# Explanations distancePigments\_nucleus\_vf.ijm

This macro should be launched after the macro *detectPigment\_quantifySignalAround\_vf.ijm*.

The associated image should have as last channel the nucleus (called cyan)

(-> This could easily be modified by asking the user the channel of nucleus)

1/ The macro asks to load a **ROI set** (generated by the macro *detectPigment\_quantifySignalAround\_vf.ijm*); if the file is not a .zip, the macro will exit with this message:

Une image contenant texte

Description générée automatiquement

From this ROI set, the macro detects the image name and the possible sub-stack performed to keep only the slices of interest.

2/ The macro asks for the XY and Z-steps in µm:

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

Description générée automatiquement

3/ On the last channel, on the maximum z-projection, a threshold (Otsu method after a subtract background) is applied to create a mask. Sequentially for each cell, an object detection is performed on the mask.

* If only one object of area over 100 pixels is detected in the ROI of the cell, it is considered as the nucleus and the corresponding Z is defined as the slice with maximum standard deviation:

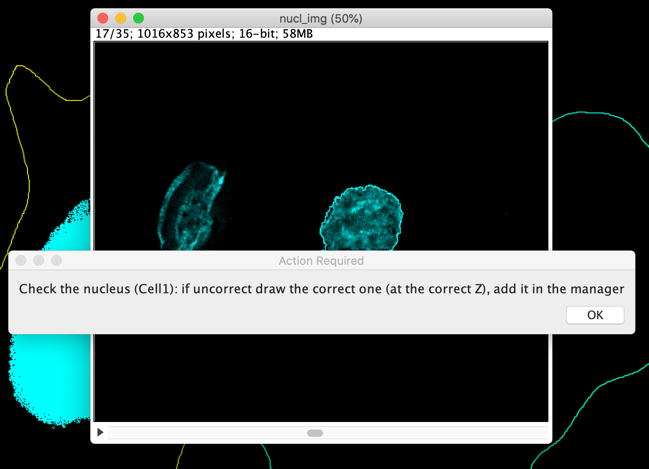
Une image contenant graphique

Description générée automatiquement Une image contenant graphique

Description générée automatiquement

*Z= 7 defined as the « focal » plane*

The user is asked to check and re-draw if necessary the nucleus for the corresponding cell:



* If more than one object (of area over 100 pixels) is detected, the user is directly asked to draw the correct nucleus (**at the correct Z**):

Une image contenant texte

Description générée automatiquement

4/ For each pigment loaded in the zip file (detected before, with the macro *detectPigment\_quantifySignalAround\_vf.ijm*), the (Euclidian) distance between this pigment, located in , and the nucleus, located in is computed thanks to:

This distance is saved in an excel file, in the folder containing the image, with the image name and finishing by “*distanceNucleus\_eachPigment.xls*”

5/ In addition some characteristics of each nucleus are computed, especially an approximative radius, as the average of the major and minor axis divided by 2:

Une image contenant texte, invertébré, léger, sombre

Description générée automatiquement Une image contenant texte, invertébré

Description générée automatiquement Une image contenant texte, invertébré

Description générée automatiquement

Original ROI Fit ellipse of the ROI Minor & Major axis

In the publication, are presented the subtraction between the positions computed in step 4/ and the average radius.

At the end of the macro are saved (in the folder containing the data):

Une image contenant texte, Police, logiciel, Bleu électrique

Description générée automatiquement

* The ROIs of cells (from previous macro) and nuclei
* The Result table with distance from the nucleus center for each pigment:

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

Description générée automatiquement

* A Result table with characteristics of the nuclei:

Une image contenant texte, capture d’écran, Police

Description générée automatiquement