

Adobe Photoshop for Scientists Pt. 1

A Brief Illustrated Guide

By Jerry Gleason

There is no shortage of material on Adobe Photoshop. Many books are available, and there are courses that can be taken. It's a very comprehensive program with many features that you may never need to learn. The focus of this article will be on the basic procedures for the preparation of digital images and figures for publication or display by research scientists. In this article, I will discuss the basics of image acquisition and manipulation, then in part 2, how to make a figure with multiple images and annotations.

Scanning and Resolution

When scanning an image, you should first consider what the final output will be. If you were preparing a figure for publication, the images within would need to be higher in resolution than if the final output were just for screen display, as in a PowerPoint presentation or web page. Note that when I talk about resolution here, I'm referring to the number of pixels in the image per inch, or ppi (dots per inch, or dpi is often used here, but that specifically refers to printer resolution). The thing you need to consider is the resolution of the final output and make sure the scan resolution is adequate to cover that. This is an important and often misunderstood concept.

For example, let's say that you are making what will become a figure to be printed on an 8.5 x 11 page. The final resolution should be at least 300 ppi. The figure will have four photographs. Each photo will be 3.5 inches by 2 inches on the page. Each photo, then, must have 1050 x 600 pixels to achieve 300-ppi resolution on the page. You don't need to know the exact number of pixels in your scan, because you probably will scale the image to fit anyway. The idea is that you can scale downward without losing anything, but you want to avoid scaling up, because then you're losing resolution. There is no need to scan at much higher resolutions than your final output (unless the same image is going to be used later for something that needs it), so pick a scan size that more than covers the number of pixels you will need. I'll talk more about resolution when we get to sizing images.

Bit Depth

RGB images are generally 24-bit, or 8 bits per primary color. Some scanner plug-ins will allow scanning at 16 bits per color channel, making a 48-bit RGB image. Working with 16-bit images (48-bit files) is not recommended, since the file size is much larger, and Photoshop's 16-bit support does not include many of the commands and filters you will need. 24-bit images have over 16 million possible colors, which I think you will find is more than enough. If you feel that you can get better scan quality at 16 bit (which is sometimes the case), you can then change modes to 8 bit, and Photoshop will do some

high-quality dithering magic, resulting in an 8-bit image that is visually indistinguishable from the original 16-bit scan.

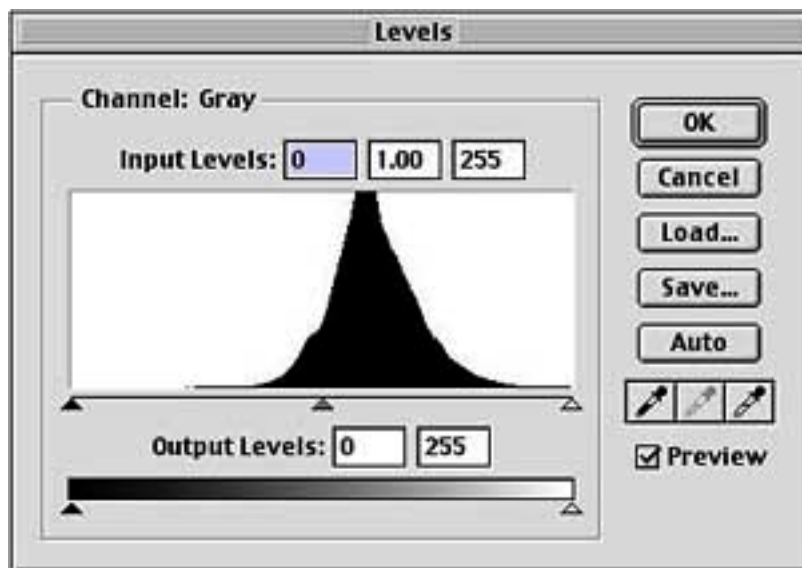
Note: If you're in grayscale, just think 8-bit vs. 16-bit, since there is only one channel, or color.

Contrast Adjustment

Okay, your pictures are scanned, but they could look better. Scanned images from prints or slides can almost always benefit from some amount of enhancement, although to what degree depends on how your scanner software is set. Generally, I prefer to do most of the enhancements such as sharpening and contrast adjustment in Photoshop rather than letting the scanner software decide for me.

Now, here is where I should point out that images captured from a digital camera should not be treated the same as scans from photos. The reason is that most digital cameras will try to optimize the image automatically before you even see the shot. Sometimes this is to your advantage, but not always. In any case, digital camera images may not tolerate the range of adjustments that you might give a scanned image without introducing serious degradation

One of the adjustments that will almost always improve a scanned image is contrast and brightness. In Photoshop, there are a number of different ways to achieve this using different commands and tools. Let's start with what I think is the easiest and simplest way, and the one I use most often, which is the levels command. (*Image > Adjust > Levels*).



I won't get into a detailed discussion of exactly how this works (refer to the Photoshop manual for this), but here is where you can make a sometimes dramatic improvement.

First, try the *Auto* button. About 50% of the time, this will look spectacular, but just as often, it can look awful. No good? Here's a little known trick; if you have made adjustments, didn't like it, and want to undo it while the dialog box is still open, hold down the option key (alt key for PC) and the *Cancel* button changes to *Reset*. Now try adjusting the *input level* controls. You can think of the three sliders as shadows, midtones, and highlights. The goal here is to get highlights that are bright, but still have detail, and shadows or dark areas that are still light enough to see what's going on. Sometimes just moving the midtone slider is all you need. Keep in mind that when you move the shadow and highlight sliders, you are throwing away pixel information that you can't get back again once the image is saved, so do this carefully. There is much more to this, but again, refer to the user guide if you want to get deeper into it.

Some people prefer using the *Brightness / Contrast* command for this, which adjusts both input and output levels. The *Curves* command is similar to levels, except with curves on a graph instead of sliders.

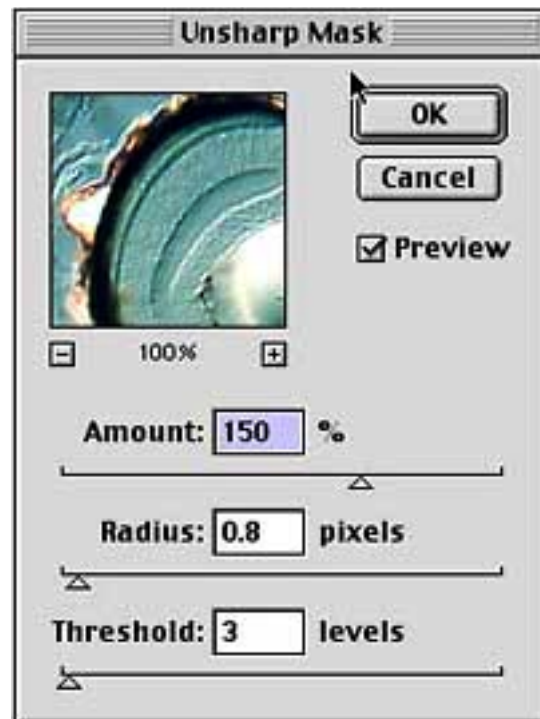
Color Balance

There are many pathways to color adjustment in Photoshop, most of which are far beyond the scope of this article. If all you need is to remove an overall color cast, the quickest and most intuitive way of adjusting color is to use the "Variations" command (*Image > Adjust > Variations*). This gives a visual comparison of different adjustments for color and tonal balance. For more precise control, the *Color Balance*, *Hue/Saturation*, *Curves*, and *Levels* controls are best, but using them correctly requires a read through the user guide. Extensive color adjustments may bring up questions about manipulating data, so ask yourself what is ethical here.

Sharpening

This is one enhancement that can really bring out hidden detail in a photograph, but also one that can easily be overdone, so proceed carefully. In Photoshop, a digital image is "sharpened" by increasing the contrast between adjacent pixels, thereby increasing the perception of sharpness and detail. There are a few different filter algorithms for this, but the one I use most often is Unsharp Mask (*Filter > Sharpen > Unsharp Mask*).

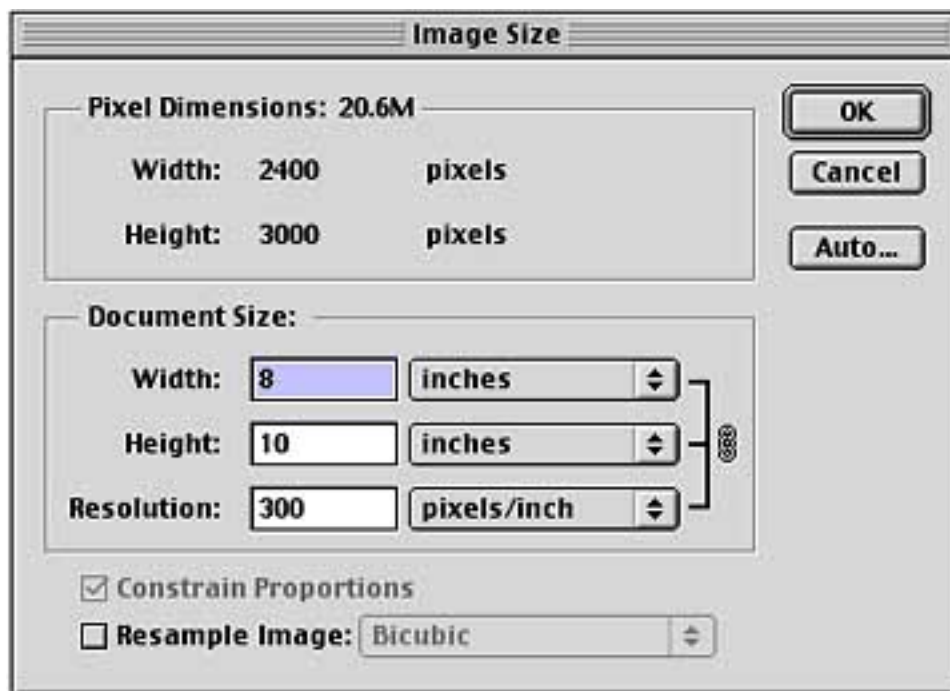
The settings illustrated here are a good



starting point, if your image is for print. The ideal pixel radius and amount of sharpening will vary with the size (number of pixels) of your image. Again, holding down the *option / alt* key changes “Cancel” to “Reset”. An image from a digital camera generally will need less sharpening than a scanned image. It is best to perform this action after your image is scaled to the desired size, and the contrast and brightness (levels, if you prefer) are adjusted.

Sizing Images

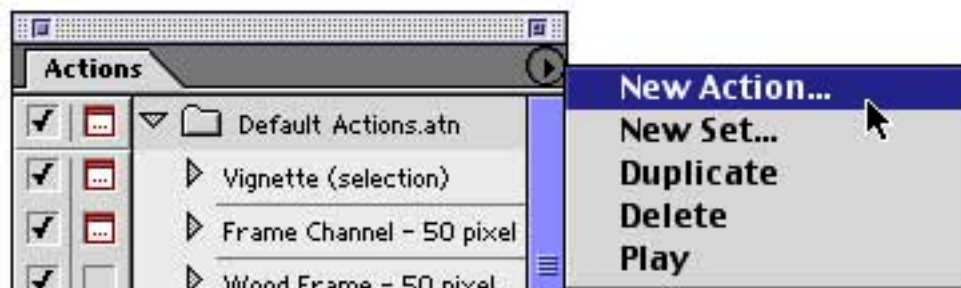
Image Size (*Image > Image Size*) is one command you will probably use on every image you work with in Photoshop. In this dialog box, you can adjust the size of the image (the print or display size in inches or centimeters) and the pixel resolution as either separate or linked parameters. This is where you can specify how big your image appears on the print and at what resolution. If your image is for screen display, consider the resolution fixed at 72 ppi, and work with the size at that resolution.



If you change the size, say in this example, the width, from 8 inches to 4 inches, the number of pixels remains the same, they just squeeze into a smaller space, and the resolution becomes 600 ppi. Now, if you were to first check the *Resample Image* box, and then change the width to 4 inches, the resolution stays at 300ppi, but the number of pixels in the image changes to 1200 x 1500. This is called *downsampling*. Half the pixels are discarded. If you resample up, new pixels are added based on the values of existing pixels, but this something you should avoid if possible, because you can't add detail to an image that wasn't there in the first place, so you are, in effect, just magnifying what is already there. Sometimes after downsampling, the image can look slightly blurry, but

applying the *Unsharp Mask* filter can restore detail. If you need to apply a scale bar to a photomicrograph, you must do it before any image resizing takes place, so that the scale stays with the image. I'll get into detail about scale bars and arrows in part 2.

By the way, since the *Image size* command is used so often, you might want to assign it to a hot key, so you can bring it up instantly. To assign this (or any other) item to a Function key, go to the Actions palette. Select *New Action* from the menu by clicking the arrow on the upper right corner.



Name the new action; in this case, call it *Image Size*. Assign a function key for it. Photoshop already has a number of Fkeys preprogrammed, so check them out before changing them. I put mine on F9. Click *Record*. Now go back to the Actions Palette, click the upper arrow again, and select *Insert Menu Item*. The dialog box will show none selected until you go to the menu item you want, in this case *Image > Image Size*. Click OK, then click the stop button at the bottom of the Actions palette.

Final Touches

You're almost done, but maybe your slide had some dust specks on it, and you want to dress it up. The *Clone tool* is invaluable for this. The clone tool picks up a place in the image when you click the alt / option key, then paints it over anyplace you select. Now obviously, this can completely alter the image content and change you data, so let your ethics be your guide here. I happen to think that getting rid of a few hairs and specks constitutes a legitimate use of this tool. Spotting is easily done by alt / option clicking on a spot right next to the defect, or at least a spot that looks the same as the spot you want to correct, then painting over the defect spot with the appropriate brush size. It takes a little practice to get it right.

That's it. You're all done. See part 2 for tips on making a multi-panel figure in Photoshop.