Protocol for installation and application of protrusion/retraction signal enrichment and protrusion-retraction cycle duration analysis according to Nanda et al.

Related to:

Nanda S., Calderon A., Duong T-T., Koch J., Sachan A., Xin X., Solouk D., Wu Y-W., Nalbant P., Dehmelt L., Crosstalk between Rac and Rho GTPase activity mediated by Arhgef11 and Arhgef12 coordinates cell protrusion-retraction cycles.

Pre-requisite: An image series with signal intensities corresponding to the activity of interest and a corresponding control image series that represents the entire cell attachment area for thresholding and segmentation. The analysis was development for TIRF microscopy-based quantifications and is optimally suited for image series pairs that correspond to 1) a sensor that is based on activity-dependent translocation from the cytosol to the plasma membrane and 2) a control sensor that is only located to the cytosol.

Dependencies: ImageJ2 version 2.3.0/1.53f and Adapt_v3.0.1. Analyses were tested on a Mac system with macOS Ventura 13.1.

License: The original ADAPT plugin was developed previously and made available as open source (https://github.com/djpbarry/adapt/wiki) via the GPL-3.0 licence. The original publication that describes the development of ADAPT :

Barry, DJ, Durkin, CH, Abella, JV and Way, M. 2015. Open source software for quantification of cell migration, protrusions, and fluorescence intensities. J Cell Biol. 209: 163-180.

According to the GPL-3.0 license, the modified ADAPT code is published under the same GPL-3.0 license and the code is made available via this repository. All changes to the ADAPT code were made by Leif Dehmelt.

Installation and analysis of the example data set:

1. Install Adapt v3.0.1 plugin in ImageJ2.

   Optional: To restrict analysis to the part of the cell edge that is inside the cell borders, replace pre-existing files in plugin folder with the adjusted adapt-3.0.1.jar and iaclasslibrary-1.0.6.jar files from this repository. If you use the original implementation, the analysis will comprise pixels near the cell border both inside and outside the cell borders.

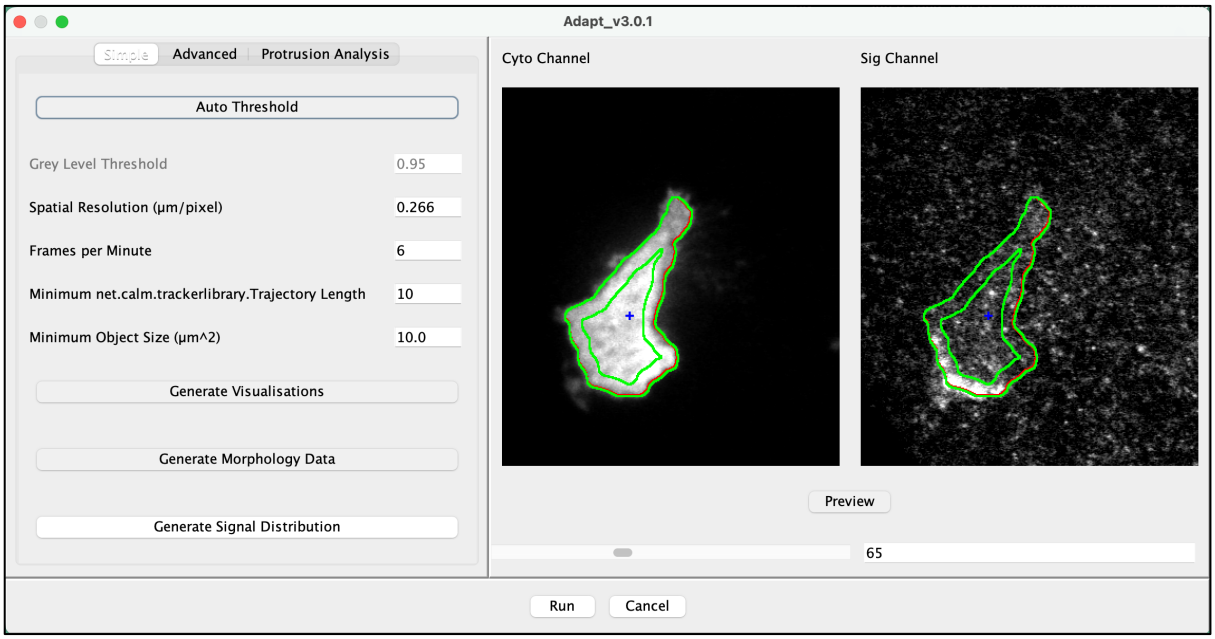
2. Relaunch ImageJ2, open raw image series to be analyzed. For an example data set, open stable_TRFPQ-BG/stable_TRFPQ-BG-1.tif and stable_TYFPQ-BG/stable_TYFPQ-BG-1.tif that can be found in the "example_rac_activity_data" subdirectory of this repository. The example data files are provided in a zip compressed format. These files therefore must first be extracted, for example with the built in unzip function of macOS. Keep the subdirectory structure of the example data set provided in the repository to make sure that the analysis script can find the data in steps 9-11.

3. Open the Adapt plugin (Plugins/Adapt/Analyse Movie).

4. Select the directory to save the output files (same main directory where the raw image series are saved. For the example data set, this is the "example_rac_activity_data" subdirectory of this repository.)

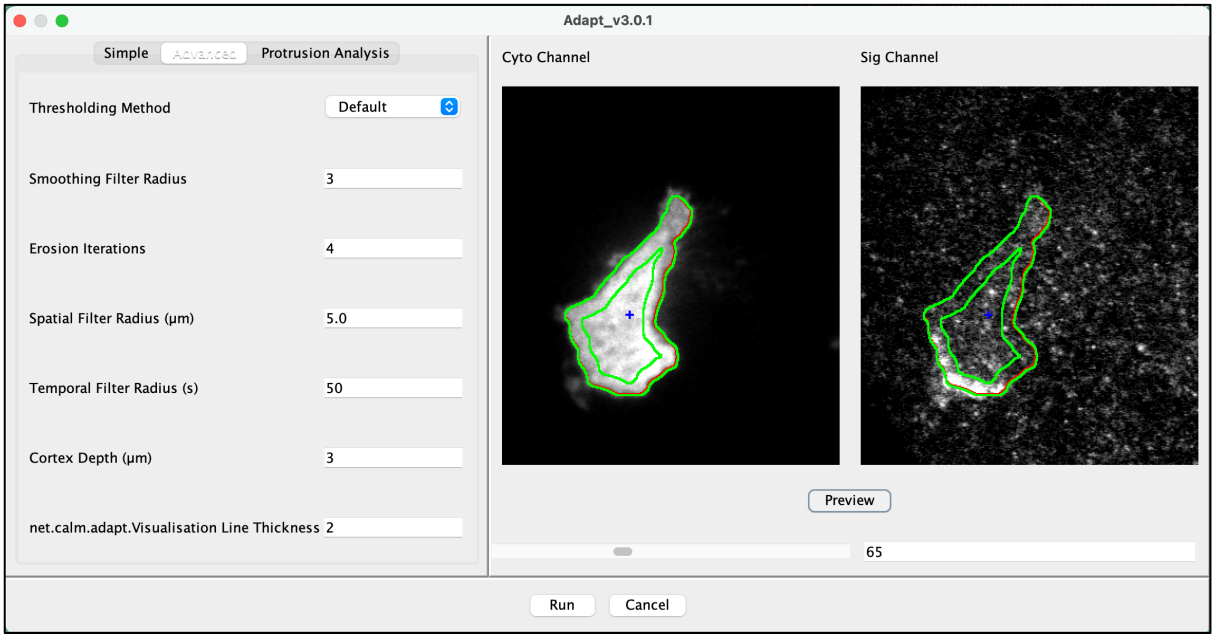
5. In the "simple" settings tab, following setting are recommended for the example dataset. Refer to the ADAPT documentation

(https://github.com/djpbarry/adapt/wiki) for details on adjusting these parameters.



For the example dataset, auto threshold can be used and the signal distribution option can be inactivated for faster processing of the data.

6. In the “Advanced” settings tab, the following setting are recommended for the example dataset.:



7. On the “Protrusion analysis” tab, individual protrusion analysis can be inactivated for faster processing of the data.
8. Run the plugin.
9. Open the pr_signal_analysis_230226.ijm macro in ImageJ that can be found in this repository. The standard settings are compatible with the example data set.
10. The header of the script file contains settings that can be adjusted for analysis. The standard settings are compatible with the example data set. However, you will need to point the script to the base directory of your system, in which the files are located. For the example dataset this would be your system path, e. g. on a Mac system this would typically be:

/Users/username/…path_to_copied_repository…/protrusion_retraction_signal_enrichment/example_rac_activity_data/

(replace “username” and “path_to_copied_repository” with your specific system information).

To adjust settings in the header file, refer to the “Macro settings” section below.

11. Run the analysis. The output files generated contains the following set of data:

protrusion_summary.xls : Data for plots of signal enrichment in protrusions

retraction_summary.xls : Data for plots of signal enrichment in retractions

protrusion_retraction_dynamics_summary.xls : Data for protrusion-retraction cycle duration.

Macro settings:

Switches (set to true or false) to perform different parts of the analysis:

analyze_protrusion_profile=true;

analyze_retraction_profile= true;

analyze_protrusion_retraction_dynamics=true;

analyze_velocity_crosscorrelation=false;

analyze_velocity_autocorrelation=false;

analysis_summary=true;

Input file information (provide strings in quotation marks):

base_path sets the system path to the directory that contains the "Adapt_v3.0.1" folder that waas generated by the ADAPT analysis. Make sure to add a closing / character in the path.

Example:

base_path="/Users/username/…/";

mask_path sets the name of the subfolder of base_path that points to the original raw data of the control signal that is used to generate cell masks. Make sure that the name of the raw data files inside this subfolder correspond to the subfolder name, with a suffix that indicates the number of the data set (e.g."-1"), followed by the tif suffix (".tif").

Example:

mask_path="stable_TYFPQ-BG";

signal1_path sets the name of the subfolder of base_path that points to the original raw data of the sensor signal that should be analysed. Follow the analgous naming convention for the raw data files inside this subfolder as for the mask_path.

Example:

signal1_path="stable_TRFPQ-BG";

numPaths sets the number of images to be analyzed in the mask_path and signal1_path subfolders.

Example:

numPaths=2;

firstPath sets the number of the first image to be analyzed in the mask_path and signal1_path subfolders.

Example:

firstPath=0; // this would start with the first image.

paths[x] sets the individual paths for the images to be analyzed in the mask_path and signal1_path subfolders, starting with x=0, and sequentially incrementing this number.

Example:

paths[0]="-1";

paths[1]="-2";

th_method[x] sets the thresholding method for the images to be analyzed in the mask_path and signal1_path subfolders, starting with x=0, and sequentially incrementing this number.

Example:

th_method[0]="Default";

th_method[1]="Default";

Other options that are typically available with ImageJ are for example: Intermodes, Otsu, Huang, IsoData or Moments

Settings for data analysis:

theshold sets the velocity threshold for detecting a protrusion or a retraction to be used for the analysis.

Example:

theshold=0.1;

filter_small_objects sets a minimal threhold for determining the cell attachement area.

Example:

filter_small_objects=100;

pixel_count_threshold sets a minimal threhold of pixels that should be available in the signal maps to include the measurement in subsequent analysis

Example:

pixel_count_threshold=20;

num_taus and **tau_shift** sets the number of time shifts to analyze and how many frames each timeshift should be apart from the next.

Example:

num_taus = 50;

tau_shift = 1;

spatial_scale_dynamics sets the spatial scale for the analysis of protrusion-retraction cycle duration. The original velocity map is rescaled to this spatial scale

with averaging, to reduce noise in the detection of protrusions and retractions and to speed up analysis.

Example:

spatial_scale_dynamics = 100;