Analysing Matching Regions of Interest in Multiple Images Using Fiji / ImageJ

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.

This procedure assumes you are familiar with thresholding and the Region of Interest (ROI) manager of Fiji / ImageJ. If not, read the 'Getting Started' pdf, which can be downloaded from: http://occm.otago.ac.nz/how/how_to.html
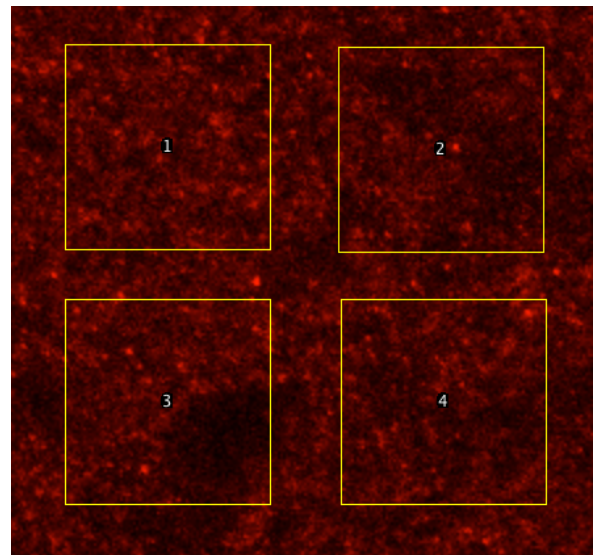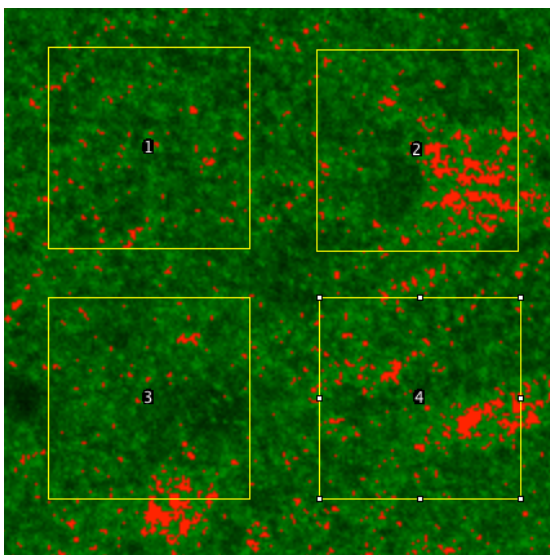
This procedure allows you to compare two (or more) images on a pixel by pixel level, if required.

Split Images (if from multi-channel confocal data)

Go to the menu Image / Colour / Split Channels. This makes two separate images, the ones you want to analyse the same regions of interest and / or thresholded pixels in.

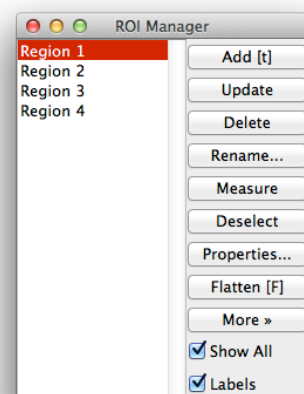**Propagating Regions of Interest to a Second Image**

Use the ROI manager (Analyse / Tools / ROI Manager) to collate multiple ROI if you want to analyse a number of areas. In this example, four regions have been added and the green image

thresholded to highlight pixels of interest.

To propagate the ROIs on the green image to the red one, click in the red image's window to make it active. Then, tick and untick the 'Show All' option in the ROI MAnager's window. This updates the ROIs and they will now appear in the red image. This process can be repeated on any number of images which have the same dimensions and resolution.
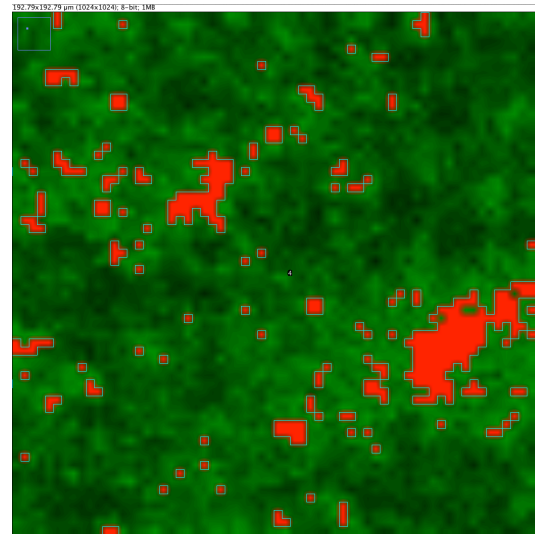

Any analysis, such as intensity, area, etc. You perform on the ROI in the first image can be replicated on the same corresponding ROI in the second image. Remember the analysis will be performed on the active window, not both simultaneously.
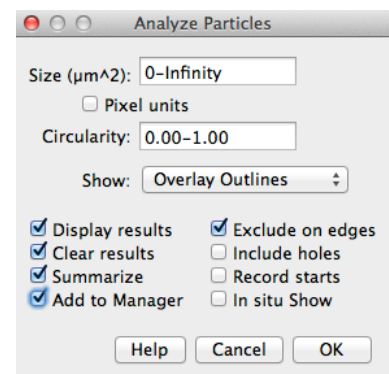
Creating Many Regions of Interest from Thresholded Pixels

In this example, each thresholded group of pixels can be treated as a particle. This means each particle can be characterised according to whichever parameters you set in the Analyse / Set Measurements window. Area, Max / Min Intensity, perimeter, etc. Make sure you have 'Analyse Threshold' ticked to ensure only thresholded (highlighted in red) pixels are counted.

After adjusting the measurement settings as appropriate, go to the Analyse / Analyse Particles menu and set it up as required. It is recommended to show Overlay Outlines as this draws a boundary around the particles being measured, which removes any doubt as to what you're measuring. (Zoom in on the image above to see how the particles are marked with a blue border.)

If you have particles bordered by a ROI, ticking 'Exclude on edges' will not count particles which are partly out of the ROI. If no ROI is used, this applies to the edge of the image instead.

Ticking 'Add to Manager' stores the particle ROIs in the ROI manager and allows their positions to be duplicated in another image as described above. The Analyse / Analyse Particles step can be run again on the second image, analysing the same position in the image as the first.

Images courtesy of Leitch Laboratory, Department of Anatomy, University of Otago.