Cellophane Manual

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Installation

Put the three files “Cellophane_.java”, “CellophaneManualNoDext_.java and “imagescience.jar” in the ImageJ/plugins directory.
Compile the Cellophane using ImageJ : Plugins-> Compile and Run... and selecting one of the two aforementioned .java files.
(You can also use another compiler that you may have)

Usage – Semi-automated version: Cellophane

This plugin assumes one of those two:
a. a stack with an even number of slices, with alternating tagged protein (eg. Pom1GFP) and dextran images or
b. a stack if an odd number if slices, with two alternating tagged protein (eg. Cdr2GFP and Pom1Tomato) and dextran images.

1. Make sure the ROI manager, if there is one, is empty.
2. When the stack is open, go to Plugins->Cellophane.
3. A projected Dextran image appears.. Click in each cell you want to phenotype with the left button. At each click a ROI appears the ROI manager. You can check that this ROI marks approximately the outline of the cell.
4. Once you have clicked on all cells, click with the middle button in the image (or space bar). This will find the profiles off the tagged-protein and the outline of the cells as well as the cell axes are displayed in the roi manager of ImageJ.
5. Tick on the “Show All” box in the Roi Manager to see the profiles and axis.
6. You can modify the cell axes, using the “Update” button on the roi manager, but not the outline.
7. Delete outlines that you want to discard
8. Press the middle button on the same Dextran image (or the space bar while the Dextran image is active) to validate the profiles
9. The plugin writes in a file called “<ImageName>_profiles.csv”, where <ImageName> is the file name of the image stack you are processing. This file is thus in the same folder as your stack and you can open it with LibreOffice (OpenOffice) or Excel. It will also save the image coordinates of the profiles in a file called “<ImageName>_position.csv” and in multiple roi filea called “<ImageName>_<N>.roi”. If those files already exist they are deleted, so remember to rename this file if you want to keep the data.

Usage – Manual version: CellophaneManualNoDext

This plugin assumes a stack with an even number if slices, alternating tagged protein.

1. Make sure the ROI manager, if there is one, is empty.
2. When the stack is open, go to Plugins->CellophaneManualNoDext
3. An average image of the stack appears. Draw the cortex of the cell (or whereever you want

to extract the signal, using ImageJ polygon selection tool. You can use the zoom if needed. Hit Ctl-t after each cortex is drawn. The selection is put into the ROI manager. Press space (with the averge window is active) when you are done.

4. The plugin extract the cell axes and puts the in the ROI manager. Correct them if necessary using the Update mode on the ROI manager. Press space when you are done.
5. The plugin writes in a file called "<ImageName>_profiles.csv", where <ImageName> is the file name of the image stack you are processing. This file is thus in the same folder as your stack and you can open it with LibreOffice (OpenOffice) or Excel. It will also save the image coordinates of the profiles in a file called "<ImageName>_position.csv" and in multiple roi filea called "<ImageName>_<N>.roi". If those files already exist they are deleted, so remember to rename this file if you want to keep the data.
6. The plugin tells you how many profiles were written. Press space to confirm. Is one is missing or 't' if this is wrong.

File Formats

Profile file:

The file profiles.csv contains four (or eight in the case of two tagged proteins) rows per profile, it is the same profile starting at two different positions (the estimated middle of the cell) and in two directions.
The first eight columns contain the following values:

1. image file name
2. profile number
3. a starting position /direction number (1 and 3 or in the same direction, 0 and 1 start at the same point)
4. The estimated length of the cell (in pixels)
5. The number of points in the profile
6. The channel used (1 in our application, for pom1 profile or 2 for cdr2 profile)
7. The external background value
8. A standard deviation of the external background value
9. The cytoplasmic background value
10. A standard deviation of the cytoplasmic background value
11. The position of the first tip of the cell
12. The position of the second tip of the cell.

The rest of the columns contain the profile values (to which the external background was subtracted).

Position file:

The file "position.csv" contains the position at which the profile where extracted. Each line of the profile file corresponds to two line in the position file, one for the x coordinate and one for the y coordinate.
The first four columns are similar to the first for columns of the profile file.

Roi file:

The roi files can be opened with ImageJ to visualize where the profile was extracted