**Adjusting Dynamic Range with the Levels Dialogue in Adobe Photoshop**

The Levels adjustment tool in Photoshop is the single most effective tool for remapping the dynamic range of an image. This adjustment tool performs a "histogram stretch" on the image. As evidenced by the pixel intensity histogram* shown below, a typical fluorescent image consists of a small amount of signal on a nearly black background. The higher intensities are generally devoid of pixels. These limited dynamic ranges are a challenge not only for creating an esthetically pleasing presentation of the structural information contained in the image, but also for separating out those structures for measurements. Remember, the human eye cannot distinguish between pixels unless their intensities differ by at least 1%-3%. The effect of the histogram stretch is to remap the intensities in the image to span the entire range of the possible intensities.

Open the Levels dialogue under the Image Menu, 'Adjust', 'Levels'. A histogram appears in the Levels dialogue box that graphs the number of pixels in the image (vertical axis) at each intensity level (horizontal axis). For example, in the dialogue at the right there is a bulge in the histogram between the shadows (black triangle) and the middletones (grey triangle). This indicates that a large portion of the image has grey values between 70% and 100% black.
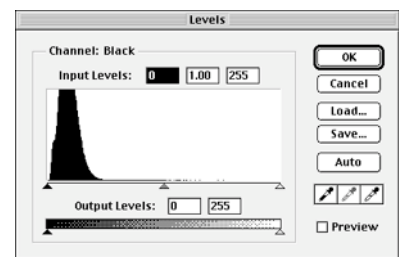
You can also see that the histogram stops before reaching the middle triangle. This means that most pixels in the image are very dark. Because the histogram stops approximately half-way between the black triangle and the middle tones triangle (50% black), we can estimate that the brightest area in the image is about 70% black. However, if you look closely, a few pixels extend past the middle tones toward the white triangle (0% black). Thus, there are very few bright pixels meaning that the pixels containing information (fluorescently labeled) will be packed together in a few intensity values.
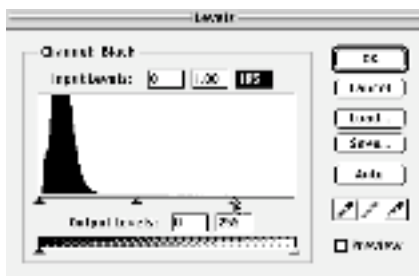
We can brighten the highlights (increase the contrast) by dragging the upper white triangle down toward the left hand end of the histogram. If the upper white triangle is moved leftwards into the region under the histogram, those pixels will all be remapped to maximum intensity. They will now become saturated.

After doing so and clicking "OK", the histogram will be stretched out so it now uses the entire spectrum of gray values. The brightest few pixels are now very close to the right edge. You can brighten highlights and darken shadows (i.e. increase contrast) by moving the UPPER highlight and shadow triangles toward the middle. However, bear in mind that any values to the left of the dark triangle or to the right of the white triangle will all

be assigned to black or white. They will be lost as distinguishable entities and the number of gray levels in the image will be reduced.

Now we need to adjust the middle tones (gamma) of the images. We can do this by adjusting the middle triangle to the left or right until the image looks good on the screen. Adjust the gamma until the image looks good and then make it just a bit lighter to compensate for loss of brightness at the printer.

We now need to re-adjust the highlights and shadows one last time. Before making the final adjustment to the highlights and shadows, open "Show Info" which is found under the "Windows" menu.

Next, position the cursor over the lightest area of the image and read the gray value (K) in the bottom of the "show info" window. If the lightest area is still not bright enough, you can brighten it further by moving the upper highlight triangle toward the middle, If the highlights are too light, you can darken them by moving the lower highlight triangles toward the middle.
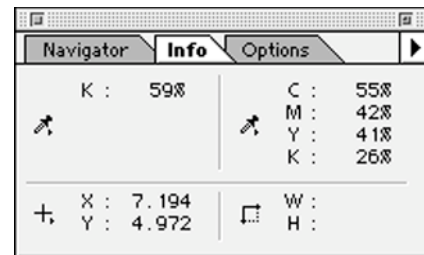
You can darken highlights and lighten shadows (i.e. decrease contrast) by moving the LOWER highlight and shadow triangles toward the middle.

Generally, the brightest highlight should not be any brighter than 3% and the darkest shadow should be no darker than 97%. There are innumerable exceptions to this rule. If there are only a few pixels at one end of the histogram, then saturating them may have little effect upon the appearance of the image. The end result will not only increase the brightness and contrast, but individual intensities will be remapped so that they are separated along the intensity scale.

*A histogram is a bar graph that demonstrates the number of pixels at each intensity level. The intensity scale is on the X-axis with black on the left, and white on the right. The Y-axis represents the number of pixels.

(after Dan Drewling, University of Washington, Printing Services)