

The goal of this exercise is to learn some basic image processing and analysis skills, using Image J (for Java), a modern version of the original NIH Image (note: do not download the old “NIH Image”, only get Image J). Image J will run on most computer platforms e.g. Windows, Mac, Linux. The homepage is [rsbweb.nih.gov/ij/](http://rsbweb.nih.gov/ij/) It is public domain, open source, with thousands users around the world continuously adding features to increase its usefulness in scientific imaging. The current version is 1.48q (Feb 2014). There is a nice tutorial at [www.fmhs.auckland.ac.nz/sms/biru/facilities/analysis\\_resources.aspx](http://www.fmhs.auckland.ac.nz/sms/biru/facilities/analysis_resources.aspx) and there is a wiki for it at [imagejdocu.tudor.lu](http://imagejdocu.tudor.lu)

Go to [rsbweb.nih.gov/ij](http://rsbweb.nih.gov/ij) and select/click the “Features”, the first item in the list, and read down and get a sense of what this is. Then go back a level, and click the “Download” item. You probably are doing this for the first time, on your own computer, so select the version appropriate to you. (If it is on a lab computer, check to see if ImageJ is already on it. If a lower version is there, then select the JAR File. Follow the instructions for installing/upgrading. (Note: current ImageJ versions requires recent Java versions – but current ImageJ versions come bundled with the necessary Java, to make it easy.)

You will be working with 3 images, all BSE: the first (B&W, 777LAVA.tif) is of a lava; the second and third (stregn\*BSE.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). The images can be downloaded from the Geology777 anonymous ftp site<sup>1 2</sup>([ftp.geology.wisc.edu/pub/g777/Images](ftp://ice2.geology.wisc.edu/pub/g777/Images)). These 3 images are also in the 777 Probe Class folder on the pc in the lab. ALWAYS work with a copy of the original image; it is very easy to make non-reversible changes.

Your assignment will be to determine the areas of the various phases present (“modal analysis”).

1. Image → Properties, and make sure Unit of Length is pixel and Pixels/Unit is 1 (because we want to use a simple measure → Note: if you wished, you could set up whatever unit you wanted, assuming you knew the conversion factor. Click OK to close.
2. Open up the first BSE image: File → Open → (Browse and locate) 777LAVA.tif --How many phases would you say are present looking at the various grayscale in the image? \_\_\_\_ Now, looking at its histogram (Analyze → Histogram), how many peaks do you see? \_\_\_\_ Take a second look --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). How many now? \_\_\_\_
3. We wish to determine the percentages of each phase present. First, we need to know the total number of pixels. In the Image → Show Info box it shows the image size in pixels; this is a 512 x 512 image, so these numbers should equal that number of pixels, right?)  
512x512 = \_\_\_\_\_ total pixels. Close the Show Info box.
4. Move the cursor over the large white phase at the top – now look at the “ImageJ” floating toolbar at the top and notice that the bottom line shows x, y and intensity value. What is the white feature's intensity? \_\_\_\_ It is 254, correct? Now drag the cursor over to the black area on the upper right

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<sup>1</sup> If you do not have an ftp program on your computer, you should. And know how to use it. In a pinch you can use a web browser, and type in instead of <http://blablah>, change http to ftp and ... <ftp://ice2.geology.wisc.edu/pub/g777/Images>

corner, what is that intensity value? \_\_\_\_ It is 1, correct? (Note that values of 0 and 255 are reserved for something else, other than minimum and maximum intensities.)

What does this tell you about the possibility that important features can be “lost” at the far right (“1”) and far left (“254”) of intensity histogram?

5. You will utilize **Thresholding** (also referred to as “segmenting” or “density slicing”). Select Analyze→Set Measurements—check “Area” and “Limit to Threshold” and Decimal Places to 0, then close with OK. Then select Image→Adjust→Threshold, then in the Threshold window, just adjust the top and bottom slides to fill in (red is nice) the phase you want to determine area for. Let’s start with the “white” phase. Click Analyze→Measure and a Results window should appear, which will now show in the area column the number of pixels for the phase you thresholded (colored in red) – you get something around 15900, right? Record this phase (give it a name, e.g. “white phase” and value), then readjust the sliders for the next phase.

Calculate the percentages of each phase. Then add them up. Do they sum to 100? Keep all your data!! And turn it in so, I can see how you did.

On a separate piece of paper, make a table with headings:

| Description of Phase (gray scale) | Number of Pixels | % pixels, |
|-----------------------------------|------------------|-----------|
|-----------------------------------|------------------|-----------|

and at bottom, total for pixels and for percentages

Go thru it once. How far off from 100 % is the total? A lot? **Then adjust your positioning of the sliders!** Iterate! If you are under 100% then you need to be more wide in your boundaries. If you are over 100%, you need to be narrower with your boundaries. Continue until you have totals that are in the 97-103% range, then take 3 sets of data in this range and average them.

You should either clearly state which phase is which in your table (e.g. “white”, “light grey”, “medium grey”, “dark grey”, “black/crack” OR include the image and tag representative phases with whatever you want to call them).

6. Now we’re going to use another feature of ImageJ, looking at orientation (e.g. compass direction) of a particle. We will examine the white phases (i.e. the brightest) in the sample.

Select Analyze→Set Measurements—and add one more check to “Fit ellipse”. Click OK.

Go back and threshold the white phases (Image→Adjust→Threshold).

Analyze→Analyze Particles, click blue Show slider and Choose Ellipses and check Display Results and Summarize, then click OK. Bang!

Several things happened: (1) you got a new image with the thresholded particles fit by ellipses; (2) a Summary box listing the number of features, area, average size (in pixels) and total area (says fraction, means % I assume); and best of all (3) under it, a Results table giving data about EACH feature: lengths of major and minor axes, and ANGLE. The table documents every particle (you could set a minimum pixel size to ignore below that Analyze→Analyze Particles, set min to say 4 ). Here we can look for a preferred angle for the particles which you could plot (e.g., in a “rose diagram”) to look for a direction fabric.

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’). Click File→Save as, and as a file the default is .xls format, although you have to tell it that it is tab delimited.

→ Put your data in a histogram. Use any program you have access to. As a last resort, you can use Excel, though it is not totally trustworthy (=not exactly accurate, but close enough for this exercise)>

Important! Excel does NOT automatically create proper histograms! The x-axis of a histogram must be the angles (i.e. 0 to 180°), with as many bins as you want e.g. if bins are every 10°, you'd have 18, and the height of each bin would be the cumulative sum of as many particles with that angle. **See appendix at end for instructions on making histogram in Excel. (Note: Excel actually is not the best way to make histograms, esp. for publication—it creates erroneous plots.)**

Is there a preferred orientation? If so, what is it? \_\_\_\_\_  
How many particles were measured? \_\_\_\_

7. Now for the second problem, for which you have 2 images, that overlap somewhat. This is a synthetic iron phosphate that apparently is heterogeneous, with 2 different compositions. I want you to determine the per cent 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 abundances (%) 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; and for the first image, the number of white phases, and whether or not (and if so, what) there is a preferred orientation of the white phases.**

**Extra credit: find an image of a material of interest to you, and perform some ImageJ operations on it, appropriate for what it is (modal percentages; orientation directions; or something else you find in ImageJ that works. Attach a printout of the image with your writeup about it.**

Revision history

10/13/06 10/19/06 9/18/07 10/1/07 11/25/08 10/9/09 3/5/12 2/25/13 2/10/14

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### **Appendix: Making histograms with Excel**

See attached sheet for details.