# Counting Number of 3D Objects Using Fiji (fiji.sc)

Import your data set into Fiji so it is a single file with multiple planes of focus. This procedure requires a 3D data set, as it measures the characteristics of the particles volumes contained within it. The plugin described below will measure surface area, volume, Feret's diameter, how spherical a particle is (compactness) and a number of other characteristics.

Preparing the Image for Counting

There is no single fail-safe method for every image, experiment with a number of options to see which is the most appropriate. *Always* work on a copy of the original image because (i) sometimes the undo button won't undo a particularly ill-advised edit and (ii) comparing the modified image to the original image will indicate if the image to be analysed has deviated too far from the original to draw sensible conclusions.

Most images contain too much background information which is not part of the structures of interest. In many cases this can be removed by using thresholding to select only the features of interest.

Thresholding selects a range of pixel intensities that correspond to only the features of interest. Check the image is either 8 or 16 bit greyscale (Image/Type to change) and then go to Image/Adjust/Threshold and move the sliders on the threshold window so that the features of interest at highlighted in red.
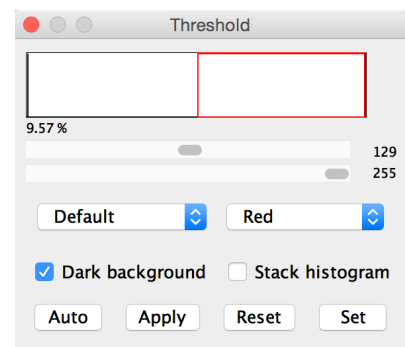
Experiment with the options under the Default drop down menu, one may suit your image better than another. Click Apply when the settings seem correct. The image needs to be white objects on a dark background, they may appear reversed if the program applies an inverted LUT (Look Up Table), where white pixels have value of 0 and black pixels 255 (for an 8 bit image). Check the image window's top edge to see if 'Inverting LUT' is written there. To correct it, go to Image/LUTs/Invert LUT to switch it back to normal.

In most cases, thresholding does not do a perfect job of isolating the features of interest. It may be necessary to further refine the image using techniques such as Process/Binary/Dilate Possibly with Erode as well, experiment with different combinations. Using Process/Filters/ Gaussian Blur can also be effective at smoothing shapes enough to remove single pixels, but retain the essential shape and size of the particle. Keep checking if you begin to corrupt the original image beyond what is sensible. The idea is to isolate the 3D objects of interest, but retain as much of their original structure.

Make a copy of the processed image to save the adjustments and to preserve the original image.

Quantifying the 3D Objects

This uses the 3D Manager in Fiji, but first choose the parameters you want to measure from its preferences under Plugins/3D/3D Manager Options menu. Choose the options you require, too many options might make the analysis slow. A reasonable configuration to try is shown here.

Note some measurements can be made in both pixel units or calibrated units. Choose one or the other to reduce data-clutter; pixels if your image is not spatially calibrated and units if it is.
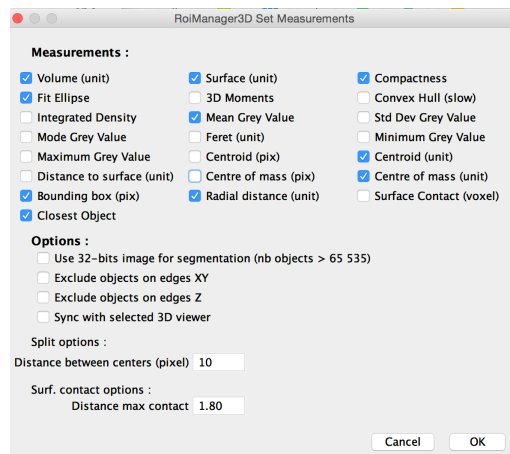
After choosing the parameters to measure, run the 3D Manager from Plugins/3D/3D Manager. The window shown here should appear.

Click on the 3D Segmentation button, this allows you to select the intensity range you are interested in. It's better, however, to have done most of the selection process before using the plugin, so this step should be analogous to speaking to the converted.

Once thresholded, click Add Image, this will fill the left hand window with a list of 3D objects detected in the volume. Click Measure 3D to generate the statistics about each of the objects, or the ones you selected from the list. Clicking Quantify 3D also generates statistics about the objects, decide if they are useful or not by trying both options.
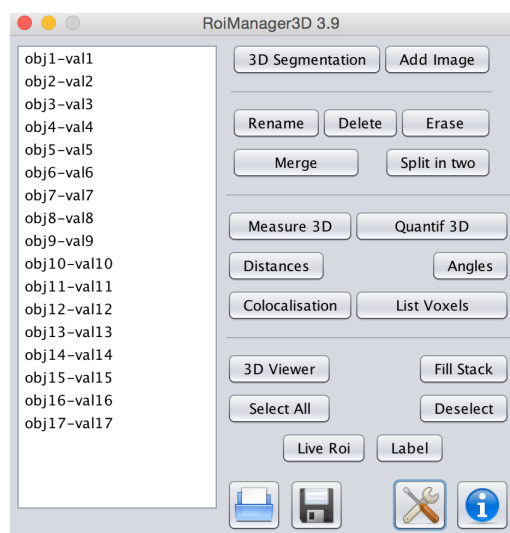
Clicking the crossed tools icon allows the settings to be changed within the plugin, rather than having to return to the settings option in Fiji's plugin menu. Explore the other buttons to see what they do, it's good way to learn stuff.

Click on the title of each column to sort on that value, click it again to reverse the order.

For a more detailed description of the measured characteristics, see the plugin's website:
http://imagejdocu.tudor.lu/doku.php?id=tutorial:working:tutorial_for_3d_roi_manager