Supplementary Information

Label-Free Imaging of Single Microtubule Dynamics Using Spatial Light Interference Microscopy

Mikhail E. Kandel[1], Kai Wen Teng[2, 3], Paul R. Selvin[2, 3, 4], and Gabriel Popescu[1,5,*]

[1] Quantitative Light Imaging Laboratory, Department of Electrical and Computer Engineering, Beckman Institute of Advanced Science and Technology, University of Illinois, Urbana, IL, USA

[2] Center for the Physics of Living Cells, University of Illinois, Urbana, IL, USA

[3] Center for Biophysics and Quantitative Biology, University of Illinois, Urbana, IL, USA

[4] Department of Physics, University of Illinois, Urbana, IL, USA

[5] Department of Bioengineering, University of Illinois, Urbana, IL, USA

1. Dispersion-relation Phase Spectroscopy (DPS)

Using SLIM, we can quantitatively measure the changes in the optical path length through the specimen. These changes are proportional to the values of the dry mass, *i.e.*, the protein concentration of the microtubule, in this case.[1] The spatiotemporal autocorrelation associated with the dry mass density, η (in pg/μm^2), is defined as:

$$g(\boldsymbol{r},\tau) = \langle \eta(\boldsymbol{r}',t)\eta(\boldsymbol{r}'+\boldsymbol{r},t+\tau)\rangle_{t,r'}\,, \tag{1}$$

where η is the density data outputted by SLIM and the angular brackets denoted averaging over a time-varying ensemble. We assume ergodicity, such that, in practice, we replace the ensemble

average with a time average. Since the intracellular transport is due to both random and deterministic contributions, η satisfies the *advection-diffusion* equation,[2,3]

$$D\nabla^2 \eta(\boldsymbol{r},\tau) - \boldsymbol{v}\cdot\boldsymbol{\nabla\eta}(\boldsymbol{r},\tau) - \frac{\partial}{\partial\tau}\eta(\boldsymbol{r},\tau) = 0. \tag{2}$$

In Eq. 2, D is the diffusion coefficient of the Brownian (diffusion) component and $\boldsymbol{v}$ is the velocity of the active (deterministic) component. In order to solve for g, we Fourier transform Eq. 2 with respect $\boldsymbol{r}$ and use the differentiation properties of the Fourier transform, to obtain:

$$(-Dq^2 + i\boldsymbol{q}\cdot\boldsymbol{v})g(\boldsymbol{q},\tau) - \frac{\partial}{\partial t}g(\boldsymbol{q},\tau) = 0, \tag{3}$$

Where $\boldsymbol{q}$ is the angular spatial frequency or mode and the variable conjugate to $\boldsymbol{r}$. Equation 3 is first order in time; thus, the solution is obtained right away,

$$g(\boldsymbol{q},\tau) = \exp(-Dq^2 + i\boldsymbol{q}\cdot\boldsymbol{v})\tau, \tag{4}$$

Where we assumed g is normalized such that $g(\boldsymbol{q},0) = 1$.

Equation 4 indicates that a mass drift at constant velocity $\boldsymbol{v}$ introduces a sinusoidal modulation to the autocorrelation function. Clearly, in a living cell, we expect a distribution of velocities, with various magnitudes and orientations, say $P(\boldsymbol{v}-\boldsymbol{v}_0)$, where $\boldsymbol{v}_0$ is the mean velocity. Averaging the autocorrelation function over the ensemble of velocity distribution yields

$$\langle g(\boldsymbol{q},\tau)\rangle_{\boldsymbol{v}} = \exp(-Dq^2\,\tau)\int P(\boldsymbol{v}-\boldsymbol{v}_0)\exp(i\boldsymbol{q}\cdot\boldsymbol{v}\tau)\,d^2\boldsymbol{v}. \tag{5}$$

Note that the integral in Eq. 5 amounts to a Fourier transform with respect to velocity $\boldsymbol{v}$. The conjugated variable is $\tau\boldsymbol{q}$. Thus, Eq. 5 can be rewritten as:

$$\langle g(\boldsymbol{q},\tau)\rangle_{\boldsymbol{v}} = \exp(-Dq^2\,\tau)\exp(i\boldsymbol{q}\cdot\boldsymbol{v_o}\tau)\tilde{P}(\tau\boldsymbol{q}), \tag{6}$$

Where $\tilde{P}$ is the Fourier transform of P shifted at $\boldsymbol{v_o}$, i.e, the Fourier transform of the zero-average velocity distribution. Again, we note a sinusoidal modulation term, $\exp(i\boldsymbol{q}\cdot\boldsymbol{v_o}\tau)$, which this time is due to the dominant (mean) velocity, $\boldsymbol{v_o}$.

If we consider the second order Taylor expansion of $\tilde{P}$ around the origin, we can obtain an analytic expression for Eq. 6 that does not dependent of the specific shape of $\tilde{P}$. We start with the expansion of $\tilde{P}$ and assuming isotropy, *i.e.*, $P(\boldsymbol{q}) = P(q)$, we find

$$\tilde{P}(\tau q) \cong \tilde{P}(0) + \left.\frac{d\tilde{P}(\tau q)}{d(\tau q)}\right|_{\tau q=0} \tau q + \frac{1}{2!}\left.\frac{d^2\tilde{P}(\tau q)}{d(\tau q)^2}\right|_{\tau q=0} (\tau q)^2. \tag{7}$$

Next, we use the *central ordinate theorem* (see, *e.g.*, Chapter 2 in Ref. [4]) to identify each term in the expansion of $\tilde{P}$ with the moments of P, namely:

$$\begin{aligned} \tilde{P}(0) &= \int P(\boldsymbol{v}) d^2\boldsymbol{v} \\ &= 1, \end{aligned} \tag{8a}$$

$$\begin{aligned} \left.\frac{d\tilde{P}(\tau q)}{d(\tau q)}\right|_{\tau q=0} &= \int i\boldsymbol{v}P(\boldsymbol{v}) d^2\boldsymbol{v} \\ &= 0, \end{aligned} \tag{8b}$$

$$\begin{aligned} \left.\frac{d^2\tilde{P}(\tau q)}{d(\tau q)^2}\right|_{\tau q=0} &= -\int \boldsymbol{v^2}P(\boldsymbol{v}) d^2\boldsymbol{v} \\ &= -\Delta v^2. \end{aligned} \tag{8c}$$

In deriving Eq. 8a, we used the fact that $P(\boldsymbol{v})$ is a probability density, such that it is normalized to unit area. For Eq. 8b, in addition to the central ordinate theorem, we also used the differentiation theorem, $\frac{d}{d(\tau q)} \leftrightarrow i\boldsymbol{v}$, where $\leftrightarrow$ indicates Fourier transformation. Deriving Eq. 8c requires the use of the differentiation theorem twice, $\frac{d^2}{d(\tau q)^2} \leftrightarrow -\boldsymbol{v^2}$. Note that Eq. 8b amounts to

the first order moment of P, which is zero (already shifted the origin of the velocity distribution at $\boldsymbol{v}_0$). Finally, Eq. 8c gives the variance of the velocity distribution, Δv^2.

Combining Eqs 7 and 8, we obtain

$$\begin{aligned}\tilde{P}(\tau q) &\cong 1 - \frac{1}{2}(\Delta v q \tau)^2 \\ &\cong \exp(-\Delta v q \tau),\end{aligned} \tag{9}$$

Therefore, the velocity averaged autocorrelation function, which is the main quantity computed from our data is:

$$\langle g(\boldsymbol{q}, \tau)\rangle_v = \exp(iqv_0\tau)\exp[-(Dq^2 + \Delta v q)\,\tau]. \tag{10}$$

In all the measurements presented here, we did not observe a dominant velocity, $\boldsymbol{v}_0$. This can be readily understood by an equal probability for the mass to be transported in opposite directions. Thus, with $\boldsymbol{v}_0 \cong 0$, Eq. 10 can be expressed in the frequency domain as

$$\tilde{g}(\boldsymbol{q}, \omega) = \frac{1}{1 + \frac{\omega^2}{\Gamma(\mathrm{q})^2}} \tag{11}$$

where Γ is the bandwidth and we used the knowledge that the Fourier transform of an exponential, g, is a Lorentzian, $\tilde{g}$. Importantly, the expression for the bandwidth (or decay rate), Γ, amounts to a (ensemble averaged) *dispersion relation*,

$$\Gamma(\mathrm{q}) = Dq^2 + \Delta v q. \tag{12}$$

Equation 12 relates the spatial frequency (mode) q, with a temporal frequency quantity, Γ, the diffusion coefficient, D, and the standard deviation of the velocity distribution, Δv. The expression shown in Eq. 12, combines the spatiotemporal frequencies associated with microtubule transport, where the active transport is expressed by the linear term and the passive

transport with the quadratic term in q. Hence, through quantitative phase imaging and dispersion-relation analysis, it is possible to retrieve information about and distinguish between both active and passive transport. Our data showed that microtubule gliding is dominated by the directed transport and the fit with Eq. 12 give access right away to the standard deviation of the velocity distribution (See Figs. 5-6).

2. Statistical comparison between the tagged and untagged microtubule populations

We looked at two categories of microtubules, those that were functionalized with biotin, and those without any labels. As in Figure 4, the parameters for consideration were the angle change and the mean gliding velocities. In short, the difference between experimental runs was too significant to support a conclusion regarding the influence of tagging on microtubule motion.

To investigate the statistical difference between biotin-tagging and the angle change behavior of gliding microtubules, we use a Mann-Whitney U-test (see, *e.g.,* Section 8.4. in [5]). This is a non-parametric test similar to an unpaired T-test. In the tagged vs. untagged case, the test yields a value of $p=0.66$, which implies there is no statistically significant difference between tagging and angle change.

To investigate the relationship between biotin-tagging and velocity we use an ANOVA test (see, *e.g.*, Chapter 10 in [5]), with each of the five experimental runs taken as a separate group.

ANOVA for Gliding Velocities					
Source	SS	df	MS	F	Prob>F
Between	4.3341	4	1.08353	49.03	2.84674E-35
Within	12.1337	549	0.0221		

Total	16.4679	553			

From the low “Prob>F” score, it is evident that the five experimental runs are not drawn from the same mean. Looking at Figure 4, there are two categories of microtubule velocity, those with mean ~0.4 and those with mean ~0.6. Further, the “Sum of Squares Between Groups” (SS, Between) parameter is smaller than the “Sum of Squares Within Groups” (SS, Within) indicating that the differences within groups outweigh the difference between biotin tagged, and untagged experiments.

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