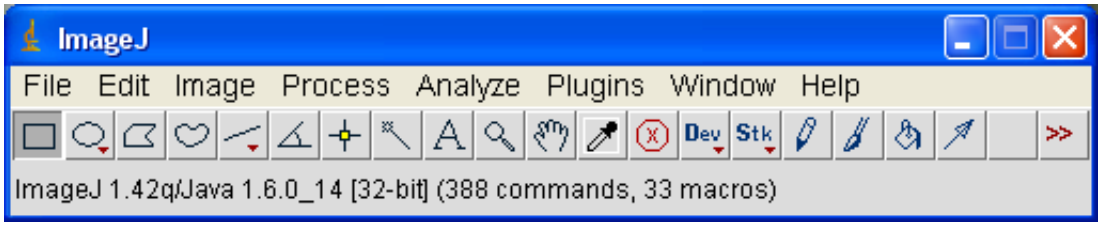
Using ImageJ to Quantify Gel Images

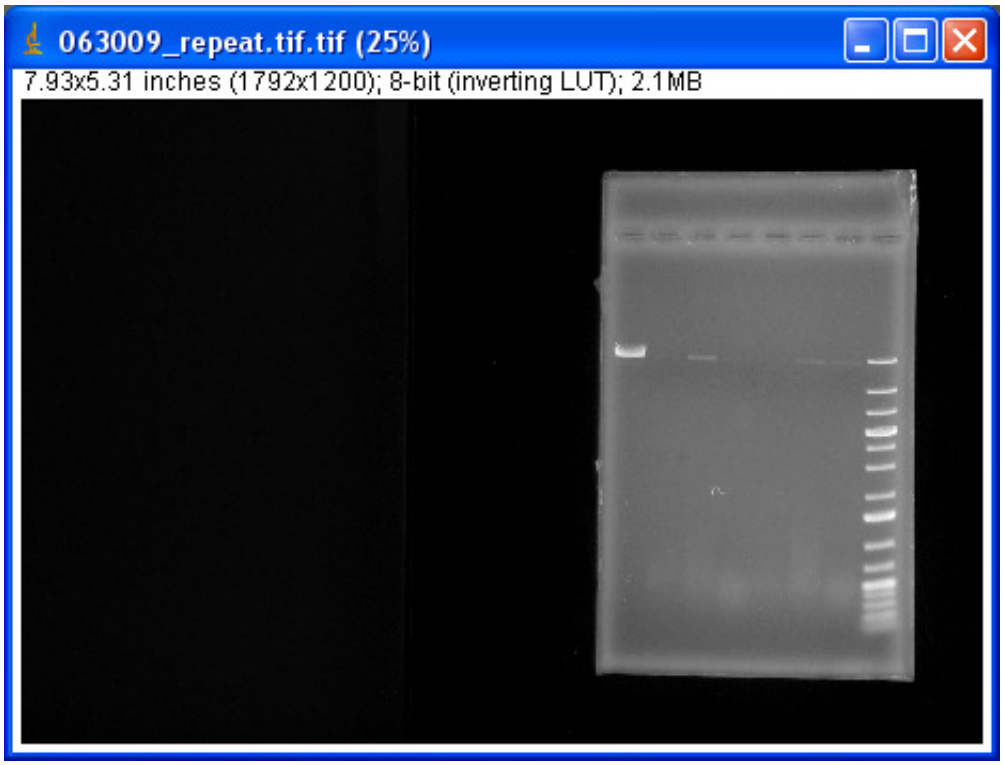
This is a quick tutorial abour using ImageJ to process gel images taken with the GelDoc. ImageJ is a free program that was originally written at NIH. ImageJ is used to analyze/process all sorts of images in biology-related research. You can use ImageJ to crop/invert/rotate/enhance your gel images. There is even a way to quantify your bands.

Rotating/Cropping Gel Images

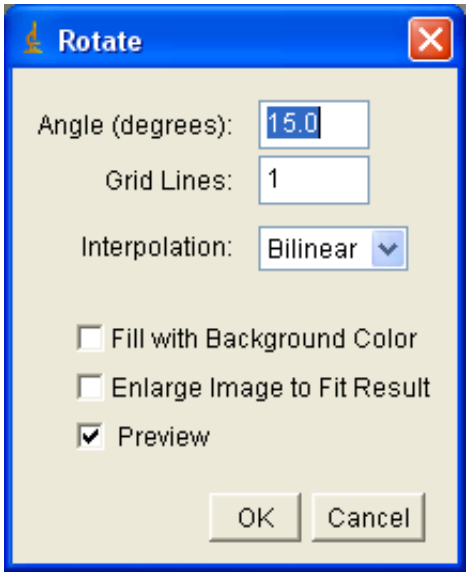
Open ImageJ using the shortcut on the desktop.



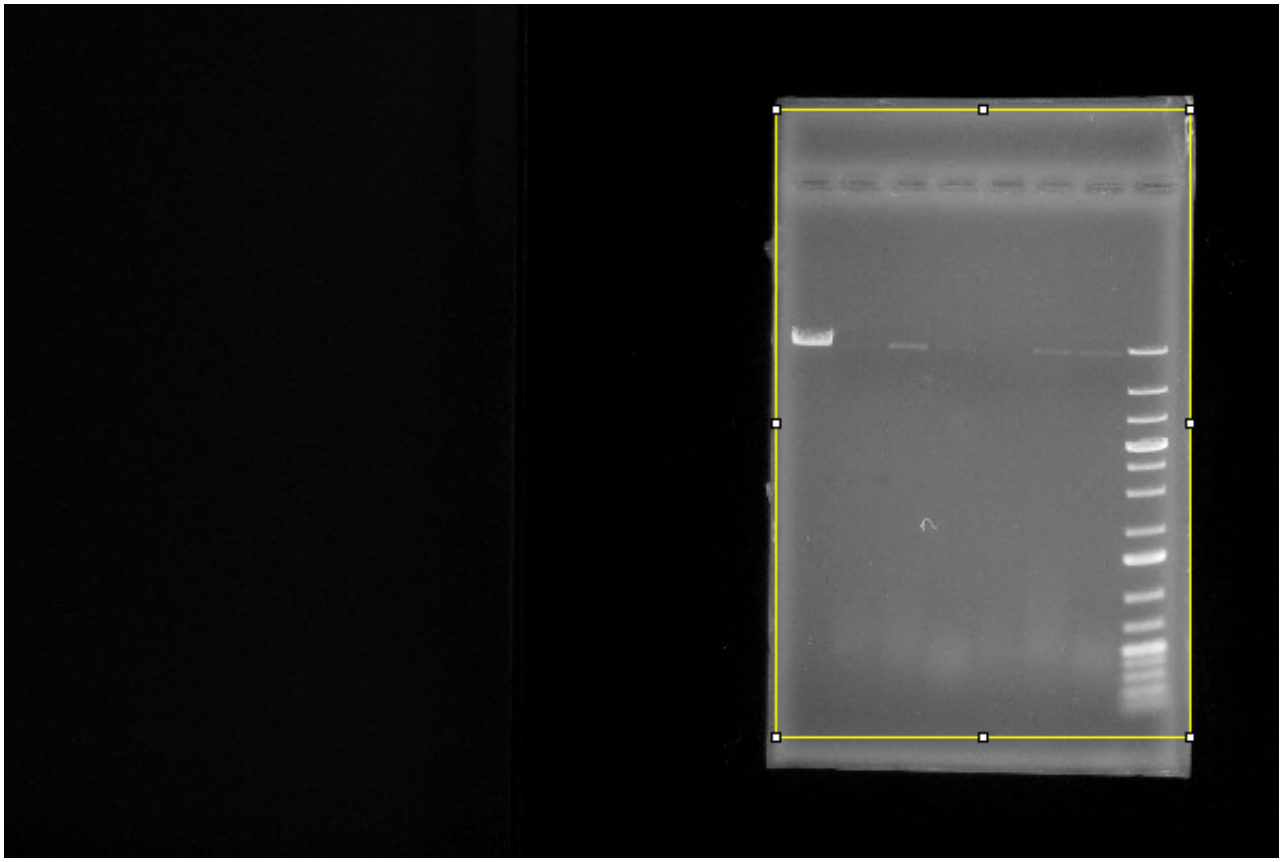
You can drag the image you want to open onto the ImageJ window. Once the gel image is open, you can zoom in with "Ctrl+" and zoom out with "Ctrl-". Your gel will look something like this (see below).



Now you can rotate the image in case it's crooked. Go to **Image/Rotate/Arbitrarily...** You will get the following dialog window. You can adjust the rotation angle (positive or negative values). To make it easy, make sure that the "Preview" box is checked, that will show you how your image is rotated, and it will also show you vertical/horizontal lines for orientation. Click OK when you're done.

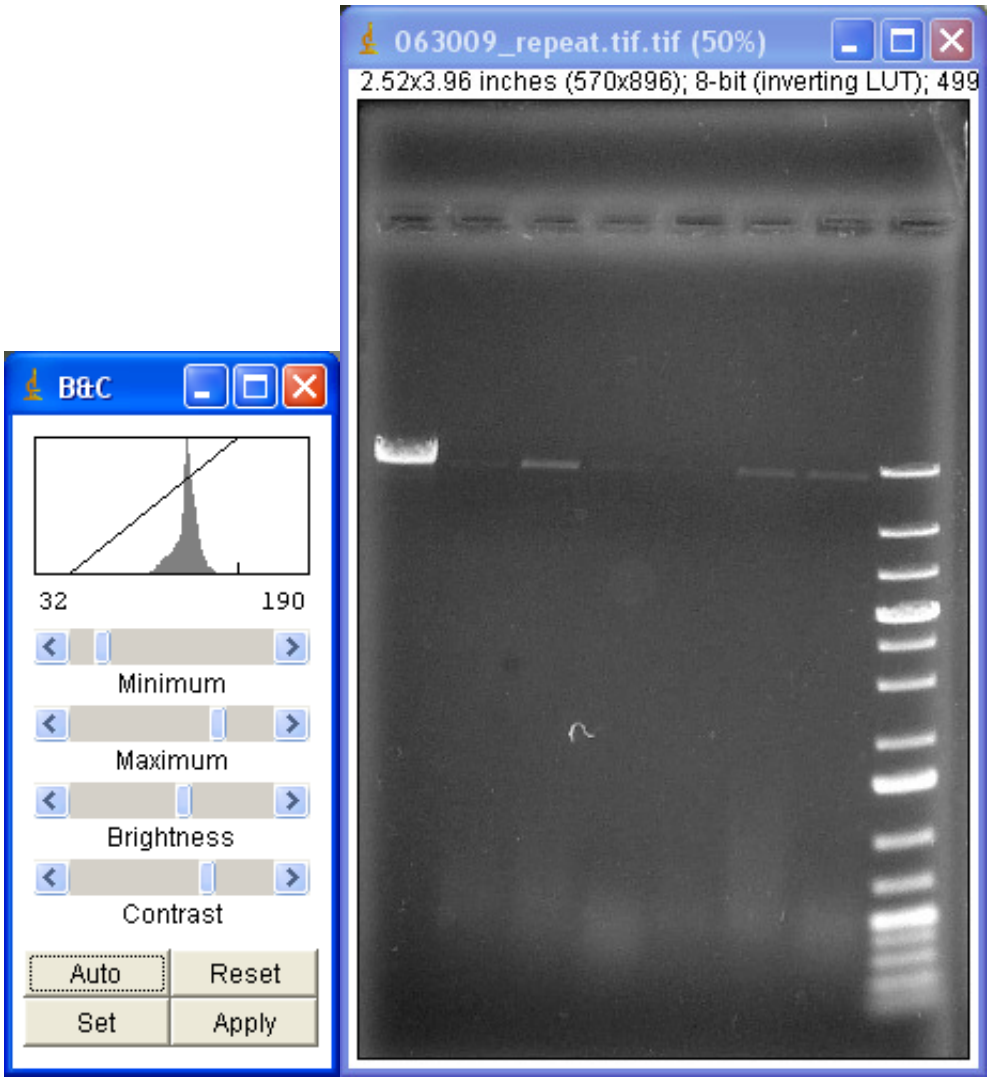


Now it's time to crop the gel image. Use the "Rectangular Selection" tool. After selecting the region of interest go to **Image/Crop** to crop the selection.See below for screenshots.



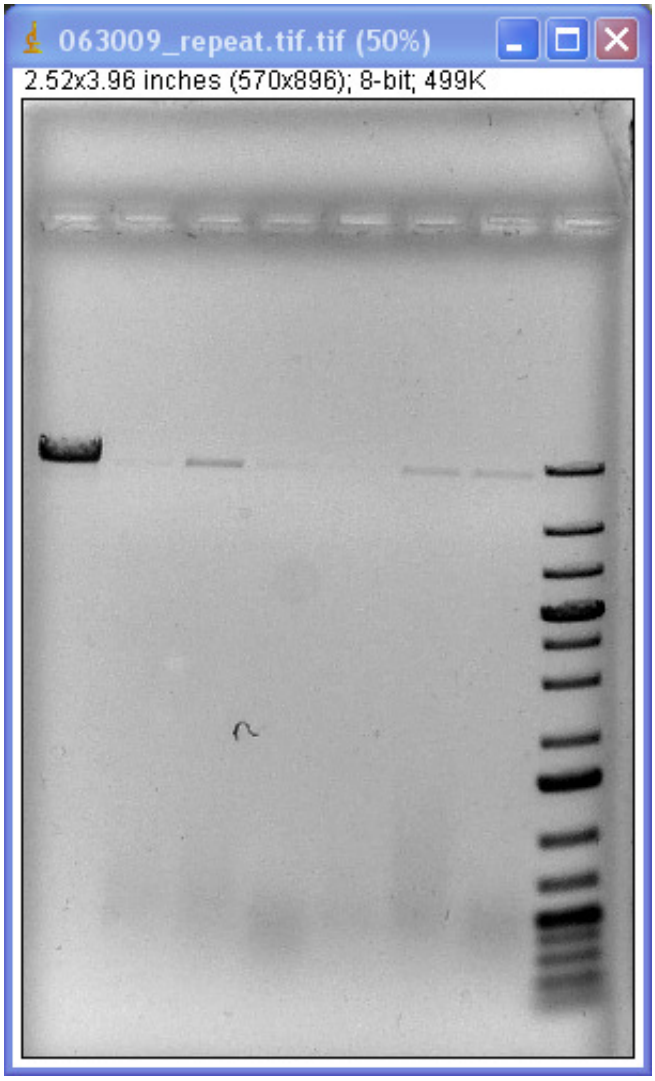
Enhancing the Gel Image

This is a typical step when dealing with gel images. You need to adjust the histogram of the image. Please make sure not to blow-out (saturate) the whites. You want to make sure your image has enough dynamic range. Talk to me if you're confused. Anyways, you can do that with **Image/Adjust/Brightness-Contrast...** , see the Brightness&Contrast window and the enhancement results below. Click on "Apply" and close the menu.



Inverting the Image

In most cases you will want to invert the EtBr gel images for convenient viewing and economic printing. Do this by going to **Image/LookupTables/InvertLUT** which will give you the result shown below. You can use Brightness&Contrast enhancement once again if you wish, but this one is good enough to move on.

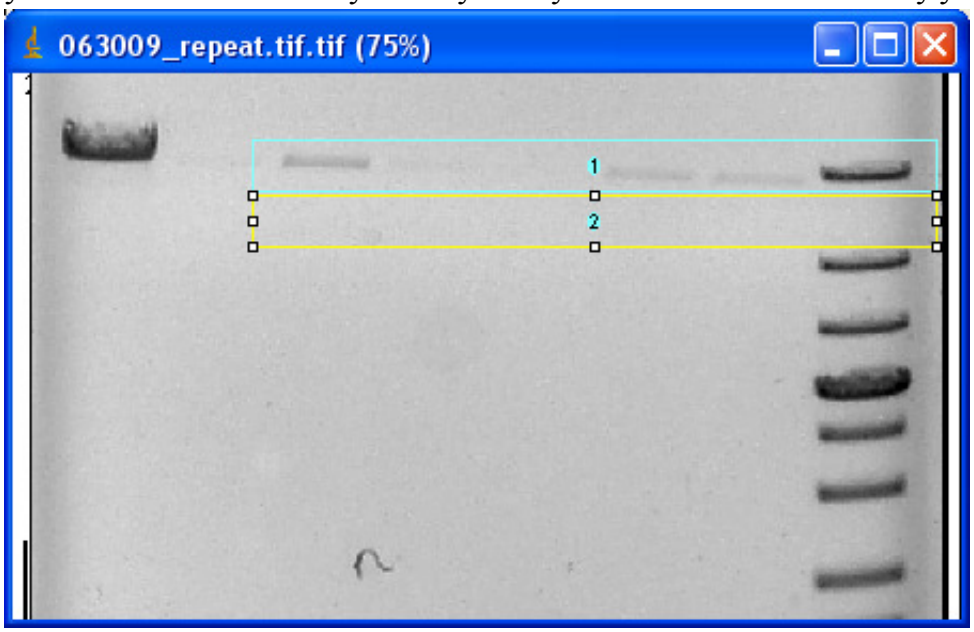


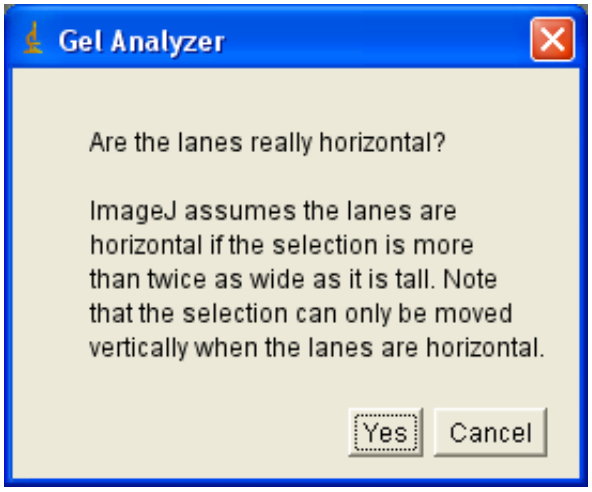
Quantifying Gel Lanes with ImageJ

Warning - this is not going to be accurate to the percent. It works and you can put some numbers on your results, but digital camera images are not going to be numerically as accurate as a Typhoon scan. You need to select your lanes first. I will do horizontal selections, but you can also do vertical selections if needed. First of all, click on the "Rectangular Selection" tool

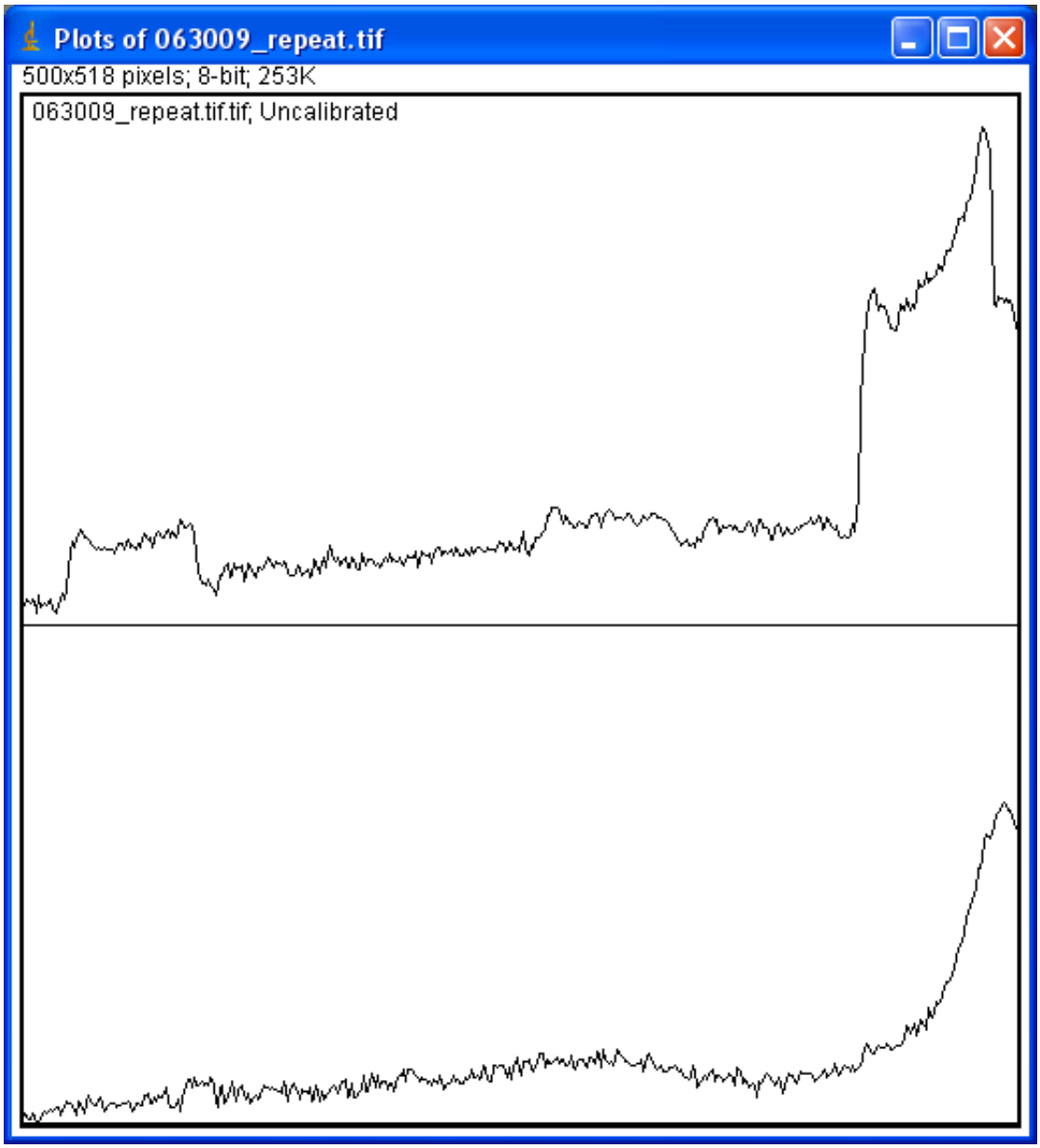
Do a tight selection around the lane of interest. Press "Ctrl+1" to designate the first lane. Grab the selection and drag it down to select the background (to use late for background subtraction). Press "Ctrl+2" to designate the second lane, then if you select a third lane, you press "Ctrl+2" for any additional lane. Once you're done, press "Ctrl+3" to plot the lane grayscale density. You can access

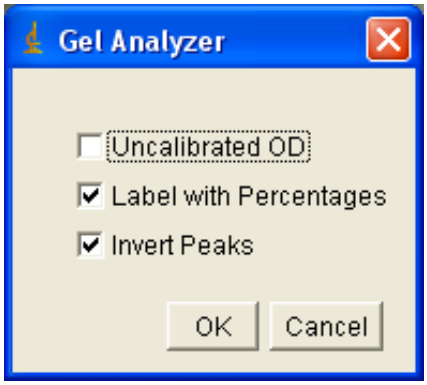
other gel-related functions in the **Analyze/Gels** menu. If you select horizontal lanes like I did here, you will be asked whether you really want your lanes horizontal. Just say yes.





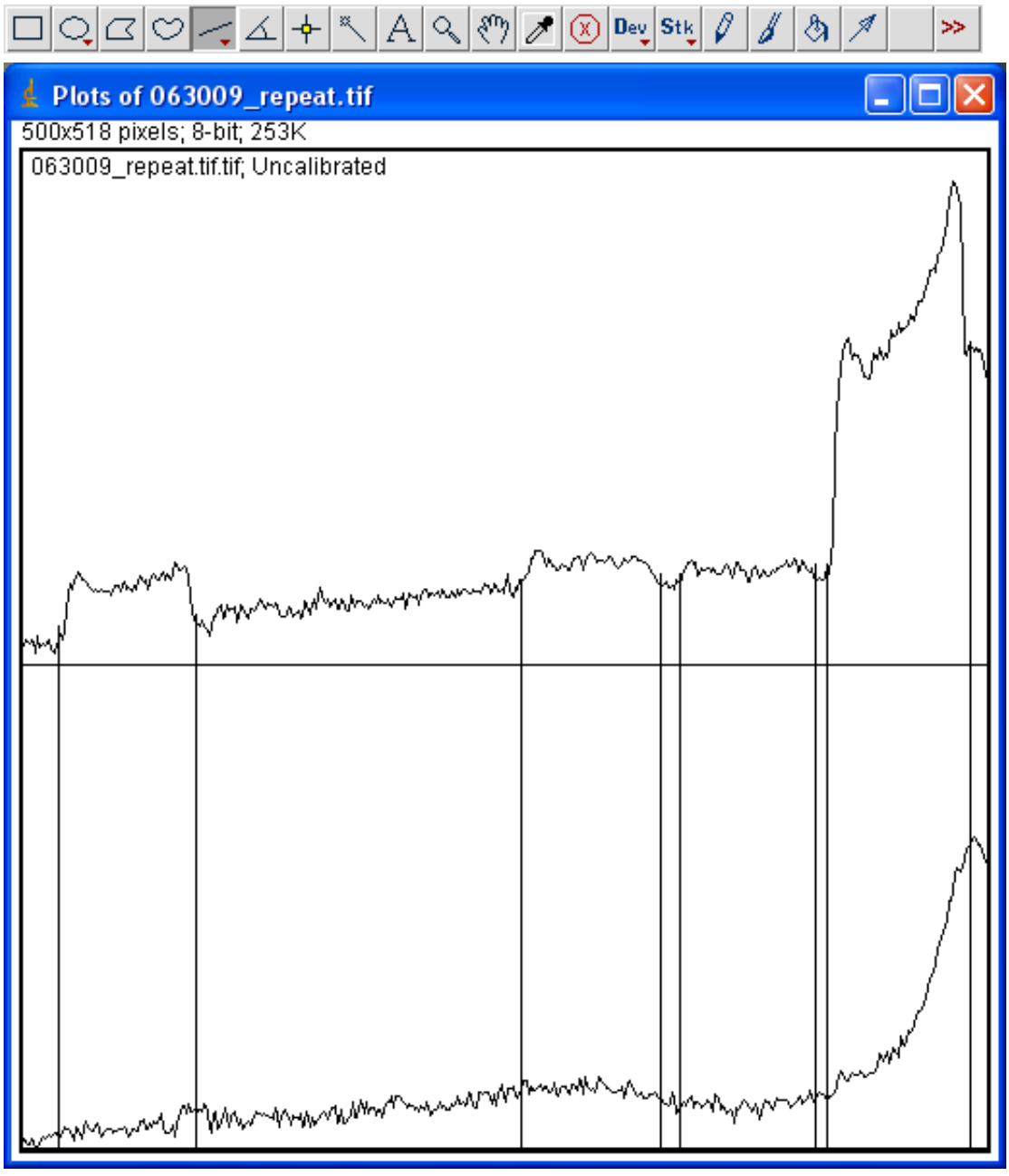
I only had two lanes - the bands of interest and the background lane. So ImageJ plots two separate grayscale profiles. You can see that subtracting the background will really make a difference. If your profiles look weird, please go to **Analyze/Gel/GelAnalyzerOptions** and make sure that the "Invert Peaks" option is checked.



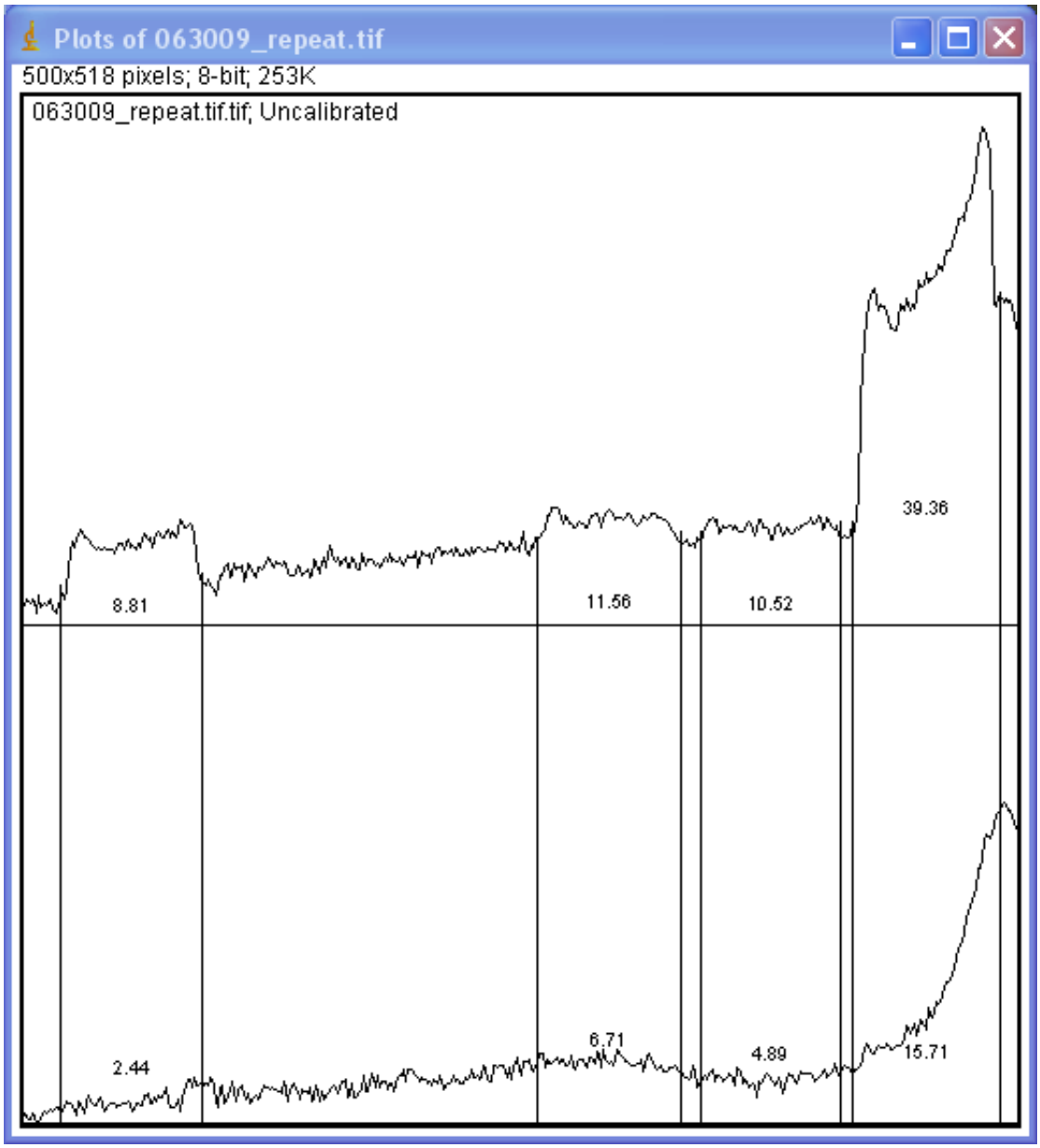


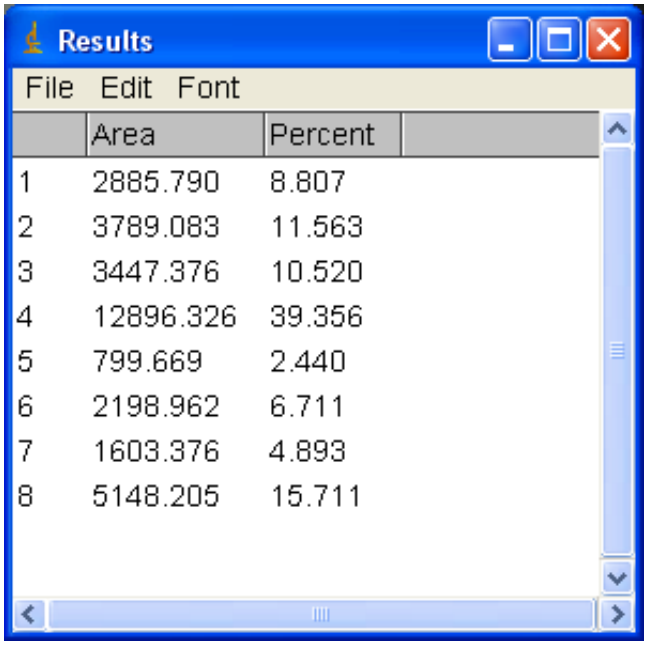
Now you will have to manually select your bands. This is done with the line select tool (press shift

key while drawing a vertical line). This is not as sophisticated as the Typhoon Software, but it offers a bit more control.



Now you can identify each band by using the "Wand Tool" (see below). Click with the Wand Tool inside each selection corresponding to a band. Then you can go to **Analyze/Gels/LabelPeaks** and ImageJ will label each selection defined with the WandTool. There is also a "Results" window that lists the results. Use the "Area" values and make sure to subtract the corresponding background offset value.


Plots of 063009_repeat.tif
500x518 pixels; 8-bit; 253K
063009_repeat.tif.tif; Uncalibrated
8.81
11.56
10.52
39.36
2.44
6.71
4.89
15.71



Saving and Printing

At the end, please save your gel image by going to **File/Save As**, I would recommend saving as TIFF or PNG (lossless). You can email the gel images to yourself. Currently there is no way to print gels directly from the GelDoc computer.