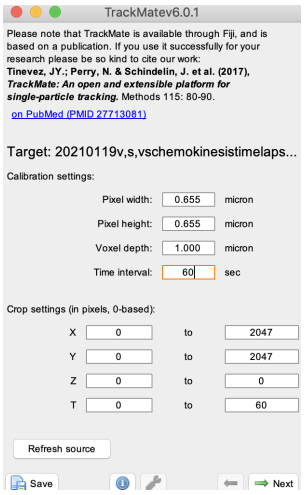
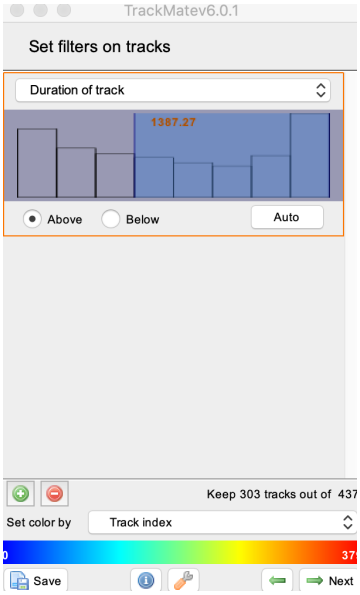
TrackMate/MATLAB chemotaxis plots and data analysis protocol

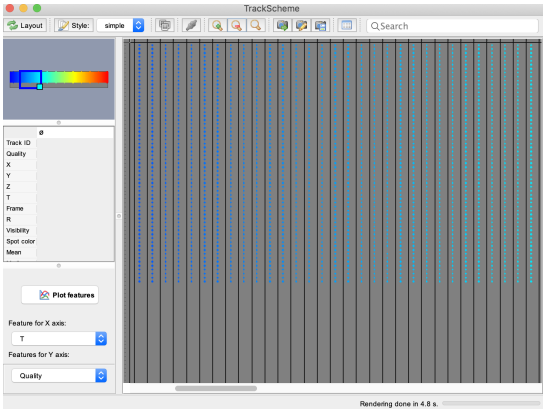
1. Open the image in ImageJ. Go to plugins > tracking > TrackMate. A window like the one below should pop up. Set the pixel width and height to 0.655 microns. Hit ‘next’.



2. Select ‘LoG detector’. Hit ‘next’ and set the Estimated blob diameter to 10.0 microns and the threshold to 0.0. Hit ‘next’ and wait for the program to detect the cells. This may take some time. Hit ‘next’ when it says it’s finished.
3. Press the ‘auto’ button. Hit ‘next’. (if in the future there aren’t enough cells, increase this number)
4. Select ‘HyperStack Displayer.’ Hit ‘next’.
5. Here, you can filter out certain cells based on their x/y coordinate, radius, etc… It’s not that important but it’s an option to keep in mind. Hit ‘next’.
6. Select ‘Simple LAP tracker’. Hit ‘next’.
7. Set the ‘Linking max distance’ to ~50 microns. This number is subject to change. If later you notice that the cell track lines are disconnected, go back to this window and increase the Linking max distance value. Hit ‘next’.
8. TrackMate should now automatically draw each cells’ movement paths. Press the play button on the bottom left to check to make sure that the cells in general are moving according to these paths. When satisfied, hit ‘next’.
9. This next window filters out certain tracks. We need to filter out tracks that do not have the maximum number of slices. Click the green + in the bottom left. In the dropdown menu of the box that pops up, select “Duration of track”. Add another filter called ‘Number of spots in track.’ Decrease the number in both of these filters until the number in the bottom right corner is closest to 0. Hit ‘next’.

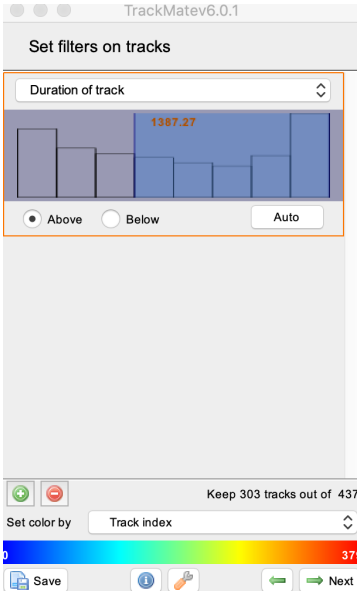


10. In the next window, click “TrackScheme”. Zoom out. Now you need to check to see that every single cell has the number of slices you want.



If there is uneven-ness, exit out, go back one window, and add new filters.
If there are too many cells, add a filter called ‘TrackID,’ which should filter out cells without any bias of location or movement.

Note: in the filtering tracks window, it records the number of tracks that you are keeping. When you feel like you have filtered out all the shorter tracks, record the final number of tracks.



Bottom right corner tells you how many tracks you are keeping and filtering out

11. Once you have filtered out the unnecessary tracks, click the “Analysis” button, and 3 windows should pop up. Find the window titled “Spots in tracks statistics” and save it as an excel file. Open the excel file and delete the first row, then save that file as a .xlsx file. Add the excel file to the same MATLAB folder as the scripts provided to you.
12. In the file named **main.m**, change the parameters in the beginning of the script to your custom needs.
    a. *Image parameters*: excel file name, number of slices, number of tracks, etc. (things that can’t be customized)
    b. *Filtering parameters*: you can filter out certain slices in the dataset, in case there are artifacts/unwanted data in the beginning/end of the dataset
    c. *Plotting parameters*: customizing the number of tracks you want to be plotted as well as the plot axes range.
    d. *Program options*: on/off switches for the different outputs of the program
13. Click ‘run’. If you selected a plot to be generated, it will pop up automatically. If you selected data to be exported as a spreadsheet, it will take some time and will appear in the same folder that the program is stored in.

Quantification characteristics definitions (taken from the Track.m file)

1. SliceNum: slice number
2. trackID: ID number that identifies each track uniquely
3. x-coordinate: coordinate along the horizontal axis relative to the cell's starting position
4. y-coordinate: coordinate along the vertical axis relative to the cell's starting position
5. xIJ: original x coordinate from the ImageJ file
6. yIJ: original y coordinate from the ImageJ file
7. indDist: individual distance between current and previous slice
8. indAngle: individual angle between current and previous slice
9. accuDist: accumulated distance the cell has traveled since the starting slice
10. speed: speed between current and previous slice
11. euDist: distance between current and starting slice
12. runsTumbles: 1 if cell moved further away from origin since the previous slice, 0 if the cell moved closer
13. accuRunsPercent: accumulated percentage of runs
14. angles: angle of cell from the positive x-axis of its starting position
15. directionality: euclidean distance divided by accumulated distance, at that slice. Measure of path straightness.
16. xFMI: horizontal distance from starting point divided accumulated distance.
17. yFMI: vertical distance from starting point divided by accumulated distance.
18. vectorPolar (two data points per slice): distance and angle between two adjacent points
19. vectorCartesian(two data points per slice): vector between two adjacent points