Densitometric Analysis using *NIH Image*

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This *Tech Corner* article aims to introduce readers to the free densitometric software offered by the NIH (http://rsb.info.nih.gov/nih-image/) and to provide a quick tutorial on how to effectively perform densitometric analysis of Western blots. Around 1993, the NIH released a free image analysis program called *NIH Image* that was only available for use on Macintosh computers. In 1997 they offered *NIH ImageJ* that could be used on a PC. The following tutorial is based on the NIH ImageJ format;

1) Densitometric analysis begins with scanning a film or gel with a desktop scanner using a grey scale mode at a DPI of 300 or greater and saving this file in a TIFF format.

2) Open the scan file (figure 1) and go to the menu under *Process>Subtract Background* to reduce background noise by starting with a rolling ball radius of 50 (figure 2). This may need to be adjusted for each densitometric analysis.

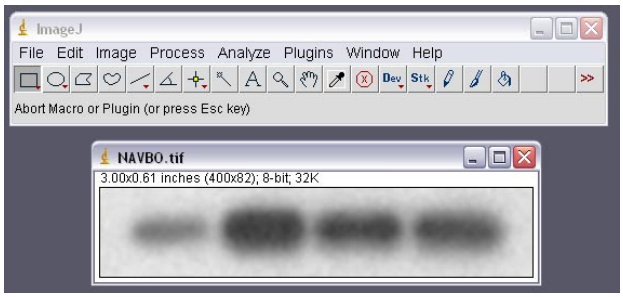

Figure 1

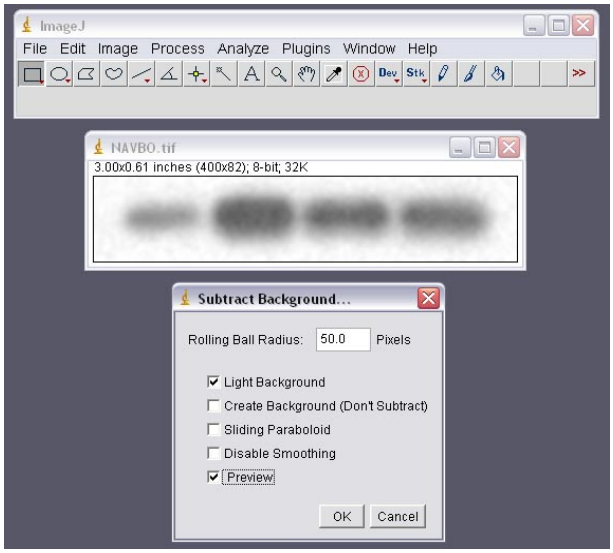

Figure 2

3) Under the menu click *Analyze>Gels>Gel Analyzer Options* and click the boxes for Label with percentages and Invert peaks (figure 3).

4) On the tool bar, choose the rectangle selection tool and draw a rectangle around the first band and go to *Analyze>Gels>Select First Lane or type the "1" key on the keyboard to designate lane #1. Move the rectangle to the next lane and go to Analyze>Gels>Select Next Lane or press the "2" key and continue to do this for each lane (figure 4).*

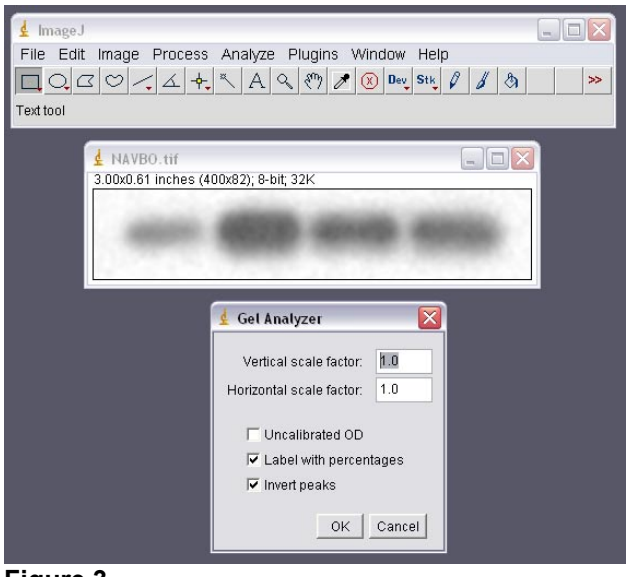

Figure 3

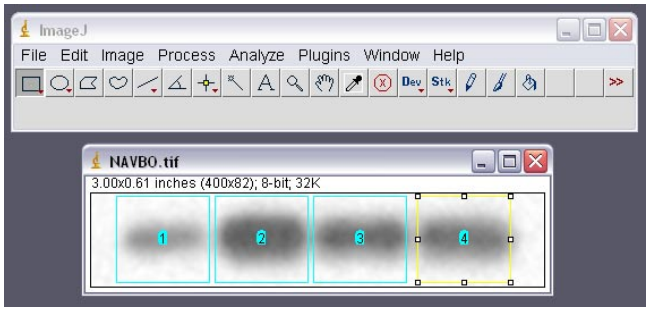

Figure 4

5) After all the lanes have been highlighted, go to *Analyze>Gels>plot lanes* which will bring up a new window displaying a histogram of each lane. Use the line tool to draw a straight line at the bottom of each peak in order to enclose the area under each peak (figure 5, red line). Each peak will need to be enclosed at the same distance from its baseline in order to equally subtract out background. When each peak has been enclosed, select the magic wand from the tool menu and click it on each peak.

6) After each peak has been selected go to *Analyze>Gels>Label Peaks* to get an analysis of the area under each peak (figure 6). This file can be copied and pasted into an excel file for further analysis.

The ability to quantify the intensity of Western blot bands for the statistical analysis of multiple blots for publication purposes makes densitometry a powerful tool for bench scientists. There are limits to this, however, as poor quality images are not suitable for this type of analysis. Moreover, the limited linear dynamic range of X-ray film and the transience of chemiluminescence present opportunities for deviations from linear relationships between target abundance and band density. Preliminary runs using target sample dilutions can help establish useful response ranges for semi-quantitative work.

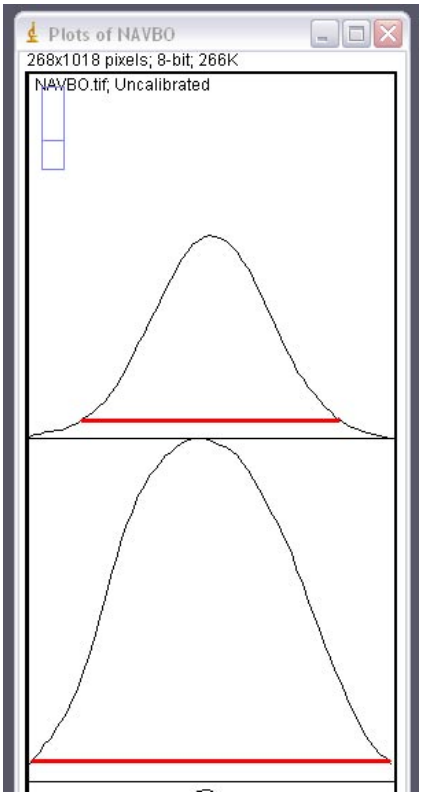

Figure 5

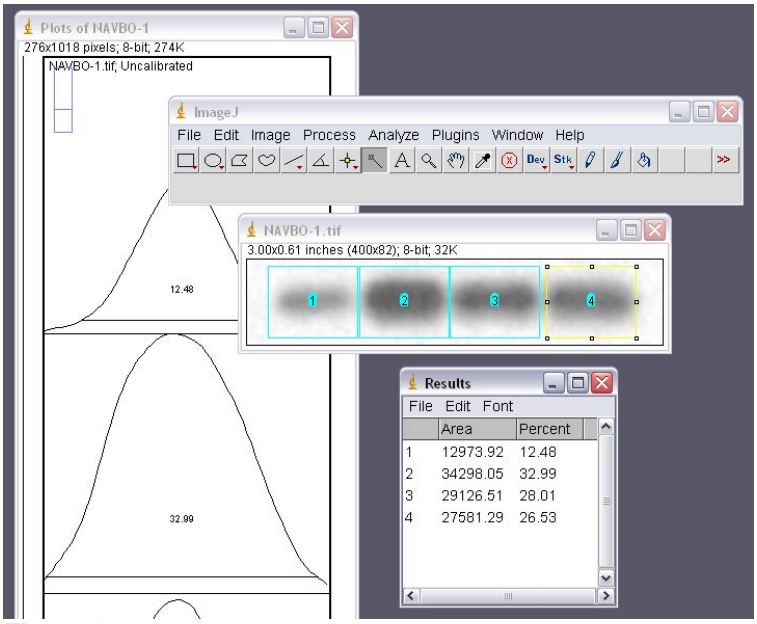

Figure 6