

Characterizing Molecular Diffusion by Image-Based Single-Particle Tracking

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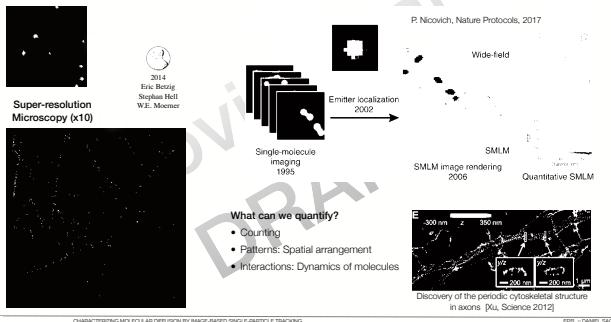
Single Molecule Localization Microscopy
Symposium – SMLMS 2025

Bonn, Germany
August 2025

EPFL

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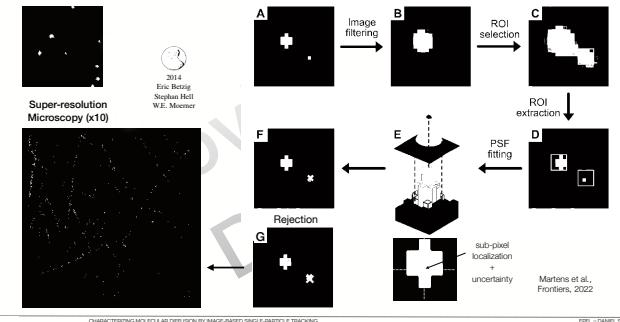
SMLM as a Quantitative Bioanalytical Tool



CHARACTERIZING MOLECULAR DIFFUSION BY IMAGE-BASED SINGLE-PARTICLE TRACKING

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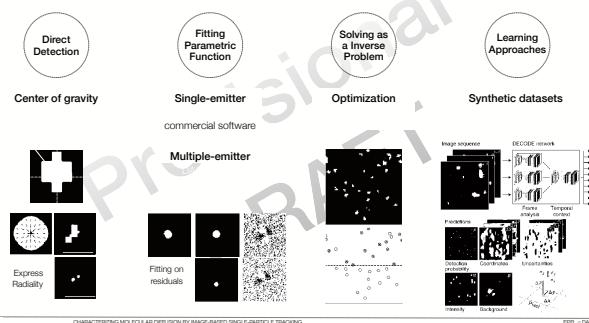
SMLM Image Analysis



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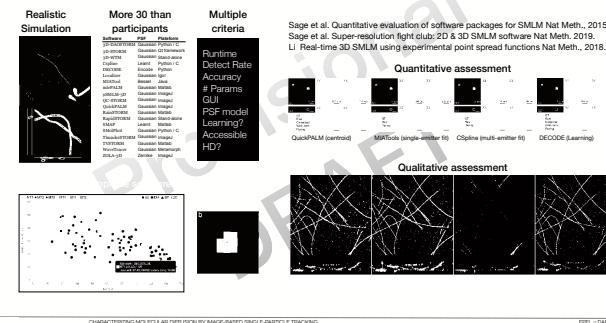
Localisation Methods



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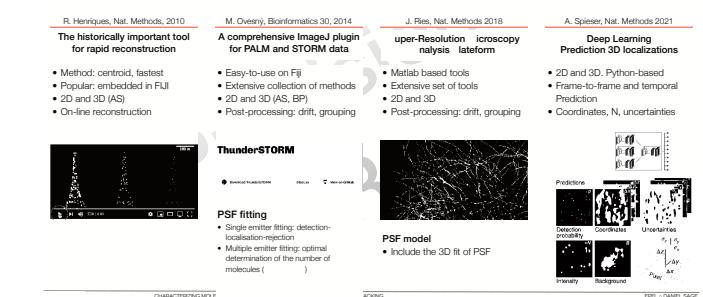
Benchmarking SMLM Software



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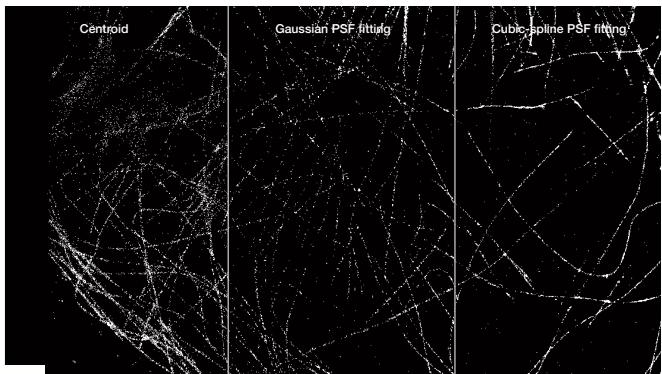
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SMLM Software

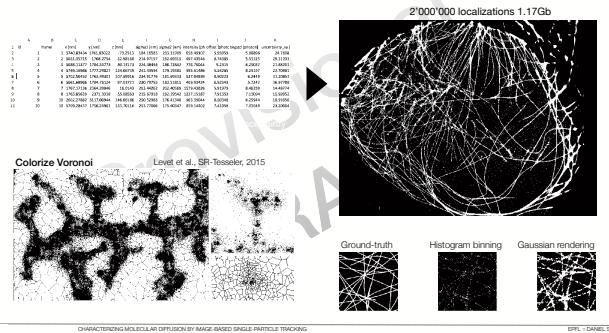


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Point Data Rendering



Point Data Processing

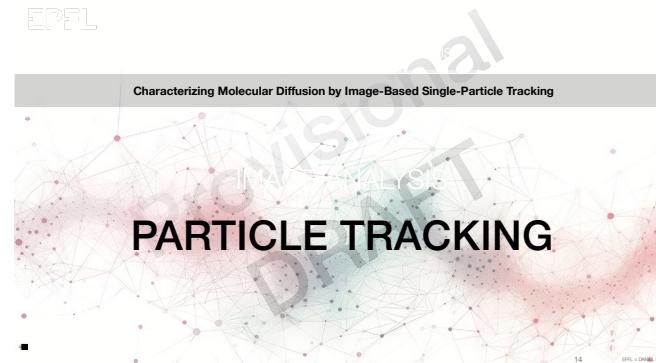
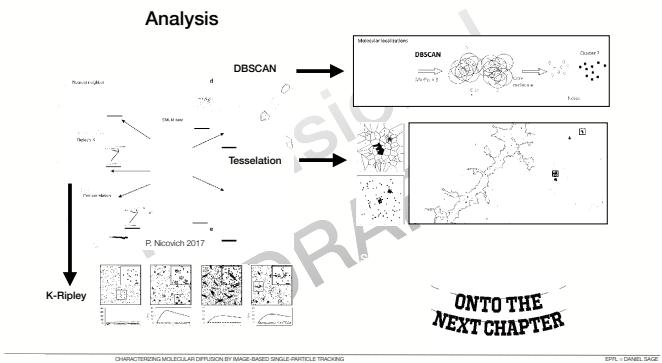
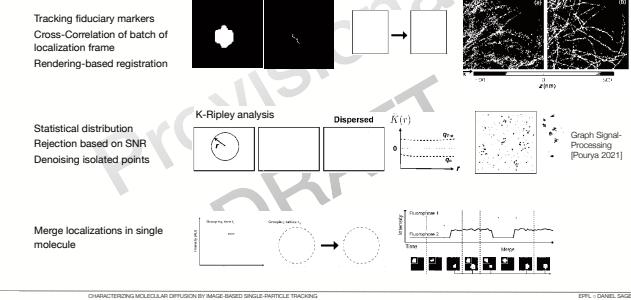
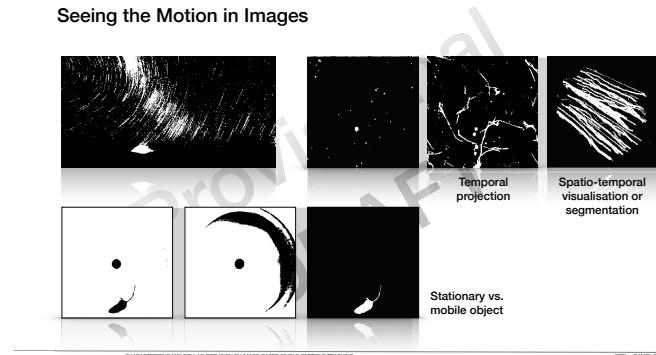
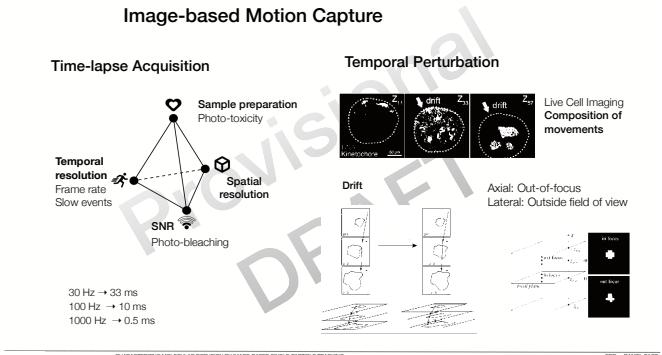
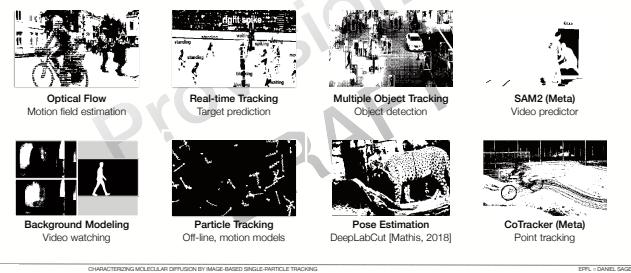
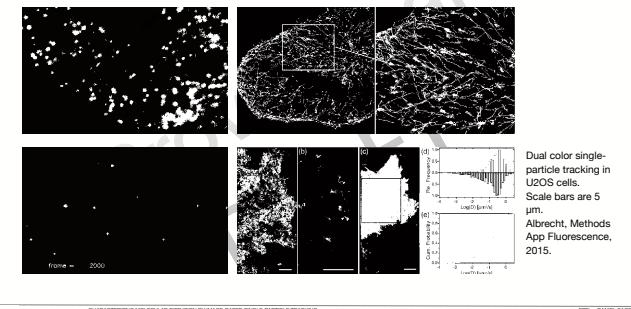


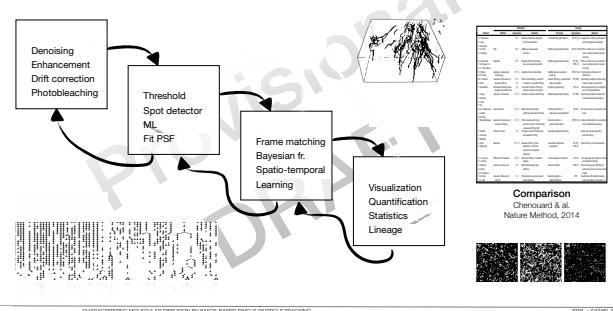
Image-Based Motion Analysis



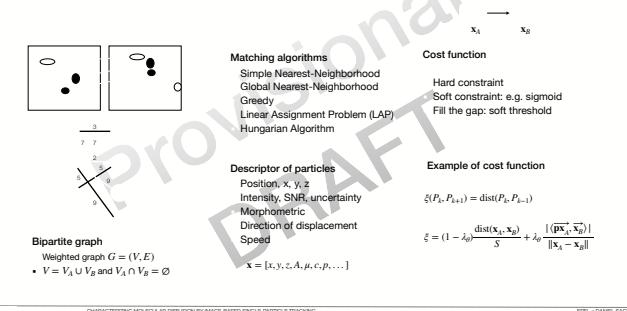
Particle Tracking



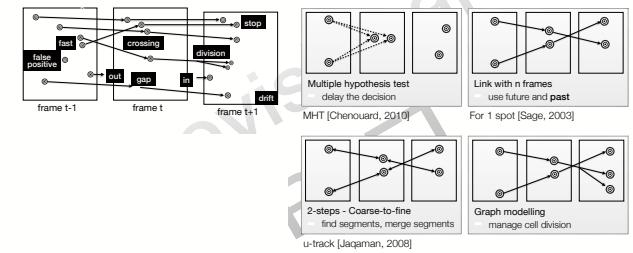
Tracking System



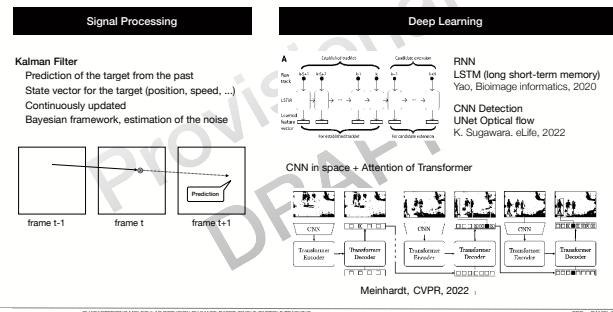
Tracking-by-Detection



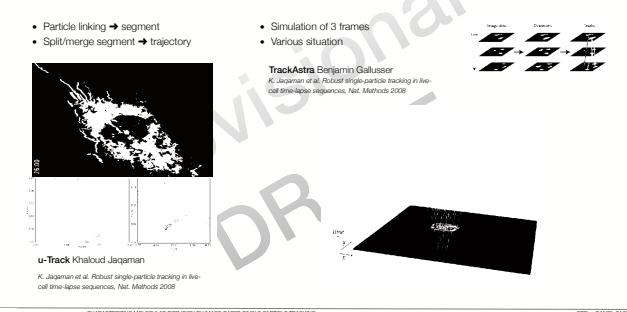
Frame-to-frame Association



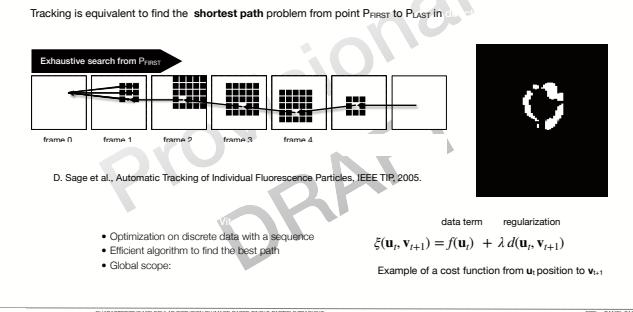
Tracking-by-Prediction



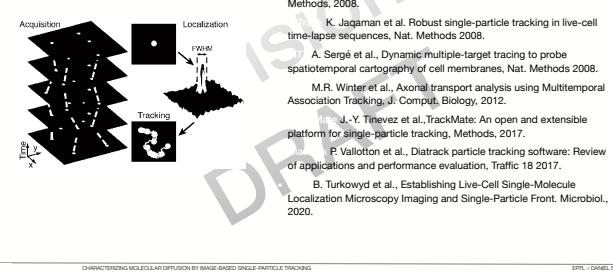
Tracking-by-Segment



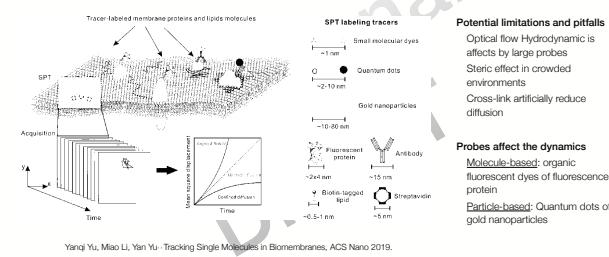
Tracking by Trajectory Reconstruction



Single Particle Tracking



Is Seeing Always Believing?

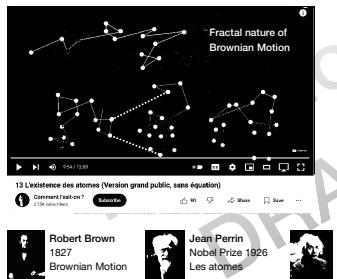


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DIFFUSION MODELS

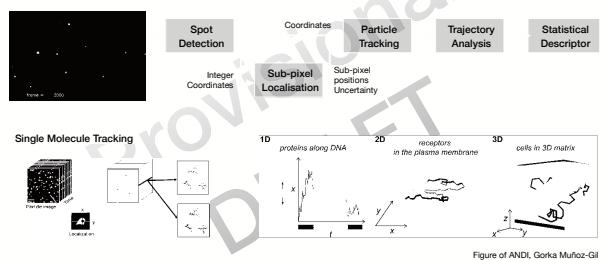
Brownian Diffusion



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Analysis of Molecular Diffusion

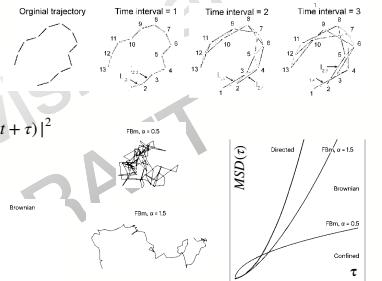


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Mean Square Displacement

- 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



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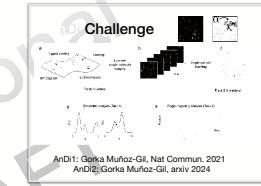
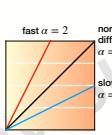
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Diffusion Models

Normal Diffusion

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

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



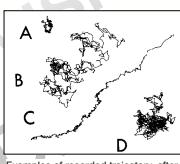
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Single-Molecule Tracking

The pioneer: Akito Kusumi

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



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

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

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Non-homogenous Diffusion

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

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



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

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

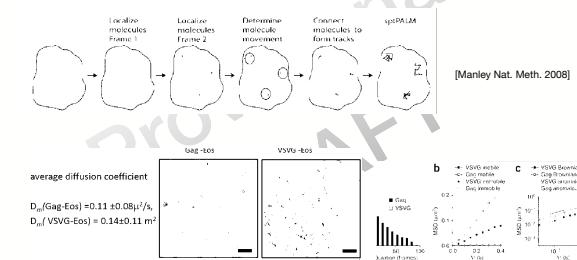
$$\text{MSD}_{zz}(\tau) \approx 4D_{zz}\tau$$

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

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Single Particle Tracking in SMLM

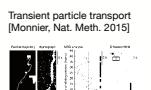


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Classification of Trajectories (1)

Diffusion coefficient in the presence of localization uncertainty? [Michalek, Physical Review 2010]



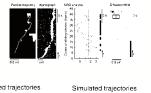
aTrack [Simon, eLife 2024]

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



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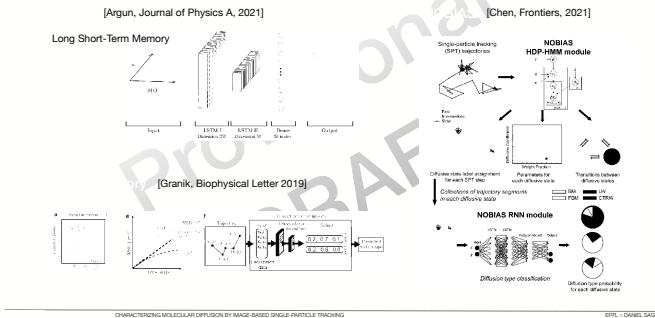
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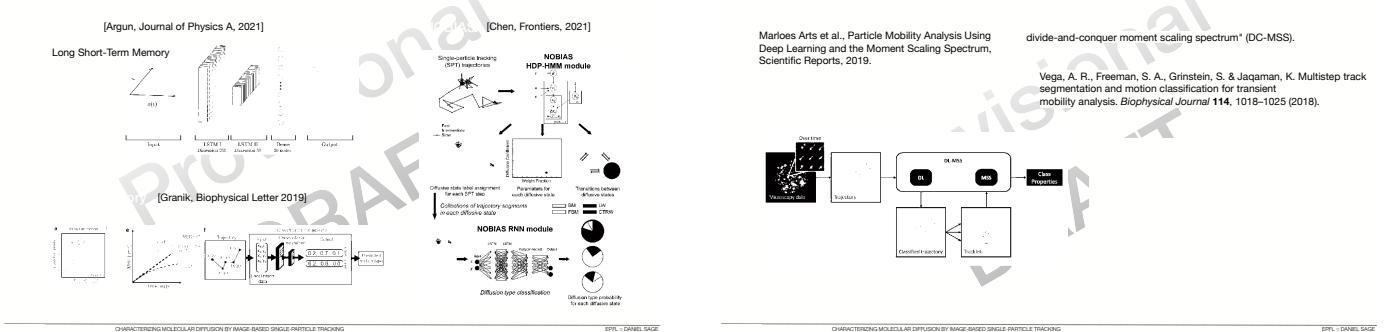
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Classification of Trajectories (2)



Multiple-states Tracklets



Trajectory Features

17 descriptive features of Pinhole
[Pinhole, PNAS 2021]

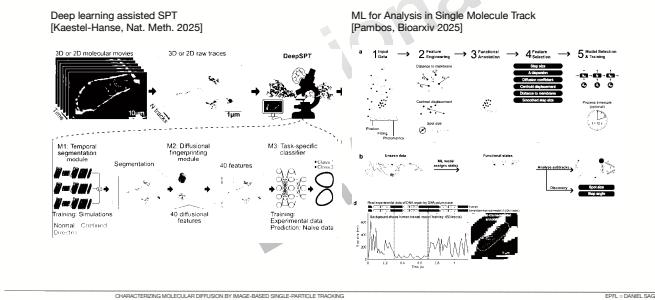
Feature name	Description
1	The total number of frames
2	The total number of frames with a particle
3	The total number of frames with a particle and a membrane
4	The total number of frames with a particle and a membrane and a hole
5	The total number of frames with a particle and a membrane and a hole and a hole radius
6	The total number of frames with a particle and a membrane and a hole and a hole radius and a hole radius
7	The total number of frames with a particle and a membrane and a hole and a hole radius and a hole radius and a hole radius
8	The total number of frames with a particle and a membrane and a hole and a hole radius and a hole radius and a hole radius and a hole radius
9	The total number of frames with a particle and a membrane and a hole and a hole radius
10	The total number of frames with a particle and a membrane and a hole and a hole radius
11	The total number of frames with a particle and a membrane and a hole and a hole radius
12	The total number of frames with a particle and a membrane and a hole and a hole radius
13	The total number of frames with a particle and a membrane and a hole and a hole radius
14	The total number of frames with a particle and a membrane and a hole and a hole radius
15	The total number of frames with a particle and a membrane and a hole and a hole radius
16	The total number of frames with a particle and a membrane and a hole and a hole radius
17	The total number of frames with a particle and a membrane and a hole and a hole radius

40 diffusional fingerprinting of DeepSPT
[Pambos, BioRxiv 2025]

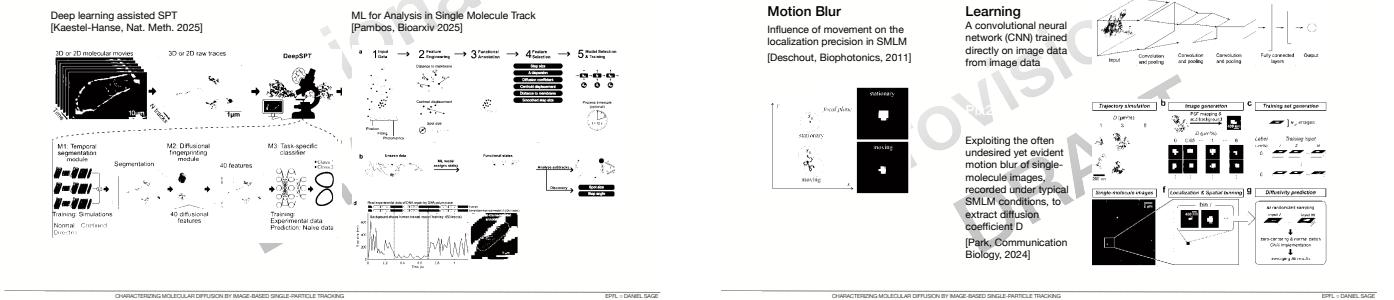
DeepSPT's features expanding on the work of Pinhole

- 60 engineered features engined TRACE
- 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
 - Comparison: comparison of two local context, dispersion

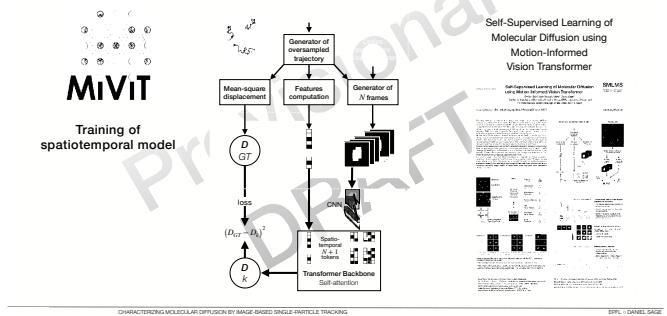
Learning from Trajectory Features



Learning from Image Patches



Motion-Informed Vision Transformer



Acknowledgments



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and many others

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