

## TIRF matlab code intro

### **peaktest.m**

Identify the coordinates of spots in the input image, under a selected signal-to-background ratio (SBR, usually 1.3~3). Function inputs are image, SBR, offset (mostly not specified). The output will be coordinates of identified spots.

### **count\_neo.m**

Plot the image with identified spots highlighted, under the SBR setting used in “peaktest.m”. This script is not used in data analysis workflow, but rather used for quick visualization to decide if the SBR threshold is reasonable. To run the script, just specify the data file name to be analyzed.

### **traj\_extract.m**

Use “peaktest.m” to identify spots from a video data file, and extract the intensity trajectory from each spot. The portion of image frames that are used to identify spots can be tuned. Function input is data file name, and a matrix “bgm” used to adjust illumination non-uniformity. Output will be a matrix containing the time trace of all molecules.

### **traj\_extract1.m**

A simplified version of “traj\_extract.m”, only identify spots from the first frame of the video data file. This works for experiment with quencher, where the intensity of molecules are partially quenched. In this case, all the molecules have already showed up in the first frame.

### **cppass\_sec.m**

Identify “good” trajectories from the output of “traj\_extract.m”, use change-point algorithm to extract the on-time and off-time of each trajectory, based on the intensity change. The on-times from all the trajectories are combined into one output matrix “on”, same for off-times with the output “off”. Trajectories with at least certain number of change-points will be categorized as “good”, and stored in the output “pass”. The average intensity of each on/off event from all the trajectories are stored in the output “sec”.

### **CPcall.m**

Called by “cppass\_sec.m”, part of the change-point algorithm that calculate the likelihood ratio. Details please refer to 2005 JPCB (Watkins et. al.) and 2006 JPCB (Montiel et. al.) papers.

## **CriVal.m**

Called by “cppass\_sec.m”, part of the change-point algorithm that calculate the critical value based on the number of data points and error rate. Details please refer to 2005 JPCB (Watkins et. al.) and 2006 JPCB (Montiel et. al.) papers.

## **cpplot.m**

Plot the trajectories with their identified change points. Serve as a visualization of the trajectory quality and the performance of change-point algorithm. Function input will be the “pass” matrix from “cppass\_sec.m” function.

## **dweltime.m**

Perform single exponential fitting to the on/off times generated from “cppass\_sec.m”, and plot the fitting curve with fitted kinetics values. Select certain portion of the code for working with on or off times. Function input will be the on/off matrix and chosen number of bins for histogram (the fitting is against the histogram of on/off values).

## **bg\_extract.m** (optional)

Provide the average background intensity of each frame, where no molecules are detected. This could be used to correct the background level, if background intensity is observed to change along with time. Under current condition, the background intensity seems to slightly decrease along with time, on the timescale of mins. To run the script, just specify the data file name to be analyzed. And the generated “bg” matrix is to be checked.

Data analysis procedures:

- (1) set a reasonable SBR by using “count\_neo.m”.
- (2) run “traj\_extract.m” or “traj\_extract1.m” to obtain all the raw trajectories.
- (3) optional: evaluate background stability using “bg\_extract.m”.
- (4) run “cppass\_sec.m” to generate passed trajectories and identified on/off times.
- (5) check the trajectories manually by running “cpplot.m”.
- (6) Fit the on/off data by running “dweltime.m”.