Project: Single-Particle Tracking & Diffusion Analysis

 # Single-Particle Tracking (SPT)

Single-particle tracking (SPT) is an experimental and analytical technique used to follow the motion of individual particles—such as proteins, nucleic acids, vesicles, or nanoparticles—over time. Unlike ensemble-averaged methods, SPT provides direct access to the dynamics of single molecules, allowing heterogeneous behaviors to be resolved that would otherwise be hidden in population averages.

In a typical SPT experiment, particles are fluorescently labeled and imaged using optical microscopy. By acquiring a time series of images at a fixed time interval, the position of each particle is localized in every frame, producing a trajectory that describes its motion in space and time.

Although a single fluorescent molecule appears as a diffraction-limited spot in an optical microscope, the center of that spot can be determined with much higher precision than the spot size itself. This is because the image of a point emitter is well described by a point spread function (PSF), which can be approximated by a two-dimensional Gaussian distribution.

The fluorescence intensity profile of a single molecule is fitted to a Gaussian function:

$$I(x, y) = I_0 \exp\left(-\frac{(x - x_0)^2 + (y - y_0)^2}{2\sigma^2}\right)$$

where (x_0, y_0) represents the true molecular position. By accurately determining the centroid (x_0, y_0), the molecular position can be localized with nanometer precision.

The key result of SPT is that localization precision is not limited by diffraction, but by photon statistics. The localization uncertainty σ_{loc} scales approximately as

$$\sigma_{\text{loc}} \sim \frac{\sigma_{\text{PSF}}}{\sqrt{N}}$$

where σ_{PSF} is the width of the point spread function, and N is the number of detected photons [1]. With sufficiently bright fluorophores and low background noise, the SPT can practically achieve localization accuracy on the order of 1–2 nm [2, 3].

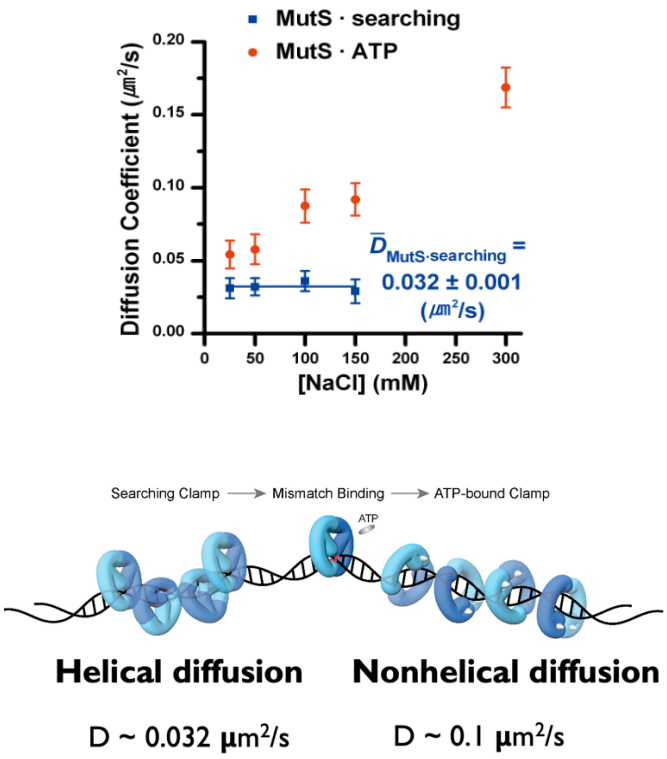
Question 1: Drive the centroid uncertainty relation $\sigma_{\text{loc}} = \frac{\sigma_{\text{PSF}}}{\sqrt{N}}$.

Assume that a single fluorescent molecule emits Ndetected photons, each photon position $x_i$is an independent random variable drawn from the point spread function (PSF), and the PSF is approximated by a normal distribution ($\mathcal{N}$) with mean x_0 and standard deviation σ_{PSF}:

$$x_i \sim \mathcal{N}(x_0, \sigma_{\text{PSF}}^2).$$

Explain physically the centroid uncertainty relation and why the centroid uncertainty decreases when the photon number increases.

Question 2:



MutS adopts two distinct conformations. In the target-searching state, MutS exhibits a constant diffusion coefficient of 0.032 $\mu m^2/s$, whereas in the ATP-bound state, it diffusion coefficient depends strongly on ionic strength (NaCl concentration), as shown in the figure above. The search-state MutS diffuses along DNA while maintain continuous contact with the DNA backbone (sliding diffusion), resulting in rotation-coupled helical diffusion. In contrast, ATP-bound MutS interacts with DNA intermittently and moves via hopping-like diffusion. Explain why the strong ionic-strength dependence of the hopping-like diffusion arises.

 # Diffusion Constant

The motion observed in SPT is often governed by diffusion, which arises from random thermal fluctuations. In one-dimensional Brownian diffusion, the probability density $P(x,t)$of finding a particle at position $x$and time $t$obeys the diffusion equation:

$$\frac{\partial P(x,t)}{\partial t} = D\,\frac{\partial^2 P(x,t)}{\partial x^2},$$

where $D$is the diffusion constant.

Assume that the particle is localized at the origin at time $t = 0$:

$$P(x,0) = \delta(x).$$

The solution of the diffusion equation with this initial condition is well known and has a Gaussian form:

$$P(x,t) = \frac{1}{\sqrt{4\pi D t}}\exp\left(-\frac{x^2}{4Dt}\right).$$

The displacement over a time interval $\Delta t$is defined as

$$\Delta x = x(t+\Delta t) - x(t).$$

Brownian motion is memoryless and independent of absolute position, so the probability of moving by Δx in time Δt is identical to the probability of being at position $x = \Delta x$ after time Δt when starting from the origin. Therefore, the displacement distribution has the same form as $P(x,\Delta t)$:

$$p(\Delta x) = \frac{1}{\sqrt{4\pi D \Delta t}}\exp\left(-\frac{\Delta x^2}{4D\Delta t}\right).$$

From this Gaussian distribution, the variance of the displacement $\langle \Delta x^2\rangle - \langle \Delta x\rangle^2$ equals to the mean squared displacement (MSD) because $\langle \Delta x\rangle = 0$ (No drift!):

$$MSD = 2D\Delta t.$$

where Δt is the time lag.

Project 1:
The movie (**PCNA-Cy5.pptm**) shows a representative PCNA protein labeled with a Cy5 fluorophore diffusing along a long DNA molecule that is stretched along the *y*-axis. The accompanying CSV files (**long_trace.csv**) contains the time-dependent positional data of the PCNA molecule obtained from SPT of Cy5-PCNA in the movie. The frame rate is 0.1 sec.

(a) Determine the diffusion constant of Cy5-PCNA on DNA using $MSD = 2D\Delta t$.
(b) You will observe a relatively broad distribution of diffusion coefficients. Discuss the possible origins of this heterogeneity.

Project 2:
Fluorescent proteins such as Green Fluorescent Protein (GFP) generally have relatively short photophysical lifetimes due to photobleaching and blinking. As a result, single-particle trajectories obtained from GFP-labeled molecules are often short, consisting of only a limited number of frames. Such short trajectories impose significant limitations on estimating the diffusion constant using the conventional mean squared displacement (MSD) method, which typically requires long trajectories to obtain reliable statistics over multiple time lags.

(a) Determine the diffusion constant from the provided short single-particle traces (**short_trace.csv**) as accurately as possible using an alternative analysis method that does not rely on long trajectories.
(b) Compare the diffusion constant obtained from this short-trajectory analysis with the diffusion constant determined in **Project 1**, and discuss any agreement or discrepancy between the two results.

References

1. R. E. Thompson et al. "Precise Nanometer Localization Analysis for Individual Fluorescent Probes." ***Biophysical Journal*** **82**, 2775-2783 (2002)
2. A. Yildiz et al. “Myosin V walks hand-overhand: single fluorophore imaging with 1.5-nm localization.” ***Science*** **300**, 2061-5 (2003).
3. https://app.jove.com/v/51774/fluorescence-imaging-with-one-nanometer-accuracy-fiona