Appropriate Image Processing

—Explanation of Submission Guidelines of Journals—

Second Edition

Japan Agency for Medical Research and Development

AMED

Images play a crucial role in the biomedical field. Appropriate processing of images allows scientific output to be shared correctly, which ensures credibility of research findings, contributing to the advancement of research and development. While image processing helps make research papers easier to read and more impactful, inappropriate image processing can lead to data distortions, misinterpretation by readers, and, in some cases, even misconduct. Because inappropriate image processing compromises the credibility of research and acts as a barrier to scientific development, it is unacceptable.

This booklet offers a commentary on image processing guidelines of scientific journals, which can be helpful when processing biological images in order to handle image data scientifically, and provides recommendations of appropriate and unacceptable practice of image processing.

As submission guidelines are reviewed often, it is necessary to check for updates as needed. In addition, you must securely store not only the images and data that are directly linked to the paper but also all the original images and other raw data properly according to the rules specified by your research institutions and the procedures recommended by the journal, if applicable.

Chapter 1　Image processing guidelines of scientific journals

In image processing of biological images, the appropriateness of the processing method depends on the research purpose or analytical approach, even when the same process is used. It might be, therefore, extremely difficult to define image processing methods uniformly, and to state "This image processing method is considered fraudulent in all cases."

The International Association for Scientific, Technical and Medical Publishers published "Recommendations for Addressing Issues of Image Integrity" as recommendations for image processing in 2021.[1] The International Committee of Medical Journal Editors (ICMJE), which is composed of editors of medical journals, such as JAMA, Nature Medicine, the New England Journal of Medicine, and the Lancet, updates "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" (hereinafter "ICMJE Recommendations") every year and provides image-related guidelines in these recommendations.[2] Springer Nature, which has owned Nature Portfolio since 2024, works with EMBO in the publication of journals,[3] and Nature Portfolio and EMBO have adapted the same guidelines.

Scientific journals explicitly state the minimum criteria in their submission guidelines. While the submission guidelines for all scientific journals are not exactly the same, the major scientific journals' image processing guidelines are in line with each other.

Taking into account the factors such as ease of access and impact of the journal, the submission guidelines of eight journals, including Nature Portfolio, are presented in this booklet (Second edition). Some of the guidelines that may require attention, especially related to image processing, are selected. As guidelines vary by journal and image type and may be revised, please check the current guidelines using the URL links, etc. along with the introductions in this chapter.

1.1　Guidelines of Nature Portfolio[4]

The following is a reorganized and summarized version of Nature Portfolio's guidelines regarding images.

URL: https://www.nature.com/nature-portfolio/editorial-policies/image-integrity

○ We recommend retaining unprocessed images and related metadata (data related to image acquisition conditions, these data are part of an image file or stored in a separate file from the image file, depending on the image format) to enable submission to the publisher upon request, ideally archiving data in perpetuity. Especially for images of gels and western blots, it is required to submit unprocessed images in the final accepted version. These unprocessed images are published in the Supplementary Information.

○ List all image acquisition tools and image processing software packages used, and document key image-gathering settings and processing procedures.

○ 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.

1) STM Working Group. (2021, December 6). *Recommendations for handling image integrity issues.* STM. https://www.stm-assoc.org/2021_12_07_Recommendations_for_handling_image_integrity_issues_V10.pdf

2) ICMJE. (2025, January). *Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals.* International Committee of Medical Journal Editors. https://www.icmje.org/recommendations/

3) Springer Nature. (2023, March 30). *Springer Nature and EMBO cooperate to publish the EMBO Press suite of journals.* Springer Nature. https://group.springernature.com/jp/group/media/press-releases/archive-2023/springer-nature-embo-partner-to-publish-journals/25171590

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

4) Nature Portfolio. *Image integrity and standards.* Nature Portfolio. https://www.nature.com/nature-portfolio/editorial-policies/image-integrity

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

- 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 or any feature that deliberately obscures manipulations is unacceptable.
- 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.

\<Electrophoretic gels and blots>
- Quantitative comparisons between samples on different gels/blots are strongly discouraged.
- Re-arranged lanes that are non-adjacent in the gel must be clearly indicated in a manner that delineates the boundary between the lanes. Rearrangement of lanes should be stated as such in the figure legend.
- Loading controls (e.g. GAPDH, actin) must be run on the same blot.
- When sample processing controls are run on different gels, they must be identified as such in the figure legend.
- Cropped gels in the paper must retain all important bands.
- High-contrast gels and blots are discouraged, as overexposure may mask additional bands.

\<Microscopic images>
- 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-coloring" and nonlinear brightness adjustment (e.g. "gamma changes") are used, this must be disclosed.
- Brightness adjustments of individual color channels are sometimes necessary on "merged" images, but this should be noted in the figure legend.
- Cells from multiple fields should be grouped into a single field.
- The type of equipment (microscopes/objective lenses, cameras, detectors, filter model, and batch number), image acquisition software, and key equipment settings should also be listed.
- Store raw image files including relevant metadata (e.g. acquisition information including time and space resolution data; image bit depth; experimental conditions such as temperature and the name and composition of culture medium for observation; fluorochromes [excitation and emission wavelengths or ranges, filters, wavelength characteristics of dichroic mirrors]).
- The display lookup table (LUT) and the quantitative map between the LUT and the bitmap should be provided, especially when rainbow pseudocolor is used.
- The measured resolution at which an image was acquired and any processing or averaging that enhances the resolution of the image should be clearly stated.
- Processing/analyzing software should be named in the Methods section, and any manipulations indicated in the relevant figure legends (such as type of algorithm for deconvolution, three-dimensional reconstructions, surface and volume rendering, gamma correction, filtering, brightness thresholding, and projection).

1.2 Guidelines of the EMBO Journal's guidelines[5]

The following is a reorganized and summarized version of the EMBO Journal's submission guidelines regarding images.

URL: https://www.embopress.org/page/journal/14602075/authorguide#figureformat

<Image Processing>
- Images submitted with a manuscript for review should be minimally processed (for instance, to add arrows to a micrograph). Unprocessed data and metadata files should be retained, 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 image acquisition tools and image processing software packages used should be listed, as well as key image-gathering settings and processing procedures.
- 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, 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.

<Electrophoretic gels and blots>
- The display of cropped gels and blots in the main paper is permitted if they retain all important bands, and space (several band-widths) should be retained above and below the relevant band(s).
- Re-arranged lanes that are non-adjacent in the original gel must be clearly indicated in a manner that delineates the boundary between the lanes. Rearrangement of lanes should be stated as such in the figure legend.
- Quantitative comparisons between samples on different gels/blots are discouraged; if this is unavoidable, the figure legend must state that the samples are derived from the same experiment and that gels/blots were processed in parallel.
- Loading controls must be run on the same blot.
- Processing gel or blot images with high contrast is discouraged, as overexposure may mask original bands.
- Exposures should be displayed with gray backgrounds. Multiple exposures should be presented in the Expanded View (a collapsible/expandible section as supplementary information to the paper) 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.

<Microscopic images>
- Original data at the resolution collected, from which their images were generated, need to be stored.

- Cells from multiple field-of-view (FOV) images should not be juxtaposed in a single image field; instead, multiple supporting fields of cells should be shown as part of the Expanded View.
- Adjustments of image quality should be applied to the entire image.
- Threshold manipulation, expansion or contraction of brightness ranges, and altering of high brightness areas should be avoided.
- If 'Pseudo-coloring' and nonlinear brightness adjustment (e.g. 'gamma changes') 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.
- Sometimes, brightness adjustment of each channel image on merged multi-channel color images may be required, and this should be noted in the figure legend.
- The type of equipment (microscopes/objective lenses, cameras, detectors, filter model, and batch number), image acquisition software, and key equipment settings should also be listed.
- The following information should be listed 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 beam splitters).
- The display lookup table (LUT) and the quantitative map between the LUT and the bitmap should be provided, especially when rainbow pseudocolor is used.
- Processing software should be named and manipulations indicated (such as deconvolution, three-dimensional reconstructions, surface and volume rendering, gamma correction, filtering, brightness thresholding, and projection).
- The measured resolution at which an image was acquired and any processing or averaging that enhances the resolution of the image should be clearly stated.

1.3   Guidelines of Science[6]

The following is a reorganized and summarized version of Science's submission guidelines regarding images.
URL: https://www.science.org/content/page/science-journals-editorial-policies#image-text

6) *Science Journals. Science Journals: Editorial Policies.* AAAS. https://www.science.org/content/page/science-journals-editorial-policies#image-text

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

- Images presented in research papers should correctly represent the original images.
- No part of a digital image may be selectively manipulated or altered.
- When figures are assembled from multiple images or non-contiguous portions of the same image, a line or space should indicate the border between the separate parts.

1.4 Guidelines of the New England Journal of Medicine (NEJM)[7]

The following is a reorganized and summarized version of NEJM's submission guidelines regarding images.

URL: https://www.nejm.org/about-nejm/editorial-policies

○ All modifications to images, selective digital adjustments to part of an image, and digital image processing enhancements to images should be described and clearly indicated.
○ No specific feature in an image has been enhanced, obscured, moved, removed, or introduced.
○ Any adjustments to brightness, color, or contrast have been made to an entire image and do not misrepresent any features of the original image.
○ No image has been duplicated within the manuscript or published elsewhere.
○ Original image metadata is available and can be provided to NEJM editors on request.

1.5 Guidelines of the Lancet[8]

The following is a reorganized and summarized version of the Lancet's submission guidelines regarding images.

The Lancet provides guidelines for the image files to be attached at submission; however, it does not provide guidelines for image processing.

URL: https://www.thelancet.com/pb/assets/raw/Lancet//authors/artwork-guidelines.pdf

1.6 Guidelines of the British Medical Journal (the BMJ)[9]

The following is a reorganized and summarized version of the BMJ's submission guidelines regarding images.

URL: https://authors.bmj.com/writing-and-formatting/formatting-your-paper/

○ Any cleaning or image enhancement should be clearly described in the submission and in the figure legend.

1.7 Guidelines of Journal of American Medical Association (JAMA)[10]

The following is a reorganized and summarized version of JAMA's submission guidelines regarding images.

URL: https://jamanetwork.com/journals/jama/pages/instructions-for-authors#SecFigures

○ Digital adjustments of brightness, contrast, or color should be applied uniformly to an entire image.

7) NEJM. *Editorial Policies*. Massachusetts Medical Society. https://www.nejm.org/about-nejm/editorial-policies

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

8) The Lancet. *Formatting guidelines for electronic submission of artwork*. Elsevier. https://www.thelancet.com/pb/assets/raw/Lancet//authors/artwork-guidelines.pdf

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

9) The BMJ. *Formatting your paper*. BMJ Publishing Group. https://authors.bmj.com/writing-and-formatting/formatting-your-paper/

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

10) JAMA. *Instructions for Authors: Format My Manuscript*: Figures. American Medical Association. https://jamanetwork.com/journals/jama/pages/instructions-for-authors#SecFigures

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

- These digital adjustments are permissible as long as they do not selectively highlight, misrepresent, obscure, or eliminate specific elements in the original figure, including the background.
- Selective adjustments applied to individual elements in an image are not permissible, and these individual elements may not be moved within an image field, deleted, or inserted from another image.
- Cropping may be used for efficient image display or to deidentify patients but must not misrepresent or alter the interpretation of the image by selectively eliminating relevant visual information.
- Juxtaposition of elements from different parts of a single image or from different images, as in a composite, must be clearly indicated by the addition of dividing lines and/or borders.
- The submission and publication of images created by artificial intelligence, machine learning tools, or similar technologies is discouraged, unless they are part of research design or methods; however, if so, details about the content generated by it as well as the name of the model or tool, version and extension numbers, and manufacturer must be described. Authors must take responsibility for the scientific integrity of the content generated by these models.
- For photomicrographs, include the type of specimen, original magnification or a scale bar, and staining method in the legend.
- For gross pathology specimens, label any rulers with a unit of measure.
- Digitally enhanced or manipulated images must be clearly identified in the figure legends (e.g., computed tomographic scans, magnetic resonance images, photographs, photomicrographs, x-ray films).

1.8   Guidelines of Journal of Cell Biology (JCB)[11,12,13]

The following is a reorganized and summarized version of JCB's submission guidelines regarding images.

URL: https://rupress.org/jcb/pages/submission-guidelines
URL: https://rupress.org/jcb/pages/fig-vid-guidelines
URL: https://rupress.org/jcb/pages/editorial-policies

<Submission Guidelines>
- Source data used to generate figures containing gels and Western blots should be provided.
  - Source Data consist of fully uncropped and unprocessed images for each gel/blot displayed in the main and supplemental figures.
  - For assays performed using capillary electrophoresis and/or immunoassay-based detection, the electropherogram graph(s) for each experiment, plotting fluorescence/chemiluminescence vs. molecular weight/size, should be provided.
  - One source data file for each figure that contains gels, blots, and/or capillary electrophoresis assays should be provided.
  - For traditional gels and blots, the lanes of the gels/blots should be labeled as they are in the associated figure, the place where cropping was applied should be marked (with a box), and molecular weight/size standards should be labeled wherever possible.
  - For capillary electrophoresis assays, each trace in the graph should be color-coded and labeled to indicate which protein, gene, or sample is

being measured. Try to avoid red/green combinations to accommodate our color-blind readers.
- ○ For microscopic images, the following information must be provided: manufacturer and model of microscope; type, magnification, and numerical aperture of the objective lenses; temperature; name and composition of culture medium for observation; fluorochromes; manufacturer and model of cameras; image acquisition software; and any software used for image processing subsequent to data acquisition; types and details of image operations and processing involved (e.g., type of deconvolution algorithm, 3D reconstitutions, surface or volume rendering, gamma correction).
- ○ If you export files from a microscope or other acquisition device, be sure to use consistent file formats (8 bit, 16 bit, etc.).

<Figure and Video Guidelines>
- ○ Screenshots should never be used to capture images.
- ○ If image file size is a concern, use only lossless image compression such as LZW; do not save images as JPEGs.
- ○ Scales in micrographs must be disclosed in the figure legend, or scale bars must be included in the images, rather than magnification factors.
- ○ Protein molecular weights or DNA marker sizes must be indicated in all figure panels showing gel electrophoresis.

<Editorial Policies>
- ○ Images should be minimally processed and accurately reflect the original data.
- ○ Any reuse of images, including control data, across multiple figures should be explicitly stated and justified in the figure legend.
- ○ Nonlinear adjustments (e.g., changes to gamma settings) must be disclosed in the figure legend or Materials and Methods section.

As mentioned previously, peer-reviewers of scientific journals verify whether or not:
- a specific feature within the image has been enhanced, obscured, moved, removed or introduced;
- dividing lines are shown between juxtaposed images taken from different parts of the same gel or from different gels, fields, or exposures;
- the adjustment of the brightness, contrast and/or color balance have been applied across the entire image;
- the information present in the original image, including the background, has been highlighted, eliminated, or misrepresented due to adjustments; and
- duplicate images are used within the manuscript.

Many journals' guidelines recommend saving the original data and recording the equipment used as well as the steps and details of the processing performed. You may be requested to submit these with your paper or during the peer review process. In serious cases, such as if the conclusion becomes obscured or altered due to inappropriate image processing, the acceptance of the paper may be withdrawn. However, even when inappropriate image processing is detected before publishing, if you follow these guidelines, many issues can be resolved, and you can move forward with the publishing of your paper. If a published paper is suspected of fraud (e.g., plagiarism, inappropriate data processing, or duplicate publication), it may result in publication of the errata sheet, recommendation of the paper retraction, or expression of the editor's concerns, based on the findings of the investigation committee.[13]

Chapter 2　Appropriate image processing

Inappropriate image processing compromises the credibility of the research. Additionally, being suspected of image fraud may affect your career as an individual researcher. If you have recorded and saved the original images and image processing procedures, you can assert that the images had been processed using an appropriate method and by taking appropriate steps. It is, therefore, essential to save the original images as separate files when processing images and to record the image processing procedures. This chapter will discuss appropriate image processing procedures.

2.1　Recording image processing procedures and retaining original images

In order to ensure the reproducibility of the results, it is important to document the image generation process and to record all the image processing procedures along with their parameter settings. Scientific journals may request that information such as the "name of software used for image processing," "software version," and "main image processing procedures and their parameter settings" be provided. For required information on the image processing to be provided at paper submissions, "Community-developed checklists for publishing images and image analyses," published by Nature Methods, is helpful.[14]

You may be requested to submit original images at the paper submission or during peer review process, or if image fraud is suspected. To avoid situations such as overwriting the original image with an edited image, losing the original image, you need to save the original image as a separate file while processing images. Since JPEG compression affects the image resolution, some scientific journals recommend saving images in TIF format.[15]

Figure 1 The original image and the processed images are saved separately[16]
(An example where file names present the image processing procedures and their parameter settings)
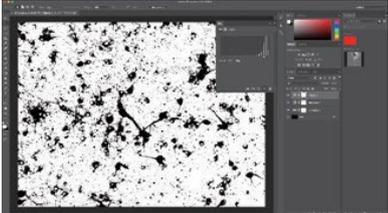
Figure 2 Retaining the original image as a layer[17]
(An example where non-destructive image processing is applied to an image and the image is saved using Photoshop)

14) Schmied, C., Nelson, M. S., Avilov, S., et al. (2024). Community-developed checklists for publishing images and image analyses. *Nature Methods*, *21*, 170-181.

15) Martin, C., & Blatt, M. (2013). Manipulation and misconduct in the handling of image data. *Plant Cell*, *25*(9), 3147-3148.

16) Provided by LPIXEL Inc.

17) Provided by iJapan. Co. Ltd

2.2　Trimming and resizing

Trimming and resizing images to enhance their composition is considered acceptable; however, trimming or resizing of images that makes the interpretation of the image complicated or that hides the original contents may be considered an inappropriate manipulation.[18] For example, when resizing the shape of an image, the common practice is to take measures, such as disabling the pixel interpolation before processing, in order to prevent artificial pixel values being added to the data.

18) Cromey, D. W. (2013). Digital images are data: and should be treated as such. *Methods Mol Biol*, *931*, 1-27.

2.3　Contrast enhancement without decorative image processing

As biological images are scientific data in the first place, you do not need to make the appearance of images more beautiful than necessary. The contrast may be enhanced in some biological images in order to improve the visibility of the images; however, it may result in removing undesirable parts. If excessive contrast enhancement creates black areas (blocked-up shadows) and/or white areas (blown-out highlights), it is not possible to distinguish whether or not such areas are a result of hiding unfavorable data. For that reason, when enhancing the contrast, remember not to manipulate excessively.

The contrast is adjusted appropriately in Figure 3, and the contrast is excessively enhanced in Figure 4, causing an excessive contrast adjustment with blocked-up shadows/blown-out highlights. Figure 5 shows the blocked-up shadows in Figure 4 in blue and blown-out highlights in red. You can see that the contrast in Figure 4 is enhanced excessively.
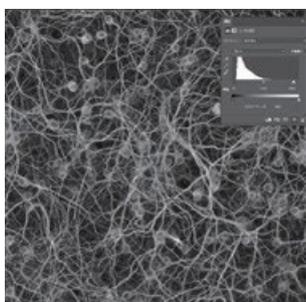
Figure 3 Appropriate contrast adjustment[19] (Adjusted so that both ends of the brightness histogram match)
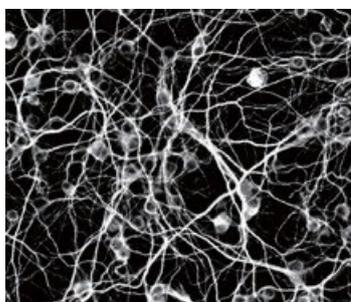
Figure 4 Excessive contrast adjustment with blocked-up shadows and blown-out highlights[20]
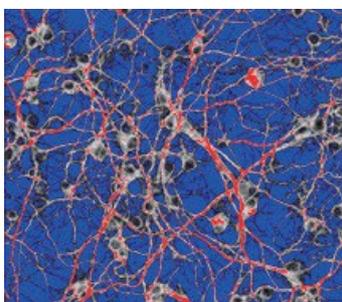
Figure 5 The blocked-up shadows in Figure 4 are shown in blue and blown-out highlights in red[21]

19)–21) Provided by LPIXEL Inc.

2.3.1　Appropriate contrast adjustment on a single still image

The following techniques are recommended for adjusting the contrast:
(1) Use both ends (minimum and maximum target signals) of the brightness histogram to make level adjustments.
(2) Adjust the contrast to a level at which the background brightness is visible.

Technique (1) is an image data visualization method in which the quantitativeness is strictly preserved. For images obtained by fluorescent microscopy, the contrasting density of the image accurately reflects the concentration of the substance, providing an image without any blown-out highlights or blocked-up shadows while reducing the background brightness, as shown in Figure 7. Technique (2) may be used when prioritizing the ease of visual recognition of the contrast density on the image of interest. This contributes to ensuring the quantitativeness even when printing the image (Figures 8 and 9).

As described above, technique (1) is used when the purpose is to strictly preserve the quantitativeness of the image, and technique (2) is used when the purpose is to prioritize the objectivity of visual recognition at the expense of the quantitativeness.

Regardless of which technique you use, it is important to include the minimum and maximum value settings used for the level adjustment (contrast adjustment) and the justification for the choice in your paper. If you choose to use a technique that causes blocked-up shadows and/or blown-out highlights similar to Figure 10, you should provide the minimum and maximum value settings and state the fact that parts of the

pixels are saturated. You also should explain the reasons and justification for the choice (for example, "to visualize the structures with higher contrast," etc.), as well as whether there are any structures that are masked due to the saturation, and if there are, explain how the paper's thesis and those masked structures are related or unrelated. As discussed in 2.1, be sure to record and save the original images and image processing procedures in a way that ensures their reproducibility, and check the submission guidelines and other relevant information before submitting your paper.
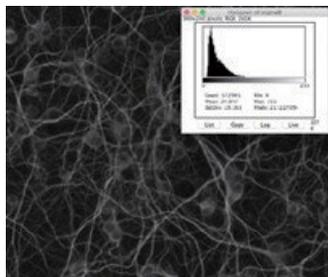
Figure 6 Original image[22]
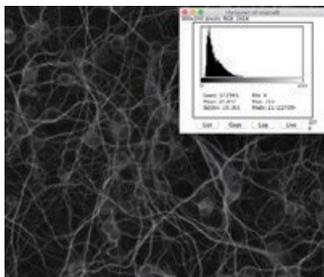
Figure 7 Contrast adjusted image[23]
(An example where the level is adjusted to both ends of the brightness histogram (the minimum and maximum target signals))
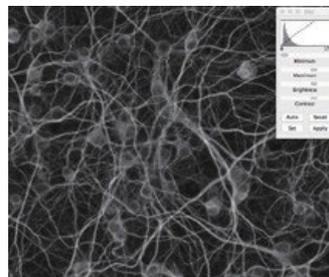
Figure 8 Contrast adjusted image[24]
(An example where the contrast is adjusted so that the signal area of interest is quantitatively visible)

22)–26) Provided by LPIXEL Inc.

Figure 9 Brightness spectral gradation of an image with 256 pixels in width[25]
(Transitions from brightness 0 (black) to brightness 255 (white), one brightness level at a time. The quantitativeness at both ends is not perceivable by human vision (or on printouts).)
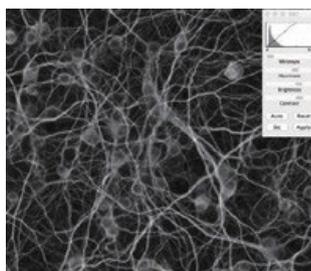
Figure 10 Contrast adjusted image[26]
(An example where about 1-2% of blocked-up shadows and blown-out highlights have occurred)

2.3.2　Contrast adjustment on multiple comparing images

If there are multiple biological images to be shown with the image being compared with, the contrast of all of the images needs to be adjusted equally. For example, on fluorescent microscopy images, even if the minimum brightness value (the background brightness of the image) is the same across images, the maximum brightness of signals (fluorescent substances and fluorescent protein) would vary by image. If you want to quantitatively evaluate the fluorescent brightness across multiple images, it is appropriate to set the upper limit brightness for the level adjustment to the maximum brightness of all images. However, when the entire image is bright, this level adjustment may offer only limited contrast improvement and may not be very effective. In such cases, you can set the minimum brightness value of all images as the lower limit brightness for the level adjustment to improve the contrast.

2.3.3 For images visible only under high contrast

It is an ideal situation that only the area or region of interest would be beautifully imaged. However, there are times when an image contains some areas that have been imaged brighter because of auto-fluorescence, non-specific fluorescence, etc. If the contrast of the image is adjusted according to the area of interest, the contrast of the surrounding areas may be excessively adjusted. In such cases, you could include displaying a series of photos with gradually adjusted contrast, or submitting the original image as supplemental information.
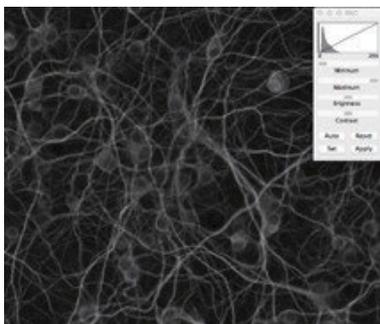
Figure 11 Original image[27]
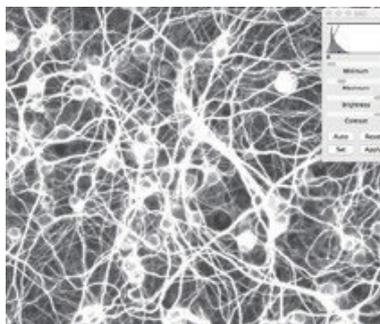
Figure 12 Contrast adjusted image[28]

27) and 28) Provided by LPIXEL Inc.

(If you want to enhance a poorly signaled area, you could either display a series of photos with gradually adjusted contrast, or submit the original image as supplemental information.)

2.4 Syntheses of gel images

If, for any inevitable reasons, you have synthesized images (for example, synthesizing multiple images to save space, etc.), you must state clearly the reasons and details of the image processing procedure applied. You should ensure the credibility of your image data by following the submission guidelines, such as including molecular markers in each image, clearly showing the borderline of the synthesized area, providing an explanation of the fact that multiple gel images have been synthesized in the text or the figure legend.
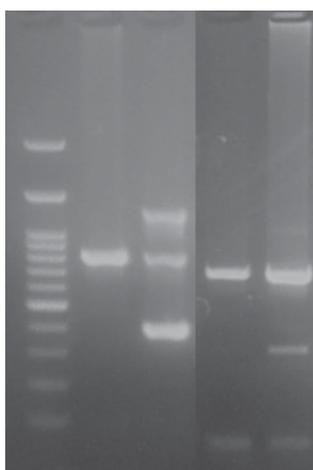
Figure 13 An example of an unacceptable synthesized gel image[29]
(The synthetic image area is not shown.)
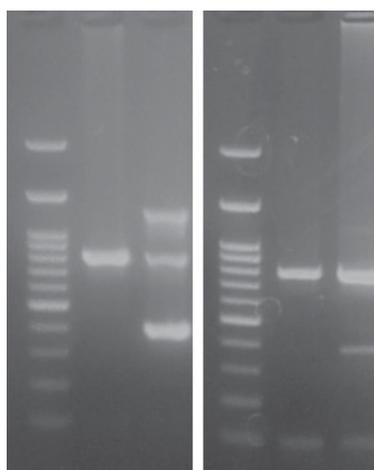
Figure 14 An example of an appropriately synthesized gel image[30]
(An example in which each gel image contains molecular markers, and a white line is added to the synthesized area)

29) and 30) Provided by LPIXEL Inc.

Chapter 3　Unacceptable image processing

There are many different techniques for biological image processing. An appropriate technique needs to be applied according to the research purpose, and the technique needs to be applied carefully as applying an inappropriate technique compromises the image's quantitativeness and objectivity. When submitting your paper to a scientific journal, check the current guidelines of the journal before processing the images because some journals request inclusion of information such as the processing methods and tools. This chapter discusses inappropriate image processing methods that researchers should refrain from using.

3.1　Copy and paste of images

Generally, copy and paste of images should not be used, regardless of whether in whole or in part. If copy and paste is necessary, it is crucial to include a statement describing which part of which image was copied and where it was pasted, as well as why such image processing was applied in the text or the figure legend within the paper. Also, the borderline between the pasted part and other parts needs to be clearly visible within the image.

An example of copy-and-paste fraud is image cloning. Figure 15 shows an example, in which a part of an image was copied and pasted into another part of the image. The cloned parts are indicated by red circles.
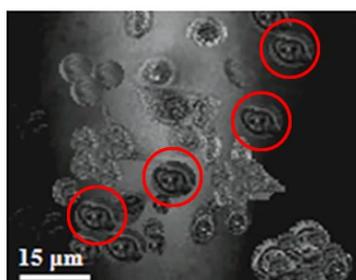
Figure 15 An example of image cloning[31]

31) Farooq, M. U., Novosad, V., Rozhkova, E. A., Wali, H., Ali, A., Fateh, A. A., Neogi, P. B., Neogi, A., & Wang, Z. (2018). Gold Nanoparticles-enabled Efficient Dual Delivery of Anticancer Therapeutics to HeLa Cells. *Scientific Reports*, *8*(1), 2907. Copyright 2018 The Author(s). CC BY 4.0

Figure 17 shows an image where the brightness and contrast of the image in Figure 16 have been adjusted. As indicated by yellow arrows, you can see outlines suggesting intentional image manipulation.
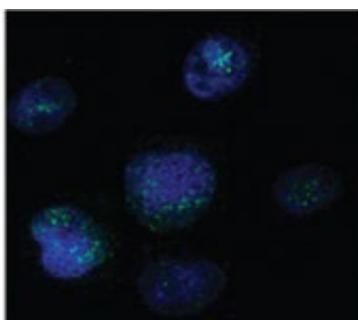
Figure 16 Original image[32]
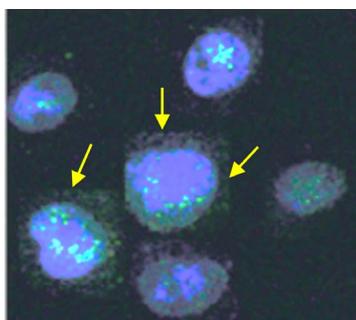
Figure 17 Contrast adjusted image[33]

32) Chen, S., Chen, X., Li, W., Shan, T., Lin, W. R., Ma, J., Cui, X., Yang, W., Cao, G., Li, Y., Wang, L., & Kang, Y. a. (2018). Conversion of epithelial-to-mesenchymal transition to mesenchymal-to-epithelial transition is mediated by oxygen concentration in pancreatic cancer cells Retraction in /10.3892/ol.2022.13227. *Oncol Lett*, *15*(5), 7144-7152. Copyright 2018 The Author(s). CC BY-NC-ND 4.0

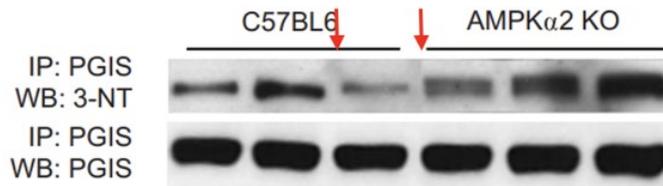33) The contrast was adjusted and the arrows were added by iJapan. Co. Ltd

Processing where inserting a band from another image or another part to a certain part on a gel image is generally considered an inappropriate manipulation. In Figure 18, you can see signs of artificial insertion of an image into the part indicated by red arrows.

Figure 18 An example of artificial insertion of a gel band[34]

34) Xie, Z., Zhang, J., Wu, J., Viollet, B., & Zou, M. H. (2008). Upregulation of mitochondrial uncoupling protein-2 by the AMP-activated protein kinase in endothelial cells attenuates oxidative stress in diabetes. *Diabetes*, *57*(12), 3222-3230.

Alterations of images using copy and paste can be detected through analysis of the noise pattern or other similar methods (see Chapter 5).

3.2 Selective elimination of part of an image

Using a touch-up feature or other advanced image editing features allows you to eliminate unwanted foreign particles (such as fine dust that shows up on images) that appear on images; however, it may be interpreted as a selective elimination of unfavorable data. Therefore, unless it is absolutely necessary, a touch-up feature should not be used. Figures 19 - 21 show an example of image manipulation where a touch-up feature was used to remove a certain part from the image. Figures 22 and 23 are not an example of elimination of foreign particles, but indicate image manipulation. Some signals on the Figure 22 image were selectively altered to the color of the background to present as the image after drug treatment shown in Figure 23 where signals appear to be reduced due to the effects of the drug. This is clearly research misconduct (falsification).
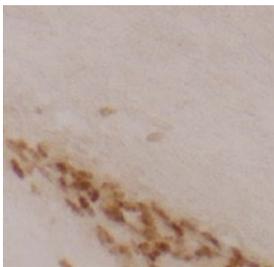
Figure 19 Original image[35]

Figure 20 The part where touch-up was applied[36]
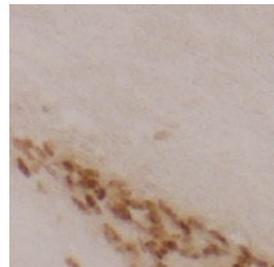
Figure 21 An image with a certain part removed[37]

35)–37) Provided by LPIXEL Inc.

Figure 22 Original image[38]

Figure 23 Processed image[39]

38) 39) Wu, Z., Zhao, G., Peng, L., Du, J., Wang, S., Huang, Y., Ou, J., & Jian, Z. (2013). Protein Kinase C beta Mediates CD40 Ligand-Induced Adhesion of Monocytes to Endothelial Cells. *PLOS ONE*, *8*(9), e72593.
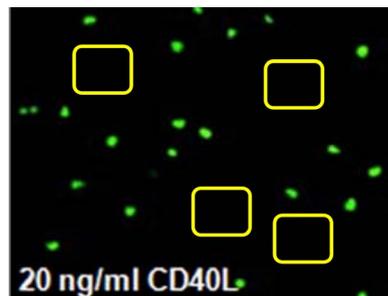
3.3 S-shaped tone curve processing (non-linear gray level transformations)

When adjusting images using image editing software (e.g., Photoshop), setting the tone curve to an S-shape brightens the dark areas and darkens the bright areas, improving the visibility of the entire image; however, applying this technique to biological images makes it impossible to evaluate the brightness quantitatively.

To be more specific, when the proportional relationship between the brightness input to the camera and the brightness displayed in the images is maintained on a gel image, and if the brightness level is almost double after background brightness subtraction is applied, you can assume the expression level to be nearly doubled as well. However, if the S-shaped tone curve is applied, this proportional relationship would turn into an S-shape, making it impossible to evaluate the brightness quantitatively.

Therefore, when quantitatively comparing the brightness, the non-linear gray level transformation should not be used. When the non-linear gray level transformation is used, you need to include a statement describing what type of brightness alteration was applied.
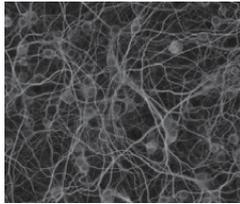
Figure 24 Original image[40]
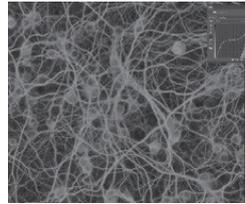
Figure 25 An image where non-linear gray level transformation has been applied[41]

40) and 41) Provided by LPIXEL Inc.

3.4　Alteration of aspect ratio

The shape of biological images must not be distorted. As the aspect ratio of images can easily be altered using presentation software, such as PowerPoint, you need to be careful not to unintentionally distort images when positioning images to fit on slides or paper.

Figures 26 and 27 show the same image with different aspect ratios. Figure 27 can be obtained by vertically stretching the original image in Figure 26.
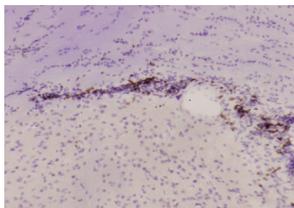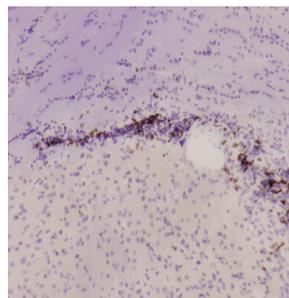

Figure 26 Original image[42]

Figure 27 Distorted image[43]

42) and 43) Provided by LPIXEL Inc.

Figures 28 and 29 show a case where an image was distorted in order to mislead the readers that the experiments were independent. A western blotting image has been vertically stretched and used as two different experiment images.
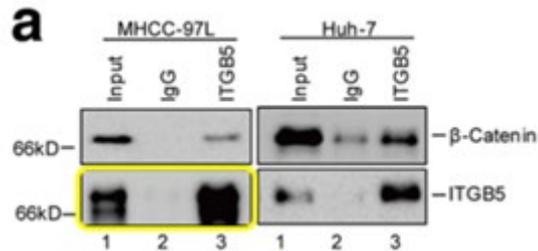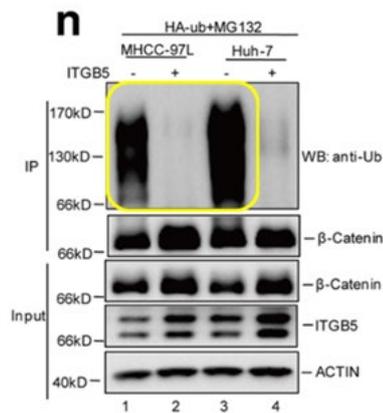
Figure 28 Image before stretching[44]

Figure 29 Stretched image[45]

Additionally, Figures 30 and 31 show an example of a certain image that had been distorted and used in another publication as a different image.
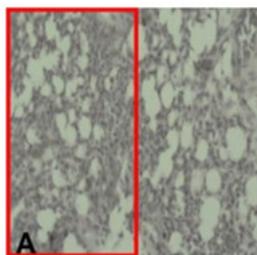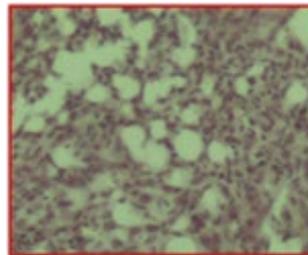

Figure 30 Original image[46]

Figure 31 An image that has been horizontally stretched[47]

3.5　Deceptive image editing

Deceptive image editing applied to an image raises a concern that the authors have removed unfavorable objects. If the contrast absolutely needs to be enhanced for reasons such as the signals of the part that is important for the research conclusion are extremely weak, you would need to have the original image available for additional submission as supplemental information.

Figure 32 shows an example of contrast adjustment. The contrast becomes higher from A to C. In image C, some features (bands) within the image have become less clear than in image B, and excessive contrast enhancement has been applied. This is not considered to be appropriate image processing.
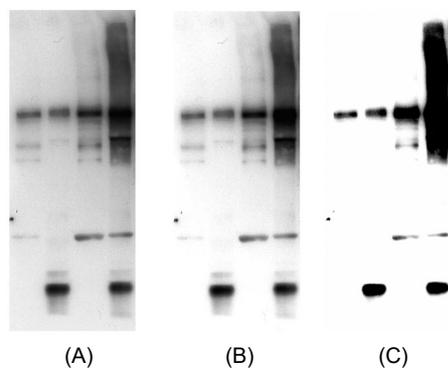

Figure 32 An example of contrast adjustment[48]

44) 45) Lin, Z., He, R., Luo, H., Lu, C., Ning, Z., Wu, Y., Han, C., Tan, G., & Wang, Z. (2018). Integrin-β5, a miR-185-targeted gene, promotes hepatocellular carcinoma tumorigenesis by regulating β-catenin stability. *Journal of Experimental & Clinical Cancer Research*, *37*, 17.
Copyright 2018 by BioMedCentral CC BY 4.0

46) Zhu, Y. T., Liu, H., Zhang, X. Q., Tang, M. M., Liu, J. Y., & Cao, G. Q. (2020). Effect of atorvastatin on pulmonary hypertension rats through regulating notch signaling pathway. *Eur Rev Med Pharmacol Sci*, *24*(9), 5118-5126.
Copyright CC BY-NC-ND 4.0

47) Liu, H., Li, Y., Zou, Y., Zhang, X., Shi, X., Yin, Z., & Lin, Y. (2021). Influence of miRNA-30a-5p on Pulmonary Fibrosis in Mice with Streptococcus pneumoniae Infection through Regulation of Autophagy by Beclin-1. *BioMed Research International*, *2021*(1), 9963700.
Copyright 2021 Hanyu Liu et al. CC BY 4.0

48) Rossner, M., & Yamada, K. M. (2004). What's in a picture? The temptation of image manipulation. *Journal of Cell Biology*, *166*(1), 11-15.
CC BY-NC-SA 4.0

3.6   Processing only one of the comparison images

If there are images to be compared (e.g., wild type vs mutant, or treated vs untreated areas), the same processing would need to be applied to all images. You must not apply image processing to just one of the comparison images. All images need to be processed using the same processing technique and parameter settings.

3.7   Processing only part of the image

Image processing needs to be applied across the entire image, and you must not apply image processing to only part of the image. For example, even when the image colors are slightly different between the colors observers perceive visibly and those expressed in the image, the color information across the entire image needs to be corrected, not only the color information of a specific part.

Chapter 4　Criteria for AI generated images

The emergence of generative AI has raised new challenges beyond those that conventional image manipulation techniques had. As there are concerns regarding the impact of AI, scientific journals limit the use of AI-generated images.

1.　Advantages of AI-generated images

Some of the known image generation tools using AI include Midjourney, Stable Diffusion, and DALL-E. Using these tools reduces the time it takes to create figures and graphs for papers, posters, and presentations, and fulfills the needs of researchers who want to make the complicated concepts of specialty fields easier to understand from the aspect of visibility.

Some researchers have already started to use AI-generated images in the explanation of their methodologies, presentation of their results on social media, and in their presentation slides that describe the concept of the paper.[49]

49) Wong, C. (2024). AI-generated images and video are here: how could they shape research? *Nature*. https://doi.org/10.1038/d41586-024-00659-8

2.　Issues of AI-generated images

Image generative AI models have the skills to generate extremely realistic biological images. For example, ChatGPT has the DALL-E3 image generation feature, and it easily generates high-quality images with a simple descriptive prompt. Figure 33 shows experiment images generated by ChatGPT. The images generated by AI are difficult to identify as fakes by visual inspection.[50]
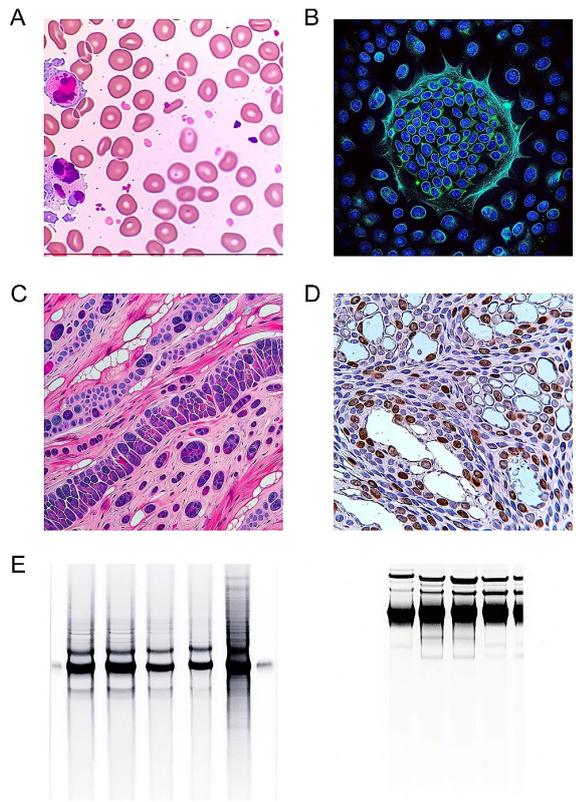
50) Zhu, L., Lai, Y., Mou, W., Zhang, H., Lin, A., Qi, C., Yang, T., Xu, L., Zhang, J., & Luo, P. (2024). ChatGPT's ability to generate realistic experimental images poses a new challenge to academic integrity. *Journal of Hematology & Oncology*, *17*(1), 27.
Copyright 2024 The Author(s) CC BY 4.0

Figure 33 Experiment images generated by ChatGTP[51]

51) Zhu, L., Lai, Y., Mou, W., Zhang, H., Lin, A., Qi, C., Yang, T., Xu, L., Zhang, J., & Luo, P. (2024). ChatGPT's ability to generate realistic experimental images poses a new challenge to academic integrity. *Journal of Hematology & Oncology*, *17*(1), 27.
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3.　Criteria for AI-generated images

In recent years, scientific journals have expressed growing concern regarding generative AI. The policy of whether or not to accept the use of AI-generated images in manuscripts differs by scientific journal. This chapter introduces the criteria for the use of AI-generated images in major scientific journals. Please see the submission guidelines of each scientific journal or other relevant information for details.

4.1   Nature Portfolio[52]

Springer Nature does not permit the use of AI because legal issues, including those relating to AI-generated images, remain unsolved. However, provided that the images are labeled as generated by AI, the journal permits the use of AI-generated images in the following three situations:

URL: https://www.nature.com/nature-portfolio/editorial-policies/ai#generative-ai-images

<Exceptions to the use of AI-generated images>
○ Images/art obtained from agencies that Springer Nature has contractual relationships with that have created images in a legally acceptable manner.
○ Images and videos that are directly referenced in a research paper about AI
○ The use of generative AI tools developed with specific sets of basic scientific data that can be attributed, checked, and verified for accuracy, provided that ethics, copyright, and terms of use restrictions are adhered to.

4.2   The EMBO Journal[53]

The EMBO Journal restricts the use of AI primarily to improve the style, accessibility, or quality of human-generated text and images. Also, you must take care to ensure that the use of AI does not lead you to plagiarize, misrepresent, or falsify content, or to infringe upon third-party rights. If AI is used, details on which parts of the paper AI was used in and on the tools employed must be disclosed, and if data generated by AI is used, this must be clearly labeled in the main text and figure, and it must also be documented in the Methods section.

URL: https://www.embopress.org/page/journal/14602075/authorguide#aitools

4.3   Science[54]

AI-generated images and other multimedia are not permitted in Science journals without explicit permission from the editors. The use of AI is permitted in certain situations such as for images in manuscripts about AI and/or machine learning; however, such exceptions will be evaluated on a case-by-case basis and should be disclosed at the time of submission.

URL: https://www.science.org/content/page/science-journals-editorial-policies

4.4   The New England Journal of Medicine (NEJM)[55]

NEJM has adopted policies specified by the International Committee of Medical Journal Editors (ICMJE) on the use of AI when preparing materials to be submitted for publication in NEJM.

URL: https://www.nejm.org/about-nejm/editorial-policies

52) Nature Portfolio. (2024, December) *Artificial Intelligence (AI)*. Nature Portfolio. https://www.nature.com/nature-portfolio/editorial-policies/ai#generative-ai-images

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

53) EMBO press. *Authorship Guidelines: Use of AI tools in text, figures and authorship. EMBO press.* https://www.embopress.org/page/journal/14602075/authorguide#aitools

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

54) Science Journals. *Science Journals: Editorial Policies*. AAAS. https://www.science.org/content/page/science-journals-editorial-policies

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

55) NEJM. *Editorial Policies*. Massachusetts Medical Society. https://www.nejm.org/about-nejm/editorial-policies

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

<Key points to consider when using AI>

*Only descriptions related to the generated image are excerpted
- ○ Whether or not AI-assisted technologies (such as image creators) were used should be disclosed.
- ○ If so, both the cover letter and the submitted work should include a description of the technologies used and what was produced.
- ○ Be prepared to assert that there is no plagiarism in images produced by AI.

4.5   The Lancet[56]

56) The Lancet. *Information for Authors*. Elsevier. https://www.thelancet.com/pb-assets/Lancet/authors/tl-info-for-authors-1690986041530.pdf

The Lancet is a signatory journal to the ICMJE Recommendations issued by the International Committee of Medical Journal Editors (ICMJE) and to the Committee on Publication Ethics (COPE) code of conduct for journal editors, and it follows COPE's guidelines.

URL: https://www.thelancet.com/pb-assets/Lancet/authors/tl-info-for-authors-1690986041530.pdf

<Key points to consider when using AI>

*Only descriptions related to the generated image are excerpted
- ○ Generative AI should be used primarily as a tool for brainstorming and suggesting image concepts.
- ○ Direct use of generative AI in creating figures or artwork should be limited to demonstrations of its capabilities in specific contexts, such as academic articles.

4.6   The BMJ[57]

57) The BMJ. *AI use*. BMJ Publishing Group. https://www.bmj.com/content/ai-use

As the World Association of Medical Editors (WAME) and the Committee on Publication Ethics (COPE) do, BMJ focuses on transparency of research and requests detailed disclosure on the use of AI.

URL: https://www.bmj.com/content/ai-use

<Key points to consider when using AI>

*Only descriptions related to the generated image are excerpted
- ○ Include an acknowledgement of AI use in the 'contributor' section.
- ○ If the AI use was in the course of research, a fuller description should be included in the Methods section.
- ○ The following information should be included in supplementary files or additional information; the name of the AI technology used, the reason for its use, the way it was used (the purpose of the task), a summary of the input, output, and the way in which the AI output was reviewed by the author.

4.7   Journal of American Medical Association (JAMA)[58,59]

JAMA provides matters that require attention when using AI, and as for images (figures), the following should be complied with:

URL: https://jamanetwork.com/journals/jama/pages/instructions-for-authors#SecFigures

URL: https://jamanetwork.com/journals/jama/pages/instructions-for-authors#SecUseofAIinPublicationandResearch

<Key points to consider when using AI>
*Only descriptions related to the generated image are excerpted

○ The submission and publication of images created by artificial intelligence, machine learning tools, or similar technologies is discouraged, unless part of research design or methods, and is not permitted without a clear description of the content that was created and the name of the model or tool, version and extension numbers, and manufacturer. Authors must take responsibility for the integrity of the content generated by these models and tools.

○ Follow relevant reporting guidelines for specific study designs when they exist and report each recommended guideline element with sufficient detail to enable reproducibility.

○ Avoid inclusion of identifiable patient information.

○ Be aware of copyright and intellectual property concerns.

○ If content protected by copyright was entered into the AI model, include a copy of the permission or license from the copyright owner and describe this permission/license in the Methods section.

○ If content generated by AI is included, indicate permissions to publish as determined by the owner or the AI service in the Methods section or in the legend(s) of any AI-generated figures.

4.8   Journal of Cell Biology (JCB)[60]

JCB requests, if AI or Large Language Model programs are used for the writing of a manuscript (including using them to polish, condense, or otherwise lightly edit the writing), generation of graphical or image elements, or in the collection or analysis of data, that it is precisely illustrated which tool was employed and how it was used in the Materials and methods section.

URL: https://rupress.org/jcb/pages/editorial-policies#ai

58) JAMA. *Instructions for Authors: Format My Manuscript*. American Medical Association. https://jamanetwork.com/journals/jama/pages/instructions-for-authors#SecFigures

59) JAMA. *Instructions for Authors: Use of AI in Publication and Research*. American Medical Association. https://jamanetwork.com/journals/jama/pages/instructions-for-authors#SecUseofAIinPublicationandResearch

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

60) JCB. Editorial Policies: Artificial Intelligence. Journal of cell biology. https://rupress.org/jcb/pages/editorial-policies#ai

Note: As of January 30, 2025
Note: Please see the submission guidelines or other relevant information for up-to-date details.

Chapter 5　Use of image processing check tool

Use of advanced image editing (photo retouching) software may eliminate unfavorable parts or unintentionally apply inappropriate image processing. Checking the images using software with a noise analysis feature and/or a duplicates detection feature before submission is also an effective measure.

5.1　Noise analyses

When inappropriate editing is applied on an image, the background noise within the image will become inconsistent. Analyzing noise using software with a noise analysis feature allows you to quickly identify signs of image editing.[61] Figure 34 shows an electrophoretic gel image before a noise analysis and Figure 35 shows the electrophoretic gel image after a noise analysis. You can see a sign of copy and paste in the third lane in the latter image and a sign of insertion in the fourth lane.
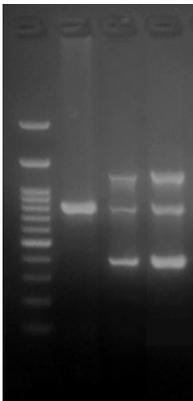
Figure 34 Image before noise analysis[62]
(Gel electrophoretic image that has been fraudulently processed)
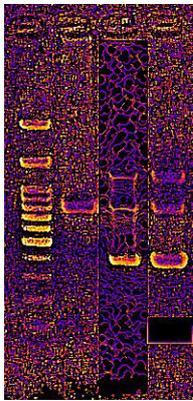
Figure 35 Image after noise analysis[63]
(A sign of copy and paste in the third lane and a sign of insertion in the fourth lane can be observed.)

5.2　Detection of duplicates

There are cases where the same image is unintentionally reused. Using software with a duplicate detection feature allows you to identify similar areas between images and within an image. Using the software, you can not only identify areas with a complete match, but also identify areas with a partial match, rotation, inversion, enlargement, reduction, elongation, or other alterations.[64] In Figures 36 - 39, red circles indicate parts that were detected as similar areas.
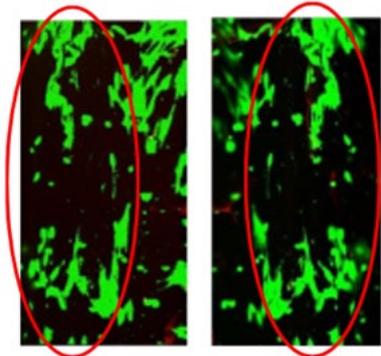
Figure 36　　　　　Figure 37

An example of inversion[65]
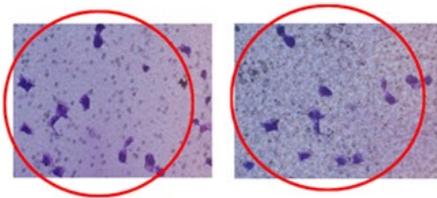
Figure 38　　　　　Figure 39

An example of rotation[66]