

If the mmicron markers have the same values, then it is relatively easy thing to do. Just scale the second image by the ratio of the two lengths of the markers and you have it. However, if the markers have the different values, e.g. 1  $\mu\text{m}$  and 5  $\mu\text{m}$ , then you have to scale by the ratios of the Length/ $\mu\text{m}$  of one image to the length/ $\mu\text{m}$  of the other image. In Photoshop, you simply go into Image-Image Size and then if the new image will be smaller, you should use resample option. If it will be larger you should take resample off. Then go to width and change to percent. Use your ratio value and multiply it by 100 to get percent and change it making sure that the link is there for the height so that the aspect ratio stays constant. In one case your file size will be smaller and in the other case, your resolution of the image will get smaller.

I don't have Photoshop, so I would have to defer to those who do. The idea of setting up one image as a reference to which all the others will be matched is absolutely correct. Others noted that the reference image could be made a layer in Photoshop and the image under repair could be stretched or shrunk until the scale bars matched. That is a valid approach, but as someone noted, you may need to be careful that the aspect ratio is not inadvertently changed.

Since the end result of such a process is changing the image size (or pixels per inch), I would recommend editing those values directly. Like I said, I don't have Photoshop, but most image programs offer the option to change DPI and print size. I favor FastStone Image Viewer for my personal use. It is robust enough for most applications and it is small enough that it loads quickly. It is NOT a PhotoShop replacement. And it has a nice batch mode where operations done on a single image can be applied to many images.

I used the resize/resample function within the program. I took as my task trying to match up images that were taken at 150x and 120x. I suppose you might also have a third or fourth magnification to reconcile.

The first issue would be to choose a magnification for the finished images. You can digitally magnify the images from lower a lower mag to a higher mag and the print size of the lower mag image will grow. You would then crop it back to the desired print size. (You could also demagnify the images taken at high magnification, but you would then have white space surrounding them compared to the other images taken at lower magnifications. You would end up cropping the low mag images as if you had magnified them in the first place.)

The next issue would be to determine the new parameters for the images. I have laid out my example calculations below.

Mag	Res	DPI	Size	Comment
150x	1024	227	4.51	150x original
120x	1024	227	4.51	120x original

150x' 1024 182 5.64 120x corrected to 150x

The only thing that changed was the print size and its attendant DPI. The existing number of pixels is stretched out to fit a wider image and DPI drops accordingly. I suppose you could redo the number of pixels at the same time, but that would be interpolating data. I would not do it unless pixels were becoming obvious in the print. Then I would increase the pixel count.

I would then crop the adjusted image back down to the same size as the high magnification images, e.g., 4.51 inches.

You didn't say how you were going to render or use the images. I inserted the images into Word and it dutifully checked the DPI and rendered the images at the proper sizes. The scale bars match up as intended. The same is true of PowerPoint.

The user needs to be careful not to sidestep the DPI rendering. If so, the same thing needs to be done to all images. For example, I tend to scale my images to fit the margins in Word. The images end up as 6.50 inches wide. I need to make sure I am stretching images from 4.51 inches to 6.50 inches and not stretching some from 4.51 inches and others from 5.64 inches. That would undo all the previous work.

I hope this helps.

Warren S.

## Micron Scale on Images

I am not sure what you are asking. The real sizes of what? Bill's reply addresses calibration of negative mags versus gratings, etc. I read your question as, "I know my negative mag is 80,000X from my TEM 'read out' and the carbon grating replica calibration. There is no micron marker on the negative anymore and so how long is one micron on that negative (or on a print I make)?"  $80 \text{ mms} = 1 \mu\text{m}$ .

This calculation works on SEM prints, TEM prints, optical micrographs, and negatives where you know the magnification.

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### GENERAL MAG CALCULATION RULE:

Calculate the known magnification in KX. That numerical KX value in millimeters is the length of one micrometer ( $\mu\text{m}$ ) on your print, negative, or whatever.

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For example, you have a final print mag of 125,000X. If you draw a line 125 millimeters in length, that will be the length of one micron on that print. For a tenth of a micron ( $0.1 \mu\text{m}$ ), just use 12.5 mm. This rule is simple to remember and it works on any photographic media for which you know the magnification.

You can reverse the procedure to calculate the magnification on a journal article micrograph without a stated magnification but with a micron scale. Calculate the length that it would take to make one micron with the scale marker shown on the micrograph. That number in millimeters, is the KX value of the magnification of the photograph in the article.

A bit more: A (no arrowheads) line scale marker can be made with Photoshop on electronically scanned images of negatives. See the reference below for a detailed discussion. Basically, select the line tool (/). Hold down the shift key. With the line option window tab set to a width of 5-10 and the preferences set to CM units in the PS preferences, mouse drag a horizontal line to the length that you need. Look in the info window to read the current line length. The length displayed will be in CM, not MMs, and so you can get confused at times. Release the mouse button to make the proper line length as shown in the info window / tab.

To make the micron,  $\mu$ , character; hold down the ALT key. Type 0181 on the NUM PAD using those keys. DO NOT use the regular number keys at the top of the keyboard. Release the ALT key and the micron symbol,  $\mu$ , will appear. This works on any PC clone and in any program running on a windows platform, even email clients. Woolley, below, also claims a method for Macs on ISO-Latin-1 characters.

10  $\mu\text{m}$   
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If the serial numbering and micron marking are both broken, use a Sanford Sharpie® extra fine marking pen to write waterproof and D-19 developer-proof numbers on your TEM film before use. They work for manually drawing line length markings on prints too.

I hope this helps you out.

Paul Beauregard

### Notes:

An Optical Microscopy example. The mag is 500X. That's 0.5 KX. So one micron is 0.5 mm. That is too small of a length to draw. So multiply by ten and use 5 mms. Label that 5 mm length as 10 microns.

Ref: Microscopy.com archive. On the home page in Microscopy.com at the bottom left, click on "Search the archives" Use author Beauregard and look in "all of 2002" or just Feb, 2002. Look for: Subject: Straight parallel micron bars in Photoshop®. Feb 4, 2002.