Analysis and classificiation of organelle trajectories in the cytoplasm

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Abstract

Organelle movement within a cell is a vital part of cellular development and metabolic processes. When cells have defects in intracellular transport, it can lead to diseases such as Alzheimer's. Organelles use active motor transport, hitchhiking, and Brownian motion to travel within the cell; however, when organelles are not in active transport, they can also be tethered to microtubules. We use the velocity autocorrelation function to analyze these movements and classify them as tethered or diffusive, and also analyze how localization error affects the accuracy of these measurements.

Background

Organelles can move via processive motion, diffuse, or simply be tethered to a stationary object such as microtubules. This tethering with other intracellular components is the cause of non-diffusive motion within the cell. Furthermore, fluctuations in temperature or cytoskeletal networks can cause diffusive motion [1].

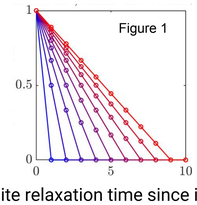
Diffusive motion follows the probability density function $P(x,t) = \frac{1}{\sqrt{4\pi Dt}} \exp\left(-\frac{(x-x_0)^2}{4Dt}\right)$,

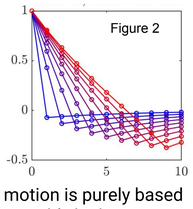
where D is the diffusion coefficient, x_0 is the initial position, and t is the time; namely, diffusive motion follows Gaussian distribution [2]. On the other hand, tethered motion occurs when an object is attached to another object with a spring. The behavior of tethered particles depends on the relaxation time τ_r, the elasticity of the medium, the length of the tether, and the size of the object. The ability to distinguish between diffusive and tethered motion is an important concept which appears greatly in the study of intracellular movement and various roles certain mechanisms play in this movement.

Methods

The velocity autocorrelation function, $C_v^{(\delta)}(t) = \langle \vec{v}(t_0+t) \cdot \vec{v}(t_0) \rangle$, is used to distinguish particles from diffusive and tethered motion, where delta δ is the step between each velocity calculation $\vec{v}(t) = \frac{1}{\delta}(\vec{P}(t+\delta) - \vec{P}(t))$, lag t is the step between each dot product in the velocity autocorrelation function, and $\vec{P}(t)$ is the position vector of the particle at time t [3]. We then normalize this over $C_v^{(\delta)}(0)$. For a diffusive particle, when the normalized velocity autocorrelation function is plotted over many values of δ and t, all values where t > δ equal 0 (Fig 1). On the other hand, a tethered particle will reach a negative local minimum before rising and converging to 0 for all δ as t increases (Fig 2).

Because of randomness and background noise, the plotted velocity autocorrelation for a realistic particle will not exactly resemble one of a perfectly diffusive or tethered particle, so a fitting algorithm is used to determine τ_r, the relaxation time of the particle, if it was attached to another object via a spring.





Theoretically, a diffusive particle has an infinite relaxation time since its motion is purely based on Gaussian distribution; however, this is normally impossible so any reasonably high τ_r indicates that the particle is diffusive, while a small τ_r indicates that the particle is tethered to another object. The cutoff value determines whether the particle is either tethered or diffusive.

When we analyze our data, we can choose how much delta δ values there are and how many lag t values there are, where the number of δ corresponds to the number of functions and the number of t corresponds to how many data points we have per function. In general, as $t \to \infty$, the accuracy of the fitting increases but so does the total runtime. It was also found that most optimal and accurate results required δ to be as close to t as possible. This data was collected with 9 δ values and 10 t values, excluding the case when $t = 0$ ($C_v^{(\delta)}(t=0)/C_v^{(\delta)}(0) = 1$ for all δ).

Background noise is a notable error which will always affect the accuracy of the tracking of particles. This is also known as localization error, or ε. Localization error is assumed to follow Gaussian distribution. This error can lead to negative values in the velocity autocorrelation function [3].

In order to minimize the amount of free variables we have and make our analysis of localization error more coherent, we perform some basic dimensional analysis by setting our time units as the time between frames, Δt, and space units as the square root of the diffusion coefficient times the time between frames, √DΔt. We get that the nondimensional relaxation time is τ_r / Δt, and that the nondimensional localization error is √(ε^2/DΔt).

Results / Analysis

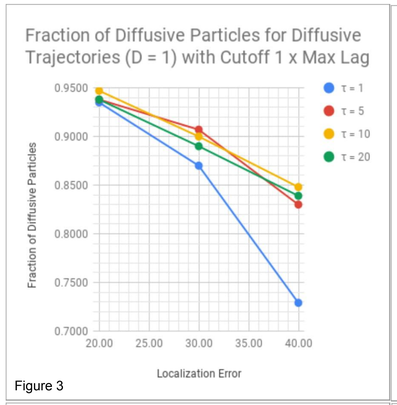


Figure 3

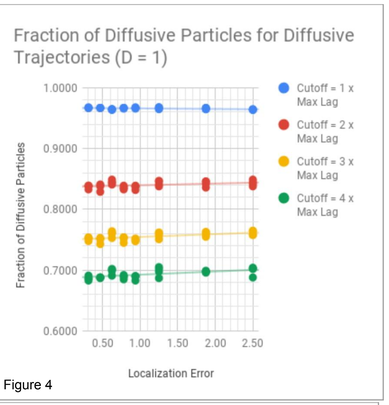


Figure 4

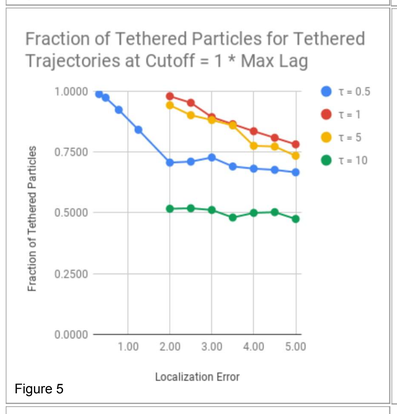


Figure 5

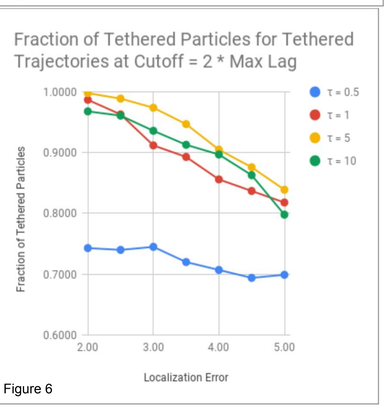


Figure 6

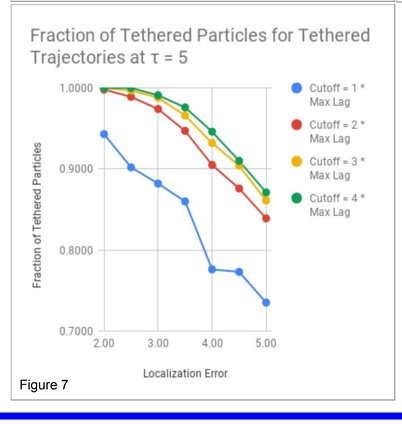


Figure 7

Figures 3 and 4 use a 100% diffusive dataset with varying cutoff values and localization errors. Unlike tethered particles, the accuracy of diffusive datasets are highly dependent on the cutoff value (Fig 4). Because of this, our real world data will be analyzed with a cutoff of 1 * maxLag, or 1 * # of t values, since this is where accuracy is still preserved when analyzing diffusive particles.
Figures 4, 5, and 6 use a 100% tethered dataset with varying cutoff values, localization errors, and relaxation times. In most cases of actual data, we will not know what the true τ_r is before analyzing it. Thus, in our analysis, we used varying τ_r values to see the effects of localization error on different τ_r.

Clearly, as cutoff increases, so does the fraction of tethered particles. When the relaxation time nears to the cutoff, a similar pattern with diffusive particles occur, where the accuracy of tethered datasets becomes extremely dependent on cutoff (Fig 5, 6). However, this is to be expected since the higher the relaxation time, the more diffusive a trajectory becomes.

Since we observed that a cutoff of 1 * maxLag gives optimal results, we now can find that the maximum localization error that allows for accurate classification. For tethered trajectories with with τ_r = 0.5, τ_r = 1, and τ_r = 5, an accuracy of 90% can be obtained with localization errors up to about 0.90, 3.00, and 3.20, respectively.

The figures below and to the right analyze the movement of peroxisomes in a fungal hypha. Our analysis finds that 42.11% of particles analyzed are diffusive, and 57.89% of particles analyzed are tethered, out of 18 total particles. 2 particles in particular are prime examples of diffusive (Fig 8, 9) and tethered motion (Fig 10, 11).

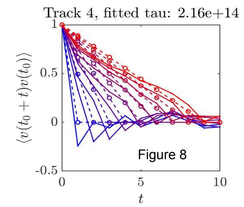


Figure 8

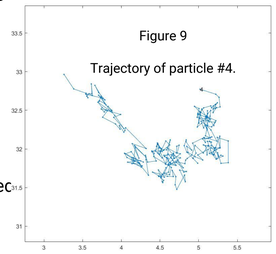


Figure 9

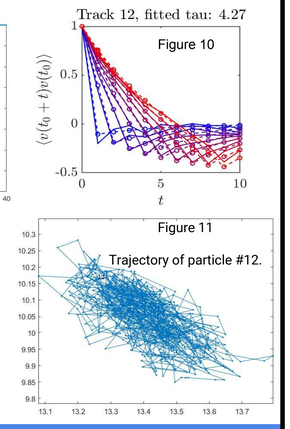
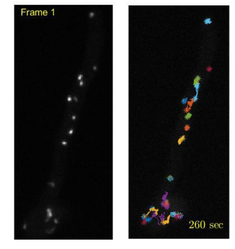


Figure 10

Figure 14

The movement of peroxisomes in the cell, and their trajectories over 260 seconds.



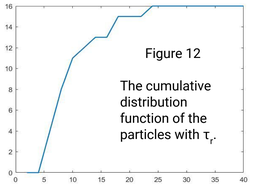


Figure 12

The cumulative distribution function of the particles with τ_r.

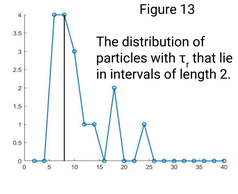


Figure 13

The distribution of particles with τ_r that lie in intervals of length 2.

Figure 11

Conclusion

In conclusion, the velocity autocorrelation function is a powerful tool in distinguishing diffusive and tethered particles. However, it is important to understand the effects that background noise can have on the study of intracellular movement.

References

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Acknowledgements

We would like to thank Saurabh Mogre and Dr. Elena Koslover for providing invaluable guidance and assistance with this project. We would also like to thank Dr. Elizabeth Komives and the UCSD Extensions Program for giving us this wonderful opportunity to work on this project. Finally, we thank Dr. Reza Aghajani for teaching us a wonderful course in probability.