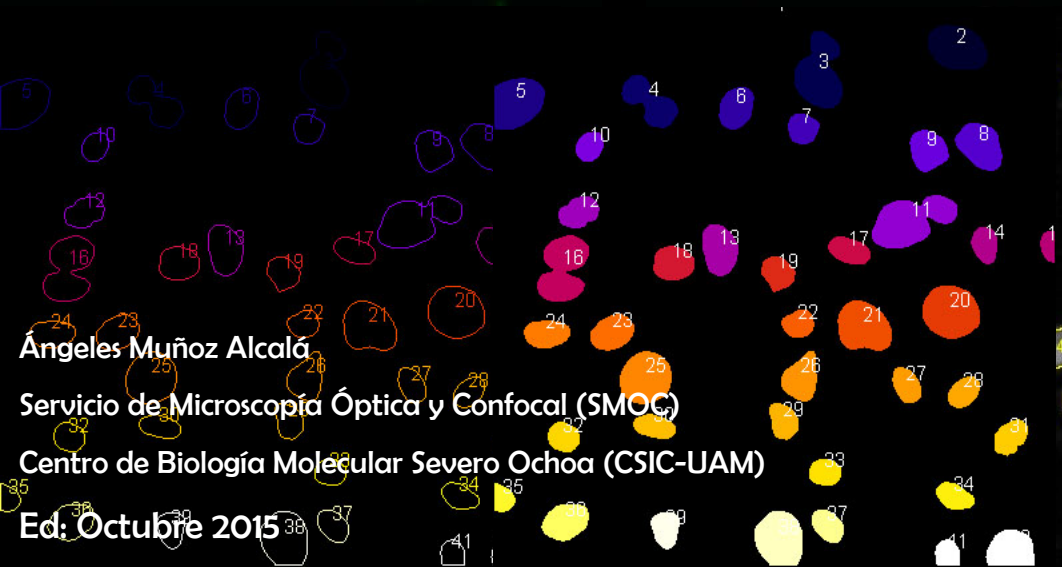
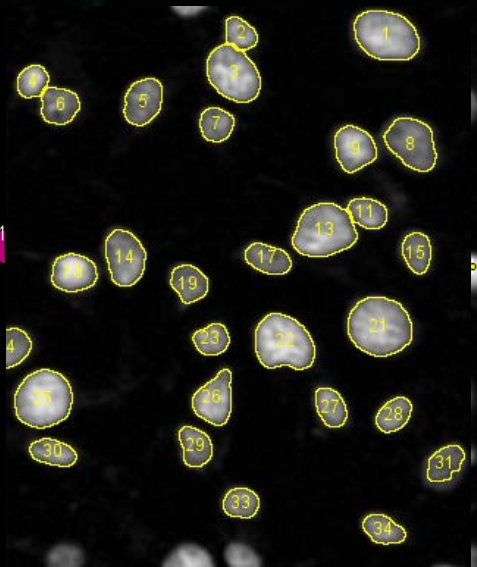
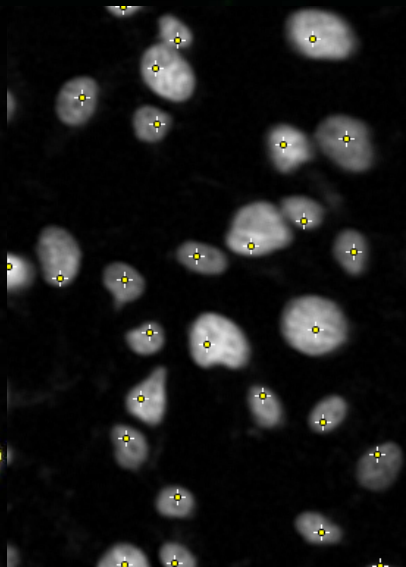
ImageJ/Fiji particle counting tools







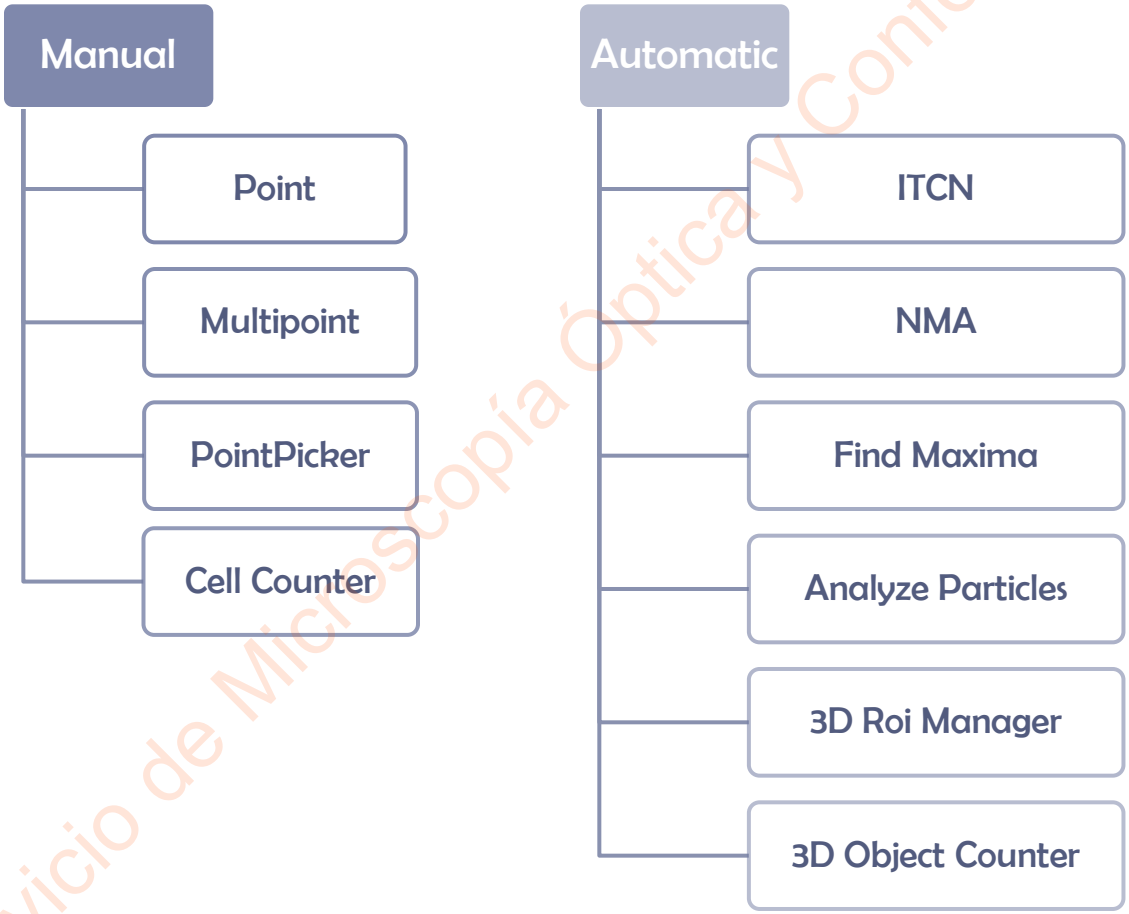
Ángeles Muñoz Alcalá

Servicio de Microscopía Óptica y Confocal (SMOC)

Centro de Biología Molecular Severo Ochoa (CSIC-UAM)

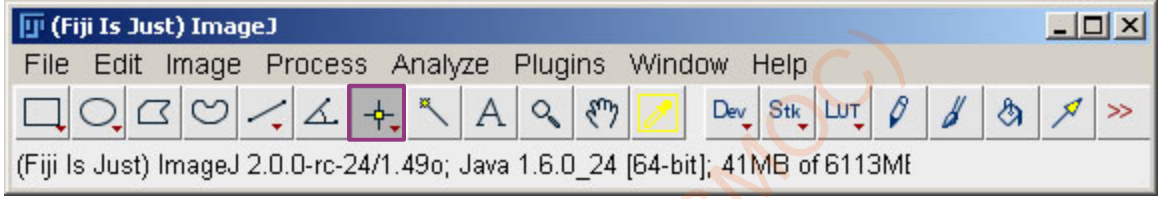
Ed: Octubre 2015

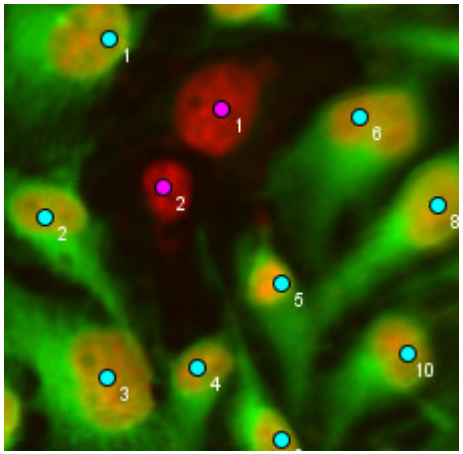
COUNTING



Manual

Point





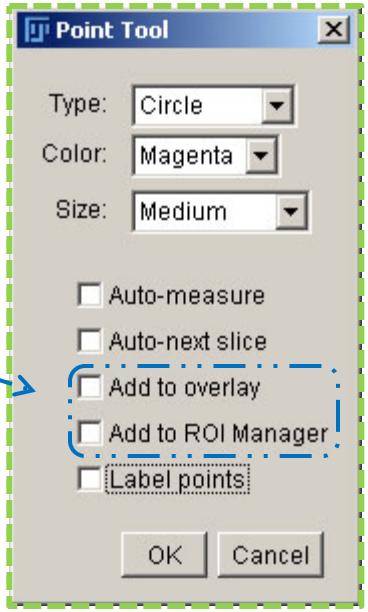
To count several points

+ Shift

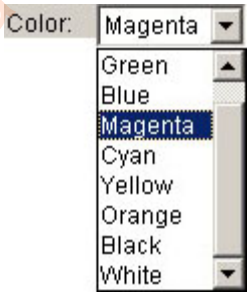
or

+

Some of these options



By double clicking you can access some additional options:



Auto-Measure: Records the pixel location and intensity.

Auto-Next Slice: Automatically advance to the next stack slice.

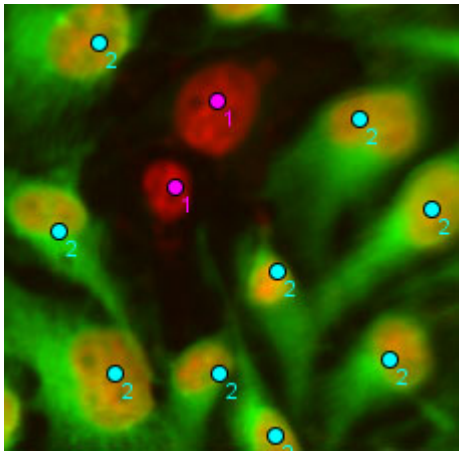
Add to ROI Manager: Points will be automatically added to the ROI MANAGER

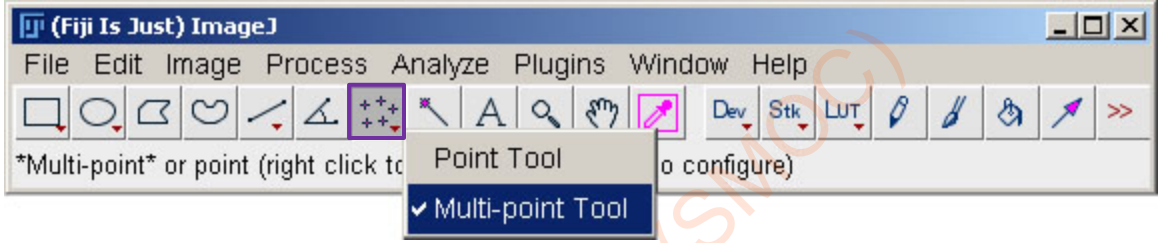
Label Points: Each point selection will be displayed with an accompanying numeric label.

http://rsbweb.nih.gov/ij/docs/guide/146-19.html#sec:Point-Tool

Manual

Multi-Point

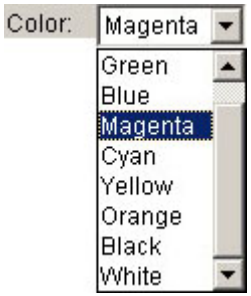


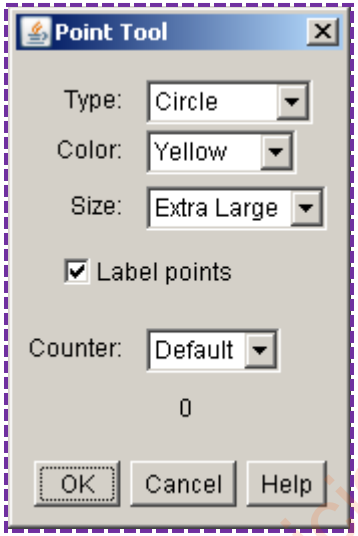


With a right-mouse click over the Point Tool  t is possible to access the **Multi-point Tool**

to mar  ral points

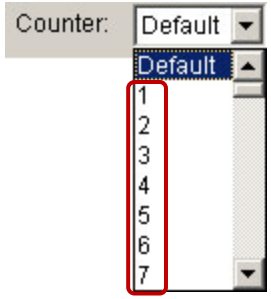
By double clicking  u can access some additional options:



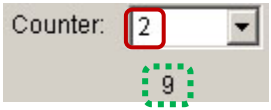


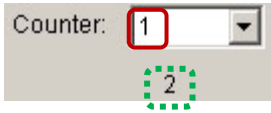
Label Points: Each point selection will be displayed with an accompanying numeric label.

Counter:



Select **counter** to group **numbers** in categories



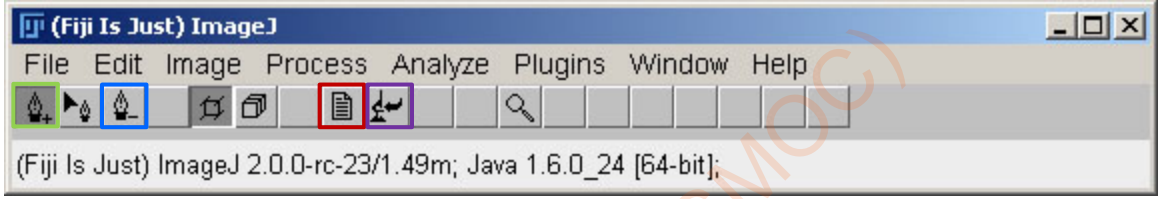


- Alt-click, or control-click, on a point to delete it.
- Press 'y' (*Edit>Selection>Properties*) to display the counts in a results table.
- Use *File>Save As>Tiff* or *File>Save As>Selection* to save the points and counts.

http://rsbweb.nih.gov/ij/docs/guide/146-19.html#sec:Multi-point-Tool

Manual

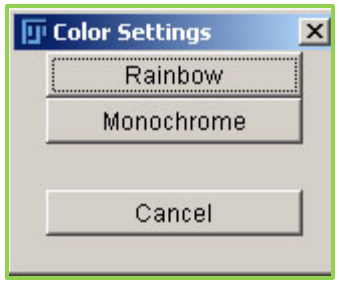
PointPicker

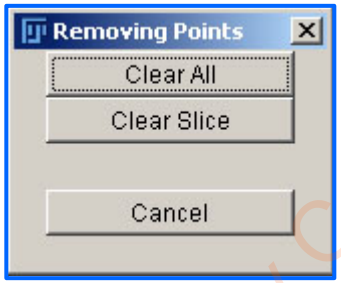


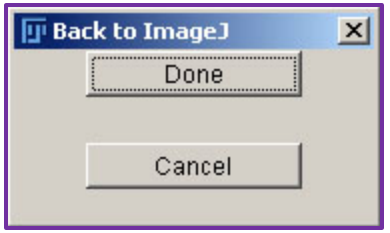

Add point

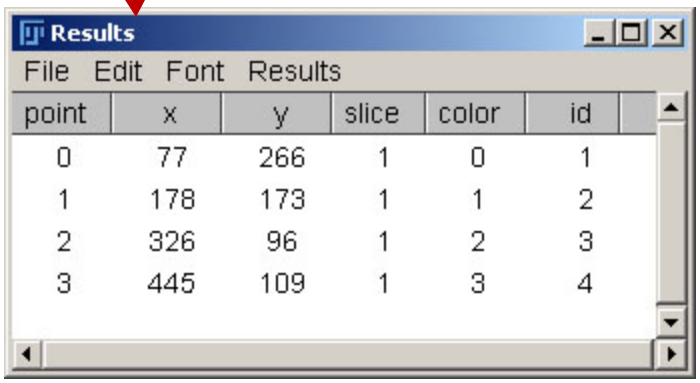

Move point

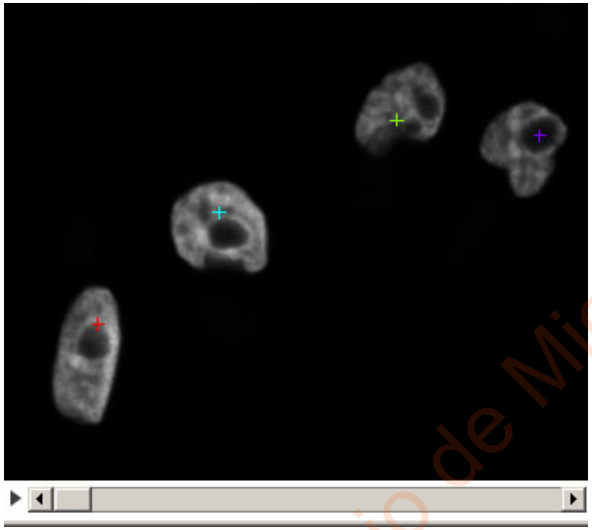

Remove point











Go to **Analyze/Tools/PointPicker**

This tool allows you to pick some points in an image or stack and to save the list of pixel coordinates as a text file.

It is also possible to read back the text file to restore marks over another image.

http://bigwww.epfl.ch/thevenaz/pointpicker/

Manual

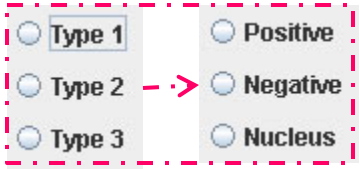
Cell Counter

1º - Go to **Plugins/Analyze/Cell Counter**

2º - Select "**Keep Original**" and "**Show Numbers**".

3º - Press "**Initialize**" (this will create a duplicate image/stack).

4º - Select **Type** (1, 2, 3, …).



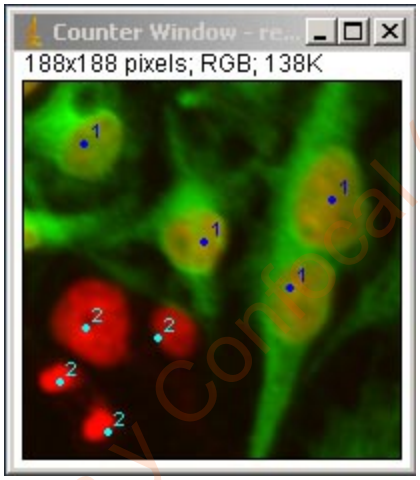
*You can **Rename** the types*

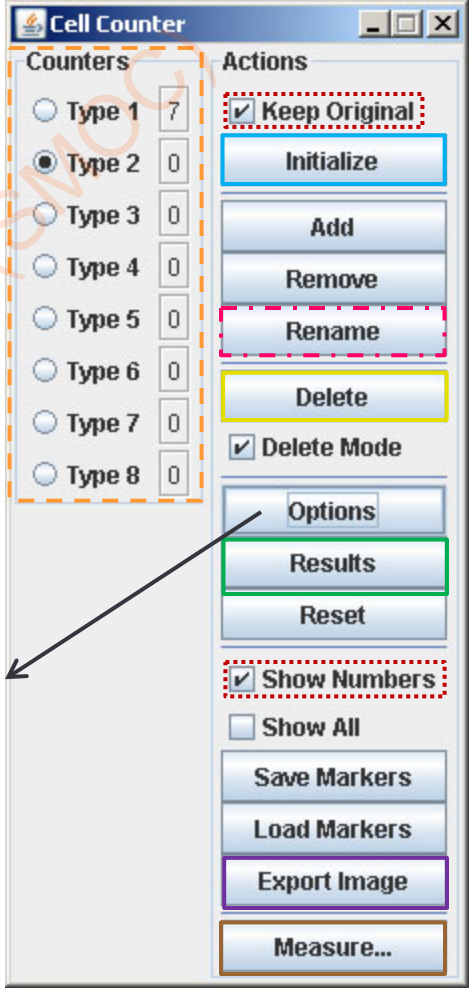
5º - Count by clicking on the feature in the image.

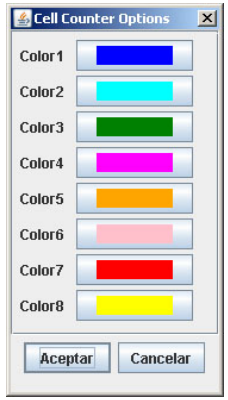
6º - Repeat steps 3 and 4 as many times as needed.

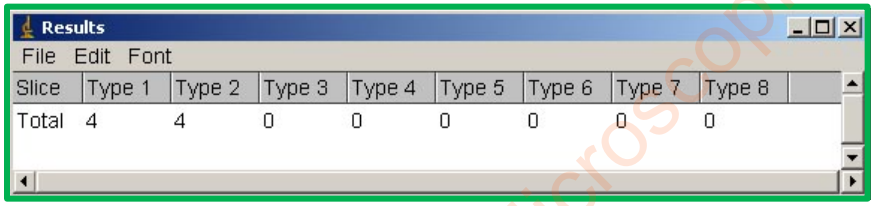
7º - **Export Image -** Makes a copy of the image with the markers written on it .

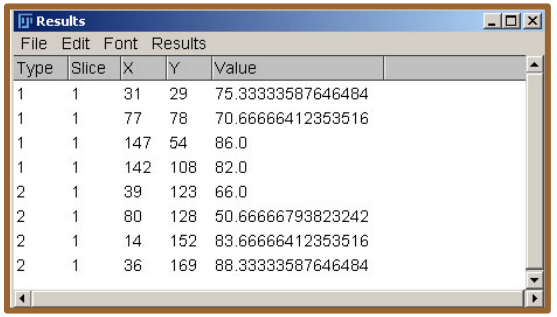
8º - Results - Shows a table with results.











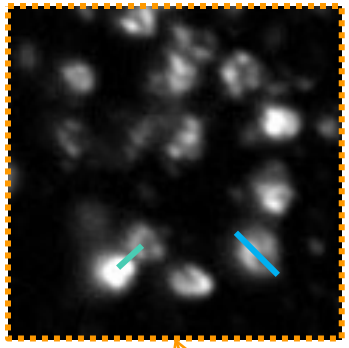
Delete - Delete the last placed marker

Measure - Measures pixels for each marker and displays a table with Type - Slice -

X coordinate - Y coordinate - Pixel Value

Only 8 bits images, NO STACKS

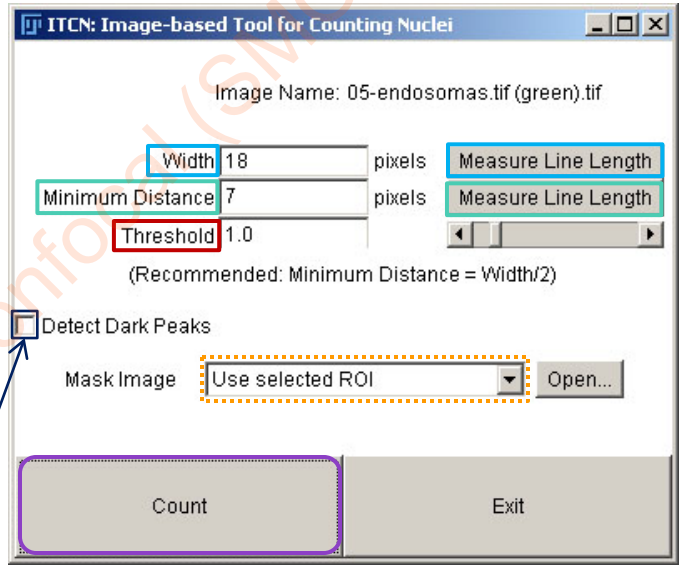
ITCN (Image-based Tool for Counting Nuclei)



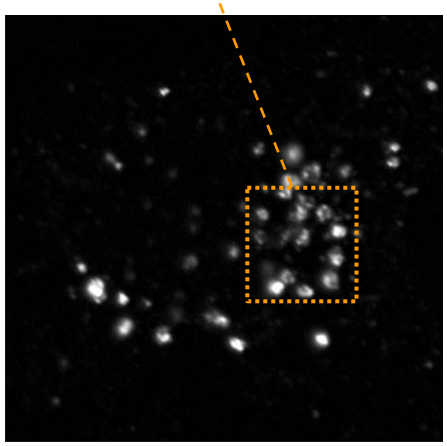
1º- Go to **Plugins/ITCN**

2º- Write a line to measure the diameter of the cell and press **Measure Line Length** in the **Width** menu.

3º- Write a line to measure the distance between cell's centers and press **Measure Line Length** in the **Minimum Distance** menu.



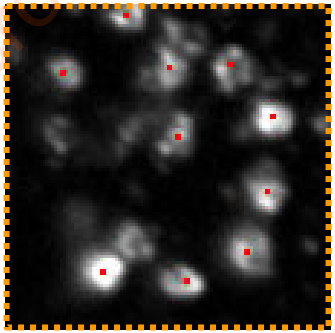
Mark checkbox if you're looking for dark peaks

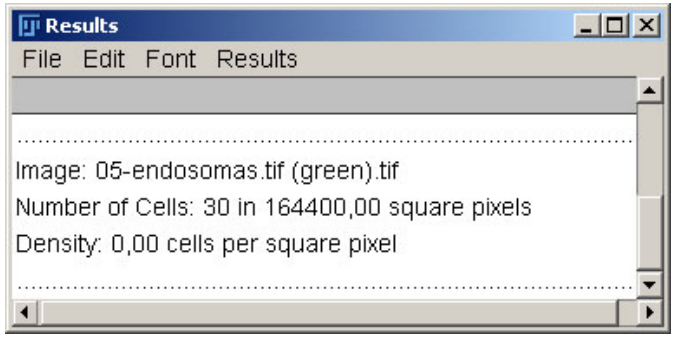


4º- Modify the **threshold** to select cells by intensity and press **Count**.

5º- Repeat step 4 as many times as needed.

For testing you can use a region of interest and select "**Use selected Roi**"





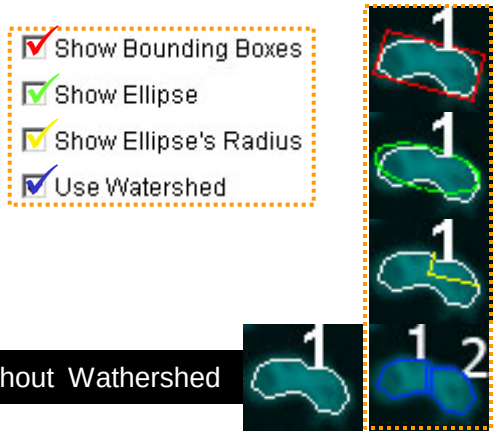
Check how to install this plugin in page 19

Automatic

NMA (Nuclear Morphometric Analysis)

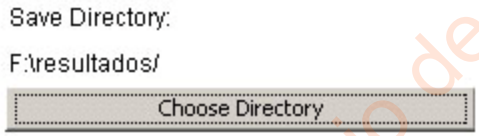
1º- Go to **Plugins/NII_Plugin/NII**

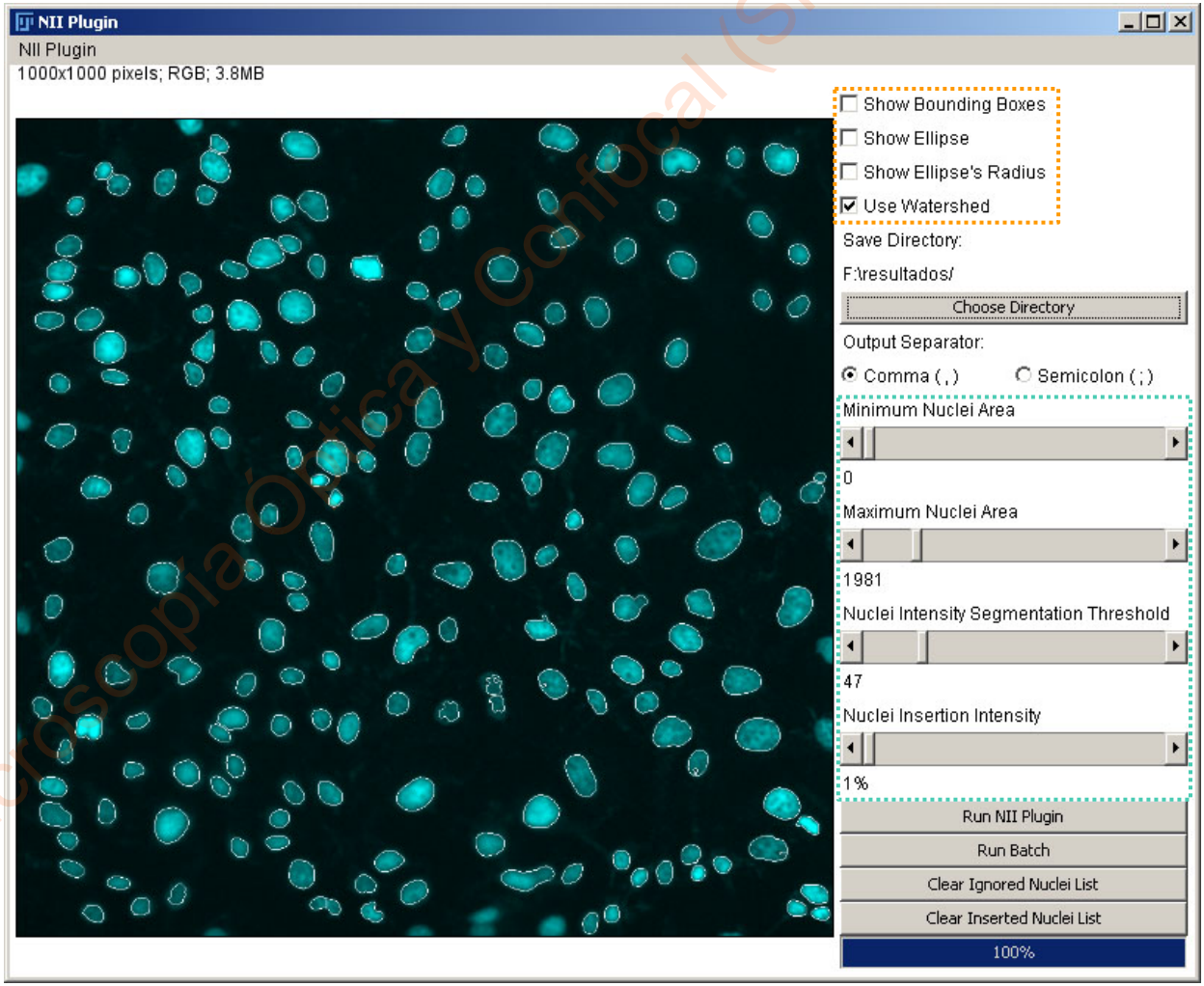
2º- Choose the options to select.



Watershed *will try to separate some of the nuclei that are touching each other.*

3º - Select directory for results



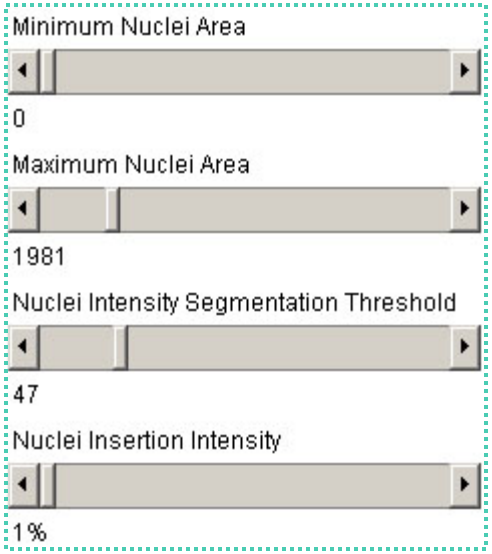


http://www.ufrgs.br/labsinal/NMA/ & Manual

Automatic

NMA (Nuclear Morphometric Analysis)

4º - Modify the next parameters to obtained a good result.

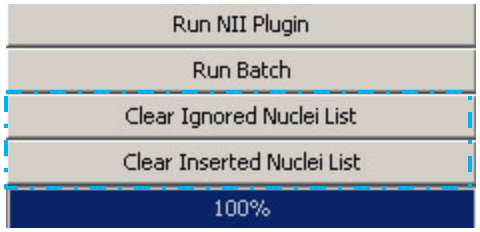


Minimum Nuclei Area: The minimum area (in pixels) of a nucleus to be considered.

Maximum Nuclei Area: The maximum area (in pixels) of a nucleus to be considered.

Nuclei Intensity Segmentation Threshold: To select nuclei by their intensity

Nuclei Insertion Intensity: This is the percentage of the Segmentation Threshold used when adding or removing an element manually.



5º - Select Run NII Plugin to process one image or Run Batch to process every image located in some folder

To add a nucleus press 

To remove nucleus press 

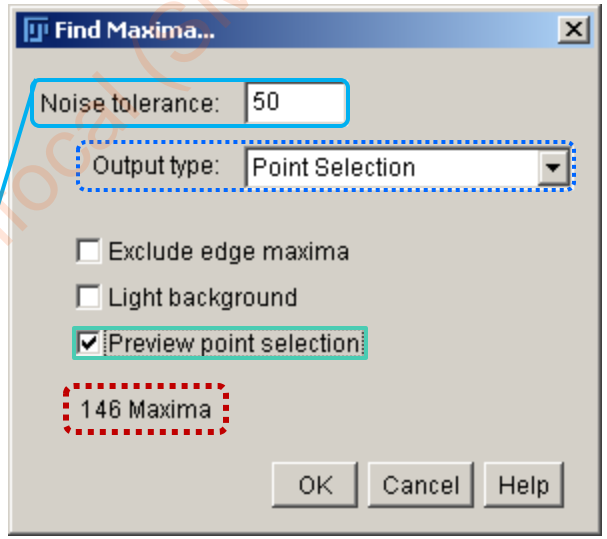
Remove all inserted nuclei or re-insert all removed nuclei

http://www.ufrgs.br/labsinal/NMA/ & Manual

Values in pixels

Automatic

Find Maxima

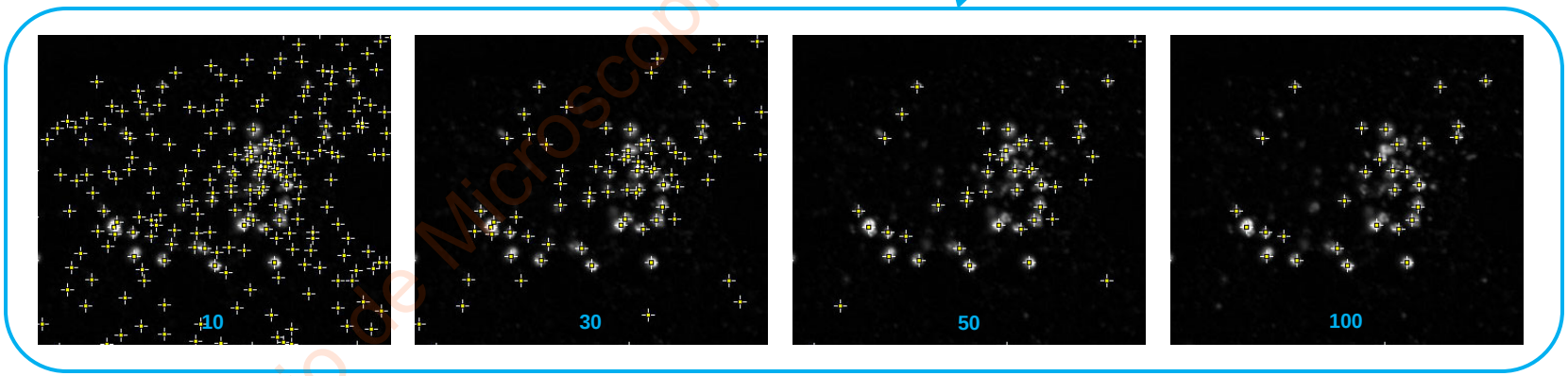


1º - Go to **Process/Find maxima**

2º - Activate **Preview point selection** to check how it works

3º - For testing you can modify **Noise tolerance** *(a threshold is set at the maximum value minus noise tolerance and the contiguous area around the maximum which is above the threshold is analyzed)*

4º - Select the **Output type** (see next page)



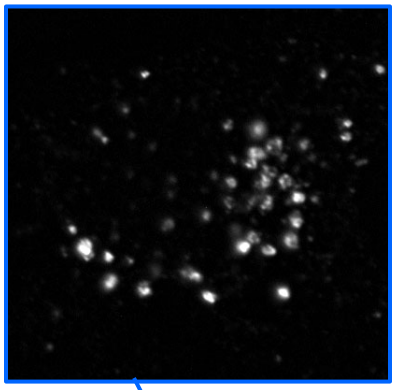
Counts: 191 **Counts: 73** **Counts: 44** **Counts: 30**

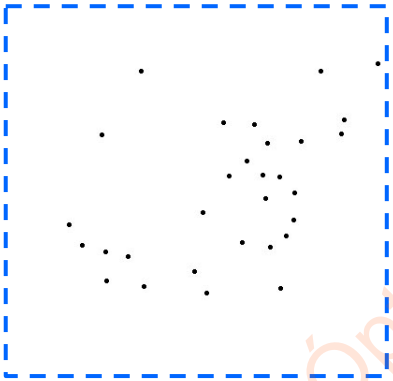
http://rsbweb.nih.gov/ij/docs/guide/146-29.html#toc-Subsection-29.4

Automatic

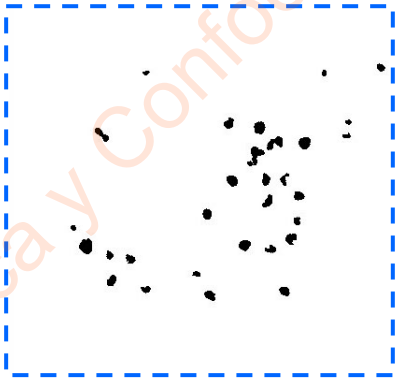
Find Maxima

Six **outputs** are possible: Single Points, Maxima Within Tolerance, Segmented Particles, Point Selection, List and Count.

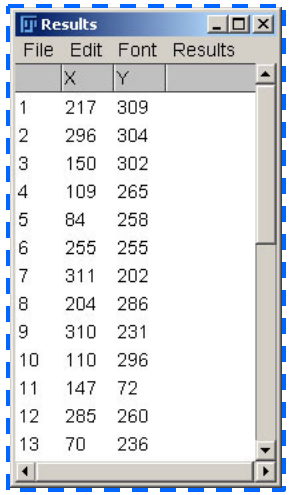




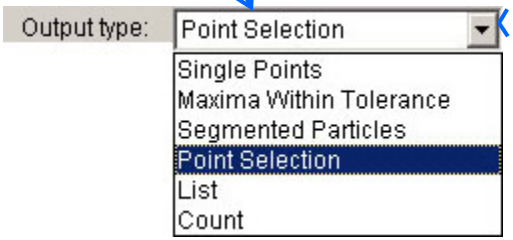
Single Points

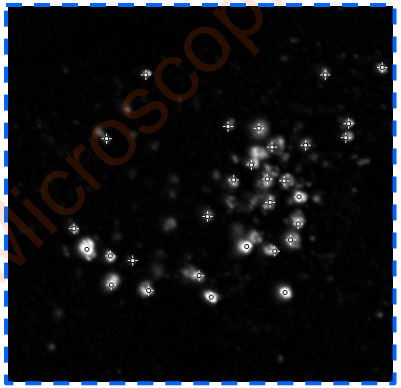


Maxima Within Tolerance

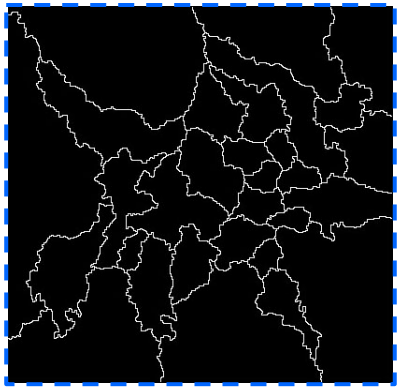


List

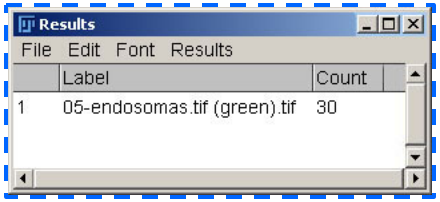




Point Selection



Segmented Particles



Count

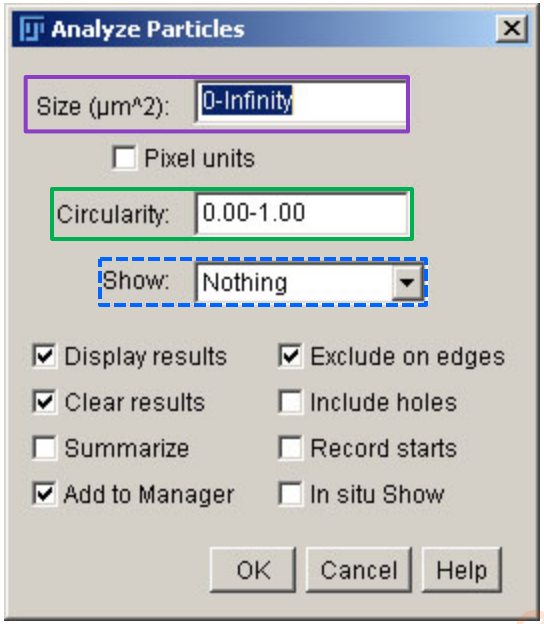
http://rsbweb.nih.gov/ij/docs/guide/146-29.html#Flo:Maxima-outputs

Analyze Particles

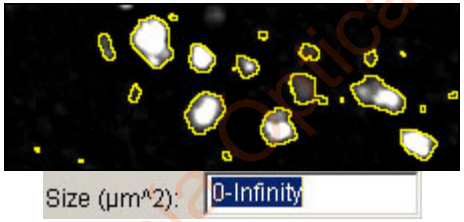
Analyze Particles counts and measures objects in binary or thresholded images

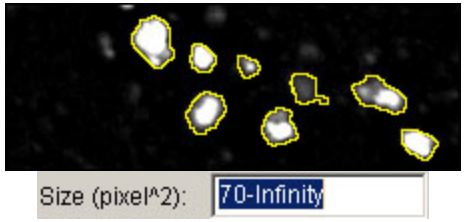
1º - Open the image, apply some **filters** to smooth surfaces (Median or Mean, usually) and check the best **threshold** (Image/Adjust/Threshold) to select the particles of interest.

2º - Go to **Analyze/ Analyze Particles** and modify the next parameters to obtain a best result

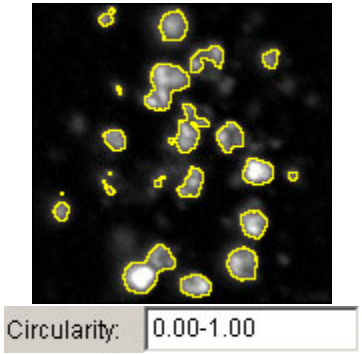


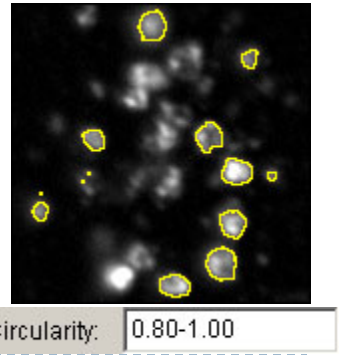
Size Particles with size (area) out of the specified range are ignored. Values may range between 0 and 'Infinity'.





Circularity Particles with size circularity values out of the specified range are also ignored. Circularity ($4\pi \times [Area][Perimeter]^2$. *R*anges from 0 (infinitely elongated polygon) to 1 (perfect circle).





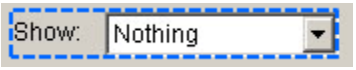
http://rsbweb.nih.gov/ij/docs/guide/146-30.html#toc-Subsection-30.2

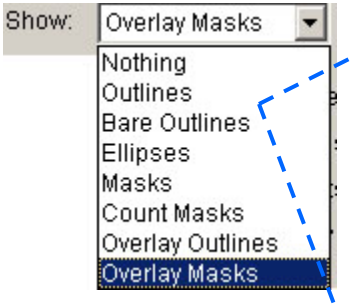
Automatic

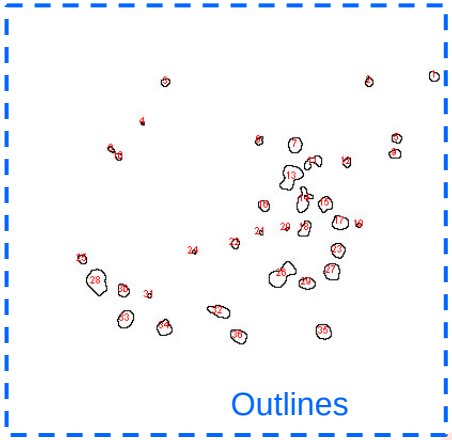
Analyze Particles

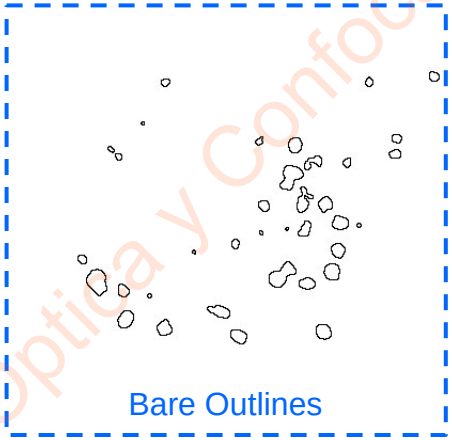
Show. This drop-down menu specifies which image (or overlay) should be displayed after the analysis.

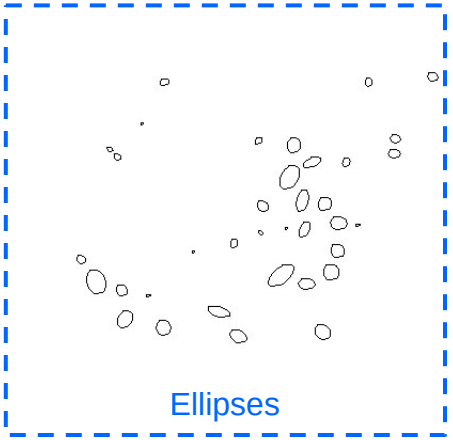
Size, color and background of text labels can be adjusted in Image/Overlay/Labels

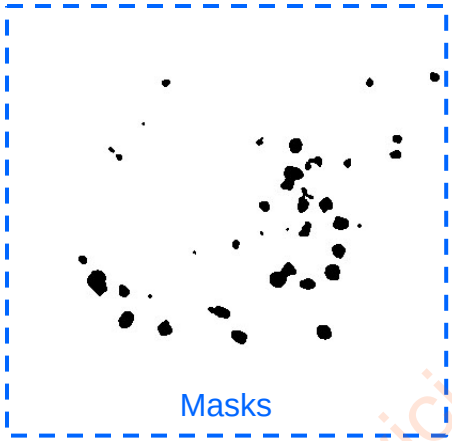





Outlines

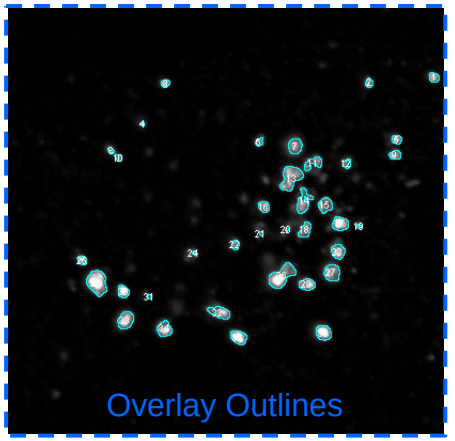

Bare Outlines


Ellipses


Masks


Count Masks


Overlay Masks


Overlay Outlines

http://rsbweb.nih.gov/ij/docs/guide/146-30.html#fig:Display-options-Particle-Analyzer

Automatic

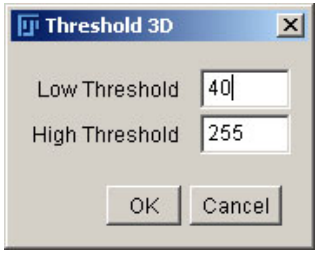
3D Roi Manager

ROI 3D manager can segment 3D objects in a stack and measure various parameters relative to each object (volume, surface, intensity parameters...) or to the objects relations (distances).

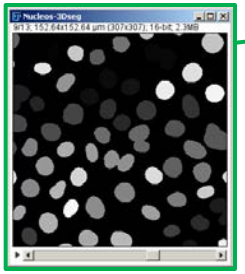
1º - Open the image, apply some **filters** to smooth surfaces (Median or Mean, usually) and check the best **threshold** (Image/Adjust/Threshold) to select the particles of interest.

2º - Go to **Plugins/3D/3D Manager**.

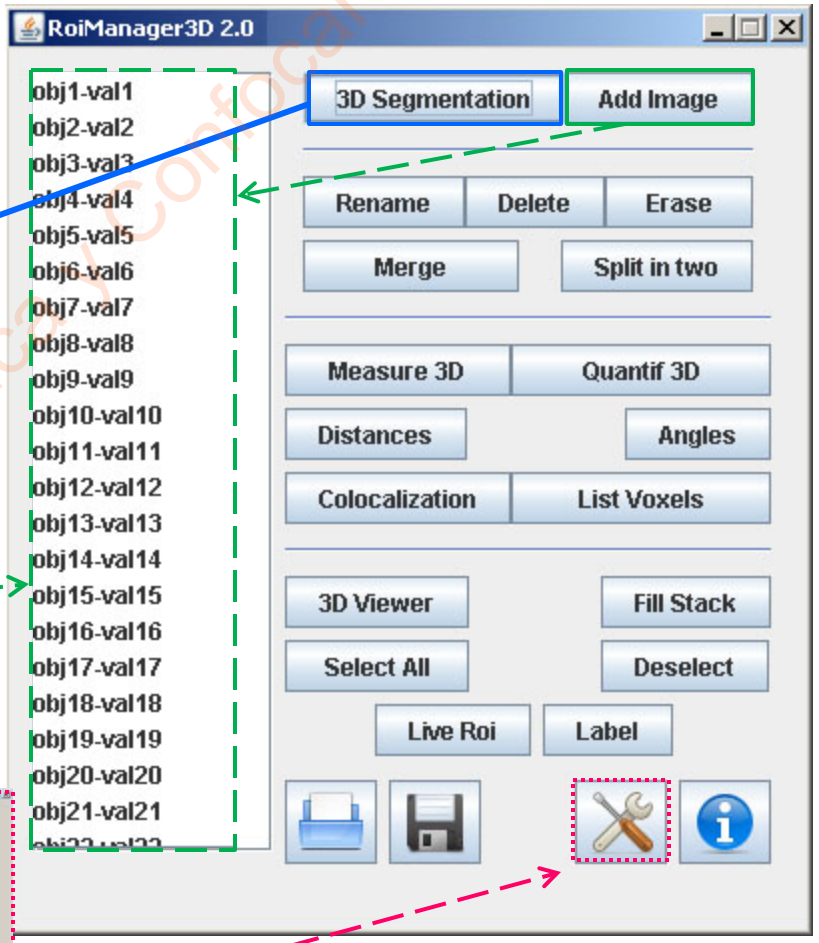
3º - Apply **3D Segmentation** and select proper threshold limits



4º - An image window with "-3Dseg" will be opened.



Select and press **Add Image** to add ROIs to the list.



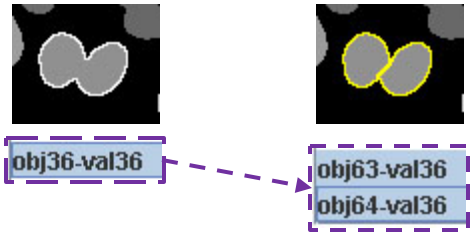
Set Measurements: Use this dialog box to specify which measurements are recorded

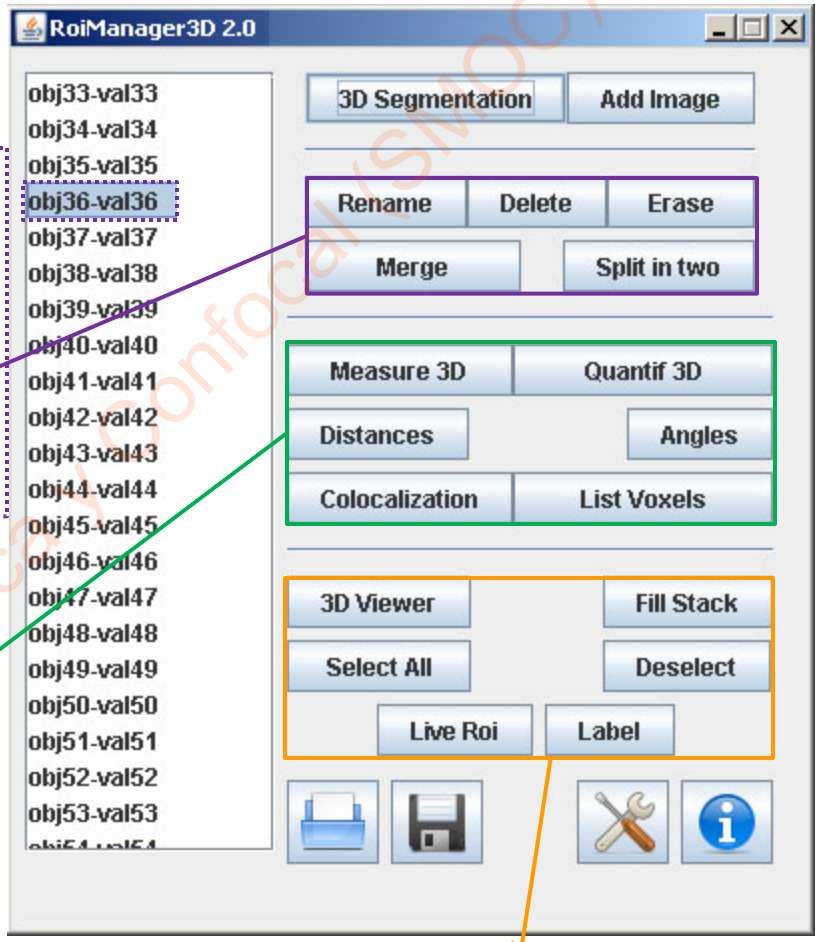
http://imagejdocu.tudor.lu/doku.php?id=plugin:stacks:3d_roi_manager:start // http://imagejdocu.tudor.lu/doku.php?id=plugin:stacks:3d_ij_suite:start

Automatic

3D Roi Manager

ROIs can be modified, merged and splitted if neccesary





Measure 3D will display a "3D Measure“ window where all the measurement are listed for the object(s) selected or for all the particles if none is selected

Quantif 3D will display a "3D quantif“ window with the grey level of the objects

Distance will display a “3D distance” window with the distance between objects previously selected.

Colocalization will compute the percentage of the volume of co-localised voxels to the object volume

Fill Stack will draw the selected objects in the current stack

Check the **Live Roi** button if the contours of the selected objects aren’t displayed in the current 3D image windows

The **Label** button will add text overlay at the selected object position in the corresponding slice

Check how to install these plugins in the last page

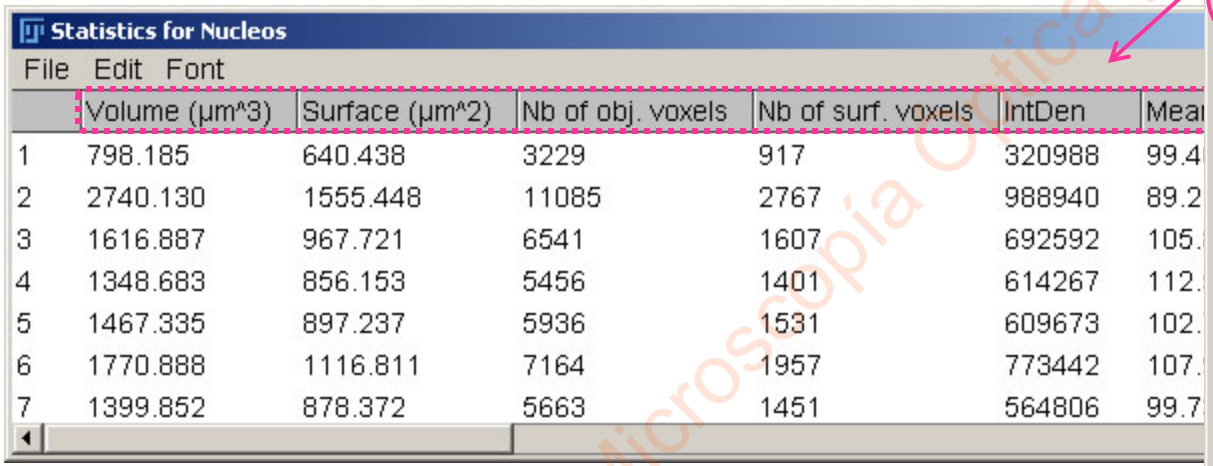
http://www.gdja2ip.upmc.fr/index.php?option=com_content&view=article&id=38&Itemid=38

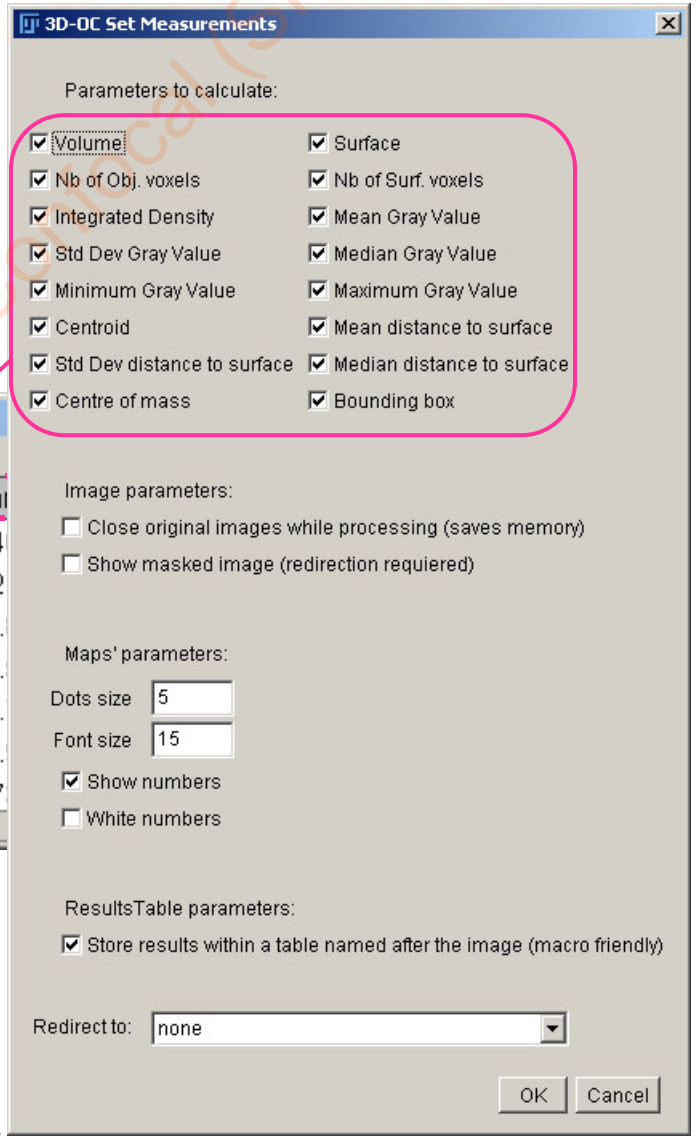
Automatic

3D Object Counter

This plugin counts the number of **3D objects** in a stack and quantifies several parameters for each found object

First of all, select **Analyze/3D OC Options** to set the parameters to calculate and other options.

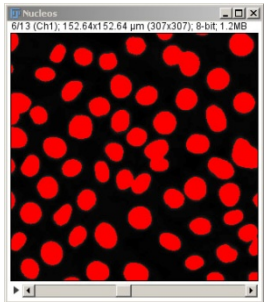




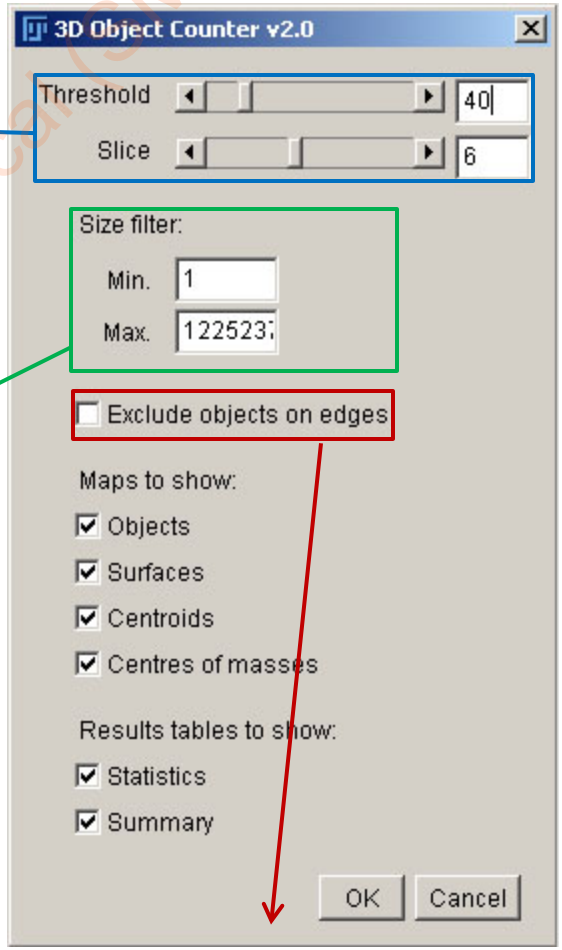
http://fiji.sc/3D_Objects_Counter & Manual

3D Object Counter

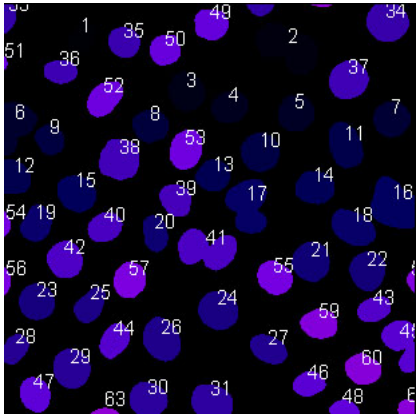
Once the options have been set, launch **Analyze/3D Objects Counter**

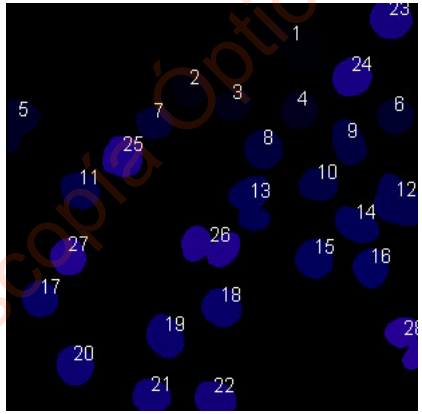


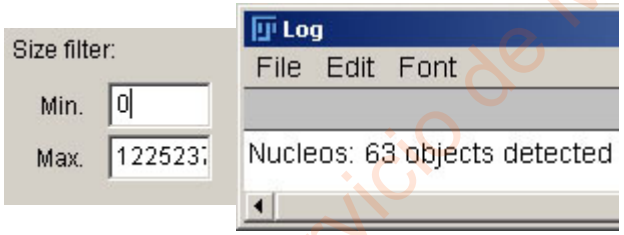
Define the **Threshold**: using this slider and navigate along the stack with **Slice**

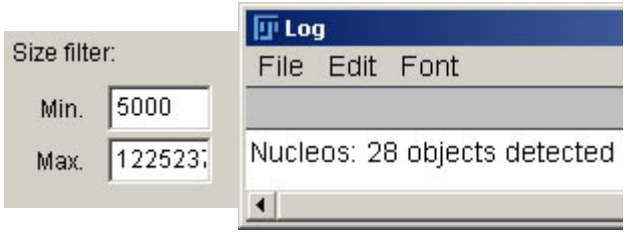


With **Size filter,** objects with size out of the defined range will be excluded









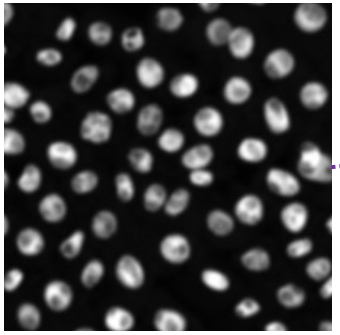
Be careful with this option. It doesn't work porperly sometimes

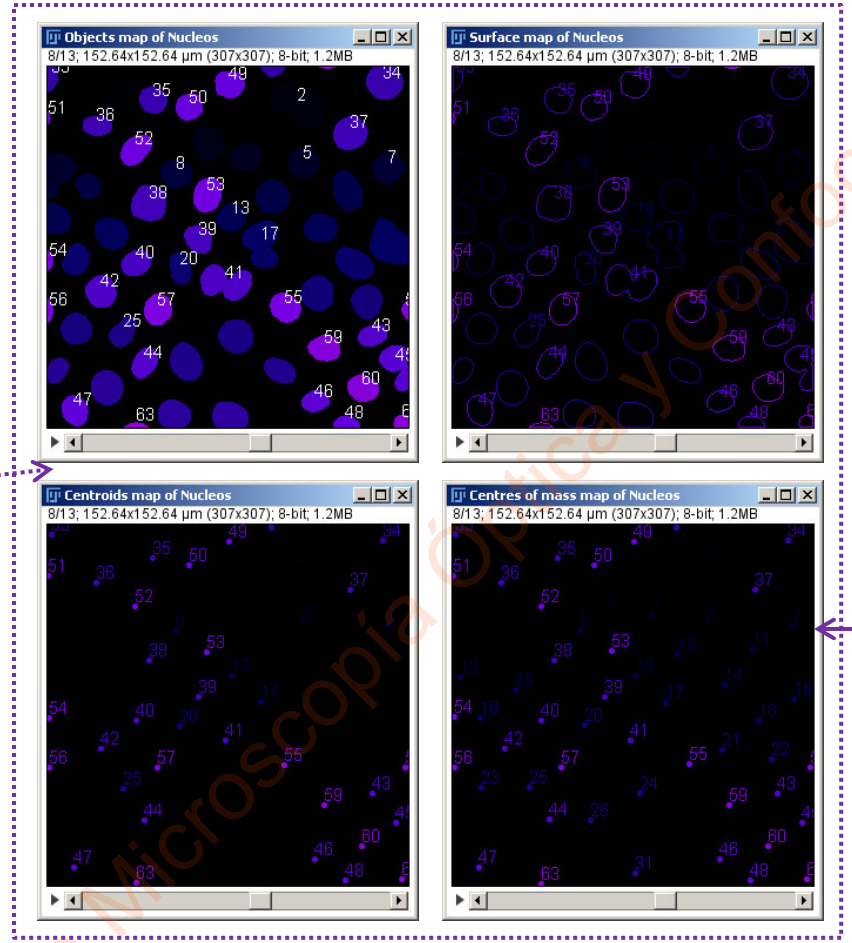
http://fiji.sc/3D_Objects_Counter & Manual

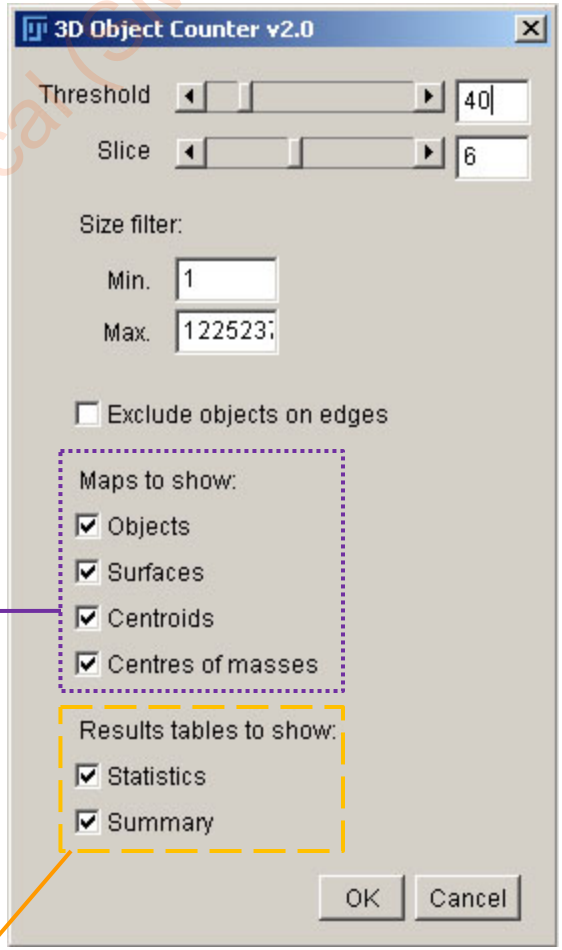
Automatic

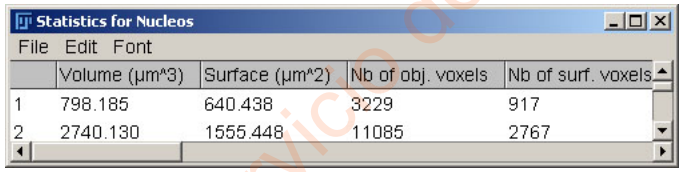
3D Object Counter

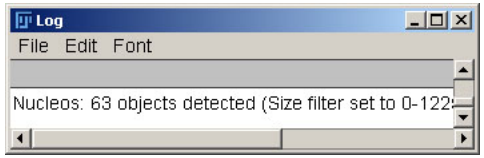
Select how do you want to show the **information**







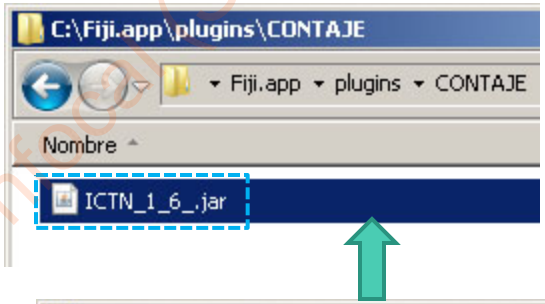




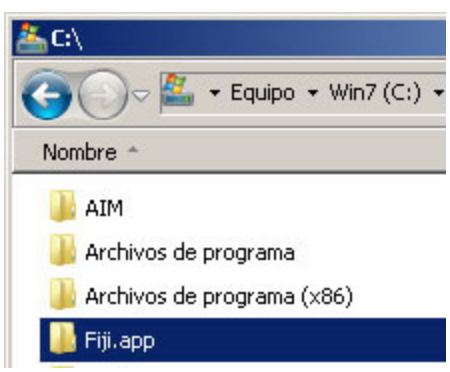
http://fiji.sc/3D_Objects_Counter & Manual

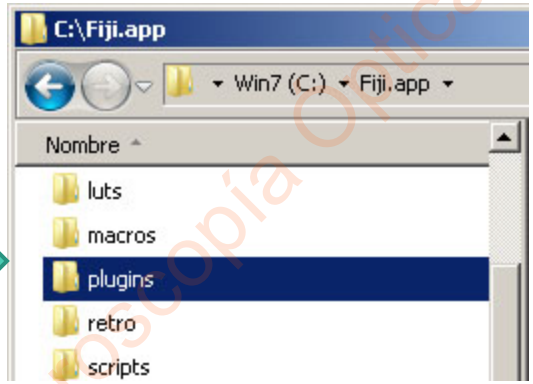
How to install ITCN plugin

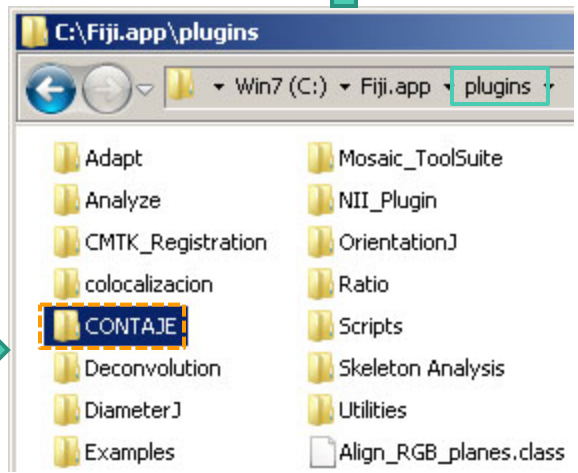
1º- Go to http://biodev.ece.ucsb.edu/projects/bioimage/downloader/download/category/7



2º- **Download** ITCN.jar and **save** it into the plugins folder of Fiji







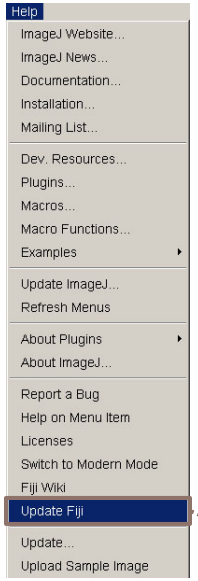
You can create some additional folder inside the plugins one to locate that plugin easily in the plugins menu

3º- Restart Fiji to become the plugin available in the plugins menu.

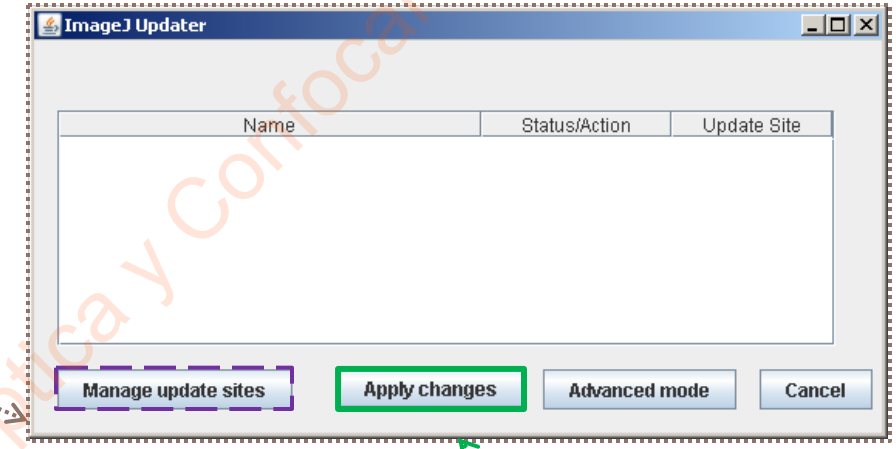
http://fiji.sc/How_to_follow_a_3rd_party_update_site // http://fiji.sc/Update_Sites // http://fiji.sc/Installing_3rd_party_plugins

How to install 3D Roi Manager

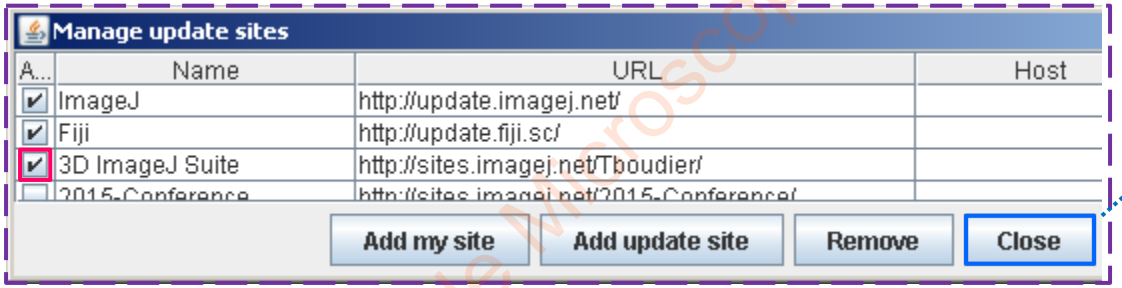
1º- Go to **Help/ Update Fiji**



2º- Open **Manage update sites**



3º- Select **3D ImageJ Suite**



4º- Select **Close**

5º- Select **Apply changes**

6º - Restart Fiji.

http://fiji.sc/How_to_follow_a_3rd_party_update_site // http://fiji.sc/Update_Sites // http://fiji.sc/Installing_3rd_party_plugins