Kymograph mt2 - Multithreaded generation of Kymographs

Description:

This plugins allows the creations of kymographs from TIFF stacks. The plugin will first "straighten" the input stack using an overlayed line (e.g. representing the nuclear envelope position). This line can be an either a straight or segmented line, without line selection the plugin will not start. The straighten approach is similar to the implemented ImageJ straighten function: The line will be fitted with a spline and then

Figure 1: Straighten the stack

for every coordinate the normal is constructed (length is variable). The intensity along the normal is then plotted in a new frame. The height of new frame corresponds to the length of the line selection (fig.1; blue line/arrow) and the width to the length of the normal (fig.1; red lines/arrow) However, **no** interpolation or smoothing is used. The straightened stack is only calculated as 3D array (to speed up the plugin) but can be shown optionally (see below).
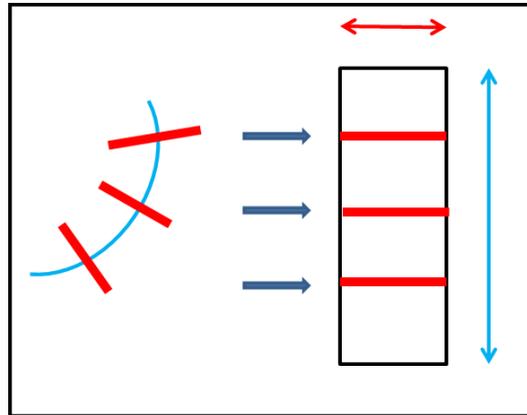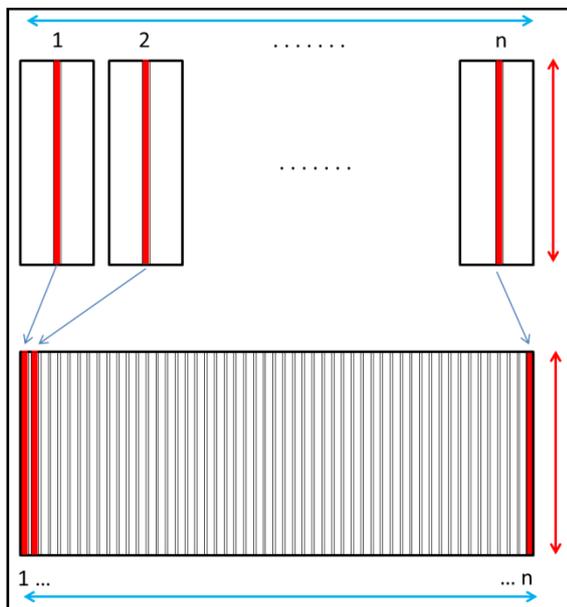
Figure 2: Creating the kymograph

The kymograph is then created according to Figure 2. By using a straightened stack several kymographs can be contructed from the input stack, e.g. a "nuclear", "membrane" and "cytosolic" kymograph. The intensity can be plotted unfiltered or the mean/ median filtered intensity (using an adjustable ROI size) is plotted (see below).

To speed up the plugin the JAVA code is optimized for multithreading according to Albert Cardona's Multithreading tutorial: http://albert.rierol.net/imagej_programming_tutorials.html
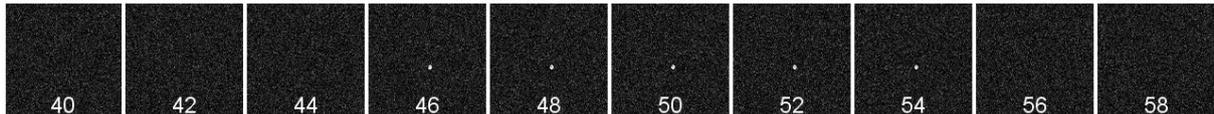
Tested with ImageJ 1.45q (64BIT, Win7).

Installation:

Place the .jar file in the ImageJ plugins directory. The new plugin is then located in Plugins-> Kymograph -> Kymograph (multithreaded)
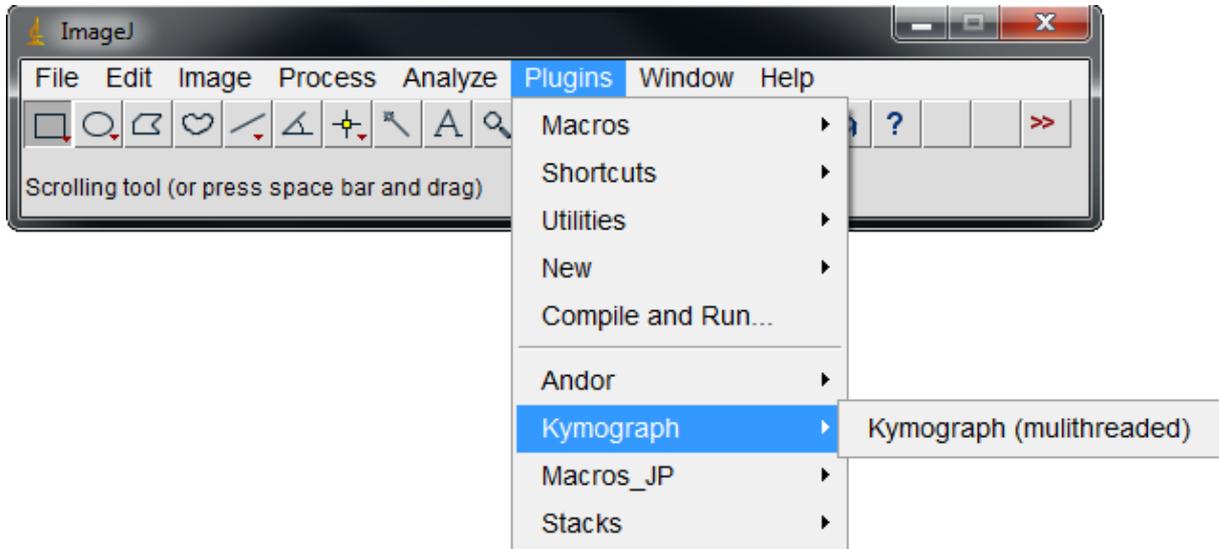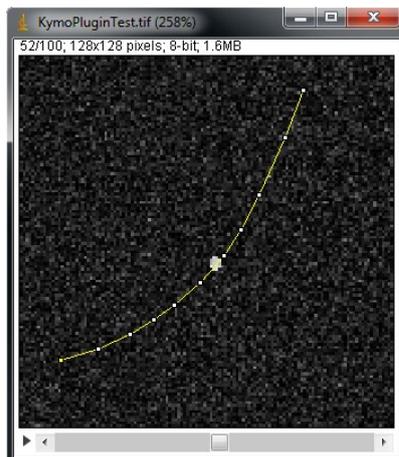

Input:

Open a TIFF stack (8, 16 or 32bit).
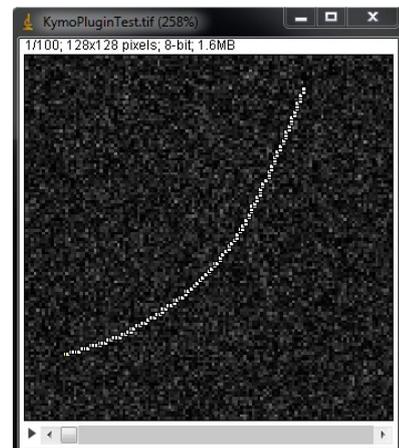
Figure 3: Sample TIFF stack with 100 frames

The stack must be overlayed with a line selection (straight or segmented; fig.4A). The line will be replace with a fitted spline later (fig.4.B)
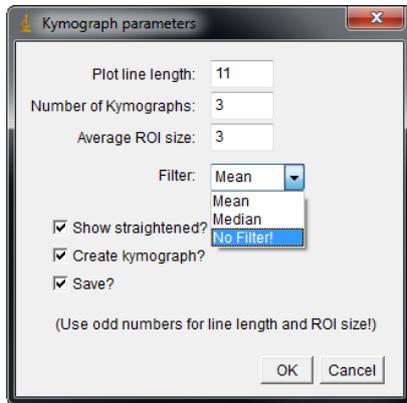
Figure 4: A) Input line overlay   B) Fitted spline

Start Menu:

Figure 5: Start menu

- *Plot line length*: Depends on the number and size of ROIs. Use odd number!
- *Number of kymographs:* At least one will be created representing the input line.
- *Average ROI size:* Use odd number!
- *Filter:* If mean or median is chosen the mean/median intensity of the ROI is used. "No Filter!" will use the intensity of the central pixel in the ROI without filtering.

Show straighten: Check to see the straighten stack which is usually only calculated as 3D array. This option will slow down the plugin!

Create kymograph: Uncheck if you only want to straighten the input stack (default for single-slice TIFFs/images).

Save: Will create a subdirectory called "kymo" in the file path of the input stack; store the kymographs as stack and montage (see *Output* section) and the parameter log file as ASCII text file.
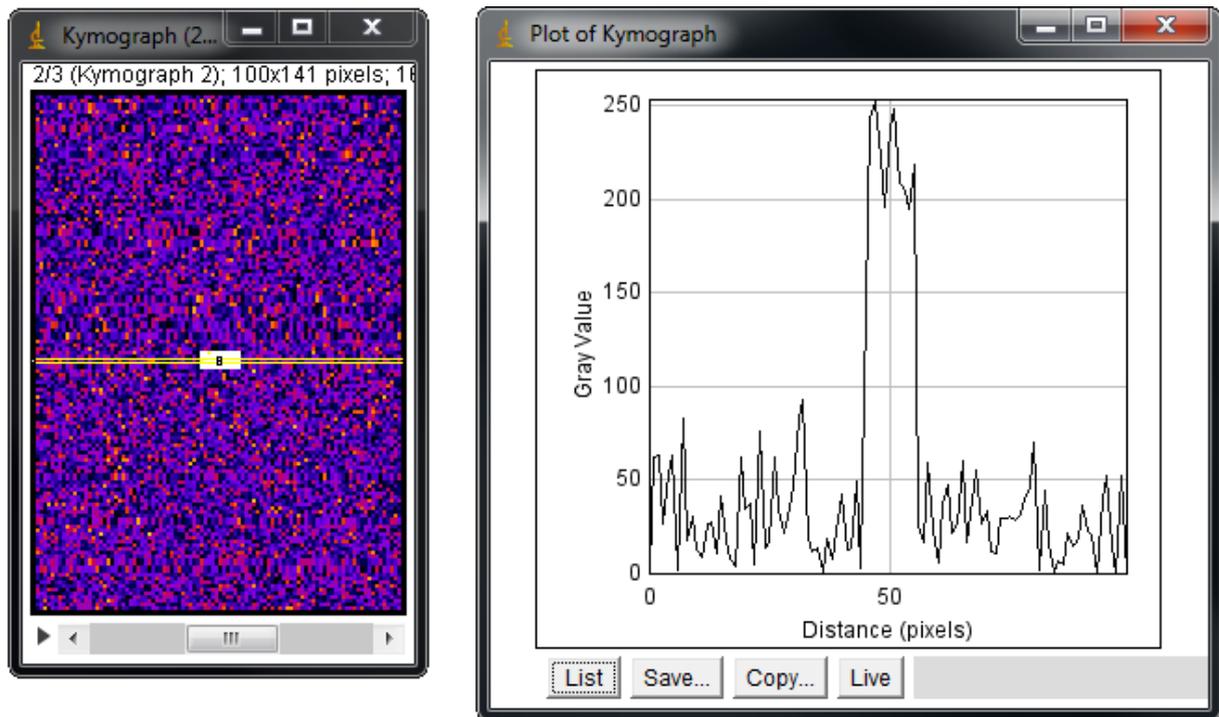
Output:

Figure 6: Output
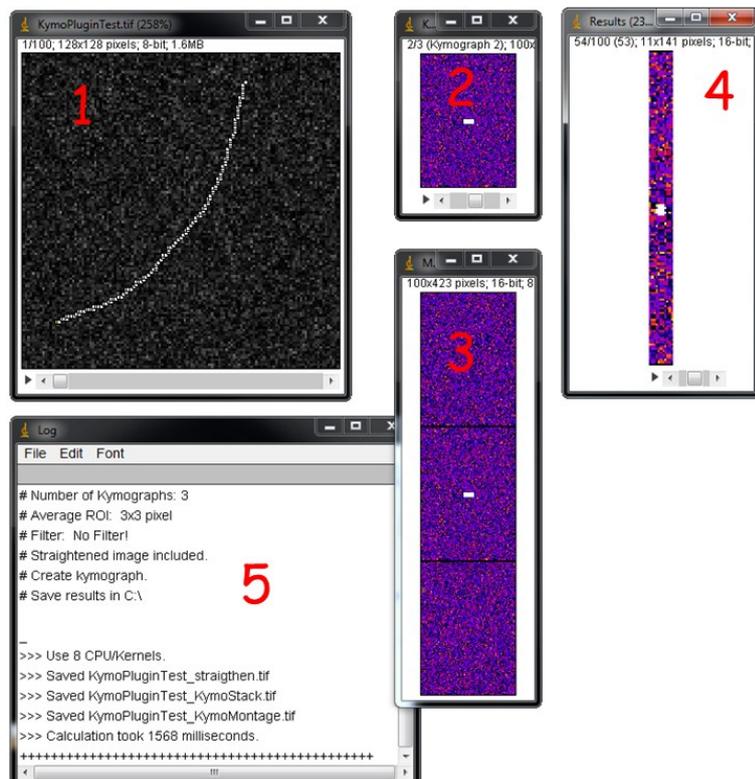
1) Input stack with fitted spline.

2) Kymograph stack

3) Kymograph montage

4) Straighten stack

5) Log window

Figure 6: Results with "No Filter!" option

The sample stack contains 100 frames; the line selection is 150 pixels long. Therefor the resulting kymograph is 100x150 pixels (x/y). The "membran test signal" from frame 45 to 55 produces a characteristic line in the kymographs.