A self-assembled microlensing rotational probe

James P. Brody and Stephen R. Quake

Department of Applied Physics, California Institute of Technology, Pasadena, California 91125

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A technique to measure microscopic rotational motion is presented. When a small fluorescent polystyrene microsphere is attached to a larger polystyrene microsphere, the larger sphere acts as a lens for the smaller microsphere and provides an optical signal that is a strong function of the azimuthal angle. We demonstrate the technique by measuring the rotational diffusion constant of the microsphere in solutions of varying viscosity and discuss the feasibility of using this probe to measure rotational motion of biological systems. © 1999 American Institute of Physics.
where \( n_2 \) is the index of refraction of the lensing microsphere and \( n_1 \) is the index of refraction of the surrounding medium (typically water). Applying Snell’s law at the bottom interface we obtain

\[
\theta' = \sin^{-1}\left(\frac{n_1}{n_2} \sin(\theta + \phi)\right).
\]

Then, by direct substitution of Eq. (3) into Eq. (2), we find that

\[
\theta'' = \phi + \theta.
\]

Using the law of sines, we can write

\[
\sin(\pi - \theta - \phi) = \frac{\sin \theta}{r + \delta},
\]

and then explicitly find the angle \( \phi \) as a function of \( r, \theta \), and \( \delta \):

\[
\phi(r, \theta, \delta) = \sin^{-1}\left(\frac{r + \delta}{r} \sin \theta \right) - \theta.
\]

Finally, we can write the exit angle \( \theta' - \theta'' \) in terms of the original angle \( \theta \), the radii of the two spheres \( r, \delta \), and the indices of refraction \( n_1 \) and \( n_2 \):

\[
\theta' - \theta'' = 2 \sin^{-1}\left(\frac{n_1}{n_2} \sin[\theta + \phi(r, \theta, \delta)]\right) - \theta - 2 \phi(r, \theta, \delta).
\]

For \( \delta \ll r \), we note that \( \phi \approx \theta \). The exit angle is then given by

\[
\phi' - \theta'' = 2 \sin^{-1}\left(\frac{n_1}{n_2} \sin \theta \right) - \theta.
\]

Typical realizable values of \( n_1 \) and \( n_2 \) are for water \( n_1 = 1.3 \), and polystyrene \( n_2 = 1.59 \). For small \( \theta \), Eq. (8) reduces to \( [2(n_1/n_2) - 1] \theta \). This gives an exit angle of 0.64\( \theta \) for a ray entering at an angle \( \theta \). Since the exit angle is always less than the original angle, we conclude that the lensing microsphere focuses rays from the fluorescent microsphere and enhances the optical signal.

The enhancement in the observed optical signal also depends on the numerical aperture (NA) of the objective. The NA is defined as \( \text{NA} = n \sin \theta_0 \), where \( \theta_0 \) is the collection angle. For our objective (20\( \times \), 0.4 NA) in air \( \theta_0 = 23.6^\circ \). Equation (8) shows that the focusing microsphere increases the angle of collection to 43.5\( ^\circ \). This corresponds to an effective NA of 0.69. The epifluorescent intensity is proportional to \( \text{NA}^4 \), so we expect an intensity enhancement of \((0.69/0.4)^4 \approx 9\) times; this is of the order of what we observed. Objectives with high NA collect almost all of the emitted light, and thus we would not expect to see any fluorescent enhancement from a high NA objective. Observations made with a 40\( \times \), 1.30 NA oil immersion objective indicate no noticeable intensity enhancement.

To construct this system we used the biotin/streptavidin binding system. Biotin is known to bind streptavidin with a very high affinity.\(^{15}\) We obtained streptavidin coated microspheres from Interfacial Dynamics (polystyrene at a volume concentration of 2.6\%, 1.1 \( \mu \)m diameter) and biotin coated yellow–green fluorescent microspheres (polystyrene 1% by volume, 0.2 \( \mu \)m diameter, excitation maximum at 505 nm, emission maximum at 515 nm) from Molecular Probes. The smaller microspheres were diluted by a factor of 1000 in distilled water. A volume of 10 \( \mu \)l of this solution was added to 10 \( \mu \)l of the large microspheres and allowed to incubate at room temperature for approximately 5 min. The solution was then diluted by a factor of 10\(^4\) in either distilled water or a glycerol/water mixture to give a final concentration of approximately 1 microsphere pair per nanoliter at three different viscosities (1, 4, and 13 cP).

The solution was placed on a microscope slide with a coverslip and the edges were sealed with a clear nail polish to prevent any evaporation or fluid flow. These specimens were examined on a Nikon fluorescent microscope with a 20 \( \times \), 0.4 NA objective. The lensing microsphere undergoes constant rotational diffusion, and when the attached fluorescent microsphere is aligned with the optical collection axis the observed intensity is substantially larger than in other positions.

Images of these intensity fluctuations were observed using a Phillips FTM-800 charge coupled device (CCD) camera and recorded on video tape. The sequence of video images was analyzed by digitizing them with a PC at a 30 Hz frame rate. The microspheres’ fluorescent intensities were measured in each frame, and their translation diffusion was slow enough that their positions could be followed from frame to frame. Data with better time resolution could be obtained by using a photodiode or photomultiplier tube to observe single microspheres, but the video camera is sufficiently fast for observing rotational diffusion and offered the advantage of being able to observe many microsphere pairs in parallel.

From the intensity fluctuations we can compute the probability \( P(t) \) that the intensity is above a threshold at time \( t \), given that the intensity was above this threshold at time \( t = 0 \). \( P(t) \) can in turn be calculated from first principles. The rotational motion of the lensing microsphere can be decomposed into orthogonal azimuthal and polar directions. Since only the azimuthal angle contributes to the intensity enhancement, it suffices to consider a one-dimensional problem. Let \( \alpha \) denote the azimuthal angle over which the signal is enhanced, which then determines the initial conditions. The solution for the one-dimensional diffusion equation with initial condition \( |\theta| \leq \alpha \) and infinite boundary conditions is\(^{16}\)

\[
P_{\text{int}}(\theta, t) = \frac{1}{2\alpha} \left[ \text{erf} \left( \frac{\alpha/2 - \theta}{\sqrt{D_r t}} \right) + \text{erf} \left( \frac{\alpha/2 + \theta}{\sqrt{D_r t}} \right) \right],
\]

where \( D_r \) is the rotational diffusion constant of a sphere

\[
D_r = \frac{k_B T}{8 \pi \eta a^3},
\]

\( \eta \) is the viscosity and \( a \) is the sphere’s radius.\(^{17}\) Equation (9) can be made periodic in \( \theta \) by summing over all multiples of 2\( \pi \). The probability density function for the distribution in angles is then

\[
p(\theta, t) = \sum_{n=-\infty}^{n=\infty} P_{\text{int}}(\theta + 2n \pi, t)
\]

and the total probability distribution is
The probability correlation function of the fluorescent intensity signal measured in three different glycerol–water solutions of varying viscosities. These measurements are the average over approximately 30 microspheres measured over approximately 1 min. The errors in the measurements are on the order of the diameter of the circles marking the spheres measured over approximately 1 min. The errors in the ties. These measurements are the average over approximately 30 microspheres measured in three different glycerol–water solutions of varying viscosities.

FIG. 2. The probability correlation function of the fluorescent intensity signal and integrating it using a trapezoid algorithm. The inset shows typical data from a single microsphere that is characterized by the angle \( \theta = 4 \)°.

The rotational diffusion coefficient \( D_r \) is determined by the optics of the system and the threshold applied to the data. The rotational diffusion coefficient \( D_r \) is given by Eq. (10). The measured diffusion coefficients are in agreement with the predicted values (Fig. 2).

We have outlined a mechanism by which a microlensing effect is used to measure rotations of individual microspheres. The microlensing can be explained with geometric optics; a complete theory will need to take into account near field optical effects. The microlensing was observed experimentally and used to measure the rotational diffusion constant of a sphere. This mechanism may have applications in studies of biomolecular rotations and fluid dynamics, especially in situations with a high rotational rate. For a rotation at a constant rate, the load can be adjusted by changing both the viscosity of the fluid and size of the larger microsphere. Precision microspheres are commercially available with diameters of less than 1 \( \mu \)m to greater than 100 \( \mu \)m giving a range of loads that differ by \( 10^6 \).

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