

Photoshop Distilled: Basic Black and White Image Adjustment for Microscopists

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Ready or not, digital imaging in the microscope lab is here to stay. While I am one of those fogies who insist that digital images are nowhere near as good as photographic prints, I've also bowed to the inevitable, and begun to find ways to assist clients in our multi-user facility with their digital imaging needs. We can acquire digital images from our FESEM, with a CCD camera on our EFTEM, from our light microscope with a CCD, and from a flatbed scanner. Clients use their images for image analysis, transport to others scientists via the Internet or World Wide Web, for reports, slides, overhead transparencies, and publication. Generally, these images need to be manipulated for use, just as one would manipulate a photographic print in the darkroom to correct any deficiencies in exposure or area of view. Adobe Photoshop is the software of choice for image adjustment, accounting for more than 80% of the digital image-editing software market. Photoshop is extremely powerful, and the ethics of using it to manipulate visual data are being hotly debated. I strongly feel that any manipulation beyond what is outlined here would be unacceptable, and I'm sure there are some who will feel that even this is too much, but that is the subject for another article! The learning curve for Photoshop is steep, and the majority of the available books deal at length with color management and effects. We as scientists, however, are more interested in faithful reproduction and presentation of our visual data. To this end, I've tried to distill the contents of many books and a two-day workshop down to the basic techniques of interest to microscopists. The following is a step-by-step mini-tutorial designed to lead you through the rudiments of Photoshop.

Image Adjustment

1. Start up Adobe Photoshop, preferably 5.0 or 5.5.
2. Select **File** → **Acquire** to obtain an image with a scanner or other plug-in, *or*
3. Select **File** → **Open**, then find and select the filename for an existing image, such as one obtained with NIH Image. If your file doesn't have an extension recognizable to Photoshop, click on Show All Files to find and then rename it.
4. **Crop** the image if necessary.
 - You may crop a few pixels around an image by selecting **Image** → **Canvas Size**, selecting a smaller size than your image, and placing your image in the center or to one side.
 - For more cropping, use the **Crop Tool**. Hold the mouse button down over the selection tool in the Toolbox (top left) to get a pop-up menu of selection tools, and select the **Crop Tool**.
 - Drag the crop marquee around the portion of the image you want to keep, then double-click inside it or go to **Image** → **Crop**.
 - In 4.0 and higher the Crop Tool may also be resized and rotated before cropping.
5. Adjust image tones:
 - **Select Image** → **Adjust** → **Levels** and get the gray levels histogram
 - Adjust input levels:
 - Move the left, black arrow under the histogram to the right to select the darkest input pixels, or enter a value in the left

most Input Levels option box (e.g., 20).

- Move the right, white arrow to the left to select the lightest pixels, or enter a value in the rightmost option box (e.g., 235).
 - For **midtone**, or **gamma correction**, move the middle, gray arrow to the left, or enter a value in the middle option box (e.g., 1.8). Be aware that in order to get a good print you will need to adjust your on-screen image until it shows the full range of grays but overall looks too light! This is due to the fact that the image you see on your monitor has already had gamma values applied to it that will not be sent to your printer.
 - Click on OK. Check image, and re-do gamma correction again, if needed.
6. Determine if image needs sharpening. Check your ethics barometer; will this change your data?!
 - View image in the size it will be printed with **View** → **Print Size**. Resampling or resizing up (not a good idea) or down changes the apparent sharpness. Best sharpening is accomplished by-
 - **Filter** → **Unsharp Mask**
 - Select a value in the dialog box (e.g., 144%). Values less than about 25 will be subtle and more than about 300 will be too dramatic.
 - Select a radius of perhaps 1 to 4 pixels.
 - Select a threshold of perhaps 0 to 16 levels.
 - Click OK.
 7. Save your file using **Save As...** and select a name, format and location for it.
 - For greatest fidelity and transferability between computer platforms, select **TIFF** with no compression.
 - TIFF with LZW compression will compress the file size some what with no loss of information, but may not be accepted by some computers.
 - You may have to try several file sizes and formats to see which works best for your printer, slidemaker, or other media.
 - Compressed formats such as JPEG are lossy compression schemes, meaning that some information is lost. Depending on the application this loss may be unnoticeable. For images to be viewed on the web, Photoshop 5.5 with Image Ready has an intelligent compression scheme that works well to shrink file sizes, and I recommend it.

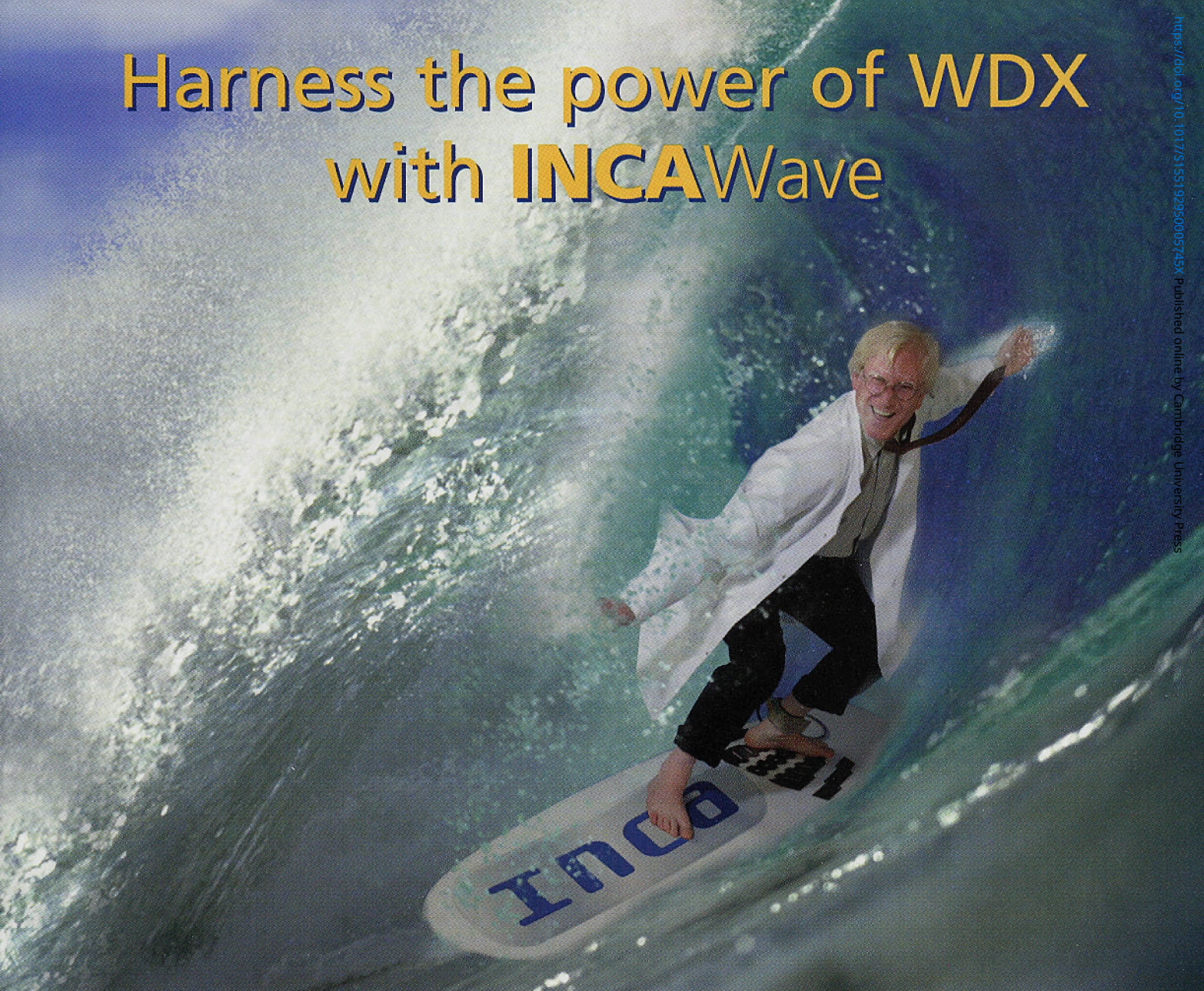
Applying Magnification Bars

Applying a magnification bar in Photoshop makes sense because then it is always available with your image, no matter what its ultimate fate, such as reduction in print or as a slide. Other labeling with text and arrows can be done either with Photoshop (the subject of the next section) or, perhaps more effectively, with additional programs such as PowerPoint, PageMaker, Quark Express, etc.

1. With your image file open, go to **Window** → **Show Rulers**. You may set the units to centimeters, inches, or picas in **File** → **Preferences** → **Units**.
2. Select the color you want your bar as your foreground color. To get true black or white, click on the tiny black and white rectangles at the bottom of the Toolbox; black will be the foreground color. White can be brought to the foreground by clicking on the curved arrow pointing between the two.
3. Double-click on the **Line Tool** (it's bundled with the pencil tool in some versions) to get the options palette. Select **Anti-aliased**, **Normal**, and 100% opacity. Enter a line width (e.g., 4 pixels) and be sure that Arrowheads Start or End are not selected.

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4. Select **Windows → Show Layers** to get the Layers palette. Click on the folded paper icon at the bottom to make a new layer. Making a new layer each time you want to add to your image allows you to move the individual elements.
5. Use the **Line Tool** cursor lines (faint dotted lines) at the top and side rulers to position and measure the length of the line that you draw by pressing the mouse button and dragging on your image. If you made it on a new layer, you may move the line around with the **Move** tool.
6. If you made a mistake or do not like your line, use **Edit → Undo** before you do anything else, or delete that particular layer.
7. Make sure you remember what this magnification bar represents. You may append notes to the image by selecting **File → File Info**. This information will be available to you each time you open the image.

Labeling images in Photoshop

Addition of type to digital images is best done with software designed for that purpose, such as QuarkExpress, Adobe PageMaker, or Microsoft PowerPoint. With Photoshop, type is treated as another bitmapped image and, as such, will exhibit pixelation at low resolutions. However, at high resolutions the jaggedness is not so apparent. Check the anti-aliasing box in the Text options palette if you wish to have Photoshop soften the edges of the type. Type applied to an image will have the same resolution as that image.

1. To apply text to an image, click on the **Type Tool (T)** in the Toolbox. Your cursor will become an I-beam. Position this cursor over the image approximately where you would like the type and click. You will get a dialog box.
 - Select Font, Size, Style(s) and Alignment.
 - Type your label in the box, then click OK.
 - Your text will appear in the foreground color and as its own layer (5.0) or as a temporary or floating layer (older versions).
 - While selected or in its own layer, text can be moved with the Move Tool or with the keyboard arrows. If you make a mistake, the type, while still selected, can still be deleted or thrown in the trash in the Layers palette.
2. To apply arrows to an image, double-click on the **Line Tool** in the Toolbox to see the Line Tool Options palette.
 - Select Line Width in pixels (e.g., 4), Anti-aliasing, Normal, and 100% Opacity.
 - Select Arrowheads Start or End, depending on whether you want the arrowhead to start where you begin to drag your cursor, or end where you stop your cursor.
 - Select Shape, Width (e.g., 400%), Length (e.g., 600%), and Concavity (e.g., 16%), the amounts of which are related to the Line Width. Click on OK.
 - The arrow will be in your foreground color, so check it.
 - Your best bet is to place arrows in their own layer. Go to **Window → Show Layers** palette and click on the little folded paper icon at the bottom, or to the right-pointing black triangle on the top right and select New Layer from the pop-up menu.
 - Drag the mouse across your image to make an arrow. You may change or rotate the arrow with **Edit → Transform**. Doubleclick to make it stick.

- Remember when you use the Move tool, it will move the entire layer.
- Save As a new filename so that the labeled image stays separate from your unlabeled one. Trust me, you may want your unlabeled image back someday!

Page Layout

Preparation of digital figure plates for publication is best done with software designed for that purpose, such as QuarkExpress or Adobe PageMaker. However, if you have only Photoshop in your repertoire, you can still tackle page layout.

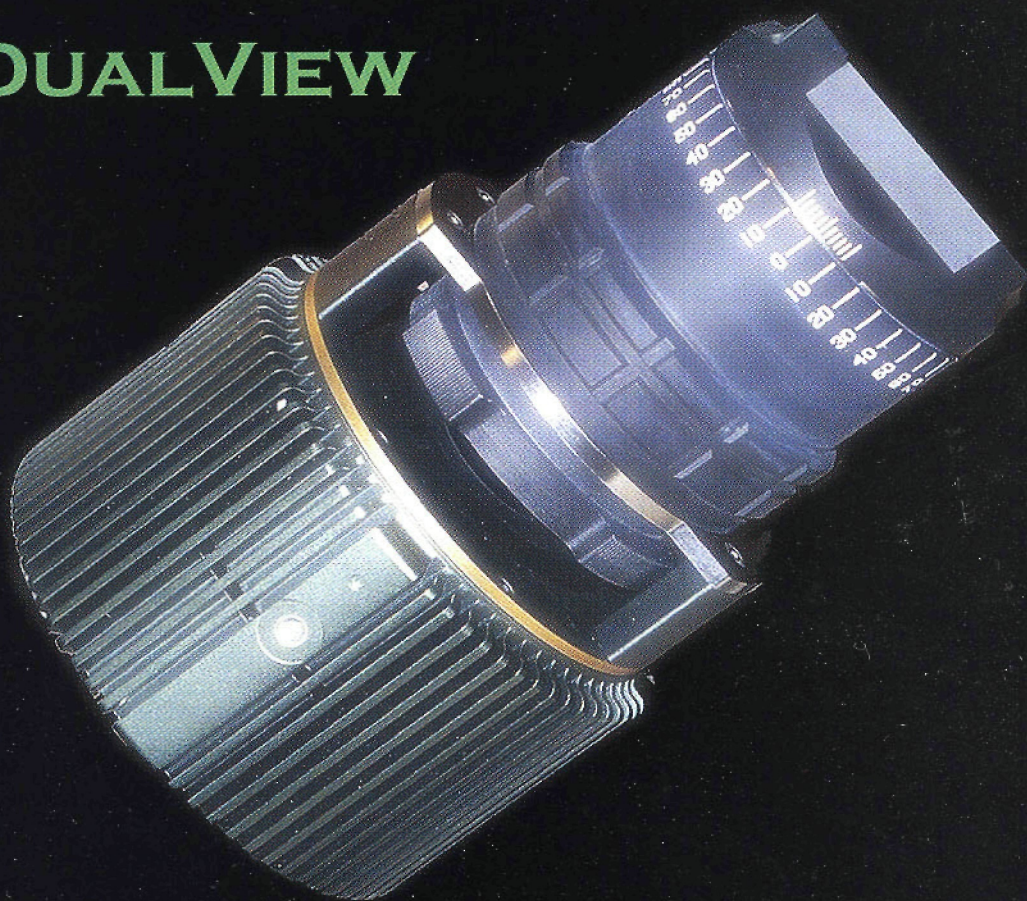
This method utilizes the Grids and Guides found in Photoshop 4.0 and higher.

1. Go to **File → New** and fill in the dialog box:
 - Name the new plate.
 - Enter the page Width in appropriate units (see **File → Preferences → Units**).
 - Enter the page Height.
 - Enter Resolution in pixels/inch.
 - Select Mode (e.g., greyscale).
 - Set the Contents (background) to white.
 - Click OK.
2. Now **File Open** all the images wanted to combine in the plate.
3. **Crop and Adjust Levels** if necessary, then size it to fit in your figure with **Image → Image Size...**
 - In 3.0 make sure Constrain: Proportions is checked and File Size is not. Enter Width and Height values.
 - In 4.0 make sure Constrain Proportions box is checked and Resample Image is not.
 - You may choose to resample (change the resolution) at this time. Remember that resampling up to a higher resolution means that pixels will be invented by Photoshop! Resampling to a lower resolution means losing pixels, but at least data is not invented. In either case, further image adjustments, such as gamma and sharpening, may be needed.
4. Set up your **Grids and Guides**:
 - Go to **Window → Show Grid (4.0)** or **View → Show Grid (5.0)** and set up the number of lines and divisions.
 - Go to **File → Preferences → Guides and Grids** and select a line type and color for guides
 - Guides are made by depressing the mouse button and dragging from a ruler line to the grid position you want the guide line(s). Make a left vertical and a top horizontal guide.
5. Go to the image you want on the top left of the plate. **Select → All** then click on the **Move Tool** and drag and drop the image to the new file. The image will have become a new Layer and can be moved with the Move cursor and/or keyboard arrows as long as that layer is selected in the **Window → Palettes → Show Layers** palette. Place the image against the upper left guide of the blank white sheet. When near the guide line, it will "snap" to it.
6. Position more guides as needed to define the gutters between the individual images. Continue to select and move images to the new plate and position them as desired. Increase magnification to see fine adjustments.
7. In 4.0 and higher each part of the plate is still a separate layer, and each may be operated on individually by selecting that layer. To operate on the plate as a whole, the layers all need to be Merged, or the image needs to be Flattened, both of which can be found by clicking on the right pointing arrow on the top right of the Layers palette.
8. The image may be saved with its layers intact as a TIFF or PSD (Photoshop) file, but must be merged or flattened for most other file formats. ■

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