# Macro\_spheroid\_3dinvasion.ijm

This macro treats a folder and measures the nuclear translocation of a given protein by computing a ratio of its mean intensity in the nucleus by its mean intensity in the cytoplasm.

Steps of the macro

1/ The macro asks for the folder to treat which contains all images that the user wants to treat. A “Translocation analysis” subfolder is created in which all measurements and data will be saved.

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

Description générée automatiquementUne image contenant texte, capture d’écran, logiciel, Logiciel multimédia

Description générée automatiquement

2/ The macro asks the user to:

* define the extension of the files to treat. In our case, by selectin .tif, the macro will disregard the .nd2 file and only open the .tif file (one after the other).
* The channel representation: the nucleus, the protein of interest (which translocates or not), and a potential channel to draw the cells (phalloidin for instance, but it can also be the same as the translocated protein channel).
* Define if the images are one slice or several (a z-stack)

Remark: this means that all images treated together (*i.e.* in the same folder) should have the same order for the channel acquisitions (or at least for nucleus and collagen).

Click on OK.

3/ The first image is loaded; if it is a z-stack, the macro asks the user to choose the plans on which the projection will be made (maximum intensity projection). Click on OK.

Une image contenant texte, capture d’écran, Police

Description générée automatiquement

4/ The channel of cell contour is displayed.

1. If the code detects cell contours drawn from a previous analysis, it will load the old cell contours in the ROIManager. The user may simply click on OK or add or delete ROIs.
2. If no previous analysis was detected, the user must draw the contours and then added to the ROIManager (by pressing the letter t on the keyboard). Several cells can be drawn and added to the ROIManager. Once done, click on OK.

Une image contenant texte, capture d’écran, graphisme

Description générée automatiquement

5/ For nuclei, two scenario may happen: A picture of the nucleus is displayed, n and an automatic threshold (MaxEntropy Dark) is applied

1. If the code detects nuclei ROIs from a previous analysis and if no modifications were done for cell contours, it will load the nuclei ROIs from the previous analysis in the ROIManager.
2. Otherwise, the nucleus channel is extracted and nuclei are counted on a maximum z-projection, thresholded (default threshold) after a blur of 2 pixels (parameter customizable at the beginning of the code). The user is asked to adjust the threshold if necessary. Too small thresholded structures (minimum size: 800 pixels, customizable at the beginning of the code) or nuclei on the border are removed from the analysis. Click on OK.

Une image contenant capture d’écran, texte

Description générée automatiquement

6/ For each cell, the nuclei are detected and the user is asked to confirm that the detected nuclei belongs to a cell. If there is more than one nuclei (multinucleated cells), either delete one or merge them together : maintaining the Shift key, select the two nuclei in the ROIManager, right click, select “OR (Combine)” and press t to add the merged nuclei ROI to the manager. Delete the ROIs for the individual nuclei.

Une image contenant texte, capture d’écran, conception

Description générée automatiquement

7/ The macro will measure the mean intensity of the translocated protein in the cytoplasm and in the nucleus and computes a ratio of the mean intensity.

8/ The next image is loaded and at the end of the folder to treat, a Results table is displayed and contains for each treated cell the cell area, the mean intensity of the translocated protein in the nuclei and in the cytoplasm and the ratio. This Results table in saved in the “Translocation analysis” directory.

Une image contenant texte, Police, capture d’écran

Description générée automatiquement