



DYNAMIC LIGHT SCATTERING

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Introduction

- When a monochromatic beam of light encounters solution containing macromolecules, light scatters in all directions as a function of the size and shape of the macromolecules.
- In static light scattering, the intensity of scattered light is analysed as time-averaged intensity, which provides useful information on molecular weight and radius of gyration of macromolecules.
- On the other hand, if the intensity fluctuations (caused due to Brownian motion of macromolecules in solution) of scattered light is analysed, the diffusion coefficient (D_{τ}) that is related to hydrodynamic size of macromolecules can be obtained.

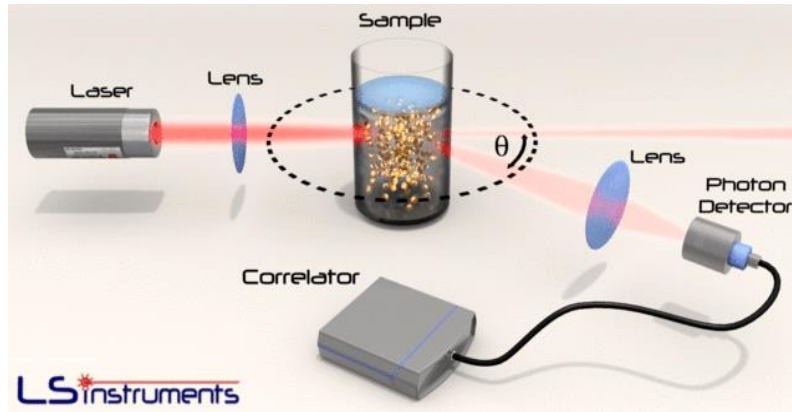


Dynamic light scattering

- Dynamic light scattering, also known as photon correlation spectroscopy or quasi-elastic light scattering, is a technique that primarily measures the Brownian motion of macromolecules in solution and relates this motion to the size of particles.
- The monochromatic incident light will undergo a phenomenon called Doppler broadening as the macromolecules are in continuous motion in solution.
- The scattered light will either result in mutually destructive phases and cancel each other out, or in mutually constructive phases to produce a detectable signal.
- The digital autocorrelator then correlates intensity fluctuations of scattered light with respect to time (ns- μ s) to determine how rapidly the intensity fluctuates, which is related to the diffusion behaviour of macromolecules.

Assumptions

- The particles follow Brownian motion in solution.



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

Stokes-Einstein Equation

- The particles are relatively spherical.

How DLS works

- In dynamic light scattering, the speed at which the particles are diffusing due to Brownian motion is measured.
- This is done by measuring the rate at which the intensity of the scattered light fluctuates when detected using a suitable optical arrangement.

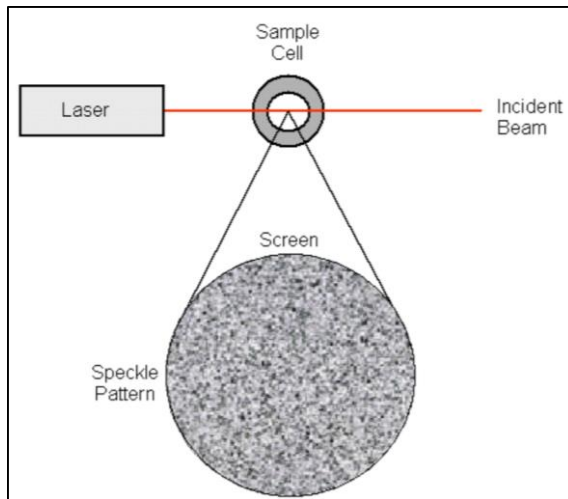


Fig: Schematic representation of a speckle pattern.

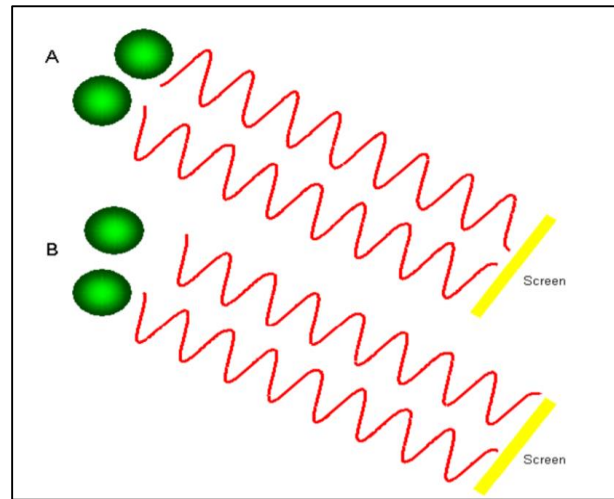


Fig: The observed signal depends on the phase addition of the scattered light falling on the detector.

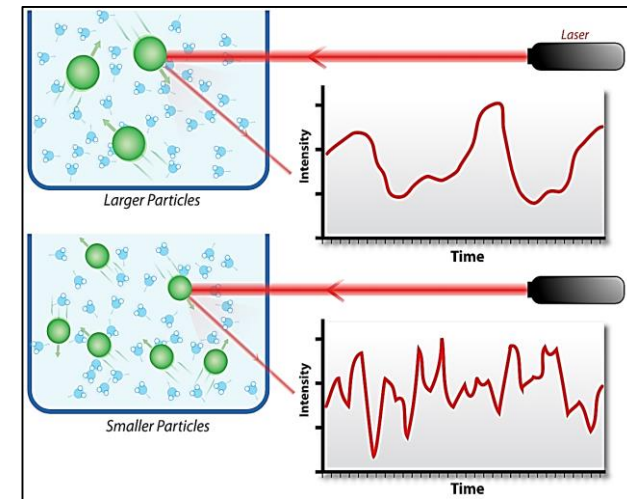
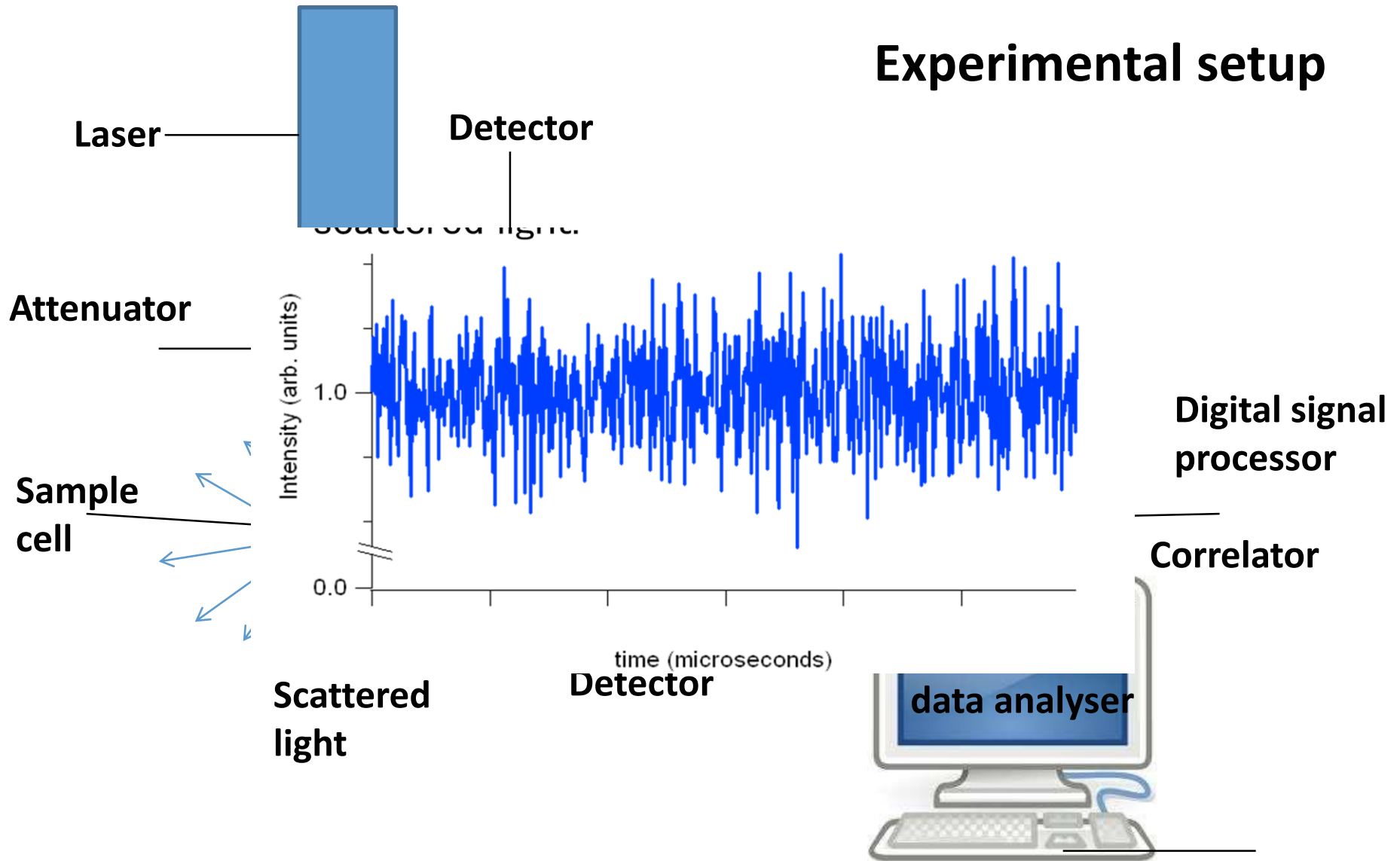
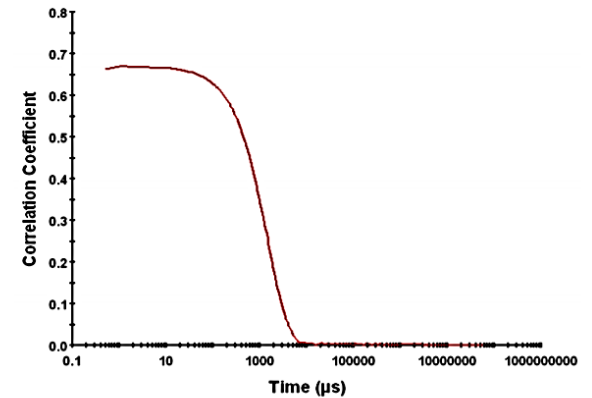
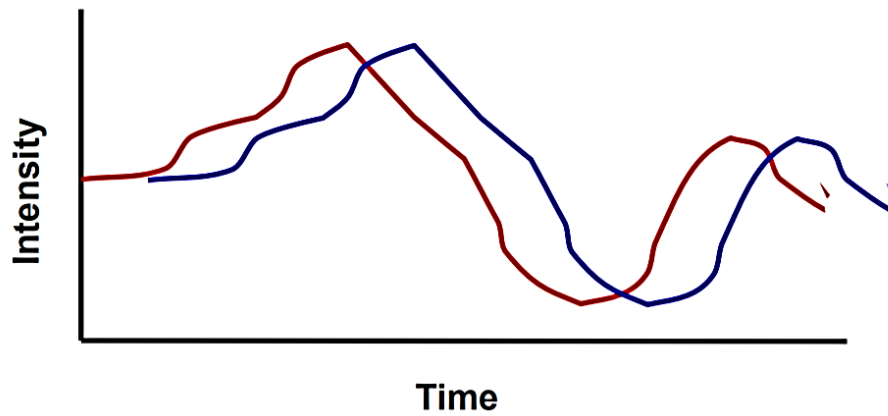
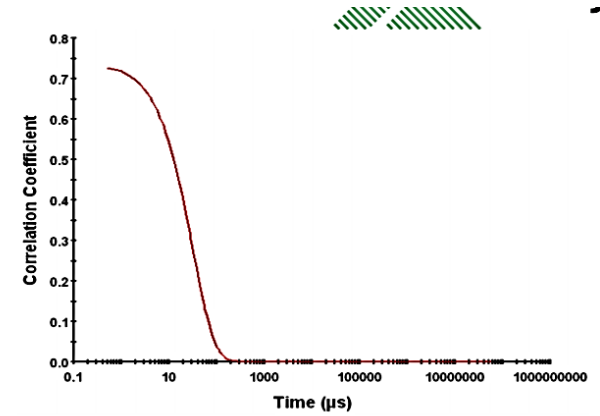
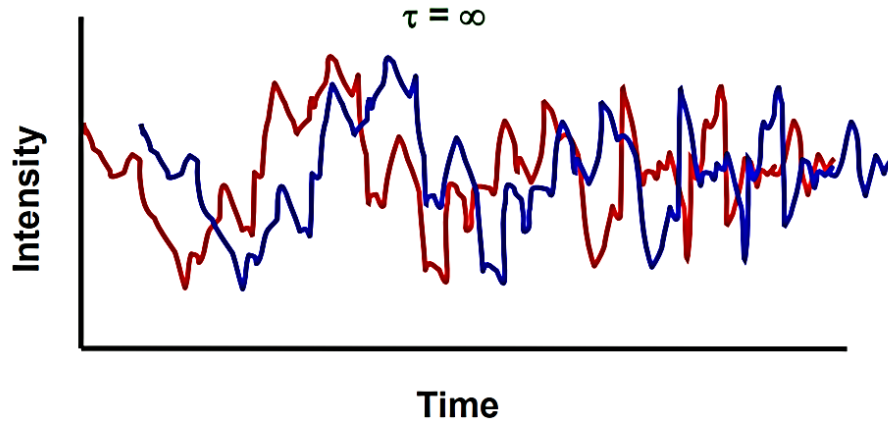


Fig: Typical intensity fluctuations for large and small particles.

Experimental setup



Correlogram





Applications

- We can measure Hydrodynamic Size of nanoparticle, protein and biomaterial
- We can also study stability of nanoparticles as function of time
- Good for detecting the aggregation of the particles

Advantages

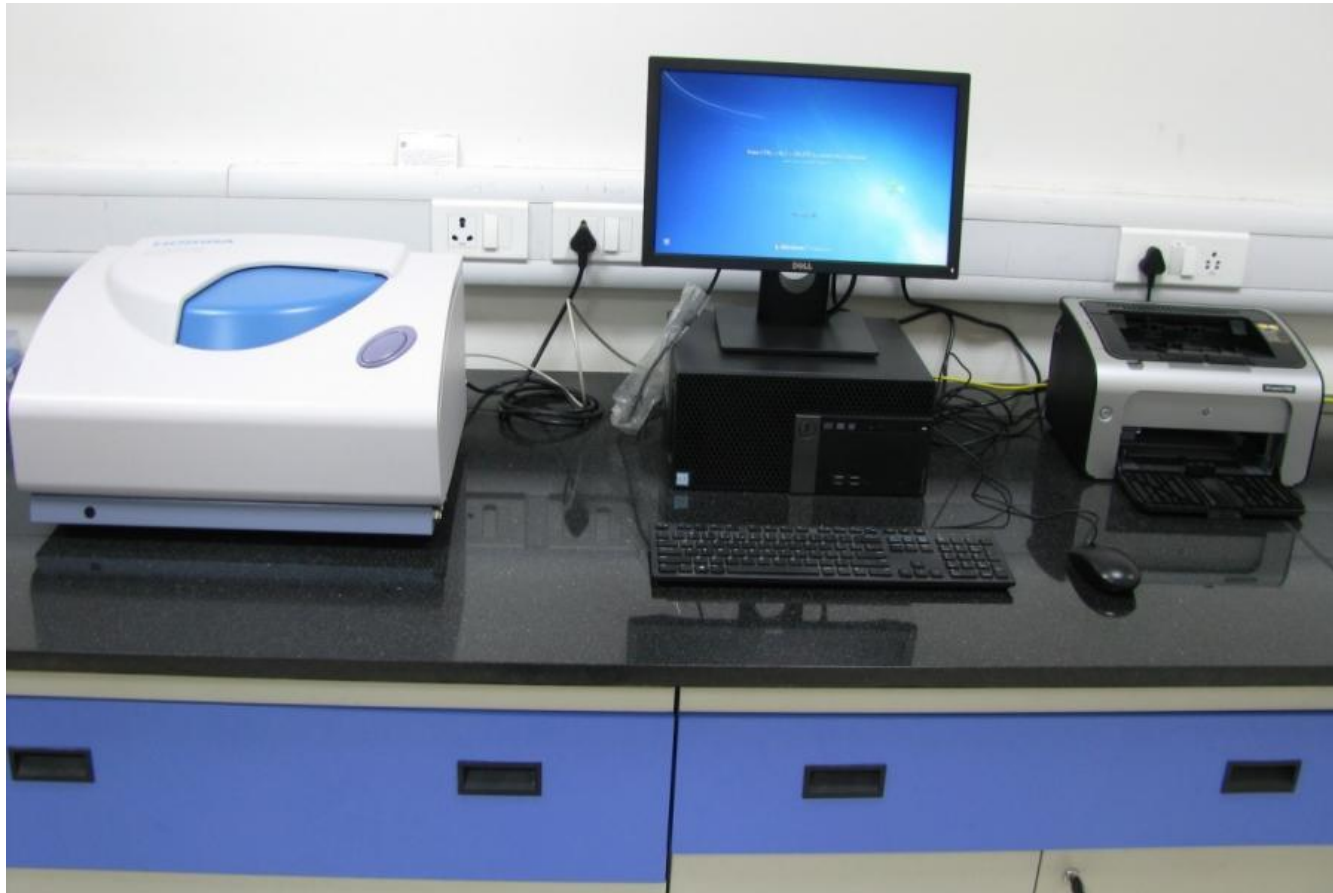
- Required small volume of sample
- Complete recovery of sample after measurement
- Sample preparation is not required for the measurement



Limitations of DLS

- We measure the hydrodynamic radius of the particle, not able to measure the actual size of the particle
- Size of solid particles are not measured by DLS
- Large particles if present in even small quantities may be accounted during data analysis
- Multiple light scattering - Multiple scattering is when the scattered light from one particle is scattered by another before reaching the detector, and it compromises the accurate calculation of particle size in more concentrated samples.

DLS instrument



Thank you