

# Using ImageJ as a Tool to Analyze and Process Biological Images

BI-102, Fall '07, Drs. J. A. LaMack & C. S. Tritt (v. 1.0)

ImageJ is a public-domain Java image processing program supported by the NIH. It is used by biologists extensively to analyze, enhance, and process biological images obtained using microscopy or other types of imaging. This document will describe how to obtain and install ImageJ, as well as how to perform a few basic tasks using the program.

## Installing ImageJ

Download ImageJ from: <http://rsb.info.nih.gov/ij/download.html>. Choose the correct platform for your machine, probably the Windows version without Java. Save the file somewhere you can find it (like c:\downloads), and then run it. Proceed through the prompts to install the program.

## Running ImageJ

Open the program. This will bring up a toolbar (**not a window**). To open an image (and a window), go to File | Open.

ImageJ supports all standard image formats, such as .jpg, .bmp, .tif, etc. For most image processing routines, it is best to use the TIFF format. This format (usually) involves no image compression, so all of the raw data is preserved, but the tradeoff is that images are large in size, particularly high resolution images.

The program allows you to open several images at the same time, so you can collect data on one image and apply it to another.

## Navigating Through the Features of ImageJ

Among the toolbar options, the Image menu provides basic functions such as viewing image properties, changing the image type (grayscale versus RGB, etc.), cropping, rotating, zooming, and making simple changes like adjusting brightness, contrast, and size (i.e., resolution).

The Process menu provides more advanced image processing functions such as smoothing, sharpening, detecting edges, and filtering the image. Using Process | Math..., you can perform operations to combine images.

The Analyze menu provides most of the functions that allow you to make measurements in and obtain data from your images. A few useful functions will be described next.

## Making Measurements within an Image

You can make measurements on regions you select using your cursor. The type of region you are using can be changed using the buttons on the toolbar. For example, you can select to analyze a rectangle by toggling the leftmost button or a line by toggling the fifth button. Some region types have certain default measurements associated with them. For example, when you make a

measurement on a line, the angle and length of the line will always be given. When you make a measurement on a rectangle, there are no default measurements. For any region type, you can add to the types of measurements performed by selecting Analyze | Set Measurements and then checking the boxes. Note that linear distances will be given in units according to the Scale, of which the default is unpredictable (see below for how to set the scale).

To make a measurement on a region you have selected using your cursor, select Analyze | Measure or type ctrl-m. The program will perform all of the default measurements, as well as those that you have selected in the Set Measurements menu. This data will appear in a new window labeled “Results”. You can make multiple measurements by selecting different regions and typing ctrl-m. A new row of results will appear in the Results window for each measurement. Data from this window can either be saved as a text file, which can later be opened in Excel, or cut and pasted directly into Excel for statistical analysis.

### **Application: Using a scale in one image to make measurements and add a scale bar to another**

Open two images, one showing your scale and the other showing your sample. This procedure only works if both images were obtained at identical resolutions using the same objective. Do not alter the size of either image prior to performing this analysis (other than zooming – see below).

First, we will calibrate the scale. Select the Line button on the toolbar. On your scale image, carefully draw a line that spans a known distance. Note: your accuracy will improve the longer you make this line. Select Analyze | Set Scale. In the “Known Distance” box, enter the actual distance of line. In the “Unit of Length” box, enter the corresponding unit (eg., um). Select the Global option, and hit OK. From that point on, all measurements you make will be in that unit.

Note: you *may* zoom in (ctrl-+) to aid in placing your line while making measurements or setting the scale. It will automatically adjust the pixel size to be that of the original, unzoomed image.

At this point, length measurements can be made in the sample image following the procedure above. Results will be shown in the “Results” window. It’s a good idea to make sure your scale was set accurately by performing a test measurement somewhere else on the scale image.

To add a scale bar to the sample image, select this image. Then, select Analyze | Tools | Scale Bar. You can choose the length of the scale bar, where it will be placed in the image, and how it will appear.

### **Further Help**

You can get more information on how to use ImageJ at <http://rsb.info.nih.gov/ij/docs/index.html>. There, you can find help in HTML format, a link to a full user manual, and a link to a Wiki helpsite.