Understanding Dynamic Light Scattering

When in solution, macromolecules are buffeted by the solvent molecules. This leads to a random motion of the molecules called Brownian motion. For example, consider this movie of 2 µm diameter particles in pure water. As can be seen, each particle is constantly moving, and its motion is uncorrelated with the other particles. (Movie courtesy of Dr. Eric R. Weeks, Emory University).

Brownian motion

Brownian motion.

As light scatters from the moving macromolecules, this motion imparts a randomness to the phase of the scattered light, such that when the scattered light from two or more particles is added together, there will be a changing constructive or destructive interference. This leads to time-dependent fluctuations in the intensity of the scattered light (Fig. 1).

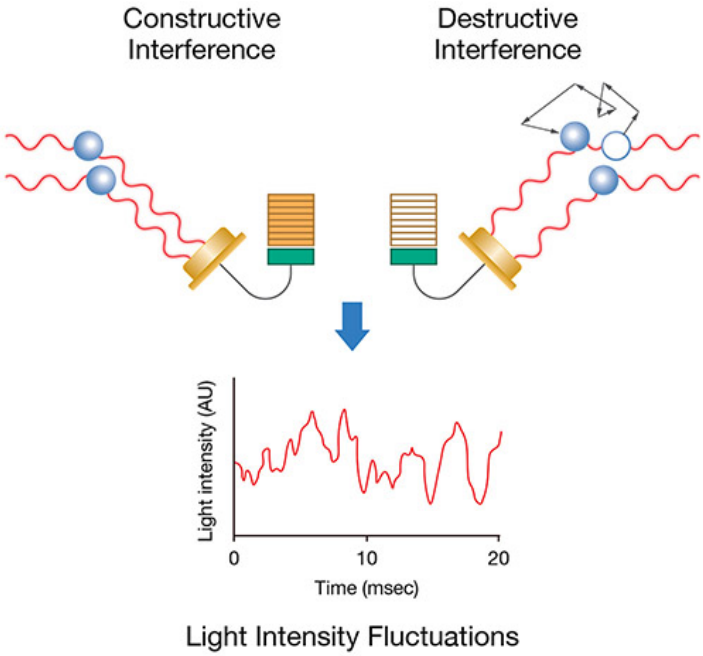

Figure 1. Brownian motion results in measurable fluctuations in the light intensity over time.

In dynamic light scattering (DLS), the time-dependent fluctuations in the scattered light are measured by a single photon counting module. The rate of fluctuations is directly related to the rate of diffusion of the molecule through the solvent, which is related in turn to the particles' hydrodynamic radii. Smaller particles diffuse faster, causing more rapid fluctuations in the intensity than larger particles. Therefore, the fluctuation in light intensity contains information about the diffusion of the molecules and can be used to extract a diffusion coefficient and calculate a particle size.

DLS is employed by the DynaPro® NanoStar®, the DynaPro® Plate Reader, the Mobius™ and the WyattQELS™ module module for MALS detectors to determine the effective particle size. The analyte's translational diffusion coefficient, D_t, is obtained by automated nonlinear least squares fitting of the autocorrelation function that quantitatively describes the measured time-dependent fluctuations in light scattering intensity. The analysis is done directly in the accompanying DYNAMICS®, DYNAMICS® Touch™ or ASTRA® software. A typical autocorrelation function for a monodisperse sample is shown in Fig. 2.

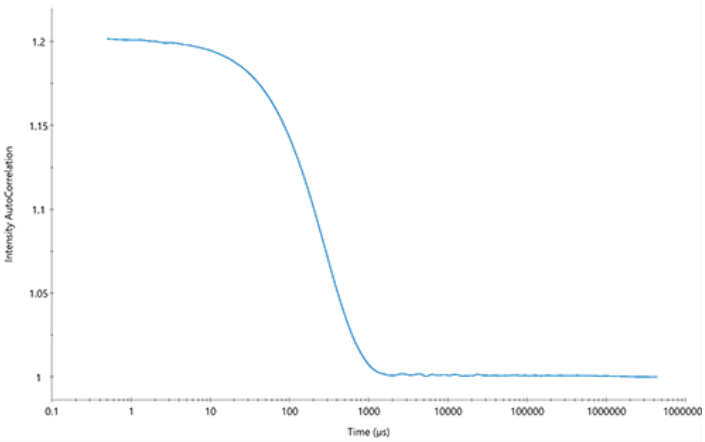

Figure 2. Autocorrelation function for a monodisperse sample with 50 nm R_h particles.

The Stokes-Einstein equation then gives the hydrodynamic radius, R_h, (Fig. 3) corresponding to the measured D_t:

$$R_h = \frac{kT}{6\pi\eta D_t}$$

where k is Boltzmann's constant, T is the temperature in K, and η is the solvent viscosity.

Different sizing techniques, e.g., DLS, small angle X-ray scattering, microscopy, and molecular modeling may report different types of radii. It is therefore important to know how a reported "size" was determined and whether it refers to the radius or diameter of the molecule. The R_h measured by DLS is the radius of a hard sphere with the same diffusion coefficient as the sample. Other measures include R_g (the radius of gyration, or root-mean-square radius, obtained by e.g., MALS), R_{Vol} (the radius of a hypothetical sphere that occupies the same volume as the macromolecule), and R_{Rot} (the radius subtended by rotating the macromolecule). Fig. 3 illustrates how these different measures compare for the compact, globular protein lysozyme.

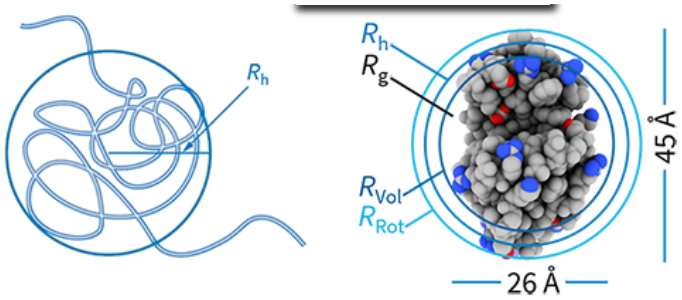

Figure 3. (Left) R_h is the radius of a hard sphere with the same diffusion coefficient as the sample. (Right) Compact molecules, e.g., globular proteins such as lysozyme, generally have $R_g < R_h$.

+ Analyzing Particle Size Distributions

+ Measuring Nanoparticle Concentration

+ Characterizing Intermolecular Interactions

− Understanding the Autocorrelation Function

In DLS, the fluctuations in light intensity measured over time are quantified via a second order correlation function $g^{(2)}(\tau)$. The function of intensity is shifted by a delay time (τ) and the autocorrelation function $g(\tau)$ is calculated. Qualitatively, the autocorrelation function is a measure of how similar the intensity function is to itself when shifted by time τ. As the value of τ increases, the function reaches a baseline of 1.

More specifically, and as described in various light scattering texts (cf. Chu, B. Laser Light Scattering: Basic Principles and Practice; Academic Press: Boston, 1991), the correlation function for a monodisperse sample can be analyzed by the equation:

$$g^{(2)}(\tau) = 1 + \beta\exp(-2\Gamma\tau)$$

where β is the correlation function amplitude at zero delay, Γ is the decay rate, and the baseline of the correlation function relaxes to a value of 1 at infinite delay.

DYNAMICS and ASTRA use a nonlinear least squares fitting algorithm to fit the measured correlation function to equation 2 to retrieve the correlation function decay rate Γ. From this point, Γ can be converted to the translational diffusion coefficient Dt for the particle via the relation:

$$D_t = \frac{\Gamma}{q^2}$$

Here, q is the magnitude of the scattering vector, and is given by:

$$q = \frac{4\pi n_0}{\lambda} \sin\left(\frac{\theta}{2}\right)$$

where n_0 is the solvent index of refraction, Λ0 is the vacuum wavelength of the incident light, and q is the scattering angle.

Finally, the diffusion coefficient can be interpreted as the hydrodynamic radius R_h of a diffusing sphere via the Stokes-Einstein equation:

$$R_h = \frac{kT}{6\pi\eta D_t}$$

where k is Boltzmann's constant, T is the temperature in K, and η is the solvent viscosity (Fig. 3).

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