**Instructions for using the CellTracking code (**[**https://github.com/dfraebel/CellTracking**](https://github.com/dfraebel/CellTracking)**) for generating trajectories from videos of *E. coli* swimming in a microfluidic chamber, tested on Matlab R2014A**

1. Getting Started
   1. Download the sample video and supplementary code from the Illinois Data Bank dataset: <https://doi.org/10.13012/B2IDB-4912922_V2>
   2. The code will expect a certain organizational structure for the files:
      1. Put the sample video in a folder called ‘Trial01’
      2. Put the ‘Trial01’ folder in a folder called ‘Round01’
   3. Unzip ‘Code.zip’ and add the root folder to your Matlab path. You will need many of the functions contained within.
   4. Download all 4 scripts from the GitHub folder. These will be used one at a time as described below.
2. Segmenting
   1. Copy the SegmentAll\_XXXXXXXX\_Sun script from the previous experiment and paste inside the date of your experiment. Modify the name so the date is correct. Your date folder should now consist of a bunch of rounds and the SegmentAll script.
   2. Open MATLAB, set the current folder to the date folder so that in the Current Folder window, you see all the rounds and SegmentAll script.
   3. Open the script. Change the baseFolder variable in the script to reflect the folder that you are in.
   4. Run the script. (Takes a while)
      1. Troubleshooting if the program crashed before finishing. It’s usually because of a corrupted video file.
      2. Locate the corrupted video file. What was the last video segmented before crashing?
      3. If ever during the experiment an error window popped up that said “A mode change was detected while recording” and you had to press record again, there is likely a bum video file in the folder for that trial.
      4. Or, if the computer crashed while taking a video, that video is likely corrupted.
      5. Relabel the trial with the bum video. If it was Trial04, rename it xTrial04. This will ensure the segmentation script will not see that video.
      6. Run the SegmentAll script again. You should change the indices on the for-loops for rounds and trials to make sure it only runs ones that need it.
   5. Manually look through every trial of every round folder, open the “Boundaries” PNG file, and ensure that the red boundary corresponds to the actual boundary.
      1. If there is a trial where this is not the case, here is what you do:
      2. Open up the MakeMaskVR function. You can do this by highlighting it in the SegmentAll script and pressing ctrl+D
      3. Change the “num” variable to some other number. I would try any number between 50 and 150. Save.
      4. Edit the segmentAll script such that the indices in the for-loops for rounds and trials only contain the ones whose boundaries you want to try for again.
      5. Make sure your current folder is the date of the experiment and run it.
      6. You may need to try a few different “num”s before you get it right.
3. Tracking
   1. Copy the TrackingScript\_XXXXXXXX\_Sun script from the previous experiment and paste inside the date of your experiment. Modify the name so the date is correct. Your date folder should now consist of a bunch of rounds, the SegmentAll script, and the TrackingScript.
   2. Set the current folder to Round01 and then to Trial01.
   3. Run every cell up to and including “save trackedB B params”
   4. Skip the “PLOT CHUNKS MANUALLY” cell.
   5. Run the “CHECK CROSSING EVENTS” cell with i =1 and then with i = 2. You do not have to run this cell if there is only one bacterium in the chamber.
   6. Run every following cell up to and including “examine basic properties of traces”
      1. If it looks good then great. Take note of where each trace begins and ends and if there were any divisions.
      2. Otherwise, you might have to switch some things around like if there is an obvious crossing event. We need to fix it. Go to the next cell, uncomment one of the lines, edit it to do the fix you want and run the cell. I would recomment the line to make sure you don’t run it when we run this script on another trial later. Run the “examine again” cell to see if your fix performed as expected. If it didn’t go back to the previous cell and try again. Take note of where each trace begins and ends and if there were any divisions.
   7. Run the “save the fixed x” cell.
   8. Edit the next cell to reflect what your traces looked like. Uncomment as many blocks as you have traces.  
      p(A).trajectory = x(B:C,:,D) Do not edit “A”. Edit “B” and “C” to say where trace started and ended. Edit “D” to be the color of the trace. It will be blue, green, or red. Rarely it will be cyan.

p(A).isFull = 0 Do not edit anything. In 5-minute videos you will never have full lifetime.

p(A).hasBeg = E Do not edit A. Set “E” to 1 if this trace includes the beginning of the bacterium’s life. This will be the case if the bacterium’s mother divided during the video. Otherwise set to 0.

p(A).hasEnd = F Do not edit A. Set “F” to 1 if this trace includes the end of the bacterium’s life. This will be the case if the bacteriu divided during the video. Otherwise set to 0.

p(A).lineage = G or [G,H] Lineage can be 1,2,3, etc. or [1 1], [1 2], [2 1], etc. If there were two bacteria in the chamber initially, label one as 1 and the other as 2. If 1 divides, label its children [1 1] and [1 2]. If 2 divides, label its children [2 1] and [2 2].

* 1. Run the “SAVE B,p,params” cell.
  2. If this trial was totally untrackable, put an “x” in front of the trial folder so that it reads xTrial02 or whatever. Clear the workspace.
  3. Change the current folder to the next trial that needs to be tracked.
  4. Scroll to the top of tracking script and run again from the top.

1. Analyze
   1. Copy the AnalyzeAll\_XXXXXXXX\_Sun script from the previous experiment and paste inside the date of your experiment. Modify the name so the date is correct.
   2. Set the current folder to the date folder.
   3. Open the Analyze\_All script. Change the baseFolder variable in the script to reflect the folder that you are in.
   4. Between baseFolder, dateString, compString, labelString, and od, change the ones that need changing.
   5. Run the script.
2. Combine pAnalyzed
   1. Copy the combinePanalyzed\_XXXXXXXX\_Sun script from the previous experiment and paste inside the date of your experiment. Modify the name so the date is correct.
   2. Set the current folder to the date folder.
   3. Open the combinePanalyzed script. Change the path variable at the top. Change the lines at the bottom that have “SET THIS MANUALLY” next to them. Note that the second of these lines has the date twice.
   4. Run the script.