

A Brief Introduction to Using ImageJ

ImageJ is a NIH resource for processing and analyzing images. The software can be downloaded from the ImageJ web site: <http://rsbweb.nih.gov/ij/>

The documentation can be found at <http://rsbweb.nih.gov/ij/docs/index.html>

Learning Objectives

The student will be able to

- load images into ImageJ
- prepare images for image analysis
- perform simple image analysis (measuring and counting)

Knowledge and Skills

- using imaging software for image analysis
- concepts: circularity

Prerequisites

- measurement of distance in different units
- measurement of area and perimeter of geometric objects, including circle, rectangle, and ellipse

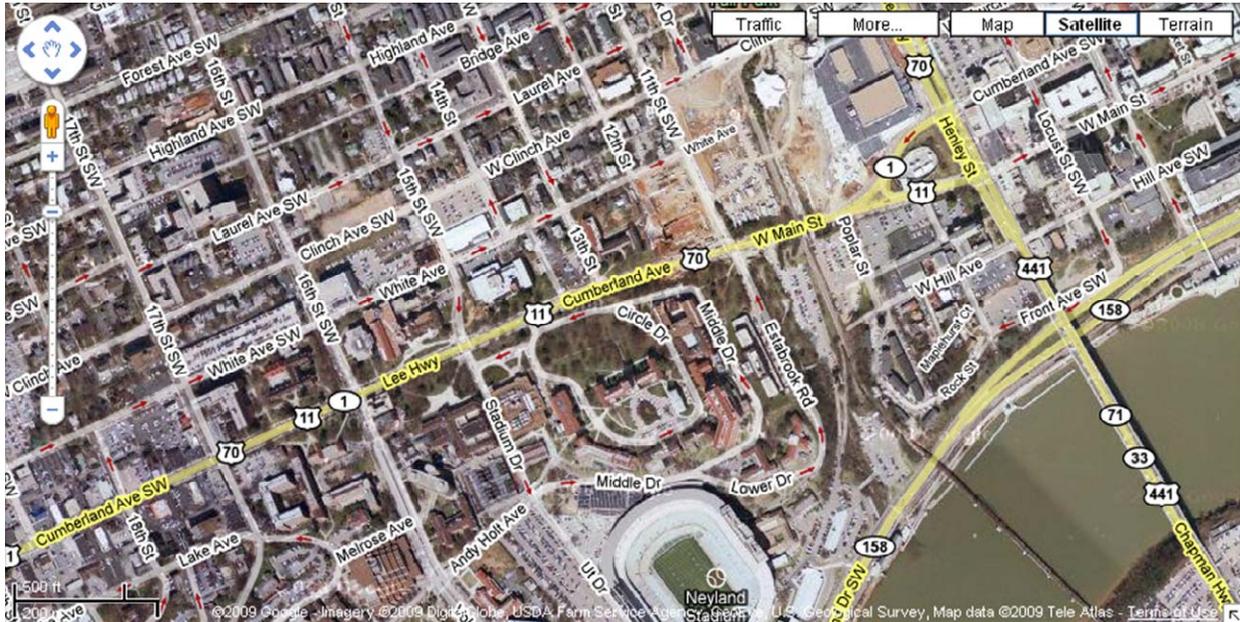
Introduction

ImageJ is a public domain software that can be downloaded on a variety of platforms, including Windows and Mac. The software can convert many image formats including GIF, JPEG, and TIFF to 8-bit, 16-bit, or 32-bit images, which can then be analyzed by ImageJ.

We will use ImageJ to measure distances and count objects. Since TIFF is the least degradable format when an image is repeatedly used for analysis, it is recommended to save any image first as TIFF before conversion to 8-bit, 16-bit, or 32-bit.

Measuring Distances

The image MapNIMBioS.tif is a Google map of the University of Tennessee campus and the adjacent area. The workshop hotel is at the corner of Estabrook Rd and White Ave. The Walters Life Sciences is on Stadium Drive. In the lower left corner of the image is the scale.



Start ImageJ and load the image MapNIMBioS.tif into ImageJ: click on the **File** menu and choose the submenu **Open**. A file manager opens. Locate the folder where the image is saved and open the folder. Click on the image and click on Open to load it into ImageJ.

The image is already in TIFF format. We need to change it to a bit format before we can use any of the tools within ImageJ: Image > Type > 8-bit

Next, we will determine how many pixels correspond to 500 ft. Note that the x- and y-coordinates of a point are in units of inches. For this, move the cursor to the beginning of the line segment that corresponds to 500 ft. Record the x-value. Move the cursor to the end of the line segment that corresponds to 500 ft. Record the x-value again. The difference in the x-values tells you how many inches correspond to 500 ft. Click on **Analyze > Set Scale**. A dialog box pops up. The dialog box tells you how many pixels correspond to 1 inch. Use this to determine how many pixels correspond to 500 ft.

Hint: If the line segment corresponding to 500 ft measures x inches, then the number of pixels corresponding to x inches is equal to (x inches)(#pixels/inch).

In the dialog box, enter the distance in pixels, the known distance (500 ft) and the unit of length (feet). You can check whether you entered the correct numbers by measuring the length of the line segment that corresponds to 500 ft. Click on the **Straight Line Selection** tool and draw a straight line from one of the segment to the other. Click Ctrl-M. A Result box pops up which should display the length 500.

We can now measure the distance between the hotel and the Walters Life Sciences building using the **Straight Line Selection** tool. Draw a straight line along the street that you would use to walk from the hotel to the Walters Science Building. To record the distance of the line segment, click Ctrl-M. A window will pop up with the record of your measurement. Continue with straight lines until you reach your destination. What is the total distance traveled? Alternatively, you can use the Freehand Line tool to measure the distance. You can choose the tool by right-clicking on the Straight Line Selection tool and selecting the **Freehand Line Selection** tool.

Counting Objects

To learn how to count objects, we will count the number of sheep in an aerial photo. Load the image sheep1.tif.



The image is already in TIFF format. We need to change it to a bit format before we can use any of the tools within ImageJ: Image > Type > 8-bit

To segment the image into the objects of interest and the background, we use thresholding

Image > Adjust > Threshold

A dialog box pops up where we can adjust the threshold so that only the pixels of the objects of interest appear red. Choose Black & White in the drop down menu within the dialog box. Click Apply. The image will change into a black and white picture where the pixels of the objects of interest are black and all other pixels are white. Close the dialog box.

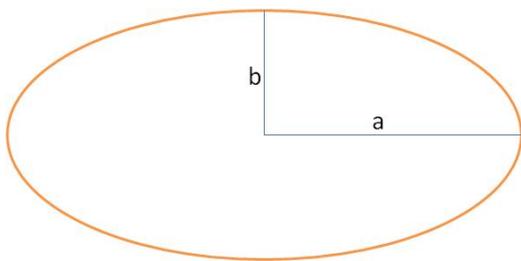
To count the objects, we use the Analyze menu

Analyze > Analyze Particles

A dialog box pops up where we need to enter the size and circularity of the objects, and what we want to show. In order to count only particles above a certain number of pixels, enter a single value into the Size field. Particles smaller than that value will be ignored. The circularity is calculated as

$$\text{Circularity} = 4\pi \frac{\text{Area}}{\text{Perimeter}^2}$$

If the object is circular, the value of the quantity Circularity is equal to 1.



To understand the concept of circularity, we calculate this quantity for ellipses with different values of the major radius a and minor radius b . The area of an ellipse is equal to $A = \pi ab$. There is no simple formula for the perimeter of an ellipse. An approximation is given by

$$P \approx 2\pi \sqrt{\frac{1}{2}(a^2 + b^2)}$$
. This implies that the circularity is

approximately

$$C \approx \frac{2ab}{a^2 + b^2}$$

The following table provides values for circularity for different ellipses:

a	b	c
1	1	1.00
2	1	0.80
3	1	0.60
4	1	0.47
5	1	0.38
6	1	0.32

Back to counting sheep: Enter **Size** and **Circularity** into the dialog box, and check **Display**, **Clear**, **Summarize**, and **Include Holes**. Experiment with different values for Size and Circularity to get a sense for how these parameters influence the total count.

How many sheep? Partitioning the image and counting by hand reveals 95 sheep.

