Kymolyzer

User Guide

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Summary

This Fiji macro uses microscopy time-series calculates kymograms and analyzes them. The analysis includes filtering of main directions and calculation of several quantification parameters, such as speed of particles (high-intensity foci), their lifetime, etc. It also analyzes the coupling (colocalization) of signals from two channels, when applicable.

Workflow

It is strongly recommended that raw microscopy images are corrected for drift and bleaching before defining regions of interest (ROIs), for example using our **Correct and project.ijm** macro, which also provides an option for different types of Z projections. Working with maximum intensity projections can be helpful when working with images with low signal. It also facilitates quick assessment of drift correction.

The required data structure and use of the code is described in detail in Zahumensky & Malinsky, 2024.

available processing options (modules): Select the appropriate operation from the list below. The available operations need to be run in the order in which they are listed. The macro will fail otherwise. After an operation is finished, the next one is preselected automatically.

1. Draw ROIs

The calculation of kymograms requires ROIs. These can be prepared either automatically, using the approach described **here**, or manually using the provided option in this macro. Draw ROIs Manually create ROIs for your images. Images are displayed one at a time, together with a prompt and details on how to proceed. The ROIs can be defined using e.g. Cellpose, as described in [1], or in any other way, but they need to be named and organized as described in [1]. They can also be defined manually using the *Draw ROIs* option of this macro, which respects these requirements.

2. Create kymograms

Create kymograms Create kymograms from defined ROIs. Error is displayed if no ROIs are defined.

Note: direct import of kymograms created externally is currently not supported

3. Display kymograms

Display kymograms Images in the specified folder are displayed one-by-one, together with kymograms for each cell (defined ROI). If direction-filtered images of kymograms and individual traces have been already calculated, they can be displayed as well. In this case, the regular kymograms are displayed as well, to facilitate comparison.

4. Filter kymograms

Filter kymograms Kymograms are filtered using Fourier transformations into dominant directions: backward (bwd), forward (fwd), static (stat). These are then thresholded and binarized to extract prominent individual traces.

5. Analyze kymograms

Analyze kymograms Kymograms are quantified. Results are saved in a csv table file, which is reffered to as *Results table*. For detailed description of the parameters reported in the Results table see the Results table legend.

6. Process the *Results table*

The Results table can be processed using our custom *R script* developed previously.

Dialog windows

Directory

Specify the directory where you want *Fiji* to start looking for folders with images. The macro works *recursively*, i.e., it looks into all *sub*folders. All folders with names *ending* with the word *data* are processed. All other folders are ignored.

Image type

Select if your images represent *transversal* (also called *equatorial*) or *tangential* sections of the cells.

Subset

If used, only images with filenames containing specified *string* (i.e., group of characters and/or numbers) will be processed. This option can be used to selectively process images of a specific strain, condition, etc. Leave empty to process all images in specified directory (and its subdirectories).

Channel(s)

Specify image channel(s) to be processed. Use comma(s) to specify multiple channels or a dash to specify a range.

Channel display

Specify LUTs (lookup tables) image channel(s) to be used for display of images. The calculated kymograms are saved using these. Note that the LUT names need to correspond with the names used by Fiji.

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Citation

When using this work, please cite the authors:

- GitHub: https://github.com/jakubzahumensky/kymolyzer
- Zenodo: https://doi.org/10.5281/zenodo.15650134
- Related work: Zahumensky J., Malinsky J. Live cell fluorescence microscopy—an end-to-end workflow for high-throughput image and data analysis. *Biology Methods and Protocols*, Volume 9, Issue 1, 2024, bpae075; doi: https://doi.org/10.1093/biomethods/bpae075