# **Single-molecule diffusion and assembly on polymer-crowded lipid membranes**

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# **List of MATLAB code files**

* 1. Details for using the MATLAB files
     1. binding\_SPT: This MATLAB code finds out the number of particles in each frame as detected in u-track. INPUT: time\_frame (integration time of image acquisition) and the analyzed binding movie folders from u-track (see step 2). OUTPUT: time (total time of movie); particle\_no (Number of particles per frame). Plot the OUTPUT results and fit it onto a kinetic model to recover the binding rate.
     2. ISD\_analyzer: This MATLAB code plots the ISDs from trajectories found out in u-track analysis as heat maps, histograms, or violin plots. INPUT: data\_all analyzed movie folder by u-track; pixel\_size size of each pixel in micrometer. Two sets are included as supplementary data. Any other input is highlighted in the code. OUTPUT: Plots of ISDs as heat maps, histograms, or violin plots.

NOTE: Files such as uipickfiles, cbrewer, boundedline, and al\_goodplot must be downloaded from MATLAB file exchange website and added to MATLAB path.

* + 1. MSD\_ISD: This MATLAB code plots the MSDs from trajectories calculated from u-track analysis as line plots. INPUT: Same as for ISD\_analyzer. (Example data included with the **Supplementary Coding File 3**. Select all the 6 folders). OUTPUT: Plots of MSDs.
    2. drift\_correct\_images: For correcting drift in photobleaching movies.

NOTE: Tested in MATLAB 2017b, and the inputs are movies in .tif format.

* + 1. Extract\_traces\_1C: Package for analyzing intensity time trace of assembled structures in photobleaching movie (Sample data is included in the **Supplementary Coding File 3**). INPUT: movie files in tiff format. OUTPUT: Intensity time trace for each movie
    2. Stepcount\_immobile: Package for mapping x-y coordinates from u-track to raw movies. The raw movie should be at the same place where the analyzed u-track folder is. INPUT: u-track analyzed folder for photobleaching movies. OUTPUT: Intensity time trace for each movie

NOTE: stepDetection has to be downloaded5 and added to the MATLAB path.

* + 1. uTrack\_Int: Package for mapping x-y coordinates from u-track to raw movies. The raw movie should be at the same place where the analyzed u-track folder is. INPUT: u-track analyzed folder for photobleaching movies. OUTPUT: Intensity time trace for each movie and steps in the detected clusters in each movie.
    2. Step\_correction: MATLAB code for binomial correction for inaccurate labeling of biomolecules. INPUT: steps obtained from the MATLAB code Stepcount\_immobile. OUTPUT: Binomially corrected mass fractions of different oligomeric species.

NOTE: This code is written for a system where the maximum possible step size is 12. Corrections can be applied to other systems with different assemblies.

## Sample Data

* + 1. Two sets of data for DNA mobility on two different membranes as u-track analyzed folders.
    2. Photobleaching movie (**Video 3**) with u-track analyzed folder.

# **Steps in u-track GUI**

NOTE: Tested in MATLAB R2014b.

* 1. Start the GUI after putting the necessary codes in MATLAB path by calling the function uTrackPackageGUI from the command window. The window below will appear. Click on **New**.

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* 1. Click on **Import Movie using Bio-Formats** to load the movie. The movie should be in tiff format with the correct metadata. It can be seen or changed in ImageJ by checking the properties of the movie file.

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* 1. The movie will be loaded with the following window. Click on **Save** button.

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* 1. The above three steps can be repeated for loading multiple movies. Here we show the rest of the steps for one movie. Click on **Continue** button.

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* 1. Select **Single particles** from the three options. Click on **OK** button.

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* 1. Click on **Setting** button to input parameters for detection of particles.

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| 1. 2.7. Select **Gaussian Mixture-Model Fitting** from the available options for the detection method. Click on **Setting** button. |
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* 1. Make sure the different input parameters are correct.
     1. Frames to use: The number of frames to analyze. If it shows only one frame, then there is a problem with the metadata of the tiff file. Correct it in ImageJ by checking its properties.

* + 1. Gaussian Standard Deviation, input 1 pixels. Click on **Apply** button.

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* 1. Click on **Tracking button** setting as seen in step 2.6. Input the parameter for Maximum Gap to close as 0.

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* 1. Click on **Run** button to analyze the movie. When multiple movies are input check on **Run all Movies** and press **Run**.

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* 1. The following dialogue box appears on the completion of the analysis. Click on **OK.**

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* 1. In the main GUI green tick mark will appear when the movie analysis is done successfully.

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**References:**

Douglas Schwarz. uipickfiles: uigetfile on steroids https://www.mathworks.com/matlabcentral/fileexchange/10867-uipickfiles uigetfile-on-steroids, MATLAB Central File Exchange. Retrieved April 29, 2022 (2022).

Charles. cbrewer: colorbrewer schemes for Matlab https://www.mathworks.com/matlabcentral/fileexchange/34087-cbrewer-colorbrewer-schemes-for-matlab, MATLAB Central File Exchange. Retrieved April 29, 2022 (2022).

Kelly Kearney. boundedline.m https://github.com/kakearney/boundedline-pkg, GitHub. Retrieved April 29, 2022 (2022).

Antoine Legouhy. al\_goodplot - boxblot & violin plot (https://www.mathworks.com/matlabcentral/fileexchange/91790-al\_goodplot-boxblot-violin-plot), MATLAB Central File Exchange. Retrieved April 29, 2022 (2022).

McGuire, H., Aurousseau, M. R. P., Bowie, D., Blunck, R. Automating single subunit counting of membrane proteins in mammalian cells. *Journal of Biological Chemistry.* **287 (43)**, 35912–21 (2012).