Photoshop and 12-bit Digital Microscope Camera Images

Brent Neal
Reindeer Graphics, Inc.
brent@reindeergraphics.com

One problem facing owners of high-end digital microscope cameras and scanners is dealing with 12-bit TIFF format images. Because of a vagueness in the TIFF specification [1], some programs do not load 12-bit TIFF images at all, or do not handle them gracefully. Unfortunately, Adobe Photoshop is one such program.

A common problem with 12-bit images that do load in Photoshop is that the image appears to be totally black once loaded. This occurs when the camera stores the most significant bits of the image in the lower 12-bits of a 16-bit data space. Photoshop, when converting to its internal 16-bit representation, does not scale these properly, resulting in a low contrast image. This can present problems with viewing the images, or performing any processing or measurement steps that you might desire.

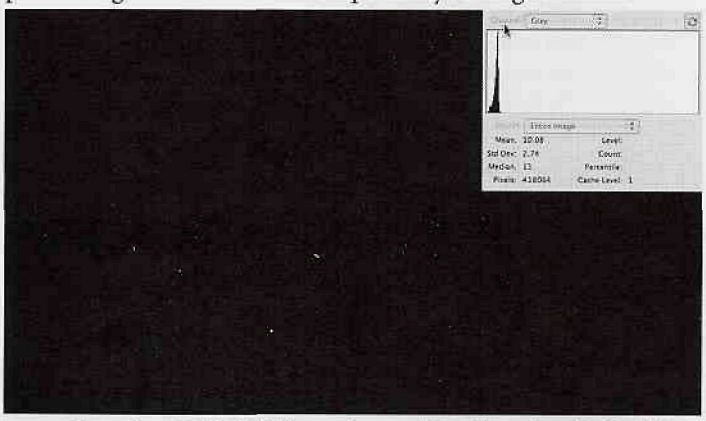

Figure 1. A 12 bit TIFF image imported into Photoshop. No detail is easily visible. The inset shows histogram of the image, indicating that most of the pixels are in the darkest portion of the image.

In this article, I will show how to use either Photoshop's built in Custom filter or a free third-party filter to properly (i.e. losslessly) adjust the contrast of the image so that the image appears correctly. You will notice in Figure 1, the histogram of the image insert shows that all the detail is compressed into a very small range of the available grey scale values. How small of a range? Figure 2 illustrates this.

Figure 2. The fraction of a 16 bit range used by a 12 bit image. Note that 4096 is about 6% of 65536.

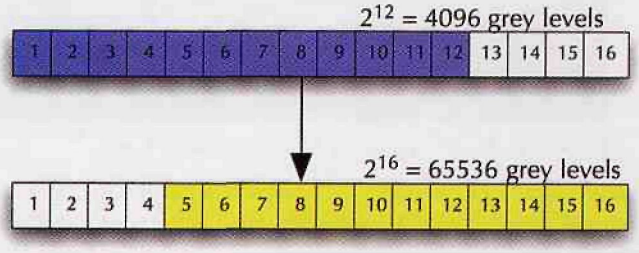

Figure 3. Correcting the image requires shifting the data from the least to most significant bits to place it in the full dynamic range available.

From Figure 2, we see that a 12 bit image can represent 4096 unique greys. A 16 bit image can represent 65536 [2], thus the 12 bit image is only using about 6% of the dynamic range available. What we want to do is move this exact image into the higher bits, in order to take advantage the larger dynamic range. Note that this does not actually add more information to the image - there will still be a maximum of 4096 discrete grey values. The values will, however, be spaced further apart, making it easier to distinguish between them. What this entails, and the result, is diagrammed in Figure 3.

Solving the problem with a kernel convolution

To solve this problem, we will use a pixel-space (as opposed to Fourier) convolution. As we saw in Figure 3, we simply want to perform a "bit shift" on the data, moving each pixel's bits four bins to the right. This is identical to a multiplication by the appropriate power of two: 2^4, or 16. Therefore, we need a tool that will let us multiply each pixel in the image by sixteen.

Photoshop has a built-in kernel convolution tool: the Custom filter, found under the Filter menu (Filter > Other > Custom....) This tool is adequate for occasional use, but lacks some key features. Reindeer Graphics, Inc. provides a free Custom plug-in that can be downloaded from http://www.reindeergraphics.com, which provides several more features than the internal Photoshop plug-in [3]. If you are using Photoshop CS, you can use the Photoshop's built-in plug-in. If not, you will need the Reindeer Graphics free plug-in, since Photoshop's version did not work in 16-bit mode before Photoshop CS.

To multiply by sixteen, we will use the following kernel:

$$\begin{pmatrix} 0 & 0 & 0 \\ 0 & 16 & 0 \\ 0 & 0 & 0 \end{pmatrix}$$

Figure 4 shows what the plug-in dialogs will look like with the appropriate values entered. You can enter the values manually, or save a text file containing the kernel for future use. This would be useful if you wanted to automate the process in a

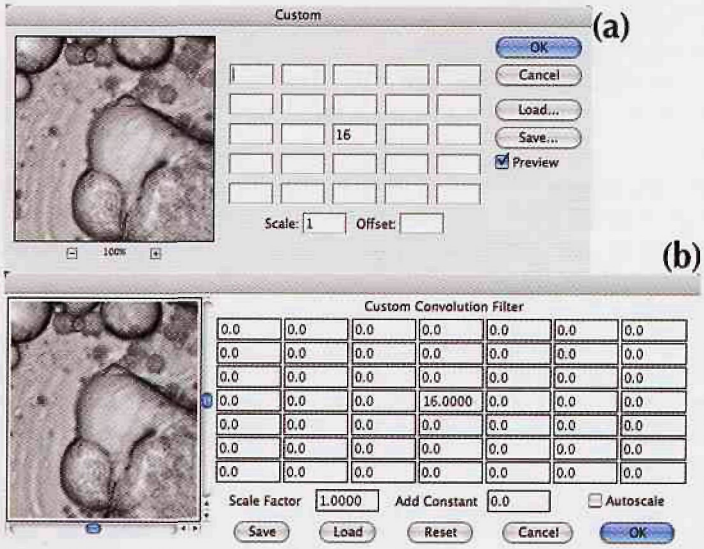

Figure 4. Convolution dialogs from a) Photoshop CS and b) Reindeer Graphics Custom. The kernel entered in each dialog will multiply the value of each pixel by 16.

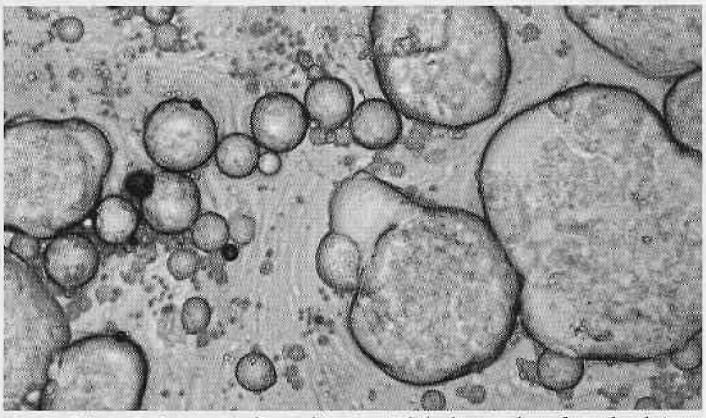

Figure 5. Contrast enhanced image. While the number of grey levels is still at most 4096, they are distributed evenly in a 16 bit (65536 grey level) space, taking advantage of the extra dynamic range available.

Photoshop Action. Applying this transform will turn the near-totally black image from Figure 1 to the image shown in Figure 5. Note the increase in contrast.

Understanding the problem and solution

The problem we solve here occurs because the TIFF specification does not provide a canonical way of handling 12-bit images. The camera manufacturers have the option of putting the image data in the least significant 12 bits of a 16 bit data field, or in the most significant 12 bits. Photoshop expects the latter, where some camera manufacturers implemented the former. Fortunately, no data is lost from this; Photoshop imports the entire 16-bit pixel. The problem is that all of the data is compressed into a very small portion of the available dynamic range.

After the multiplication, the 4096 grey levels that are available in the image are now spaced 16 grey levels apart. This increases the contrast in image, but does not add any new data. Savvy users of Photoshop will wonder why a simple Auto Levels procedure would not work just as well. Auto Levels goes a bit further than what we did, and stretches the highest value in the histogram to the maximum grey scale value. For a single image, this might be fine, assuming you didn't want to measure the image afterwards.

If you had taken a time series of images under the same conditions, and used Auto Levels on each frame, any continuity in the meaning of a grey level between frames would be destroyed. Further, any attempt to analyze the image quantitatively on the basis of luminance would be meaningless unless there was a calibration standard in each image. This means that Auto Levels is unsuitable for images from fluorescence microscopy, scanning probe microscopy and profilometry, and densitometry, among others. The technique described here will preserve the ability to quantitatively analyze such images. ■

References and Notes:

1. The TIFF 6 specification can be found at http://partners.adobe.com/asn/developer/pdfs/tn/TIFF6.pdf
2. Photoshop, in actuality, does not support a true 16-bit space, but rather a 15 bit + 1 space. In 16 bit mode, the maximum grey value for a pixel is 32768. There have been several discussions of this in the Photoshop forums at the Adobe website.
3. The Reindeer Graphics Custom plug-in provides 16-bit support in Photoshop 7 and earlier, a larger neighborhood, the ability to enter floating point kernels, and an optional autoscale function. It also works in Photoshop-compatible programs as well.