Output of ”FindTrajects”

The tracking algorithm runs from the script ”runToAnalyse_1” and consists of the functions; frameAverage, FindTrajects, linkTrajSegments, goThroughTracksVideo and ShowManyTrajAnalysis. The function FindTrajects finds spot centres on each frame in turn and then joins them with spots from the previous frame. FindTrajects calls the following functions:

1. extract_image_sequence_data
2. getCellMaskAndBoundary
3. findCandidateSpots
4. eliminateCoincidentSpots (used for all frames apart from the start frame)
5. findSpotCentre1frame

and then goes on to link spots into trajectories.

In the function’findSpotCentre1frame’ there are three parameters that define whether a spot is accepted or not:

1. signal to noise ratio (SNR) defined as $\frac{I0\ of\ gaussian\ fit}{\sigma\ of\ background\ noise}$
2. r^2 of the gaussian fit
3. minimum sigma of the fit
4. maximum sigma of the fit

I noticed that the tracking algorithm was having difficulty picking up the dimmer spots in the in-vivo data, so I decided to run the in-vitro data through the algorithm. The in-vitro data is a bit cleaner and therefore it is easier to see if the algorithm is detecting the dimmer spots or not. I used the first two frames from one image sequence of in-vitro data, frame 10 and frame 11.

I left the parameters set to the default settings:

1. signal to noise ratio (SNR) = 2
2. r^2 of the gaussian fit = 0.2
3. minimum sigma of the fit = - inner circle mask radius
4. maximum sigma of the fit = inner circle mask radius

and put the first two frames of in-vitro data through the FindTrajects part of the tracking algorithm. I uncommented some of your ”show graphically” sections so I could see what the algorithm was doing at each stage. Figures 1 - 6 show the positions of the candidate spots and accepted spots for frames 10 and 11 for the default parameter settings.

Mark thought this has somethings to do with the SNR ratio and asked me re-run the data with a reduced SNR of 0.2, but this didn’t make any difference.

Then I reduced the parameters excessively:

1. signal to noise ratio (SNR) = 0.2
2. r^2 of the gaussian fit = 0.1
3. minimum sigma of the fit = - inner circle mask radius
4. maximum sigma of the fit = inner circle mask radius

The accepted spot centres for these parameters are shown in Figures 7 - 8. This results in the detection of almost all spots. I think the only spots that haven’t been detected are those that don’t have a candidate spot to start with. I don’t know if these are good parameters to work with however. Perhaps I should increase the parameters up to the point where spots stop being detected?

I have looked at the function findCandidateSpots. I have uncommented some of your ”show graphically” sections and have compiled the output for each stage in a separate document called ”findCandidateSpots”. The output is only for frame 11 and the parameters for finding the spot centres are the default settings.

I noticed there are lots of steps in the findCandidateSpots function and I have written my understanding of each step in each figure title. Please do correct any mistakes!

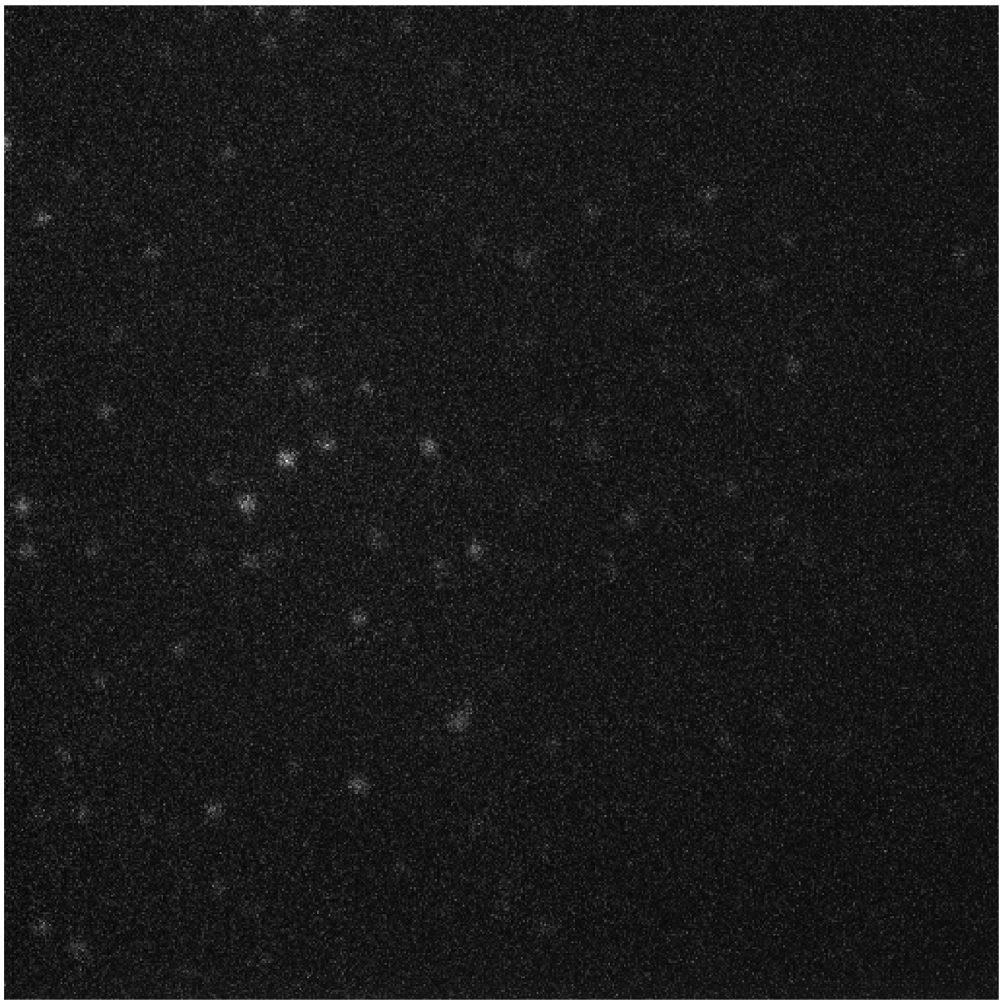

Figure 1: Frame 10

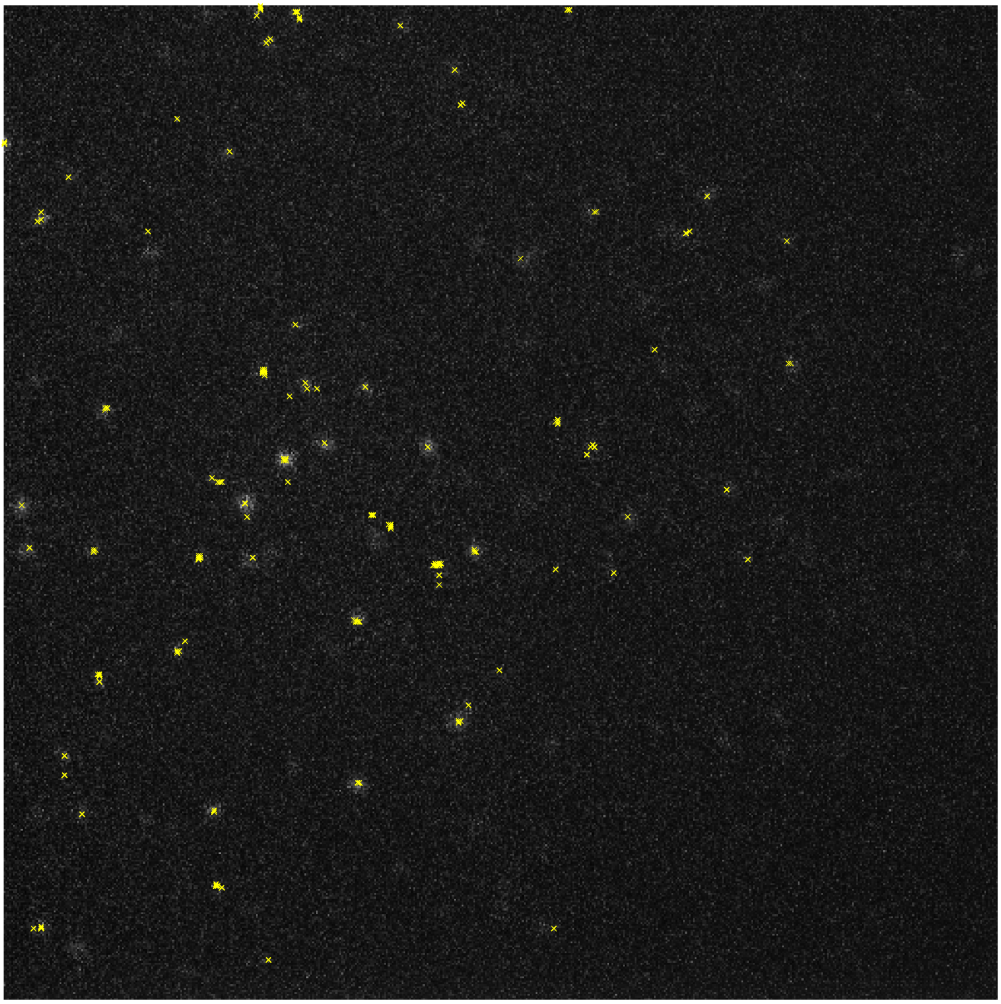

Figure 2: Frame 10 - positions of candidate spots (SNR = 2, r^2 fit = 0.2)

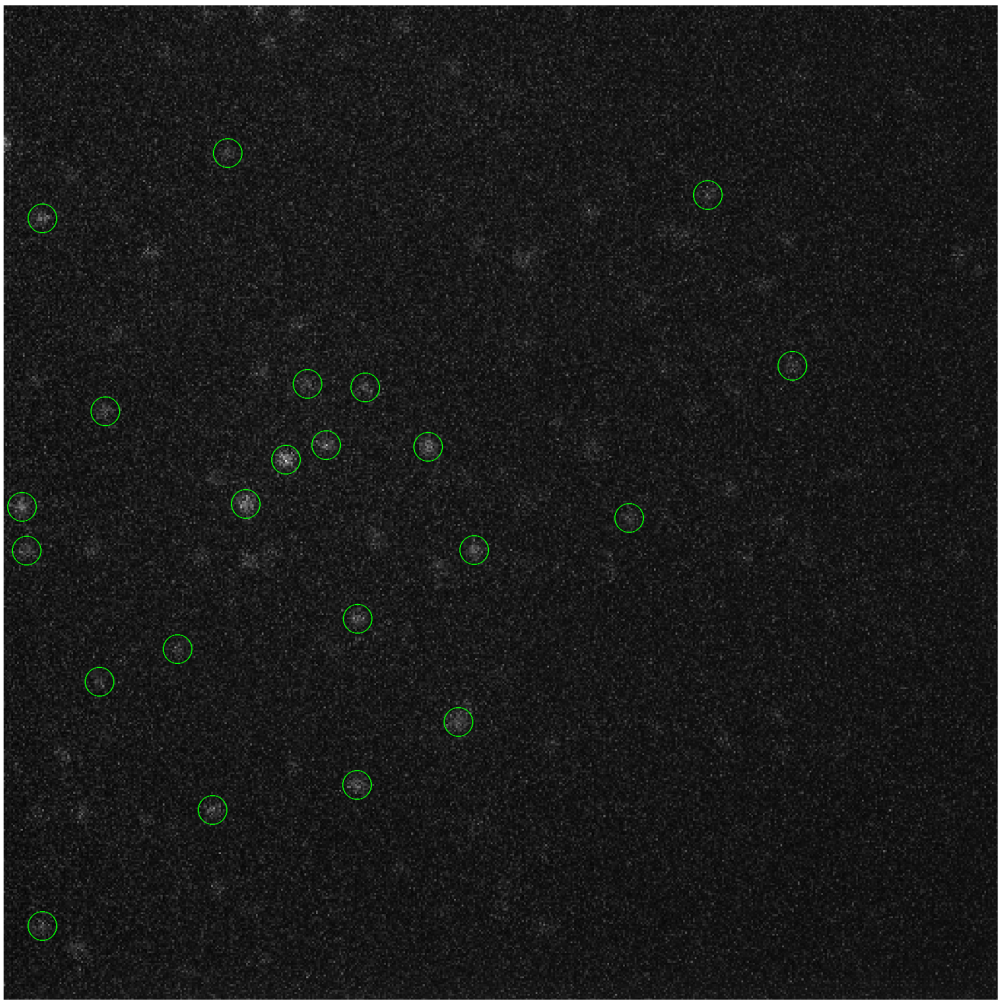

Figure 3: Frame 10 - accepted spot centres (SNR = 2, r^2 fit = 0.2)

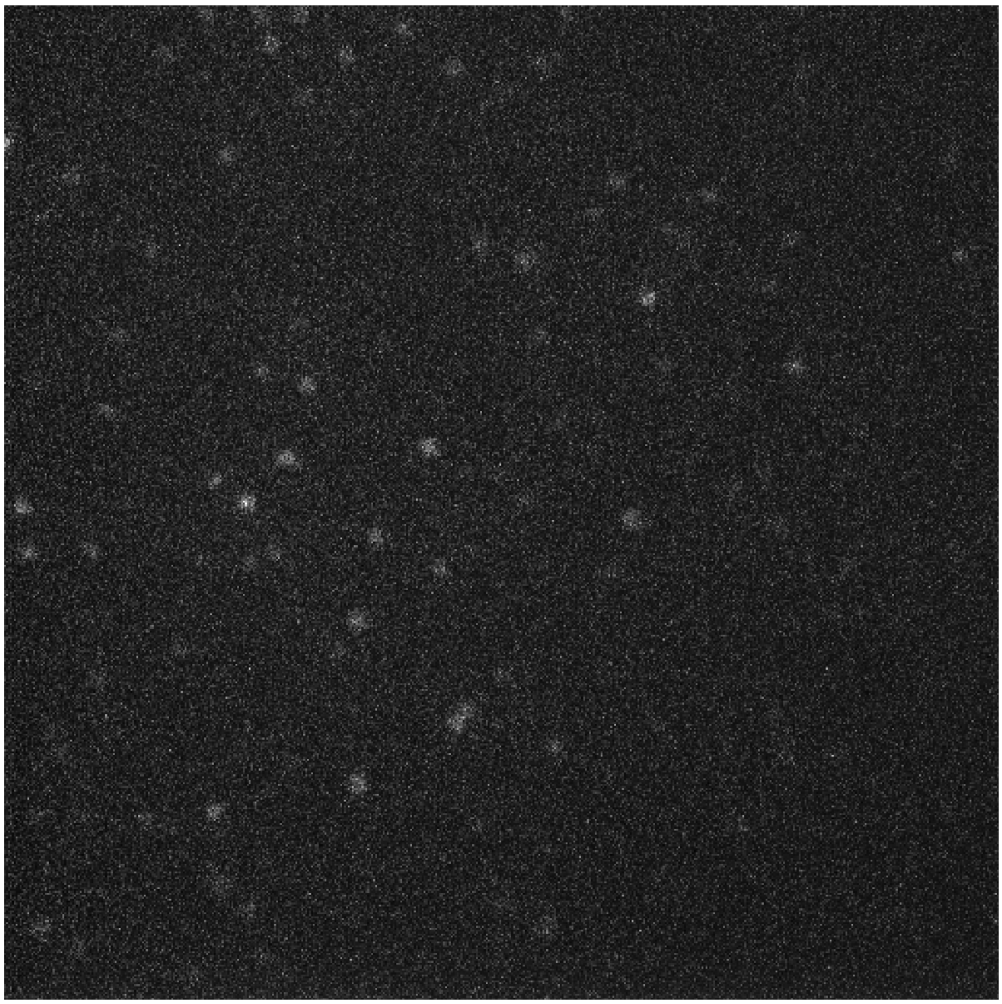

Figure 4: Frame 11

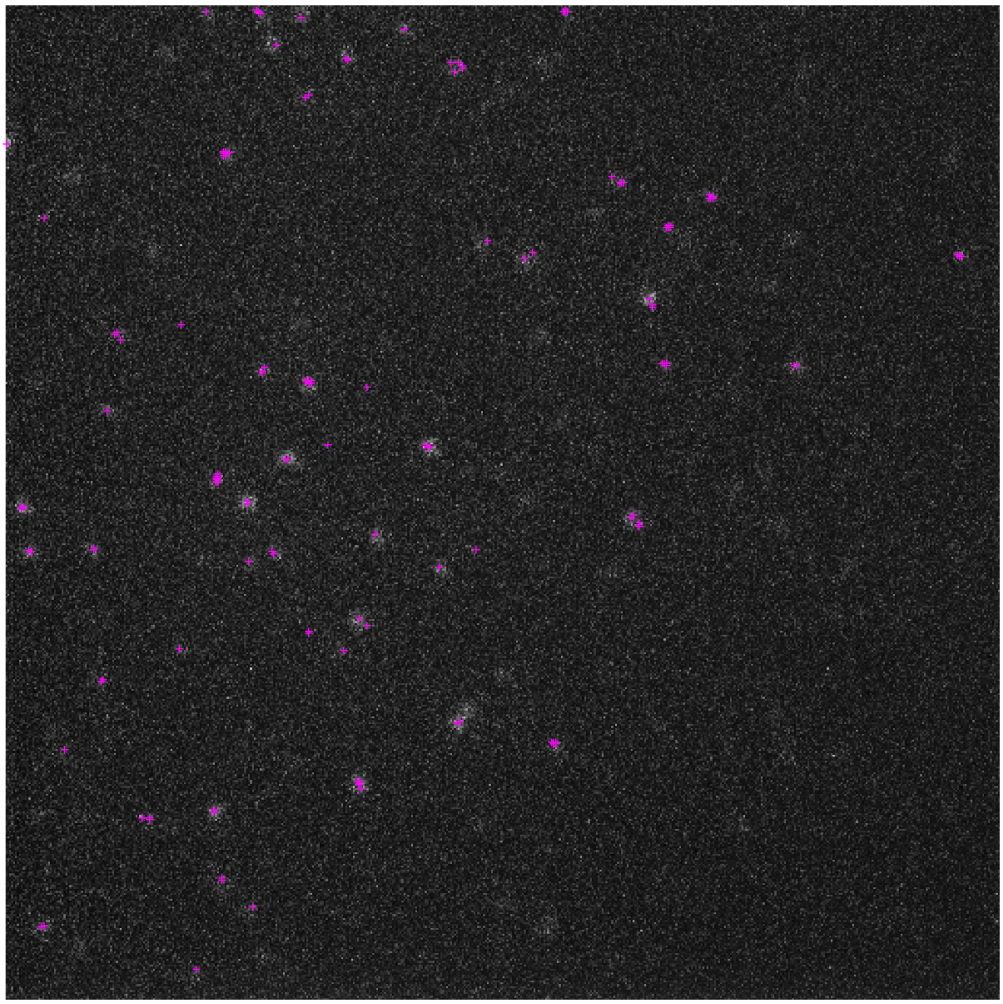

Figure 5: Frame 11 - Candidate spots after eliminating coincidences (SNR = 2, r^2 fit = 0.2)

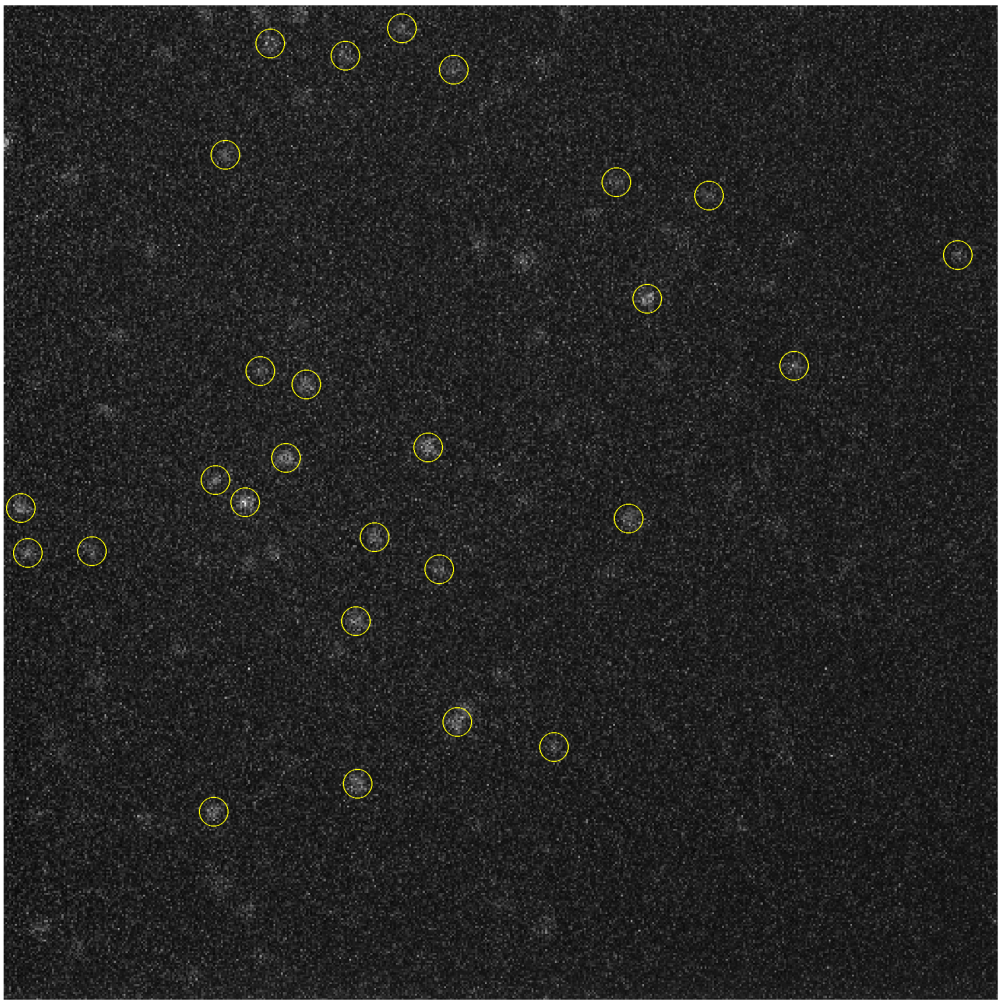

Figure 6: Frame 11 - accepted spot centres(SNR = 2, r^2 fit = 0.2)

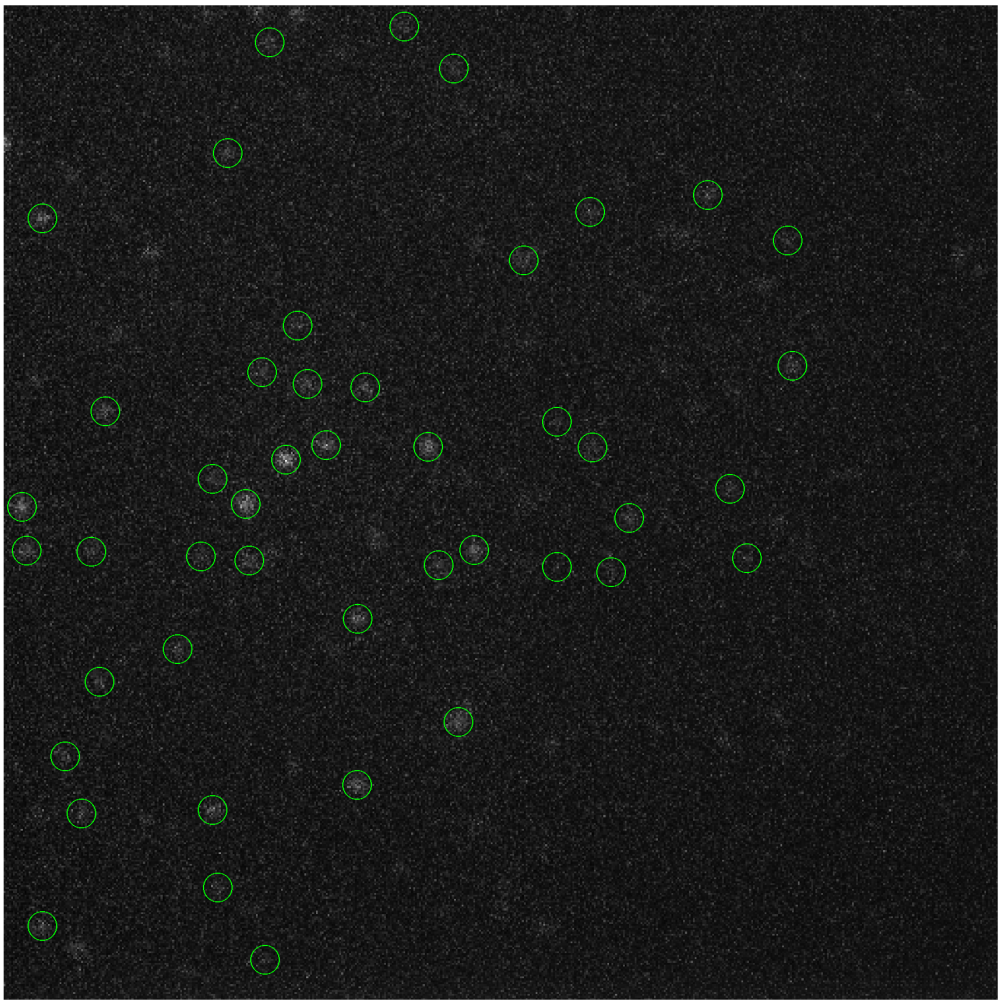

Figure 7: Frame 10 - accepted spot centres (SNR = 0.2, r^2 fit = 0.1)

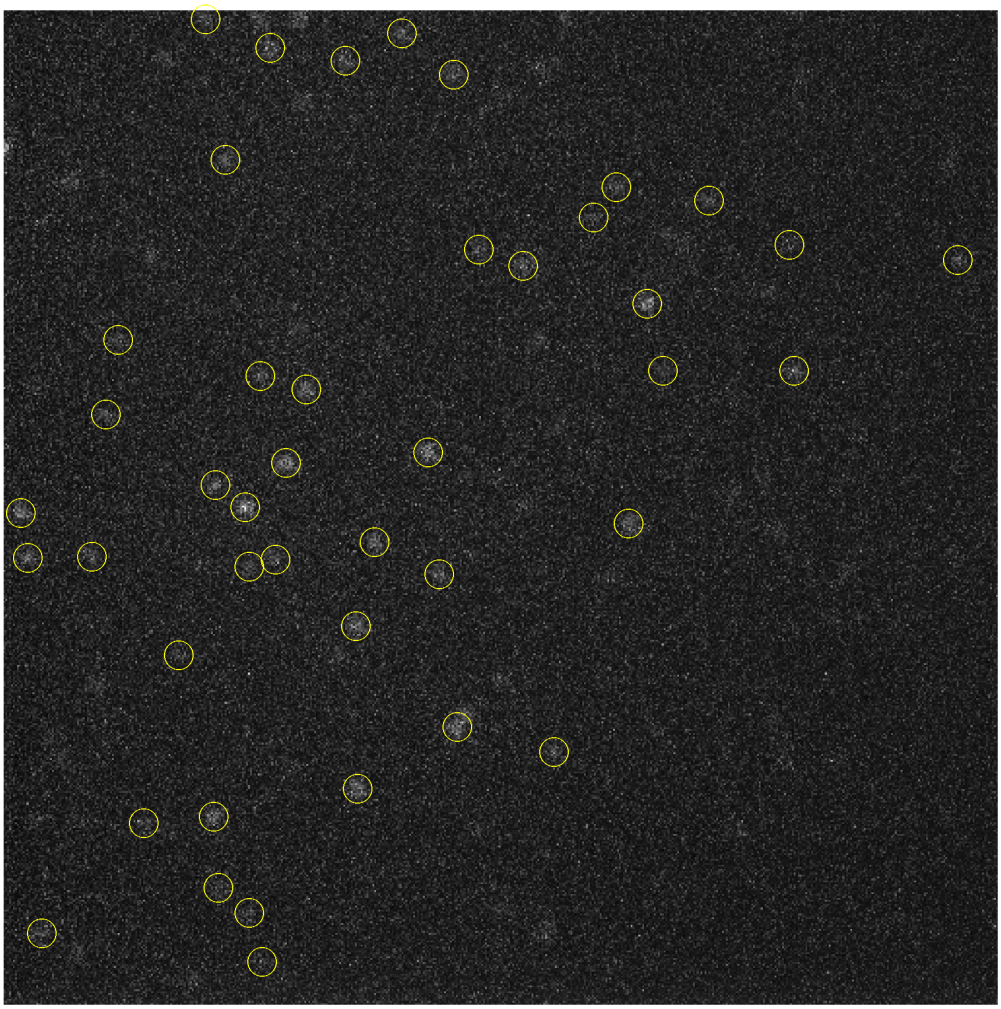

Figure 8: Frame 11 - accepted spot centres(SNR = 0.2, r^2 fit = 0.1)