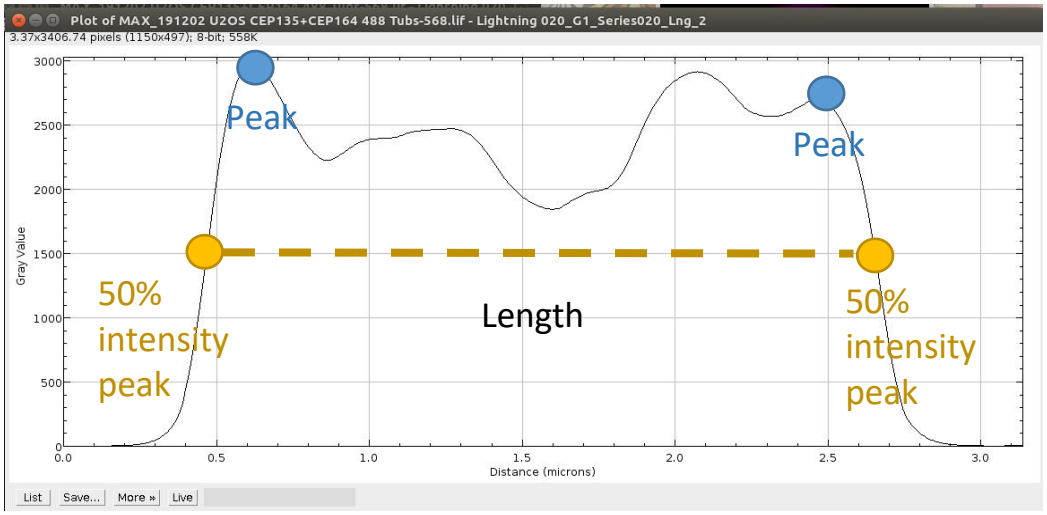
pickCentrioleDim plugin : How to use

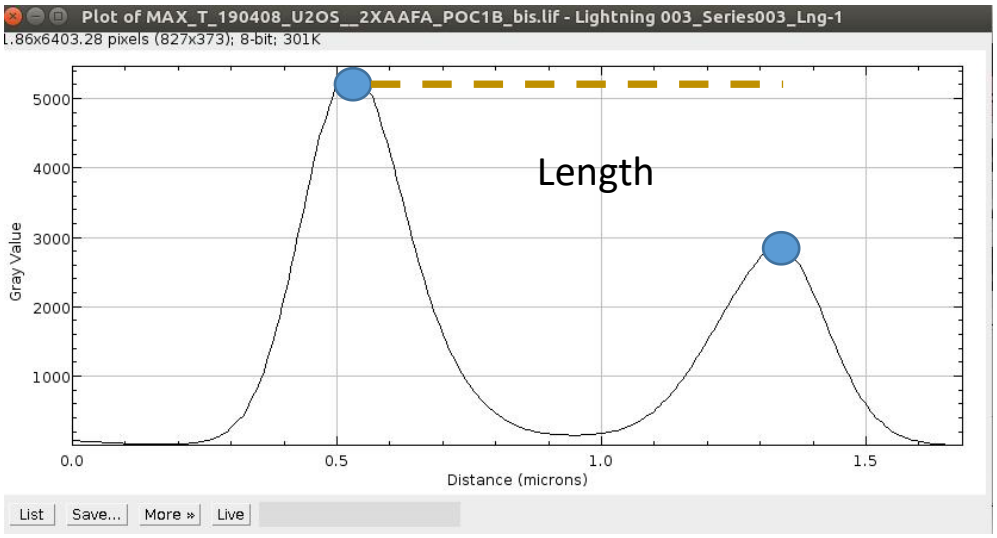
Aim :

This plugin allows the user to easily pick the extremities of a centriolar protein signal taken by immunofluorescence considering that :

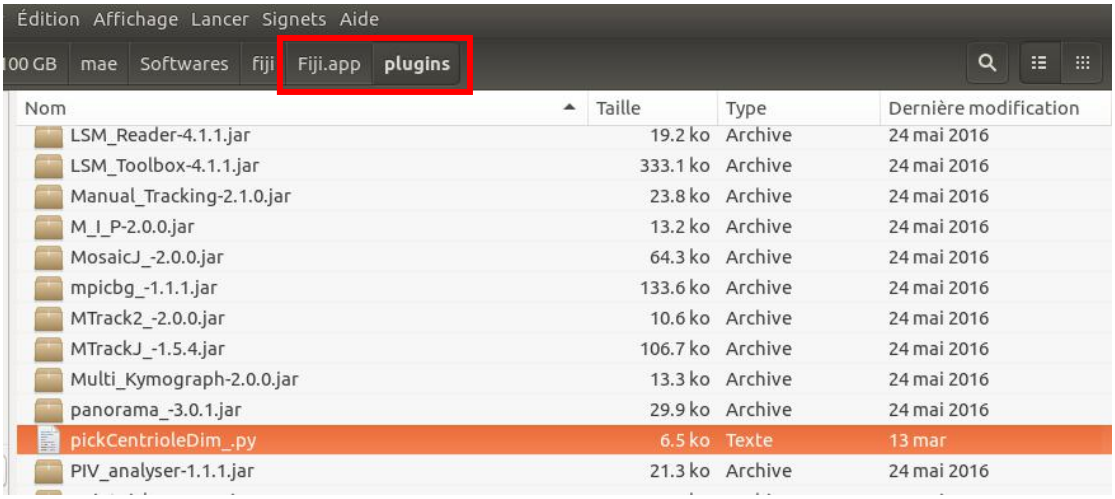
- the **length** of the signal (for a side view centriole) is measured by taking the 50% peak intensity of the border of the plot profile;



- the **diameter** of the signal (for a top view centriole) is measured as the distance between two peaks of the plot profile.

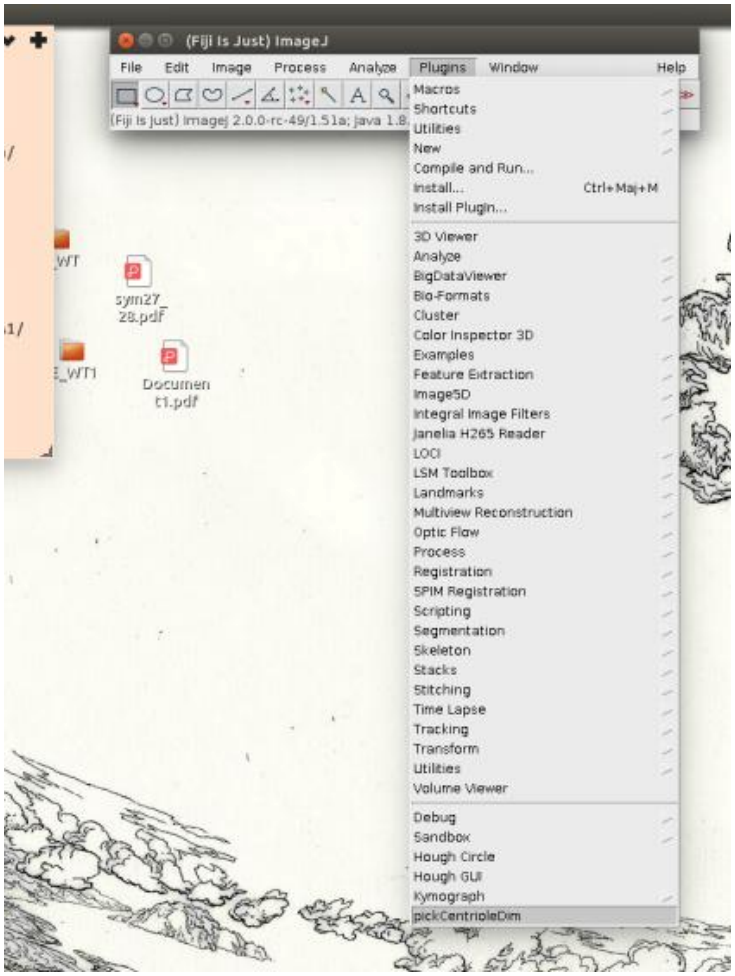


Step 0 : Installation

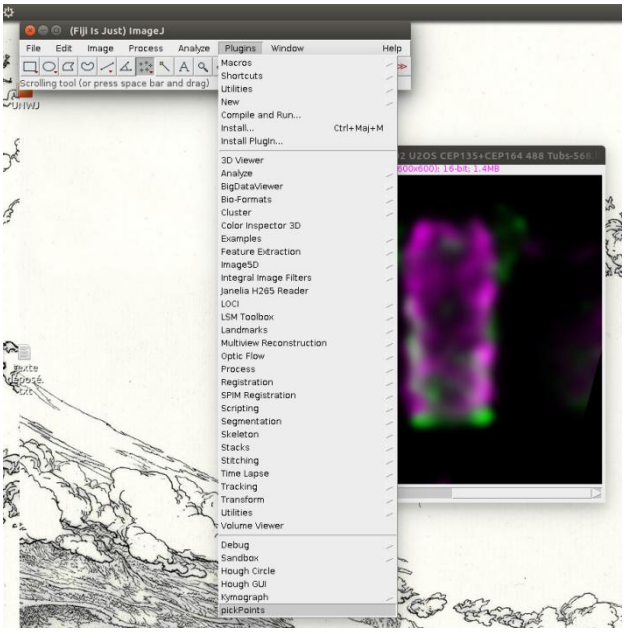


Put the file “pickCentrioleDim_.py” in the Fiji/plugins folder.

When you’ll open Fiji again, the plugin will be available in the list :

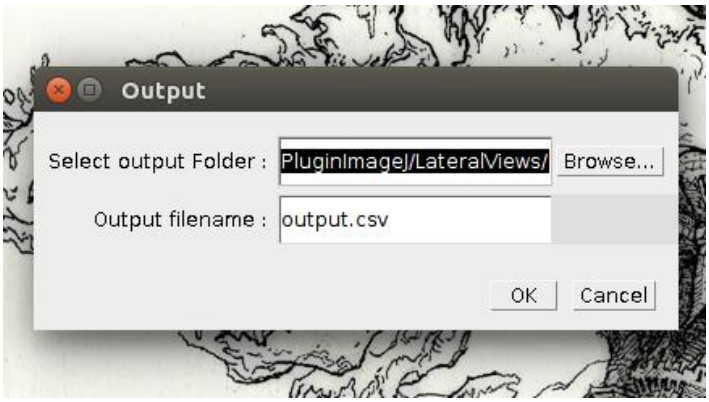


Step 1 : File opening and creating the output



Open the image you want to quantify.
Launch the plugin.

Select the output folder (“Browse...”) and name the output file :



We advise to put a .csv extension as the results will be written in this format.

In case the file already exists, this warning appears :
Be careful, if you keep the same name, the previous file will be replaced.

The output file is created, its content is :

```
Label, prot1_pk1, prot1_pk2, prot2_pk1, prot2_pk2
```

Where :

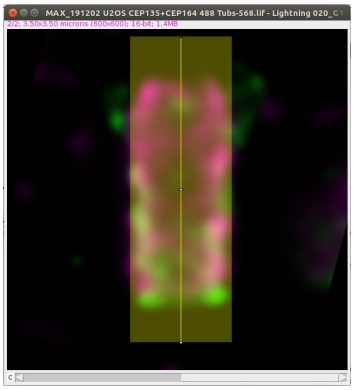
- **Label** : the identifiant of the centriole, chosen by the user
- **prot1_pk1** : the position of the 1st peak of the 1st protein signal analyzed
- **prot1_pk2** : the position of the 2nd peak of the same signal
- **prot2_pk1** : the position of the 1st peak of the 2nd protein signal analyzed
- **prot2_pk2** : the position of the 2nd peak of the same signal

In case, there is only one signal to quantify, the columns **prot2_pk1** and **prot2_pk2** will be left empty as soon as the user doesn't use the "2nd Prot" option (see later).

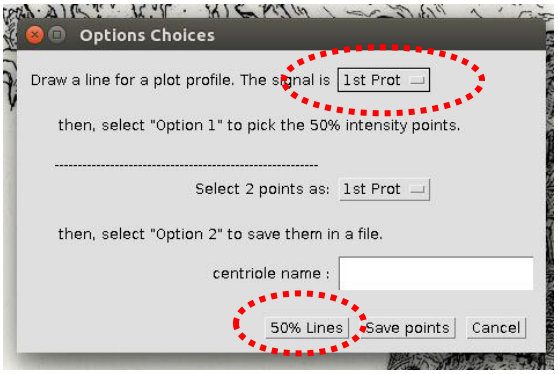
Case 1 - Signal length quantification

Step 2 : Preparing the plot profile

Make a linear selection of the longitudinal signal (if the centriole is oriented differently, rectangular selection can also works). Here, the signal that will be quantified is the magenta one.

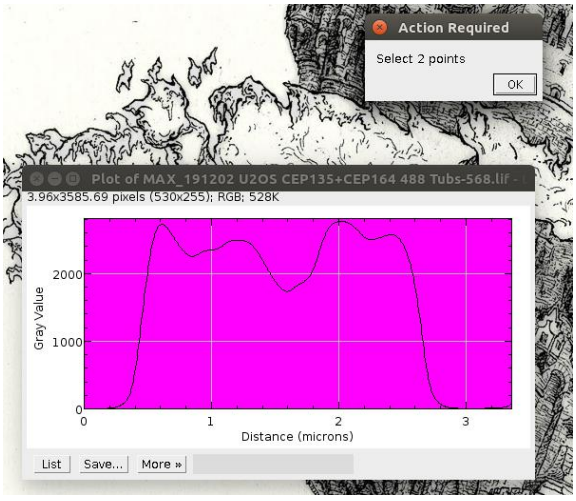


Be sure, the option "1st prot" is selected.
Then, click on "50% Lines".

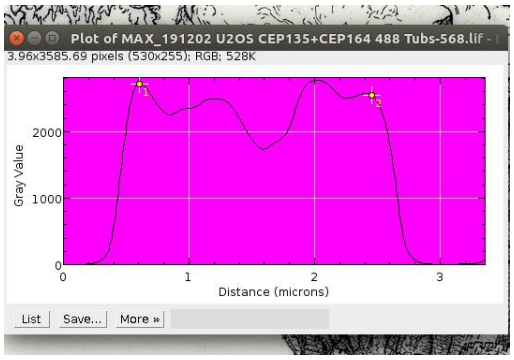


Step 3 : Interacting with the plot profile

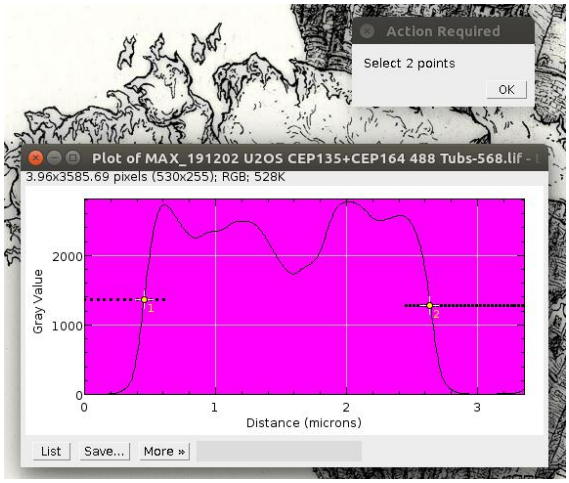
The plugin draws the plot profile of the selection made and propose the user to select 2 points on this plot.



Select 2 points and click on “Ok”.

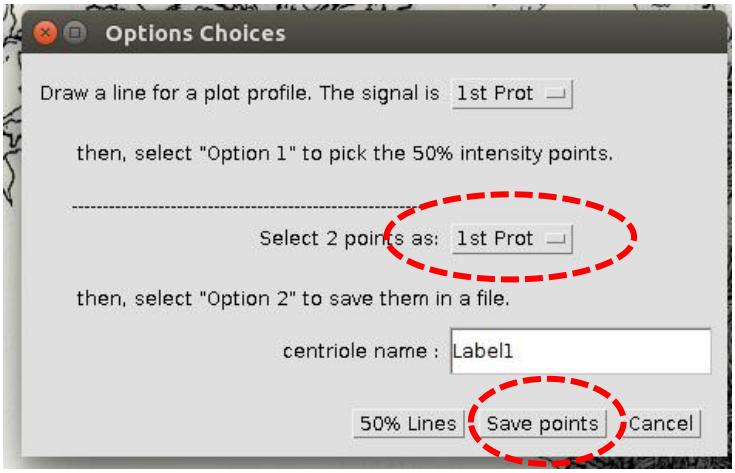


Two lines are drawn representing the 50% signal intensity of each point previously selected. Select the two points corresponding to the 50% intensity signal of the curve and click on “Ok”.



Step 4 : Save selected points

The main window appears again Be sure you’ve selected “1st Prot” for the 2nd Option and indicate a label to identify the centriole. Then, click on “Save points”.



The content of the output file is now :

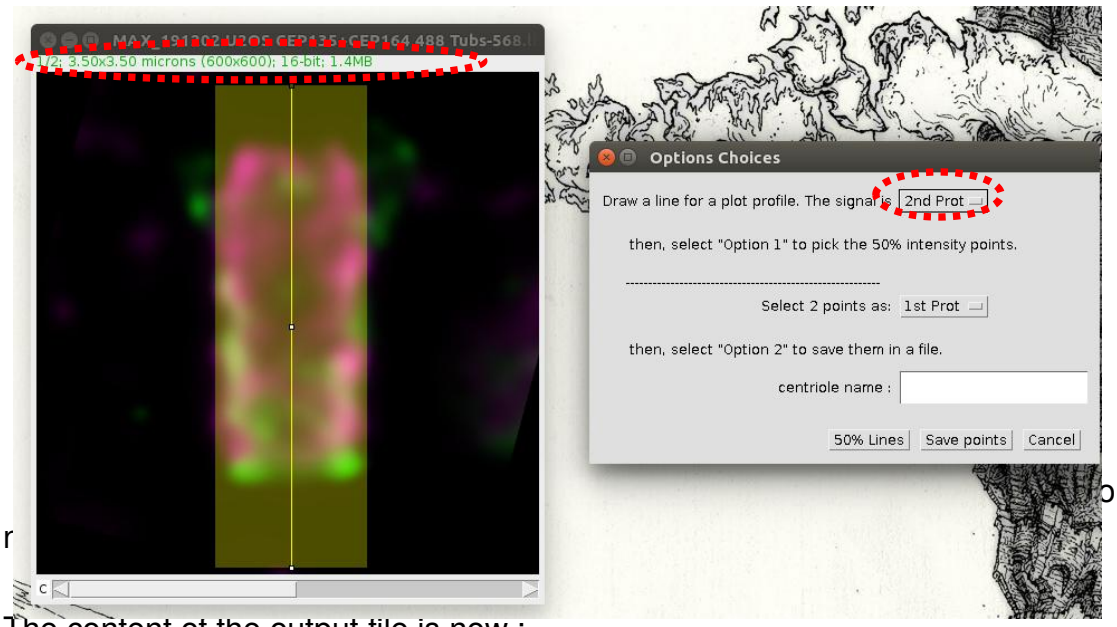
```
Label, prot1_pk1, prot1_pk2, prot2_pk1, prot2 pk2
Label1, 0.455291419029, 2.63471919537
```

Where 0.45 and 2.6 indicate the x position of the signal extremities as selected by the user.

Step 5 : Do the same for the 2nd signal

Repeat steps 2-4 with slight differences :

- Select the 2nd channel for the plot profile and the “2nd Prot” option before clicking on “50% Lines”

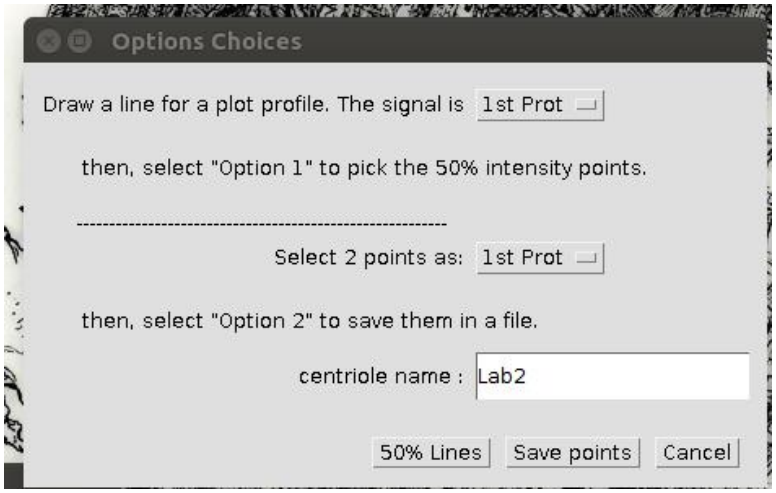


The content of the output file is now :

```
Label, prot1_pk1, prot1_pk2, prot2_pk1, prot2_pk2
Label1, 0.455291419029, 2.63471919537, 0.574712119102, 2.73921230793
```

(Optional) Step 6 : Add a new centriole

If you have several centrioles you want to quantify in a row, keep the “Options Choices”/main window open (don’t click on “Cancel”) and open a new image. You can work as previously but remember to add a different label to identify easily your centrioles.



After the 2nd signal is also picked and written, the content of the file will be :

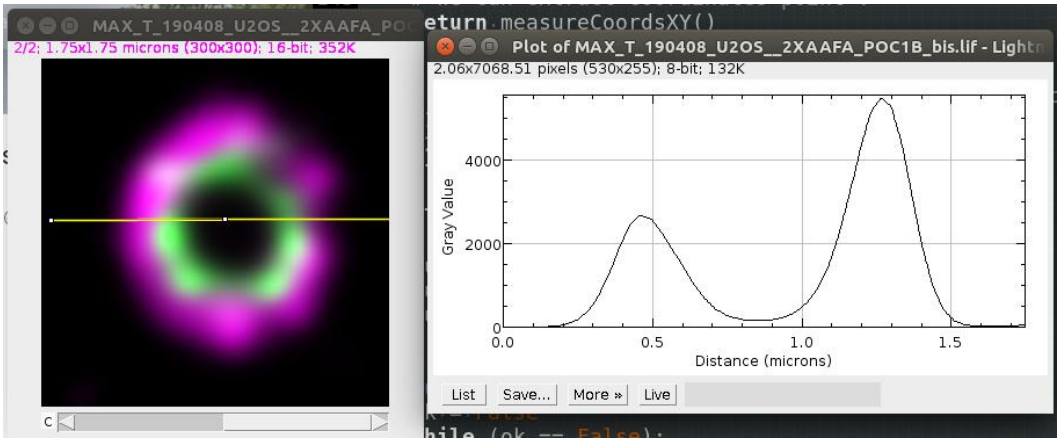
```
Label, prot1_pk1, prot1_pk2, prot2_pk1, prot2_pk2
Label1, 0.455291419029, 2.63471919537, 0.574712119102, 2.73921230793
Lab2, 0.340158716838, 2.2053623475, 0.48189151552, 1.96725124571
```

Case 2 - Signal diameter quantification

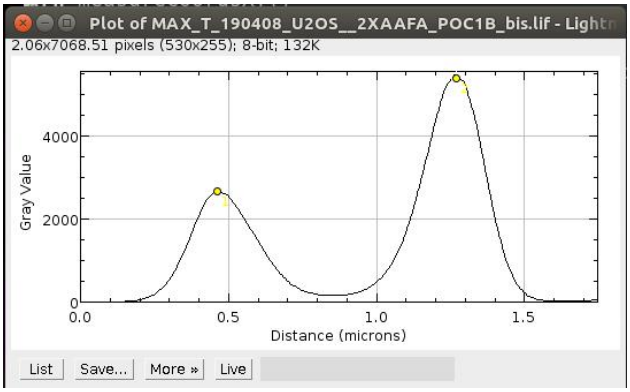
Step 1 : Preparing the plot profile

In this case, you don't need to use the "50% lines" option.

Open your image, and make the plot profile.

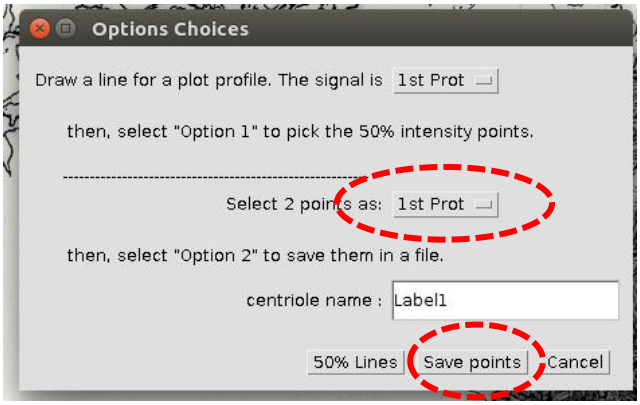


Select the 2 peaks.



After selecting the “1st prot” parameters and labeling the centriole, click on “Save points”.

Do the same for the 2nd protein.



Additional notes

You can process several images/centrioles that will be saved in the same file (*as long as you don’t close the plugin*) with one centriole per line. It is advised to label the centriole to keep track of them (when you **Save points** of the **1st Prot**). If you picked several centrioles, **always keep the same order of protein picking** ! Do not put the tubulin as a “2nd prot” if you started picking it as a “1st prot”.

The main dialog window is non-modal one, it means it allows you to interact with ImageJ as usual, so you can open/close images and play with them with the login still open. This way you don’t have to open all the centrioles you want to process before launching the plugin, you can do it one by one.

You can pick several “2nd prot”, for example, if you have three proteins to pick or a protein with two different positioning. To do so, pick the first two proteins as usual, then prepare your 3rd entry as if it was a 2nd protein and Save the points as 2nd protein. You’ll see in the output file that 2 additional columns for this new protein/signal.