**CODE TO ANALYSE MICROTUBULE STABILITY**

Bioformat for Matlab has to be downloaded from <https://www.openmicroscopy.org/bio-formats/downloads/>, it has to be placed in a “bfmatlab-2” folder where the “MainAnalyse.m” file is.

It measures the evolution of the total intensity along time.

The intensity is normalized to correspond to 100% at time 0.

The method makes a segmentation to detect the cell.

=> It needs to have sufficient contrast.

The code allows measuring the intensity taking in consideration control videos (for dye accumulation and photo-bleaching).

**/!\ WARNINGS :**

- To have significant results, we need to have at least 10 videos/conditions.

- Deconvolve your videos on the microscope software before analysing them.

- Always crop your videos to avoid to keep interphase cells on it and to avoid having too big videos. (If possible, try to have videos less than 50000 Ko.)

- Don't touch any windows displaying figure before the saving windows appears.

- The program may not work on cells which rotate during the video time. However, it works on misoriented cells.

- The program was developed on cells whose microtubules were marked with SIR-tubulin. It may not work with other dye (different contrast)

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**\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* ANALYSE OF MANY CELLS \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\***

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The main program to use is **<MainAnalyse()>**

-> Open Matlab

2 ways to open it

-> Open the file MainAnalyse.m

-> Click on RUN in the Editor menu.

-> If a windows open with

«File [...] is not found in the current folder ...»,

-> Click on 'Change Folder'

-> Change the current folder to the ‘Code Microtubule Stability folder’

-> Write I=MainAnalyse() in the current folder

-> You can follow the progress of the code on the 'Command Window'

-> If errors appear, they will be displayed in the ‘Command Window’ and stop the program

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**First parameters to set:**

- Kind of projection: the code use a Z-projection to pass from a 3D object along time to a 2D object along time. The segmentation is done on the maximum projection but then, the measure of intensity can be done in different projection

*Maximum projection* (default):

Take the maximum intensity of all stacks by pixel

*Sum projection*:

Make the sum of all stacks’ intensities by pixel

- Microscope: you can use this code with videos from Nikon or DeltaVision

*Nikon videos*: On Nikon software, convert the videos on '.ome.tif'

format to be able to analyse them

*Deltavision videos* (default):

The files must be in '.dv' format (software default)

- Allow intensity increase (>15%): Sometimes, when the contrast isn't sufficient, the segmentation work wrongs. To avoid having, you can stop the measure of intensity if the increase is biggest than 15% in one step time. The last video times won't be measured.

(Default: not allow big increase)

- Number of Repetitions

- Number of conditions by repetitions (must be the same for all repetitions)

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**Configurations of the folders containing videos:**

- Each repeat should be in a distinct folder

- Each condition should be in distinct folder

- IF you don't have control for this repeat, each condition should be a subfolder of the repeat folder

- IF you have control for this repeat,

- create one subfolder which will contain folders for each control

- create one subfolder for the experimentations (which will contain folders for each condition)

(You can have different control for each condition or a unique one.)

*EXAMPLE with control*

*-> Repeat 1*

*-> Control*

*-> Condition 1*

*-> Condition 2*

*-> Experiments*

*-> Condition 1*

*-> Condition 2*

*-> Repeat 2 ….*

*EXAMPLE without control*

*-> Repeat 1*

*-> Condition 1*

*-> Condition 2*

*-> Repeat 2 ….*

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-> Enter the pathway to the repeat folder in the dedicated frame

-> Set the check box on if you want to take in consideration control videos files (you can have one set of control videos for both conditions or a set of control videos for each condition)

-> Click on ‘Apply settings’ when it's over (you can't come back after)

**IF you have control**:

A window will open for each condition asking you the pathway to control folder containing the videos for this condition/repeat or ask if they're the same as for the precedent condition.

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**For each video**,

A window will open, asking you to select all the colourful shapes corresponding to part of cells.

You just have to click on the different colourful shapes.

-> When it's over, press the 'Return' key.

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It will display all the intensity curves in a separate graph for each condition.

For each condition, you can exclude outliers. When all the videos of this condition are analysed and their curved are displayed, a window opens giving you instructions and a cross appears on the graph. Click on the curves you want to exclude and press return when it’s over.

If you have control videos, you must select the curve on the graph without correction. You can also exclude outliers from control itself.

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The code will display 3 statistics tools on a same graph for the several conditions (in a separate window, if you have control or in the same one if not)

- MEAN: show the mean of all curves, with its confident interval compute with the bootstrap method (random sampling with replacement of the intensities, repeated 1000 times, applied for each time step)

- MEDIAN: show the median of all curves, with its confident interval compute with the bootstrap method

- Two-exponential model: the model fit the equation

**'Intensity = a exp (b\*t) + c exp (d\*t)'**

*Where a, b, c, d are fitting parameters*

This model is used to calculate the *'half-life time'* (time to access 50% intensity, i.e. half the microtubules depolymerise)

If you compare two conditions, the test of Mann-Whitney is applied at each time to test if the difference is significant

The result of the test is displayed on the graph of the two-exponential model with the usual convention:

\*\*\*: p-value<0.001; \*\*: p-value<0.01; \*: p-value<0.05

IF you have controls, the control graph will be displayed in a graph and the curves with and without correction in separate graphs

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**Final comparison**:

A final comparison of all repeats is displayed in a new window.

It shows the mean, average and two-exponential model for all the videos of all repetitions.

It also calculates and displays the Mann-Whitney statistics on all videos for each time.

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**Saving information:**

The program permits to save all results in an excel file (only on Windows).

-> Indicate the pathway to the folder in which saving it

-> Indicate the name of the experiment.

It will create a new folder with this name containing the excel file and all the figures if you decide to save them.

-> You can also quit and finished the program without saving the data.

You can always save the figure when the program is finished as they are still open until you lunch the program again.

The intensities can be found in I (or ans, depending the way you open the code) in the Workspace windows. It’s an array where each column corresponds to a condition and each row to a repetition. Each cell is a matrix of all intensities (by row) over time.

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**Modifying display:**

When the program is finished you can access the figures and modify them thanks to the *'Figures Properties' in Edit Menu.*

From 'Figures Properties', you can also access to the name of each independent videos.

-> Click on a curve

-> Click on *‘More properties’*

-> The name is in the *‘DisplayName' field.*

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**\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* ANALYSE OF 1 CELL \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\***

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The main program to use is **<analyse1cell()>**

-> Open Matlab

-> Open the file analyse1cell.m

-> Click on RUN in the Editor menu.

-> If a windows open with " File [...] is not found in the current folder or on the MATLAB path. ", click on 'Change Folder'

-> You can follow the progress of the code on the 'Command Window'

-> If errors appear, they will display in the ‘Command Window’ and stop the program

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**First parameters to set**:

- Select the name of the video to analyse (via browser or copying the pathway + name in the dedicated frame)

- Choice to display the result of segmentation

If you choice to display and save it, it will be saved in the subfolder ‘*VideoSeg’* in the Code Microtubule Stability folder

- Kind of projection

- Microscope

- Allow intensity increase (>15%)

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A window will open, asking you to select all the colourful shapes corresponding to part of cells.

You just have to click on the different colourful shaped.

When it's over, press the 'Return' key.

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The program only displays the curve of intensity along time.

It will be saved in the subfolder ‘VideoSeg’