Lab 2 Assignment – Part 2: (Due two weeks following the fluorescence lab) (10 points)

Each individual should prepare one set of corresponding phase contrast and fluorescent images and an accompanying figure legend. The file containing the figure will be submitted to as JPEG files to the "Lab assignment" page on the course web site. The figure legend should be written separately and printed out and handed in to your lab instructor.

From the digital images that your group produced, each member of the group should select a fluorescent image and its corresponding phase contrast image to use in preparation of the figure. You will have used two different fluorescent stains; choose the image that appeals to you most. A scale bar should be drawn on the images and a figure legend (as a Word document) should be prepared. These figures can be prepared using Adobe Photoshop as described below. They should be "ready for printing," but will not be actually printed.

How to write a figure legend:

A **figure legend** should contain the figure number, an informative title, and all the information a reader would need to understand the figure. This information includes the identity of the sample (e.g., what was the cell type?), the treatment it has undergone, the method of data collection (or how it was viewed in the case of microscopy), a description of the information that appears in the figure, a definition of any symbols used in this description, and in the case of a micrograph, the magnification of the final image (although this is not necessary if the size of the scale bar is indicated on the image). Double space the figure legend.

Criteria for evaluating assignment:

The micrographs/digital images:

- Are all the required items included?
- Do the pictures portray accurately the sample being shown, and reveal a level of detail normally visible in such a preparation?
- Are the images prepared according to the instructions given?
- Are the images properly labeled?

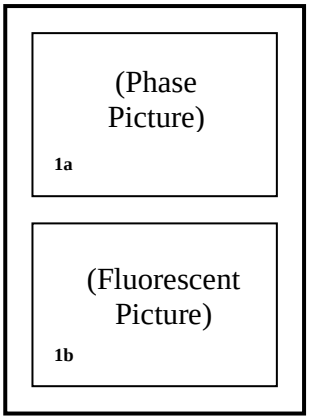
Figure legend:

- Are all elements as described in "How to write a figure legend" above included?
- Do these clearly, accurately and completely describe what is shown in the figures?
- Are both the phase contrast and fluorescence images described?
- Are all symbols identified?
- Is the magnification or scale bar information accurate?

Preparing Figures for Bio 29 using Photoshop CS3

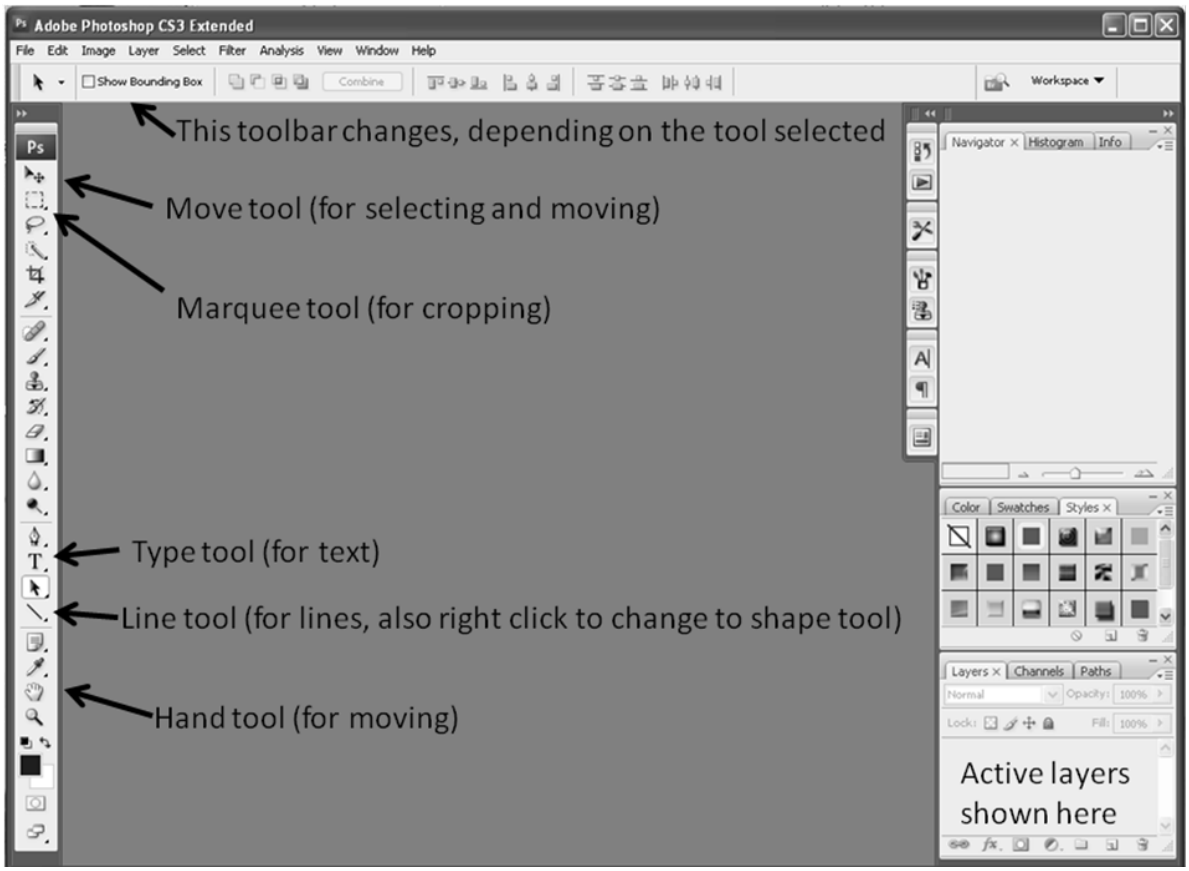
(Photoshop is available on college computers, and you can load it from the college web site, for instructions see https://www.amherst.edu/offices/it/help/software/college-install)

As a group, you have collected images of cultured cells using various fluorophores and fluorescence microscopy. You should individually prepare a set of figures and figure legends from the images your group collected, using Adobe Photoshop as described herein. You will submit the figure to your lab instructor via the course web site, and you should print out the figure legend separately to hand in to your lab instructor.



Each of you should pick out one pair of corresponding phase and fluorescence images from one of the stains your group used. You should make one figure for each pair of pictures (example is shown at the left). The two pictures that are part of each figure would probably look best if aligned one above the other. You should also include a figure legend for the figure, making sure that you describe and refer to both pictures. The completed assignment will therefore contain an image file in Photoshop format and a Word document with the figure legend.

Below is an open window of Photoshop, showing windows, toolbars, and some of the commonly used tools. You may wish to refer to this as you begin working with the program:



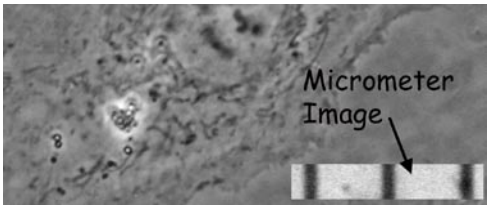
Using Photoshop CS3to make figures:

1.Start **Adobe Photoshop** (download from K drive, or start from the "Programs" menu on other computers). **Note**: Be sure that you are opening Photoshop CS3. A new file window opens. This will be the page that you paste your final figures into. Set the size at 8.5 inches wide, 11 inches high, 300 pixels/inch resolution, and RGB color, 8 bit. **Click OK.** (300 pixels/inch resolution is typical for a photo-quality printer; even though we won't be printing these files, work in this resolution). Be sure the rulers show on the image (**View > Ruler**) and are in inches (on a PC, right click on ruler to change to inches; on a Mac, double click on the ruler to change to inches).

2. Open the first picture you want to work with **(File>Open).** Save this as the "original image" and from now on work on a copy. To do this, duplicate the image **(Image> Duplicate)** and give the new copy of the image a new name. Close the window with the original (unduplicated) image. (This way you can always go back to the original image and re-do any manipulations from the beginning). Be sure the rulers show on your working copy of the image (**View > Ruler**) and are in inches

3. Determine the size of the image **(Image > Image Size)**. The image size will be several inches on a side at 72 pixels/inch. This is typica**l resolution for a comp**uter monitor, but makes the image very large. Therefore, change the resolution to 300 pixels/inch for print quality. Be sure that the "Resample image" box is *unchecked*. The image size should now be about 7 X 5 inches, a more reasonable size to work with (you will notice that the ruler size at the top of the window has changed to accommodate the new scale). *Note*: Remember to save the image along the way, so that your hard work won't be lost! You may want to save intermediate stages of the figure preparation as distinct files so that you can go back to them if necessary.

4. Now open an image of a *stage micrometer* taken with the same objective lens and microsope you used to take the picture you are currently editing. Overlay this image onto your working image before you do any further resizing of your image. Images taken with each objective are available in the "Micrometer" folder on the computer next to the microscope you used. These images have been cropped for ease of use, but are at the same resolution as your images (300 pixels/inch). Copy these to your network space if you will be working on these images outside of the lab. Open the appropriate micrometer image **(File>Open). Select>All, Edit>Copy.** Switch to the picture file; **Edit>Paste.** Use the Move Tool (the arrow at the top of the tool bar on the left side of the window) to select and reposition the micrometer image by clicking and dragging it. You will eventually delete this micrometer image, but for now, keep it with your picture so if you resize the picture, the micrometer image will resize as well.



Notes on working with Layers: Notice that each element you add to the image, whether it is a new picture, text, or drawing, etc., is treated as a separate *layer*. These are visible on the right side of the window (if you don't see them, select **Window > Layer** and they will show). These layers are convenient in figure preparation because they allow you to manipulate one layer without disrupting the others. Click on a layer to activate and change it; right click on a layer and select "delete" to remove that layer (alternately, use the "layer" dropdown menu and select "delete." However, when you need to manipulate all parts of the figure at once, you must flatten the layers into one (see step 12). Don't flatten the image until you are sure you want to keep all changes you have made to it! Layers cannot be changed or removed once the image is flattened.

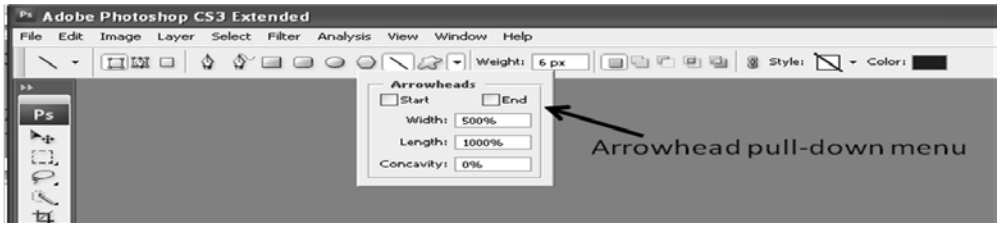
5. If desired, crop the image to eliminate unnecessary parts. Make sure you have highlighted the layer that is that image, use the Rectangular Marquee tool in the toolbox to select the region you'd like to keep, and then go to **Image > Crop** to execute the crop function. You should take care to crop corresponding fluorescence/phase images in the same area and with the same dimensions (use the rulers to determine where to crop so you crop both images over the same areas). Also, do not crop the image of the stage micrometer! Move it to an area of the picture you will keep before you crop the image.

6. If you have cropped the image, resize it to the size you would like it to be in the final document **(Image > Resize > Image Size)**, keeping the resolution at 300 pixels/inch (the "Constrain Proportions" and "Resample Image" (Bicubic) boxes must be checked to do this). We suggest approximately 6 x 5 inches. You may not be able to get these dimensions exactly, but a similar size should be fine. The scale micrometer will change along with the rest of the image.

7. Adjust the brightness and contrast if desired. Go to **Image > Adjustments > Brightness/Contrast.** Make sure preview box is checked and move the small triangles back and forth to try to improve the image. Clicking "Cancel" will return the picture to its original state. If your phase image is colored, be sure to convert it to grayscale, use "image> mode> grayscale" then "discard."

8. Add labels using the Type Tool (T) in the tool bar. Click on the Type Tool and notice that various options appear at the top of the image. Use black or white font color and Arial font in an appropriate size (start with 12 pt.; you can always change it later). Select the desired spot to label on the image, click and start typing. Notice these two icons that appear at the top right: ⊘ ✓ . The circle/slash is the "cancel" icon and the checkmark is the "commit" icon. If you change your mind about where you want to place the type, click on the "cancel" icon; once you are satisfied with the label you have typed (its size, color, placement, etc.), you must click on the "commit" icon before you can move on. If you want to change the location of the text after you have committed, select it with the Move Tool and drag. Alternately, select the layer that contains that text, delete it (right click, delete) and start again. Use abbreviations to label structures (e.g., N for nucleus) and then explain in your figure legend what the abbreviations represent. You may want to label prevalent features on the image, but be sure that the labels don't detract from the overall quality of the image!

9. You can also use arrows if you'd like to point to different features. Draw a line using the Line Tool in the tool box. If the Line Tool isn't showing, click on the Shape or Rectangle Tool. A shape tool bar now becomes visible in the upper left of the screen; select the line. (Alternatively, you can right click on the Shape Tool and select the Line). To add arrow-heads to the line, before you draw it, click the small arrow at the left side of the shape tool bar at the top of the screen to find a pull-down menu labeled "Arrowheads." Choose the arrowhead settings you would like to use. To change the thickness of the line, change the "Weight" of the line. Remember that to delete or change an item, you must select the layer it is in and then you can act on it.



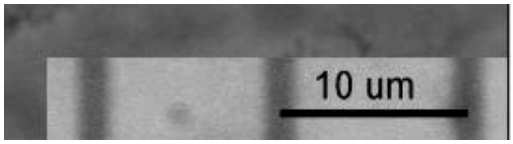
Other changes you may want to make to a layer before the image is flattened:

To change size, font, color, etc. of text, select from the Layers Palette the layer that contains the text you want to change. Then use the text tool bar at the top of the screen to select the changes you want to make.

Sometimes it is helpful to use a contrasting color on an arrowhead e.g., use a white arrow against a dark background or vice versa. To do this, select the appropriate layer and use the tool bar above to select a color. Feel free to experiment with your images, but save intermediate images along the way! Also, use the "Undo" command if you don't like the result!

Remember that if it seems Photoshop isn't letting you "move on," you may have to click on the "commit" or "cancel" icon first. ⊘ ✓

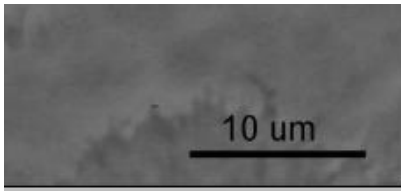
10. Now use the line tool (without an arrowhead!) to generate a scale marker. Here is where you use the image of the scale micrometer that you added earlier. Position it somewhere near the lower right corner of the image. Each division on the micrometer equals 10 μm. From the upper toolbar, select white or black for your line, depending on the darkness in that part of your image, and a thickness (weight) that will be easily visible. Click in the center of one of the scale markers and draw a line across one or more of the divisions, ending in the center of another marker. The number of divisions you cover will depend on the magnification of your image; don't make it so long that it is distracting. Note that this is just a small corner of a much larger image:



If you can't see the line you have drawn, it is because that layer is behind the layer containing the micrometer image. Select the layer containing the micrometer image, then **Layer>Arrange>Send to Back.** This should place that layer under all of the others, except the background layer.

11. Using the text tool, type a line of text just above the line, indicating what its length represents (e.g., 10 μm).

12. **Select the layer containing the original image of the scale micrometer and delete it by right-clicking on that layer and selecting "delete."** The scale marker and text that you added will remain, because they are in separate layers:



13. Flatten the image when the image looks exactly how you want it to **(Layer > Flatten Image)**. Remember that this will condense all the layers into one, and you will no longer be able to work on individual layers. (Be sure you have deleted the image of the scale micrometer before you do this!)

14. As you finish working with each picture, copy and paste the 2 images for one organelle (a phase contrast and a fluorescent) into a new blank document. You opened one when you started the program, and you can use that for the set of figures. If you need to open another blank file, use **(File > New)**. This document should be 8.5 x 11 inches, 300 pixels/inch, and have a white background. To copy and paste images into the blank document, after you have flattened the image, **Select > All, Edit > Copy.** Switch to the blank document, **Edit > Paste.**

15. Flatten the image when the image looks exactly how you want it to **(Layer > Flatten Image)**.

16. Save the final image (corresponding phase contrast and fluorescence images for a particular stained organelle/structure) as a JPEG file (.jpg extension). Also prepare a Figure Legend as a text document. Submit the figures by uploading them onto the course website "Laboratory>Lab assignments" page, submit the figure legend by handing them in.

    To Upload:

    In contents toolbar click on "add" then on "file upload" in the menu that appears. Browse for and attach your file. Important: remember to give it a title (your name), otherwise they will all look the same on the page.