Getting started

There is no installation program. Nevertheless, there is one program that must be called only once before SPTrack_v6 is run for the first time. This program saves the path of the parameters needed to perform the tracking.

After setting the path in MatLab:

Type in the command window:

```
> SPTrackinit
```

If the initialization was successful, you will have the confirmation:

```
SPTrack_v6 path saved in C:\Matlab\SPTinit_v6.mat
SPTrack initialization completed
```

Don't forget to re-start MATLAB after the initialization before running SPTrack_v6.

SPTrack_v6

The programs called by SPTrack_v6.m perform the detection, tracking and basic diffusion analysis of fluorescent signals coming from single-emitters like single fluorophores or quantum dots. See the help file 'Analysing SPT data' and 'Insights to SPT procedures' to learn how to run analysis procedures.

The help file 'Insights into SPT' describes single particle tracking more deeply. Briefly, the fluorescent signals (peaks) are fitted to a two-dimensional Gaussian surface. The peaks are compared to cut-off values to select the ones arising from single molecules. The retained peaks are tracked along the series of images for an initial tracking. The initial trajectories are reconnected to overcome the blinking periods or the periods were the molecule went out of the focal plane.

The positions of the particles can be compared to a localization matrix (an image of domains, i.e. synaptic labelling). This localization procedure assigns a value to each point of the trajectory depending on its localization.

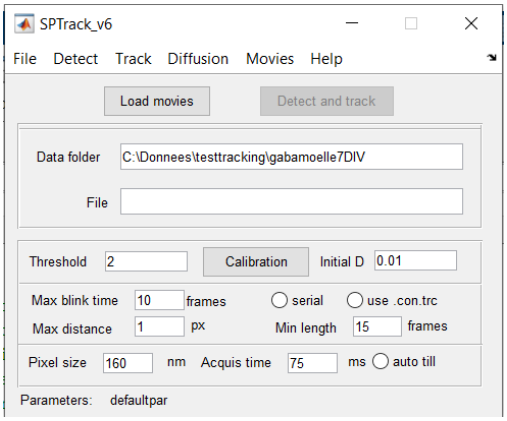
The tracking results can be visualized overlying trajectories on top of the images used for the analysis. See 'Visualization' for more details.

The final trajectories are used to calculate parameters for diffusion analysis such as the mean square displacement (*MSD*) and the diffusion coefficient (*D*). There are several options to analyse diffusion with the tracking results of SPTrack_v6. Some typical analyses are described in the help file 'How to survive the flood of numbers'.

1) Main window

The main window displays the name of the file/s that are analysed and parameters needed for detection, tracking and diffusion analysis. See 'Choosing parameters' for an explanation of each one of them.

Click on '**Detect and track**' to perform detection and tracking at once. It is also possible to launch separately the different parts of the analysis using the menus (see below).



2) Menus

1 – File

File operations:

- **Load parameters**: To load a previously saved set of parameters.
- **Save parameters**: Save the parameters of the main window with a given name.
- **Save report**: save a *.txt file containing data about parameters and results of tracking.
- **Quit**

2 – Detect:

- **Detection of particles**:

  It finds single particles on a .stk or .tif movie using the **threshold** value entered in the main window. There are some other parameters that can be set using **Calibrate**. It saves the results on a .pk file in pk folder. Note: not all the peaks that are detected will be used to create the trajectories: there are quality cut-offs to select only those (most probably) coming from single molecules.

- **Test of signals**:

  It makes histograms of intensity of peaks, background intensity (offset) and the width of the peaks using the data of *.pk files. These tests are useful to control the quality of labelling.

3 – Track:

- **Initial tracking**:

  Once the detection done, this option does the initial tracking on the objects (detections) that were saved in *.pk files. It connects objects that are present in consecutive images. The resulting trajectories are left short on purpose to increase computing efficiency. It creates a *.trc and a *.traj file (non-linked), and saves them in the \trc and \traj folders, respectively. It uses the **Initial D** parameter.

- **Automatic reconnection**:

  It calls the script that elongates automatically the trajectories, using the corresponding parameters **Max blink time** and **Max distance** that appear on the

main window. It creates .con.trc files and appends .traj files with the reconnected (linked) trajectories.

- **Manual reconnection**:

  It opens another window to perform the manual reconnection of trajectories. This program connects between them the trajectories that were selected manually. It also allows the correction of the trajectories.

- **Localize over domains**:

  It adds the information of localization on .traj files, by comparing the (x,y) position of the particle with respect to a localization image (previously segmented).

- **Clean trajectories**:

  It is useful to discard badly constructed trajectories or trajectories constructed over objects that are not the desired ones (but spurious detections due to autofluorescence, non-specific labelling, etc.). It also allows to select a given group of trajectories to be analysed; for example, on a particular cell or cellular compartment.

3- Diffusion:

- **Analysis on individual trajectories**:

  It calculates diffusion parameters for each trajectory. If the trajectory has information about the localization, the different segments of the trajectory that correspond to each localization are analysed separately. It saves the results in a separate folder called ‘diff’.

- **D vs time:**

  It calculates D over a sliding window to display instantaneous D in function of time.

- **Statistics**:

  Creates cumulative frequency histograms, performs Kolmogorov-Smirnov and Student t tests on excel or .txt files.

4- Movies:

The **Visualization** option allows plotting the trajectories on top of images (movies or not) or just visualizing the images, merged or not.

5- Help

That’s how you are reading this!