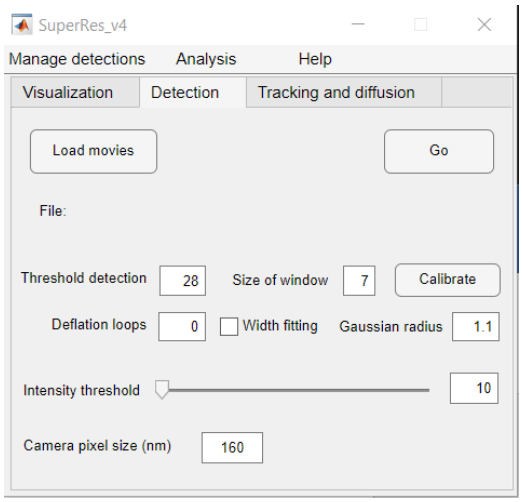
Detection



Tab for the detection of single molecules, based on Multiple-target tracing (MTT) algorithm (Arnaud et al 2008, Nat Methods. 2008 5:687-94. doi: 10.1038/nmeth.1233).

Load Movies: select one or more .tif files. The name of the **File** that is being analysed appears on the window. Please check the **Camera pixel size**.

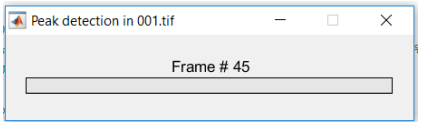
The **Intensity threshold** allows to keep only the detections above a certain intensity (level calculated on the whole image).

Regarding the quality of the detection, there are several parameters to adjust in the window:

- **Threshold detection**: it is the minimum relative intensity value; it allows to exclude detections that are too weak. It is calculated only in the surroundings (detection window) of the detection.
- **Size of window**: size in pixels of the detection window (side). The gaussian fitting will be done on intensity data from this window.
- **Gaussian radius** and **Width fitting**: The half-width of the Gaussian curve used to fit the signals is set by Gaussian radius. It can be fixed or set automatically from the data with **Width fitting**.
- **Deflation loops**: increasing this number may improve detection when the labelling density is high.

The best way to set these parameters is to use **Calibrate** (see below).

Go will launch the detection sequence using the parameters on the window. When the detection procedure starts, a waiting bar indicates the progression of the detection process. Close it to stop the run if needed.



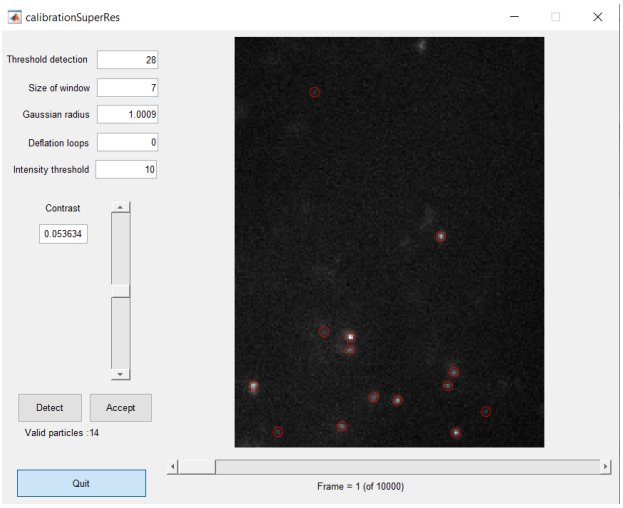
Calibration of the detection

Detection parameters should be verified and optimized for each series of movies. In order to verify the detection parameters, use **Calibrate**. This opens a new window where it is possible to visualize image per image seeing the detected peaks.

Use the **Contrast** value/bar to adjust the visualisation. Each time you change the frame number, click on **Detect** to see the result.

The number of valid particles is shown on the window, together with the identification of detections on the image (red circles).

The parameters selected with Calibrate will be transferred to the Detect window when you click on **Accept**.



The successive parameters and number of valid detections (particules) found in the present image are shown in the command window:

```
Calibration on file g2-001a.tif

Frame # 1
Threshold alpha: 10
Threshold detection: 28
Window size: 7
Fit of gaussian free, radius obtained: 1.0009
Deflation loops: 0

Valid particles: 14
```

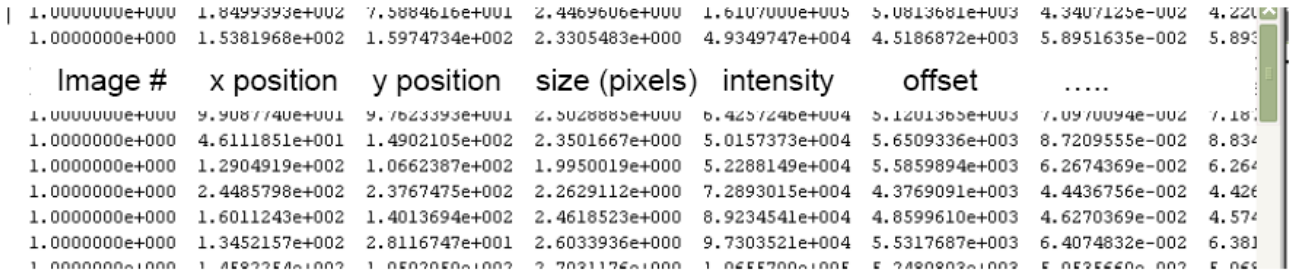
Results

Format of results:

1) .mat file, readable only by Matlab. Saved in the main folder.

2) .pk files (in pk folder): .txt file with the information about the peaks that were detected (all of them, independently of cut-offs).

The format of this files (moviename.pk) is:



If there is a drift of the microscope stage, perform Correct of stage drift before pursuing the analysis!