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Community-developed checklists for publishing images and image analyses

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Abstract

Images document scientific discoveries and are prevalent in modern biomedical research. Microscopy imaging in particular is currently undergoing rapid technological advancements. However for scientists wishing to publish the obtained images and image analyses results, there are to date no unified guidelines. Consequently, microscopy images and image data in publications may be unclear or difficult to interpret. Here we present community-developed checklists for preparing light microscopy images and image analyses for publications. These checklists offer authors, readers, and publishers key recommendations for image formatting and annotation, color selection, data availability, and for reporting image analysis workflows. The goal of our guidelines is to increase the clarity and reproducibility of image figures and thereby heighten the quality and explanatory power of microscopy data in publications.

Introduction

Images and their analyses are widespread in life science and medicine. Microscopy imaging is a dynamic area of technology development, both in terms of hardware and software. This is especially true in the area of light microscopy with great recent improvements in sensitivity, and spatial and temporal collection. Resources developed by scientists help researchers to navigate designing microscopy experiments and obtaining image data [1–3], and cover aspects such as sample preparation [1], microscope usage [1,4], method reporting [5–8], or fluorophore and filter usage [9,10]. Despite widespread adoption of microscopy as a tool for biology and biomedical research, the resulting image figures in publications at times fail to fully communicate results or are not entirely understandable to audiences. This may be because authors do not include comprehensive imaging method statements[11], or because they omit basic information in figures such as specimen size or color legends [12], which are key to fully understanding the data. To ensure that images are presented in a clear, standardized, and reproducible manner, it is essential that the scientific community establishes unified and harmonized guidelines for image communication in publications.

Images document biological samples and ranges of their phenotypes. Increasingly, microscopy images are also a source of quantitative biological data and variables are measured with a growing number of image analysis software packages FIJI/ImageJ [13], CellProfiler [14], KNIME [15], Python software libraries [16](scikit-image.org[17]), and commercial software packages such as ZEN, LAS X, NIS-Elements, Amira-Avizo, Imaris, Arivis

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Vision 4d, Huygens [18]. Image analysis is often a workflow of many steps, such as image reconstruction, segmentation, processing, rendering, visualization and statistical analysis, many of which require expert knowledge [19,20]. A comprehensive publication of quantitative image data should then not only include basic specimen and imaging information, but additionally the image processing and analysis steps that produced the data plot and statistics. Towards fully reproducible image analysis it is also essential that images and workflows are available to the community, e.g., in image repositories or archives [21–23], and code repositories such as Github [24].

To ensure that image figures provide insights to their readership, any supportive experimental metadata and image analysis workflows must be clear and understandable ("what is the pixel size", "what does the arrow mean"), accessible ("are colors visible to colorblind audiences"), representative (no cherry picking), and reproducible ("how were the data processed", "can one access and re-analyze the images"). In the framework of the initiative for 'Quality Assessment and Reproducibility for Instruments and Images in Light Microscopy', QUAREP-LiMi [25,26], the 'Image Analysis and Visualization workgroup' established community consensus checklists to help scientists publish understandable and reproducible light microscopy images and image analysis procedures. Where applicable, the checklists are aligned with the FAIR principles, which were developed as recommendations for research data (Findability, Accessibility, Interoperability, and Reusability [27]).

Scope of checklists

The scope of the checklists is to help scientists publish fully understandable and interpretable images and results from image analysis (Fig. 1). In this work the term images includes raw or essentially unprocessed light microscope data, compressed or reconstructed images, and quantification results obtained through image analysis (see glossary). While the focus of QUAREP-LiMi is on light microscopy images in life sciences, the principles may also apply to figures with other images (photos, electron micrographs, medical images) and to image data beyond life sciences. The intended audience of the checklists are novices or non-experts occasionally using light microscopy, and also experts (core facility staff, global bioimage community) who review image data or teach image handling.

The checklists do not include principles for designing imaging experiments or recommendations to avoid image manipulation. Previous literature covers experimental design for microscopy images, including accurate and reproducible image acquisition and ensuring image quality [2,28], examples and recommendations for avoiding misleading images [1,29–33], detection of image manipulation [34–37], appropriate image handling and analysis [7,19,38,39], guidelines for writing materials and methods sections for images [40], and recommendations for general figure preparation [41]. These topics are therefore not covered in the checklists.

The checklists cover image (Fig. 2) and image analysis (Fig. 6) publication and are structured into three levels that prioritizes legibility and reproducibility.

- The first reporting level ("Minimal") describes necessary, non-negotiable requirements for the publication of image data (microscopy images, data

obtained through image analysis). Scientists can use these minimal criteria to identify crucial gaps before publication.

- The second reporting level ("Recommended") defines measures to ensure the understandability of images and aims to reduce the efforts toward evaluating image analysis. We encourage scientists to aim for the "Recommended" level as their image publication goal. However, we acknowledge that some aspects (e.g., large data in repositories) may be unattainable today for some authors.
- The third reporting level ("Ideal") are recommendations we encourage scientists to consider adopting in the future.

Checklists for image publication

Image Formatting.

After exploring, processing and analyzing image data, authors then communicate insights in publications with figures as visual evidence. Preparing a figure begins with the selection of representative images from the dataset which illustrate the message. When quantitative measurements are reported in a chart, an example of the input image should be shown; when ranges of phenotypes are described, several images may be necessary for illustrating the diversity. To quickly focus the audience on key structures in the image, it is permitted to crop areas without data or with non-relevant data (Fig. 3A). As a rule, cropping, similar to selecting the field-of-view on the microscope, is allowed as long as this does not change the meaning of the conveyed image.

Next, specimens are often presented in consensus orientation in figures (e.g., apical side of cells upwards, tree top upwards), which may require image rotation. When such rotation is done in vector software, pixel interpolation is not necessary, since the square representing a pixel can be rotated and size-changed as is, preventing any optical data modification. When rotation is done in a pixel-based image processing software, any rotation that is not a multiple of 90 degrees, however, changes the intensity values through interpolation and therefore alters the information in the image [31,42,43]. The effect of interpolation, while it may be negligible in large images composed of many pixels, can greatly distort the information in small or zoomed images composed of fewer than 100x100 pixels.

When cropping and rotating, authors should ensure that the operation does not affect the original information contained in the image; while loss in image quality may be acceptable, quantifications, especially intensity measurements, should be performed beforehand [39]. In a figure, individual images should be well separated (spacing, border, see Fig. 3B) to avoid misleading image-splicing [29,31].

When presenting two magnification views of the same image (e.g., a full- and a zoomed/inset view), the position of the inset in the full-view image should be made clear; if the inset is placed on top of the full-view image, e.g., to save space, it should not obstruct key data (Fig. 3C). If an inset is digitally zoomed, the original pixels should not be interpolated but "resized" to maintain the original resolution. Overall, the image should be sufficient in size so that audiences can identify all relevant details.