



# Editorial: *Histochemistry and Cell Biology* implements new submission guidelines for image presentation and image analysis

Douglas J. Taatjes<sup>1</sup> · Takehiko Koji<sup>2</sup> · Michael Schrader<sup>3</sup> · Jürgen Roth<sup>4</sup>

Published online: 25 October 2023

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Journal “Instructions for authors” typically describe in more or less detail how manuscript figures should be prepared for submission. This issue is certainly of paramount importance for journals such as *Histochemistry and Cell Biology* where images (from various microscopes, Western blots, flow cytometry, etc.) are part and parcel for the majority of the manuscripts we receive. However, it seems that those instructions are not always rigorously enforced. Similarly, the amount of detail provided in a manuscript’s Methods section to describe image analysis protocols is often cursory at best. Have you ever been frustrated attempting to repeat a published imaging or image analysis experiment only to be thwarted by the lack of details provided in the manuscript? What if standardized recommendations for publishing images acquired by different iterations of light microscopy, as well as accompanying image analyses, formulated by a global community of microscopists were in existence? Would these be useful tools for authors, reviewers, and editors alike? Further, what if these tools appeared in the form of a simple checklist proffering “minimal,” “recommended,” and “ideal” levels for image format, image colors and channels, image annotation, and image availability for microscopy images, or for established, novel, or machine learning image analysis protocols accompanying a manuscript submission? Would this serve to enhance the rigor and reproducibility of published microscopy images and image analyses? We hope that the answer to these questions is a

resounding “yes”, as such guidelines were just published in the November issue of *Nature Methods* (Schmied et al. 2023). These community-developed checklists represent the culmination of more than 2 years of effort from the “Image Analysis and Visualization” workgroup of the international organization Quality Assessment and Reproducibility for Instruments & Images in Light Microscopy (QUAREP-LiMi). QUAREP-LiMi was established as a forum to discuss ways to introduce general quality control protocols to improve the reproducibility and rigor of images acquired through the multifarious types of light microscopy instrumentation (Boehm et al. 2021). The “Image Analysis and Visualization” workgroup of QUAREP-LiMi was tasked with developing guidelines regarding how images should be presented, and image analysis protocols described in published manuscripts [Disclosure: one of us (DJT) is a member of this workgroup, and a co-author of the resulting manuscript]. As mentioned, the guidelines were created to present as easy-to-use checklists for authors, reviewers, and editors, and could simply be added to the journal’s Instructions for Authors, as well as to manuscript reviewer portals (Figs. 1 and 2; PDF version of guidelines provided in the supplementary material for convenience). Although the checklist may be perceived by some as “just an additional form,” if used appropriately, it should simplify manuscript preparation and review by succinctly providing a series of visually appealing “check” boxes for the different aspects of image presentation and image analyses. Importantly, the checklists were designed to be aligned with the popular concept of findability, accessibility, interoperability, reusability (FAIR) principles for overall research data (Wilkinson et al. 2016), and recently specified for bioimaging data in particular (Kemmer et al. 2023).

We are all aware of the perceived “rigor and reproducibility” crisis in science, which has even been highlighted in the popular press (see for instance Harris 2017; Ritchie 2020). In response, over the past few years, several publications in the microscopy imaging field have advocated for the

✉ Douglas J. Taatjes  
douglas.taatjes@med.uvm.edu

<sup>1</sup> Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA

<sup>2</sup> Office for Research Initiative and Development, Nagasaki University, Nagasaki 852-8521, Japan

<sup>3</sup> Faculty of Health and Life Sciences, Biosciences, University of Exeter, Exeter EX4 4QD, UK

<sup>4</sup> University of Zurich, 8091 Zurich, Switzerland

## Checklist for image publishing

### Image format

	Focus on relevant image content (e.g. crop, rotate, resize)
	Separate individual images
	Show example image used for quantifications
	Indicate position of zoom-view/inset in full-view/ original image
	Show images of the range of described phenotype



Minimal



### Image colors and channels

	Annotation of channels (staining, marker etc.) visible
	Adjust brightness/contrast, report adjustments, use uniform color-scales
	Image comparison: use same adjustments
	Channel colors: high visibility on the background Best visibility: grayscale
	Multi-colors: provide grayscale for each color channel
	Multi-color: if channels are merged, make accessible to color blind
	Provide intensity scales (calibration bar) for greyscale, color, pseudo color...
	Pseudo-colored images: additionally provide grayscale version for comparison.
	Gamma adjustments: additionally provide linear-adjusted image for comparison



Minimal



### Image annotation

	Add scale information (scale bar, image length; in figure/figure legend)
	Explain all annotations (in figure/figure legend)
	Annotations should be legible (line width, size/point size, color)
	Annotations should not obscure key data
	Annotate imaging details important for interpreting the figure. <i>Depending on the main message and imaging technique this may be e.g., image pixel size, imaging intervals (time-lapse in movies), exposure time, or anatomical section.</i>



Minimal



### Image availability

	Images are shared (lossless compression/microscope images)
	Image files are freely downloadable (public database)
	Image files are in dedicated image database (added value database or image archive)



Minimal



Recommended

Ideal

**Fig. 1** Checklist for image publication. (From Schmied et al. 2023)

## Checklists for publication of image analysis workflows

Established workflows			
	Cite workflow & platform	<input type="checkbox"/>	Minimal
	Key settings	<input type="checkbox"/>	
	Example data	<input type="checkbox"/>	Recommended
	Manual ROIs	<input type="checkbox"/>	
<b>193</b>	Exact version	<input type="checkbox"/>	Ideal
	All settings	<input type="checkbox"/>	
	Public example	<input type="checkbox"/>	Ideal
	Document usage (e.g. screen recording or tutorial)	<input type="checkbox"/>	
	Cloud hosted or container	<input type="checkbox"/>	
Novel workflows			
	Cite components & platform	<input type="checkbox"/>	Minimal
	Describe sequence	<input type="checkbox"/>	
	Key settings	<input type="checkbox"/>	Recommended
	Example data & code	<input type="checkbox"/>	
	Manual ROIs	<input type="checkbox"/>	Ideal
<b>193</b>	Exact versions	<input type="checkbox"/>	
	All settings	<input type="checkbox"/>	Ideal
	Public example data & code	<input type="checkbox"/>	
	Rationale	<input type="checkbox"/>	Ideal
	Limitations	<input type="checkbox"/>	
	Screen recording or tutorial	<input type="checkbox"/>	Ideal
	Easy install & usage, container	<input type="checkbox"/>	
Machine learning workflows			
	Cite original method	<input type="checkbox"/>	Minimal (All models)
	Access to model	<input type="checkbox"/>	
	Example or validation data	<input type="checkbox"/>	Recommended (Pre-trained & novel models)
	Train, test & metadata	<input type="checkbox"/>	
	Code available	<input type="checkbox"/>	Ideal (Novel models)
	Limitations	<input type="checkbox"/>	
	Cloud hosted or container	<input type="checkbox"/>	Ideal (Novel models)
	Standardized format	<input type="checkbox"/>	

**Fig. 2** Checklist for image analysis workflows. (From Schmied et al. 2023)

need for enhanced detail in the methods section of scientific manuscripts where microscopy-based images and image analysis are included (Schmied and Jambor 2021; Jambor et al. 2021; Miura and Norrelykke 2021; Heddleston et al. 2021; Faklaris et al. 2022). We hope that the guidelines reproduced in this Editorial will complement and render operational in a simplified manner the ideas promulgated in these manuscripts. In this spirit, we are pleased to announce that *Histochemistry and Cell Biology* (a Springer Nature journal) will now implement these checklists for all submitted manuscripts with accompanying images and image analyses, and we encourage authors to use the guidelines in the manuscript preparation stage. Since Springer Nature is a

signatory to the Center for Open Science “Transparency and Openness Promotion (TOP) Guidelines” (<https://www.cos.io/initiatives/top-guidelines>) to promote rigor in the published scientific literature (Announcement 2017), this appears a natural next step for our journal to take to ensure the highest levels of rigor and reproducibility in the images and analyses we publish. We believe that these are certainly praiseworthy goals that we as scientists, authors, reviewers, and publishers should enthusiastically support.

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