Installing FIJI and Kymolyzer

1. Download and install FIJI:
   1. Download from: <https://imagej.net/Fiji/Downloads>
   2. Install Fiji from installer
2. Update Fiji:
   1. With the first run of FIJI, it will automatically ask for permission to update.
      1. Press “Yes, Update Now” and let the update happen
      2. Switch off FIJI and switch it back on, and update again
      3. After the first two initial updates, the basics of FIJI should be ready
3. Run kymolyzer installer:
   1. Download the zip file and extract it in a separate folder anywhere on your computer (preferably on your desktop, so that you can access it easily. You can delete this folder after installation).
      1. In the folder named ***“Kymography\_Macro\_TLS\_Lab\_\*date\*”***, there should be the subfolders named as *Kymolyzer\_HimanishBasu\_SchwarzLab,* the installer file named as *installer\_Kymolyzer\_HimanishBasu\_SchwarzLab\_.ijm,* and the *instructions.docx* file.
   2. From FIJI, go to Plugins>Macros>run
      1. Select the file named ***installer\_Kymolyzer\_HimanishBasu\_SchwarzLab\_***
   3. Restart FIJI
   4. You should be able to see a new tab under Plugins>Macros called “kymolyzer”
4. In this case you are ready to run kymolyzer!

Running Kymolyzer:

\*\* Note: For running kymolyzer, preferably use single channel movies. Refer to the example (in the example folder) where I have the mitochondria movie is saved separately from the image of the GFP channel. This is not necessary but makes for an easier time using the macro

\*\* Note: Preferably do not pre-open the images before running the STEP 1. As soon as you run Step1, it will ask you to open an image. In this way the macro can read the metadata of the image.

1. Run step 1 of kymolyzer: Plugins>Macros>Kymolyzer>Step1\_MakeKymograph
   1. Select the movie
      1. if it is a dual channel movie, the macro will ask the user to select any one channel, which has the moving particles that are to be traced
   2. The macro will then give a maximum projection image of that channel and ask to user to trace a line to make the kymograph.
      1. At this stage the user can ask the macro to open up a file where drawing the line will be easier (such as the GFP channel)
   3. Macro will then make the kymograph and ask the user to change the brightness and contrast of the kymograph as necessary
   4. Step 1 is finished!
2. Run step 2 of kymolyzer: Plugins>Macros>Kymolyzer>Step2\_Track
   1. A folder selection window will open up as soon the step 2 is run.
      1. Select the folder with the name of the image
   2. Macro will open up the kymograph and ask the user the following:
      1. Puncta Start Count:
         1. At the start of each image this number should be 1
         2. If, for some reason the step 2 has to be restarted with some particles being already tracked in that image, then change the puncta start count to whatever number of particles that have already been tracked +1 (so that it represents the present particle number being tracked)
      2. Show box size: This is the size of the box used to display the tracked particle. Start with show box size as 5 and adjust as necessary
      3. After the setting the puncta start count and show box size press OK to start the tracking
   3. During the tracking process you can do the following at any point of the image:
      1. Shift+LeftClick : adds points on a track
      2. Cntrl+leftClick: finishes the track
      3. Alt+leftClick: drops a straight line (depicting a stationary particle) till the end of the movie
         1. It is advisable not to use the Alt+LeftClick function to begin with. Only use it once you know what to depict as stationary and what as moving (after going through the step 3 atleast once).
      4. After the tracking of each puncta, a movie will popup showing how well the puncta was tracked. Press OK if you are satisfied with the tracking.
      5. Then go on the next puncta, by clicking “Yes” in the “For another track” dialog
      6. After the final puncta, click “No” on the same dialog box
3. Run step 3 of kymolyzer: Plugins>Macros>Kymolyzer>Step3\_Measure
   1. A folder selection window will open up as soon the step 3 is run.
      1. Select the folder with the name of the image
   2. After folder selection, a window will pop-up asking for the image parameters:
      1. Pixel Scale in um/pixel: length of each pixel in micro after maginification
      2. timescale (s): Time gap between two frames
      3. Kymograph width (in pixels): generally the length of the line selection made
      4. Kymograph height (in pixels): generally, total number of frames in the movie.

*For most cases, these fields should populate themselves, after the macro reads the metadata. If for some reason the macro hasn’t read the metadata (due to a file formatting issue) or has retrieved the wrong metadata, feel free to change it.*

* 1. After setting the scale parameters, the following two options have to be selected:
     1. *Calculate step (every ?? time points):* Each track is divided into segments. This value defines the number of frames to skip in between recording the position of each particle, to divide the tracks (for example, in a movie with a 100 time points, a value of 5 will divide the track into 20 segments).
     2. Lower limit of velocity: lowest velocity of a particle at any segment below which, it is considered as stationary.
  2. The default options are the ones that are typically used by the Schwarz Lab to track mitochondria in distal axons. But feel free to change it.
  3. The best way to decide the values is to track two test particles, one that is stationary and one that is motile. Then set the frame step gap and lowest velocity limit to the lowest possible values such that the stationary particle is indeed recorded to be stationary while movement is detected for the motile particle
     1. The raw data for all the particles in a kymograph can be found in the following excel file: …pathTofolderWithImageName/RawPunctaFiles/Summary.xls
     2. Open up this file and check the tab “Move %” to see whether the stationary particle is indeed recorded to be stationary.
     3. If not repeat the third step by increasing the lower velocity limit or the frame skip gap.
  4. This value should be changed in between different samples of the same experiment.

1. Run step 4 of kymolyzer: Plugins>Macros>Kymolyzer>Step4\_CollateDataMeansOnly
   1. A folder selection window will open up as soon the step 3 is run.
      1. Select the folder with the name of the image
      2. To add more folders press “yes”
      3. After all the image folders have been added, a dialog box will appear asking the location and name of file to be saved.
   2. This creates a file in the desired place which has the mean per kymograph of the various measurements.
   3. If the single particle measurements are needed instead of the mean of all the particles per kymograph, the particle-wise raw data for each kymograph is stored in :

…pathTofolderWithImageName/RawPunctaFiles/Summary.xls