Anomalous Diffusion of mRNA in the Cytoplasm of HeLa Cells

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SCHOOL OF BIOMEDICAL ENGINEERING
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Talk Outline

- Motivation
- Diffusion & Mean Square Displacement (MSD)
- Microscopy & Single Particle Tracking (SPT)
- Results and Analysis
- Conclusion / Future Aims

Motivation

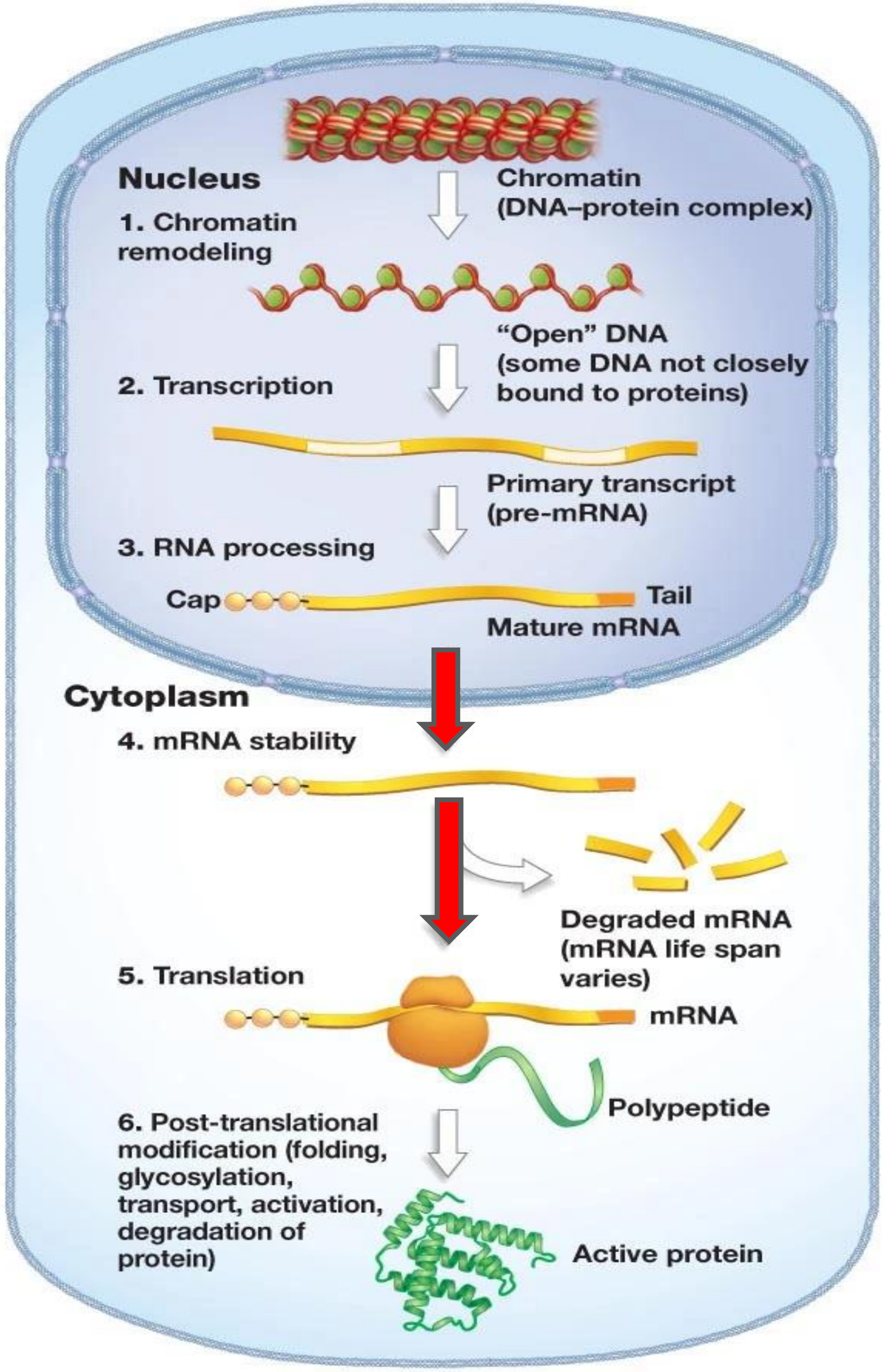
- DNA → mRNA → Protein Synthesis

- mRNA must diffuse out of the nucleus, then find ribosomes
  - Ribosomes may also be moving

- miRNA regulation of gene expression
  - miRNA must locate mRNA and gene seq.

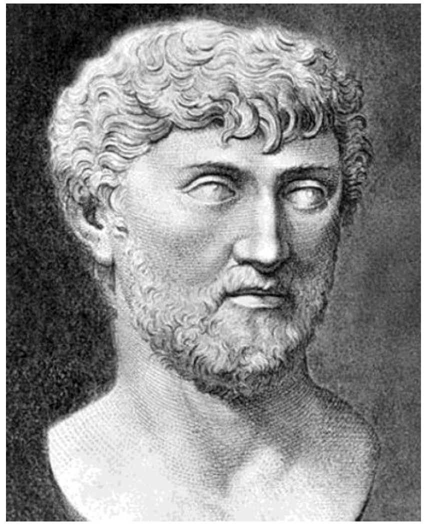
- Intracellular structures can dictate diffusive behavior

- We study HeLa cells because they are human cells



Russell, Peter J. *IGenetics: A Molecular Approach* (2010).

Diffusion

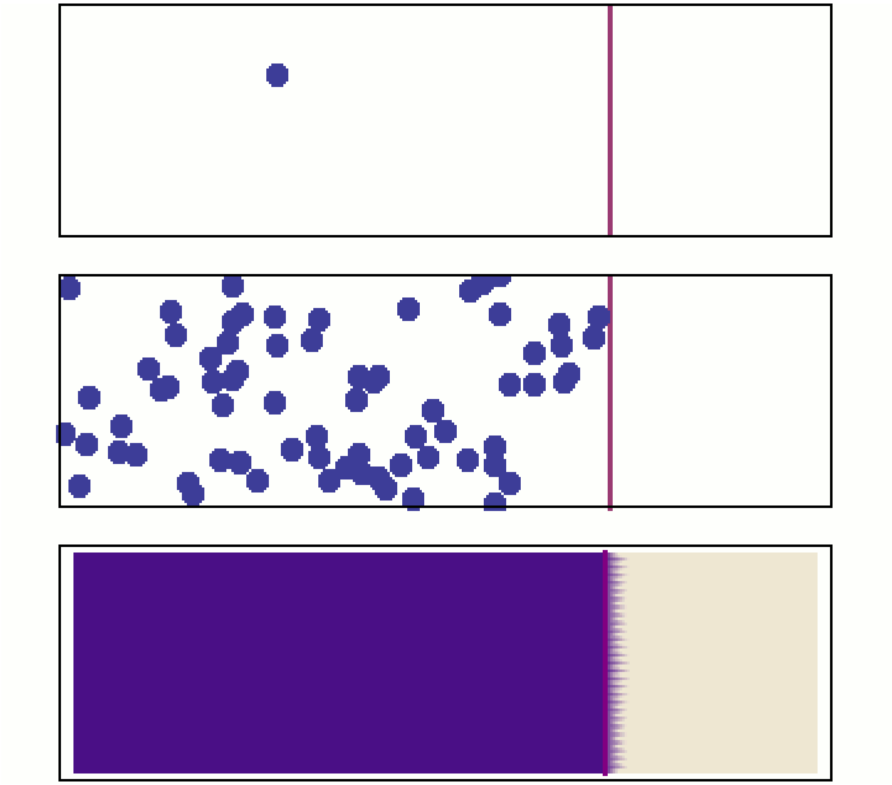


"*Thus motion ascends from the primevals on, and stage by stage emerges to our sense, until those objects also move which we can mark in sunbeams, though it not appears what blows do urge them.*" **–Titus Lucretius Carus (60 BCE)**

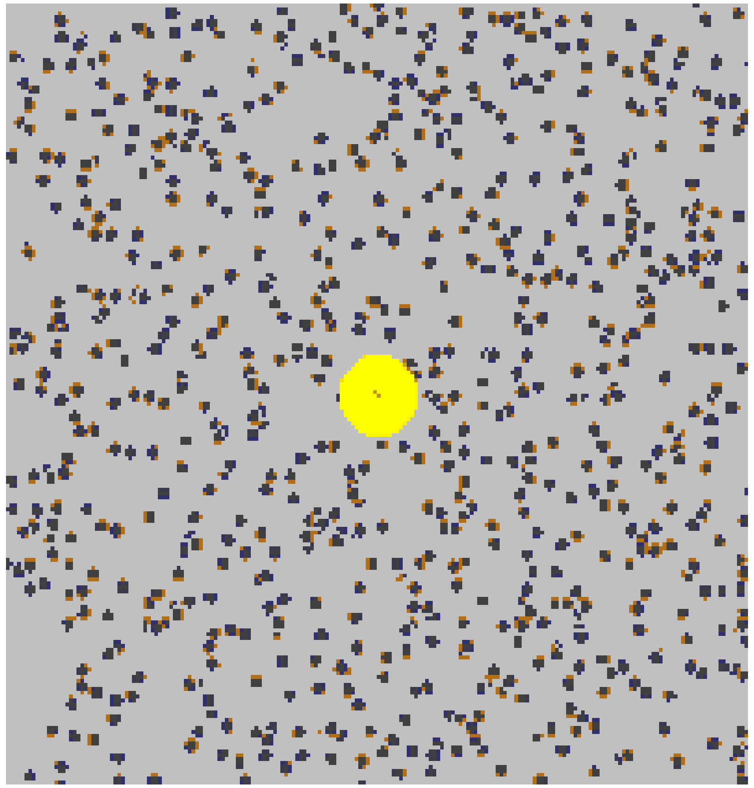
Robert Brown (1827) observed when a pollen granule (~5 μm) is immersed in water, the microscopic movement of the water molecules (~3 Å) gives rise to the random motion of the pollen.

Titus Lucretius and William Ellery Leonard. (2008)
Helmut Mehrer and Nicolaas A Stolwijk. (2009)

Diffusion



Macroscopic



Microscopic

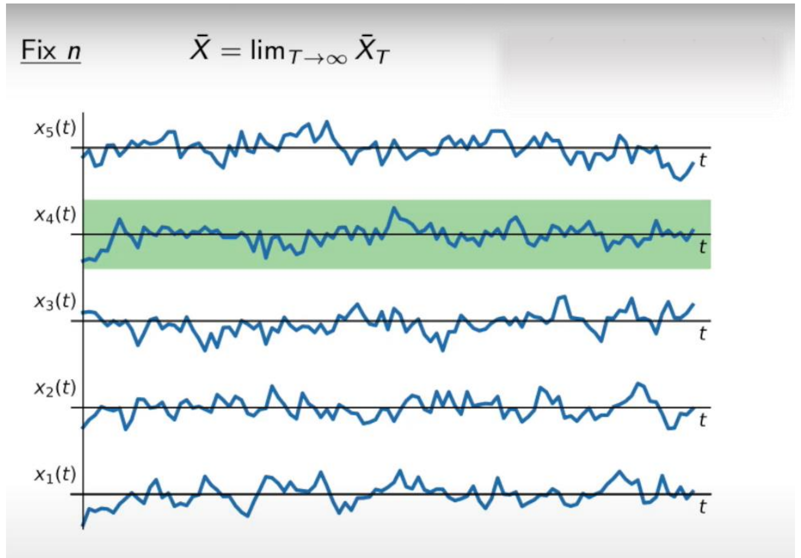
Brownian motion:

- Driven by thermal fluctuations
- Independent, uncorrelated displacements
  - No memory
- Ergodic

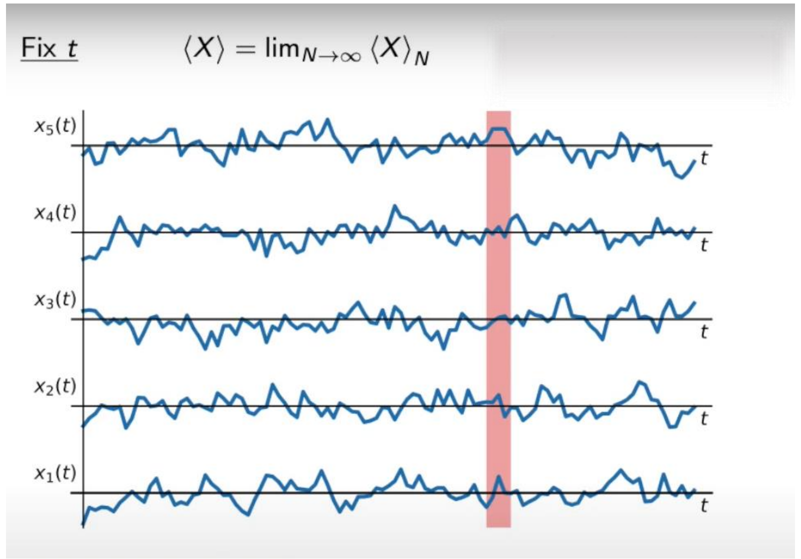
jemdoc + MathJax, *MIT.edu*, (2017).

Ergodicity

Time-averaged (TA)

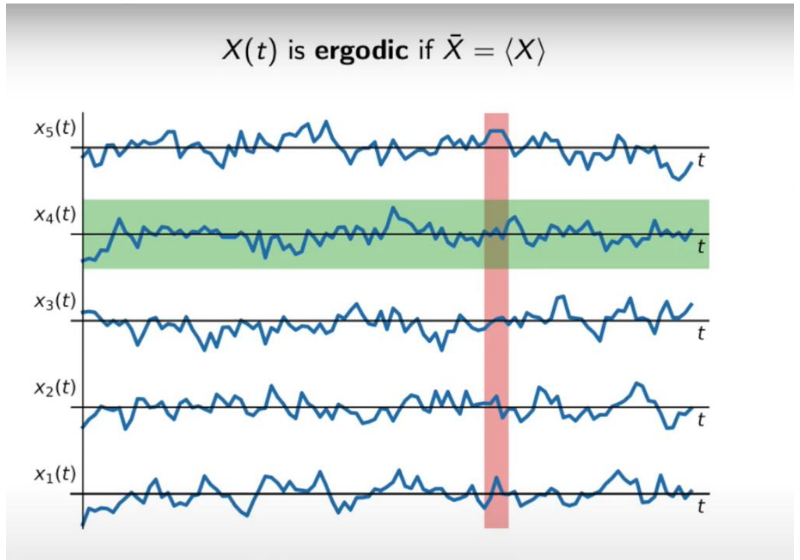


Ensemble-averaged (EA)



Ergodic when TA = EA

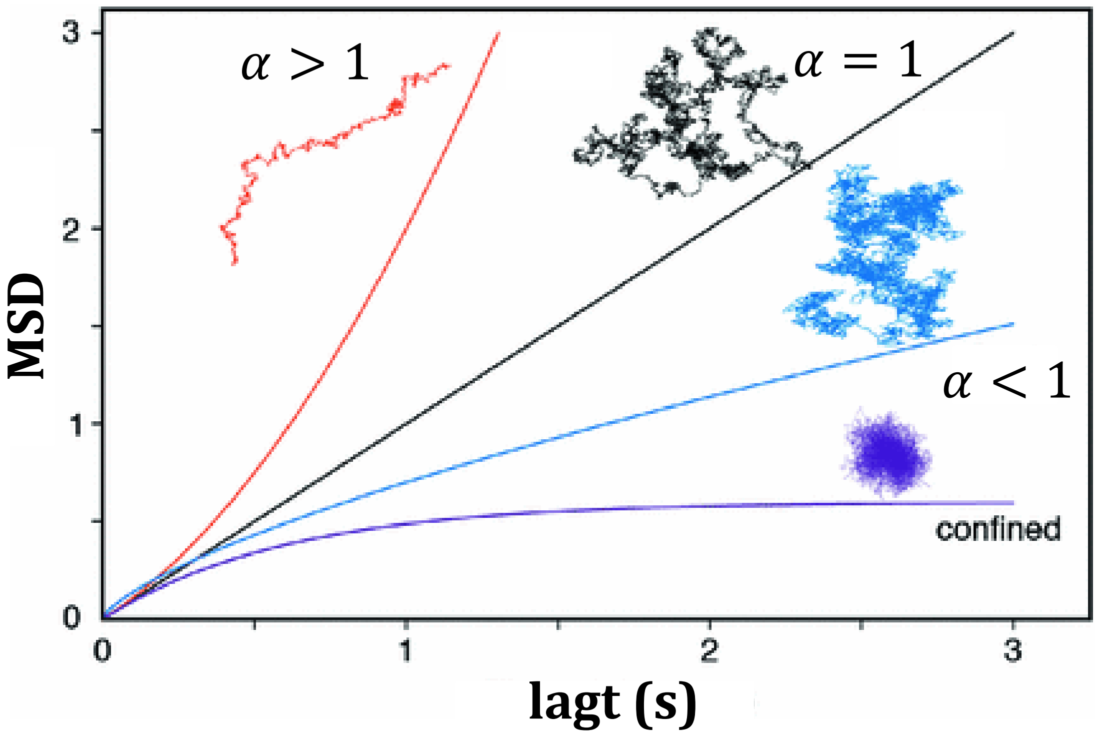
for T $\longrightarrow$ ∞ and N $\longrightarrow$ ∞



Alex Adamou, *Ergodicity TV*, (2021).

Mean Square Displacement (MSD)

TA-MSD: $\overline{\delta^2(t_{\text{lag}})} = \frac{1}{T_m - t_{lag}} \int_0^{T_m - t_{lag}} \left[r(\tau + t_{\text{lag}}) - r(\tau)\right]^2 \mathrm{d}\tau$



Brownian motion:

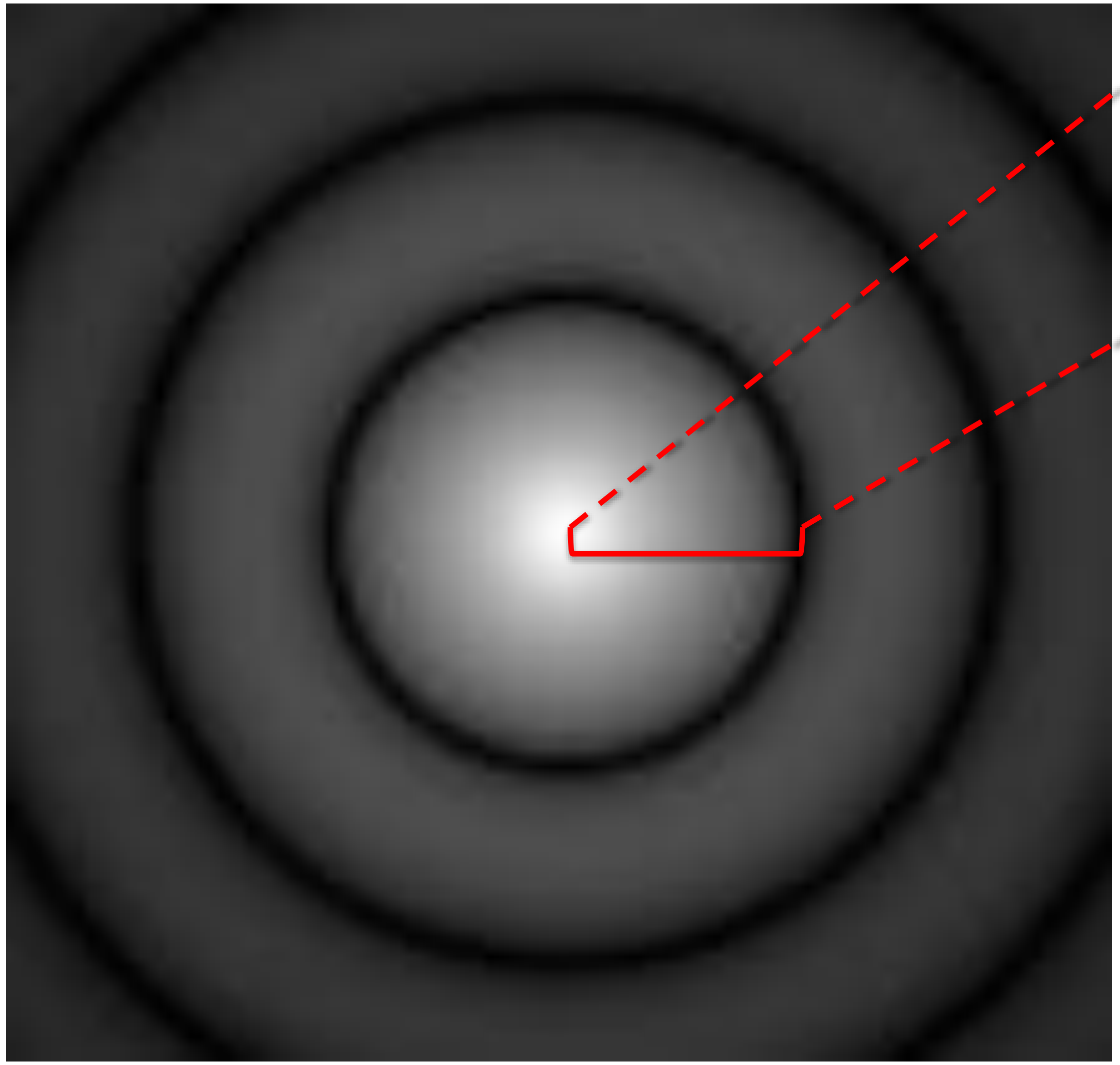
- $< x^2(t) > \sim$ t
- MSD linear w/ time

Anomalous Diffusion:

- $< \boldsymbol{x^2(t)} > \sim \boldsymbol{t^{\alpha}}$
- Where $\alpha \neq 1$
- Does not obey the definitions of Brownian motion

Adapted from Ortega Arroyo, J. *Springer Theses*, (2018).

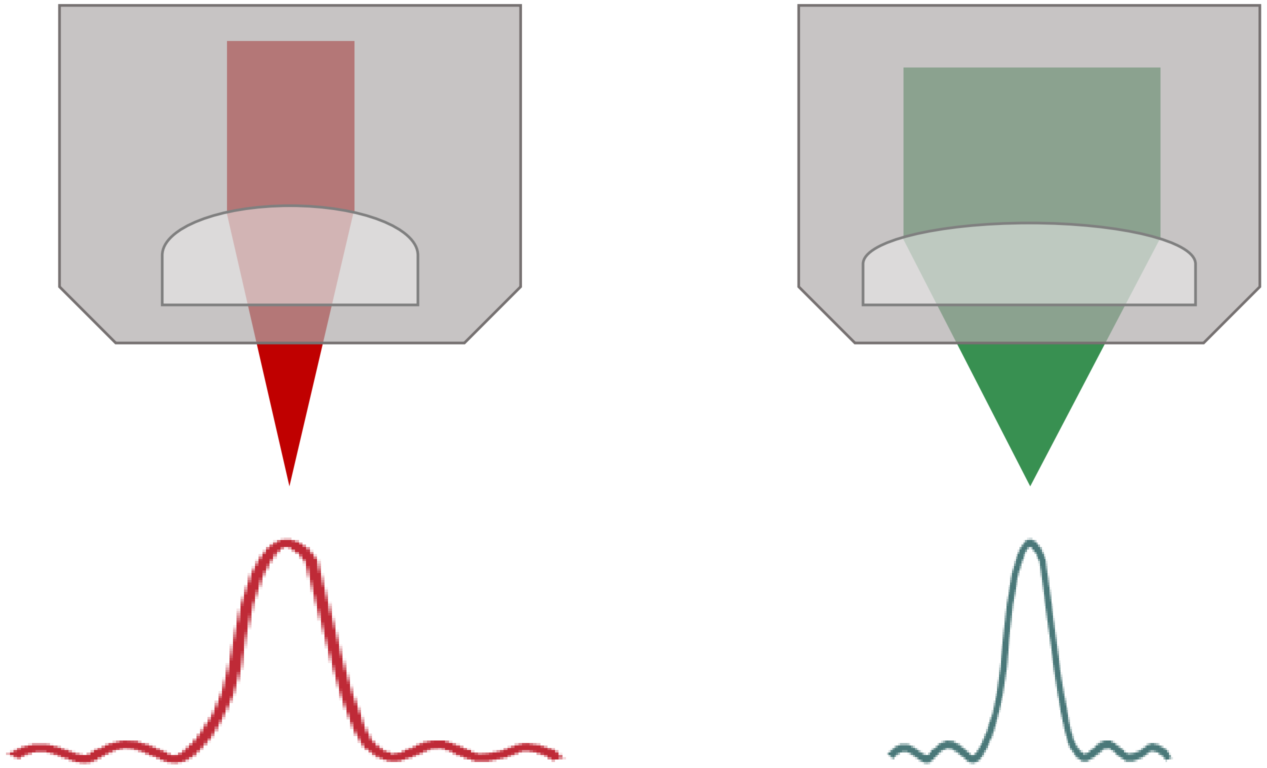
Microscopy: Diffraction Limit



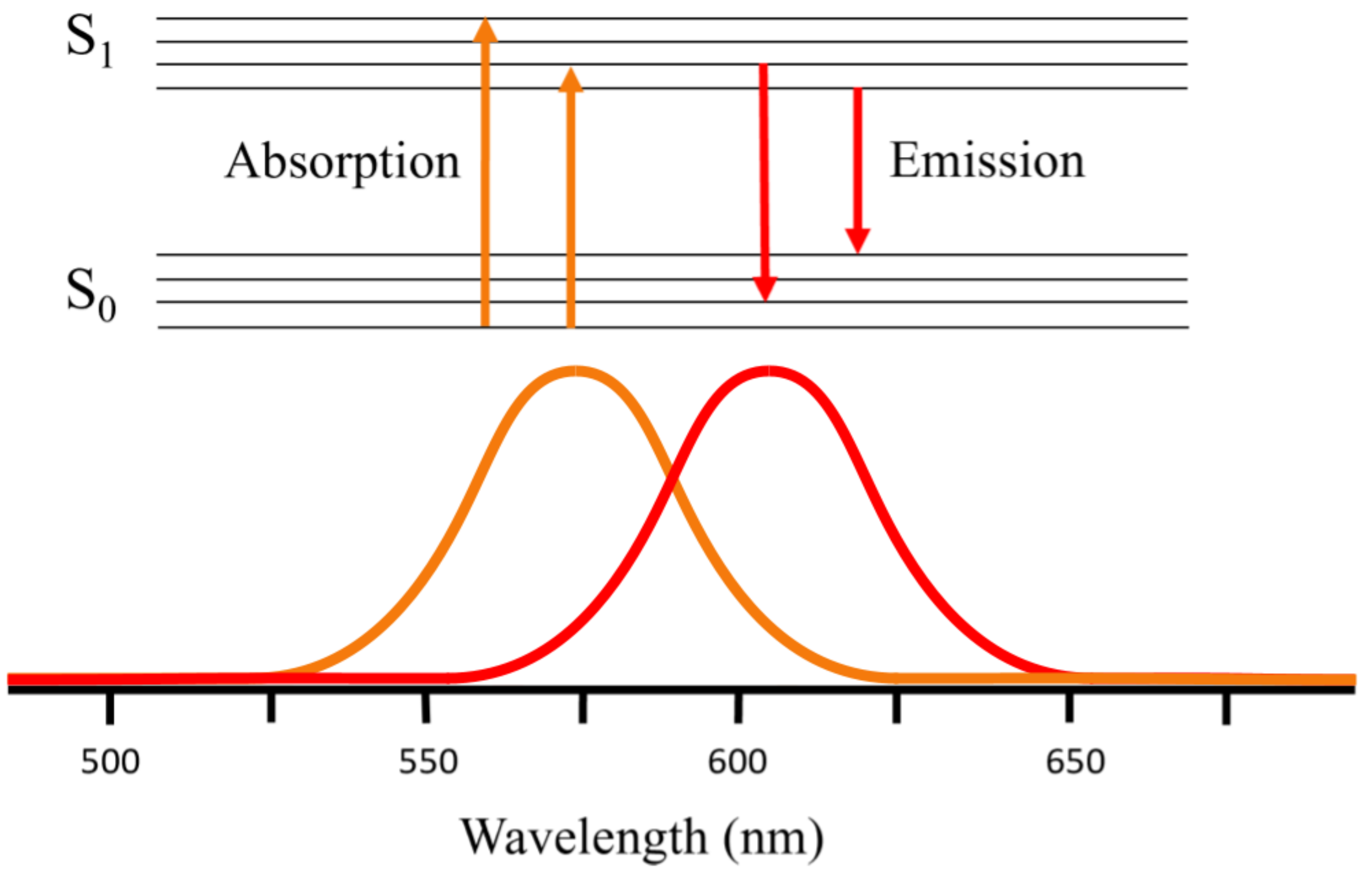
Greg Hollows, Nicholas James, *Edmund Optics*

$$r_{Airy} \sim \frac{\lambda_0}{NA_{obj}}$$

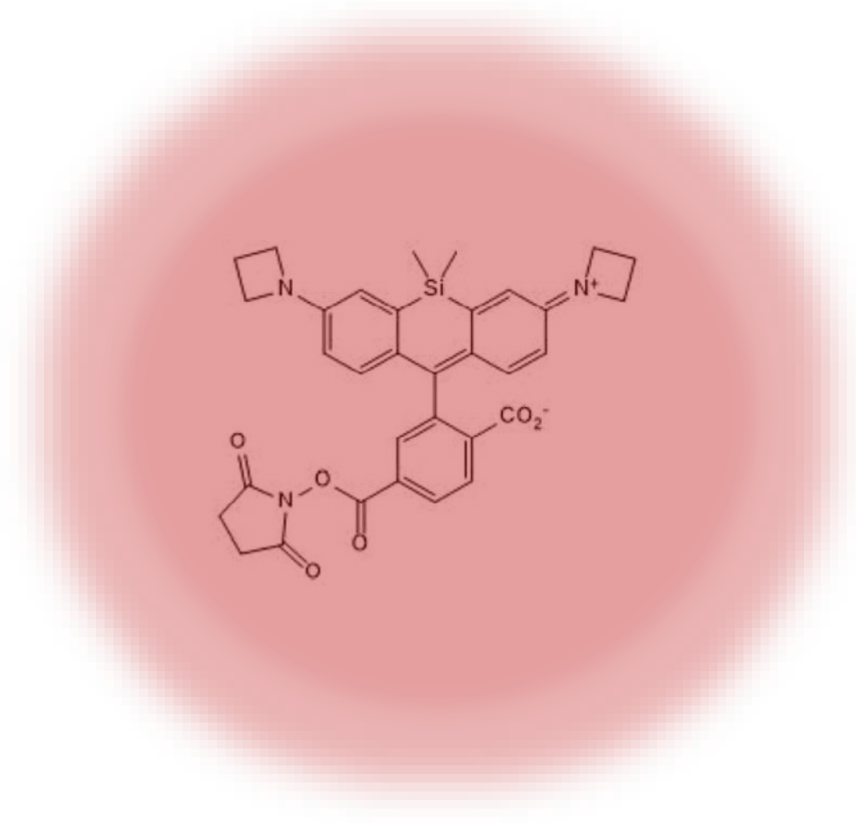
- λ_0 - Incident wavelength of light
- NA_{obj} - Numerical aperture of objective lens



Fluorescence Microscopy



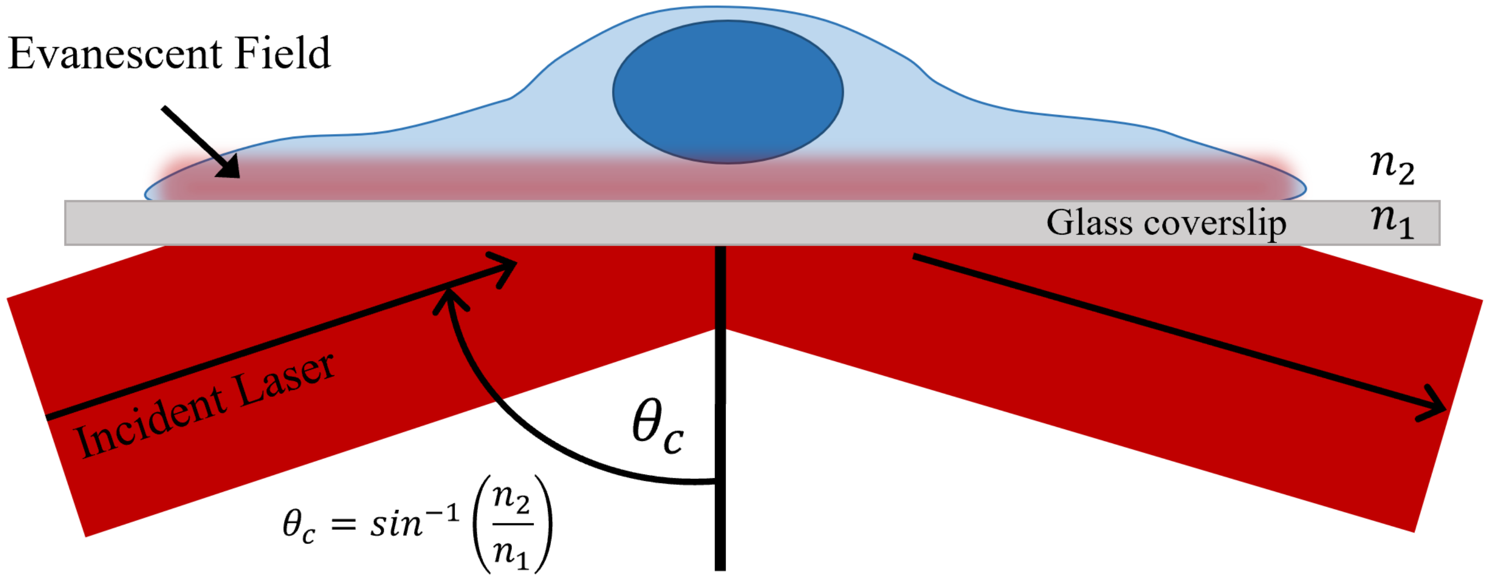
Janelia Fluor (JF) 646



Adapted from Lichtman, Jeff W. and Conchello, Jose Angel. (2005)

Microscopy: TIRF

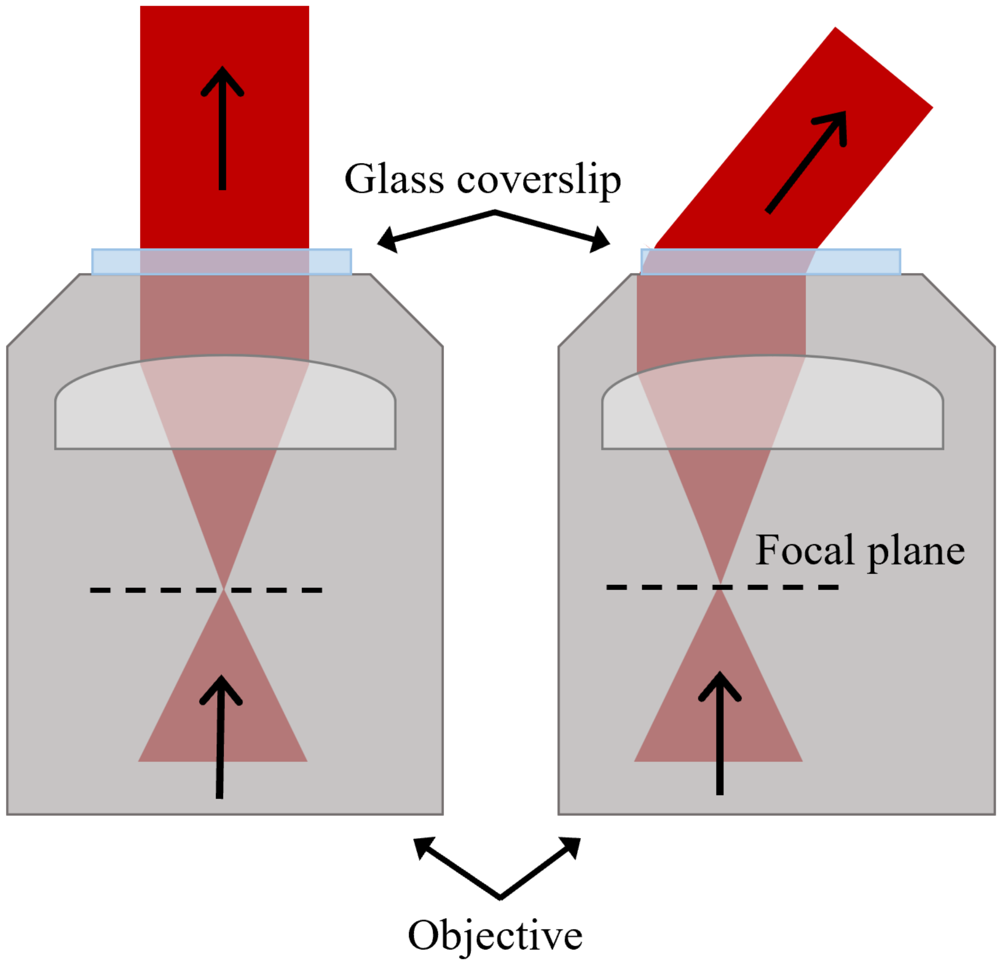
Total Internal Reflection (TIRF)



TIRF Variations

Epifluorescence

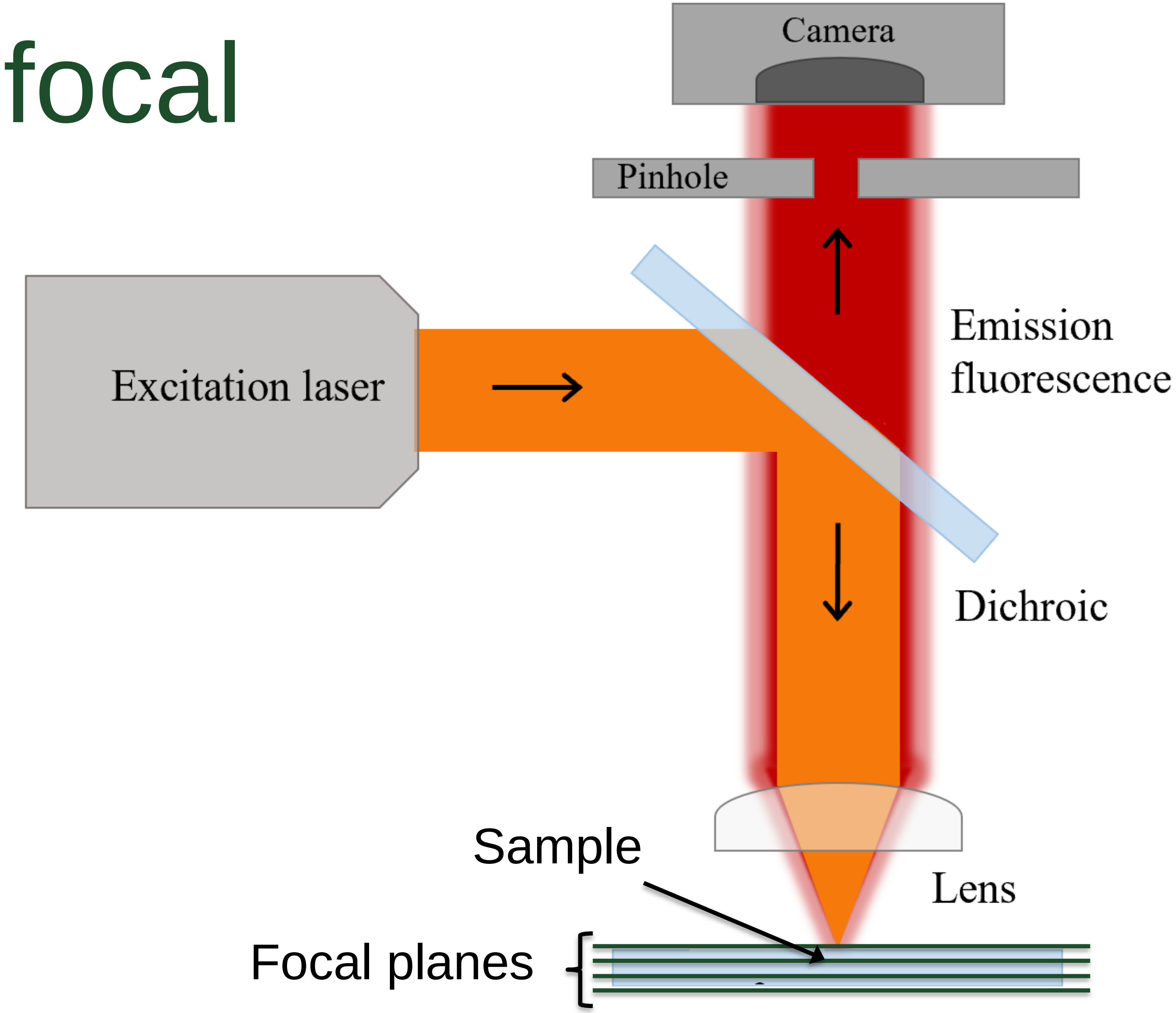
Oblique Illumination



Adapted from Adrien Mau et al. *bioRxiv*. (2020).

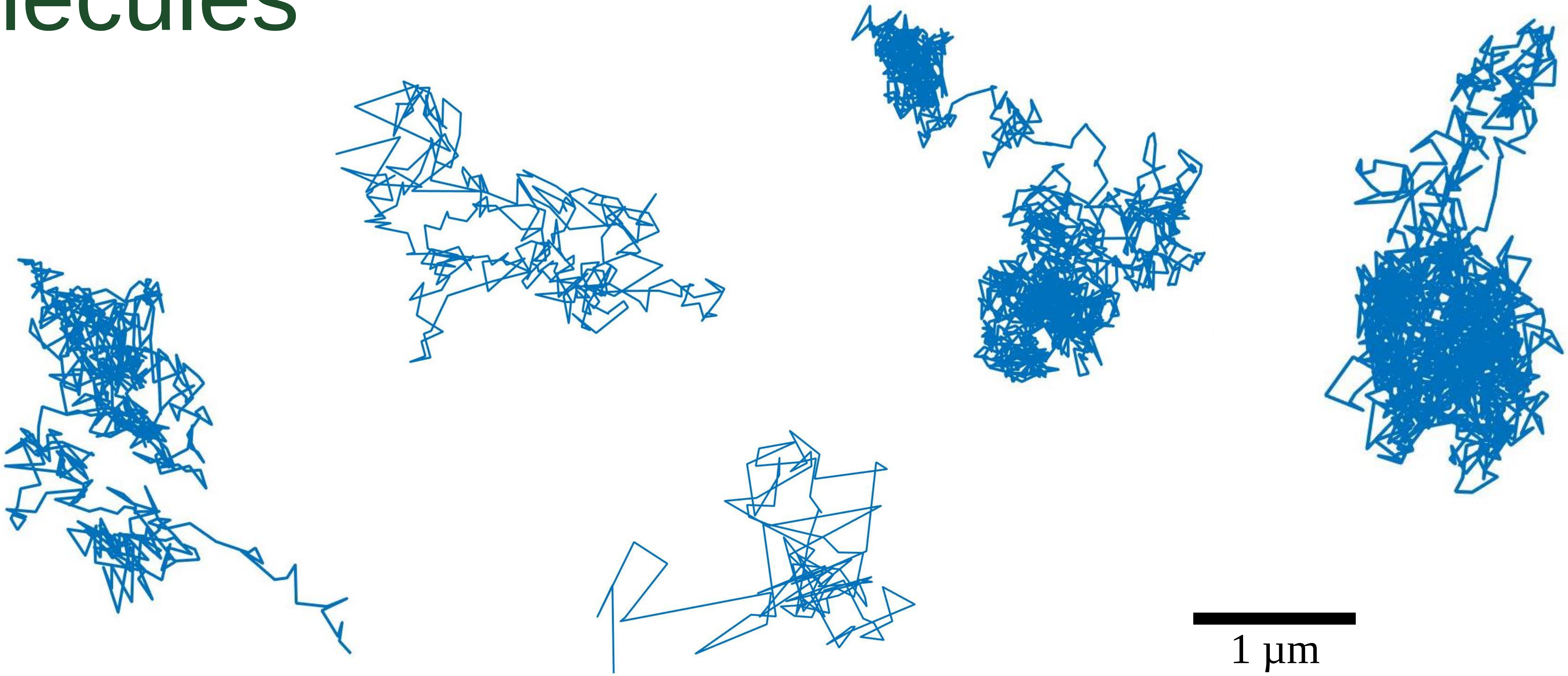
Microscopy: Confocal

Confocal Fluorescence Microscopy

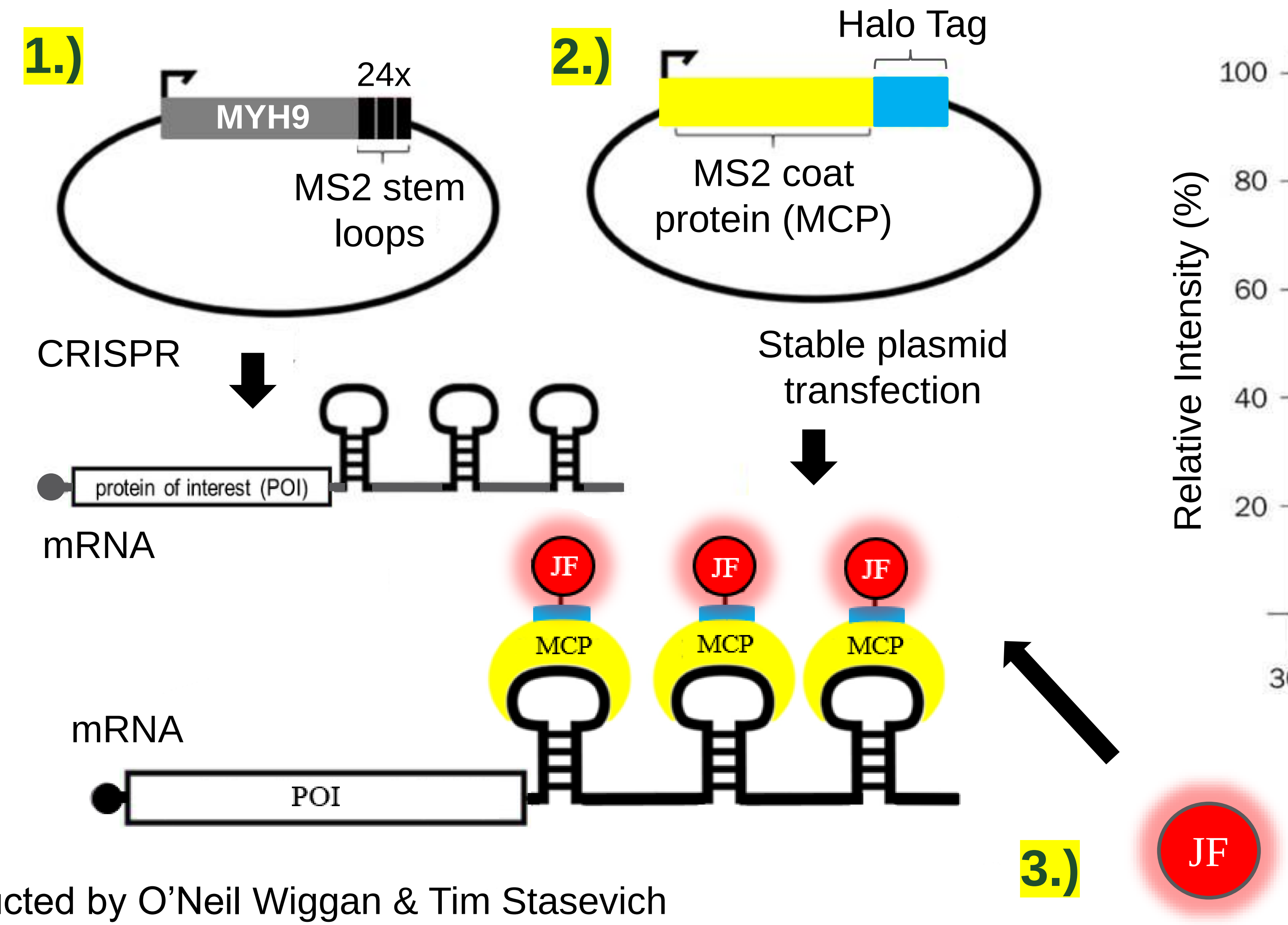


Adapted from Adaobi Nwaneshiudu et al. *Journal of Investigative Dermatology* (2012)

Single-particle Tracking of mRNA Molecules

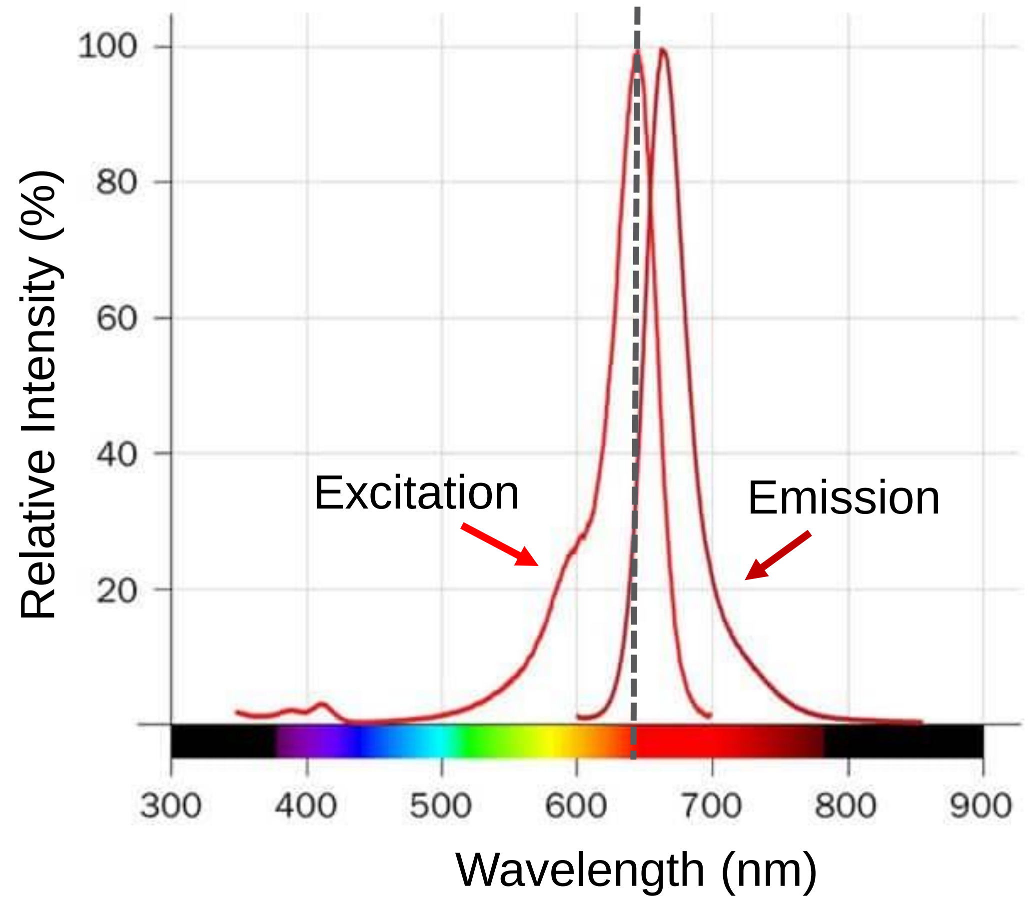


SPT: Labeling



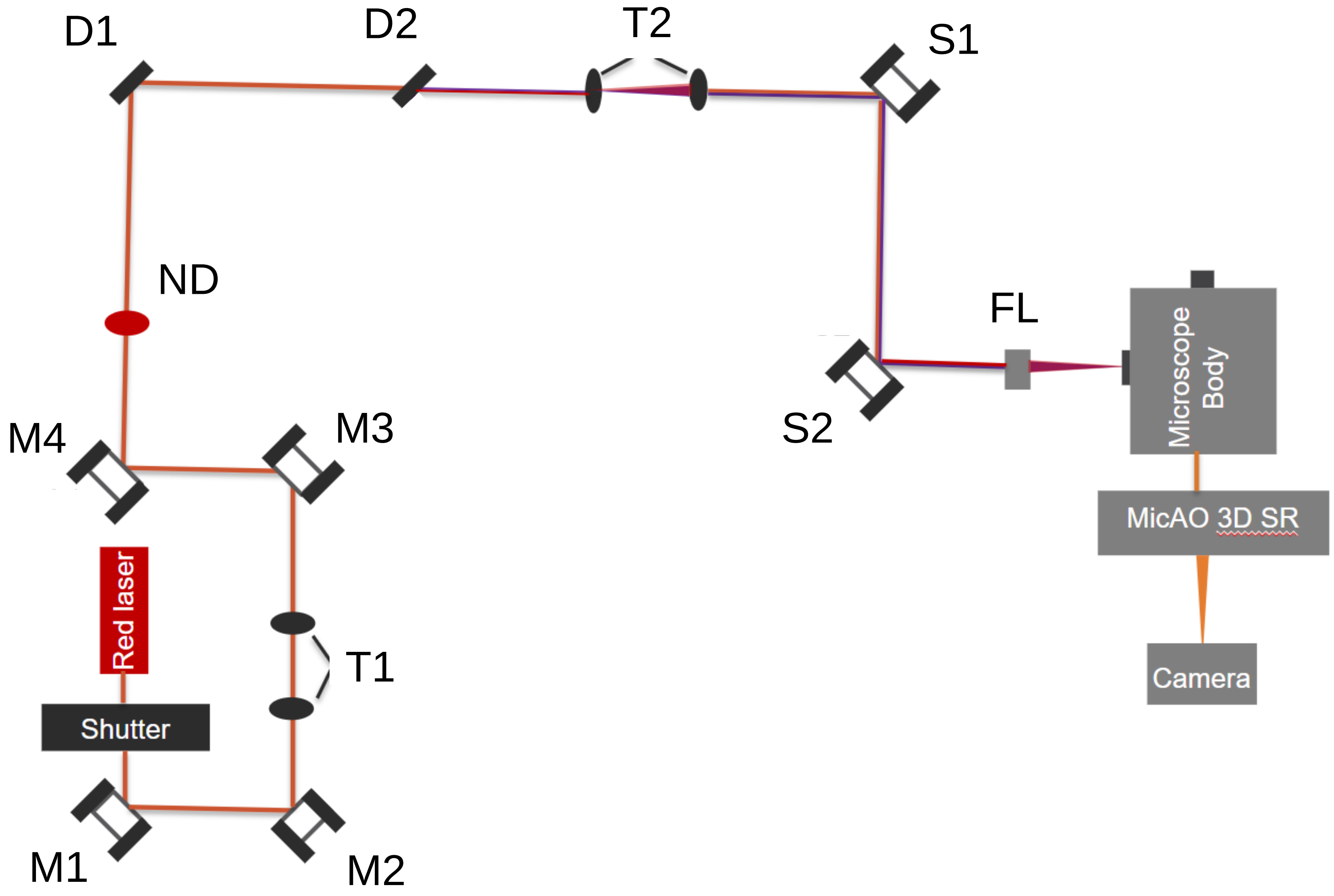
JF – Halo ligand

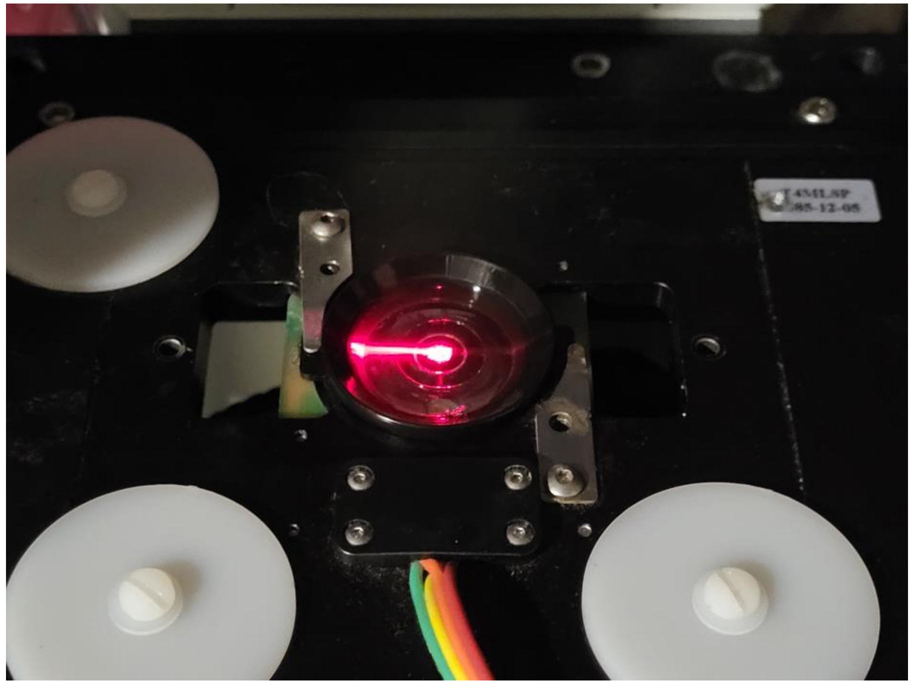
Janelia Fluor (JF) 646 Spectrum



Constructed by O’Neil Wiggan & Tim Stasevich
Morisaki T, et al.TJ. *Science*. (2016)

SPT: Imaging Setup (TIRF)

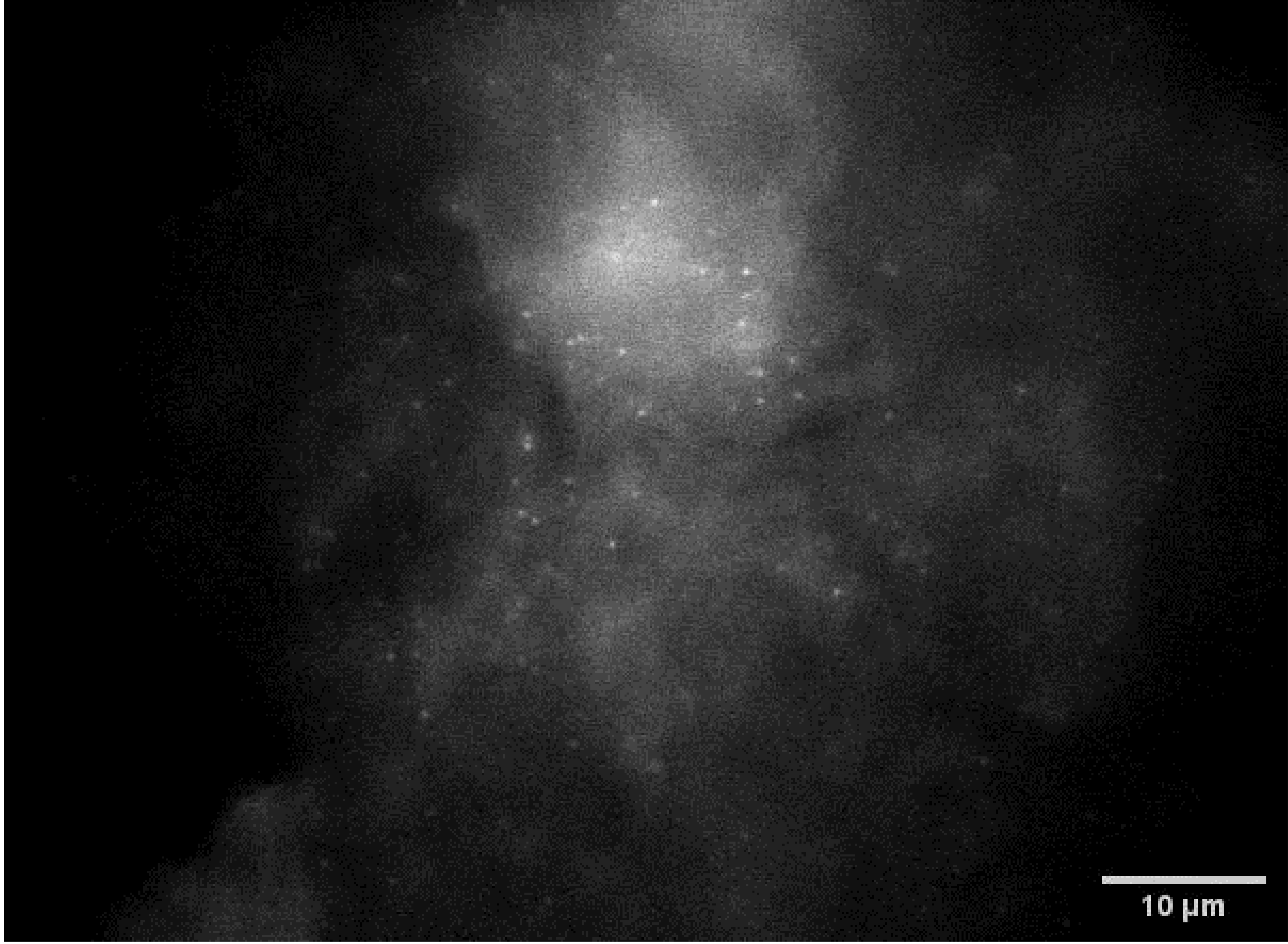




Adapted from Reshma Sunny, (2022)

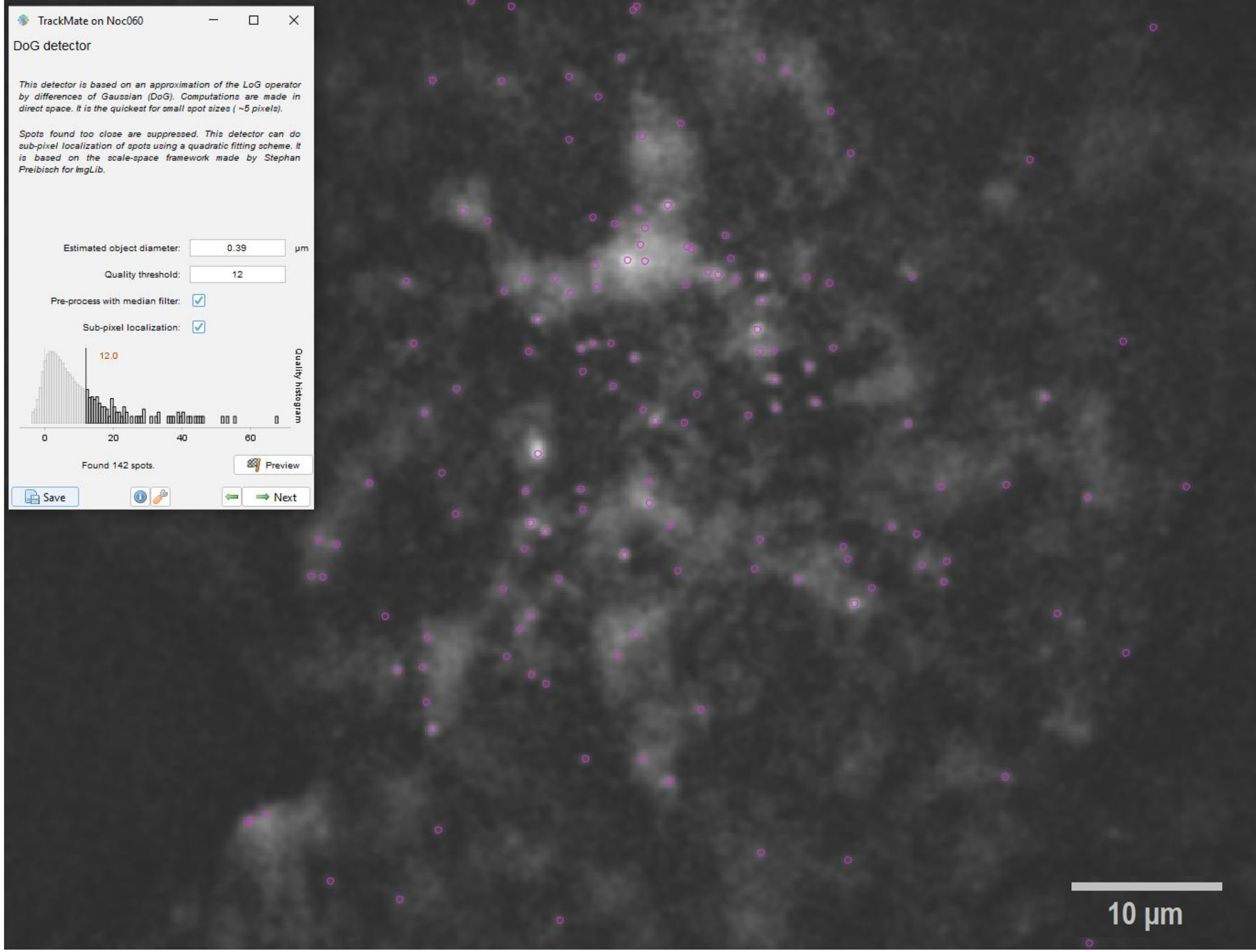
SPT: Imaging

- Data from our TIRF microscope in Scott
- 10 fps
- Tracking:
  - TrackMate plugin (ImageJ) for automated tracking of RNAs



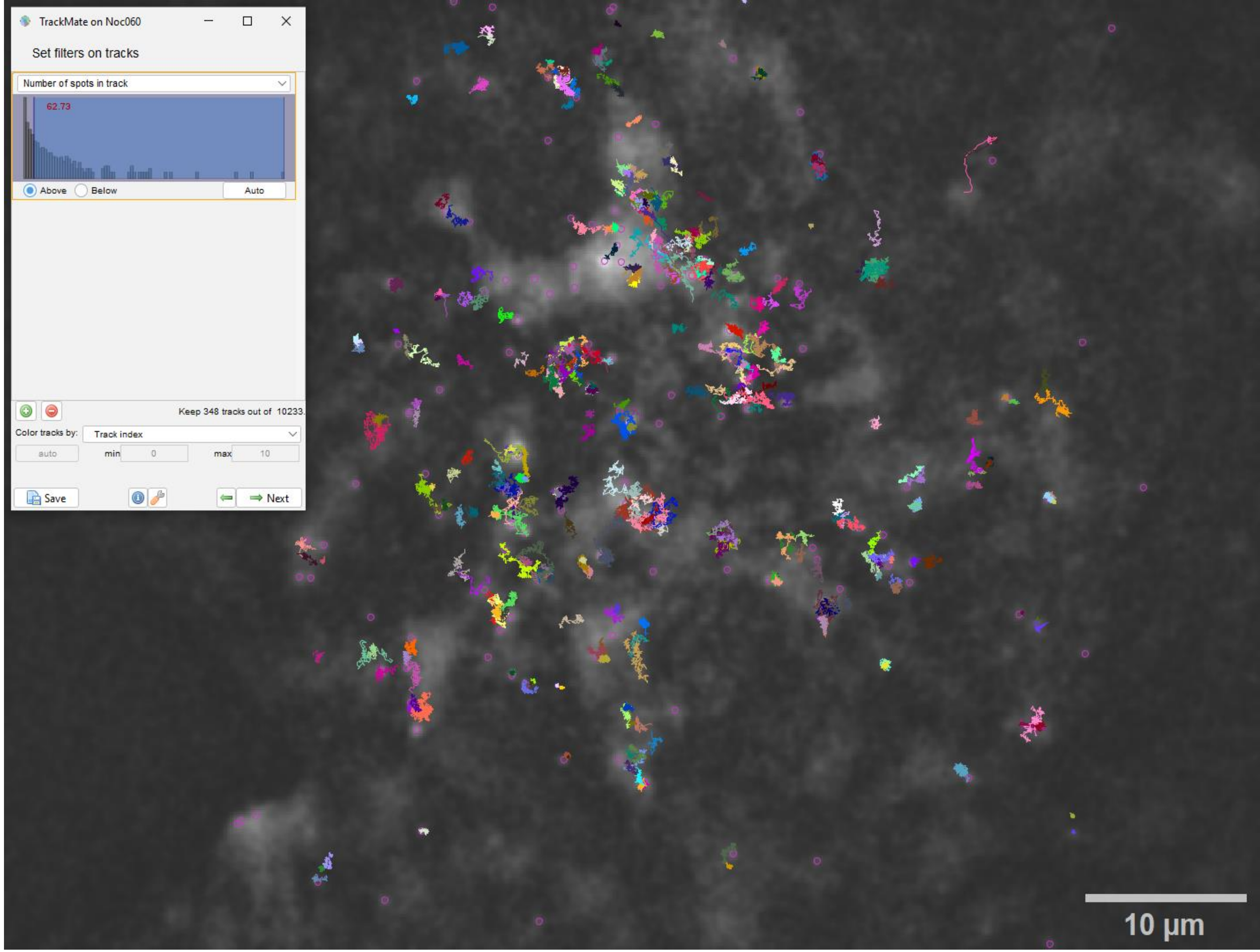
Tracking

- TrackMate (ImageJ)
  - Background subtraction
  - Gaussian blur
  - Particle diameter
    - ~0.39 µm
    - 1 px = 0.13 µm
  - Intensity threshold



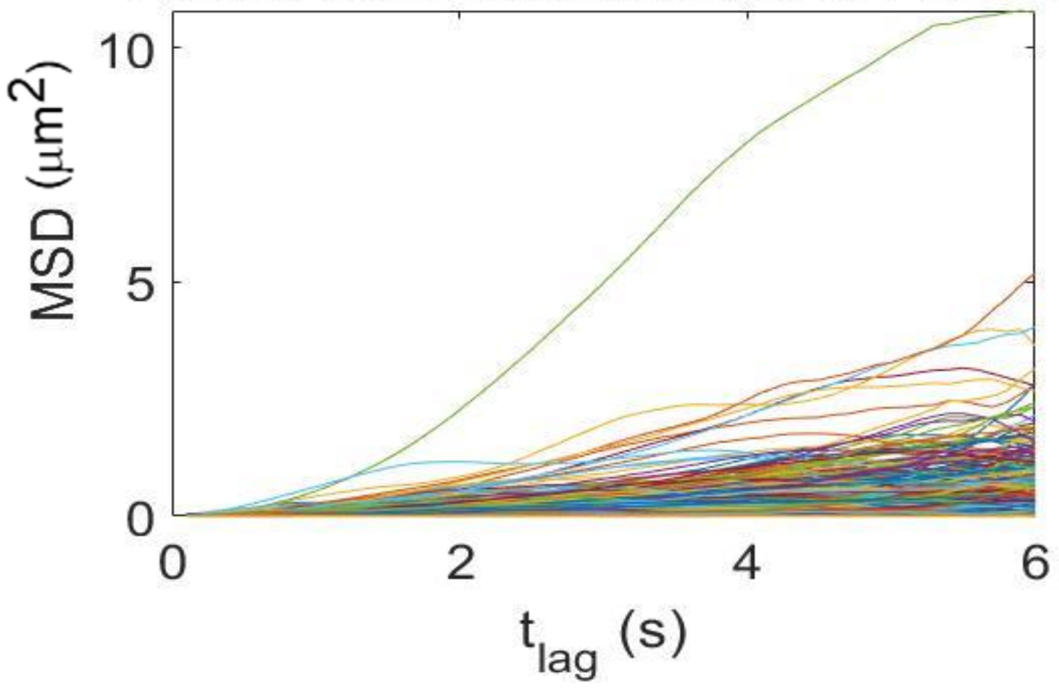
Tracking

- TrackMate (ImageJ)
  - Linking max distance
  - Gap-closing max frame gap
  - Gap-closing max distance
  - Trajectories filtered for > 62 frames in length, colored by ID

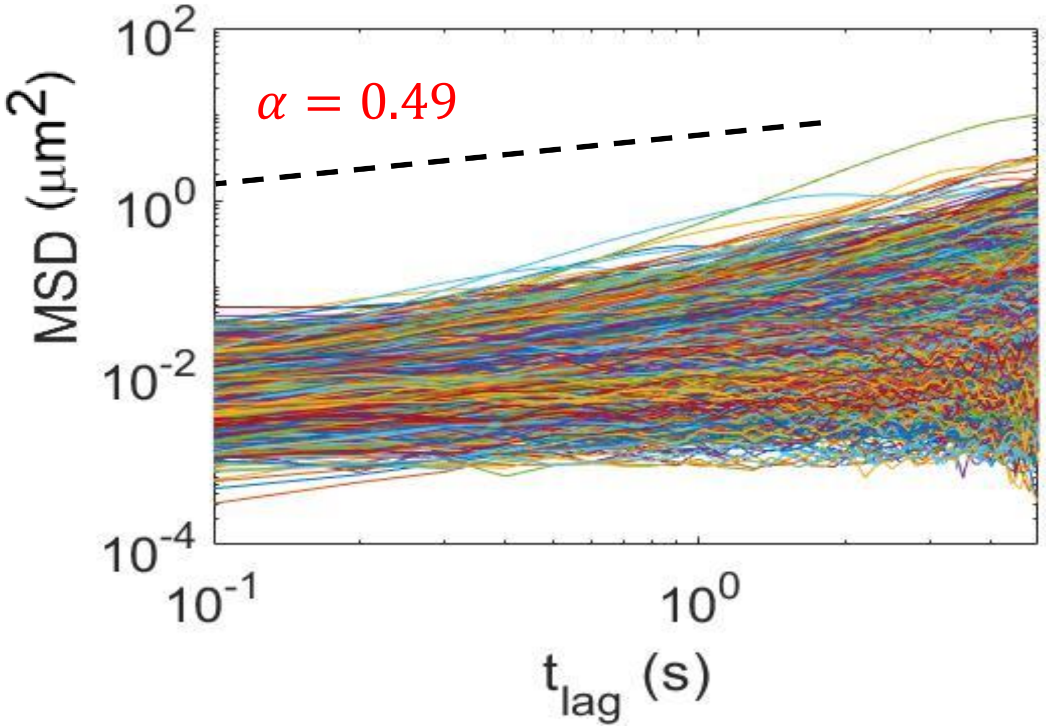


Analysis

Individual TA-MSDs
(5,820 trajectories)



Individual TA-MSDs
(5,820 trajectories)

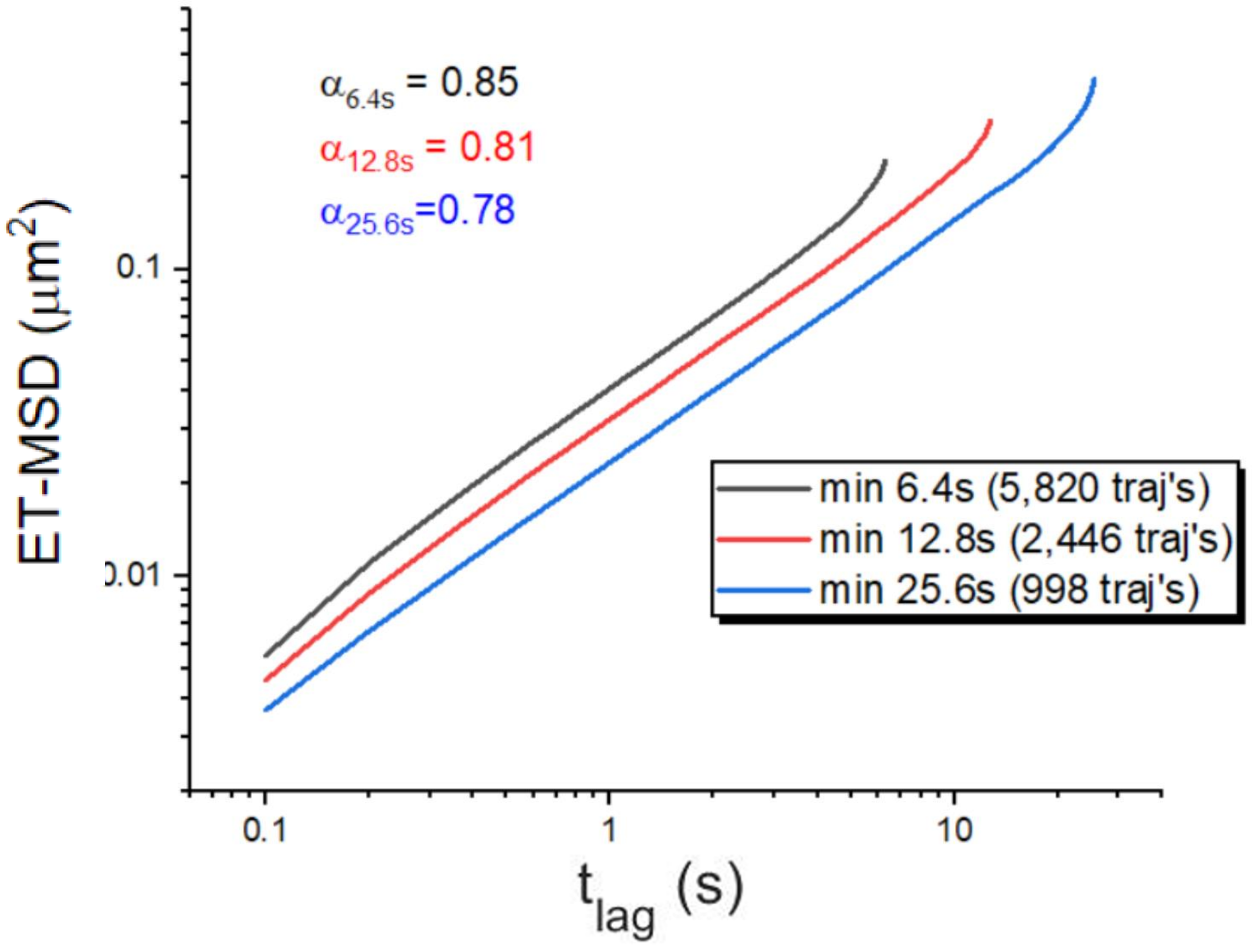


- Anomalous diffusion → Subdiffusive on average
- Ergodicity breaking (*D* varies for each trajectory)

Analysis

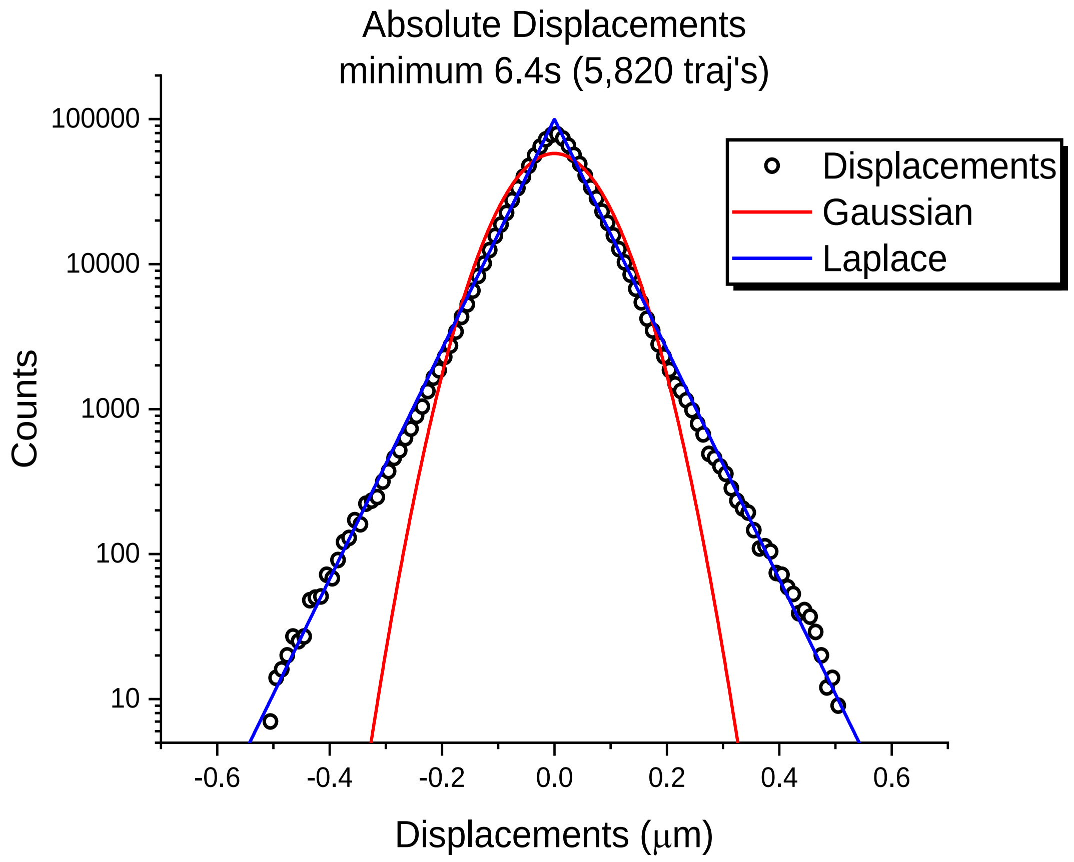
ET-MSD: $$\overline{\langle \delta^2 (t_{\text{lag}}) \rangle} = \frac{1}{T_m - t_{\text{lag}}} \left\langle \int_0^{T_m - t_{\text{lag}}} [\mathbf{r}(\tau + t_{\text{lag}}) - \mathbf{r}(\tau)]^2 \mathrm{d}\tau \right\rangle$$

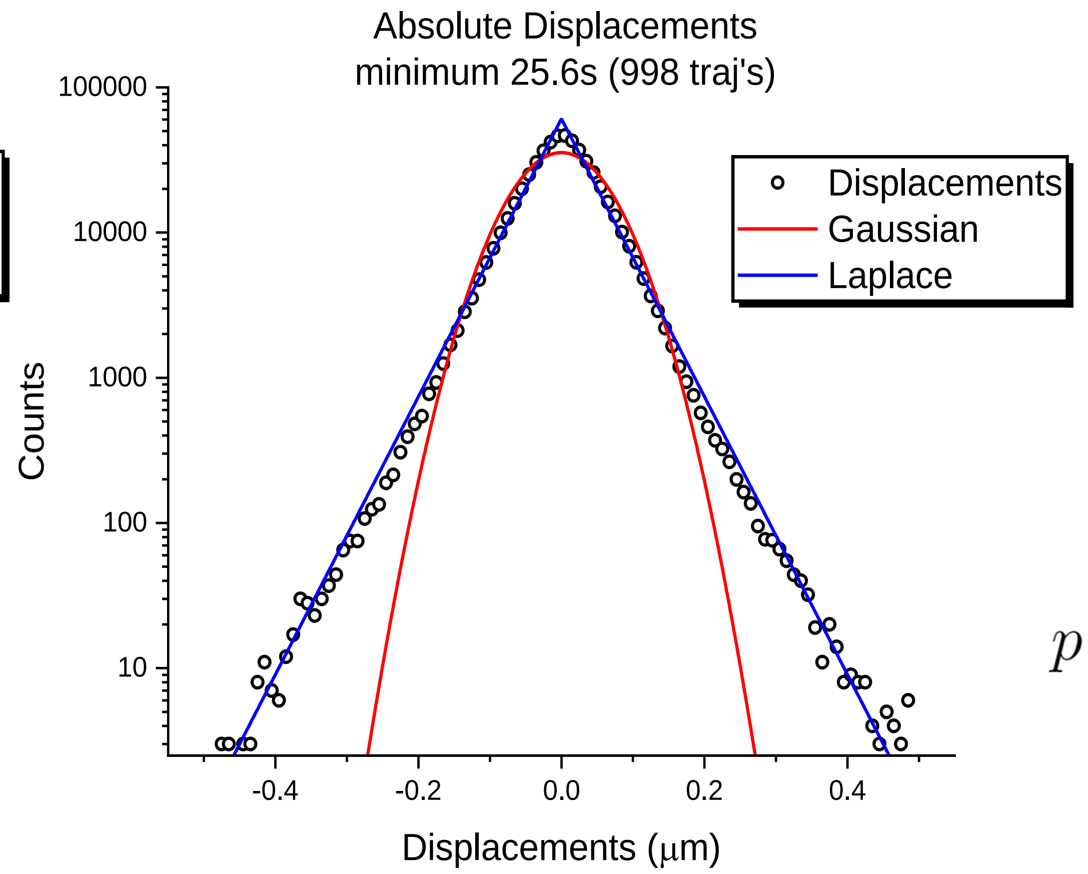
mRNA ET-MSDs



- Subdiffusive
- Maybe a bias for confined trajectories to be longer because TrackMate doesn’t lose them as fast

Distribution of Displacements





Gaussian:

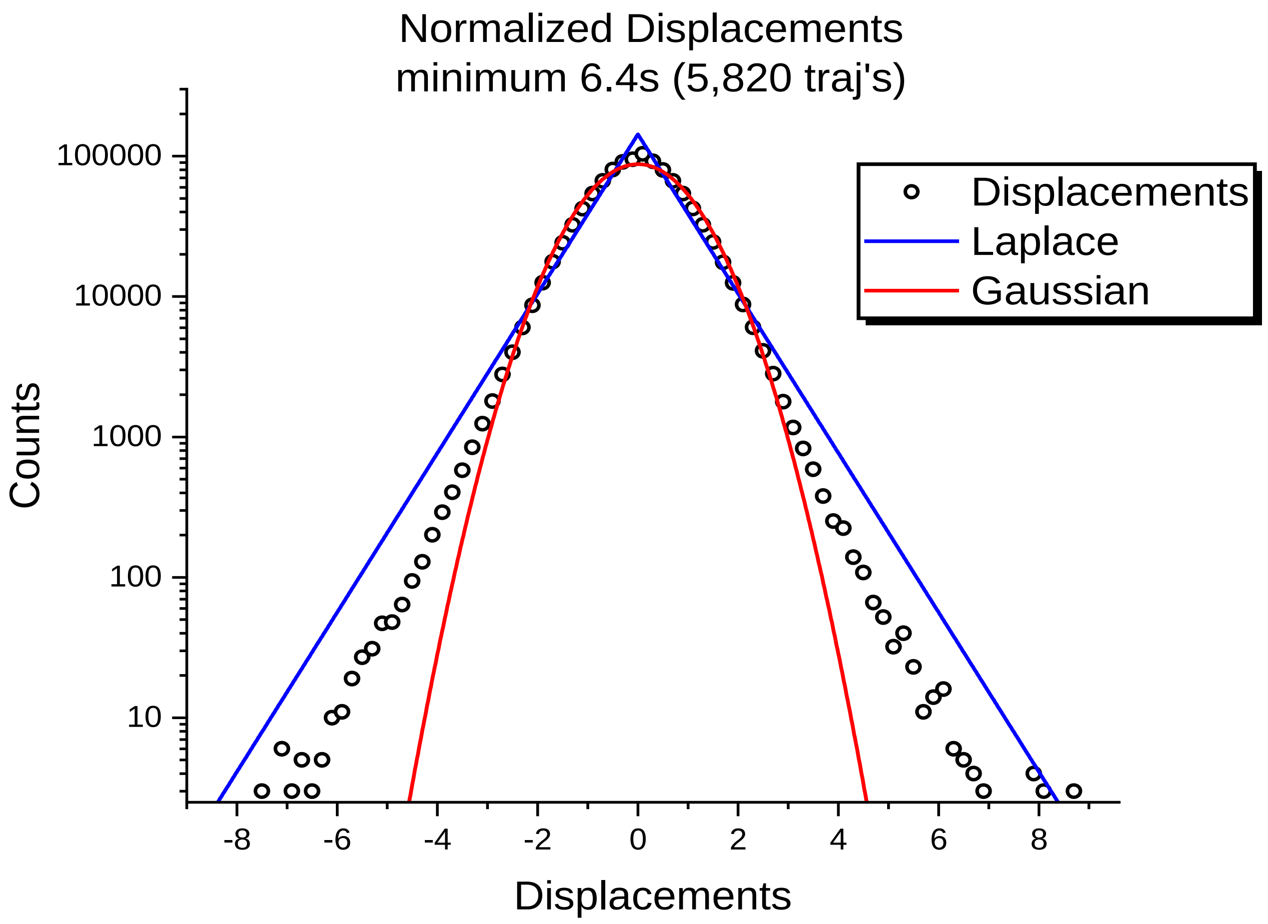
$$p\left(x\right) = \frac{1}{\sigma\sqrt{2\pi}}e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

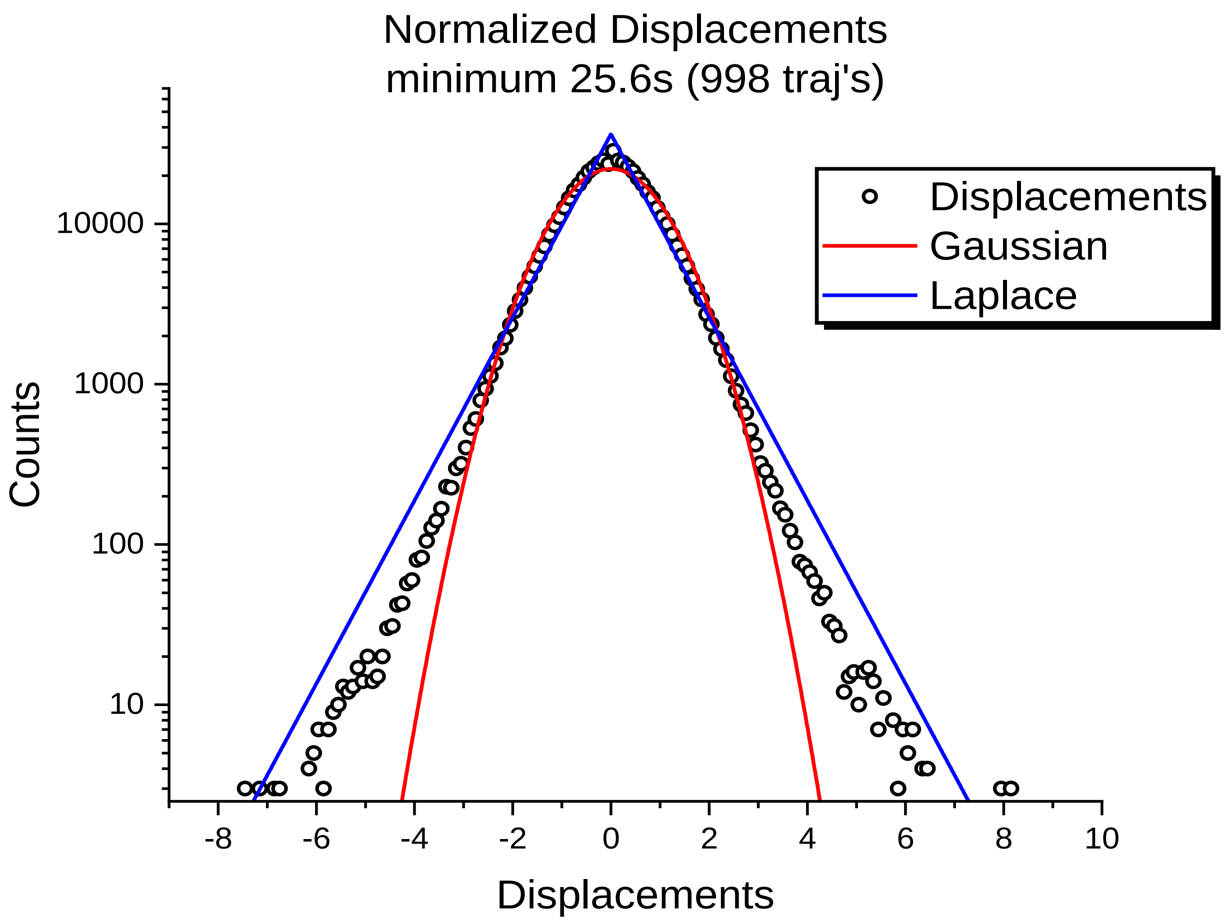
Laplace:

$$p\left(x\right) = \frac{1}{2}e^{-|x|}$$

Laplace distribution: heterogeneity across trajectories

Distribution of Displacements

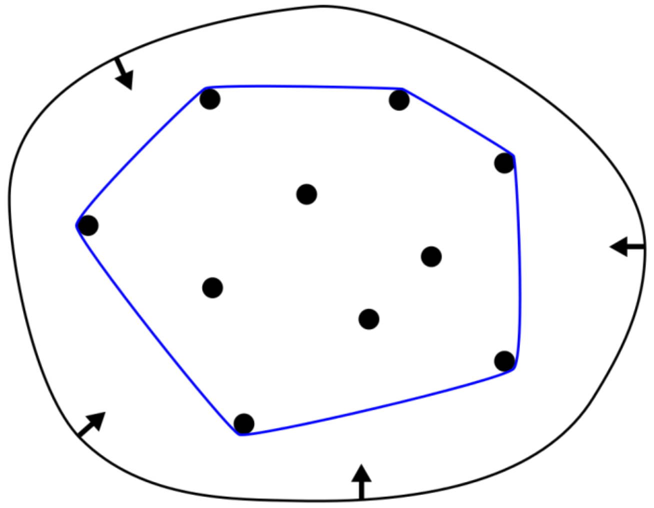




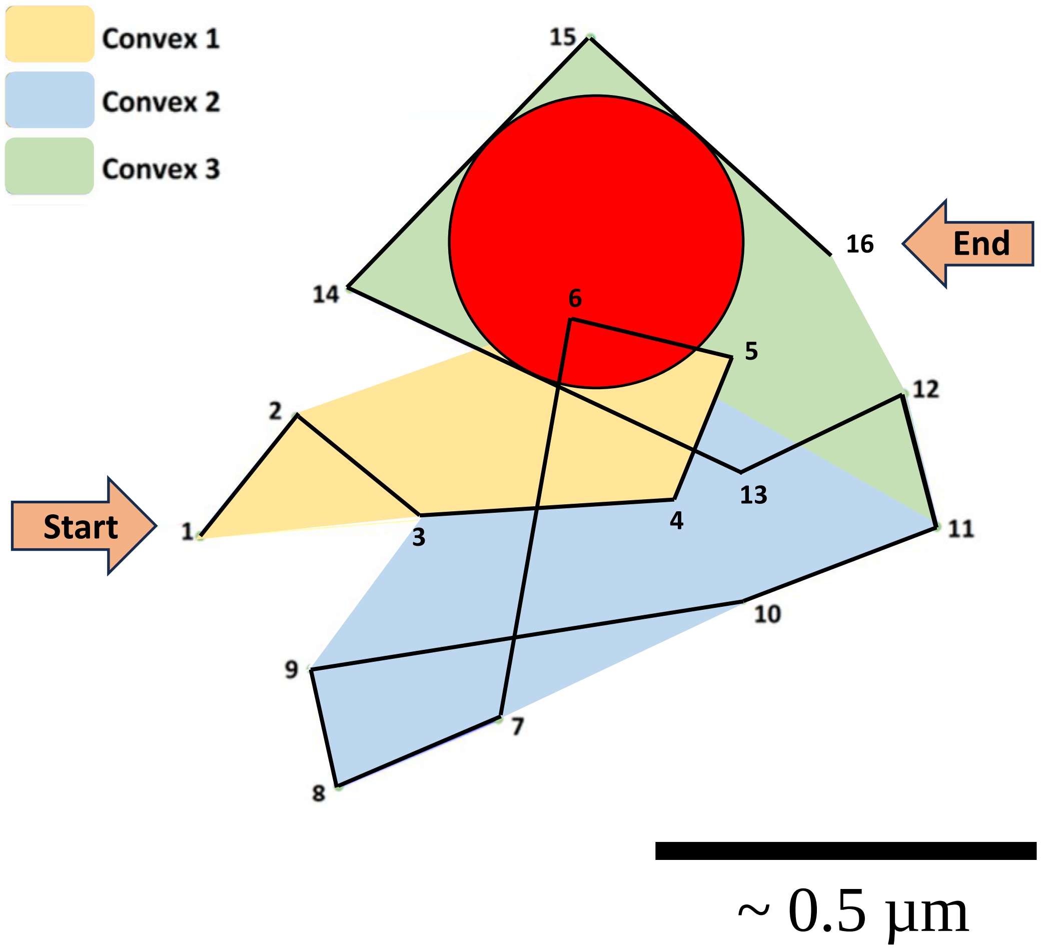
Some heterogeneity within trajectories

Segmentation

Convex Hull



Parameters:
- Window size (frames)
- Threshold for two states

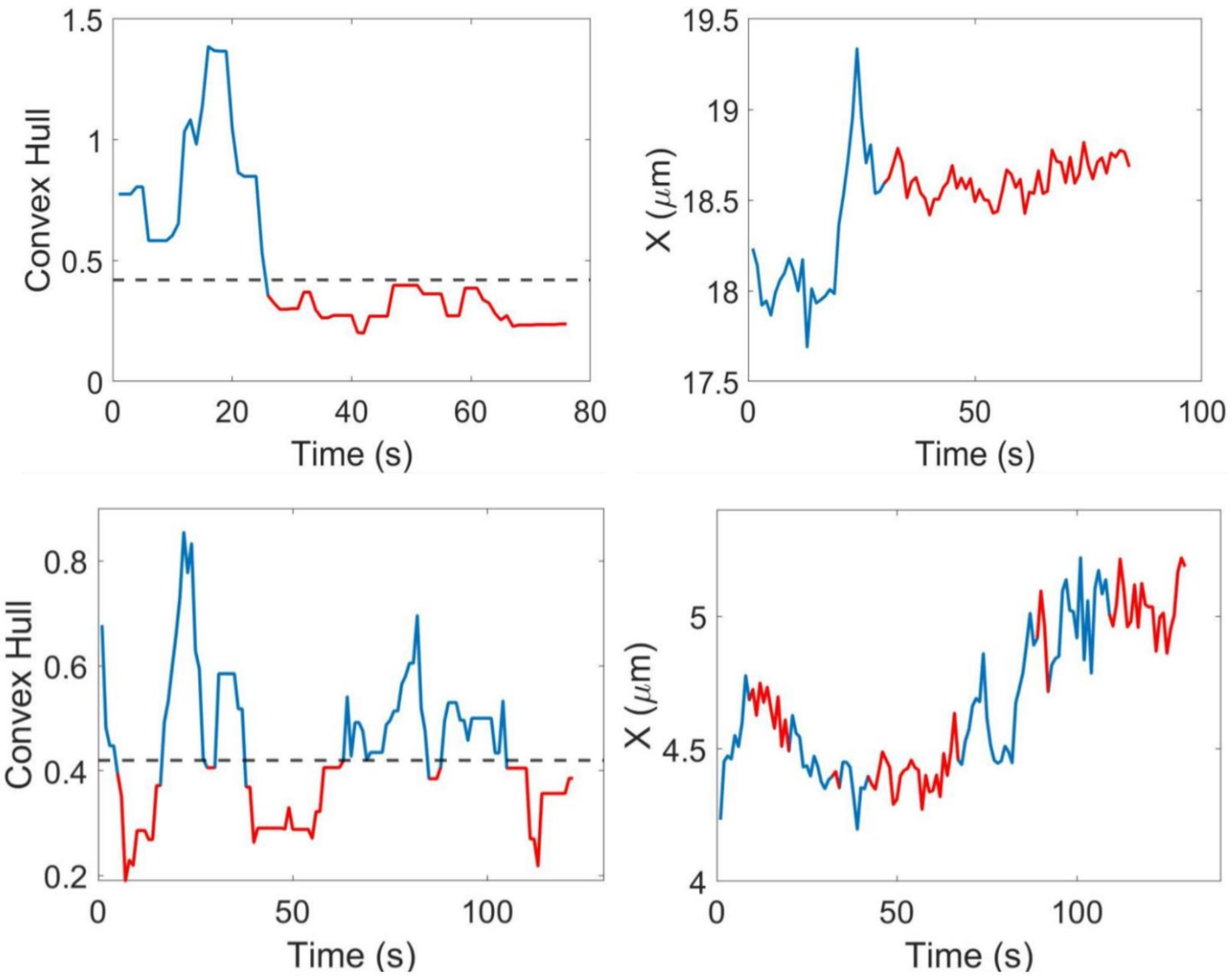


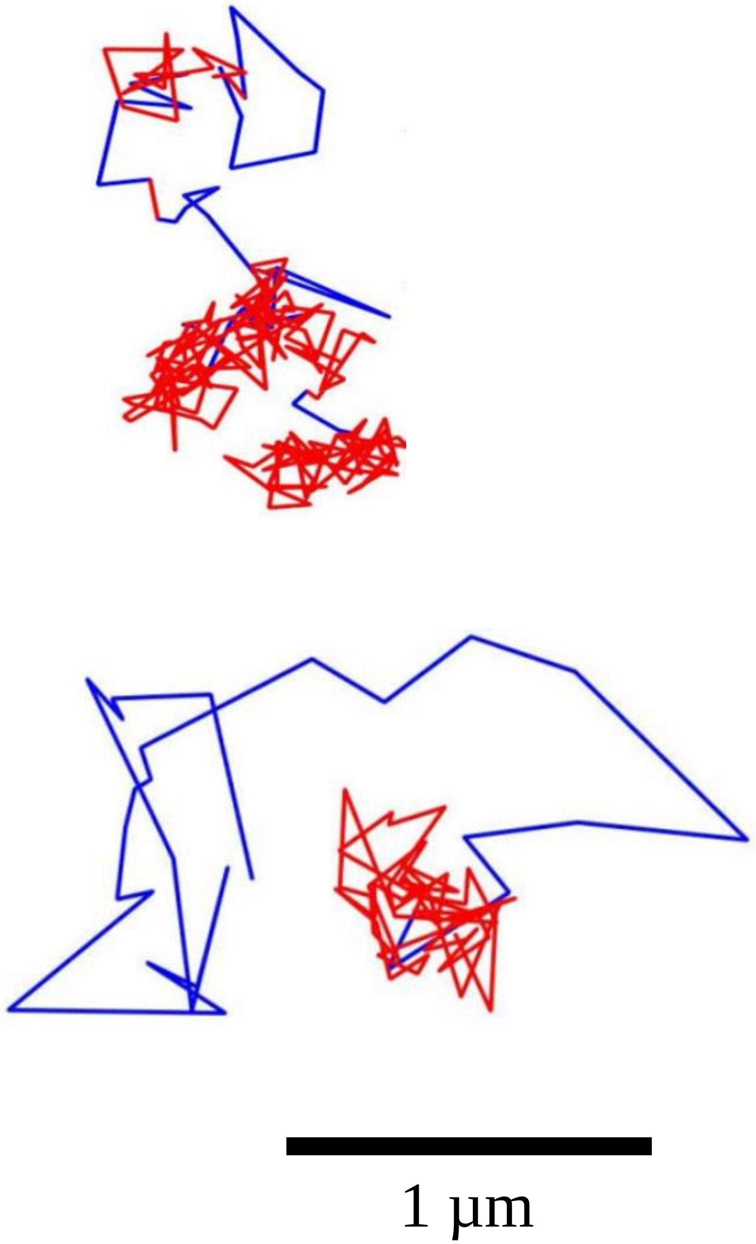
Lanoiselee and Grebenkov, *PRE* 2017

Segmentation

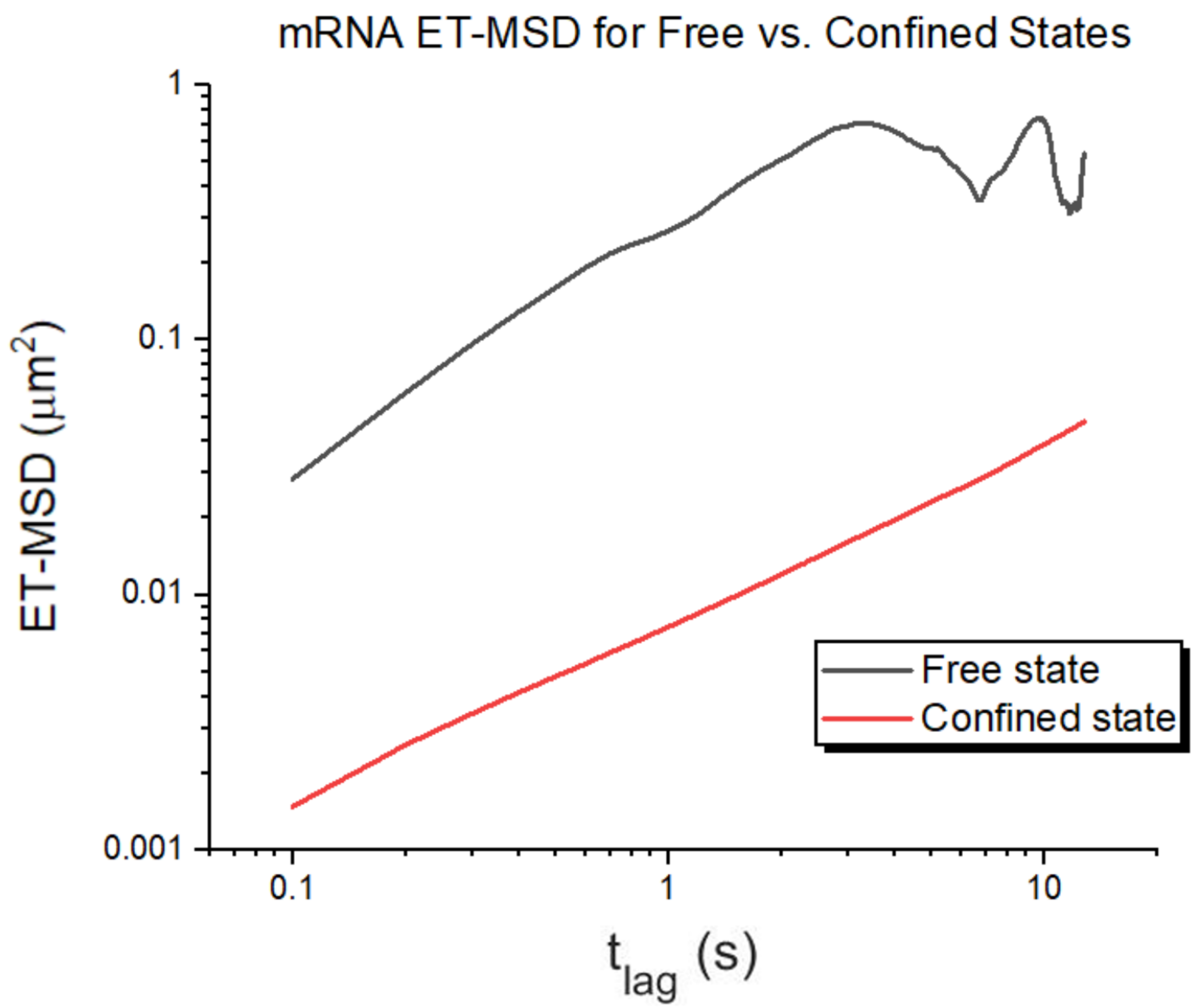
Th = 0.42

“Free” > Th
“Confined” < Th



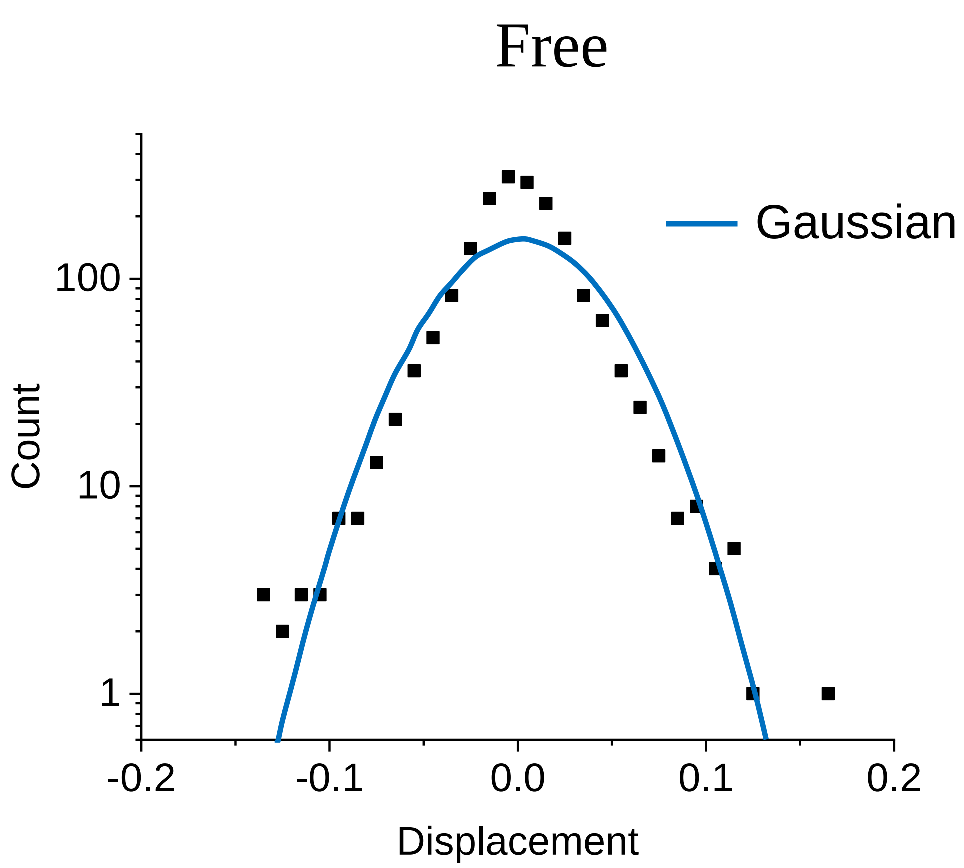


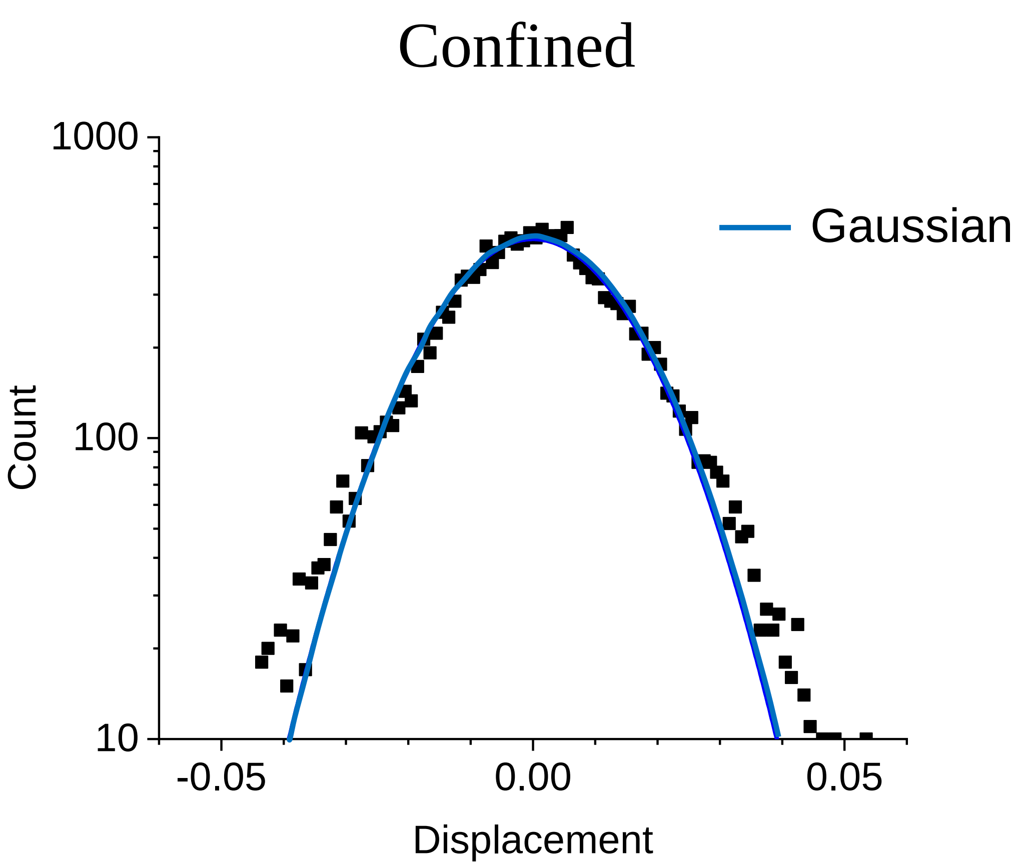
Segmentation



- Free state ET-MSD ~1 order of magnitude higher than confined state
- Quantitative confirmation of segmentation

Distribution of Displacements by State

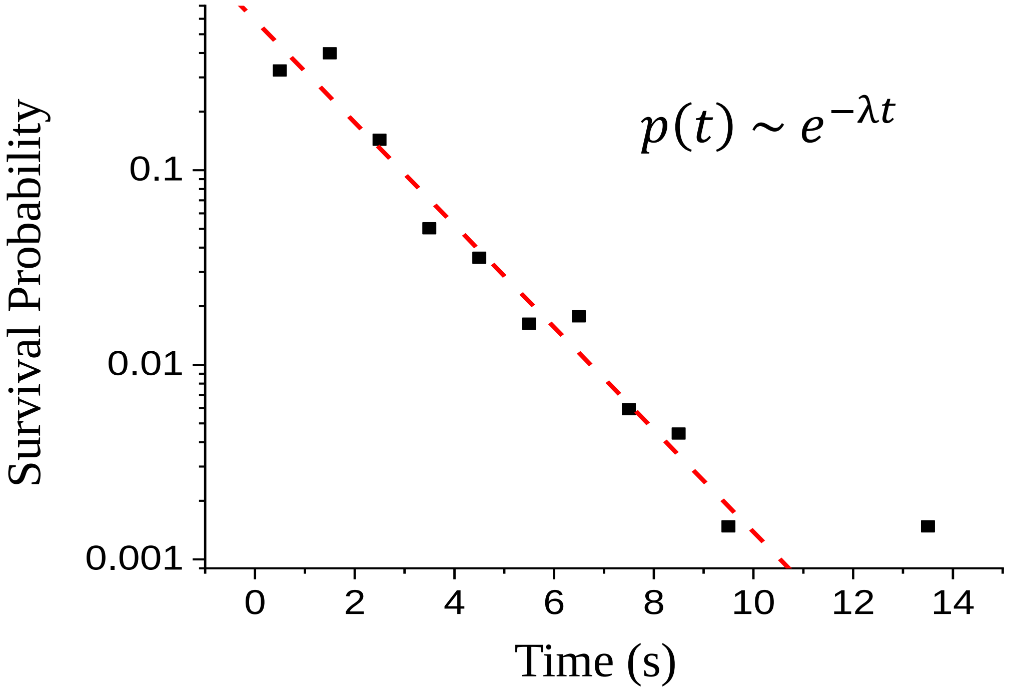




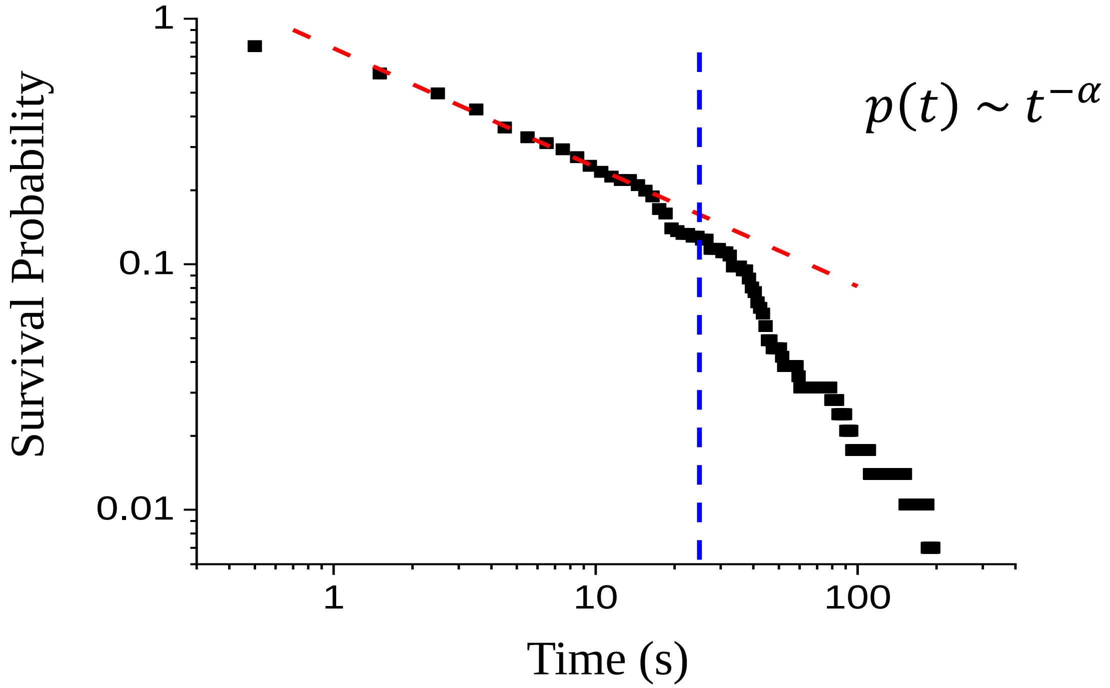
Free state: Deviation from Gaussian: more heterogeneous

Sojourn Times

Free



Confined



Free state: Exponential distribution

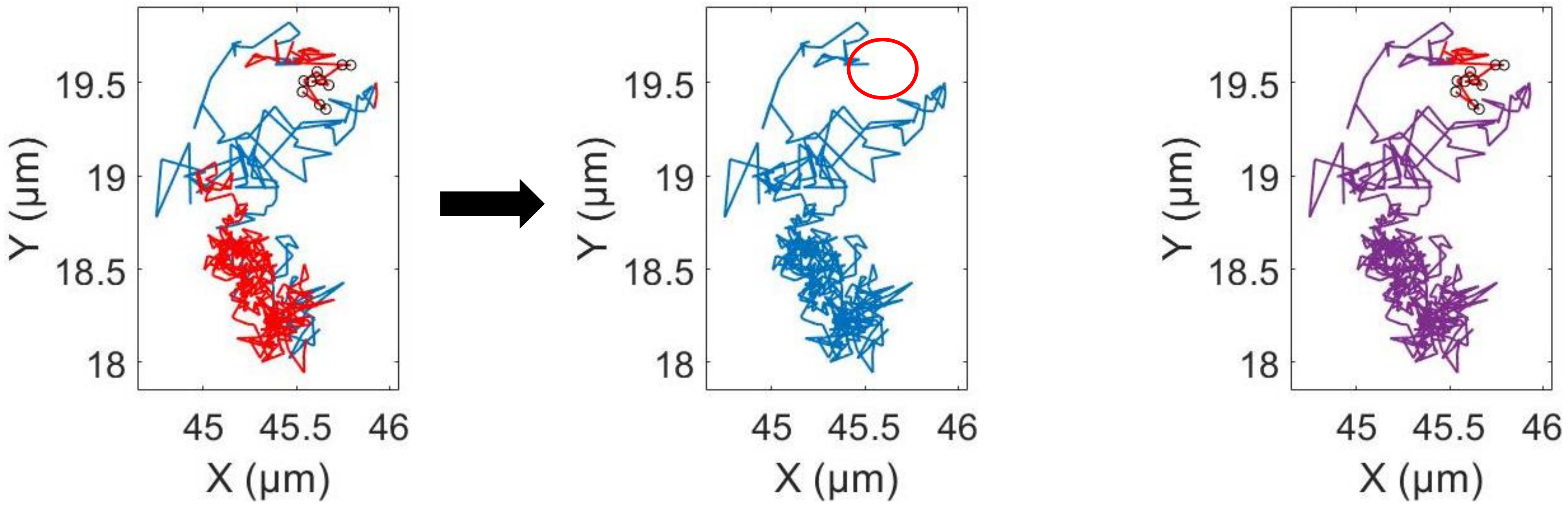
Confined state: Power-law distribution
(times between jumps/states can be very long)

Removal of First Confined Segment

- Free state exhibits an exponential distribution of waiting times (no memory/correlations)
  - Probability of each waiting time is independent of previous ones

- Thus, we want trajectories to start in a free state

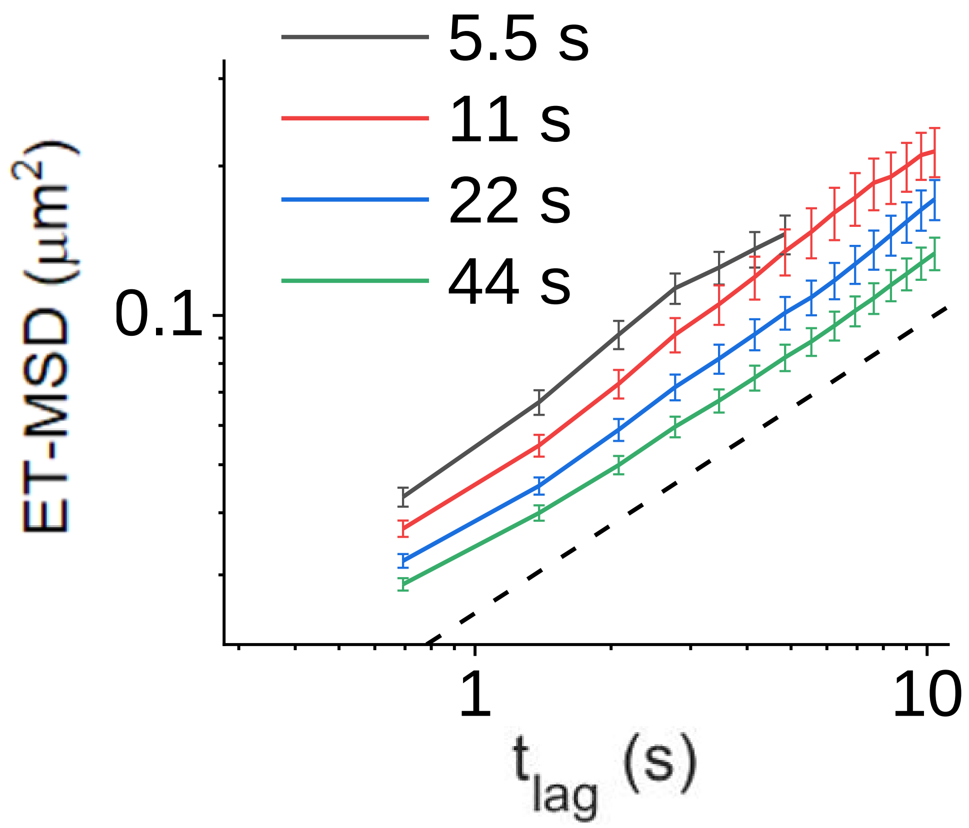
Original　　Removed　　Overlayed



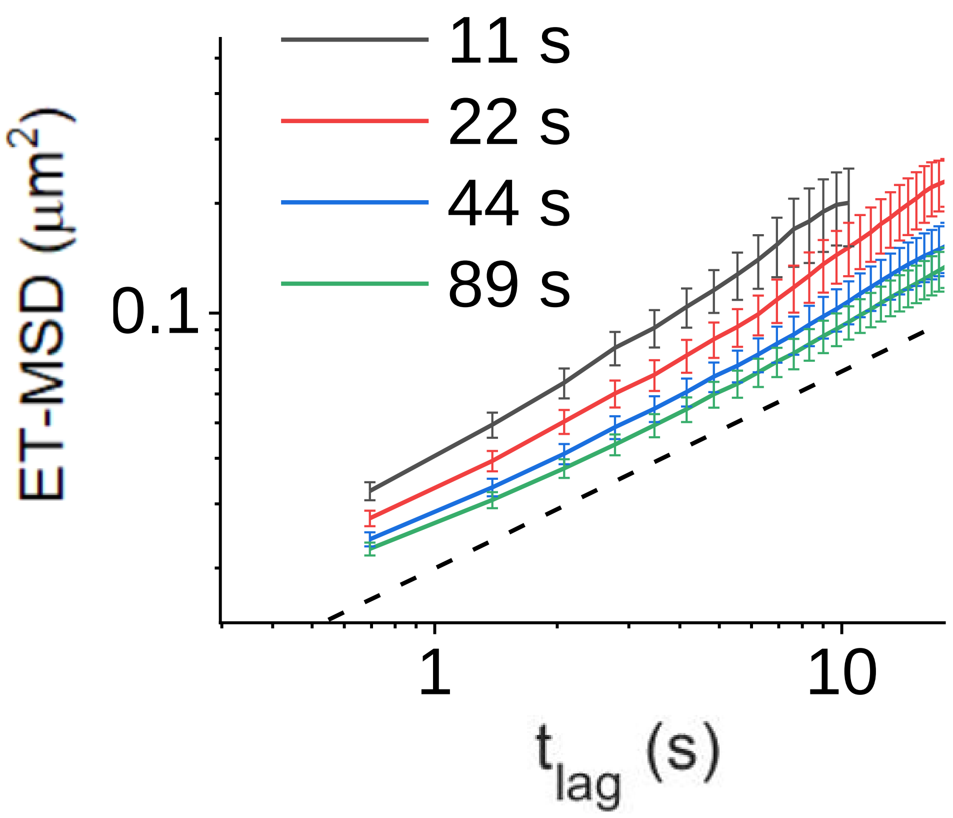
Analysis

$$\textbf{ET-MSD}: \quad \langle\overline{\delta^2(t_{lag})}\rangle = \frac{1}{T_m - t_{lag}} \int_0^{T_m - t_{lag}} [r(\tau + t_{\mathrm{lag}}) - r(\tau)]^2 \mathrm{d}\tau$$

- Confocal data
  - 4 focal planes (0.5 µm depth)
  - ~ 2 µm depth & 1.5 fps
  - Maximum intensity projection
- Exhibits aging



248 trajectories

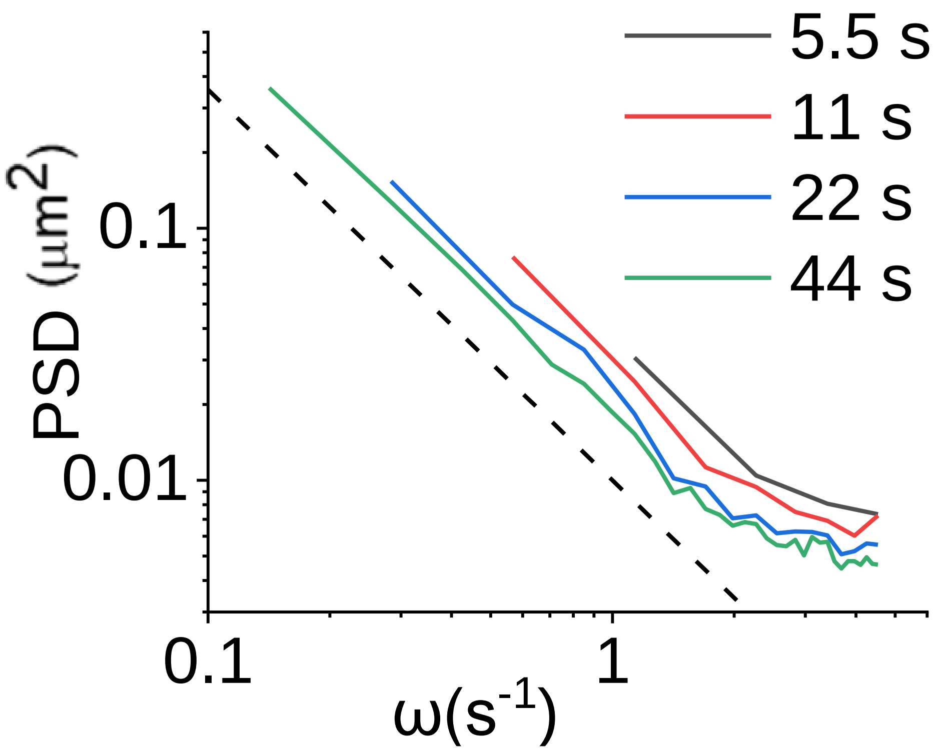


103 trajectories

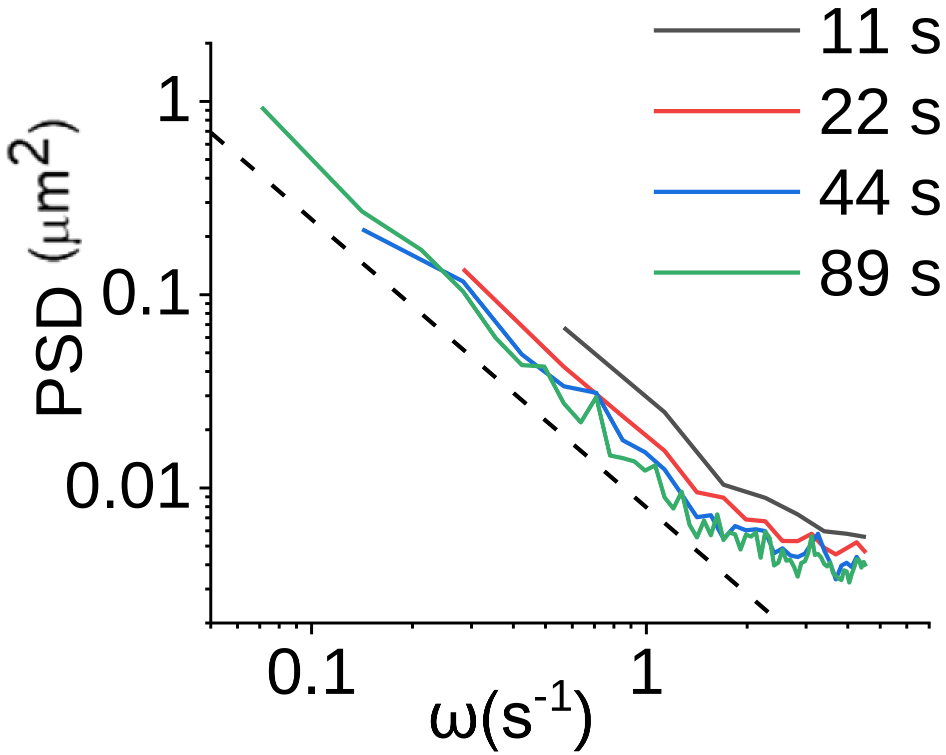
Analysis

PSD: $S_T(\omega) = \left\langle \frac{1}{T} \left| \int_0^T e^{i\omega t} X(t) dt \right|^2 \right\rangle$

- Confocal data
  - 4 focal planes (0.5 μm depth)
  - ~ 2 μm depth & 1.5 fps
  - Maximum intensity projection
- Exhibits aging



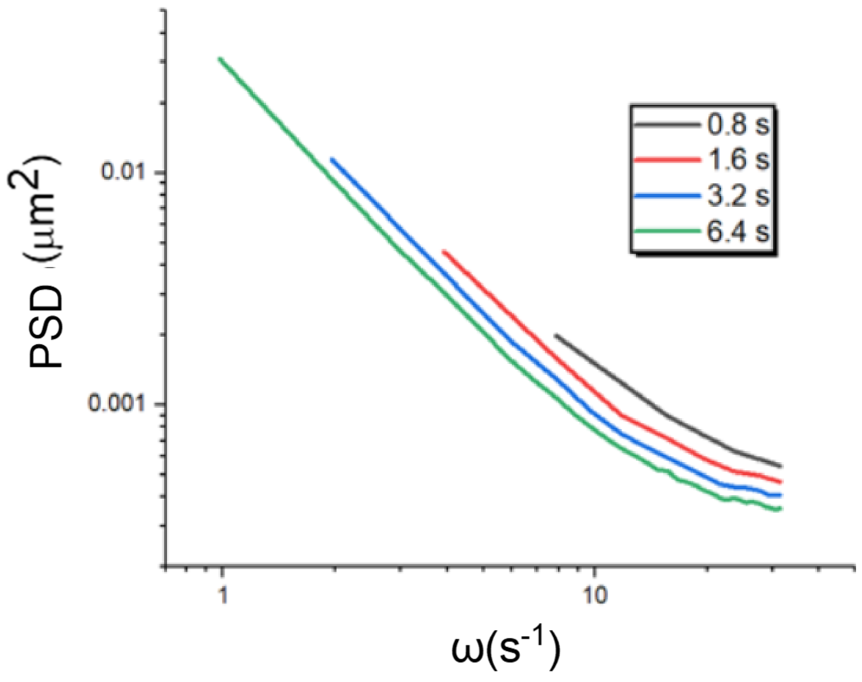
248 trajectories



103 trajectories

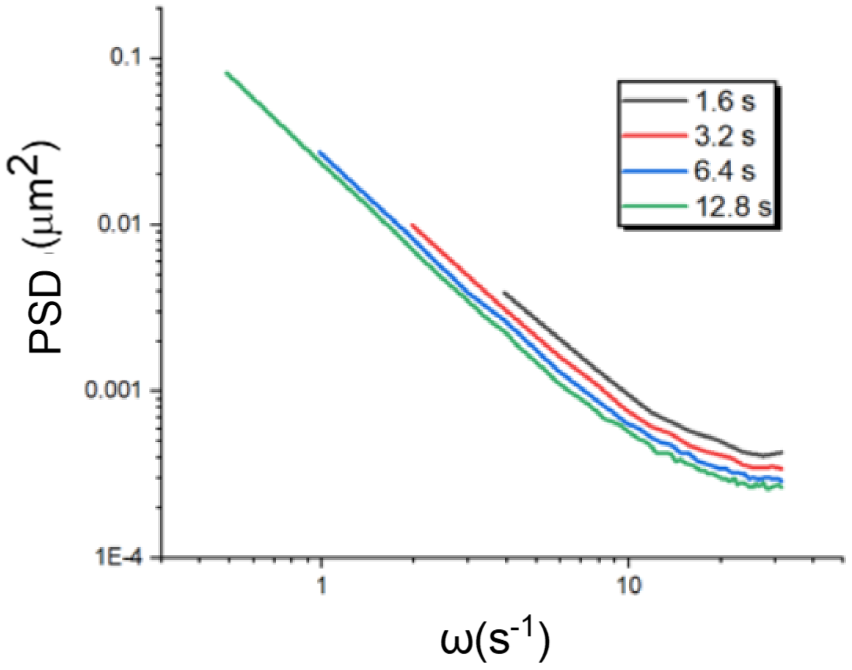
Analysis

mRNA PSD vs. Observation Time
Minimum Trajectory Length of 6.4 s



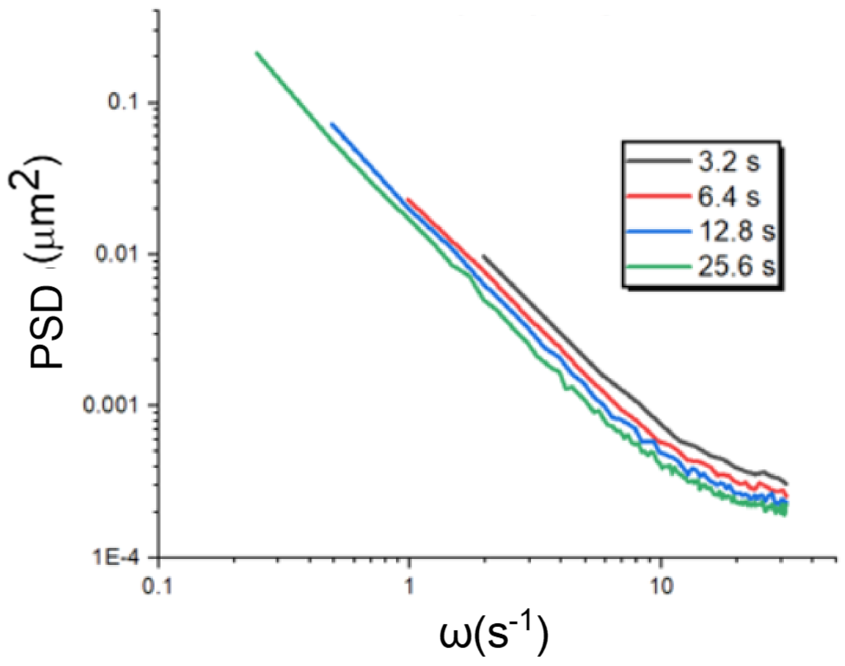
5,820 trajectories

mRNA PSD vs. Observation Time
Minimum Trajectory Length of 12.8 s



2,446 trajectories

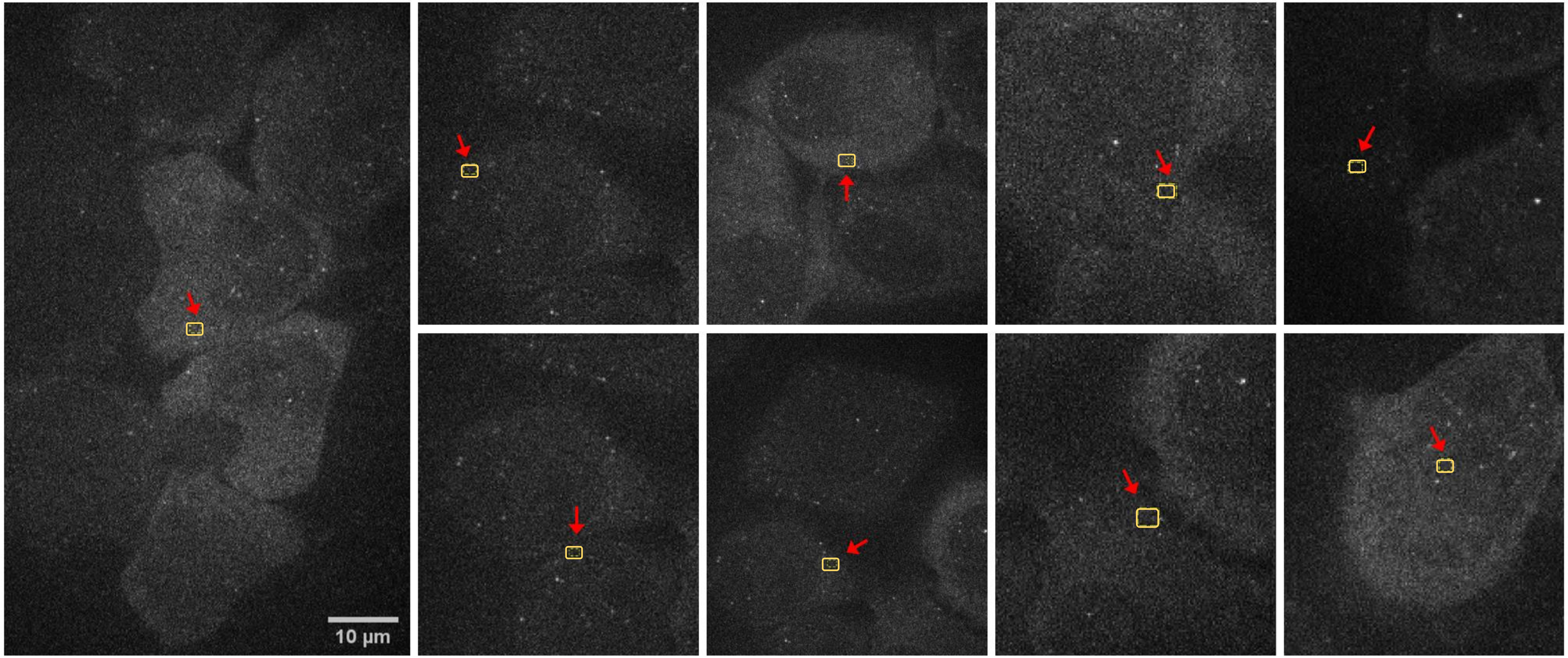
mRNA PSD vs. Observation Time
Minimum Trajectory Length of 25.6 s



998 trajectories

- TIRF data
    - All minimum length trajectories exhibit aging PSDs

Long Free States (> 27 s)

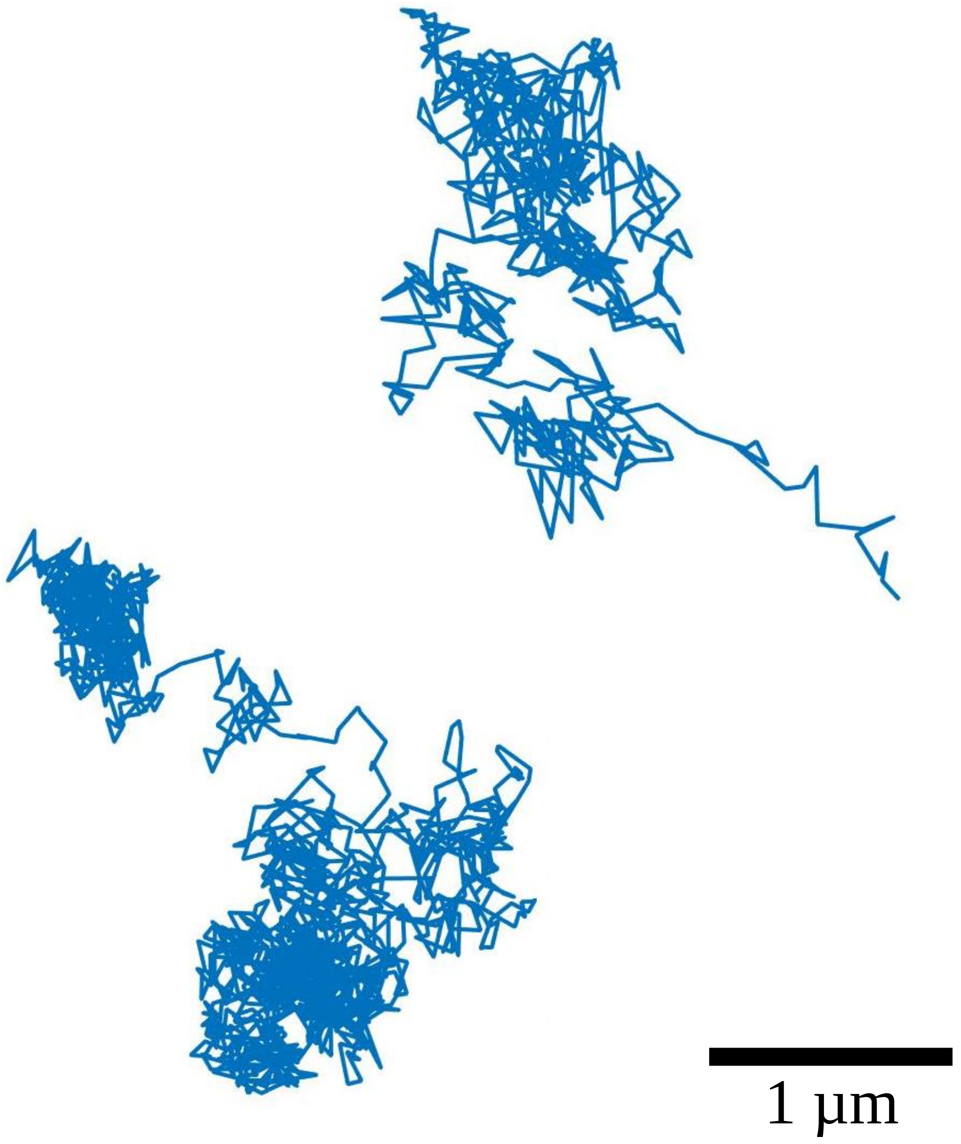


78% of these RNAs are found near cell periphery

Conclusions

- mRNA is subdiffusive and non-ergodic with stochastic switching between “free” and “confined” mobility states, and exhibits aging
- Mobility states differ by an order of magnitude in MSD
- Free state is more transient, whereas the confined state has power-law waiting times
- Molecules that experience “long” free states tend to be near the cell periphery

- Hypothesis: confined states represent translation



Future / Ongoing Work

- Disruption of translation & intracellular structures
- See how miRNA interacts with mRNA
- How does the introduction of miRNA change transcription and translation?

Acknowledgements

- Diego Krapf
- O’Neil Wiggan
- Carsten Dietvorst
- Snehal Patil
- Tim Stasevich

NSF

WALTER SCOTT, JR.
COLLEGE OF ENGINEERING
COLORADO STATE UNIVERSITY

Thank you

Colorado State University