



W125 Veterinary Medicine Bldg • Columbia, MO 65211-5120
voice: (573) 882-8304 • fax: (573) 884-2227
URL: www.emc.missouri.edu

Magnification Issues in Electron Microscopy

When you take an image on an electron microscope, what does the stated magnification value for that image actually represent? Although this seems to be a straightforward question, we find that there is a certain amount of confusion on this issue.

For example, when you set the microscope to record an image at a magnification of 10,000x, what does this number really mean?

Unfortunately, in practice it usually doesn't mean much more than "that's where the microscope was set".

Here's the problem: electron microscopes have long been designed so that the indicated magnification is only valid for images recorded on a film negative or other photographic image generated by the microscope itself. On the transmission electron microscopes (TEMs) in the Electron Microscopy Core Facility, we have always used a special film of a certain size designed for these scopes. **On these negatives**, the reported magnification is indeed 10,000x if that is how you set the magnification. In other words, you can measure a structure directly on a negative with a ruler and divide it by the stated magnification and get very close to the actual size of the structure.

But images taken on microscopes seldom end up being used at the same size as the negative. Some are enlarged when printed or viewed on a computer screen. For example, to make an 8x10 inch photo enlargement from a TEM negative involves a 2.8x magnification of the original image. That means the final magnification is 28,000x, even though the stated magnification on the negative is 10,000x. Sizes are also changed during publication of images in research journals. This means that the final viewed image has a magnification other than 10,000x.

To complicate matters further, when using an image recorded on a digital camera, as most images are these days, that **image will not be at the stated magnification**. *In fact, on our JEOL 1400 TEM with the Gatan digital camera, the digital image is magnified about 9.25 times more than the magnification the microscope is set for!*

So how do you deal with this?

Rule #1: Rely on the magnification scale recorded with your image (the line with a value written next to it). This is accurate at any size, **as long as the scale is part of the image and is magnified or reduced along with it**. If that bar says it represents 1.5 micrometers, that is true whether the final image size is 2 centimeters square, 2 meters square, or 2 kilometers square (in case you have a REALLY big hard drive).

Rule #2: It is best to ignore the stated microscope magnification figures. The only times they are useful are if you are using the image at the original size that the scope would record on film or if you know the magnification or reduction factor of your final image. In the latter case, if you know that you enlarged the final image 3.0x and your stated magnification was 10,000x, then you know your final magnification is 30,000x.

It is best NOT to report a magnification figure when publishing micrographs, because they will almost always be wrong for the final image (unless you know the exact final size of the image in the publication and somebody adjusts the magnification number for that size).

So....what if you have an image and really need to know the magnification of that image for some reason, but no value is listed?

Easy. Measure the length of the magnification marker and divide that value by what it represents. That's your magnification for that image.

Example: Your magnification bar measures 2.2 cm. The value written above or below that line says 1.5 μm . 2.2 cm equals 22,000 μm . $22,000 \div 1.5 = 14,666\text{x}$. This is just another way of saying that an object 1.5 μm in length will measure 2.2 cm when magnified 14,666x.

Round off as appropriate---no microscope is accurate enough to get exactly 14,666x, so 14,600x or even 15,000x is fine. Manufacturers generally guarantee magnification accuracy to $\pm 5\%$, although they are usually more accurate than that. Also, we perform our own in-house calibrations using special, very expensive, standard specimens.

The take home lesson is: ***Don't take magnification figures at face value. Trust magnification markers embedded in an image.*** Mag bars are your friends. They are loyal and generally don't lie to you, if the microscope and software are working properly (which is another story altogether.....)