Creating Clear and Informative Image-based Figures for Scientific Publications

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Abstract

Scientists routinely use images to display data. Readers often examine figures first; therefore, it is important that figures are accessible to a broad audience. Many resources discuss fraudulent image manipulation and technical specifications for image acquisition; however, data on the legibility and interpretability of images are scarce. We systematically examined these factors in non-blot images published in the top 15 journals in three fields; plant sciences, cell biology and physiology (n=580 papers). Common problems included missing scale bars, misplaced or poorly marked insets, images or labels that were not accessible to colorblind readers, and insufficient explanations of colors, labels, annotations, or the species and tissue or object depicted in the image. Papers that met all good practice criteria examined for all image-based figures were uncommon (physiology 16%, cell biology 12%, plant sciences 2%). We present detailed descriptions and visual examples to help scientists avoid common pitfalls when publishing images. Our recommendations address image magnification, scale information, insets, annotation, and color and may encourage discussion about quality standards for bioimage publishing.

Keywords: microscopy; imaging; images; photographs; colorblind; transparency; good bioimaging practices

Introduction

Images are often used to share scientific data, providing the visual evidence needed to turn concepts and hypotheses into observable findings. An analysis of 8 million images from more than 650,000 papers deposited in PubMed Central revealed that 22.7% of figures were “photographs”, a category that included microscope images, diagnostic images, radiology images and fluorescence images.[1] Cell biology was one of the most visually intensive fields, with publications containing an average of approximately 0.8 photographs per page.[1] Plant sciences papers included approximately 0.5 photographs per page.[1]

While there are many resources on fraudulent image manipulation and technical requirements for image acquisition and publishing,[2-4] data examining the quality of reporting and ease of interpretation for image-based figures are scarce. Recent evidence suggests that important methodological details about image acquisition are often missing.[5] Researchers generally receive little or no training in designing figures; yet many scientists and editors report that figures and tables are one of the first elements that they examine when reading a paper.[6, 7] When scientists and journals share papers on social media, posts often include figures to attract interest. The PubMed search engine caters to scientists’ desire to see the data by presenting thumbnail images of all figures in the paper just below the abstract.[8] Readers can click on each image to examine the figure, without ever accessing the paper or seeing the introduction or methods. EMBO’s Source Data tool (RRID:SCR_015018) allows scientists and publishers to share or explore figures, as well as the underlying data, in a findable and machine readable fashion.[9]

Image-based figures in publications are generally intended for a wide audience. This may include scientists in the same or related fields, editors, patients, educators and grants officers. General recommendations emphasize that authors should design figures for their audience rather than themselves, and that figures should be self-explanatory.[7] Despite this, figures in papers outside one’s immediate area of expertise are often difficult to interpret, marking a missed opportunity to make the research accessible to a wide audience. Stringent quality standards would also make image data more reproducible. A recent study of fMRI image data, for example, revealed that incomplete documentation and presentation of brain images led to non-reproducible results.[10, 11]

Here, we examined the quality of reporting and accessibility of image-based figures among papers published in top journals in plant sciences, cell biology and physiology. Factors assessed include the use of scale bars, explanations of symbols and labels, clear and accurate inset markings, and transparent reporting of the object or species and tissue shown in the figure. We also examined whether images and labels were accessible to readers with the most common form of color blindness.[12] Based on our results, we provide targeted recommendations about how scientists can create informative image-based figures that are accessible to a broad audience. These recommendations may also be used to establish quality standards for images deposited in emerging image data repositories.

Results

Using a science of science approach to investigate current practices: This study was conducted as part of a participant-guided learn-by-doing course, in which eLife

Community Ambassadors from around the world worked together to design, complete, and publish a meta-research study.[13] Participants in the 2018 Ambassadors program designed the study, developed screening and abstraction protocols, and screened papers to identify eligible articles (HJ, BA, SJB, VB, LHH, VI, SS, EMW). Participants in the 2019 Ambassadors program refined the data abstraction protocol, completed data abstraction and analysis, and prepared the figures and manuscript (AA, SA, TLA, IF, MAG, HL, SYM, MO, AV, KW, HJ, TLW).

To investigate current practices in image publishing, we selected three diverse fields of biology to increase generalizability. For each field, we examined papers published in April 2018 in the top 15 journals, which publish original research (Table S1, Table S2, Table S3). All full-length original research articles that contained at least one photograph, microscope image, electron microscope image, or clinical image (MRI, ultrasound, X-ray, etc.) were included in the analysis (Figure S1). Blots and computer-generated images were excluded, as some of the criteria assessed do not apply to these types of images. Two independent reviewers assessed each paper, according to the detailed data abstraction protocol (see methods and information deposited on the Open Science Framework (RRID:SCR_017419) at https://osf.io/b5296/).[14] The repository also includes data, code and figures.

Image analysis: First, we confirmed that images are common in the three biology subfields analyzed. More than half of the original research articles in the sample contained images (plant science: 68%, cell biology: 72%, physiology: 55%). Among the 580 papers that included images, microscope images were very common in all three fields (61 to 88%, Figure 1A). Photographs were very common in plant sciences (86%), but less widespread in cell biology (38%) and physiology (17%). Electron microscope images were less common in all three fields (11 to 19%). Clinical images, such as X-rays, MRI or ultrasound, and other types of images were rare (2 to 9%).

Scale information is essential to interpret biological images. Approximately half of papers in physiology (49%) and cell biology (55%), and 28% of plant science papers provided scale bars with dimensions (in the figure or legend) for all images in the paper (Figure 1B, Table S4). Approximately one-third of papers in each field contained incomplete scale information, such as reporting magnification or presenting scale information for a subset of images. Twenty-four percent of physiology papers, 10% of cell biology papers, and 29% of plant sciences papers contained no scale information on any image.

Some publications use insets to show the same image at two different scales (cell biology papers: 40%, physiology: 17%, plant sciences: 12%). In this case, the authors should indicate the position of the high-magnification inset in the low-magnification image. The majority of papers in all three fields clearly and accurately marked the location of all insets (53 to 70%, Figure 1C left panel), however one-fifth of papers appeared to have marked the location of at least one inset incorrectly (17 to 22%). Clearly visible inset markings were missing for some or all insets in 13 to 28% of papers (Figure 1C left panel). Approximately half of papers (43 to 53%, Figure 1C right panel) provided legend explanations or markings on the figure to clearly show that an inset was used, whereas this information was missing for some or all insets in the remaining papers.

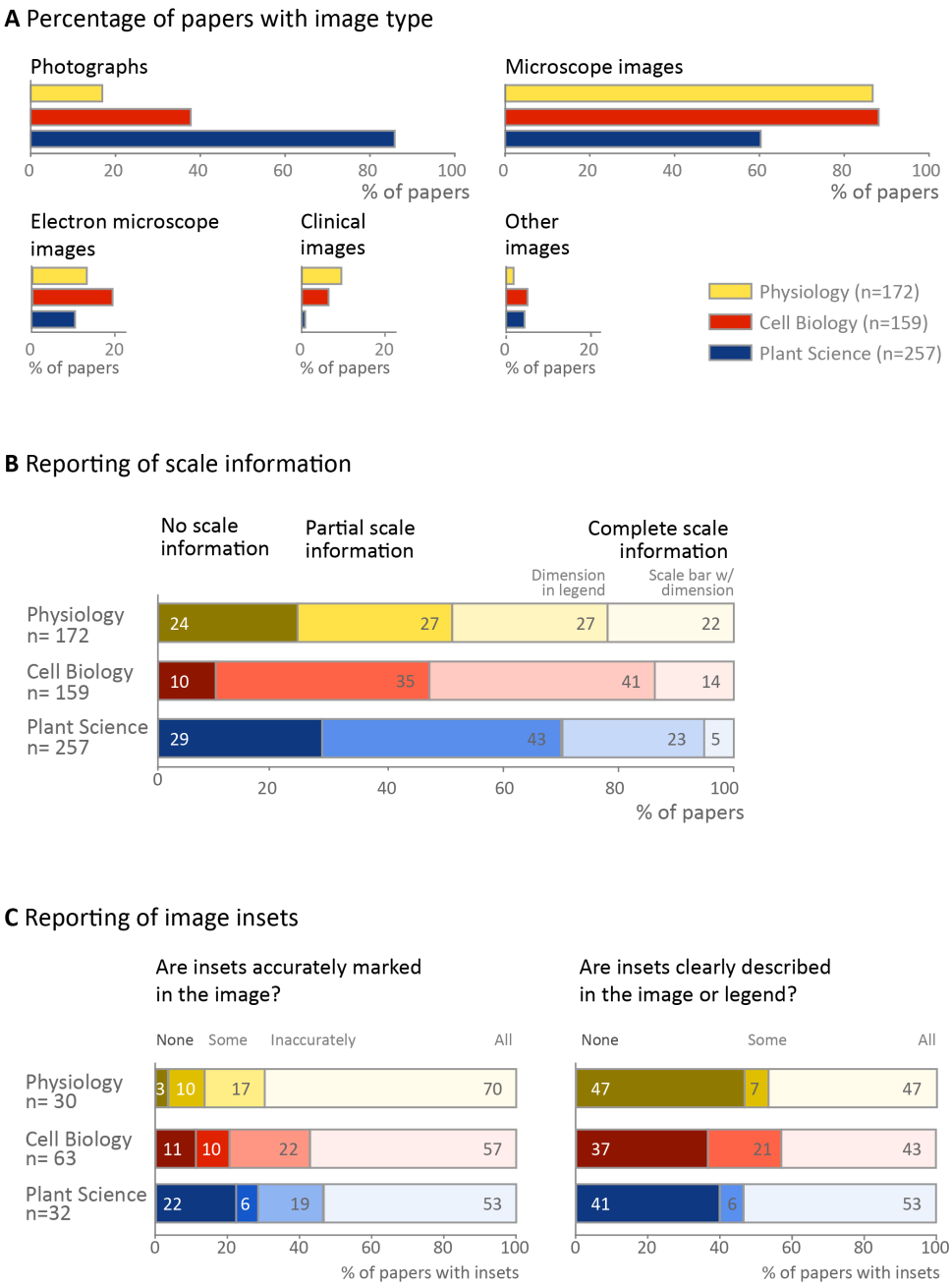


Figure 1: Image types and reporting of scale information and insets
A: *Microscope images and photographs were common, whereas other types of images were used less frequently.*
B: *Complete scale information was missing in more than half of the papers examined. Partial scale information indicates that scale information was presented in some figures, but not others, or that the authors reported magnification rather than including scale bars on the image.*
C: *Problems with labeling and describing insets are common.*
Totals may not be exactly 100% due to rounding.

Many images contain information in color. We sought to determine whether color images were accessible to readers with deuteranopia, the most common form of color blindness, by using the color blindness simulator Color Oracle (https://colororacle.org/, RRID: SCR_018400). We evaluated only images in which the authors selected the image colors (e.g. fluorescence microscopy). Papers without any colorblind accessible figures were uncommon (3 to 6%), however 45% of cell biology papers and 21-24% of physiology and plant science papers contained some images that were inaccessible to readers with deuteranopia (Figure 2A). 17 to 34% of papers contained color annotations that were not visible to someone with deuteranopia.

Figure legends and, less often, titles typically provide essential information needed to interpret an image. This text provides information on the specimen and details of the image, while also explaining labels and annotations used to highlight structures or colors. 57% of physiology papers, 48% of cell biology papers and 20% of plant papers described the species and tissue or object shown completely. 5-17% of papers did not provide any such information (Figure 2B). Approximately half of the papers (47-58%, Figure 1C, right panel) also failed or partially failed to adequately explain that insets were used. Annotations of structures were explained better. Two-thirds of papers across all three fields clearly stated the meaning of all image labels, while 18 to 24% of papers provided partial explanations. Most papers (73 to 83%) completely explained the image colors by stating what substance each color represented or naming the dyes or staining technique used.

Finally, we examined the number of papers that used optimal image presentation practices for all criteria assessed in the study. Twenty-eight (16%) physiology papers, 19 (12%) cell biology papers and 6 (2%) plant sciences papers met all criteria for all image-based figures in the paper (data not shown in figure). In plant sciences and physiology, the most common problems were with scale bars, insets and specifying in the legend the species and tissue or object shown. In cell biology, the most common problems were with insets, colorblind accessibility, and specifying in the legend the species and tissue or object shown.

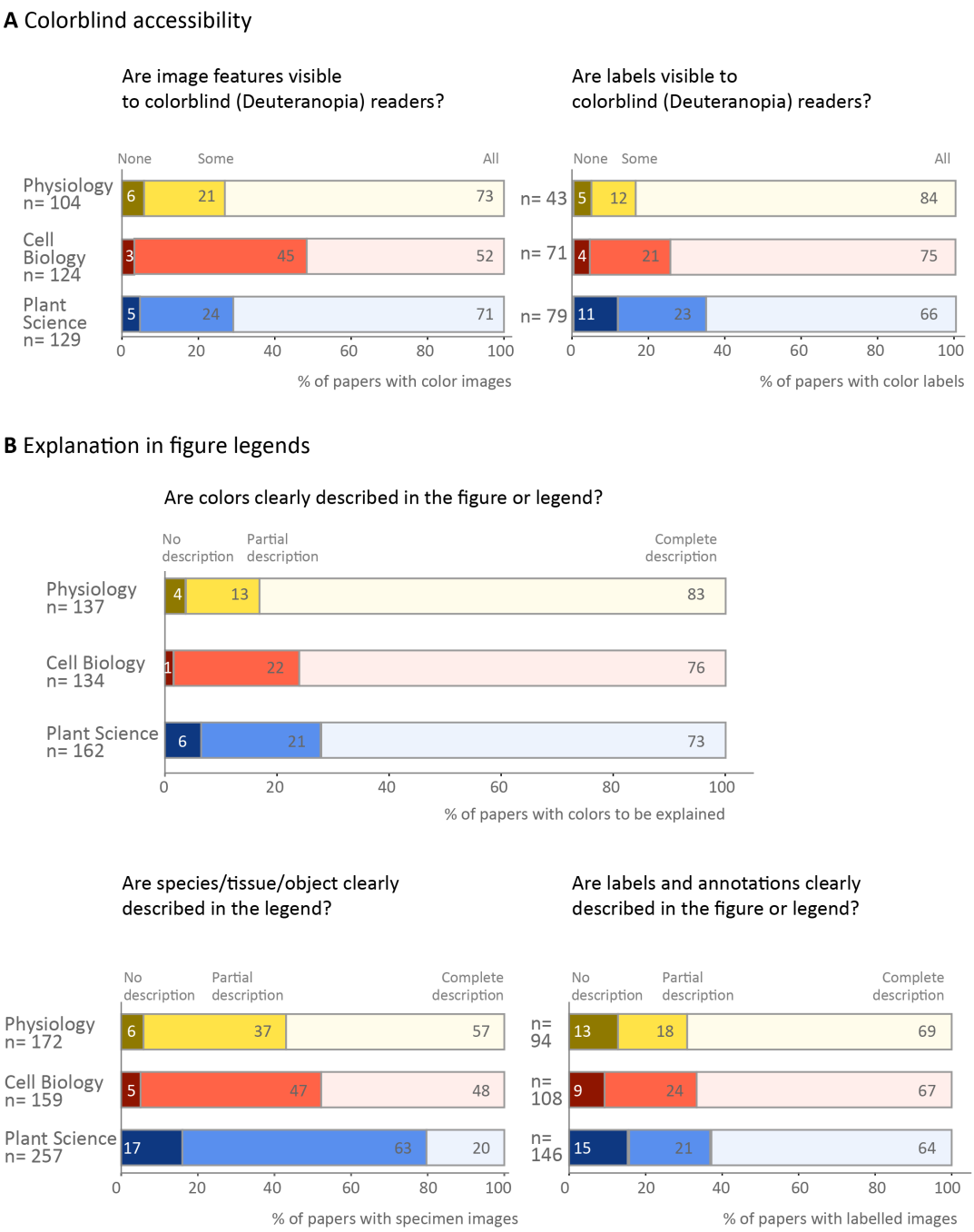


Figure 2: Use of color and annotations in image-based figures
A: *While many authors are using colors and labels that are visible to colorblind readers, the data show that improvement is needed.*
B: *Most papers explain colors in image-based figures, however, explanations are less common for the species and tissue or object shown, and labels and annotations.*
Totals may not be exactly 100% due to rounding.

Designing image-based figures: How can we improve?

Our results obtained by examining 580 papers from three fields provide us with unique insights into the quality of reporting and the accessibility of image-based figures. Our quantitative description of standard practices in image publication highlights opportunities to improve transparency and accessibility to readers from different backgrounds. We have therefore outlined specific actions that scientists can take when creating images, designing multipanel figures, annotating figures and preparing figure legends.

Throughout the paper, we provide visual examples to illustrate each stage of the figure preparation process. Other elements are often omitted to focus readers' attention on the step illustrated in the figure. For example, a figure that highlights best practices for displaying scale bars may not include annotations designed to explain key features of the image. When preparing image-based figures in scientific publications, readers should address all relevant steps in each figure. All steps described below (image cropping and insets, adding scale bars and annotation, choosing color channel appearances, figure panel layout) can be implemented with standard image processing software such as FIJI[15] (RRID:SCR_002285) and ImageJ2[16] (RRID:SCR_003070), which are open source, free programs for bio-image analysis. A quick guide on how to do basic image processing for publications with FIJI is available in a recent cheat sheet publication[17] and a discussion forum and wiki are available for FIJI and ImageJ (https://imagej.net/).

1. Choose a scale or magnification that fits your research question

Scientists should select an image scale or magnification that allows readers to clearly see features needed to answer the research question. Figure 3A shows *Drosophila melanogaster* at three different microscopic scales. The first focuses on the ovary tissue and might be used to illustrate the appearance of the tissue or show stages of development. The second focuses on a group of cells. In this example, the "egg chamber" cells show different nucleic acid distributions. The third example focuses on subcellular details in one cell, for example, to show finer detail of RNA granules or organelle shape.

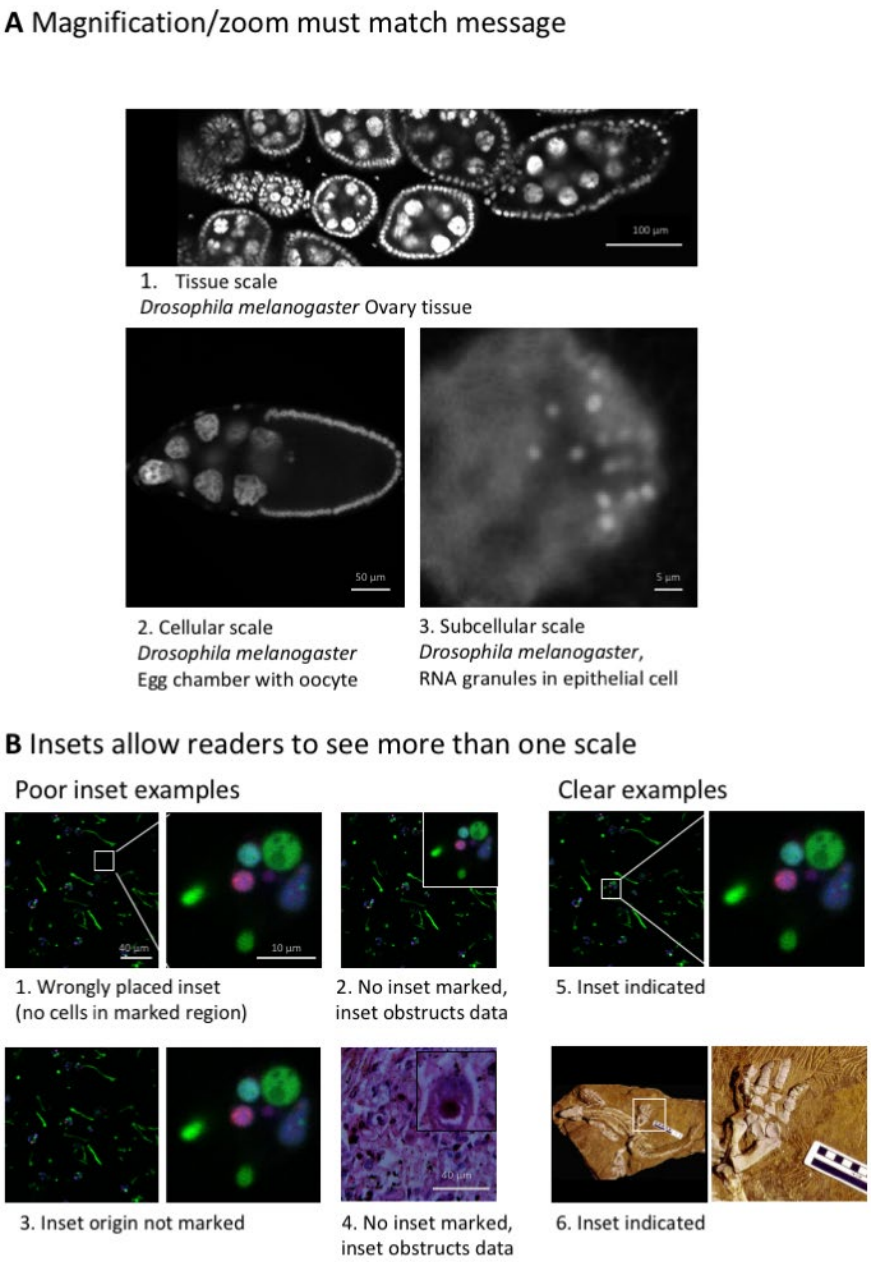


Figure 3: Selecting magnification and using insets

A. *Magnification and display detail of images should permit readers to see features related to the main message that the image is intended to convey. This may be the organism, tissue, cell, or a subcellular level.*

Microscope images[18] show D. melanogaster ovary (A1), ovarian egg chamber cells (A2), and a detail in egg chamber cell nuclei (A3).

B*. Insets or zoomed-in areas are useful when two different scales are needed to allow readers to see essential features. It is critical to indicate the origin of the inset in the full-scale image. Poor and clear examples are shown.*

Example images were created based on problems observed by reviewers. Images show B1, B2, B3, B5: Protostelium aurantium amoeba fed on germlings of Aspergillus

fumigatus D141-GFP (green) fungal hyphae, dead fungal material stained with propidium iodide (red), and acidic compartments of amoeba marked with LysoTracker Blue DND-22 dye (blue); B4: Lendrum-stained human lung tissue (Haraszti, Public Health Image Library); B6: fossilized Orobates pabsti.[19]

When both low and high magnifications are necessary for one image, insets are used to show a small portion of the image at higher magnification (Figure 3B). The inset location must be accurately marked in the low magnification image. We observed that the inset position in the low magnification image was missing, unclear, or incorrectly placed in approximately one third of papers. Inset positions should be clearly marked by lines or regions-of-interest in a high-contrast color, usually black or white. Insets may also be explained in the figure legend. Care must be taken when preparing figures outside vector graphics suits, as insert positions may move during file saving or export.

2. Include a clearly labeled scale bar

Scale information allows audiences to quickly understand the size of features shown in images. This is especially important for microscopic images where we have no intuitive understanding of scale. Scale information for photographs should be considered when capturing images as rulers are often placed into the frame. Our analysis revealed that 10-29% of papers screened failed to provide any scale information and another third only provided incomplete scale information (Figure 1B). Scientists should consider the following points when displaying scale bars:

- **Every image type needs a scale bar:** Authors usually add scale bars to microscope images, but often leave them out in photos and clinical images, possibly because these depict familiar objects such a human or plant. Missing scale bars, however, adversely affect reproducibility. A size difference of 20% in between a published study and the reader's lab animals, for example, could impact study results by leading to an important difference in phenotype. Providing scale bars allows scientists to detect such discrepancies and may affect their interpretation of published work. Scale bars may not be a standard feature of image acquisition and processing software for clinical images. Authors may need to contact device manufacturers to determine the image size and add height and width labels.

- **Scale bars and labels should be clearly visible:** Short scale bars, thin scale bars and scale bars in colors that are similar to the image color can easily be overlooked (Figure 4). In multicolor images, it can be difficult to find a color that makes the scale bar stand out. Authors can solve this problem by placing the scale bar outside the image or onto a box with a more suitable background color.

- **Annotate scale bar dimensions on the image:** Stating the dimensions along with the scale bar allows readers to interpret the image more quickly. Despite this, dimensions were typically stated in the legend instead (Figure 1B), possibly a legacy of printing processes that discouraged text in images. Dimensions should be in high resolution and large enough to be legible. In our set, we came across small and/or low-resolution annotations that were illegible in electronic versions of the paper, even after zooming in. Scale bars that are visible on larger figures produced by authors may be difficult to read when the size of the figure is

reduced to fit onto a journal page. Authors should carefully check page proofs to ensure that scale bars and dimensions are clearly visible.

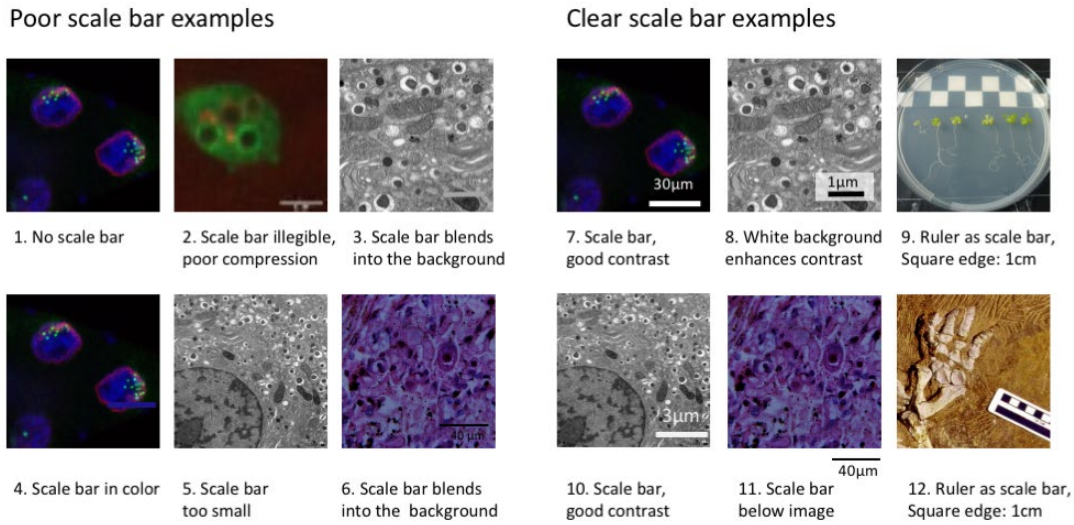


Figure 4: Using scale bars to annotate image size
Scale bars provide essential information about the size of objects, which orients readers and helps them to bridge the gap between the image and reality. Scales may be indicated by a known size indicator such as a human next to a tree, a coin next to a rock, or a tape measure next to a smaller structure. In microscope images, a bar of known length is included. Example images were created based on problems observed by reviewers.
Poor scale bar examples (1-6 bottom), clear scale bar examples (7-12). Images 1, 4, 7: Microscope images of D. melanogaster nurse cell nuclei;[18] 2. Microscope image of Dictyostelium discoideum (see Figure 7); 3, 5, 8, 10:. Electron microscope image of mouse pancreatic beta-islet cells (Andreas Müller); 6, 11: Microscope image of Lendrum-stained human lung tissue (Haraszti, Public Health Image Library); 9. Photo of Arabidopsis thaliana; 12: Photograph of fossilized Orobates pabsti.[19]

3. Use color wisely in images

Colors in images are used to display the natural appearance of an object, or to visualize features with dyes and stains. In the scientific context, adapting colors is possible and may enhance readers' understanding, while poor color schemes may distract or mislead. Images showing the natural appearance of a subject, specimen or staining technique (e.g. images showing plant size and appearance, or histopathology images of fat tissue from mice on different diets) are generally presented in color (Figure 5). Images showing electron microscope images are captured in black and white ("grayscale") by default and may be kept in grayscale to leverage the good contrast resulting from a full luminescence spectrum.

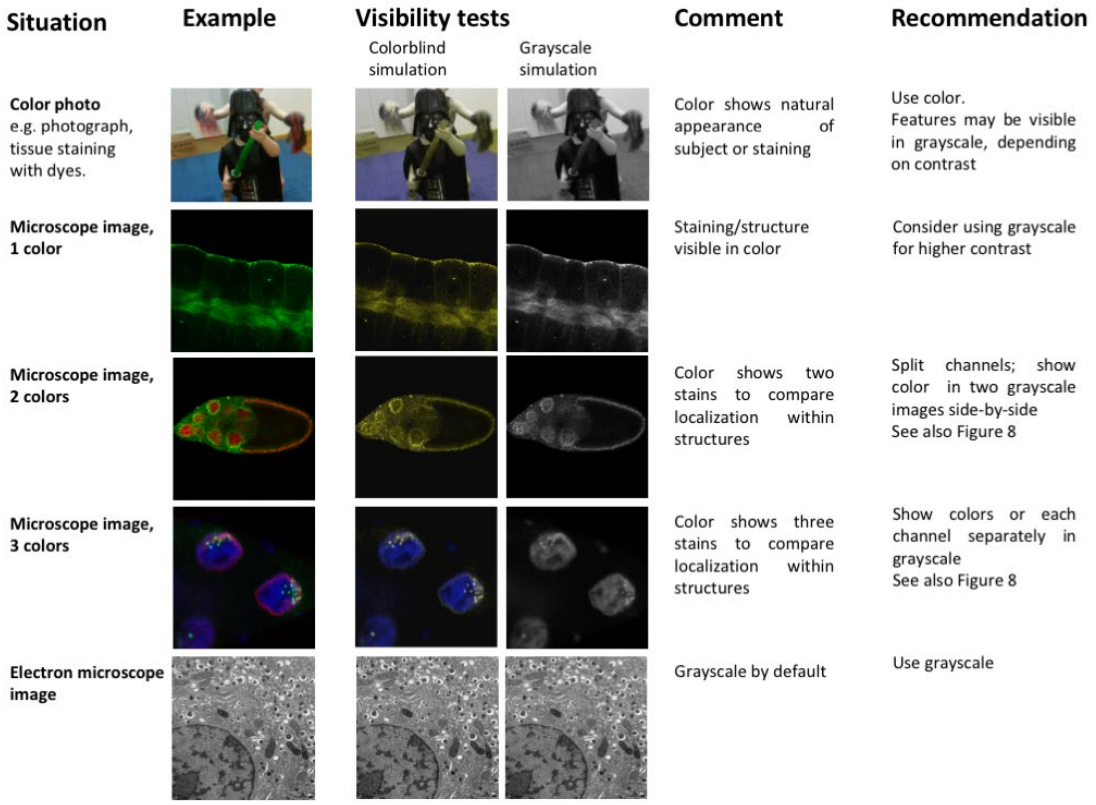


Figure 5: Image types and their accessibility in colorblind render and grayscale mode

Shown are examples of the types of images that one might find in manuscripts in the biological or biomedical sciences: photograph, fluorescent microscope images with 1-3 color hues/Look-up-tables (LUT), electron microscope images. The relative visibility is assessed in a colorblind rendering for deuteranopia, and in grayscale. Grayscale images offer the most contrast (1-color microscope image) but cannot show several structures in parallel (multicolor images, color photographs). Color combinations that are not colorblind accessible were used in rows 3 and 4 to illustrate the importance of colorblind simulation tests. Scale bars are not included in this figure, as they could not be added in a non-distracting way that would not detract from the overall message of the figure.
Images show: Row 1: Darth Vader being attacked, Row 2: D. melanogaster salivary glands,[18] Row 3: D. melanogaster egg chambers,[18] Row 4: D. melanogaster nurse cell nuclei,[18] and Row 5: mouse pancreatic beta-islet cells.

In some instances, scientists can choose whether to show grayscale or color images. Assigning colors may be optional, even though it is the default setting in imaging programs. When showing only one color channel, scientists may consider presenting this channel in grayscale to optimally display fine details. This may include variations in staining intensity or fine structures. When opting for color, authors should use grayscale visibility tests (Figure 6) to determine whether visibility is compromised. This can occur when dark colors, such as magenta, red, or blue, are shown on a black background.

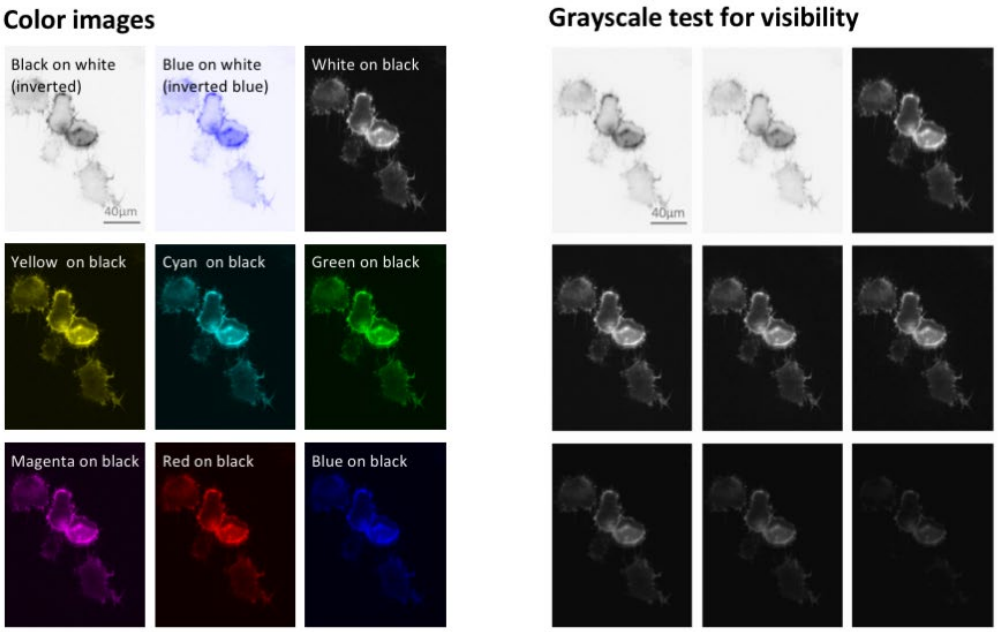


Figure 6: Visibility of colors/hues differs and depends on the background color
The best contrast is achieved with grayscale images or dark hues on a light background (first row). Dark color hues, such as red and blue, on a dark background (last row) are least visible. Visibility can be tested with mock grayscale.
Images show actin filaments in Dictyostelium discoideum (LifeAct-GFP). All images have the same scale. Abbreviations: GFP, green fluorescent protein.

4. Choose a colorblind accessible color palette: Fluorescent images with merged color channels visualize the co-localization of different markers. While many readers find these images to be visually appealing and informative, these images are often inaccessible to color blind co-authors, reviewers, editors, and readers. Deuteranopia, the most common form of colorblindness, affects up to 8% of men and 0.5% of women of northern European ancestry.[12] A study of articles published in top peripheral vascular disease journals revealed that 85% of papers with color maps and 58% of papers with heat maps used color palettes that were not colorblind safe.[20] We show that approximately half of cell biology papers, and one third of physiology papers and plant science papers contained images that were inaccessible to readers with deuteranopia. Scientists should consider the following points to ensure that images are accessible to colorblind readers.

- **Select colorblind safe colors:** Researchers should use colorblind safe color palettes for fluorescence and other images where color may be adjusted. Figure 7 illustrates how four different color combinations would look to viewers with different types of color blindness. Green and red are indistinguishable to readers with deuteranopia, whereas green and blue are indistinguishable to readers with tritanopia, a rare form of color blindness. Cyan and magenta are the best options, as these two colors look different to viewers with normal color vision, deuteranopia or tritanopia. Green and magenta are also shown, as scientists

often prefer to show colors close to the excitation value of the fluorescent dyes, which are often green and red.

- **Display separate channels in addition to the merged image:** Selecting a colorblind safe color palette becomes increasingly difficult as more colors are added. When the image includes three or more colors, authors are encouraged to show separate images for each channel, followed by the merged image (Figure 8). Individual channels may be shown in grayscale to make it easier for readers to perceive variations in staining intensity.

- **Use simulation tools to confirm that essential features are visible to colorblind viewers:** Free tools, such as Color Oracle (RRID:SCR_018400), quickly simulate different forms of color blindness by adjusting the colors on the computer screen to simulate what a colorblind person would see. Scientists using FIJI (RRID:SCR002285) can select the “Simulate colorblindness” option in the “Color” menu under “Images”.

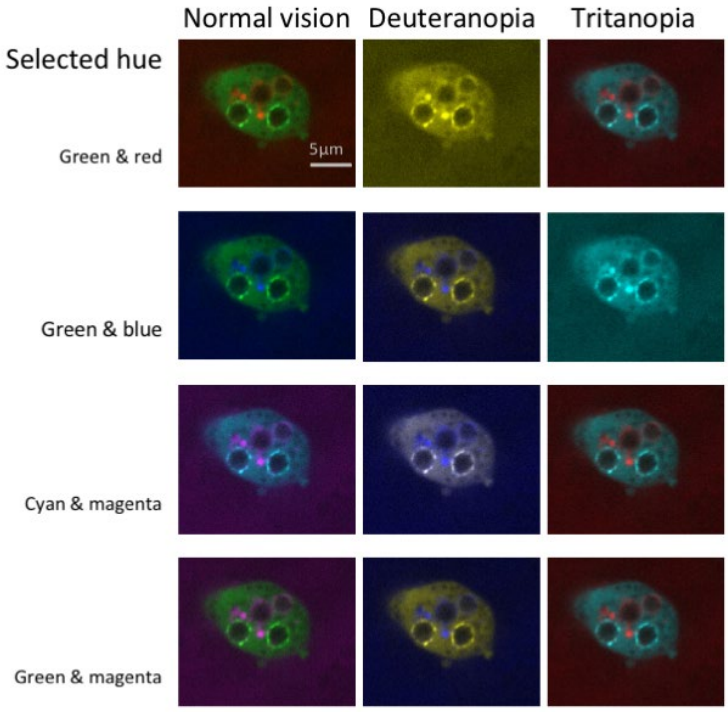


Figure 7: Color combinations as seen with normal vision and two types of colorblindness
The figure illustrates how four possible color combinations for multichannel microscope images would appear to someone with normal color vision, the most common form of colorblindness (deuteranopia), and a rare form of color blindness (tritanopia). Some combinations that are accessible to someone with deuteranopia are not accessible to readers with tritanopia, for example green/blue combinations.
Microscope images show Dictyostelium discoideum expressing Vps32-GFP (Vps32-green fluorescent protein shows broad signal in cells) and stained with dextran (spotted signal) after infection with conidia of Aspergillus fumigatus. All images have the same scale.

Abbreviations: GFP, green fluorescent protein.

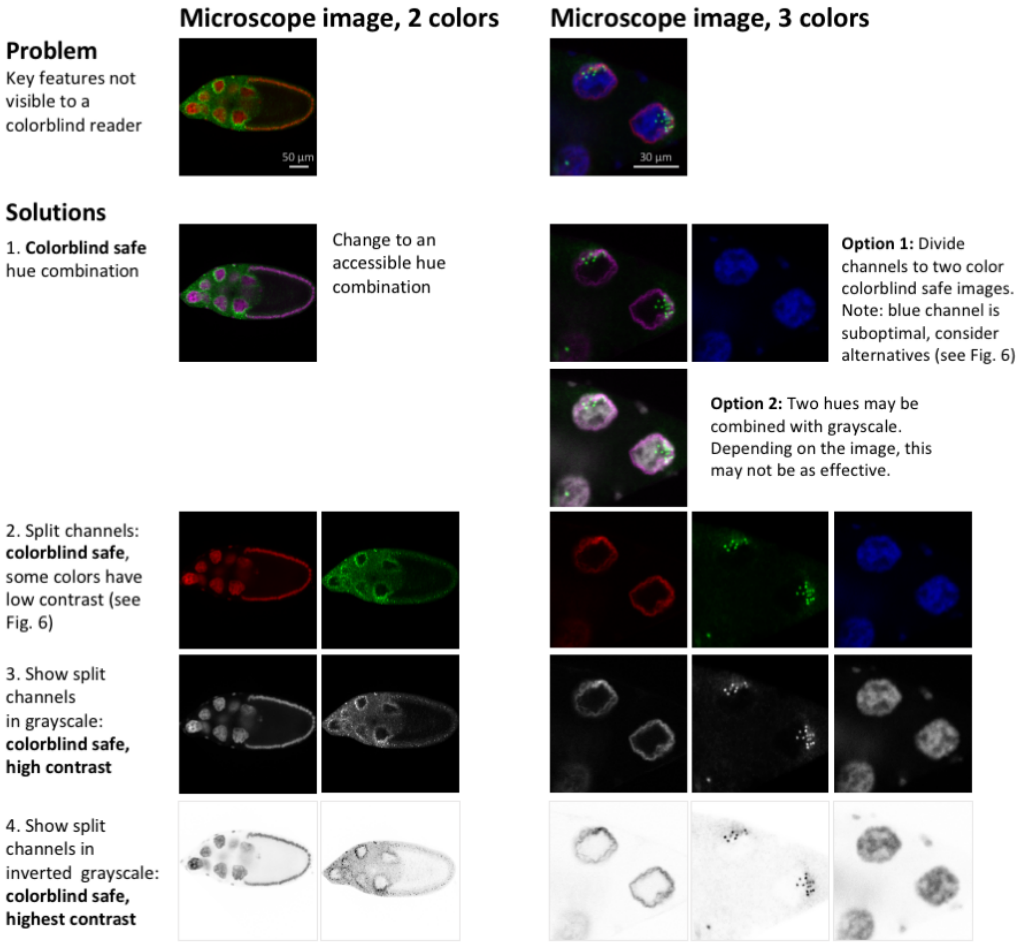


Figure 8: Strategies for making 2- or 3-channel microscope images colorblind safe
Images in the first row are not colorblind safe. Readers with the most common form of colorblindness would not be able to identify key features.
Possible accessible solutions are shown: changing colors/LUTs to colorblind friendly combinations, showing each channel in a separate image, showing colors in grayscale and inverting grayscale images to maximize contrast. Solutions 3 and 4 (show each channel in grayscale, or in inverted grayscale) are more informative than solutions 1 and 2. Regions of overlap are sometimes difficult to see in merged images without split channels. When splitting channels, scientists often use colors that have low contrast, as explained in Figure 6 (e.g. red or blue on black).
Microscope images show D. melanogaster egg chambers (2 colors) and nurse cell nuclei (3 colors).[18] *All images of egg chambers and nurse cells respectively have the same scale.*
Abbreviations: LUT, look-up table.

5. Design the figure

Figures often contain more than one panel. Careful planning is needed to convey a clear message, while ensuring that all panels fit together and follow a logical order. A planning table (Figure 9A) helps scientists to determine what information is needed to answer the

research question. The table outlines the objectives, types of visualizations required, and experimental groups that should appear in each panel. A planning table template is available on OSF.[14] After completing the planning table, scientists should sketch out the position of panels, and the position of images, graphs, and titles within each panel (Figure 9B). Audiences read a page either from top to bottom and/or from left to right. Selecting one reading direction and arranging panels in rows or columns helps with figure planning. Using enough white space to separate rows or columns will visually guide the reader through the figure. The authors can then assemble the figure based on the draft sketch.

A Organize and plan figures with a "**Figure planning table**"

Example for a study of mouse placenta genetics and test of a treatment.

Panel	Panel objective	Visualizations	Experimental groups	Notes
A	Illustrate differences in pup phenotype	Photograph, chart	1. Control group + placebo 2. Animal model + placebo 3. Control group + treatment 4. Animal model + treatment	Photo with scale (ruler for pups) Box plot: fetal weight
B	Illustrate differences in placenta phenotype	Photograph, chart	see above	Photo with scale (ruler) Box plot: placental weight
C	Illustrate histological differences in placenta e.g. staining for two biomarkers	Micrograph	see above	One image per group; separate rows for each biomarker

B Organize panels into **"Figure layout sketch"**, exemplary for Figure planning table in A

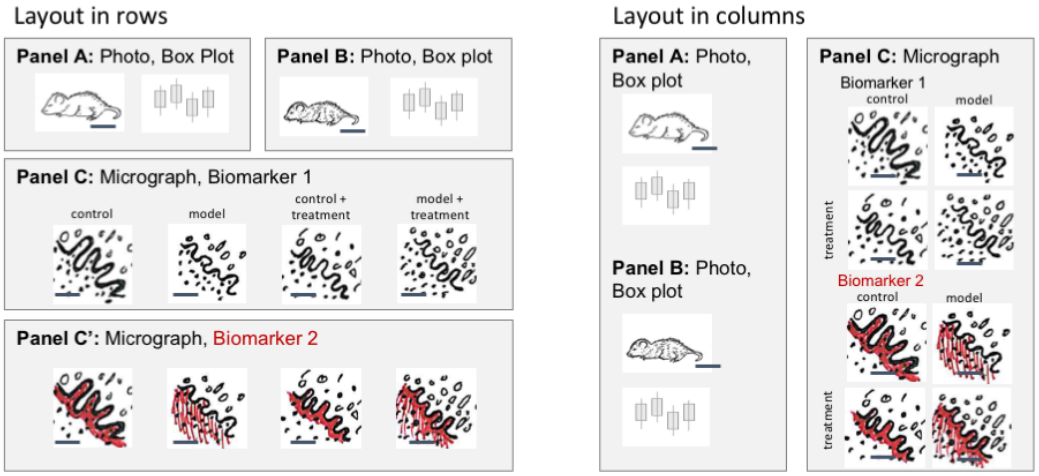


Figure 9: Planning multipanel figures

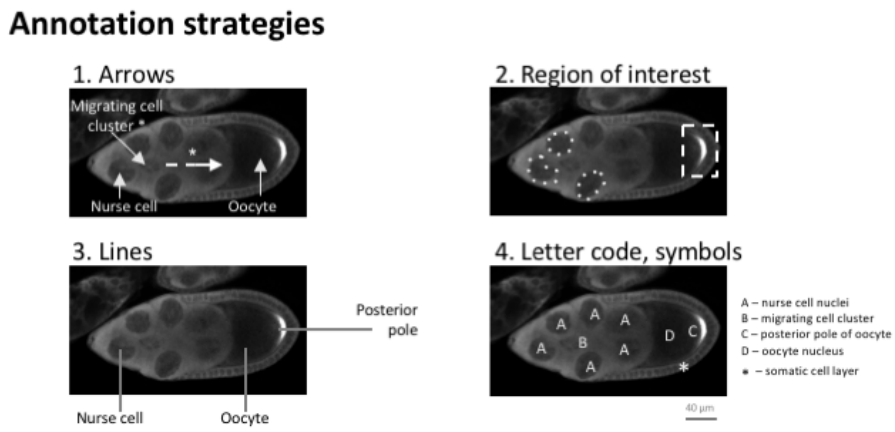
Planning tables and layout sketches are useful tools to efficiently design figures that address the research question.

A. *Planning tables allow scientists to select and organize elements needed to answer the research question addressed by the figure.*

B. *Layout sketches allow scientists to design a logical layout for all panels listed in the planning table and ensure that there is adequate space for all images and graphs.*

6. Annotate the figure

Annotations with text, symbols or lines allow readers from many different backgrounds to rapidly see essential features, interpret images, and gain insight. Unfortunately, scientists often design figures for themselves, rather than their audience.[7] Examples of annotations are shown in Figure 10. Table 1 describes important factors to consider for each annotation type.



	Use	Caution	Tips
1. Arrows	Points to structure May indicate direction of movement (e.g. *)	Do not concurrently use arrows for pointing to structure and indicating movement (example *) Arrowheads alone often have no clear direction	Avoid crossing arrows Align arrows
2. Region of interest	Delineates entire structure	May obstruct image features (especially when fill color is used)	Careful when saving: dashed lines may be too thin
3. Lines	Direct labeling of structure at line end	Label may be outside of image to not obstruct image features	Avoid crossing lines Align lines
4. Letter code, symbol	Labels many features clearly where lines and arrows would confuse	Legend is critical, requires large space. Labels may obscure image features	Choose suitable font e.g. sans serif

Figure 10: Using arrows, regions of interest, lines and letter codes to annotate structures in images

Text descriptions alone are often insufficient to clearly point to a structure or region in an image. Arrows and arrowheads, lines, letters, and dashed enclosures can help if overlaid on the respective part of the image.

Microscope images show D. melanogaster egg chambers,[18] with the different labelling techniques in use. The table provides an overview of their applicability and common pitfalls. All images have the same scale.

Table 1: Use annotations to make figures accessible to a broad audience

Feature to be Explained	Annotation
Size	Scale bar with dimensions
Direction of movement	Arrow with tail
Draw attention to:	
• Points of interest	Symbol (arrowhead, star, etc.)
• Regions of interest: Black & white image	Highlight in color if this does not obscure important features within the region OR Outline with boxes or circles
• Regions of interest: Color image	Outline with boxes or circles
• Layers	Labeled brackets beside the image for layers that are visually identifiable across the entire image OR A line on the image for wavy layers that may be difficult to identify
Define features within an image	Labels

When adding annotations to an image, scientists should consider the following steps.

- **Choose the right amount of labeling.** Figure 11 shows three levels of annotation. The barely annotated image (11A) is only accessible to scientists already familiar with the object and technique, whereas the heavily annotated version (11C) contains numerous annotations that obstruct the image and a legend that is time consuming to interpret. Panel 11B is more readable; annotations of a few key features are shown, and the explanations appear right below the image for easy interpretation. Explanations of labels are often placed in the figure legend. Alternating between examining the figure and legend is time consuming, especially when the legend and figure are on different pages. Figure 11D shows one option for situations where extensive annotations are required to explain a complex image. An annotated image is placed as a legend next to the original image. A semi-transparent white layer mutes the image to allow annotations to stand out.

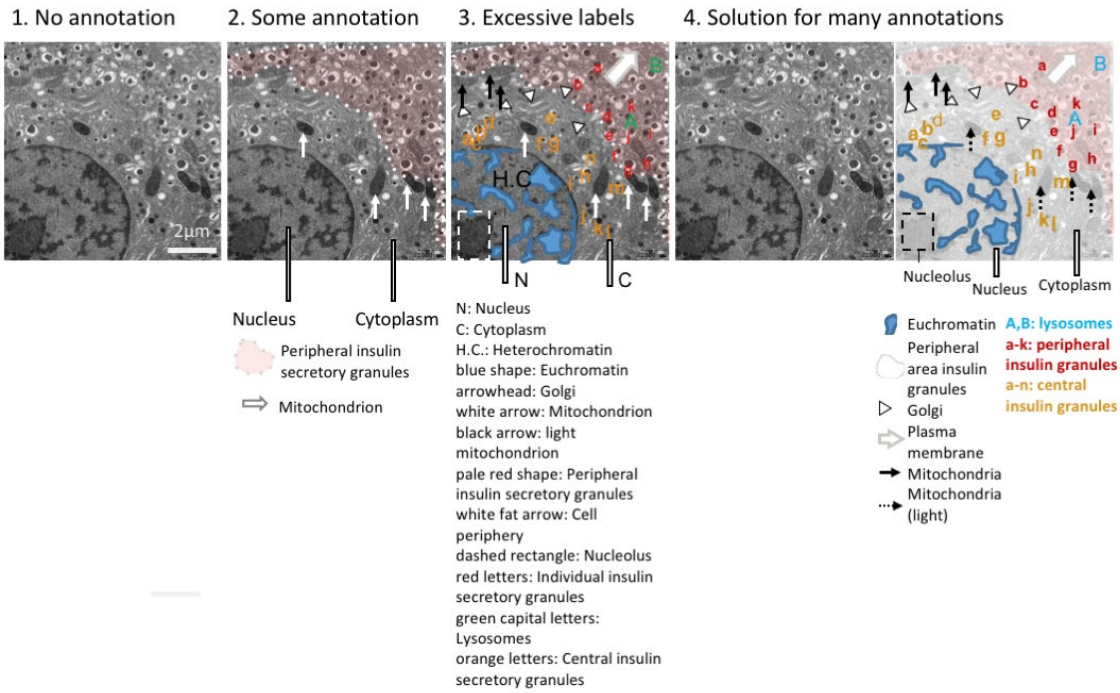


Figure 11: Different levels of detail for image annotations
Annotations help to orient the audience but may also obstruct parts of the image. Authors must find the right balance between too few and too many annotations. 1. Example with no annotations. Readers cannot determine what is shown. 2. Example with a few annotations to orient readers to key structures. 3. Example with many annotations, which obstruct parts of the image. The long legend below the figure is confusing. 4. Example shows a solution for situations where many annotations are needed to explain the image. An annotated version is placed next to an unannotated version of the image for comparison. The legend below the image helps readers to interpret the image, without having to refer to the figure legend. Note the different requirements for space. Electron microscope images show mouse pancreatic beta-islet cells.

- **Use abbreviations cautiously:** Abbreviations are commonly used for image and figure annotation to save space, but inevitably require more effort from the reader. Abbreviations are often ambiguous, especially across fields. Authors should run a web search for the abbreviation.[21] If the intended meaning is not a top result, authors should refrain from using the abbreviation or clearly define the abbreviation on the figure itself, even if it is already defined elsewhere in the manuscript. Note that in Figure 11, abbreviations have been written out below the image to reduce the number of legend entries.

- **Explain colors and stains:** Explanations of colors and stains were missing in around 20% of papers. Figure 12 illustrates several problematic practices observed in our dataset, as well as solutions for clearly explaining what each color represents. This figure uses fluorescence images as an example; however we also observed many histology images in which authors did not mention which stain was used. Authors should describe how stains affect the tissue shown or

use annotations to show staining patterns of specific structures. This allows readers who are unfamiliar with the stain to interpret the image.

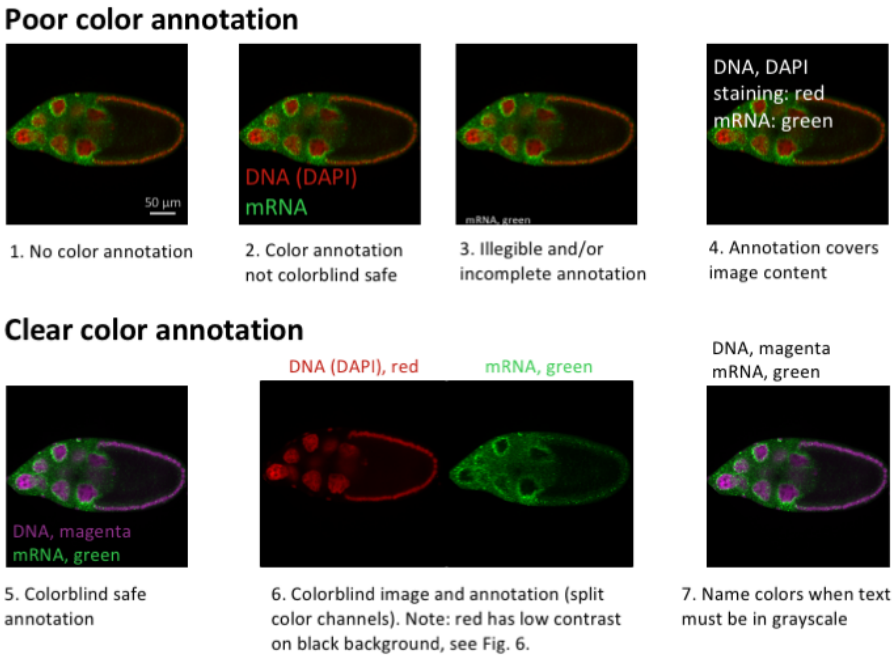


Figure 12: Explain color in images
Cells and their structures are almost all transparent. Every dye, stain, and fluorescent label therefore should be clearly explained to the audience. Labels should be colorblind safe. Large labels that stand out against the background are easy to read. Authors can make figures easier to interpret by placing the color label close to the structure; color labels should only be placed in the figure legend when this is not possible. Example images were created based on problems observed by reviewers.
Microscope images show D. melanogaster egg chambers stained with the DNA dye DAPI (4',6-diamidino-2-phenylindole) and probe for a specific mRNA species.[18] All images have the same scale.

- **Ensure that annotations are accessible to colorblind readers:** Confirming that labels or annotations are visible to colorblind readers is important for both color and grayscale images (Figure 13). Up to one third of papers in our dataset contained annotations or labels that would not have been visible to someone with deuteranopia. This occurred because the annotations blended in with the background (e.g. red arrows on green plants) or the authors use the same symbol in colors that are indistinguishable to someone with deuteranopia to mark different features. Figure 13 illustrates how to annotate a grayscale image so that it is accessible to color blind readers. Using text to describe colors is also problematic for colorblind readers. This problem can be alleviated by using colored symbols in the legend or by using distinctly shaped annotations such as open vs. closed arrows, thin vs. wide lines, or dashed vs. solid lines. Color blindness simulators help in determining whether annotations are accessible to all readers.

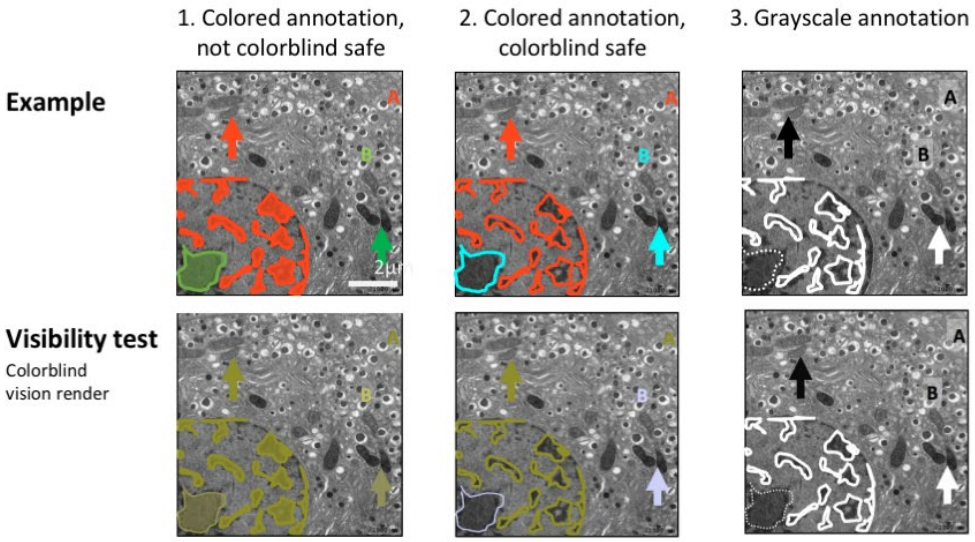


Figure 13: Annotations should be colorblind safe
1. The annotations displayed in the first image are inaccessible to colorblind individuals, as shown with the visibility test below. This example was created based on problems observed by reviewers. 2-3. Two colorblind safe alternative annotations, in color (2) and in grayscale (3). The bottom row shows a test rendering for deuteranopia colorblindness. Note that double-encoding of different hues and different shapes (e.g. different letters, arrow shapes, or dashed/non-dashed lines) allows all audiences to interpret the annotations.
Electron microscope images show mouse pancreatic beta-cell islet cells. All images have the same scale.

7. Prepare figure legends

Each figure and legend are meant to be self-explanatory and should allow readers to quickly assess a paper or understand complex studies that combine different methodologies or model systems. To date, there are no guidelines for figure legends for images, as the scope and length of legends varies across journals and disciplines. Some journals require legends to include details on object, size, methodology or sample size, while other journals require a minimalist approach and mandate that information should not be repeated in subsequent figure legends.

Our data suggest that important information needed to interpret images was regularly missing from the figure or figure legend. This includes the species and tissue type, or object shown in the figure, clear explanations of all labels, annotations and colors, and markings or legend entries denoting insets. Presenting this information on the figure itself is more efficient for the reader, however any details that are not marked in the figure should be explained in the legend.

While not reporting species and tissue information in every figure legend may be less of an issue for papers that examine a single species and tissue, this is a major problem when a study includes many species and tissues, which may be presented in different

panels of the same figure. Additionally, the scientific community is increasingly developing automated data mining tools, such as the Source Data tool, to collect and synthesize information from figures and other parts of scientific papers. Unlike humans, these tools cannot piece together information scattered throughout the paper to determine what might be shown in a particular figure panel. Even for human readers, this process wastes time. Therefore, we recommend that authors present information in a clear and accessible manner, even if some information may be repeated for studies with simple designs.

Discussion

A flood of images is published every day in scientific journals and the number is continuously increasing. Of these, around 4% likely contain intentionally or accidentally duplicated images.[3] Our data show that, in addition, most papers show images that are not fully interpretable due to issues with scale markings, annotation, and/or color. This affects scientists' ability to interpret, critique and build upon the work of others. Images are also increasingly submitted to image archives to make image data widely accessible and permit future re-analyses. A substantial fraction of images that are neither human nor machine-readable lowers the potential impact of such archives. Based on our data examining common problems with published images, we provide a few simple recommendations, with examples illustrating good practices. We hope that these recommendations will help authors to make their published images legible and interpretable.

Limitations: While most results were consistent across the three subfields of biology, findings may not be generalizable to other fields. Our sample included the top 15 journals that publish original research for each field. Almost all journals were indexed in PubMed. Results may not be generalizable to journals that are un-indexed, have low impact factors, or are not published in English. Data abstraction was performed manually due to the complexity of the assessments. Error rates were 5% for plant sciences, 4% for physiology and 3% for cell biology. Our assessments focused on factors that affect readability of image-based figures in scientific publications. Future studies may include assessments of raw images and meta-data to examine factors that affect reproducibility, such as contrast settings, background filtering and processing history.

Actions journals can take to make image-based figures more transparent and easier to interpret

The role of journals in improving the quality of reporting and accessibility of image-based figures should not be overlooked. There are several actions that journals might consider.

- **Screen manuscripts for figures that are not colorblind safe:** Open source automated screening tools[22] may help journals to efficiently identify common color maps that are not colorblind safe.
- **Update journal policies:** We encourage journal editors to update policies regarding colorblind accessibility, scale bars, and other factors outlined in this manuscript. Importantly, policy changes should be accompanied by clear plans for implementation and enforcement. Meta-research suggests that changing journal policy, without enforcement or implementation plans, has limited effects on author behavior. Amending journal policies to require authors to report RRIDs,

for example, increases the number of papers reporting RRIDs by 1%.[23] In a study of life sciences articles published in Nature journals, the percentage of animal studies reporting the Landis 4 criteria (blinding, randomization, sample size calculation, exclusions) increased from 0 to 16.4% after new guidelines were released.[24] In contrast, a randomized controlled trial of animal studies submitted to PLoS One demonstrated that randomizing authors to complete the ARRIVE checklist during submission did not improve reporting.[25] Some improvements in reporting of confidence intervals, sample size justification and inclusion and exclusion criteria were noted after Psychological Science introduced new policies,[26] although this may have been partially due to widespread changes in the field. A joint editorial series published in the Journal of Physiology and British Journal of Pharmacology did not improve the quality of data presentation or statistical reporting.[27]

- **Re-evaluate limits on the number of figures:** Limitations on the number of figures originally stemmed from printing costs calculations, which are becoming increasingly irrelevant as scientific publishing moves online. Unintended consequences of these policies include the advent of large, multipanel figures. These figures are often especially difficult to interpret because the legend appears on a different page, or the figure combines images addressing different research questions.
- **Reduce or eliminate page charges for color figures:** As journals move online, policies designed to offset the increased cost of color printing are no longer needed. The added costs may incentivize authors to use grayscale in cases where color would be beneficial.
- **Encourage authors to explain labels or annotations in the figure, rather than in the legend:** This is more efficient for readers.
- **Encourage authors to share image data in public repositories:** Open data benefits authors and the scientific community.[28-30]

How can the scientific community improve image-based figures?

The role of scientists in the community is multi-faceted. As authors, scientists should familiarize themselves with guidelines and recommendations, such as ours provided above. As reviewers, scientists should ask authors to improve erroneous or uninformative image-based figures. As instructors, scientists should ensure that bioimaging and image data handling is taught during undergraduate or graduate courses, and support existing initiatives such as NEUBIAS[31] (Network of European Bioimage Analysts) that aim to increase training opportunities in bioimage analysis. Scientists are also innovators. As such they should support emerging image data archives, which may expand to automatically source images from published figures. Repositories for other types of data are already widespread, however the idea of image repositories has only recently gained traction.[32] Existing image databases, which are mainly used for raw image data and meta-data, include the Allen Brain Atlas, the Image Data Resource[33] and the emerging BioImage Archives.[32] Springer Nature encourages authors to submit imaging data to the Image Data Resource.[33] While scientists have called for common quality standards for archived images and meta-data,[32] such standards have not been defined, implemented, or taught. Examining standard practices for reporting images in scientific publications, as outlined here, is one strategy for establishing common quality standards.

In the future, it is possible that each image published electronically in a journal or submitted to an image data repository will follow good practice guidelines, and will be accompanied by expanded “meta-data” or “alt-text/attribute” files. Alt-text is already published in html to provide context if an image cannot be accessed (e.g. by blind readers). Similarly, images in online articles and deposited in archives could contain essential information in a standardized format. The information could include the main objective of the figure, specimen information, ideally with research resource identifier[34] (RRID), specimen manipulation (dissection, staining, RRID for dyes and antibodies used), as well as the imaging method including essential items from meta-files of the microscope software, information about image processing and adjustments, information about scale, annotations, insets, and colors shown, and confirmation that the images are truly representative.

Conclusions

Our meta-research study of standard practices for presenting images in three fields highlights current shortcomings in publications. Pubmed indexes approximately 800,000 new papers per year, or 2,200 papers per day (https://www.nlm.nih.gov/bsd/index_stats_comp.html). Twenty-three percent,[1] or approximately 500 papers per day, contain images. Our survey data suggest that most of these papers will have deficiencies in image presentation, which may affect legibility and interpretability. These observations lead to targeted recommendations for improving the quality of published images. Our recommendations are available as a slide set via the Open Science Framework and can be used in teaching best practice and avoid misleading or uninformative image-based figures. Our analysis underscores the need for standardized image publishing guidelines. Adherence to such guidelines will allow the scientific community to unlock the full potential of image collections in the life sciences for current and future generations of researchers.

Methods

Systematic review: We examined original research articles that were published in April of 2018 in the top 15 journals that publish original research for each of three different categories (physiology, plant science, cell biology). Journals for each category were ranked according to 2016 impact factors listed for the specified categories in Journal Citation Reports. Journals that only publish review articles or that did not publish an April issue were excluded. We followed all relevant aspects of the PRISMA guidelines.[35] Items that only apply to meta-analyses or are not relevant to literature surveys were not followed. Ethical approval was not required.

Search strategy: Articles were identified through a PubMed search, as all journals were PubMed indexed. Electronic search results were verified by comparison with the list of articles published in April issues on the journal website. The electronic search used the following terms:

Physiology: ("Journal of pineal research"[Journal] AND 3[Issue] AND 64[Volume]) OR ("Acta physiologica (Oxford, England)"[Journal] AND 222[Volume] AND 4[Issue]) OR ("The Journal of physiology"[Journal] AND 596[Volume] AND (7[Issue] OR 8[Issue])) OR (("American journal of physiology. Lung cellular and molecular physiology"[Journal] OR "American journal of physiology. Endocrinology and metabolism"[Journal] OR "American journal of physiology. Renal physiology"[Journal] OR "American journal of physiology. Cell physiology"[Journal] OR "American journal of physiology. Gastrointestinal and liver physiology"[Journal]) AND 314[Volume] AND 4[Issue]) OR ("American journal of physiology. Heart and circulatory physiology"[Journal] AND 314[Volume] AND 4[Issue]) OR ("The Journal of general physiology"[Journal] AND 150[Volume] AND 4[Issue]) OR ("Journal of cellular physiology"[Journal] AND 233[Volume] AND 4[Issue]) OR ("Journal of biological rhythms"[Journal] AND 33[Volume] AND 2[Issue]) OR ("Journal of applied physiology (Bethesda, Md. : 1985)"[Journal] AND 124[Volume] AND 4[Issue]) OR ("Frontiers in physiology"[Journal] AND ("2018/04/01"[Date - Publication] : "2018/04/30"[Date - Publication])) OR ("The international journal of behavioral nutrition and physical activity"[Journal] AND ("2018/04/01"[Date - Publication] : "2018/04/30"[Date - Publication]))

Plant science: ("Nature plants"[Journal] AND 4[Issue] AND 4[Volume]) OR ("Molecular plant"[Journal] AND 4[Issue] AND 11[Volume]) OR ("The Plant cell"[Journal] AND 4[Issue] AND 30[Volume]) OR ("Plant biotechnology journal"[Journal] AND 4[Issue] AND 16[Volume]) OR ("The New phytologist"[Journal] AND (1[Issue] OR 2[Issue]) AND 218[Volume]) OR ("Plant physiology"[Journal] AND 4[Issue] AND 176[Volume]) OR ("Plant, cell & environment"[Journal] AND 4[Issue] AND 41[Volume]) OR ("The Plant journal : for cell and molecular biology"[Journal] AND (1[Issue] OR 2[Issue]) AND 94[Volume]) OR ("Journal of experimental botany"[Journal] AND (8[Issue] OR 9[Issue] OR 10[Issue]) AND 69[Volume]) OR ("Plant & cell physiology"[Journal] AND 4[Issue] AND 59[Volume]) OR ("Molecular plant pathology"[Journal] AND 4[Issue] AND 19[Volume]) OR ("Environmental and experimental botany"[Journal] AND 148[Volume]) OR ("Molecular plant-microbe interactions : MPMI"[Journal] AND 4[Issue] AND 31[Volume]) OR ("Frontiers in plant science"[Journal] AND ("2018/04/01"[Date - Publication] : "2018/04/30"[Date - Publication])) OR ("The Journal of ecology" ("2018/04/01"[Date - Publication] : "2018/04/30"[Date - Publication]))

Cell biology: ("Cell"[Journal] AND (2[Issue] OR 3[Issue]) AND 173[Volume]) OR ("Nature medicine"[Journal] AND 24[Volume] AND 4[Issue]) OR ("Cancer cell"[Journal] AND 33[Volume] AND 4[Issue]) OR ("Cell stem cell"[Journal] AND 22[Volume] AND 4[Issue]) OR ("Nature cell biology"[Journal] AND 20[Volume] AND 4[Issue]) OR ("Cell metabolism"[Journal] AND 27[Volume] AND 4[Issue]) OR ("Science translational medicine"[Journal] AND 10[Volume] AND (435[Issue] OR 436[Issue] OR 437[Issue] OR 438[Issue])) OR ("Cell research"[Journal] AND 28[Volume] AND 4[Issue]) OR ("Molecular cell"[Journal] AND 70[Volume] AND (1[Issue] OR 2[Issue])) OR("Nature structural & molecular biology"[Journal] AND 25[Volume] AND 4[Issue]) OR ("The EMBO journal"[Journal] AND 37[Volume] AND (7[Issue] OR 8[Issue])) OR ("Genes & development"[Journal] AND 32[Volume] AND 7-8[Issue]) OR ("Developmental cell"[Journal] AND 45[Volume] AND (1[Issue] OR 2[Issue])) OR ("Current biology : CB"[Journal] AND 28[Volume] AND (7[Issue] OR 8[Issue])) OR ("Plant cell"[Journal] AND 30[Volume] AND 4[Issue])

Screening: Screening for each article was performed by two independent reviewers (Physiology: TLW, SS, EMW, VI, KW, MO; Plant science: TLW, SJB; Cell biology: EW, SS) using Rayyan software (RRID:SCR_017584), and disagreements were resolved by consensus. A list of articles is uploaded into Rayyan. Reviewers independently examined each article and marked whether the article was included or excluded, along with the reason for exclusion. Both reviewers screened all articles published in each journal between April 1 and April 30, 2018 to identify full length, original research articles (Table S1, Table S2, Table S3, Figure S1) published in the print issue of the journal. Articles for online journals that do not publish print issues were included if the publication date was between April 1 and April 30, 2018. Articles were excluded if they were not original research articles, or if an accepted version of the paper was posted as an “in press” or “early release” publication; however, the final version did not appear in the print version of the April issue. Articles were included if they contained at least one eligible image, such as a photograph, an image created using a microscope or electron microscope, or an image created using a clinical imaging technology such as ultrasound or MRI. Blot images were excluded, as many of the criteria in our abstraction protocol cannot easily be applied to blots. Computer generated images, graphs and data figures were also excluded. Papers that did not contain any eligible images were excluded.

Abstraction: All abstractors completed a training set of 25 articles before abstracting data. Data abstraction for each article was performed by two independent reviewers (Physiology: AA, AV; Plant science: MO, TLA, SA, KW, MAG, IF; Cell biology: IF, AA, AV, KW, MAG). When disagreements could not be resolved by consensus between the two reviewers, ratings were assigned after a group review of the paper. Eligible manuscripts were reviewed in detail to evaluate the following questions according to a predefined protocol (available at: https://osf.io/b5296/).[14] Supplemental files were not examined, as supplemental images may not be held to the same peer review standards as those in the manuscript.

The following items were abstracted:

1. Types of images included in the paper (photograph, microscope image, electron microscope image, image created using a clinical imaging technique such as ultrasound or MRI, other types of images)
2. Did the paper contain appropriately labeled scale bars for all images?
3. Were all insets clearly and accurately marked?
4. Were all insets clearly explained in the legend?

5. Is the species and tissue, object, or cell line name clearly specified in the figure or legend for all images in the paper?
6. Are any annotations, arrows or labels clearly explained for all images in the paper?
7. Among images where authors can control the colors shown (e.g. fluorescence microscopy), are key features of the images visible to someone with the most common form of colorblindness (deuteranopia)?
8. If the paper contains colored labels, are these labels visible to someone with the most common form of color blindness (deuteranopia)?
9. Are colors in images explained either on the image or within the legend?

Questions 7 and 8 were assessed by using Color Oracle[36] (RRID:SCR_018400) to simulate the effects of deuteranopia.

Verification: Ten percent of articles in each field were randomly selected for verification abstraction, to ensure that abstractors in different fields were following similar procedures. Data were abstracted by a single abstractor (TLW). The question on species and tissue was excluded from verification abstraction for articles in cell biology and plant sciences, as the verification abstractor lacked the field-specific expertise needed to assess this question. Results from the verification abstractor were compared with consensus results from the two independent abstractors for each paper and discrepancies were resolved through discussion. Error rates were calculated as the percentage of responses for which the abstractors' response was incorrect. Error rates were 5% for plant sciences, 4% for physiology and 3% for cell biology.

Data processing and creation of figures: Data are presented as n (%). Summary statistics were calculated using Python (RRID:SCR_008394, version 3.6.9, libraries NumPy 1.18.5 and Matplotlib 3.2.2). Charts were prepared with a Python-based Jupyter Notebook (Jupyter-client, RRID:SCR_018413,[37] Python version 3.6.9, RRID:SCR_008394, libraries NumPy 1.18.5[38] and Matplotlib 3.2.2[39]) and assembled into figures with vector graphic software. Example images were previously published or generously donated by the manuscript authors as indicated in the figure legends. Image acquisition was described in references (*D.melagenoster* images[18], mouse pancreatic beta islet cells: A. Müller personal communication, and Orobates Pabsti[19]). Images were cropped, labeled, and color-adjusted with FIJI[15] (RRID:SCR_002285) and assembled with vector-graphic software. Color-blind and grayscale rendering of images was done using Color Oracle[36] (RRID:SCR_018400). All poor and clear images presented here are 'mock examples' prepared based on practices observed during data abstraction.

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Supplemental Tables

Table S1: Number of articles examined by journal in physiology

Journal	Articles Screened (n = 431)	Original Research Articles (n = 312, 72%)	Included Articles (n = 172, 40%)
Journal of Pineal Research	7	6 (86%)	5 (71%)
Acta Physiologica	21	10 (48%)	5 (24%)
Journal of Physiology	39	22 (56%)	12 (31%)
International Journal of Behavioral Nutrition and Physical Activity	9	9 (100%)	0
AJP: Lung, Cellular and Molecular Physiology	15	12 (80%)	6 (40%)
Journal of General Physiology	10	4 (40%)	3 (30%)
AJP: Endocrinology and Metabolism	9	8 (89%)	6 (67%)
Frontiers in Physiology	142	107 (75%)	47 (33%)
Journal of Cellular Physiology	88	55 (63%)	47 (53%)
AJP: Renal Physiology	15	15 (100%)	10 (67%)
AJP: Cell Physiology	11	11 (100%)	9 (82%)
Journal of Biological Rhythms	9	8 (89%)	2 (22%)
AJP: Gastrointestinal and Liver Physiology	6	6 (100%)	5 (83%)
Journal of Applied Physiology	31	31 (100%)	10 (32%)
AJP: Heart and Circulatory Physiology	19	8 (42%)	5 (26%)

Values are n, or n (% of all articles). Screening was performed to exclude articles that were not full-length original research articles (e.g. reviews, editorials, perspectives, commentaries, letters to the editor, short communications, etc.), were not published in April 2018, or did not include eligible images.

Abbreviations: AJP, American Journal of Physiology

Table S2: Number of articles examined by journal in plant science

Journal	Articles Screened (n = 502)	Original Research Articles (n = 377, 75%)	Included Articles (n = 257, 51%)
Nature Plants	13	3 (23%)	0
Molecular Plant	14	7 (50%)	6 (43%)
Plant Cell *	15	9 (60%)	8 (53%)
Plant Biotechnology Journal	12	10 (83%)	6 (50%)
New Phytologist	73	53 (73%)	31 (42%)
Plant Physiology	39	34 (87%)	27 (69%)
Plant Cell and Environment	14	11 (79%)	7 (50%)
Plant Journal	31	24 (77%)	19 (61%)
Journal of Experimental Botany	74	55 (74%)	41 (55%)
Journal of Ecology **	0		
Plant and Cell Physiology	21	13 (62%)	9 (43%)
Molecular Plant Pathology	21	16 (76%)	15 (71%)
Environmental and Experimental Botany	17	17 (100%)	12 (71%)
Molecular Plant – Microbiome Interactions	8	7 (88%)	4 (50%)
Frontiers in Plant Science	150	118 (79%)	72 (48%)

* This journal was also included on the cell biology list (Table S3).

** No articles from the Journal of Ecology were screened as the journal did not publish an April 2018 issue.

Values are n, or n (% of all articles). Screening was performed to exclude articles that were not full-length original research articles (e.g. reviews, editorials, perspectives, commentaries, letters to the editor, short communications, etc.), were not published in April 2018, or did not include eligible images.

Table S3: Number of articles examined by journal in cell biology

Journal	Articles Screened (n = 409)	Original Research Articles (n = 222, 54%)	Included Articles (n = 159, 39%)
Cell	50	33 (76%)	19 (38%)
Nature medicine	32	10 (31%)	6 (19%)
Cancer Cell	21	12 (57%)	5 (24%)
Cell Stem Cell	18	7 (39%)	5 (28%)
Nature Cell Biology	20	9 (45%)	9 (45%)
Cell Metabolism	20	9 (45%)	8 (40%)
Science Translational Medicine	25	18 (72%)	17 (68%)
Cell Research	13	6 (46%)	5 (38%)
Molecular Cell	38	26 (68%)	13 (34%)
Nature Structural and Molecular Biology	12	7 (58%)	2 (17%)
EMBO Journal	23	17 (74%)	16 (70%)
Genes and Development	13	8 (62%)	5 (38%)
Developmental Cell	22	15 (68%)	15 (68%)
Current Biology	87	36 (41%)	26 (30%)
Plant Cell *	15	9 (60%)	8 (53%)

* This journal was also included on the plant science list (Table S2).

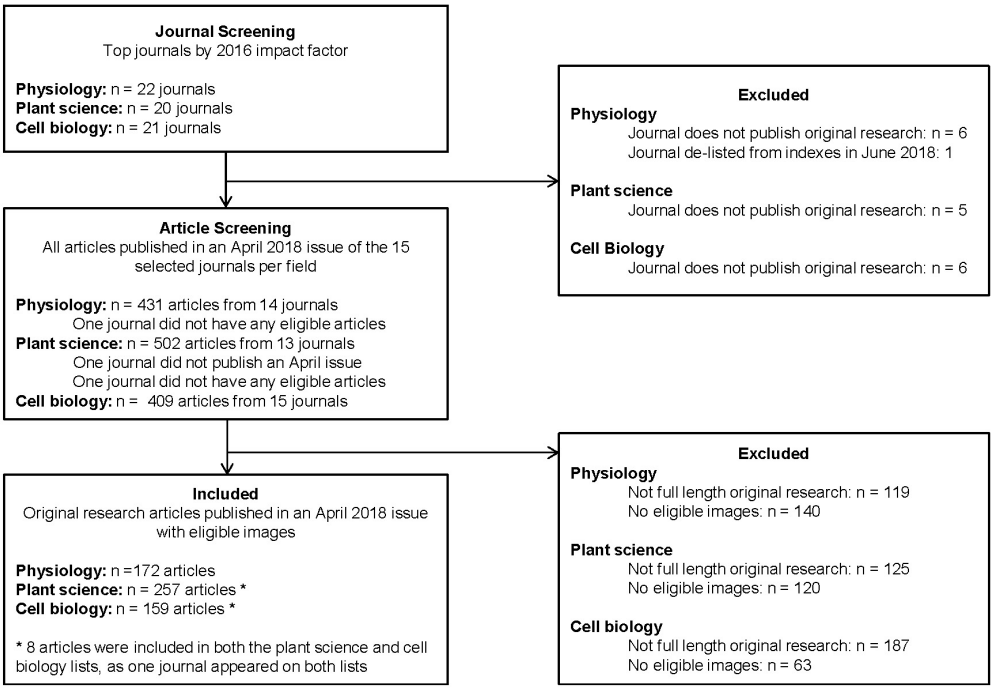
Values are n, or n (% of all articles). Screening was performed to exclude articles that were not full length original research articles (e.g. reviews, editorials, perspectives, commentaries, letters to the editor, short communications, etc.), were not published in April 2018, or did not include eligible images.

Table S4: Scale information in papers

Field	No scale information in any figure	Some scale information				Complete scale information	
		Some figures, magnification in legend	All figures, magnification in legend	Some figures, scale bar with dimensions in legend	Some figures, scale bar with dimensions	All figures, scale bar with dimensions in legend	All figures, scale bar with dimensions
Physiology	24.4	5.2	1.7	10.5	9.3	26.7	22.1
Cell biology	10.1	0.0	1.3	22.0	11.9	40.9	13.8
Plant science	29.2	0.4	0.4	31.5	10.5	23.3	4.7

Values are % of papers.

Figure S1: Flow chart of study screening and selection process



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