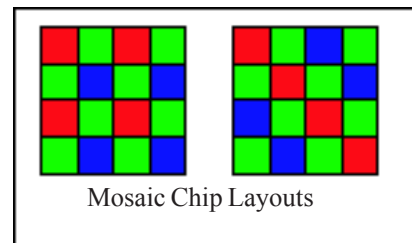


The most persistent misconception made in digital imaging among those who use our core facility involves the understanding of digital resolution; and the greatest mistakes result from a mis-use of the “Image Size” dialogue box in Adobe Photoshop. Add to that an intractable desire to use Microsoft PowerPoint and to somehow make Photoshop and PowerPoint agree about the nature of images that open in both programs, weighted toward the miserable resolutions and compression required by PowerPoint, changes in original data become as common as dandelions on a lawn. Yet, changes in data that result from increasing or, more commonly, decreasing pixels (what is called *re-sampling*), can be avoided.

Resolution, as many of us have learned, is the ability to discriminate between two points in space, a phenomenon made easier by high contrast objects versus those that are low in contrast. Following that definition, the caveat for digital imaging would then be a desire for 1) more pixels that are 2) physically smaller in size and 3) spaced more closely together, since the pixel itself can be the limiting component of an image after optics and scattering of light. Thus, in the simplest sense, more pixels equals greater possibility for resolution.

Digital camera manufacturers are well aware of that, especially those who market for mass consumption. Every month we read about mega amounts of megapixels, with the implicit understanding that more megapixels are good. The reported resolution from many of these cameras differ, however, from the advertised resolution because of two things:

1. Only two-thirds of the pixels are typically used
2. Visual information is “grouped” using lossy compression.



Many of these cameras use the cheaper “mosaic” chip. Since the photosites (the physical photon detection sites that correlate 1:1 with subsequent pixels on a chip (when looking at advertised optical resolution--NOT “interpolated”) simply read photons which are “colorless” (since chips only “see” in grayscale), red, green and blue microfilters are placed on each photosite. These filtered photosites are subsequently divided into what are called “channels” in Photoshop, and intensities of each channel are averaged together by taking several photosites and averaging the color in that location. In that process, fully a third of all pixels are “thrown out.” Thus, it is a good idea when buying a camera to ask if a mosaic chip is used.

Resolution can be further reduced by compression into the commonly used JPEG format. Some JPEG compression is non-lossy—in essence, no pixels are averaged together—but most JPEG compression averages color (versus intensities) even further. Camera manufacturers have found that the human is more offended by changes and alterations in contrast than in color. Thus, resolution loss along the lines of finely divided areas of color and consequent detail again takes the back seat.

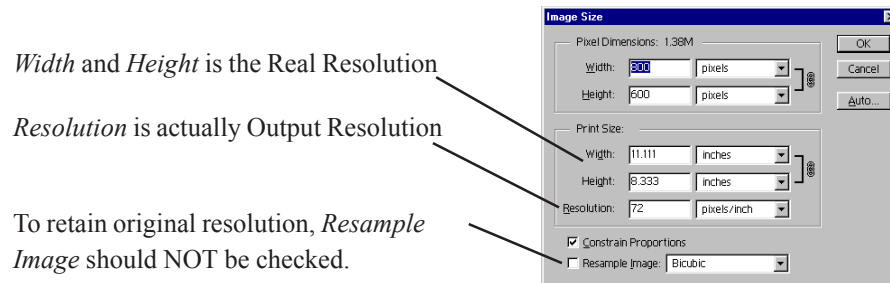
Often a camera with less pixels across and down better fits our needs. Those who work with fluorescently labeled tissue at magnifications greater than what is achieved with a 40x lens need LESS pixels than those who work at lower magnification with brightfield, or those who work in electron microscopy. The upper end of magnification in light microscopy suffers a loss of resolution far more from optics and the nature of the material that is imaged than by how many pixels are used. In other areas of imaging, such as when video-rates of 30 frames per second need to be achieved and that digital information stored on a computer, less pixels across and down becomes a necessity because of current limitations with fast imaging and the practicality of wading through immense amounts of data and massive amounts of disk space.

Whatever the beginning resolution is, and how that was maintained through a chip whose sum of advertised pixels across and down often operates as a megalie, the result is an image on the computer screen that now has a resolution that is fixed. That resolution has only one reference: the number of pixels across and down, viewed in Photoshop’s *Image Size* dialogue box as the height and width (seen only when *resample* is checked in Photoshop 5x-7x).

It should be stated that the inherent resolution of an image is not necessarily what a person discriminates on a computer screen, for that is limited by the resolution of the screen itself, something that has far less resolution than a typical printer. Do not be fooled by the magnification indicator at the top, left of the image window, either: that percentage is simply the size of the image in relation to the dimensions of your computer screen display settings (thus an image that is 800 pixels in

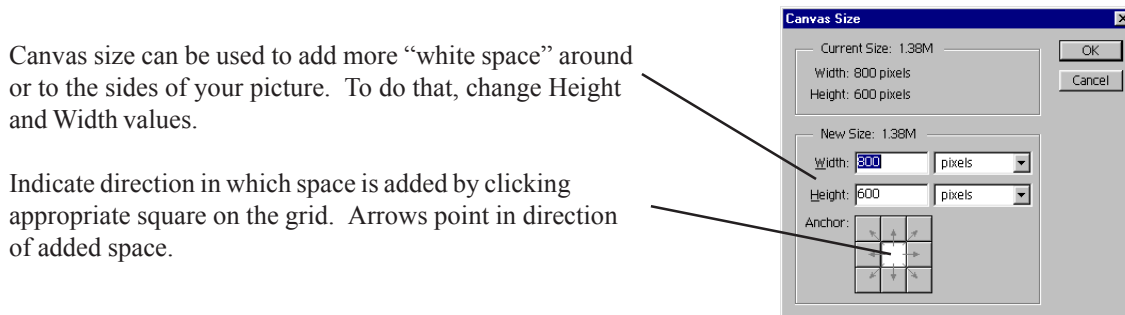
width reads at a magnification of 100% when it fills a screen in which the screen settings are also set to a width of 800 pixels).

The *Image Size* dialogue box is the great pitfall for many. Again, we encounter a sort of lie: in the *Image Size* dialogue box we read “resolution” and that is a measurement of pixels per inch. Fully aware that the inherent resolution of the image is its height and width in pixels, and then seeing another definition of resolution called “pixels per inch” immediately creates confusion. The makers of Photoshop call pixels per inch (ppi) “resolution” and so that must be true. But it isn’t. The makers of Photoshop should have labeled ppi “output resolution,” because pixels per inch refers to what the resolution will be when it is *printed*, and then only when it is printed to a printer that uses ppi as a parameter (what is true, for example, with the Fuji Pictography printer). When a printer uses dots per inch or lines per inch as a means for measurement, then, once again, we encounter a measurement that is untrue because pixels don’t correlate 1:1 with dots made by laser and ink jet printers, nor do pixels correlate with the lines per inch measurement used by output devices for printing presses.



Furthermore, if images are acquired with a digital camera or a laser/PMT system such as a confocal, rather than a flatbed scanner, the value placed by Photoshop in *Resolution* is arbitrary. Because you were not given the option to set output resolution from the beginning as pixels or dots per inch, as you would do when using a flatbed scanner, Photoshop has no way to figure it, and so an arbitrary value of either 72 or 96 ppi magically appears. Many have had the dictum of “72 dots per inch is too low” hammered into their consciousness, and so the immediate response is to raise that value to a more reasonable 300 ppi. Don’t do it! You should retain original data if you wish to follow Good Laboratory Practices.

What is listed as the Height and Width in pixels in the *Image Size* dialogue box should not change until just before the file is sent to a printer. To be sure change doesn’t accidentally happen, uncheck *Resample*. If two or more images need to be added to make a figure for publication or for presentation, make extra space by using the *Canvas Size* function under *Image* in the menu. You should figure what extra space is needed by adding pixels, making additional room for spaces between images. The location of the first image can be fixed by using the grid in the *Canvas Size* dialogue box.



Pixels should not be thrown away by consciously creating less pixels in height and width for the sake of squeezing images into PowerPoint, or because of a fear of large file sizes (hard disk space and faster computers are reasonably affordable). If a smaller image is needed for PowerPoint, keep the original as a TIFF or PSD (Photoshop) file in a folder entitled “original images” and save a duplicate image in the JPEG format (PICT format for earlier Macintosh computers) for PowerPoint and Microsoft Word.

Just before printing, open the *Image Size* dialogue box under *Image*, and then **ONLY** check *Resample* when creating a file for electronic submission to a publication at their recommended output resolution settings, or when printing to a photographic or dye sublimation printer in which pixels per inch may also need to be set. When printing to an ink jet printer, do **NOT** check *Resample*. Instead, put in the Height and Width values and let the resolution setting change arbitrarily since

the ppi is not translatable into the spitting of ink on paper in this context. Printing to a laser jet to evaluate images from Photoshop is not recommended, since these printers reinterpret the image in ways that do not show accurate results.

By following these steps, original pixel resolutions from your source can be maintained, and chunky or pixellated results can be avoided (with the exception of blowing up images captured at <500 pixels in one dimension to full page sizes and with the exception of blowing these images up far too great on a computer screen). Even better, when the time comes to publish, that one special image can be at the appropriate resolution to make the perfect cover photo for a journal.

*Jerry Sedgewick is the director of a light microscopy/digital imaging facility at the University of Minnesota. He is the author of the book "Quick Photoshop for Science: A Guide to Digital Imaging" which will be available from Kluwer/Plenum press in August, 2002. He also hosts a website: [www.quickphotoshop.com](http://www.quickphotoshop.com). This article will be published in "Microscopy Today."*