Positions opening in the fall 😊
When light interacts with matter, it can….

- Be absorbed
  - and re-emitted at modified $\lambda$ (fluorescence)
- Change polarisation
- Be scattered
  - Reflected/refracted/diffracted from ordered matter
  - Inelastically (change of $\lambda$) e.g. Raman
- - all disregarded here

Rayleigh scattering: $\lambda$ of light is significantly larger than the dimensions of the scattering particles (point scatterers)

- Be scattered
  - Elastic (same $\lambda$) **SLS**
  - Quasi-elastic (nearly same $\lambda$) **DLS or QELS**
  - movement of particles modifies I (Doppler effect)
Why is light scattered?

Electrodynamics:
An oscillating dipole emits electromagnetic radiation in all directions
Induced dipole momentum (oscillating)

$$\mu = \alpha \cdot m \cdot E_{0,laser}$$

Polarizability  Mass of dipole

The scattering intensity is proportional to the square of the particle molecular weight.

The scattered light is proportional to the concentration of the particle.

$I_{s,1}(r) = I_0 \cdot \frac{\pi^2 \alpha^2}{r^2 \lambda^4}$

$\tau$: turbidity
$x$: pathlength
$I_0$: incoming intensity
Monochromatic
Collimated

**Intensity**: Reflects the molecular weight of the particles

**Fluctuations**: Reflect the diffusion coefficient of the particles
Basics: SLS and DLS

**Intensity:** Reflects the molecular weight of the particles. SLS measures at many different angles (typically 10-100), intensity is averaged over time (1 sec or more)

**Fluctuations:** Reflect the diffusion coefficient of the particles. DLS employs measurements in a time series, averaging over very short time intervals (typically 100 nsec).
Basic comparison

• **SAXS, SANS, SLS:**
  - Same theory
  - Same experimental setup but different light sources
  - Measures the **structural characteristics** of the sample at different resolutions
  - Structure including both the form factor and structure factor

• **DLS**
  - Different theory
  - Different experimental setup
  - Measures the **diffusion** of the particles in the sample
Small Angle Scattering/Static light scattering

\( \lambda \): Wavelength of X-ray, neutron or light
\( n_0 \): Refractive index of sample (=1.33 for water)

**Beam:**

**Neutron (SANS)**

**X-ray (SAXS)**

**or light (SLS)**

\( | Q_{SAS} | = \frac{4\pi \sin \theta}{\lambda} \)

\( | Q_{SLS} | = \frac{4\pi n_0 \sin \theta}{\lambda} \)

**SAXS/SANS:** \( \theta_{\text{min}} \approx 0.03^\circ, \theta_{\text{max}} \approx 3^\circ, Q=[0.001-0.5 \text{ 1/Å}], 1-200 \text{ nm} \)

**SLS:** \( \theta_{\text{min}} \approx 8^\circ, \theta_{\text{max}} \approx 160^\circ, Q=[0.0004-0.001 \text{ 1/Å}], 200-2000 \text{ nm} \)
Static light scattering

- Intensity depends on:
  - The molecular weight of the particles
  - The concentration of the particles
  - The size of the particles
  - The refractive index of the pure solvent
  - The refractive index of the suspended molecules
  - Interaction forces between particles

\[ I_{\text{total}} = K I_0 V C M / r^2 \]

- \( C \): mg/ml
- \( V \): volume
- \( M \): mass
- \( r \): distance to detector
- \( K \): optical contrast constant

Amyloid(-like) fibrils

- Amyloid diseases (Alzheimers, Parkinsons...)
- Functional Fibrils (Antimicrobial, Biofilm, Spider silk)
- Biopharmaceutical stability (insulin, glucagon, ...)
- Self-assembly bio-systems
  - Drug delivery (Degarelix)
  - Nano-material: the strength of steel
Applications: monitoring aggregate growth of ConA

- Qualitative information
- Easy analysis
- Complemented with other techniques

Vetri V. et al. (2013) PloS One
Coupling with Size Exclusion Chromatography

• Separate the molecular species according to size on a HPLC column
• Measure light scattering and derive molar mass on individual fractions
• Measure conc. of individual fractions via the refractive index
Therapeutically relevant insulin oligomerization

Protein based drugs:
• Typically proteins in solution to be injected
• Control of release profile is desirable
Tuning experimental conditions by SEC-MALS

Tuning experimental conditions by SEC-MALS

Dynamic Light Scattering

Pinholes

Intensity

$T_0$ $T_1$ $T_2$ $T_3$

Larger Particles

Smaller Particles

Intensity

Time
Dynamic light scattering – principle of measurement

• Fluctuations: Reflect the diffusion coefficient of the particles. DLS employs measurements in a time series, averaging over very short time intervals (typically 100 nsec).

• Frequency of fluctuations depends on how fast the particles move (large particles move slowly – small particles move faster ...)

• Amplitude of fluctuations depends on particle size, contrast, and concentration (for a given fixed $\lambda$)
The Stokes-Einstein relation for spherical particles:

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

The hydrodynamic radius

Measure of the diffusion coefficient \( D \)

Then calculate equivalent hydrodynamic radius:

Range: Down to \( r_h \sim 1 \text{ nm} \)
Up to \( r_h \sim 1000 \text{ nm} \)

The characteristic decay time

Diffusion in one dimension:

\[ \langle x^2 \rangle = 2D \cdot t \]

\( T \): absolute temperature
\( k_B \): Boltzmann's constant
\( \eta \): Viscosity of liquid

D: Diffusion coefficient
\( t \): time
\( x \): displacement

Characteristic diffusion distance for change in interference:

\[ \left( \frac{1}{q} \right)^2 = 2D\tau_0 \Rightarrow \tau_0 = \frac{1}{2Dq^2} \]
Dynamic light scattering – principle of measurement

\[ G_2(\bar{q}, t) = \frac{\langle |A(\bar{q}, t)|^2 \rangle}{\langle |A(\bar{q}, t)|^2 \rangle} > \langle N \rangle^2 + \langle N \rangle^2 e^{-2Dq^2 \tau} \]

The Auto-correlation function: Cross-correlation of a signal with itself over time (similarity as a function of the time-lag between signals)
Size distribution for macromolecules in solution

- Quantitative information
- Easy analysis (if the software automatically does it)
Back to the growing oligomers of insulin analogues

Note complementarity and advantages with DLS versus SLS?
Analysis of IgG subclass structure and aggregation properties

Identical light chains and identical variable regions in the heavy chain

Low-pH: relevant during production of therapeutical antibodies (affinity chromatography and virus deactivation)

Low-pH induced aggregation study

Low-pH: relevant during production of therapeutical antibodies (affinity chromatography and virus deactivation)

Skamris, Tian et al. (2016) Pharm Res
Low-pH induced aggregation study

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Thank you for your attention

Questions?

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