

## **Restrictions on Image Manipulation**

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## Introduction

Recent years have seen the emergence of a growing number of applications that automatically process photographs taken with a cellphone or similar device to create an image of the user's preference. In scientific research, too, there are occasions when images obtained as a result of experiments are manipulated. However, when doing so, you must fully understand the boundary



between appropriate and inappropriate image manipulations and carry out such manipulation with the greatest care in accordance with the regulations of your field of research, to ensure that the resultant images do not mislead readers and others. This is because improper, arbitrary manipulations of images and their publication distort the heart of scientific research — the findings of which must be both objective and reproducible — and can thus have a seriously adverse impact on scientific development.

For example, the White Paper on Publication Ethics published by the Council of Science Editors, a group of scientific journal editors, contains a section entitled Digital Images and Misconduct. If you read this, you will understand what constitutes inappropriate manipulation of images, how it is regarded, and how it is handled. In addition, many academic journals have regulations concerning image manipulation. Therefore, when submitting an article, you must check that every image manipulation has been carried out in accordance with these regulations or declare as such.

The objective of this module is to understand “the background of inappropriate image manipulation” and “inappropriate and appropriate image manipulations.” The three basic principles are as follows:

- 1) The original unprocessed image data should always be retained and be available for review by the publisher.
- 2) The imaging conditions and all image processing carried out should be recorded, so that the main forms of image processing can be detailed in the article.
- 3) Images in articles should always properly reflect the original images and be produced using the image processing methods accepted in the research field in question.

## Learning Objectives

- Understand the background to inappropriate manipulation of images.
- Understand the inappropriate manipulation of images.
- Understand the appropriate manipulation of images.

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### Inappropriate Manipulation of Images on the Rise

Photoshop and other image editing software began to be widely used in the late 1990s, giving rise to the problem of fraudulent manipulation of experimental images obtained in research. When carried out appropriately, the manipulation of experimental images is useful in presenting research findings in an appealing way, but inappropriate image manipulation constitutes a formidable problem, as it distorts the information obtained from experiments. Regardless of whether it is carried out intentionally or not, inappropriate image manipulation has a highly adverse impact on the development of scientific research, as it is deemed to fall within the scope of fabrication, falsification, and plagiarism — collectively known as “misconduct in research.”

Mike Rossner, formerly the Managing Editor of *The Journal of Cell Biology* (JCB), published an article about the JCB’s response when this problem surfaced (Rossner 2006).

- About 25% of manuscripts accepted by the JCB had at least one figure with inappropriate image manipulation that violated the journal’s guidelines, even though it did not affect the interpretation of the data.
- Around 1% of papers accepted by the JCB had to have their acceptance revoked after they were found to have fraudulent image manipulation that distorted the interpretation of the data.

In particular, the first fact tells us that the authors of the articles themselves do not always fully understand where the boundary between appropriate and inappropriate image manipulations lies.

Rossner also published an article listing typical cases of inappropriate image manipulation using Photoshop (Rossner and Yamada 2004) in which many examples of impropriety are highlighted. This article has come to be regarded as a landmark article, providing guidance for authors and readers alike.

A study of 20,621 articles published in 40 academic journals over the 20 years or so between 1995 and 2014 found that 3.8% of those articles contained inappropriate image duplications. The findings of the study were reported in a 2016 article (Bik et al. 2016). One example of malpractice described in this article was the reuse of images of electrophoretic gel loading controls as images of the results of a different experiment. Many such cases have been detected since 2003. Also highlighted are examples of habitual fraud, in which an author who has used an inappropriate image often uses inappropriate images in other articles. This kind of problem goes beyond the individual author. For example, if a laboratory director does not fully understand the boundary between appropriate and inappropriate image manipulations, they can pass on bad habits to the students under their supervision. In the case of collaborative research involving multiple researchers, this can lead to a chain of misconduct.

Facing this situation, many academic journals have drawn up various regulations concerning the handling of images in articles. The following excerpt from the JCB's Editorial Policies lists a number of rules around images.

- Images should be minimally processed and accurately reflect the original data.
- Specific features within an image may not be enhanced, obscured, moved, removed, or introduced.
- Dividing lines should be added between juxtaposed images taken from different parts of the same gel or from different gels, fields, or exposures.
- Adjustments of brightness, contrast, or color balance should have been applied to the entire image and should not enhance, erase, or misrepresent any information present in the original, including the background.

What constitutes fraudulent image manipulation may be different depending on the research field and type of experiment (electrophoretic gel images, microscope images, etc.) as well as on the academic journal to which you submit your article. However, the basic principles are the same as that described above. It also goes without saying that it is important for you to appropriately handle images obtained from your experiments in all situations, not only when giving conference presentations and submitting articles, but also at lab meetings and joint research conferences.

Image data play a very important role in articles because they convey a large amount of information within a small space. However, they alone do not guarantee the objectivity of research. The person who creates images can intentionally bring bias into the images, arbitrarily inducing their desired conclusion. To avoid such "fraudulent manipulation," some

academic journals require details of the manipulation performed on the experiment images to be described in the article, including the name of the software used and the numerical settings used to adjust the images.

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### **Basic Points Concerning Image Manipulation**

The following describes “the basic points about image manipulation.” We recommend that you use [JCB Data Viewer](#) if you wish to see how your experiment images change as a result of image manipulation. This site also enables you to view original and manipulated images from articles published in the JCB.

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#### **1. Apply Image Manipulation Only to a Copy of an Image and Save the Original, Unmanipulated Image**

Saving original, unmanipulated images is an essential part of proper research conduct. If you have the original, unmanipulated images, you can check them later, no matter what problems may arise. For example, an academic journal’s editor or a peer reviewer may ask you to submit the original images. This may be a requirement at the time of submission, or it may be due to suspicions that an image used in the submitted article are fraudulent. Of course, the request may simply be inspired by a desire to see the results in greater detail. Either way, it is important to appropriately save original images in their unmanipulated form so that you can respond to such requests.

It is also important to bear in mind the need to retain original images when putting together the results of experiments. For example, even if there is nothing intrinsically wrong with the manipulation applied to an image, any errors arising in the manipulation process cannot be undone later if you save over the original. To prevent such accidents, image manipulation should be applied only to a copy of the image, or, if the software you are using permits, you should save the original image as a layer and then carry out processing on another layer.

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#### **2. “Simple” Adjustments Applied to the Whole Image Are Not a Problem**

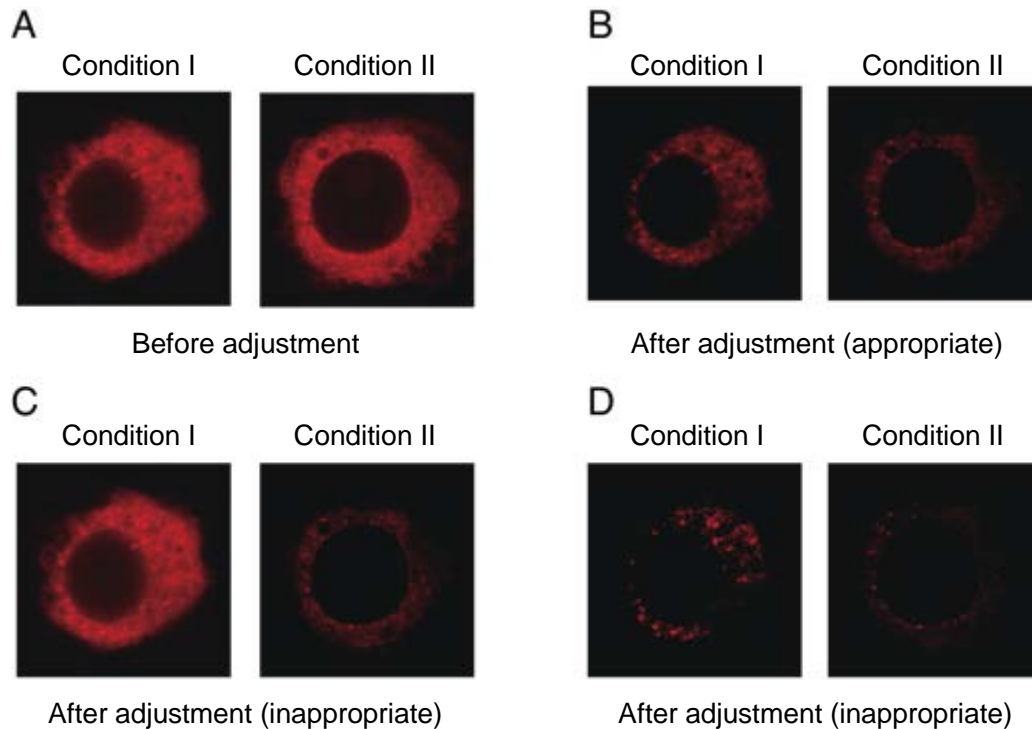
Adjustments of brightness and contrast are not a problem, as long as they are applied to the whole image. When adjusting brightness and contrast, the same processing is applied to all pixels in a linear manner. It is recommended that you view a brightness histogram and adjust the brightness level in such a way that the minimum and maximum brightness of each pixel in the image falls at either end of the scale displayed. The shape of the histogram will change if the pixels with high or low brightness are gathered at one end of the scale. This

kind of manipulation has the potential to cause the loss of important information because it becomes harder to see the bright and dark parts of the image (excessive adjustment, such as blown-out highlights and blocked-out shadows, is regarded as inappropriate).

In the case of gel images, the appropriate level of contrast is one in which a little of the background color still remains. It goes without saying that manipulation that involves adjusting brightness in only one of two images to be compared is regarded as fraudulent. For example, making only the unstained control group darker or the stained group brighter in an image with fluorescent staining is fraudulent.

In gamma correction of images, each pixel is not processed in the same way, but undergoes different correction in a nonlinear way according to the brightness of each pixel. Since it mainly corrects neutral colors, the shade balance could become inappropriate. Care is required when using gamma correction. If you need to correct the image, it would be wise to specify the gamma value in the legend to the figure. Another issue you must be careful with when setting the gamma value is ensuring that the PC monitor settings of all collaborating researchers are identical. If researchers view the same image in different environments, for example, if they see colors differently, discrepancies could arise in their interpretations and understanding of the image.

Even if the manipulation is simple and applied to the whole image, changing the height-to-width ratio of an image is inappropriate, as it distorts the object's original shape. This manipulation results in different vertical and horizontal magnifications in microscopic images.



**Fig. 1 Inappropriate Adjustment of Brightness and Contrast**

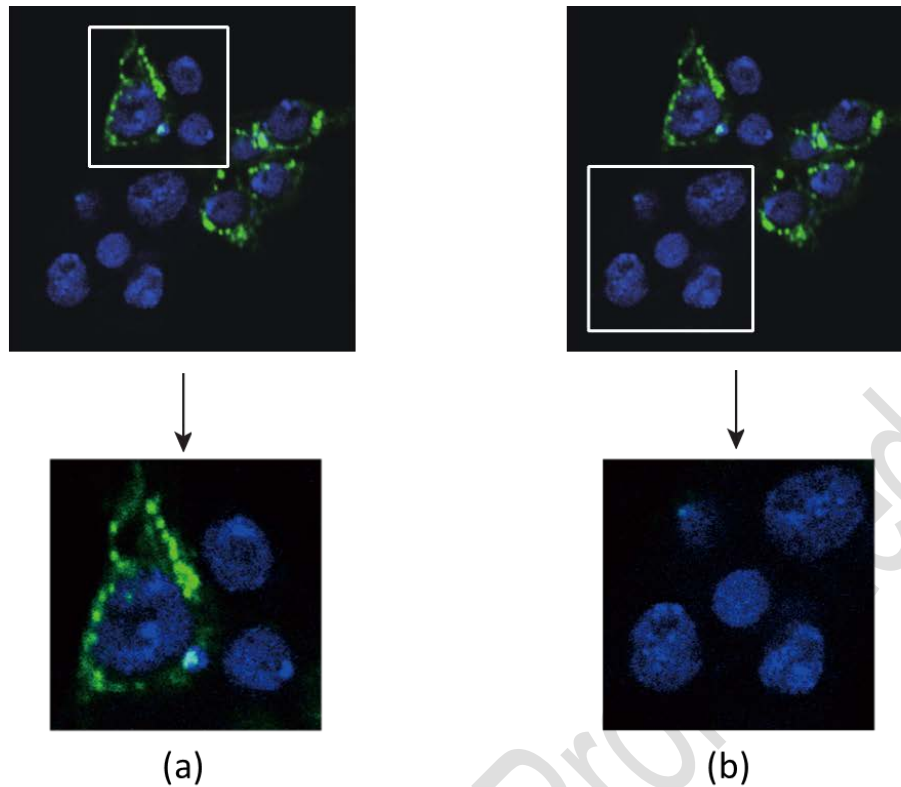
- A. Images before adjustment.
- B. Images after appropriate adjustment: The contrast for both conditions I and II has been altered in the same way.
- C. Images after inappropriate adjustment: Only the contrast for condition II has been adjusted.
- D. Images after inappropriate adjustment: The contrast for both conditions I and II has been adjusted too drastically.

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### 3. Image Cropping That Affects the Interpretation of the Data Is a Problem

Cropping, which involves trimming the edges off an image, is basically not a problem as long as it is solely done for the purpose of deleting blank space. Adjusting the display area is appropriate and necessary because the space is limited in academic journals. However, cutting out dead cells from a microscopic image to show only living cells or removing from electrophoretic gel images any bands that are not consistent with the hypothesis are actions that constitute “falsification” because as they involve hiding results that are disadvantageous to your argument. You must ensure that there is no alteration in the information provided to readers.





**Fig. 2 Example of Inappropriate Cropping**

Images of immunostaining of a protein (green) are presented.

Since the expression level of this protein varies from one cell to another, there is no problem with cropping it in the way shown in (a).

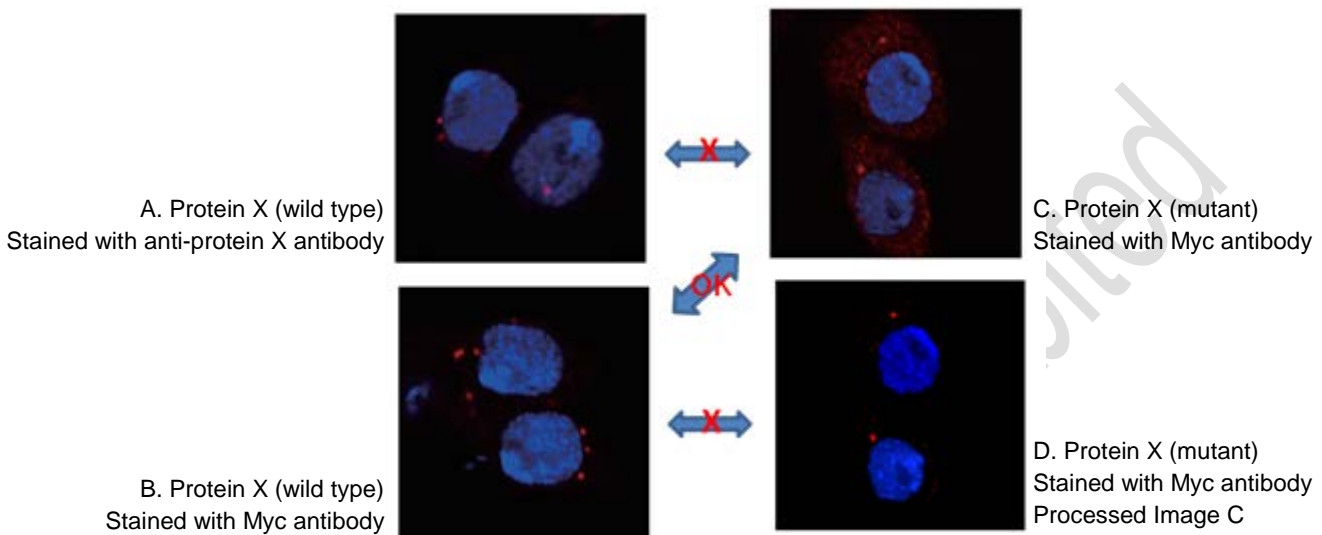
However, cropping only cells where the protein is not expressed, as in (b), gives the reader incorrect information and therefore constitutes “falsification.”

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#### **4. Images That Will Be Compared with Each Other Must Be Obtained under Identical Conditions and Subject to Identical Manipulation**

Comparing images obtained under different experimental conditions or subjected to different manipulation constitutes inappropriate behavior. Let us look at an example of quantitative evaluation of images from tissue sections and comparisons with the control group. Here it is assumed that the staining efficiency is identical in all sections. Thus, we compare images obtained using the same microscope with the same settings from sections of equal thickness, prepared with the same fixing method and time, and stained at the same time in the same operation with the same reagent. It will be fabrication of experimental results if you use images from a different experiment as those from the control group on the assumption that the results would be just about the same. The procedures used for analysis should also be identical. Suppose you are processing images obtained under the same conditions: the

color balance is adjusted, the background brightness is subtracted from the brightness of the color that you wish to determine, the threshold value is specified to determine the area to be quantified, and the measured area is selected. This type of image processing must be done identically on all images to be quantitated, including those of the control group because if it is not identically done, you will obtain different results, even from the same images.



**Fig. 3 Examples of Appropriate and Inappropriate Image Comparisons**

- A: Intracellular localization of protein X was observed using anti-protein X antibody. The endogenous protein was detected as red dots.
- B: Wild type protein X with a myc tag was expressed by means of gene transfer and its localization was observed using the myc tag antibody.
- C: Mutant protein X with a myc tag was expressed by means of gene transfer and its localization was observed using the myc tag antibody.
- D: The image C was processed to leave only the fluorescence of the red dots by suppressing the red fluorescence in all other areas.

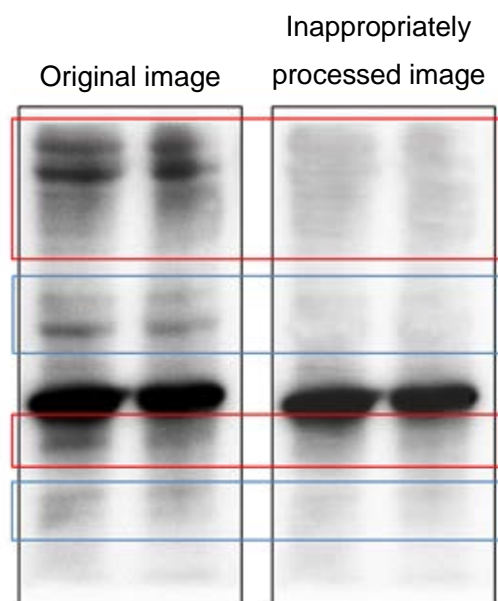
Because the conditions of the experiments and immunostaining were different, including the use of different antibodies for staining and the use of gene transfer to forcibly express the protein, it is inappropriate to use the image A as the control group for the image C, even though the results shown in the images A and B were very similar. The appropriate control group for the image C is the image B, which was obtained from an experiment carried out under the same conditions. Another important point is the fact that the images B and C were obtained under the same microscopic conditions.

On the other hand, if the images B and D are compared, the intracellular localization of protein X appears the same for the wild type and the mutant. However, the image D is a version of the image C with its contrast intensely “processed.” In other words, comparing the images B and D is inappropriate for two reasons: weak signals have been removed through excessive contrast adjustment, and it involves processing of only one of the images.

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### 5. Manipulation Applied Only to a Specific Part of an Image Will Be Regarded as Fraudulent

Manipulating parts of an image, such as selectively altering the shade of a specific band or lane in an electrophoretic gel image or selectively altering the color in only one part of a fluorescent-stained image, is fraudulent. These acts create experiment results that do not actually exist, raising the question of data fabrication. It constitutes fabrication and falsification to cut objects from elsewhere in the same image or from another image and to past them into a specific part of the image where they did not originally exist. Typical examples of this include use of tools such as “Healing Brush” and “Copy Stamp” to add or delete bands in gel images. Such changes in image contrast cause differences in the background, showing that the image has been partially edited. Using “the eraser” tool to delete part of the background is also problematic because it is not possible to tell whether what was erased was just a noise within the image or something inconvenient. There is also the possibility that a reaction of great significance that the author has overlooked might be erased.



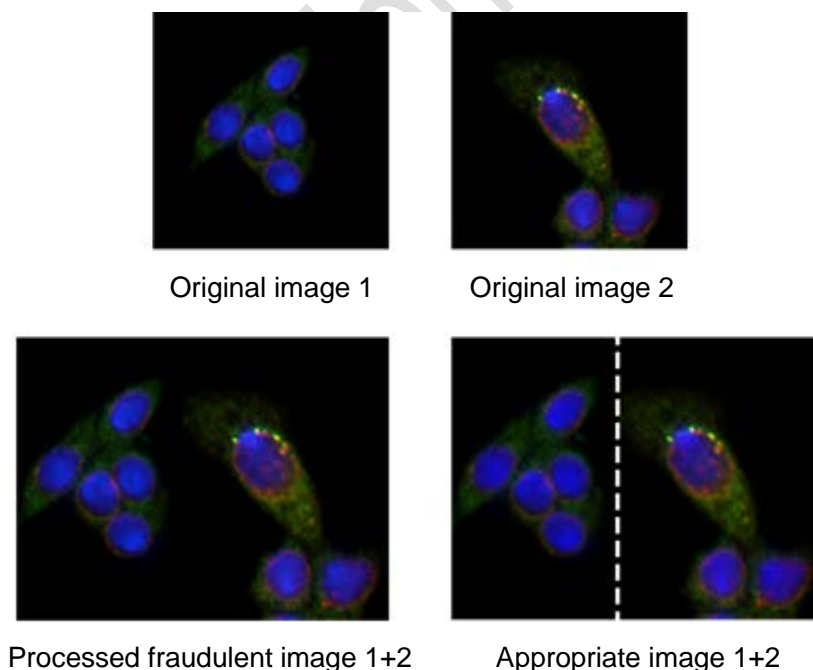
#### Fig. 4 Inappropriate Manipulation of Blots: Making the Background Clearer

The “Copy Stamp” tool in Photoshop is sometimes used to clean up the background image in original data. If you look closely at the inappropriately processed image, you can see traces of this tool’s use in the left-hand lane.

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#### 6. Caution Is Required When Cutting and Pasting from within a Single Image or from a Separate Image

Cutting parts from multiple images and pasting them together into a “seamless” single image is inappropriate manipulation. The type of manipulation frequently seen to date involves cut-and-paste of separate lanes of an electrophoretic gel image or of gel images obtained from several experiments. This kind of manipulation is permitted only if a border line is drawn between the pasted fragments to show that they were from different gel images. Failing to show the boundary gives the reader the impression that they were obtained at the same time from the same experiment and is therefore regarded as inappropriate manipulation. In microscopic images, gathering together cells from different fields of view and presenting them as a single picture without a border is similarly regarded as inappropriate manipulation.



**Fig. 5 Appropriate Way of Presenting Joined Images**

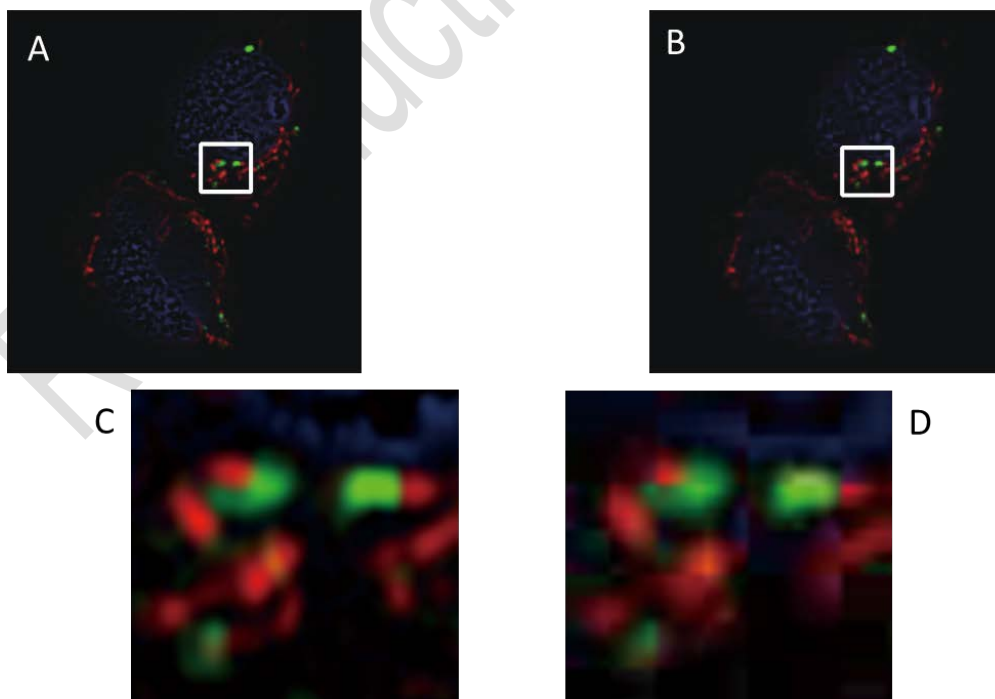
The processed fraudulent image 1+2 is inappropriate because it does not clearly show the boundary or distinction between the two original images that have been joined

together. On the other hand, the appropriate image 1+2 has a white dotted line inserted along the border between the two original images, showing that two separate images have been placed side by side.

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## 7. Avoid Using Lossy Compression

The choice of file format for image files is important. Currently, JPEG is generally used as the format for saving images from digital cameras and the like. However, this format reduces file size by means of lossy compression, and block noise can find its way into the image when compression takes place. TIFF is recommended for saving image data in scientific research. While files saved in this format tend to be large, any compression is lossless unless you select JPEG compression, avoiding deterioration in image quality. In scientific research, image quality is very important because various information is obtained from image data. It is preferable to save images at the highest possible level of quality to ensure that noise does not result in inappropriate interpretation or that compression does not cause the loss of important information. Because of data transfer quantity issues, you may be asked to submit images as small files (JPEG, etc.) when first submitting an article to an academic journal, and then to resend them later as large images (TIFF, etc.). When doing so, you should retain the original image data and separately save the image files for submission.



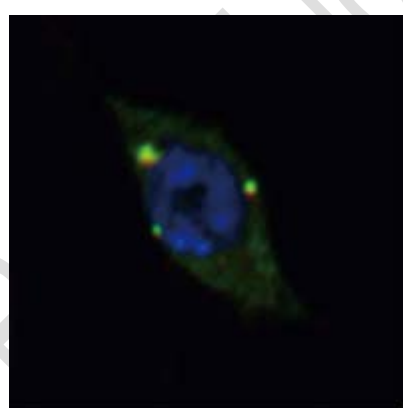
**Fig. 6 Differences Between TIFF (Lossless Compression) and JPEG (Lossy Compression) Formats**

- A. Image saved in TIFF.
- B. Image saved in JPEG (image quality is poorer than the image saved in TIFF).
- C. Enlarged view of the area in the white square in Fig. A.
- D. Enlarged view of the area in the white square in Fig. B.

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### 8. Be Cautious about Changing the Resolution and Size (Number of Pixels)

In scientific research, the spatial size and shape of the study subject are important. Therefore, it is necessary to record images of an appropriate resolution and size (number of pixels). Many academic journals describe restrictions in their Author Information on the resolution of images presented in articles. For example, the edges of the study subject are indistinct in blurred, low-resolution images, making it impossible to appropriately estimate its size or distance. On the other hand, there are occasions when you may want to reduce the size of a large (high number of pixels), high-resolution image for practical reasons, such as the space available on the published page. While this kind of manipulation is permitted, you should keep any reductions in size or resolution to a level at which the study subject can still be shown appropriately. On the other hand, enlarging small images (those with few pixels) is not appropriate because it involves adding pixels that were not originally there. If the original image is too small and you wish to show it in an appropriate size, you need to recapture the image data at an adequate size and resolution.



Original image

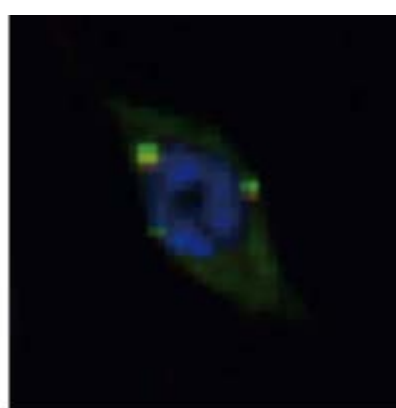


Image with a lower resolution

**Fig. 7 Change in Resolution**

Reducing the resolution makes the image rougher than the original, and the harder it is to identify the reactive sites, the more difficult it becomes to spot details.

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## 9. Images of Graphs

Graphs are a means of expressing your research data in a way that is easy to understand. There was a time when researchers prepared their graphs by hand and there were cases of deliberate fraud that went beyond mere errors in drawing. For example, some authors adjusted the length of error bars to make the accuracy of the results look better. Thanks to advances in computer software, graphs can now be prepared automatically from numerical data. Many academic journals require graphs to be drawn automatically using software, to prevent fraud arising in hand-drawn graphs. However, there is still a possibility of falsification, such as by selecting and entering only the data the author wants when manually entering data into the graph-drawing software. In addition, editing software can be used to modify the resultant graph image (by such means as altering the position of error bars or deleting data points). Therefore, we cannot say that graphs drawn using software are necessarily reliable. As described below, authors are required to submit graphs that include the data, to prevent such fraud.

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## 10. Diagram Formats

There are several image formats for graphs and diagrams used in articles. Formats, such as TIFF, PNG and JPEG, create Bitmap images which are portrayed as a collection of dots and use different methods for compression to reduce file size. For example, the JPEG format divides an image into small files and reduce its data size by regarding pixels of closely similar colors as being the same. Because it uses this method to compress files, lines in JPEG images become blurred or look jagged when enlarged.

On the other hand, lines in diagrams remain sharp even after enlarged when the EPS format (vector format), a standard in graphics software such as Adobe Illustrator and Corel Draw, is used.

In vector format images, the start and finish points are assigned coordinates and the line is then drawn to link them. Since the type of line to be drawn is specified by means of a formula, there is no change in its appearance, even if enlarged or compressed. Accordingly, some academic journals recommend that diagrams are submitted as vector format images.

These characteristics of graphs drawn as vector format images are important in research activities. Specifying coordinates and formulas, rather than portraying an image with dots, makes it possible to reconstruct the original experiment data from the image. In fact, recent years have seen cases of research misconduct allegations raised after analyzing vector

format images. This said, you might think that authors are asked to prepare graphs as vector format images in order to identify research misconduct. However, this feature of vector format images is actually to protect researchers, as it enables images to be compared with appropriate records of experiment results, such as experiment notes.

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## Conclusion

This module presented some of the key points relating to image manipulation. However, these are not the only points to bear in mind. You must obtain further information as needed, by such means as consulting the regulations of academic journals.

The readers of academic articles do not expect the images that they see to have undergone fraudulent processing. The publication of research findings in articles involves mutual trust between researchers. Consequently, the use of inappropriately treated images is immoral conduct that will impair trust in scientific research.

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JCB Data Viewer

<http://jcb-dataviewer.rupress.org>