# Measuring Cell Fluorescence using ImageJ

Image J can be downloaded for free from here

Here is a very simple guide for determining the level of fluorescence in a given region (e.g nucleus)

1. Select the cell of interest using any of the drawing/selection tools (i.e. rectangle, circle, polygon or freeform)
2. From the Analyze menu select "set measurements". Make sure you have AREA, INTEGRATED DENSITY and MEAN GRAY VALUE selected (the rest can be ignored).
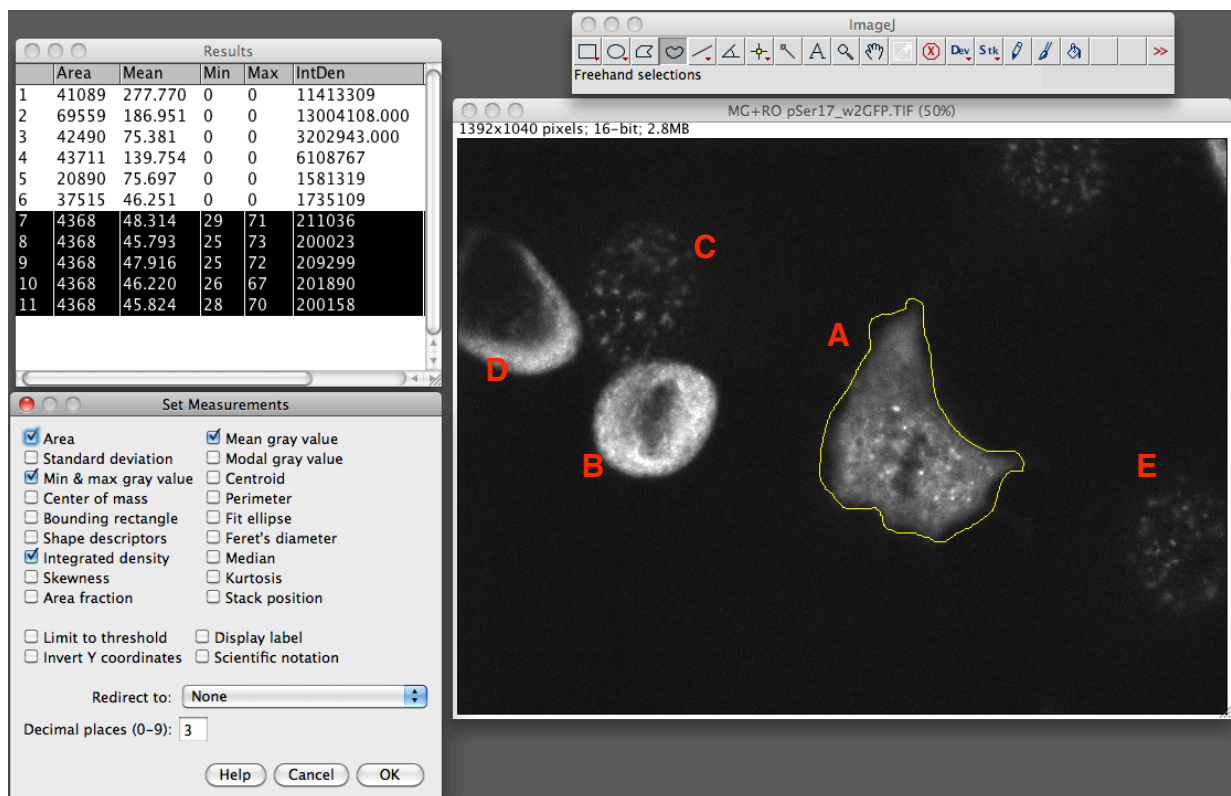
3. Now select "Measure" from the analyze menu or hit cmd+m (apple). You should now see a popup box with a stack of values for that first cell.
4. Now go and select a region next to your cell that has no fluroence, this will be your background.
   NB: the size is not important. If you want to be super accurate here take 3+ selections from around the cell.
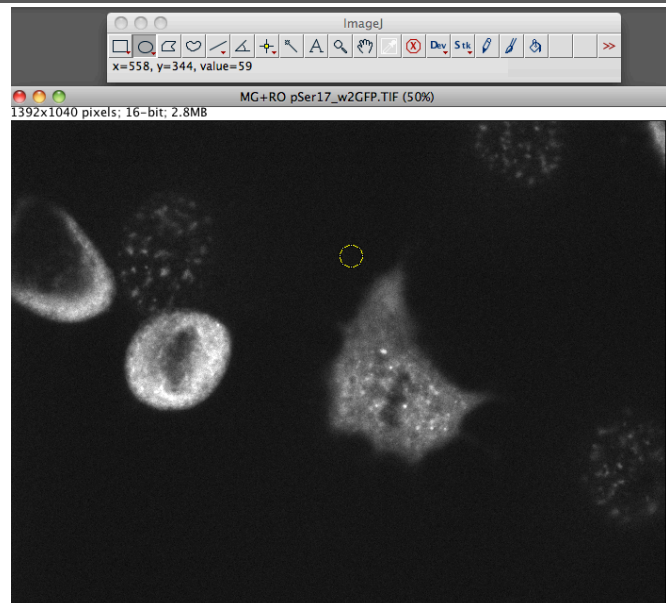
5. Repeat this step for the other cells in the field of view that you want to measure.
6. Once you have finished, select all the data in the Results window, and copy (cmd+c) and paste (cmd+v) into a new excel worksheet (or similar program)
7. Use this formula to calculate the corrected total cell fluorescence (CTCF). NB: You can use excel to perform this calculation for you.

CTCF = Integrated Density - (Area of selected cell X Mean fluorescence of background readings)

8. Make a graph and your done. Notice that in this example that the rounded up mitotic cell appears to have a much higher level of staining, but this is actually due to its smaller size, which concentrates the staining in a smaller space. So if you just used the raw integrated density you would have data suggesting that the flattened cell has less staining then the rounded up one, when in reality they have a similar level of fluorescence.