

# A Brief Introduction to Using ImageJ

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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>

Tutorials are found at: <http://rsbweb.nih.gov/ij/docs/examples/index.html>

## Learning Objectives

The student will be able to

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

## Knowledge and Skills

- using imaging software for image analysis
- concepts: circularity

## Prerequisites

- 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.

## Counting Objects

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

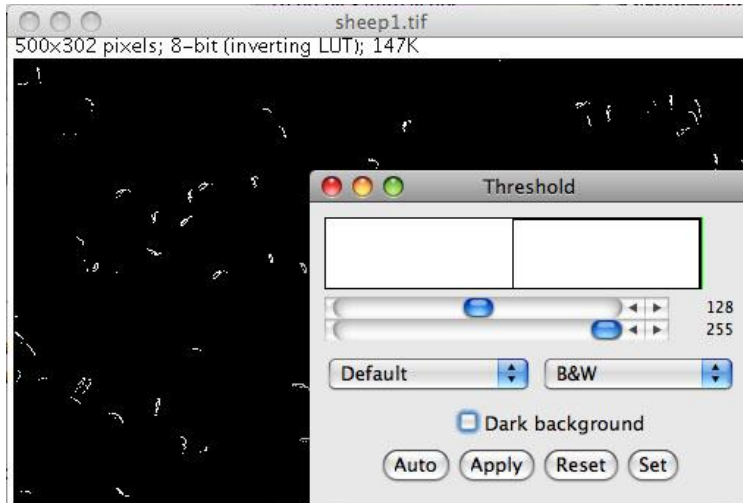


The image is already in TIFF format. We need to change it to a bit format before we can use any of the tools within ImageJ: **Image > Type > 8-bit**

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. Choose **Black & White** in the drop down menu within the dialog box. Choose ellipses in the menu. Click **Apply**. The image will change into a black and white picture. Adjust using the sliders so that where the pixels of the objects of interest are white and all other pixels are black.



Under **Edit** in the menu, choose **Invert**. The background will appear white emphasizing the ellipses (sheep).

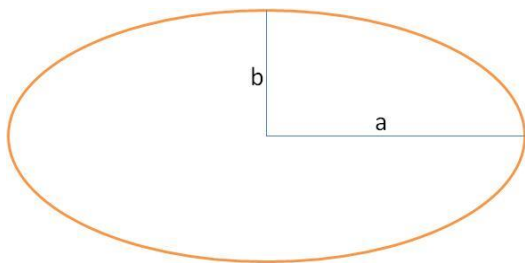
To count the objects, we use the Analyze menu

#### 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.



approximately

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

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

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

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**, and **Include Holes**. Use **5 to infinity** values for **Size** and and **0.3** for **Circularity** to get a total count. Try to increase the accuracy of your total count.

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

