Computational methods attached

ENDIS: ENDosomes positioning relatively to nuclei DISplacement vector.

The software can be run from Matlab, with the Image Processing Toolbox.

The main matlab file to be launched is PipelileENDIS.m, but depends on all files

It takes as input 2D + time tif stack, one for each channel (nuclei and endosomes).

Several nuclei can be studied at the same time.

List of parameters:

FirstFrame(included), LastFrame,

To let the user define a subsequence of the movie showing nuclei

%(since division or appearance are not handled). Disappearance is well handled.

Cut off speed (min) (pixel per frame)

While a nuclei is not moving, i.e having a displacement vector below the cut off speed defined in this parameter, the endosomes positioning is ignored.

Search Radius

This parameter defines the bandwidth of around the nuclei perimeter in which endosomes are looked for and associated to this nuclei (this is obtained by a dilation of the nuclei with a disk structuring element of radius Search Radius)

A 64 bits precompiled exe file is also provided with the link to the corresponding Matlab emulator exe.

Warning: The code as it is requires the naming of the files to be: ‘1 GFP','1 mCherry’

This can be changed

- linescan analysis and splinefitting of the MT1 phLuorin experiments MOCK/GM6001

- analysis of the position of endosomes according to the nucleus displacement

To analyze nuclei speed and corresponding endosomes positioning for each frame, the software starts by segmenting all nuclei. At the first frame only, it let the user select the nuclei to track by removing the other ones. Several nuclei can be followed at the same time. For each pair of frame, perform the nuclei association, dealing with disappearance or loss of the nuclei, and return the associated speed vector to give an orientation to a new system of coordinates. Nuclei are processed only if they are moving, i.e. have a speed superior to min speed parameter. Image is cropped around the center of the nuclei by 2x Search radius pixels for memory purposes. This system of coordinate is oriented such that the center is the center of gravity of the nuclei, and the ordinate axis is parallel to the speed vector of the processed nuclei. For each nuclei, in addition to its speed, an index called SCI gives the changes of orientation. It should be 1 for a perfect line, -1 is the nuclei changed its direction collinearly, and 0 for a 90 degree turn. The endosomes images are smoothed by median filtering, and then transformed in the same coordinate system that nuclei image. The transform is also applied to the nuclei mask.

This mask is dilated by the search radius and applied to the endosomes movie frame, to segment the endosomes: After applying a Laplacian of Gaussian filter of sigma 0.5 pixels, all regional maxima not touching the border of the mask above a predefined intensity threshold of 40% of the max value of the image are defined as watershed seeds. A marker-control watershed segmentation based on these seeds is then applied, using a structuring element disk of radius 1 . The coordinates of endosomes are then converted to the Cartesian coordinate system as defined by the centroid of the nuclei and its vector of displacement, to polar coordinate system (where 0 degree means in the direction of the nuclei displacement).