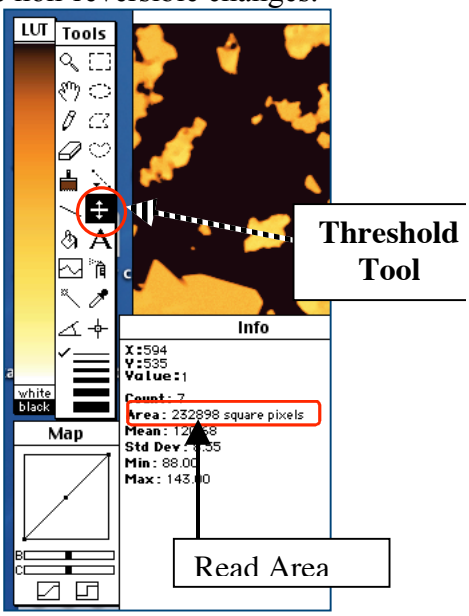
Image Processing Exercise Geology 777 11/18/2003

The goal of this exercise is to learn some basic image processing and analysis skills. You will use the (free) NIH/Scion Image software package, which was initially developed for the Mac, but has been ported to Windows ("Scion"), and now has a Java version (Image J). The homepage is <rsb.info.nih.gov/nih-image> for the Mac and Java versions as well as a lot of other good information and links. For the windows version try <www.scioncorp.com:8080/Downloads>

For the second year running I have tried Image J but cannot seem to get it to do what I want it to do, though some students apparently got it working OK last year (see attached sheet from Jake Eaton). The mac OS 9 version (v 1.63, runs under Classic fine)

ALWAYS work with a copy of the original image; it is very easy to make non-reversible changes.



NB: Image processing can take up lots of RAM. You probably need to allocate at least 10 MB to NIH Image if you want to have many images open at once (the rule is that you need to have 3 times the size of each file opened allocated to RAM). The lab Mac has lots of memory so that is one advantage of using that. You may get memory warnings: I generally ignore them if on the lab Mac--until forced to deal with them because the computer crashes.

There is a pretty good manual available as either hard copy in lab, a file version on the Hard Disk, and an html version you can download. Balloon help is available.

Tools (Vertical menu, shown here): see page 59 (and 65-66, 68-69) of manual for description

You will be working with 3 images, all BSE: the first (B&W, 777test.tif) is of a lava; the second and third (stregnite*.tif) are BSE images (false color), 2 views of the same sample, just different regions (I couldn't image it all in one field of view). Your assignment will be to determine the areas of the various phases present.

1. Start up Image (Mac NIH Image, PC Scion Image). Go to Options menu, Preferences, check Invert Pixel Value if working with Mac version. This is because (for some reason), the mac settings have black as 255 and white as 0, whereas this is the opposite of what our image values correspond to.) Thus you see later under the Info box, there will be two intensity values for a pixel—the first is the one we are concerned with. (Do not need to do with ImageJ).

2. Open up the first BSE image (777test.tif), look at its histogram (Analyze > Show Histogram). How many phases would you say are present looking at the image? ___ Looking at the histogram? ____ Don't forget the lowest and the highest intensities, as in fact there is a one pixel wide black peak (cracks) and a one pixel wide white peak (white phase).

3. We wish to determine the percentages of each phase present. We need to know the total number of pixels. Edit>Select All, then Analyze>Measure and look in the Info box (bottom left) for Area, write this number down.

4. You will utilize Thresholding (also referred to as “segmenting” or "density slicing"), using the slider Tool (6th from top on right column tools), to select (color in Red) one particular phase -- first you need to play around and determine which grayscale is which phase. Pick a phase and threshold it—double click the slider and a red bar should be activated on the LUT to the left. Slide it up and down and watch it work. Then go to Analyze>Measure, then read off the area (sq.pixels) in the Info window, and write it down. (If you go to Histogram now, it will show you the thresholded region in black.) Repeat the thresholding for each phase. Calculate the percentages of each phase. How far off from 100 % is the total? Now redo the thresholding 2 more times for all the phases, and calculate the averages for each phase.

5. Now examine the white phases in the sample. Go back and threshold the white phases. Go to Analyze > Options, and check Area, Ellipse Major Axis, Minor Axis, Angle. Then go to Analyze > Analyze Particles and check Label particles, Outline particles and Reset counter. Then OK and Continue, and Voila! Neat, huh? How many particles are recorded in the Info box summary? ___ Now go to Analyze > Show Results and there each particle has its own data. The angle is the strike of the long axis of the particle, with 0 degrees being due east, and going counterclockwise (thus 90 is straight up, ‘north’). Copy the data to excel or another program and make a histogram of the angles. Is there a preferred orientation? If so, what is it?

6. Now for the second problem, for which you have 2 images, that overlap somewhat. This is a synthetic iron phosphate that apparently is heterogenous, with 2 different compositions. I want you to determine the abundance of each (bright and dark) phosphate in each sample (remember to exclude the dark epoxy in which it is embedded), and compare the two images (fields of view): are the proportions of each the same in each image, or within a certain margin of error (sigma)?

Turn in a brief report that answers all the questions above, particularly the percentage of each phase in the sample, the number of white phases, and whether or not (and if so, what) there is apreferred orientation of the white phases.

Extra credit: Get Image J to work and show me how you do it!

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Appendix-Tips for using Image J (by Jake Eaton, Fall 2002 777 class)

ImageJ Tutorial for OSX & Jaguar

The tutorial for ImageJ on the web is really the way to figure out the program, but for a few shortcuts, here goes:

You do not need to invert the screen as this is not a problem in OSX or later. There are several problems with running ImageJ about which I am not aware as I’m running Jaguar. You can get an update on these problems on the ImageJ website <http://rsb.info.nih.gov/ij/>.

First you should set up your desktop with all the tools that you’re going to need to use. Select:

- Analyze>Histogram
- Analyze>Measure (you can always clear the data later “Edit>Clear Results” when that screen is selected.

Thresholding

To set up thresholding, go to:

- Image>Adjust>Threshold

This will give you the screen that is, in previous versions, a sidebar. Though here it is another window. The concept is pretty much the same – slide the two sidebars to adjust the thresholding region. It is important that if you are clicking through all the open windows on the page, some of the changes you make in your thresholding window ("Threshold") will not register unless you have previously clicked the image. There are some buttons in the "Threshold" window itself:

- Auto – This button automatically selects the threshold level based on contrast of the current image.
- Apply – This button allows the user to convert the selected area to black.
- Reset – Allows the user to negate the selected thresholding area in either the image window or the histogram window.
- Set – Allows the user to define the threshold limits numerically.

One caveat of the thresholding function is that it is possible to make changes in either the thresholding window or the histogram window. I did not discover a way to make changes in both at the same time. However, it is possible to make changes in the image window and then open up a new histogram.

After the area, that you want segmented, has been selected, you want to measure it, so go to:

- Analyze>Measure (your new data appears in the "Results" window)

When you have finished with all the data in the "Results" screen, you can put it into an excel file (or other) by cutting and pasting or by saving that data file (saved as text – tab-delineated I think). Coincidentally, you can change the measurements that are being made (adding more characteristics) by selecting:

- Edit>Set Measurements in the data window

or

- Analyze>Set Measurements in the other windows

Particle Analysis

In this function, it is possible to examine certain phases. You'll want to go to:

- Analyze>Analyze Particles – make sure that display results and clear results table are selected. You can adjust "show" to be either nothing, outline, filled, or ellipses. I only played around with the last function. If you check size distribution as well, it will display a size distribution histogram automatically. When you have pressed okay, it will ask if you would like to save your old data. Voilá. If you don't get what you want displayed, my recommendation is to play around with the selections in Analyze>Set Measurements window.