# Explanations macro quantif\_cometes\_EB1\_vf

1/ Choose your image to treat (you can either choose a .nd file -but all TIF files from acquisition should also be present in the same folder- or directly a reconstructed TIF):

Une image contenant texte, logiciel, Icône d’ordinateur, affichage

Description générée automatiquement

2/ Enter the parameters for the analysis:

Une image contenant texte, capture d’écran, Police, nombre

Description générée automatiquement

* The channel for detection (containing the comets)
* The slices on which you want to study the comets (for the first pole you will analyse); the maximum z-projection will be applied on the kept slices if more than one
* The parameter for Find Maxima on GFP channel for comets detection
* The maximum size in pixels of one comet (-> this is to remove the really wrong detection, see step 5/ for more precision)

-----> If you do not know exactly, you can measure on the image, with the line tool, check the size of several comets and enter a slightly bigger value in the interface:



3/ The macro asks to a/ draw the **pole** (that will be removed of the analysis). The macro will show again and again this message if there is not exactly one ROI (should be the pole one) in the Manager:

Une image contenant capture d’écran, Logiciel multimédia, logiciel, Logiciel de graphisme

Description générée automatiquement

This ROI must be **a polygon ROI** so that the step 5/ works correctly.

and b/ draw the region of analysis (on which the comets will be detected). The macro will show again and again this message if there is not exactly two ROIs (the pole from before and this one) in the Manager:

Une image contenant capture d’écran, Caractère coloré, sphère

Description générée automatiquement

4/ The comets detection is performed on the channel specified in the interface (step 2/) with the prominence parameter chosen. You can remove or add some points, but you **must click on “Update”** in the ROI Manager (otherwise the change may not be considered!!):

Une image contenant texte, capture d’écran, Police, blanc

Description générée automatiquement Une image contenant texte, Police, nombre, capture d’écran

Description générée automatiquement

On a PC:

* to add a point: click on a point
* to remove a point, click on the point with **Ctrl** key pressed

on a Mac:

* to add a point: click on a point
* to remove a point: click on the point with **Command** key pressed

At this point, is saved a first result containing for each comet the distance to the pole; careful the value computed is to the pole contour (drawn in step 3/) and **not to its centroid** !

5/ The comet size is find automatically using Gaussian fit (see below for more explanation). Because sometimes the Gaussian fit is totally wrong (comets close to each other, background with high intensity), the macro checks two things: that the sigma of the gaussian is positive and that the corresponding size of the comet is smaller than the last value entered in the interface of step 2/.

6/ The user can check the size of the comets automatically detected; be careful, you cannot add new ROIs (they will not be considered by the macro), but you can remove some (if it is totally wrong) and modify the displayed ones:

Une image contenant capture d’écran

Description générée automatiquement

*Automatic detection was wrong on this comets -> it is removed from the analysis*

7/ For the comets which size has been detected, a file is created, containing the distance to the pole and the size.

Une image contenant texte, capture d’écran, logiciel, affichage

Description générée automatiquement

8/ The macro offers the possibility to treat the other pole; if yes, it goes back to step 1/ for a second pole.

Une image contenant texte, capture d’écran, Police, nombre

Description générée automatiquement

Are saved:

* For **all** comets, distance to the pole
* For **the comets kept at the end of step 6/**, the distance to the pole and the size of the comet
* The ROI file containing: pole, ROI of analysis, final comets (after modification of the user), final line that represent the comet size:
* Une image contenant texte, capture d’écran, diagramme, conception

  Description générée automatiquement

## **To test the FindMaxima parameter to put:**

You have to:

* Duplicate the channel and slices you want to study:

Une image contenant texte, capture d’écran, logiciel, affichage

Description générée automatiquement

* Apply the Maximum z-projection: Image > Stacks > Z-projection and choose Max Intensity
* Test prominence parameter by launching Process > Find Maxima (do not hesitate to zoom on the area containing your comets before launching the Plugin -> after the image is not accessible) and change the Prominence parameter to find the best one (3 first check boxes should be unclicked as shown, and preview activated to compare the results):

Une image contenant capture d’écran, Logiciel multimédia, logiciel, Logiciel de graphisme

Description générée automatiquement

Do not forget that the analysis will be performed only the ROI you drawn so this is OK if at this step there are some detections outside, they will be removed in the macro.

## **How are computed comets parameters in step 5/?**

1/ For the **comet distance to the pole**, we use the Plugin "Distance Transform 3D" which from a binary image (0, background, 255 pole), creates a distance map to the structure. The mean intensity of the points (i.e. the comets centroid) on this image gives the distance to the pole.

2/ For the **closest pole point to the comet**, we also use the Plugin "Distance Transform 3D", made from one pixel at 255: the one corresponding to the comet centre. We create a stack image, one slice per comet, containing this distance map. The ROI contour of the pole is resampled into a fixed number of equally spaced points and on each slice; the points of this re-sampled contour are measured and the one with minimal intensity (i.e. distance) is kept (might be different for each comet of course), representing the closest point of the pole to the studied comet !

3/ For the **Gaussian fitting** :

a/ We use the closest point to the comet in the pole contour as explained in step 2:

Une image contenant collier, Accessoire de mode, Bijoux, art

Description générée automatiquement Une image contenant vert, Caractère coloré, léger

Description générée automatiquement

b/The line between the pole and the comet is “doubled” (to be sure to have the full comet):

Une image contenant vert, Caractère coloré, léger, laser

Description générée automatiquement

c/ On the profile of this line, the Gaussian fit is performed: we choose a model where only the additive factor, multiplicative factor and sigma are unknown. The mean of the gaussian is forced to be the position of the comet (on the line).

Sigma value is approximated and FWHM computed as show here:

Une image contenant texte, capture d’écran, Police, ligne

Le contenu généré par l’IA peut être incorrect.

From Wikipedia (https://en.wikipedia.org/wiki/Full\_width\_at\_half\_maximum)

Ex of correct fit:

Une image contenant texte, capture d’écran, ligne, Tracé

Description générée automatiquement

Two additional macros were written to help concatenate the results obtained after launching the macro on several (different) images in a same folder:

* *macro\_nbComets\_perImage*: for each treated image, gives the number of comets per pole
* *macro\_concatenate\_EB1\_distance\_size*: for all treated image, concatenates the required parameter(s) (can be distance and/or length)

See the dedicated documentation for more information.

## **Remarks:**

* For the step 5/ a/ an interpolation of the pole ROI is made and has been only tested on the polygon tool, which is why for the step 3/a/ will refuse a ROI that is not a polygon.
* At step 6/ it is impossible to add ROI because the name of the ROI contains the comet number (and enables to make the connexion with the points detected during the FindMaxima to have in the same result table distance to the pole and comet size