Merging images to form a color composite

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An article entitled "Combining Pictures for Electron Microscopists" appears in the July 2008 issue of Microscopy Today. Unfortunately, as was the case for a previous series of articles printed in this magazine purporting to show how to use Photoshop, the procedure shown and the explanation of the steps is entirely wrong. The authors have devised an extraordinarily cumbersome sequence of operations for accomplishing a very simple result, and in the process have confused themselves and any potential reader about the important differences between (and the correct uses of) layers and channels in Photoshop.

The premise is that three different images of the same field of view have been recorded using an SEM, for example by using different accelerating voltages, or different detectors. However, the same procedure is useful when three images are acquired from a light microscope (or any other imaging device), for instance with different colored filters. It can also be useful when acquiring images using different modes in an AFM. The goal is to merge the individual images into a color composite, and if necessary perform minor alignment amongst the three images. The correct, simple and direct procedure is this:

1. Open the three images in Photoshop. It is assumed that they have the same pixel dimensions and are each gray scale images. (Some instruments record the images as "indexed color'; if that is the case, convert each one to gray scale by selecting Image -> Mode -> Grayscale.)

2. In the Channels palette, click on the icon at the upper right corner to produce a drop-down menu. (If the Channels palette is not visible, you can bring it up by selecting Window -> Channels in the menu bar.)

The Channels palette in different versions of Photoshop. Click here for the drop-down menu.

3. Select Merge Channels in the drop-down menu, and in the dialog that appears select RGB Color mode. Then assign each of the three images to one of the color channels (red, green and blue) and click OK.
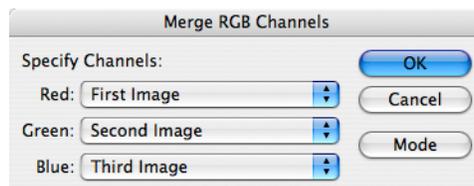
4. If the images are well aligned, and the relative brightness and contrast of each are acceptable, the job is finished and the final image can be saved, printed, etc. If not, the individual channels can be adjusted. This can be done most easily by clicking on the name of the channel to be adjusted (e.g., 'Red') and then making all three channels visible for a full color presentation. That is done by clicking on the eye icon for the top line, labeled 'RGB'.
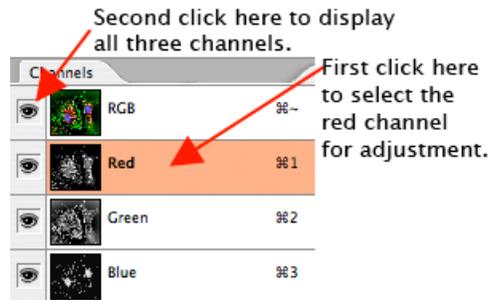
5. The selected channel can be adjusted in contrast and brightness, without altering any of the other channels, using any of the usual Photoshop tools such as Image -> Adjustments -> Levels or Image -> Adjustments -> Curves. Since the color composite is being viewed while the one selected channel is being adjusted, the overall appearance can be seen.

6. To align the highlighted channel image to the others, choose Select -> All from the menu. Depressing the Control (Windows) or Command (Mac) key and dragging the image in the highlighted channel can now be done with the mouse, but for greater control hold down the Control (Windows) or Command (Mac) key and use the arrow keys to move the highlighted channel image one pixel at a time in any direction. It is best to enlarge the image to at least 100% to facilitate comparing the position of features in the different channels. Since the color composite is being viewed while the one selected channel is being shifted, comparison is made easy.

7. Once the channel image has been highlighted and selected as above, it is also possible to use the Edit -> Transform -> Scale function to resize, shift, rotate or skew the image for alignment purposes. This can be done either by clicking and dragging on the bounds of the image, or (more easily in most cases) by entering numbers in the options bar.
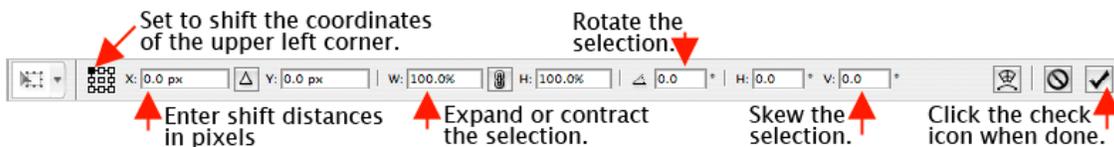
Note that this procedure applies to merging images as red, green and blue. It does not address the interest that is often expressed in colorizing SEM images by selecting specific features and applying a 'color wash' to give different structures different, distinctive color tones. For a step-by-step tutorial on that technique, and a complete introduction to the proper use of Photoshop for scientific imaging, please go to the "downloads' page on my website <DrJohnRuss.com> and download the tutorial pdf files on these topics.