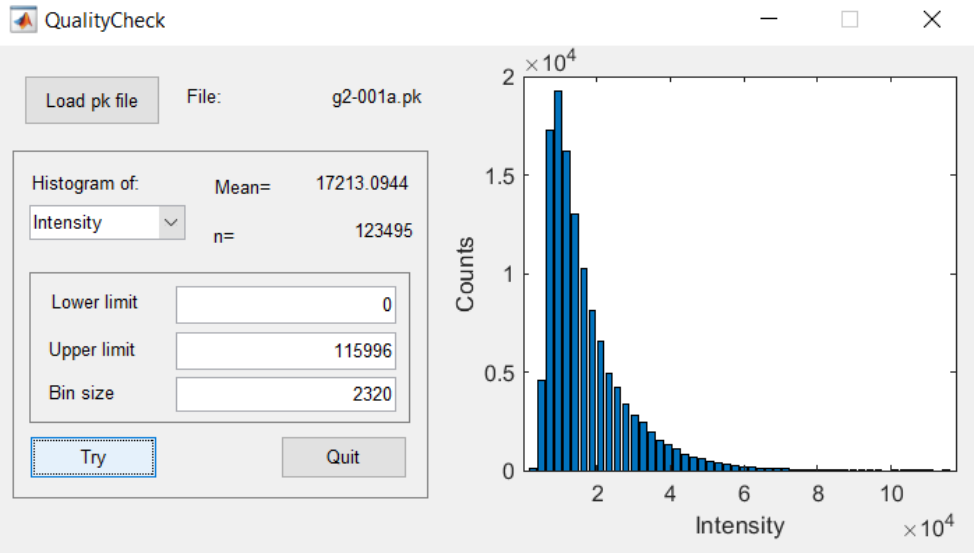
Manage detections

1. Quality of detections

This program plots histograms of the intensity of the peaks, their width and the offset (noise). These are important features to assess the quality of labelling and detection.



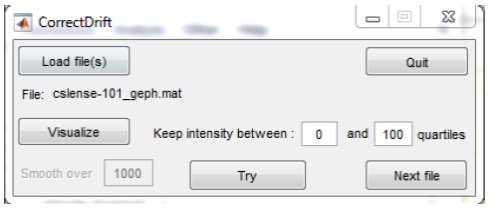
Load the .pk file
Choose the data (**Histogram** of intensity, offset or width)
Try a first histogram
Set another limits and bin size if necessary.

Intensity histogram:
Ideally it has to be a normal distribution with only one local maximum that corresponds to the intensity of single particles. The appearance of another peak at higher intensities is an indication of the presence of detections of multiple tracers together.

Width histogram:
The detection procedure is optimized for objects that are 2-4 pixels wide. If you do not get similar values, you may have detected objects that are not single particles, or your single particles do not have the good size (check the pixel size).

Offset histogram:
It shows the distribution of intensities of the background: the infamous noise.

2. Correct stage drift

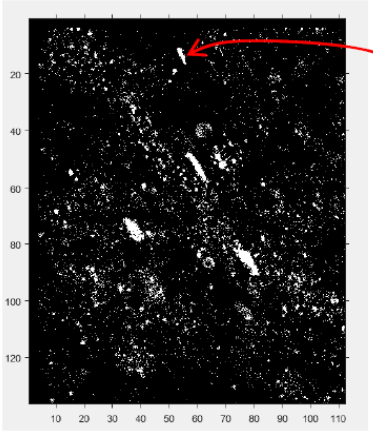


This step is needed to verify and correct the movements of the stage. The correction is based on the alignment of detections of immobile objects (for example beads).

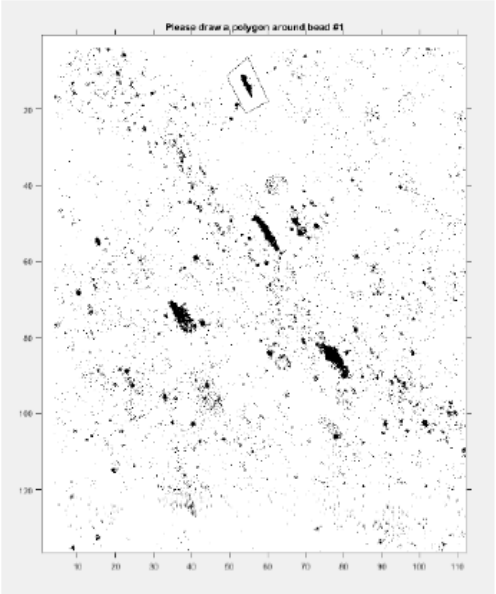
This correction has to be done after the detection and before any analysis!

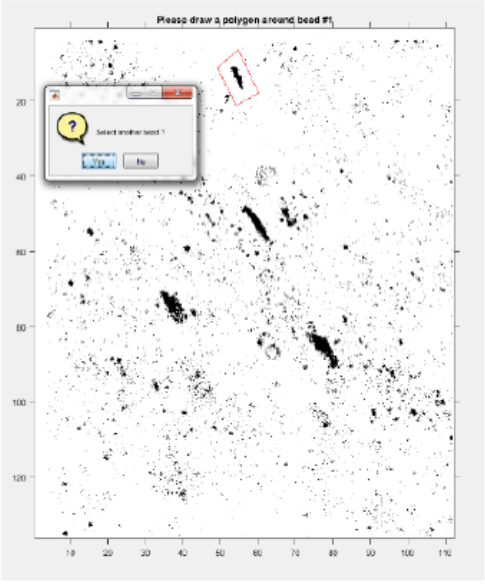
After loading the .mat file (after detection) with **Load file**, use **Visualize** to see the original distribution of detections. Identify beads or objects that can be used as a reference for the drift. To eliminate too weak or too strong detections, use **Keep intensity between**…

Try starts the alignment routine: draw an ROI around the detections to be used as reference (i.e. fluorescent beads). Close the ROI double clicking.

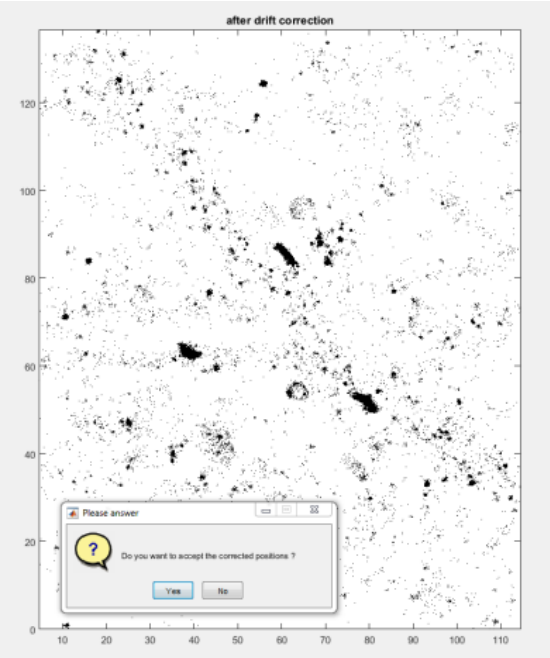


Detections from a fluorescent bead

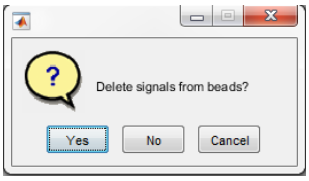




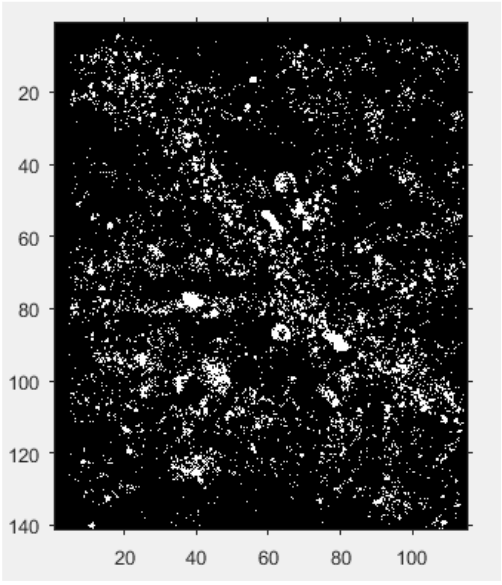
Select as many objects (containing enough number of detections each) as needed, one at a time. More objects means better correction.
Once the selection done, the result is shown in a new figure. If the result is not satisfying, start again...



If the correction could not be done (i.e. the selection does not contain enough points), a message will give you the posibility to make other selection, to save the data as it is or to quit this image.
Before saving the results, you may want to delete signals from the objects (beads…). However, take into account that these signals may be helpul afterwards, for example in case of color correction.



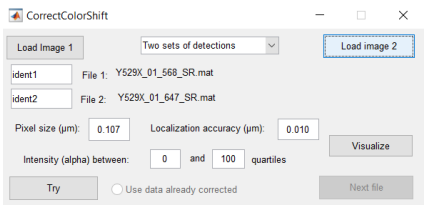
If the result is accepted, the new .mat and .pk files are saved in a folder “driftcorrected”. Remember to continue the analysis using this corrected file!



If several files were selected with Load file, **Next file** will load the next one.

3. Correct color drift

This routine allows the colocalisation of two super-resolved images or one super-resolved image and one regular fluorescence image. As different colors have different paths through the optics of the microscope, and extra stage drifts could happen between the registering of two sets of data, it is necessary to verify and correct any shift between the images before co-localization analysis.



Select one among the two possible datasets:

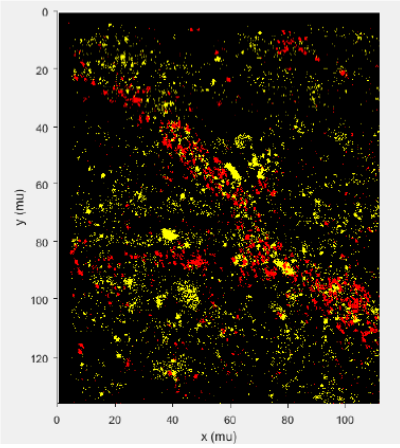
- **Two sets of detections**: data from two single molecule detection files (.mat).

- **One set of detections vs one image**: data from one single molecule detection files (.mat) and one regular fluorescent image. In this case, file 1 is a .mat file (detections) and file 2 a .tif file (image).

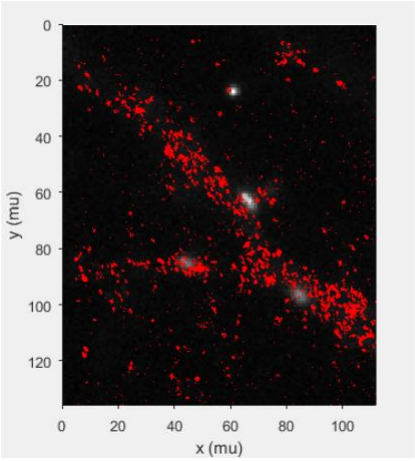
The identification of both images (file 1 and 2) is done thanks to the identifiers that you can enter through the window. If the identifiers (in the example, "_568" and "_647" in the example) are correctly chosen, when you chose the first file or series of files with **Load Image 1**, the second file should be found automatically. If this is not the case, load the second file with **Change image 2**.

To just see data, use Visualize.

Example for "two sets of detections"

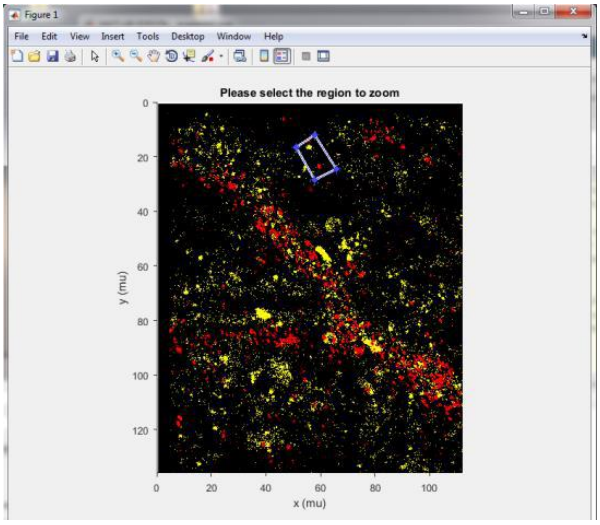


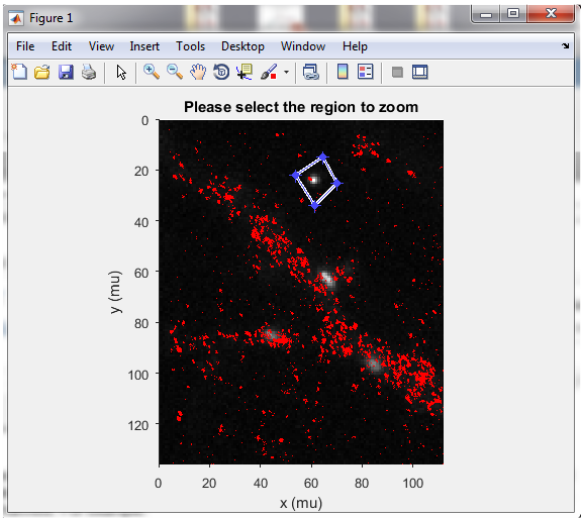
Example for "one set of detections and one image"



To proceed to the correction, press "Try". You will be asked to select the region that will be used to calculate the shift.

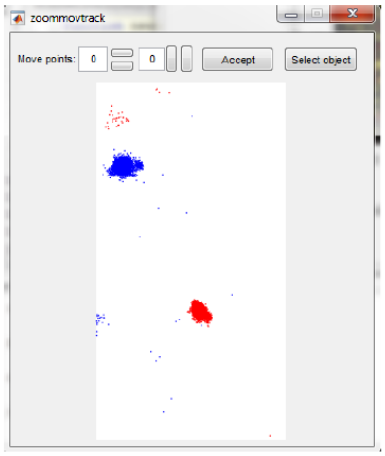
Typically this corresponds to the area where a fluorescent bead is observed, on both channels. In the previous examples:



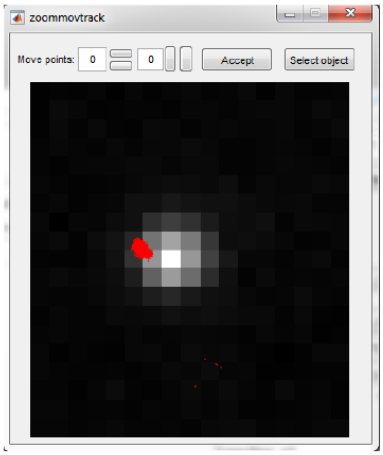


Double click to finish the selection. A new window will open showing the zoomed region:

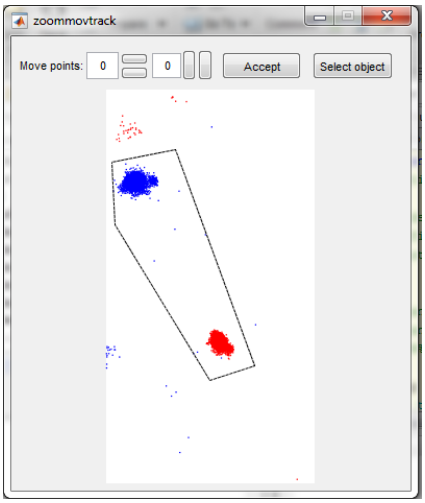
Two sets of detections

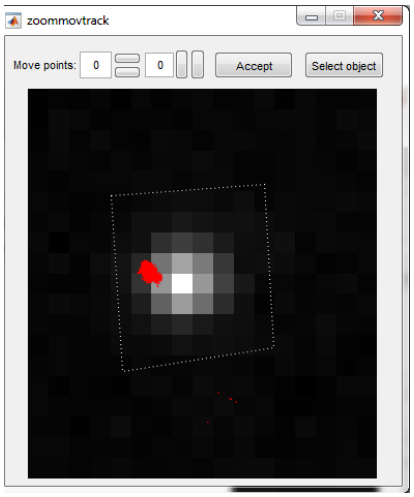


One set and one image

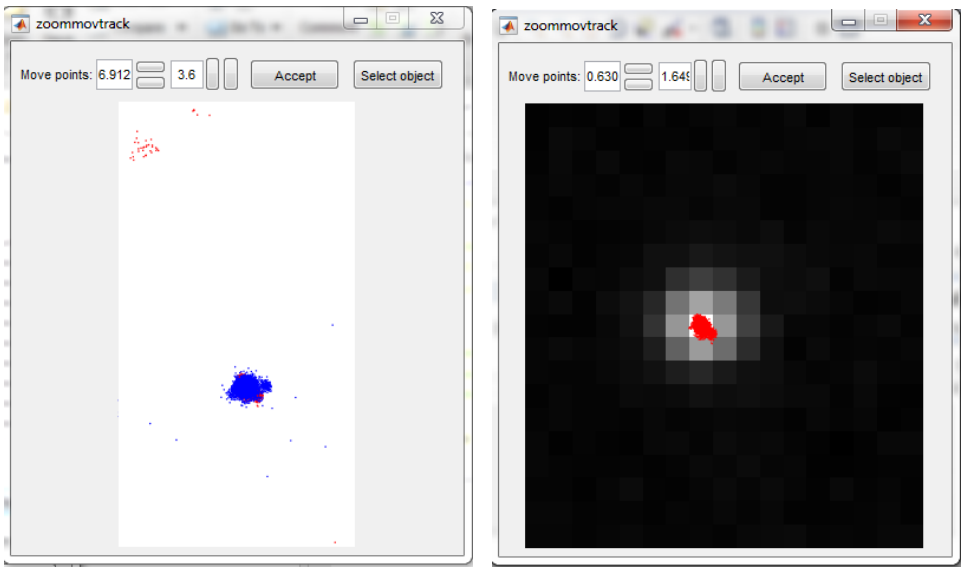


Move detections using the buttons on the top (**Move points**), or select and area containing a clear cloud of points for the/each set of detections using **Select object**:





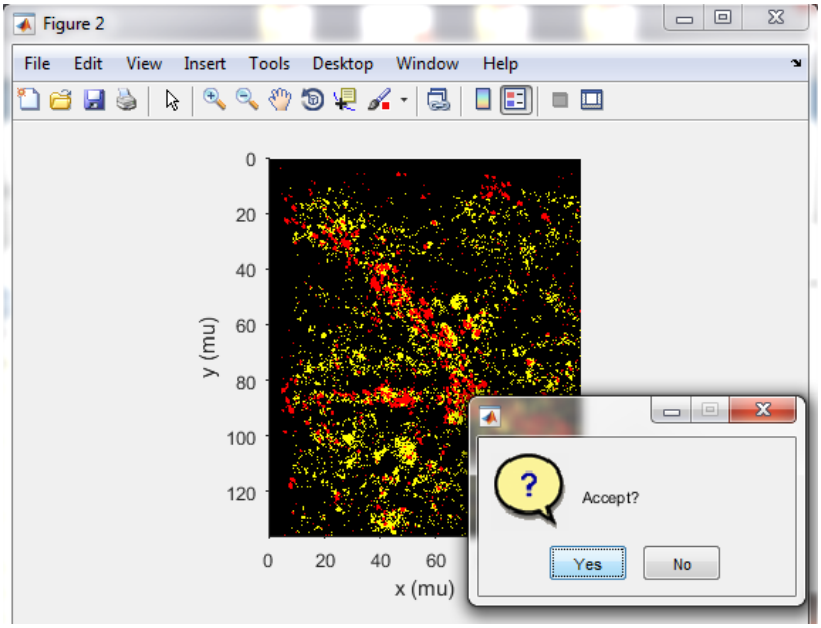
In this case the correction will be done automatically (both clouds of points will be overlaid):



If you keep the correction, press **Accept**. Otherwise, just close the window.

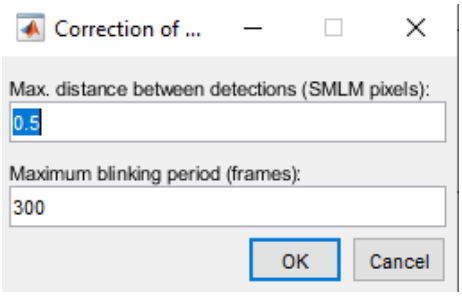
A final verification will be shown on the entire image. Clicking “Yes” will create a new file(s) of detections in the folder “colorcorrected”.

Do not forget to use this folder for the analysis!



To further verify the correction, choose “**Use data already corrected**” and click on “**Visualize**”. Sometimes, the correction is not perfect at once. You can correct again the positions of detections selecting “**Use data already corrected**” and making “**Try**” again.

4. Correct multiple detections



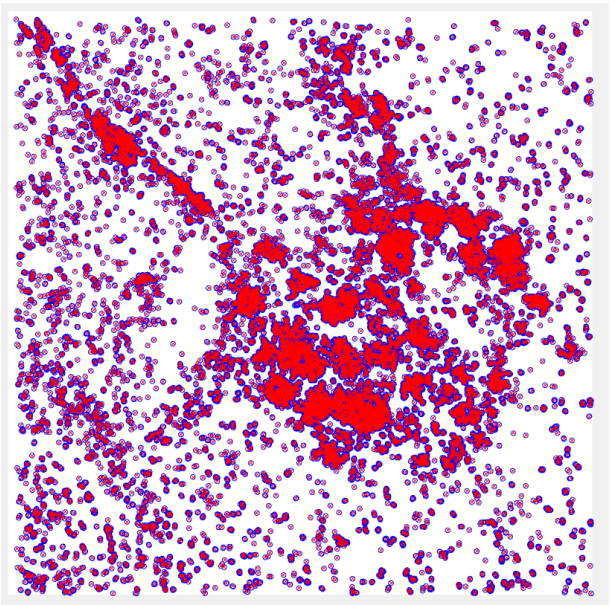
This algorithm selects detections that are likely to belong to the same fluorophore, based on the localization precision (maximum distance between detections) and on the period in which this fluorophore could be detected (maximum blinking period). The values depend upon the type of fluorophore.

Please note that here you correct multiple detections of a fluorophore, but not necessarily multiple detections of the target molecule: this depends on how many fluorophores are attached to the same molecule.

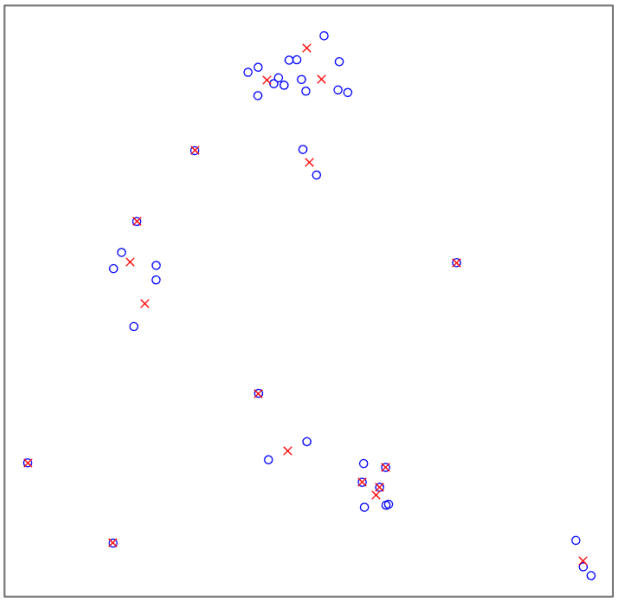
The detections selected in this way will be replaced by only one located at the mean x and mean y position.

The result is shown in a figure, where the original detections are shown with blue cercles, and the retained ones by red crosses.

The full image:

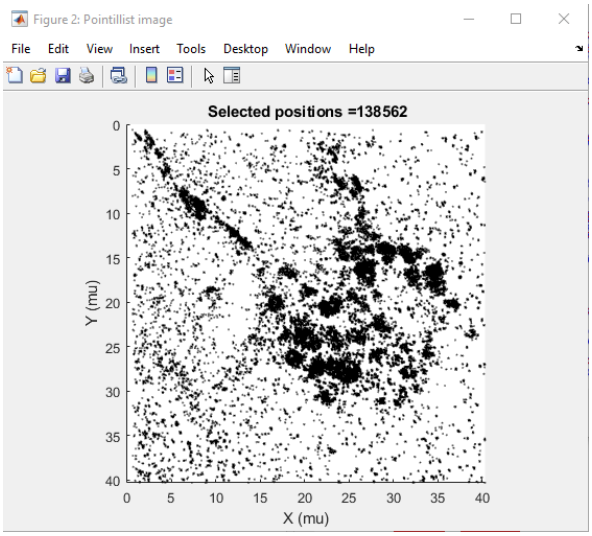


A detail:

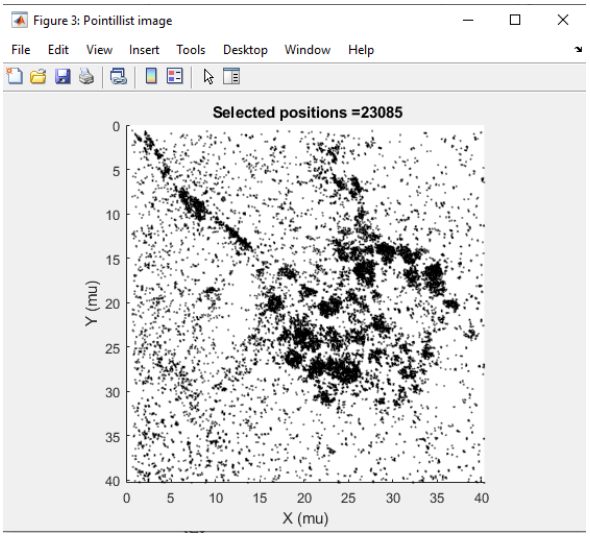


Corrected data will be saved as *name*-corr.mat.

Exemple before correction:



After correction:



5. Concatenate data detections:

When there are more than one movie corresponding to one experiment, this option allows to merge all the detections in one file (.mat). Select all the files (.mat) that you want to concatenate. Data will be added to the previous one following the order of the file names. Frames are renumbered to obtain a single sequence.

Results are saved as .mat and .pk files, with the name of the first file followed by “concat” to differenciate it from the orginal file.