

Spot Tracking and Trajectory Analysis Solution in Fluorescence Microscopy Images

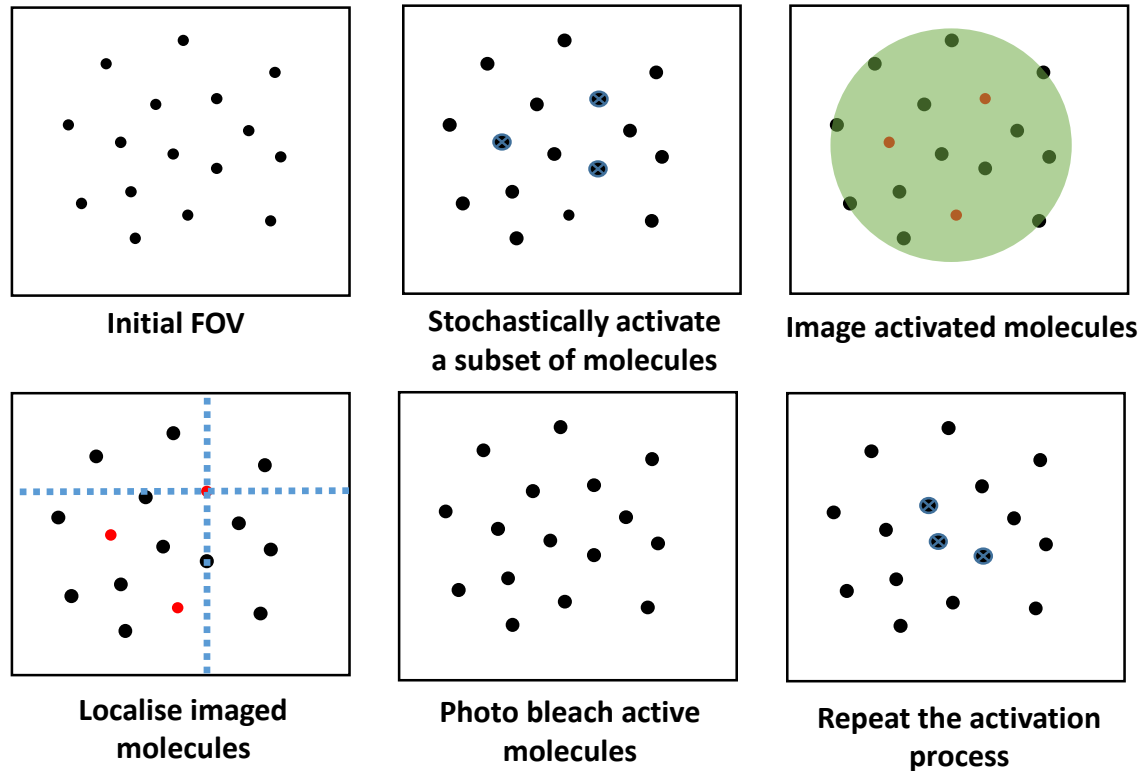
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(sa753@cam.ac.uk)

Overview

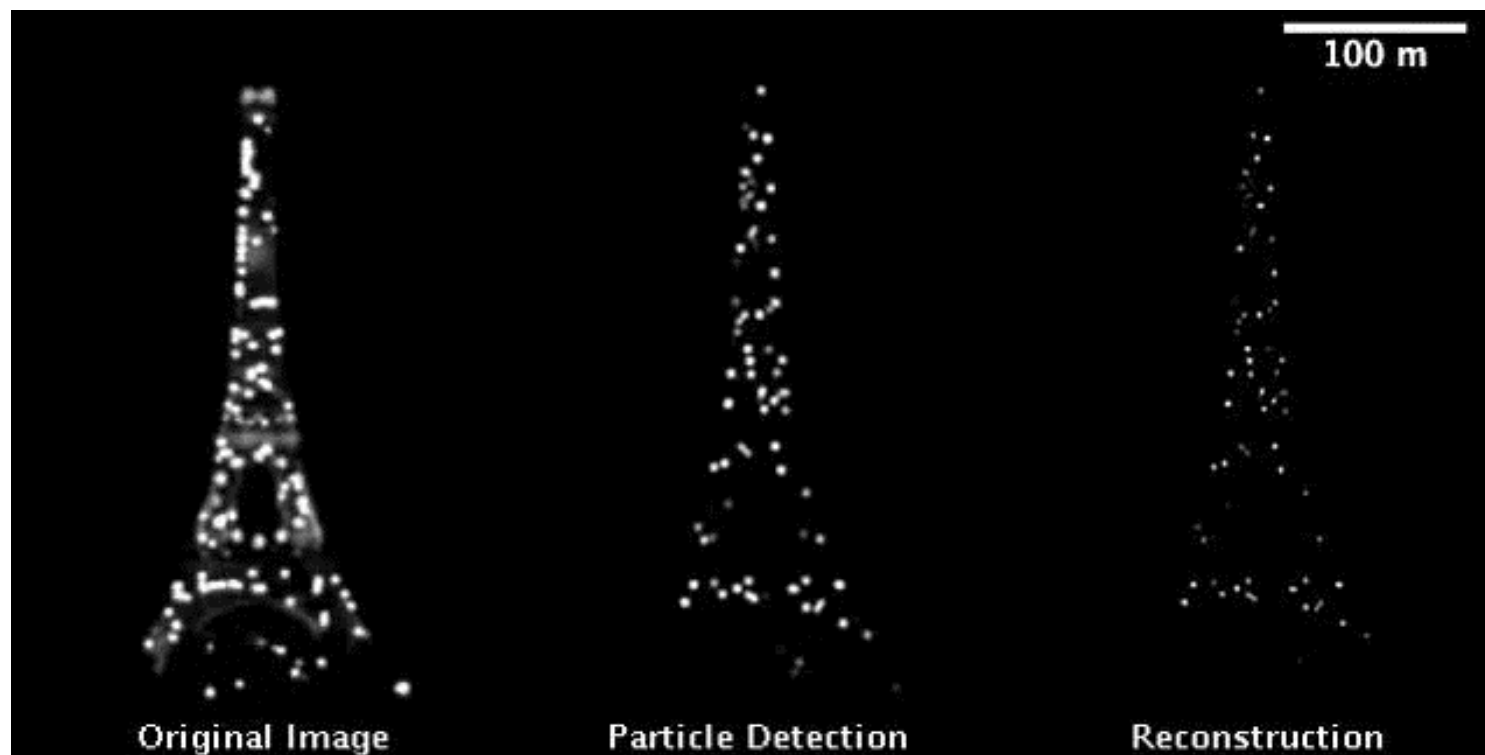
- Localisation microscopy
- Spot detection – icy plugin
- Tracking – icy plugin
- Trajectory analysis – MATLAB GUI

Localisation Microscopy

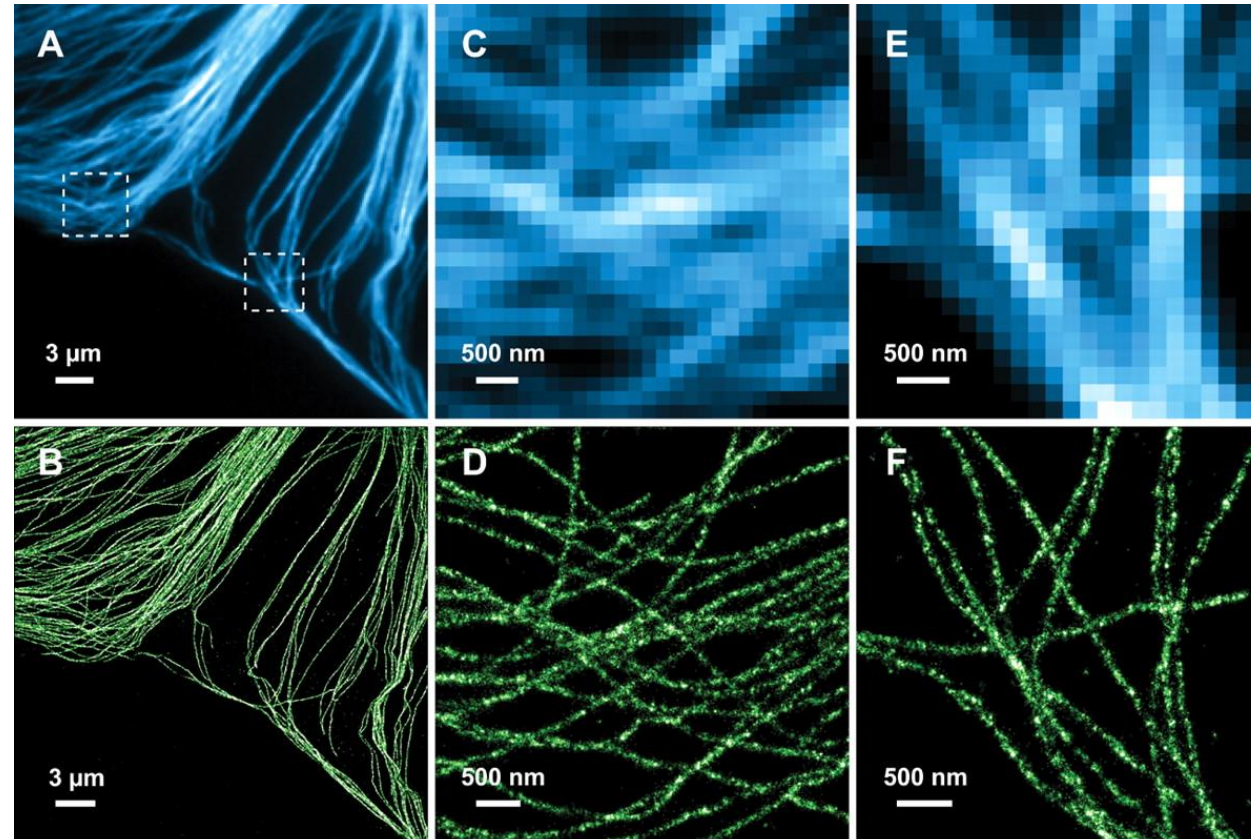
- Super-resolution technique to overcome the diffraction limit of light
- Resolves molecules within the diffraction limited region by separating them in time
- High resolution, ~ 20 nm, in all spatial dimensions



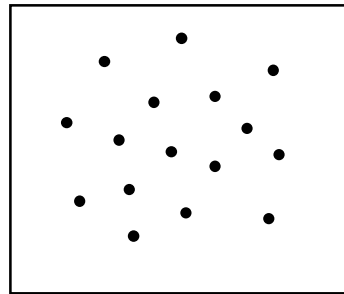
Can be used for localisation and tracking of single molecules



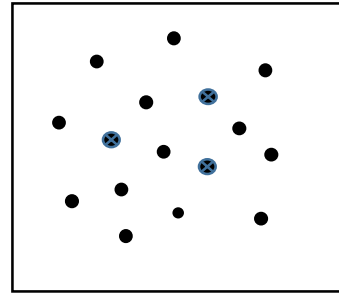
<https://www.youtube.com/watch?v=RE70GuMCzww>



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



Initial FOV



Stochastically activate
a subset of molecules

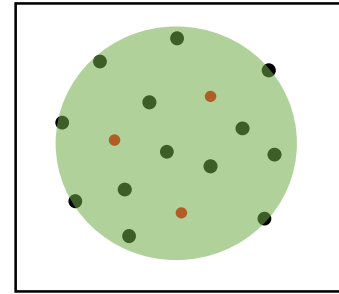
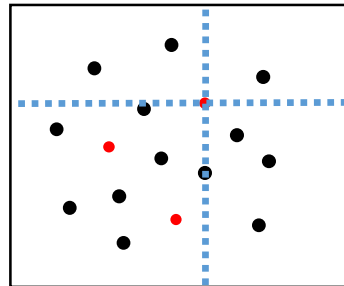


Image activated molecules



Localise imaged
molecules

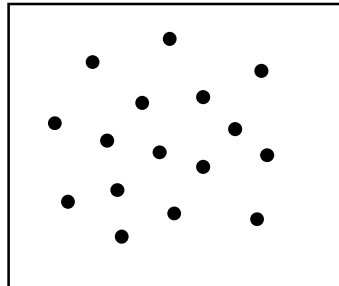
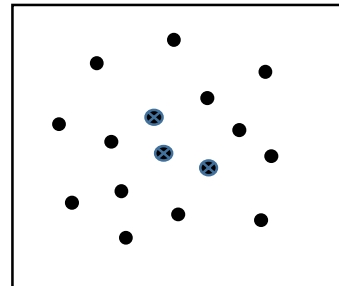


Photo bleach active
molecules



Repeat the activation
process

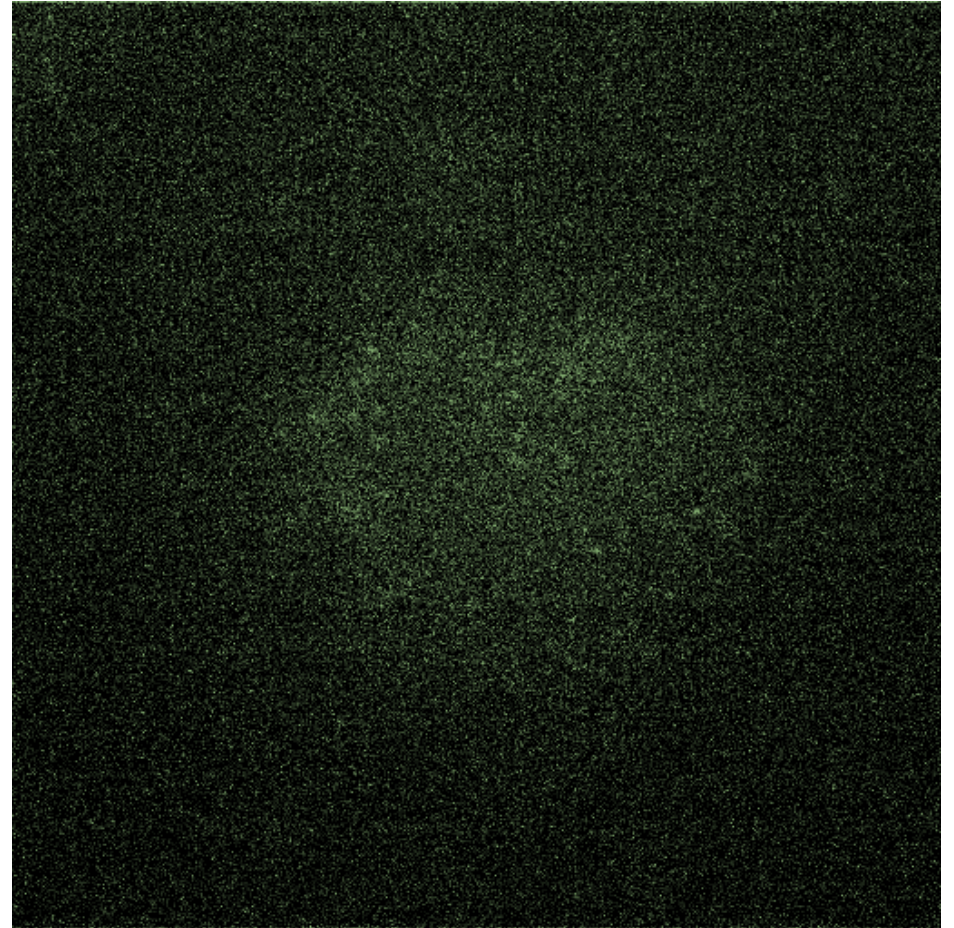
Stochastic activation of single molecules can be used for tracking of single molecules

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

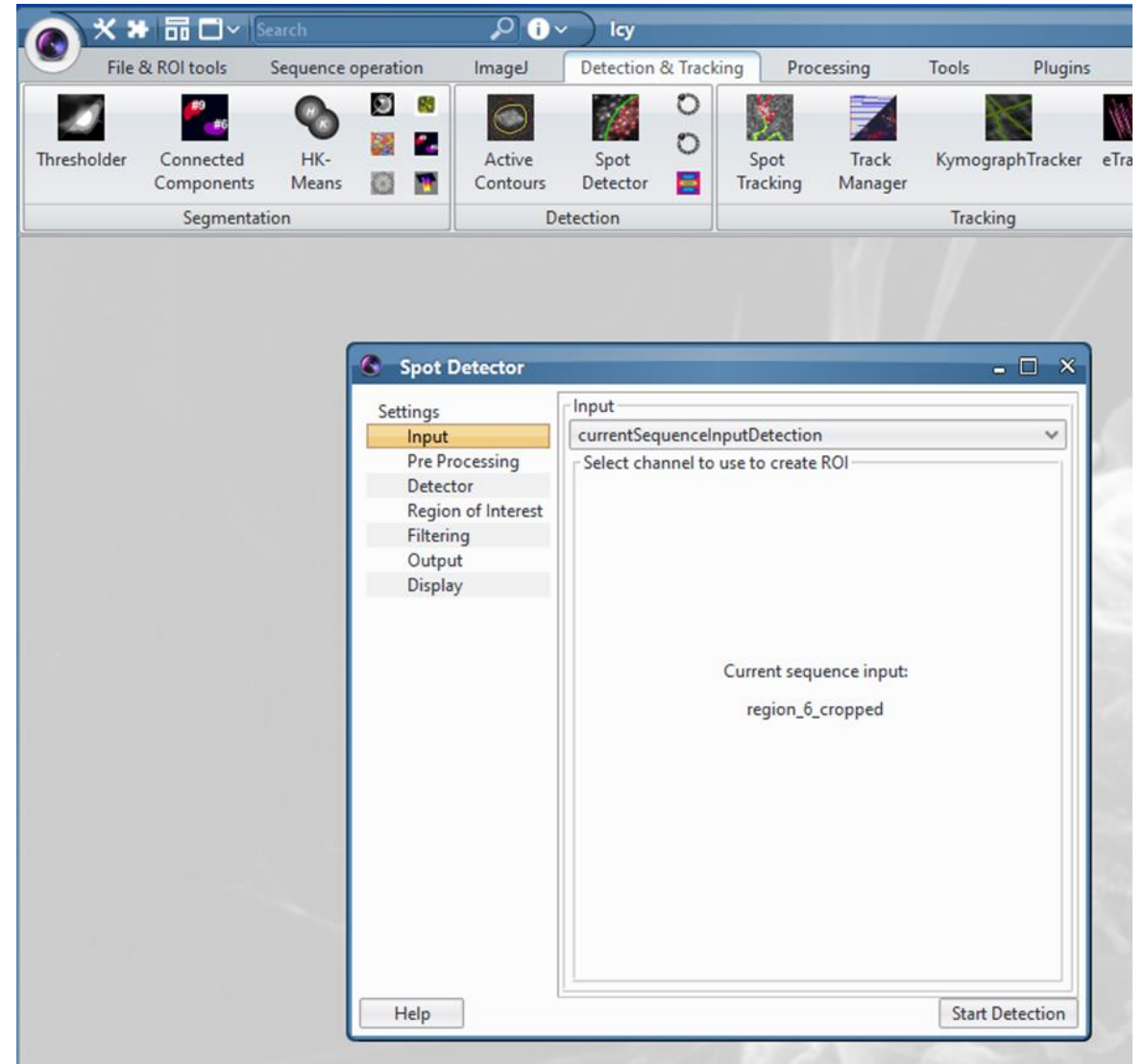
- Labelled with a photo-convertible protein: mEOS
- Nuclei were imaged 20-40 um deep inside the tissue



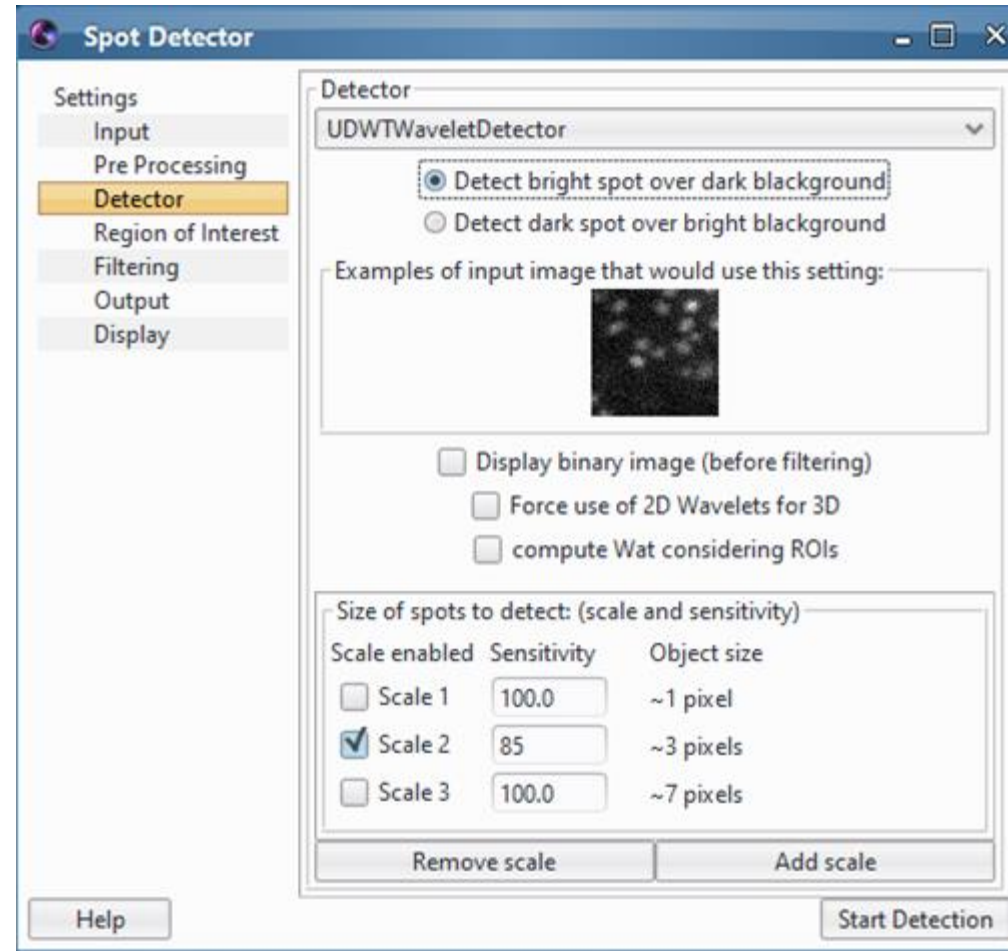
Goal: Detect and track single molecules

Spot Detection

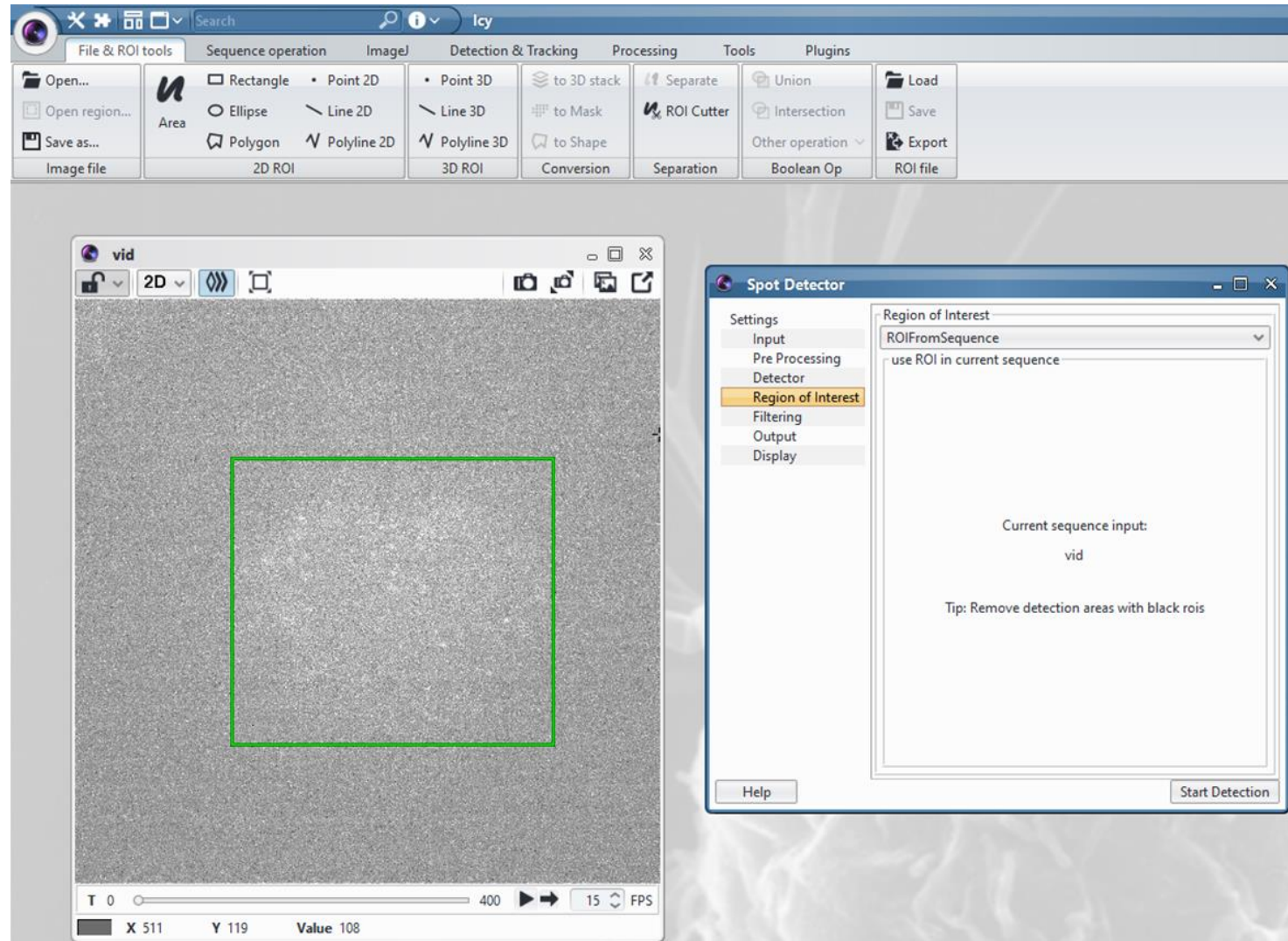
- Freely available as an Icy-plugin
- Based on wavelet transforms
- Important step before tracking



Detector



Spot Detection – ROI



Spot Detection – Display and Output

Settings

- Input
- Pre Processing
- Detector
- Region of Interest
- Filtering
- Output**
- Display

Output

Excel output settings:

Automatic XLS file naming

☒ Enable Automatic

The XLS file will be saved in the 'save' folder of the original image. The file name will be 'originalfilename.xls'. If an XLS file already exists, it will be replaced.

☐ Append data to existing files.

Append all data to a single file

☐ Enable Specific file

The XLS data will be appended to this file. If the file does not exist, it will be created. Each image will consist in one page in the XLS file. If it becomes slow consider using XML. Watch how to use it on the online documentation.

no file selected

XML output settings:

☐ Enable XML export

The XML data will be appended to this file. If the file does not exist, it will be created.

no file selected

☐ Export to ROI

☒ Remove previous spots rendered as ROI

☐ Export original image with ROIs and detection

☐ Export binary image

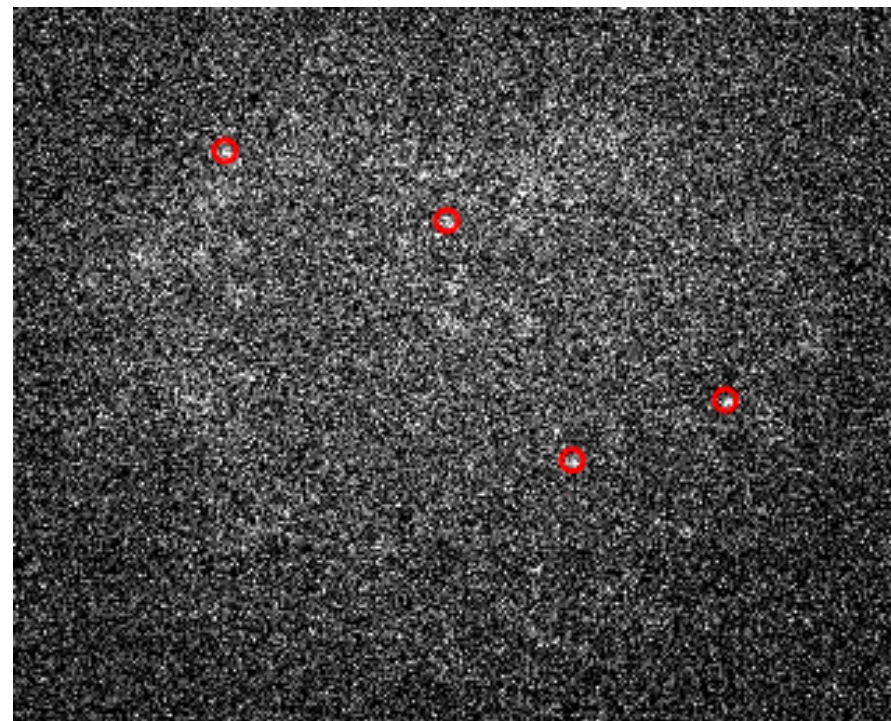
☒ Export to SwimmingPool

Help

Start Detection

27										
28	Detection	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	

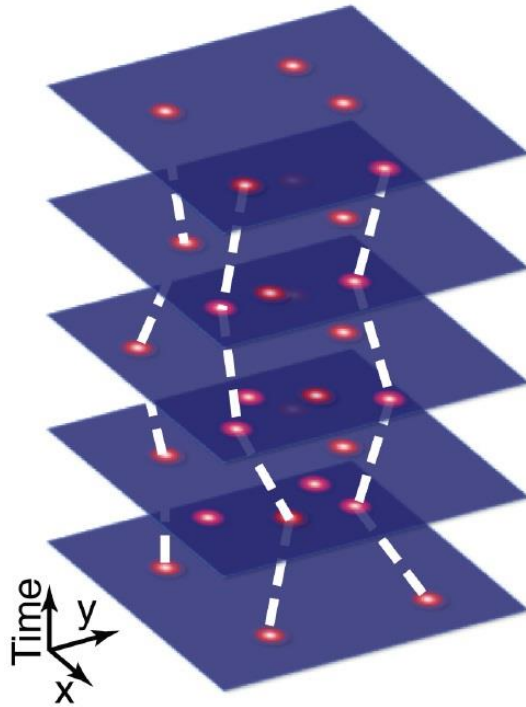
Detection Results, Excel file



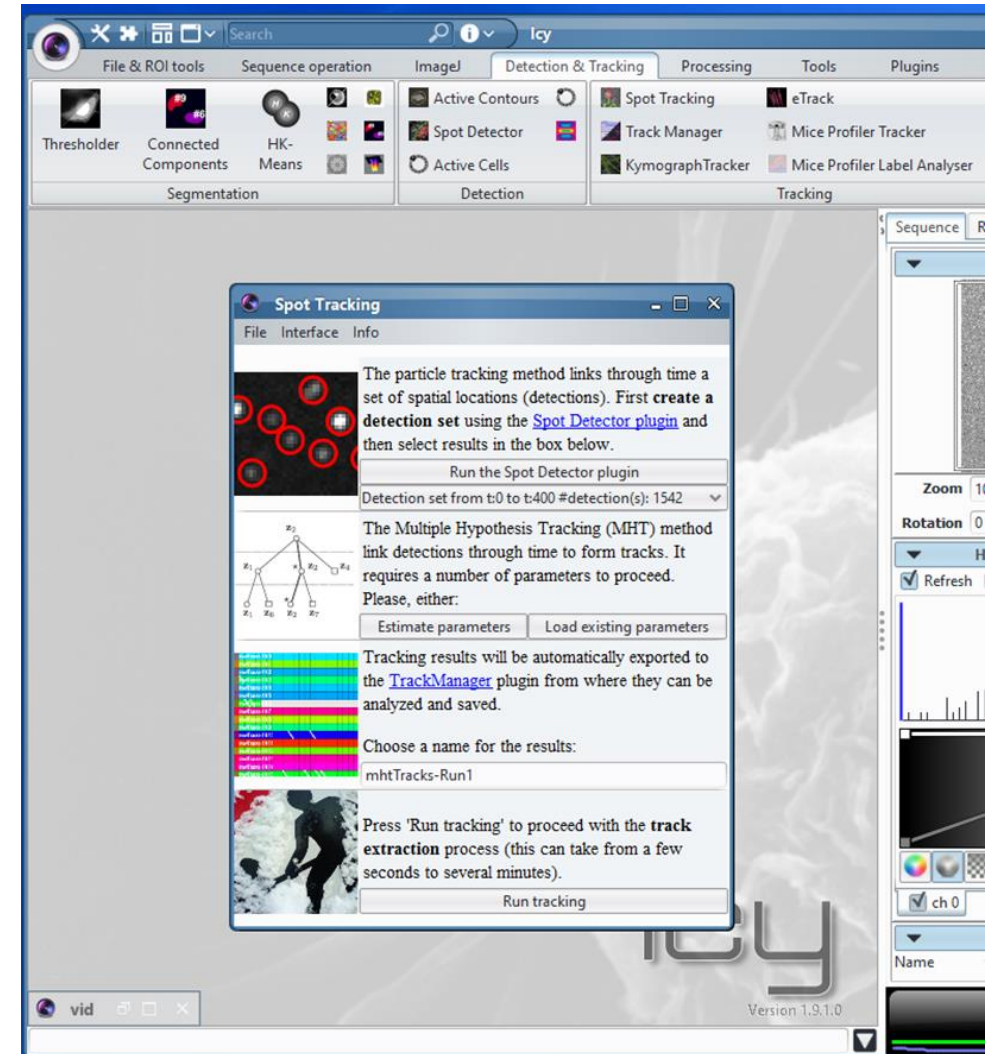
Next Step: Tracking

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

Parameter...timation

Target motion

- ☒ is mainly diffusive (default).
- ☐ is mainly directed.
- ☐ is both diffusive and directed.

Parameters for target motion

- ☐ are kept to their initial values (default).
- ☒ are re-estimated online.

Run parameter estimation procedure

Spot Tracking

File Interface Info

The particle tracking method links through time a set of spatial locations (detections). First **create a detection set** using the [Spot Detector plugin](#) and then select results in the box below.

Run the Spot Detector plugin

Detection set from t:0 to t:400 #detection(s): 1542

The Multiple Hypothesis Tracking (MHT) method link detections through time to form tracks. It requires a number of parameters to proceed. Please, either:

Estimate parameters Load existing parameters

Tracking results will be automatically exported to the [TrackManager](#) plugin from where they can be analyzed and saved.

Choose a name for the results:

mhtTracks-Run1

Press 'Run tracking' to proceed with the **track extraction** process (this can take from a few seconds to several minutes).

Run tracking

Spot Tracking

File Interface Info

Detection source

Tracking

Target existence

Motion model

Tracking algorithm

Output

Configuration file

Reference

This plugin implements the Multiple Hypothesis Tracking algorithm described in:
Nicolas Chenouard, Isabelle Bloch, Jean-Christophe Olivo-Marin, Multiple Hypothesis Tracking for Cluttered Biological Image Sequences, IEEE Transactions on Pattern Analysis and Machine Intelligence, vol. 35, no. 11, pp. 2736-3750, Nov., 2013.
Pubmed link : <http://www.ncbi.nlm.nih.gov/pubmed/23689865>.
Please reference and cite properly.

Quick settings

Expected number of new tracks per frame

0.753

Expected number of objects in the first frame

5

Expected track length

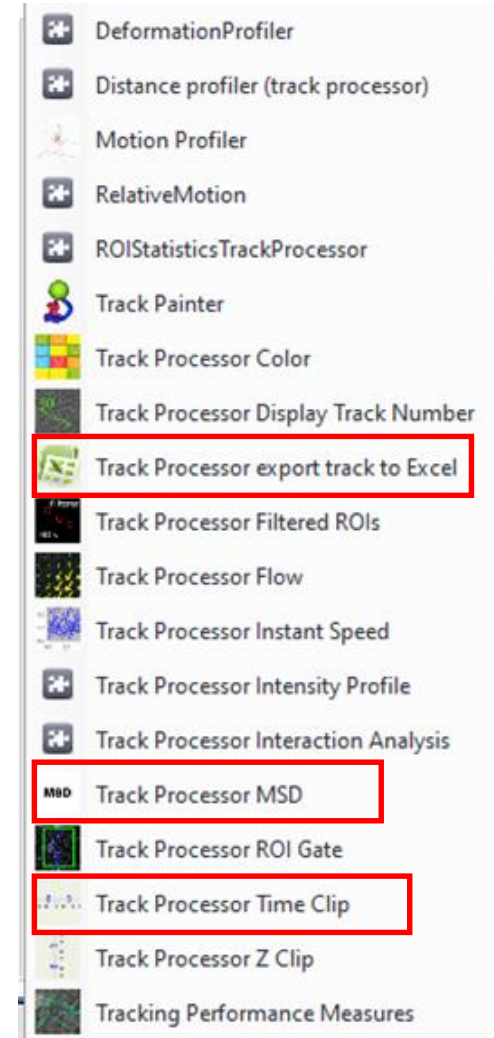
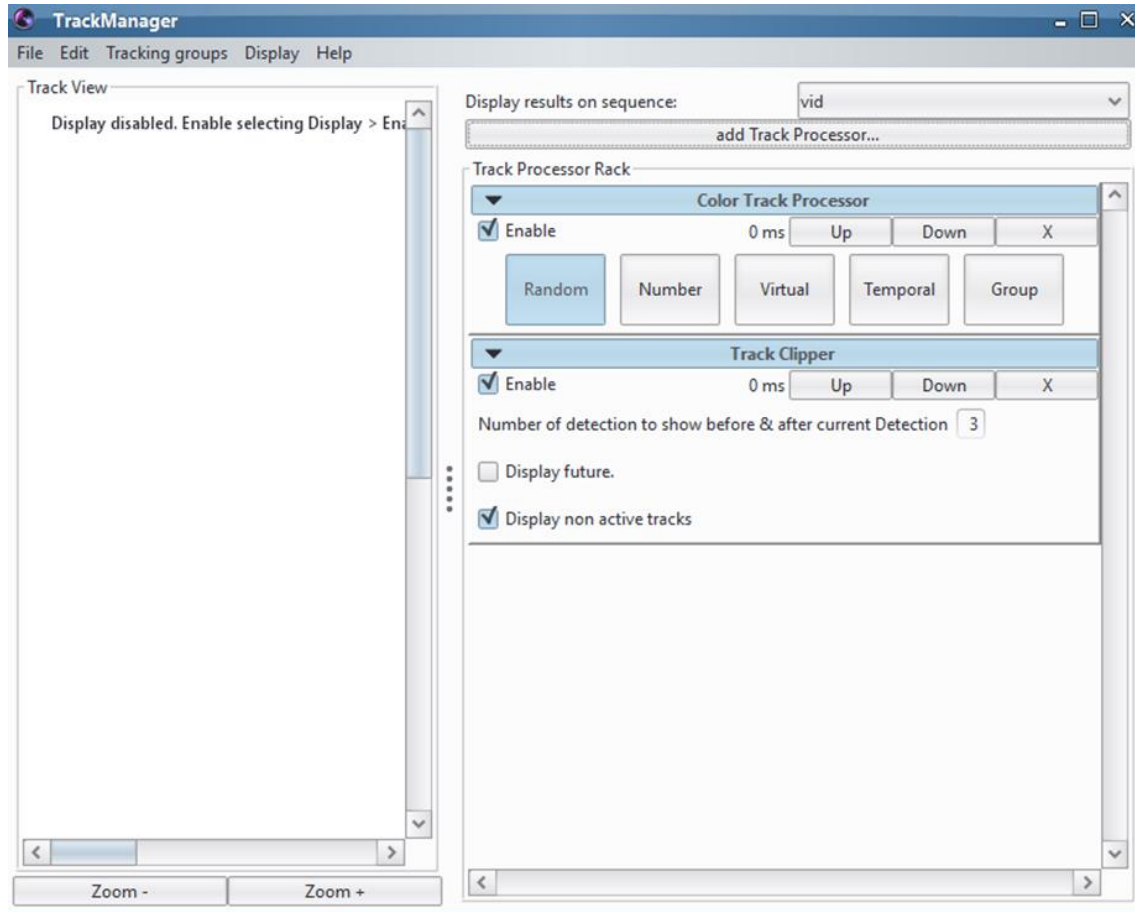
5

Average length of displacement between two frames

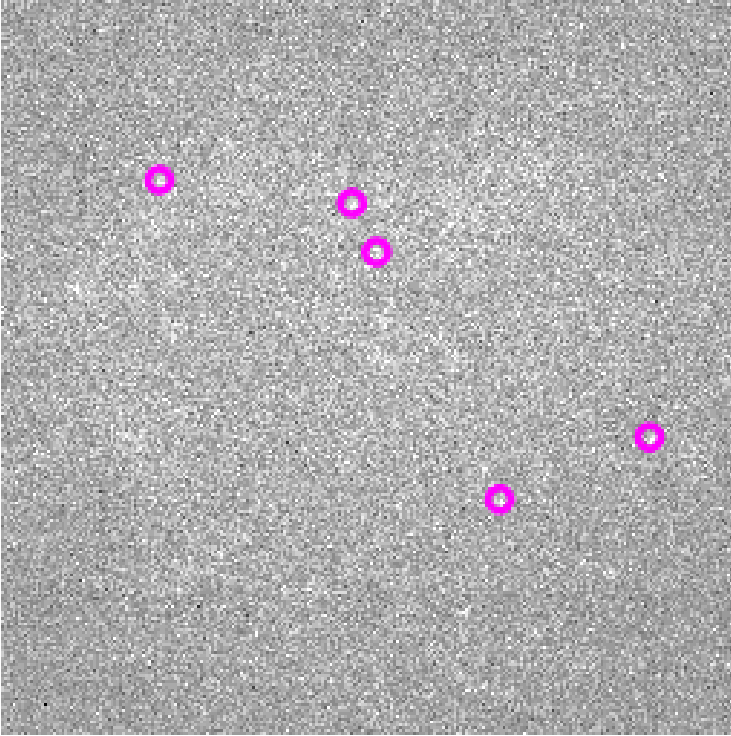
1.955

Detection source	Select the input for detections in the Swimming Pool
Tracking	Detection set from t:0 to t:400 #detection(s): 1889
Target existence	Expected number of false detections per frame
Motion model	
Tracking algorithm	4
Output	Probability of detection for each particle
Configuration file	0.793

Simple Analysis: Track Manager



Output



1	version	description	id	classname	color	t	type	x	y	z	linklist
2	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	0	1	237.8333333	152.1666667	0	
3	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	1	1	239.6666667	151.3333333	0	
4	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	2	1	237.8888889	151.3333333	0	
5	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	3	1	239	152.5	0	
6	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	4	1	239	152.2857143	0	
7	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	5	1	239.6666667	152.6666667	0	
8	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	6	1	239.5	152.5	0	
9	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	7	1	239.125	153.875	0	
10	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	8	1	239	153.5	0	
11	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	9	1	239.5	153	0	
12	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	10	1	239.1111111	153.3333333	0	
13	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	11	1	239.5	153.5	0	
14	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	12	1	238.5	153.5	0	
15	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	13	2	238.7300306	153.4863628	0	
16	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	14	1	239	153	0	
17	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	15	1	239.5	153.5	0	
18	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	16	1	239.3333333	153.3333333	0	
19	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	17	1	239.2222222	153.4444444	0	
20	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	18	1	238.5	152.5	0	
21	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	19	1	240.1111111	152.6666667	0	
22	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	20	1	239	153.2857143	0	
23	1	tracks_1	1085845567	plugins.nchenouard.particletracking.DetectionSpotTrack	-16776449	21	1	240.25	155	0	

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

Create Runinputfile

Settings

Input data: .all_tracks.mat

Trajectory field: all_tracks

Output data: .loutput.mat

☒ Run in parallel mode

☒ Additional estimates

Bootstraps: 100 ☒ FULL Bootstrapping

Number of runs: 25

Max # hidden states: 10

Min. trajectory length: 2

Advanced users can find additional settings in the generated script.

Parameters

Unit of length: nm Dimensionality: 2

Timestep [s]: 0.01

Ranges for initial guesses:

Diff. coeff. [$\mu\text{m}^2/\text{s}$]: 0.01 to 5

Dwelltime [timesteps]: 2 to 20

Choose a reasonable range for initial guesses. Within an order of magnitude from the expected values.

Job ID: Data from all_tracks.mat :: all_tracks from_26-May

Generate ID

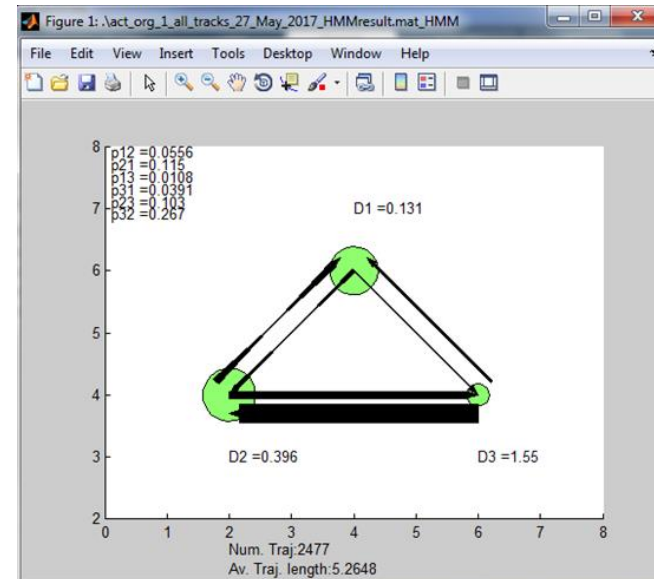
Script: runinput_26_May_2017.m

Local folder: C:\Users\Sohaib\Dropbox\PhD_Lab\used_alltracking_molecults_maria\input_segmenting_track

About Save Run Show Result Load Script Close

Results

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



Command Window

```

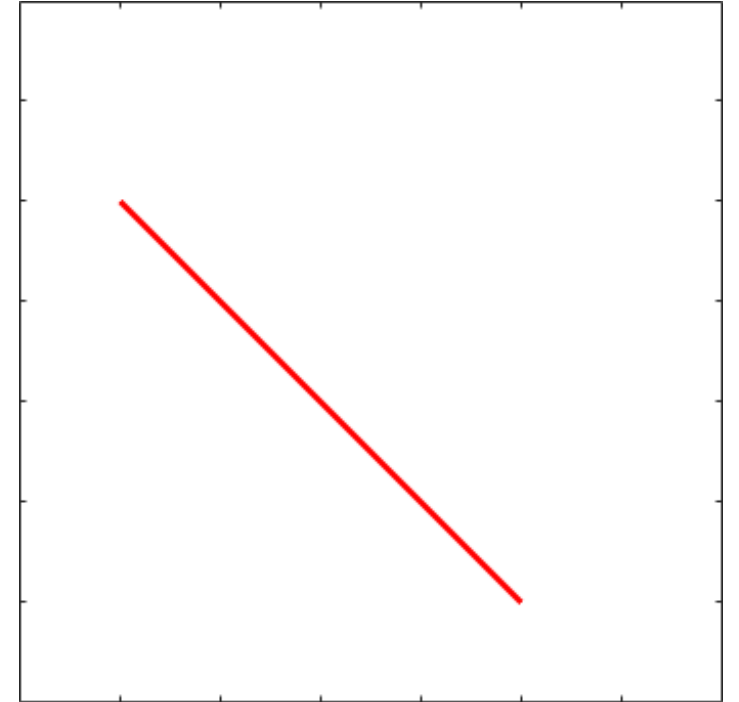
Num. Traj.:
2477
Av. Traj. length:
5.2648
Diffusion Coeff [um^2/s]:
0.13107    0.39573    1.5521
0.0020373  0.0058751    0.035637
Total Occupation:
0.39146    0.42926    0.17929
Dwell time [s]:
0.15014    0.046068    0.033025
Transition probability per timestep:
0.9336     0.055571    0.01083
0.11498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

Different States within a Track

- Each step in the track is allocated a diffusion coefficient

e.g.

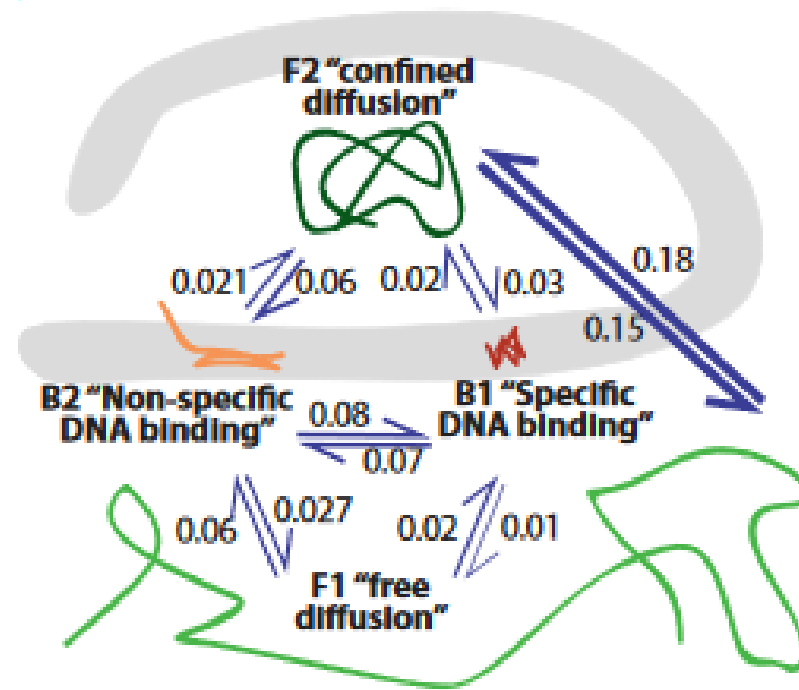
[1.6,1.6,1.6,1.6,1.6,0.1,0.1,0.1,0.1] $\mu\text{m}^2/\text{sec}$



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