QUANTITATION OF AN ELECTROPHORETIC GEL

In the previous experiment, you used the electrophoretic gel to get an idea of the **molecular weight** of various proteins. It is also possible to get some information about the **amounts** of proteins in a gel. What you can measure is the amount of dye that is bound by each of the bands in the gel. In this set of experiments, you will use a computer program, ImageJ, to attempt to measure the amount of protein in specific bands in an electrophoretic gel. The gel, similar to those you prepared in lab last week, has been scanned on a densitometer, and stored as a digital representation. The ImageJ program is designed to allow you to select specific regions of the image and count the number and intensity of the pixels in the image. You will use this program to determine the relative quantities of protein in each experimental sample.

1. The Process of Digitization.
In the densitometer, a laser beam is passed through the sample, and the intensity of the light that passes through any position in the sample is determined. In order to process the image in a computer, this output is divided into individual units, called pixels, and each pixel is assigned a numerical value, depending on how dark it is. Fully transparent regions are given the value of "0", and areas of maximum density are given the value of "4.000". These values are expressed as units of optical density, or absorbance.

In the following figure, you can see how an apparently continuous image is in fact made up of pixels of different intensities.
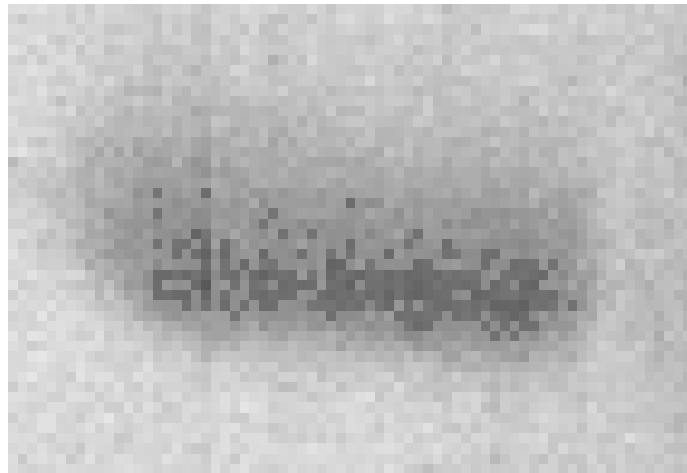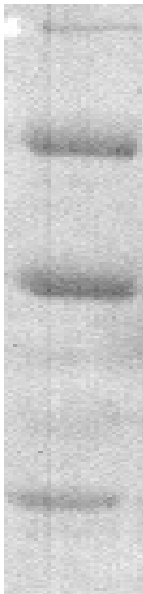
The intensity of each pixel is proportional to the amount of absorbance of the laser beam as it scans through the sample. Although this machine can detect absorbancies from 0 (completely transparent - white), to 4.000 (Absolutely opaque - black), in practice most samples use a smaller range, as you will see.

The analysis that you will do requires several major steps:

1. Start the program and load the file containing the scanned image.

2. Determine the range of intensities of the sample.

3. Outline the region of the sample that you want to analyze.

4. Make the measurements.

5. Report and summarize your data.

What follows is a brief "cookbook" set of instructions. You can get more information about the process by choosing the "Help" item from the ImageJ menu. Look at either the help files or the tutorial.

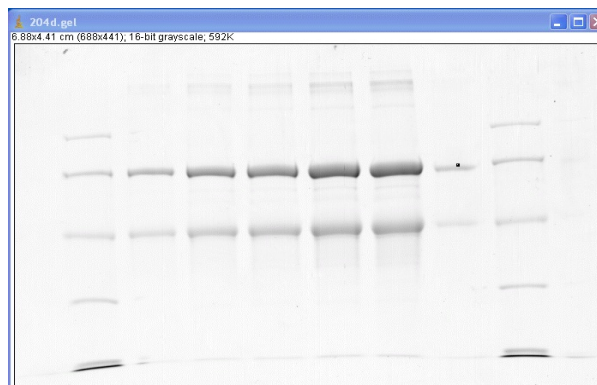**1. Start the program and load the image file.**

Look for the "ImageJ" icon and double-click on it.

You should see the following image:
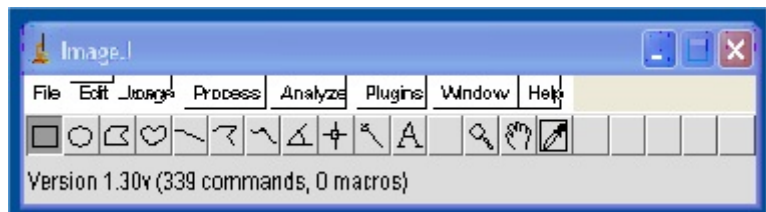
Click on the "File" menu and then "Open". The menu should appear with the option to select a file. Choose a file beginning with "204", and double click it. You should now an image of an electrophoretic gel,   similar to the one below.

2. Determine the range of intensities of the sample. As you move the cursor over the image, you will see it change into a plus symbol. If you look at the lower left corner of the program window, you will see the X and Y coordinates of the cursor, and a "value" number which is proportional to the intensity of the sample. Note that darker regions give larger numbers. Move the cursor around the sample and record the highest and lowest number that you find. Note that even if you can't see them, there are differences throughout the sample.

3. Draw a rectangle on the sample. You can use the computer to outline the region of the sample that you want to analyze. For today's exercise, we will draw a long rectangle that includes all of the bands of the same molecular weight.
Answering the following question may help you decide which rectangle to draw:

From the ImageJ program bar, click on the rectangle selection.

 Move the cursor to the upper left corner of the rectangle that you want to draw. Then, hold down the left mouse button and drag out the rectangle horizontally and down, to include just those bands that you will analyze.
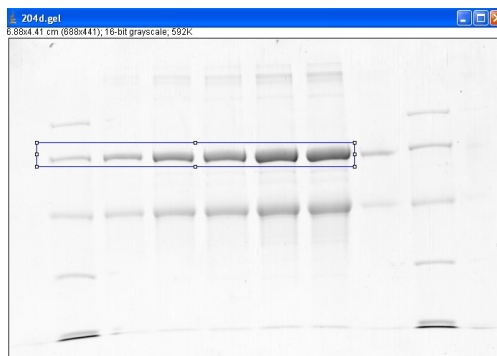

As you make this selection, you should think about how much of each band should be within the rectangle. Similarly, you should think about how much background to include. You might try several different rectangles and decide which is best. When you are satisfied, type <control>1. (This means hold the control key down, and type a 1). You will see no change, but the area has been noted by the computer.

4. Make the measurements.

a. Plot the curve. This is done by typing <control>3. (Note it is <control >3, not 2).

You should see two changes on the screen. First, you will get the following graph:
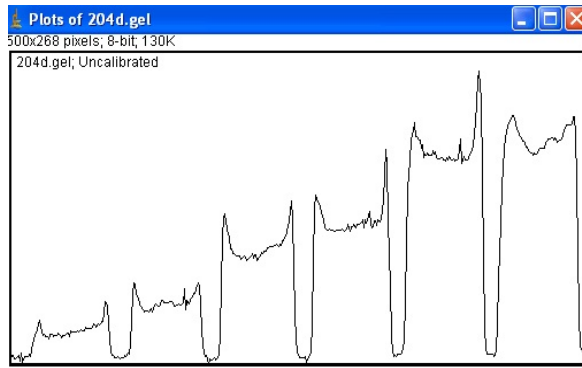
Second, you will see that the "line selection" box has been chosen in the ImageJ menu bar.

ImageJ

File Edit Image Process Analyze Plugins

Straight line selections

(b) Your goal now is to use the "line" tool to: (i) draw the appropriate baseline for this graph, and then (ii) select the peaks for analysis.

(i) To draw the baseline, move your cursor to where you want the baseline to begin. Think about this. Should the baseline begin at 0 on the Y axis, or at the beginning of the tracing? How do you make the decision? Once you have decided, left click and draw out the baseline to fit the rest of the curve. If you feel that it is appropriate, you can make the line in several sections (Why would you want to do that?). When you are finished, release the mouse button.

(ii) It is time for you to set up the peaks that you want to measure. Note that many of the peaks in the graph look like doubles. How do you account for this? Look at the original image, and see if the individual bands are uniform. Use the line tool to draw vertical lines that separate the peaks that you want to measure. Notice how in this example, the minimum values come very close to the baseline. In some gels, this is not the case, and the vertical lines represent a decision that you have made about where the boundaries of a peak are located.

(c) The area under the curve represents the density of the band that you have selected. To obtain this data, select the "wand" tool on the ImageJ menu. Do NOT choose the "point" tool which is at the left of it.
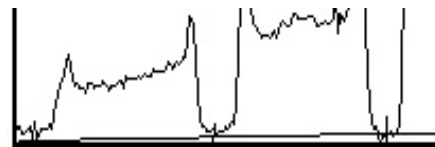
Now, when you click in each one of the peaks, you will see the peak outlined, and the area will be reported in a "results" table. Click on each peak in turn, and the results table will grow with the additional data.

ImageJ

File Edit Image Process Analyze Plugin

Wand (tracing) tool

Plots of 204d.gel

500x268 pixels; 8-bit; 130K

204d.gel; Uncalibrated

Results

File Edit

	Area
1	1899