# Two Ways to Count Cells with ImageJ

Figuring out how many cells are in a given image or group of images is a common need in image analysis. There are several ways to go about this, some more involved than others. These instructions cover two very basic ways to get cell counts, one manual and one automated. The automated version also allows other information about each cell, such as pixel intensity, area and/or diameter, to be read out at the same time.

Manual Cell Counting and Marking (plugin required)

This set of instructions allows you to count cells by clicking in the cell image. Each click marks the cell with a colored square and adds the cell to a tally sheet. You can mark up to four different groups of cells, and each group is tallied separately and marked with a different color square.

1) First, you must install the Cell Counter plugin, which is available on the microscopy server under ~ImageJ → plugins → 1 analysis. To install the plugin, copy the Cell Counter .jar file into the ImageJ plugins folder on your computer. You can also choose to install the entire "1 analysis" folder. Either way, you must re-start ImageJ after plugin installation for the Cell Counter plugin to appear under the Plugins menu.
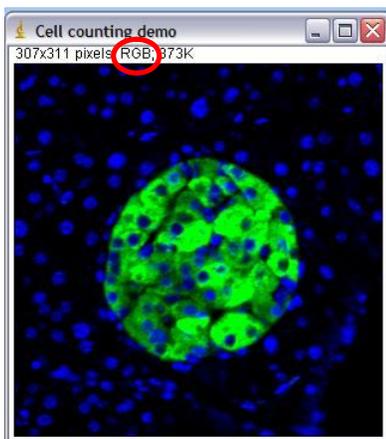

2) Open the image you want to count. Cell counter only works on single images, not stacks. You can use Image → Stacks → Stack to Images to convert a .tiff stack or .stk to single .tif files.

It does not matter if the image is greyscale, single color or multiple colors. However, if your image is in greyscale (you can tell because it will say "8-bit" or "16-bit" at the top, ours is a color image, so it says "RGB") and you want your clicked squares to be in color, you must convert the image to an RGB color image with Image → Type → RGB color. Greyscale images are supported by the counter plugin, but the squares are white or black, so difficult to distinguish between types. .

3) Select Plugins → 1 analysis → Cell Counter (or Plugins → Cell Counter). Two new windows will open, a counter window with your image on top of a row of buttons, and a results window where cells will tally.

4) To begin counting, click one of the buttons at the bottom of the counter window. Then click directly on a cell/object you wish to count. A white square will be
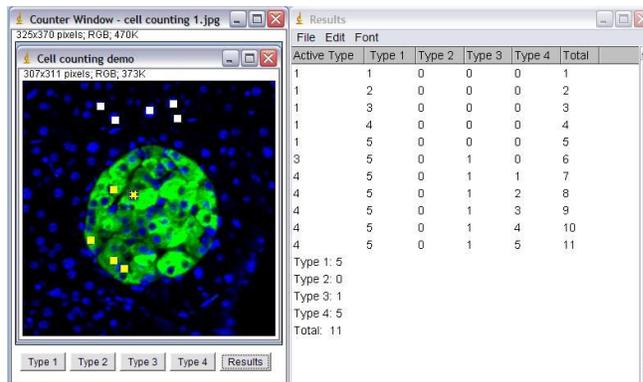
left behind on the object, and a tally will start in the results window. If you click on an object by mistake, you can undo it by selecting Edit → Undo. You must undo right away, you cannot undo more than one object in a row. To change the type of cell counted, click another button at the bottom. Type 1 = white, type 2 = green, type 3 = blue and type 4 = yellow markers. In this example we distinguish between nuclei inside the green area (type 4, yellow markers) and outside the green area (type 1, white marks). You can switch back and forth between types at any time, the tally will keep track. Cells may be counted as more than one type.

5) When you are finished counting, click the Results button. You will get a total for each cell type plus a grand total of all clicks at the bottom of the Results window. The results log can be copied and pasted and/or saved as .xls Excel spreadsheet. Unfortunately, the only way we know of to save the image with the marks is to create a screenshot (Print screen), open this in ImageJ with File → New → System Clipboard (Cntl + Shift + V) and save this as a .tif or .jpg file. This is essentially a snapshot of the image plus marks, it cannot be altered.

Automated Counting of Single Color Images (no plugin required)

   Sometimes counting by hand is just not practical, such as when there are many cells per image or you have many images to process. Counting can be automated, although depending on your image, this can get to be a complicated process. The example below details a simple count from a single color fluorescence image.

For more complicated counts (multi-color images, counts from histology or brightfield image, counting cells within a region, etc.) the Microscopy Core Facility staff can help you develop a custom counting routine or macro.

1) Open the image to be counted. If it is a color image (RGB), as in our example above, it will have to be converted to greyscale before proceeding. Check that you have set Edit →

Options → Conversions to "scale when converting." Then use Image → Type → 16-bit to convert to greyscale.

2) Once the image is in greyscale (8-bit or 16-bit) use Image → Adjust → Threshold (Cntl + Shift + T) to highlight all of the structures you want to count. 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).

Some particles may be touching already, shown here, or they may run together during the threshold. This is somewhat fixable. Process → Subtract background with rolling ball may

modified blobs sample
256x254 pixels; 8-bit (inverting LUT); 64K
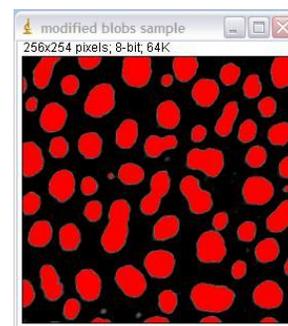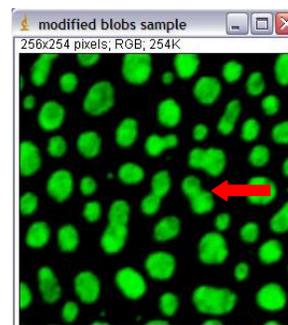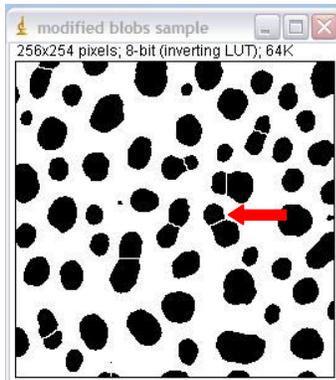
help if you find you are highlighting too many "noise" or background pixels.

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/index.html.

4) Once you have a binary image of the particles you wish to count, go to Analyze → Analyze Particles. There are some choices here that can effect the counts from your images. Size will effect 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 (Cntl + Shift + P). To count all particles, leave it at the default of 0 – Infinity. If you are getting too many small "noise" pixels counted as pixels, or you want to exclude particles based on size, adjust these numbers.
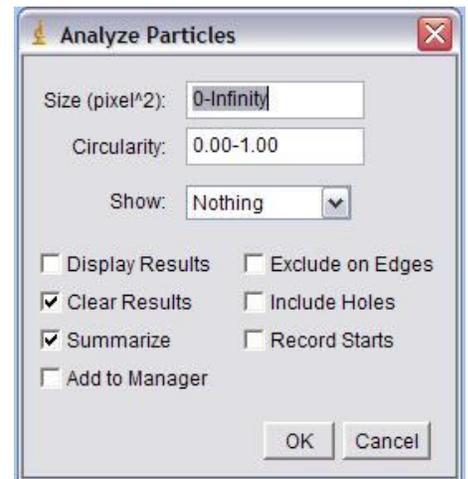
Circularity excludes particles based on how close to perfectly round they are. To include everything, keep at the default 0.00 – 1.00. To exclude things, adjust these numbers, keeping in mind that 1.00 is a perfect circle and 0.00 is a straight line.

"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. "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
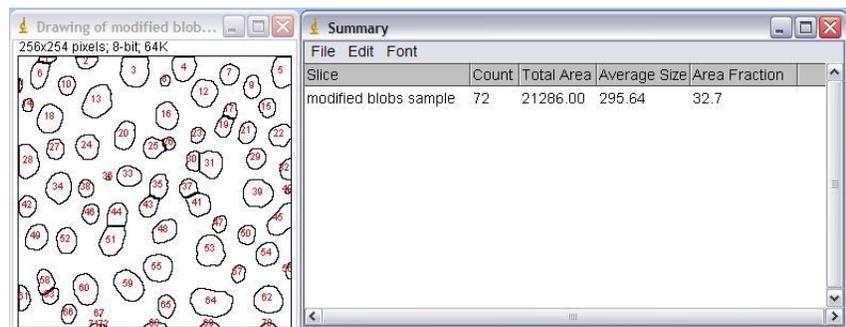
name of the image, total counts and other information for the whole image (shown). If you count multiple images, all counts remain listed in the Summary window, even if you clear the Results window.

"Add to Manager" adds the particle outline to the region of interest manager, which can be useful if you plan to do something else with the position information of the particle, such as use it to figure out how many foci are in a nucleus, or what other colors may be present in the particle. "Exclude on Edges" will not count particles that are touching the edge of the image (for example, #5 in the upper righthand corner of the example would not be counted with the exclude box checked). "Include Holes" will disable counting of particles inside other particles and "Record Starts" is a way of keeping track of the particles so they can be recreated with the "do Wand" function in a plugin or macro.

5) To get other information from the image besides just area, go to Analyze → Set Measurements. Check the boxes next to the information you want. For an explanation of any of these parameters, see the ImageJ documentation page: http://rsbweb.nih.gov/ij/docs/menus/analyze.html#set

6) To get information about another image based on the outlines in the counted image (such as an accurate pixel intensity instead of the 255 binary image pixel intensity) you can "redirect" to the original image or another, related image. Use the "Redirect To" section on the Set Measurements window, putting in the name of the image you want to use with the pulldown (image must be open in ImageJ for this to work. Specify the number of decimal places you want for your numerical data (2 is usually enough).
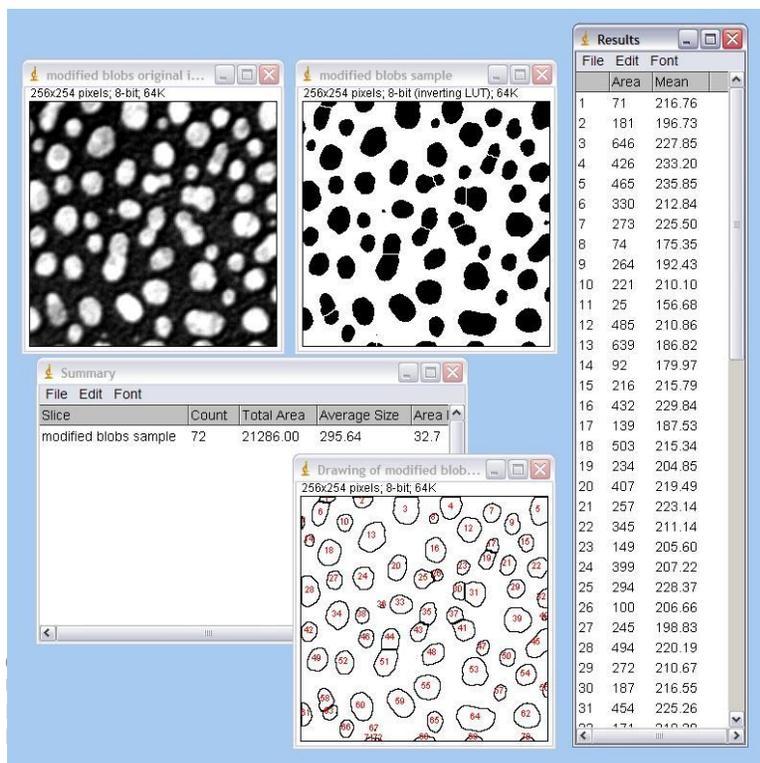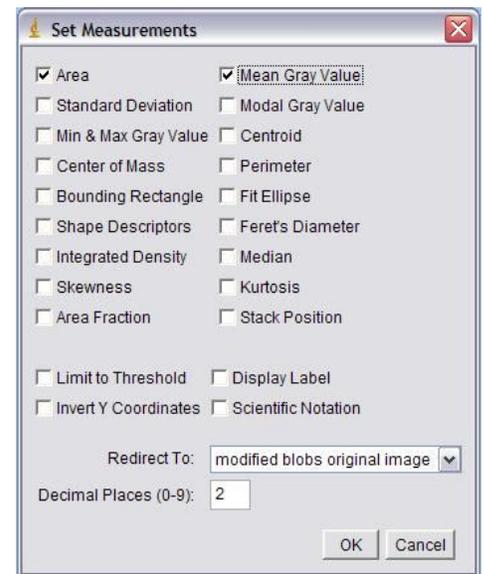
This is an example of a count that "redirected" to the original 8-bit greyscale image. The results window lists mean pixel intensities for the blobs on the original image (top left), based on outlines applied from the binary image (top right and outline drawing).