

Protocol for “Bacterial motility patterns adapt smoothly in response to spatial confinement and disorder”, Zhang, Wetherington, Ko, FitzGerald, Munro & Nirody.

### **Bacteria Tracking Protocol**

#### **Bacteria tracking via ImageJ:**

1. Load the image sequences as a virtual stack:
  - a. Go to file/import/bio-formats and choose the file (for example, the recording of C = 6, D = 0)
  - b. Click ‘Use virtual stacks’ and then Ok
  - c. Go to image/adjust/brightness/contrast and a window will pop up and hit ‘auto’
2. Create a background intensity from the video:
  - a. Go to image/stacks/z\_project
  - b. In the pop-up window, click ‘Average Intensity’ next to ‘projection type’ and OK
3. Subtract the background image sequences
  - a. With both windows open in the program (i.e., original stack and AVG image), go to process/image\_calculator
  - b. Choose the original video images to subtract the AVG video images and click ‘open a new window’ and OK. Close the other two windows
4. Record the regions of interest
  - a. Go to file/import/image\_sequence, and find the folder where the brightfield images are located and open the folder
  - b. Go to analyze/tools/ROI\_manager. Use a rectangle to draw a rectangle on the image for the region of interest and click “Add” in the ROI Manager, then click ‘Rename’ to change the name of the region (for example “1-1”). Then click “update”, and repeat for all the pillar positions. Then click More/Save/ to save a zip file named RoiSet.zip. Close the window
5. Load saved ROIs and crop the videos based on ROIs
  - a. Open a new window, go to analyze/tools/ROI\_manager ROI\_manager and click more/open/RoiSet.zip
  - b. With the window ‘Result of...’ open, click on the corresponding region “1-1” in the ROI Manager. The rectangle will appear in the video
  - c. Go to image/crop, the cropped video will replace the original video
6. Track bacteria using TrackMate
  - a. Go to plugins/tracking/trackmate to open the program. Check if TrackMate is about to use the correct target image. Then hit ‘next’
  - b. Use DoG detector for spot segmentation. Enter 10-15 pixels for ‘estimated object diameter’ and 3-5 pixels for ‘quality threshold’. Then enable ‘sub-pixel localization’. Check ‘Preview’ to see if the objects are under/over-detected. If it reaches the desired initial result, then hit ‘next’
  - c. Click on next again to pass the initial thresholding and then click on next
  - d. Choose LAP tracker. Enter 10-20 pixels for ‘max distance’ and 10-25 pixels for ‘max frame gap’. Then hit ‘next’ and ‘next’ again
  - e. Use TrackScheme to manually edit the tracks. Remove the repetitive tracks, tracks that have little displacement, and tracks that are in and out of the boundaries
  - f. In the TrackMate window, click on ‘Tracks’ and export the file to CSV and save.