Spot Tracking and Trajectory Analysis Solution in Fluorescence Microscopy Images

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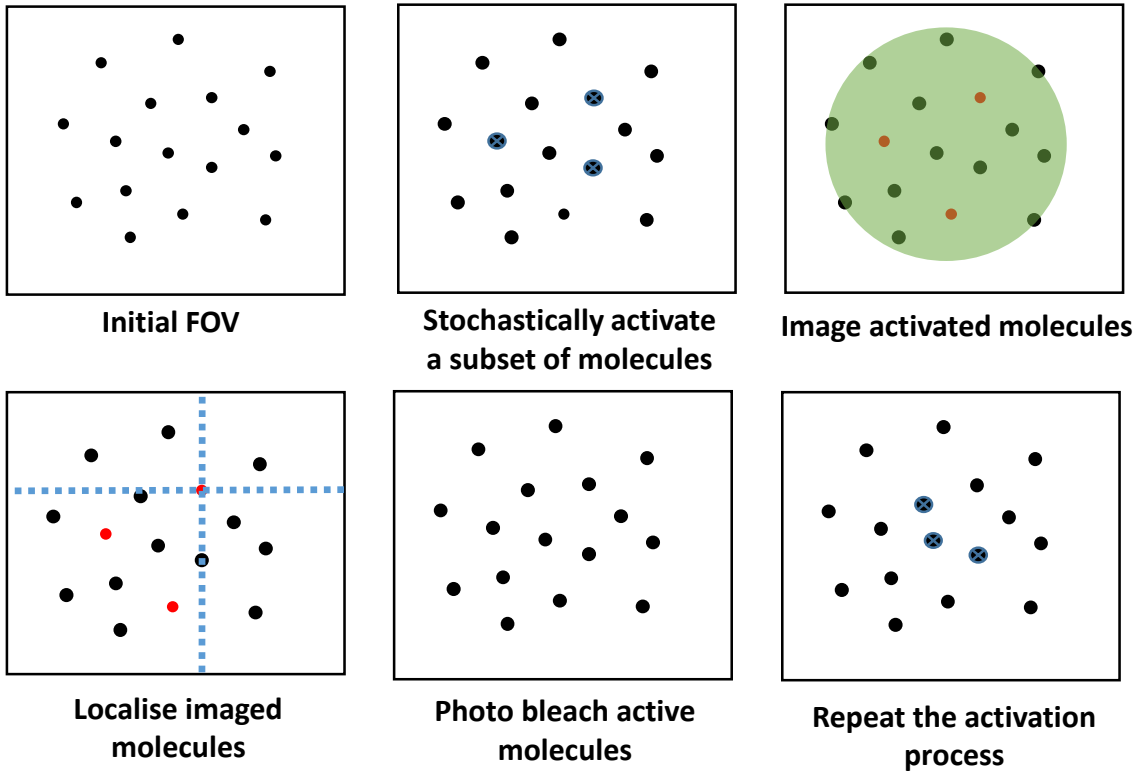
Overview

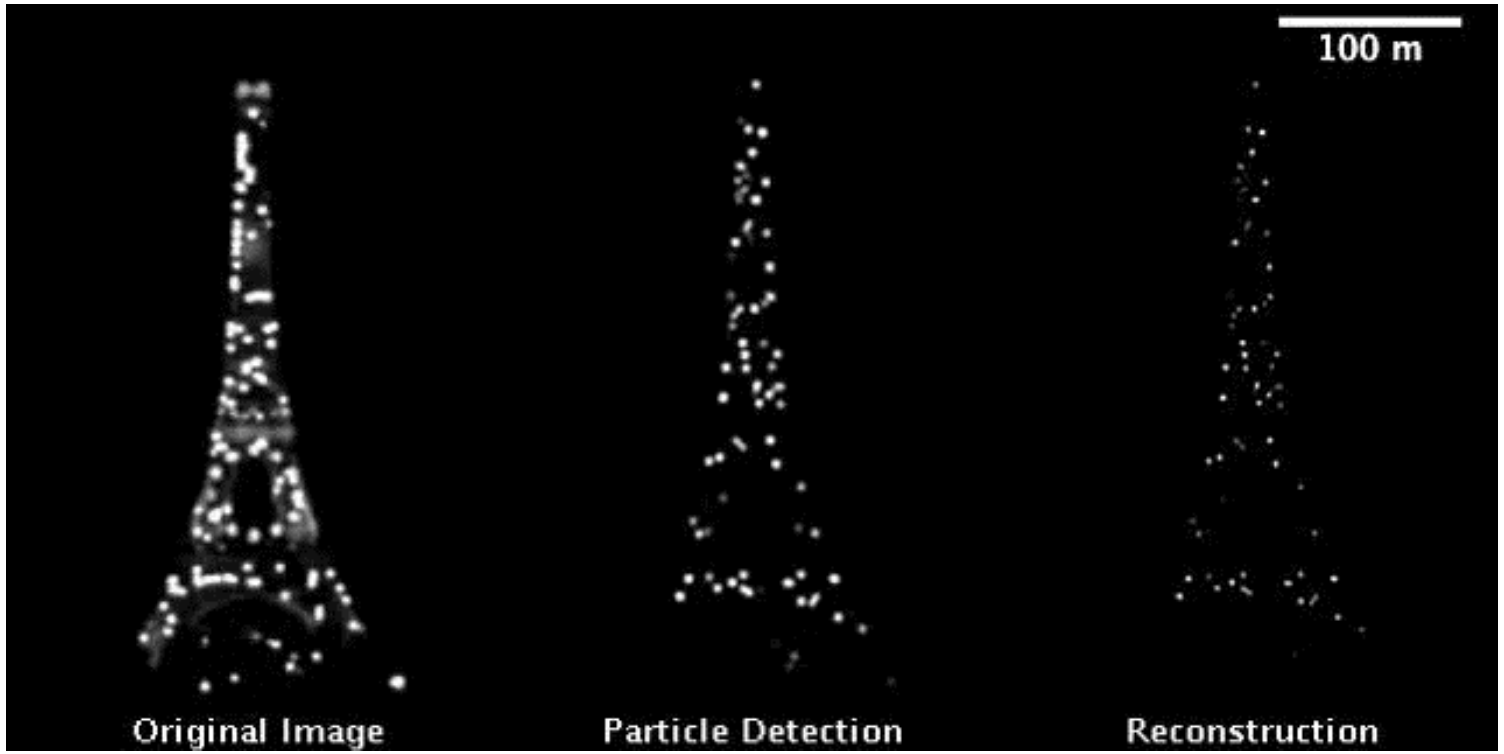
- Localisation microscopy
- Spot detection – icy plugin
- Tracking – icy plugin
- Trajectory analysis – MATLAB GUI

Localisation Microscopy

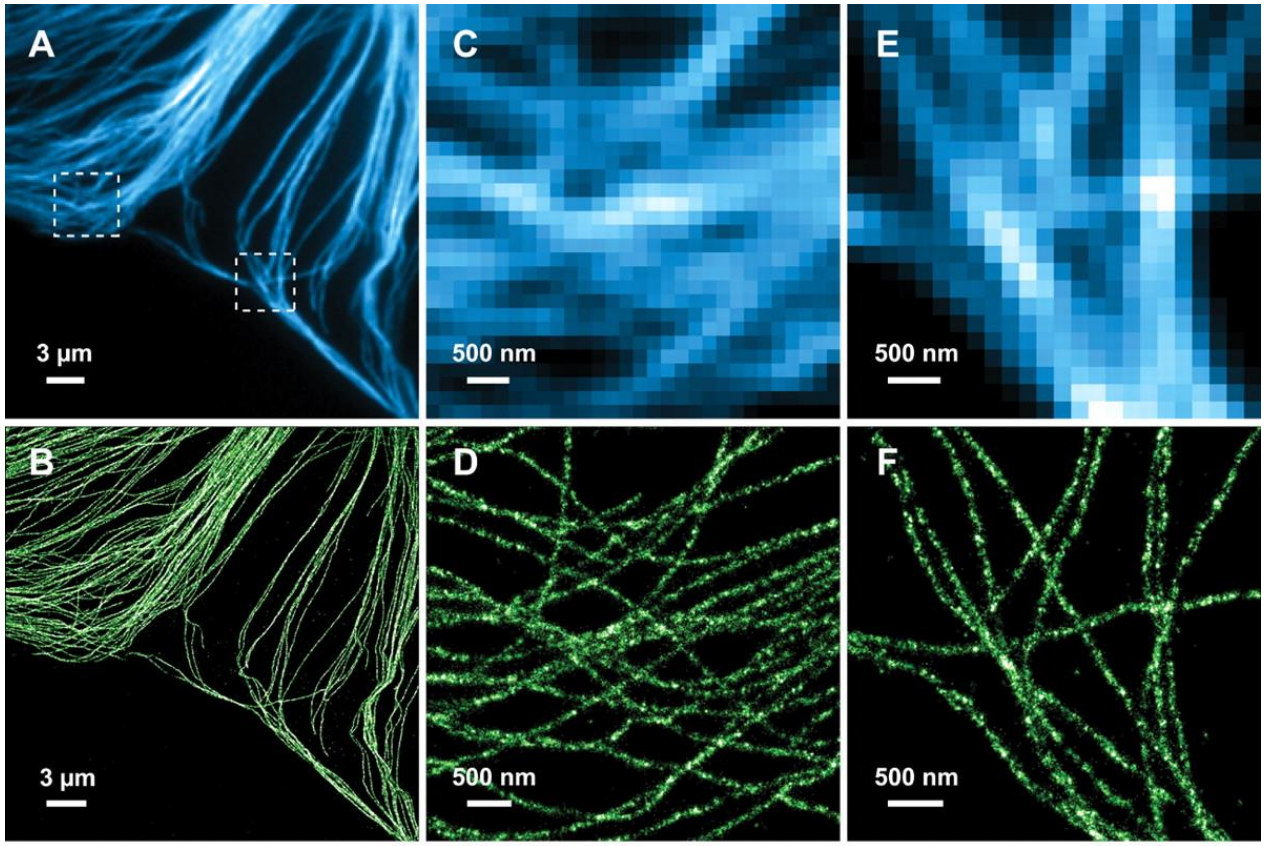
- Super-resolution technique to overcome the diffraction limit of light
- Resolves molecules within the diffraction limited region by separating them in time
- High resolution, ~20 nm, in all spatial dimensions



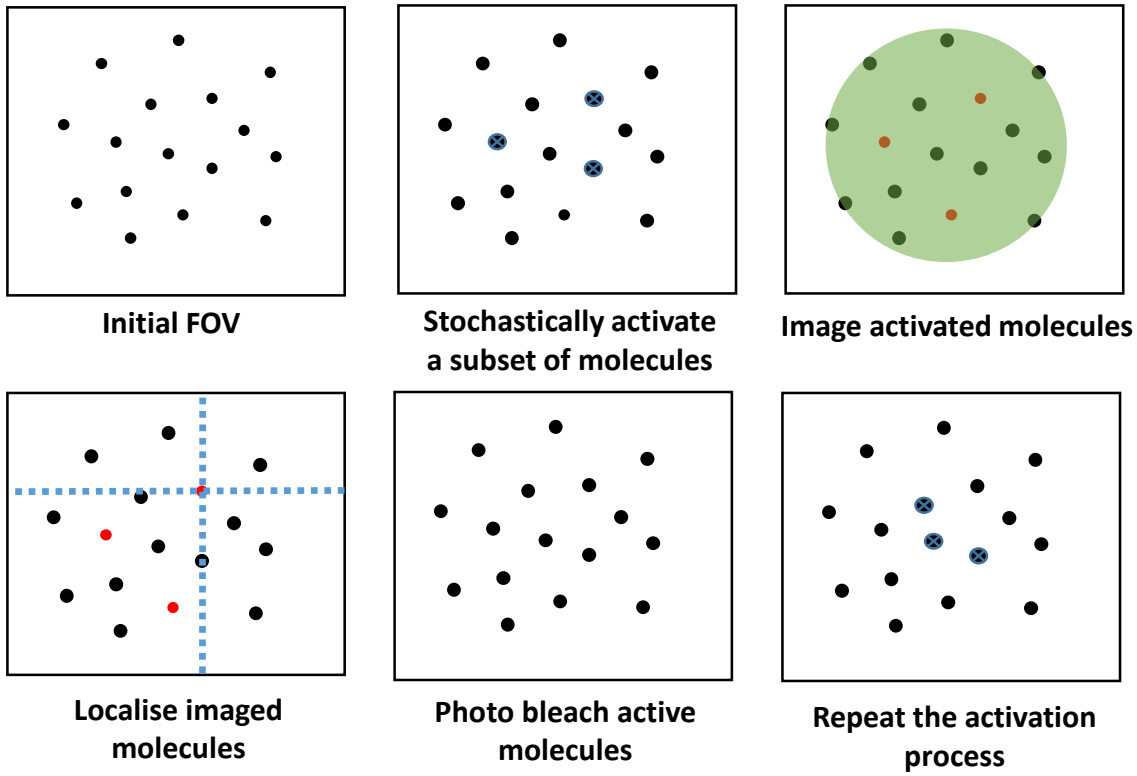
Can be used for localisation and tracking of single molecules



https://www.youtube.com/watch?v=RE70GuMCzww



Mark Bates et al. Science 2007;317:1749-1753



Stochastic activation of single molecules can be used for tracking of single molecules

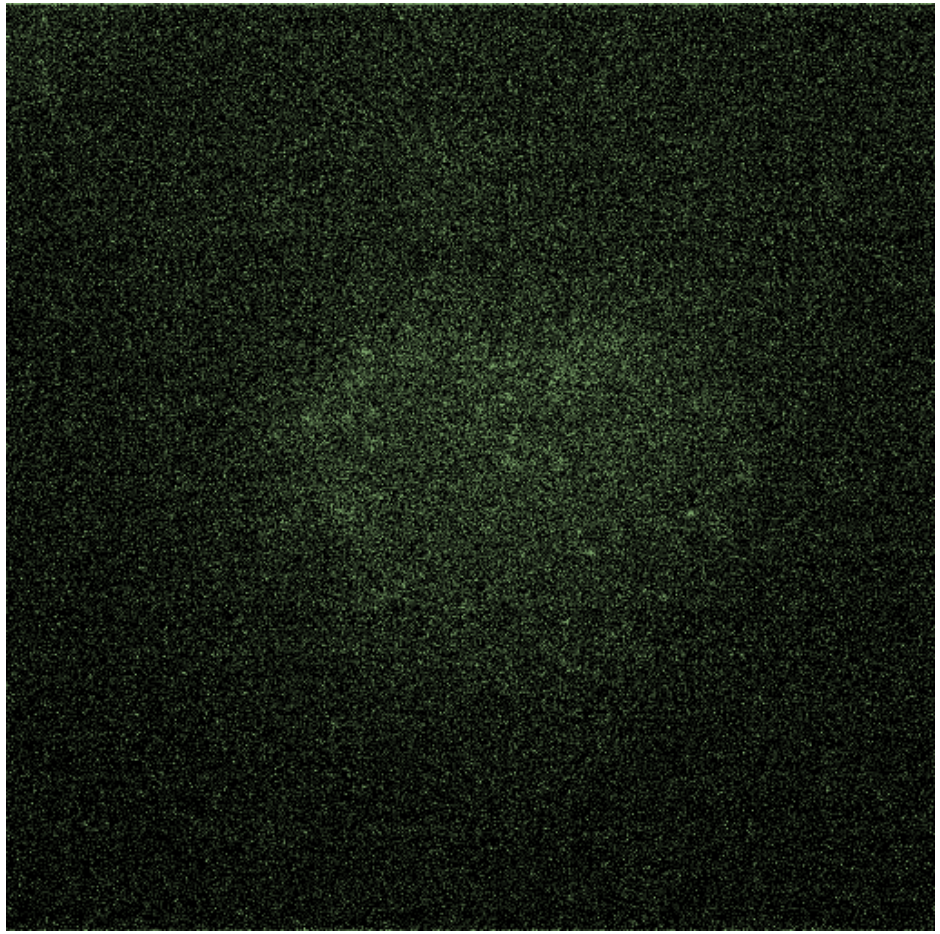
Question: Dynamics of transcription factor

- Study the dynamics of DNA-binding transcription factor for Notch pathway in *Drosophila* salivary glands to calculate:
  - Different diffusion states
  - Occupation of these diffusion states
  - Dwell times and transition probabilities of these states

- Study these parameters under different conditions i.e. when the pathway is active or inactive

Raw Data

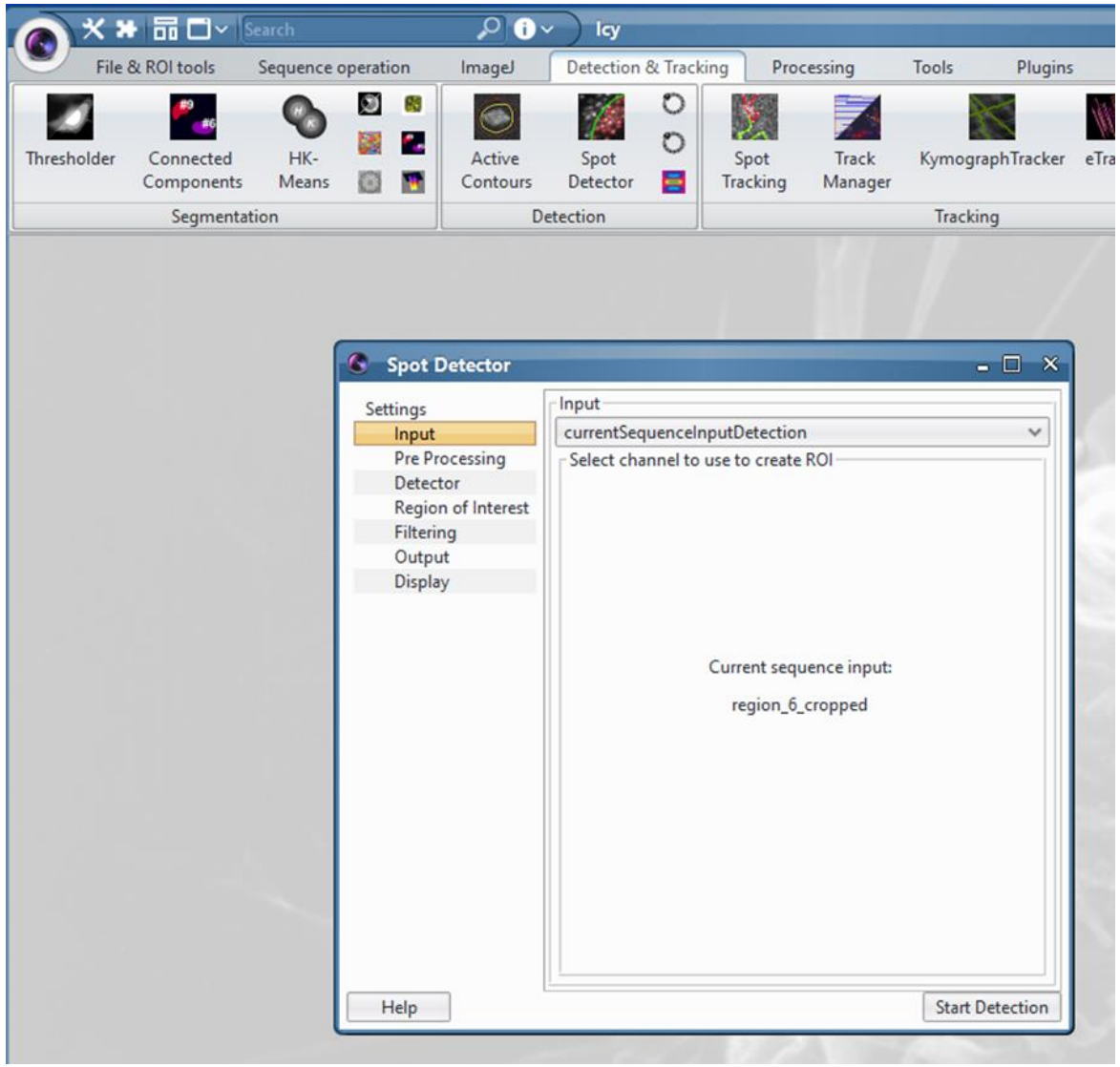
- Labelled with a photo-convertible protein: mEOS
- Nuclei were imaged 20-40 um deep inside the tissue



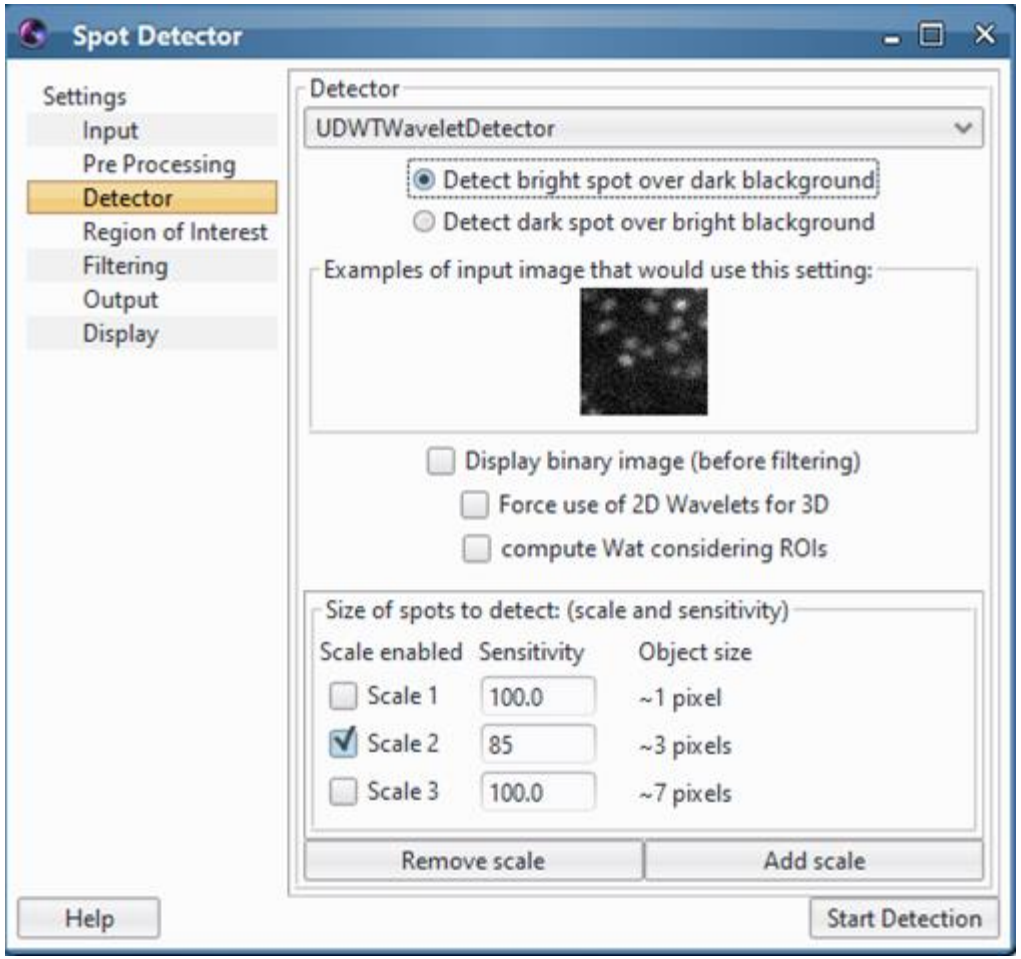
Goal: Detect and track single molecules

Spot Detection

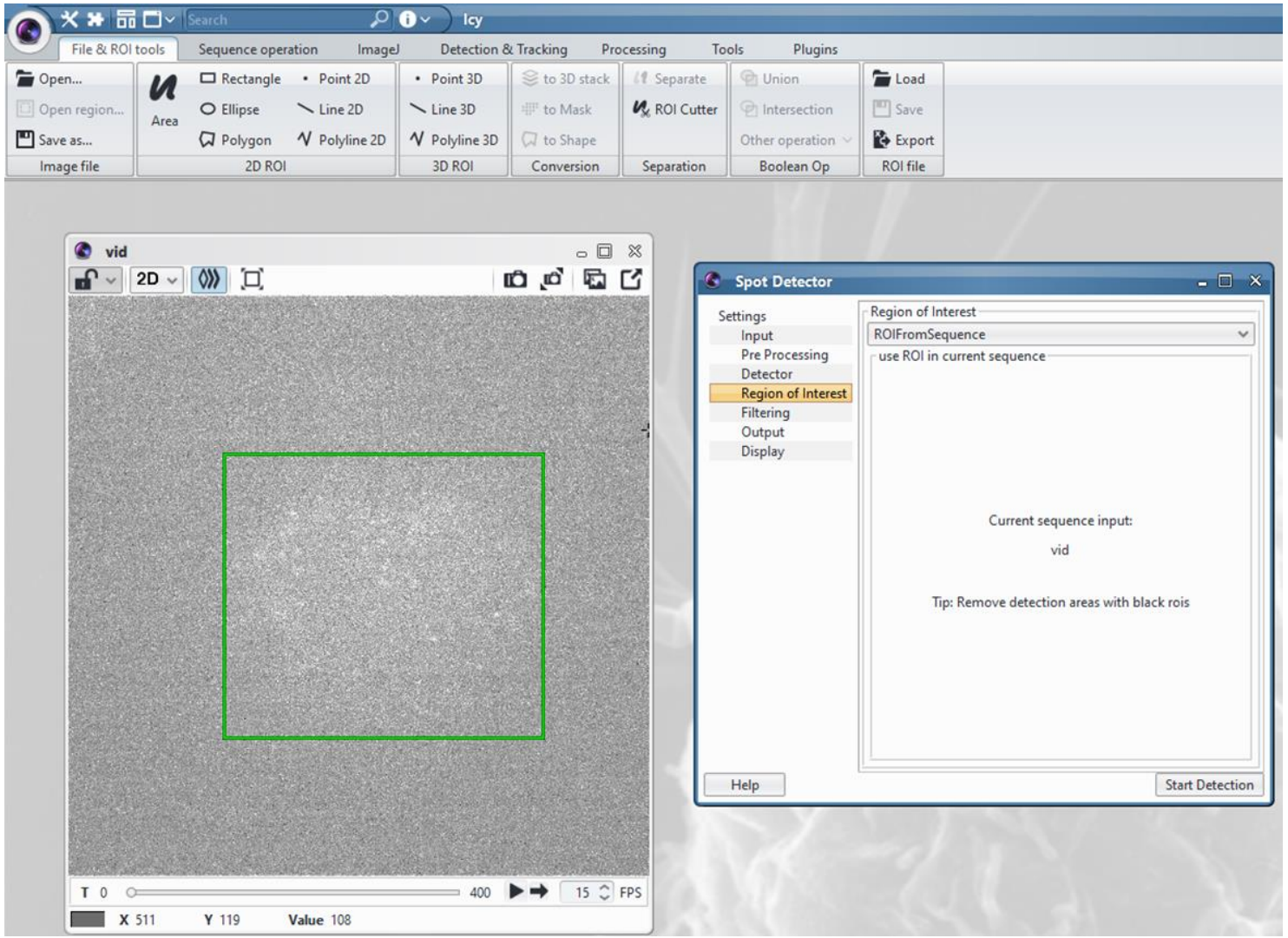
- Freely available as an Icy-plugin
- Based on wavelet transforms
- Important step before tracking



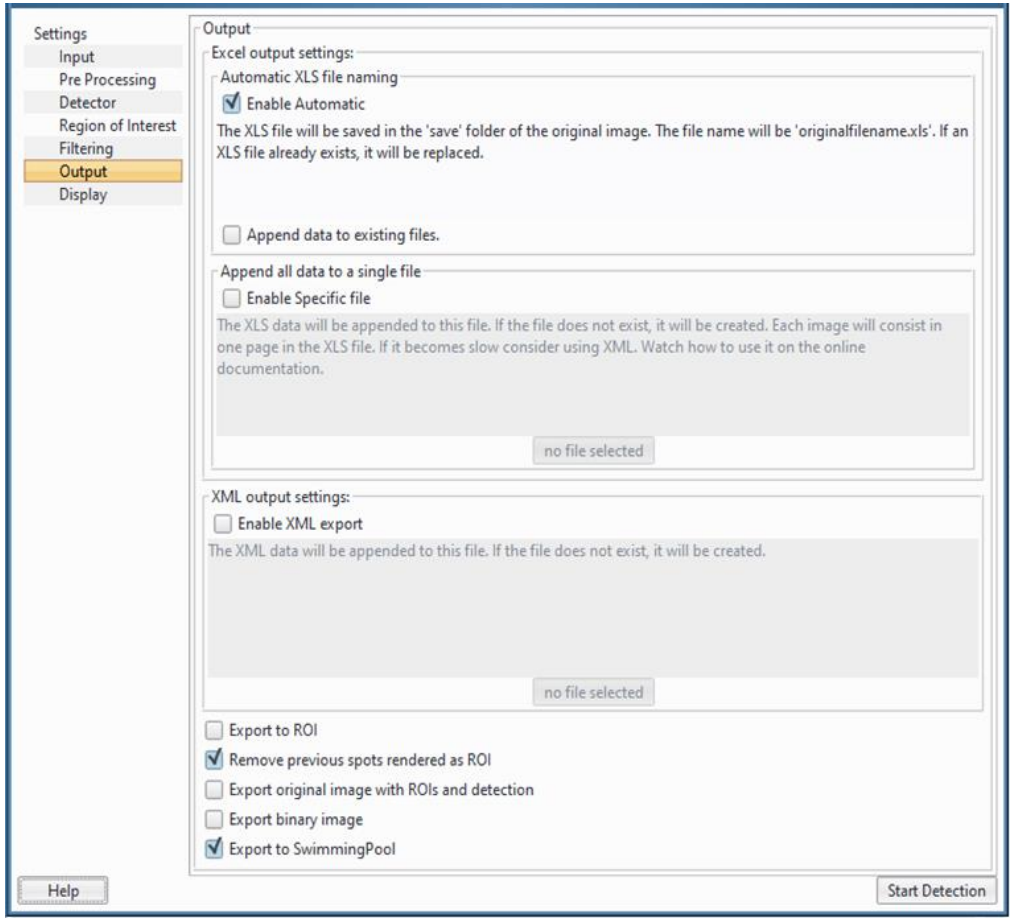
Detector


Spot Detector
Settings
Input
Pre Processing
Detector
Region of Interest
Filtering
Output
Display
Detector
UDWTWaveletDetector
Detect bright spot over dark blackground
Detect dark spot over bright blackground
Examples of input image that would use this setting:
Display binary image (before filtering)
Force use of 2D Wavelets for 3D
compute Wat considering ROIs
Size of spots to detect: (scale and sensitivity)
Scale enabled
Sensitivity
Object size
Scale 1
100.0
~1 pixel
Scale 2
85
~3 pixels
Scale 3
100.0
~7 pixels
Remove scale
Add scale
Help
Start Detection

Spot Detection – ROI

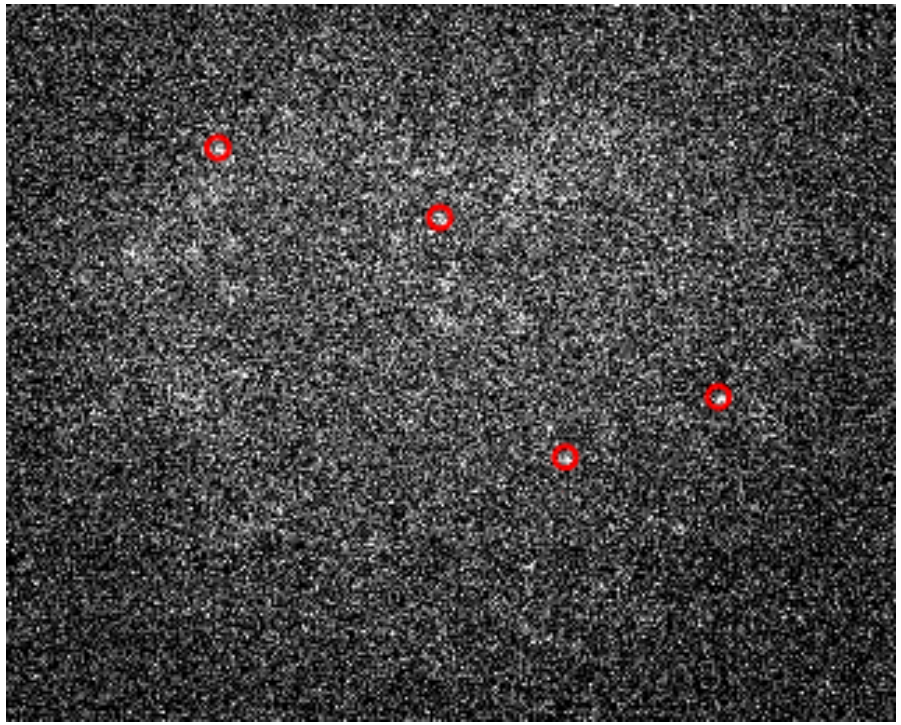


Spot Detection – Display and Output



	Detection i	Surface	x	y	z	t	min intens	max intens	average intensity
27									
28	Detection i	Surface	x	y	z	t	min intens	max intens	average intensity
29	0	4	322	302.75	0	0	236	255	248.25
30	1	6	376.8333	281.1667	0	0	200	255	242.6667
31	2	1	277	217	0	0	205	205	205
32	3	2	197.5	192	0	0	255	255	255
33	4	2	361.5	328	0	1	205	246	225.5
34	5	1	321	303	0	1	225	225	225
35	6	3	391.6667	287.3333	0	1	220	241	230.3333
36	7	4	378.5	280.5	0	1	195	255	237.75
37	8	1	302	255	0	1	255	255	255
38	9	3	164.6667	229.6667	0	1	210	255	240
39	10	3	201.6667	224.6667	0	1	189	251	227
40	11	1	276	218	0	1	255	255	255
41	12	4	198.5	192.5	0	1	236	255	246.75
42	13	1	323	304	0	2	241	241	241
43	14	9	376.8889	280.3333	0	2	220	255	245.8889
44	15	1	382	273	0	2	255	255	255
45	16	3	256.3333	230.6667	0	2	225	255	245
46	17	2	164.5	230	0	2	255	255	255
47	18	2	202.5	226.5	0	2	251	255	253
48	19	1	386	218	0	2	255	255	255
49	20	1	223	179	0	2	251	251	251
50	21	4	378	281.5	0	3	230	255	242.5
51	22	2	382	274.5	0	3	246	255	250.5
52	23	6	255.1667	231.8333	0	3	255	255	255

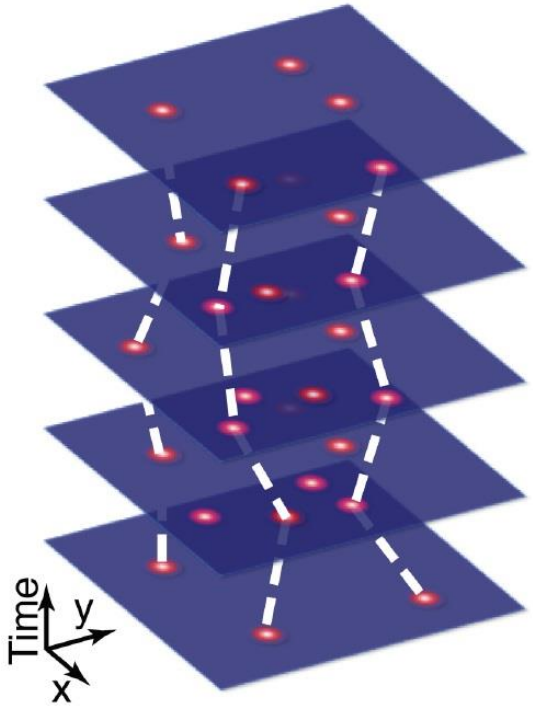
Detection Results, Excel file

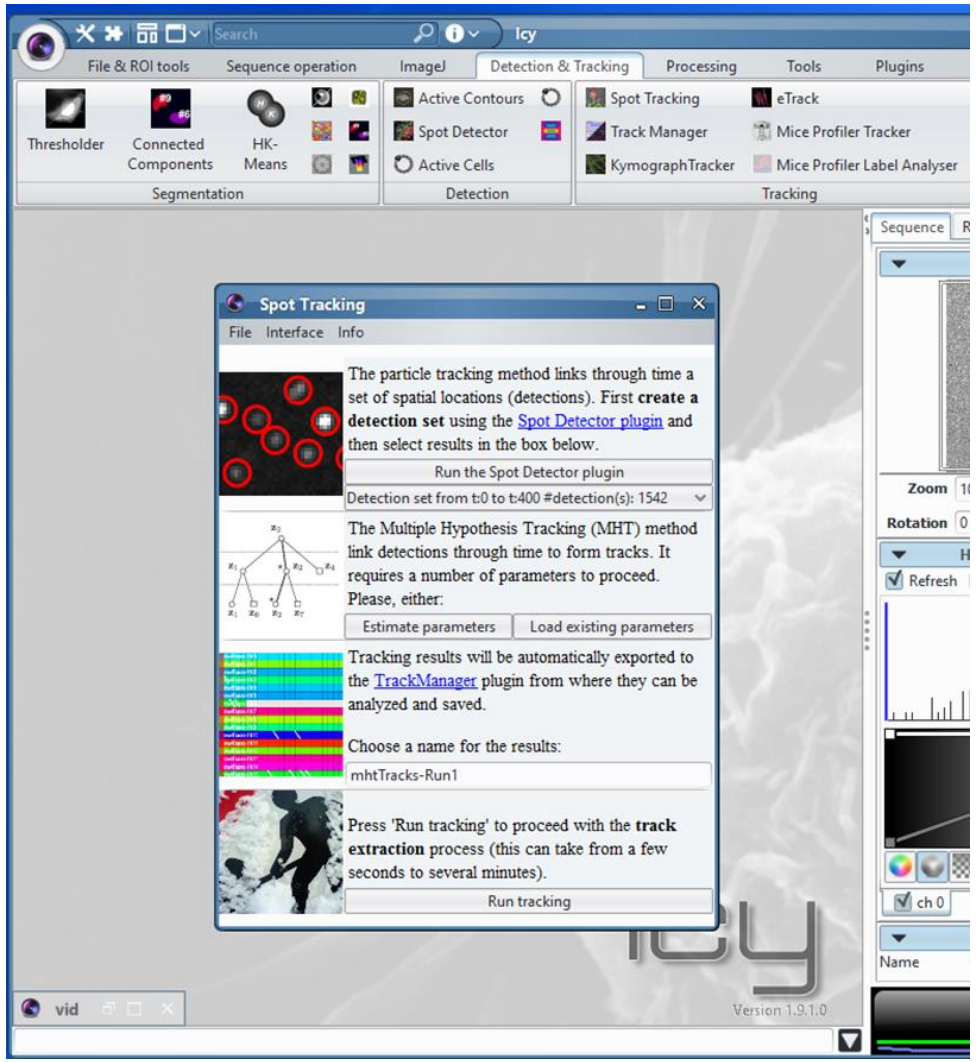


Next Step: Tracking

Tracking – ICY Plugin

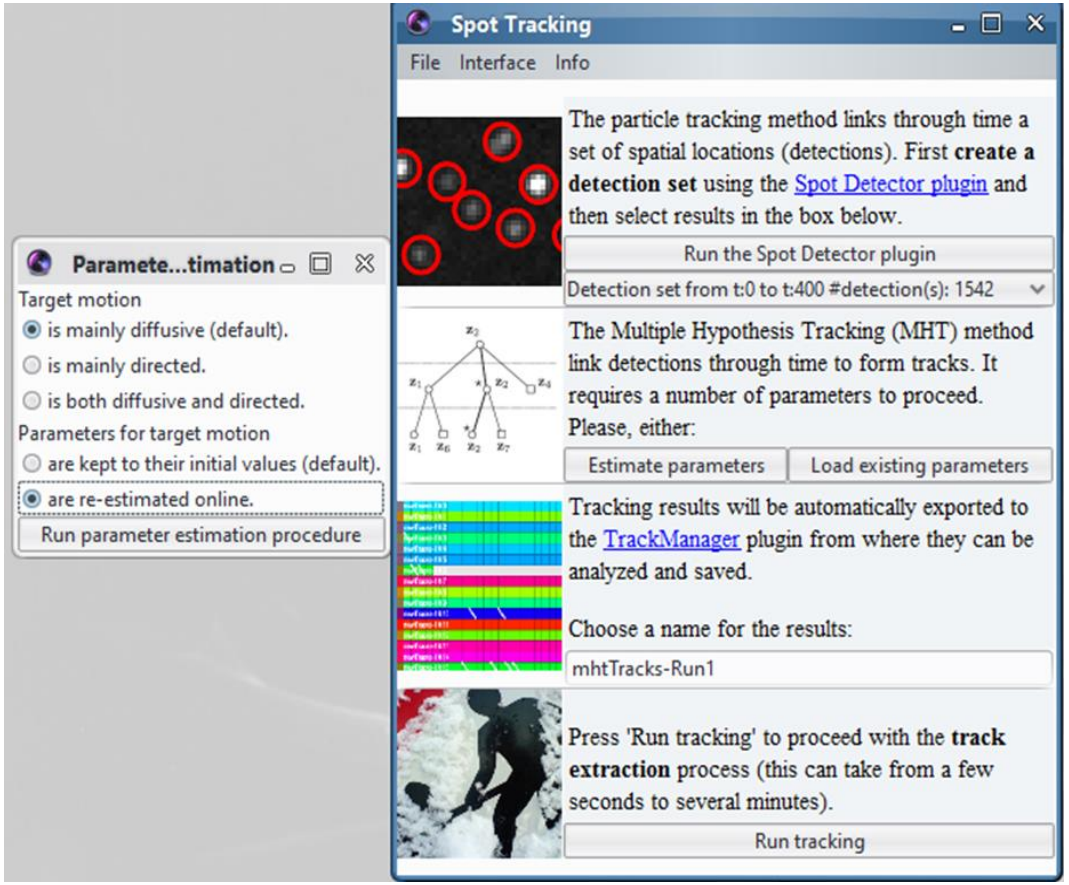
- Based on Multiple hypothesis tracking
- Imports detection results from ‘Spot Detection’ plugin

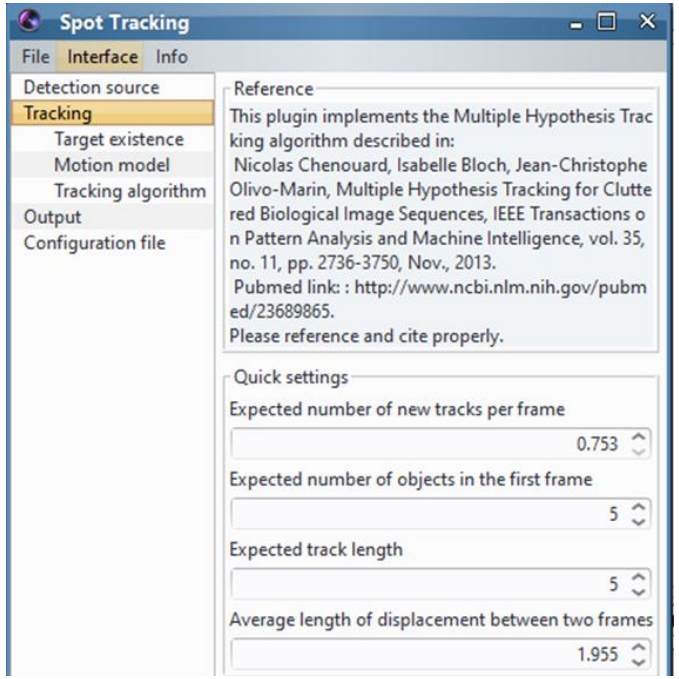


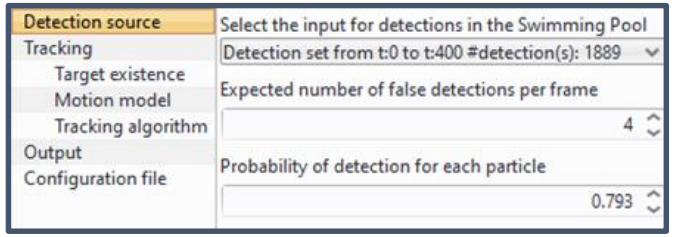


Carlo Manzo and Maria F Garcia-Parajo, “A review of progress in single particle tracking: from methods to biophysical insights,” Reports on Progress in Physics **12**, (2015).

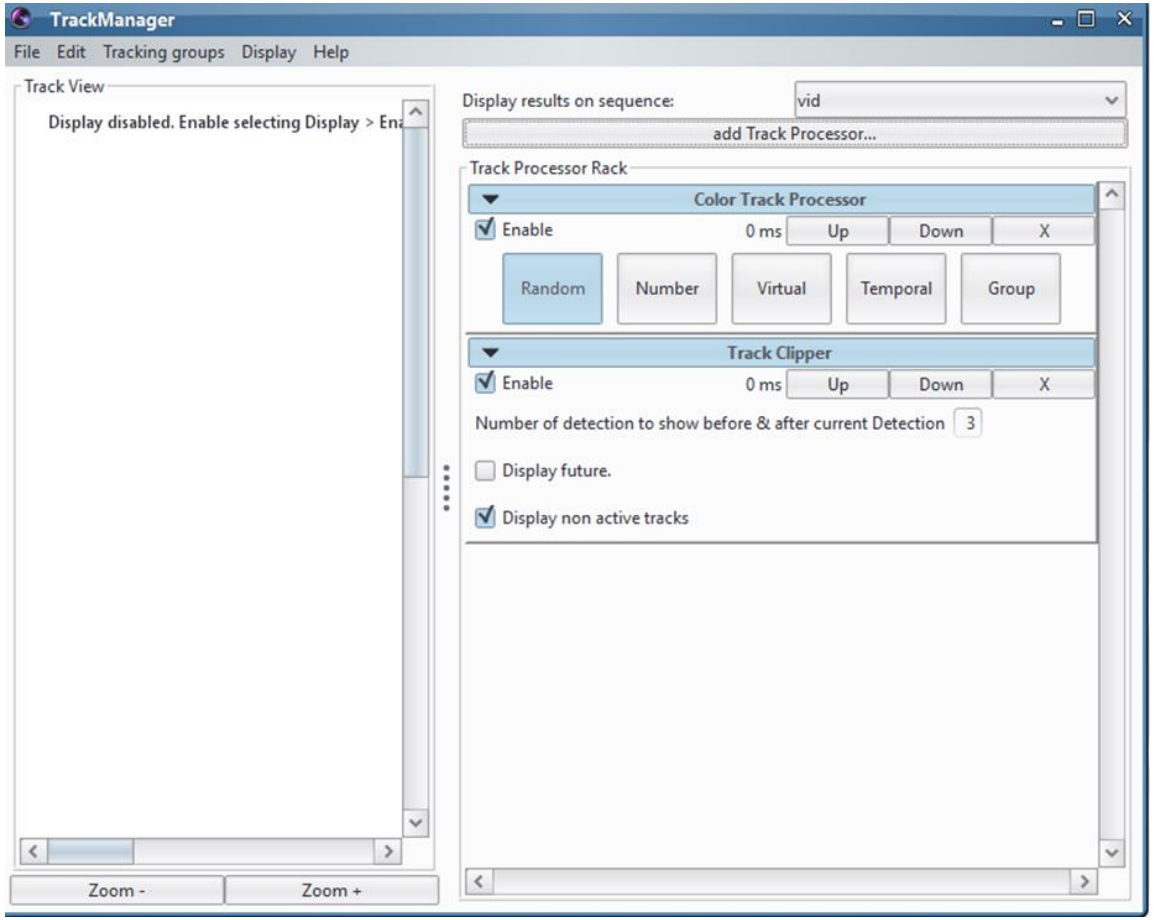
Parameter Estimation and Tracking

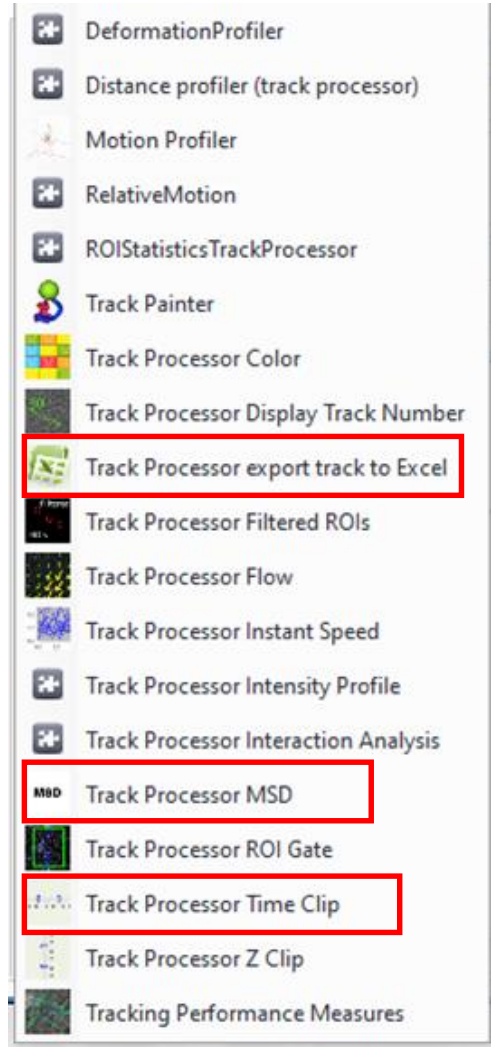




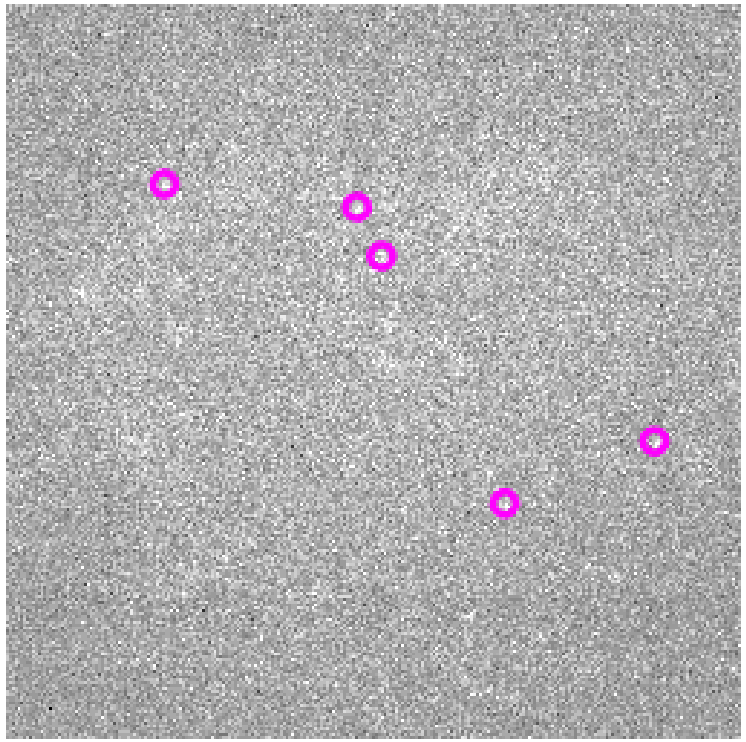


Simple Analysis: Track Manager





Output



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Different Diffusing Populations

Bound Molecule

Unbound Molecule

Multiple Behaviours

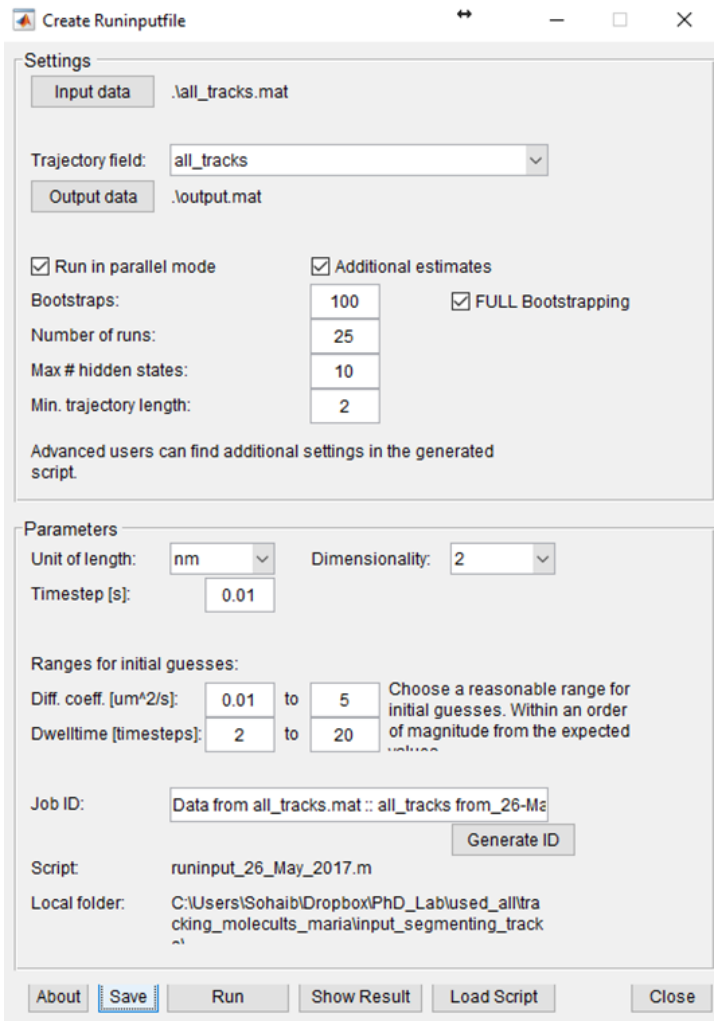
Next Step: Analyse tracks to calculate dynamics of the transcription factor

Analysis - vbSPT

- Implemented in MATLAB
- Tracking data from ICY (or other tracking software)
- Data should be converted to a MATLAB cell array with each index containing track co-ordinates
- Based on Hidden Markov Models, where different states represent different diffusion coefficients

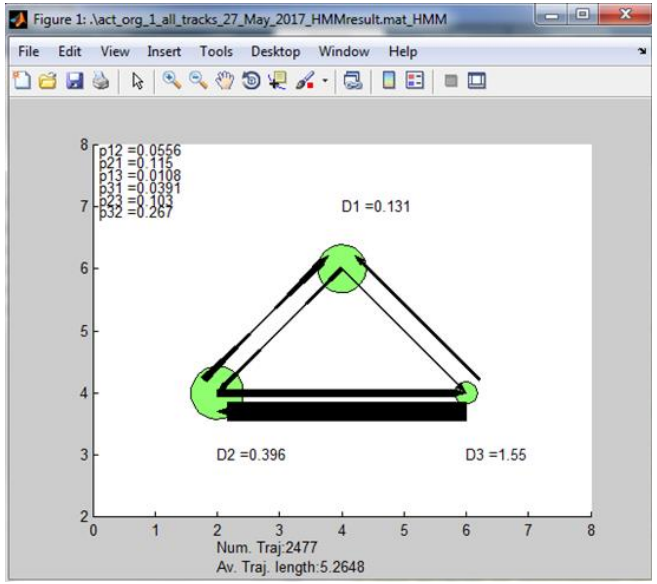
$$x(t+1) = x(t) + \sqrt{2D_{st}\Delta t}\ w_t$$

Different Parameters



Results

- Diffusion Coefficients
- Occupation of different diffusing states
- Dwell times
- Transition probabilities



Command Window

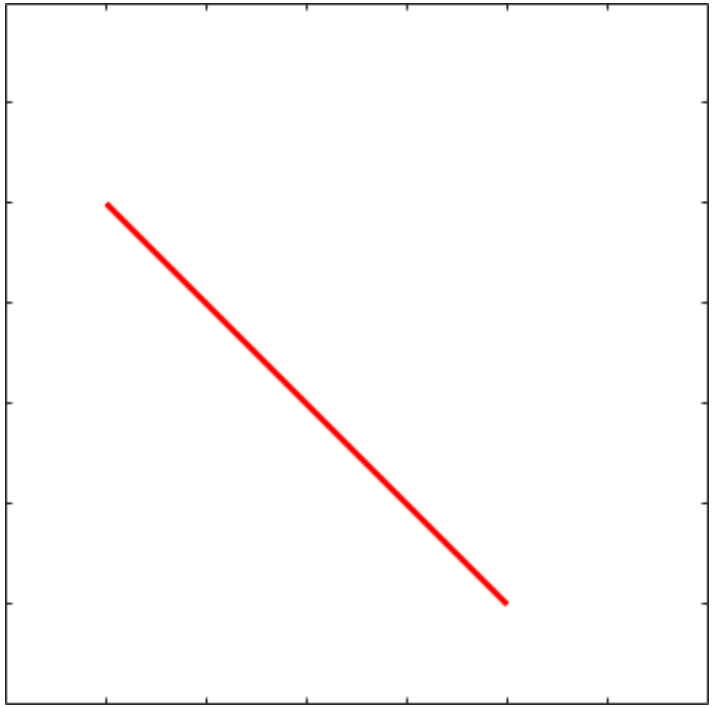
```
Num. Traj.:
2477
Av. Traj. length:
5.2648
Diffusion Coeff [um^2/s]:
0.13107     0.39573      1.5521
0.0020373   0.0058751    0.035637
Total Occupation:
0.39146     0.42926     0.17929
Dwell time [s]:
0.15014    0.046068    0.033025
Transition probability per timestep:
  0.9336    0.055571     0.01083
 0.11498     0.78225     0.10277
0.039115     0.26665     0.69423
>>
```

Different States within a Track

- Each step in the track is allocated a diffusion coefficient

e.g.

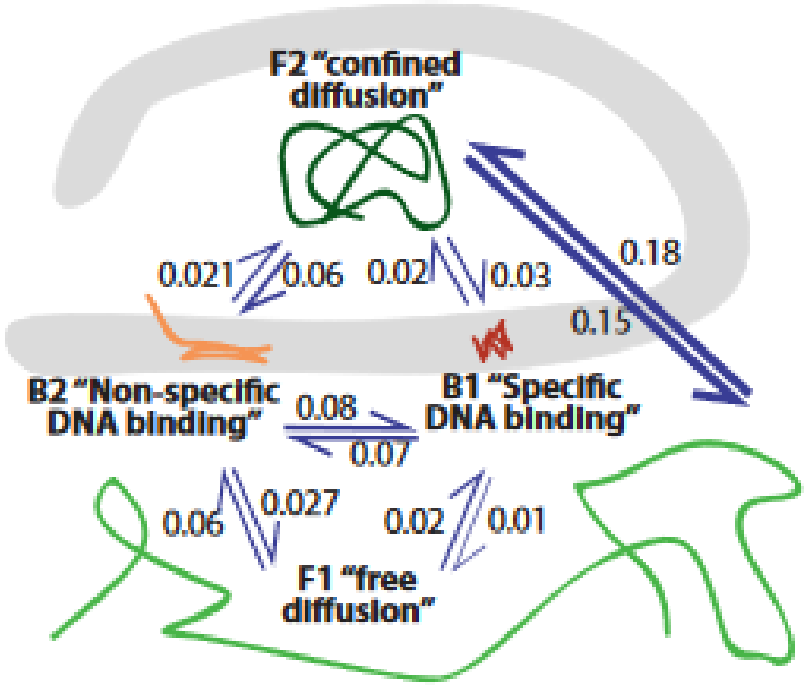
[1.6,1.6,1.6,1.6,1.6,0.1,0.1,0.1,0.1] um^2/sec



Number of States

Number of tracks	Diffusion Coefficients	Occupation
~7000	[0.12 0.40 1.8]	[0.29 0.40 0.31]
~15000	[0.09 0.21 0.47 1.85]	[0.15 0.30 0.30 0.25]
~40000	[0.08 0.19 0.36 0.52 1.83]	[0.13 0.31 0.17 0.14 0.26]

For small number of tracks different diffusion states are merged



Knowledge about the system helps in determining the number of states

Conclusion

- Overview of localisation microscopy
- Spot detection and tracking using ICY plugin
- Trajectory analysis using vbSPT in MATLAB

Thank you