Dynamic Light Scattering from Colloidal Spheres and Proteins in Femtoliter Volumes

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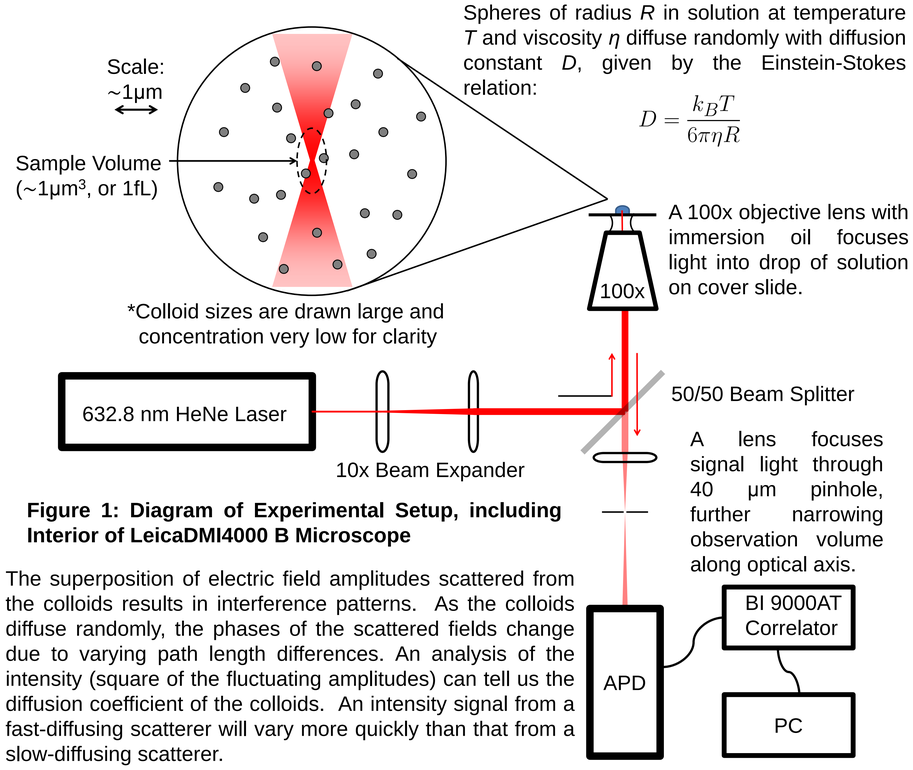
Abstract

We have built a setup for performing Dynamic Light Scattering (DLS) and Fluorescence Correlation Spectroscopy (FCS) experiments on femtoliter volumes. A 632.8nm Helium Neon laser was directed through an objective lens on a LeicaDMI4000 B microscope and focused onto an aqueous drop of colloidal spheres (on the order of 100nm in diameter) of varying glycerol concentration. The light backscattered from the sample was focused through a pinhole, and the diffracted light was collected by an Avalanche Photodiode (APD) detector. The raw signal from the APD was hardware-processed with a Brookhaven Instruments 9000AT Digital Autocorrelator. The correlation function was analyzed using second-order Cumulant Analysis in order to yield a measure of effective diameter of the colloidal spheres. We find that this technique produces results consistent with our standard DLS setup, which uses milliliter-size sample volumes. This technique was also used to measure the effective diameter of Hen Egg White Lysozyme (HEWL) protein, which cannot be done on the previous setup.

Apparatus



Spheres of radius *R* in solution at temperature *T* and viscosity *η* diffuse randomly with diffusion constant *D*, given by the Einstein-Stokes relation:

$$D = \frac{k_B T}{6\pi\eta R}$$

A 100x objective lens with immersion oil focuses light into drop of solution on cover slide.

A lens focuses signal light through 40 μm pinhole, further narrowing observation volume along optical axis.

Figure 1: Diagram of Experimental Setup, including Interior of LeicaDMI4000 B Microscope

The superposition of electric field amplitudes scattered from the colloids results in interference patterns. As the colloids diffuse randomly, the phases of the scattered fields change due to varying path length differences. An analysis of the intensity (square of the fluctuating amplitudes) can tell us the diffusion coefficient of the colloids. An intensity signal from a fast-diffusing scatterer will vary more quickly than that from a slow-diffusing scatterer.

Theory

We analyze the intensity fluctuations with an autocorrelation function (ACF):

$$G(\tau) = \int I(t)I(t+\tau)dt$$

If τ is small compared to the fluctuations, the signal is correlated. For large, τ it is decorrelated:

$$G(0) = \langle I(t)^2 \rangle \qquad G(\infty) = 0$$

For a monodisperse solution, where every scatterer is the same size, $G(\tau)$ decays exponentially with decay constant Γ, where *q* is the magnitude of the scattering vector:

$$G(\tau) = e^{-\Gamma\tau} \qquad \Gamma = Dq^2 \qquad q = \frac{4\pi n}{\lambda} sin(\frac{\theta}{2})$$

Here, *n* is the refractive index of the solution (dependent on glycerol concentration), *λ* is the wavelength of the light (632.8nm), and *θ* is the angle of reflection (180º in the micro-DLS setup). A linearized correlation function will have slope –Γ, which can be used to find the diffusion constant D, which relates to the scatterer's effective diameter by the Einstein-Stokes relation.

Analysis

For a perfectly monodisperse sample, a straight line would fit the linearized data:

$$\ln(G(\tau)) = -\Gamma\tau$$

Typically, there is a distribution of diameters centered on a mean. For a distribution, the linearized ACF is not a straight line. We have used Cumulant Analysis to more accurately measure the mean diameter. Cumulant Analysis uses a Taylor series expansion of the linearized data with respect to τ:

$$\ln(G(\tau)) = -\Gamma\tau + (\frac{\mu_2}{2!})\tau^2 - (\frac{\mu_3}{3!})\tau^3 + (\frac{\mu_4 - 3\mu_2^2}{4!})\tau^4 - \ldots$$

The first term on the right is unchanged, but we have allowed for higher order terms of $G(\tau)$. The second order coefficient gives the polydispersity of the size distribution (the width of a Gaussian peak), the third order coefficient gives the skew, and the fourth order term gives the kurtosis. We have limited our analysis to a second order fit, which defines our distribution as Gaussian with a given mean and width only. We are interested in the mean diameter, and the second order term is the highest to have a nontrivial effect on the mean derived from the fit.

Sample of Data

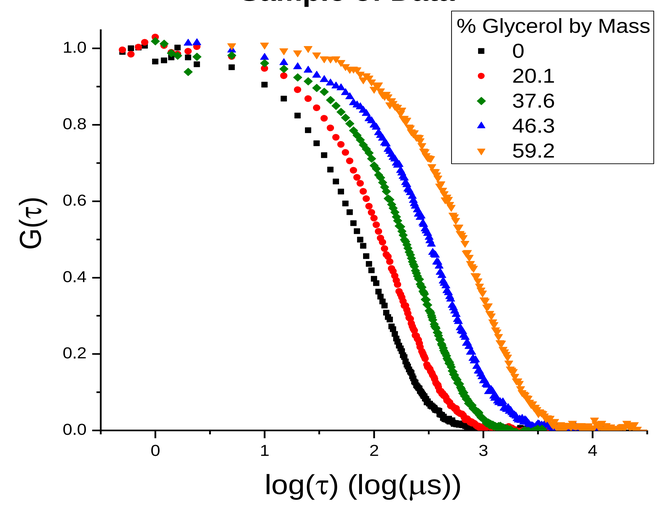


Figure 2 (above): Linear-Log Scale ACFs of 60nm Colloids in Glycerol Mixtures, Normalized and Background Subtracted

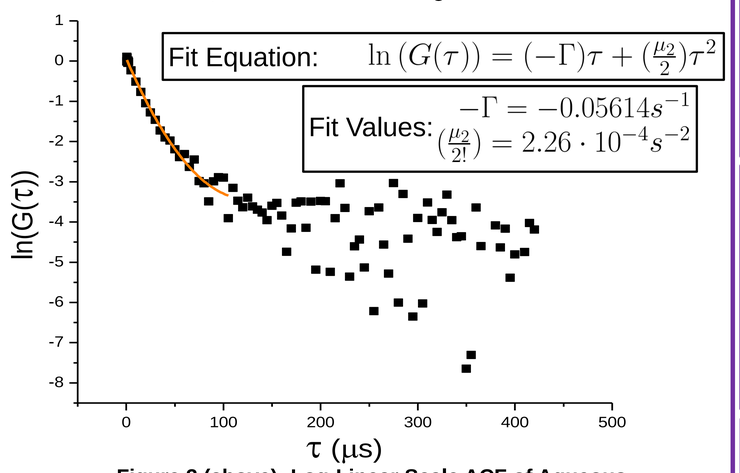


Figure 3 (above): Log-Linear Scale ACF of Aqueous Lysozyme with Second-Order Polynomial Fit

Results

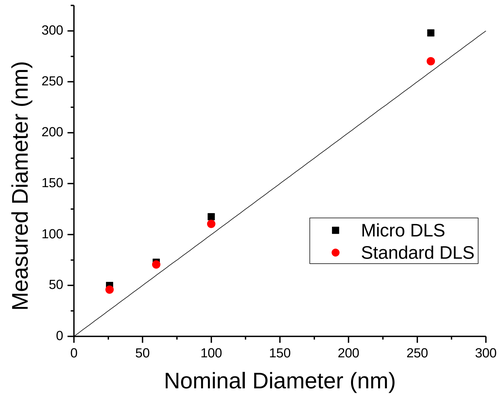
We measured the diameter of a 60nm colloid in five different glycerol solutions with the result:

$$d = [66 \pm 3]nm(4\%)$$

The small relative uncertainty confirms reproducibility of results with Micro-DLS.

%Mass Glycerol	Viscosity (mPa*s)	Diameter (nm)
0	0.94	67.96
20.1	1.61	65.58
37.6	2.93	61.97
46.3	4.46	69.59
59.2	9.12	67.31

Table 1: 60nm Colloids in Glycerol Solutions



We have compared the Micro-DLS measurements with the standard setup by measuring effective diameters of four different colloids, with nominal sizes of 26nm, 60nm, 100nm, and 220nm. Our data from the two DLS experiments are consistent with each other, but both yield systematically higher diameters than the nominal values. We have also found that the Micro-DLS gives inconsistent results for large spheres, presumably because the spheres are large with respect to the sample size.

Figure 4: Four Colloid Sizes Measured with Two DLS Apparatus

Discussion and Future Work

The new femtoliter-scale DLS system produces results consistent with the standard system but has unique advantages. The standard DLS apparatus cannot collect enough scattered light from lysozyme to perform an autocorrelation analysis because the proteins are too small. Also, the micro-DLS can perform localized measurements of diffusion. Since the cross-sectional area of the observation volume is about a square micron, we can perform diffusion measurements on lysozyme at a particular location, such as near a growing crystal. Another advantage of the Micro-DLS is its adaptability to Fluorescence Correlation Spectroscopy (FCS). We have used the device in this regime to measure the size of fluorescing colloids with a different model. With a stronger laser, more sensitive detector, and more refined optics, it may be possible to perform FCS on proteins labeled with Alexa 633 Dye or on the dye itself as it diffuses through a protein crystal. Currently, we are developing software to use with a NI PCI-6602 high speed (80MHz) counter, which will allows us access to the raw pulse train from the detector. This will allow us to perform not only a correlation function analysis, but also Photon Arrival-time Interval Distribution (PAID) analysis, which yields more information than DLS and FCS experiments.

References

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Acknowledgments

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