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, Germany**
**August 2025**


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

# SMLM WORKFLOW

# DATA ANALYSIS

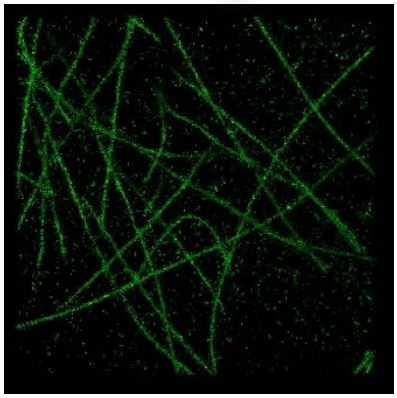


## SMLM as a Quantitative Bioanalytical Tool

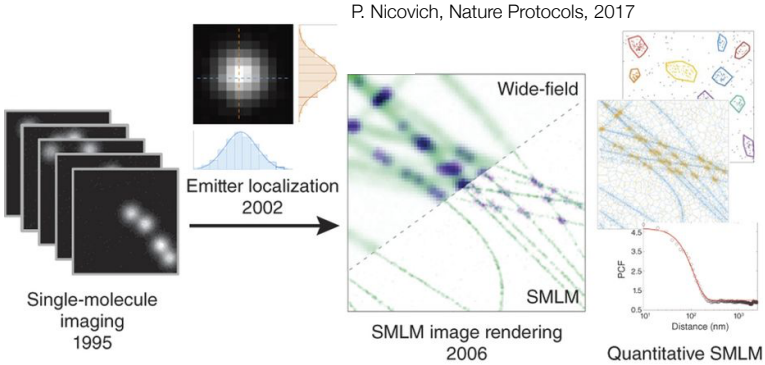
Breaking the Light Diffraction Limit

2014
Eric Betzig
Stephan Hell
W.E. Moerner

Super-resolution Microscopy (x10)

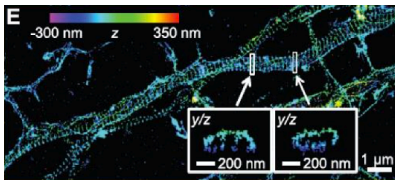


P. Nicovich, Nature Protocols, 2017



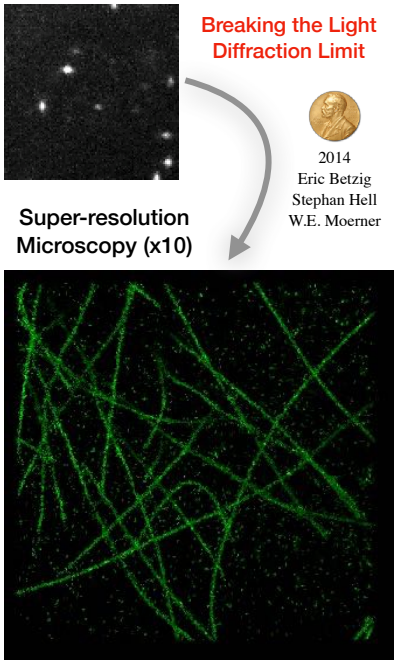

**What can we quantify?**

- 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
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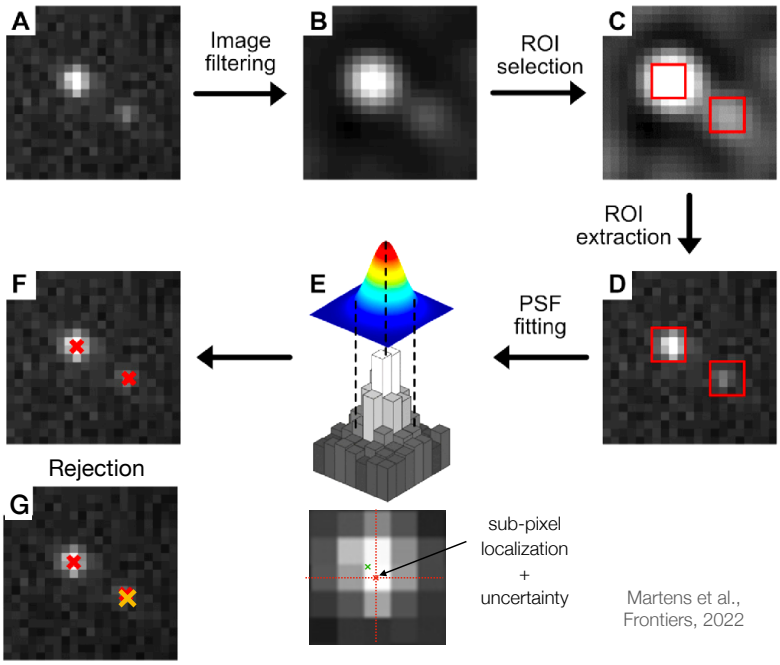
Super-resolution Microscopy (x10)

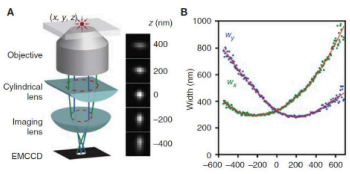



Martens et al., Frontiers, 2022



# 3D SMLM

*3D can be performed by putting a cylindrical lens into the imaging path to create astigmatism*
*Huang 2008*



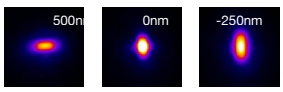

| Interferometry | |
|---|---|
| iPALM | Shtengel, PNAS 2009 |
| SELFI | Bon, Nat Methods 2018 |
| **EngineeredPSF** | |
| Cylindric lens | Huang, Science 2008 |
| Astigmatism AO | Izzedi, Optics Express 2011 |
| Double-helix | Pavani, PNAS 2009 |
| Saddle | Shechtman, Phys Rev, 2014 |
| Tetrapod | Aristov, 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 |

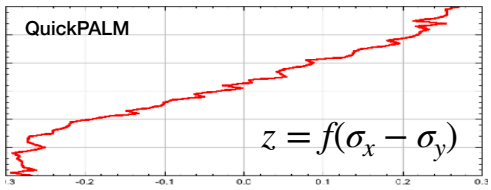
Astrid Magenau 2014 and Bassam Hajj 2018

## Calibration

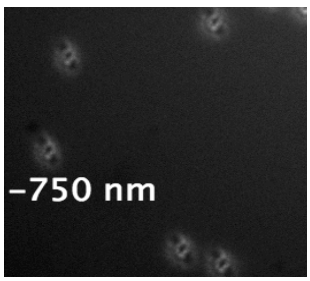
PSF dependence on z using immobilized nanobead z-scan

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

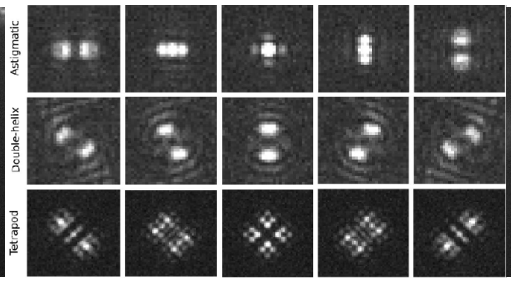



Acquisition in 2D, Stacking all the 2D-PSF, 3D PSF signature gives Z:




Double Helix PSF

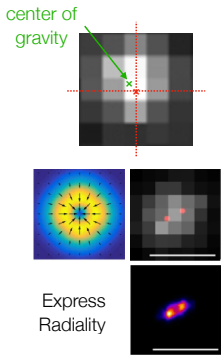



Fazel et al. Biophysics and Bioengineering, 2022

# Localisation Methods

**Direct Detection**

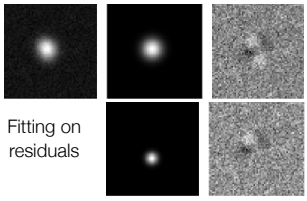
**Center of gravity**
QuickPALM




**Fitting Parametric Function**

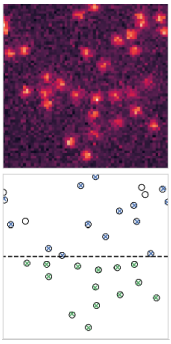
**Single-emitter**
RapidSTORM
commercial software

**Multiple-emitter**
DAOSTORM
ThunderSTORM



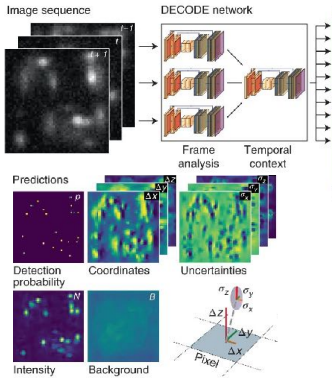

**Solving as a Inverse Problem**

**Optimization**
ADCG



**Learning Approaches**

**Synthetic datasets**
DECODE

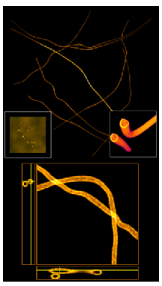





# Benchmarking SMLM Software

http://srm.epfl.ch/

**Realistic Simulation**



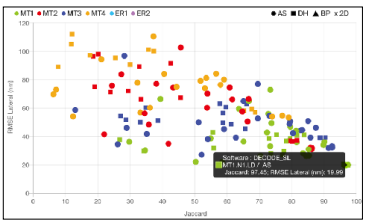
**More 30 than participants**

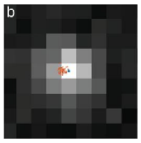
| Software | PSF | Plateform |
|---|---|---|
| 3D-DAOSTORM | Gaussian | Python / C |
| 3D-STORM | Gaussian | Qt framework |
| 3D-WTM | Gaussian | Stand-alone |
| Cspline | Learnt | Python / C |
| DECODE | Encode | Python |
| Localizer | Gaussian | Igor |
| MIATool | Bessel | Java |
| mlePALM | Gaussian | 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?

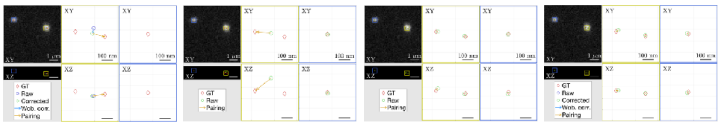
**Choose the appropriate software affects results**





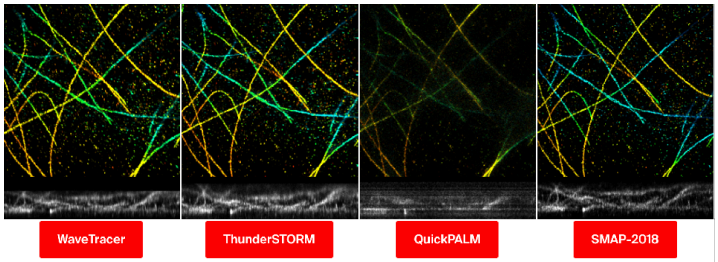

Sage et al. Quantitative evaluation of software packages for SMLM Nat Meth., 2015.
Sage et al. Super-resolution fight club: 2D & 3D SMLM software Nat Meth. 2019.
Li Real-time 3D SMLM using experimental point spread functions Nat Meth., 2018.

**Quantitative assessment**



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

**Qualitative assessment**



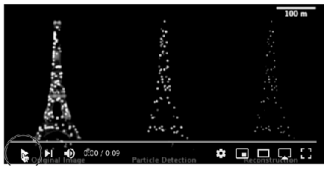
# SMLM Software

## 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**

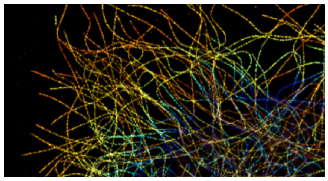
- Single emitter fitting: detection-localisation-rejection
- Multiple emitter fitting: optimal determination of the number of molecules (high-density)

## 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**

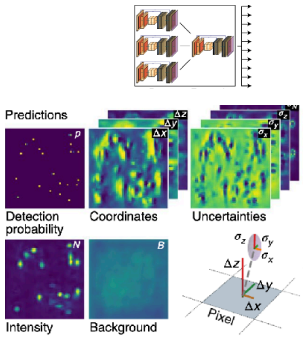
- Include the 3D fit of PSF fit3Dspline

## DECODE

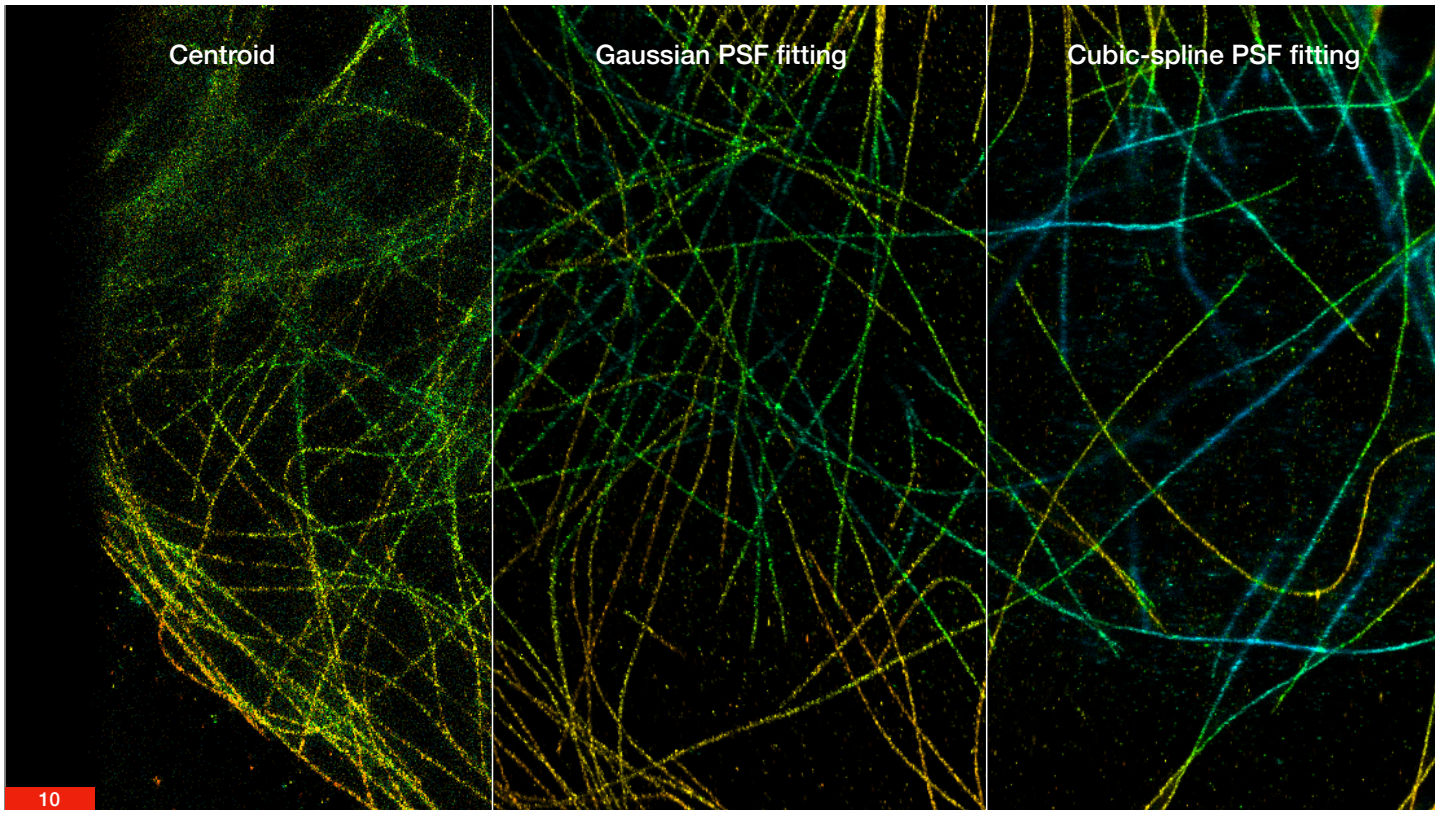
A. Spieser, Nat. Methods 2021

**Deep Learning Prediction 3D localizations**

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





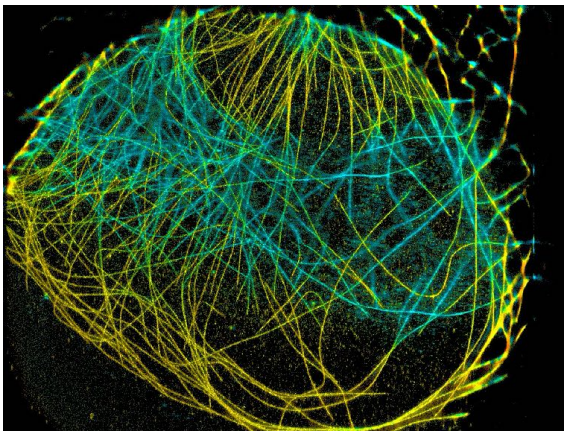



# Point Data Rendering

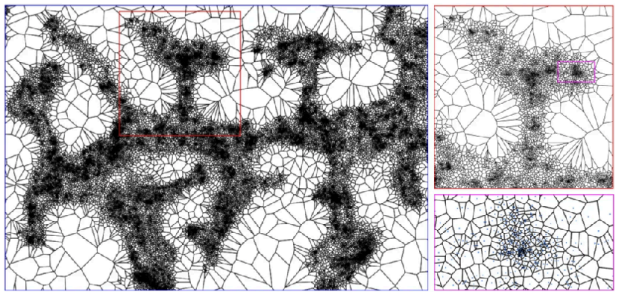
| | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | id | frame | x [nm] | y [nm] | z [nm] | sigma1 [nm] | sigma2 [nm] | intensity [ph] | offset [photc | bkgstd [photon] | uncertainty_xy [ |
| 2 | 1 | 1 | 5740.83434 | 1761.83022 | -73.2513 | 184.16585 | 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

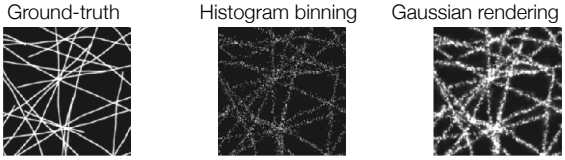
2'000'000 localizations 1.17Gb



**Colorize Voronoi** Levet et al., SR-Tesseler, 2015



Ground-truth Histogram binning Gaussian rendering

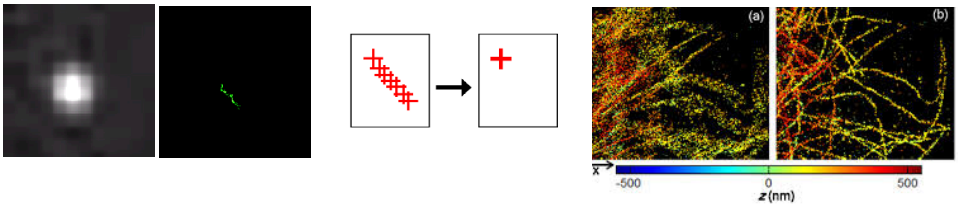




# Point Data Processing

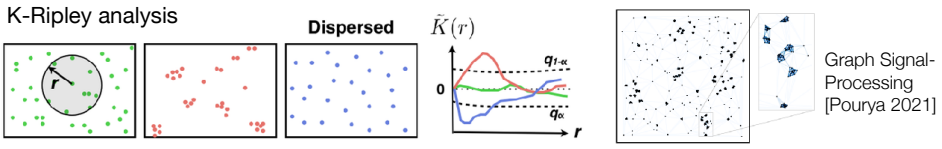
## Drift Correction

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



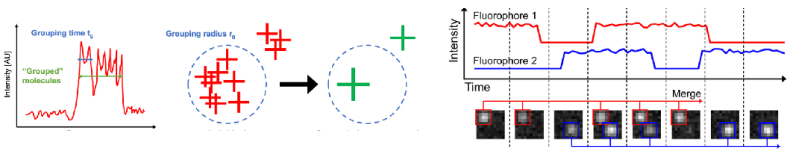

## Denoising / Rejection

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

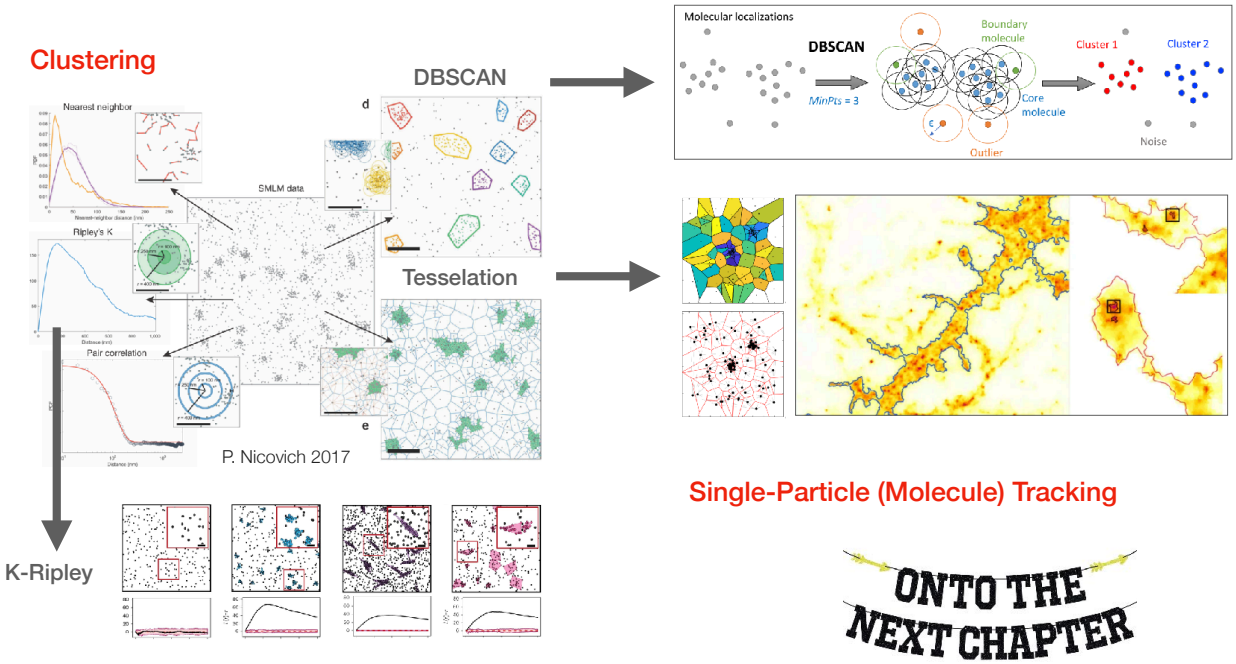



## Temporal Grouping

- Merge localizations in single molecule



# Point Data Analysis






EPFL

WORKSHOP SMLM DATA ANALYSIS

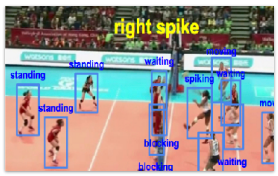
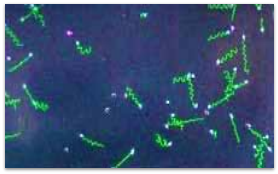
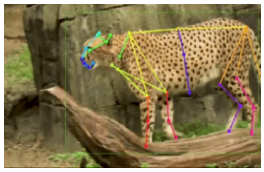
**Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking**

## IMAGE ANALYSIS

# PARTICLE TRACKING

SMLMS 2025

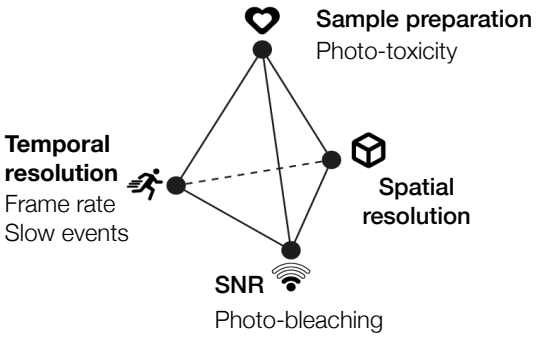
# Computer Vision Image-Based Motion Analysis

| Pixel-based Method | Feature-based Method | Spatio-temporal Inference | AI Foundation Models |
|---|---|---|---|
|  |   |  |  |
| **Optical Flow**<br>Motion field estimation | **Real-time Tracking**<br>Target prediction | **Multiple Object Tracking**<br>Object detection | **SAM2 (Meta)**<br>Video predictor |
|  |  |  |  |
| **Background Modeling**<br>Video watching | **Particle Tracking**<br>Off-line, motion models | **Pose Estimation**<br>DeepLabCut [Mathis, 2018] | **CoTracker (Meta)**<br>Point tracking |



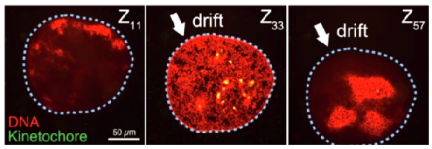
# Imaging Image-based Motion Capture

## Time-lapse Acquisition



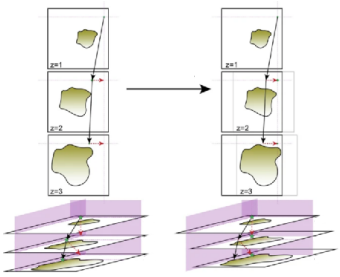

- 30 Hz → 33 ms
- 100 Hz → 10 ms
- 1000 Hz → 0.5 ms

## Temporal Perturbation

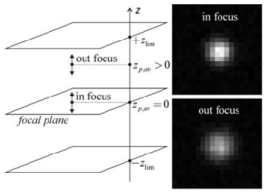



Live Cell Imaging
**Composition of movements**

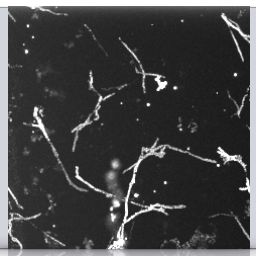
**Drift**

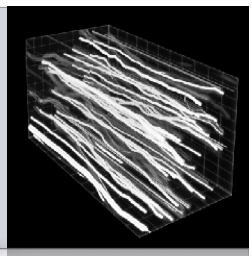


Axial: Out-of-focus
Lateral: Outside field of view



# Seeing the Motion in Images





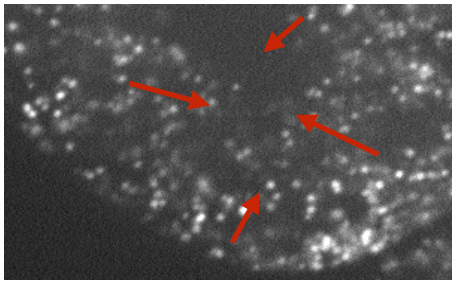
Temporal projection

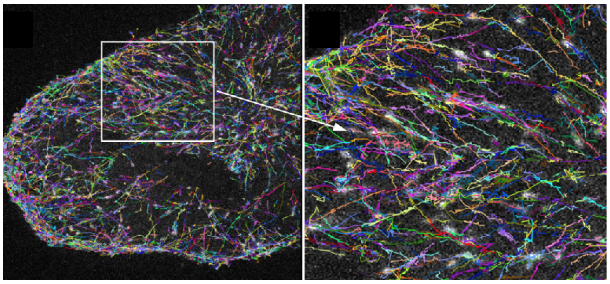
Spatio-temporal visualisation or segmentation

Stationary vs. mobile object



# Particle Tracking





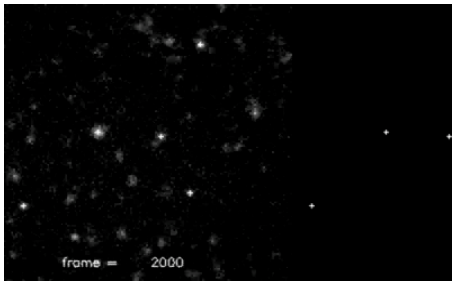
**Motion model**

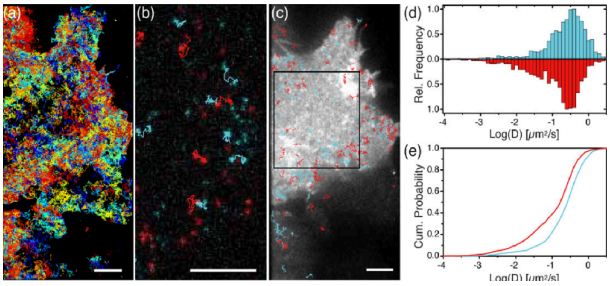
**Event type**

**Ambiguities**

**Occlusion**

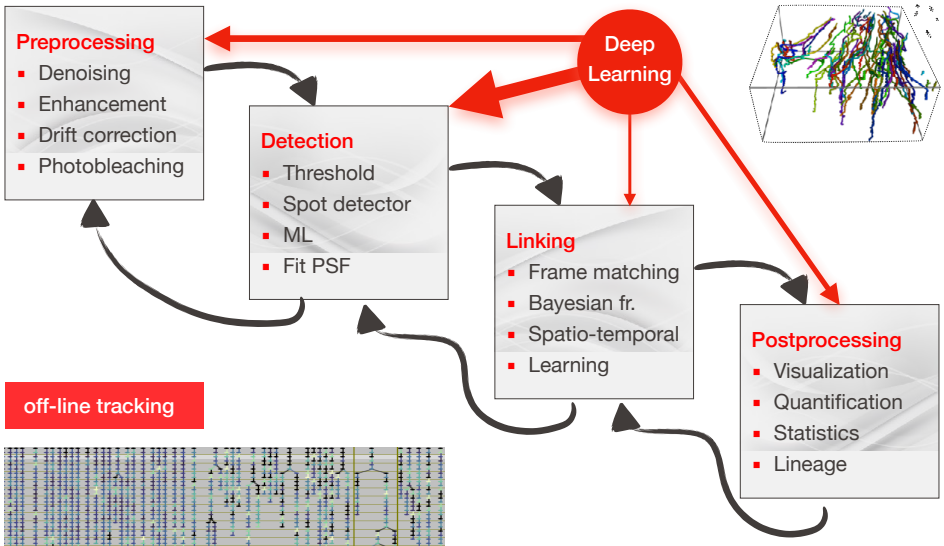
**Density**




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

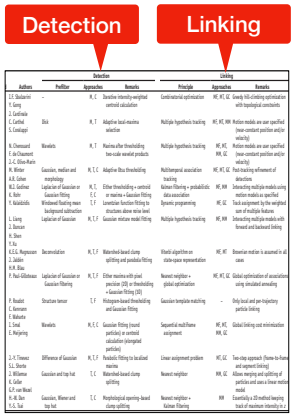
# Tracking System



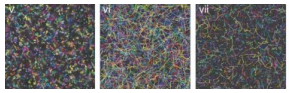





Challenge



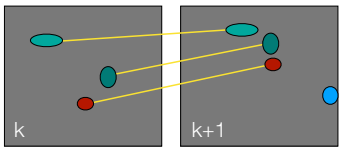

**Comparison**
Chenouard & al.
Nature Method, 2014

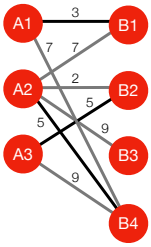




# Tracking-by-Detection

**Linking / Association**




**Bipartite graph**

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

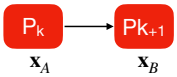
**Matching algorithms**

- Simple Nearest-Neighborhood
- Global Nearest-Neighborhood
- Greedy
- Linear assignment problem (LAP)
- Hungarian Algorithm

**Descriptor of particles**

- Position, x, y, z
- Intensity, SNR, uncertainty
- Morphometric
- Direction of displacement
- Speed

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




**Cost function**

**Weighted sum and regularization**

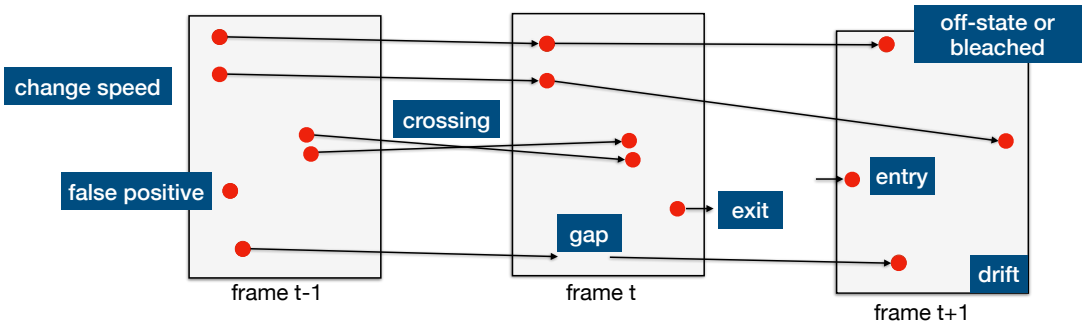
- Hard constraint
- Soft constraint: e.g. sigmoid
- Fill the gap: soft threshold

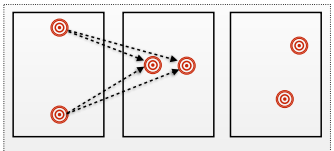
**Example of cost function**

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

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

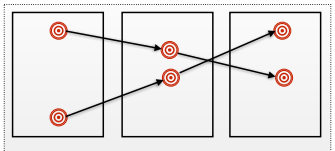
# Frame-to-frame Association



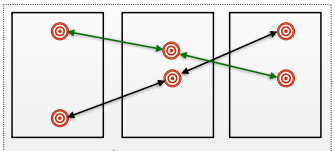
Multiple hypothesis test
➡ delay the decision

MHT [Chenouard, 2010]



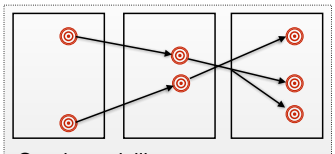
Link with n frames
➡ use future and **past**

For 1 spot [Sage, 2003]



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

u-track [Jaqaman, 2008]



Graph modelling
➡ manage cell division

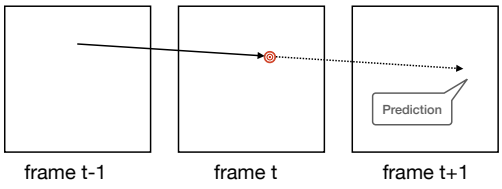


# Tracking-by-Prediction

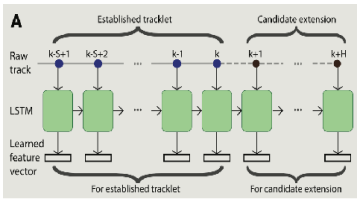
## Signal Processing

**Kalman Filter**

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



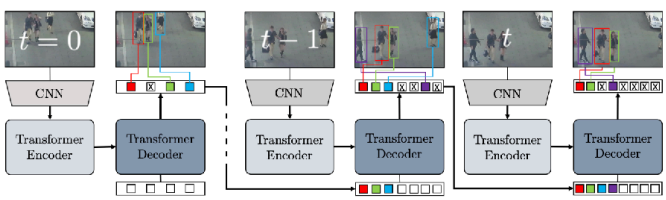

## Deep Learning




RNN
LSTM (long short-term memory)
Yao, 2020

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

CNN in space + Attention of Transformer

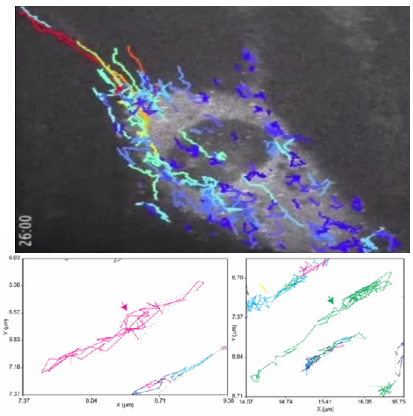



TrackFormer Meinhardt, CVPR, 2022

# Tracking-by-Segment

## Coarse-to-fine tracking

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

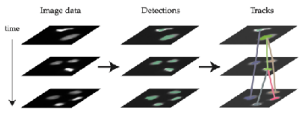


**u-Track** Khaloud Jaqaman

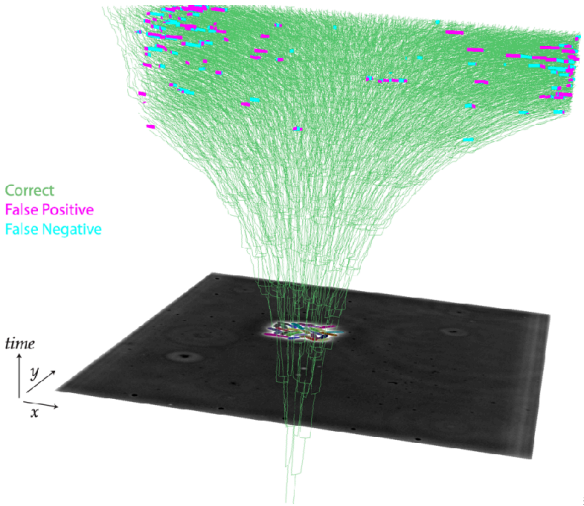
*K. Jaqaman et al. Robust single-particle tracking in live-cell time-lapse sequences, Nat. Methods 2008*

## Learning Association

- Simulation of 3 frames
- Various situation



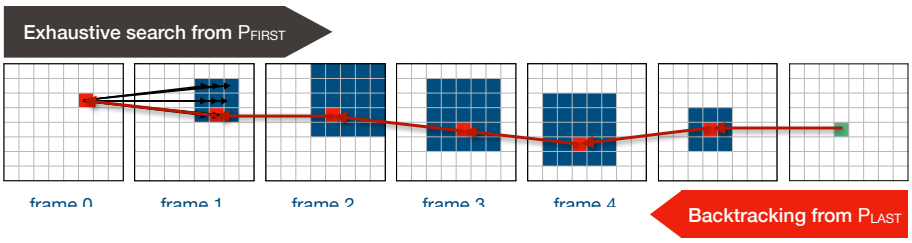

**TrackAstra** Benjamin Gallusser



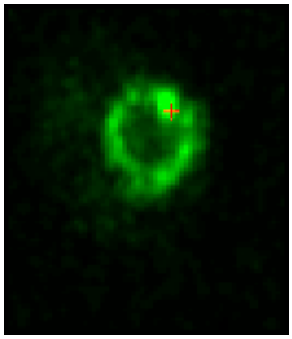



# Tracking by Trajectory Reconstruction

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

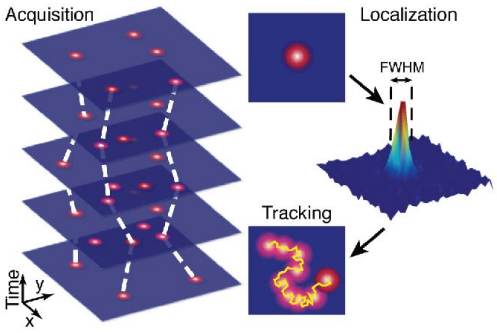
- Optimization on discrete data with a sequence
- Efficient algorithm to find the best path
- Global scope: **past + future**

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 $\mathbf{v}_{t+1}$

# Single Particle Tracking




- **sptPALM** S. Manley et al., High-density mapping of single-molecule trajectories with photoactivated localization microscopy, 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 of cell membranes, 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: Review of applications and performance evaluation, Traffic 18 2017.
- **swift** B. Turkowyd et al., Establishing Live-Cell Single-Molecule Localization Microscopy Imaging and Single-Particle Front. Microbiol., 2020.



EPFL

WORKSHOP SMLM DATA ANALYSIS

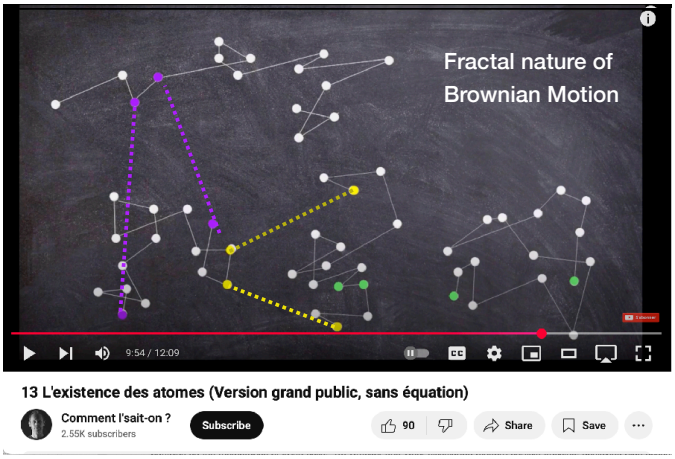
**Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking**

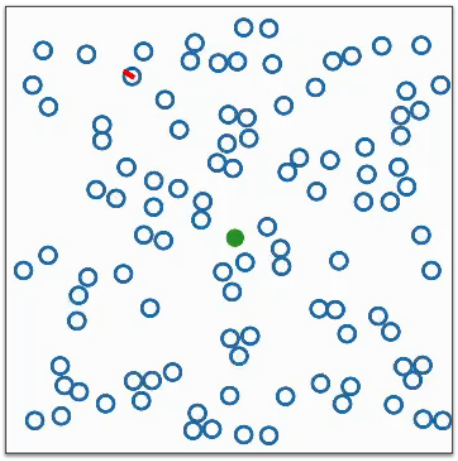
BIOPHYSICS

# DIFFUSION MODELS

SMLMS 2025

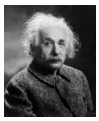
# Brownian Diffusion



**Robert Brown**
1827
Brownian Motion

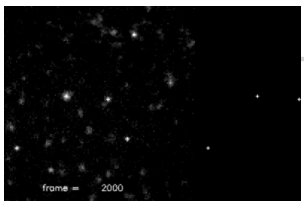
**Jean Perrin**
Nobel Prize 1926
Les atomes



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



# Analysis of Molecular Diffusion

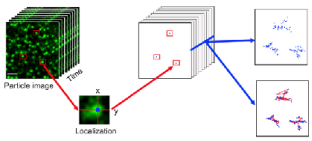


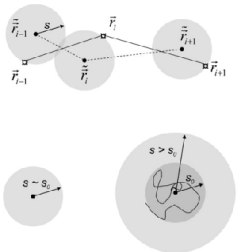

Spot Detection → Coordinates → Particle Tracking → Trajectory Analysis → Statistical Descriptor

Spot Detection → Integer Coordinates → Sub-pixel Localisation → Sub-pixel positions Uncertainty → Particle Tracking

Statistical Descriptor: **Mean-square Displacement**

**Single Molecule Tracking**



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

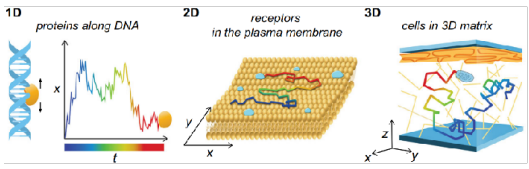



Figure of ANDI, Gorka Muñoz-Gil

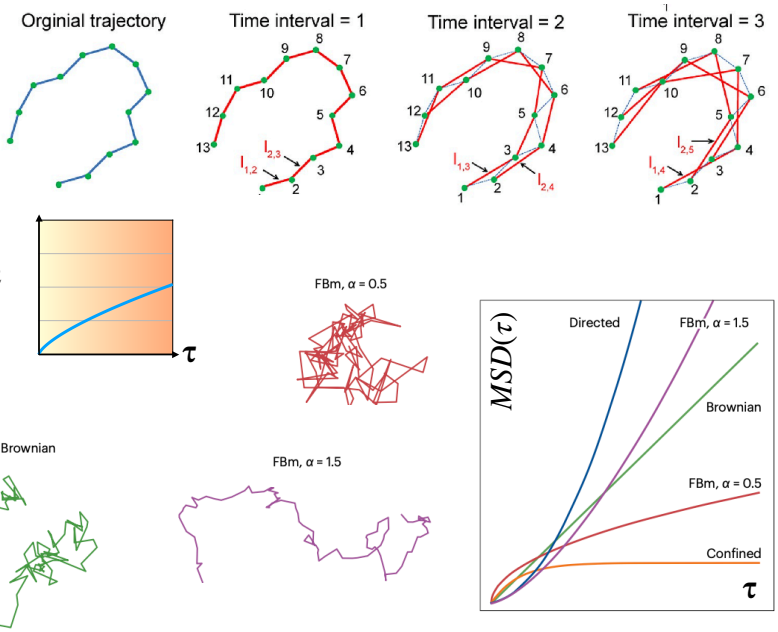
# Mean Square Displacement

**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

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

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



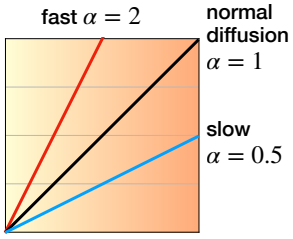


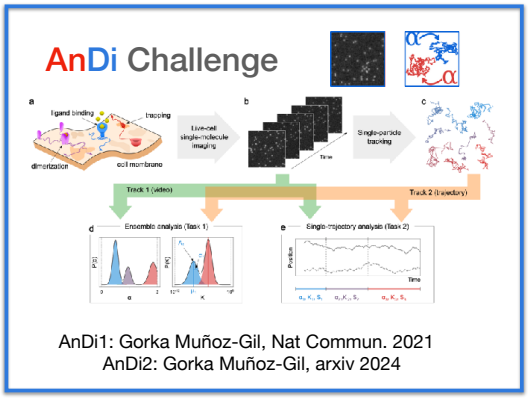

# Diffusion Models

**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




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

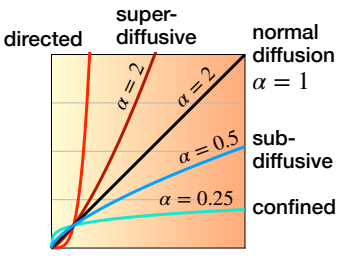
**Anomalous Diffusion**

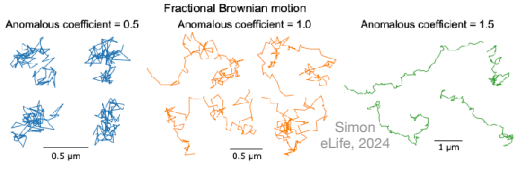
$$MSD(\tau) \propto 2^d\, \tau^{\alpha}$$

Fractional Brownian motion (fBM)

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

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

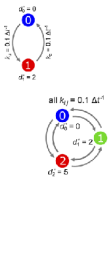


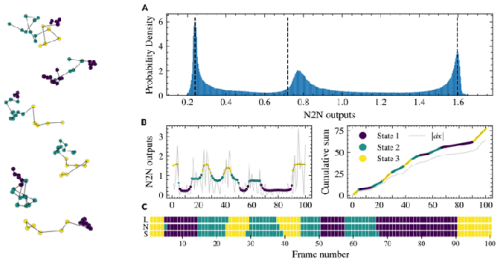
# Non-homogenous Diffusion

## Time-varying diffusion

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

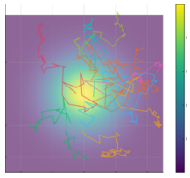


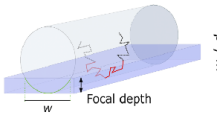

F. Simon, ExTrack, JCB, 2023



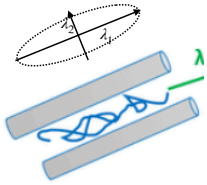

## Spatial-varying diffusion

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






**Anisotropic diffusion**



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

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



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ALGORITHMS

# ESTIMATING FFUSION

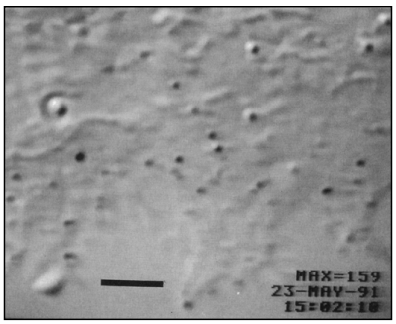
SMLMS 2025

# Single-Molecule Tracking

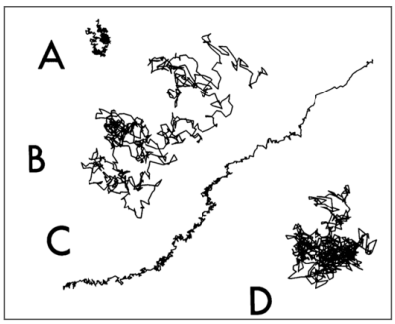
**The pioneer: Akihito Kusumi**

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.

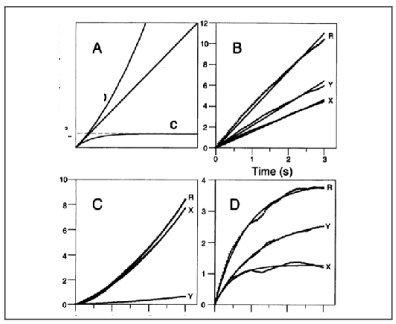
Single Molecule Tracking (SMT) provides unprecedented ability to directly observe molecular behaviors and interactions in living cells [Kusumi, 2024]




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




Examples of recorded trajectory, after detection particles and tracking them.

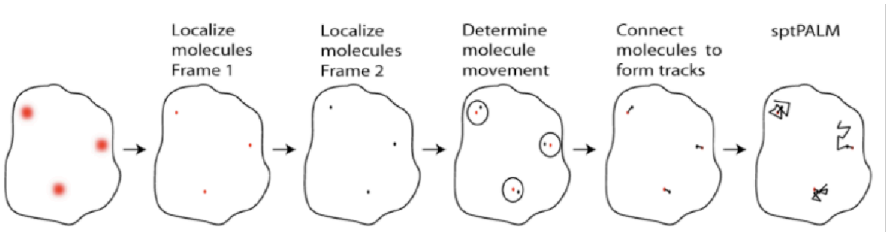



Classification of rancor by analyzing the Mean-Square Displacement (MSD)



# Single Particle Tracking in SMLM

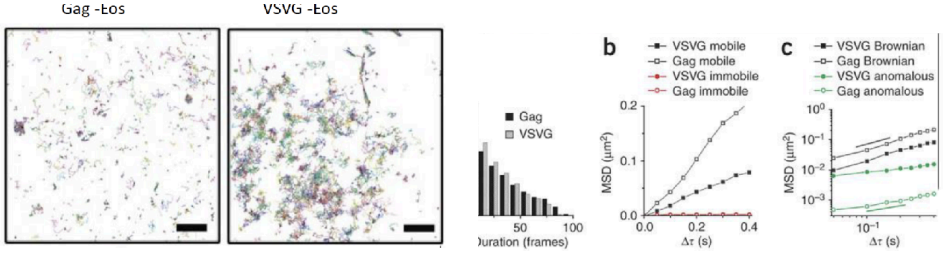
## sptPALM



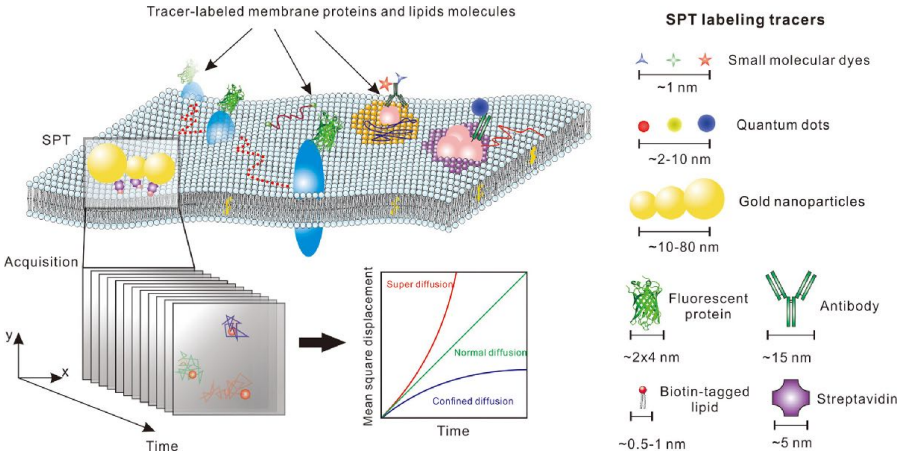

[Manley Nat. Meth. 2008]

average diffusion coefficient

$D_m$(Gag-Eos) =0.11 ±0.08μ²/s,
$D_m$( VSVG-Eos) = 0.14±0.11 m²



# SPT Is Seeing Always Believing?




**Potential limitations and pitfalls**

- Optical flow Hydrodynamic is affects by large probes
- 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

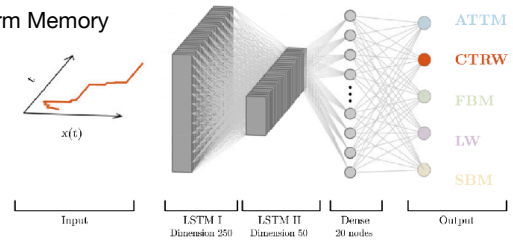
Yanqi Yu, Miao Li, Yan Yu · Tracking Single Molecules in Biomembranes, ACS Nano 2019.



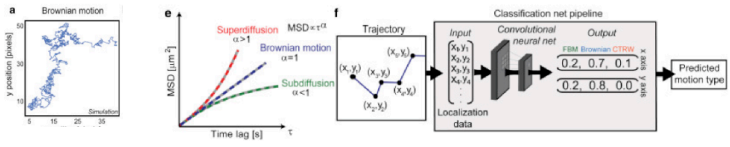
# Classification of Trajectories

**RANDI** [Argun, Journal of Physics A, 2021]

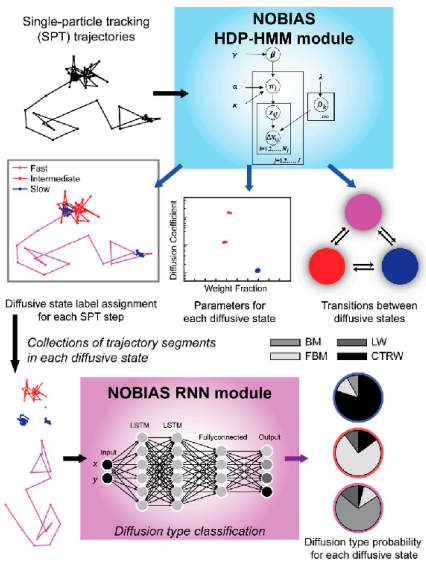
Long Short-Term Memory




**CNN Trajectory** [Granik, Biophysical Letter 2019]



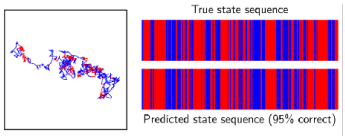

**NOBIAS** [Chen, Frontiers, 2021]



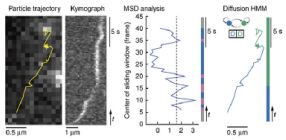
# Multiple Diffusion States

## Hidden Markov Models (HMM)

HMM for quantifying dynamic
[Das, PLoS 2009]



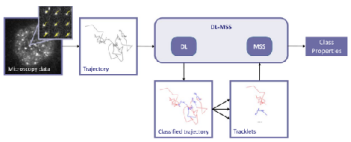

Transient particle transport
[Monnier, Nat. Meth. 2015]




## Divide-and-conquer

Moment scaling spectrum (MSS)

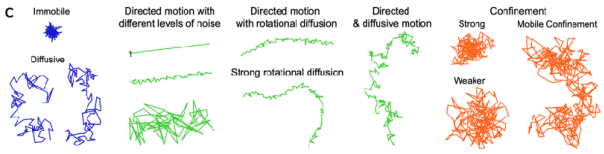
Particle Mobility Analysis Using DL-MSS, [Arts 2019]



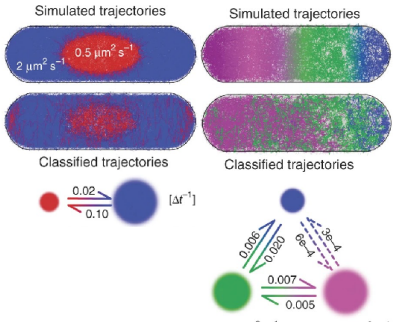

## Probabilistic

**aTrack** [Simon, eLife 2024]

Modeling true variables as hidden variables.
Applies likelihood ratio tests to classify the trajectory




**vbSPT**
[Persson, Nat. Meth. 2013]
Diffusive states and transition rates






# Trajectory Features

**17 descriptive features of Pinholt**

[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 coefficient (<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 coefficient (from statistics of trajectory rather than fit, 0 for Brownian motion) |
| N (track duration) | Bleaching rate or binding affinity to the substrate |

**60 engineered features DeepTRACE**

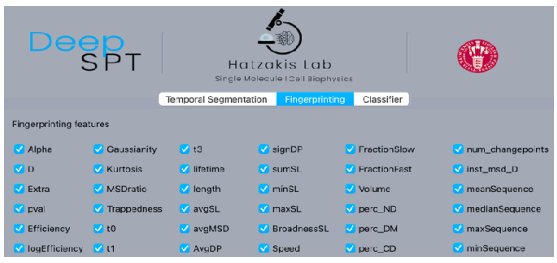
[Kaestel-Hanse, Nat. Meth. 2025]

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

**40 diffusional fingerprinting of DeepSPT**

[Pambos, Bioarxiv 2025]

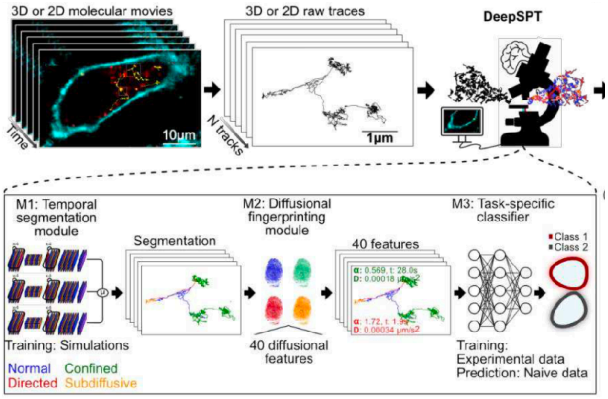
DeepSPT's features expanding on the work of Pinholt



# Learning from Trajectory Features

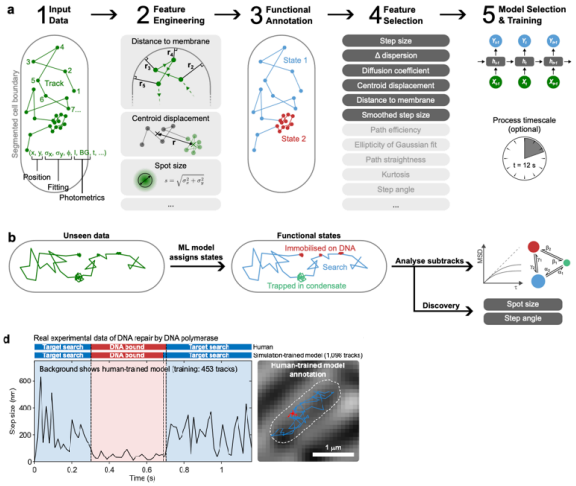
## DeepSPT

Deep learning assisted SPT
[Kaestel-Hanse, Nat. Meth. 2025]




## DeepTRACE

ML for Analysis in Single Molecule Track
[Pambos, Bioarxiv 2025]

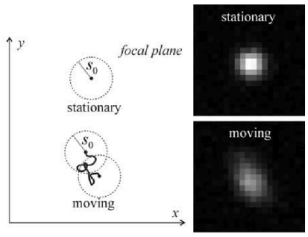





# Learning from Pixel Patches

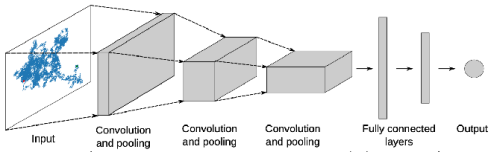
## Motion Blur

Influence of movement on the localization precision in SMLM
[Deschout, Biophotonics, 2011]




## Learning

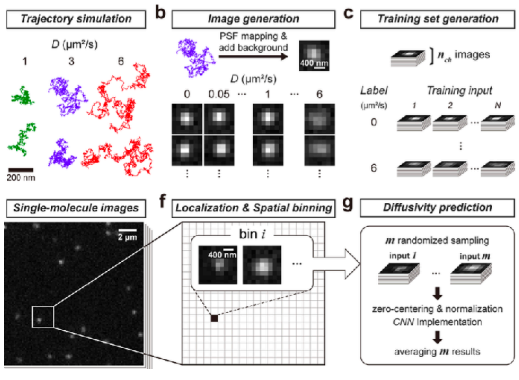
A convolutional neural network (CNN) trained directly on image data from image data




## Pix2D

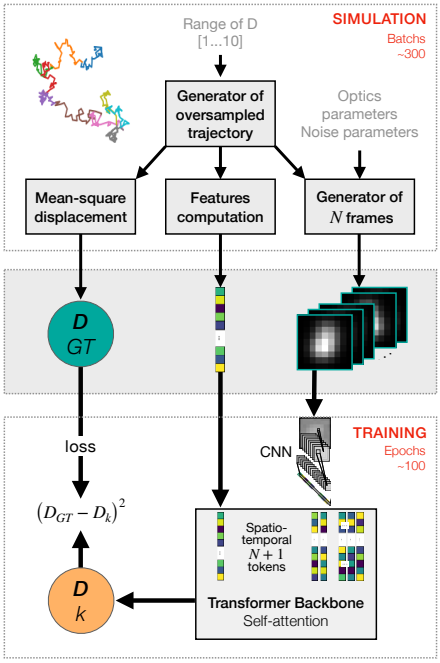
Exploiting the often undesired yet evident motion blur of single-molecule images, recorded under typical SMLM conditions, to extract diffusion coefficient D

[Park, Communication Biology, 2024]



# Motion-Informed Vision Transformer MiViT

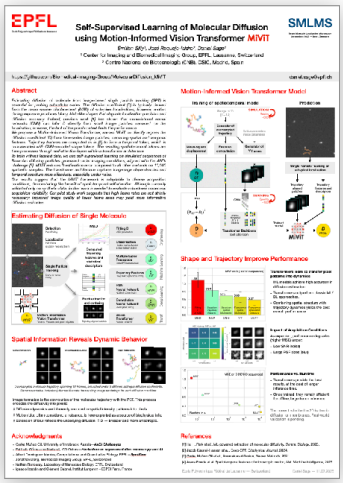
Training of spatiotemporal model




**Poster at SMLMS 2025**

Self-Supervised Learning of Molecular Diffusion using MiVit

*Émilien Silly, José Requejo-Isidro, Daniel Sage*





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