Brownian modulated optical nanoprobes

C. J. Behrend, J. N. Anker, and R. Kopelman
Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1055

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Brownian modulated optical nanoprobes (Brownian MOONs) are fluorescent micro- and nanoparticles that resemble moons: one hemisphere emits a bright fluorescent signal, while an opaque metal darkens the other hemisphere. Brownian motion causes the particles to tumble and blink erratically as they rotate literally through the phases of the moon. The fluctuating probe signals are separated from optical and electronic backgrounds using principal components analysis or images analysis. Brownian MOONs enable microrheological measurements on size scales and timescales that are difficult to study with other methods. Local chemical concentrations can be measured simultaneously, using spectral characteristics of indicator dyes embedded within the MOONs. © 2004 American Institute of Physics. [DOI: 10.1063/1.1637963]

Rotational viscosity is a fundamental property of materials relevant to fields from biophysics to materials and chemical processing. Its measurement is important for understanding molecular behavior, cell function, colloidal properties, and bulk materials. For non-Newtonian and homogeneous fluids, the measured viscosity is a complex function of timescales over which forces are applied, and the size scale of the instrument used to probe the viscosity. On the microscopic scale, measurements of viscosity in the cell cytosol have varied over six orders of magnitude depending on the size of the probe used. Recent work suggests sieve-like structures within subdomains of cytoplasm that allow small particles to diffuse through while resisting the motion of larger particles. In addition, liquid filled structures such as endosomes may allow particles to rotate within but not translate, causing divergence between rotational and translational Brownian diffusion. Macroscopic instruments determine viscosity by measuring the resistance of a fluid to mechanical movement. Similarly, thermal fluctuations of microscopic particles can be used to mechanically probe viscosity on a much finer scale.

In 1828, Robert Brown first observed pollen grains translating in unpredictable directions and tumbling around their own axes. This “Brownian motion” was explained in 1906 by Albert Einstein who derived the following equations for the rotational and translational diffusion coefficient, \(D_{\text{rot}}^m\) and \(D_{\text{trans}}^m\) of a spherical particle:

\[
D_{\text{rot}}^m = \frac{k_B T}{8 \pi \eta r^3}, \quad D_{\text{trans}}^m = \frac{k_B T}{6 \pi \eta r},
\]

where \(k_B\) is Boltzmann’s constant, \(T\) is the temperature, \(\eta\) is the viscosity, and \(r\) is the particle radius. Jean Perrin employed studies of Brownian motion of 13 \(\mu m\) particles to demonstrate the existence of molecules and evaluate Avogadro’s number. Most current studies of microscopic rotation continue to use larger particles (>2 \(\mu m\)) with observable landmarks.

Molecular translational and rotational diffusion is studied by optical techniques such as fluorescence anisotropy, fluorescence recovery after photobleaching (FRAP), and fluorescence correlation spectroscopy. To measure translational diffusion on larger size scales (50 nm–5 \(\mu m\)), the motion of nanospheres and microspheres are observed while they are pulled through the solution with magnetic tweezers, or move under the influence of Brownian forces. Rotational motion of microspheres and nanospheres has proven more difficult to measure. Dynamic light scattering and polarized FRAP are used to determine rotational diffusion coefficients for ensembles of particles; however, these techniques generally acquire an average over a large number of particles and are effected by scattering. Alternatively, particle orientation may be observed directly, but only if each particle has an observable landmark or is not spherical in shape. Particle orientation may also be determined by measuring the average magnetic moment from magnetized particles. However, the magnetic methods provide information only on the average particle and require a relatively large number of magnetic particles to obtain an accurate reading.

Brownian MOONs provide a method for measuring rotational viscosity in the vicinity of single particles. Brownian MOONs have one hemisphere coated with an opaque metal cap so that only the other hemisphere is able to emit light. The particles blink erratically as Brownian motion causes them to tumble. Fluorescent detection of this modulated signal allows the dynamics affecting small particles to be observed, with high accuracy and on short timescales. A simple process is used to prepare large batches of particles, and their size can be controlled over a wide range, including particles far smaller than the diffraction limit of visible light.

The autocorrelation function of the intensity fluctuations from a randomly rotating Brownian MOON provides a measure of the rotational diffusion rate. Experiments using magnetic fields to rotate 4.4 \(\mu m\) magnetically modulated optical nanoprobes (MagMOONs) indicated the fluorescence intensity is proportional to the cosine of the azimuthal angle of the particles, with weaker harmonics also appearing. Assuming that the fluorescence intensity from Brownian MOONs is also proportional to the cosine of the azimuthal angle, the autocorrelation function, \(G_{\text{rot}}(\tau)\), of the fluctuations is expected to decay exponentially [Eq. (2)].

\[
G_{\text{rot}}(\tau) = e^{-\tau/\tau}, \quad \tau = \frac{k_B T}{2 k_B T}.
\]
For a spherical particle, the shape factor $k$ is 6; for aspherical particles, the shape factor depends on the relative dimensions of the particle. The rotational diffusion time depends on volume, $V$, of the particle and is proportional to the viscosity of the surrounding solvent environment. If harmonics are present in the angular intensity distribution, the autocorrelation function is expected to decay more quickly.

Brownian MOONs are produced using simple physical processes. Fluorescent nano- or microspheres are allowed to settle, forming a monolayer on a microscope slide. Using vapor deposition of aluminum, or sputter-coated gold, one hemisphere of the particles is coated with around 100 nm thick layer of an opaque metal, as shown in Fig. 1. This metal capping layer prevents excitation light from reaching the fluorophores and also blocks emitted fluorescence from leaving through the coated hemisphere of the particle. After completing the coating, the particles are removed from the glass slide by gentle brushing with an artists brush. Sonication function is expected to decay more quickly. This signal separation dramatically improves signal to background levels. The main sources of light contributing to the observed spectra were determined using PCA and are shown in Fig. 3. The time series, or “score,” for each of the components in Fig. 3(c) is calculated by projection of the principal components onto the original data space. The random fluctuation rate for the Brownian MOONs, with a characteristic correlation time, $\tau$, is directly proportional to the rotational viscosity. Brownian MOONs thus provide a measurement of viscosity in their local environments.

The autocorrelation time for 850 nm aluminum coated Brownian MOONs in each solution was measured and used to prepare the calibration curve shown in Fig. 4. Figure 4(a) shows typical autocorrelation functions from particles in different viscosity liquids. Figure 4(b) shows a calibration curve from the average autocorrelations for a number of particles and trials. The autocorrelation function decayed exponentially and, as expected, the decay rate increased linearly with increasing viscosity. Overall, the observed values for $\tau$ were systematically 25% shorter than predicted from theory. The theoretical values were calculated using Eq. (2) and included the volume change from the 100 nm aluminum capping (which increases $\tau$ by 18%) and the aspherical shape factor (which increased $\tau$ by 5%). Additional sources of error in-
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18See EPAPS Document No. E-APPLAB-84-006401 for a video of Brownian MOONs. A direct link to this document may be found in the online article’s HTML reference section. The document may also be reached via the EPAPS homepage (http://www.aip.org/pubservs/epaps.html) or from ftp.aip.org in the directory/epaps/; see the EPAPS homepage for more information.