



Bioimage Informatics

Daniel Sage & Arne Seitz

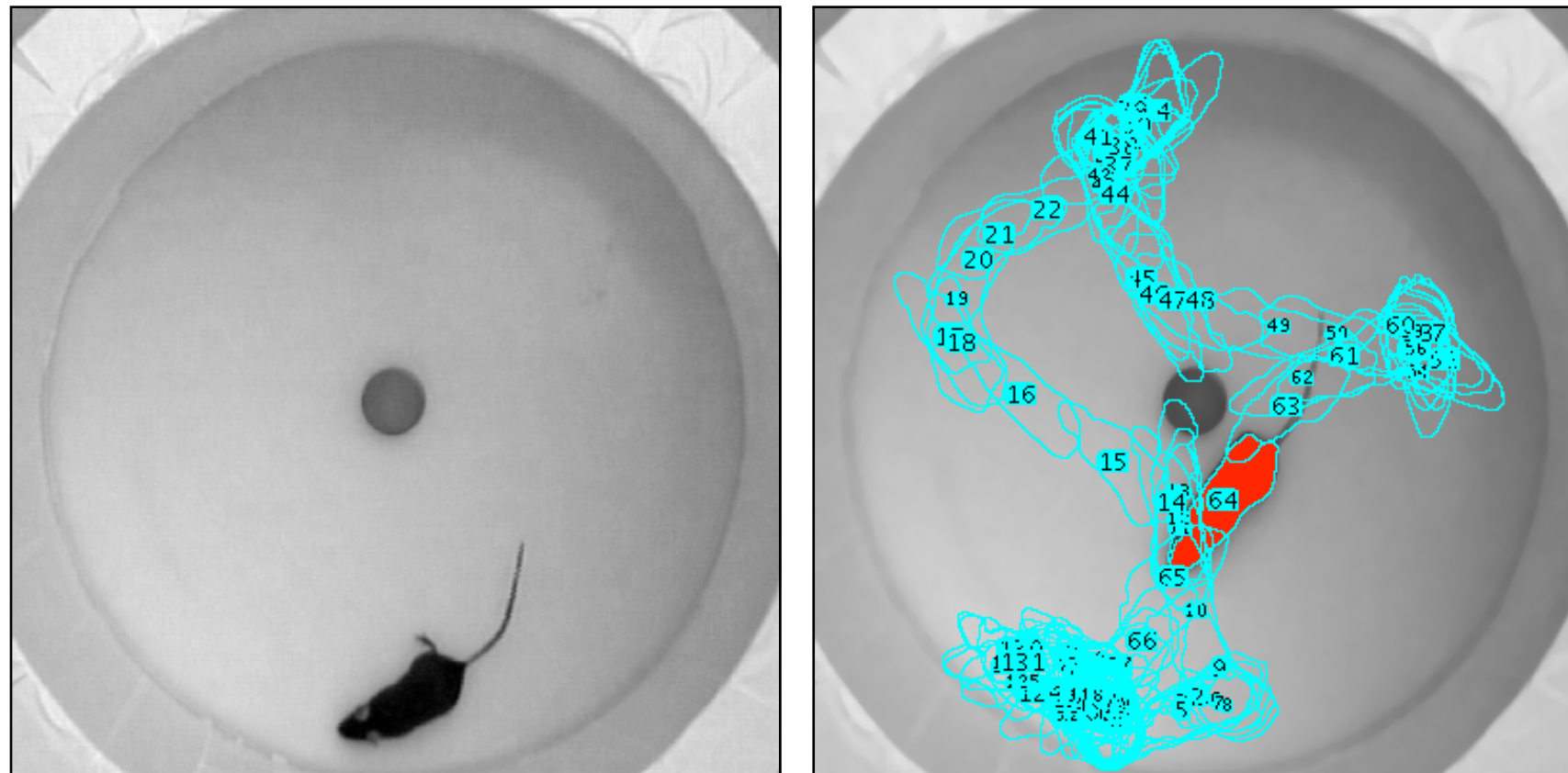
Course

Tracking Particles

Complexity of the Problem



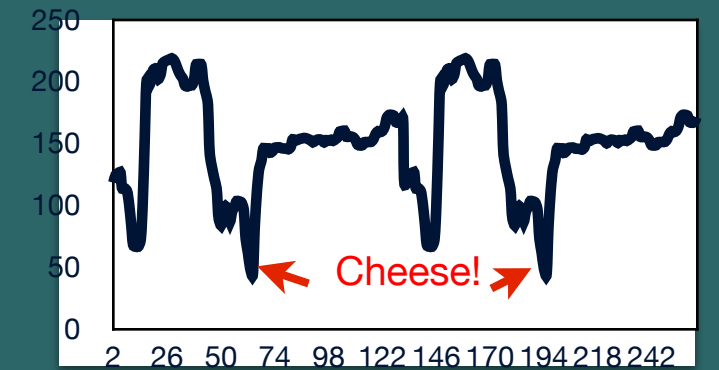
Standard tool



- One moving object
- No cross trajectory
- Stationary background
- High SNR
- Orthogonal view 2D
- Small displacement

Piece of cheese! 10 lines of macro

```
setThreshold(0, 41)
run("Median...", "radius=3 stack")
run("Analyze Particles...", "add stack display")
run("Set Measurements...",
    "size=500-Infinity area center fit circularity")
for (i=0; i<nSlices;i++) {
    roiManager("Select",i);
    run("Measure");
}
x=newArray(nResults)
y=newArray(nResults)
for (i=0; i<nResults;i++) {
    x[i]=getResult("XM", i);
    y[i]=getResult("YM", i);
}
makeSelection("polyline", x, y);
```



Visual feature

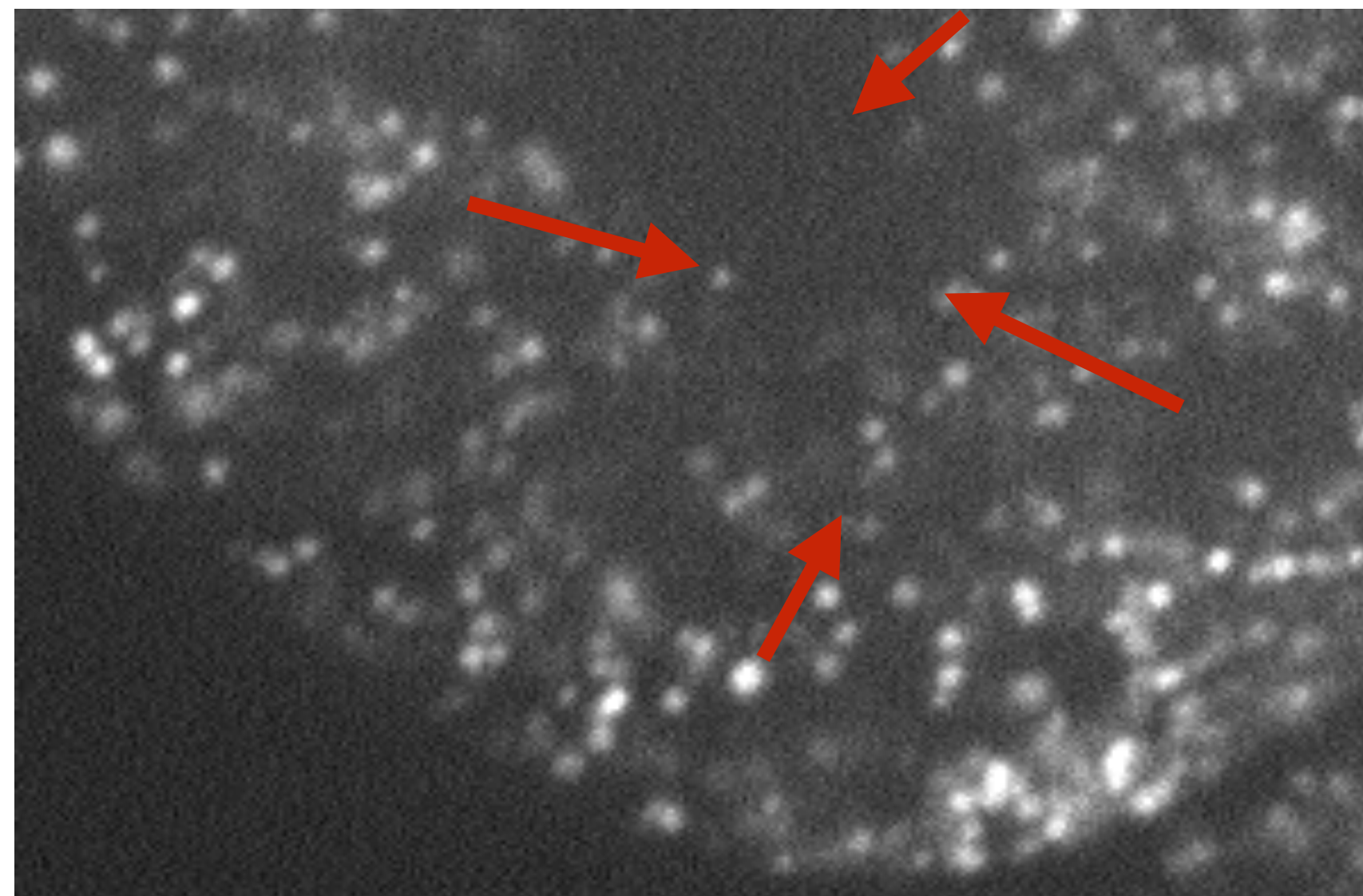
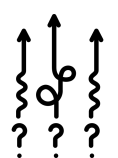
- Density: disconnected regions
- Discriminative target: shape, intensity, 2D or 3D

Motility model

- Motion: directional, brownian or physical constraint
- Events (fusion, division, interaction, out of FoV, occlusion)
- Ambiguities: crossing, occlusion

INTEGRATION OF THE MOTION MODEL IN THE TRACKING ALGORITHM

Require a
specific dev.



Motion Analysis in Imaging

Motion field estimation

Pixel-based method

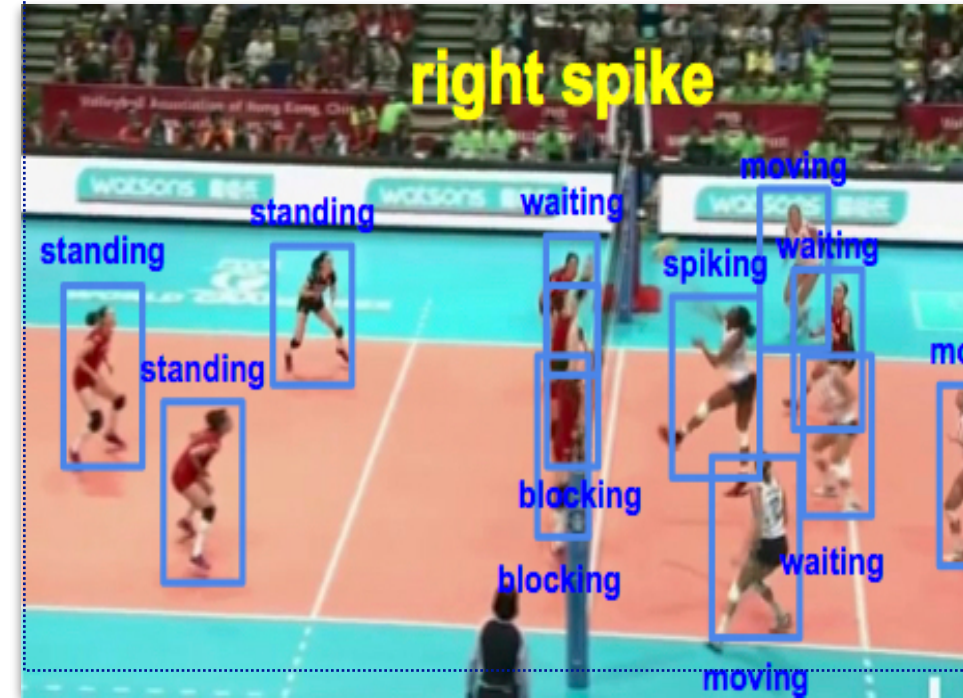
- Optical flow
- Solve inverse problem



Target tracking

Real-time tracking

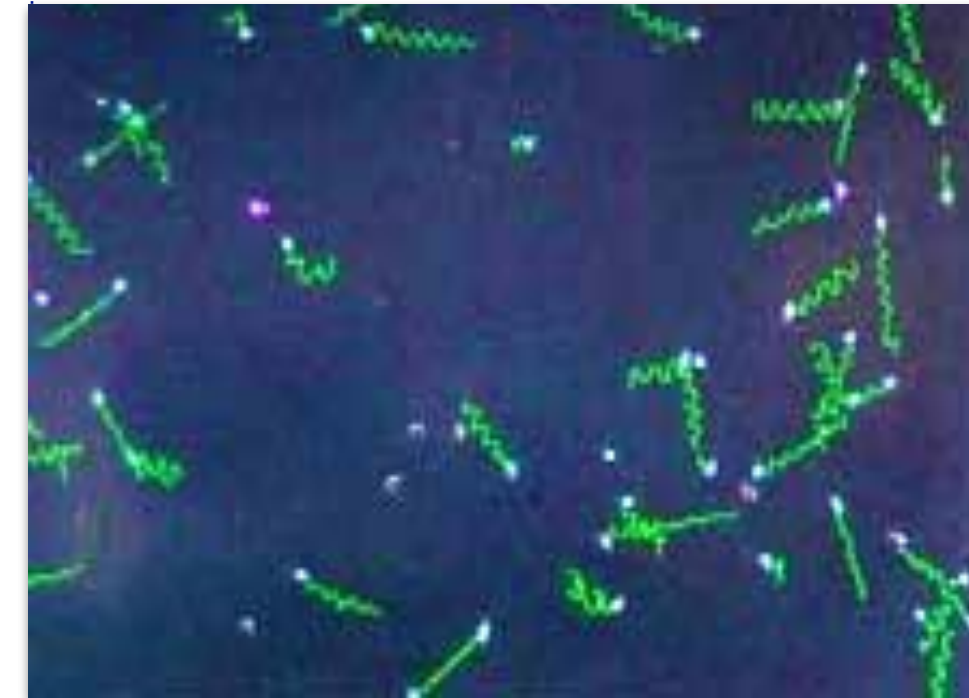
- Feature extraction
- Prediction of position



Particle tracking

Off-line tracking

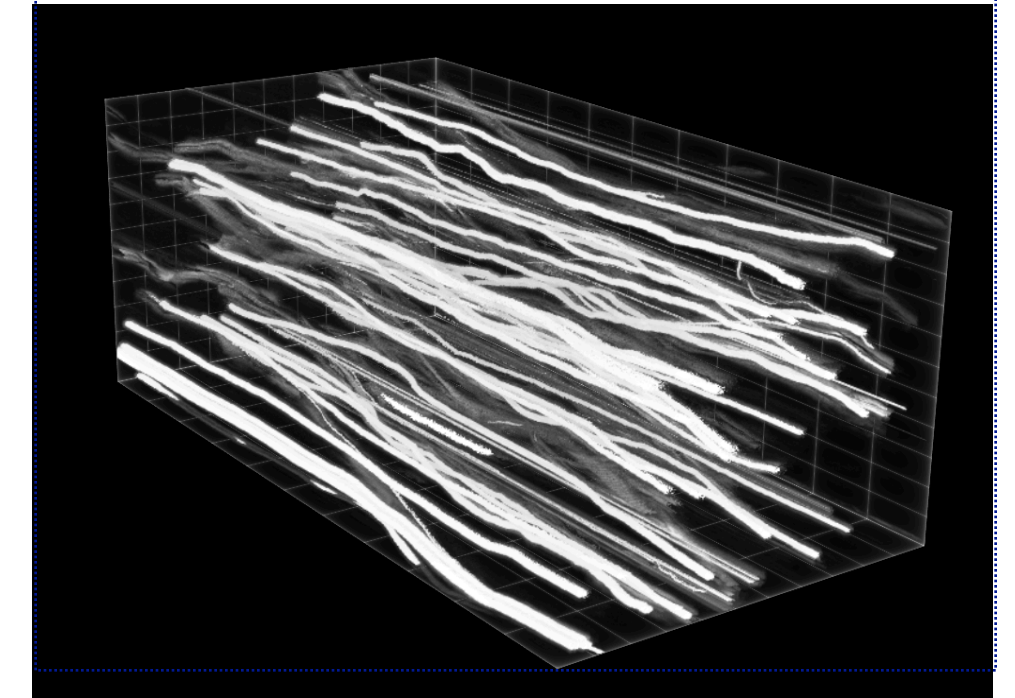
- Detection
- Linking with a cost



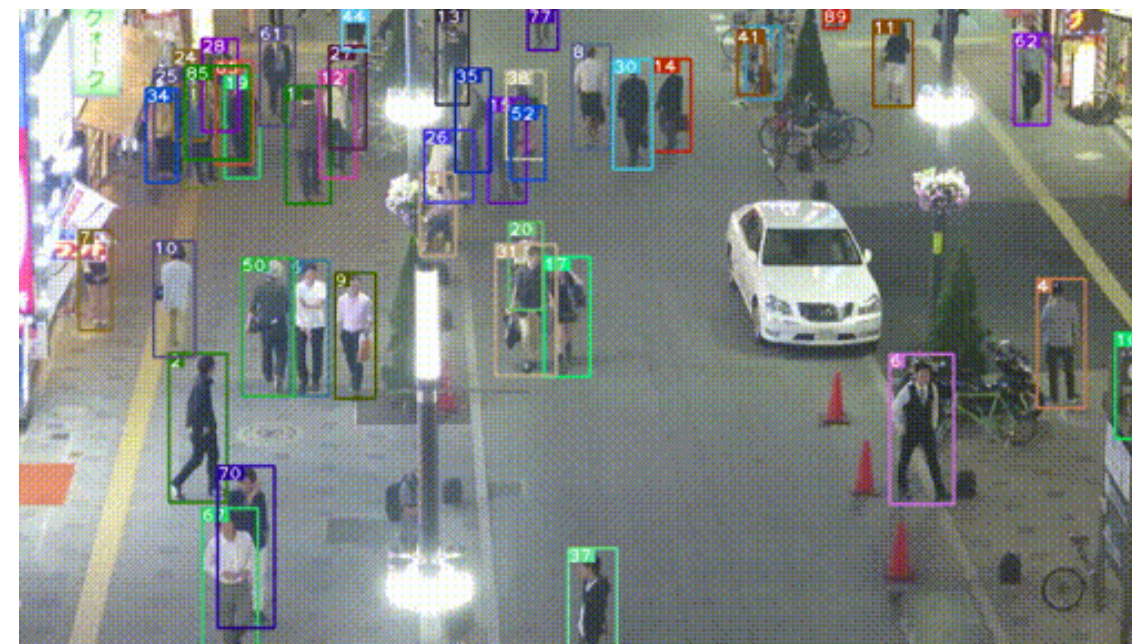
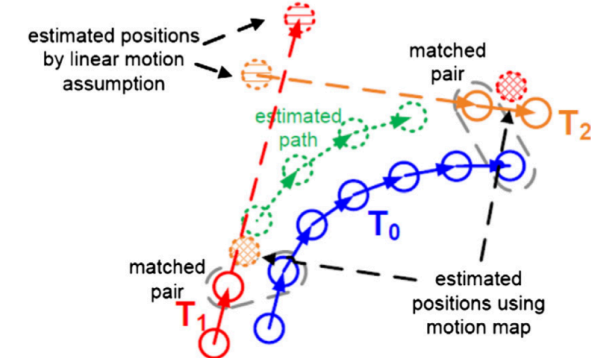
Spatio-temporal data

Trace as an object

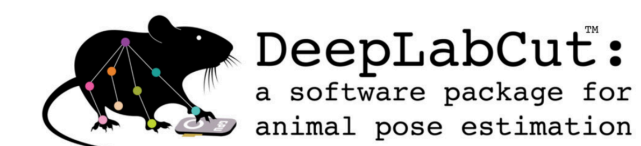
- Segmentation 2D+T



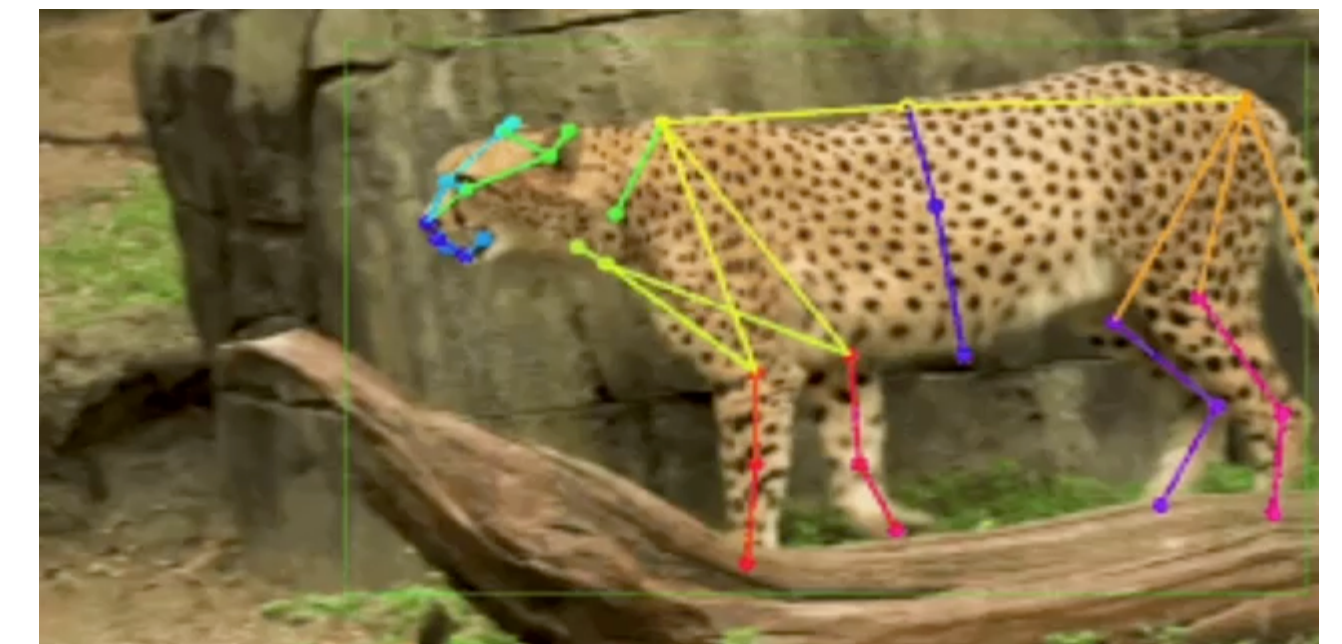
Multiple Objects Tracking (MOT)



Pose Estimation



Mathis et al, [Nature Neuroscience](#) 2018

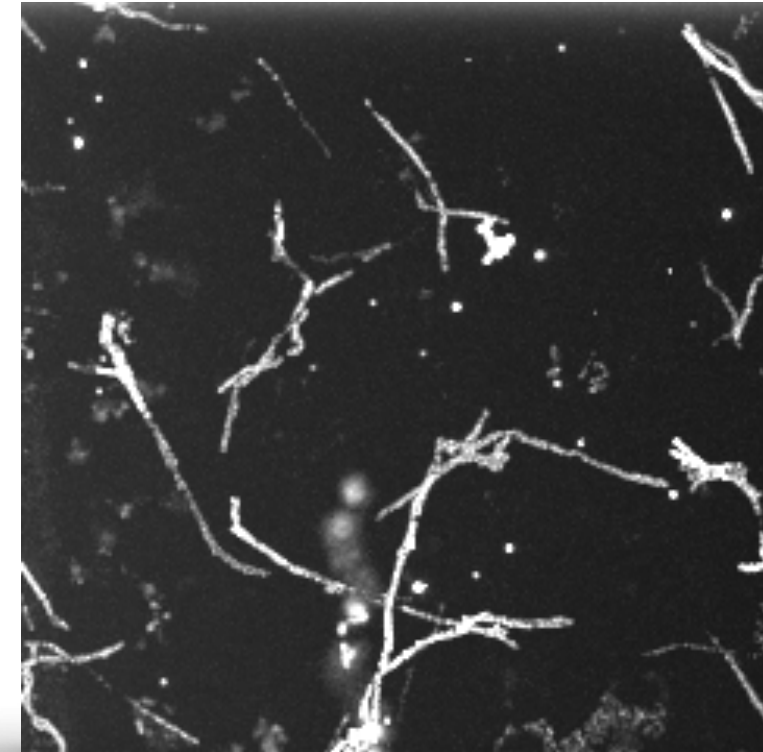




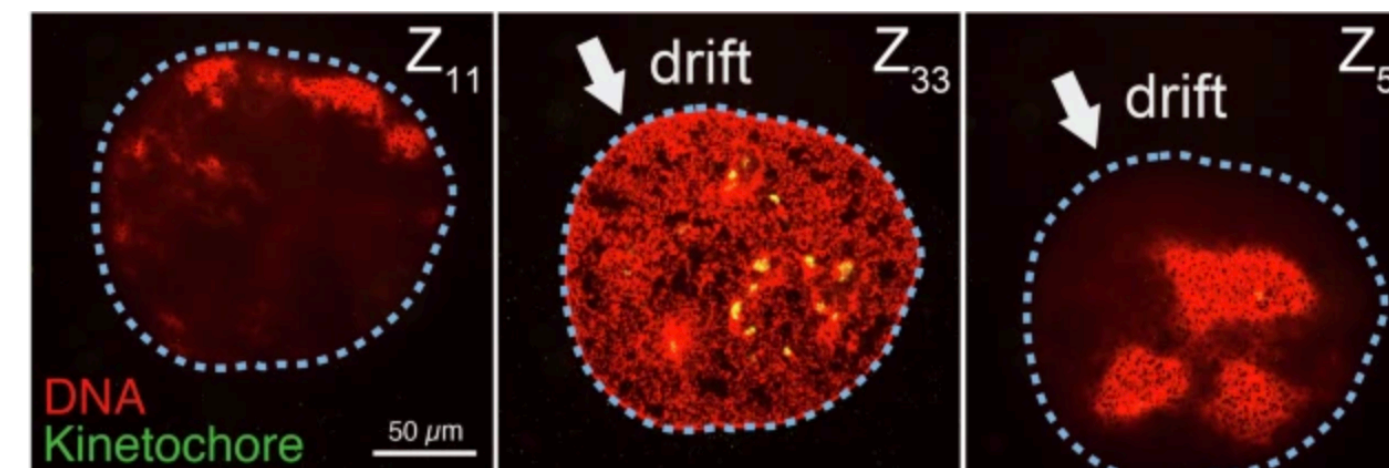
Moving Objects in Images



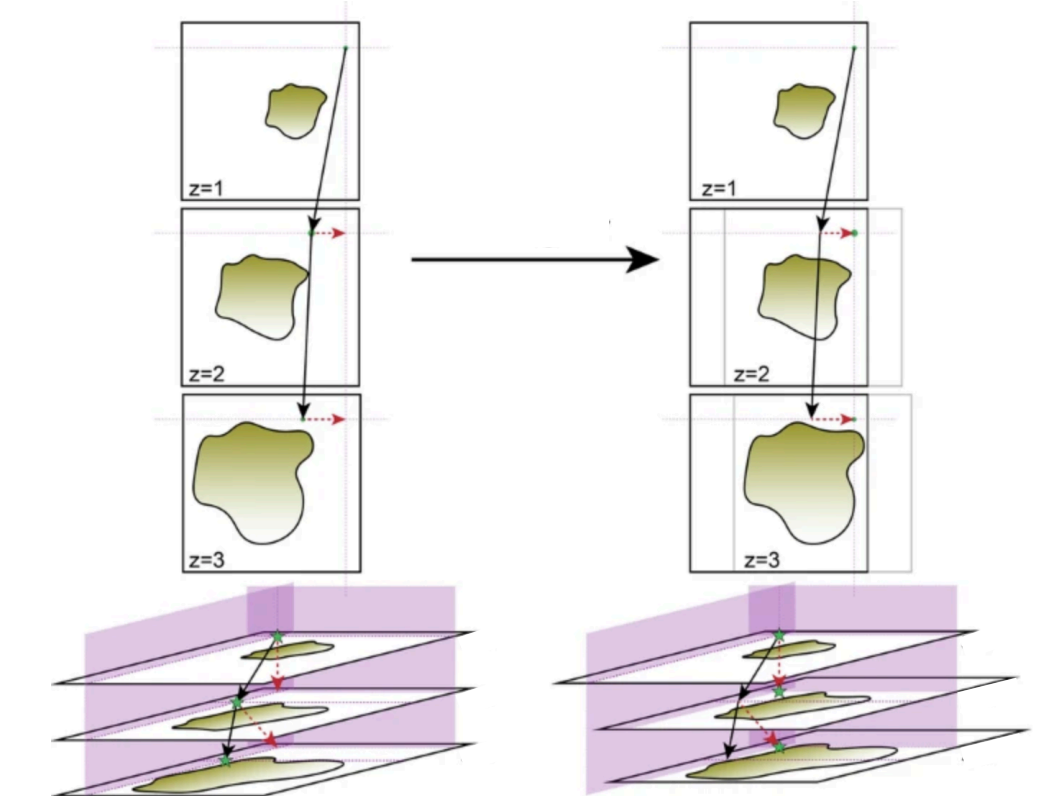
Movie



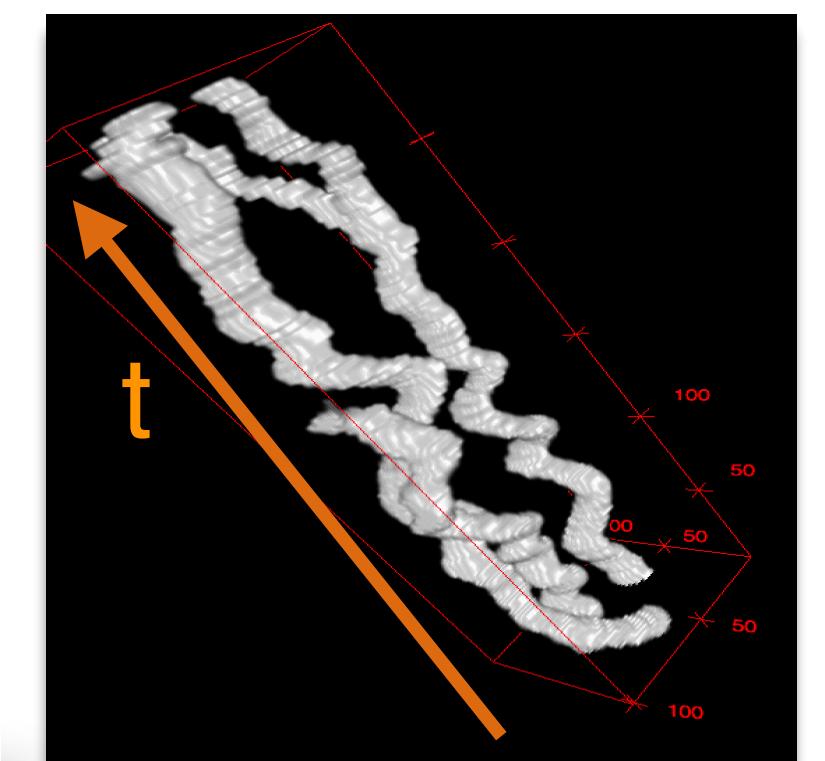
Time projection



Composition of movements: life sample, acquisition drift
➔ Drift correction



MIP in time
Projection



Volume rendering
Viewer 3D



Particle Tracking

Goals

- Diffusion: record individual trajectory
- Cell lineage: record cell division of mobile particle
- Interaction of molecules

Motility model

- Motion model: pathways or biophysical constraint
- Life events: mitosis, interaction
- Handling trajectory: crossing, ambiguities, occlusion

Time-lapse acquisition

Sample preparation

Photo-toxicity

Temporal resolution

Frame rate
Slow transition

Perturbation

Drift, vibration
Out-of-focus
Field of view

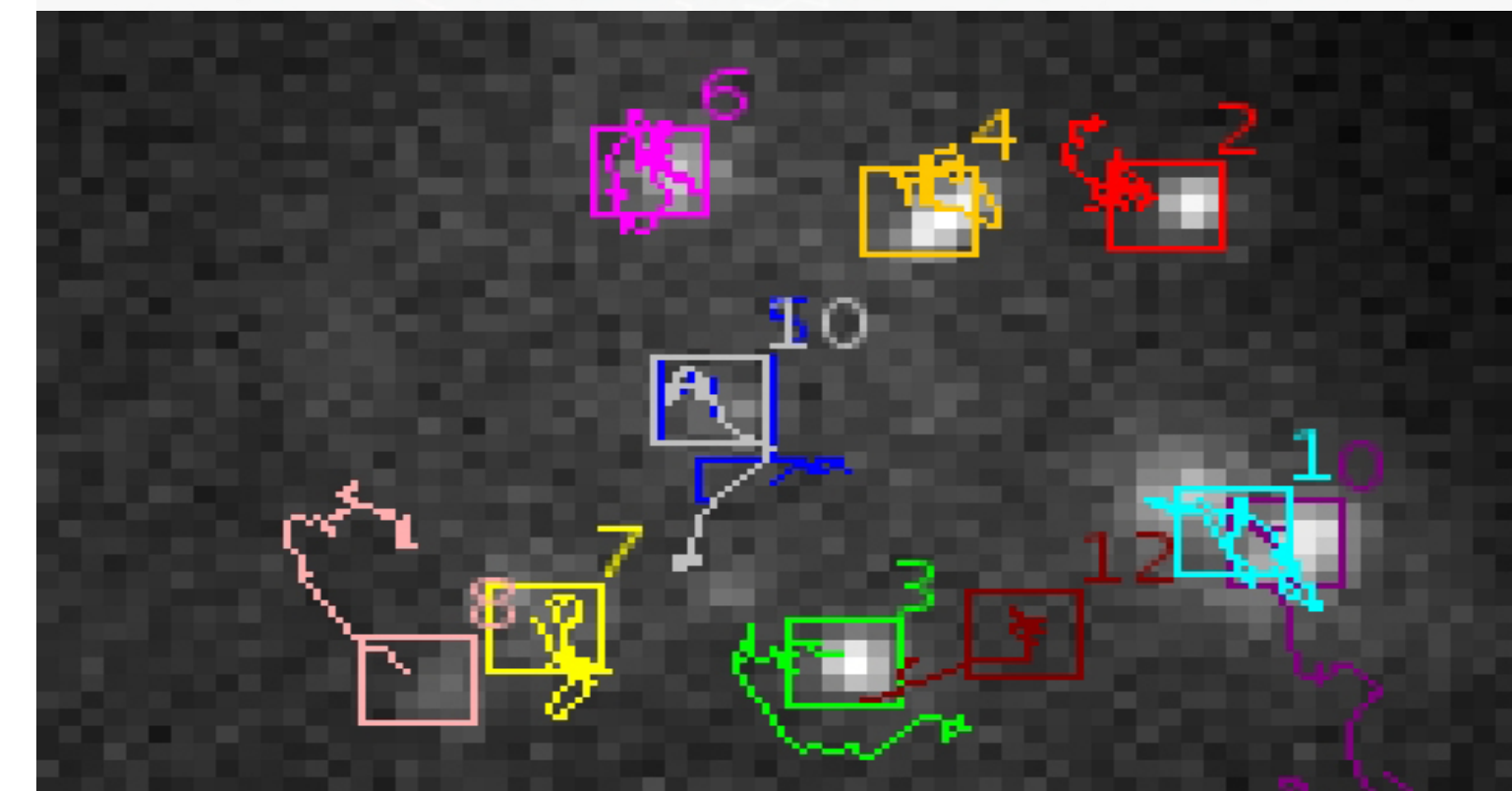
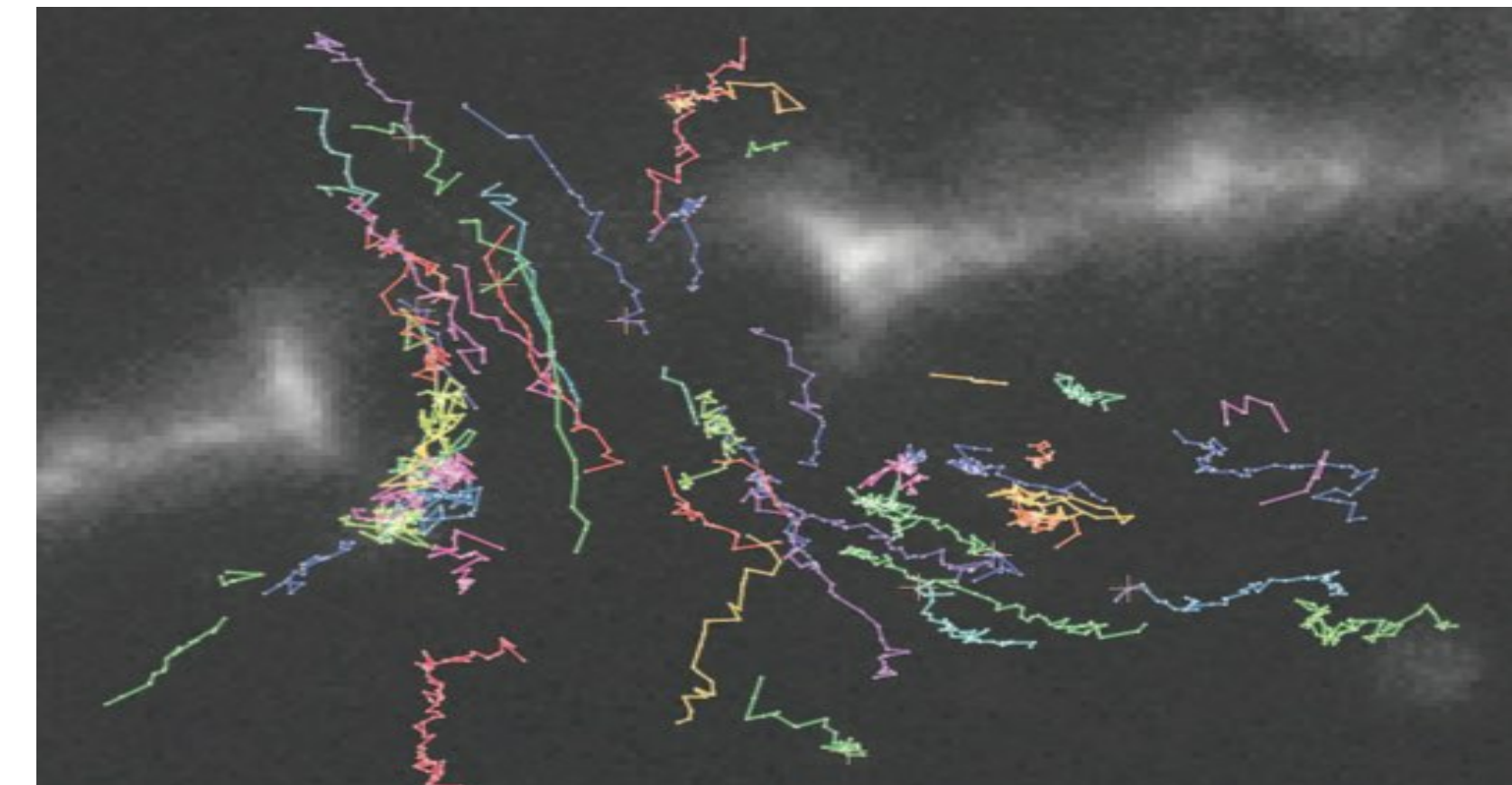


Spatial resolution

SNR

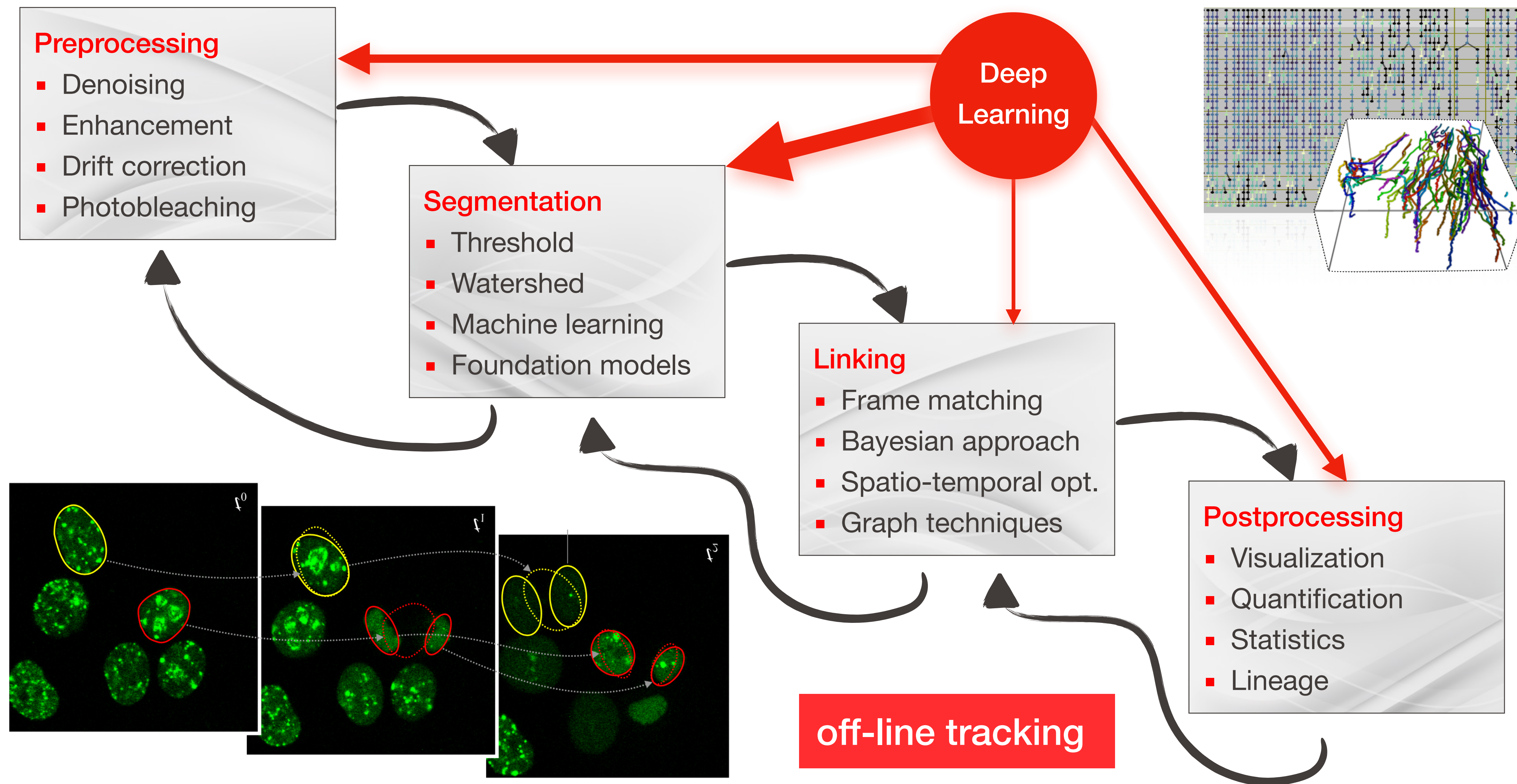


Photo-bleaching





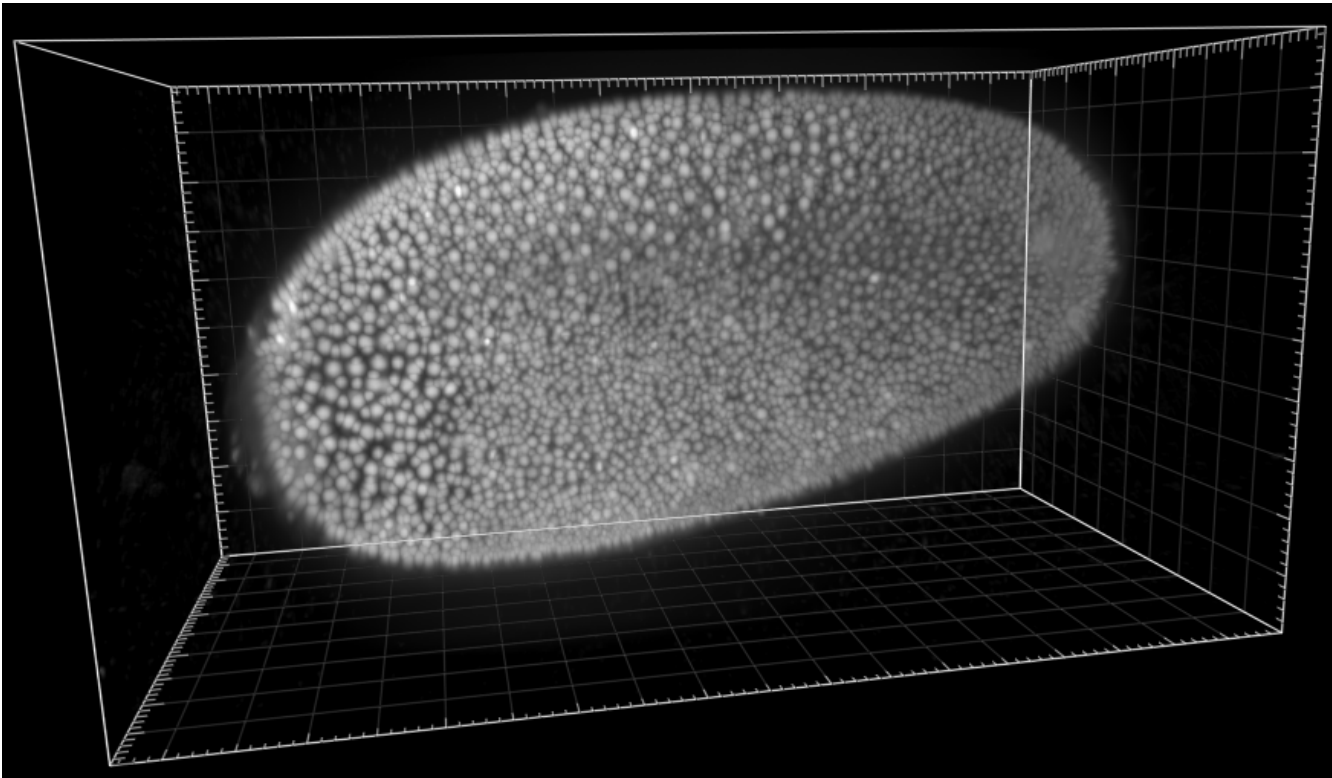
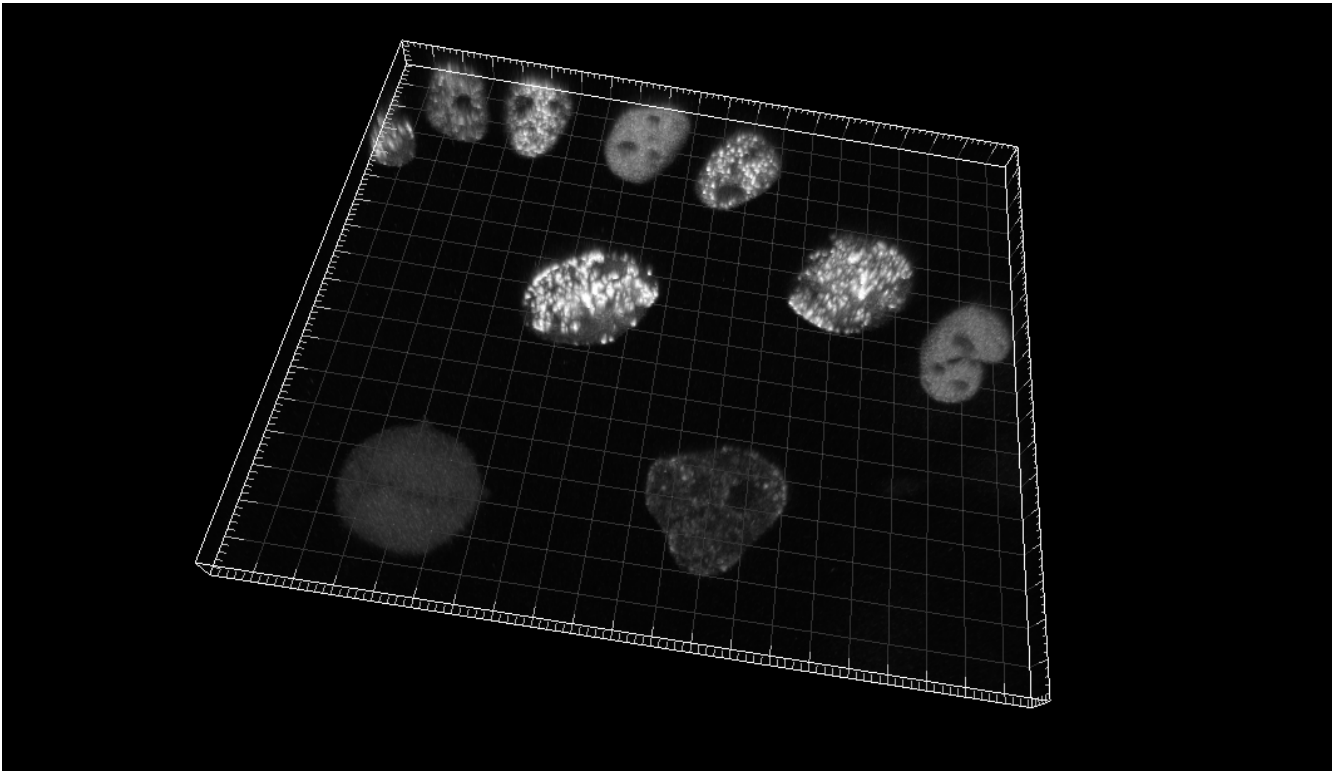
Tracking System





Tracking Methods

<http://celltrackingchallenge.net/>



Cell tracking challenge

Maska & al.

Nature Methods, 2023



Methods

Structure

Detection

Linking

Name	Available	Platform	Source	Cell	Particle	Multiple	Dimensions	Automation	Author or Reference
Braincells	Free	Win	✓	✓	✓	✓	2D	Manual	Gabor Ivancsy
CellProfiler	Free	Win/Lin/Mac	✓	✓	✓	✓	2D	Auto	Carpenter et al. (2006)
CellTrack	Free	Win	✓	✓	✓	✓	2D	Auto	Sacan et al. (2008)
CellTracker	Free	Win	✓	✓	✓	✓	2D	Semi	Shen et al. (2006)
ClusterTrack	Free	Matlab	✓	✓	✓	✓	2D	Auto	Matov et al. (2010)
DCellQ	Free	Matlab	✓	✓	✓	✓	2D	Auto	Li et al. (2010)
DIAS	Paid	Win/Mac	✓	✓	✓	✓	3D	Auto	Wessels et al. (2006)
DiaTrack	Paid	Win	✓	✓	✓	✓	3D	Auto	Semasopht (Switzerland)
DYNAMIK	Free	Matlab	✓	✓	✓	✓	2D	Auto	Mosig et al. (2009)
FARSIGHT	Free	Win/Lin/Mac	✓	✓	✓	✓	3D	Auto	Bjornsson et al. (2008)
GMinPro	Free	Win	✓	✓	✓	✓	2D	Auto	Mashanov & Molloy (2007)
ICY	Free	Java	✓	✓	✓	✓	3D	Auto	de Chaumont et al. (2011)
Image-Pro Plus	Paid	Win	✓	✓	✓	✓	3D	Auto	Media Cybernetics (USA)
ImarisTrack	Paid	Win/Mac	✓	✓	✓	✓	3D	Auto	Bitplane (Switzerland)
LevelSetTracker	Free	Matlab	✓	✓	✓	✓	3D	Auto	Dzyubachyk et al. (2010)
LineageTracker	Free	ImageJ	✓	✓	✓	✓	2D	Auto	Till Bretschneider
ManualTracking	Free	ImageJ	✓	✓	✓	✓	3D	Manual	Fabrice Cordelières
MetaMorph	Paid	Win	✓	✓	✓	✓	3D	Auto	Molecular Devices (USA)
MTrack2	Free	ImageJ	✓	✓	✓	✓	2D	Auto	Nico Stuurman
MTrackJ	Free	ImageJ	✓	✓	✓	✓	3D	Manual	Erik Meijering
MTT	Free	Matlab	✓	✓	✓	✓	2D	Auto	Sergé et al. (2008)
Octane	Free	ImageJ	✓	✓	✓	✓	2D	Auto	Ji Yu lab
Oko-Vision	Paid	Win	✓	✓	✓	✓	2D	Semi	Okolab (Italy)
ParticleTracker	Free	ImageJ	✓	✓	✓	✓	3D	Auto	Sbalzarini et al. (2005)
ParticleTracking	Free	IDL	✓	✓	✓	✓	2D	Auto	Crocker & Grier (1996)
plusTipTracker	Free	Matlab	✓	✓	✓	✓	2D	Auto	Danuser lab
PolyParticleTracker	Free	Matlab	✓	✓	✓	✓	2D	Auto	Rogers et al. (2007)
QuimP	Free	ImageJ	✓	✓	✓	✓	2D	Auto	Bosgraaf et al. (2009)
SpeckleTrackerJ	Free	ImageJ	✓	✓	✓	✓	2D	Semi	Smith et al. (2011)
SpotTracker	Free	ImageJ	✓	✓	✓	✓	2D	Auto	Sage et al. (2005)
StarryNite	Free	Win/Lin	✓	✓	✓	✓	3D	Auto	Murray et al. (2006)
TIKAL	Request	Win/Lin	✓	✓	✓	✓	3D	Auto	Bacher et al. (2004)
TLA	Free	Matlab	✓	✓	✓	✓	2D	Auto	Huth et al. (2011)
u-track	Free	Matlab	✓	✓	✓	✓	2D	Auto	Jagaman et al. (2008)
Volocity	Paid	Win/Mac	✓	✓	✓	✓	3D	Auto	Perkin Elmer (USA)

Review

Meijering & al.

Methods in Enzymology 2012

Comparison

Chenouard & al.

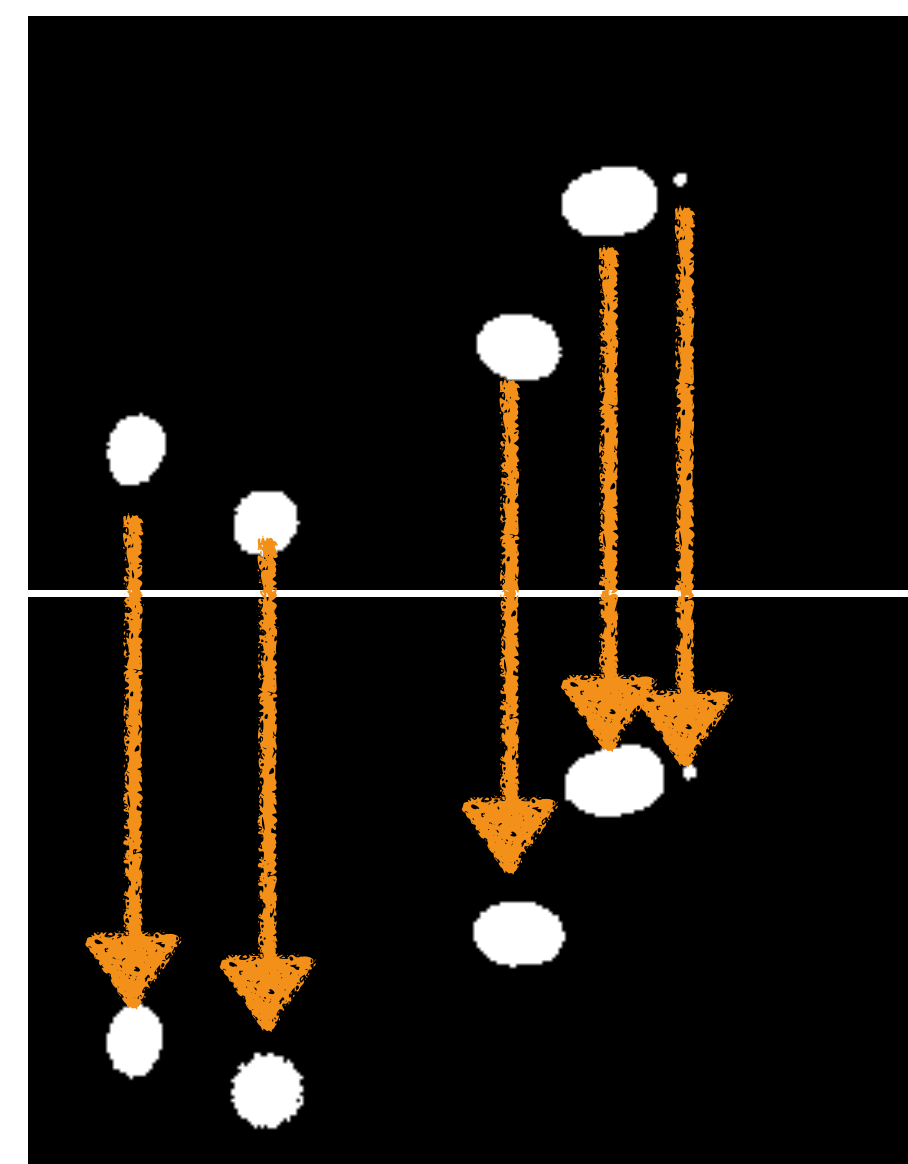
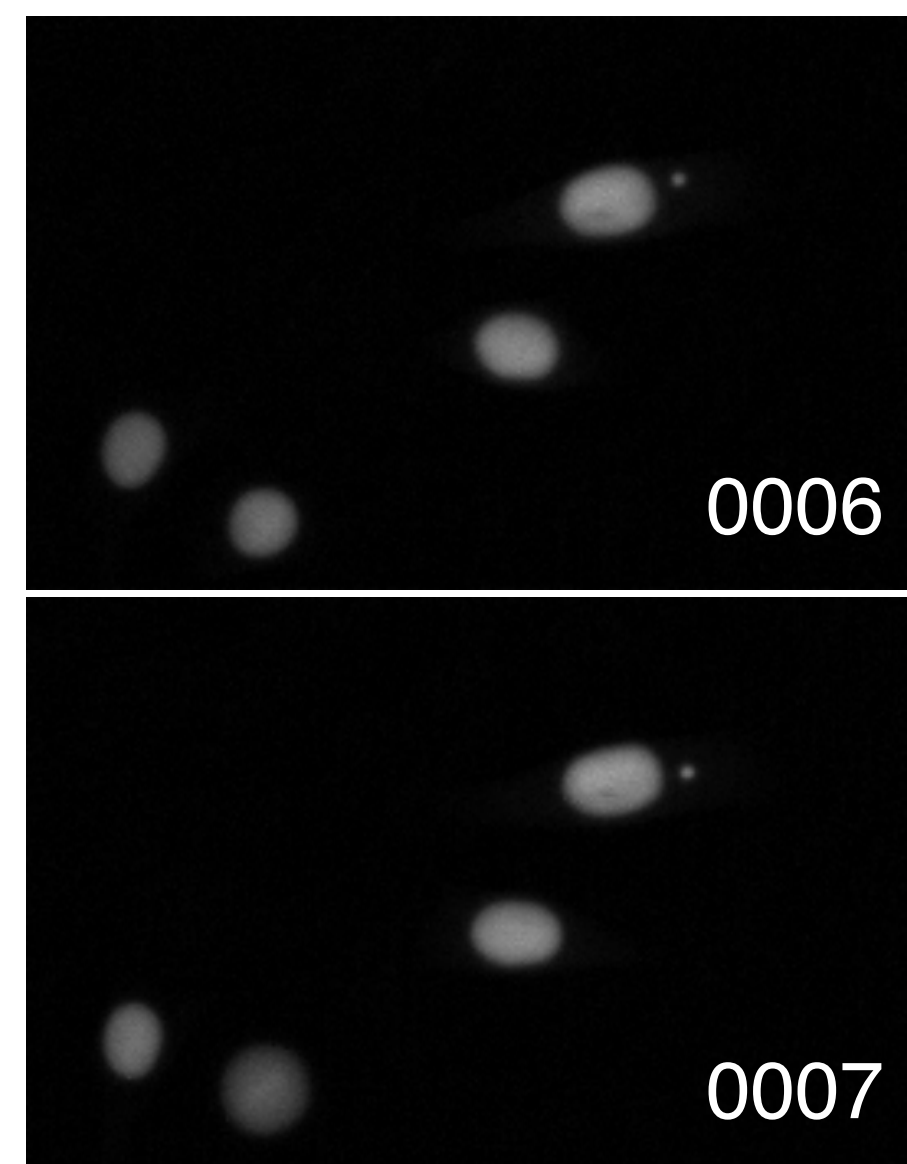
Nature Method, 2014

Table 1 Participating teams and tracking methods									
Method	Authors	Detection			Linking			Dim.	Refs.
		Prefilter	Approaches	Remarks	Principle	Approaches	Remarks		
1	I.F. Sbalzarini Y. Gong	-	M, C	Iterative intensity-weighted centroid calculation	Combinatorial optimization	MF, MT, GC	Greedy hill-climbing optimization with topological constraints	2D & 3D	32
2	J. Cardinale C. Carthel S. Coraluppi	Disk	M, T	Adaptive local-maxima selection	Multiple hypothesis tracking	MF, MT, MM	Motion models are user specified (near-constant position and/or velocity)	2D & 3D	33,34
3	N. Chenouard F. de Chaumont J.-C. Olivo-Marin	Wavelets	M, T	Maxima after thresholding two-scale wavelet products	Multiple hypothesis tracking	MF, MT, MM, GC	Motion models are user specified (near-constant position and/or velocity)	2D & 3D	35-37
4	M. Winter A.R. Cohen	Gaussian, median and morphology	M, T, C	Adaptive Otsu thresholding	Multitemporal association tracking	MF, MT, GC	Post-tracking refinement of detections	2D & 3D	38,39
5	W.J. Godinez K. Rohr	Laplacian of Gaussian or Gaussian fitting	M, T, F, C	Either thresholding + centroid or maxima + Gaussian fitting	Kalman filtering + probabilistic data association	MF, MM	Interacting multiple models using motion models as specified	2D & 3D	29,40
6	Y. Kalaidzidis	Windowed floating mean background subtraction	T, F	Lorentzian function fitting to structures above noise level	Dynamic programming	MF, GC	Track assignment by the weighted sum of multiple features	2D	41
7	L. Liang J. Duncan H. Shen Y. Xu	Laplacian of Gaussian	M, T, F	Gaussian mixture model fitting	Multiple hypothesis tracking	MF, MM	Interacting multiple models with forward and backward linking	2D	42
8	K.E.G. Magnusson J. Jaldén H.M. Blau	Deconvolution	M, T, F	Watershed-based clump splitting and parabola fitting	Viterbi algorithm on state-space representation	MF, MT	Brownian motion is assumed in all cases	2D & 3D	43,44
9	P. Paul-Gilloteaux	Laplacian of Gaussian or Gaussian filtering	M, T, F	Either maxima with pixel precision (2D) or thresholding + Gaussian fitting (3D)	Nearest neighbor + global optimization	MF, MT, GC	Global optimization of associations using simulated annealing	2D & 3D	45,46
10	P. Roudot C. Kervrann F. Waharte	Structure tensor	T, F	Histogram-based thresholding and Gaussian fitting	Gaussian template matching	-	Only local and per-trajectory particle linking	2D	47-49
11	I. Smal E. Meijering	Wavelets	M, F, C	Gaussian fitting (round particles) or centroid calculation (elongated particles)	Sequential multiframe assignment	MF, MT, MM, GC	Global linking cost minimization	2D	35,50,51
12	J.-Y. Tinevez S.L. Shorte	Difference of Gaussian	M, T, F	Parabolic fitting to localized maxima	Linear assignment problem	MT, GC	Two-step approach (frame-to-frame and segment linking)	2D & 3D	52,53
13	J. Willemse K. Celler G.P. van Wezel	Gaussian and top hat	T, C	Watershed-based clump splitting	Nearest neighbor	MM, GC	Allows merging and splitting of particles and uses a linear motion model	2D & 3D	54,55
14	H.-W. Dan Y.-S. Tsai	Gaussian, Wiener and top hat	T, C	Morphological opening-based clump splitting	Nearest neighbor + Kalman filtering	MM	Essentially a 2D method keeping track of maximum intensity in z	2D & 3D	56,57

See **Supplementary Note 1** for further details on methods 1-14. Dim, dimensionality. Detection approaches: M, maxima detection; T, thresholding; F, fitting; C, centroid estimation. Linking approaches: MF, multiframe; MT, multitrack; MM, motion models; GC, gap closing.



Tracking-by-Detection



Trivial case

	x		199.1	149.9	177.8	33.0	72.5
	x	y	49.9	101.0	56.7	132.2	154.5
1	179.3	56.1	20.8	53.7	1.6	165.0	145.3
2	202.0	53.4	4.5	70.6	24.4	186.5	164.3
3	149.8	102.7	72.3	1.7	53.9	120.5	93.1
4	32.6	135.2	187.1	122.1	165.0	3.0	44.3
5	73.1	150.6	161.4	91.4	140.7	44.1	3.9

Algorithm {
Cost Spatial distance
Linking Nearest-Neighborhood

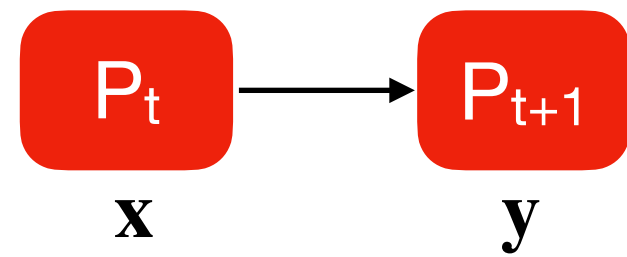


Designing a Cost Function

Cost of the link

- Energy
- Lost function
- Edge weight

$$\xi(P_t, P_{t-1}) = \text{dist}(P_t, P_{t-1})$$



Weighted sum

$$\xi_{xy} = \lambda_f \frac{|f(\mathbf{x}, \mathbf{y})|}{N_f} + \lambda_g \frac{|g(\mathbf{x}, \mathbf{y})|}{N_g}$$

$$\lambda_g = 1 - \lambda_f$$

Control the penalization

- Soft constraint: exp, sigmoid
- Hard constraint: soft threshold

Example of cost function

Particle Feature $\mathbf{x} = [x, y, z, A, \mu, c, p, \dots]$

- Position, x, y, z
- Intensity, probability of detection p
- Morphometric parameters, area A, ..

$$\xi_{xy} = \lambda_d \frac{\text{dist}(\mathbf{x}, \mathbf{y})}{N_d} + \lambda_\mu \frac{|\mathbf{x}_\mu - \mathbf{y}_\mu|}{N_\mu} - \lambda_p \mathbf{y}_p$$

$\mathbf{y}_p$ penalize low probability of detection

$|\mathbf{x}_\mu - \mathbf{y}_\mu|$ penalize change of mean intensity

Trajectory Feature

- Speed
- Direction of displacement
- Fill the gap

$$\xi_{xy} = \lambda_s \frac{|T_{\text{speed}} - \text{dist}(\mathbf{x}, \mathbf{y})|}{S} + \lambda_\theta \frac{|\langle \overrightarrow{\mathbf{p}\mathbf{x}}, \overrightarrow{\mathbf{x}\mathbf{y}} \rangle|}{\|\mathbf{x} - \mathbf{y}\|}$$

$|\langle \overrightarrow{\mathbf{p}\mathbf{x}}, \overrightarrow{\mathbf{x}\mathbf{y}} \rangle|$ co-linearity from the previous point $\mathbf{p}$

Prior constraints

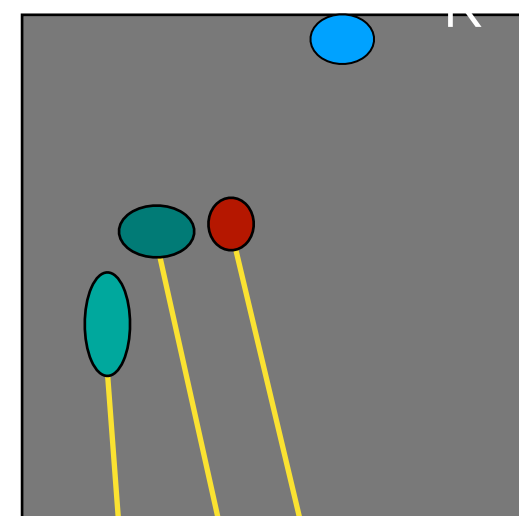
- Motion model
- Shape prior
- Life cycle

$$\xi_{xy} = \lambda_d \frac{\text{dist}(\mathbf{x}, \mathbf{y})}{N_d} + \lambda_a \frac{\|A_y - A_{\text{known}}\|}{N_a}$$

A area of the particle

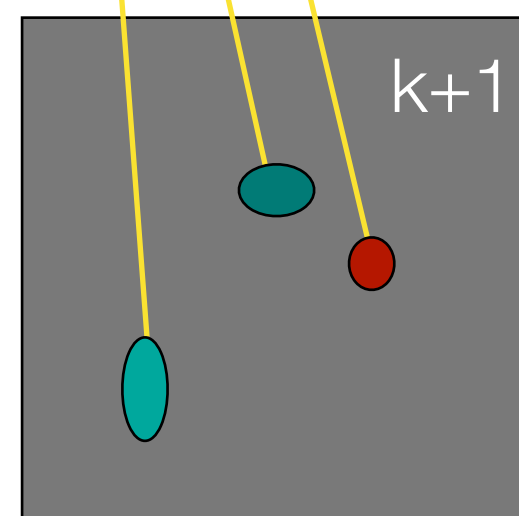
$$\xi_{xy} = \lambda_d \text{dist}(\mathbf{x}, \mathbf{y}) + \lambda_a \|\mathbf{y} - \mathbf{O}\|^\alpha \quad \alpha > 1 \text{ favored confined trajectory}$$

👁 Frame-to-frame Association

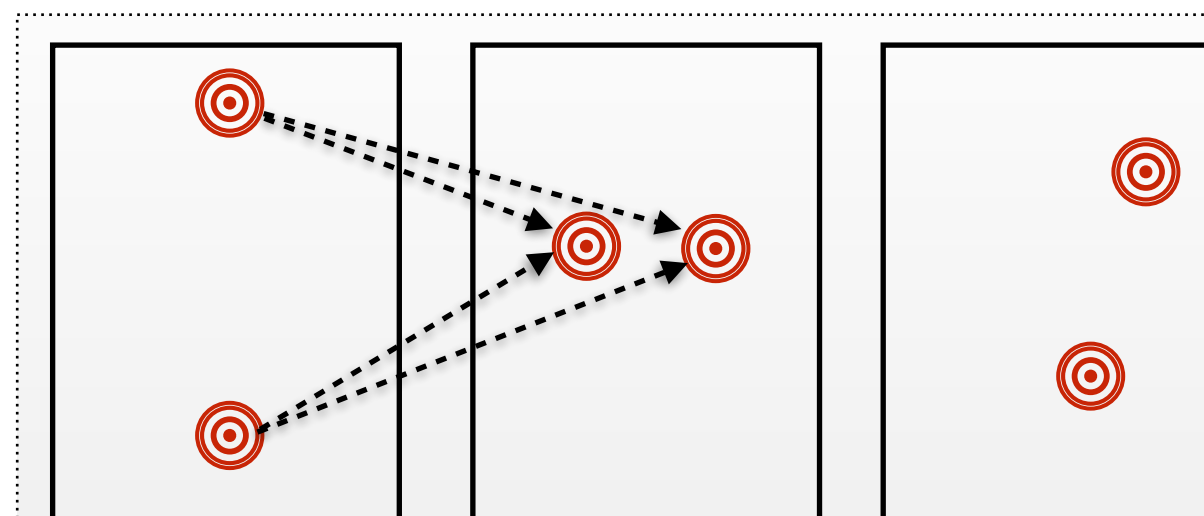
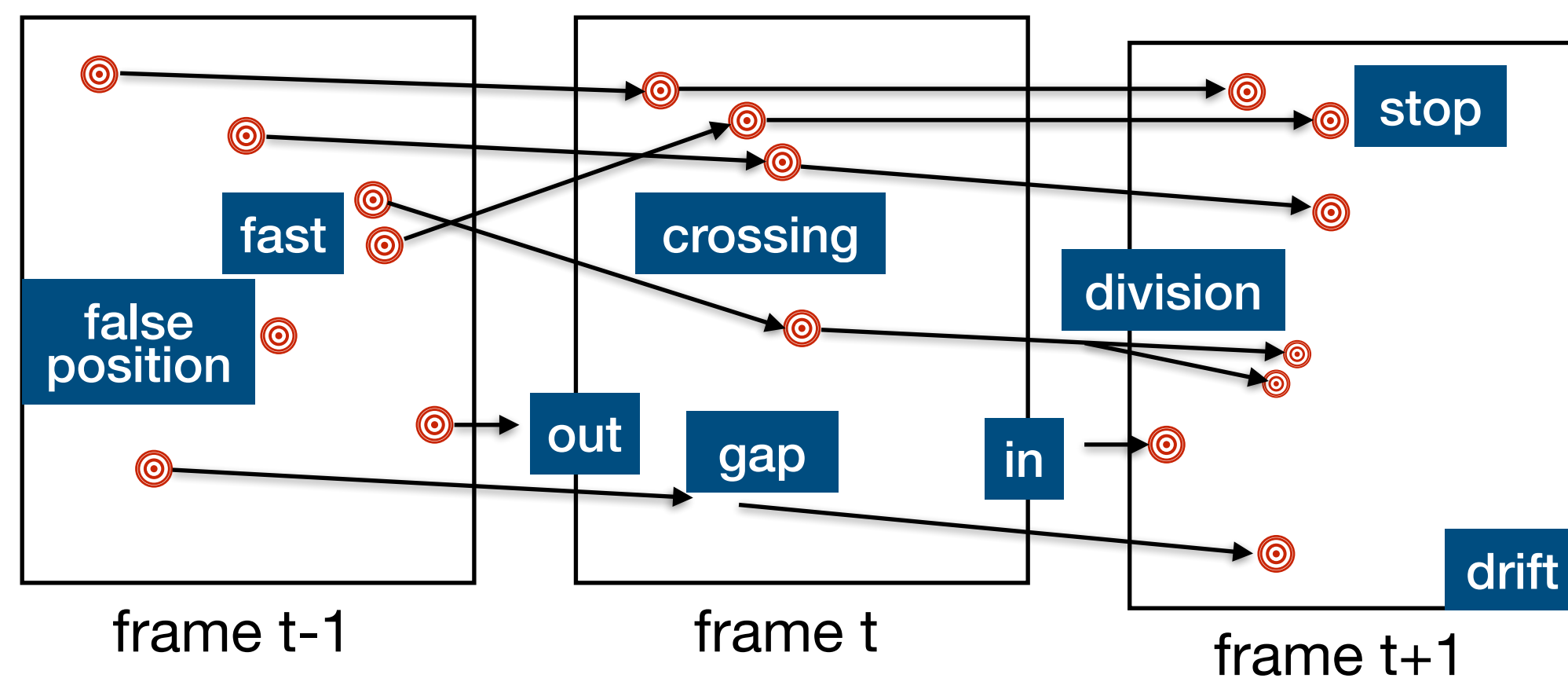


Bipartite graph (unbalanced)

- Weighted graph $G = (V, E)$
- $V = V_A \cup V_B$ and $V_A \cap V_B = \emptyset$



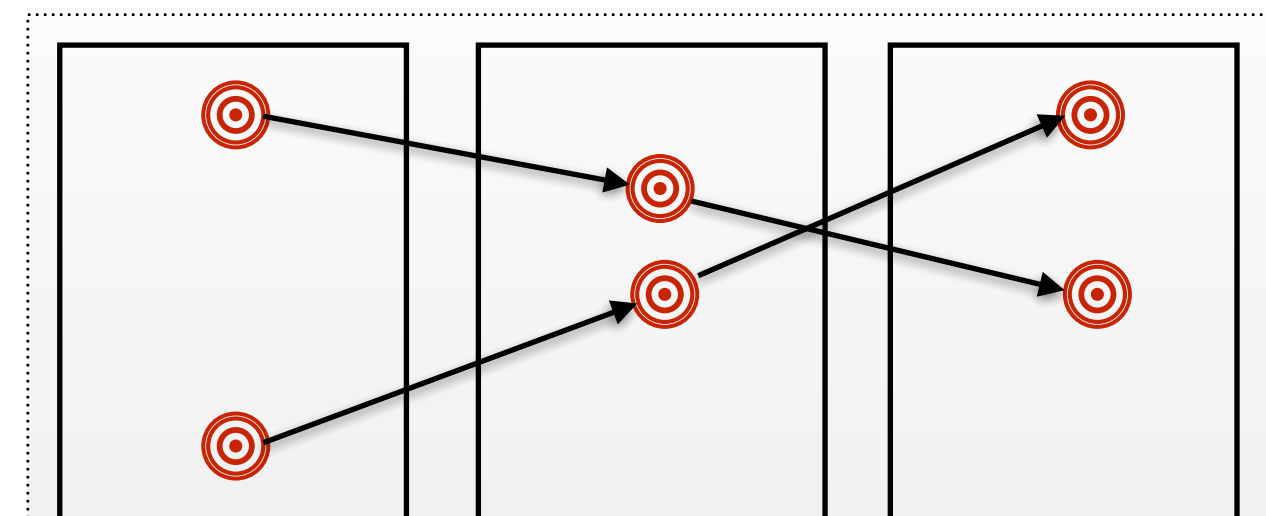
Solving the linear assignment problem LAP



Multiple hypothesis test

➡ delay the decision

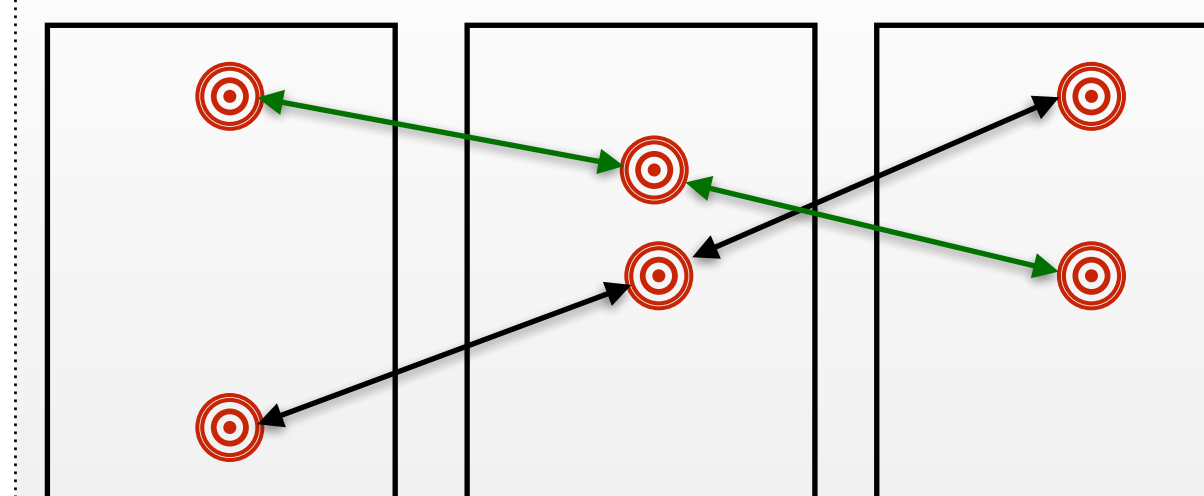
MHT [Chenouard, 2010]



Link with n frames

➡ use future and **past**

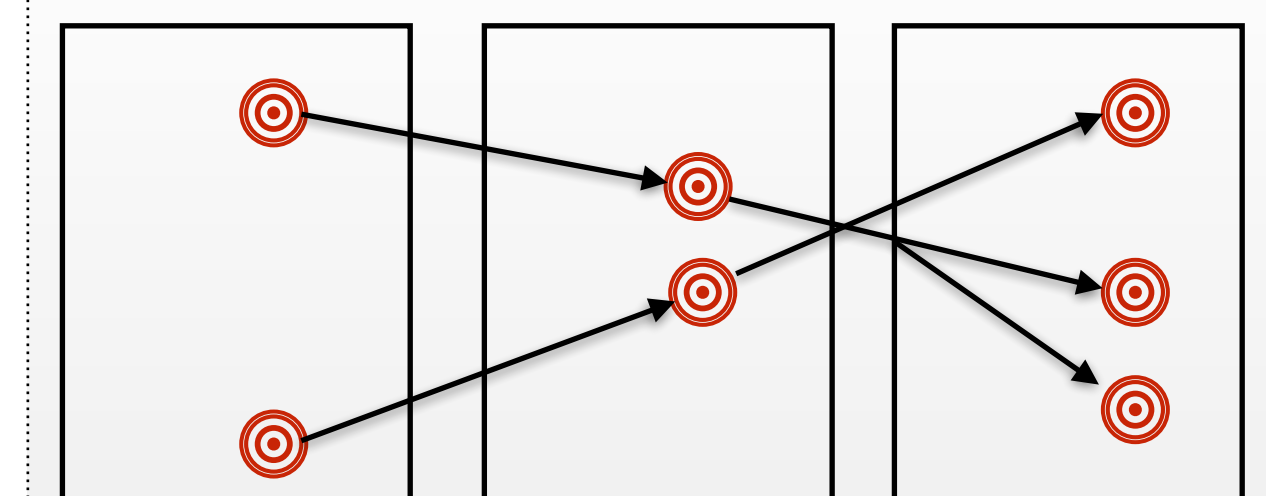
For 1 spot [Sage, 2003]



2-steps - Coarse-to-fine

➡ find segments, merge segments

u-track [Jaqaman, 2008]



Graph modelling

➡ manage cell division

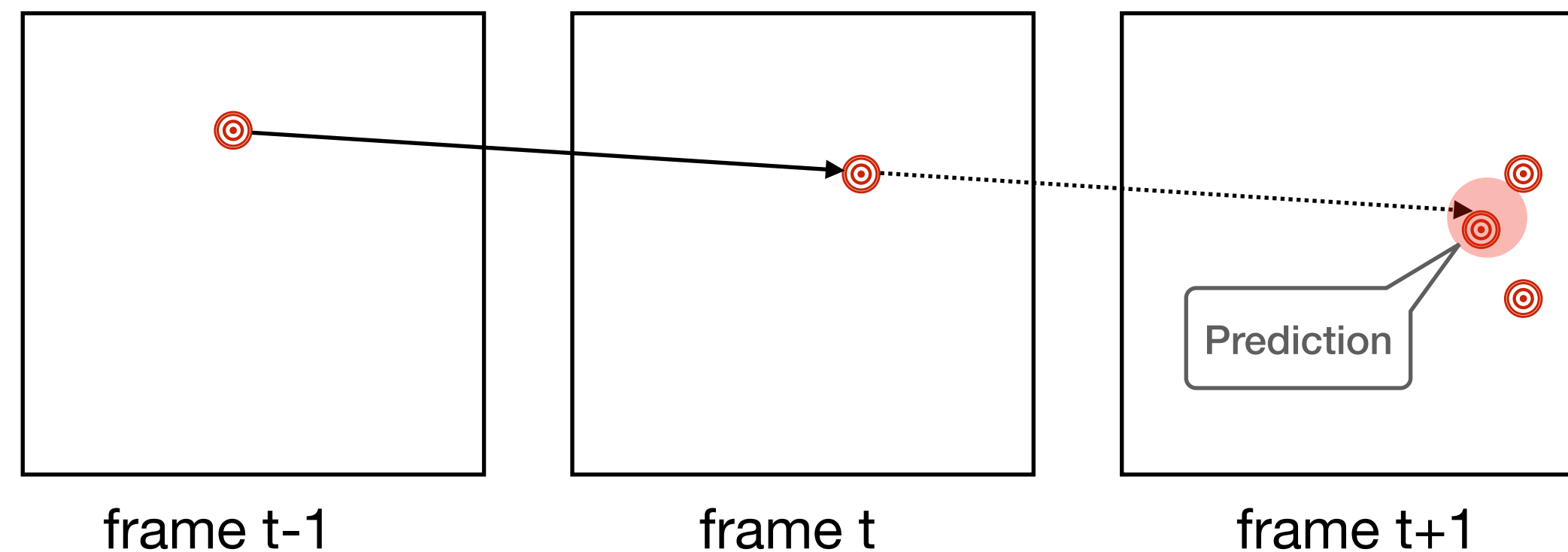


Tracking by Prediction

Signal Processing

Kalman Filter

- Prediction of the position of the target from the past
- State vector describing the target (position, speed, ...)
- Continuously updated
- Bayesian framework, estimation of the noise



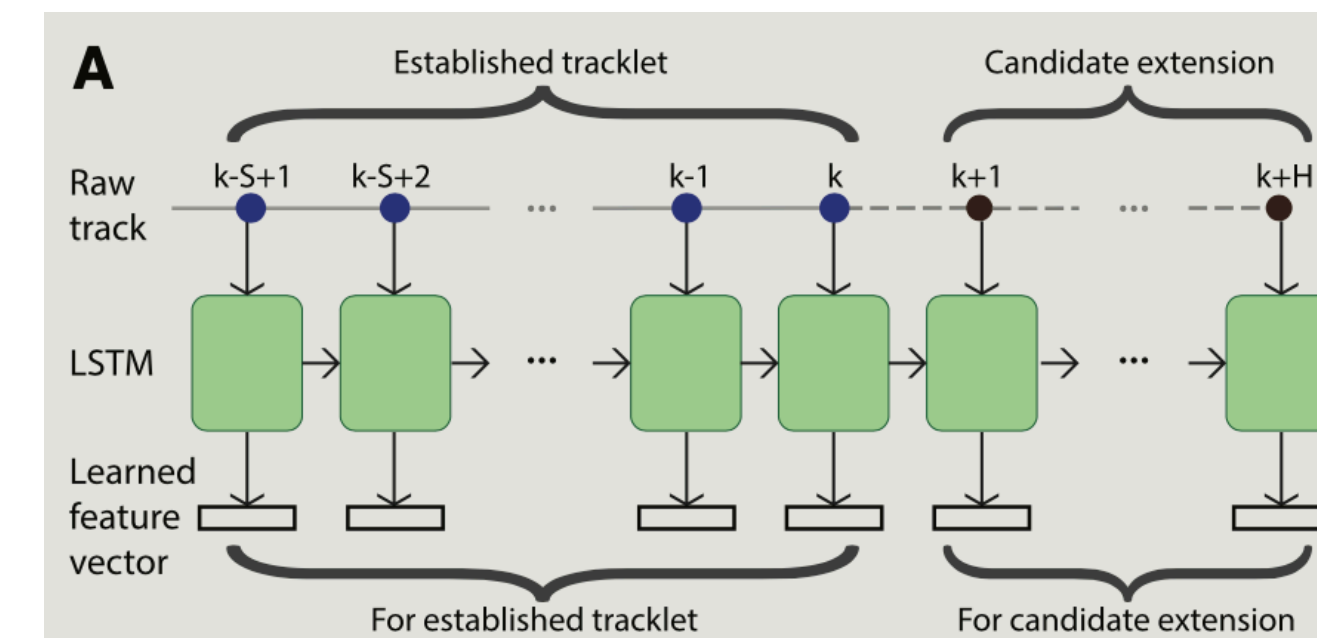
Deep Learning

RNN, LSTM (long short-term memory)

Yao and Meijering, Bioimage informatics, 2020

CNN Detection, UNet Optical flow

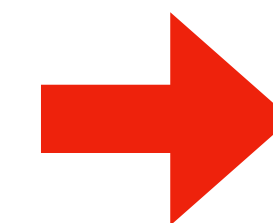
K. Sugawara. eLife, 2022



«DL don't outperform classic methods»

«DL aren't able to learn the motion models»

«Need more sophisticated network designs»



Vision Transform

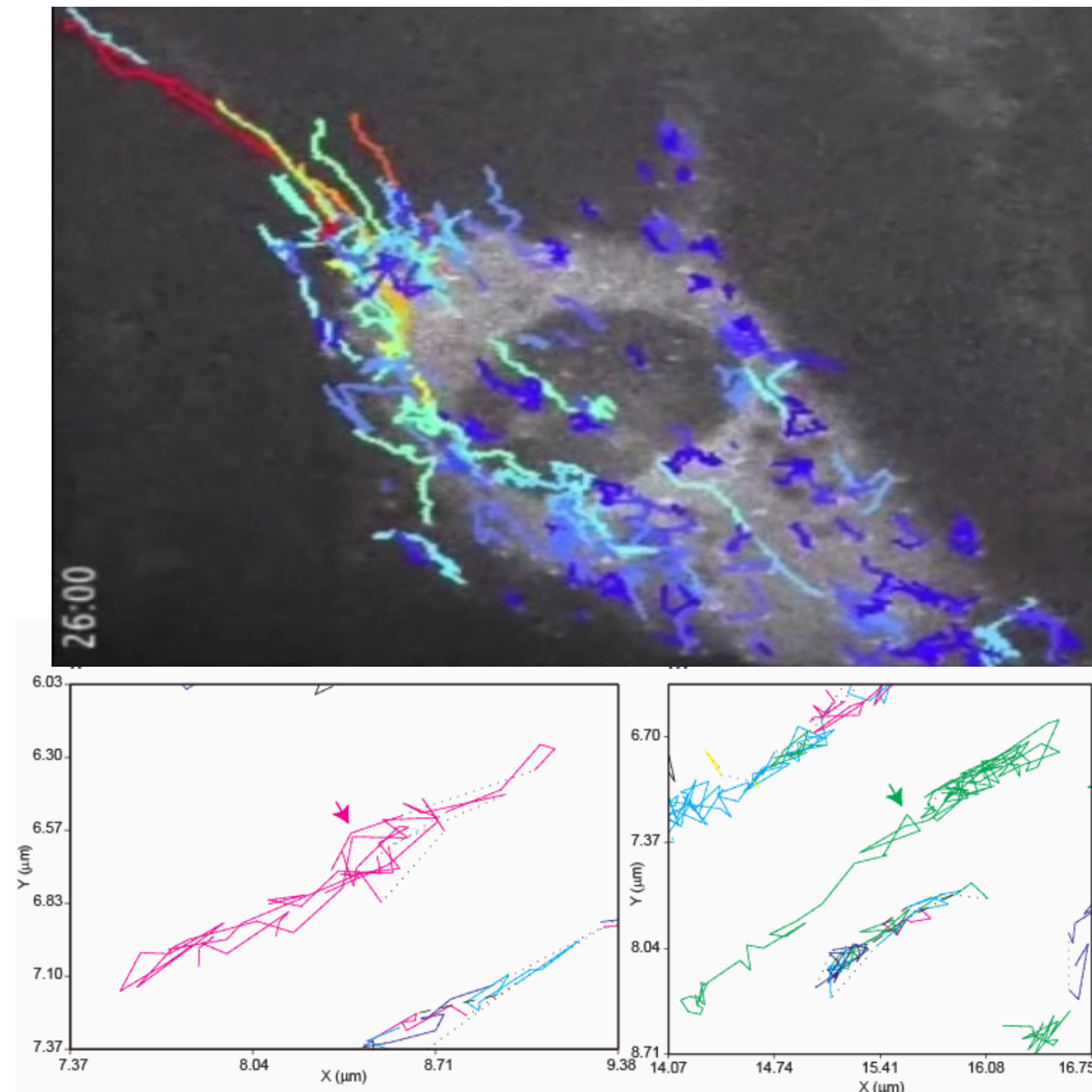
Attention mechanism over patch and frames



Tracking-by-Segment

Coarse-to-fine tracking

- Particle linking → segment
- Split/merge segment → trajectory

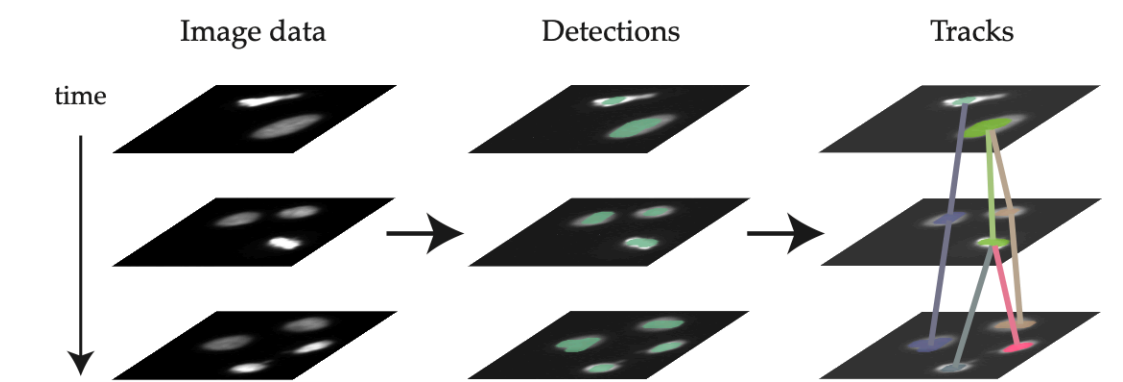


u-Track Khaloud Jaqaman

K. Jaqaman et al. Robust single-particle tracking in live-cell time-lapse sequences, Nat. Methods 2008

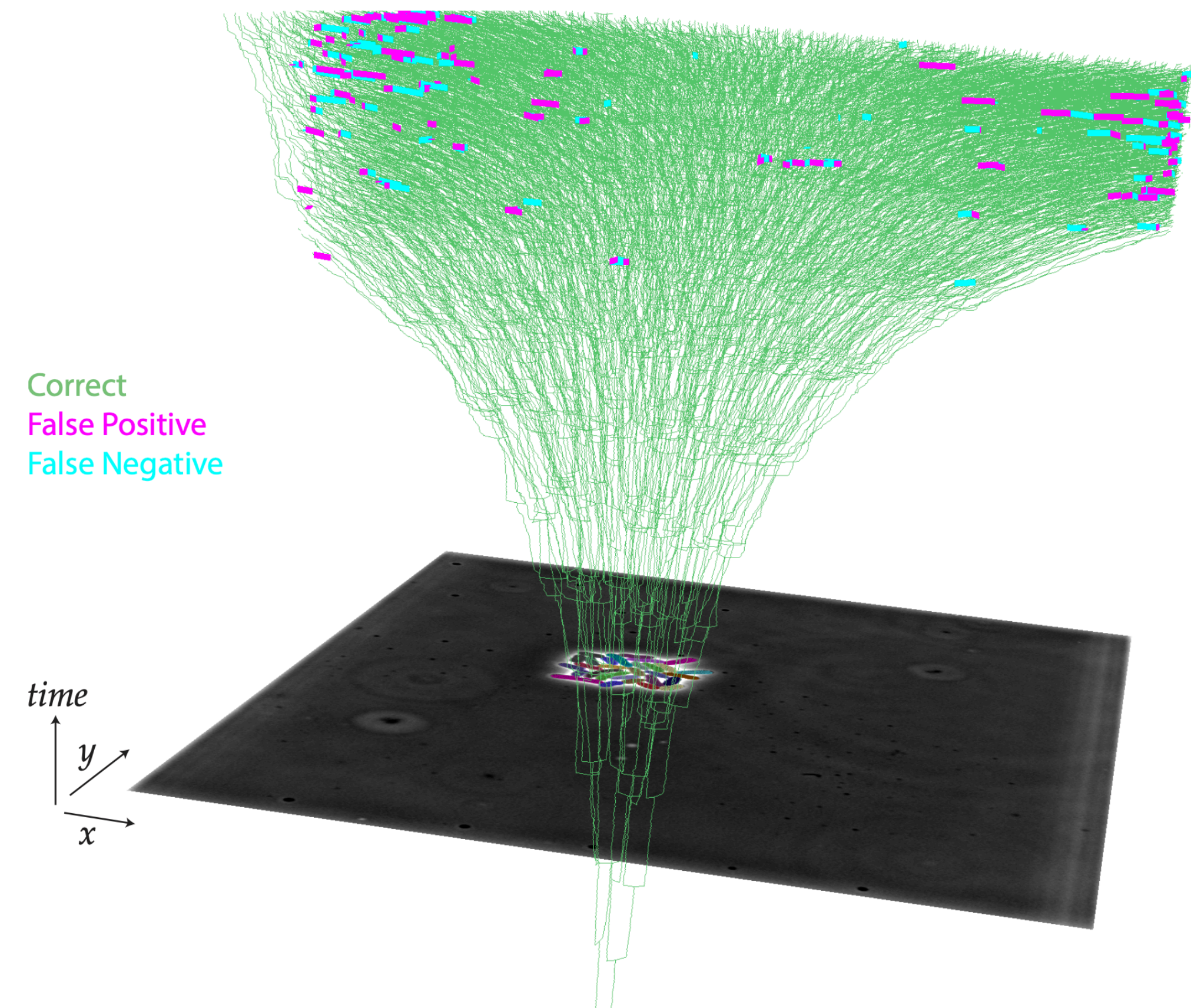
Learning Association

- Simulation of 3 frames
- Various situation



TrackAstra Benjamin Gallusser

K. Jaqaman et al. Robust single-particle tracking in live-cell time-lapse sequences, Nat. Methods 2008



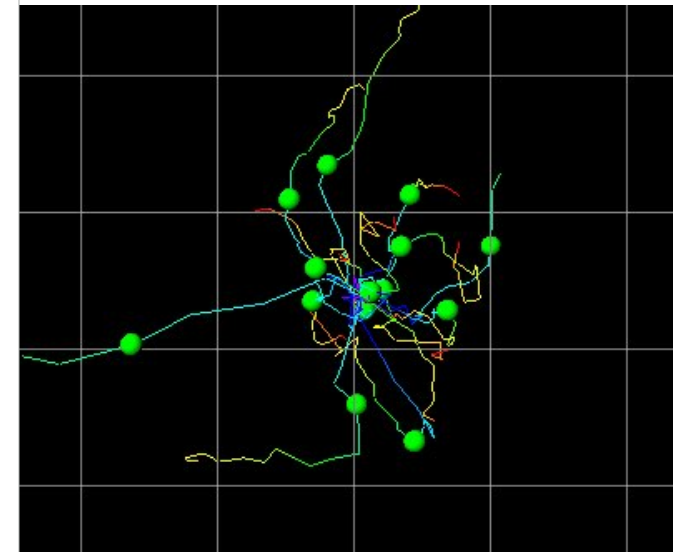


Software Packages

Imaris Tracking

Bitplane

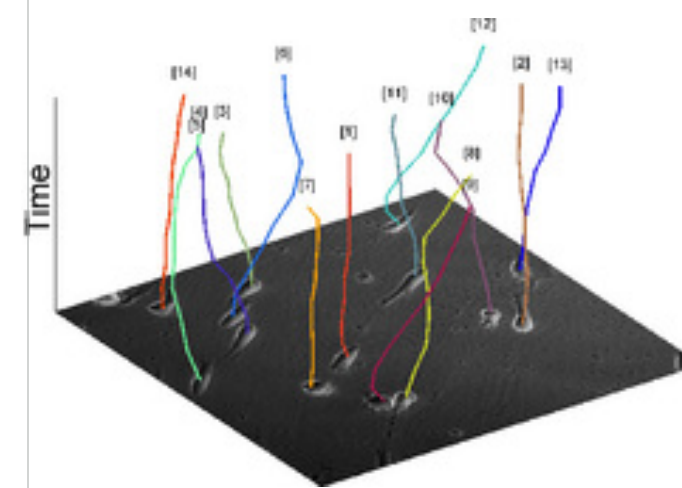
- Spot tracking
- Visualisation
- Commercial



CellTracker

Horvarth Lab

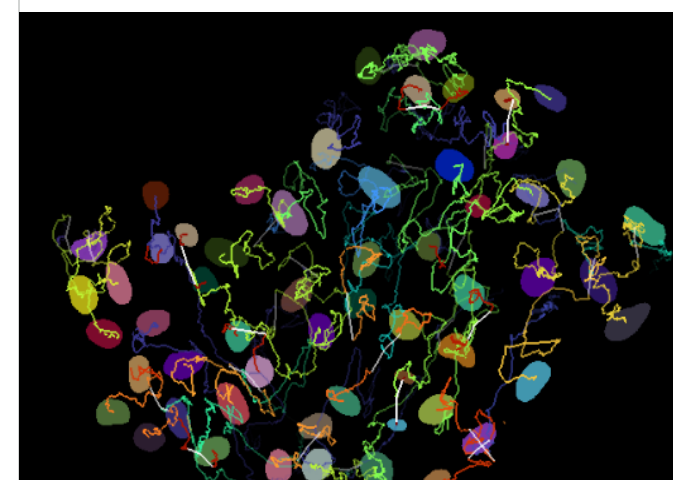
- Matlab Script
- User-friendly
- multi-modalities



b-Track

Kristina Ulicna UCL

- Python / napari
- Bayesian framework
- Multi-object tracking

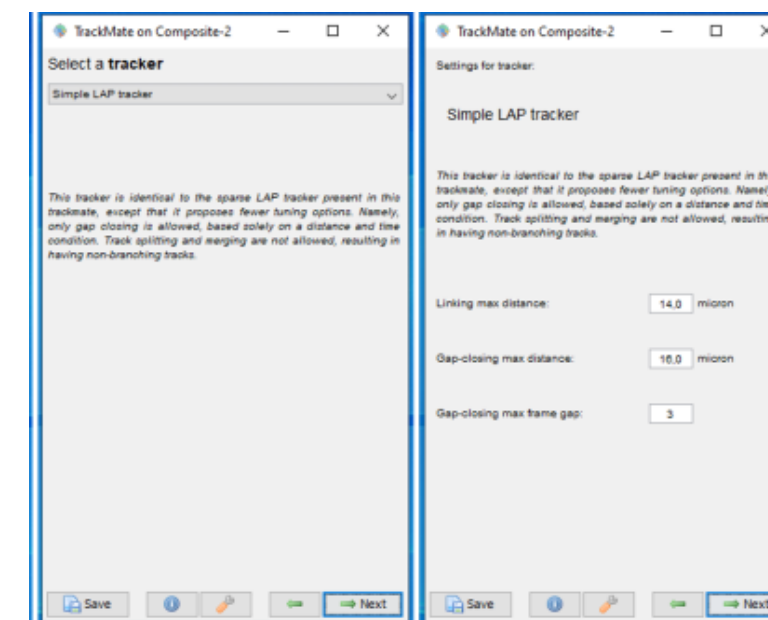


TrackMate of Fiji

Pasteur Institute

- Several detectors
- Several algo.

Tivenez et al., *TrackMate*, Elsevier *Methods* 2017.



- B&W masks
- Label images
- Thresholded images

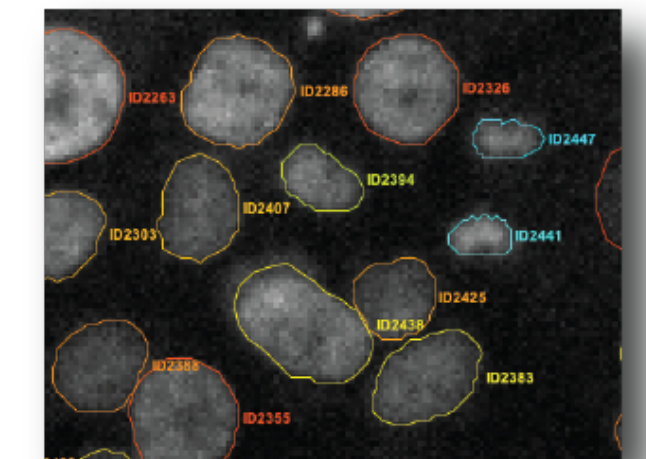
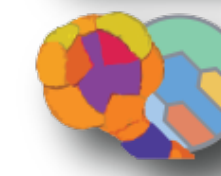
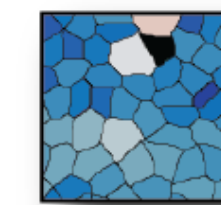
MorphoLibJ
morphological segmentation

Weka
custom model

Ilastik
custom pixel classification project

StarDist
built-in nuclei model & custom models

Cellpose
built-in models & custom models



New TrackMate API:

Interoperate with external segmentation components. Store, create and analyze object contours.

- Tracking cells
- Lineage tracing
- Changes in 2D shape over time
- Changes in intensity over time
- 2D to 3D segmentation

User-friendly interface that allows for performing tracking, data visualization, editing results and track analysis.

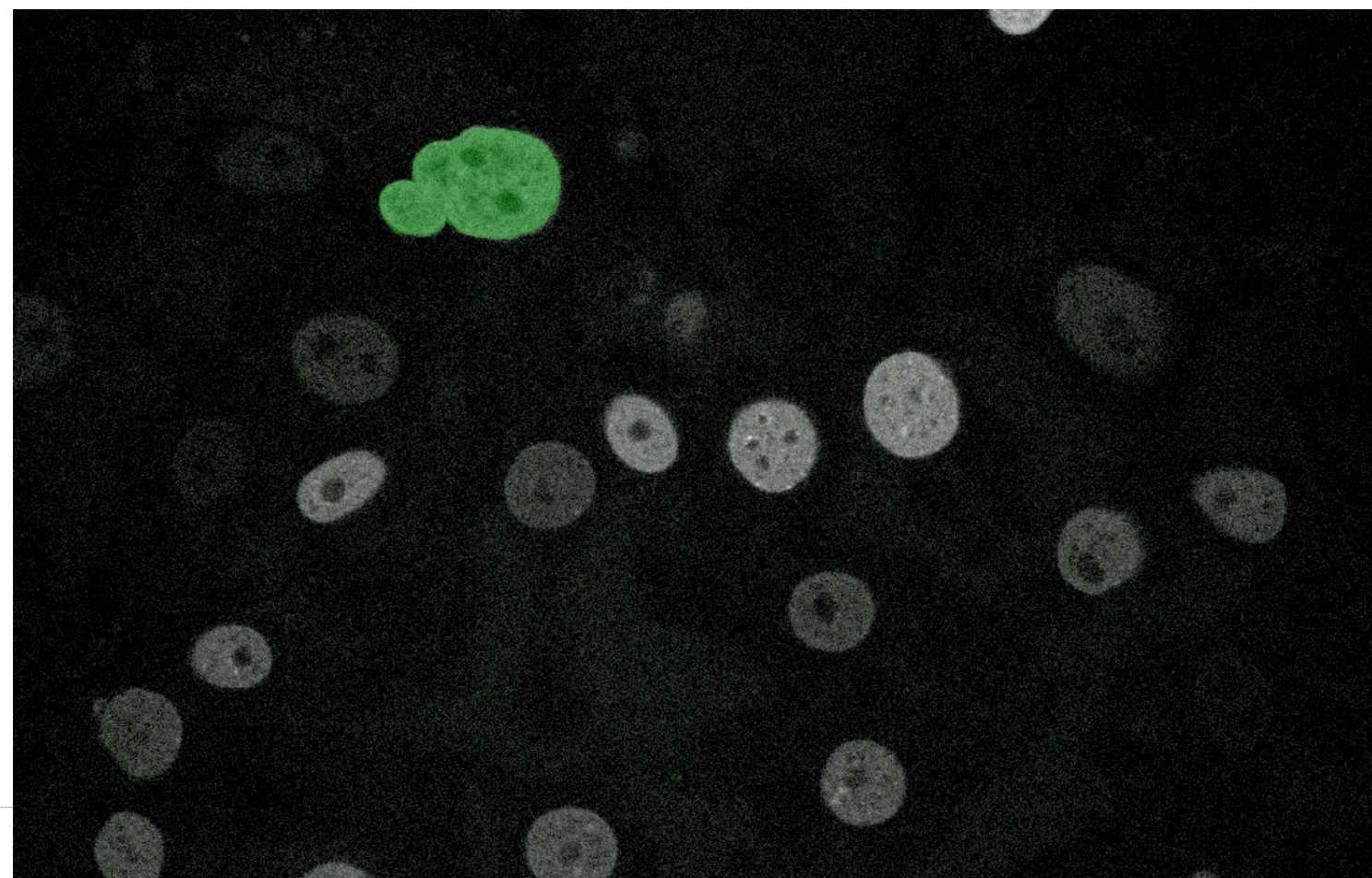
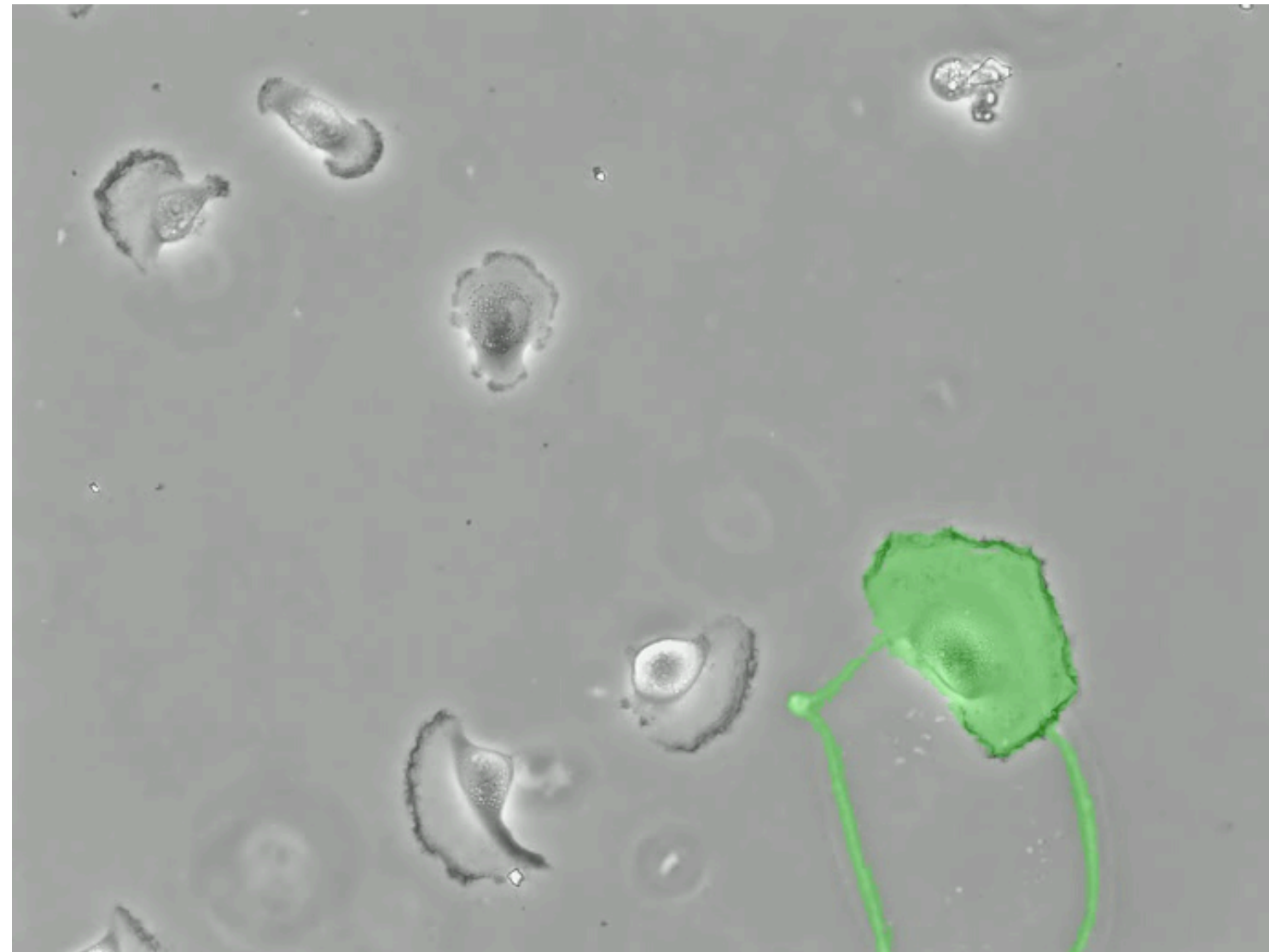
J.Y. Tivenez, Institut Paster, Paris

Promptable Models of Computer Vision

Segment-Anything Model SAM

MetaAI 

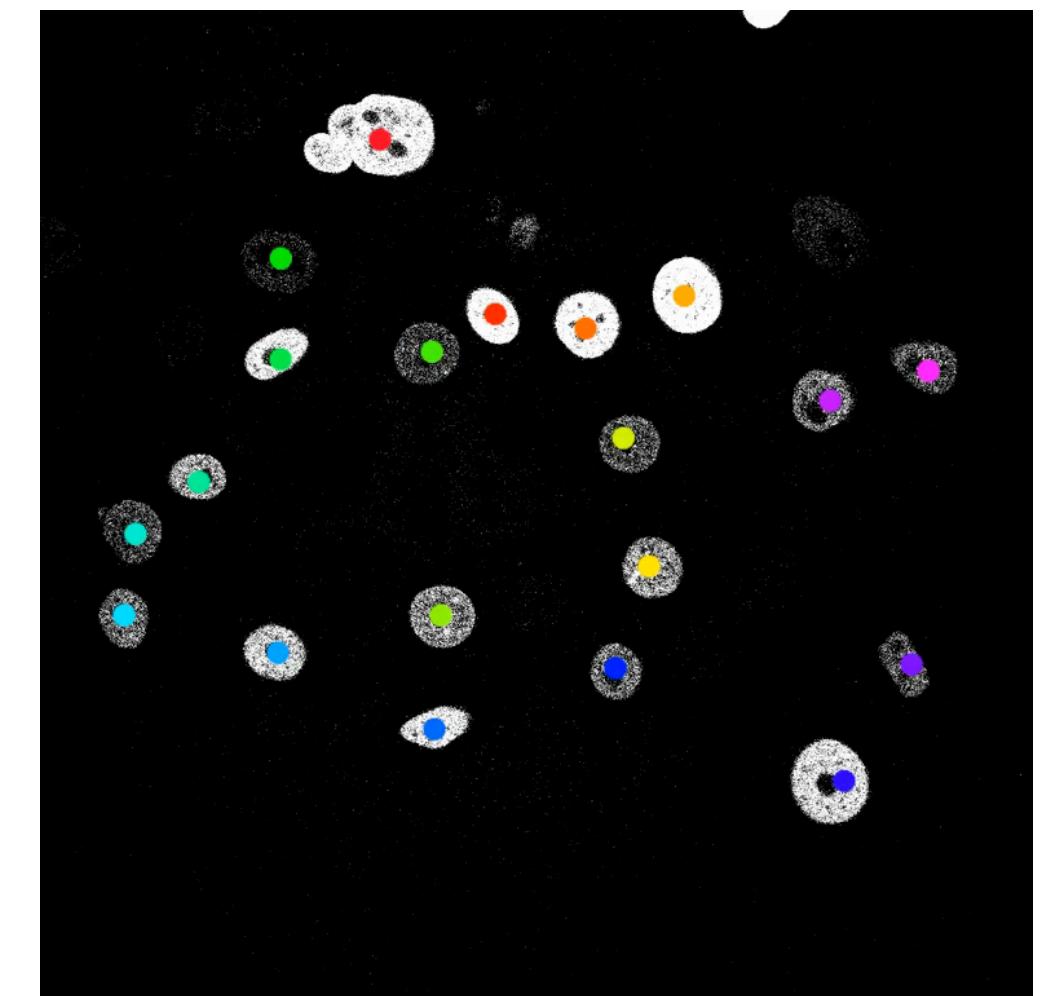
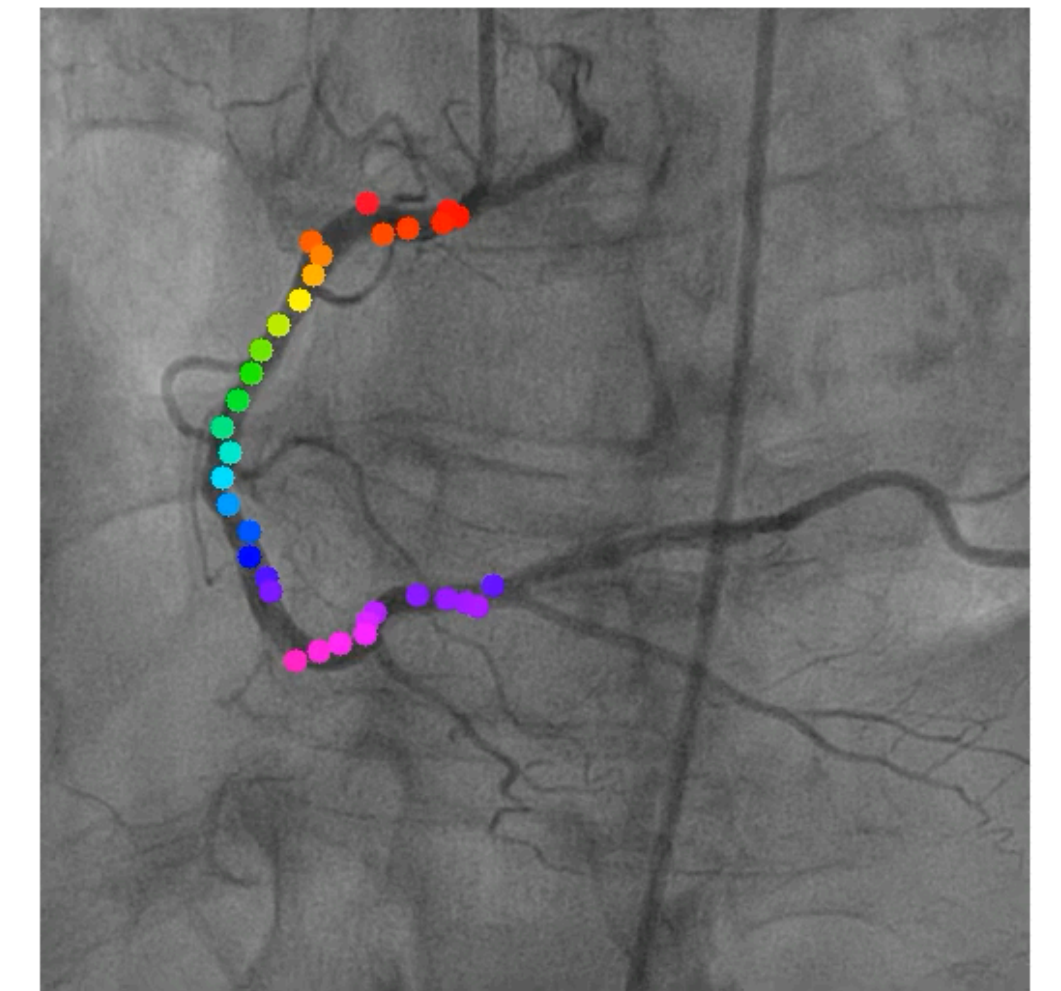
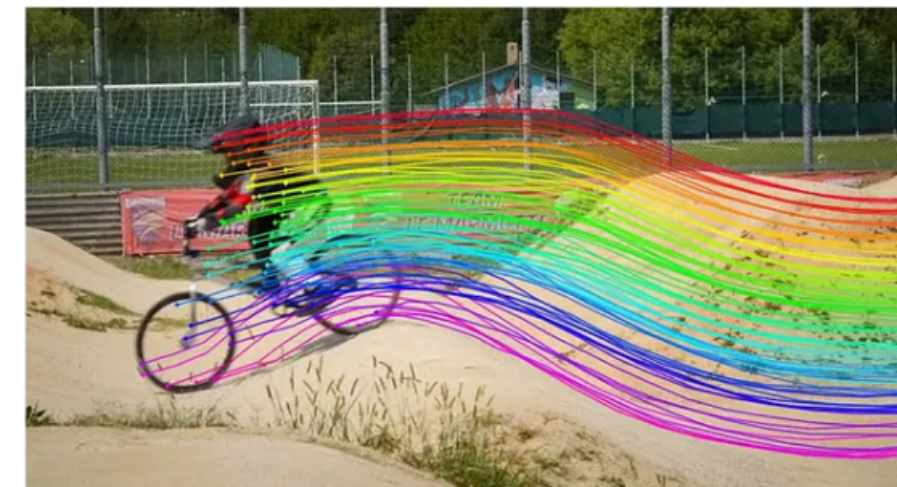
- Segmentation
- Video predictor
- SAM2



CoTracker3

MetaAI 

- Points of the 1st frame
- Occlusions
- Scaling effect



Vision Transformer
Foundation Model
Attention Mechanism

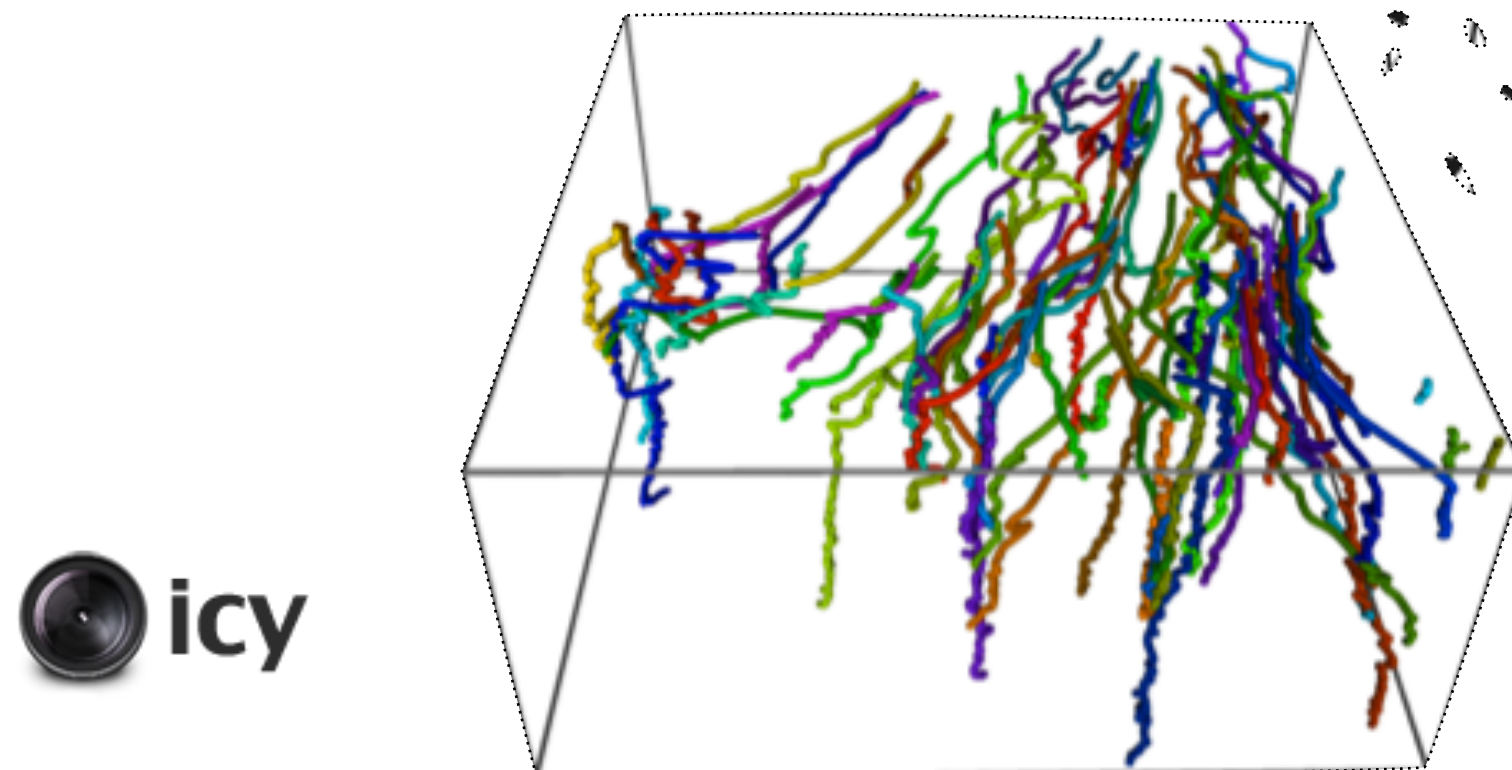
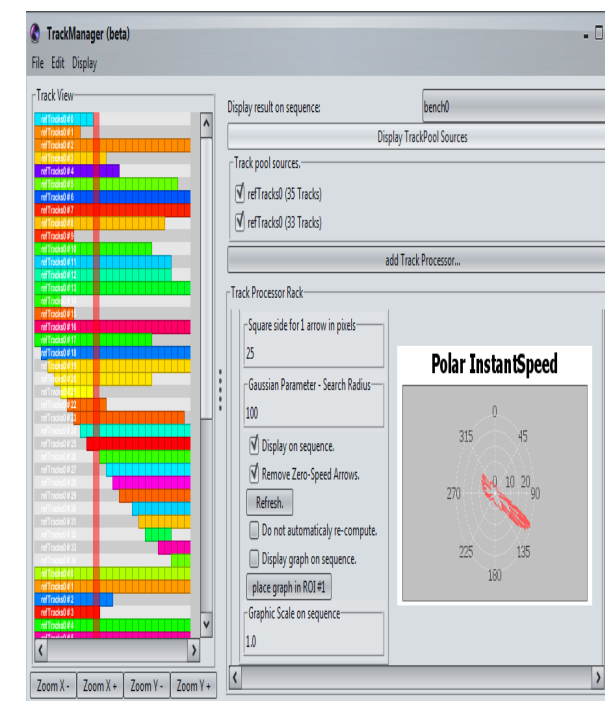


Analysis of Trajectory

- ✓ Visualization “Random color”
- ✓ Curation of the trajectories
- ✓ Measure: speed, diffusion, growth
- ✓ Mean Square Displacement (MSD)

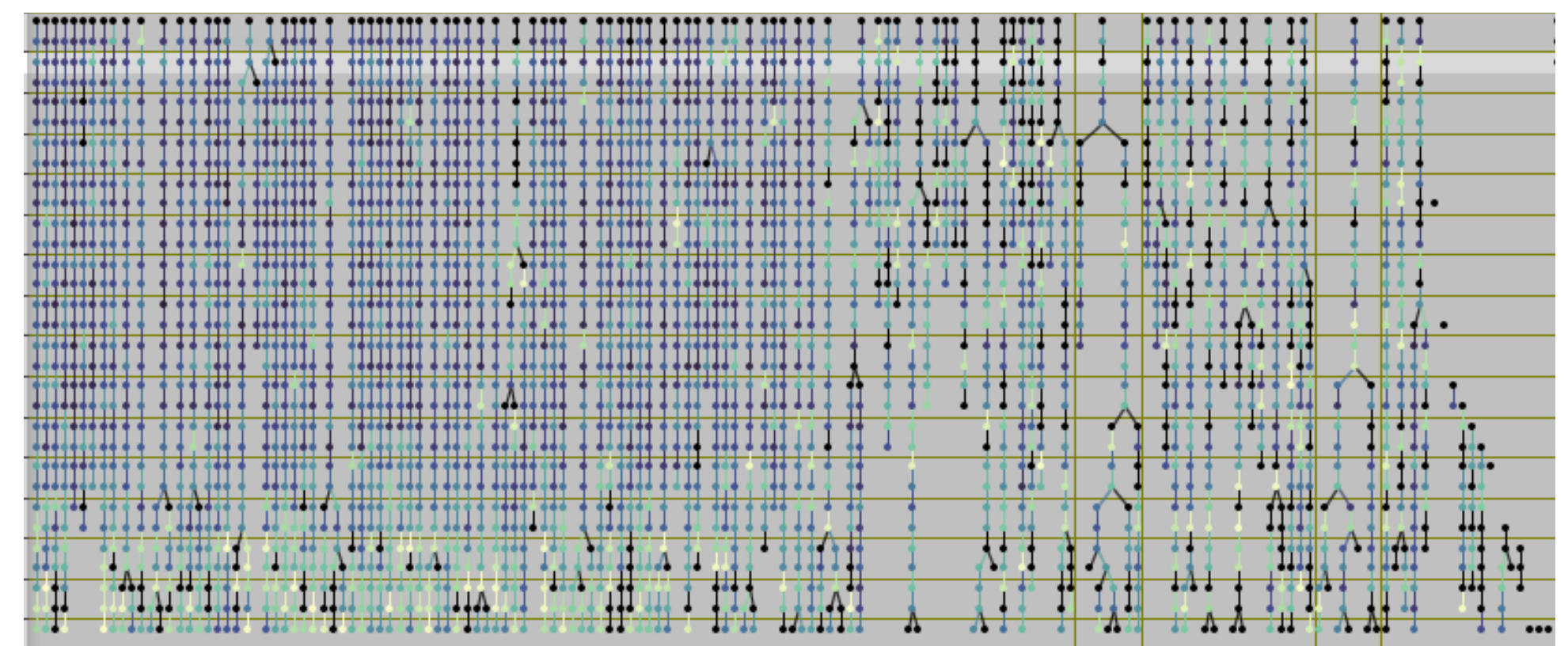
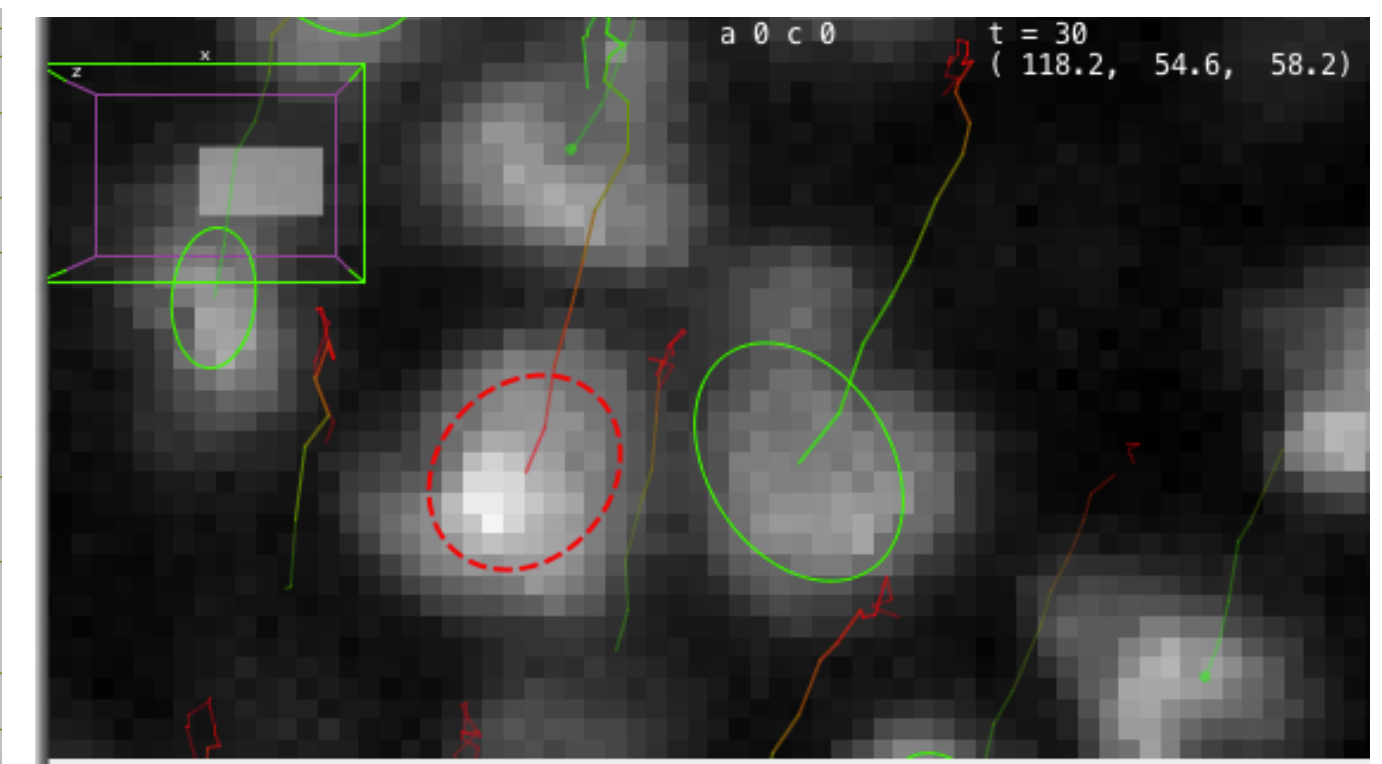
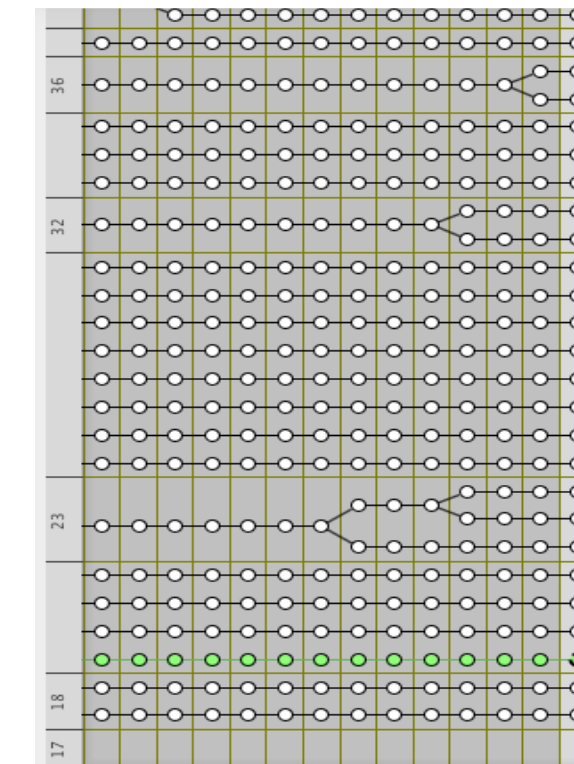
TrackManager

- Interactive trajectory edition
- Companion of SpotTracking



Mastodon [Wolff 2018]

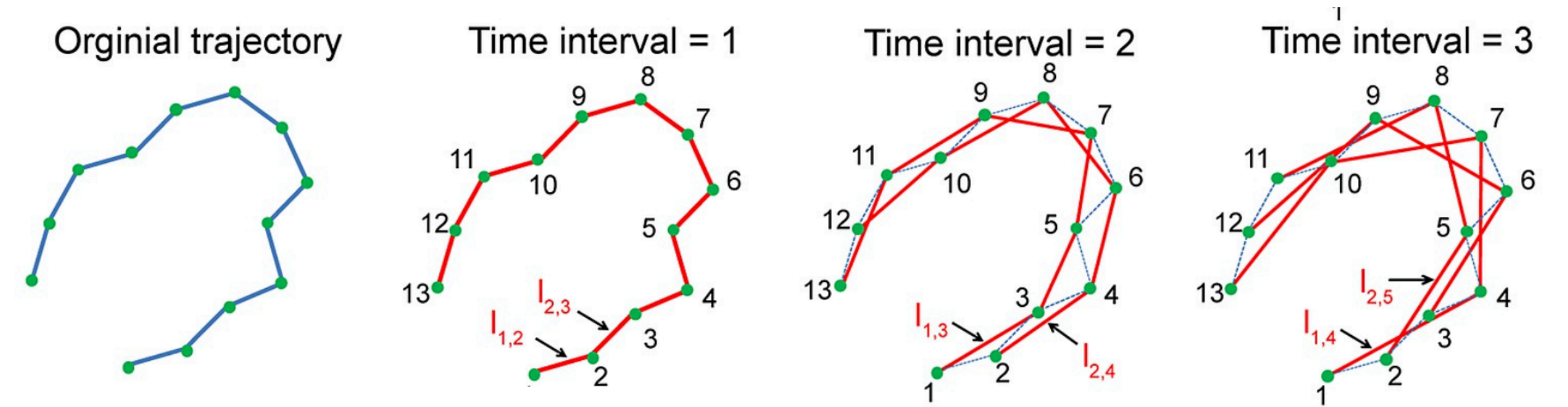
- Large-scale tracking and track editing
- Companion of TrackMate



Mean Square Displacement

MSD

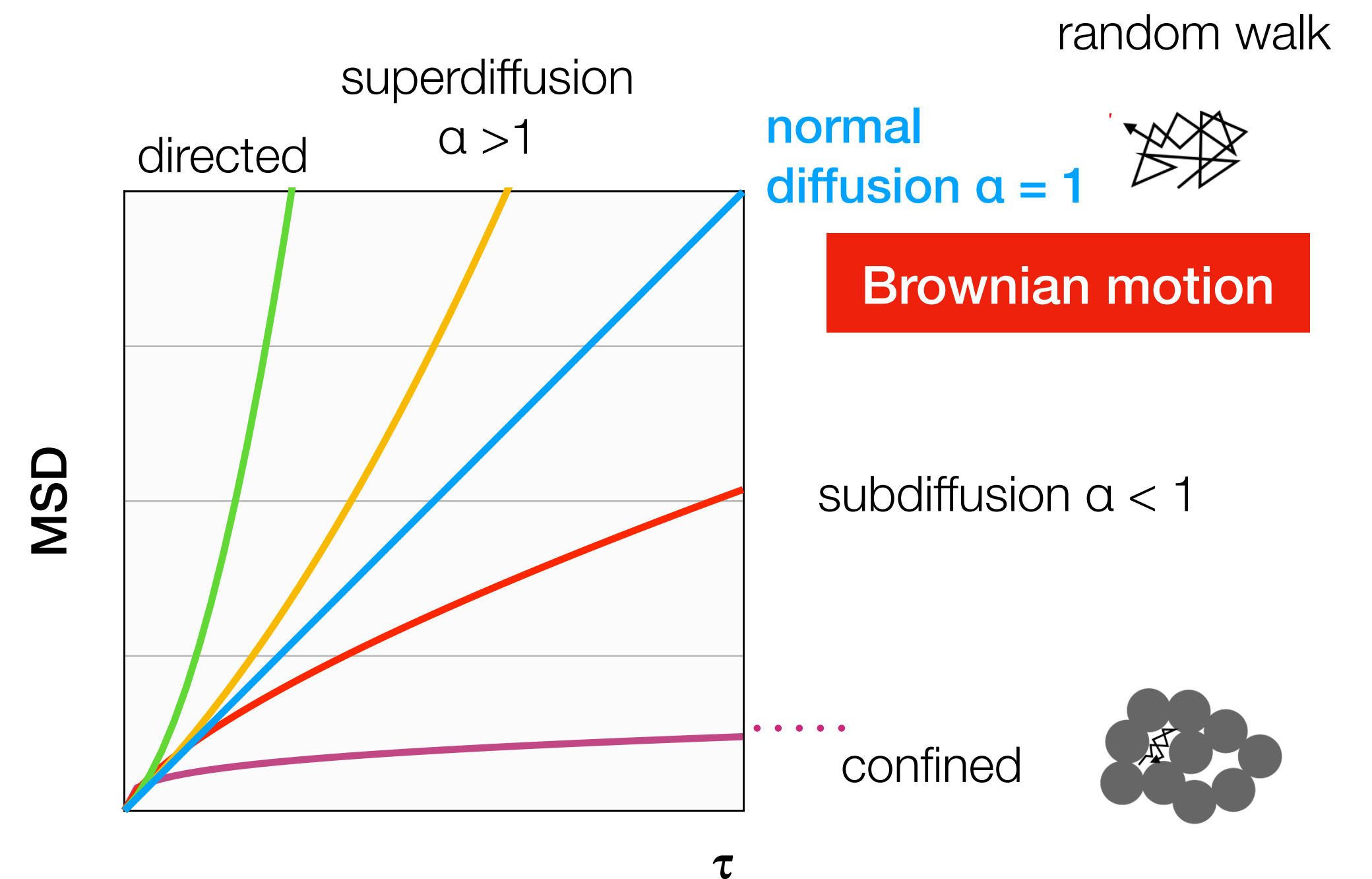
- Deviation of the position with respect to a reference position over time
- Characterisation random motion in biology
- Estimation of the spreading solely to diffusion



$$MSD(\tau) = \frac{1}{N - \tau + 1} \sum_{t=0}^{N-\tau} |\mathbf{x}(t) - \mathbf{x}(t + \tau)|^2$$

Diffusion model

- Normal diffusion $MSD(\tau) \propto 4 D \tau$
- Anomalous diffusion $MSD(\tau) \propto 4 D \tau^\alpha$

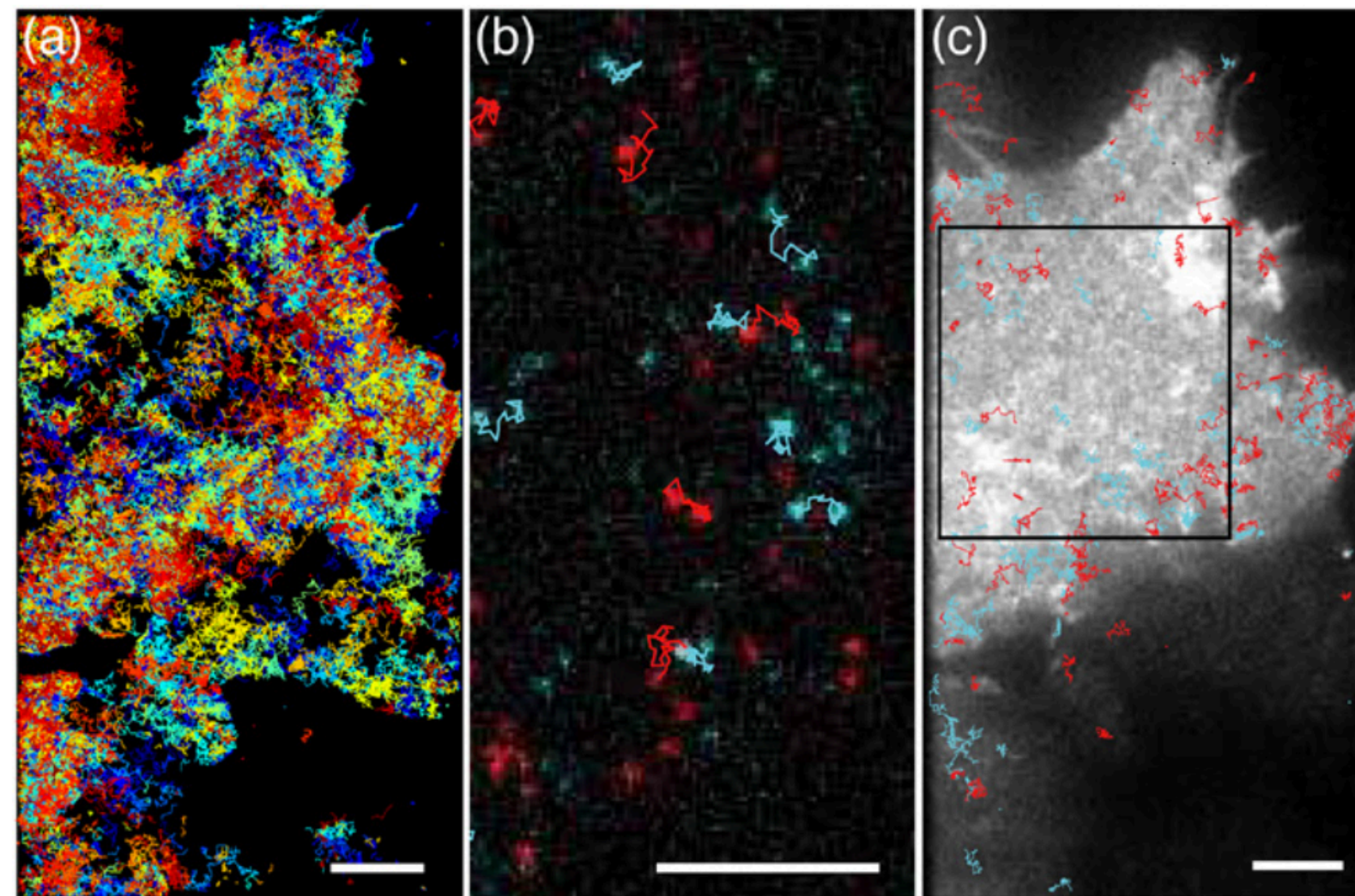




Single Particle Tracking

Spot-like protein of interest

- Diffusion



Dual color single-particle tracking in U2OS cells.

(a) Trajectories longer than 5 frames.

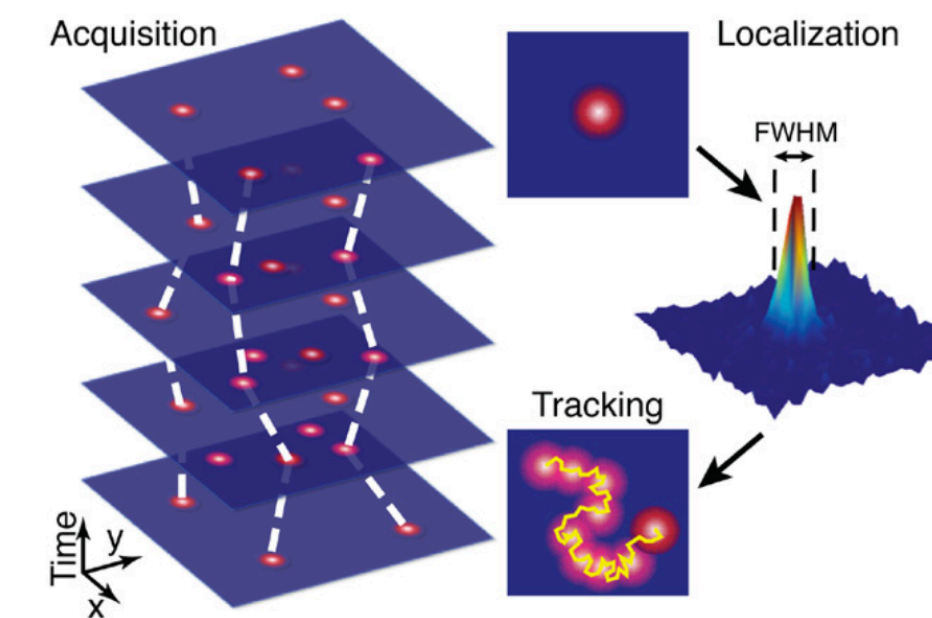
(b) Connected particle positions over the 25 frame

(c) 46 trajectories of track lengths of 30–40 frames. Scale bars are 5 μm .

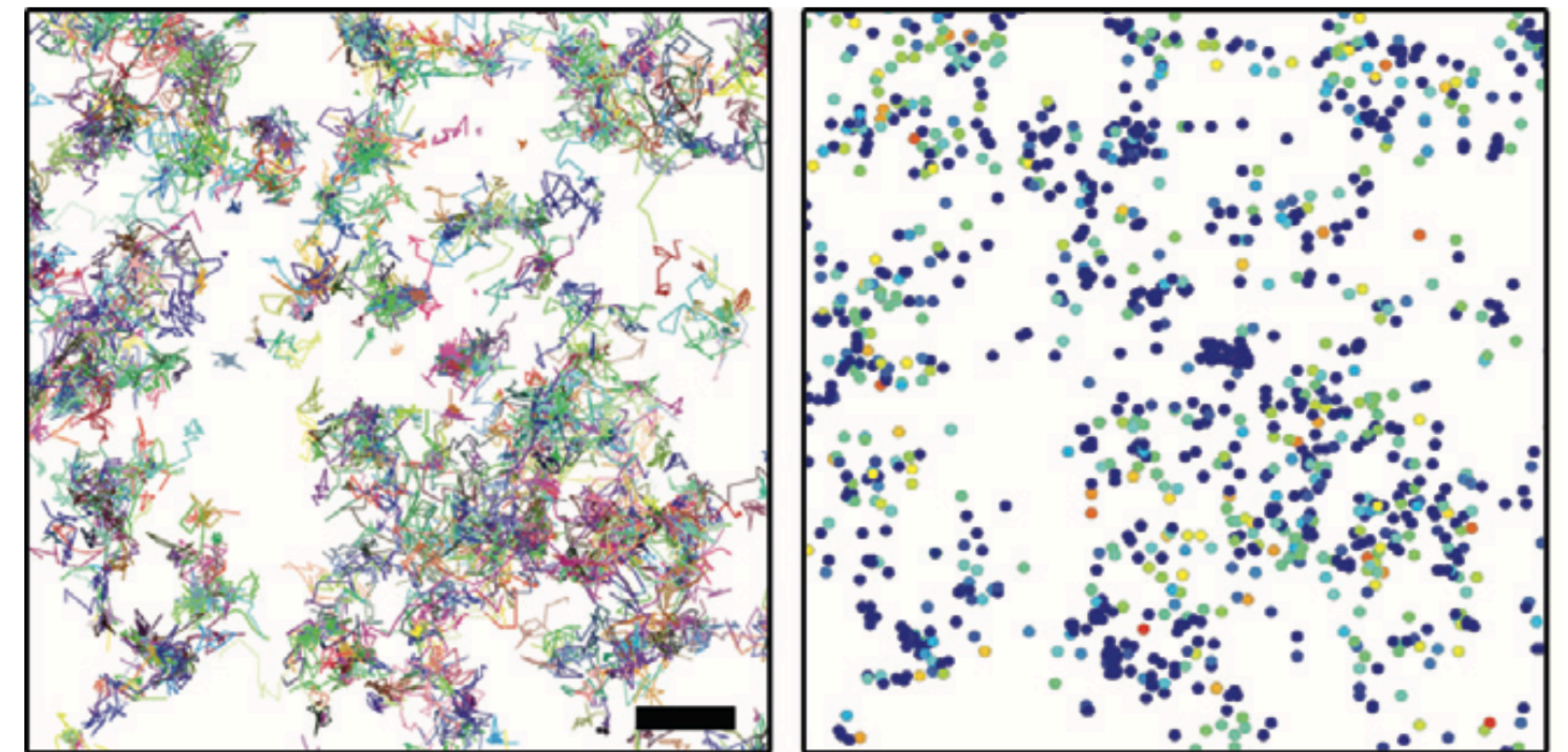
Methods Appl Fluoresc. 2015 Albrecht D, Winterflood CM, Ewers H.

Super-resolution localisation microscopy

- PALM or STORM



sptPALM



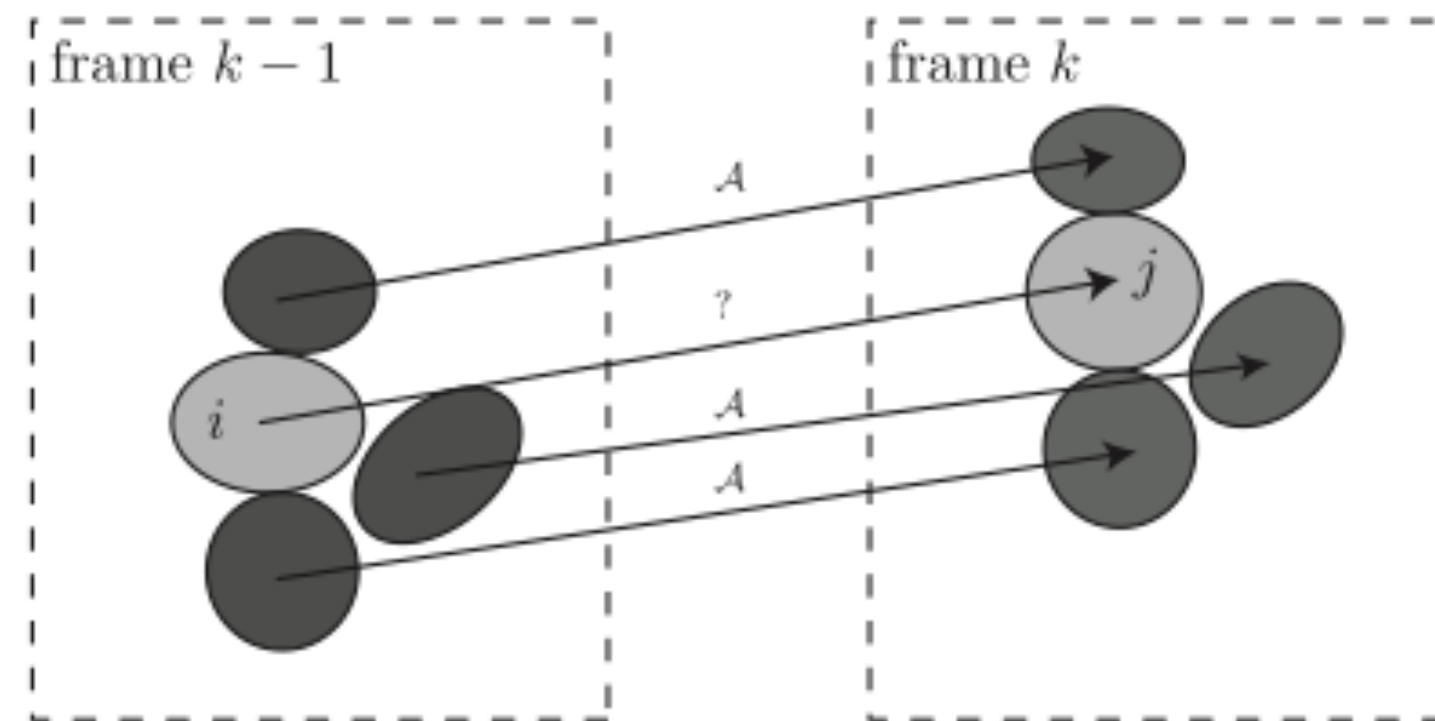
Single-Particle Tracking Photoactivated Localization Microscopy for Mapping Single-Molecule Dynamics, Suliana Manley, Jennifer Gillette, and Jennifer Lippincott-Schwartz, 2010



Tracking of Similar Cells

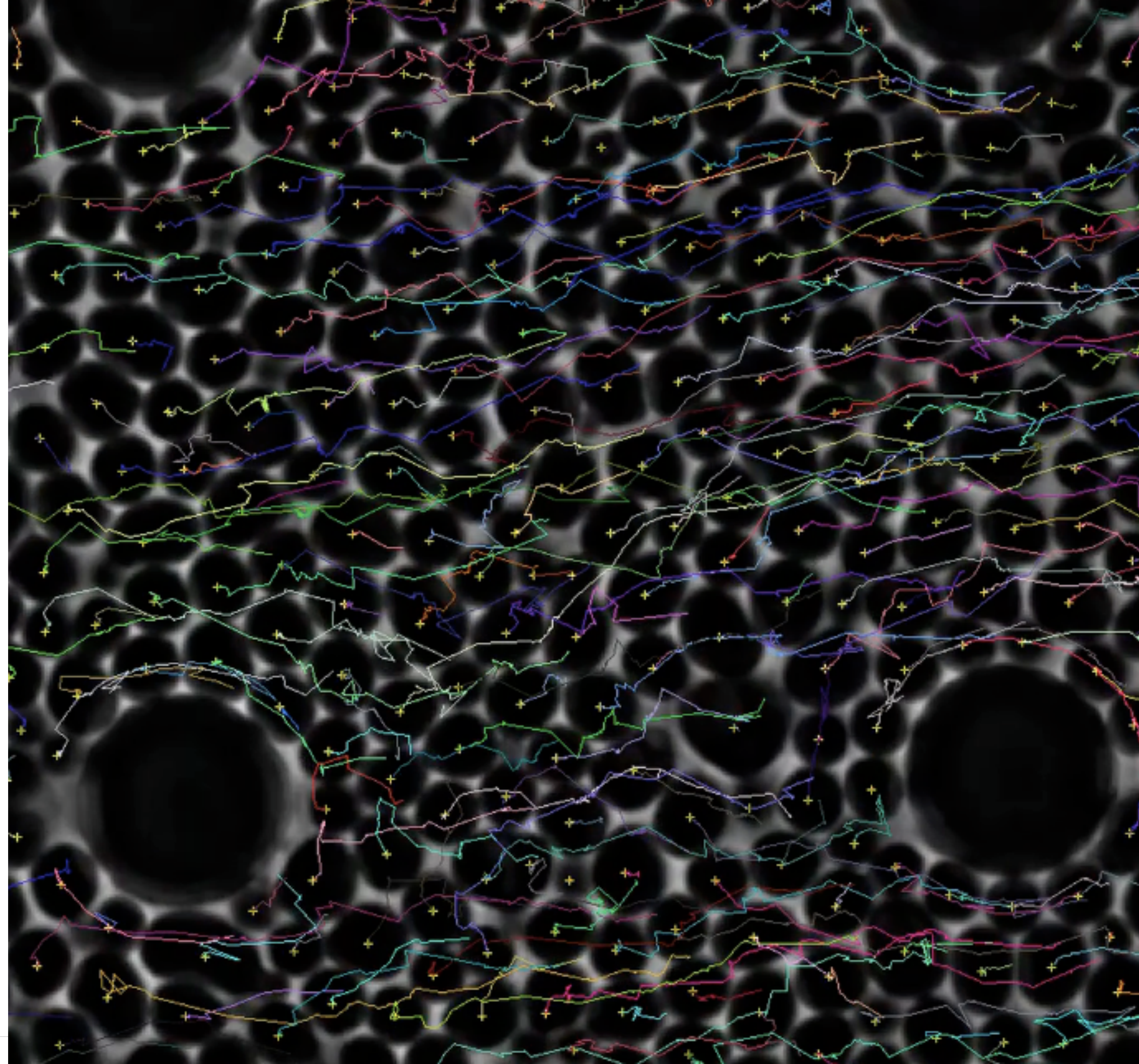
Methods

- Watershed
- Linking frame-to-frame



*Yeast cells population strains in
microfluidic chamber. Sebastian
Maerkl, LBNC, EPFL.*

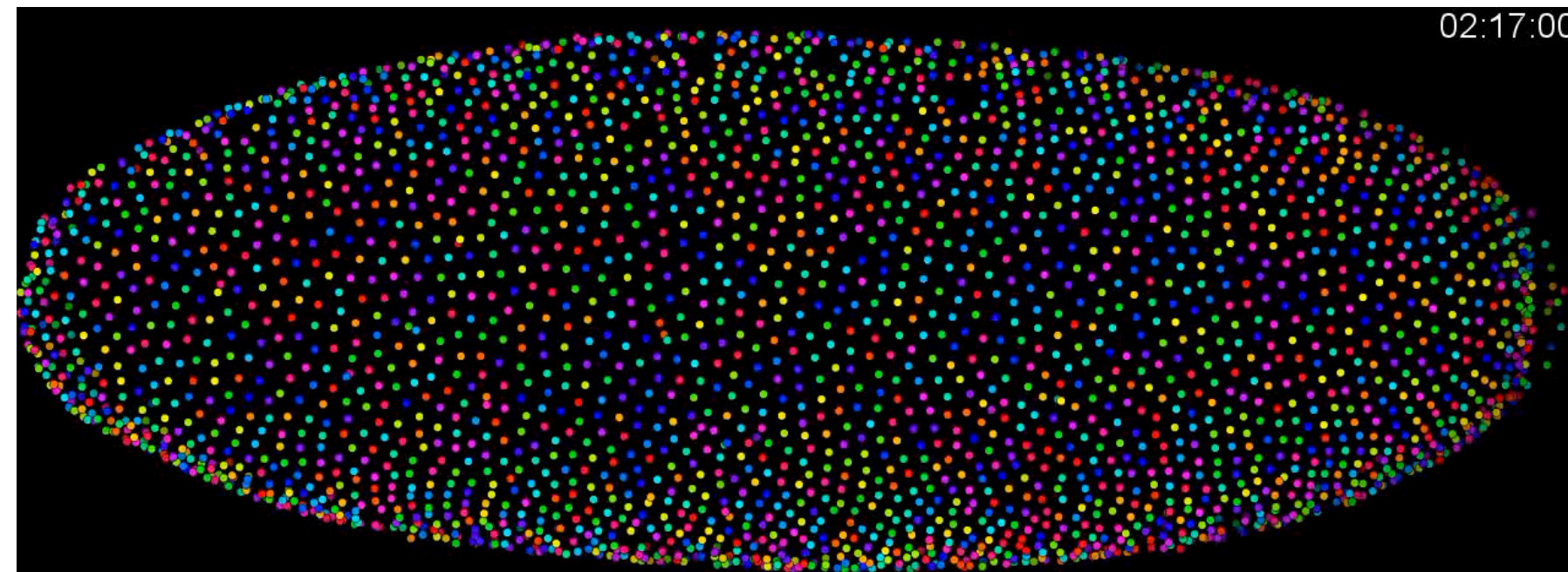
[Delgado 2010]



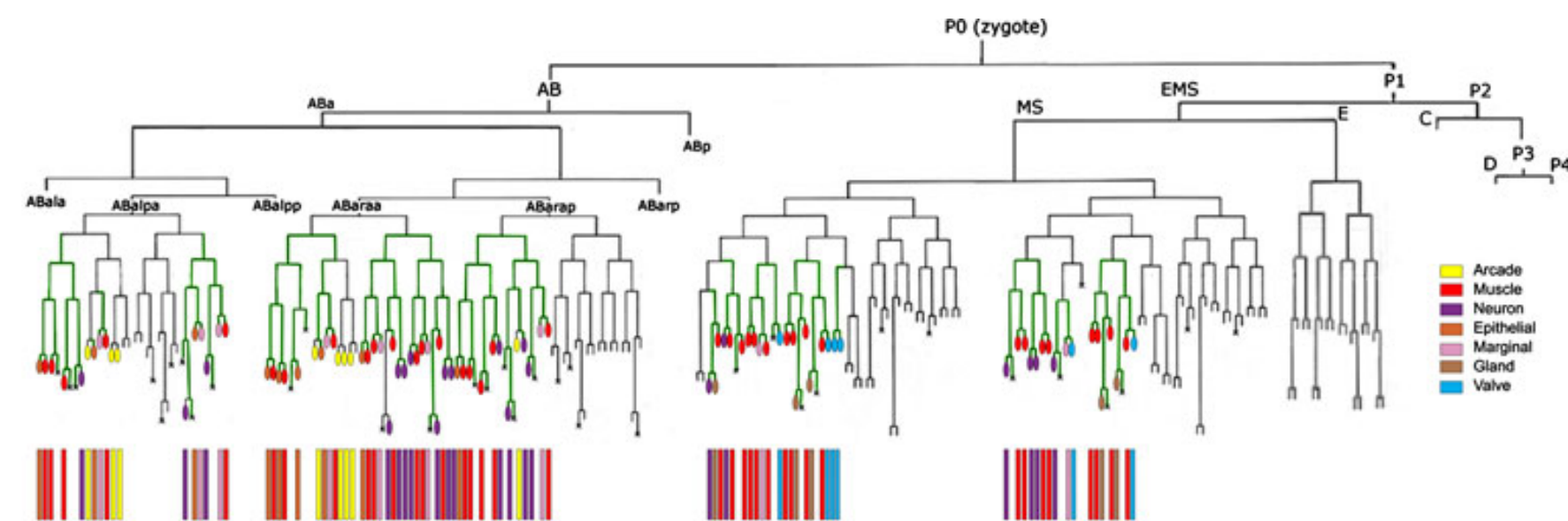


Cell Lineage

Construction of lineage

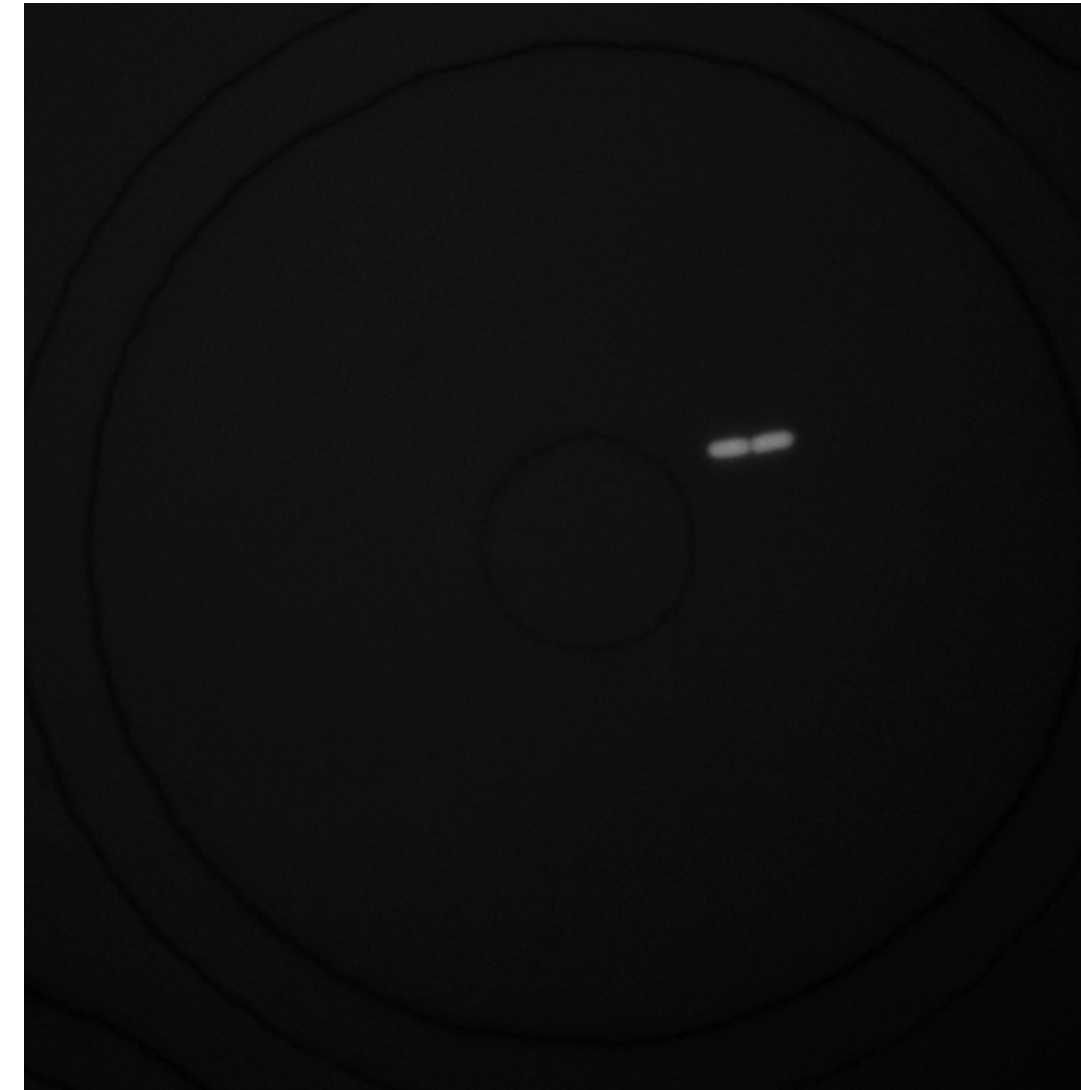


- Track cells and identity cell divisions
- Build the graph with erroneous detections

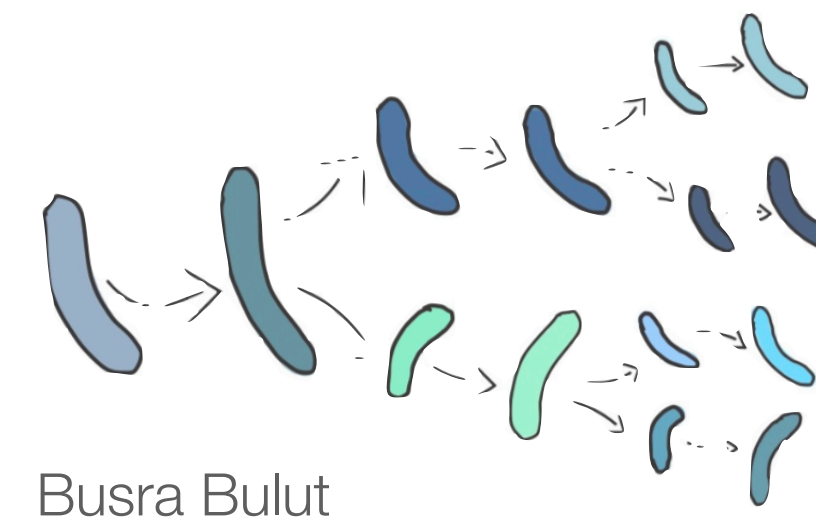


The C. elegans pharynx: a model for organogenesis Susan E. Mango

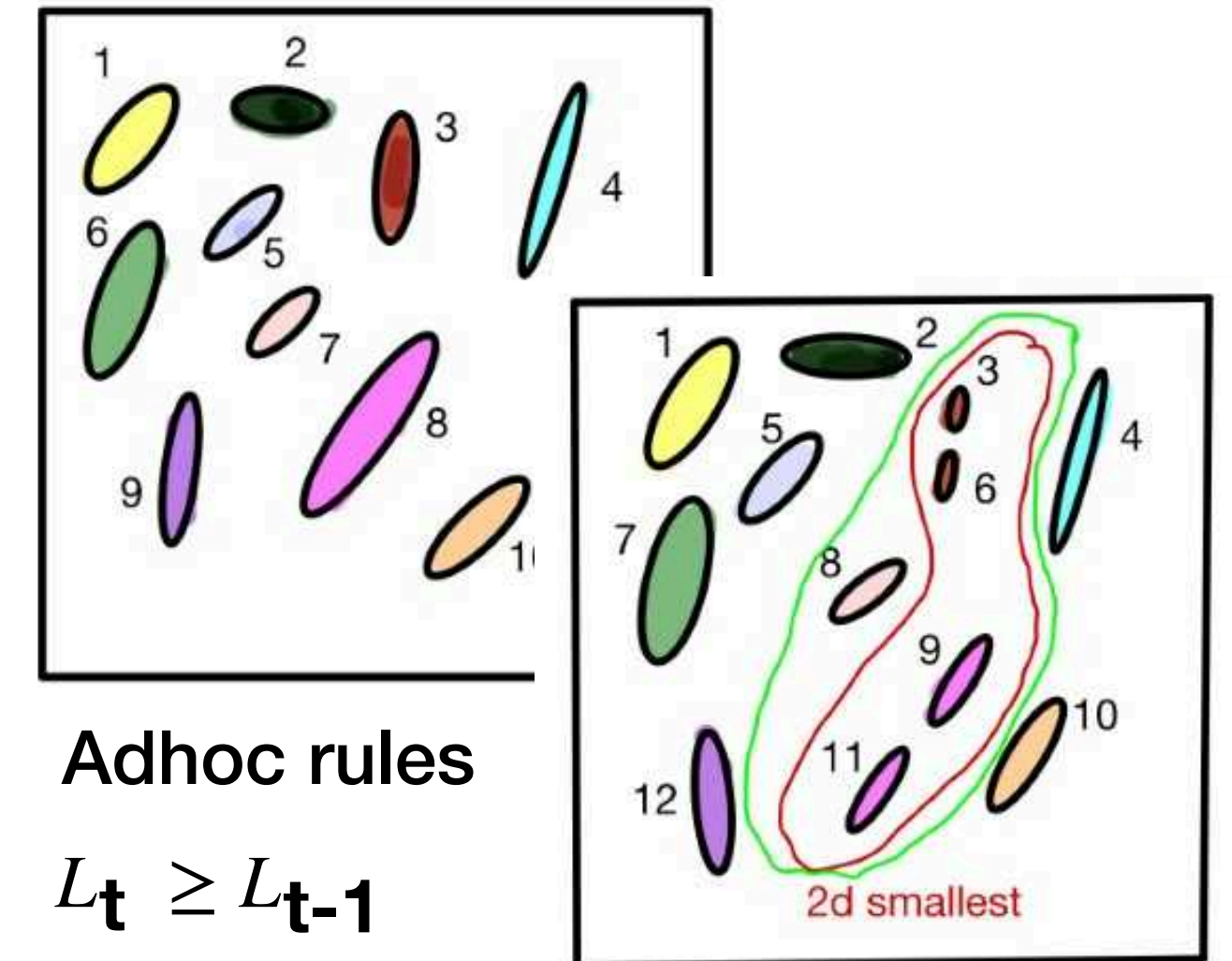
Colony of e-coli bacteria



Time-lapse fluorescence microscopy ~5h
Frédéric Normandeau, McKinneyLab, EPFL



Busra Bulut

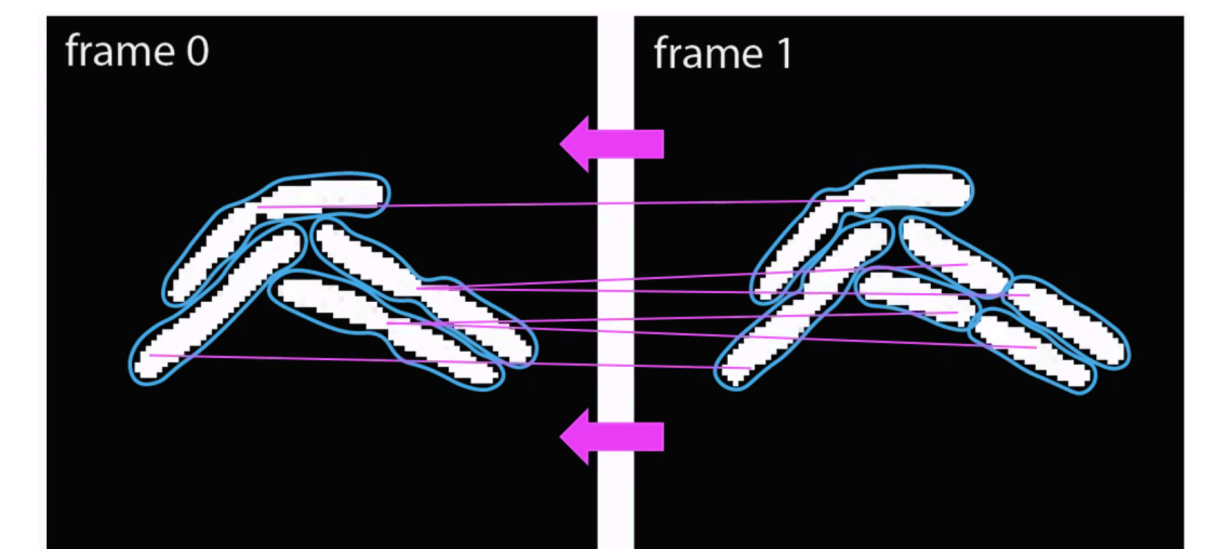


Adhoc rules

$$L_t \geq L_{t-1}$$

$$| \langle \mathbf{x}_t, \mathbf{x}_{t-1} \rangle | > T$$

$$L_{\text{mother}} \approx 2 L_{\text{Daughter}}$$



RECAP

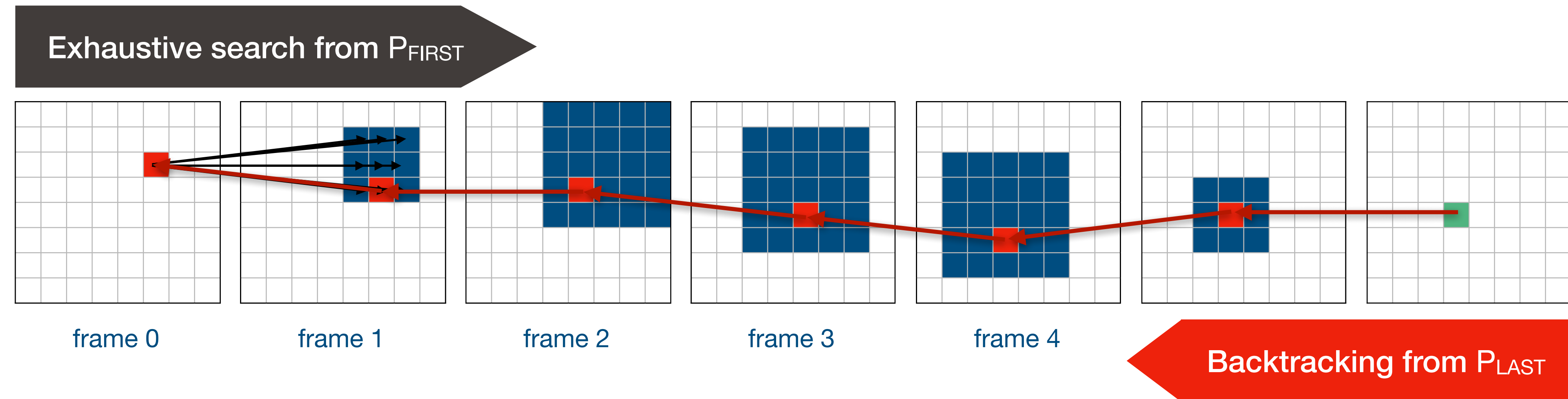
- IDENTIFY A GRAPH PROBLEM AND A GRAPH SOLVER
- CODE A GRAPH STRUCTURE (HOMEWORK)
- CHARACTERIZE A TRACKING PARTICLES APPLICATION
- DESIGN A COST FUNCTION FOR TRACKING
- TRACKING-BY-DETECTION FRAME-TO-FRAME
- OTHER STRATEGY OF TRACKING
- MEAN SQUARE DISPLACEMENT – BROWNIAN MOTION
- STANDARD SOFTWARE – TRACK MATE

Extra Material

Application Case **SpotTracker**

Solving a Shortest-Path Problem

Tracking is equivalent to find the **shortest path** problem from point P_{FIRST} to P_{LAST} in **directed acyclic graph (DAG)**



Dynamic Programming - Viterbi

- Optimization on discrete data with a sequence
- Efficient algorithm to find the best path
- Global scope: **past + future**



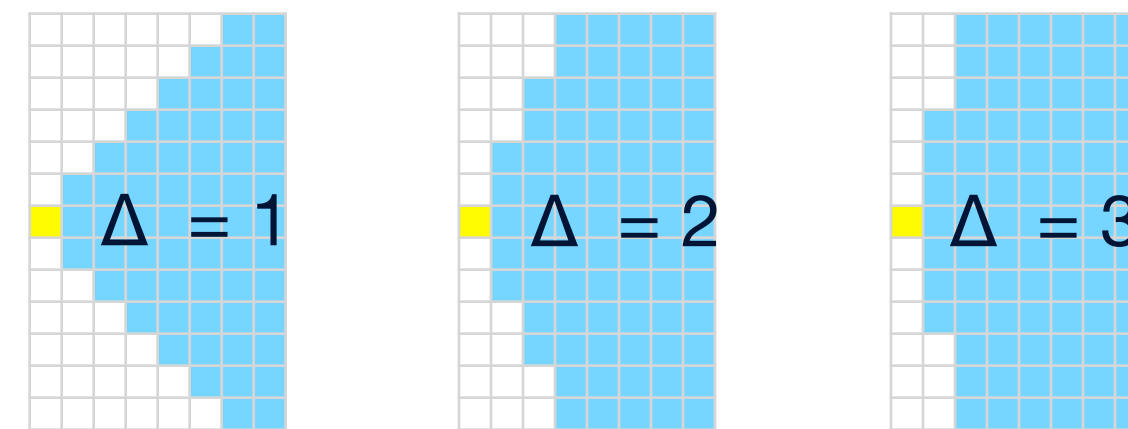
$$\xi(\mathbf{u}_t, \mathbf{v}_{t+1}) = \underbrace{f(\mathbf{u}_t)}_{\text{data term}} + \underbrace{\lambda d(\mathbf{u}_t, \mathbf{v}_{t+1})}_{\text{regularization}}$$

Example of a cost function from $\mathbf{u}_t$ position to $\mathbf{v}_{t+1}$

Dynamic Programming

Aperture

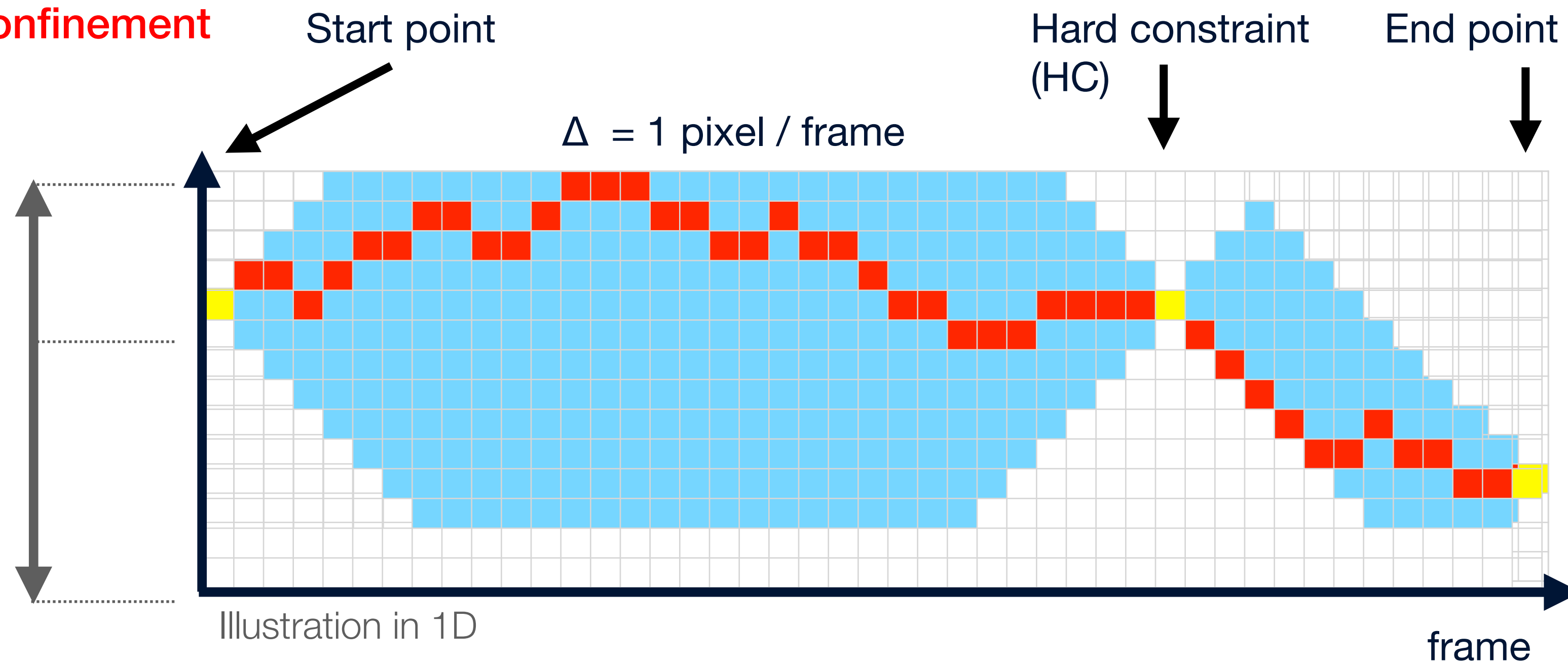
- Maximum speed: Δ pixel/frame
 - User input: start point
- } Admissible range



2D/3D

Up to the complexity limitation

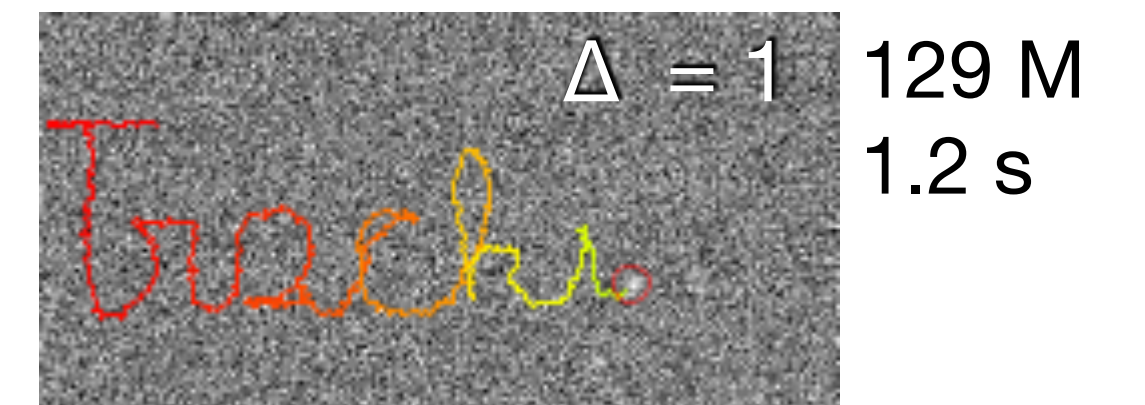
Confinement



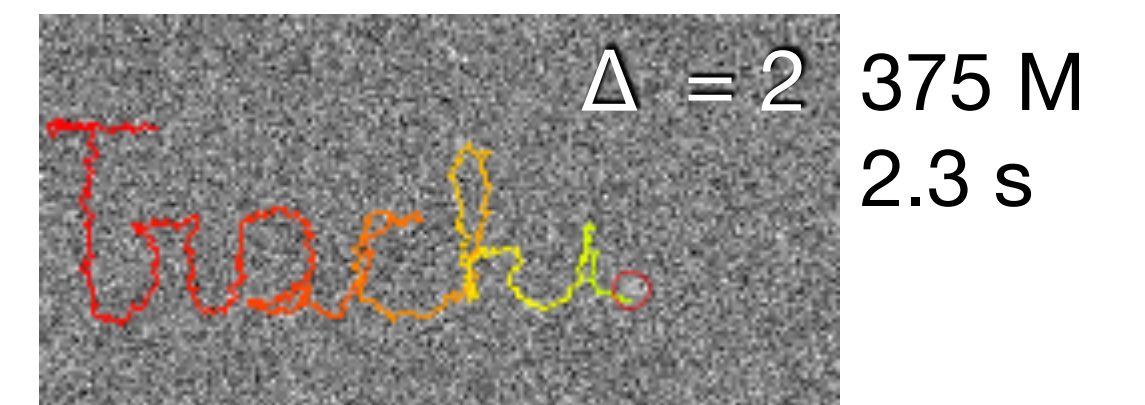
Complexity in 2D

Dim: 200x200x830

Full area: 16'600'00 px



Adm. area: 14'286'564 (80%)



Adm. area: 15'068'740 (90%)

HC	Conn.	ms
0	129 M	1028
1	113 M	670
2	93 M	581
3	70 M	404
4	54 M	364

👁 Dynamic Programming **Spot Tracker**

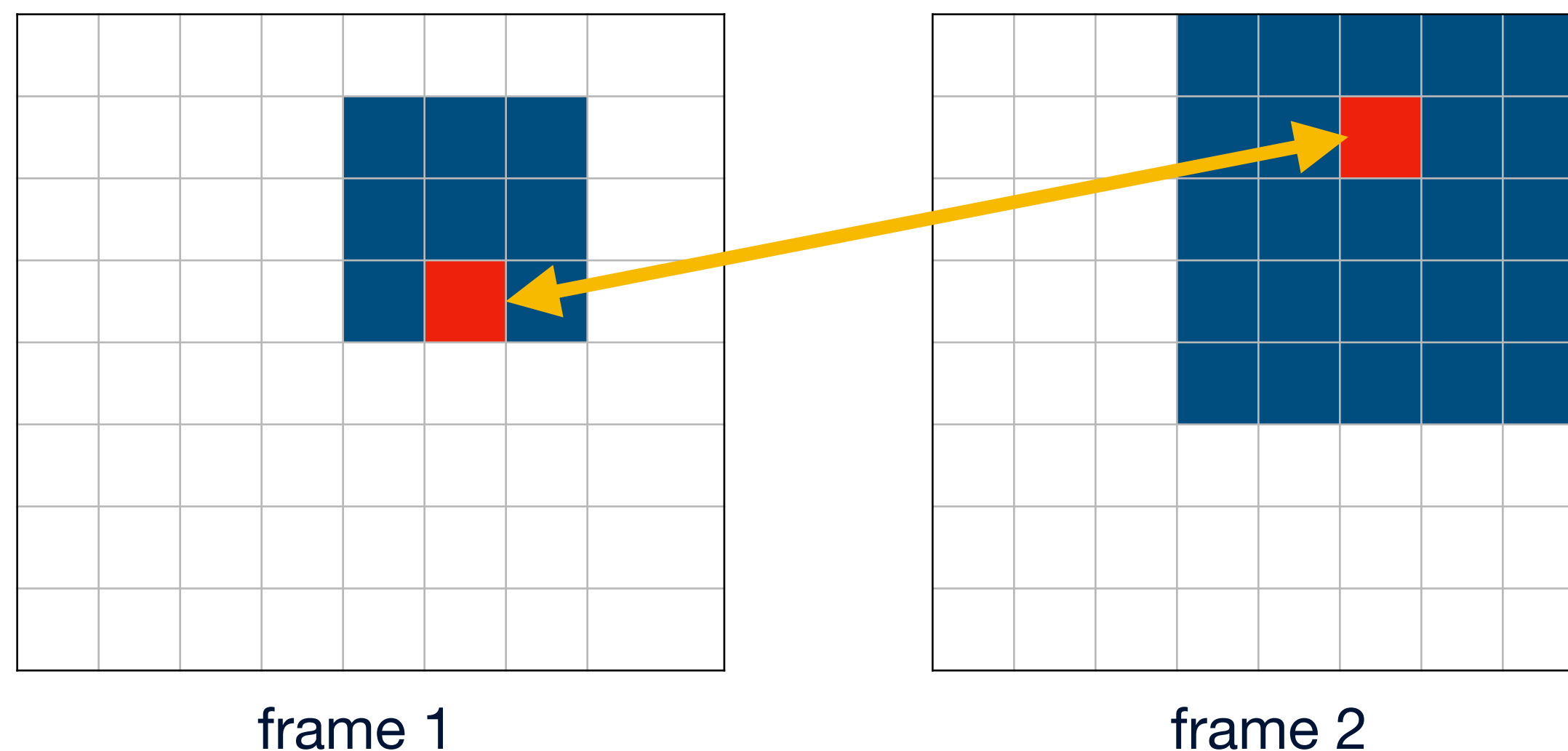
Tracking: Dynamic Programming Algorithm (DPA)

- Optimal shortest path, global: past + future **Bellman principle**
- Cost function from $\mathbf{u}_t$ position to $\mathbf{v}_{t+1}$

$$\xi(\mathbf{u}_t, \mathbf{v}_{t+1}) = \lambda_1 |f(\mathbf{u}_t) - f_c| + \lambda_2 |\mathbf{u}_t - \mathbf{v}_{t+1}|^2 + \lambda_3 |f(\mathbf{u}_t) - f(\mathbf{v}_{t+1})| + \lambda_4 |f(\mathbf{u}_t - \mathbf{p}_c|$$

Promote intensity close to f_c Promote short displacement Promote short intensity variation Attraction to a specific position

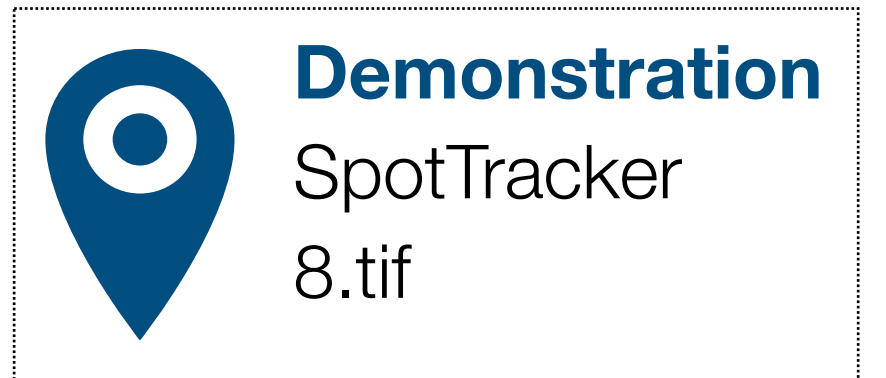
$\lambda_1, \lambda_2, \lambda_3, \lambda_4$ parameters: weighted and normalized



SpotTracker ImageJ

- Fast
- Tunable
- Interactive
- Global: past + future
- Spatio-temporal segmentation

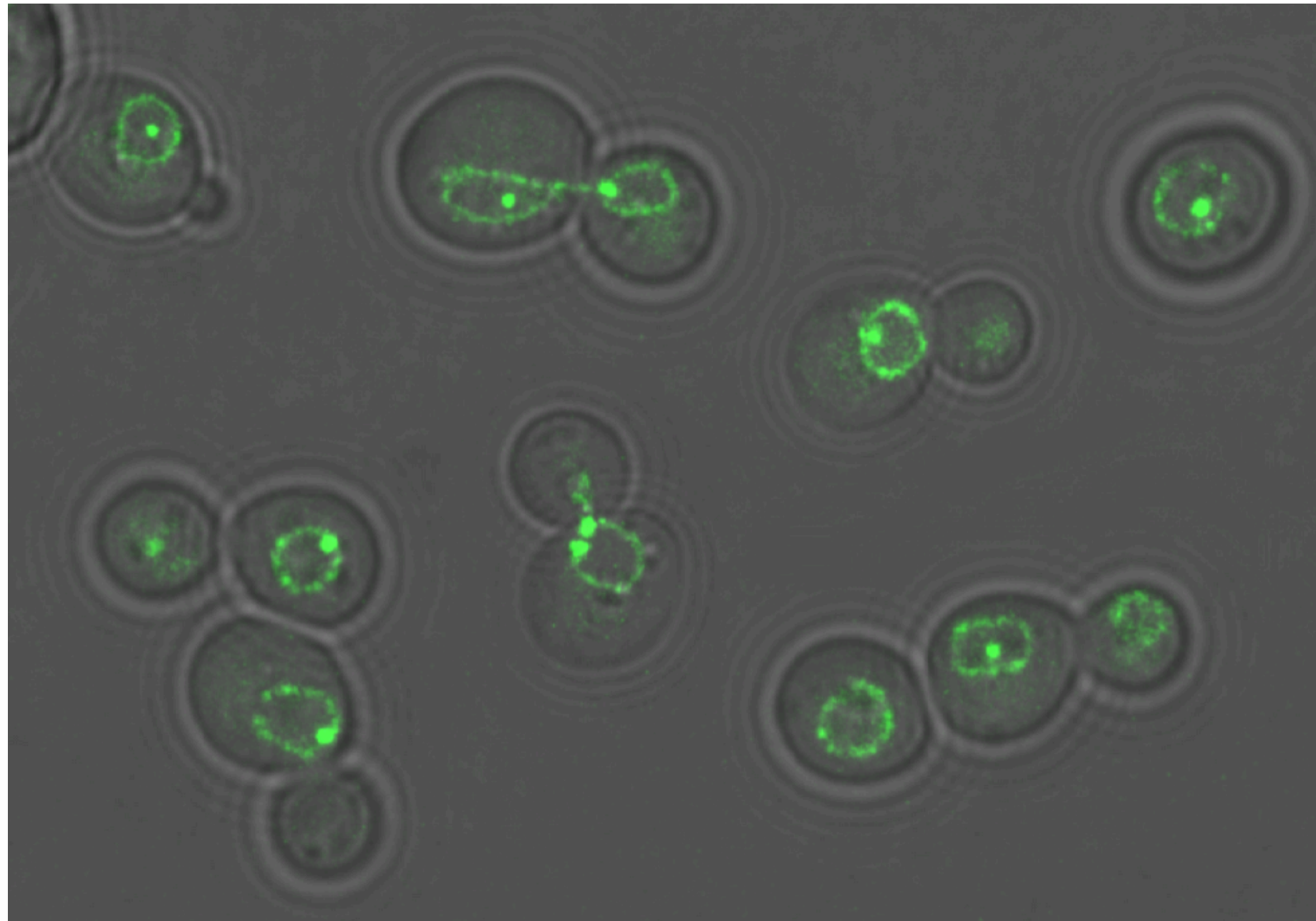
 <http://bigwww.epfl.ch/sage/soft/spottracker/>



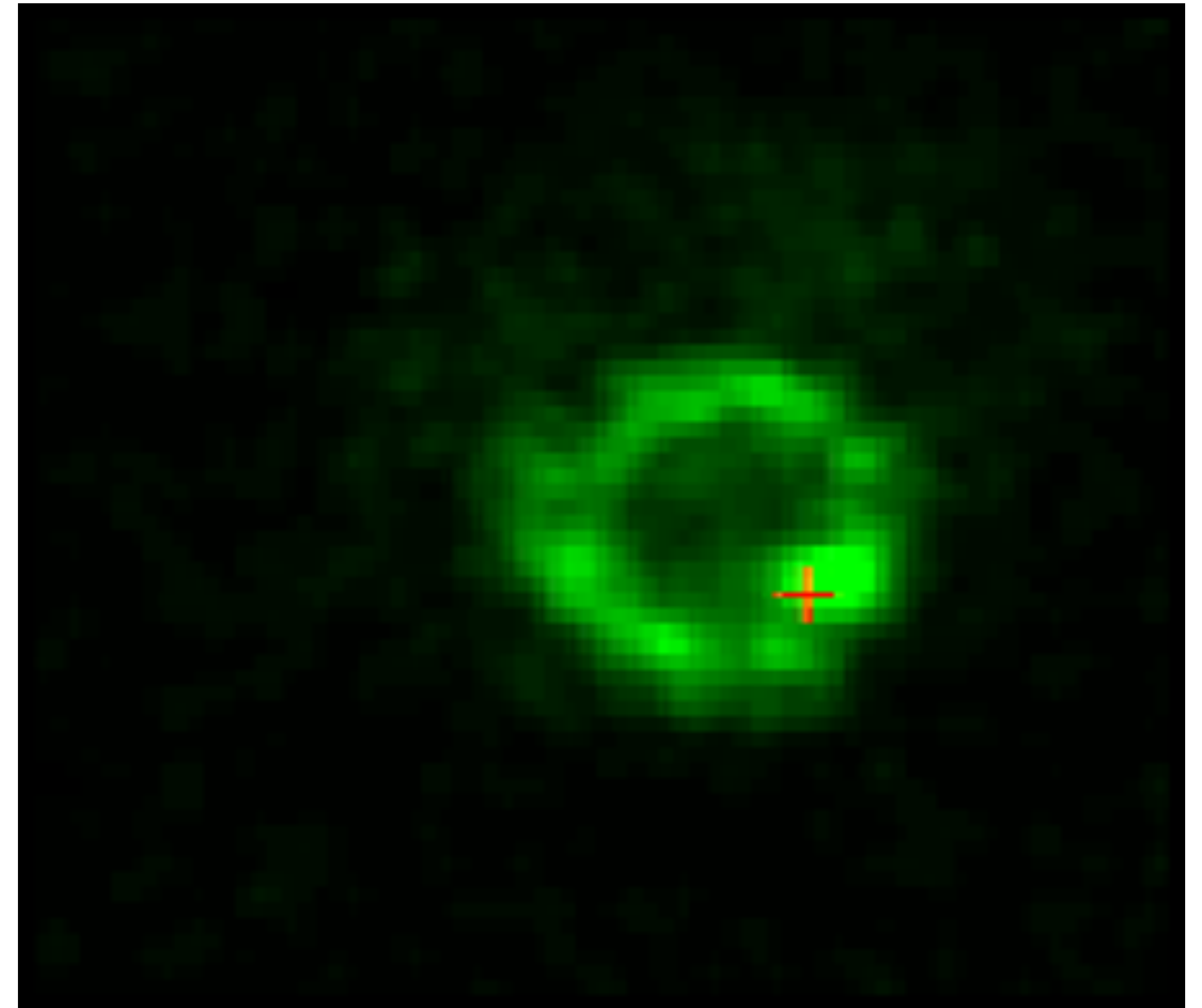
👁 Application Tracking Telomere

Subnuclear organisation and dynamics

- Telomere tagging in yeast cell
- In vivo localizing chromosomal domains

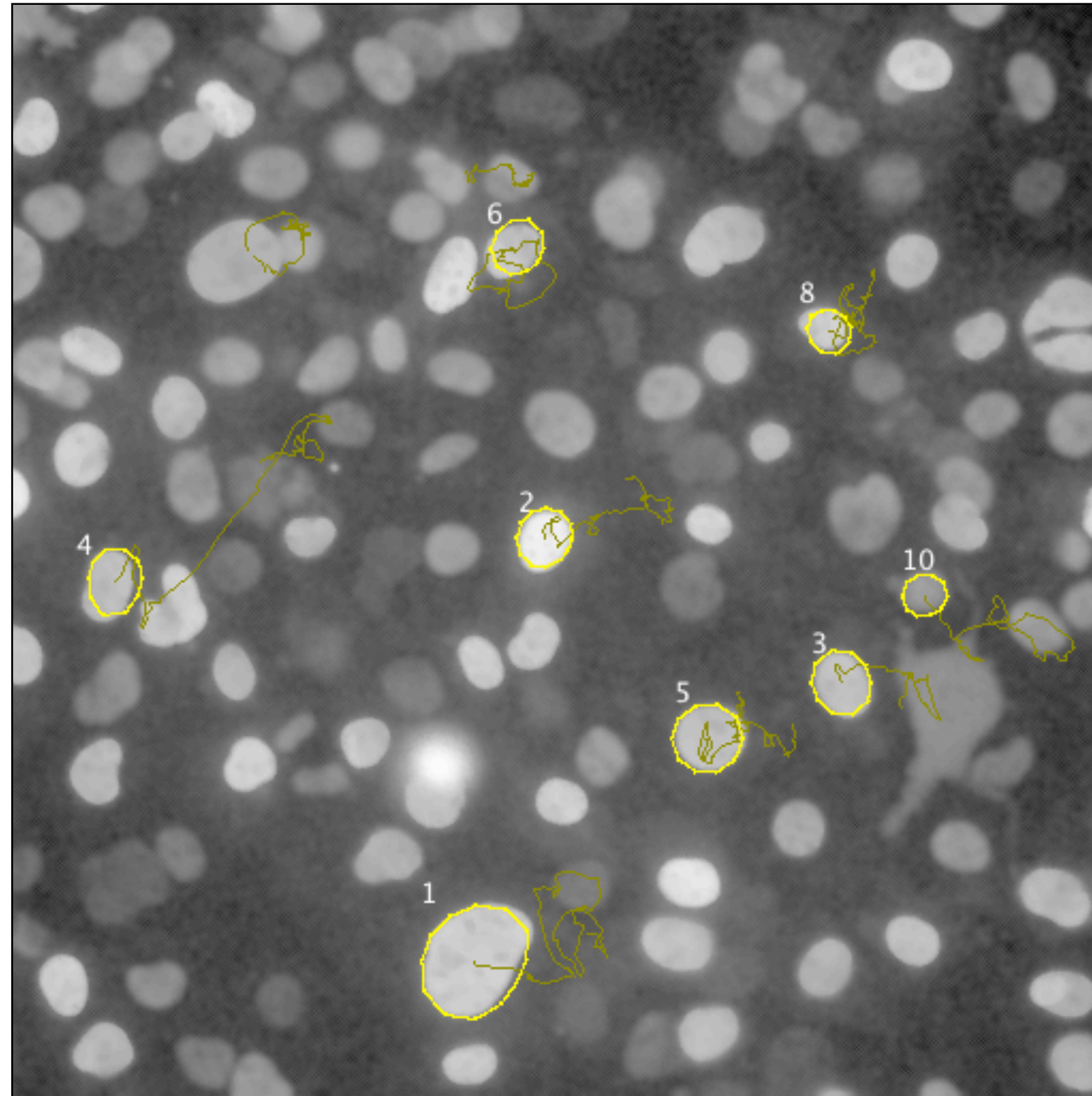
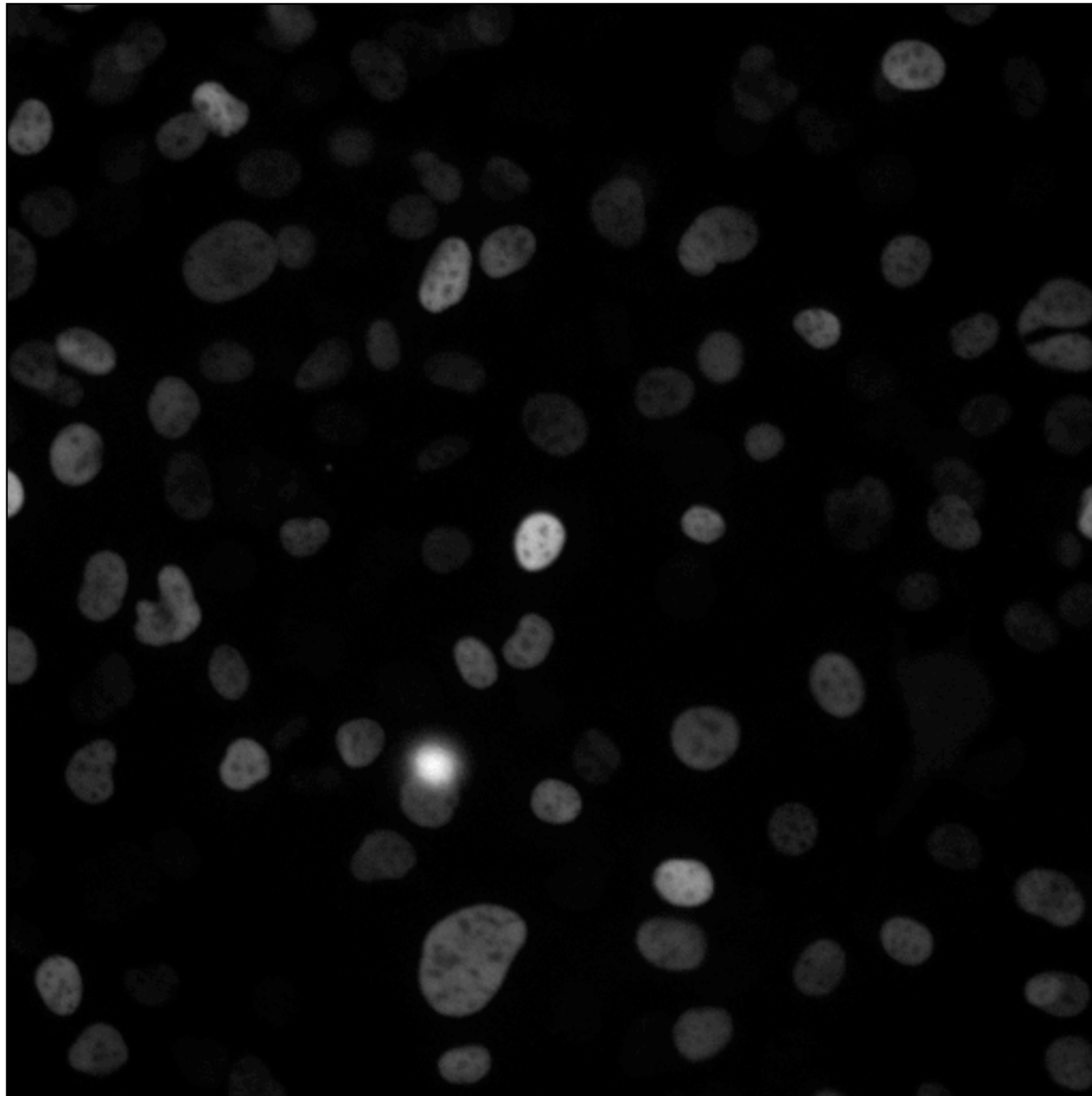


Nuclear membrane and telomere visualise through a GFP. Confocal Microscopy. Source: Susan Gasser, University of Geneva and FMI Basel



Application Tracking of Oscillating Cells

Circadian cycle



Source: Bioluminescence. Charna Dibner, HUG, Geneva

