Image Processing using the Histogram Tool

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One of the most useful tools in any image processing program is the histogram tool *(located on the IMAGE menu in Adobe Photoshop™)*. A histogram shows the distribution of intensity values in an image.

Histogram A is of a low-contrast black & white image. Notice that the histogram does not show any values at the extremes of the scale. There are no true blacks or whites in this image, only mid-range grays. This is a "muddy" looking image.
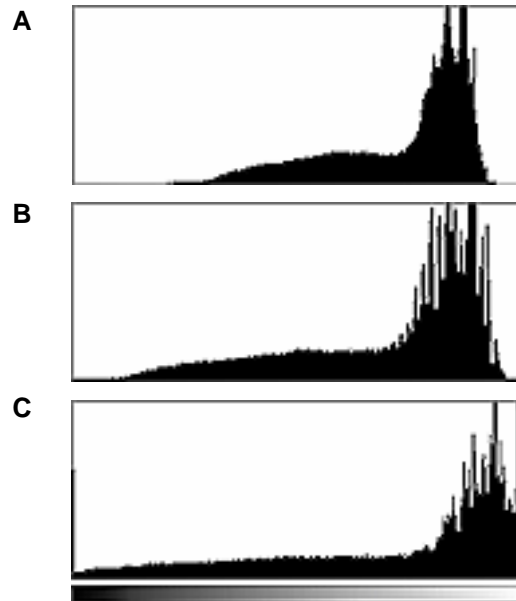
Histogram B shows the image *(after processing with Adobe Photoshop™)* with much better contrast. The image uses most of the available range from blacks to whites.

The best place to improve image contrast is at the beginning, when the user is sitting at the microscope. Try to capture the best images possible, ones that will require little or no image processing. There are several things that users can do to improve image quality, they include:

- Make sure that the microscope is properly aligned *(see our handout on Köeller Illumination)*

- Use only high-quality specimen preparations *(i.e., no artifacts, low background staining, etc.)*

- Use the full dynamic range of the image acquisition device *(e.g., CCD or film-based camera)* without over-saturating or over-exposing the device.

Over-exposing a device causes problems in the image that are similar to those caused by over-exposing film. In certain areas of a film-based image, information is lost because of the over-saturation of the film *(e.g., outdoor photos where someone's face has too much sunlight on it)*. Detection devices *(e.g., cameras, photo-multipliers)* can be over-saturated and lose information as well. Another problem is that the images themselves can also be over-processed such that they lose information.

Histogram C shows what happens when the original image from A was over-processed in Adobe Photoshop, although it could easily be from a piece of over-exposed film or an over-saturated detector. Notice that the information represented by the far left and right sides of the histogram have now been truncated.