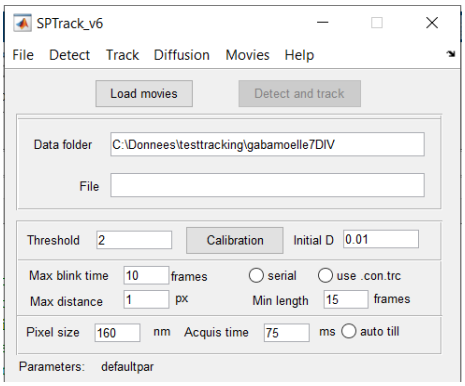
Choosing the parameters



When SPTrack_v6 starts, it automatically loads the default parameters, normally those used in the previous analysis session. It is possible to load other previously saved parameters using **File: Load Parameters** (files *.par). The values can be changed in the window, and saved with **File: Save Parameters**. Each time the analysis is finished, the actual values showed on the window are saved as default (file defaultpar.par).

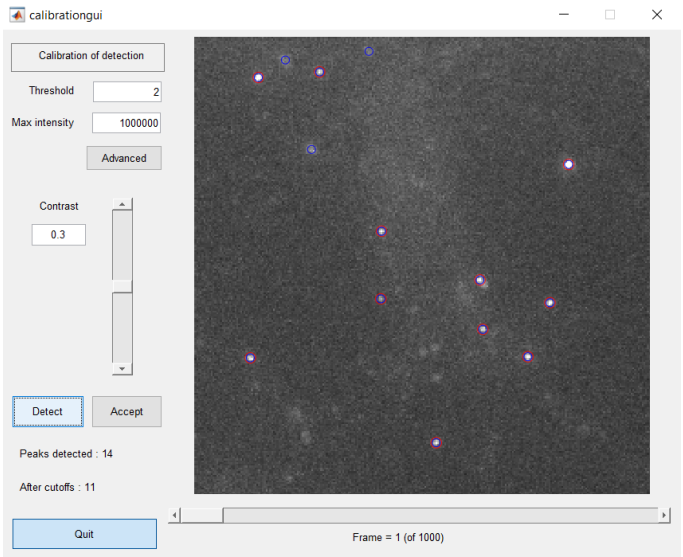
Other parameters are saved in a Matlab file (detecoptions.mat). These are the parameters for doing the Gaussian fitting and various statistical tests for the detection and tracking.

Please note that some of them can be changed by the 'advanced' button on the calibration window. These values will not be conserved once the SPTrack_v6 window is closed.

Peak detection

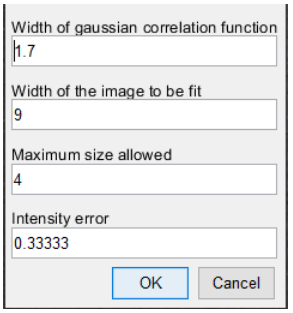
Threshold: changes the number of peaks detected (the sensibility of the detection).

On the **Calibration** window: **Max intensity** is the maximum intensity allowed for peaks.



The **Advanced parameters** are:

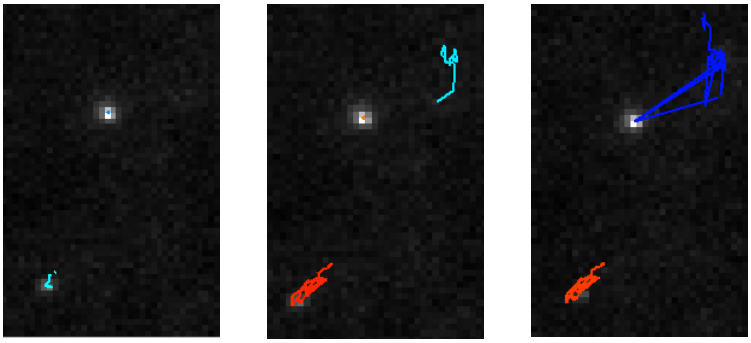
- Width of gaussian correlation function: FWHM (full width at half maximum) of the 2D gaussian function used to fit signals (corresponds to the PSF of the set-up), in pixels.



- Width of the image to be fit: the size of the square containing the signal to be fit, in pixels.
- Maximum size allowed: maximum size of the detected peak, in pixels.
- Intensity error: maximum difference between the expected peak (PSF) height and that of the detected peak.

Initial tracking

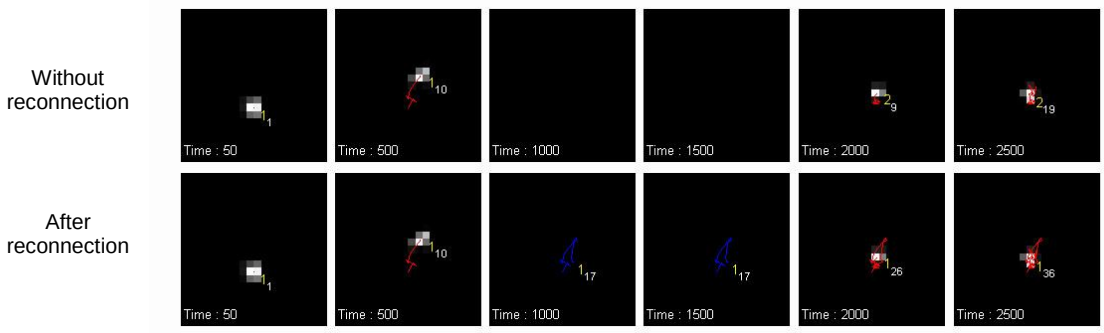
Initial D: It indicates the area around the peak where the next one (on the next image) will be looked for. This parameter MUST be controlled. A value too big can lead to wrong connections between different objects and a value too low will cause losing of the molecules that move faster:



The tracking results of the same quantum dots using different values of initial D. With D=0.001 (left), the trajectories are short and the faster particles are lost. D=0.04 (center) shows a correct tracking. D=1 (right) caused wrong connections between different particles.

Reconnection

- **Max blinking**: is the maximum number of images without signal. A trajectory that end at time t1 will be connected to a trajectory that starts at time t2 only if the difference between t2 and t1 is less than this value.
- **Max dist**: the maximum distance to look for the next point of the trajectory after the max blinking time.



Example of reconnection. The blue trajectory indicates the blinking period. The number in yellow is the number of the trajectory and the number in white, the amount of points of the trajectory at this image.

- If you choose to perform **serial** reconnections, you are asked to enter the series of values for the previous parameters (in this order: Max blinking, Max dist). In general,

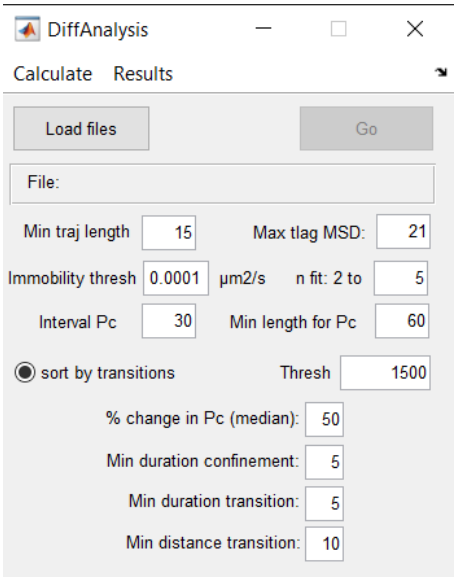
serial reconnections (with progressively growing Max blinking and Max dist) provide better results than single reconnections.

- Using **.con.trc**, you can complete a previous reconnection. When this option is not clicked, the reconnection is done on the initial tracking .trc file.
- **Min lenght**: after the reconnection, all the trajectories having less that this number of points will be discarded. This value depends upon the type of diffusion analysis that will be performed. See the help file “Surviving the flood of numbers”.

Diffusion analysis

- **Acquis. Time**: time between frames, in ms. Depending on the metadata of the movie file, it may be extracted from the movie if you run the detection with the option ‘**auto till**’ clicked.
- **Pixel size**: in nm.

Other parameters (in DiffAnalysis window):



- **Points to fit**: number of points of the MSD function used to perform the linear fit to calculate D. The common agreement is that the best number of points is 5.
- **Immobility threshold**: The smallest value of D considered to keep the trajectory. It corresponds to the value obtained after doing SPT analysis on immobile tracers (for example, quantum dots dried on a coverslip).

Packing coefficient analysis

Packing coefficient (Pc) calculation is done on a sliding window, the length (in frames) of this window is set with **Interval Pc**. As in the case of the calculation of D, a short window will provide more noisy values but it may detect transient changes in diffusive behaviour. A length of 30 frames is set as default but you can reduce this size up to 5-10 frames. Depending on the length of this window, you may change the **Min length for Pc**, that obviously must be longer.

The choice of Pc threshold (Thresh) depends on which kind of transition in diffusive behaviour you want to detect. The first thing to do is to try one (for example, the default one) to have a glimpse on the values of Pc observed in your data. Use **Plot trajectories** option in the menu of DiffAnalysis window to have a graphical display of Pc over time for each trajectory. With this in mind, the pointing accuracy of your set-up and the relationship L-Pc, you can find a threshold that is meaningful. If you want to detect immobilizations, you will choose the Pc value corresponding to a confinement area size L = pointing accuracy.

However, Pc will also vary due to statistical variations that are present also in perfectly Brownian trajectories. Thus, to avoid spurious classification, trajectories are considered as displaying transitions only if the Pc values are above the threshold during **Min duration confinement** frames, with a change in Pc values above **% change in Pc**, with a transition that lasted at least **Min duration transition** frames, and a minimum distance between transition of **Min distance transition** (in nm). All these values have to be set to be meaningful to the transitions that you want to detect.