BacFormatics v1.x

MATLAB open-code platform to analyze bacterial biofilms, and more

User-Guide

A straightforward user-guide for the unfamiliar user ☺

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NOTES-

Programmer is Dr. Raz Shimoni
Direct supervision: Prof. Cynthia Whitchurch
Financial support and funding: Prof. Cynthia Whitchurch, Prof. Liz Harry

•If other programmer modify or add new capabilities to BacFormatics, only the new prospective code should credited to the corresponding programmer. To sharp the points above, if a developer made X contribution to BacFormatics (where X stands for a specific function, modification, new code, new module, new tool, etc), a statement such as the following shall be provided:
"Using BacFormatics as a platform, I programmed/wrote/coded/etc X in order to….."
Thus, the credit shall be towards the X contribution but not towards BacFormatics.

• Support and feedbacks see home page: http://BacFormatics.com/

• Special thanks to the contributors of open-code functions used in BacFormatics and biologists who provided data input. To see a list of all current contributors **Go to: >Acknowledgments** at the Main Manu.

• Since BacFormatics uses third party open-code library (i.e. tracking algorithms, filters, GUI tools, etc), MATLAB developers who wish to modify BacFormatics to create a new program should be aware of and acknowledge the relevant contribution.

• BacFormatics use requires a MATLAB license.

• BacFormatics users are not expected to share co-authorship, however when BacFormatics is used for publications, please refer to BacFormatics publications in xxx**.** New components customized by the requirements can be easily set up (not necessarily in exchange for co-authorship).

• BacFormatics version 1.0 is based on the open-code of TACTICS Toolbox Version 3.0.

•This user guide was written by Raz Shimoni and contain some text from TACTICS Toolbox. Since Raz Shimoni is the sole programmer of the two programmers, there are no copyrights issues.

Before you start: input data for BacFormatics

• File format – The files accessible by BacFormatics are tif (.tiff and .TIF are also common tags for TIFF files), high-quality graphics files that contain the raw data in a lossless compression format. In each subfolder the microscopy images must be named in the format:
Data Management Code

Experiments that generate data files on another device such as the Olympus microscopes computer, should bear a file name with the same basic code structure as the experiments write up in the book. When naming and saving files, they should be named using the same code for the experiment in your lab book as well as the date the image/file was generated on:

YYMMDD_[Your Initials]YY_001_[NAME]_*

As an example, if Bruce Wayne was using the Olympus microscope as his first experiment on the 13th of April 2013 and was observing a growing biofilm over time he should name his images like this:

130420_BW13_001_BIOF_t001_ch00
[Date]_[Initials][Year]_[Experiment Number]_[Short Title]_[Image Number] _[channel]

And subsequent images:

130420_BW13_001_BIOF_t002_ch00
130420_BW13_001_BIOF_t003_ch00

And so on, noting that depending on the system used, some programs may automatically increment the number as further images are taken. This keeps track of the date the data was produced and the BW13_001_BIOF code allows you or anyone else to easily correlate the data you generate to the experiment in your lab book the data relates to. For example if someone looks at your microscope images labelled 130420_BW13_001_BIOF_1 to 9 and finds an issue or observation worth investigating, it becomes easy to cross reference your data with the experiment you performed in your lab book, which would have the title BW13_001_BIOF.

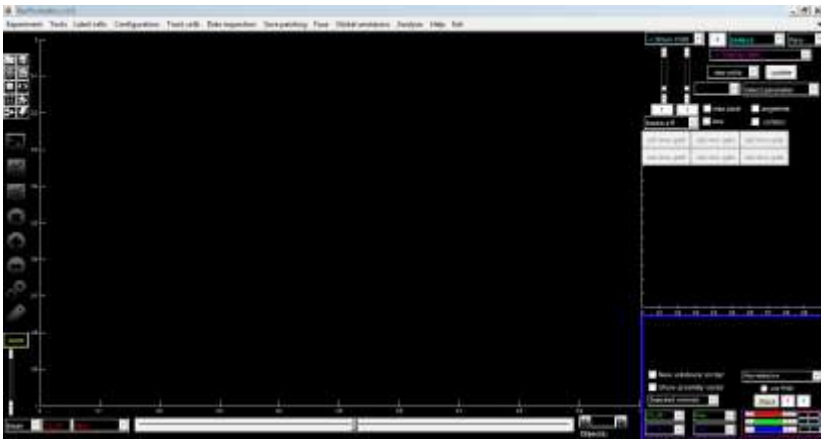
If a microscope cant directly save the files in this format use renaming tool such as included in BacFormatics.
To process multiple positions from several experiments create subfolders:
[Date]_[Initials][Year]_[Experiment Number]_[Short Title]_[\colony number][\pos number]
For example:

C:\130420_BW13_001_BIOF\colony_01\pos_01\
Contains the files:
130420_BW13_001_BIOF_t001_ch00
130420_BW13_001_BIOF_t002_ch00
130420_BW13_001_BIOF_t003_ch00

…and so on

Installation and implementation

1. Download the BAC.zip,
2. Open the BAC.zip file and create new directory (does not have to be placed on a specific location).
3. Run MATLAB.
4. **To run BacFormatics** change the current MATLAB directory to where BacFormatics is located, Input 'run_BAC' in the command window of MATLAB, and press ***Enter*** to execute. BacFormatics main interface will appear:



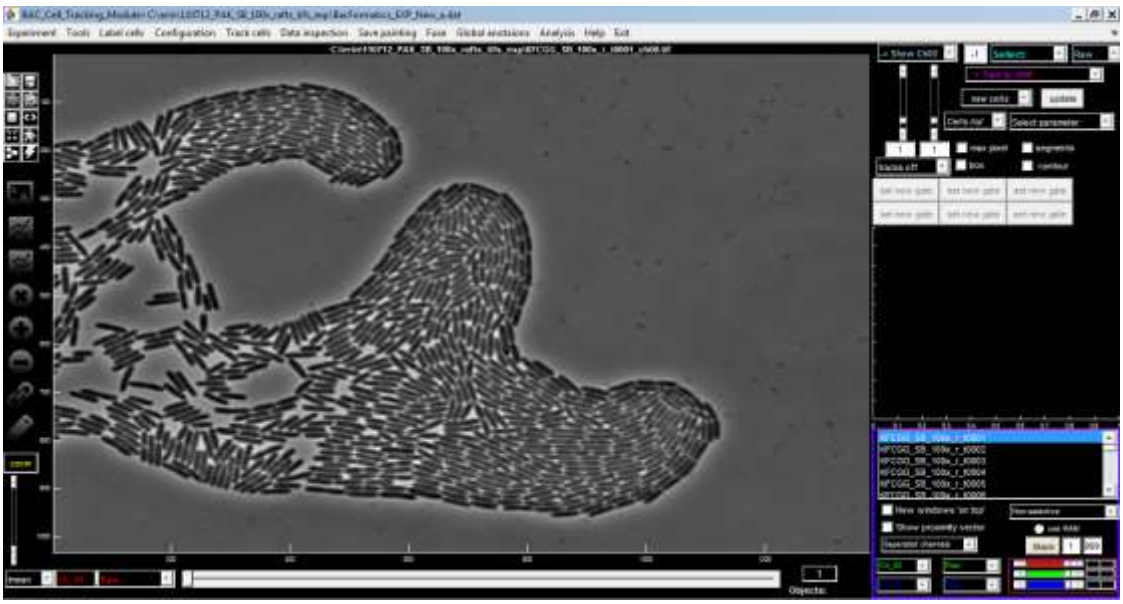
>1minute

Many users will intuitively think to load the movie (the '.tif' images files), but the approach BacFormatics is slightly different and is based on loading experiment file.

Follow the steps in page 3 (the next page) to create new experiment file

~1minute

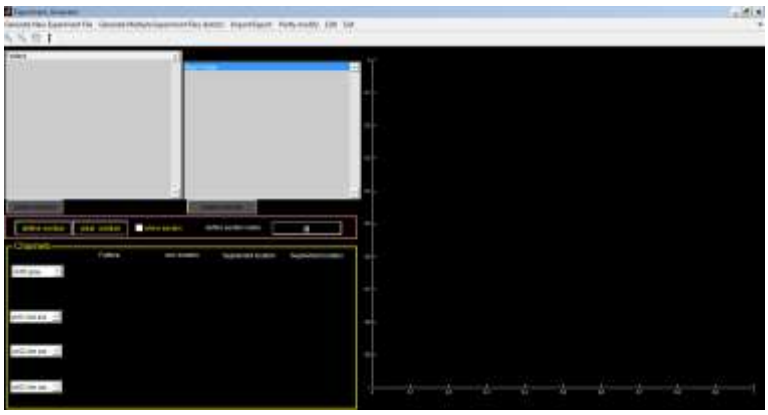
To load experiment file **Go to: >Experiment >>Load File**



The analysis pipeline is explained page 4

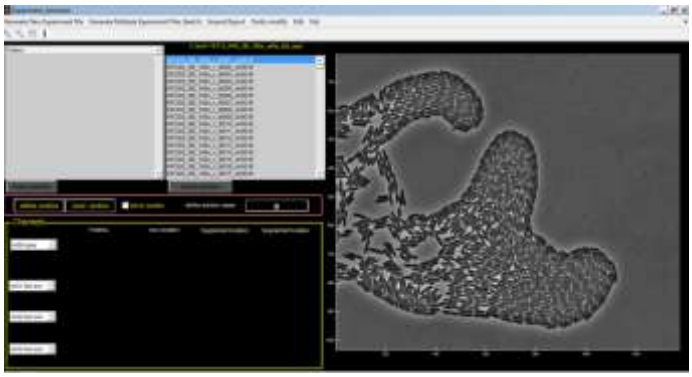
Starting a new experiment

Instructions:
To run the Experiment generator. : **Go to>Experiment>> New Experiment**

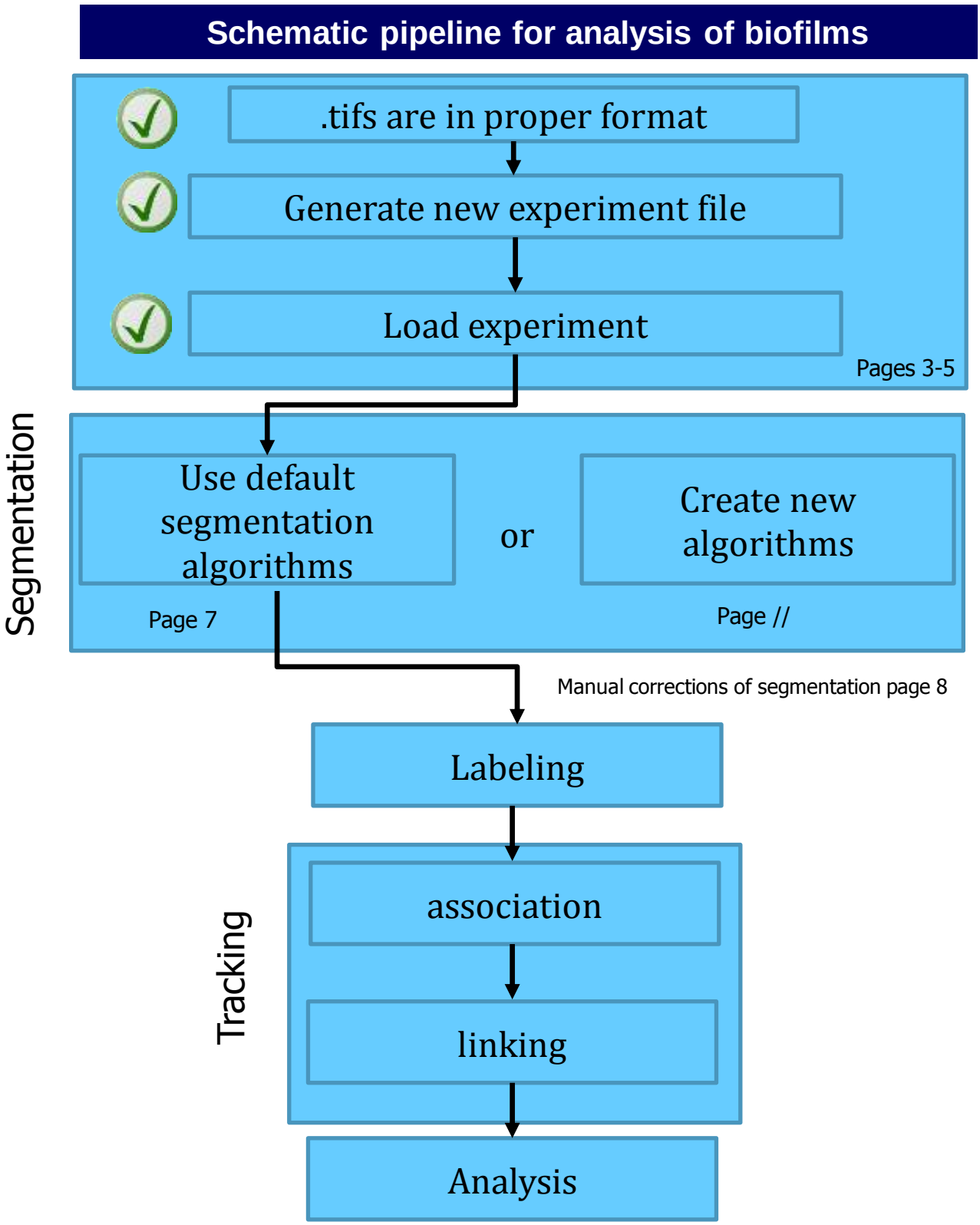


For first time users it might look complicated, but most of the options are for advance use. There are only two most basics steps that are required. Follow the next steps:

1. Load a sequential series of 2-D images by:
Go to: >Generate New Experiment File>> >>Load .tifs>>>Select By Folder
Wait a few seconds. The tifs will be loaded into the listbox:

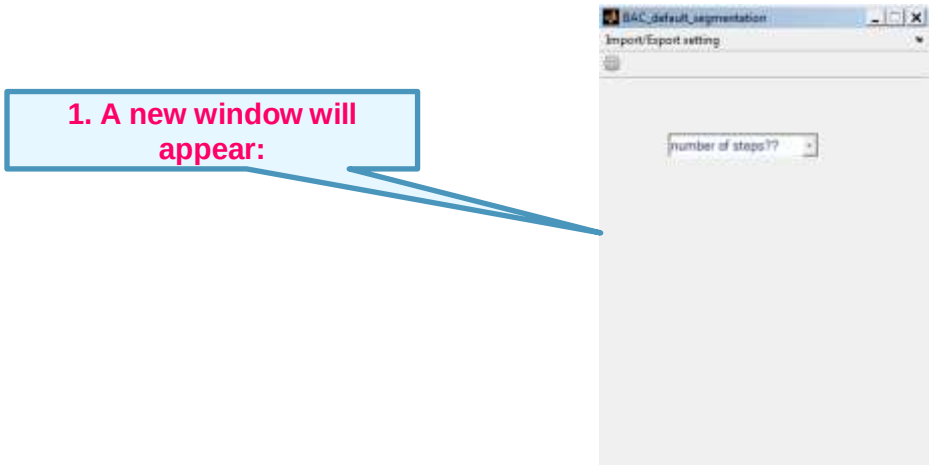


2. **Go to: >Generate New Experiment File>> >>Save New Experiment File**
Wait a few seconds. A new experiment data will be created in the format 'BacFormatics_EXP_##.dat', whereas ## is the tag name. In addition, new subfolders for each corresponding channel for the filtered and segmented images will be created. For example:


Schematic pipeline for analysis of biofilms
.tifs are in proper format
Generate new experiment file
Load experiment
Pages 3-5
Segmentation
Use default segmentation algorithms
Page 7
or
Create new algorithms
Page //
Manual corrections of segmentation page 8
Labeling
Tracking
association
linking
Analysis

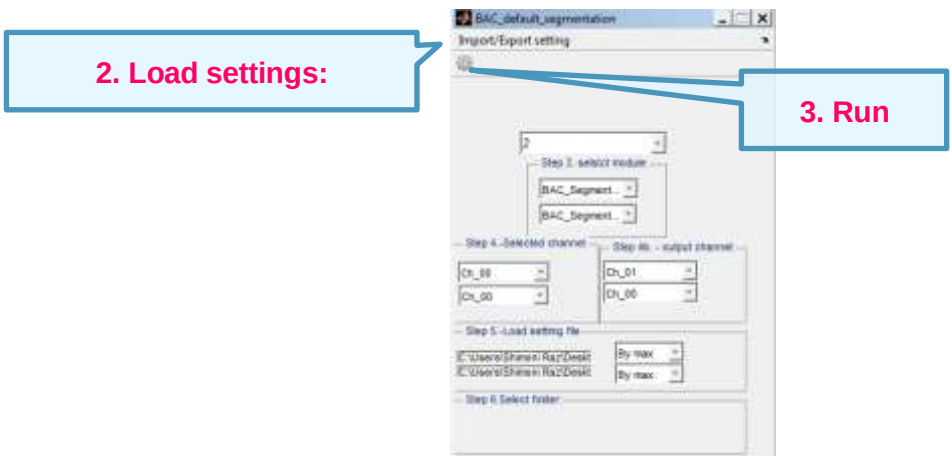
Use default segmentation algorithms

From BacFormatics menu, go to >Segment>>run segmentation

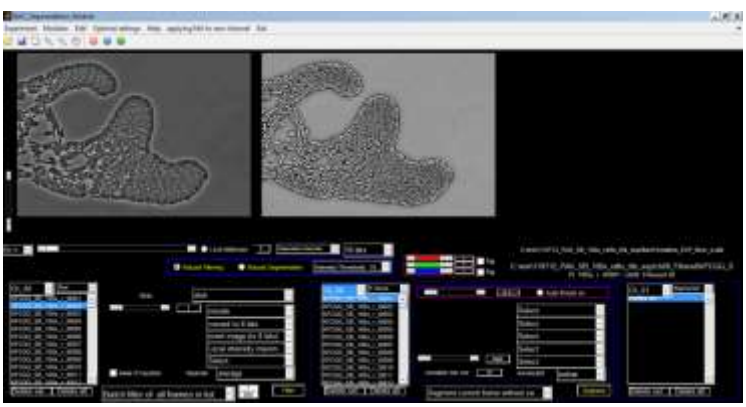


From the BAC_default_segmentation menu, go to >Import/Export setting>>Load Settings

Select BAC_Robust_Module_SETTINGS_Pseudomonas_aeruginosa.dat
The file is located in ../BAC/Segmentation setup files folder



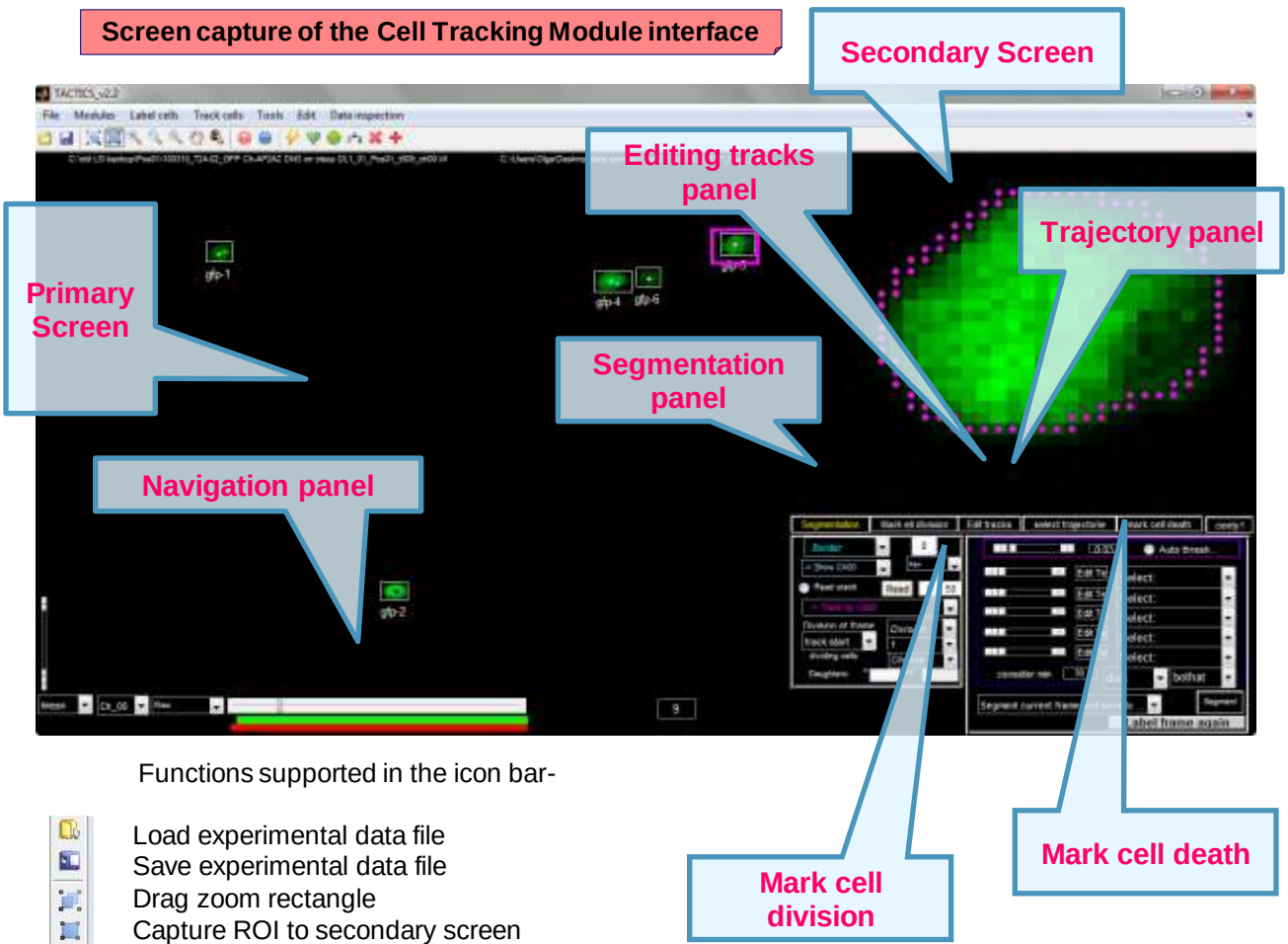
The Segmentation Module will start to segment all frames automatically. This can take few hours. Strong computer is recommended, and it is required to leave the computer until the segmentation is completed.



Manual corrections of segmentation

Once the images were successfully segmented, the next step in the processing scheme, is the cell tracking and manual correction interface:
From the main menu, go to >Cell Tracking Module
The Cell Tracking Module is used to correct segmentation, label cells and tracking. The GUI has several selection methods for manual image segmentation that allow the user to inspect and correct cell segmentation manually, and allows for multiple objects tracking correction.

Screen capture of the Cell Tracking Module interface



Functions supported in the icon bar-

Load experimental data file
Save experimental data file
Drag zoom rectangle
Capture ROI to secondary screen
Zoom in
Zoom 100%
Zoom out
Pan
Error Zoom (temporary solution for Zoom issues)
Open new MATLAB figure
Run the Linker
Split cells after divisions
Save manual correction of cell associations
Show lineage tree
Mark cell division
Mark cell death

Cell Tracking Module – 1 (user interface)

Screen capture of the Cell Tracking Module interface

Change the channel and the display mode (raw, filtered etc.) of the Primary screen using this pull down menu

Channel and display mode of the secondary screen

Read and upload images within selected range to RAM (dependent on the computer memory)

Keyboard shortcuts:

Uparrow - Next frame
Downarrow - Previous frame
Space - label frame again
Enter - associate frame overpassing selective operator
F - Format painting
S - Segmentation mode
M - Mark division mode
E - Next frame
T - Trajectory mode
A - Association mode

D - Dead cell mode
E - Next frame
R - Remove segment mode
U - uncheck/check
P - Paint Tool mode

Segmentation

The Segmentation Module is used for image processing-based filtering functions and segmentation. The GUI is comprised of three listboxes, which contain the raw, filtered, and segmented images.

From BacFormatics menu, go to >Tools>>Segmentation Module

To load and segment a movie:

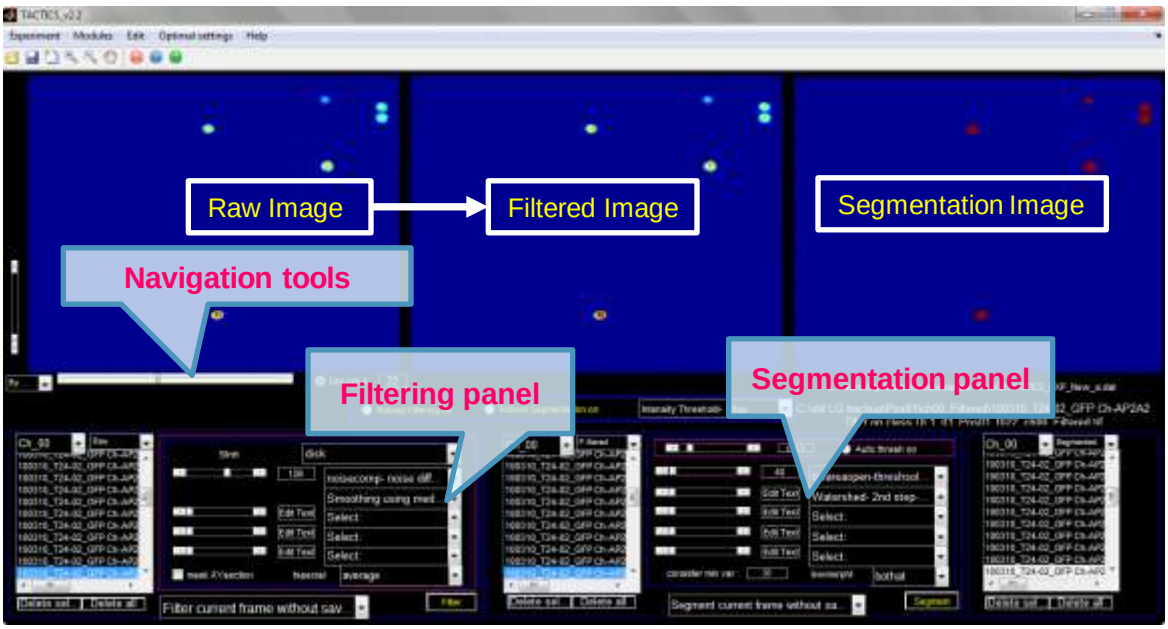
1.Load an experiment data file to BacFormatics by clicking on the open file icon . Navigate through to your experimental folder. This folder will contain a series of .tif files and other files and folders. Within the folder choose the experiment .dat file.

2.The left listbox will show all the raw .tif file images that are associated with the experimental data file. The user can navigate through the images using functional options that define the channel and section, and changing the current frame using an interactive scrollbar. Both filtering and segmentation can be performed for each z section or for the mean projected image.

3.The images must be filtered to remove noise prior to the cell segmentation. The left scrollbar panel allows the user to set up a combination of MATLAB built-in functions or customized functions using the interactive scrollbar, which maximizes the efficiency of manual customization of specific settings for each experiment. This step can be applied for selected frames without saving, with saving, or in batch mode for selected frames. An image of the filtered file will appear.

4.All saved frames are added into the middle listbox, dedicated to the filtered images. The next step is to segment the cells to convert to binary images. The default for segmentation is threshold-based fluorescence intensity. The user can define the threshold value manually or to apply automated global image threshold using Otsu's method (Otsu, N., "A Threshold Selection Method from Gray-Level Histograms," IEEE Transactions on Systems, Man, and Cybernetics, Vol. 9, No. 1, 1979, pp. 62-66.). Briefly, Otsu`s method gives the threshold value that gives minimum interclass variance of the cells and the background. Operations on the binary image such as dilation, erosion, and removal of segments by size can be applied to remove false objects. MATLAB users can easily adopt other methods.

Screen capture of the Segmentation Module interface



Segmentation Module - 2

Channel selection (also available in the Cell Tracking Module)
You can view the cells in different channels (representing different florescent wavelengths or transmitted light images) and also choose whether to view the data in raw, filtered, segmented, F&S (filtered AND segmented for background elimination), and R&S (raw images AND segmented for background elimination).

Instructions for changing channels:

1.To choose how you would like to view the data in both the Primary screen and Secondary Screen, you will use the pull down menu.

2.To change the channels, each screen has its own (linked) pull down menu.

•Virtual channels
The user can create a virtual channel (for instance, by selecting a new destination channel. The current number of supported channels is 14, but can be extended.
•Save/Load optimal settings
The settings for each frame will be saved to the RAM memory of the computer. After processing the raw .tif, the logged information about the image processing procedure must be saved as a new/overwritten experiment file. Otherwise, this information will be lost and the operation will have to be repeated again. In addition, once a suitable setting is defined, the user can load and save optimal settings in log file that contain information about the settings. **Go to >Optimal setting>>Save optimal setting** .The log file format is BacFormatics_SEG_###.dat, where ### is any name chosen by the user.
To retrieve settings for specific processed frame, select a frame using the raw listbox (the left hand listbox), then **Go to >Optimal setting>>Setting for selected file**

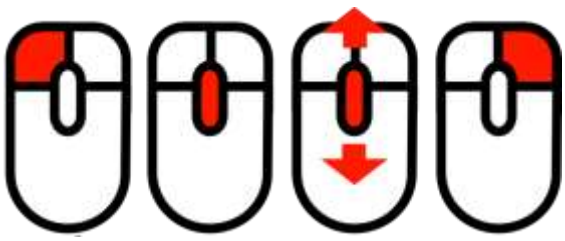
•Supported in the icon bar-

Load experimental data file.
Save experimental data file.
Create new experimental data file (run the Experiment Generator GUI).
Zoom in.
Zoom out.
Pan.

Open new MATLAB figure showing the data in the left axes. The data is stored in the 'userdata' property of the figure.

Controls

Channel selection (also avail



See mouse order.pptx

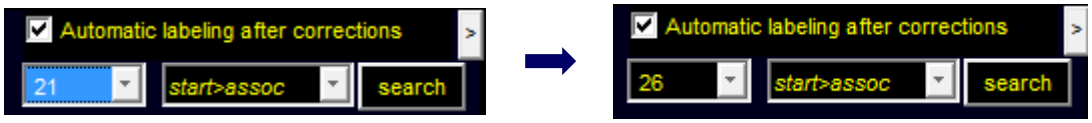
Segmentation Module - 2

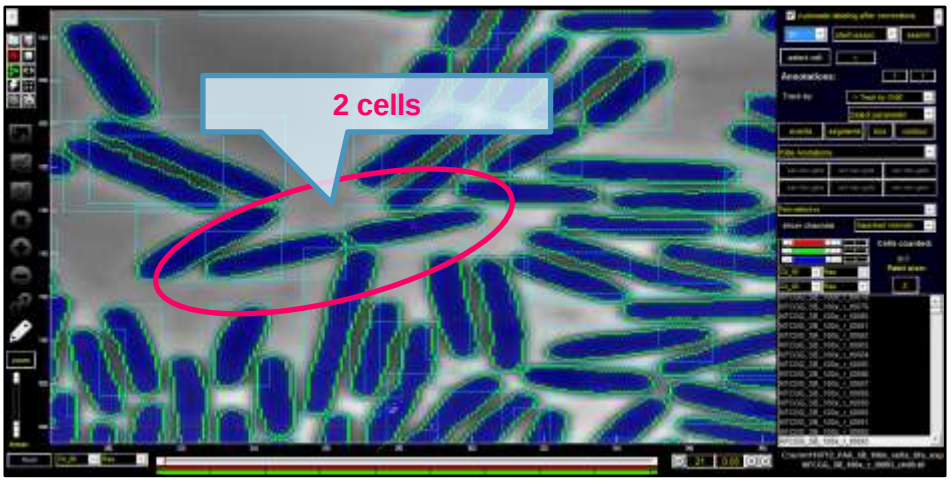
Automatic labeling after connections see examples below

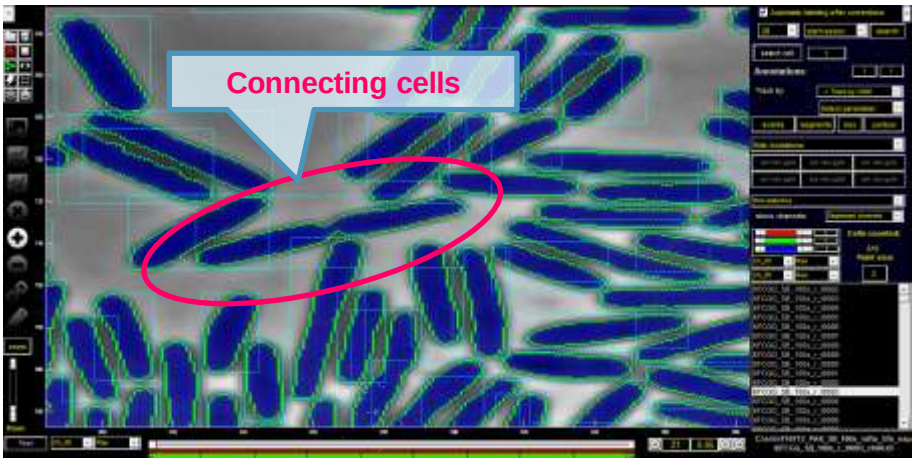
Obvious corrections:
Without updating list of errors
Fast but can be hard to find errors

Searching corrections:
Using updating list of errors
Easy to find errors but slower

Enable **Automatic labeling after connections**







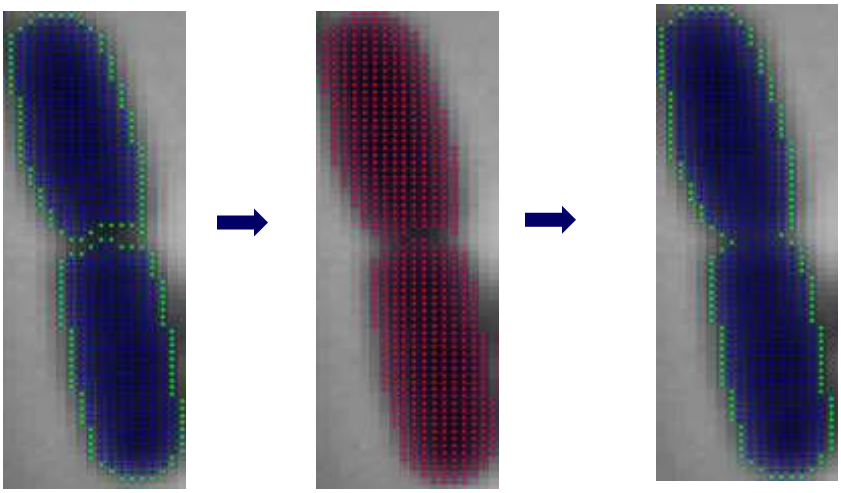
Manually fixed the segmentation

Segmentation Module - 2

Channel selection (also available in the Cell Tracking Module)
You can view the cells in different channels (representing different florescent wavelengths or transmitted light images) and also choose whether to view the data in raw, filtered, segmented, F&S (filtered AND segmented for background elimination), and R&S (raw images AND segmented for background elimination).

Instructions for changing channels:

1. define spot size as distance between selected objects that can be connected:



2. Right mouse click to red-select

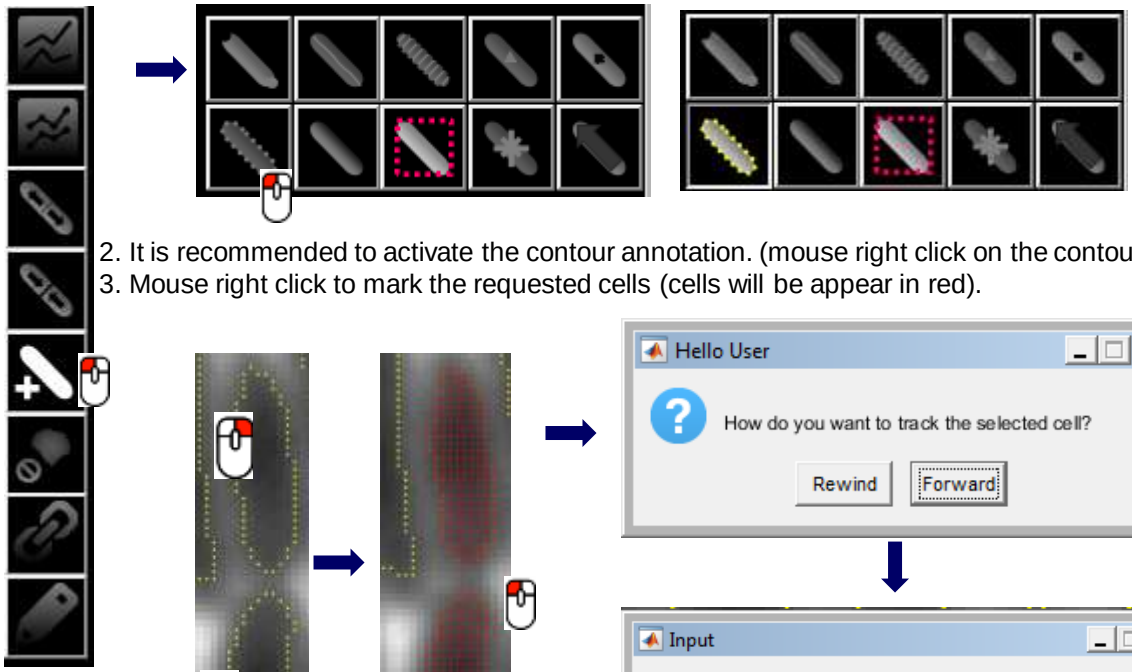
3. Left mouse click To connect

Tracking Module - 2

Add Cells mode:
You can select a particular sequence of a cell splitting or merging and automatically apply or correcting split/merge operation.

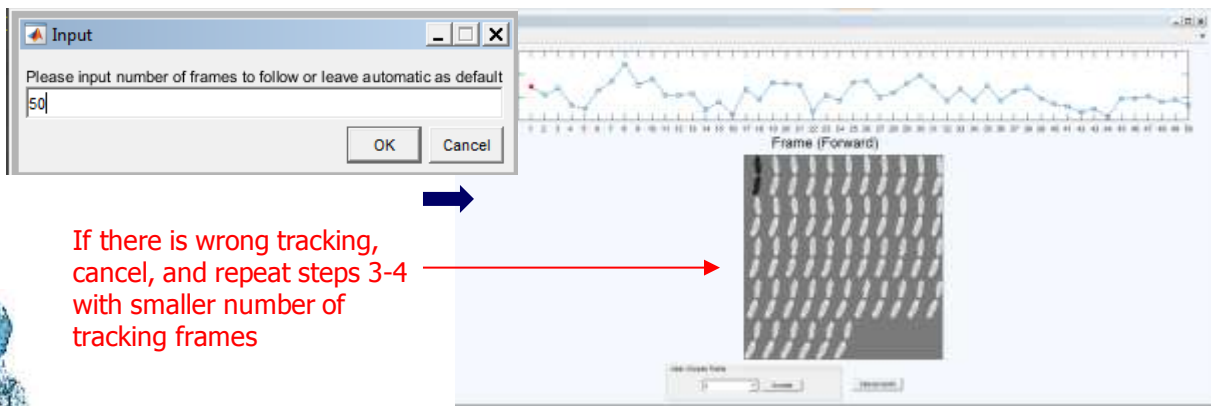
Instructions:

1. Mouse left click on the 'Add Cells' icon. The icon will appear in white color to indicate that this mode is active. In addition, the 'Show Box' icon will automatically get activated (but can be turn off by the user). The red box will indicate for the location of the selected cell in the following steps.



2. It is recommended to activate the contour annotation. (mouse right click on the contour icon).
3. Mouse right click to mark the requested cells (cells will be appear in red).

4. Mouse left click will open a dialog box asking the user if the tracking should be backwards (Rewind) or forward. A second dialog box will be opened asking the user to input the number of frames to track the cell/s in question. As default, 'Automatic' option track the cell the maximum number of frames, but can overloading the commutating power in some cases. Therefore, in the example below, the number 50 was input instead of 'automatic'.
5. The computer will run tracking algorithm in after few seconds (depended on the number of frames that were selected by the user) a new window gets opened, showing a graph of the cells area over time and a montage of the tracked sequence:

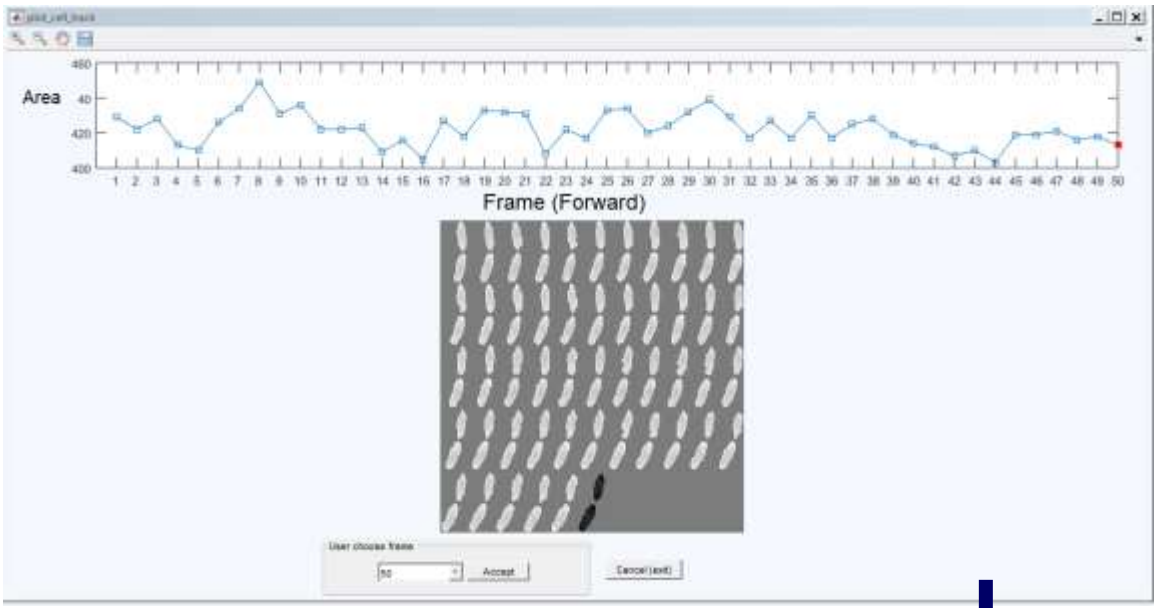


If there is wrong tracking, cancel, and repeat steps 3-4 with smaller number of tracking frames

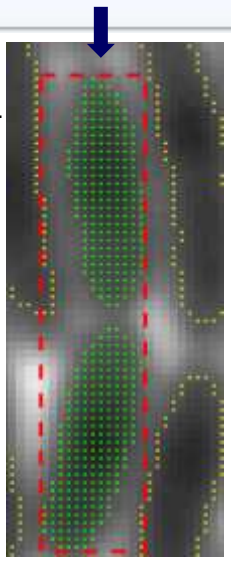
Tracking Module - 2

Add Cells mode:
Instructions:

6. Select the frame where you want to stop the tracking of the cell/cells. In the example shown below, two cells were chosen and tracked for 50 frames:



7. The cell/s will be marked by a red box for the next frames (or previous frames, depending whether the selection of step 4 was 'rewind' or 'forward'). At this stage the user can go between frames and inspection the tracking.



If there is wrong tracking, repeat steps 3-7 with smaller number of tracking frames.

8. Ctrl+O will open a new selection window. Choose one of the following: