Dynamic light scattering (DLS)

https://www.khanacademy.org/science/in-in-class10th-physics/in-in-the-human-eye-and-the-colourful-world/in-in-scattering-of-light-and-tyndall-effect/v/scattering-of-light-tyndall-effect

The principles of dynamic light scattering

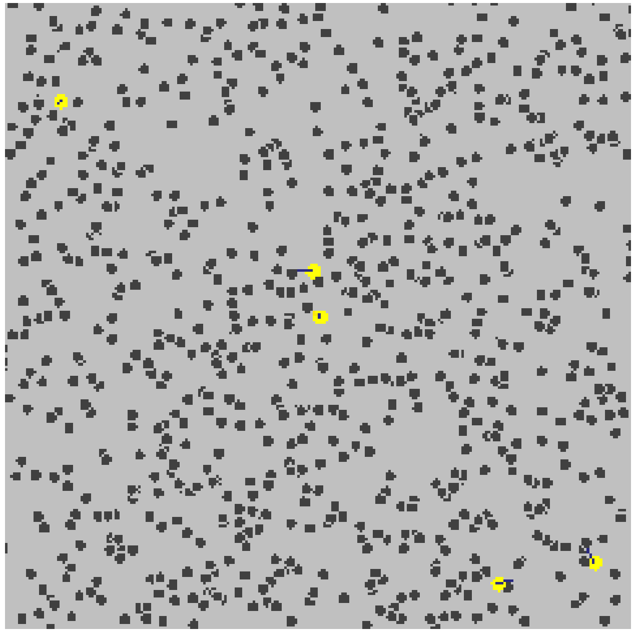
The method of dynamic light scattering (DLS) is the most common measurement technique for particle size analysis in the nanometer range.

Theoretical background of dynamic light scattering

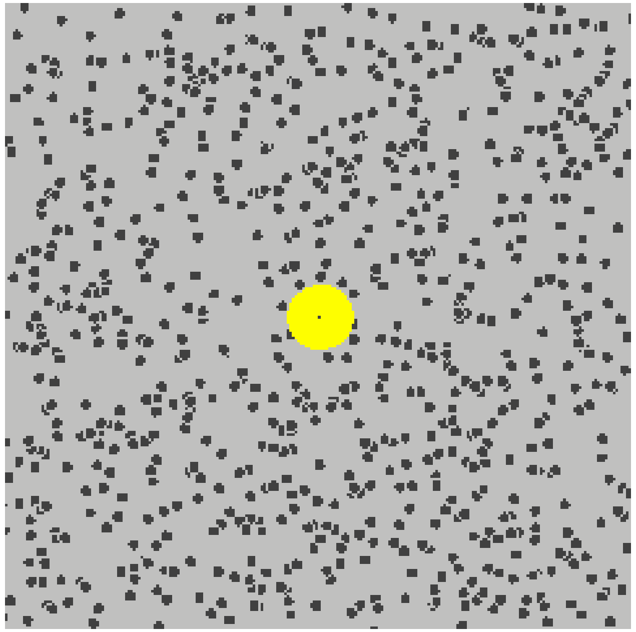
- When light hits small particles, the light scatters in all directions (Rayleigh scattering) as long as the particles are small compared to the wavelength (below 250 nm).
- This fluctuation is due to small molecules in solutions undergoing Brownian motion, and so the distance between the scatterers in the solution is constantly changing with time.
- This scattered light then undergoes either constructive or destructive interference by the surrounding particles, and within this intensity fluctuation, information is contained about the time scale of movement of the scatterers.

Brownian motion

- **Brownian motion** or **pedesis** (from [Ancient Greek](): πήδησις"leaping") is the random motion of [particles]() suspended in a [fluid]() (a [liquid]() or a [gas]()) resulting from their collision with the fast-moving [molecules]() in the fluid



Simulation of the Brownian motion of 5 particles (yellow) that collide with a large set of 800 particles. The yellow particles leave 5 blue trails of random motion and one of them has a red velocity vector.



Simulation of the Brownian motion of a big particle (dust particle) that collides with a large set of smaller particles (molecules of a gas) which move with different velocities in different random directions.

Theoretical background of dynamic light scattering

- ***The principle of Brownian motion***: smaller particles are moving at higher speeds than larger particles.

If you know all other parameters which have an influence on particle movement, you can determine the hydrodynamic diameter by measuring the speed of the particles.

- ***Stokes-Einstein equation*** relates the speed of the particles and the particle size:

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

D Translational diffusion coefficient [m²/s] – "speed of the particles"

k_B Boltzmann constant [m²kg/K s²]

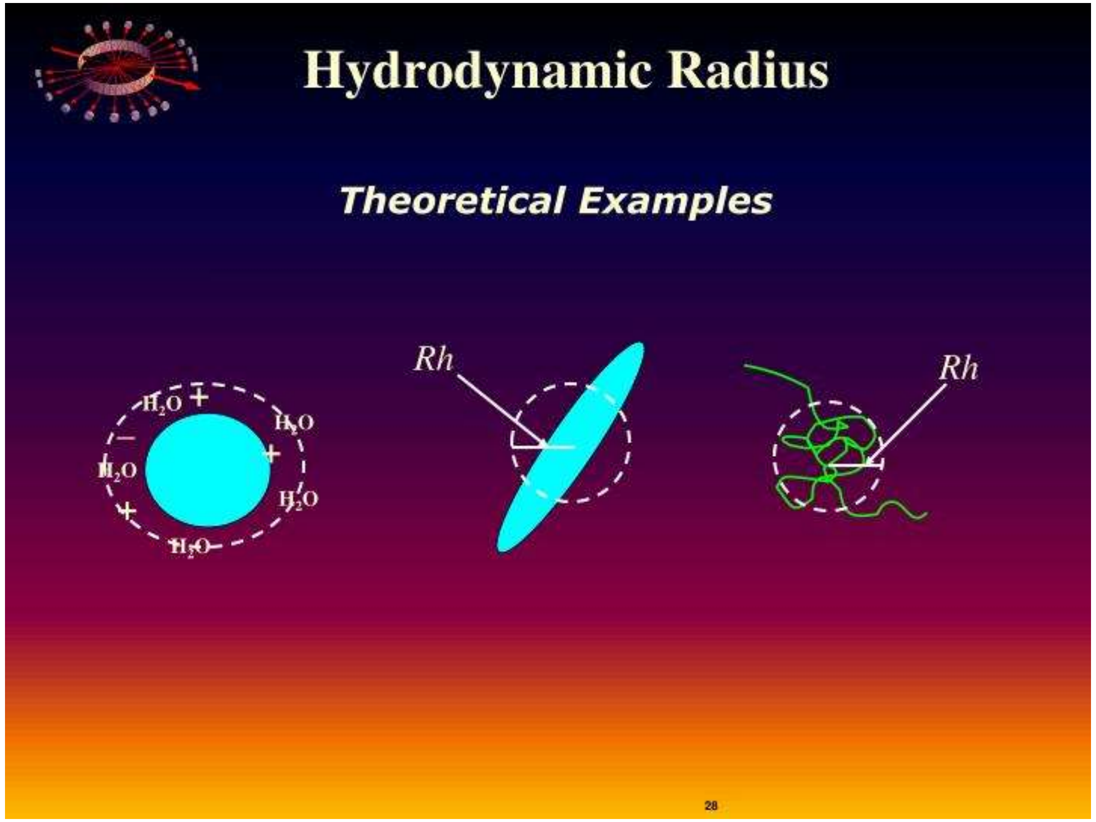
T Temperature [K]

η Viscosity [Pa.s]

R_H Hydrodynamic radius [m]

What is the hydrodynamic radius (R_H)?

By definition, the DLS measured radius is the radius of a hypothetical hard sphere that diffuses with the same speed as the particle under examination. This definition is somewhat problematic with regard to visualization however, since hypothetical hard spheres are non-existent. ***In practice, macromolecules in solution are non-spherical, dynamic (tumbling), and solvated***. As such, the radius calculated from the diffusional properties of the particle is indicative of the *apparent* size of the dynamic hydrated/solvated particle. Hence the terminology, **‘hydrodynamic’ radius**.



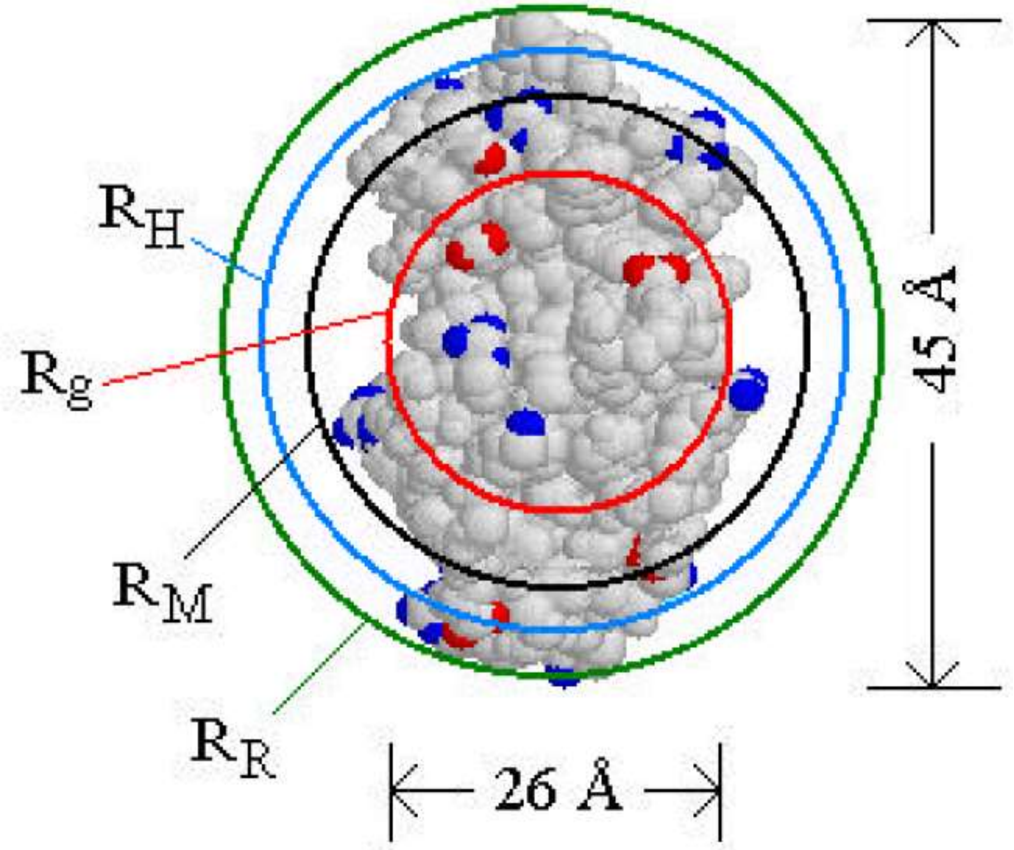
What is the hydrodynamic radius (R_H)?

Comparison of the hydrodynamic radius to other types of radii for lysozyme.

From the crystallographic structure, lysozyme can be described as a 26 x 45 Å ellipsoid with an axial ratio of 1.73. The molecular weight of the protein is 14.7 kDa, with a partial specific volume or inverse density of 0.73 mL/g.

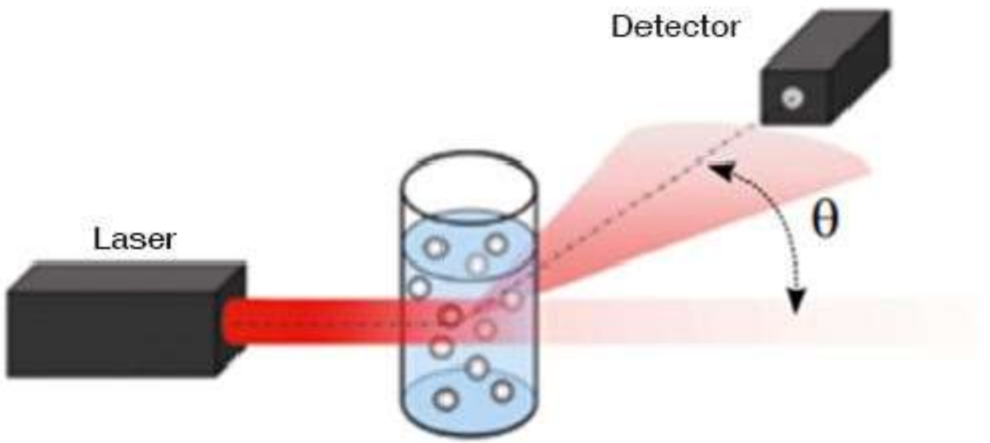
- The radius of gyration (R_g) is defined by the expression given in the figure, where m_i is the mass of the i^{th} atom in the particle and r_i is the distance from the center of mass to the i^{th} particle.
- R_M is the equivalent radius of a sphere with the same mass and particle specific volume ($\upsilon = V/m = \rho^{-1}$) as lysozyme, and
- R_R is the radius established by rotating the protein about the geometric center.

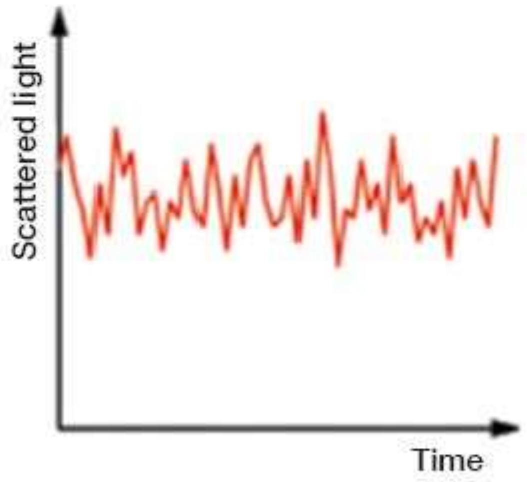
$$R_g^2 = \frac{\sum m_i r_i^2}{\sum m_i}$$



The basic DLS setup

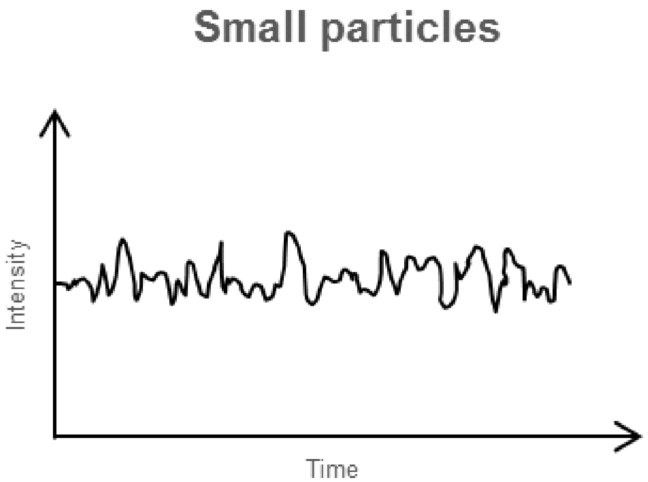
The scattered light is detected at a certain angle over time and this signal is used to determine the diffusion coefficient and the particle size by the Stokes-Einstein equation.

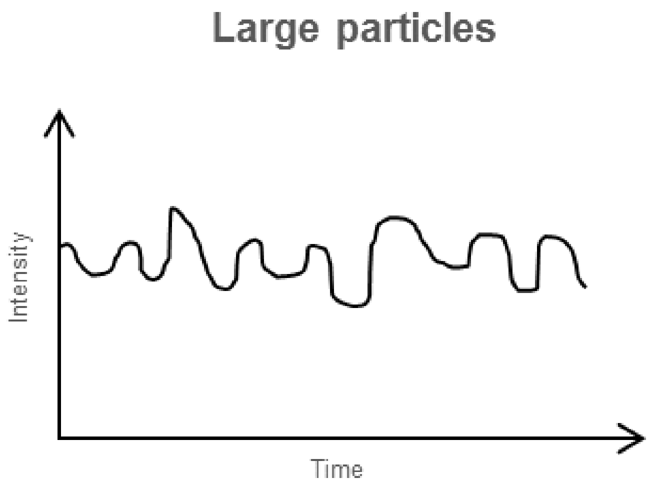




Intensity trace

- The scattered light is detected over a certain time period in order to monitor the movement of the particles.
- The intensity of the scattered light is not constant but will fluctuate over time.
- Smaller particles, which are moving at higher speeds, show faster fluctuations than larger particles.
- Larger particles result in higher amplitudes between the maximum and minimum scattering intensities





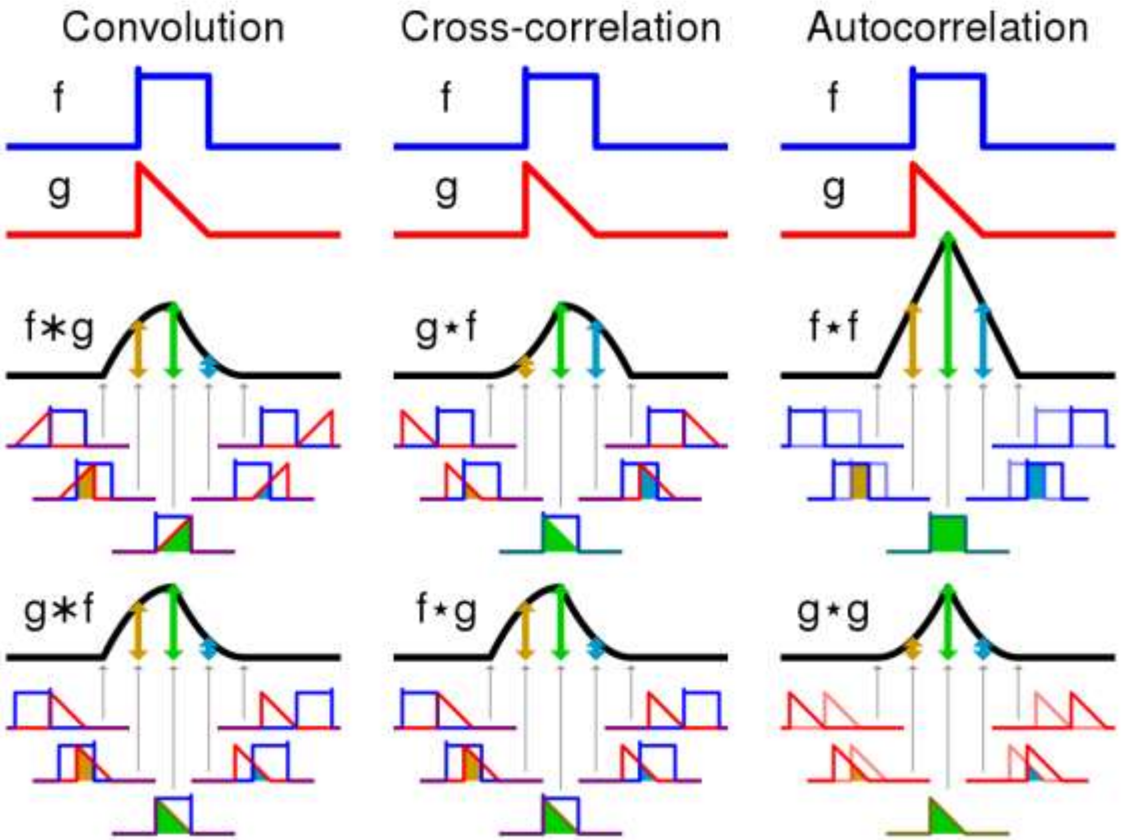
Intensity trace and correlation function

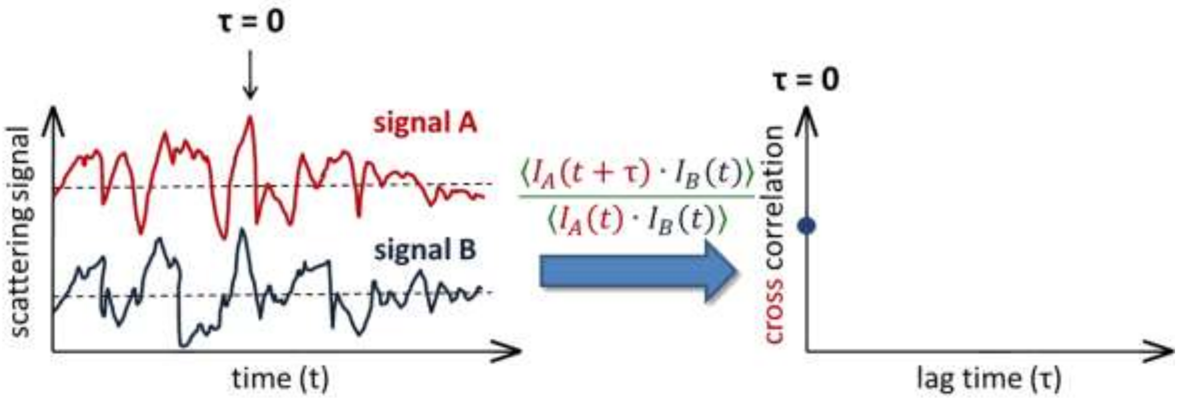
- The dynamic information of the particles is derived from an autocorrelation of the intensity trace recorded during the experiment. The second order autocorrelation curve is generated from the intensity trace as follows:

$$g^2(q;\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}$$

where $g^2(q;\tau)$ is the autocorrelation function at a particular wave vector, q, and delay time, τ, and I is the intensity.

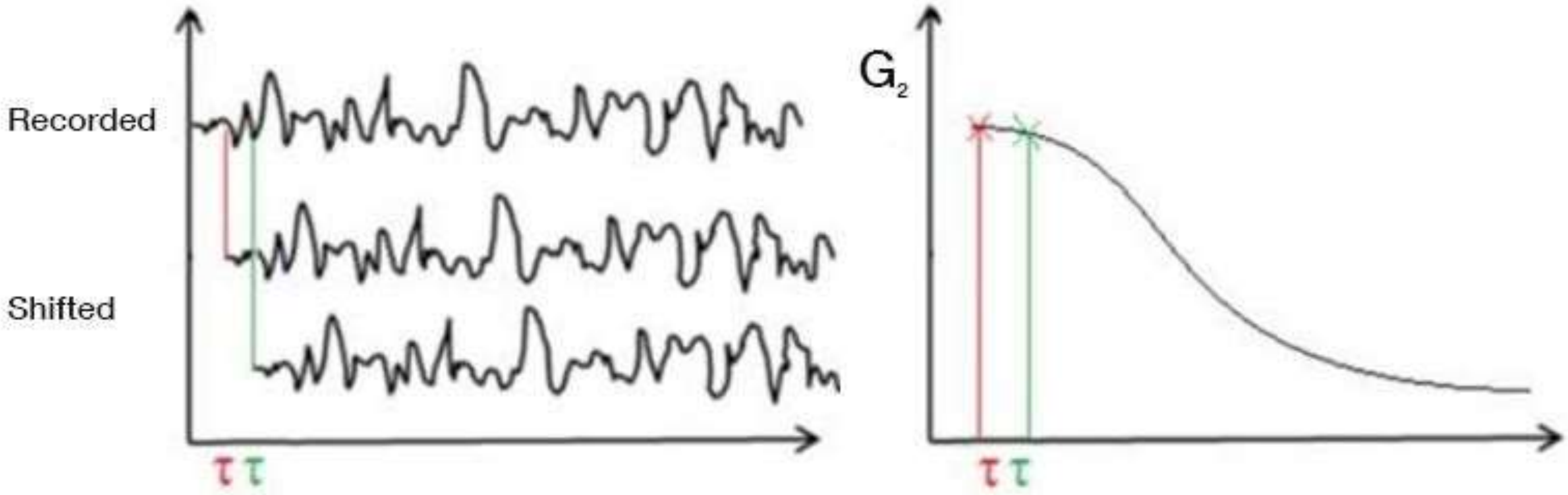
Autocorrelation, is the correlation of a signal with a delayed copy of itself as a function of delay. Informally, it is the similarity between observations as a function of the time lag between them. The analysis of autocorrelation is a mathematical tool for finding repeating patterns, such as the presence of a periodic signal obscured by noise, or identifying the missing fundamental frequency in a signal implied by its harmonic frequencies.



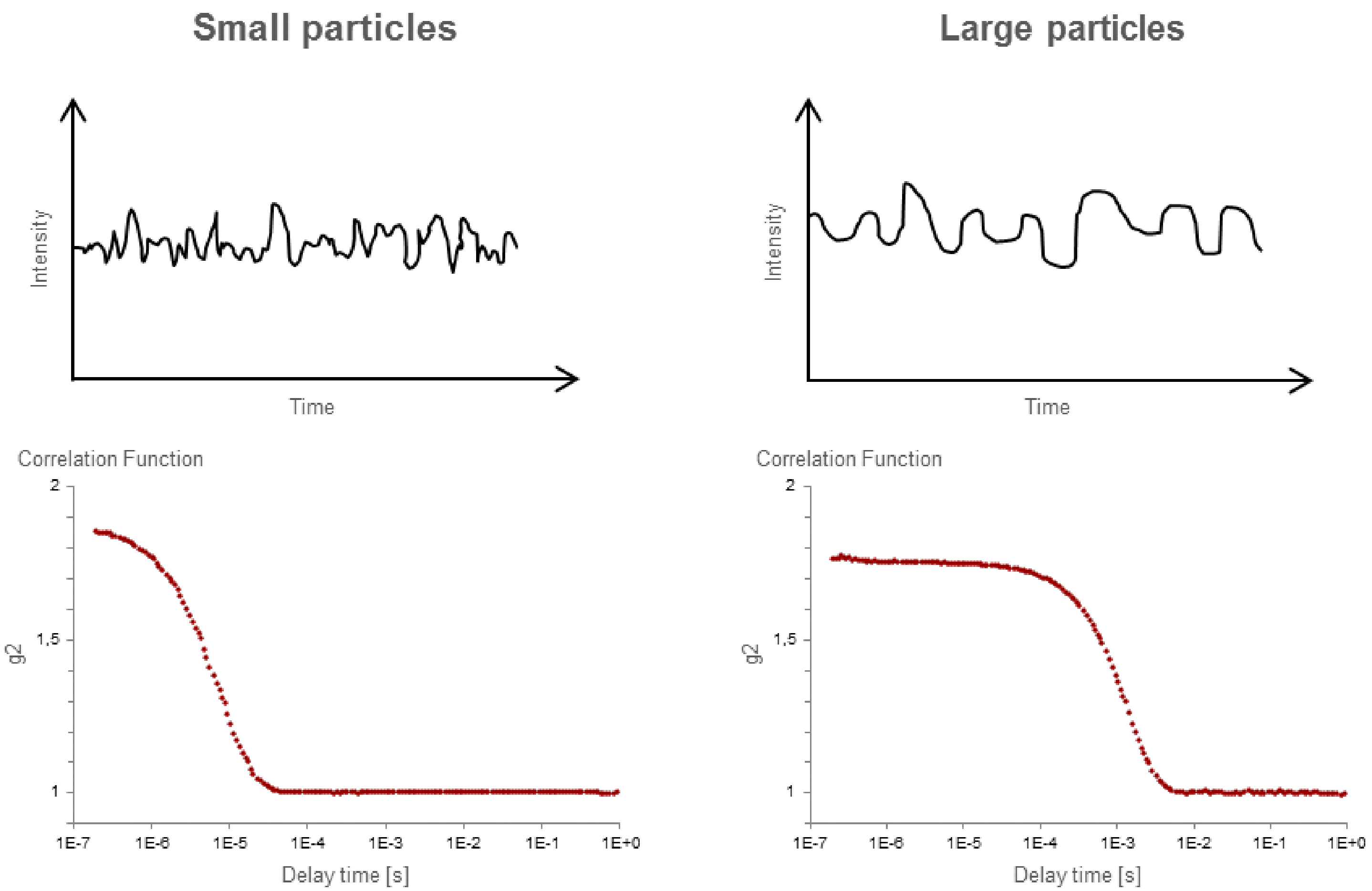


Intensity trace and correlation function

In fact, the correlation function is a mathematical description of the fluctuations of the scattered light. It is used to determine the translational diffusion coefficient. To do this, the intensity of the scattered light at a time t is compared with the intensity of the same intensity trace shifted by the delay time τ.



Intensity trace and correlation function



In general, the correlation function describes how long a particle is located at the same spot within the sample.

At the beginning the correlation function is linear and almost constant, indicating that the particle is still at the same position as it was the moment before.

Later, you can see an exponential decay of the correlation function, which means that the particle is moving. If there is no similarity to the initial spot, the correlation function shows a linear behavior again. This part of the correlation function is known as the baseline.

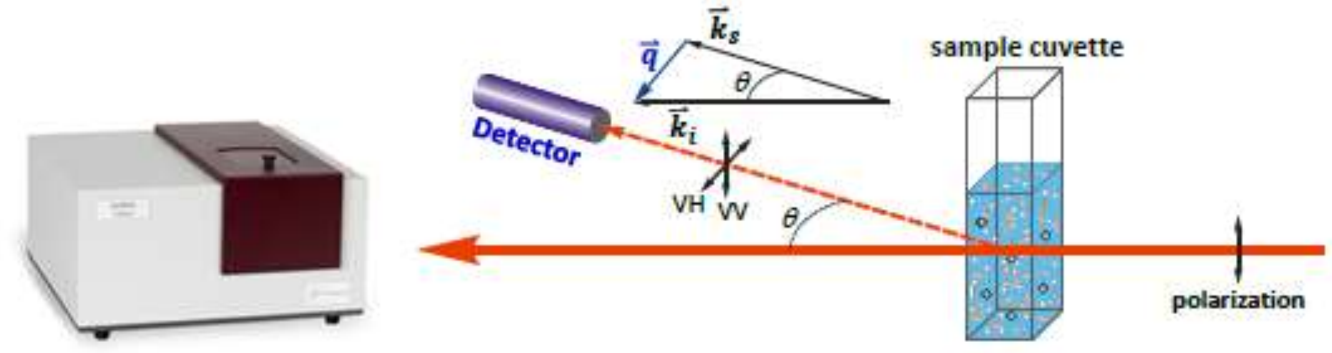
The information of the size-dependent movement is included in the decay of the correlation function. The decay represents an indirect measure of the time that the particles need to change their relative positions. Small particles move quickly so the decay is fast. Larger particles move more slowly and therefore the decay of the correlation function is delayed.

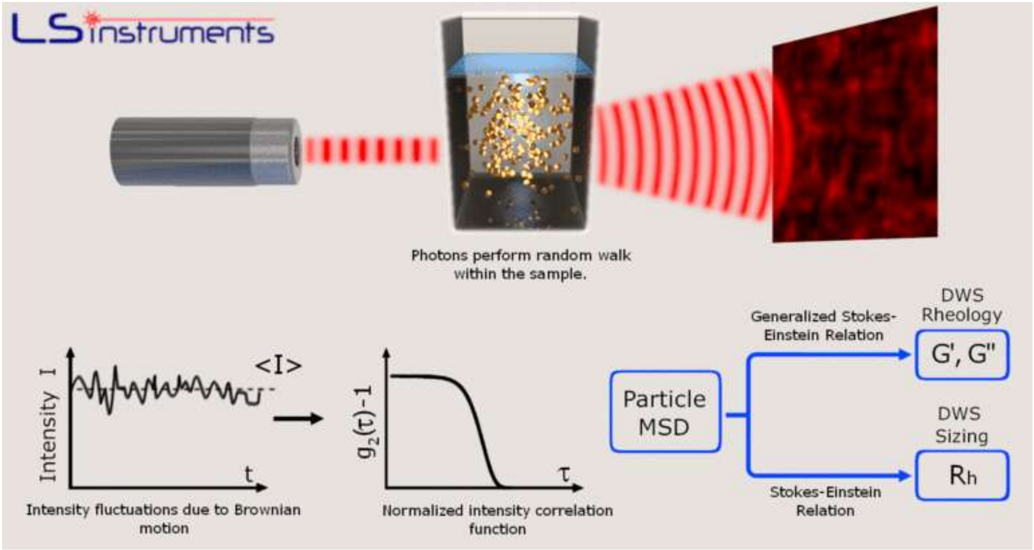
Measurement results

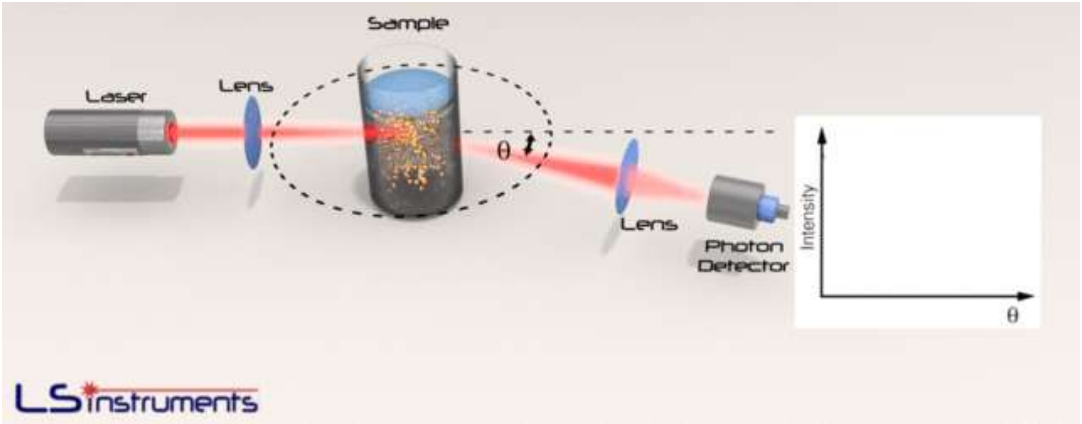
The particle size of the sample is not measured directly but is based on the movement of the particles. The term hydrodynamic diameter refers to the particle size of smooth, spherical particles which diffuse at the same speed as the particles of the sample.

The polydispersity index (PDI) is given in order to describe the broadness of the particle size distribution. The polydispersity index is also calculated by the cumulant method. A value below 10 % reflects a monodisperse sample and indicates that all of the measured particles have almost the same size.

Summary and conclusion







- Dynamic light scattering is a well-established, standardized technique for particle size analysis in the nanometer range and has been used for about 40 years.
- DLS provides information on the mean particle size as well as on particle size distribution.
- It covers a broad size range from the lower nanometer range up to several micrometers.
- Only low sample volumes are required and the sample can be re-used after the measurement.