Basic Intensity Quantification with ImageJ

Pretty pictures are nice, but many times we need to turn our images into quantifiable data. ImageJ is useful for getting information from images, including pixel intensity. There are a number of different ways to get intensity information from images using the base package of ImageJ (no plugins required).

Quantify Gray Levels Across an Entire Image or Single Object/Region

If you want to know the intensity of the entire image or a defined area within the image, there are several ways to do this:

1) You can simply hover the cursor over a given area in the image and read out the pixel intensity at that pixel on the toolbar. For RGB images, there will be three numbers, red, green and blue.

2) You can use the Analyze → Measure tool. First go to Analyze → Set Measurements. Check the boxes next to the information you want. You can get information on area, diameter, perimeter and other factors as well as information about intensity. For an explanation of any of these parameters, see the ImageJ documentation page:

http://rsbweb.nih.gov/ij/docs/guide/userguide-27.html#sec:Analyze-Menu
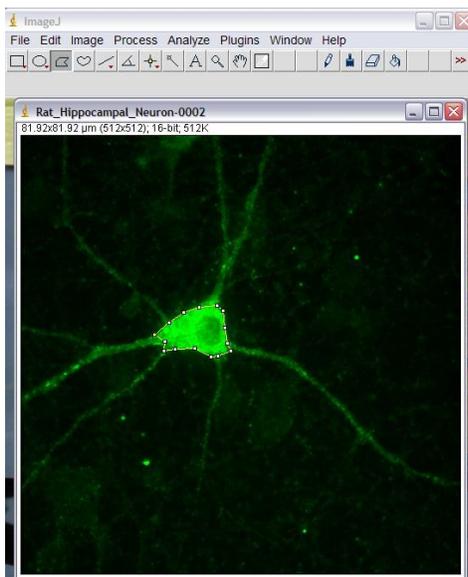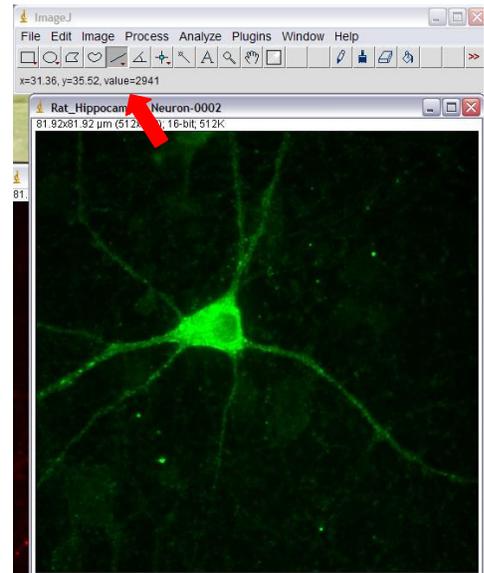
If you simply set your parameters and then choose Analyze → Measure, you will get information on the entire image, so average pixel intensity for your object plus background.

If you want to limit your measured area to just your object you have a couple of choices: first, you can draw a region of interest (ROI) around your object with one of the drawing tools (in the toolbar) and then Analyze → Measure will limit it's measurement to that area. Use Edit → Selection → Restore Selection to copy/paste that area onto another image to analyze the same size/shape area in another image.

Alternatively, you can go to Analyze → Set Measurements and check off the box next to "Limit to Threshold." Then use Image → Adjust → Threshold to highlight the area you want to analyze, and then Analyze → Measure will give you intensity measurements in just your thresholded area. In this example the thresholded area is highlighted with red. Note that thresholding works best on grayscale images, although there is an experimental color threshold tool built in to ImageJ that you can try as well.
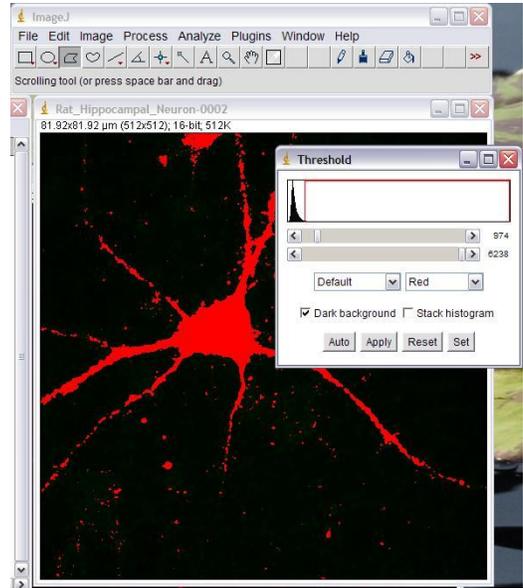

3) You can use Analyze → Plot Profile to create a plot of intensity values across features in your image. In the example below, the plot gives the intensity values along the line drawn across three cell processes. Image → Stacks → Plot z-axis profile will give a similar plot for intensity values through a z or time stack or within an ROI drawn on a stack. In both the Plot Profile and Plot z-axis Profile cases, pushing the "List" button gives a list of the intensity values used to create the graph. These values can be copied into another graphing program – particularly helpful if you want to graph two or more images on the same axis/scale.
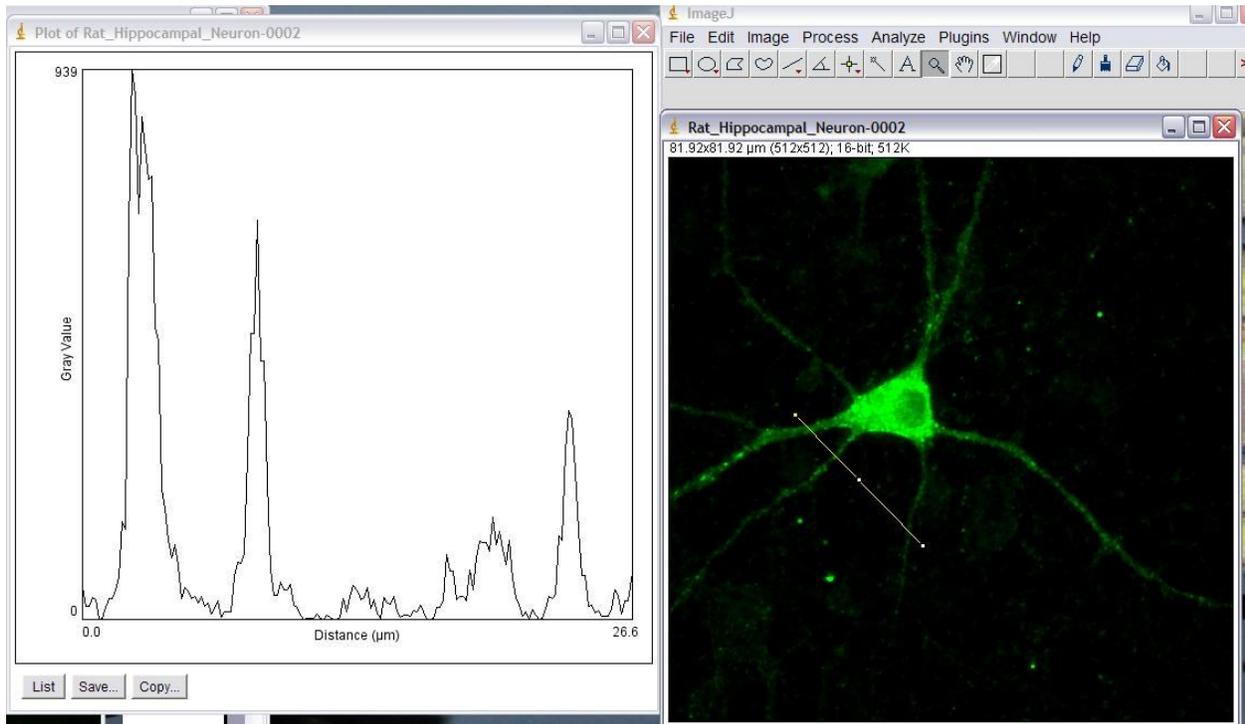
To Quantify Gray Levels for Each Object in Images with Multiple Objects

   If you have multiple objects in your image and you want intensity information for each individual object, begin as you would for object counting.

1) If your image is a color image (RGB), as in our example here, it is easiest to split multi-color images into single channels and convert single channel color images to grayscale before proceeding. Check that you have set Edit → Options → Conversions to "scale when converting." Then use Image → Type → 16-bit to convert to grayscale.
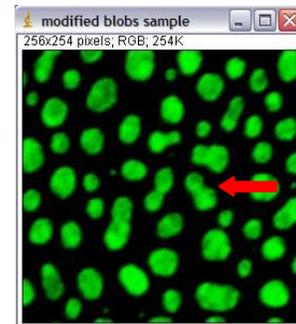
2) Make a copy of your image using Image → Duplicate.

Leave one copy as-is for now, and use your other copy to create a binary image. To create a binary image, use Image → Adjust → Threshold to highlight all of the structures you want to measure. In our example, the structures of interest are highlighted in red. To highlight, either use the sliders or use the "set" button to type in a known range of pixel intensities (if you want to threshold a whole set of images the same way, for instance).
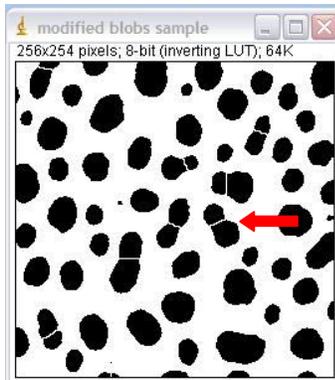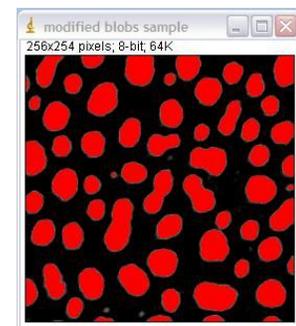
Process → Subtract background with rolling ball may help if you find you are highlighting too many "noise" or background pixels.

Once you have the area highlighted as well as you can, click "apply." This will create a binary version of the image with only two pixel intensities: black = 0 and white = 255.

3) If you have particles that have merged together, Process → Binary → Watershed can often (but not always) accurately cut them apart by adding a 1 pixel thick line where it feels the division should be. The example at right has been thresholded, turned into a binary image with "apply" and then run through the watershed program. For more information on other binary image tools, such as fill holes, see the Menu Commands section of the ImageJ Documentation page at: http://rsbweb.nih.gov/ij/docs/guide/userguide-26.html#sec:Process
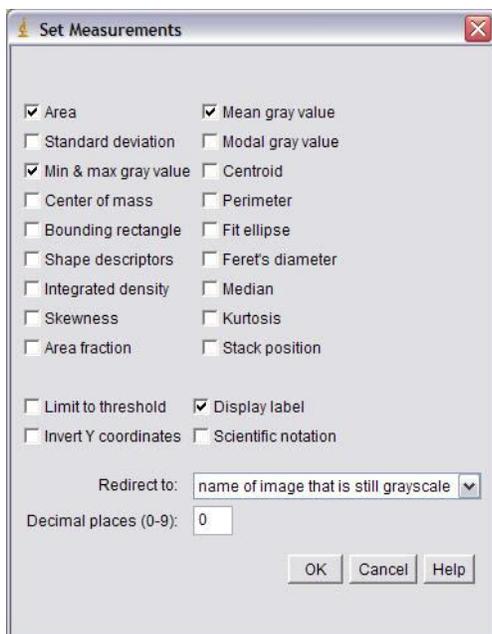
4) Use Analyze → Set Measurements and set the "Redirect to" line to the name of the copy of the image that is still grayscale. If you don't do this, your intensity values will be read from the binary image, and they will all be 255! Checking "display label" will label your data table with the image name and particle number. Use the checkboxes to select which gray value statistics you want from your image. You can get information on area, diameter, perimeter and other factors as well as information about intensity. For an explanation of any of these parameters, see the ImageJ documentation page:

http://rsbweb.nih.gov/ij/docs/guide/userguide-27.html#sec:Analyze-Menu

5) Click on the binary image to select it, then go to Analyze → Analyze Particles. There are some choices here that can affect the counts from your images. Size will affect what size particles to count. It will either be in pixels, or, if your image is calibrated, in a unit of measurement^2 (check under Image → Properties to see if your image is calibrated). Calibration is also important if you want any measurements involving size to have units of something other than pixels (um^2, for example).
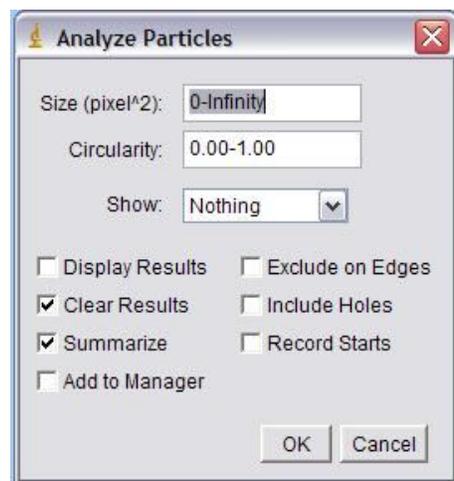
The ImageJ documentation page does a good job of explaining what each of the choices in this menu does, plus they are better at keeping up with updates than I am. Documentation can be found on the web at: http://rsbweb.nih.gov/ij/docs/guide/userguide-27.html#sec:Analyze-Menu

Below is an example of a count that "redirected" to the original 8-bit greyscale image. The results window lists mean pixel intensities for the blobs on the original image (top left), based on outlines applied from the binary image (top right and outline drawing).
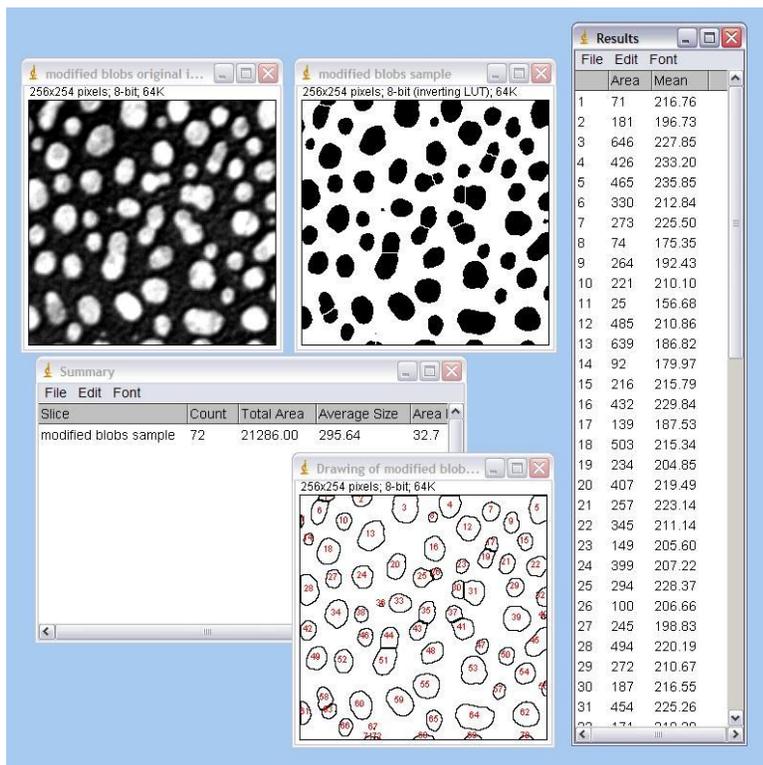
"Show" is helpful for troubleshooting. The example below shows the results of the "outline" choice. A copy of the image is made, and all counted particles are shown as numbered outlines. These numbers correspond to data for individual particles that is listed in the "Results" window if you check the "Display Results" option. If you want information on each particle, you want "Display Results" checked. "Clear Results" clears the Results window before a new run (save this window as an Excel file if the results are important). "Summarize" gives a summary window with the name of the image, total counts and other information for the whole image (shown). If you count multiple images, all counts remain listed in the Summary window, even if you clear the Results window.