The related programs have been updated to fit with the v1.53q version imageJ and version7 Trackmate.

Save the “spots.csv” file and “Track\_statistics.xlm” file from Trackmate for each movie either manually or using macro “Trackmate\_Levy\_Droplet\_tracking.ijm”. Pay attention to the ‘radius’ input in the macro which is different compared to the ‘diameter’ input in the user interface of Trackmate, since ‘radius’ should be one half of ‘diameter’. After trajectory ‘xlm’ file has been saved, open it individually using Excel (**open as read-only workbook**) and save them as ‘xlsx’ file for the next step. Sometimes, if error occurs during saving matrix into .mat file when running “result\_diffusion\_modi\_Tong(dt,1)”, try reducing the length of the modified excel names at this step.

First run “Filter\_traj\_exclude\_x\_y\_0.m”, this would search through x,y positions of all tracks within trajectory xlsx files and delete any trajectories that have either x or y positions recorded as 0, which is unreal in the tracked trajectories. The output is another xlsx file with the name as “<original filename>\_modi.xlsx”. Due to the naming requirements of Matlab variable, the head of this modified excel file cannot be set directly as read head of original table. This would require opening, replacing the first or first & second row with the original file first two rows and saving as the same modified file name after modified excel file has been saved.

Then run “result\_diffusion\_modi\_Tong(dt,1)”, dt is the time interval of the movie in the unit of second. 1 represents the unit conversion, which is not used (different from Mosaic) since the Trackmate output file has already included the real position unit instead of number of pixels. This will give the “result\_....mat” files which include the effective diffusion constant information.

Then run “Read\_TrackMate\_traj\_spots\_csv.m” this will extract spots information as averaged values across all time points within the trajectories, including Mean intensity, max intensity, and total droplet intensity. The output will be saved as “spots\_....mat”.

To connect spots intensity with their diffusion constants, ‘example\_plot\_D\_intensity.m’ can be used, where the output figures are Deff vs total\_intensity of selected spots (trajectory length > 10) and the histogram of total\_intensity of those selected spots. The input would require the name of the trajectory modified xlsx file and the name of “result\_” and “spots\_” mat files.