Nuclear shape change analysis manual

# Installation

You can use the macro in two ways: load it before every use or install it.

To load it directly, simply start Fiji and drag-and-drop the Nuclear\_shape\_change\_analysis.ijm file on the Fiji toolbar to open the script editor.

To install the macro, copy the Nuclear\_shape\_change\_analysis.ijm file to the plugins subfolder of your Fiji install folder and restart Fiji. The plugin can now be found at the bottom of the Plugins menu. As an alternative, store it in the plugins/Macros subfolder instead. After restarting Fiji, the macro can then be found at the bottom of the Plugins > Macro menu (which is often a lot shorter).

# Requirements

The Nuclear-shape-change-analysis macro needs two image stacks to work properly.

The first image stack is the nuclear-channel image sequence. This can be the original image or a pre-thresholded mask of the nucleus channel. If you use a thresholded mask, please pay attention to the CREATE\_MASK setting below.

The second required image sequence contains the labelled tracks. Each instance of a specific nucleus needs to be identically labelled in each frame and each label needs to be unique to that nucleus. The labelled nuclei will be used as starting points for a watershed, so it is advisable to make sure that the labeled nuclei are smaller than the original nucleus. The easiest to create an image stack like this is to use TrackMate (<https://imagej.net/plugins/trackmate/>). As output for TrackMate export the labeled spots stack and this macro will work very well on it.

# Settings

At the top of the macro are a few settings that you can change to configure the macro to your preference. These settings are written in CAPITALS and only need to be adapted at the top of the macro; i.e., there is no need to find these back in the rest of the macro and adapt them. The settings all appear before the “//----------------------” line.

The settings are:

* Default names settings

The macro needs two image stacks (nucleus and tracked spots) to be open in order to work. How to identify these can be configured by these settings:

* + USE\_DEFAULT\_NAMES If this setting is set to false, the user has to manually identify the images (see ‘Use’ section below). If set to true, the macro will automatically identify the images by means of the standard TITLE settings below.
  + MASK\_TITLE If default names are used, this is the expected name of the nucleus image stack (mask or not).
  + SPOTS\_TITLE If default names are used, this is the expected name of the tracked spots image.
* Threshold creation settings

The macro needs a binary mask image stack of the nuclei. With these settings you can configure how this mask will be created from your original image stack. Alternatively, you can provide your own masked stack and disable the macro thresholding here:

* + CREATE\_MASK If the nucleus image already is a binary mask, you can set this setting to false to avoid double thresholding. For none-binary nucleus image stacks, leave this this setting on true.
  + PREPRO\_FILTER The filter that will be used before thresholding the nucleus stack. If you want to use another filter than the default Median, use the macro recorder (Plugins > Macros > Record…) to find the appropriate name.
  + FILTER\_RADIUS The radius in pixels of the filter used. The larger the radius, the higher the impact.
  + THRESHOLD\_METHOD The thresholding algorithm used to create the mask. This is the setting that is most depending on the particulars of your own nucleus stack, so experiment with the options in Image > Adjust > Threshold if you are not satisfied with the default results. The method names of the threshold can be found in the left drop-down menu of the threshold dialog.

Note: do not use the Auto Threshold names here, as this is a different method.

* Roi save setting

Just one setting to determine to save the tracked ROIs or not:

STORE\_TRACKED\_ROIS If set to true, the ROIs of the nucleus segments will be saved as a separate zip file per track. If false, no ROIs will be saved.

# Use

Make sure that you have the nucleus image and the tracked nuclei image open (see Requirements). Start the macro by either selecting it from the Plugins menu (if installed) or by pressing Run in the macro editor (if you loaded the macro).

If default names are not used (see Settings), you will be asked to identify the nucleus stack. Select the nucleus stack so it becomes the active image and press the Ok button.

Next you will need to identify the labeled-track image stack. Select it so it becomes active and press ok.

Please note that if the OK button is hidden and you need to select the dialog in order to see it, better just to move the dialog (or the covering image) and reselect the required image and only then press Ok. This prevents the selection of the active image going wrong.

After selecting the images (or directly if default names are used), you will be asked to pick a folder to store the results in. Please note that the results will overwrite earlier results for the same image!

After selecting the results folder, the macro will start to run. You will see Results popup and the image stacks will be scrolled through. Just wait for the log window to show that the macro is finished.

# Results

The results of the macro can be found in the picked results folder. The results consist of

* A .csv file containing the nuclear change features through time. Each line of the .csv file will give you the features for a specific nucleus (identified by its Track ID) at a specific time (identified by the Frame Nr). A nucleus that appears in multiple frames will therefor get multiple lines of features, each with the same Track ID and each with a different Frame Nr. The features not only contain the NII score for a particular nucleus, but also the Delta NII score which gives the (absolute) difference between the current NII and that of the previous frame. The Delta NII is NaN (Not a Number) for the first frame a nucleus appears in.
* The second results artifact is the labeled-track segmentation. This is the mask stack of the nucleus channel which was either produced by the macro or given as input, but now each nucleus segment is labeled by its Track ID in each frame (i.e., the segment intensity value is identical to the Track ID).
* Finally, if the STORE\_TRACKED\_ROIS is set to true (see Settings), for each nucleus the outlines of segments will be saved in a separate .zip file. This means that there will be one zip file for each nucleus! In order to use these, just drag-and-drop the files to the Fiji toolbar and the outlines will be read as ROIs to the roimanager. Multiple files can be read simultaneously.

Each ROI will identify the track and frame that it applies to.