

Counting Particles in Cells Using Fiji

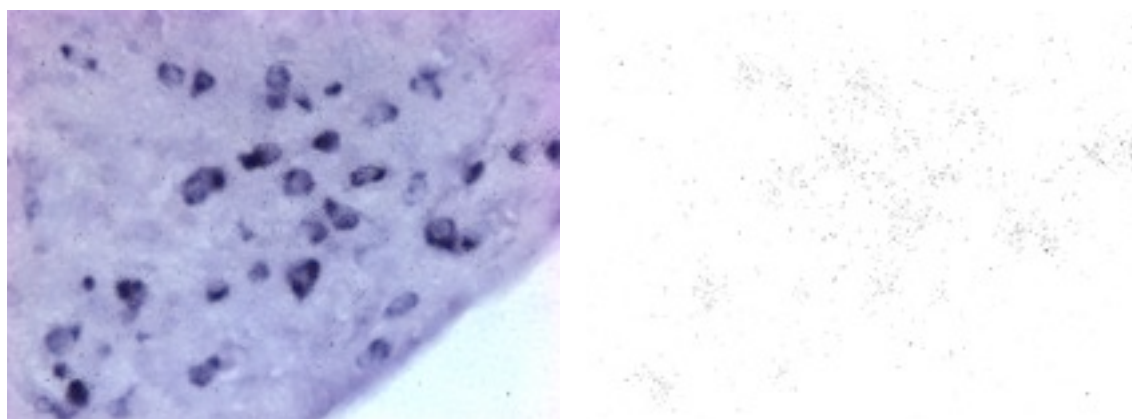
Download the open source software Fiji from <http://fiji.sc/wiki/index.php/Fiji> It's essentially the same as ImageJ, but comes with many pre-installed plugins and can update itself automatically.

Introduction

This is a method to count the number of dark particles which occur within the boundaries of cells. Treat it as a guide, as you may find your images need slightly different methods to obtain a reliable result.

Highlighting Particles of Interest

This procedure may vary wildly depending upon what the contrast is between the particles of interest and the background. This step can be done in Fiji by manipulating the contrast, brightness Image / Adjust / etc. Or, it could be performed in a program such as Photoshop or Gimp. It's assumed you have a method to transform your starting image to the one shown below which has the particles of interest separated from the rest of the image. In this case the number of particles per cell will be counted.



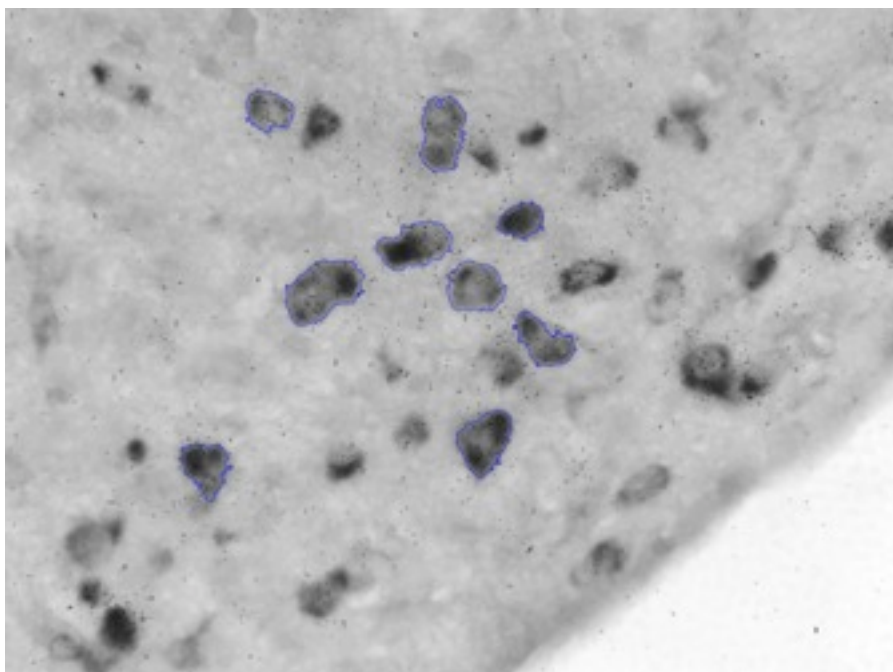
The first image has been processed in Photoshop to highlight the particles of interest to produce the second.

Selecting the Cell Boundaries

The cell boundaries set the region of interest (ROI) for the particle counting, this is done by selecting the boundary of each cell and adding each one to the ROI Manager: Analyse / Tools / ROI Manager.

The first step is to open the original image again, as shown on the left above, and convert it into an 8 bit greyscale image if it isn't already so. Image / Type 8 Bit. You may want to further enhance the particles contrast by thresholding, Image / Adjust / Threshold and then turning into a binary image by pressing Apply in the thresholding window.

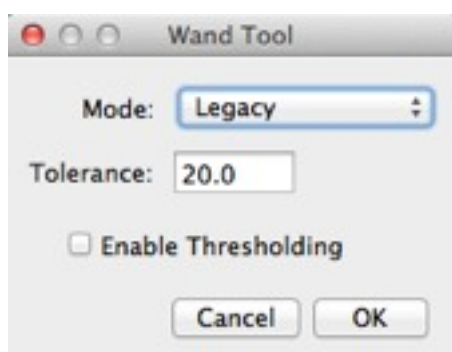
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Use the wand / tracing tool in the main menu bar (looks like something a fairy would use) and click on one of the cells. A portion of the cell will be selected, if you need to expand the selection zone, hold down the shift key and click just outside the selected area. Another portion of cell should be added. Repeat until you have the cell selected.

Add the ROI to the Region of Interest Manager and repeat for the other cells.

If the selection doesn't work as well as expected, you may have to adjust the tolerances of the wand tool. Double click its icon and change as appropriate.



The tolerance refers to how steep the threshold is between neighbouring area of grey. A low tolerance will limit the spread of the selection to area close to the value of the pixel you click on. A higher number will allow the selection to spread further.

The trick is to experiment to find a setting which suits your sample.

Applying ROIs to Particle Image

With the cell boundaries marked, apply them to the particle image by selecting it and then in the ROI Manager toggling Show All. This should make the ROIs appear.

To remove particles outside the ROIs, highlight them all in the list in the ROI Manager and go to the More option. Choose OR Combine, which treats the ROIs as one.

With the ROIs highlighted, go to the Edit / Clear Outside option and all other particles will be removed.

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Counting Each Cell's Particles

This uses the Analyse / Find Maxima ... option, which can count the number of particles (cells) corresponding to defined defined contrast relative to the background. It can be performed on a binarised thresholded image (a binary image consists of only black and white pixels, the white pixels indicating the features of interest) or a greyscale image.

If the cells have not all separated from each other, which would cause an underestimation of their numbers. By applying a Watershed filter, Process/ Binary / Watershed many of the cells can be separated. Potentially this still underestimates the cell number, but this is comes down to a judgement call. Is it a consistent error, or insignificant between treatments if the numbers counted are large enough? An alternative would be to measure the total area covered by cells, rather than their number, as this removes most of the problem produced by overlapping cells.



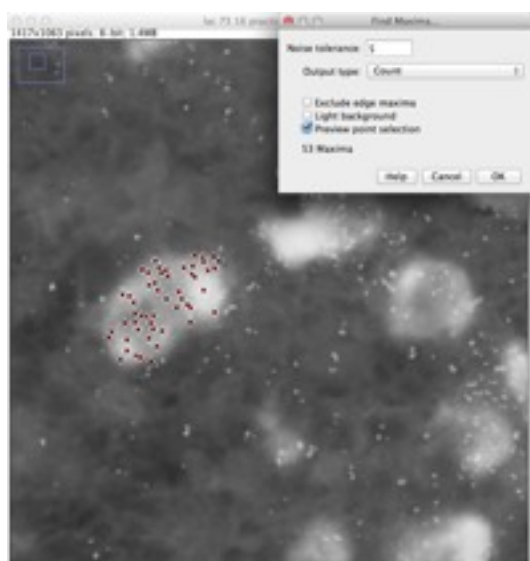
After removing all the particles outside the ROIs the image is inverted, Edit / Invert to produce white particles on a black background.

To count the particles in each cell cluster, you need to draw a ROI around each one in turn. Perform the steps described below and then repeat for the next one.

To count the number of cells, go to the Process / Find Maxima ... Choose the Points Selection option. Tick the Preview Point Selection box and assuming you have sufficient contrast, the cells will be highlighted and the cell count displayed in the window.

Record the number and repeat for the next cell cluster.

Alternative Selection Method



If you prefer to work on a greyscale image, that is, not thresholded and binarised, the Noise Tolerance option (default is 10) can be altered to vary the selection points.

The particles of interest must be lighter than the background, so inverting the image may be necessary.

Trace a ROI around a cell, use the Process / Find Maxima option as described above and by adjusting the tolerance highlight the particles and note the count.

It works by setting the threshold at which points must stand out from the background region.