BASIC IMAGE ENHANCEMENT FOR DIGITAL MICROGRAPHS (DRAFT)

Introduction

As good as your microscope and camera system may be you still might not be completely satisfied with your original image. It may contain defects such as dust specs, uneven illumination, be exposed incorrectly, some areas may be too light or dark and the detail in these areas not easy to see, or it may simply not look right in your final output. This article will describe a few things you can do to improve your image.

Ethics

Before you start working with your image stop to consider the ethical issues related to what is essentially a process of altering your data. This topic comes up regularly on microscopy Internet news nets and at meetings. People ask if enhancing an image isn't equivalent to fudging the data. Others counter that when done correctly it is no different than smoothing or averaging procedures commonly applied to traditional numerical data, but point out that moving a line or points on a graph is never acceptable. Clearly one would not be allowed to enhance an image when is used to represent the performance of a microscope, camera or film. On the other hand it may be acceptable to enhance an image to correct for defects caused by the microscope such as uneven illumination or dust and scratches on film. Not everyone agrees, however, on whether one can dodge and burn to bring out details, or to enhance contrast, in selected areas of an image. These matters are not limited to scientific applications. The well known photos of O.J. Simpson on the covers of Time and Newsweek for example, where the editors defended their "enhancements" on the grounds of artistic license. It really all boils down to how the image will be used. If there is one hard and fast rule and that is one cannot enhance the image in a way that misleads the viewer or misrepresents the data, and many insist that you keep copies of your original images.

Example

The following is an example of how one can use Adobe Photoshop 6.0 to enhance an image of a microstructure that will be printed on a standard laser printer.

1. Convert to Grayscale

Convert the color image to grayscale. You will loose color information as the image is converted from 24-bit color (8-bits per color: red, blue, green, 256 levels each) to 8-bit gray scale (one color, 256 levels) and your file will shrink to one-third the size of the original.

2. Orientation

If desired, rotate the image so that the long side is vertical. This makes it easier to fit larger images on the printed page (portrait orientation).

3. Size

Resize the image to the size that you want in the final print. Select *Image: Image Size* and make sure the "Proportions" box is checked so that your image does not become distorted and make sure the

"Reseample Image" box is not checked so that the actual image is not changed in any way, either by interpolation to create a larger image at the same resolution or by averaging to create a smaller image at the same resolution. By not resampling no image information is lost and none is added (artificially). Only the size of the printed pixel is changed. Adding pixels by interpolation does not actually add any new information to your image. It will help eliminate the "jaggies" but it will also make the image look a little less sharp and may introduce annoying artifacts such as halos around sharply defined features.

4. Correcting for Uneven Illumination
Correct for uneven illumination or spots in the microscope or camera.

Essentially a background correction procedure.

Uses the layers feature of Photoshop.

Create the background image on a new layer by taking an out-of-focus image in the same microscope, or severely softening the image. Create a duplicate layer.

Subtract one layer from the other.

5. Bringing Out the Details in Dark and Light Regions
Dodging and burning.

6. Liven up a Dull or "Flat" Image
Dull of "flat" images are those that suffer from low contrast or a very limited density range. There are several ways to address this problem.

First, do not use the Photoshop's brightness and contrast adjustment tool. To many people this is the obvious first step but there is another way that offers much more flexibility and produces much better results – adjusting the levels.

Second, use adjustment layers instead of the image adjustments available under the *Image: Adjust* menu. With an adjustment layer the original image is not altered and one can always undo or change and adjustments changes, or one can delete the adjustment layer altogether to undo all changes.

The best place to start is to create an adjustment layer by selecting *Layers: New Adjustment Layer: Levels*. This will display a histogram of levels of gray. Slide the maximum and minimum levels in to the edge of the main part of the histogram. If the distribution is skewed to one end or the other you might try adjusting the average level. If you ever need to change these settings simply double-click this layer in the layers dialog box. If you want to remove it from the image simply delete the layer, or click the visibility check box so that it is not displayed.

Can also make gamma adjustments – *Layers: New Adjustment Layer: Curves*

You may still prefer to use the brightness and contrast adjustments. They are after all, simpler to use and more familiar. Create a new adjustment layer – *Layers: New Adjustment Layer: Brightness/ Contrast* and adjust the brightness and contrast to suit you needs. What normally works well for images printed on a laser printer is to increase the contrast to a setting of about 25-30 and then

increase the brightness to a setting of around 10.

Whichever method you use you'll have to make several test prints since it is the quality of the printed image that is important here, not the appearance on the computer's monitor.

7. Removing Dust Spots and Correcting for Defective Pixels
Try Photoshop's *Dust and Scratches* filter.

Another method involves copying small regions next to a spot or defect to the clip board and pasting it over the defect. Work with the smallest areas possible to minimize problems of color or density gradients.

8. Flatten the Image
Few programs can import or display a Photoshop image file (*.PSD), especially one that contain any of the unique Photoshop features. Layers, for instance, do not exist in the more common image file formats, so if you plan to export to one of these file formats you'll need to flatten your image. To flatten your image select *Layers: Flatten Image*.

9. Exporting to a Common Image File Format
You'll need to export the file to one of the more common image file formats such as tif (*.tif), jpeg (*.jpg), GIF (*.gif) and bit-map (*.bmp). Of the many image file formats in use all can be placed into one of two groups: lossless, or lossy. Lossless formats do not alter the image in any way. Examples of lossless formats are tif and bmp. Lossy formats use compression algorithms that alter, (degrade) the original image. The jpg format is a good example. When exporting to this format you have a choice of optimizing for image quality, which leaves you with a relatively large file, or optimizing for the smallest file size, in which case the image quality is degraded.

Often factors other than file size and image quality must be considered, for instance, when creating images for display in web pages, or to be inserted into video clips, or when the image will go to a print shop that has very specific color requirements. The recommended, and pretty much the standard format in microscopy circles, is the tif format. It is a lossless format and it is supported by many software packages. The optional LZW compression, however, may not be as widely supported.

10. Color Correction
If keeping the color, may want to adjust balance. Two methods.

The first is the Versace method (Rick Oldano, Digital Camera, p38, Oct 2000) involves noting the colors in regions that should be white and black and making corrections using the *Levels* dialog to make the corrections. With *Info* palette visible, use the *Color Sampler* tool to determine the RGB values for a region you want to be white and another you want to be black. Bring up the *Image:Adjust:Levels* dialog and adjust the values of R (red) B (blue) and G (green) by replacing the "0" values with the corresponding color value from the white spot and the "255" values from the black spot.

The second method requires a bit more skill and judgement, and reminder that color imbalance can be due to excess or deficiency in specific colors. Use *Info* palette and *Color Sampler* to determine the values of colors in your image and use the *Image:Adjust:Color Balance* tool to make the

adjustments. Look at the colors in an area where the colors are neutral (a shade of gray). This will tell you what changes need to be made. After the adjustments have been made the R, G and B values should be the same, within 2-3. Different adjustments in the midtones, highlights and shadows may be required, and adjusting the contract and density using the *Image:Adjust:Levels* tool. (Darryl Nicholas, eDigitalPhoto, pp.48-51May/June 2001)

Figures to follow in the final revision.

1. Original color image
2. Converted to a grayscale image
3. Rotated
4. Resized
5. Correction for uneven illumination
6. Livened up image