I. EXPLAINING TRANSLATION-ROTATION COUPLING FOR ASYMMETRIC PARTICLES

A particle suspended in a fluid like water undergoes both translational and rotational Brownian motion due to random collision with solvent particles surrounding it which have been detected using video camera [1] and other techniques [2]. However, an important aspect of Brownian dynamics that remained almost unattended is the coupling between the translational and rotational Brownian motion inside an optical trap except occasionally [3]. When we are dealing with a complex shaped particle like deformed RBC the coupling term should be prominent as opposed to a sphere or ellipsoid. Any complex body with a propeller like shape will have a coupling term, which causes a complex Brownian motion. A typical example has been shown in Fig. 1.

FIG. 1. The shape of a random particle can be described a simple model as shown in the figure. There are two parts of the particle which can be assumed to be symmetric about the centre of the incident beam. Both of the sections scatter trapping light at different angles and thereby get different amounts of recoil in the horizontal plane. It is this asymmetry that causes both the extra force in translational and the extra torque in rotational dynamics of the particle, thereby correlating motion in these degrees of freedom.

If there is an asymmetry in the two sections of the body, it can be assumed to have an equivalent shape as in the Fig. 1. The net torque and the force acting on the object (assuming the mid point of the object to be origin) are then given by the following equations.

\[ P_1 \cos(\alpha_1) - P_2 \cos(\alpha_2) = F_1 \]  
\[ (P_1 \cos(\alpha_1) + P_2 \cos(\alpha_2)) a = \tau_1,00 \]

Where the length of the RBC is approximately 2a. The \( P_1 \) and \( P_2 \) indicate the fraction of light from the incident light beam which are incident on both the parts of the particle as indicated in the Fig. 1. If the particle is symmetrically located about the trapping beam, the \( P_1 \) and \( P_2 \) are equal, when. As soon as the mid point of the particle is displaced from the center of the beam, it shall experience different \( P_1 \) and \( P_2 \). This will then cause a torque that tries to cause further translational displacement to the particle. Thus the translational motion gets coupled to the rotational motion through the asymmetry of the particle.

II. AUTOCORRELATION OF \( x \) AND \( \theta \)

FIG. 2. The red curve indicates the normalised translational and blue curve the normalised rotational autocorrelation functions (NACF). These functions are almost identical but their amplitude is about 0.01.

In our case, the Brownian motion - both rotational and translational - can be described by the Langevin equation [4], where we add two coupling terms to cross-couple the rotation and the translation equations. Therefore, the final equation becomes:

\[ \gamma_1 \frac{dx(t)}{dt} + \kappa_x x(t) + \gamma_2 \omega_1 \theta(t) = (2k_BT\gamma_1)^{1/2} \zeta_x(t) \]  
\[ \gamma_2 \frac{d\theta(t)}{dt} + \kappa_\theta \theta(t) - \gamma_1 \omega_2 x(t) = (2k_BT\gamma_2)^{1/2} \zeta_\theta(t) \]

where \( \kappa_x \) denotes the force constant of the optical trap for translational motion, while \( \kappa_\theta \) is that for rotational motion that is polarization-dependent (i.e. it tries to orient...
TABLE I. Table showing the comparison of $D$ from fit and from theory for Normal (NRBC), moderately deformed (MoRBC), and maximally deformed (MaRBC) RBC.

<table>
<thead>
<tr>
<th>RBC type</th>
<th>$D$ from fit (rad)</th>
<th>Calculated $D$ (rad)</th>
<th>$D$ from fit (rad)</th>
<th>Calculated $D$ (rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRBC</td>
<td>-0.05(02)</td>
<td>-0.48(13)</td>
<td>-0.08(02)</td>
<td>-0.40(06)</td>
</tr>
<tr>
<td>MoRBC</td>
<td>-0.30(08)</td>
<td>-0.48(13)</td>
<td>-0.25(06)</td>
<td>-0.46(04)</td>
</tr>
<tr>
<td>MaRBC</td>
<td>0.9(1)</td>
<td>0.56(12)</td>
<td>0.75(08)</td>
<td>0.56(06)</td>
</tr>
</tbody>
</table>

an object with optics axis along the polarization of the input laser beam for both birefringence-driven rotation and that due to asymmetric scattering. $(2k_B T \gamma_1)^{1/2} \zeta_x(t)$ and $(2k_B T \gamma_2)^{1/2} \zeta_0(t)$ are two independent Gaussian random noise $(k_B$ being the Boltzmann constant), which represent the Brownian forces at absolute temperature $T$ for both $x$ and $\theta$ coordinate systems. In the above equations, $\gamma_2 \omega_1 \theta(t)$, and $\gamma_1 \omega_2 x(t)$ denote the coupling between $x$ and $\theta$. We solve for $x$ and $\theta$ by taking the derivative of eq. 3 and plugging into eq. 4 to get a second order differential equation. We approximate the solution to

$$x = x_0 e^{iPt}$$

and then solve for $P$ and $Q$.

We then proceed to compute the autocorrelations for translational and rotational motion in the method outlined in Ref. [5], so that

$$<x(t)x(t+\delta t)> = \frac{\alpha k_B T}{\kappa_x} e^{-(\kappa_x/\gamma_1+\kappa_\theta/\gamma_2)/2|\delta t|} \cos(C\delta t),$$

$$<\theta(t)\theta(t+\delta t)> = \frac{\alpha k_B T}{\kappa_{\theta,\text{thea}}} e^{-(\kappa_x/\gamma_1+\kappa_\theta/\gamma_2)/2|\delta t|} \cos(C\delta t),$$

where, $A$ is the amplitude whose functional form we are not able to determine presently, but which we find from experiments to be extremely sensitive as a measure of asymmetry, changing by a large amount for different types of RBCs. $B = (\kappa_x/\gamma_1 + \kappa_\theta/\gamma_2)/2$, and $C = \sqrt{\omega_1 \omega_2 - (\kappa_x/\gamma_1 - \kappa_\theta/\gamma_2)^2}/4$ is a coupling factor. The correlation functions have been normalised as follows.

$$NACF(x) = \frac{<x(t)x(t+\delta t)>}{<x^2(t)>},$$

$$NACF(\theta) = \frac{<\theta(t)\theta(t+\delta t)>}{<\theta^2(t)>}.$$  

III. DETERMINATION OF $\omega_1 \omega_2$

We have

$$C = \sqrt{\omega_1 \omega_2 - (\kappa_x/\gamma_1 - \kappa_\theta/\gamma_2)^2}$$

Now, we assume that $(\kappa_x/\gamma_1) >> (\kappa_\theta/\gamma_2)$, so that

$$\omega_1 \omega_2 = B^2 + C^2$$

IV. CONSISTENCY CHECK OF $D$

The cross-correlation function (CCF) between the translational and rotational Brownian motions [6] is given by:

$$<x(t)\theta(t+\delta t)> = \frac{\alpha k_B T}{\kappa_x} e^{-(\kappa_x/\gamma_1+\kappa_\theta/\gamma_2)/2|\delta t|} \cos(C\delta t + D),$$

$$<x(t)\theta(t+\delta t)> = \frac{\alpha k_B T}{\kappa_{\theta,\text{thea}}} e^{-(\kappa_x/\gamma_1+\kappa_\theta/\gamma_2)/2|\delta t|} \cos(C\delta t + D),$$

where, $\tan(D) = B/C$. We also have the differential cross-correlation, $DCCF$, the difference of two CCFs [7], as

$$<x(t)\theta(t+\delta t) - \theta(t+\delta t)x(t)> = \frac{\alpha k_B T}{\sqrt{\kappa_x \kappa_\theta}} e^{-B|\delta t|} (\cos(C\delta t + D) - \cos(C\delta t - D)).$$

Now, the value of $D$ acts as a consistency check for the values of $B$ and $C$, since $\tan(D) = B/C$, and the fit values of $D$ can thus be compared independently to that obtained from this relation. Table I shows that we obtain matches for all three types of RBCs considering the overlaps of error bars at the $3\sigma$ level$^1$ for both the CCF and DCCF.

V. EXPERIMENTAL SETUP AND SAMPLE PREPARATION

The experimental setup is described in detail in Ref. [8]. The trapping laser is a linearly polarized 1064 nm diode laser with maximum power 500 mW, coupled into the back port of an inverted microscope using coupling optics. The scattering from trapped objects is measured through the side port using a quadrant photodiode (QPD) that has been extracted from a commercial CD player head (chip Sony KSS-213C) [8]. Imaging is also done through this side port using a video camera. The sample chamber is constructed using a standard microscope glass slide and a glass coverslip which are stuck together using a double sided tape. About 50 µl of the aqueous solution sample is loaded in the chamber. For isolating individual RBCs, we collected about 50 µl blood sample from a healthy donor and diluted it in 1 ml of saline (0.9 percent w/v NaCl in $H_2O$) water to prepare...
the stock solution. 10 µl of anti-coagulating agent Heparin was added into this stock solution to prevent clumping of RBC. The solution was further diluted by mixing 200 µl of the stock solution into 1 ml of 2 percent w/v saline solution. The degree of salinity was varied to control the shape of the RBC. The sample was kept at 4 degC so that RBC did not disintegrate within a day. Data for translational and rotational Brownian motion are acquired at a sampling frequency of 40KHz taken for 5 seconds using NI PCIe-6361 data card.

VI. MEASUREMENT OF POWER SPECTRAL DENISTY

The measurements of Brownian motion are performed with a quadrant photodiode, and we use the technique we recently developed to detect rotational and translational motion simultaneously by measuring the sum and difference of the signals from the diagonal quadrants of the QPD [9]. The rotational and translational data are acquired at a sampling frequency of 40KHz taken for 5 seconds. Typical time series for translational and rotational Brownian motion for normal RBC are given in Fig. 1(d) of the main paper. The histograms of the time series data fit well to Gaussian distributions - a sample of which is shown in Figs. 1(e) in the main paper. Corresponding power spectral densities (PSD) for both translational and rotational motion are obtained by averaging over 25 individual spectra, as we show in Fig. 3(a). We also check that our signal PSD for RBCs is substantially higher than that of the noise floor of the setup, as is clear in Fig. 3(b). As is clear from the comparison of the two figures, the S/N of both the rotational and translational PSD are between $10^2 - 10^4$ times higher than that of the noise floor PSD.

![FIG. 3. (a) PSD for translational and rotational Brownian motion. (b) PSD of noise floor of our setup. It is clear that the S/N of both the rotational and translational PSD are between $10^2 - 10^4$ times higher.](image)

VII. DISCUSSION ON RBC MEMBRANE FLUCTUATIONS

The red blood cell membrane is a lipid bilayer under tension. This membrane can exhibit excitations due to the thermal effects. While membrane fluctuations in RBCs have led to interesting and intriguing research presently, our technique of studying the cross-correlation between rotational and translational Brownian motion is not really sensitive to those. To explain why, let us take a closer look at our rotational detection system. We take the light scattered in all four quadrants of a QPD and then take the difference in diagonal quadrants. Thus, any possible motional modes which may be radially symmetric would get cancelled out in this way. Now, the theory of thermal membrane fluctuations are well established. Let us take examples of Refs. [10–12]. It is clear that the fluctuation modes considered are well fitted to a function

$$\langle |u(q)|^2 \rangle = \frac{k_B T}{\sigma^2 + kq^4}$$

(13)

where, $\sigma$ is the surface tension, $k$ is the membrane bending modulus, and $q = n/R$, $n$ being an integer and $R$ the radius of the spherical object. The backscatter pattern for all of these modes are going to be radially symmetric and hence would lead to zero signal when used with the diagonal difference technique. The non-radial modes are only much higher order modes and their contribution to the power spectrum would be much smaller than the modes mentioned in Eq. 13. Further, if the RBC becomes ellipsoidal, it will line up with the sides being along opposite halves of the QPD. The technique of detection of rotational motion will still cancel out such breathing modes.

The fact that we have an offset in the cross correlation function while the autocorrelation functions have no offset in time tells us that the diagonal difference technique is indeed picking up some non-zero signal which is correlated to the translational motion. The translational modes are essentially due to Brownian motion as the size of the RBC is comparable to the laser focal spot. Besides, membrane fluctuations are typically detected by focusing a laser beam at the sides of RBCs [11, 12], while in our case we typically focus the beam near the RBC center. Hence, the probability of detecting translational Brownian motion is overwhelmingly stronger. Finally, in our technique, only the rotational Brownian motion could be correlated to the translational Brownian motion and not the membrane fluctuations.


