

## Creating pseudocolored images in Photoshop

The method for creating pseudocolor in Photoshop can be done in few steps, but the image will not be colored in the conventional way. The conventional means for producing pseudocolor comes by using a look up table (LUT). This LUT is an overlay for making a range of colors according to choices provided by the software. The colors are matched to levels of brightness and darkness in the image (grayscale pixel values), typically from a short to long wavelengths. In that scenario, violet colors are matched to the black and near-black pixels and red to those close to absolute white. Blue, yellow, green and orange become the mid-range pixel values.

The beauty of the LUT method for coloring images lies in the ability to see pseudocolor “live” while imaging. Also, many software programs provide a means for editing the range of colors to amplify differences in pixel values when shifts in pixel densities/intensities are subtle, or when researchers want to show differences at thresholds where colors change (a pseudocolor bar included with the image also needs to be edited in the same way and shown).

The bane lies both in the difficulty understanding how to make these changes in software programs and in the frustrating inability to save the pseudocolor image with the *Save* function. The latter doesn't make sense until one understands that the pseudocolor is really a temporary overlay: the computer is “looking up” the colors for immediate display on the monitor. Therefore, the pseudocolor does not exist as a change to the image itself.

You can capture these images, however, by simply holding down the control key on a desktop PC and by pressing the *Print Screen* button, often at the top, right of the keyboard. On a Macintosh, you can hold down the Apple, Shift and F3 key at the same time (at least in pre-OS X operating systems). You will hear a sound like the click of a camera shutter to denote that your screen has been saved.

In both cases, the image is copied to random memory. You will have to open Photoshop, under *File* choose *New*, click *Okay* without changing any settings; and then, under *Edit*, choose *Paste*. An image of the entire screen will appear on your new file. Use the crop tool to surround the pseudocolored image and double click inside the cropped area to complete the crop. This image will contain as many pixels across and down as it did on the computer screen and that resolution depends on the settings for your monitor. Thus, the greater the settings for pixels across and down for the monitor, the greater the resolution will be in your copied image.

If you would like, instead, to color these images in Photoshop, and to make changes to the pseudocolor (and to the image itself), you can do so by following these steps:

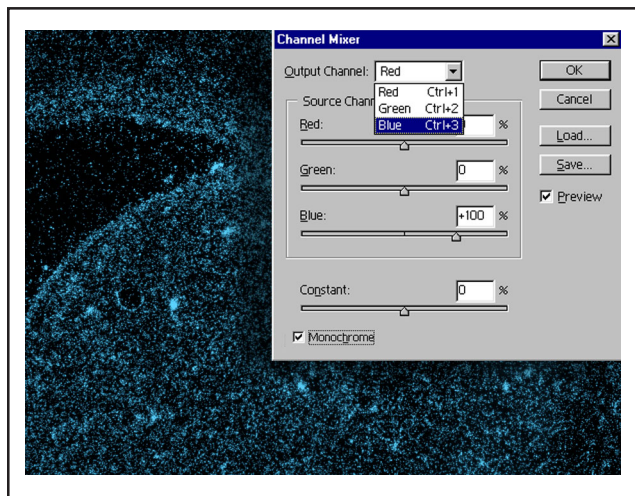


Figure 1: Use Channel Mixer when changing the mode of RGB color, fluorescent images to grayscale. The image above was collected with blue illumination and saved as RGB color. Blue is selected as the Output Channel and the Monochrome box is checked to make the image into grayscale values.

1. **Make the grayscale image Indexed Color.** Under *Image* on the menu, choose *Mode* then *Indexed Color*. Indexed colored images contain 256 grayscale or color values, as many as contained in an 8-bit image.

This assumes that the image is grayscale. If the image is in color, then change the image to grayscale first. For variously colored images, such as those collected on a brightfield microscope, simply change the image to RGB: under *Image* on the menu, choose *Mode* then *RGB Color*. In this scenario, the green channel is chosen as the grayscale image, what most matches what we see by eye.

If the image contains shades of one color only (such as fluorescent images), then change to grayscale first by using the appropriate channel. Under *Image*, choose *Mode* then *Channel Mixer*. Select the *Output Color* that matches the color of your image (e.g., if your image is red, choose *Red* as the *Output Color*) and check the *Monochrome* box at the bottom, left of the dialogue box

Note: you may get a dialogue box indicating a “palette” recommended by Photoshop. It should read “Exact” and

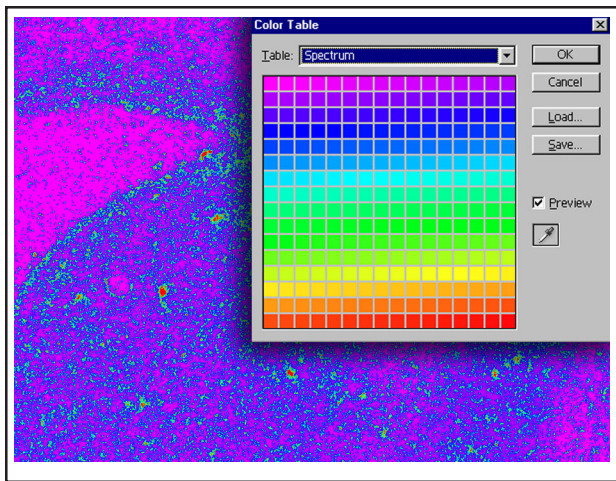


Figure 2: To pseudocolor the grayscale image, change the mode to *Indexed Color* and then choose *Color Table* in the *Image > Mode* menu. Select *Spectrum* for the Table.

the same figure.

Changes in either the image or in the pseudocolor table require the inclusion of a color bar so that any alterations can be done on both the image and the bar. Viewers can then make sense of these changes by looking at the bar.

**3. Make a color bar.** Under *Image* on the menu, choose *New* and specify the height and width of the bar (400 X 30 pixels works for me). Choose *Grayscale* for *Mode*. Near the bottom of the toolbar, click the small black and white squares under and to the left of the foreground/background squares. That will force the foreground to be black (0 on an 8-bit scale) and the background to be white (255 on an 8-bit scale). On the toolbar, click on the gradient tool. Under the menu, click on the gradient icon in the long rectangle to reveal drop down box. Choose the black to white gradient icon. Carefully find the extreme left end of the new image and, while holding down the shift key, click and hold while moving the cursor to the extreme right end of the new image. Unclick when the right end is found and a gradient from black to white will form with all the gray values in between. To make sure you found the left and right ends, check the image by moving the cursor across the gradient while looking at values in the *Info* box. The black end should read 0 and the right 255 with all other values in fixed increments along the gradient.

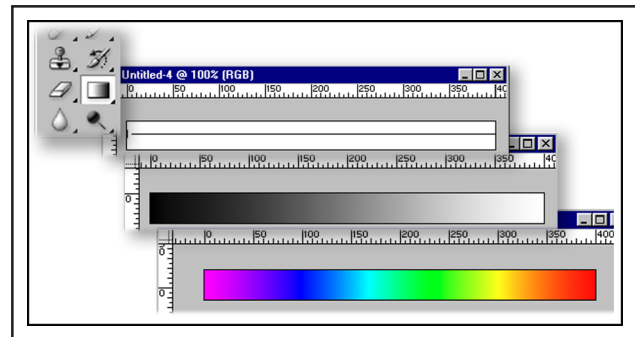


Figure 3: To make a pseudocolor bar, choose the gradient tool from the toolbar (top left), and drag the cursor from one end of a *New* file to the other while holding down the shift key. A grayscale gradient from black to white with all values between will be created. Change the *Mode* of the image to *Indexed Color*, then choose *Color Table* and *Spectrum* to pseudo-color gradient.

Now make the grayscale into pseudocolor by changing the *Mode* to *Indexed Color* (under *Image > Mode > Indexed Color*). Choose *Color Table* under *Image > Mode* and choose the *Spectrum* table to make into ranges of colors. You may also want to label the bar to indicate where incremental grayscale values lie.

Make sure that you place the color bar on the same page as your image so that all changes to the color table will affect both.

the forced output should be *Black and White*. In that manner, the grayscale values will be preserved. Other palettes adapt grayscale values for various outputs, such as the black and white values that best fit those of the web.

**2. Choose pseudocolor table.** Under *Image* on the menu, choose *Mode* and then *Color Table* to choose the pseudocolor table. The only available table that is typically used in biology would be *Spectrum*. If your image contains values from deep black to near white, then the colors may appear as desired.

If, however, the colors do not convincingly reveal desired regions of interest, then two choices follow: either change the image itself to fit the pseudocolor table, or change the pseudocolor table to fit the image. Most researchers would choose the latter, because the image is raw data and it should not be tampered with. If grayscale values are altered in the image, then only linear changes in histogram values can be made: the white and black sliders can be used in the *Levels* dialogue box. The gamma (middle) slider cannot be used because it results in logarithmic changes. Any changes to one image must also be made to other images included in

Note that the colors are not fixed by equal increments: blue and green and red take up an inordinate amount of grayscale values compared to violet, cyan and yellow.

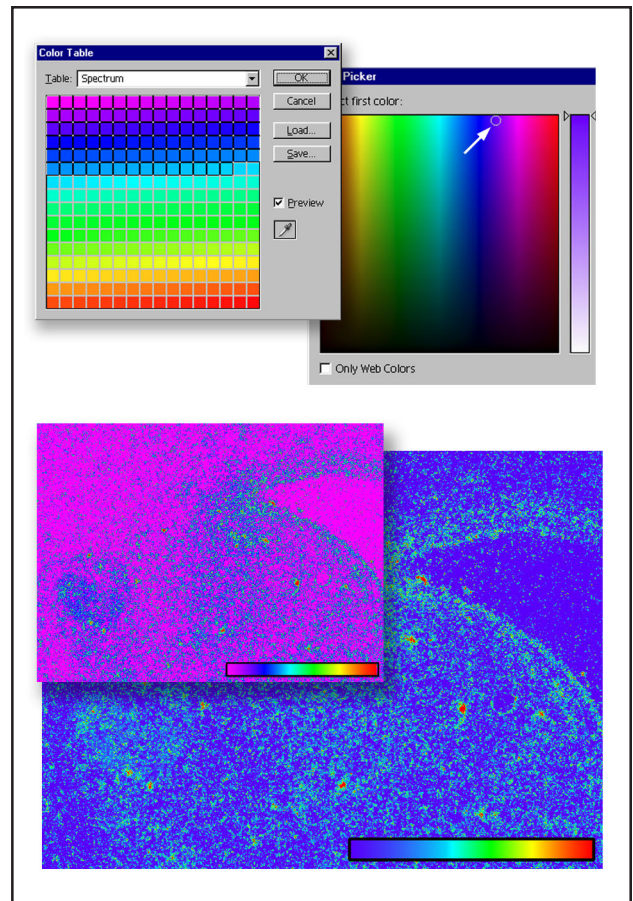
4. **If desired, change the pseudocolor table.** Changes in the table will require an investment of time and trial and error. Perhaps the best way to begin would be to visually determine what values would be regarded as background on the image and then change those colors in the image and the pseudocolor bar. These values are often placed between violet and the blue just shy of cyan.

To alter the colors on the table, under *Image* on the menu, choose *Mode* and then *Color Table*. Drag the cursor over the violet-to-blue or violet-to-cyan squares to highlight all of them. You will get a second dialogue box to indicate the first color (if you see *Web Colors* checked at the bottom of the *First Color* dialogue box, click to uncheck to make it easier to navigate the color spectrum). Click on violet color to choose it (or color of your choice), click *Okay*. Then you will get a second dialogue box asking you to choose the second color. Choose a blue near cyan or cyan (or another color of your choice), click *Okay*. The image will now show large areas of violet and blue showing background levels. Be sure to save the color table before exiting, because you will want to apply this table to subsequent images in a figure.

If you did not get what you desired, click cancel and start again, this time choosing different ranges of color squares in the Color Table dialogue box or choose different First and Second colors. Continue to re-color other grayscale levels by choosing colors of your choice in the same manner.

With a little work, this method should give you the color table you desire without the necessity for changing grayscale values on the image itself. Be sure to alter colors for publication photos when you are required to submit CMYK images to obtain good reproduction according to methods described in earlier editions of *Microscopy Today*.

Jerry Sedgewick, copyright 2004.



*Figure 4: To change colors in the pseudocolored image, edit the Spectrum color table. For the images above, the violet color made the background visually confusing. To re-color the background, the cursor is dragged across color squares matched to darkest pixel values in the image (top left). The Select First Color dialogue box appears and blue color is selected from a color swatch by clicking on it (top right). Another dialogue box appears (Select Second Color) and a color close to cyan is selected from the color swatch (not shown). Bottom left image shows background colors before changes, bottom right image shows new background colors.*

