrity to help you navigate the process of generating figures that accurately represent your findings.

How image integrity is assessed by reviewers and journal staff

Journals often have an expert specialized in image processing who manually reviews the images and flags them as "potentially manipulated." Based on these notifications, journal editors may request unprocessed or raw data files anytime during or after peer review. Some journals also do random "spot checking" of published papers, and suspected manipulations may lead to retraction of the article. But increasingly, journals are moving to supplement manual review by implementing image integrity screening using AI-based software and tools making it possible to screen all submissions instead of spot checking.

In January 2024, the *Science* family of journals made a public announcement that they will use the AI-powered software, Proofig, to evaluate image integrity of their submissions, specifically after the submitted manuscript is revised by the authors. Many journals combine this type of active monitoring with a proactive, transparent policy on image integrity. For example, most journals like *Nature*, *Journal of Immunology*, and ASM Journals post their image integrity guidelines and standards to ensure the reliability of scientific literature. The EMBO Press provides guidelines for image integrity and classifies image aberrations (see Supplemental File) into three categories, which are used by editors to flag the manuscript: Level 1, inadvertent edits; Level 2, extreme edits that lead to suspicion of an intent to mislead; and Level 3, edits clearly indicative of fabrication. Also, the Committee on Publication Ethics (COPE) has developed a systematic flowchart to guide journal editors and reviewers in dealing with inappropriate image manipulation in a published article.

Image modifications that *are* or *are not* acceptable

Here is a list of do's and don'ts to help you in editing and processing images from your research. The guiding philosophy is that all images should be minimally processed. Although the practice is to show representative data, the data should not be cherry-picked. Any manipulation that changes the interpretation of data is inappropriate.

Do the following:

- Retain raw image files, including unprocessed data and metadata (both digital and analog) in uncompressed formats (e.g., TIFF, not JPG). Also, maintain a back-up copy of all raw data.
- Be judicious in converting images that use file compression (jpg, jpeg, etc.) because conversion often leads to data loss.
- When adjusting for brightness, color, or contrast, do so for the whole image, including controls. Adjustments for a part of an image are unacceptable and easily detected. Also, make sure that such alterations do not make faint bands or faintly stained cells disappear.
- Show blots and gels without adjusting the background. Given that blots and gels invariably have background, and that researchers sometimes "even out" the background in image editing software, such "beautification" of data or images is not a recommended practice. A wide variation in background is indicative of technical error and invalidates the experiment. If the background confounds or complicates the interpretation, the experiment should be repeated.
- All procedures for normalization or correction, if used, must be applied consistently, and explained.
- Submit whole gels or blots so that controls can be compared directly to experimental samples, i.e., run controls (positive and negative) on every gel.
- Make sure that all images, especially gel bands, show signals that are within the linear range of the assay, e.g., overexposed bands should not be compared with underexposed bands.
- When juxtaposing images from different sources (gels, blots, fields, exposures), clearly separate individual images using dividing lines and explain the context of each image in the text and/or figure legend.
- For microscopy images, include scale bars and magnification.
- Disclose any non-linear adjustment of luminescence, e.g., gamma correction, in image or figure legend.
- Use arrows or add color to grayscale microscopy images (known as "pseudo-coloring") to highlight the key features.
- For complex images from fluorescence microscopy experiments, disclose any use of "filter", "digital masking", or "background subtraction" in the figure legends.

Don't do the following:

- Duplicate part of an image, background, gel bands, control bands (or whole panel). Duplication, including rotation and/or flipping, is detected by screening

scans and flagged as a deliberate attempt to manipulate an image.
- Make contrast adjustments that eliminate background fluorescence.
- Rearrange, juxtapose, and/or stitch different parts of one or many images to create a new image.
- Insert or "layer on" an image of signal over background.
- Paste or stitch images of control bands from another gel or blot.
- Use images previously processed in pdf or ppt format for publication.
- Adjust the intensity of individual gel bands.
- Use clean-up tools like "rubber stamp" in Photoshop.
- Manipulate contrast while merging fluorescence microscopy images.
- Adjust dimensions or size in a way that results in distortion of pixels and reduces quality of image.
- Crop images in such a way that information is deleted. This raises suspicion of deliberate "data hiding" or "data manipulation."

Considerations when writing legends for image-based figures:

- Describe the software/hardware used to generate the images, including the software version number.
- Fully disclose enhancement tools that were used to edit the images, e.g., "filter," "digital masking," "background subtraction."
- Describe the purpose of arrows or other symbols, as well as any pseudo-coloring and its purpose.

For additional guidelines on image integrity, see the [Online Learning Tool for Research Integrity and Image Processing] developed by the Office of Research Integrity (ORI) under the US Department of Health and Human Services.

In an era when science is routinely brought under attack, it is crucial to maintain a high level of scientific integrity. This means ensuring that individuals at all levels—from trainees to institutes—are responsible and accountable for the data they publish. The goal is to maintain the transparency, rigor, and reproducibility of scientific research.

Stay warm and Go Hawks!

Kshitija Kale (Scientific Editing Intern) and the SERCC Team

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Announcements

Upcoming changes to NIH Applications and Peer Review

The NIH is implementing multiple changes in 2025 that will affect the submission and review of grant applications. Click the link above for a summary of the changes and links to additional information.

Broader Impacts/Research Impacts Seminar

The Training Team from the NSF-supported Advancing Research Impact in Society (ARIS) organization will conduct three interactive virtual workshops that will cover the history of the Broader Impacts criterion and will provide strategies for conceptualizing, developing, implementing, and evaluating Broader Impact activities.

Upcoming Opportunities

Have a question about writing grants or research articles? Contact us and we will answer it in a future newsletter.

MATFab Seed Grant Program
Application deadline: January 3
The Office of the Vice President for Research is sponsoring a seed grant program to encourage researchers to use the Materials Analysis, Testing, and Fabrication (MATFab) Facility to help secure further external funding. Funds may be used to develop new research directions, make use of instrumentation not previously used, or employ new applications of instruments. Open to University of Iowa faculty, staff, postdoctoral scholars, and graduate students.
More information and online application

Burroughs Wellcome Fund (BWF) Climate and Health Excellence (CHEX) Centers
Internal submission deadline: January 9
These are new institutional awards that aim to understand and mitigate the impact of climate change on human health by stimulating the development of strong research, education, and public communication connections between fields. Institutions or Consortia supported by these awards will develop new lines of research, training, and graduate education that will both energize faculty collaboration and expand interdisciplinary research opportunities for trainees.
More information

Maximizing Pivot to Find Funding and Collaborators
January 31 |11:00 a.m.– 12:00 p.m. | Hardin Library
Do you want to maximize your time spent searching for funding opportunities or potential project collaborators? Join us to learn how Pivot can save you time and

effort in identifying the right funding opportunities for your research, as well as in identifying collaborators with the relevant expertise.
Additional information

Obermann End-of-Year Writing Retreat
May 12–16 | 9:30 a.m.–4:00 p.m. | North Ridge Pavilion, Coralville, IA
Jumpstart your summer writing project! Fifteen participants will enjoy a quiet week away from the distractions of campus. Each day will include structured blocks for writing, breaks, and small group "creative moments and check-ins," as well as opportunities for discussion with other writers across campus. Funded by the P3 Writing for the Public Good Initiative.
More information | Registration