Explanation of program main_tracking.m

The program is modular and consists of the main file (main_tracking.m) which calls separate functions. All these functions are quickly described in the following, and the parameters which influence the performance of these functions are given plus a note where to change them.

After running of main_tracking.m, all parameters, setup, and results are saved in a Matlab Data file 'Results' in the created Results folder. These files can be opened and inspected in Matlab.

If you have any questions/comments/suggestions, please email Laura Weimann (laura.weimann@gmail.com).

1) The first function to be called is:
[setup]=spot_detection_real_data(parameters,stack_directory);

Description: It reads in TIFF video files, bandpass filters the files, and identifies spots in the filtered image data of each frame. The image of the first image frame (filtered data) pops up, and identified spot candidates are shown as red crosses. Then the corresponding raw data image pops up, with all spots which passed the SNR and size threshold. SNR values of the filtered and raw data are plotted onto the image. Spots are then localised in each frame via calculating their centroid position

Results: in the 'Results SPT Analysis' folder a subfolder with the experiment name is created, which contains a subfolder called 'spot detection Results', in which the coordinates of all identified spots are saved in .dat files. Per spot, 6 data entries are saved: x,y coordinate, Max Intensity filtered data, Max Intensity raw data, SNR filtered data, SNR raw data. A copy of the analysed image stack is saved in this folder as well.

Further, 6 control plots for the respective image stack are created and saved in the same folder: A histogram of the maximum intensity for all identified spots (1) raw and 2) filtered data), a histogram of the SNR for all identified spots (3) raw and 4) filtered data), the number of spots identified in the respective image frame over the frame number 5) after applying the initial intensity threshold and 6) after SNR and size exclusion.

Further, in the 'spot detection Results' folder 3 results plot comparing the respective image stacks are saved: 1) The absolute threshold (mean(image_stack) + Parameters.initialthreshold*std(image_stack), with image_stack containing the intensity values of the respective stack) is plotted over the image stack, 2) the mean value of the spots identified in the first image frame (raw data) is plotted over the image stack, 3) the mean particle-particle distance of particles identified in the first image frame is plotted as a function of the image stack.

Output: setup is a Structure Array containing the following information

M	Image Height
N	Image Width
K	# of images in video
density	Mean value of nearest neighbour distances of spots identified in the first image frame, first value refers to first video etc
directory	Directory of folder in which spot detection results

	are being saved
SNR	SNR of all identified spots in the first frame, SNR = (max_intensity-mean(background))/(sqrt(std(background)^2)); The background is defined as the 20*20 pixel image corners in the first filtered image frame. Applied to filtered data
SNR_raw	Same as SNR, but applied to raw data
SNR_mean	Mean value of SNR_raw
spots_initial_th	# spots identified in all frames after initial thresholding
spots_second_th	# spots identified in all frames after exclusion based on SNR and size
threshold	Absolut threshold value used for spot identification, see Parameters.initialthreshold below
stack_directory	Directory of folder in which data to be analysed is

Parameters:

Parameter Name	Where to change	Description	Recommendation
Parameters.lobject	Header of main_tracking.m	defines parameter for bpass function to cancel out the long wavelength noise	Integer length in pixels somewhat larger than a typical object.
Parameters.lnoise	Header of main_tracking.m	defines parameter for bpass function to cancel out the short wavelength noise	Characteristic lengthscale of noise in pixels. Additive noise averaged over this length should vanish. Set to 1.
Parameters.initialthreshold	Header of main_tracking.m	threshold for spot detection, number of std deviations above mean of background of filtered image. Background is defined as the mean intensity value of the whole image stack.	A low value will slow down analysis, a high value may miss true events. Try something between 3 and 10, and check the thresholding visually.
Parameters.pkfnd_sz	Header of main_tracking.m	defines diameter of spots in which	Odd integer only, cntrd_sz – 2

		only brighter one will be selected	
Parameters.cntrd_sz	Header of main_tracking.m	defines diameter of area of spots for centroid calculation defines	Odd integer only, number should be big enough to capture the whole particle but not so big that it captures others. A larger value makes the localisation more accurate. (recommended: 5,7) or even larger if density is very low
parameters.max_spot_size	Header of main_tracking.m	Maximum Spot Size [pixel] Which is still accepted	10-100
parameters.SNR = 2	Header of main_tracking.m	Minimum SNR of the filtered data which is still accepted	2-10, depends on data quality

2) The second function to be called is:
[results] = tracking_laura_real_data(parameters,setup);

Description: It reads in .dat files of the spot coordinates and connects particles in subsequent frames with their nearest neighbours. All particle trajectories regardless of the image frame in which they appear are recovered, and particle blinking can be taken into account as well (set parameters.memory accordingly, see below).

Results: In the respective results folder creative by function 1) another folder is created, called 'Results Tracking' which contains .dat files of all particle trajectories (6 entries: image frame, x,y coordinate, Max Intensity raw data, SNR filtered data, SNR raw data) . A matlab figure (Trajectory Ensemble) showing all trajectories is created and an .avi file (track.avi) showing the tracking results on raw data. The number refers to the trajectory number. Two control histograms are created and saved in the respective folder: 1) a histogram of the track lengths [frames] and 2) of the mean SNR raw data per trajectory.

Output: results is a Structure Array containing the following information

meanSNRrawpertrack	Mean SNR per trajectory, raw data
meantracklength	Mean length of trajectories

Parameters:

Parameter Name	Where to change	Description	Recommendation
parameters.minLength	Header of	defines minimal	3-10

	main_tracking.m	length of tracks to be kept [frames]	
Parameters.max_step	Header of main_tracking.m	defines maximal distance at which 2 spots are linked in subsequent frames [pixels]	an estimate of the maximum distance that a particle would move in a single time interval
Parameters.memory	Header of main_tracking.m	# of time steps a particle can be lost and recovered again	0-4, depends on the data quality
Parameters.withvideo	Header of main_tracking.m	Will create .avi video file if set to 1	
Parameters.fbs	Header of main_tracking.m	defines Frames per second for output videos	10

3) [results] = msd_main_laura_real_data(parameters,param_guess1,param_guess2,param_guess3,results,setup);

Description: This function performs an MSD and subsequently a JD analysis on the trajectory data. The MSD analysis yields a single MSD value for each trajectory, whereas the JD analysis analyses an ensemble of trajectories. The advantage of the JD analysis is that it allows to resolve different mobility populations in the data. For more information see: http://www.plosone.org/article/authors/info%3Adoi%2F10.1371%2Fjournal.pone.0064287;jsessionid=074F3CF35E284B5DDC8D6037A82D4542

Results: In the 'Results SPT Analysis/experiment name' folder two subfolders, MSD Analysis Results and JD Analysis Results are created.

MSD Analysis Results:

MSD-values per trajectory are obtained and summarised in a histogram, on a normal and on a logarithmic scale. The MSD intercept can be used to estimate the localisation precision obtained, and a histogram of all standard deviations as a measure for the localisation precision is obtained. Further two scatterplots are created and saved, testing for a potential correlation between the respective standard deviation/Diffusion coefficient and the respective trajectory length. Finally, the average over the MSDs of all trajectories is obtained and an ensemble MSD value is obtained by fitting the average MSD using a weighted linear fit (MSD_mean).

JD Analysis Results:

Histograms and cumulative histograms of jump distances are obtained and the fit results are saved. Depending on the setting of parameters.number_populations, 1-3 mobility populations are fitted to the data and the resulting figures are saved.

Output: results is a Structure Array containing the following information

meanSNRrawpertrack	Mean SNR per trajectory, raw data
meantracklength	Mean length of trajectories
D_JD_1pop	Diffusion coefficient assuming only 1 mobility population
f_JD_1pop	Fraction of particles in mobility mode 1, 1 by definition
D_JD_2pop	Diffusion coefficients assuming 2 mobility populations
f_JD_2pop	Fraction of particles in mobility modes defined by D_JD_2pop
D_JD_3pop	Diffusion coefficients assuming 3 mobility populations
f_JD_3pop	Fraction of particles in mobility modes defined by D_JD_3pop
jump_distances	All jump distances obtained from the trajectory data
D_MSD	Mean diffusion coefficient of all individually obtained diffusion coefficients, as obtained by MSD analysis
D_ensemble	Diffusion coefficient obtained by averaging MSD-values over whole ensemble, and subsequent linear fit
diff_coefficients	All diffusion coefficients obtained from the trajectory data by means of the MSD approach
n_cell	# video stacks for analysis
n_tracks	# trajectories for analysis

Parameters: MSD Analysis

Parameter Name	Where to change	Description	Recommendation
parameters.threshold_tracklength	Header of main_tracking.m	Defines minimum trajectory length for tracks considered for MSD analysis	5-10
Parameters.step	Header of main_tracking.m	Number of time points for calculating MSD and for linear fitting.	A small value (2-5) yields short term diffusion coefficients, large values yield long-term diffusion coefficients, set to approximately of the average

			trajectory length
parameters.n_fit	Header of main_tracking.m	Number of points for fitting the ensemble plot averaged over all trajectories	Should be smaller or equal to parameters.step
parameters.bool_D	Header of main_tracking.m	If set to 1, for every single trajectory Parameters.step is determined individually by step = trajectory_length/4. If trajectory_length/4 < 3, step is set to 3. If set to 0, the same step as defined by Parameters.step is used.	
parameters.show_single_MSD_plots		If set to 1, the linear fits of all trajectories are shown	Set to 1 only if total numbers of trajectories is small, otherwise too many Matlab figures are created.

Parameters: JD Analysis

Parameter Name	Where to change	Description	Recommendation
parameters.number_populations	Header of main_tracking.m	Number of mobility populations expected in the data	1-3, fitting for values larger than 4 not implemented
param_guess1(1)	Header of main_tracking.m	Expected value for diffusion coefficient D of population [in mu^2/s]	0.01 – 1
param_guess2(1)	Header of main_tracking.m	Expected value for diffusion coefficient D of first mobility population [in mu^2/s]	0.01 – 1

param_guess2(2)	Header of main_tracking.m	Expected value for particle fraction of first mobility population	0.1-0.9
param_guess2(3)	Header of main_tracking.m	Expected value for diffusion coefficient D of second mobility population [in mu^2/s]	0.01 – 1
param_guess3(1)	Header of main_tracking.m	Expected value for diffusion coefficient D of first mobility population [in mu^2/s]	0.01 – 1
param_guess3(2)	Header of main_tracking.m	Expected value for particle fraction of first mobility population	0.1-0.9
param_guess3(3)	Header of main_tracking.m	Expected value for diffusion coefficient D of second mobility population [in mu^2/s]	0.01 – 1
param_guess3(4)	Header of main_tracking.m	Expected value for particle fraction of second mobility population	0.1-0.9
param_guess3(5)	Header of main_tracking.m	Expected value for diffusion coefficient D of third mobility population [in mu^2/s]	0.01 – 1