WHAT MAKES A TECHNICALLY GOOD MICROGRAPH?

Tom Harnish

I posted a couple of images of prepared slides online and asked for feedback because as a rank amateur I didn't know what constituted a good micrograph. As a photographer, I know what makes a pleasing photograph but my interest was technical not artistic.

Since I didn't get any feedback, I went searching for guidelines or standards and found nothing—perhaps because of where and how I was looking, admittedly. What I found was a lot of discussion about the relatively new field of Whole Slide Imaging (WSI), but surprisingly all I found was some standards for slide preparation not slide imaging.

Finally, I found a paper from 2003 titled "Digital Imaging Guidelines for Pathology" which led me to author Dr. Pamela Gibson who put me in touch with the director of the Microscopy Imaging Center at the College of Medicine at the University of Vermont, Dr. Douglas Taatjes. He was kind enough to send me an author's manuscript for a chapter in a book he recently edited.

The chapter by Douglas W. Cromey, with an oddly punctuated title "Digital Images Are Data: And Should Be Treated as Such", makes it clear that the issue of imaging quality, at least in pathology, is an important one not just because a bad slide can lead to a bad diagnosis but because falsified images can taint research for years to come. A turning point, he asserts, was the fraudulent stem cell paper peer-reviewed and published in *Science* magazine by Huang *et al* which contained digitally modified images.

A survey found that 20-25% of journal images need to be remade because they didn't follow guidelines and 1% are suspicious enough that the publishers contacted the author's institution. Unfortunately, of almost 450 journals the same survey found that only about half had guidelines and 2% had none. Many of the journals that did have guidelines referred to digital images as "art" or "illustrations" and only 10% had explicit guidelines for digital images.

The problem is that manipulated images lead to an inaccurate record, or as George Orwell put it in the book titled "1984", "The past was erased, the erasure was forgotten, and the lie became truth."

But the problem doesn't have to involve malfeasance. The failure to collect a representative sample of images can lead others to misinterpret associated data, and 'artistic' changes to an image can alter the factual content or viewer's interpretation. Even how you store digital images can be a problem if, as just one example, EXIF data is lost.

So what guidelines are there for digital imaging? The author of the article suggests (with

references to authority) that at least two to three times oversampling of the smallest resolvable elements is required to avoid artifacts, including in the z dimension. Temporal oversampling is important too for specimens that change over time. Note that oversampling with noisy techniques such as confocal microscopy, a specialized form of standard fluorescence microscopy, should be avoided, he writes, because it just makes the noise worse.

Aliasing is a digital image artifact that is easy to create in post-processing by over-sharpening an image, and even when converting a RAW or TIFF image to a JPEG. Bit depth over-saturation can cause problems, too, if the information at the ends of a histogram is lost. Moiré patterns can be a problem, as well, if something with a pattern is under-sampled. And, naturally, noise is always an issue.

Here are science publisher Springer's very specific guidelines:

Policy on Manipulation of Images

> Scientific digital images are composed of pixels of varying intensities and shades of color and are to be considered data. Any image manipulations must adhere to strong guidelines to ensure data integrity. We adhere to the following modified guidelines first expressed by The Rockefeller University Press:
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> • No specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Cropping of an image is acceptable to center a feature of interest but must adhere to the previous statement.
> • Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the entire image (not selected regions of interest) and as long as they do not obscure, eliminate, or misrepresent any information present in the original. Identical adjustments must be performed on images from control samples as well.
> • The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (e.g., dividing lines) and in the text of the figure legend.
> • If the original data cannot be produced by an author when asked to provide it, acceptance of the manuscript may be revoked. Always maintain an original unprocessed copy of the image.

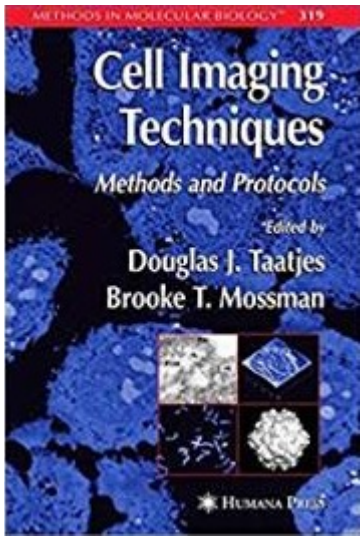
Additional important aspects regarding image manipulation:

> • Provide the name and version of any software used for image processing.
> • Mention in the text any image manipulations which have been applied. Alternatively, a blanket statement declaring that "all images have not been manipulated" would suffice.

• Additional pixels should not be added by a software program in order to produce 300 dpi resolution at the desired image size. For instance, the “Image Size” feature in Adobe Photoshop should not be used to “Resample Image”; doing so will add additional pixels not present in the original image.
• Software filters such as “sharpening” and “blurring” should not be used to improve image quality.

An inexpensive reference for amateur microscopists interested in professional quality results can be found in a book written in 2005 by Dr. Tattjes. It's available used on Amazon for the price of a latté at $2.50 plus shipping. A newer 2012 edition is available for $45.

https://www.amazon.com/Imaging-Techniques-Methods-Molecular-Biology/dp/158829157X



Comments to the author are welcomed, email: tdharnish AT gmail DOT com

The author's article 'What Makes an Artistically Good Micrograph?' is also featured in the May 2019 issue of *Micscape*.

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