Confleezers Analysis Manual

V0.5

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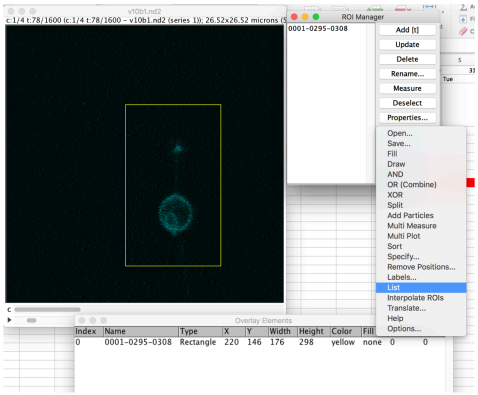
0 Movie Preprocessing

Startup:

- Make a new folder for the movie you want to process
- Copy the TEMPLATE_info.csv (on git) into that folder and rename it _info.csv

Preprocessing:

1) Open the large movie in Fiji and determine the cropping (make sure that the vesicle does not drift out of the cropping area).
2) Draw a rectangle, add it to the ROI manager, save the ROI as 'movie_ROI.roi'
3) Then show the 'List' in the ROI manager
4) transfer the values for x, width, y and height into the _info.csv
    a. `movie_crop_xStart, MANUAL eg: 220`
    b. `movie_crop_yStart, MANUAL eg: 146`
    c. `movie_crop_xwidth, MANUAL eg: 176`
    d. `movie_crop_yheight, MANUAL eg: 298`
5) change the path information in _info.csv
    a. `vesicleID_of_that_day, MANUAL eg: v7`
    b. `date, MANUAL eg: 2018-07-13`
6) change the movie names according to the movie file
    a. `movie_originalFilename, MANUAL eg: v7_OH_MY_GOOOOODDDD!!!! Movie 001.nd2`
    b. `movie_dataShareFilename, MANUAL eg: 2018-06-05_raw_movie.nd2`
7) open 'tube__0_processMovieFile.ipynb'
8) input the correct path to the parameter file
9) run the notebook



0.1 bleach correct the movies

1 Timing

Manual steps:

1) copy the force file into the analysis folder
2) change the filenames in the _info.csv accordingly
   `forceFile_raw_originalFilename`
   `forceFile_raw_dataShareFilename`
3) obtain parameters from the log file:
   `UVstart_seconds_fftime`
   `UVend_seconds_fftime`

These two parameters are manually recorded in the logbook during the experiment. Transfer the values from the logbook into the _info.csv

3) Input the correct path into the notebook to the parameter file
4) Run the Notebook

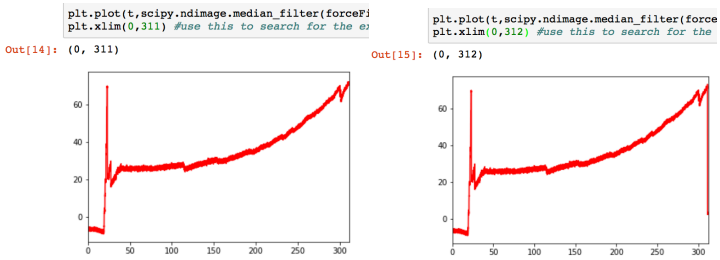
2 Force File Analysis

Manual steps:

1) Optain parameters in the input file:
   a. `forceFile_alpha_y`
   b. `forceFile_kappa_y`

2) Input the correct path into the notebook to the parameter file
3) Determine the tube breaking time

`tube_break_seconds_fftime`



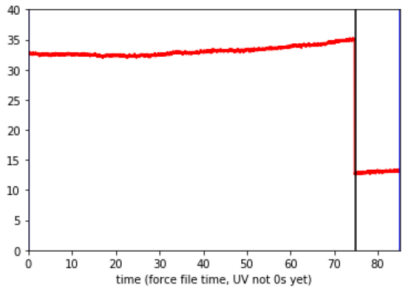
Find the time at which the tube breaks by manually cropping the xlimit of the plot

4) Determine the zoom time if the force file goes too long (cropping)

   a. ffprocessing_zoomTime0
   b. ffprocessing_zoomTime1

force file processing

```
In [22]: #INTERACTIVE:
         # play with the plot ranges until you found the exact br
         # and the zoom in x that you like

         forceFile_alpha_y = float(inproc.getInputParameter(input
         forceFile_kappa_y = float(inproc.getInputParameter(input

         plt.plot(t,scipy.ndimage.median_filter(forceFile_alpha_y


         plt.xlim(0,85) #use this to search for the exact break t
         plt.ylim(0,40)

         tube_break_seconds_fftime = 75
         ffprocessing_zoomTime0 = 0
         ffprocessing_zoomTime1 = 85

         plt.axvline(tube_break_seconds_fftime,c='k')
         plt.axvline(ffprocessing_zoomTime0,c='b')
         plt.axvline(ffprocessing_zoomTime1,c='b')
         plt.xlabel('time (force file time, UV not 0s yet)')
Out[22]: Text(0.5,0,'time (force file time, UV not 0s yet)')
```



These parameters allow you to crop the force file e.g. in case the recording was not stopped in time and the relevant time has to be cropped out of a larger trajectory

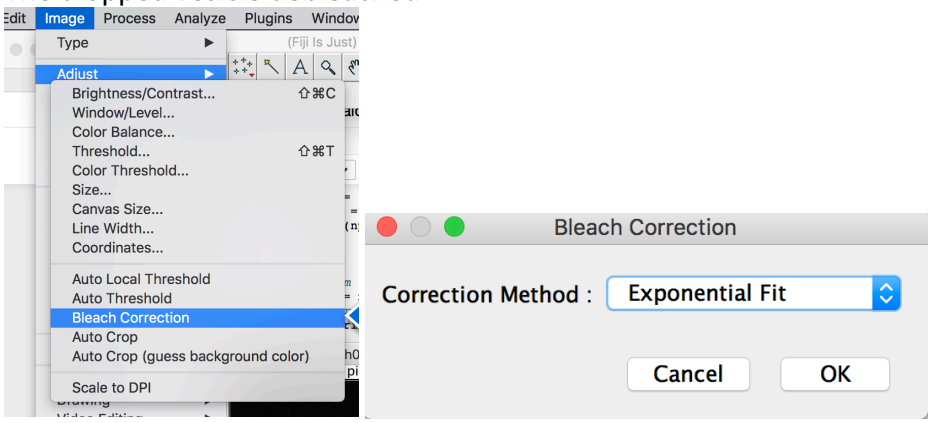
5) If you want to do fitting, put in these parameters

```
ffprocessing_fitStart
ffprocessing_fitEnd
```

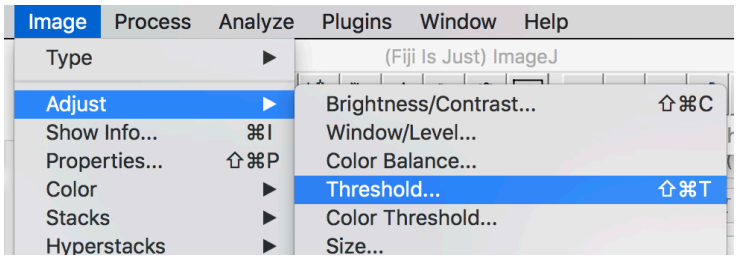
3 Calculate Tube Vector

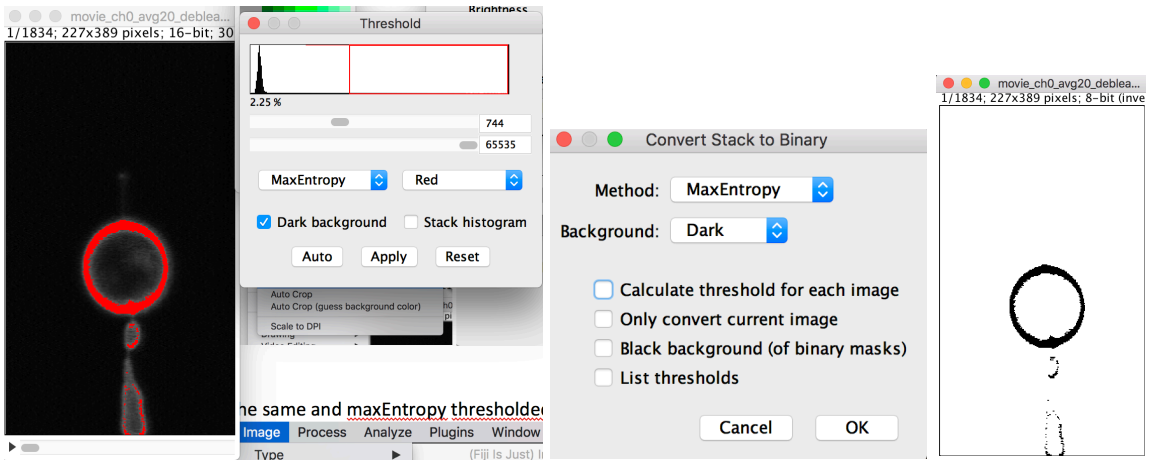
Prepare the following movies:

From channel 0: (membrane channel)

- The cropped vesicle debleached
- 

- The same and maxEntropy thresholded (auto) to find the vesicle
    - **Don't** 'Calculate threshold for each image'

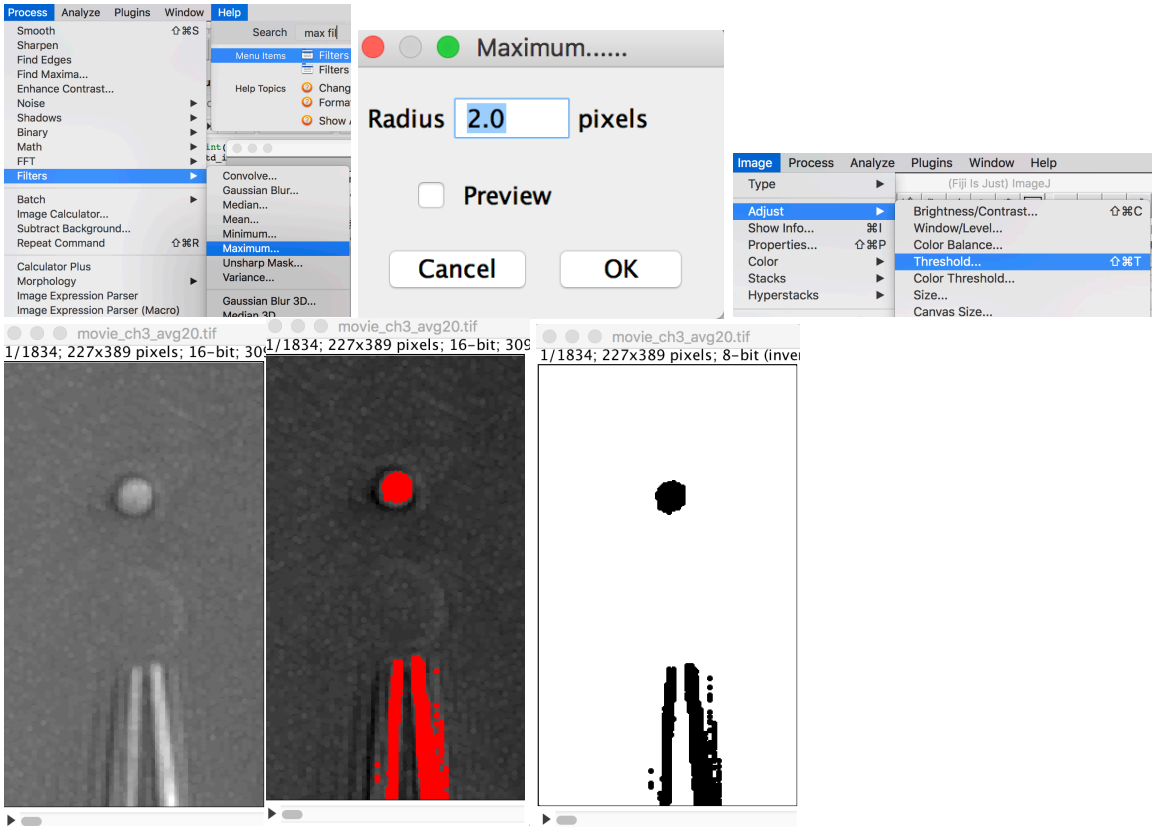




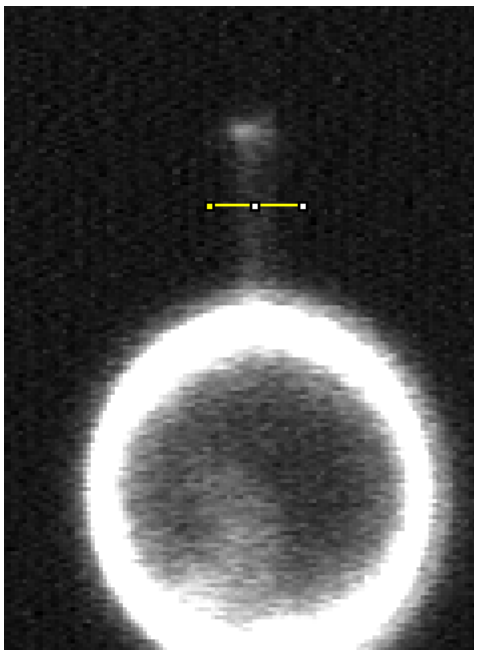
From channel 3: (transmitted detector)

- The cropped channel

- The same and maximum filteredwith 2px and yen Thresholded (auto)



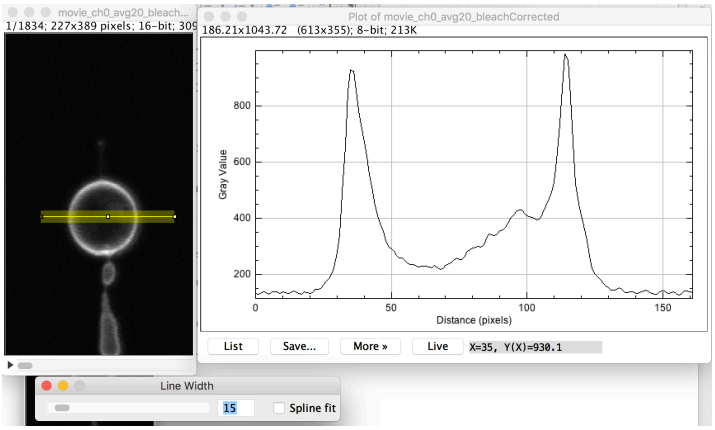
- find a good y value where the tube is ok throughout the movie, put that value into the input parameters:



- `tubeVector_tubemidpointY_px`
    - this value is the starting point in y for the algorithm to try and find a tube signature from which it expands the tube automatically.
- `tubeVector_beadAdditionalMargin_px`
    - this value determines how many pixels away from the bead boundary you define the start of the tube. For example 5 px
- `tubeVector_vesicleAdditionaMargin_px`
    - this value determines how many pixels away from the vesicle boundary you define the start of the tube. For example 5px
- `tubeVector_tubeWindoxY_px`
- this value defines the number of pixels in y that you can say that the tube is ok. The resulting initial tube from midpoint +- `tubeVector_tubeWindoxY_px` is then used for an initial fit of the tube from which the whole tube is determined.

4 Calculate the channel intensities along the tube

5 Calculate the tube radius from the tube membrane intensity



- You need the experimental value of the membrane intensity.
    - Draw a 15px line average across the vesicle
    - Hit ‘k’ to display a histogram
    - Hover with the mouse over the peaks to determine their value
    - Take 3 measurements like this and input it into the radius calculations