WORKSHOP SMLM DATA ANALYSIS

Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking

Daniel Sage

Biomedical Imaging Group

Center for Imaging

EPFL, Lausanne, Switzerland

Single Molecule Localization Microscopy Symposium

SMLMS 2025 — Bonn

25 Aug. 2025

EPFL

WORKSHOP SMLM DATA ANALYSIS

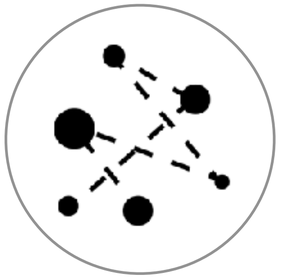
Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking

SMLM WORKFLOW

Data Analysis

IMAGE ANALYSIS

Particle Tracking



BIOPHYSICS

Diffusion Models

ALGORITHMS

Estimating Diffusion

https://go.epfl.ch/workshop-smlms

https://github.com/dasv74/workshop-tracking-diffusion-single-molecule

Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking

SMLM WORKFLOW

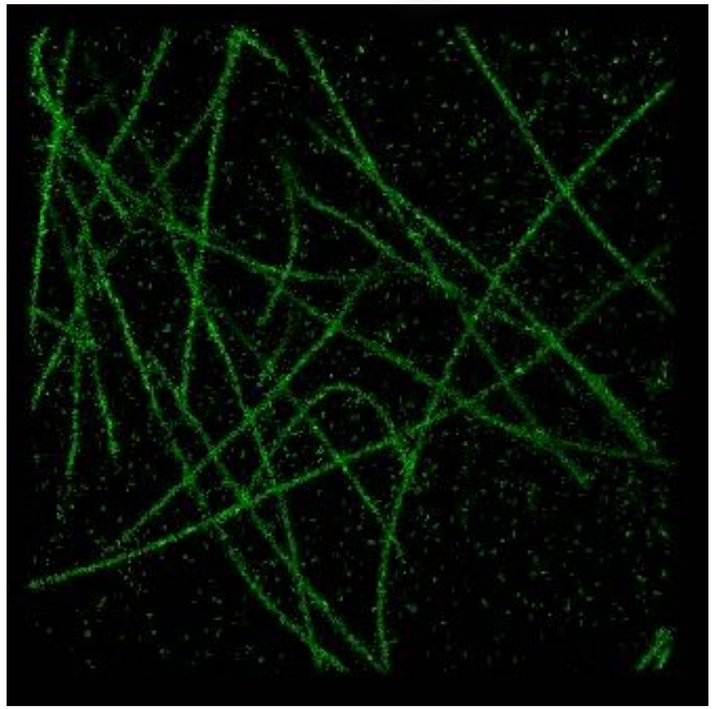
DATA ANALYSIS

SMLM as a Quantitative Bioanalytical Tool

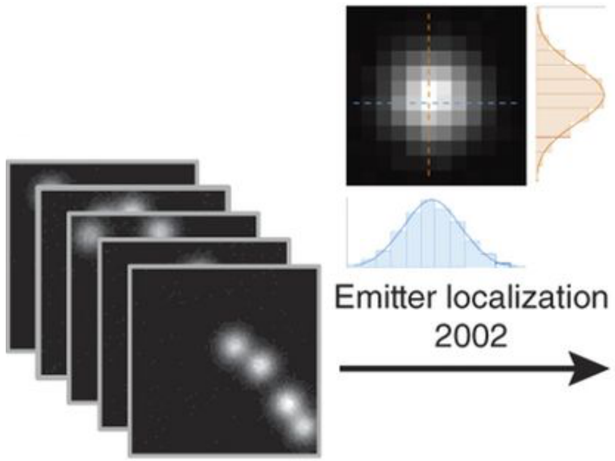
Breaking the Light Diffraction Limit

2014
Eric Betzig
Stephan Hell
W.E. Moerner

Super-resolution Microscopy (x10)

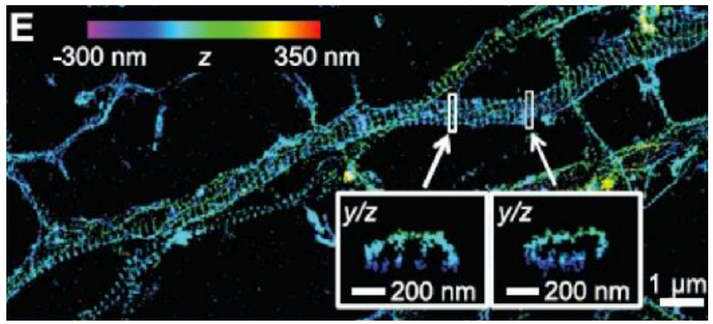


P. Nicovich, Nature Protocols, 2017



What can we quantify?

- Counting: molecules
- Pattern: Spatial arrangement
- Interaction: Dynamics of proteins



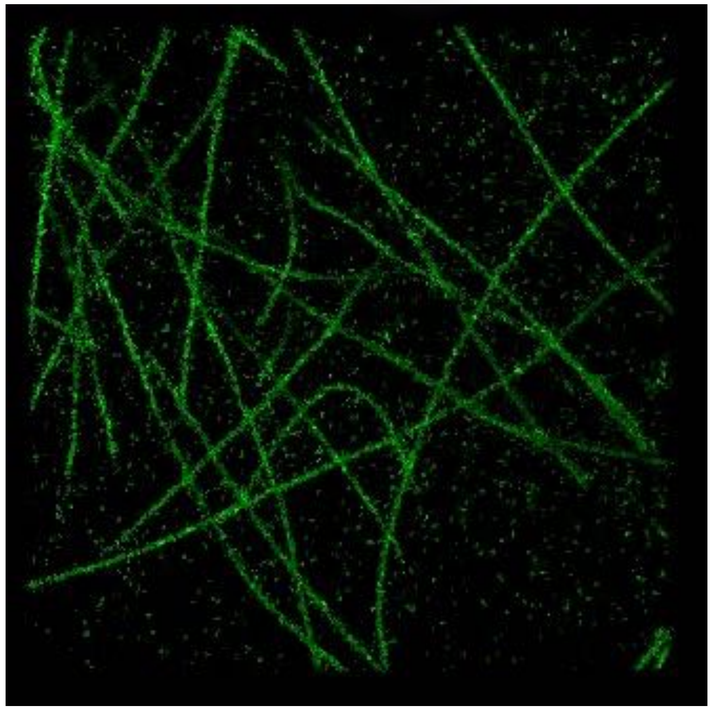
Discovery of the periodic cytoskeletal structure in axons [Xu, Science 2012]

SMLM Image Analysis

Breaking the Light Diffraction Limit

2014
Eric Betzig
Stephan Hell
W.E. Moerner

Super-resolution Microscopy (x10)



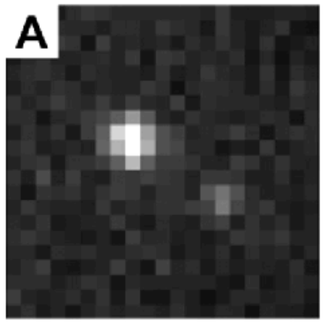
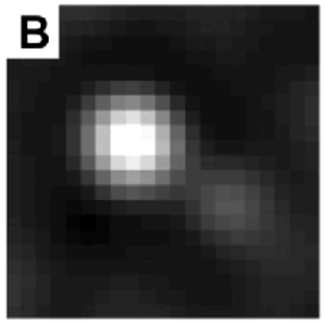
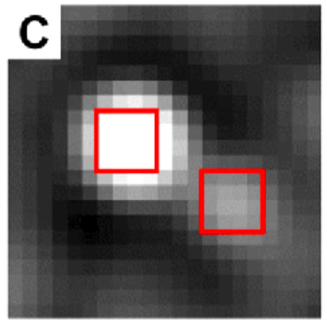


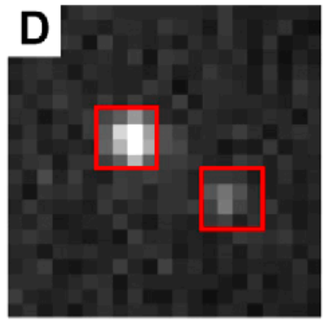
Image filtering



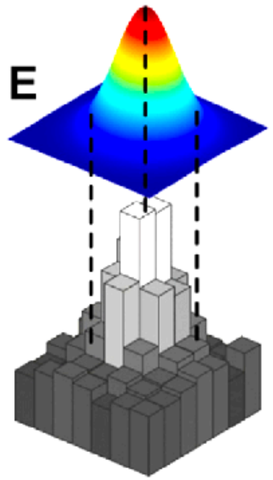
ROI selection

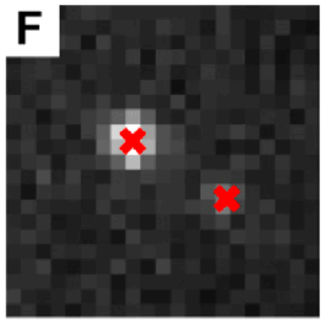


ROI extraction

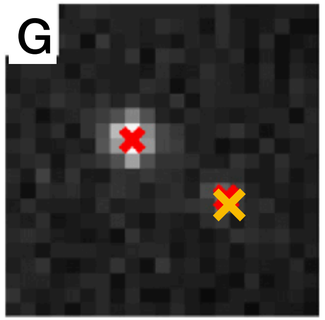


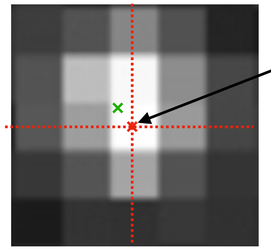
PSF fitting





Rejection





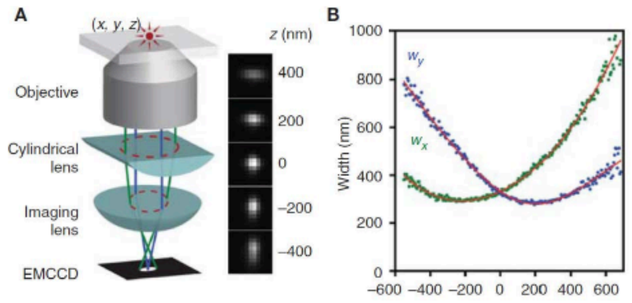
sub-pixel localization + uncertainty

Martens et al., Frontiers, 2022

3D SMLM

3D can be performed by putting a cylindrical lens into the imaging path to create astigmatism

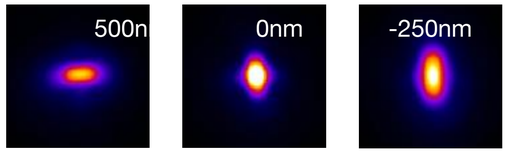
Huang 2008

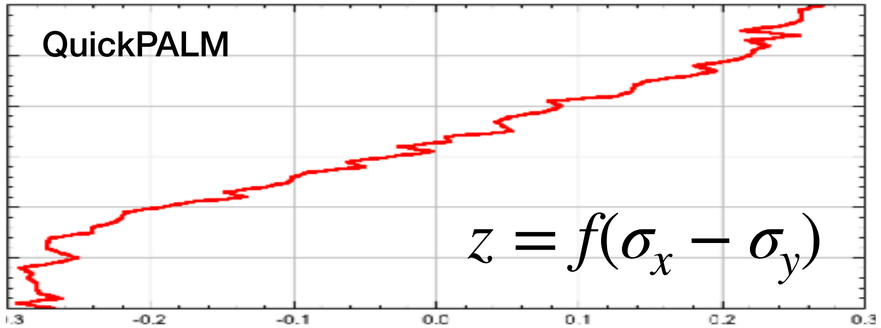


Calibration

PSF dependence on z using immobilized nanobead z-scan

$$z = f(\alpha_1, \alpha_2, \dots)$$

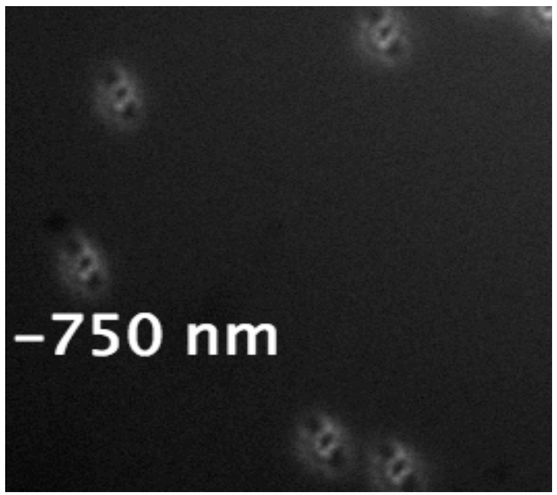




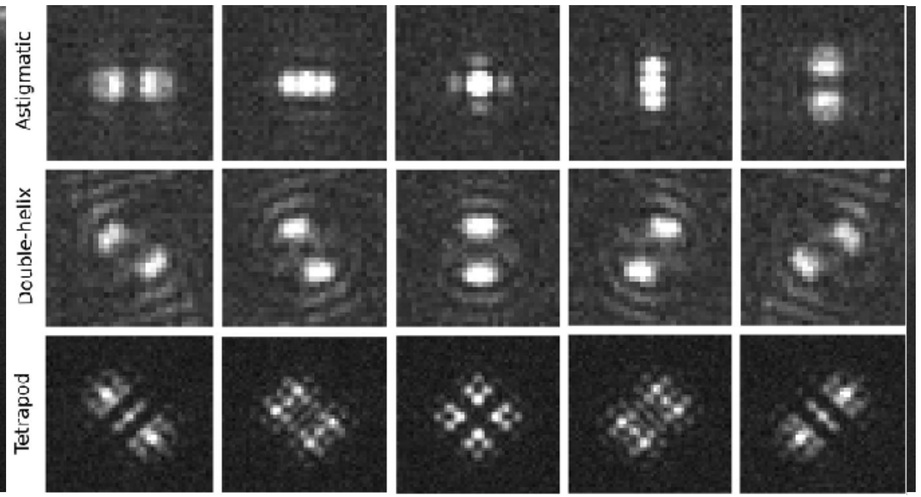
Interferometry	
iPALM	Shtengel, PNAS 2009
SELFI	Bon, Nat Methods 2018
EngineeredPSF	
Cylindric lens	Huang, Science 2008
Astigmatism AO	Izzedi, Optics Express 2011
Double-helix	Pavani, PNAS 2009
Saddle	Shechtman, Phys Rev, 2014
Tetrapod	Aristov, Nat. Comm. 2018
Multiplane	
Biplane	Juette, Nat. Methods 2008
Multiplane, MUM	Ram, Biophysical J 2008
Multifocus, MFM	Abrahamsson, Nat. Meth, 2013
Beam Splitter	Geissbuelher, Nat. Comm. 2018

Astrid Magenau 2014 and Bassam Hajj 2018

Acquisition in 2D, Stacking all the 2D-PSF, 3D PSF signature gives Z:



Double Helix PSF



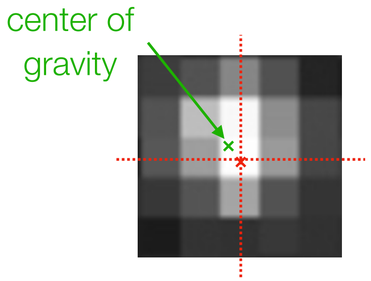
Fazel et al. Biophysics and Bioengineering, 2022

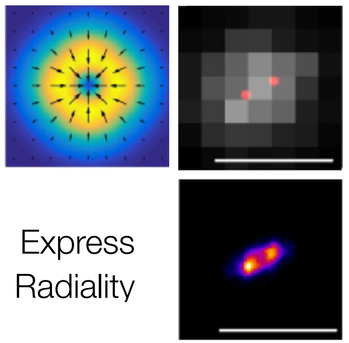
Localisation Methods

Direct Detection

Center of gravity

QuickPALM





Express Radiality

Fitting Parametric Function

Single-emitter

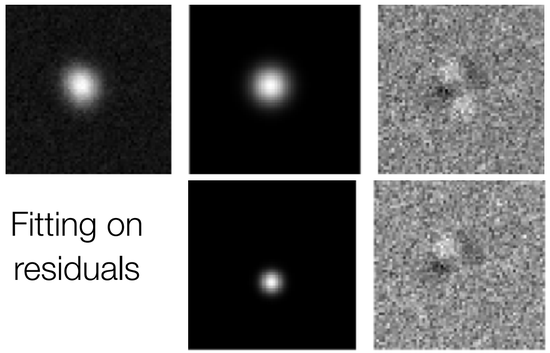
RapidSTORM

commercial software

Multiple-emitter

DAOSTORM

ThunderSTORM

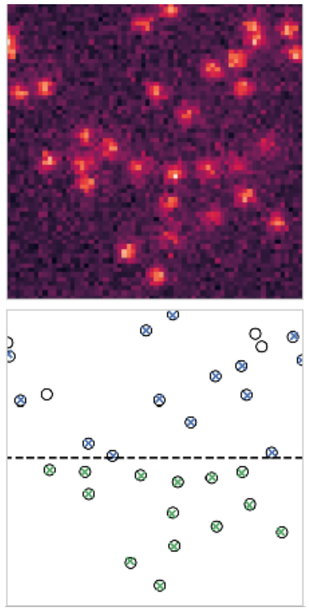


Fitting on residuals

Solving as a Inverse Problem

Optimization

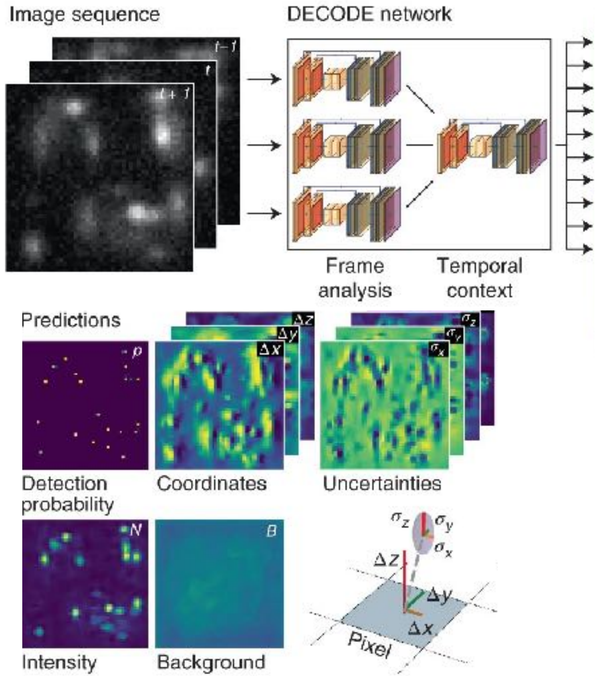
ADCG



Learning Approaches

Synthetic datasets

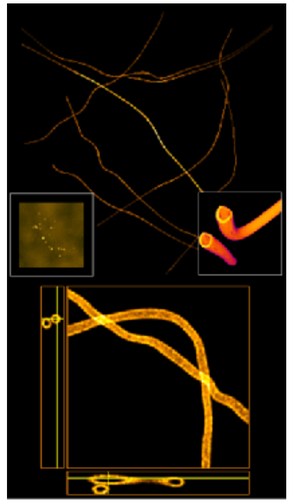
DECODE



Benchmarking SMLM Software

http://srm.epfl.ch/

Realistic Simulation



More 30 than participants

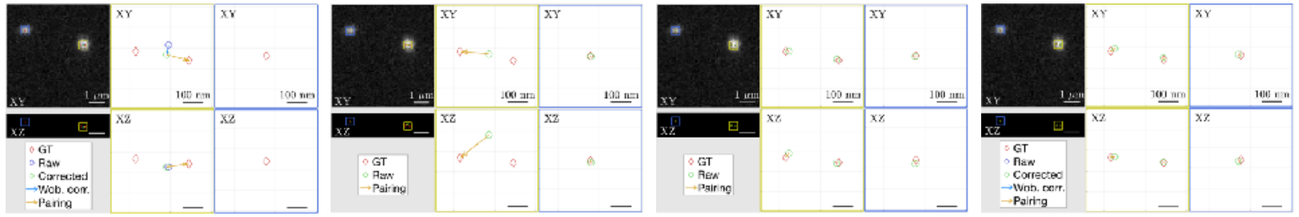
Software	PSF	Plateform
3D-DAOSTORM	Gaussian	Python / C
3D-STORM	Gaussian	Qt framework
3D-WTM	Gaussian	Stand-alone
Cspline	Learnt	Python / C
DECODE	Encode	Python
Localizer	Gaussian	Igor
MIATool	Bessel	Java
mlePALM	Gaussian	Matlab
pSMLM-3D	Gaussian	ImageJ
QC-STORM	Gaussian	ImageJ
QuickPALM	Gaussian	ImageJ
RainSTORM	Gaussian	Matlab
RapidSTORM	Gaussian	Stand-alone
SMAP	Learnt	Matlab
SMolPhot	Gaussian	Python / C
ThunderSTORM	Gaussian	ImageJ
TVSTORM	Gaussian	Matlab
WaveTracer	Gaussian	Metamorph
ZOLA-3D	Zernike	ImageJ

Multiple criteria

Runtime
Detect Rate
Accuracy
Params
GUI
PSF model
Learning?
Accessible
HD?

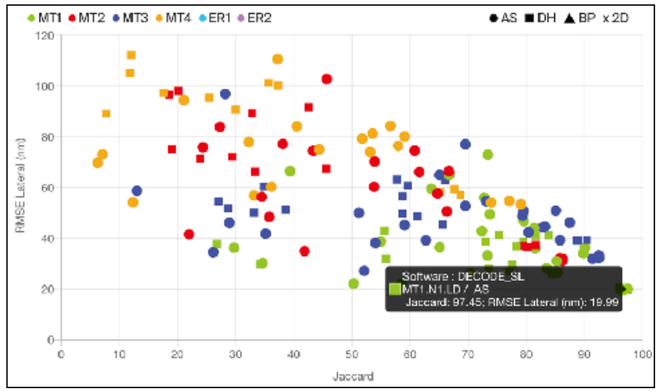
Sage et al. Quantitative evaluation of software packages for SMLM Nat Meth., 2015.
Sage et al. Super-resolution fight club: 2D & 3D SMLM software Nat Meth. 2019.
Li Real-time 3D SMLM using experimental point spread functions Nat Meth., 2018.

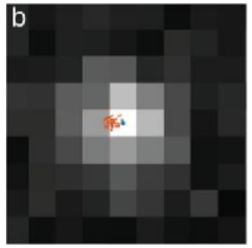
Quantitative assessment



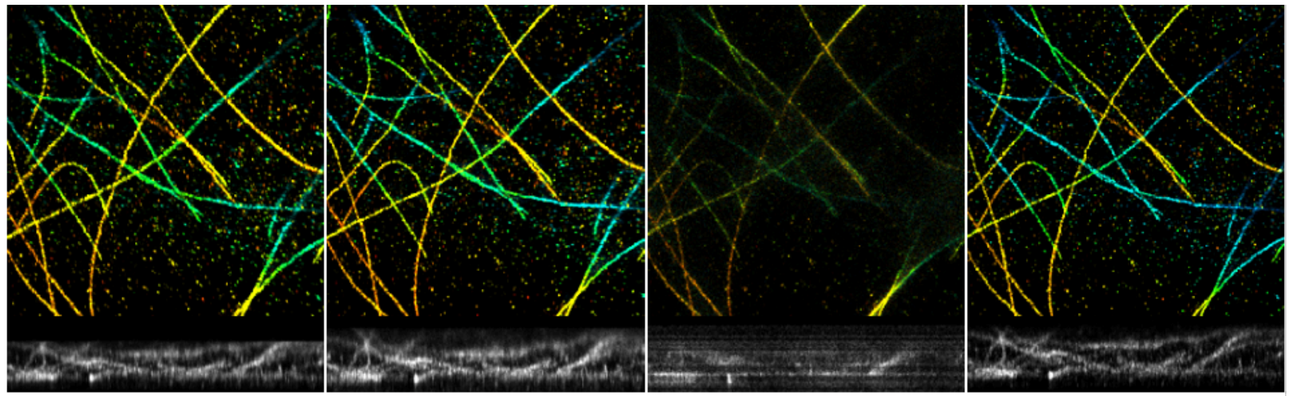
QuickPALM (centroid) | MIATools (single-emitter fit) | CSpline (multi-emitter fit) | DECODE (Learning)

Choose the appropriate software affects results





Qualitative assessment



WaveTracer | ThunderSTORM | QuickPALM | SMAP-2018

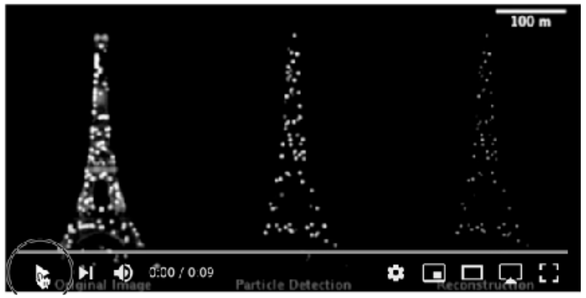
SMLM Software

QuickPALM

R. Henriques, Nat. Methods, 2010

The historically important tool for rapid reconstruction

- Method: centroid, fastest
- Popular: embedded in FIJI
- 2D and 3D (AS)
- On-line reconstruction



ThunderSTORM

M. Ovesný, Bioinformatics 30, 2014

A comprehensive ImageJ plugin for PALM and STORM data

- Easy-to-use on Fiji
- Extensive collection of methods
- 2D and 3D (AS, BP)
- Post-processing: drift, grouping

PSF fitting

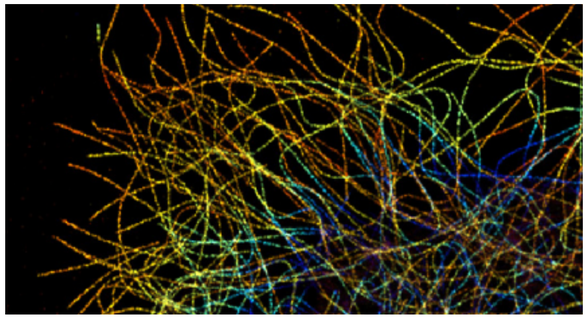
- Single emitter fitting: detection-localisation-rejection
- Multiple emitter fitting: optimal determination of the number of molecules (high-density)

SMAP

J. Ries, Nat. Methods 2018

Super-Resolution Microscopy Analysis Plateform

- Matlab based tools
- Extensive set of tools
- 2D and 3D
- Post-processing: drift, grouping



PSF model

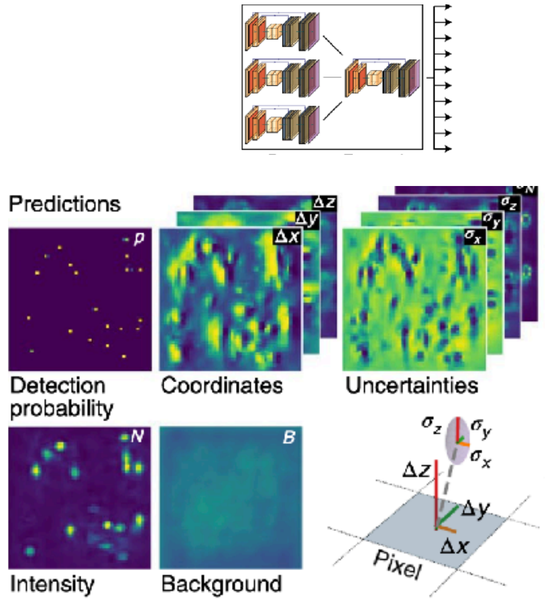
- Include the 3D fit of PSF fit3Dspline

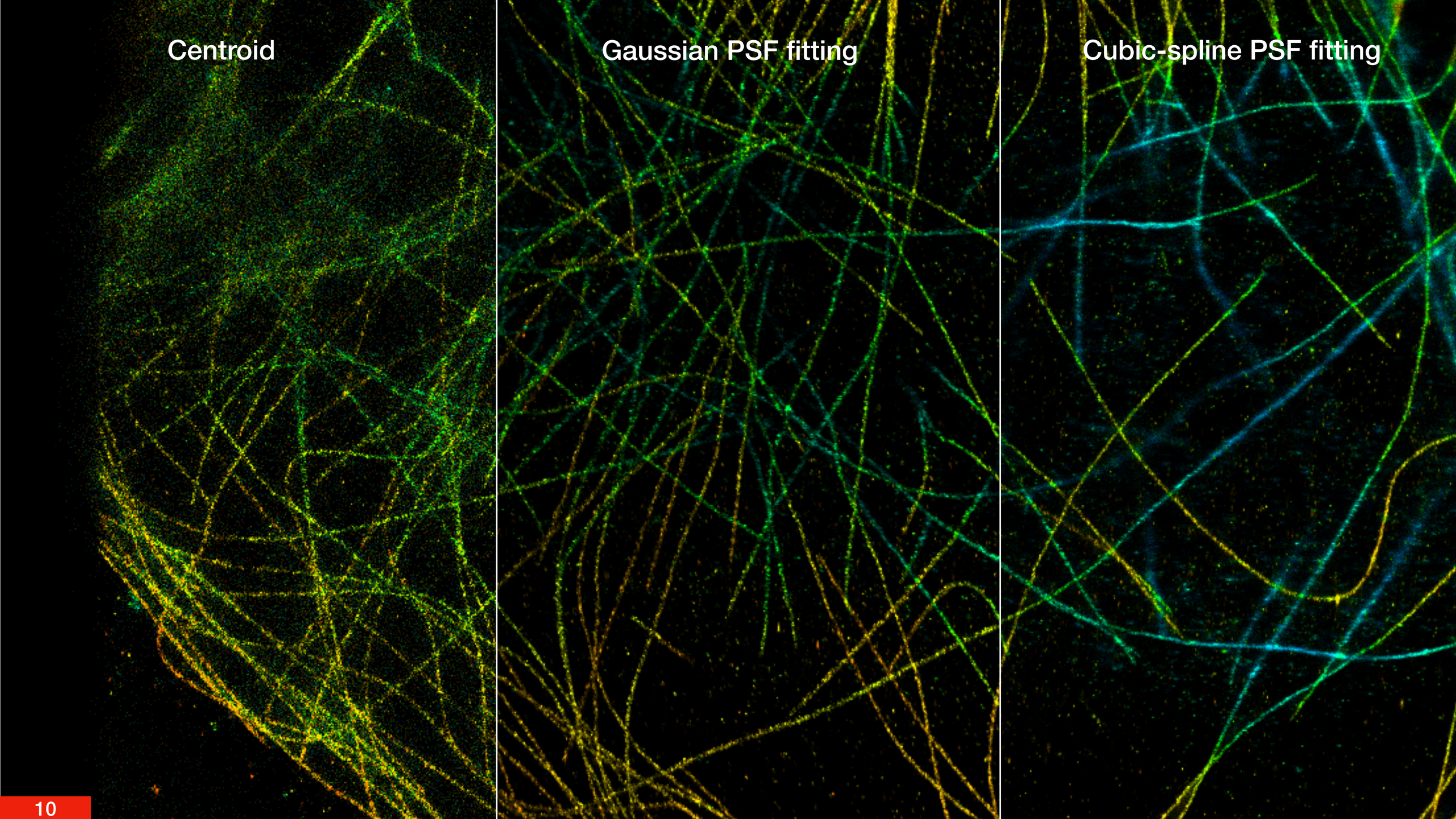
DECODE

A. Spieser, Nat. Methods 2021

Deep Learning Prediction 3D localizations

- 2D and 3D. Python-based
- Frame-to-frame and temporal Prediction
- Coordinates, N, uncertainties



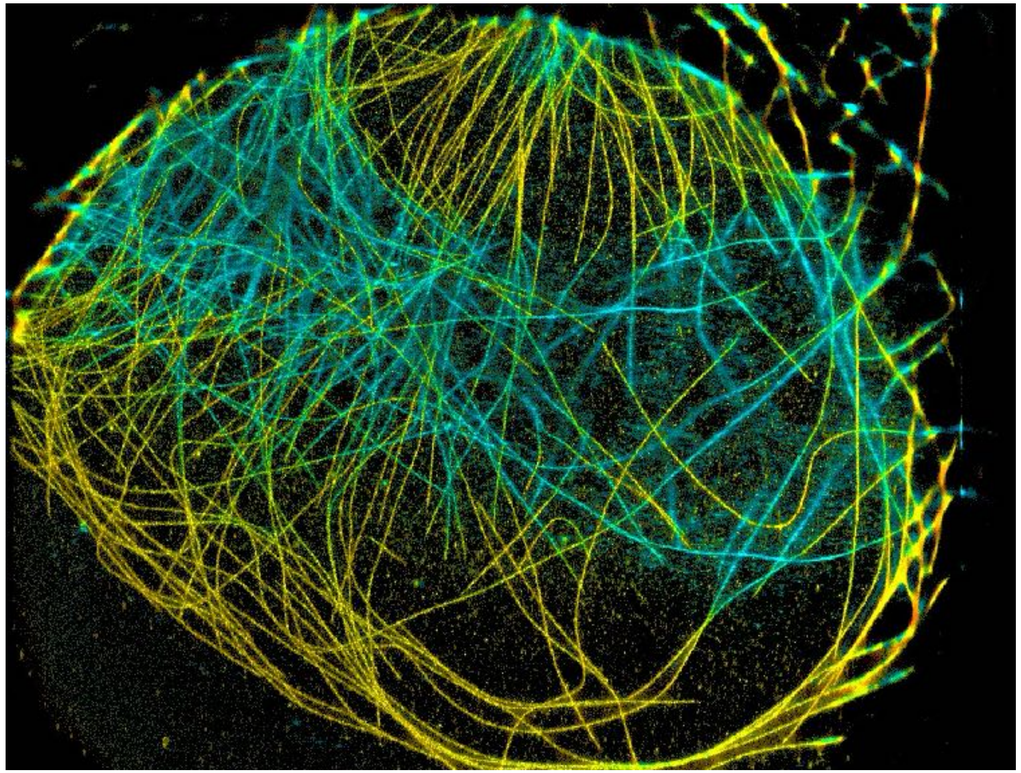

Centroid
Gaussian PSF fitting
Cubic-spline PSF fitting

Point Data Rendering

	A	B	C	D	E	F	G	H	I	J	K
1	id	frame	x [nm]	y [nm]	z [nm]	sigma1 [nm]	sigma2 [nm]	intensity [ph	offset [photc	bkgstd [photon]	uncertainty_xy [
2	1	1	5740.83434	1761.83022	-73.2513	184.16585	233.31909	658.49107	5.95959	5.88896	24.7608
3	2	2	5651.35715	1768.2754	52.98168	214.97157	192.69315	497.43546	8.74365	5.51125	29.31233
4	3	3	5688.11377	1784.34773	80.13173	224.48493	186.72842	776.76044	9.2315	6.25032	21.88203
5	4	4	5749.16986	1777.29827	124.06739	241.43594	179.19385	995.61486	5.54265	8.29197	22.70981
6	5	5	5702.50452	1763.49301	107.69916	234.91776	181.69333	537.04899	8.90223	6.2449	31.20853
7	6	6	5661.68966	1784.76124	97.03721	230.79755	183.51815	409.93424	6.92543	5.7242	36.97788
8	7	7	1767.17136	2364.39846	16.0143	203.44262	202.40569	1579.43826	5.91979	8.46239	14.48774
9	8	8	1763.85639	2371.3338	55.08563	215.67918	192.19542	1227.15187	7.91353	7.11094	15.92052
10	9	10	2602.27882	5117.00944	146.06186	250.52983	176.41348	963.39044	8.50348	6.25944	18.91056
11	10	10	5709.28437	1756.24961	153.70116	253.77066	175.60347	859.14402	7.41058	7.01649	23.20004

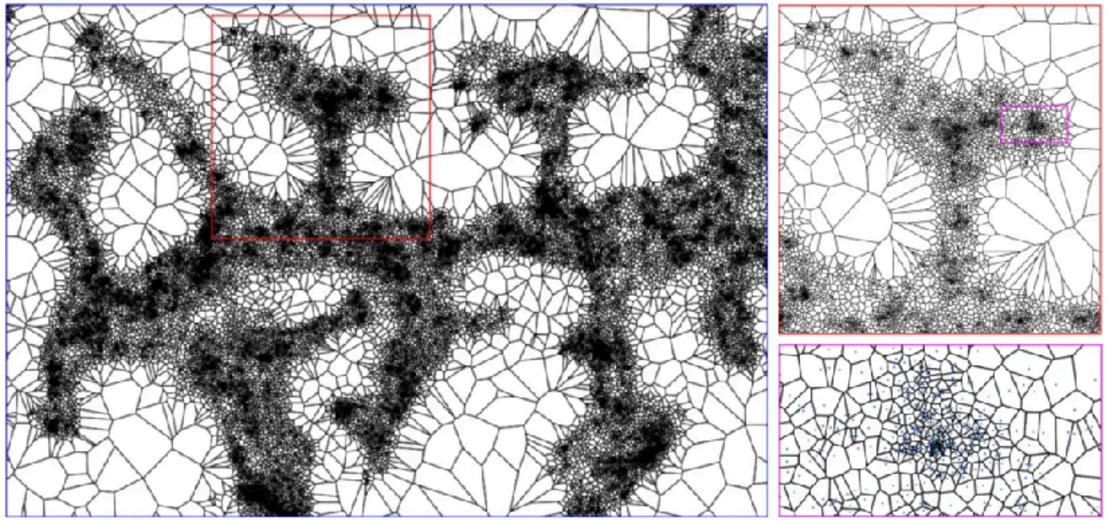
Frame XY or XYZ Nphotons Uncertainty

2’000’000 localizations 1.17Gb

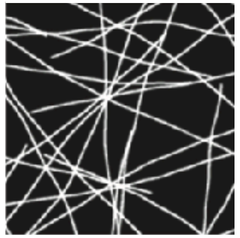


Colorize Voronoi

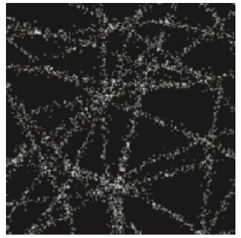
Levet et al., SR-Tesseler, 2015



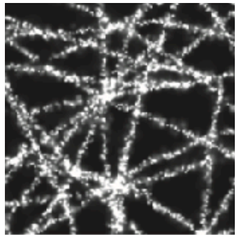
Ground-truth



Histogram binning



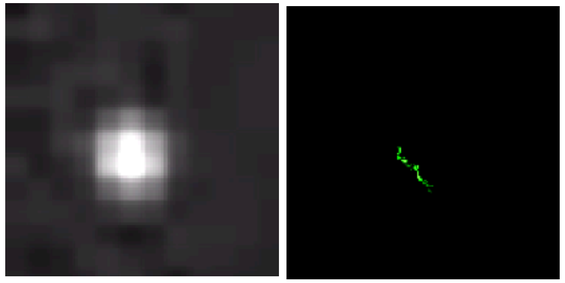
Gaussian rendering

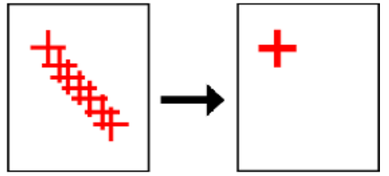


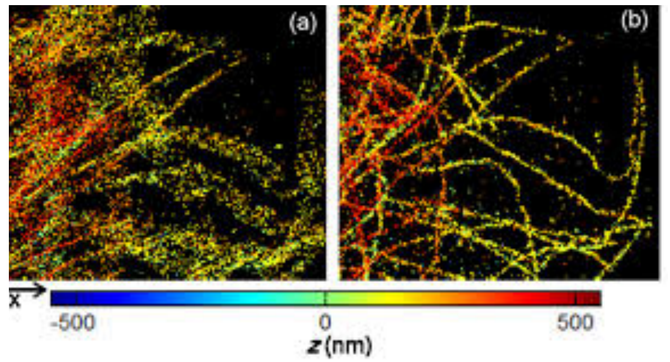
Point Data Processing

Drift Correction

- Tracking fiduciary markers
- Cross-Correlation of batch of localization frame
- Rendering-based registration



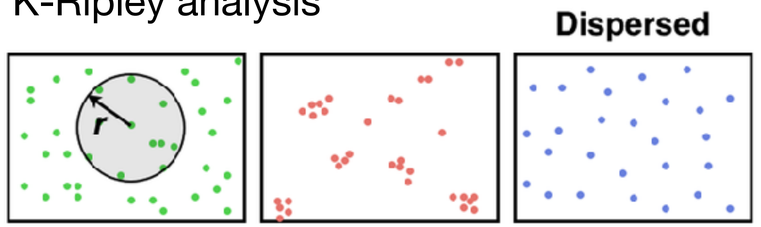


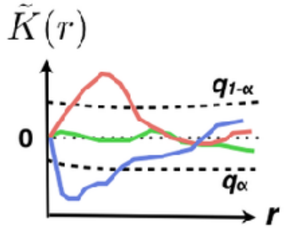


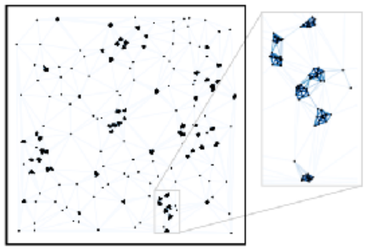
Denoising / Rejection

- Statistical distribution
- Rejection based on SNR
- Denoising isolated points

K-Ripley analysis



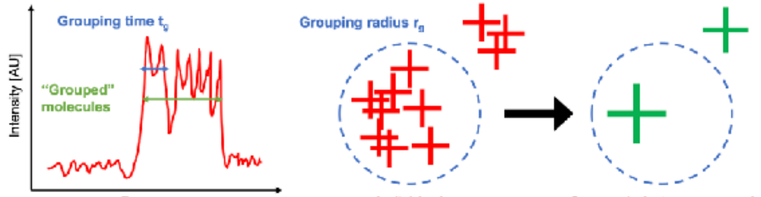


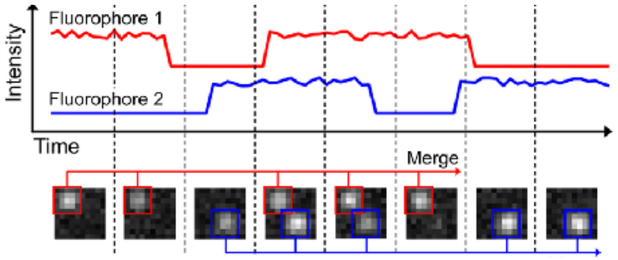


Graph Signal-Processing [Pourya 2021]

Temporal Grouping

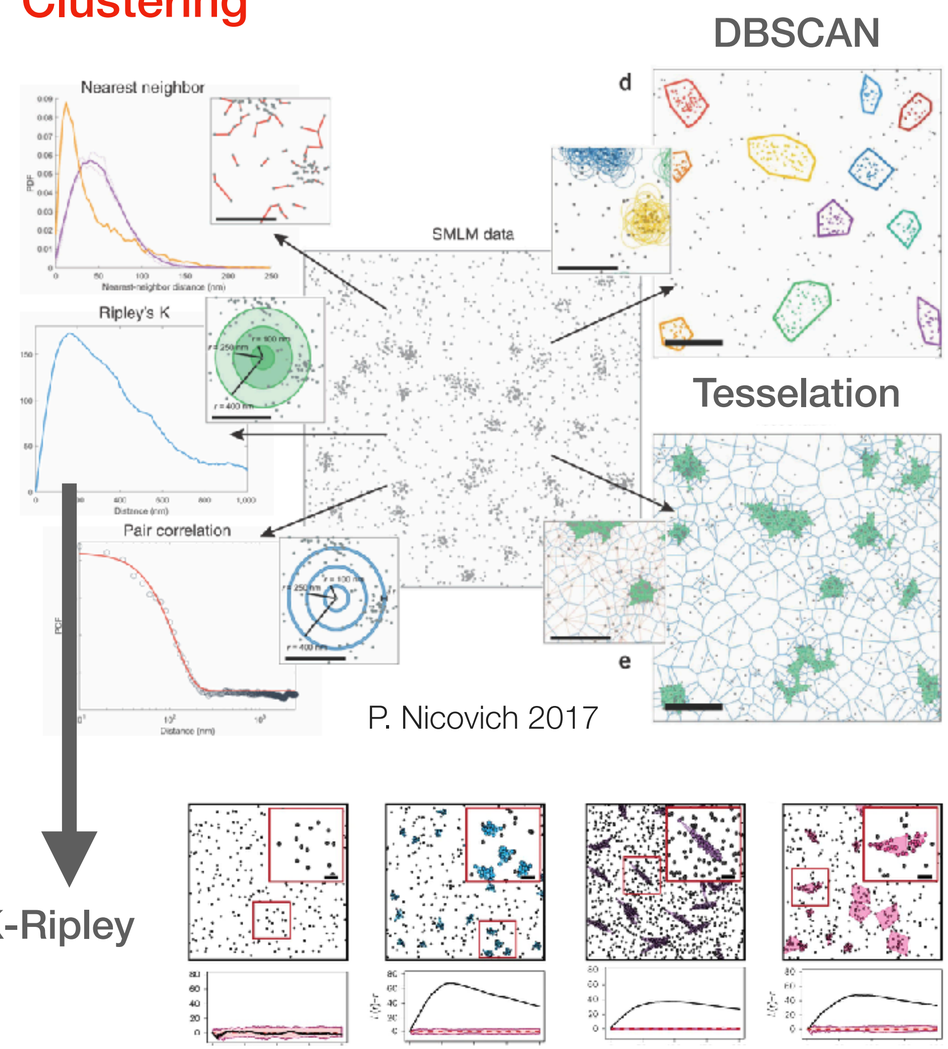
- Merge localizations in single molecule

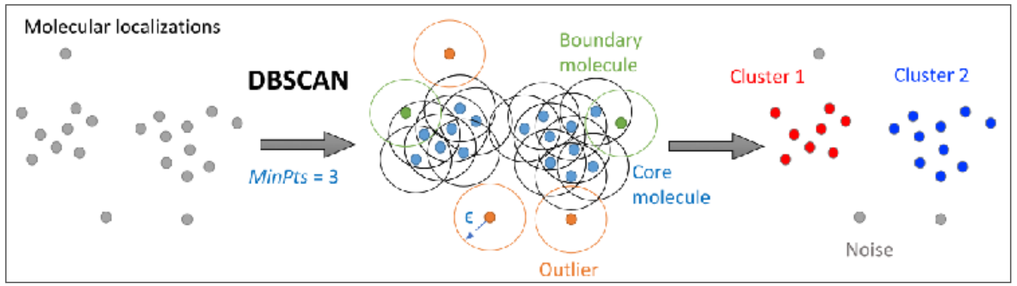


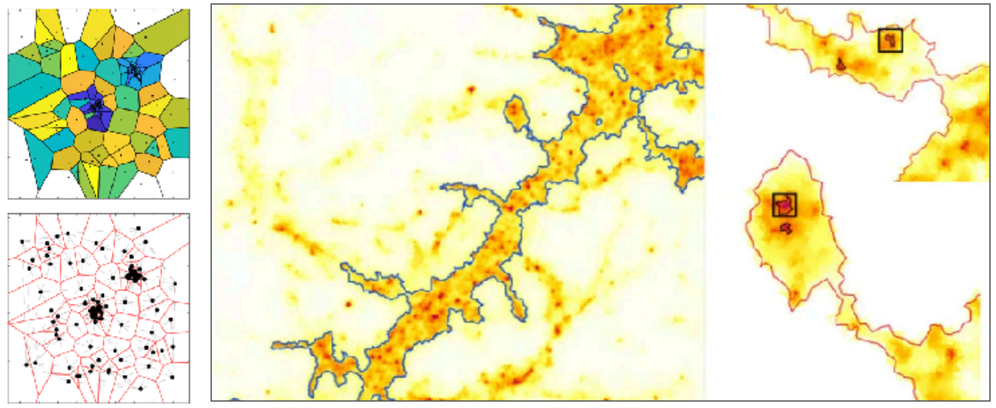


Point Data Analysis

Clustering







K-Ripley

Single-Particle (Molecule) Tracking

Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking

IMAGE ANALYSIS

PARTICLE TRACKING

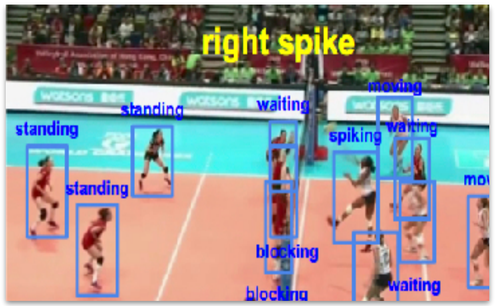
Computer Vision Image-Based Motion Analysis

Pixel-based Method

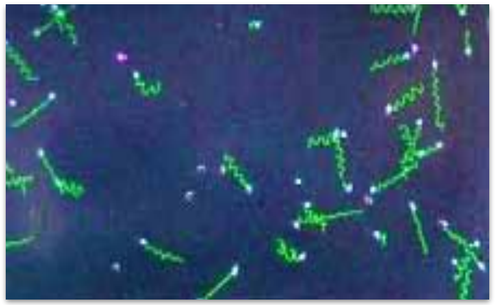
Optical Flow
Motion field estimation

Background Modeling
Video watching

Feature-based Method



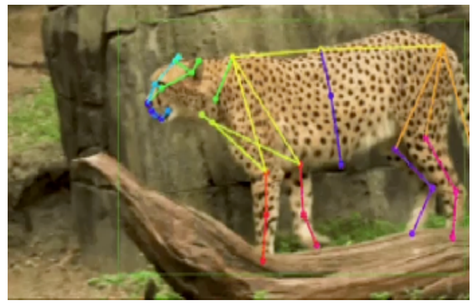
Real-time Tracking
Target prediction



Particle Tracking
Off-line, motion models

Spatio-temporal Inference

Multiple Object Tracking
Object detection



Pose Estimation
DeepLabCut [Mathis, 2018]

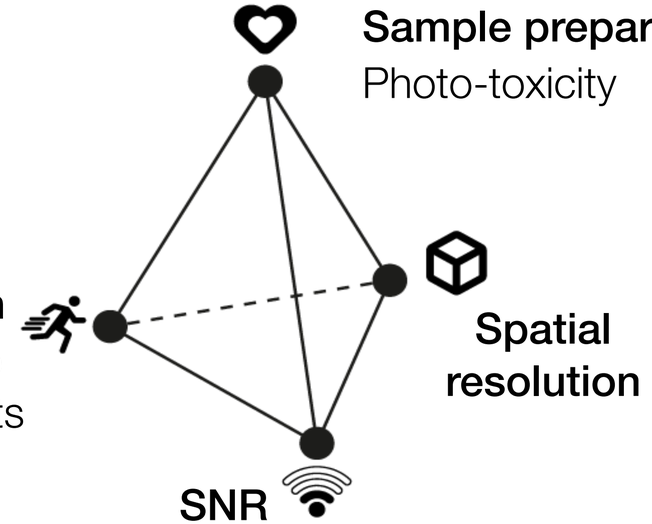
AI Foundation Models

SAM2 (Meta)
Video predictor

CoTracker (Meta)
Point tracking

Imaging Image-based Motion Capture

Time-lapse Acquisition



Sample preparation
Photo-toxicity

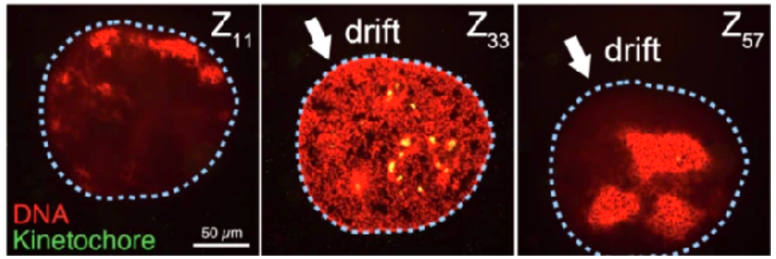
Temporal resolution
Frame rate
Slow events

Spatial resolution

SNR
Photo-bleaching

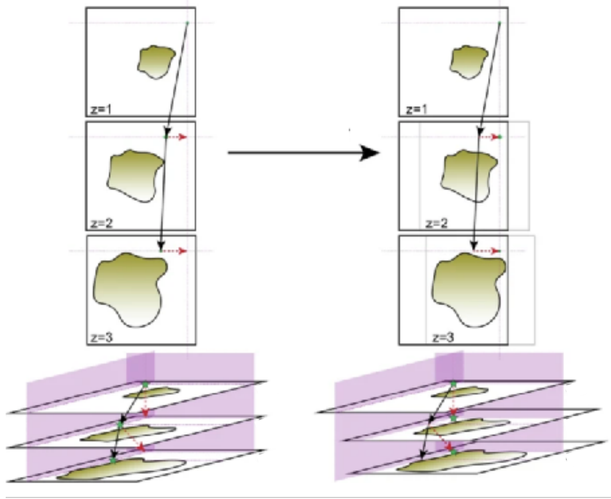
- 30 Hz → 33 ms
- 100 Hz → 10 ms
- 1000 Hz → 0.5 ms

Temporal Perturbation

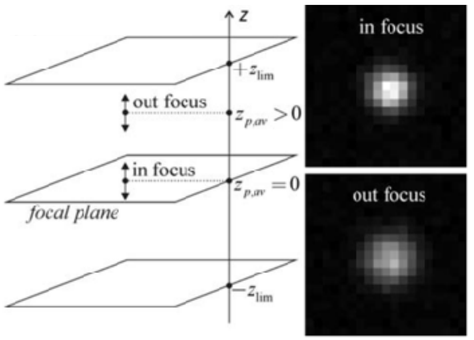


Live Cell Imaging
Composition of movements

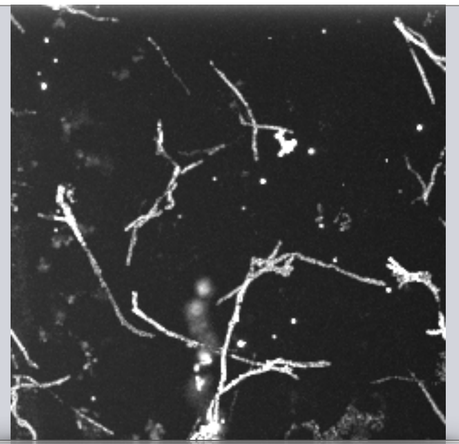
Drift



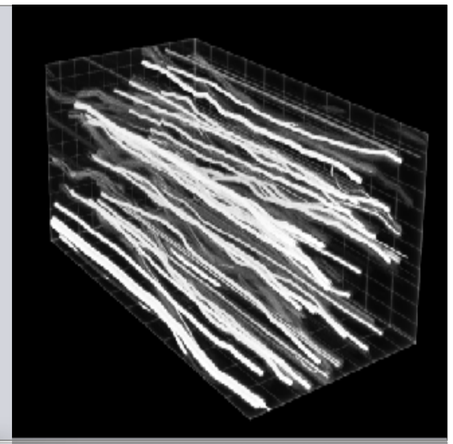
Axial: Out-of-focus
Lateral: Outside field of view



Seeing the Motion in Images



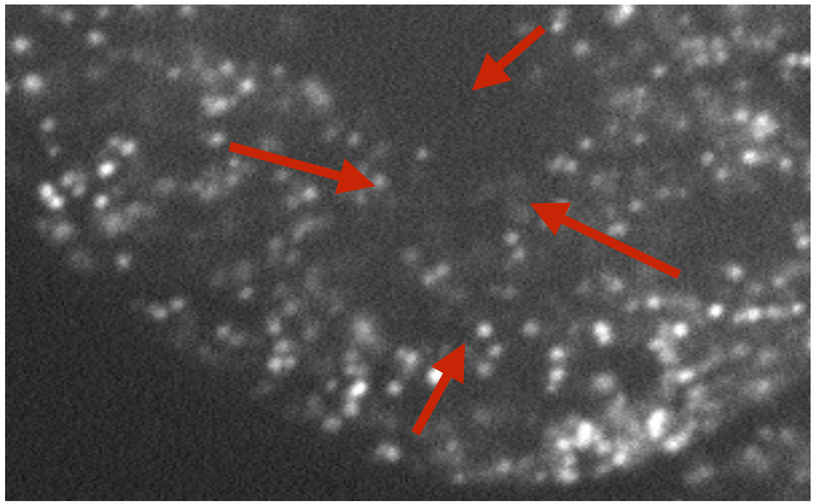
Temporal projection

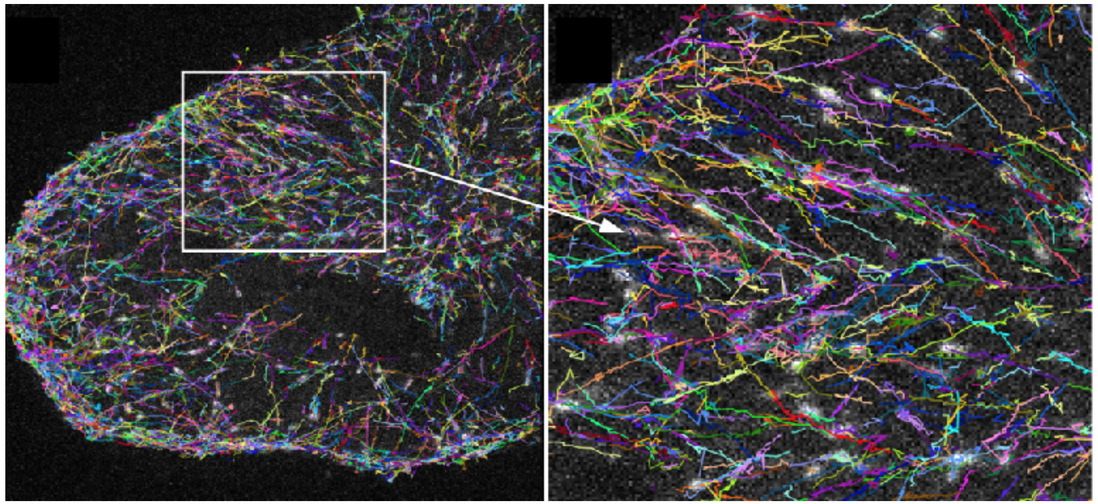


Spatio-temporal visualisation or segmentation

Stationary vs. mobile object

Particle Tracking





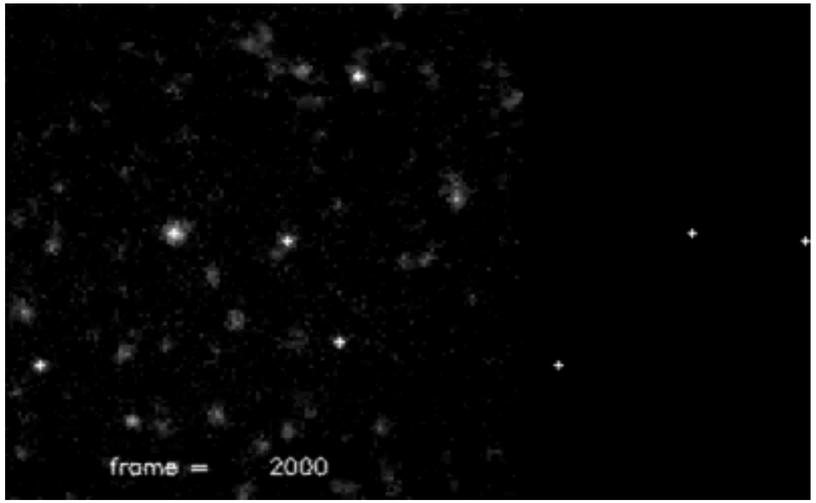
Motion model

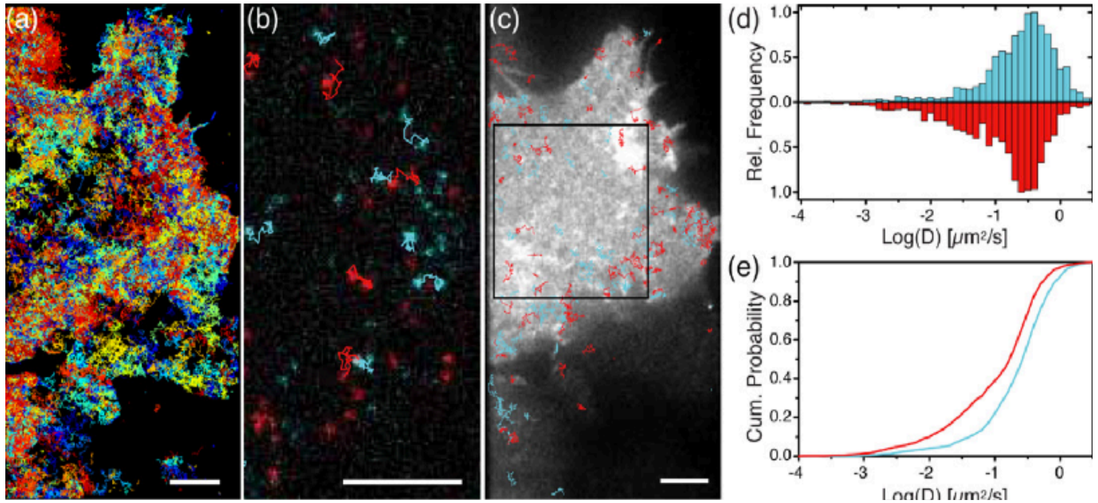
Event type

Ambiguities

Occlusion

Density





Dual color single-particle tracking in U2OS cells.
Scale bars are 5 μm.
Albrecht, Methods App Fluorescence, 2015.

Tracking System

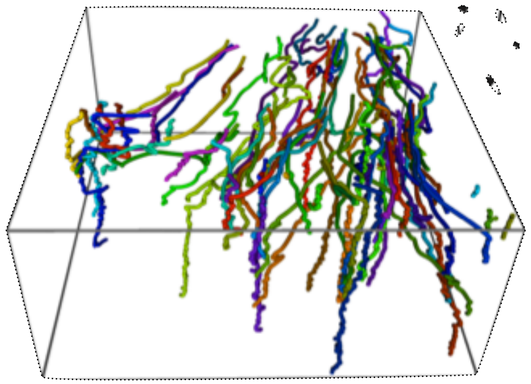
Preprocessing
- Denoising
- Enhancement
- Drift correction
- Photobleaching

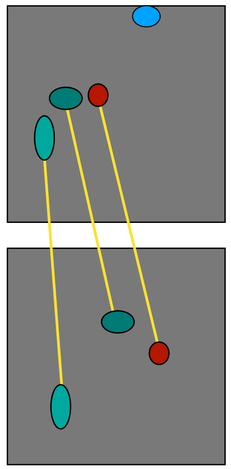
Detection
- Threshold
- Spot detector
- ML
- Fit PSF

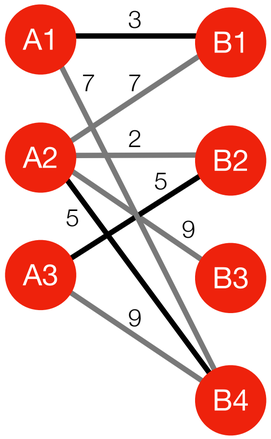
Linking
- Frame matching
- Bayesian fr.
- Spatio-temporal
- Learning

Postprocessing
- Visualization
- Quantification
- Statistics
- Lineage

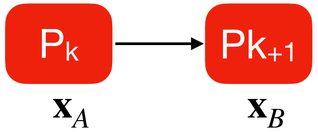
off-line tracking







Matching bipartite graph
- Simple Nearest-Neighborhood
- Global Nearest-Neighborhood
- Greedy
- Assignment problem (LAP)
- Hungarian Algorithm

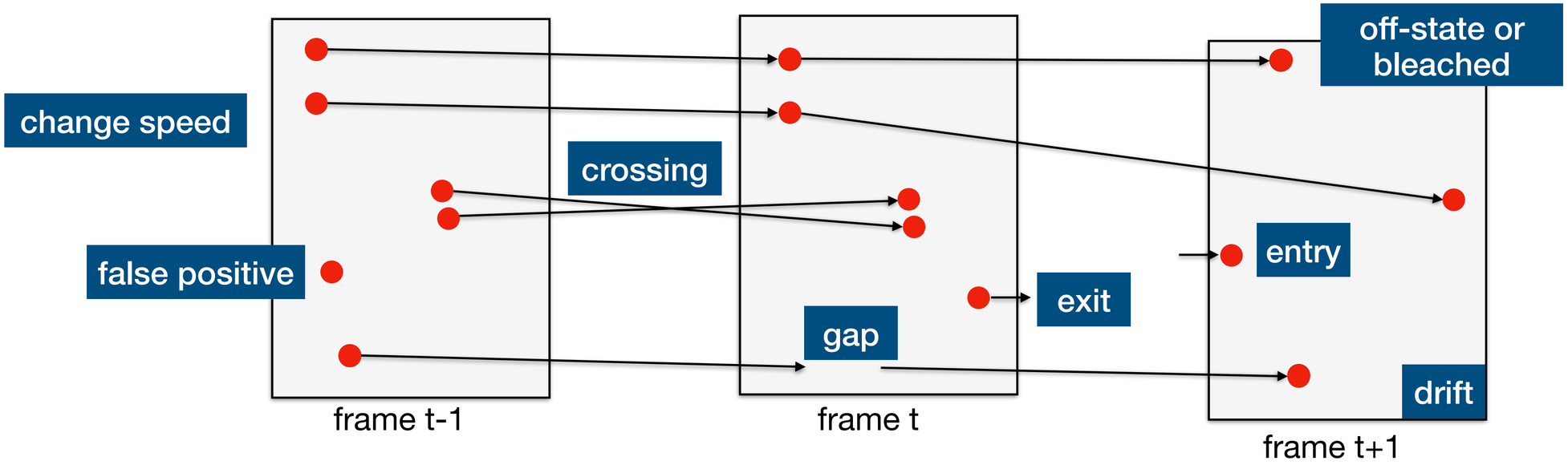


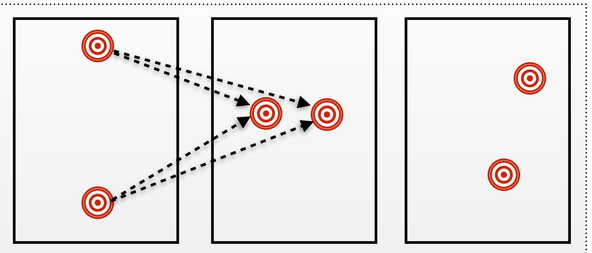
$$\xi(P_k, P_{k+1}) = \mathrm{dist}(P_k, P_{k-1})$$

Cost function: data term and regularization
- Hard constraint or soft penalization
- Fill the gap:

$$\xi = (1-\lambda_\theta)\frac{\mathrm{dist}(\mathbf{x}_A, \mathbf{x}_B)}{S} + \lambda_\theta \frac{|\langle \overrightarrow{\mathbf{px}_A}, \overrightarrow{\mathbf{x}_B} \rangle|}{\|\mathbf{x}_A - \mathbf{x}_B\|}$$

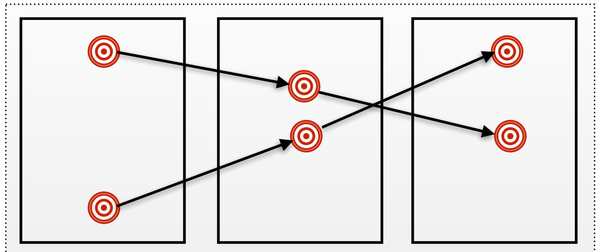
Frame-to-frame Association





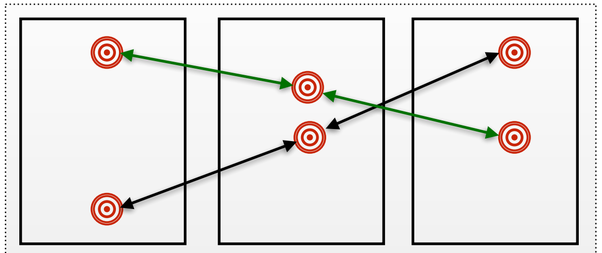
Multiple hypothesis test
➡ delay the decision

MHT [Chenouard, 2010]



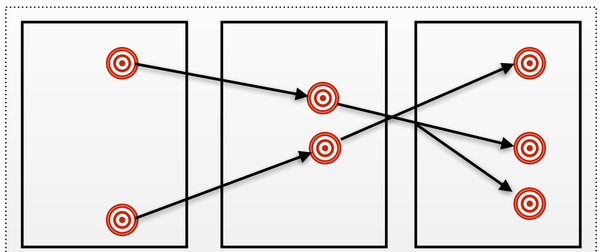
Link with n frames
➡ use future and **past**

For 1 spot [Sage, 2003]



2-steps - Coarse-to-fine
➡ find segments, merge

u-track [Jaqaman, 2008]

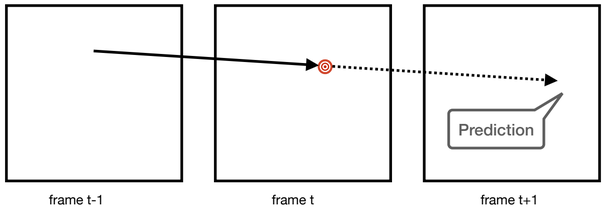


Graph modelling
➡ manage cell division

Particle Tracking Methods

Tracking-by-Prediction

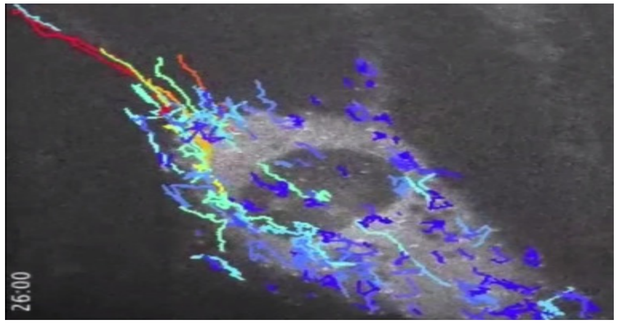
Kalman filter



Tracking-by-Segment

Coarse-to-fine tracking

u-Track [Jaqaman 2008]

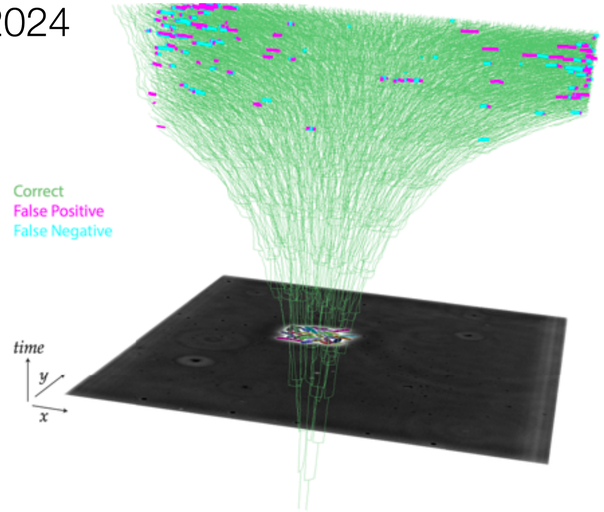


Tracking by Learning Association

TrackAstra B. Gallusser 2024

RNN Yao, 2020

CNN Detection
UNet Optical flow
K. Sugawara. eLife, 2022



- **ptPALM** S. Manley et al., High-density mapping of single-molecule trajectories with PALM, Nat. Methods, 2008.
- **u-Track** K. Jaqaman et al. Robust single-particle tracking in live-cell time-lapse sequences, Nat. Methods 2008.
- **MTT** A. Sergé et al., Dynamic multiple-target tracing to probe spatiotemporal cartography, Nat. Methods 2008.
- **MAT** M.R. Winter et al., Axonal transport analysis using Multitemporal Association Tracking, J. Comput. Biology, 2012.
- **TrackMate** J.-Y. Tinevez et al.,TrackMate: An open and extensible platform for single-particle tracking, Methods, 2017.
- **Diatrack** P. Vallotton et al., Diatrack particle tracking software:, Traffic 18 2017.
- **swift** B. Turkowyd et al., Establishing Live-Cell SMLMImaging and Single-Particle Front. Microbiol., 2020.

Detection **Linking**

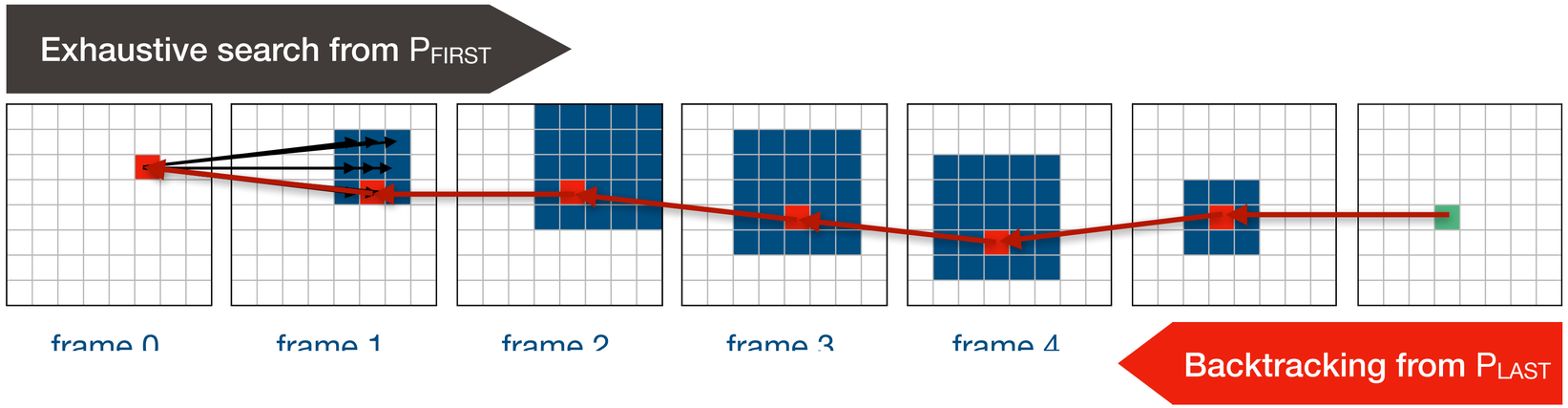
Authors	Prefilter	Approaches	Remarks	Principle	Approaches	Remarks
E.F. Shalzarini Y. Gong J. Cardinale	-	M, C	Iterative intensity-weighted centroid calculation	Combinatorial optimization	MF, MT, GC	Greedy hill-climbing optimizati with topological constraints
C. Carthel S. Coraluppi	Disk	M, T	Adaptive local-maxima selection	Multiple hypothesis tracking	MF, MT, MM	Motion models are user specifie (near-constant position and/ velocity)
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 specifie (near-constant position and/ velocity)
M. Winter A.R. Cohen W.J. Godinez K. Rohr Y. Kalaidzidis	Gaussian, median and morphology Laplacian of Gaussian or Gaussian fitting Windowed floating mean background subtraction	M, T, C M, T, F, C T, F	Adaptive Otsu thresholding Either thresholding + centroid or maxima + Gaussian fitting Lorentzian function fitting to structures above noise level	Multitemporal association tracking Kalman filtering + probabilistic data association Dynamic programming	MF, MT, GC MF, MM MF, GC	Post-tracking refinement of detections Interacting multiple models usi motion models as specified Track assignment by the weight sum of multiple features
L. Liang J. Duncan	Laplacian of Gaussian	M, T, F	Gaussian mixture model fitting	Multiple hypothesis tracking	MF, MM	Interacting multiple models wit forward and backward linking

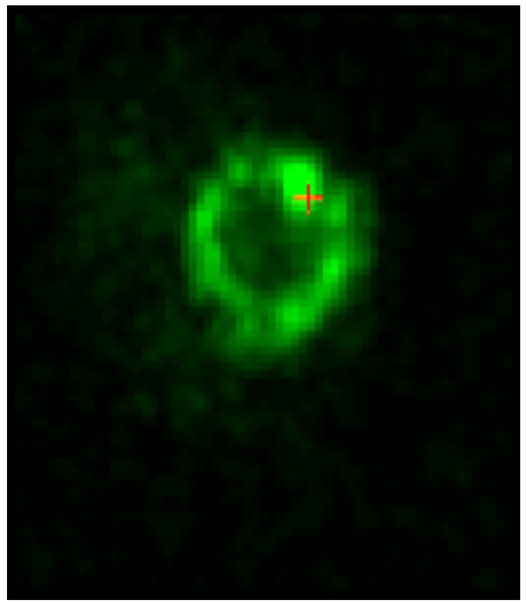
Challenge

Chenouard & al. Nat. Meth., 2014

Tracking by Trajectory Reconstruction

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





D. Sage et al., Automatic Tracking of Individual Fluorescence Particles, IEEE TIP, 2005.

Dynamic Programming - Viterbi

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

data term regularization

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

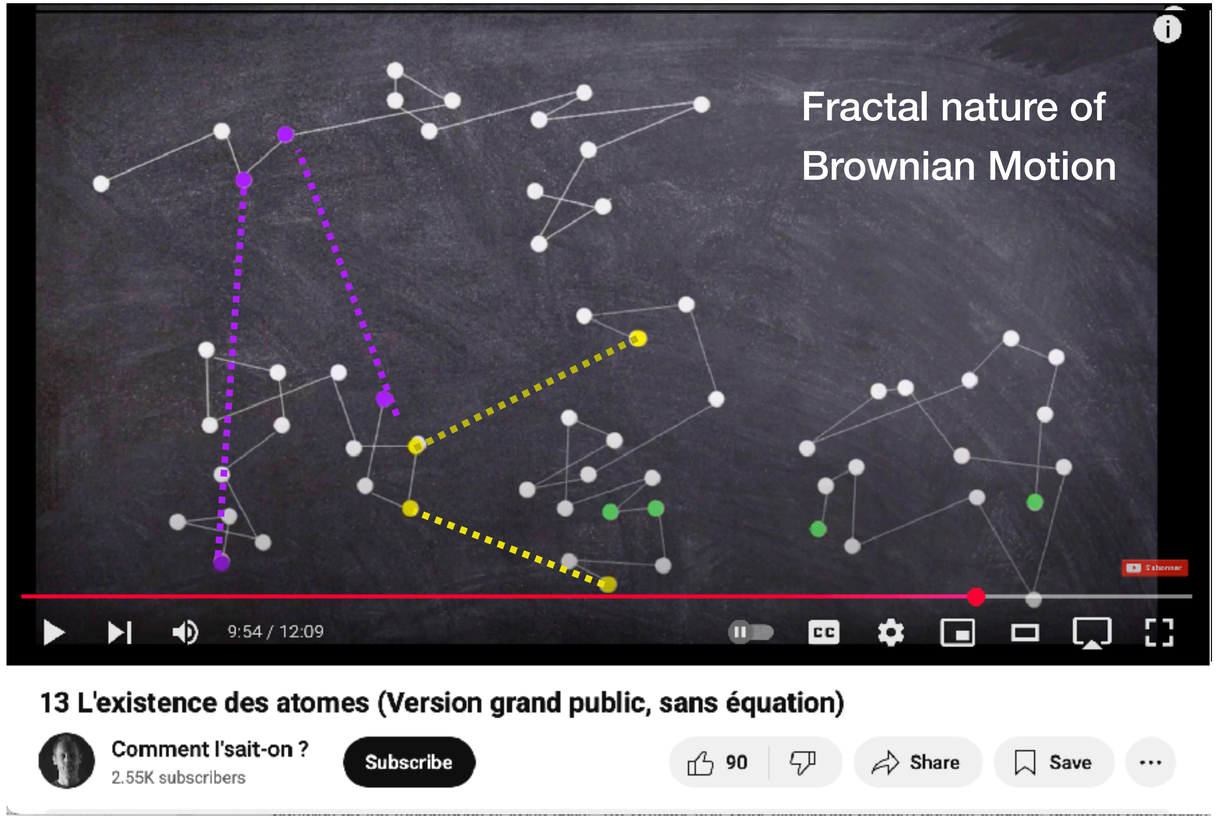
Example of a cost function from $\mathbf{u}_t$ position to $\mathbf{v}_{t+1}$

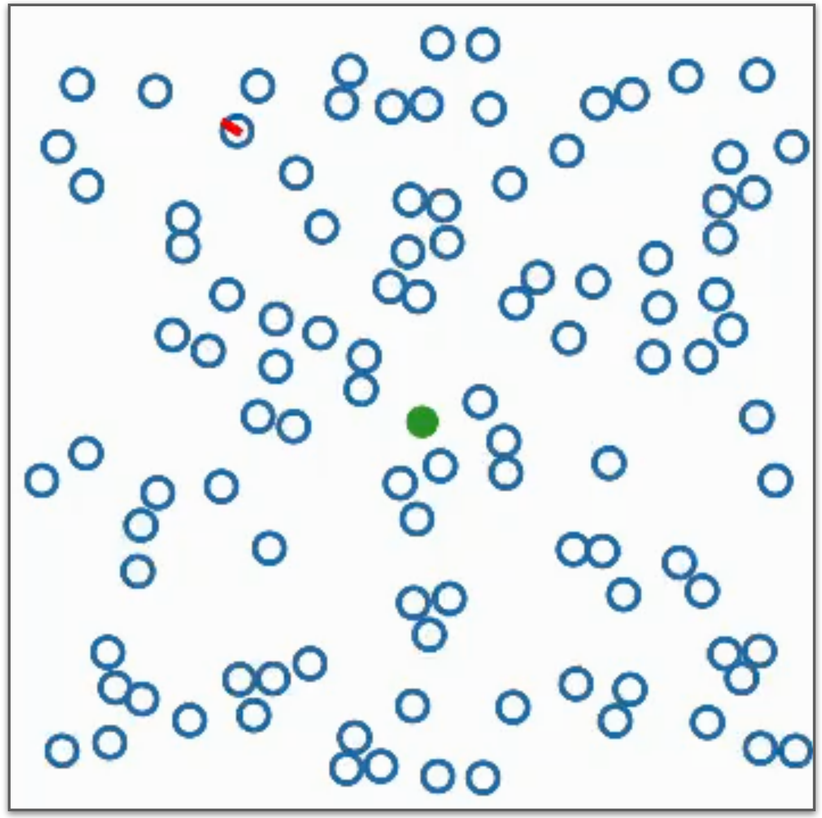
Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking

BIOPHYSICS

DIFFUSION MODELS

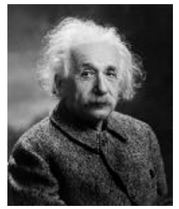
Brownian Diffusion





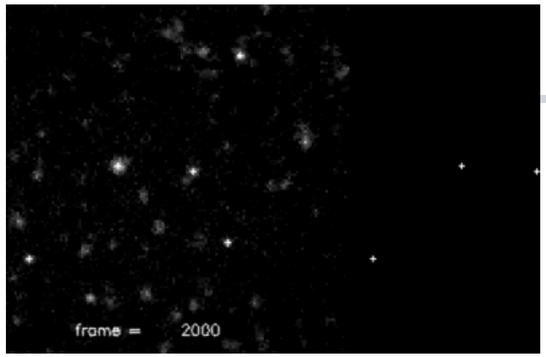
Robert Brown
1827
Brownian Motion

Jean Perrin
Nobel Prize 1926
Les atomes



Einstein (1905)
used Brownian motion to provide evidence for the existence of atoms.

Single Molecular Tracking

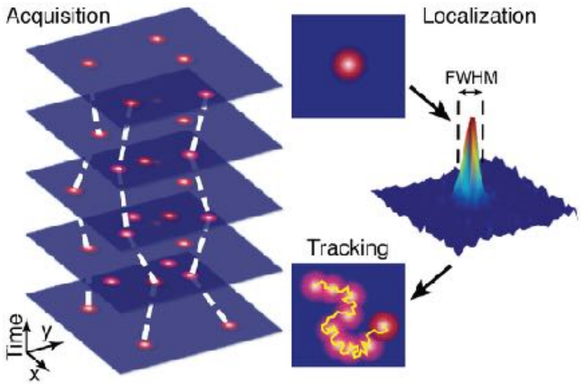


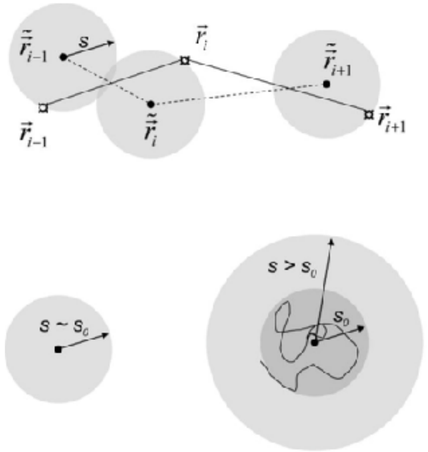
Spot Detection → Particle Tracking → Trajectory Analysis → Statistical Descriptor

Coordinates

Integer Coordinates → Sub-pixel Localisation → Sub-pixel positions Uncertainty

Mean-square Displacement





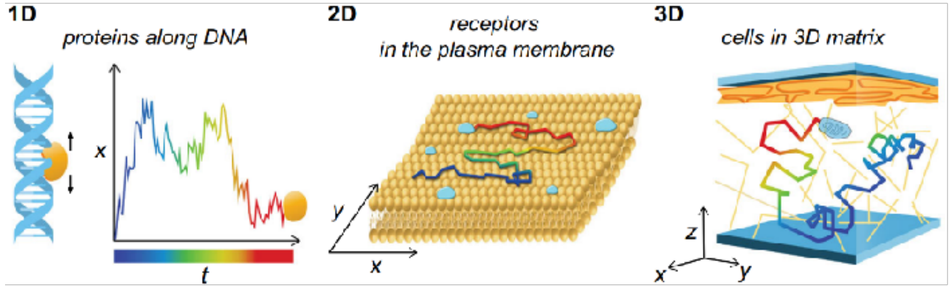


Figure of ANDI, Gorka Muñoz-Gil

Diffusion coefficient in the presence of localization uncertainty? [Michalet, 2010]

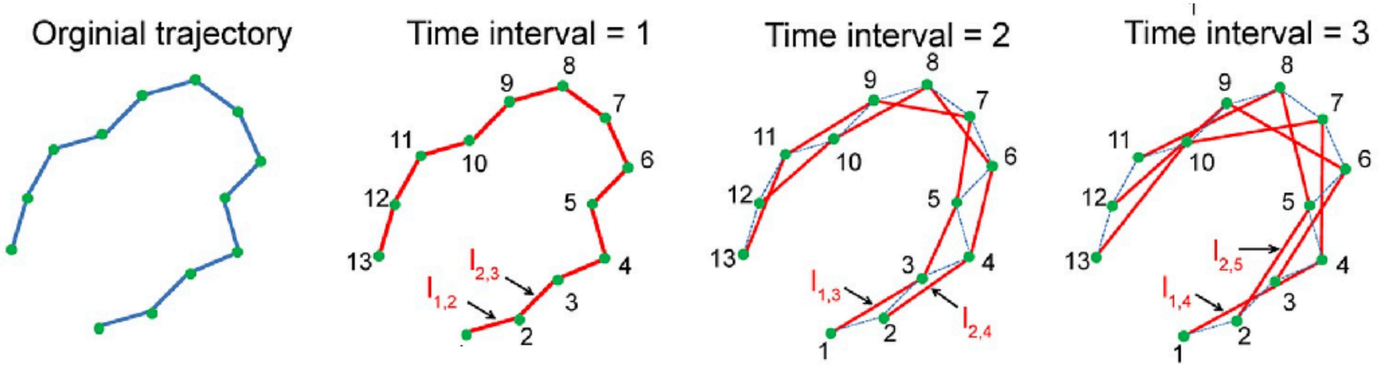
Mean Square Displacement

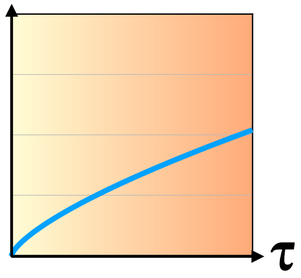
MSD: The core tools to analyze dynamics

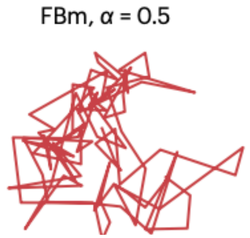
- Deviation of the position to a reference
- Characterization random motion in biology
- Estimation of the spreading solely to diffusion

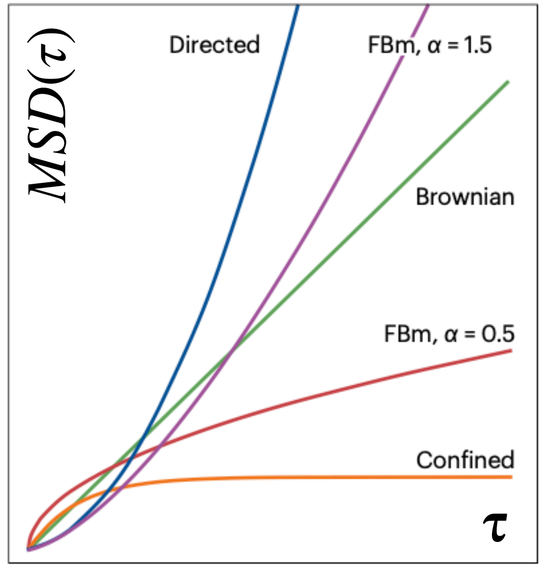
$$\mathbf{x} = (x, y)$$

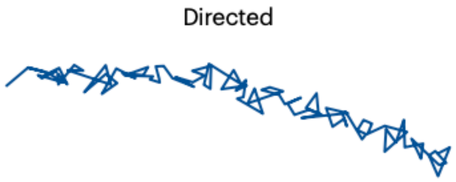
$$MSD(\tau) = \frac{1}{N-\tau}\sum_{t=0}^{N-\tau} |\,\mathbf{x}(t) - \mathbf{x}(t+\tau)\,|^2$$

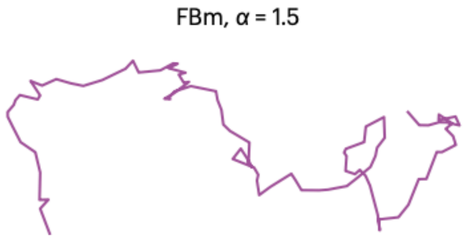










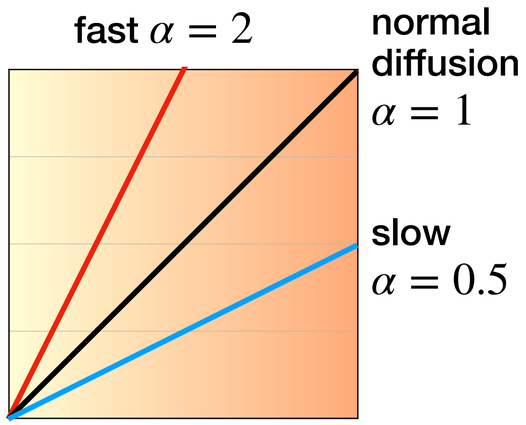


Diffusion Models

Normal Diffusion

$$MSD(\tau) \propto 2^d D \tau$$

- ✓ Trajectory: Brownian motion
- ✓ D constant in space and time
- ✓ Homogeneous medium
- ✓ Memory effect: Memoryless
- ✓ Self-similaritiy → fractal
- ✓ Ergodicity



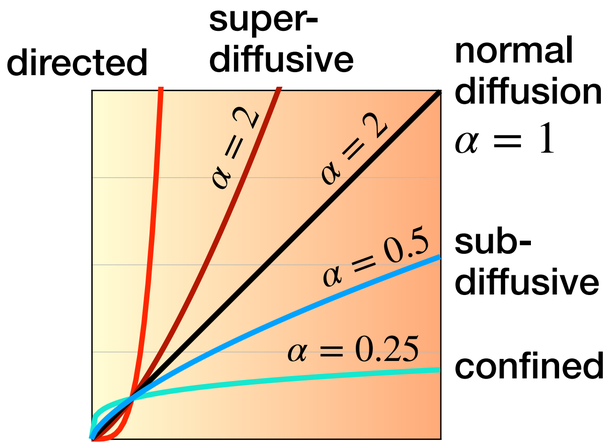
Anomalous Diffusion

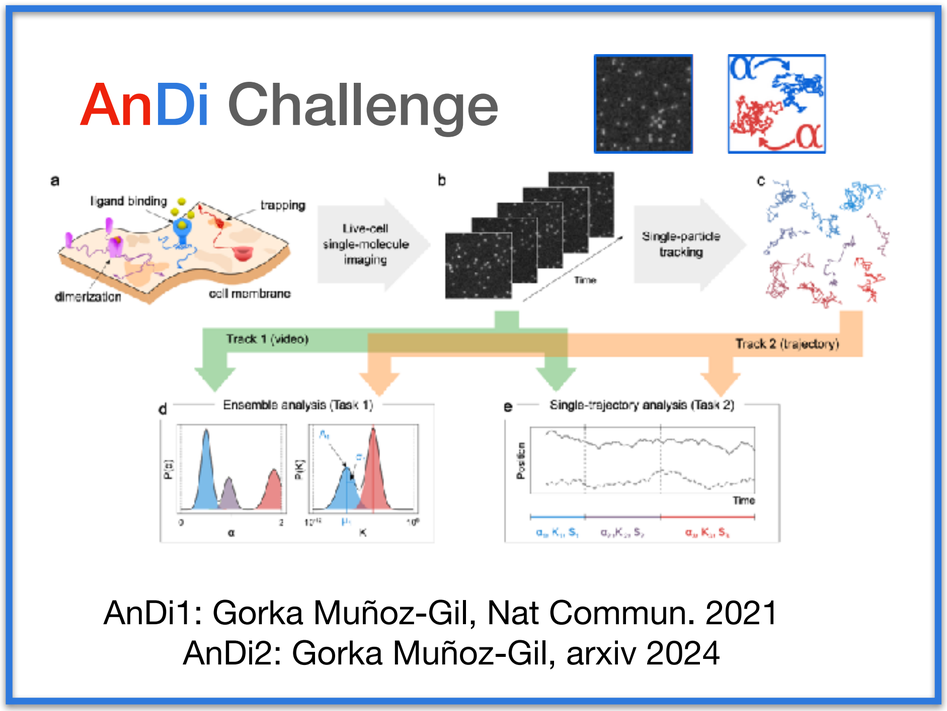
$$MSD(\tau) \propto 2^d \tau^\alpha$$

Fractional Brownian motion (fBM)

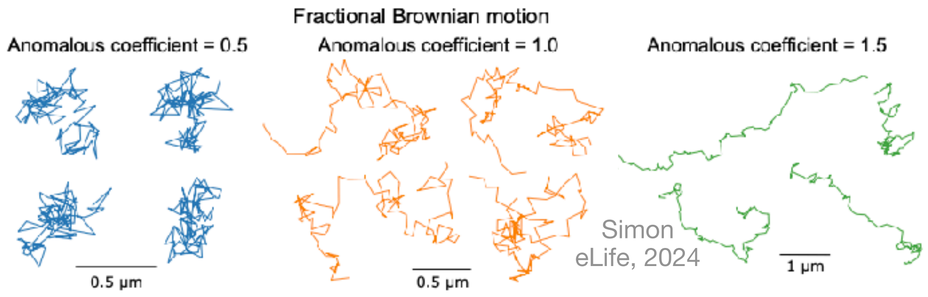
$$MSD(\tau) \propto 2^d \tau^{2H}$$

Hurst coefficient $H = \frac{\alpha}{2}$





AnDi1: Gorka Muñoz-Gil, Nat Commun. 2021
AnDi2: Gorka Muñoz-Gil, arxiv 2024

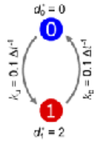


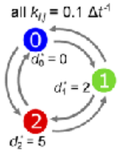
Simon
eLife, 2024

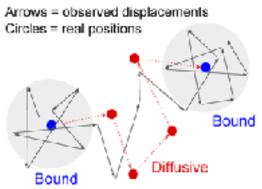
Non-homogenous Diffusion

Time-varying diffusion

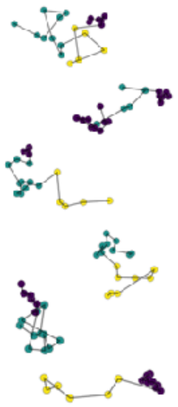
- Two-state or multiple mobility states
- Binding–unbinding interactions
- Switching to transient states
- Time-varying diffusivity
- Mechanical stress
- Non-ergodicity

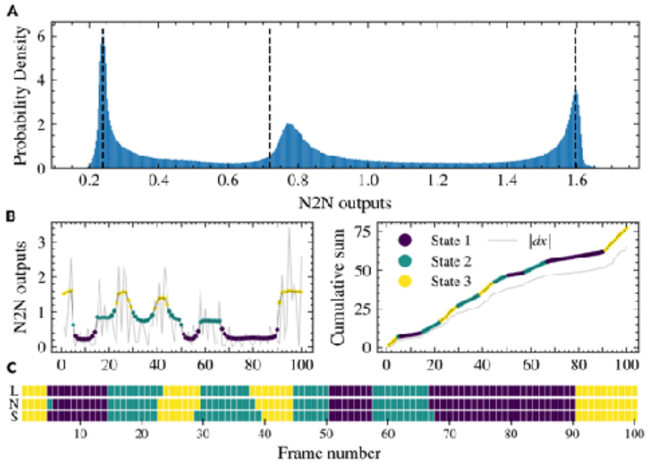






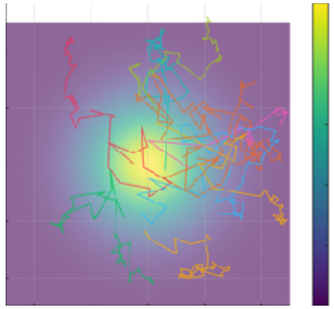
F. Simon, ExTrack, JCB, 2023

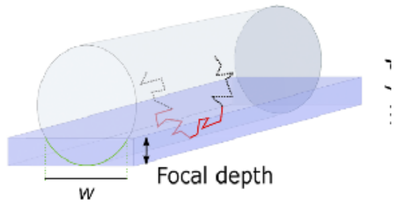




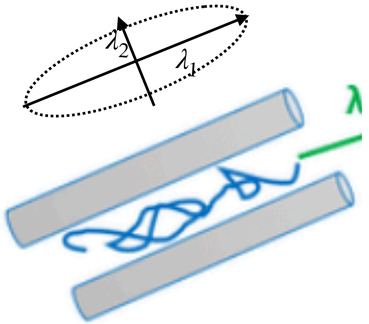
Spatial-varying diffusion

- Non-homogenous medium
- Confined environment
- Axial — z-dependency
- Cytoskeletal barriers
- Trapped trajectory
- Dimerization





Anisotropic diffusion



$$\mathrm{MSD}_{xx}(\tau) \approx 4D_{xx}\tau$$

$$\mathrm{MSD}_{yy}(\tau) \approx 4D_{yy}\tau$$

$$\mathrm{MSD}_{xy}(\tau) \approx 4D_{xy}\tau$$

$$\text{Tensor} = \begin{bmatrix} D_{xx} & D_{xy} \\ D_{xy} & D_{yy} \end{bmatrix}$$

Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking

ALGORITHMS

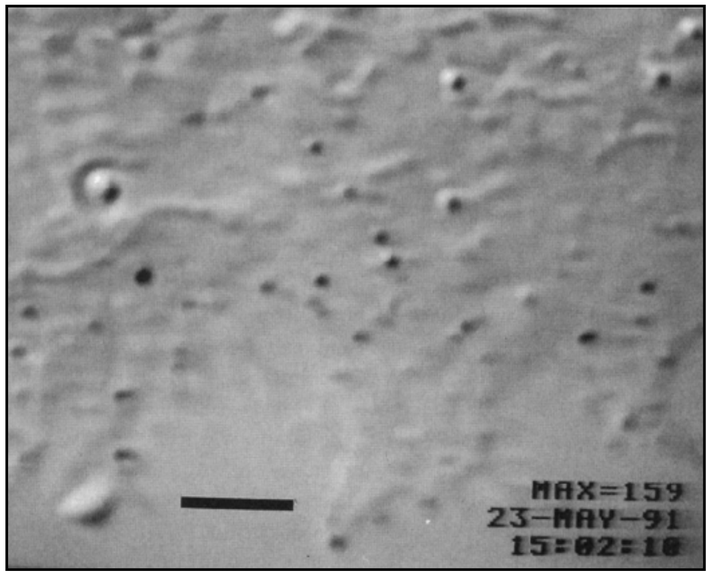
ESTIMATING FFUSION

Single-Molecule Tracking

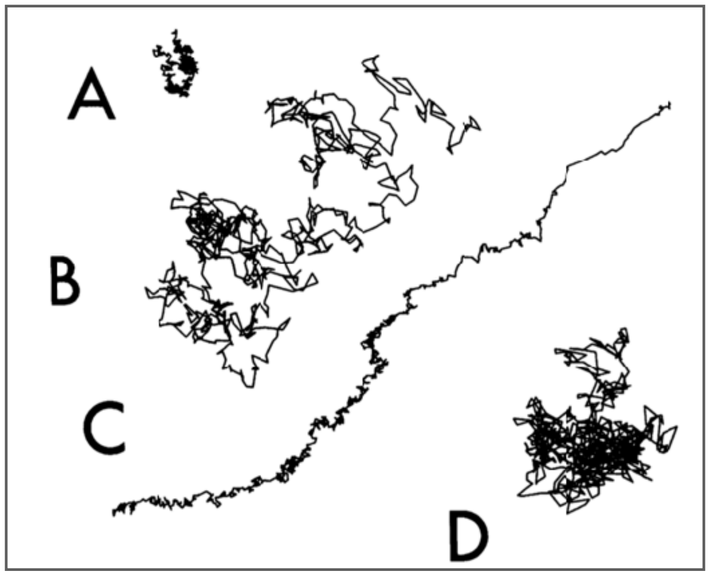
The pioneer : Akihito Kusumi

A Kusumi, Y Sako, M Yamamoto, Confined lateral diffusion of membrane receptors as studied by SPT - Effects of calcium-induced differentiation in cultured epithelial cells, 1993.

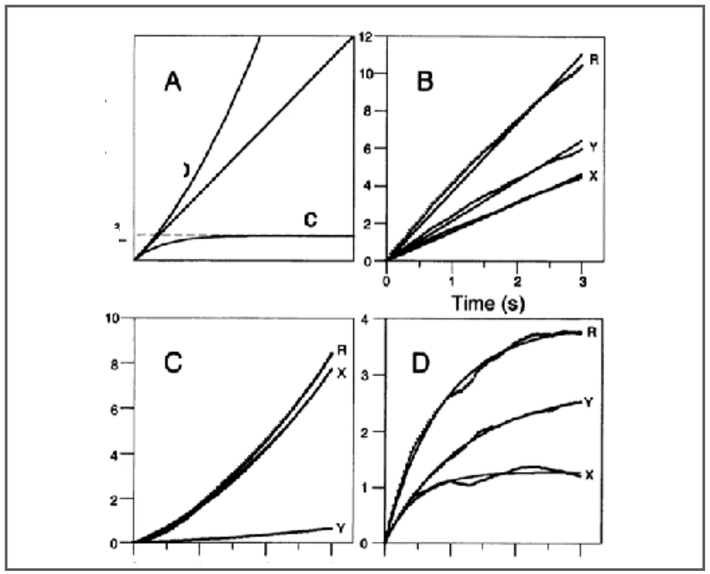
Single Molecule Tracking (SMT) provides unprecedented ability to directly observe molecular behaviors and interactions in living cells [Kusumi, 2024]



A video-enhanced DIC of cultured mouse keratinocyte labeled with gold particles. bar 3 um



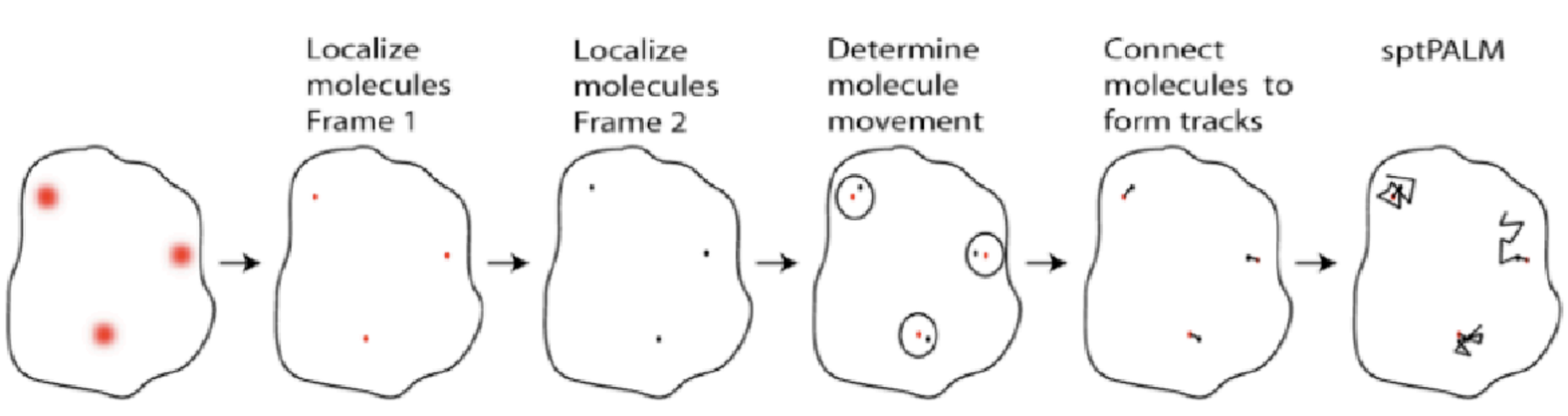
Examples of recorded trajectory, after detection and tracking.



Classification by analyzing the MSD

Single Particle Tracking in SMLM

sptPALM

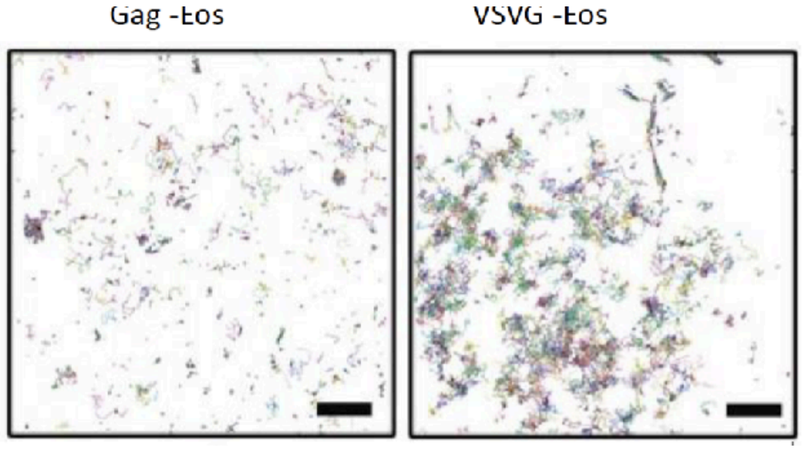


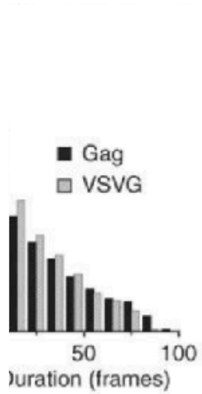
[Manley Nat. Meth. 2008]

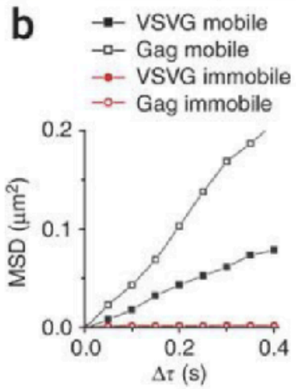
average diffusion coefficient

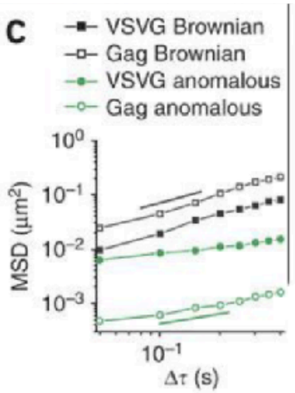
D_m(Gag-Eos) =0.11 ±0.08μ²/s,

D_m(VSVG-Eos) = 0.14±0.11 m²

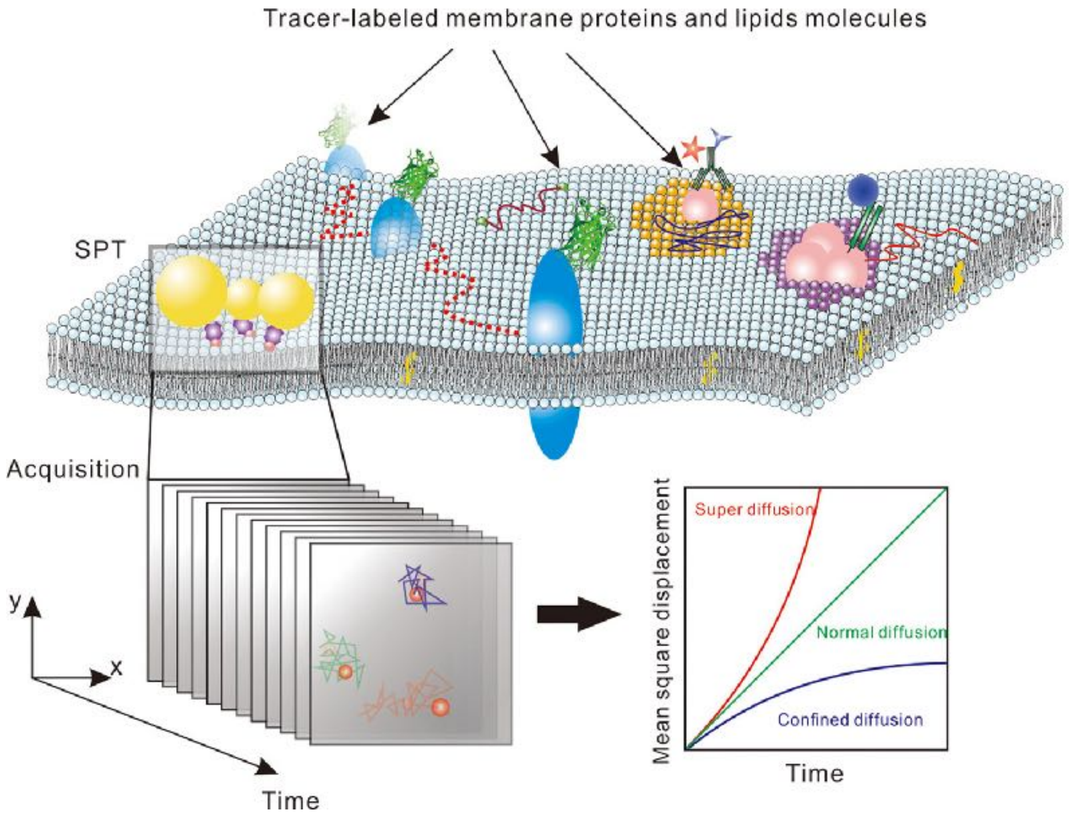


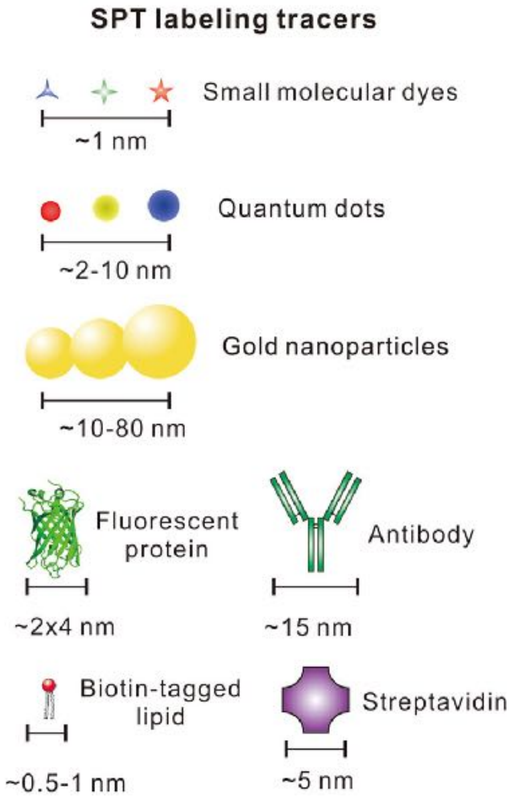






Is Seeing Always Believing?





Potential limitations and pitfalls

- Large probes affects hydrodynamic
- Steric effect in crowded environments
- Cross-link artificially reduce diffusion

Probes affect the dynamics

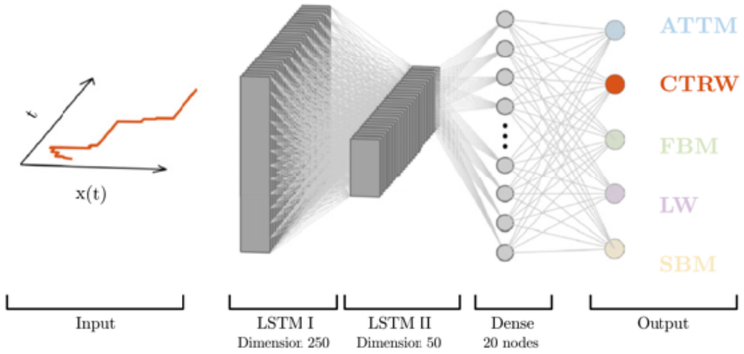
- Molecule-based: organic fluorescent dyes of fluorescence protein
- Particle-based: Quantum dots of gold nanoparticles

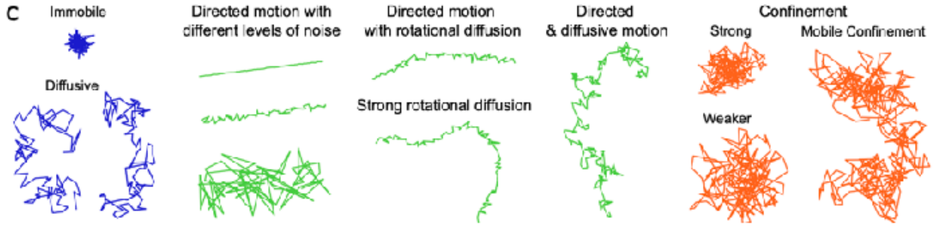
Yanqi Yu, Miao Li, Yan Yu, , Tracking Single Molecules in Biomembranes, ACS Nano 2019.

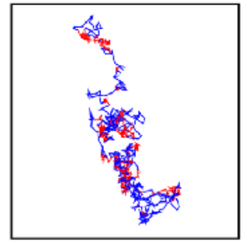
Analysis of Motility

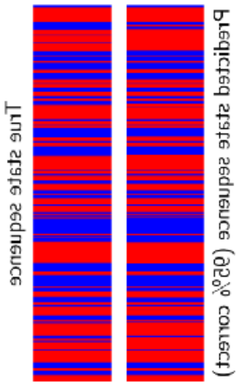
Classification of Trajectory

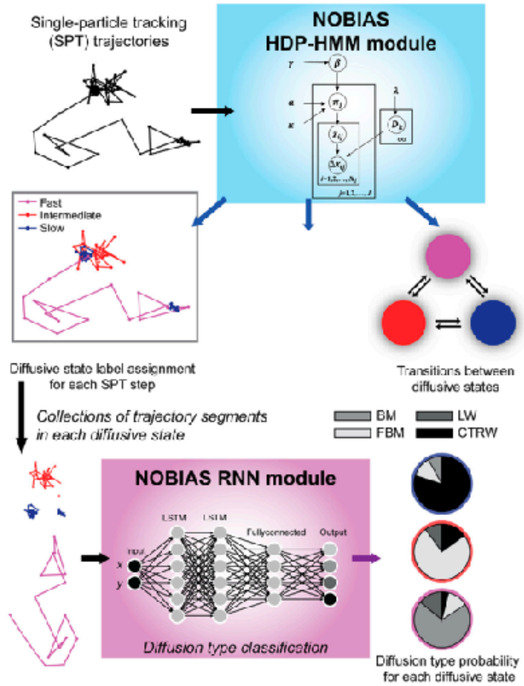
- CNN Trajectory [Granik 2019]
- Long Short-Term Memory RANDI [Argun 2021]
- Probabilistic- hidden variables aTrack [Simon 2024]
- Diffusive states and transition rates vbSPT [Persson2013]

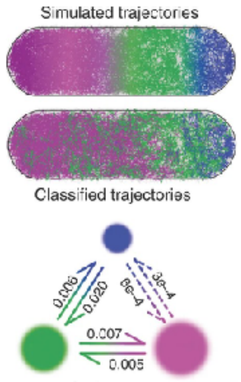












Multiple Diffusion States

- HMM for quantifying dynamic [Das, PLoS 2009]
- Transient particle transport [Monnier, Nat. Meth. 2015]
- Divide-and-conquer Moment scaling spectrum (MSS)
- Particle Mobility Analysis Using DL-MSS, [Arts 2019]
- NOBIAS [Chen, Frontiers, 2021]

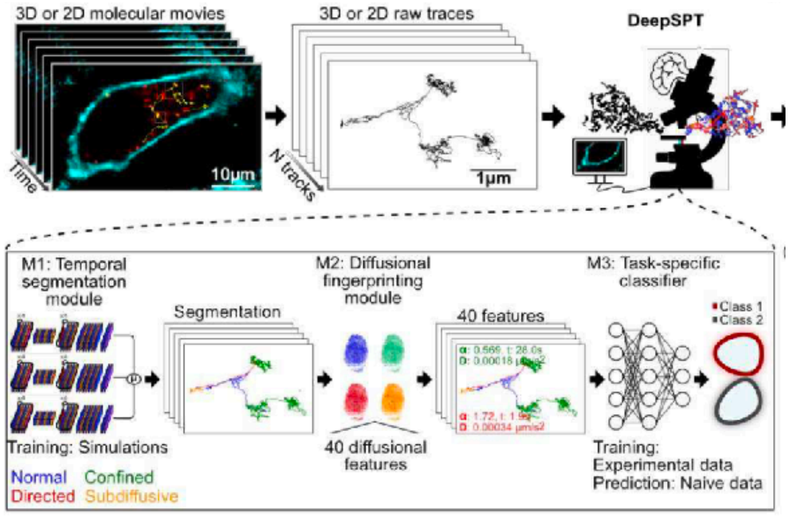
Trajectory Features

17 descriptive features [Pinholt PNAS 2021]

Feature name	Description
T0	Time in the slowest diffusion state
T1	Time in the second slowest diffusion state
T2	Time in the second fastest diffusion state
T3	Time in the fastest diffusion state
<tau>	Average residence time in a diffusion state
meanSL	Average step length for the trace
Pval	Quality of a power law fit to the msd curve
alpha	MSD power law scaling coefficent (<1 for subdiffusive and >1 for superdiffusive)
D	Diffusion constant from power law fit
Trappedness	Estimates whether the walker is trapped (≈ 0.5 for Brownian motion)
meanMSD	Intermediate time spread of the trajectory
Kurtosis	Heaviness of the tails in the distribution of points in the entire trajectory (≈ 2 for Brownian motion)
Gaussianity	Heaviness of the tails of the distribution for the steps in the trajectory (1 for Brownian motion)
Fractal dimension	Space-filling-ness of the trajectory (slightly less than 2 for Brownian motion)
Efficiency	Linearity of the trajectory (~7 for Brownian motion)
MSD ratio	MSD power law scaling coefficent (from statistics of trajectory rather than fit, 0 for Brownian motion)
N (track curation)	Bleaching rate or binding affinity to the substrate

40 diffusional fingerprinting of DeepSPT

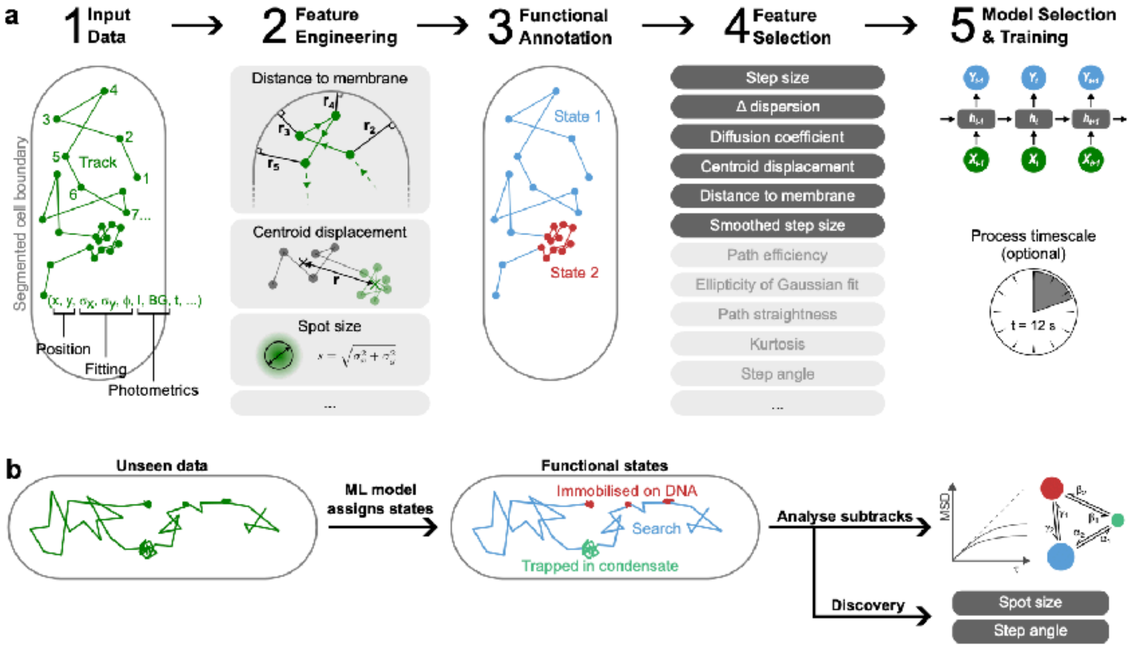
[Kaestel-Hanse, Nat. Meth. 2025]



60 engineered features DeepTRACE

[Pambos, Bioarxiv 2025]

- Raw features: Localization, photometric, tracking
- Static feature: current localization: Step size Distance to cell membrane
- Local window features: step size, step angle, straightness, fractal dimension, local trappedness, velocity
- Delta features: comparison of two local context, dispersion

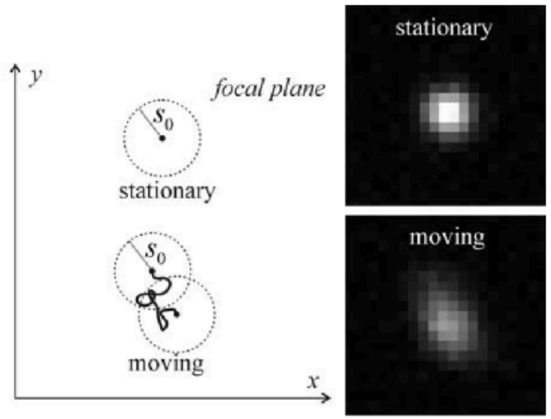


Learning from Pixel Patches

Motion Blur

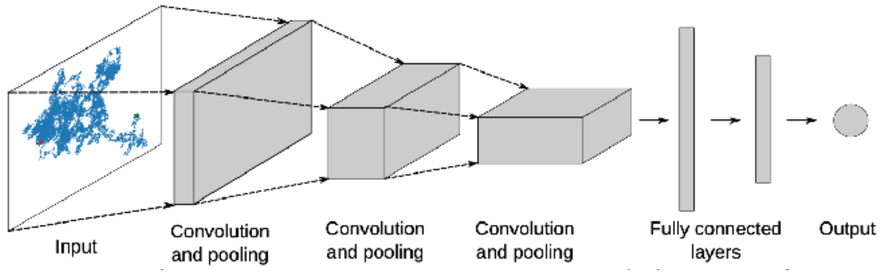
Influence of movement on the localization precision in SMLM

[Deschout, Biophotonics, 2011]



Learning

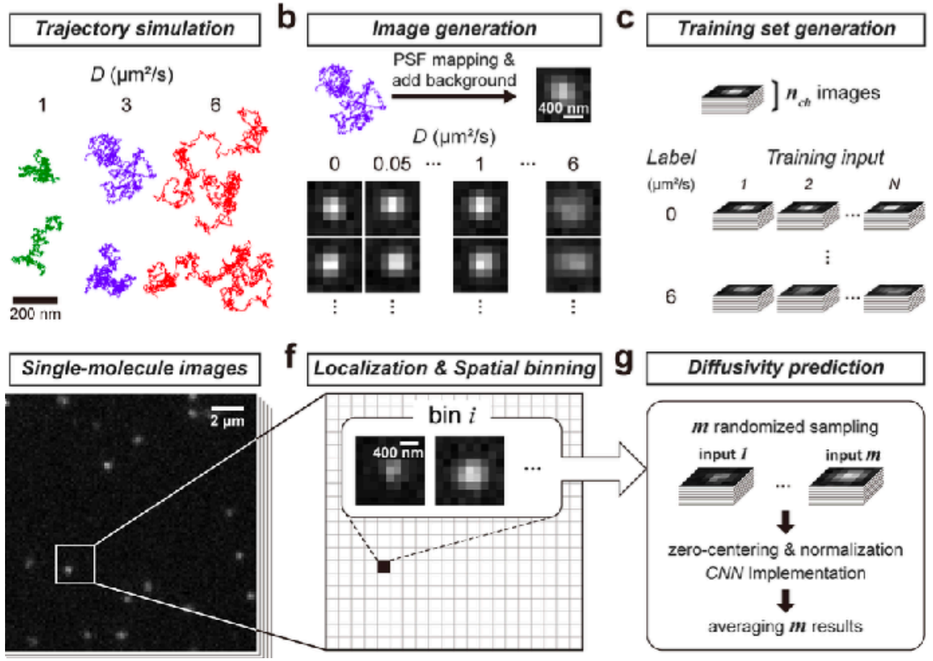
A convolutional neural network (CNN) trained directly on image data from image data



Pix2D

Exploiting the often undesired yet evident motion blur of single-molecule images, recorded under typical SMLM conditions, to extract diffusion coefficient D

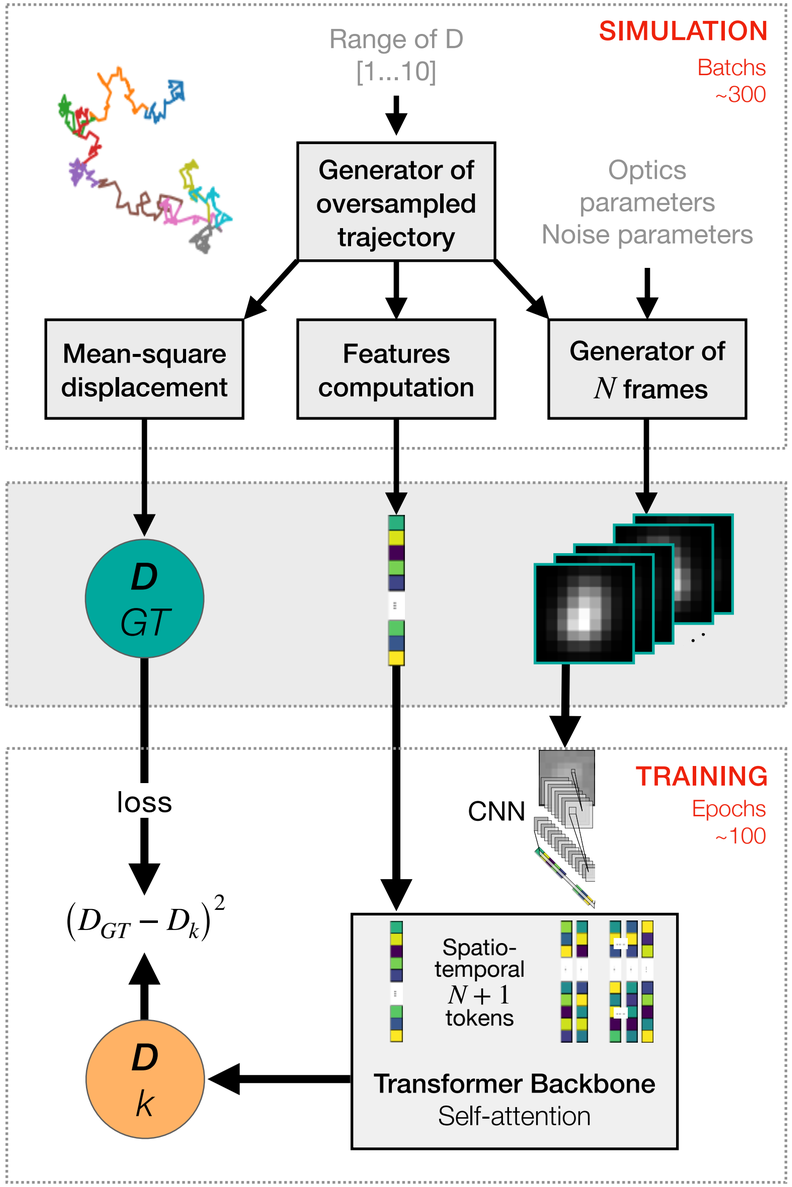
[Park, Communication Biology, 2024]



Motion-Informed Vision Transformer MiViT

MiViT

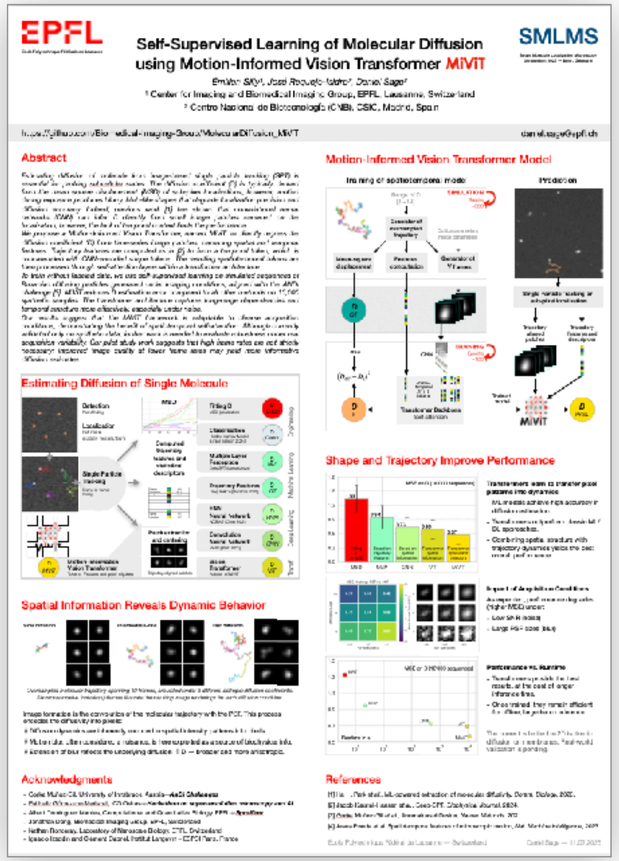
Training of spatiotemporal model



Poster at SMLMS 2025

Self-Supervised Learning of Molecular Diffusion using MiVit

Émilien Silly, José Requejo-Isidro, Daniel Sage



Acknowledgments

BIG
Michael Unser
Jonathan Dong
Mehrsa Pourya
Pakshal Bohra
Aleix Boquet Pujadas
Pablo Garcia Amorena
Emmanuel Soubies
Yan Liu
+ PhD Students

Emilien Silly, Master Student, EPFL
José Requejo-Isidro, CNB, CSIC, Madrid
Estibaliz Gómez-de-Mariscal, ICG Oeiras
G. Muñoz-Gil, Innsbruck, Austria—***AnDi***
Jonathan Dong, BIG, EPFL, Switzerland
I. Izeddin, Institut Langevin Paris, France
C. Cabriel, Institut Langevin Paris, France
Nathan Ronceray, LNB, EPFL, Switzerland
A. Dominguez Mantes, CQB, EPFL—**Spotiflow**

Roman Guiet
Guillaume Witz
Sebastian Herbert
Szymon Stoma

Rita Sarki
Olaia Naveiras
Mathis Krause
Lilly-Flore Celma
Cathrin Brisken
Pranay Day

Laurène Donati
Edward Ando
Florian Aymans
Mallory Wittwer
Sepand Kashani
Melissa Caloz
Ludovica Verde
Vasiliki Stergiopoulou

Arne Seitz
Olivier Burri
Romain Guiet
Martin Weigert
Benjamin Galluser
Nicolas Chiaruttini
Rémy Dornier

SMLM
A. Radenovic
Juliette Griffié
Inês Cunha
Suliana Manley
Thanh-an Pham
Seamus Holden

DeepImageJ/SAMJ
Arrate Muñoz-Barrutia
Caterina Fuster Barcelo
E. Gomez-de-Mariscal
C. Garcia-Lopez-de-Haro
Daniel Franco-Barranco
Lucia Moya Sans
Jonathan Heras
Curtis Rueden
Vladimir Ulman
Adrian Ines

CNRS/MAIIA/FBI
Bertrand Vernay
Christine Terryn
David Rousseau
Pejman Rasti
Anaïs Badoual
Jean-Daniel Fauny
Rémy Flores-Flores
Christophe Chamot
Fabrice Cordelières
+ many others
J.-C. Olivo-Marin
Jean-Yves Tinevez

EUROBIOIMAGING
Serrano-Solano
Aastha Mathur
Wei Ouyang
Florian Jug
Anna Kreshuk
Constantin Pape
Fynn Beuttenmüller
Arrate Muñoz-Barrutia
E. Gomez-de-Mariscal
Ricardo Henriques
Matthew Hartley
and many others