Workflow for Zymoliase contour analyses:

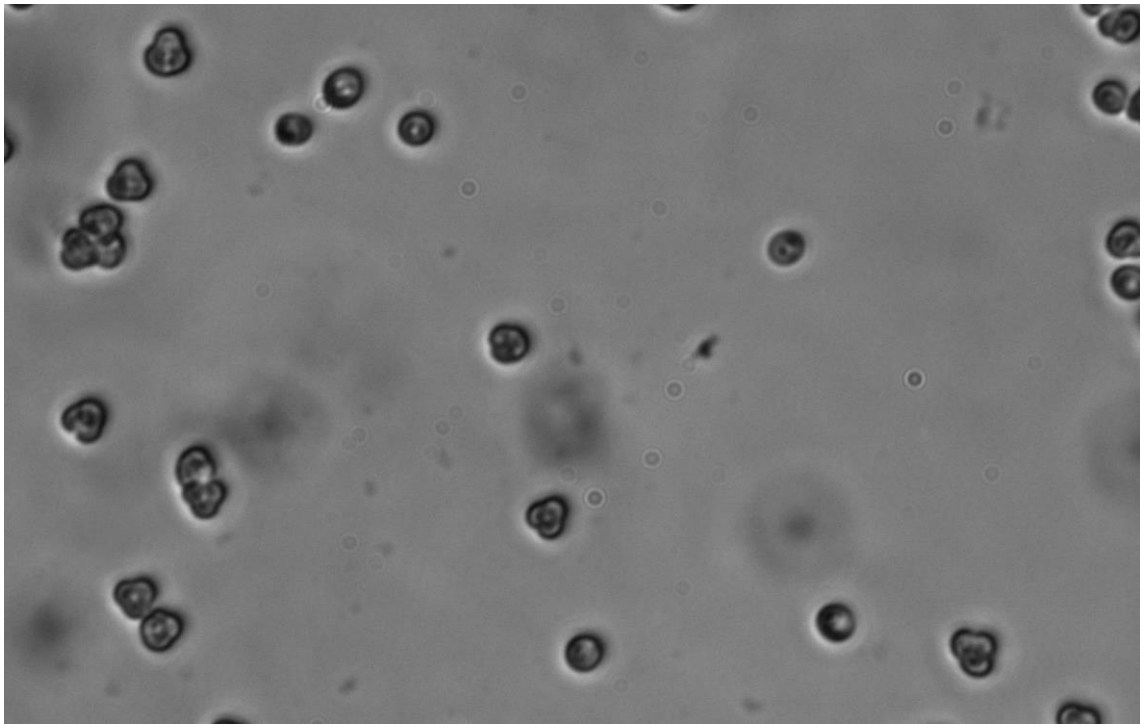
For this workflow we'll need:

1. Images separated by timelapse.
2. TrackMate plug-in in FIJI.
3. plot_boundaries_from_roi_ordered_by_frame.py script

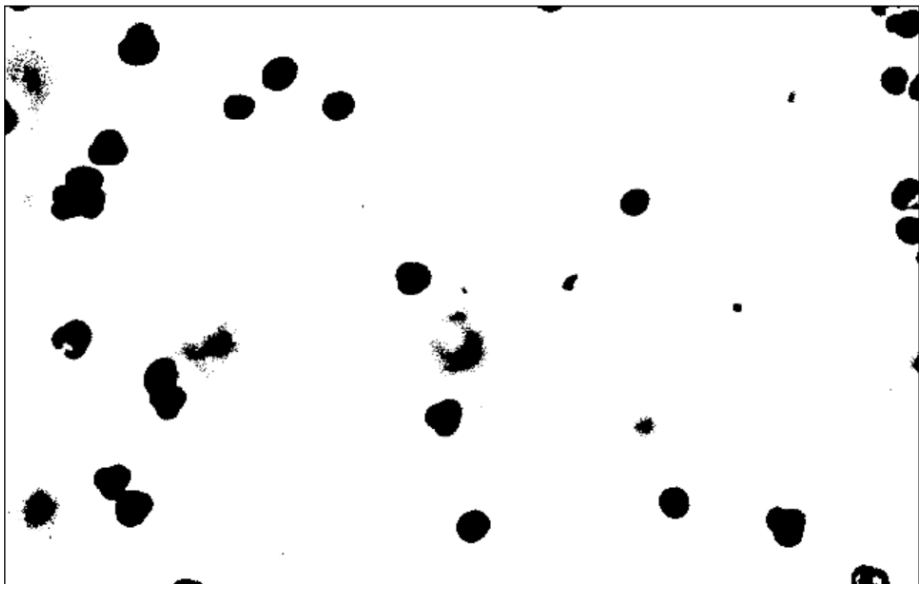
Fiji part:

1 – Must have images separated by TimeLapse. Images must be Tif.

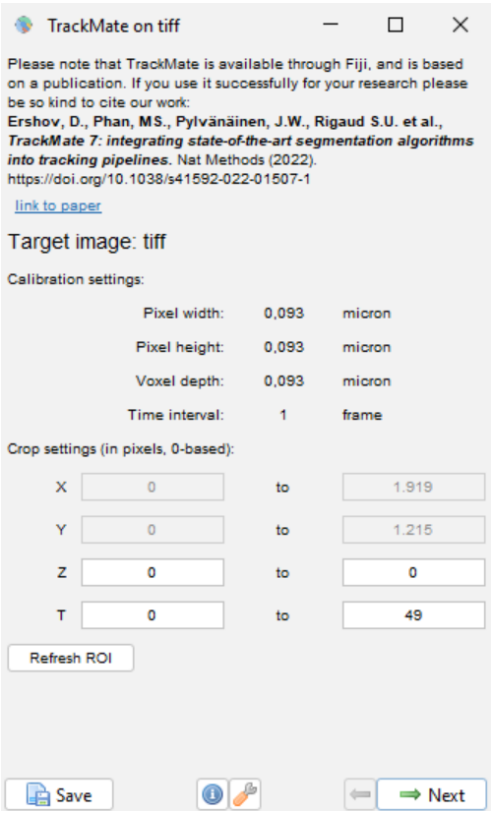
2 – Open images in Fiji as timelapse:



3 – Set the best threshold and fill holes:

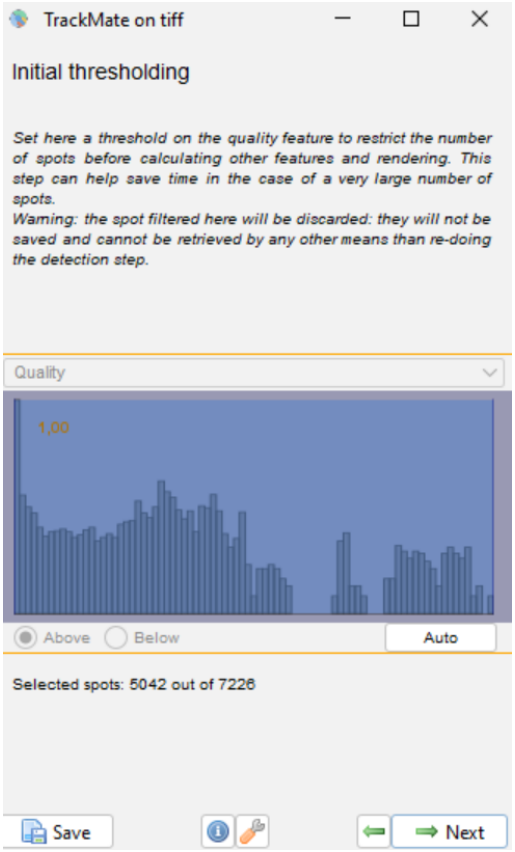


4 – Open TrackMate plugin. If some advertise of swapping from Z to T appears, select Yes.

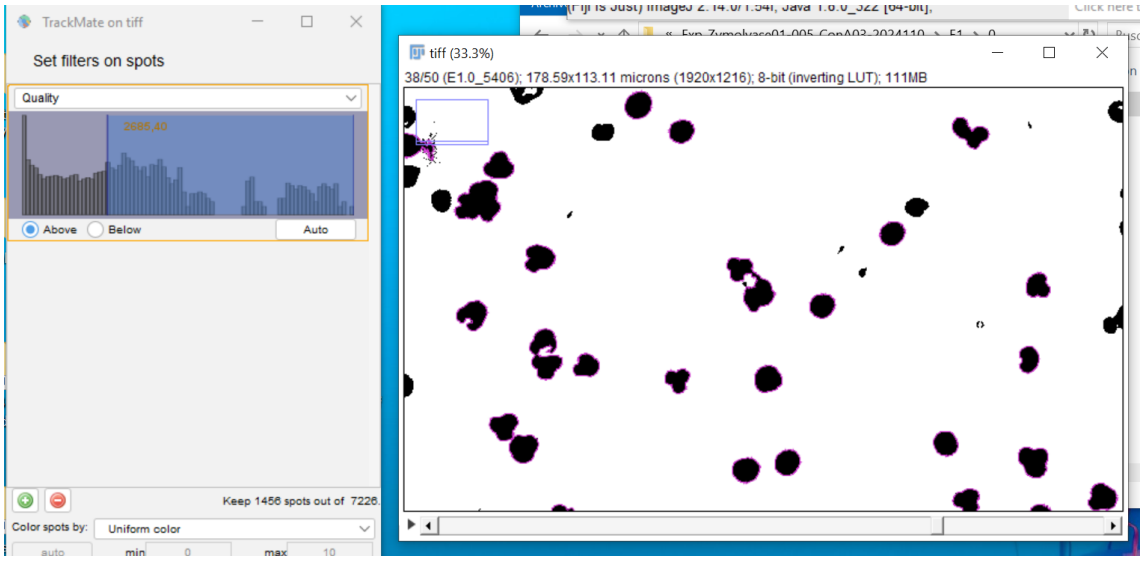


5 – Select Yes, then Mask Detector:

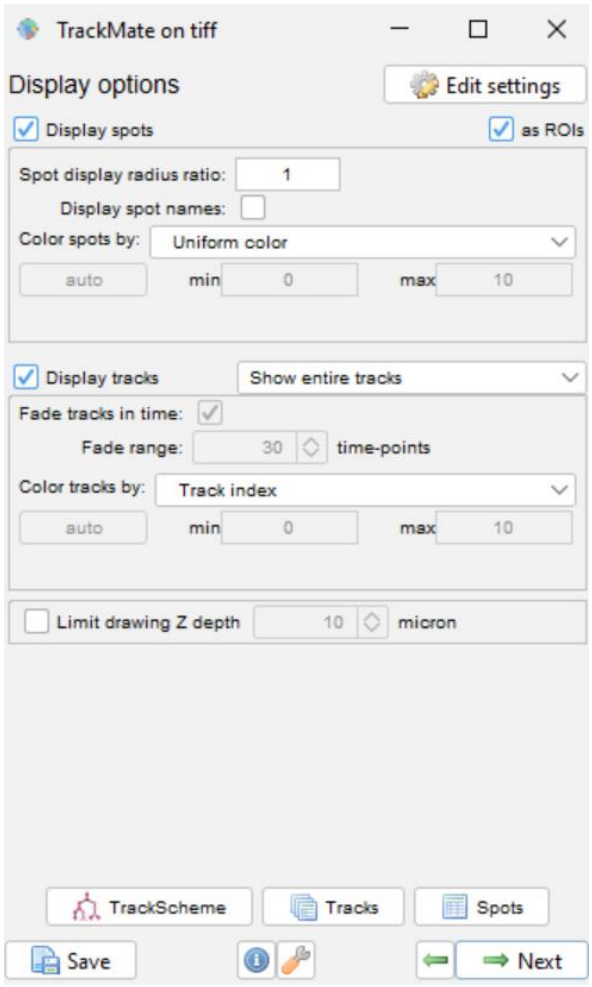
6 – Then next until you get here and go next:



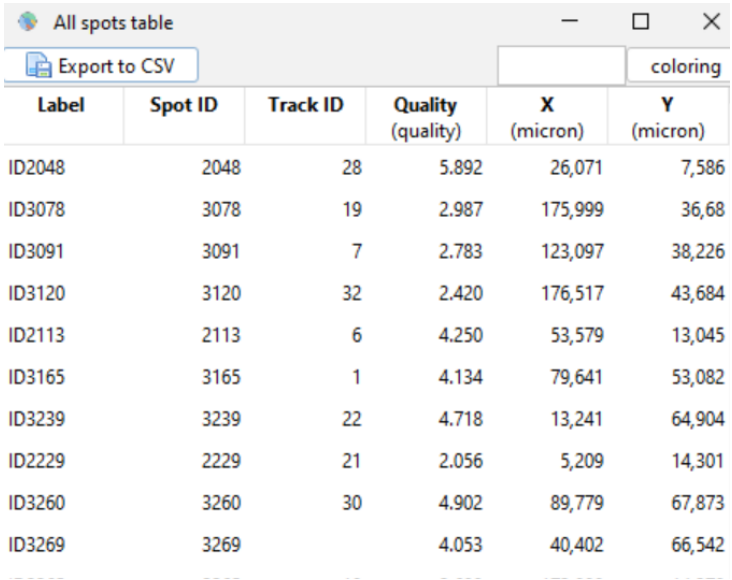
7 – It will leave you to select the spots you want to keep tracking. Move the blue shaded area until all your cells of interest are circled.



8 -Then select next. On the tracker I've decided to use LAP tracker (it seems to work fine). And next until here:

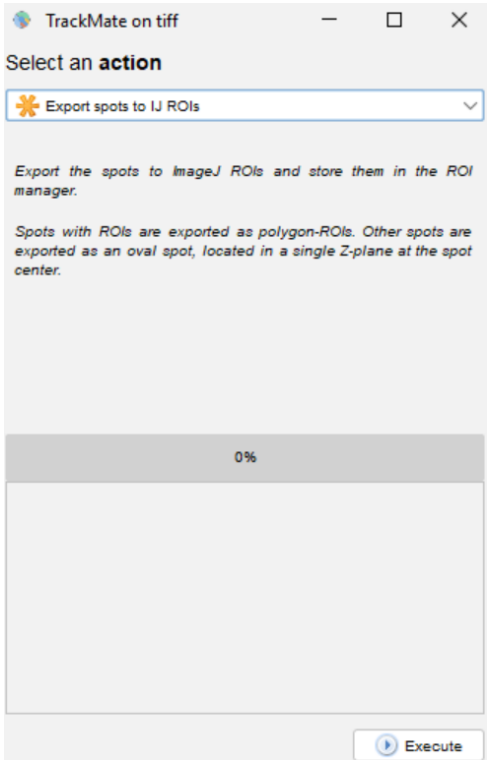


Here you have to click on spots. It will show a table that contains all the cells tracked, identified by ID and TRACK ID (which we'll be using later) and others features of interest. Export it to csv in the same folder as the images.

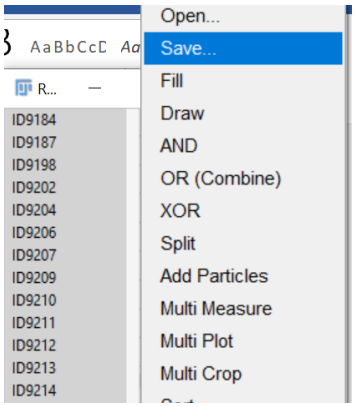


Label	Spot ID	Track ID	Quality (quality)	X (micron)	Y (micron)
ID2048	2048	28	5.892	26,071	7,586
ID3078	3078	19	2.987	175,999	36,68
ID3091	3091	7	2.783	123,097	38,226
ID3120	3120	32	2.420	176,517	43,684
ID2113	2113	6	4.250	53,579	13,045
ID3165	3165	1	4.134	79,641	53,082
ID3239	3239	22	4.718	13,241	64,904
ID2229	2229	21	2.056	5,209	14,301
ID3260	3260	30	4.902	89,779	67,873
ID3269	3269		4.053	40,402	66,542

9 – Go next until get here and select ‘Export Spots to IJ ROIs’, then ‘execute’ and select ‘all spots’:



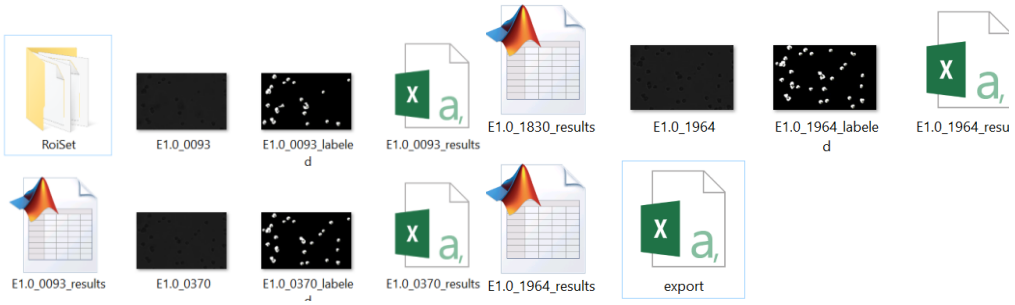
It will appear a ROI selection. Select them all, go to more and save them in the same folder as the images.



At last, you can save all images separately in the same folder as the input. It is not necessary though.

Python part:

Once you have the Roi set and the csv file saved, you should have something like this:



Maybe the RoiSet is in .zip format, just extract it in a new folder.

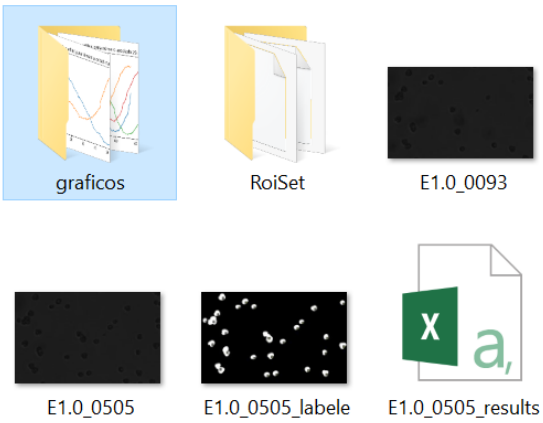
Once you've opened the plot_boundaries_from_roi_ordered_by_frame.py file, you'll find this:

```python
# -*- coding: utf-8 -*-
"""
Created on Tue Feb 13 09:03:26 2024

@author: uib
"""

import os
import numpy as np
from matplotlib import pyplot as plt
import pandas as pd
from read_roi import read_roi_file
import shutil

# Simulando la función calculate_mass_center()
def calculate_mass_center(x, y):
    x_center = np.mean(x)
    y_center = np.mean(y)
    return x_center, y_center

# Simulando la función angulos()
def angulos(x, y, x_center, y_center):
    x_rel = x - x_center
    y_rel = y - y_center
    r = np.sqrt(x_rel ** 2 + y_rel ** 2)
    theta = np.arctan2(y_rel, x_rel)
    return x_rel, y_rel, theta, r

# Ruta a la carpeta que contiene los archivos de ROI
carpeta_input = "C:/Users/uib/Desktop/prueba_script_python/prueba_zymoliasa/tiff"
```

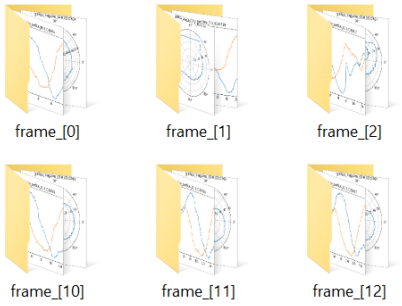
```python
for files in os.walk(carpeta_input):
    carpeta_roi = os.path.join(carpeta_input, 'RoiSet')
    ruta_csv = os.path.join(carpeta_input, 'prueba_zymoliasa.csv')
    break

if carpeta_roi is None or ruta_csv is None:
    print("No se encontró ninguna carpeta 'RoiSet' o el archivo 'csv' en la carpeta de entrada.")
    exit()

df = pd.read_csv(ruta_csv, sep=';')
```

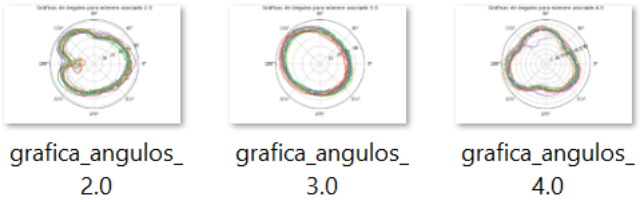
What's important here are the carpeta_input, where you should address the folder where you have your images and RoiSet and csv file. Also, in carpeta_roi, you must indicate the name of the folder with all the ROI (RoiSet by default) and in ruta_csv, the name of the csv file with the data (prueba_zymoliasa.csv in this case)

Once you've adjusted these items, just run the script. When it ends running, there should've appeared a new folder in your input folder:



Containing all the plots ordered by frame and the plots overlapped by cell:





WARNING: Be sure all the packages imported at the beginning of the file are installed in your python environment.