How to use DiLiPop code

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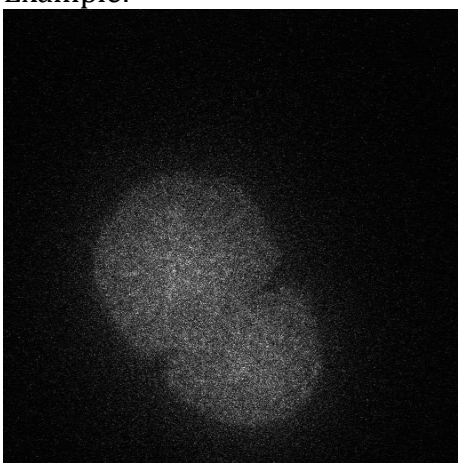
I- Preliminary steps before performing the DiLiPop analysis

Before doing the DiLiPop statistical analysis on the track duration to identify distinct dynamical behaviors, some preliminary steps are required.

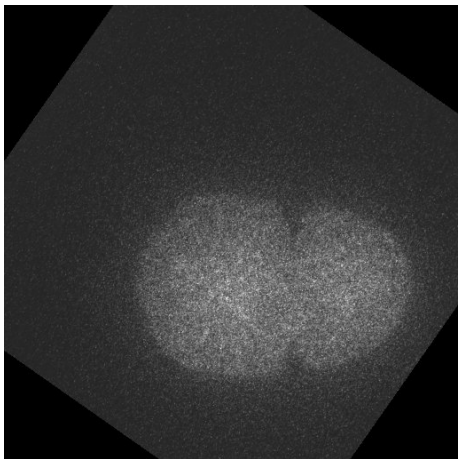
1/ Orientation and cropping of the raw image stack (manually)
First of all, you need to have your image stack as tif stack. The images should be rotated to have the embryo aligned along the AP axis horizontally and the posterior side at the right side. You may use Fiji or Icy to do this. Then, you may crop your image stack to consider only the embryo.

Example:

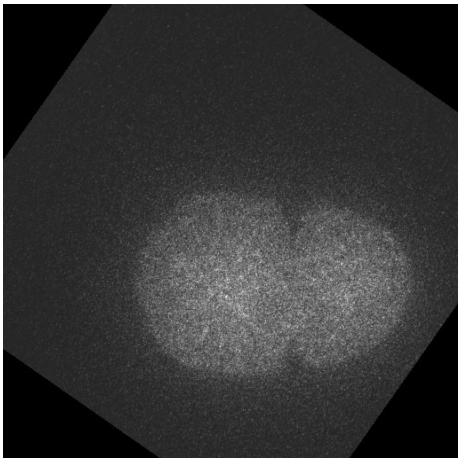


Raw frame

→

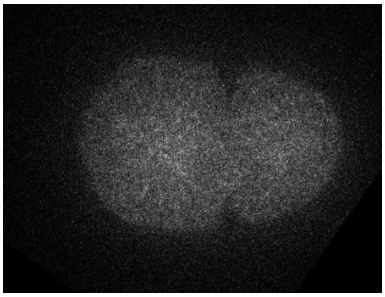


Rotated frame



Rotated frame

→



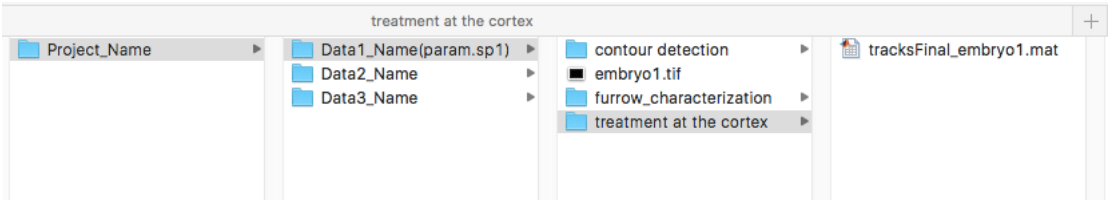
Cropped frame

2/ Generation of the parameter structure

The DiLiPop analysis requires several parameters that are saved as a structure composed of general_param, common to all embryos, and param fields for each embryo. Some fields have to be updated to your dataset. This has to be done using the following script and will generate a mat file.

to_generate_DiLiPop_parameter_structure.m

We advice you to organize your data files/folders as following, since it is how the different scripts expect your data to be present.



In the above parameter structure :

- param.basepath is the path of Project_name folder.
- param.sp1 is the name of the folder called here Data2_name.
- param.stem_name is the name of the file called here embryo1.

3/ ROI to exclude other structures possibly present in the frames.

The next step (contour detection of the embryo) will be impaired in the presence of other fluorescent signals within the image. We thus give the possibility to exclude these additional fluorescent structures by performing ROI selection using the folowing function:

to_perform_DiLiPop_ROI.m

For each embryo of the dataset, the user will be asked to do a ROI on the reference image and it will save a tif image of the binary mask generated: embryo_name_mask.tif

4/ Tracking of the embryo contour along time

Before doing the DiLiPop analysis, we need to have the contour shape of the embryo along time, which will enable to compute the area of the embryo along time, as well as its limits and lengths along AP axis. This will generate the three following mat files in the subfolder "contour detection": regionArea.mat, regionXlength.mat and regionXlimit.mat

It will also enable you to characterize the furrow ingression that happens during mitosis. For that general_param.furrow_detection.status_perform has to be equal to 1. It will generate the file of interest in the subfolder "furrow_characterization": furrow_position_convexity.mat, which contains the image of reference for the furrow onset and its position along mitosis.

This script will also save an image stack of the raw frames with a mask preventing tracking of structures found outside the embryo.

For all these tasks, the function to use is:

main_before_DiLiPop.m

5/Denoising and tracking of fluorescent sports (manually)
Firstly (if needed), you will perform the **denoising** on the stack of the raw image multiplied by a mask stack, using the detection of the embryo from above.
Secondly, you will do the **utrack** analysis to detect fluorescent spots and link them. This will generate a file called tracksFinal.mat, that we advice to save in "treatment at the cortex" subfolder

NB: these two steps (denoising and utrack) might be done automatically, adding them at the end of the main_before_DiLiPop.m script.

6/ Generation of the dataTrack_rotated.mat file needed for DiLiPop analysis
The function "**fromUtrack_to_DataTracksRotated.m**" enables to generate a structure of the data tracks extracted from tracksFinal.mat file. This structure named "dataTracks_rotated.mat" is saved in "treatment at the cortex" subfolder and contains information regarding the duration of the tracks (field: lengthTracks), their coordinates along X (tracksX) and Y (tracks Y), and their first image (indexXStart) and last image (indexXEnd).

II- The main functions of the DiLiPop code

Below are described the four functions of the DiLiPop analysis, which were used for the paper (Bouvrais et al., EMBO reports, 2021):

- **to_get_DiLiPop_parameters.m**

This functions enables to do the DiLiPop statistical analysis in the whole embryo or in the anterior or posterior regions, set by the furrow ingression position. From the durations of the tracks, it reveals the number of distinct dynamical behaviors and their parameters using Bayesian inference approach.
This function was used in Figures: 1EF, 3AB, S2C, S4BD, S6.

- **to_get_DiLiPop_parameters_3regions.m**

This functions enables to do the DiLiPop statistical analysis, investigating the difference in the 3 cortical regions of the embryo.
From the durations of the tracks, it reveals the number of distinct dynamical behaviours and their parameters using Bayesian inference approach.
This function was used in Figures: 2C, 4B, 5, EV1B, EV3BC, S10BC, S11A, S12B.

- **to_resolve_DiLiPop_spatioTemp.m**

This functions enables to do the DiLiPop statistical analysis, with a maximal resolution in time and space of the parameters of the model best fitted the experimental data.
This function was used in Figures: 4A, 6A-C, EV1A, EV2, EB3A, EV4, EV5E, S10A, S12A, S13CD.

- **to_get_DiLiPop_density_maps.m**

This function enables to generate : 1/ the number of contacts per frame that are assigned to short-lived or long-lived populations, 2/ the DiLiPop density maps, i.e. the density of MT contacts assigned to short-lived and long-lived populations for each embryo of the studied xmlfile, and the averaged density maps for the given condition.
This function was used in Figures: 2AB, S14.

- **inSilico_fabricated_dataset.m**

This function enables to generate fabricated datasets composed of X embryos of given dynamics. Then, the simulated embryos (duration distributions) are analysed using the DiLiPop stastistical analysis. The results are saved in txt file 'parameters_inSilicoTracksDurations.txt' and in mat file 'fitting_results-BayesianInference_sum_mle-' to compare assigned values to recovered values and to test whether best model is correctly found.
This function was used in Figures: S7, S8, S9.