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