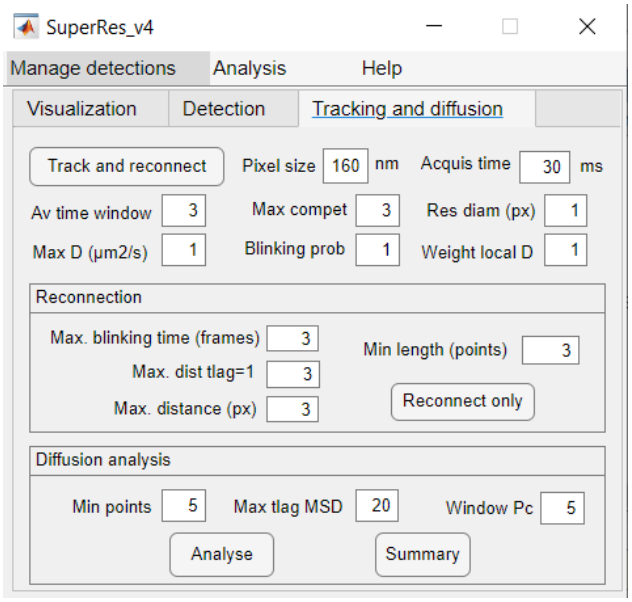
Tracking and diffusion



This tab launches the tracking and reconnection of trajectories for live cell SMLM (sptPALM, uPAINT, etc) acquisitions. It also calls a routine to analyse lateral diffusion.

The tracking routine is based on the MTT algorithm (Arnaud et al 2009). Several additional parameters will be needed:

Average time window:

In frames. Time window to average previous D value (and decide which is the most probable next position).

Max. diffusion coefficient:

Maximum allowed D to look for the next position.

Max competitors:

Maximum number of candidates for the next position.

Blinking probability:

From 0 (no blinking) to 1 (100% probability to have at least one blinking period).

Research diameter:

Maximum distance around a detection to look for the next position.

Weight max. local diffusion:

From 0: D is perfectly homogeneous all over the space, to 1: diffusion can vary locally.

After tracking, the algorithm tries to connect (reconnect) trajectories that are likely to belong to the same molecule. To allow the connection, the algorithm uses criteria in time (number of images without signal) and space (the distance roamed during blinking). More precisely, in each iteration, the last position of the molecule of the first trajectory is connected to the first position of the closest following trajectory, only if it is within a

time and a spatial limit. The same procedure is used for all trajectories and repeated iteratively until no connexions are allowed.

The reconnection parameters are:

Max. blinking time:

Maximum time allowed for one period of blinking.

Max. distance during blinking:

Maximum distance allowed to travel during a blinking period.

Maximum distance for tlag=1:

Maximum distance allowed for the first displacement.

Minimum length of trajectories:

Minimum length required to keep a trajectory, in frames.

Format of results:

Files with the information about the trajectories are saved in the trc folder.

There can be several .trc files, depending on the different steps of the analysis that were performed.

1) Initial tracking : *name*.trc

molecule #	image #	x position	y position	intensity
1.0000000e+000	1.0000000e+000	1.5381968e+002	1.5974734e+002	1.9923065e-001
1.0000000e+000	2.0000000e+000	1.5390920e+002	1.5970688e+002	1.9902105e-001
1.0000000e+000	5.0000000e+000	1.5407786e+002	1.5973974e+002	1.9938376e-001
1.0000000e+000	6.0000000e+000	1.5401896e+002	1.5974573e+002	1.9800292e-001
1.0000000e+000	7.0000000e+000	1.5425990e+002	1.5977814e+002	1.9771094e-001
1.0000000e+000	8.0000000e+000	1.5450888e+002	1.5968371e+002	1.9281971e-001
1.0000000e+000	9.0000000e+000	1.5400512e+002	1.5981614e+002	1.9728599e-001
1.0000000e+000	1.0000000e+001	1.5428252e+002	1.5971045e+002	-1.0000000e+000
2.0000000e+000	1.0000000e+000	1.3310520e+002	6.1945936e+001	1.9910794e-001
2.0000000e+000	2.0000000e+000	1.3321505e+002	6.1895810e+001	1.9945456e-001
2.0000000e+000	3.0000000e+000	1.3320545e+002	6.1871880e+001	1.9905264e-001
2.0000000e+000	4.0000000e+000	1.3326024e+002	6.1989354e+001	1.9899402e-001

2) Reconnection: the initial trajectories are connected if they are likely to belong to the same molecule (important if the fluorescent tracers blink, for exemple). Name: *name*.con.trc. These files have the same format than .trc files.

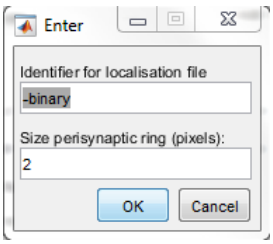
3) Localization: *name*.loc.trc. The colocalization or not with domains in an image is coded in the sixth column (see below)

Localize over domains:

It adds the information of localization on .trc files. The localization is coded in the sixth column of trc files:

0: out of domains

1..n: colocalizes with the domain number 1…n.



The image of domains is automatically recognized thank to the **identifier**. Be sure that this image has the name of the movie followed by the identifier: in the example above *name*-binary.tif.

A peri-domain (perisynaptic) ring can be defined, with the size introduced in the window above.

This routine saves also a .mat file (*name*-**identifier**-num.mat) containing the information of the domains (number) as it appears in the figure that pops up.

Diffusion analysis:

For each trajectory of the .trc file/s selected with **Load files**, this program will calculate:

- MSD and D for the whole trajectory
- The packing coefficient and the area per trajectory (convex hull).

If there is the information about localization (in and out domains) trajectories will be sorted (and eventually chopped) into the two groups (in and out) and the diffusion data will be calculated on each of these segments. Dwell time (time spent in the domain) is calculated as well.

Only trajectories with more than the minimum length **Min points** are considered. Only the first **Max tlag MSD** points will be kept (useful to accelerate analysis if trajectories are long).

Window Pc sets the duration in frames of the sliding window for Pc calculation. There will be only one value of Pc for each trajectory (mean value).

Click on **Analyze** to start. Results are stored in .tns file (one per movie), saved in \diff folder. **Summary** will compile results (for one or more experiments), creating the following files:

- Name-Dtotalextra.txt, Name-Dtotalsyn.txt :

# movie	# traj	# of traj	D	localization
1	5	1	1.8563974e-02	0
1	8	1	6.1774565e-02	0
1	9	2	4.9718861e-02	0
…	…	…	…	…

- Name-msdtotalextra.txt, Name- msdtotalsyn.txt : All the kept msd values for each trajectory or trajectory segment (each segment on one column).

Tlag (ms)	MSD traj 1	MSD traj 2	MSD traj 3
0	# of syn	# of syn	# of syn
0	0	0	0
30	1.9321813e-02	1.2708363e-02	1.9684515e-05
60	1.9321813e-02	3.0052050e-02	4.6548877e-05
…	…	…	…

- Name-meanmsdextra.txt, Name-meanmsdsyn.txt: MSD averaged over all the trajectories.

tlag	Mean MSD	S.D.	s.e.m.	Median MSD	n
0	0	0	0	0	0
30	1.9321813e-02	1.2708363e-02	1.9684515e-05	2.0913985e-02	4.1680200e+05
60	4.1123675e-02	3.0052050e-02	4.6548877e-05	4.1453423e-02	4.1680200e+05
90	6.0897525e-02	4.8641526e-02	7.5342894e-05	5.8989343e-02	4.1680200e+05
…	…	…	…	…	

- Name-length.txt: length of all the trajectories.