

Image J: Free image analysis tools from the NIH

Find a free and safe Image J download at: <http://rsb.info.nih.gov/ij/>

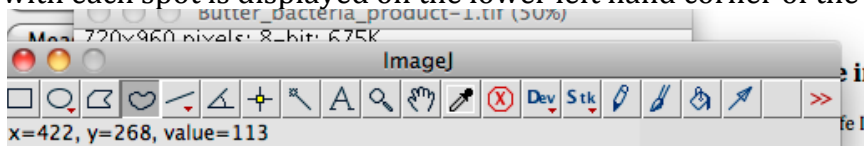
A quick guide to image intensity analysis using ImageJ

Here is a screenshot of the ImageJ dashboard:



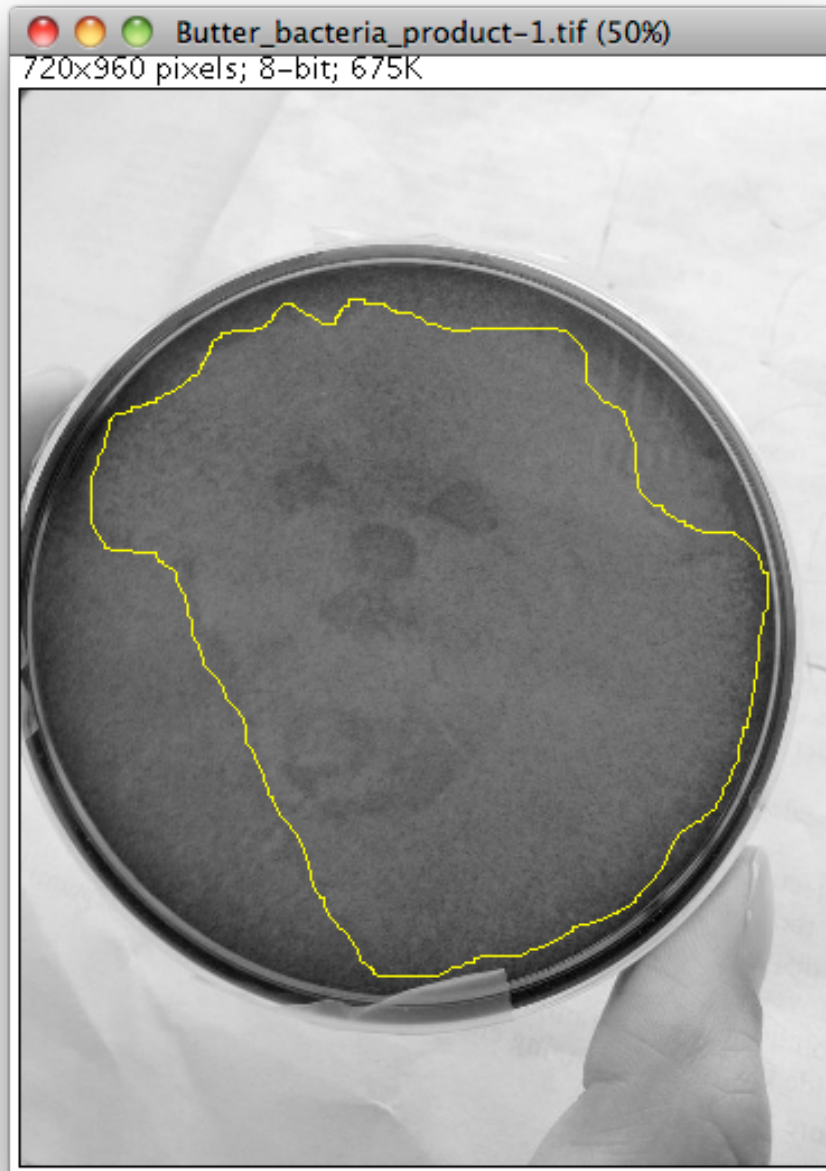
ImageJ is the most widely used free image analysis software in the biological sciences. The best way to really learn ImageJ is to read the extensive help files on the ImageJ website. However, for the purposes of the 20.109 F12 Mod2, here is a quick way to measure the pixel intensity associated with your B&W images.

1. Open the image by dragging it to the ImageJ icon.
2. Convert your image to an 8-bit file under Image → Type → 8 bit.
3. Notice that when you move your cursor around the image, the pixel intensity associated with each spot is displayed on the lower left hand corner of the dashboard.



4. You could move the cursor slowly and record numbers, but an easier way to quantify will be to define an ROI (region of interest) using the Square, Circle, Polygon, or Freehand tools at the upper left hand corner of the dashboard. In this example I've used the freehand tool

to outline Butterstick.



5. Now, go to Analyze → Tools → ROI Manager. You can Add the ROI so that you can use it across several images (for example, to compare WT to mutant).
6. Finally, go to Analyze → Measure. The Results window will contain the area (in pixels) of your ROI, the average pixel intensity, and the total pixel intensity. These are the quantities that you are interested in.
7. Play around with ImageJ to determine how to calculate the contrast of your image.

Remember – to directly compare your images they must have been prepared in a similar manner (i.e. on the same day) and the images captured with the same camera settings! There is a way to compare across days – can you think it?