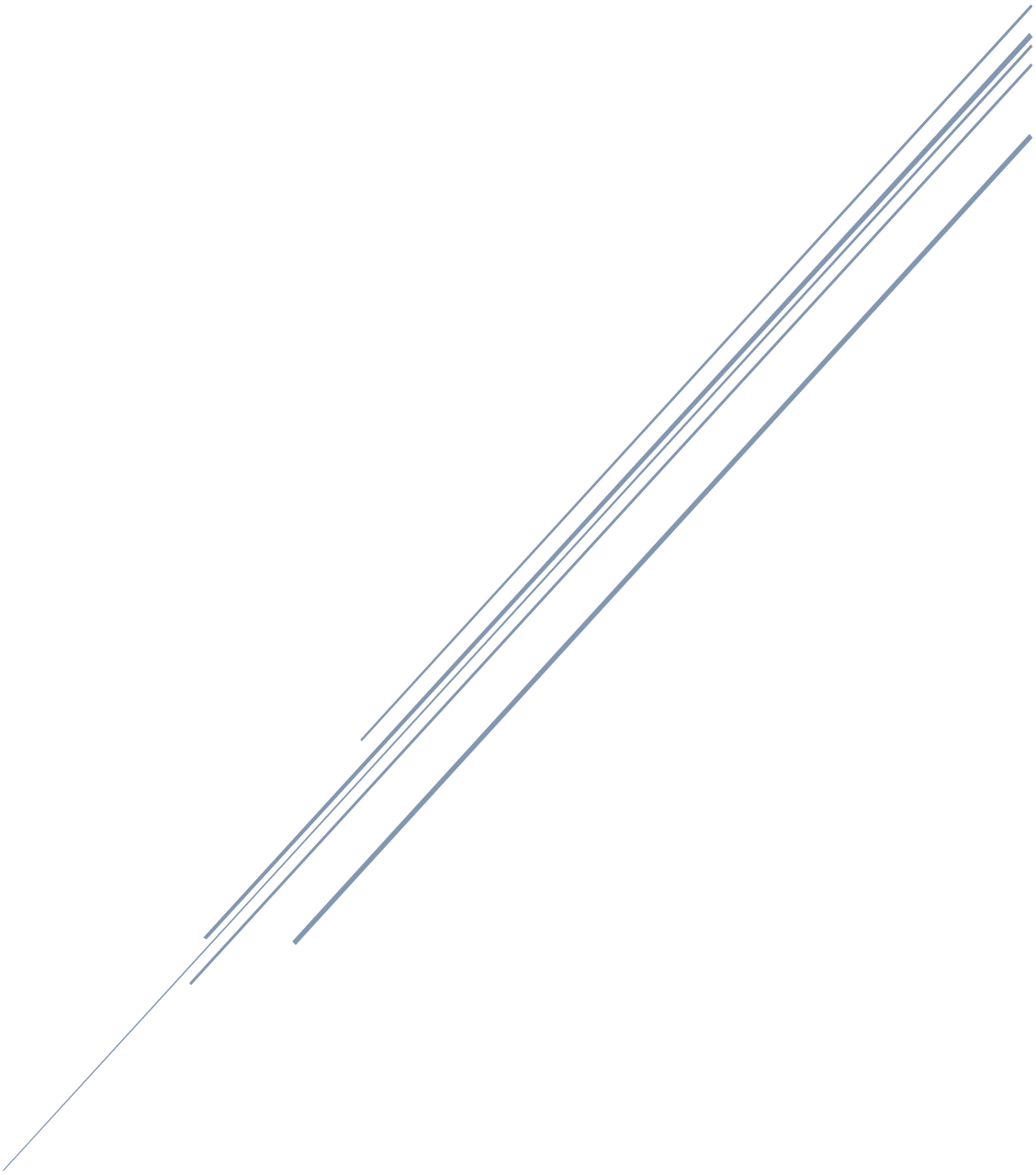
IMAGEJ MACRO

ANALYZING SYNAPTONEMAL COMPLEXES (SCs) AND CROSSOVERS (COs)



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Version 20.1

1. GETTING STARTED: INSTALLING AND MAINTAINING THE PLUGIN

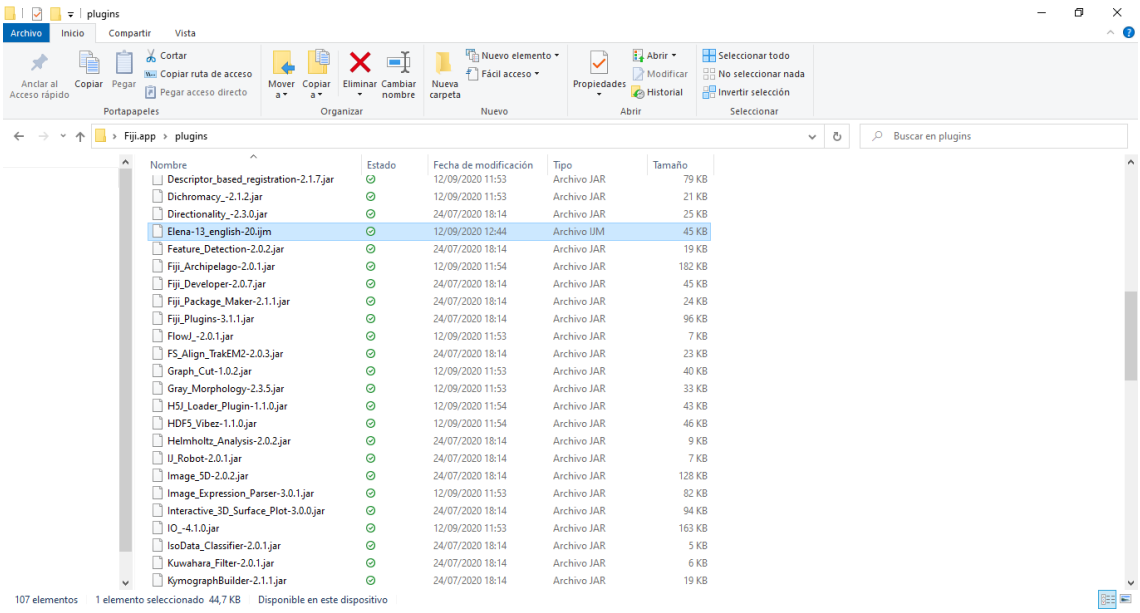
Install ImageJ-Fiji

Install Morphology Plugin Package

To check the installation, we will open Fiji and select in the upper panel: Help> Update> Manage update site. In the drop-down menu we will find “Morphology” and activate the selection box on the left.

Install the plugin and the plugin actualizations:

- We will download the plugin on our computer
- We will go to the Fiji> Plugins folder. Here we will paste our plugin.



Attention: the plugin must be copied with Fiji closed. If it is open, close and reopen to update it.

2. GENERAL CONSIDERATIONS ON THE OPERATION OF THE MACRO

• The macro can be stopped at any given time by selecting a window and pressing the "i" key on your keyboard

• To better edit and analyze the images, we can use FIJI's magnifying glass and moving tool. The magnifying glass button allows us to zoom in (left click) and zoom out (right click) the image. The move tool (with a drawing of a hand), allows the field of view up or down the image.

• The macro cannot go to previous steps. In case you want to correct drawing errors of the SCs, the macro itself will warn us of an error and allow us to fix it before finishing the measurement.

• To analyze several images in a row, we must place them all in the same folder. The results will be displayed in a folder created by the same program called "Results", in the source folder of our images.

• If the source folder of our images already contains a folder called “Results”, the program will warn us that, when it has finished analyzing, it will overwrite it. To keep it, rename or relocate it.

• The “Results” folder will consist of a subfolder for the analysis of each image. Within it the following files will appear:

- An image of the analyzed kernel in .tif format.
- A file of type .lsm with the measurements of the SCs. This file can be imported into Excel for analysis. In the event that we have also analyzed the COs, the partial distances will also appear (distance between each of them and the centromere).
- Data folders. In the event that we have done the analysis of SCs and COs, 4 folders will appear in .zip format, with the data of the position of the centromeres, the recombination sites (COs), the nuclei and the synaptonemal complexes (SCs). These folders can be opened in FIJI and can be very useful, especially for quick checking of results when we have doubts. If we have only done the analysis of the SCs, 3 folders will appear (position of the centromeres, SCs and nucleus).

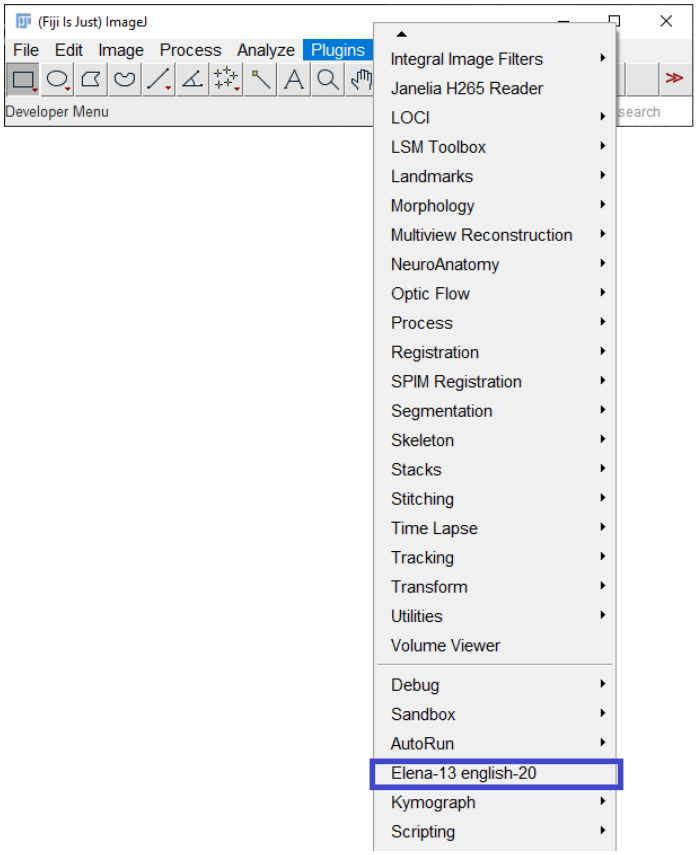
3. WORKING WITH IMAGEJ MACRO: ANALYSIS OF SCs and COs.

The steps to follow to start up the macro are as follows:

1. Open Fiji and load the plugin.

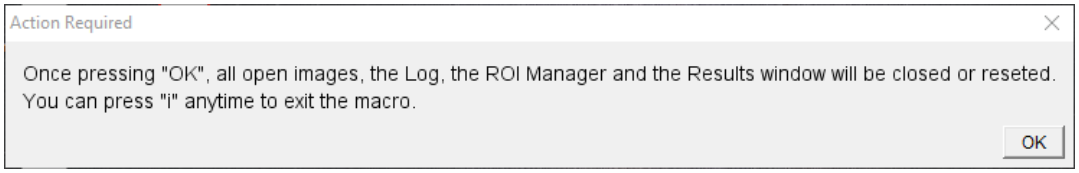
We will load the plugin by selecting "plugins" in the upper toolbar and in the list, we will click on the latest version of our plugin (for the moment: "Elena-13_english-20")

a. A pop-up window will appear, warning that all the modifications we have made so far in FIJI will be removed. We press "OK".



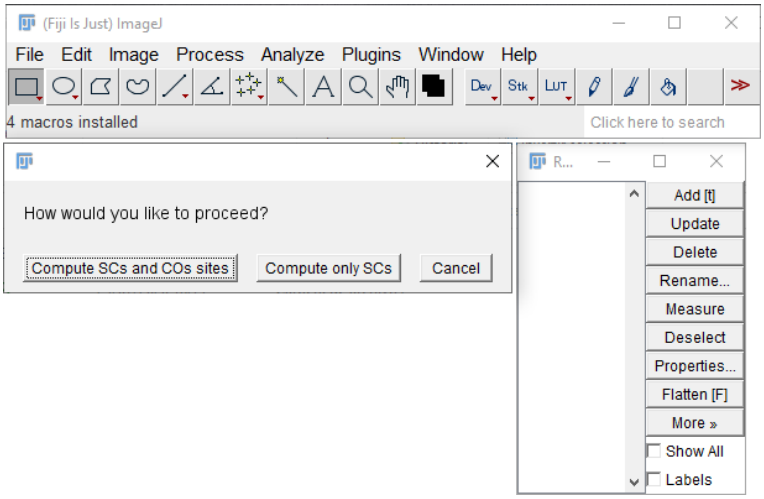
2. Selection of the image folder.

An explorer window will appear, where we can find the source folder of our images. If there is already a "Results" folder created previously, it will warn us that it will be overwritten. If you want to keep it, change its name or change its location.



3. Procedure selection.

When loading the image, the program will ask us to select the type of analysis we want to do; We can select to analyze SCs and COs at the same time or only SCs.

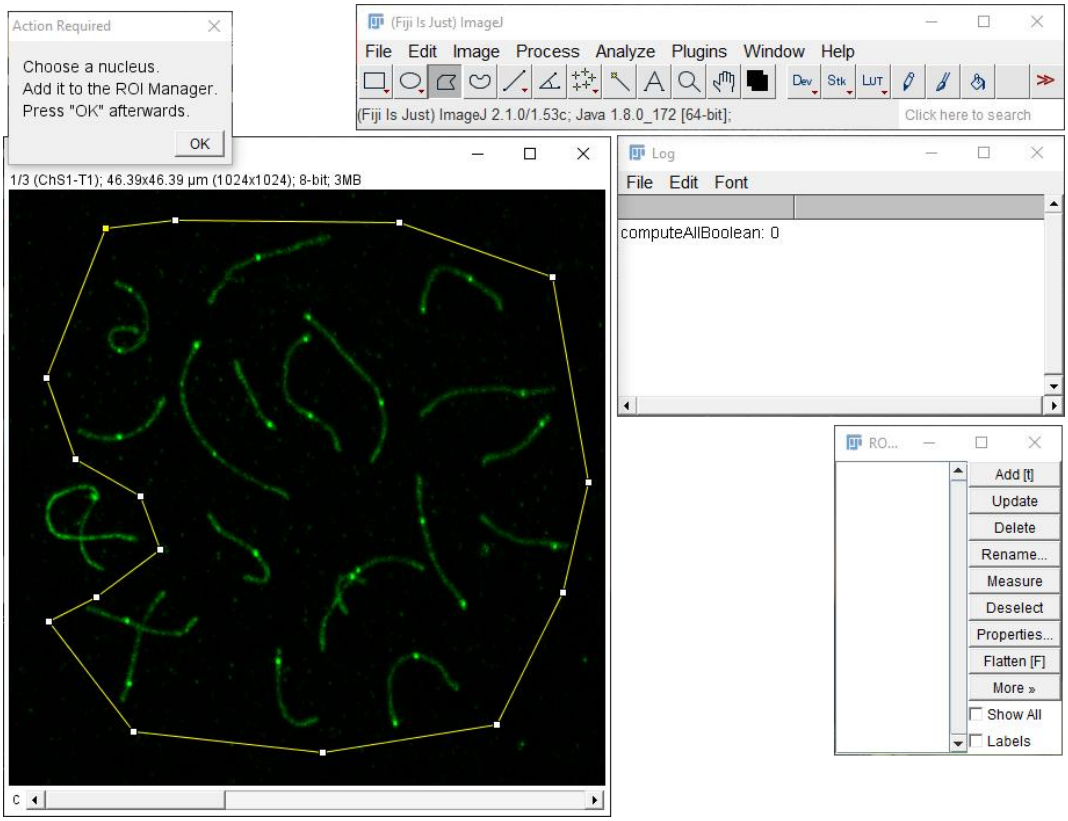


a. If you're doing only the analysis of SCs, carry out up to step 8 of this manual.

4. Selection of a nucleus.

A pop-up window will appear asking us to select a nucleus. We must select a tool from the top bar of Fiji. It is recommended to select the polygon drawing tool (third bottom from the left).

a. In this step we can leave the XY chromosome out of our selection.
b. In the case that there is more than one nucleus or chromosomes that belong to more than one nucleus, we must be careful to select the chromosomes corresponding to one of them.

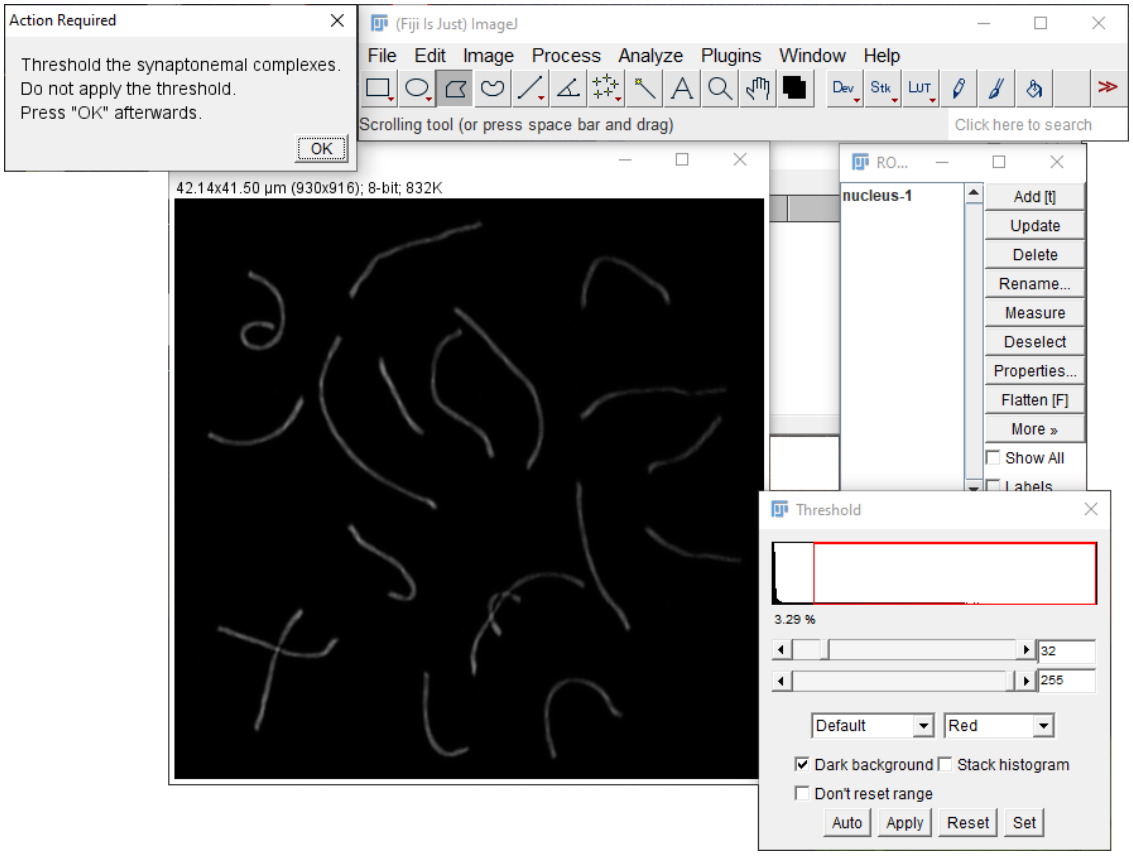


Once the selection is made, we add the nucleus in ROI manager using the “Add (f)” button and then we click “OK” to the pop-up window.

5. Image signal adjustment.

A new pop-up screen will appear, where we can adjust the image parameters.

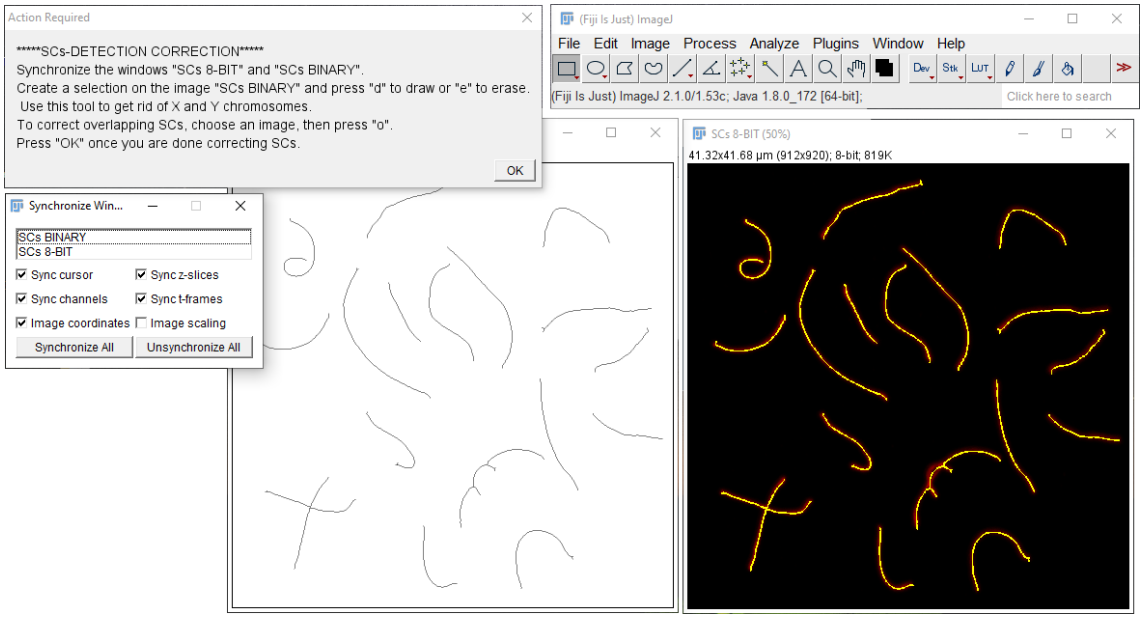
We can slide the upper bar to give or remove gain to the signal. We will eliminate as much background signal as possible, trying not to eliminate too much signal from the SCs. The “auto” function allows us to return to the automatic signal detection carried out by the program.



Once the signal has been adjusted, we will click on the “OK” button in the signal adjustment pop-up window.

6. Window synchronization.

In this new step a panel and a window will appear. The new pop-up panel will allow us to adjust the two images that we see on our screen, so that we will have an original image (where we can perfectly see the signal of the SCs, 8-BIT) and another, where we will see the edition that we are doing (BINARY).



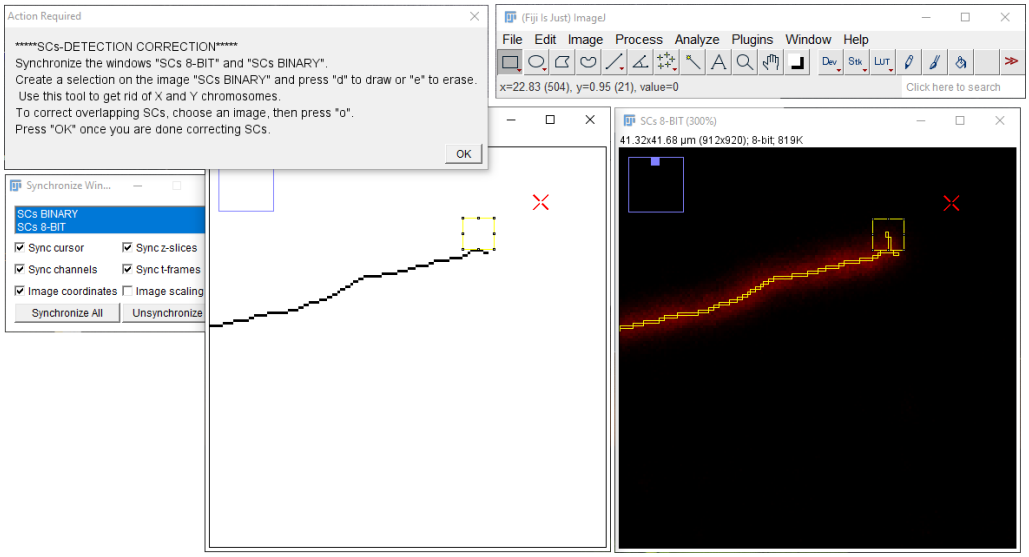
To synchronize we must give the option "synchronize all" in the pop-up panel. The window will give us the information on how to edit the SCs.

7. Edition of the SCs.

Following the prompts in the pop-up window, we will edit the SCS:

a. Eliminate portions of SCs.

To do this, we will select a drawing tool from the Fiji bar and press the “e” key on our keyboard. We recommend selecting squares and polygons for this step. If it hasn't been done before, this would be the time to remove the sex chromosome (XY) from the image.



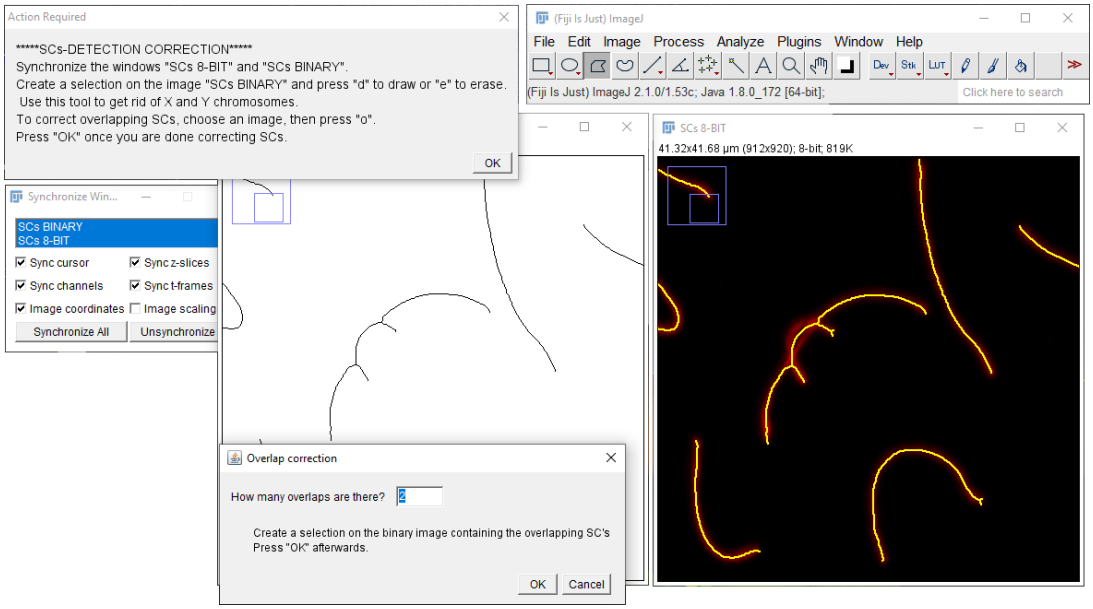
b. Create fragments of SCs.

If we want to create portions of SCs that the program has not drawn or has done wrongly, we must draw them by hand. We recommend the "freehand line" tool, which can be selected by right clicking on the "straight line" tool. To draw freely, you have to hold down the left mouse button.

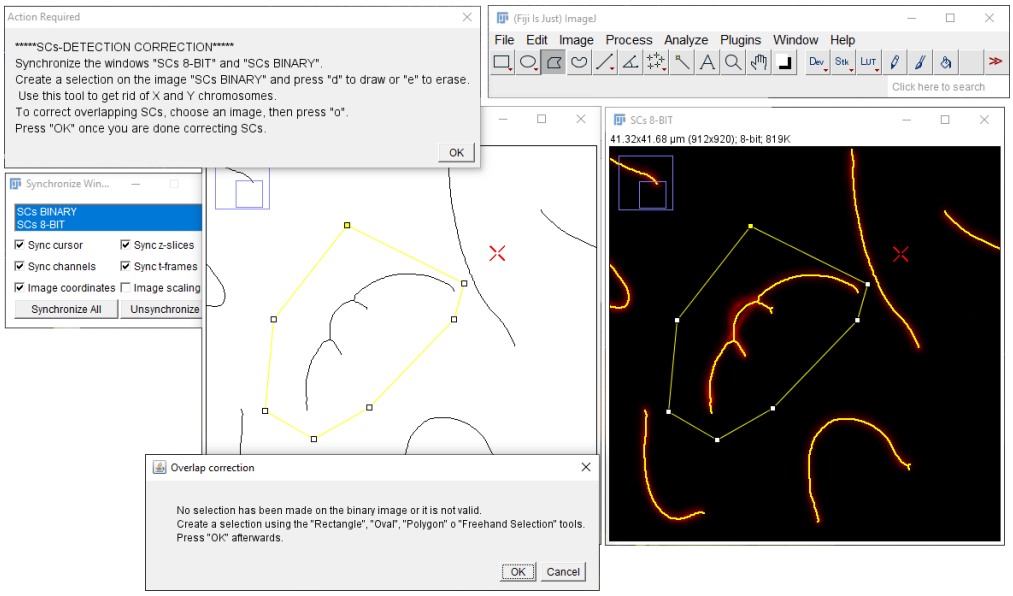
We will select the desired tool; we will draw our CS and a finished view and we will press "d" on our keyboard.

c. Indicate overlaps.

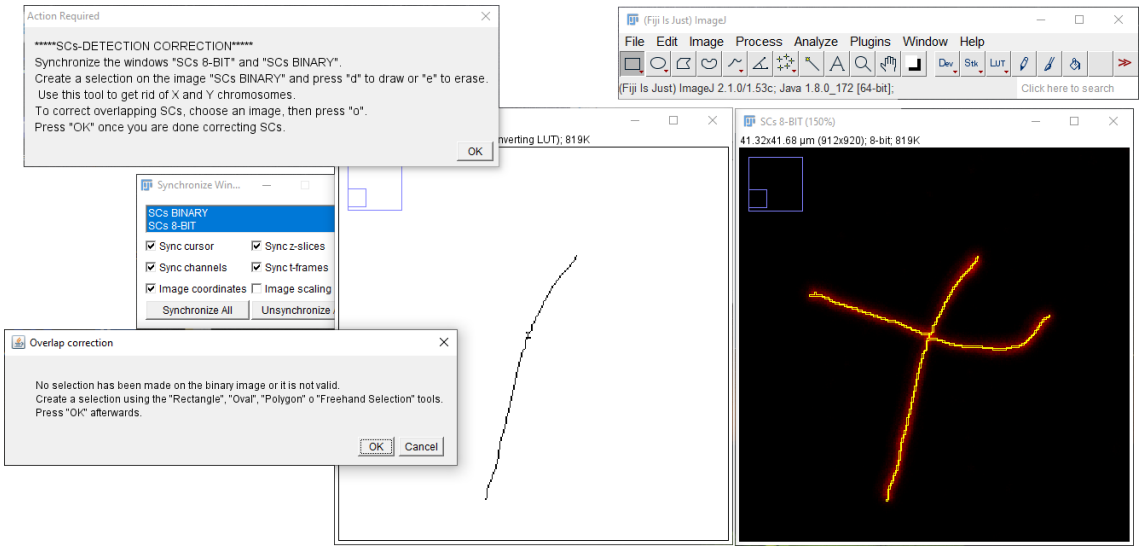
To indicate that two or more SCs are superimposed, we must press the "o" key on our keyboard. A pop-up window will appear asking us to indicate how many SCs are overlapped; when we do, we press "OK".



The next pop-up window asks us to select the area where the overlapping SCs are located. We select with the rectangular or polygonal tool and press "OK".



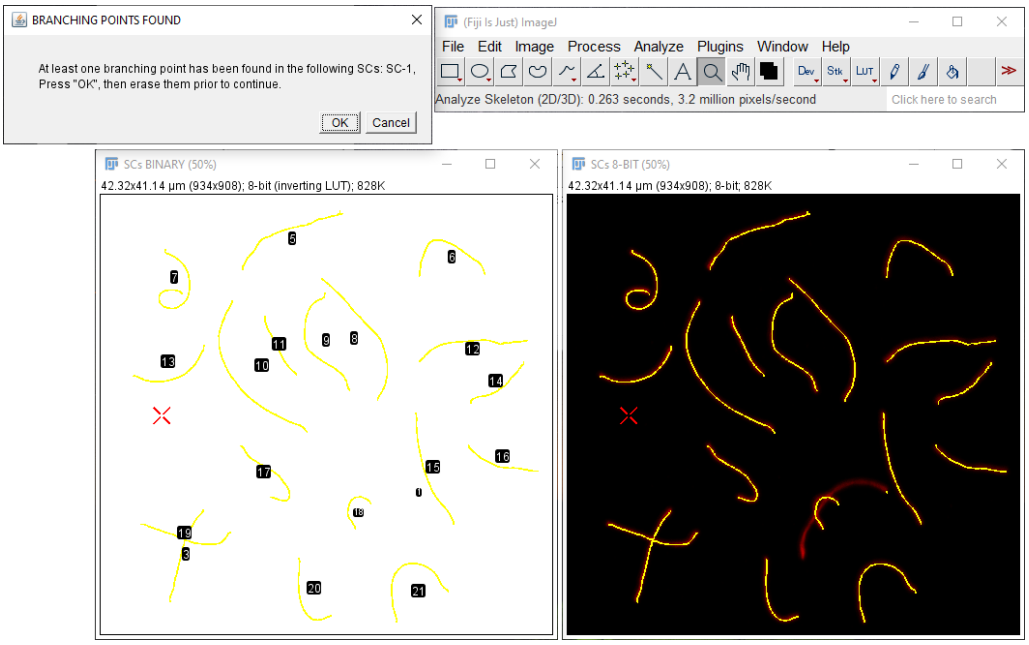
The next window will tell us to draw one of the SCs. To do this, we will retouch the direction of our SC if necessary, eliminating and drawing lines according to the instructions in the two previous steps.



Once we have finished, we will press "OK" in the pop-up window. Another pop-up window will appear, very similar, in which it will ask us to draw the next SC of the overlay. This step will be repeated successively and we will have to select the address of each of the SCs of the overlay. When we're done, no more pop-up windows will appear.

With this, we will have finished the editing step of the SCs. Now, we must go to the pop-up window that asks us to retouch the SCs and gives us the instructions to do so, and we will press "OK".

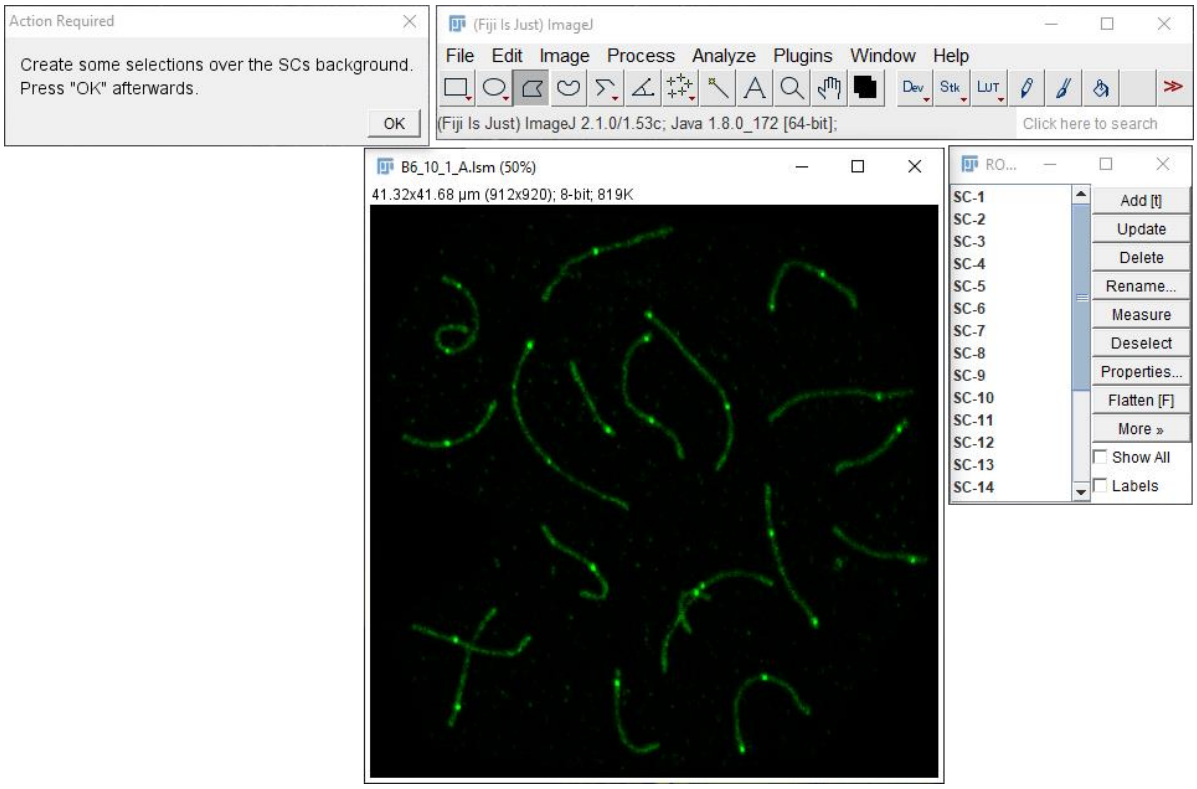
8. Correct mistakes. In the case that the program detects bifurcation errors (branching), it will show us a pop-up window indicating the number of SCs in which there are bifurcations and it will give us the option to go back to editing the SCs.



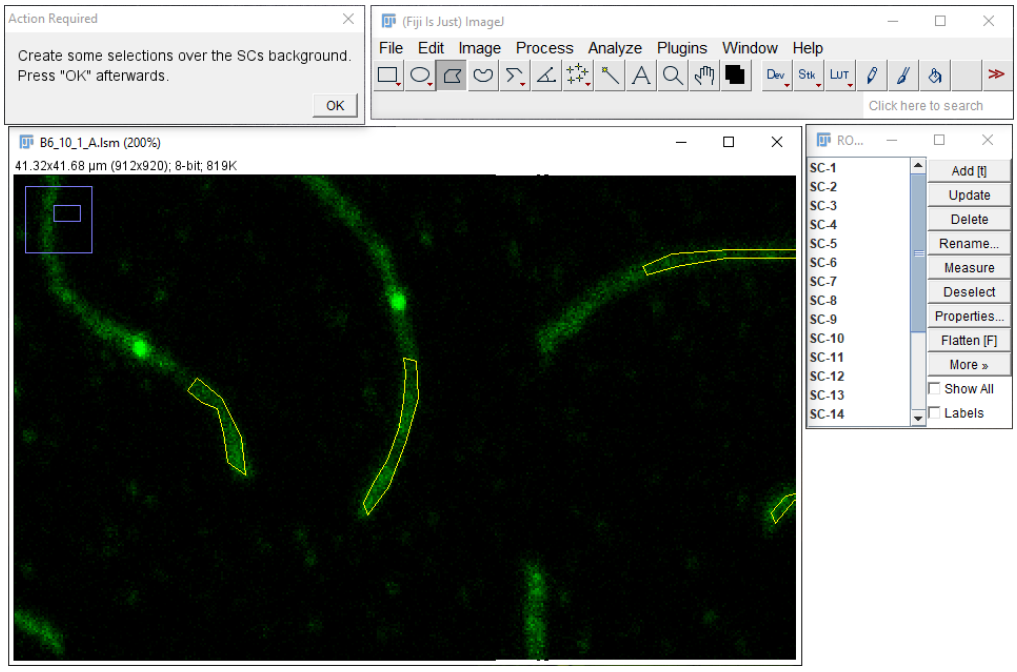
a. In case we have to do it, we will follow the same indications of step 7 and when finished we will press the "OK" key of the window with the editing instructions.
b. In case there is no bifurcation and we have selected only the SCs analysis, the program will analyze the image, show the results on our screen and create the folder “Results” in the source folder of the images.
c. If we have selected the complete analysis, which includes the COs, the program will not show the results, but will continue to the next point.

9. Selection of the SCs background.

Once the analysis of the SCs is finished, if we have selected the option to analyze SCs and COs, another pop-up window will appear. In it, we are asked to select the background of the SCs, that is, fragments of SCs that lack COs. This will be done with the polygon tool from the FIJI toolbar.



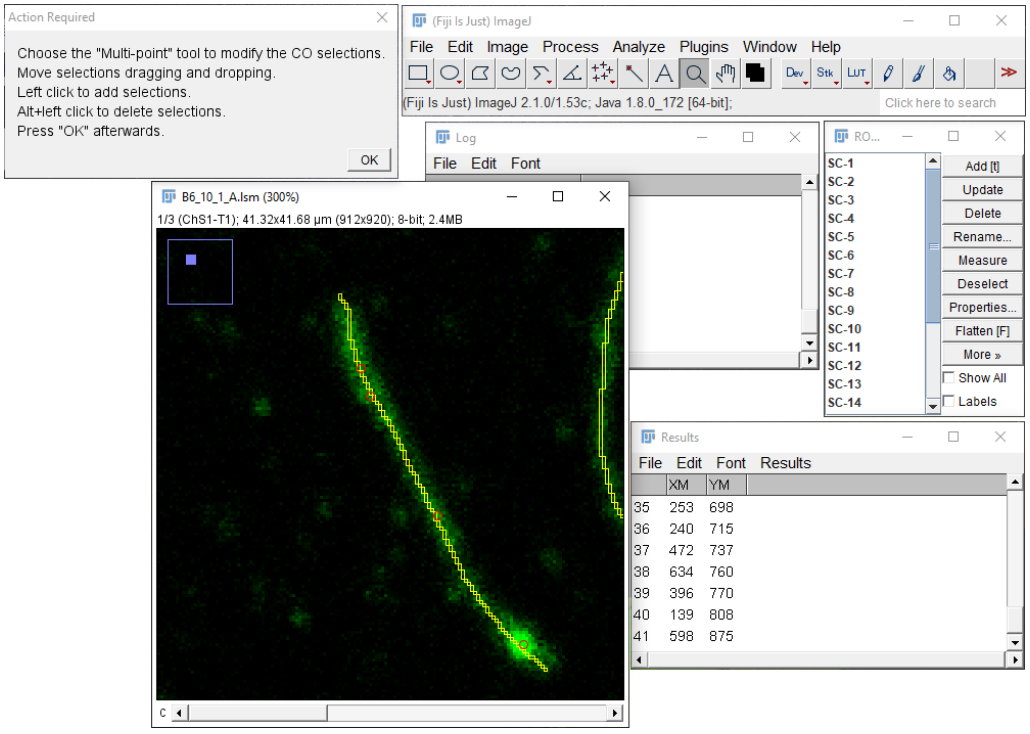
a. It is recommended to take fragments with a fairly bright signal, so that the program is able to effectively differentiate the COs from the rest of the SC signal.
b. To select several fragments, you will have to hold down the shift key (↑) on your keyboard while selecting.
c. Once we have finished, we will press the "OK" button in the pop-up window.



10. Selection of COs.

In the next step, the macro shows us the automatic detection of foci or COs that it has carried out. We will check that the program has detected all the foci and, if necessary, we will eliminate or add new COs. For this we must:

a. Select the FIJI multipoint tool (button with several stars).
b. To add a CO: we will left click on the image where we want to add a CO.
c. To delete a CO: hold down the alt key and left click on the CO that we want to delete.



When we have finished, we will press the "OK" button in the pop-up window.

11. Finishing the analysis.

If the macro does not detect any problem, it will return the results and create the results folder in the source folder of our images.