PatternJ

User Manual

An ImageJ macro toolset for the automated analysis of images with patterns

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Content

Introduction

PatternJ was developed to help researchers extract automatically pattern features from their images obtained with fluorescence microscopy as well as electron or bright-field microscopy.

https://sites.google.com/view/patternj

PatternJ is a macro toolset for ImageJ and Fiji. The algorithms used in this macro toolset have been presented in

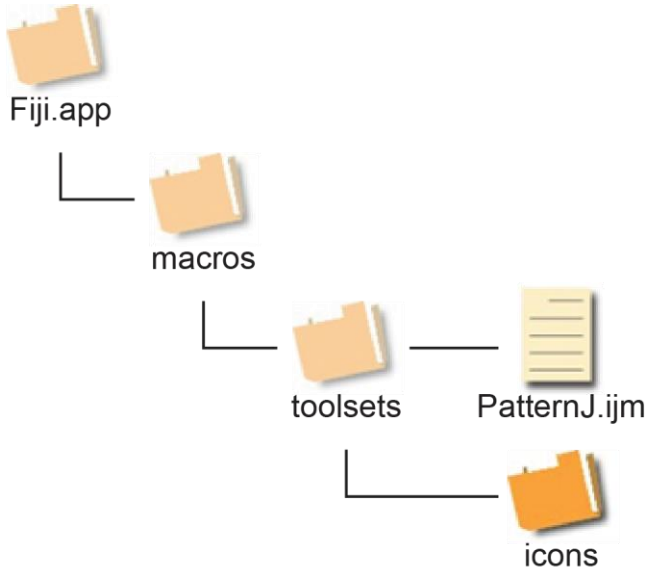
PatternJ: an ImageJ toolset for the automated and quantitative analysis of regular spatial patterns found in sarcomeres, axons, somites, and more
Mélina Baheux-Blin, Vincent Loreau, Frank Schnorrer, Pierre Mangeol. bioRxiv 2024.01.17.576053

Please cite this reference if you intend to publish data analyzed with this tool.

ImageJ can be downloaded at http://rsbweb.nih.gov/ij/
Fiji can be downloaded at http://fiji.sc/Fiji

Installation

1. Download the PatternJ macro toolset from the PatternJ website. The toolset consists of the ImageJ macro file PatternJ.ijm and a folder containing PatternJ icons in a zip file. Extract the files.
2. Move the file and folder into your ImageJ macro toolset folder:



3. For **Mac users only**, there is no title bar in file chooser dialogs since the OS El Capitan (2016). This is limiting for many tools in ImageJ/Fiji, but there is a workaround: **check "Use JFileChooser to open/save" in the *Edit › Options › Input/Output...* dialog**. This does not affect anything else and it will make the use of PatternJ (and potentially other tools) much easier.

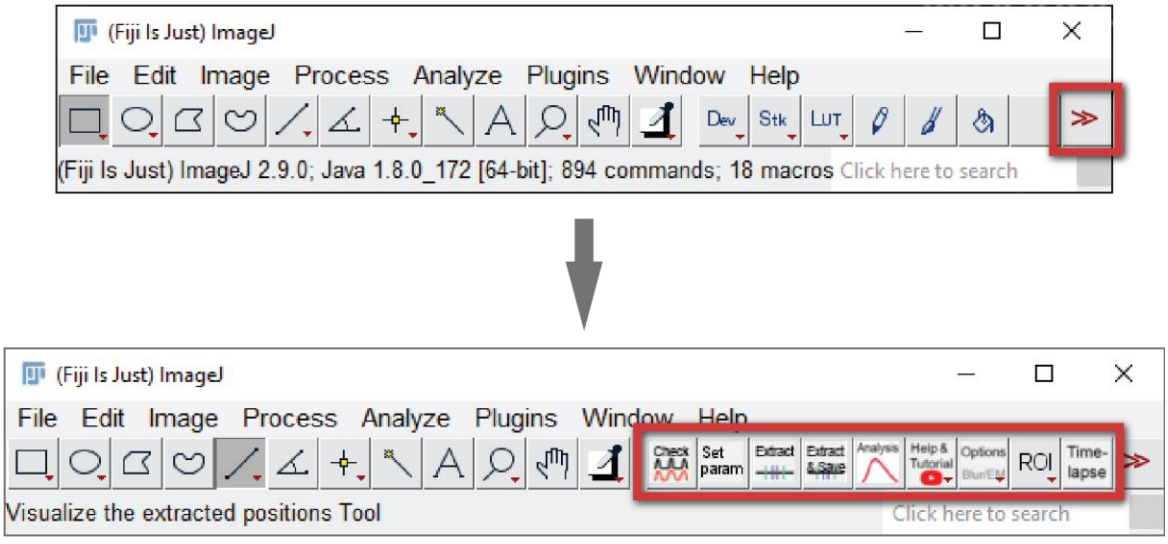
4. Start ImageJ/Fifi, the macro is ready to use.

Tutorial 1 – Extract features from a single image

This tutorial will show you how to use PatternJ in Fiji. You will need an image to follow this tutorial; you can use one of your own or use the image you will find on the PatternJ website which simulates a repeated pattern. This image is used in this tutorial.

Step 1 – Choose the PatternJ macro toolset in Fiji

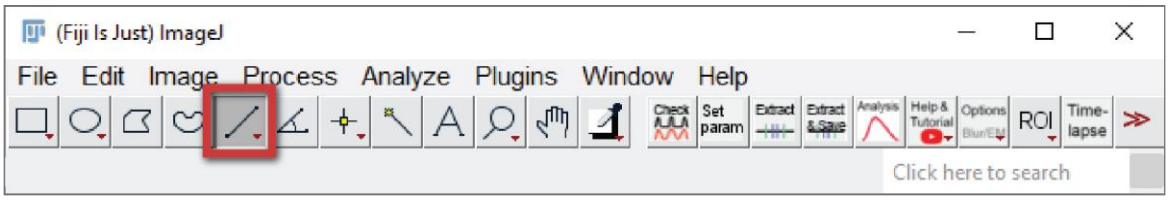
To open the PatternJ macro toolset, **click the "More tools"** menu button and **select "PatternJ 2.0"** from the drop-down menu:



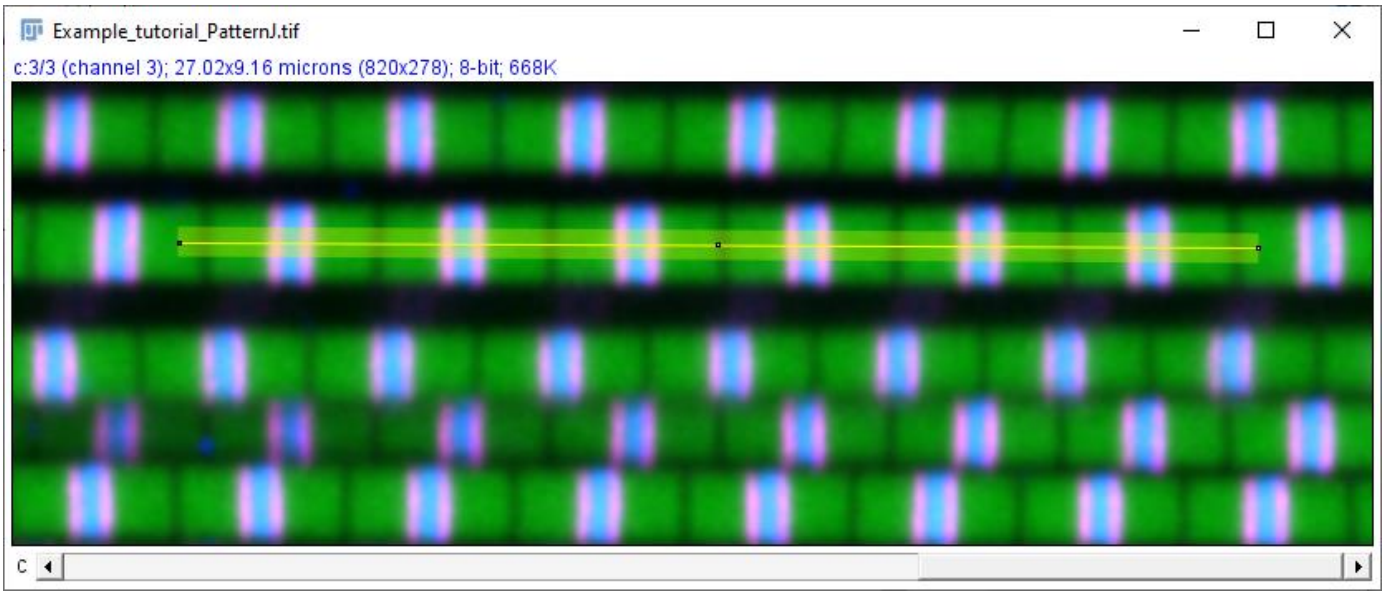
After selecting PatternJ, you will get new buttons as shown above.

Step 2 – Select a region of interest on the image

Once you have opened an image, select the line or segmented line tool from the user interface:



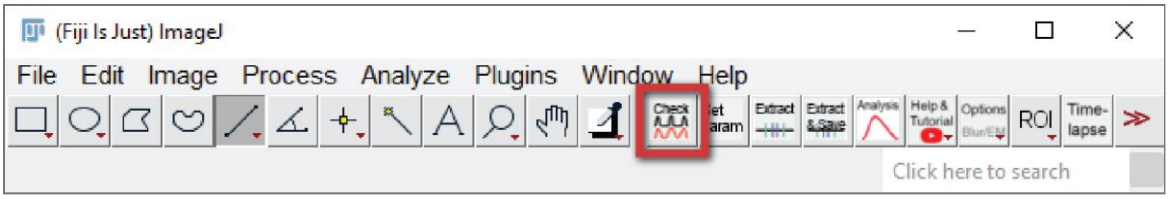
You can then draw a selection on your image as seen below. The linewidth can be modified by double-clicking the line button; in the example we provide, we used a linewidth of 18 pixels. To get the patterns on the edge extracted properly later on, you can draw the selection a bit larger than the pattern size at the edges. It is possible to use a curved selection by selecting the segmented line and clicking spline fit in the Line Width window.



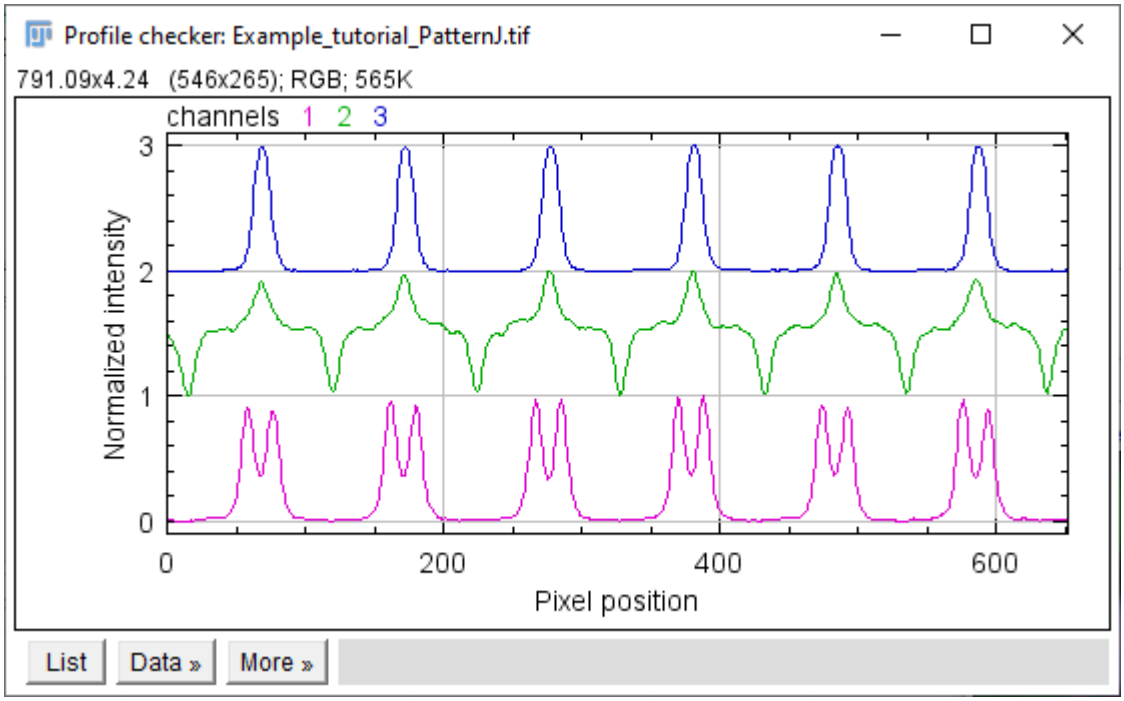
Tutorial image example: muscle from *Drosophila melanogaster*. A kind gift from Vincent Loreau, Schnorrer Lab, Marseille, France.

Step 3 – Check the intensity profiles in all channels

Once the selection is drawn, you can check the intensity profiles in all channels by clicking on “Check”:



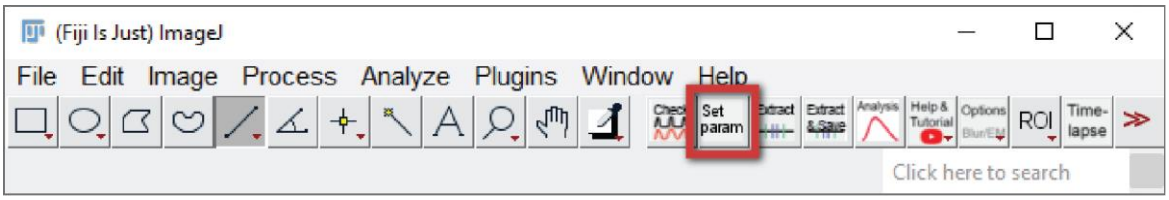
Then you should see the intensity profile in a new window:



The intensity profile of the first channel is at the bottom of the graph and the following ones are displayed above, one after the other.

Step 4 – Set the parameters of the analysis

In this example, we observe that there are two bands in channel 1, a band on a block in channel 2 (typical of actin labeling in muscle cells), and a single band on channel 3. To help the automated algorithm extract relevant features from the image, you need to define the features of each channel as seen in the profile. To achieve this, click on "Set param":



First, PatternJ will ask where the image is located to make sure everything will be saved at the right location. Then a new window will open, in which you will select the features you observe in each channel. You will be asked whether the features are on the edges of the pattern or its center. There are multiple ways to define a pattern, the only important thing here is to be consistent and select accordingly how the selection was drawn (last input at the bottom of the window). An error here only impacts how patterns are analyzed at the edges of the selection.

Here we filled up the number of bands according to what we observed for channels 1 and 3 and selected "block with middle band (sarcomeric actin)" for channel 2 (see figure below).

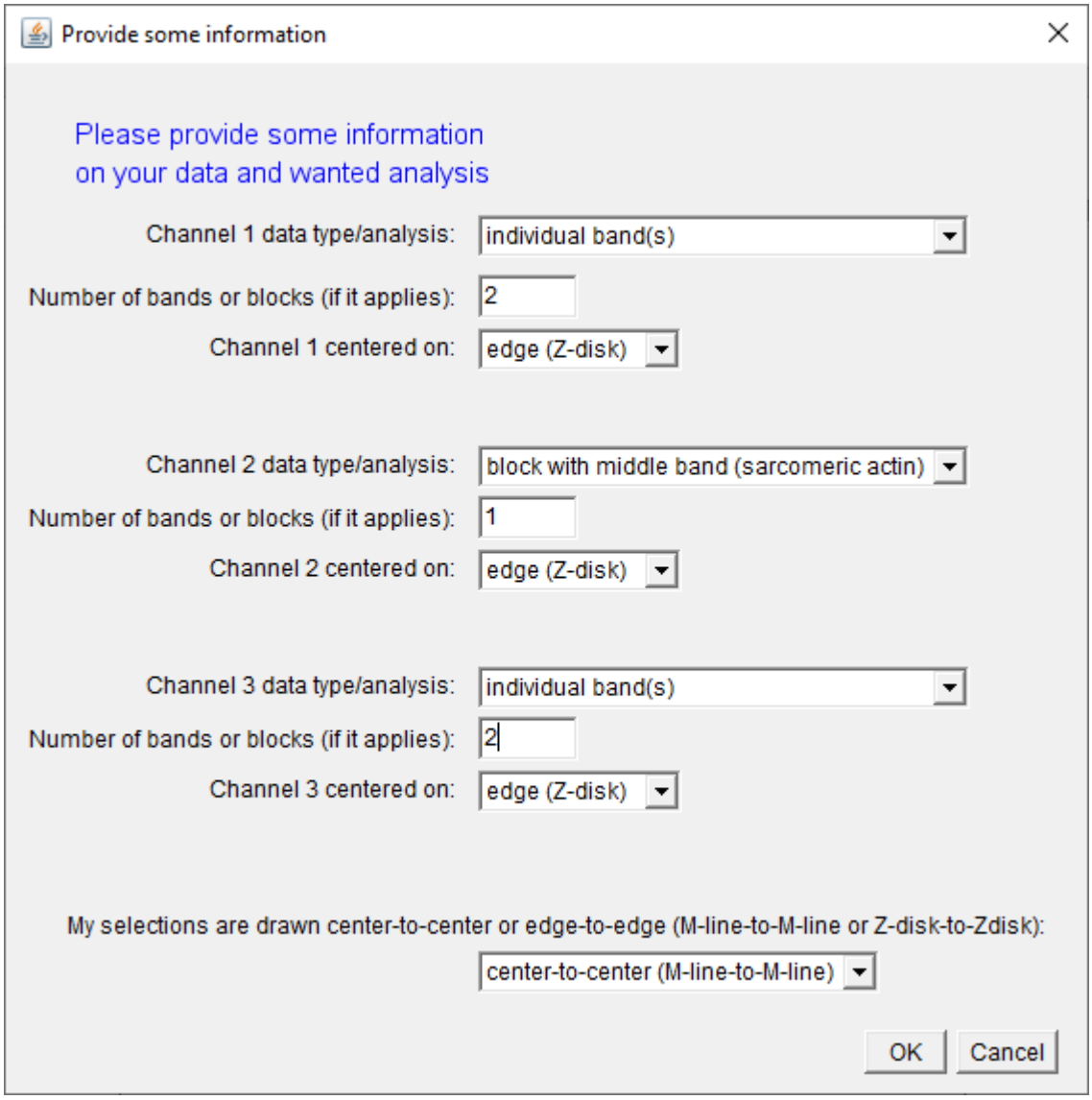
After pressing "Ok" you are ready for the first feature extraction.

In the process, a new folder named "Analyses" will be created in the folder you indicated the image is located, as well as a subfolder "internal". There will be a file with the information you provided for the different channels. Future files useful for the analysis will also be located in this internal folder. Avoid deleting its content before you are finished with the analysis.

NB: **1. This step is only required for the first selection you analyze the image. Next, selections will not require this step.**

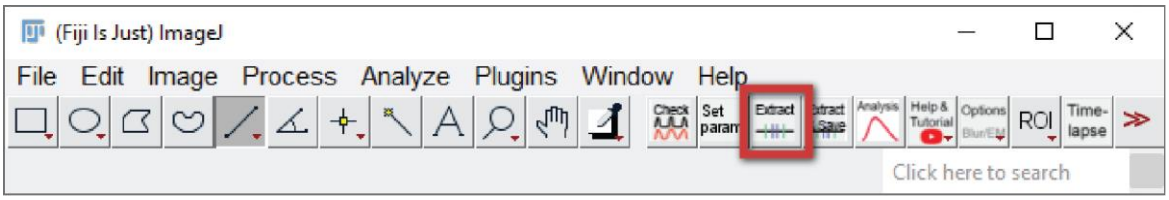
2. If you think parameters should be changed, simply click on "Set param" and modify to your liking.

3. You can define other types of features such as "block" if your pattern consists of in single large band. In case the next steps of analysis do not work for you, it might be because the features are a bit too far from what is expected. You can still use "single pattern", that should extract the rough position of each pattern. This is not as precise as the other algorithms, but this can still be useful. These other pattern types can be tested with the other example you can find on the PatternJ website.

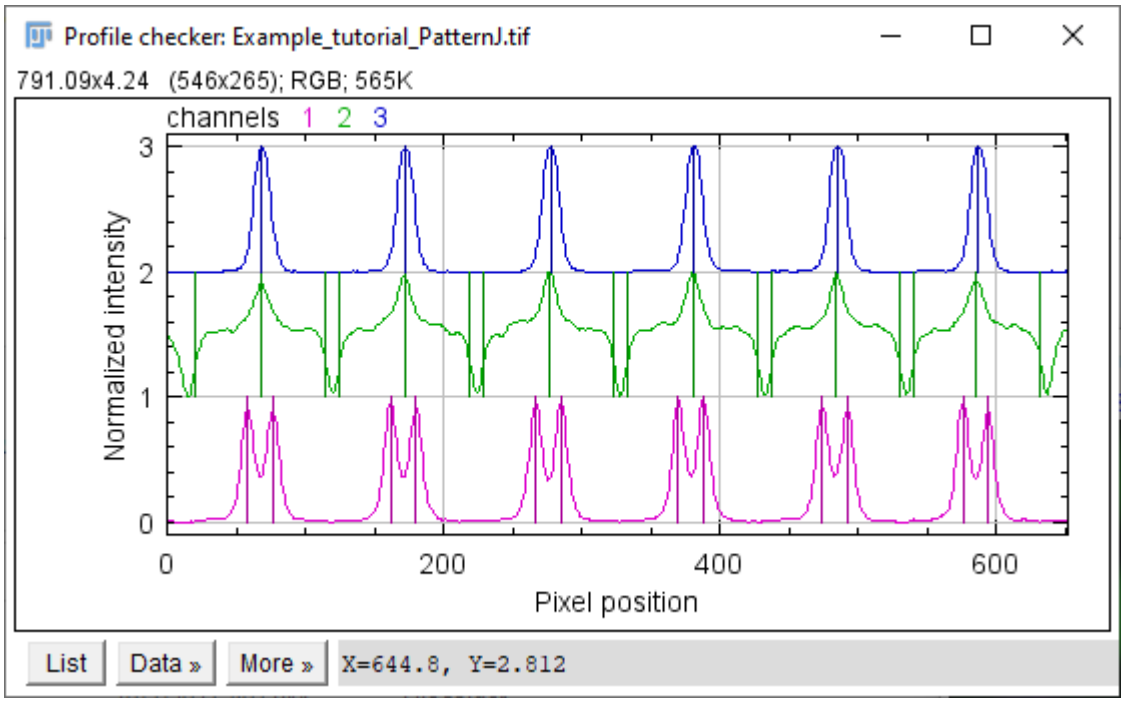


Step 5 – Extracting the features

To extract the features from the image, click on "Extract":



The window with the intensity profiles will be updated and the positions of the features extracted will depicted with a vertical line:



In this example, the center of each band was found, as well as the edges and the center of each of the actin label.

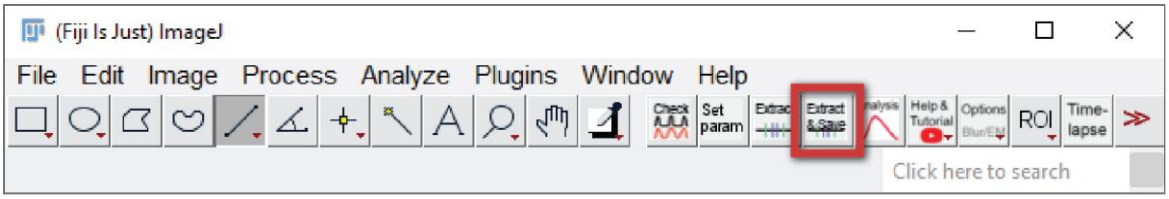
The algorithm may not perform well on your image. In our experience, it happens mostly when multiple bands need to be extracted within one pattern and when the signal-to-noise ratio is too low. To solve this, you can either click on "**Gauss blur**" (towards the right of the interface), to apply a Gaussian blur to your image, or deconvolve your image if you know how to do it. Deconvolution is better in case your features are very close, but otherwise Gaussian blurring is often sufficient. Using a thicker linewidth for the selection can also help.

If you use images from an **electron microscope**, consider clicking on "**EM prep**" (at the right of the interface), which will correct for background and invert colors to make your image look like a fluorescence image.

It is important at this stage to check that the features you expect are well-extracted. If yes, you are ready for the next step.

Step 6 – Save the extracted features

For this step, click on "Extract and Save".



Your results will be saved in the folder "internal" folder and the region of interest is automatically saved. You will

be able to retrieve the results in a user-friendly file at Step 8 of this tutorial.

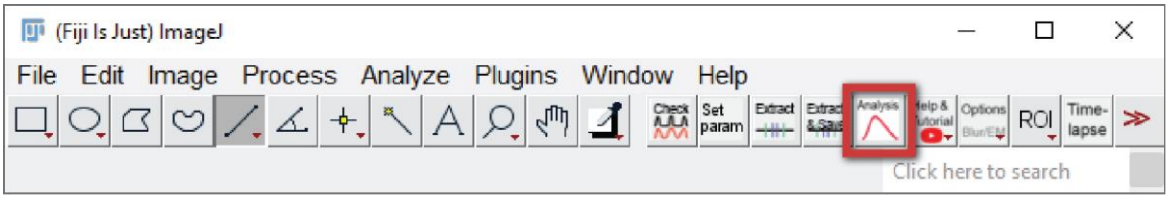
Step 7 – Continue with more selections and more images

If you want to get more data extracted on the same image, repeat steps 5 and 6 (NB: setting the parameters is only required the first time you analyze your image).

Once you have extracted and saved these, you are ready for the last step, the analysis.

Step 8 – Analyzing the extracted features and generation of an average pattern

Once you extracted features on all the selections you picked, PatternJ provides user-friendly files with all the extracted positions and a first analysis, by clicking on “Analysis”.

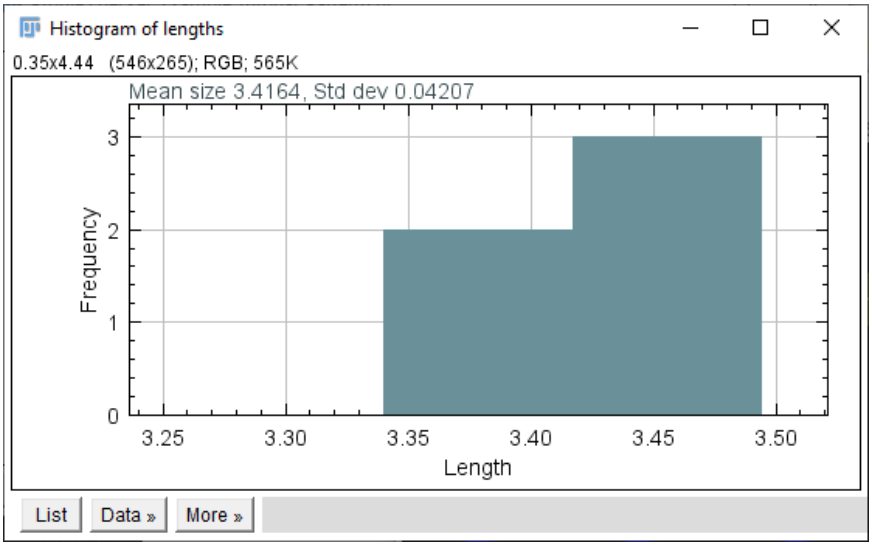


This analysis will provide:

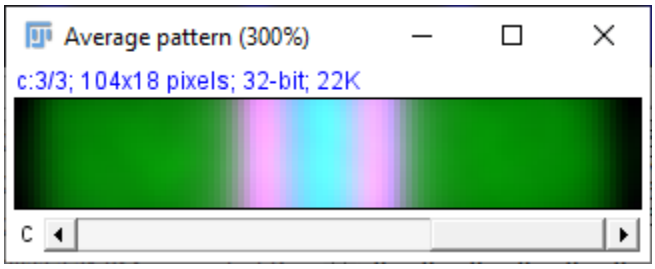
- the positions of features found for each channel (one file per channel)
- the spatial repeat length of each pattern couple (or sarcomere length in the muscle field). A file is generated and a histogram is displayed in Fiji.
- the image of the average pattern based on all patterns selected. The image is displayed, but it is not saved as default.

The spatial repeat length and the image of the average pattern require picking one channel as a reference.

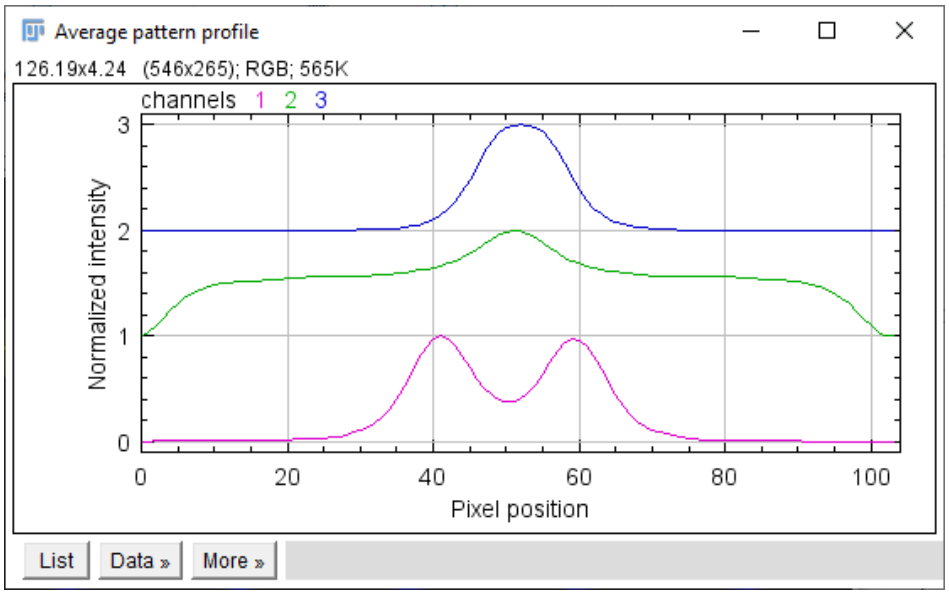
Histogram of spatial repeat length (or sarcomere length):



Average pattern:

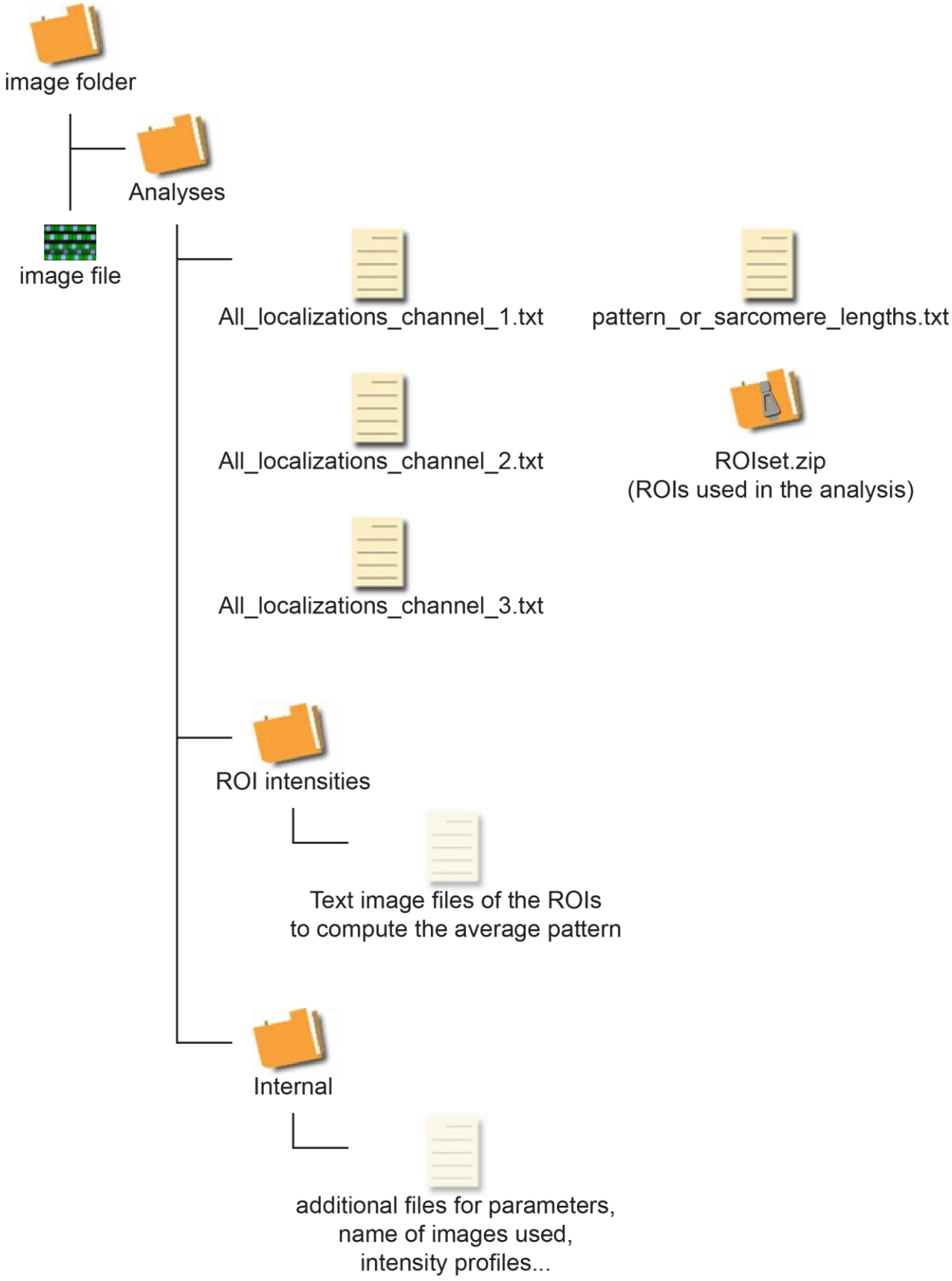


and average intensity profile:



From the file recapitulating all extracted features, one can easily obtain the distance between bands on a given pattern, the width of a block, and correlate feature characteristics from different channels.

To recapitulate the creation of new folders and files, the folder tree of your image should now be the following:



Tutorial 2 – Retrieving ROIs

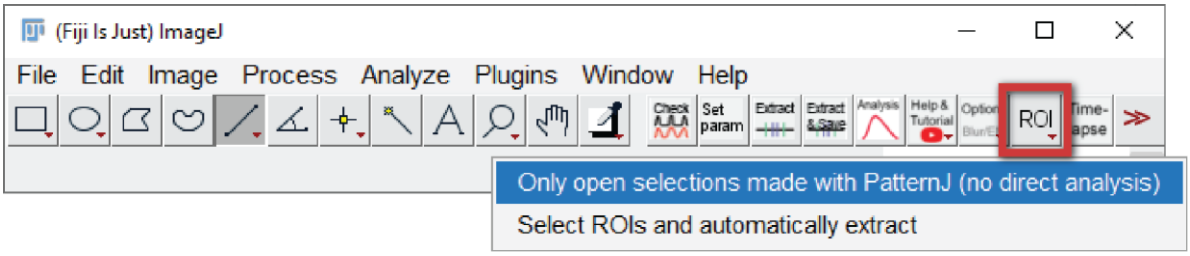
It is very useful to be able to retrieve **selections that were previously used with PatternJ** to extract features. This is very simple to do.

Step 1 – Reopen your image

If you did not open the image used previously it is time to do it. Just open it as you usually do.

Step 2 – Retrieve ROIs

Open the ROI menu and click “Only open selections made with PatternJ (no direct analysis)”.



You are first prompted to select the folder containing your image. Then, the ROI manager will open with the list of ROIs previously used on this image.

Tutorial 3 – Extract features from a list of saved ROIs

This next function is more advanced. It will allow you to extract features based on a list of ROIs. This can be achieved on ROIs previously obtained from PatternJ or another application if needed. It behaves exactly like the “Extract and save” function introduced in Tutorial 1.

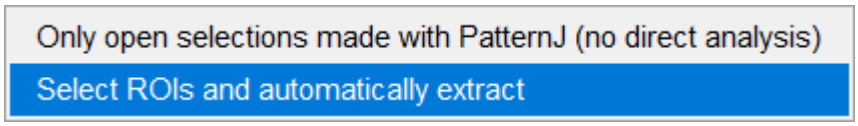
NB: if you reuse ROIs generated by PatternJ, be aware that if the “Analyses” folder is already created with saved analysis, the use of this function will duplicate the results already obtained. If you want to avoid duplication, create a new folder to be selected at step 2 of this tutorial.

Step 1 – Reopen your image

If you did not open the image used previously it is time to do it. Just open it as you usually do.

Step 2 – Retrieve ROIs

Open the ROI menu and click “Select ROIs and automatically extract”.

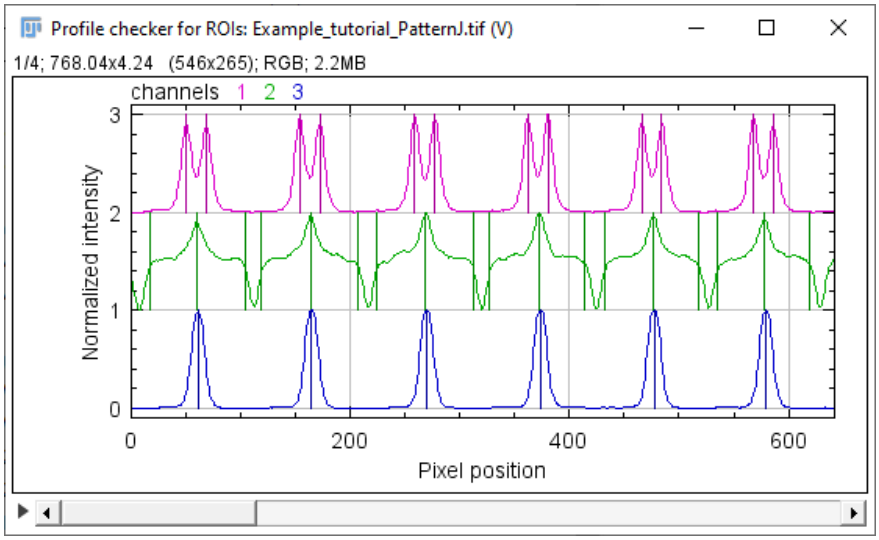


You are first prompted to select the folder containing your image (as written above, select another folder in case your image has already been analyzed). This is to make sure that the features to extract will be correct. If parameters were not set previously, you will be requested to specify them (see step 4 of Tutorial 1 if unsure). Next, you are prompted to specify which file contains your ROIs (if your ROI manager is not empty you will be

asked if it can be emptied before proceeding). If this is only one ROI, it is likely to be a file with the ".roi" extension. Otherwise, it is a zip file.

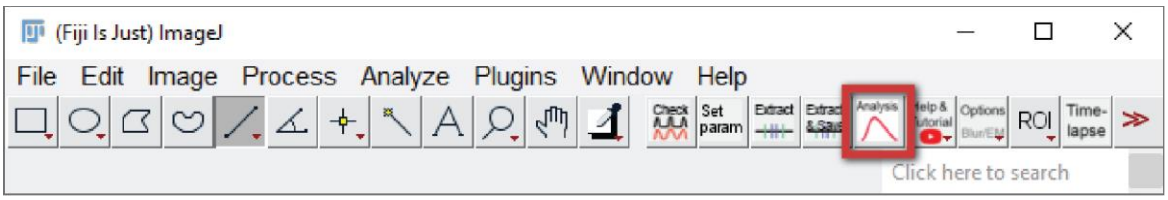
Press the "Browse" button and select your file. If your file is one that was generated with PatternJ, you can find it in the "Analyses" folder of your image, under the name "ROIset.zip". After selecting your file, press "OK".

Similar to the "Extract and save" function, the results of the analysis are displayed in a stack of graphs. The results of the first selection are in the first graph and so on until the last selection:



Step 3 – Analyze ROIs

In this last step, you can display all results in a series of user-friendly files, just like in Step 8 of Tutorial 1. Just click on "Analysis".



By clicking on this button, you will obtain:

- the positions of features found for each channel (one file per channel)
- the spatial repeat length of each pattern couple (or sarcomere length in the muscle field). A file is generated and a histogram is displayed in Fiji.
- the image of the average pattern based on all patterns selected. The image is displayed, but it is not saved as default.

For more details, please refer to the Step 8 of Tutorial 1.

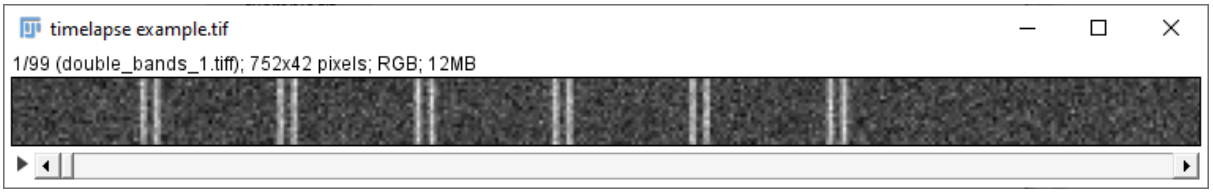
Tutorial 4 – Working with time-lapses

PatternJ can allow you to analyze time-lapse series. It can extract positions just like in the previous tutorials, but with additional features: you will not have to draw selections on each image and the analysis is part of the Time-lapse tool, so everything is done in a single step. In this case, we did not implement the average pattern image as it is less meaningful in this situation.

Make sure that your Image/Fiji version is 1.54i or later. It is likely to work poorly on earlier versions.

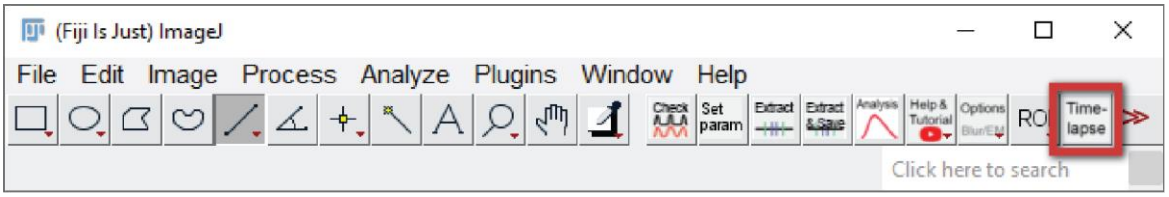
Step 1 – Open an image series

Open an image series containing a time-lapse. Such a series can be a multichannel image. It will need to have multiple frames or multiple slices. You can use our example, provided on the PatternJ website:

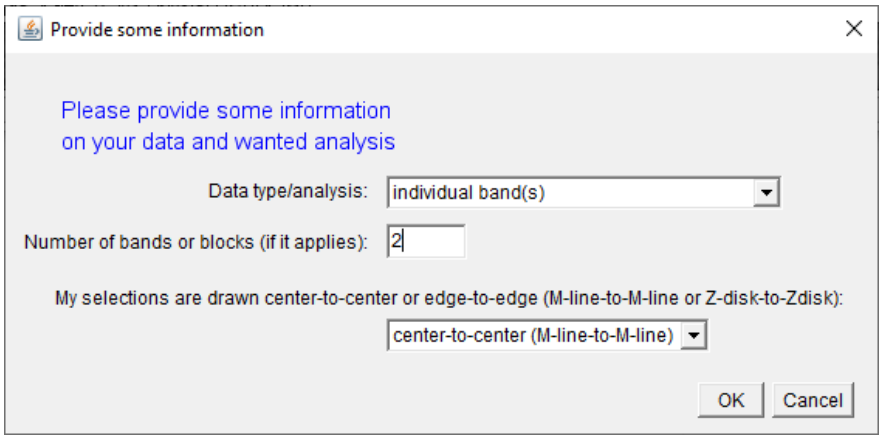


Step 2 – The "Time-lapse" function

Press the "Time-lapse" function button:

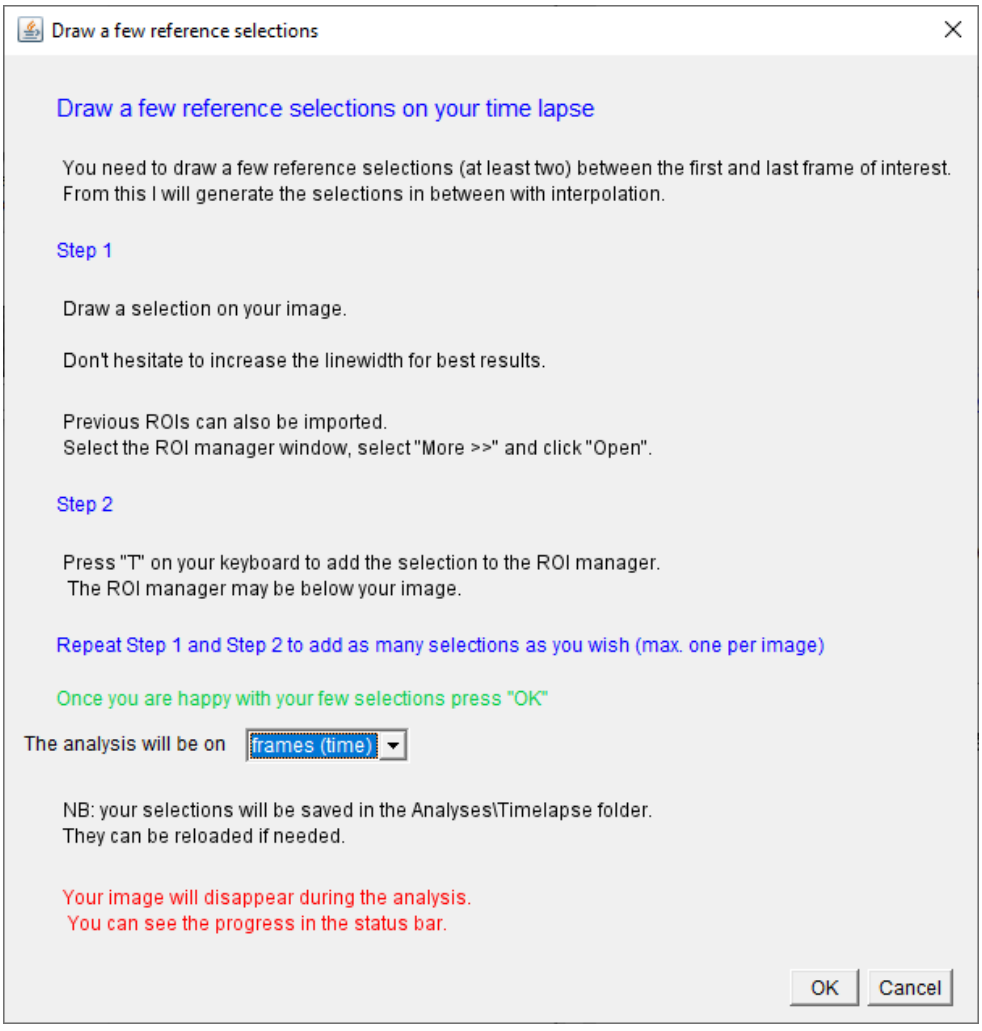


You are first prompted to select the folder containing your image (to make sure analysis will end up at the right place) and then you will need to specify the features in your image. If unsure, check the Step 4 of Tutorial 1. For the tutorial image series, you can set the parameters as follows:



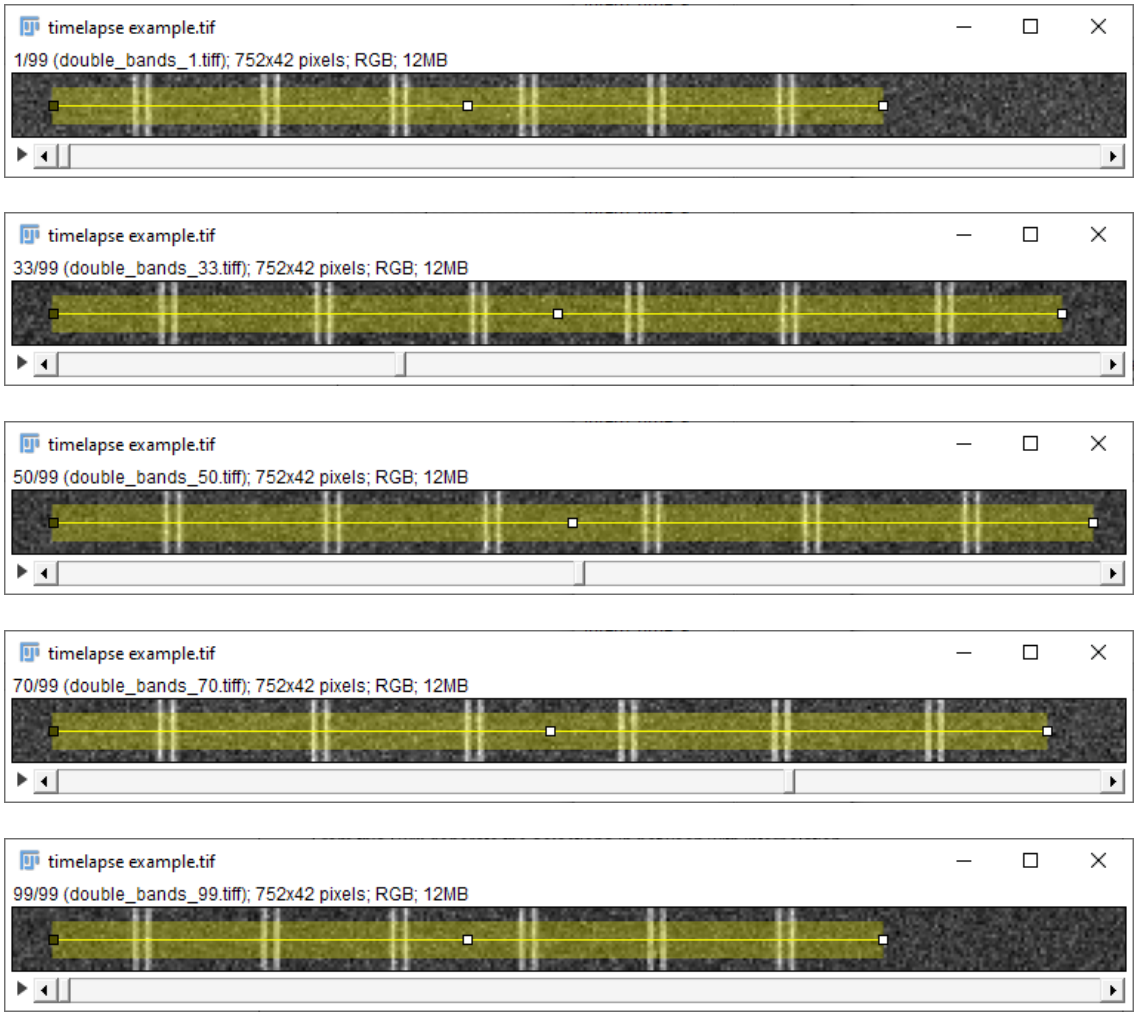
Note that if your ROI manager is not empty you will be asked if it can be emptied before proceeding.

Then a window guides you through the steps to make selections:



Follow the simple steps described in the window. In the example provided, you can draw selections on the first image, and then at images ~30, 50, 70, and 99. After each selection press “T” on your keyboard, as mentioned in the window above. Note that you can also select a list of ROIs previously saved using the ROI manager. If you already followed this tutorial, a file with ROIs is saved in the “Analyses\Timelapse” folder.

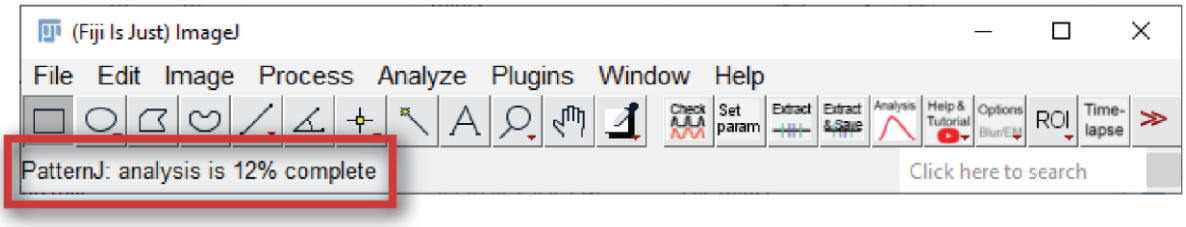
Selections drawn:



In case your series is multidimensional, leave the selection at “frames (time)” if you are interested in time analysis. Otherwise, select “slices (z)”.

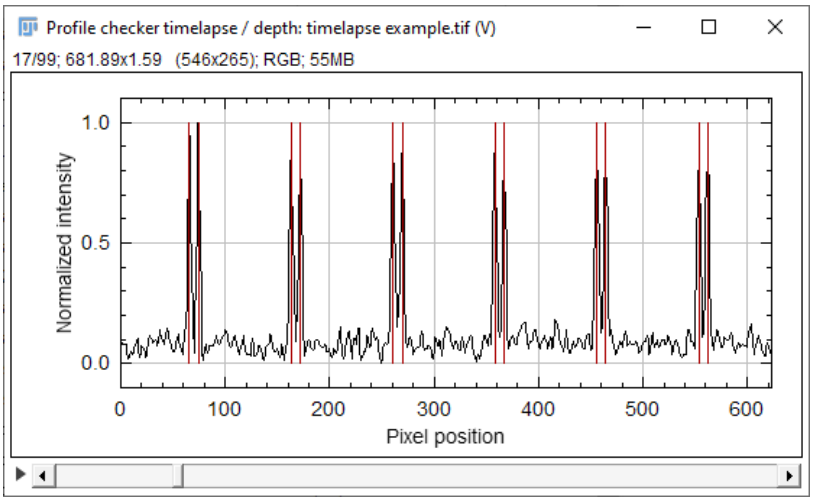
Then press “OK”.

After pressing OK, your image will disappear during the time of the analysis. You can follow the progress of the analysis in the status bar:

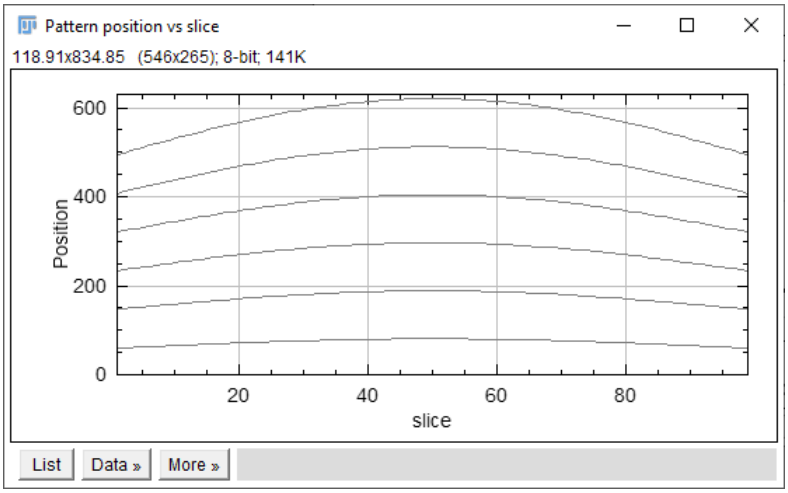


Once the analysis is completed, your image will reappear, together with:

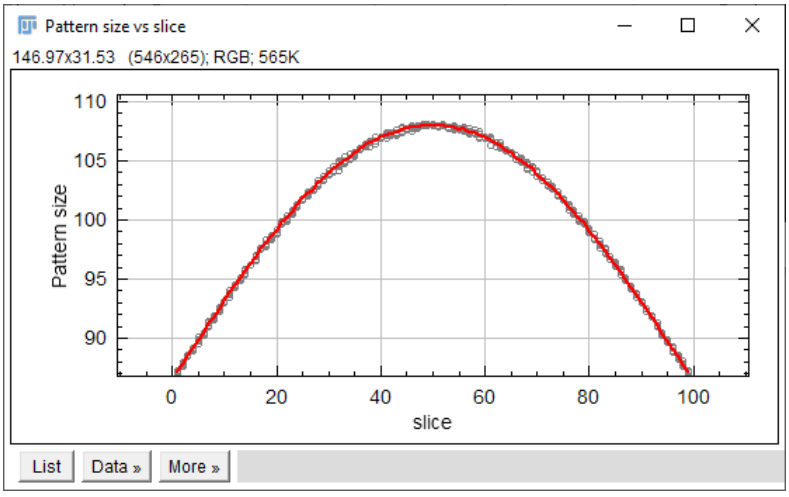
- a stack of profiles with extracted positions:



- the tracked positions for all patterns (for simplification purposes, only one position per pattern is given). Only the channel chosen as a reference is tracked (here with only one channel, there is no choice):



- the average pattern versus time (or z if selected) in red, single pattern as grey dots:



As an output, PatternJ provides user-friendly files with all the extracted positions, ROIs drawn by the user, and interpolated ROIs (ROIs on images the user did not give input):