DYNAMIC LIGHT SCATTERING

Objectives of the exercise:

- Perform dynamic light scattering measurements and determine the hydrodynamic radius of a molecule.

Caution! Laser light is dangerous to the eyes. It is therefore very important that the laser light is blocked everytime the solutions is changed.
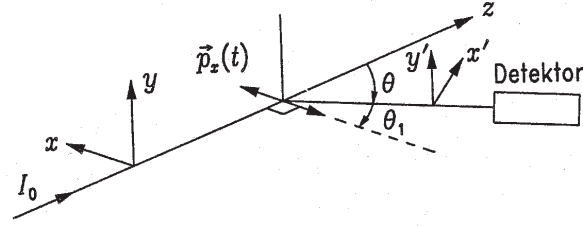
Figure 1: Radiation from induced dipole $p_x(t)$ oscillating in x-direction. The angle θ is the scattering angle. The detector is moving in the xz-plane.

1 Theory

Light scattering is an important method for the determination of molecular weight, size and geometry of macromolecules in solution. The theory of light scattering from macromolecule solutions is given in some detail in the lectures in Molecular Biophysics. Here the aspects that are directly relevant to the understanding of this exercise are repeated.

In static light scattering the scattered intensity is measured over several second so we obtain an *averaged* scattered intensity. In dynamic light scattering the scattered intensity is measured over a very short time interval (200 ns) such that we can see the *fluctuations* in the scattered intensity.

1.1 Dipole radiation

In classic electromagnetic theory a flux of photons is described as a continuous harmonic electromagnetic wave with wave number

$$k = \frac{2\pi}{\lambda} = \frac{2\pi\nu}{c} \tag{1}$$

where λ is the wavelength, c is the phase velocity and ν is the frequency of the light. For visible light λ is between 400 and 700 nm. If an atom is placed in such a harmonic electromagnetic field, the applied electric field $E(t)$ will perturb the electronic orbitals and induce a harmonically oscillating dipole momentum $p(t)$, see Fig. 1. If the electromagnetic field E is polarized in x-direction, also the induced dipole momentum p is in x-direction:

$$p_x(t) = \alpha E_x(t) \tag{2}$$

where α is the polarisability of the atom. The harmonic time variation of the induced dipole momentum implies that charges are accelerated, which always leads to emission of electromagnetic radiation. For radiation from a harmonic oscillating electric dipole the electric field of the resulting *dipole radiation* is polarized in the same direction as the dipole, in our case along the x-axis. With a detector in scattering direction θ the component of the dipole radiation in the x'-direction is measured (see Fig. 1), and this is given by the expression

$$E_{x'}(\theta) = \frac{p_x(t)\pi\nu^2}{\epsilon_r\epsilon_0 c_0^2 r}\cos\theta \tag{3}$$

where

ν = $\omega/2\pi$ = the frequency of the radiation
ϵ = relative permittivity in the surrounding medium
ϵ_0 = permittivity in vacuum
c_0 = light velocity in vacuum
r = distance between the dipole and the detector
θ = scattering angle (see Fig. 1)

By further assuming $\epsilon = 1$ (the light path between cuvette holder and detector is mostly air) and using the relation $\lambda_0 = c_0/\nu$, we obtain the following expression for the intensity, $I_{x'}(\theta)$, of the scattered light in direction θ

$$I_{x'}(\theta) \propto E_{x,0}^2 \frac{\alpha^2 \pi^2}{\epsilon_0^2 \lambda_0^4 r^2} \cos^2 \theta \qquad (4)$$

The equation above was developed for point scatterers (atoms), but it turns out to be also valid for macromolecules that are small compared with the wavelength of light if α is replaced by α_M, the polarization of the macromolecule. For macromolecule solutions where N is the number of scattered macromolecules per unit volume, the intensity of the scattered light *per unit volume of the macromolecule solution* will be equal to

$$I_{x'}(\theta) = N I_0 \frac{\alpha_M^2 \pi^2}{\epsilon_0^2 \lambda_0^4 r^2} \cos^2 \theta \qquad (5)$$

when the incoming light with intensity I_0, is polarized in the x-direction (Fig. 1).

For incoming light polarized in the y-direction the scattered light will be polarized in the y'-direction and has the intensity

$$\boxed{I_{y'}(\theta) = N I_0 \frac{\alpha_M^2 \pi^2}{\epsilon_0^2 \lambda_0^4 r^2}} \qquad (6)$$

which is independent of the scattering angle.

If the incoming light is unpolarized, the intensity is the same for all polarization directions. This gives

$$I_{\text{upol}}(\theta) = N I_0 \frac{1}{2} \frac{\alpha_M^2 \pi^2}{\epsilon_0^2 \lambda_0^4 r^2} (1 + \cos^2 \theta) \qquad (7)$$

Equations 5 – 7 are graphically illustrated in Fig. 2.

1.2 Dynamic light scattering

In dynamic light scattering the normalized intensity auto-correlation function of the scattered light, $g^{(2)}(\tau)$, is measured

$$g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}. \qquad (8)$$

It is related to the auto-correlation function of the electric field of the scattered light, $g^{(1)}(\tau)$

$$g^{(1)}(\tau) = \frac{\langle E(t)^* E(t+\tau)\rangle}{\langle E(t)^* E(t)\rangle}, \qquad (9)$$

via the Siegert relation
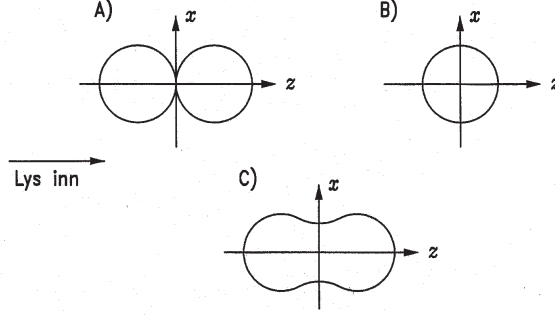
$$g^{(2)}(\tau) = 1 + [g^{(1)}(\tau)]^2. \qquad (10)$$

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Figure 2: Polar diagram that qualitatively shows the intensity of light scattered in the xz-plane (Fig. 1) from point scatterers when the incoming light is polarized in A) x-direction, B) y-direction and C) when the incoming light is unpolarized, respectively.

Here $E(t)$ and $E(t+\tau)$ means the electric field and $I(t)$ and $I(t+\tau)$ are the scattered intensity at time t and $t+\tau$, and the star means complex conjugate.

The auto-correlation function gives information concerning the diffusion in the sample. For a thin solution with monodisperse particles $g^{(1)}(\tau)$ will decay exponentially

$$g^{(1)}(\tau) = e^{-\Gamma\tau} \tag{11}$$

with a decay $\Gamma = D_t q^2$. Here q is the scattering vector and D_t is the translational diffusion coefficient. The Stoke's-Einstein relation

$$D_t = \frac{k_b T}{6\pi\eta R_h} \tag{12}$$

can be used to relate the translational diffusion coefficient to the hydrodynamic diameter, R_h, of the particle.

For a polydisperse sample $g^{(1)}(\tau)$ will no longer only consist of a single exponential function, because particles with different size have different diffusion coefficients, and thus different decay rates. The auto-correlation function will now consist of a distribution, $G(\Gamma)$, of decay rates given as

$$g^{(1)}(\tau) = \int_0^\infty G(\Gamma)e^{-\Gamma\tau}d\Gamma. \tag{13}$$

CONTIN is an analysis program that via inverse Laplace transformation of the equation above calculates the distribution function, $G(\Gamma)$, from the measured $g^{(1)}(\tau)$.

2 Instrumentation

We use a high precision light scattering instrument from ALV, purchased and installed october 2000. A schematic drawing of the instrument seen from above is shown in Fig. 3. The light source is a known air cooled He-Ne laser with wavelength 633 nm, vertical polarization and power output 22 mW. The intensity in on the sample can be varied with a shutter system. The lens L_1 is slightly biconvex. It is applied to reduce the diameter of the ray as it passes through the scattering volume. This makes it easier to devide between light scattered from the macromolecules and the dust particles, respectively.

The cuvette containing the sample is cyllindrical and contains approximately 5 ml. Around the cuvette there is placed a concentrical temperature control plate ("Vat") that contains toluen.
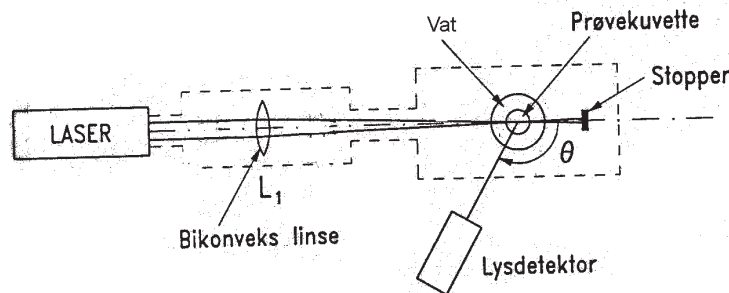
Figure 3: Schematic drawing of the light scattering instrument seen from above.

Light measurements are also performed on a standard with toluen in the cuvette (see above). Toluen is a fluid with small molecules that scatteres equally for all angles, in addition the fluid has a refractive index very close to glass. It is important to notice that at all transitions between glass and air we have approximately 4% light reflection.

The sample cuvette is placed with high precision in the center of the plate in a locked position. The sample cuvette always has to be tight in the top to prevent dust from the surroundings to enter the molecule solution. The scattered laser light is detected by a light diode ("avalanche photo diode") at different angles by a step motor controlled by the computer.

For dynamic light scattering the result from the measurements are arranged in two columns. The first column states τ in ms while the other states $g^{(2)}(\tau) - 1$.

3   Solutions

1. Latex particles (uniform polymer spheres) dissolved in water.

2. BSA (protein) dissolved in water.

3.-11. Skimmed milk, hole milk and full cream dissolved in water (vesicles).

We try to remove dust and macromolecular aggregates from the sample by filtrating the samples and all the buffer through a double set of filters with either 0.22 μm or 5 μm pores. All glass equipment and, not least, the measuring cuvette have to be flushed with filtered water. The sample cuvette has to be closed at all times.

4   Tasks

The teaching assistant will give instructions on how to use of the instrument and the computer program that controls the measurements.

A)   Perform dynamic light scattering measurements on the latex particles. How does the size distribution look like in the analysis software CONTIN? Comment on the results. Fit the correlation function with an exponential function using for example Sigmaplot. Does the obtained radius agree with the results from CONTIN?

F)   Measure the dynamic light scattering of scleroglucane, BSA and the milk products (solutions 7-11). How does the size distribution look like? Comment.

5 Report

A report is to be delivered with answers to all the questions stated above. All plots are to be attached, as well as the tables of the recorded data.