Making Light Work

Dynamic Light Scattering
Electrophoretic Light Scattering
Static Light Scattering

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Contents

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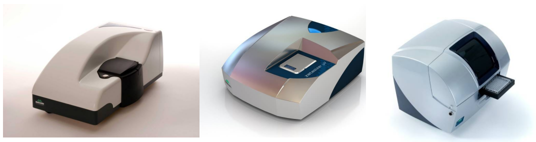
- **Instruments using light**
- Light scattering
- Nanoparticle Tracking Analysis (NTA)
- Dynamic light scattering (DLS)
- Electrophoretic light scattering (ELS)
- Static light scattering (SLS)

Instruments Using Light



Zetasizer Range
(DLS, ELS, SLS)

OMNISEC
SLS (LALS, RALS, MALS)

Nanosight
(NTA)

Mastersizer
SLS
(LALLS)

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Light Scattering

- The phenomenon of light scattering can be used in multiple ways to characterise a macromolecule/particle in solution/suspension
- A photon from an incident beam is absorbed by a macromolecule/particle and re-emitted in all directions

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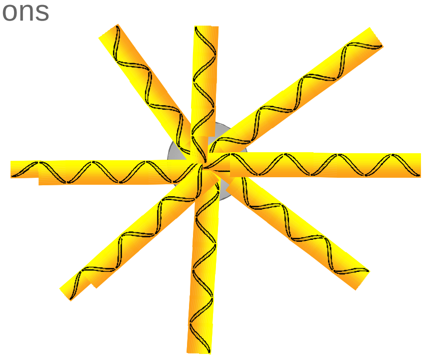


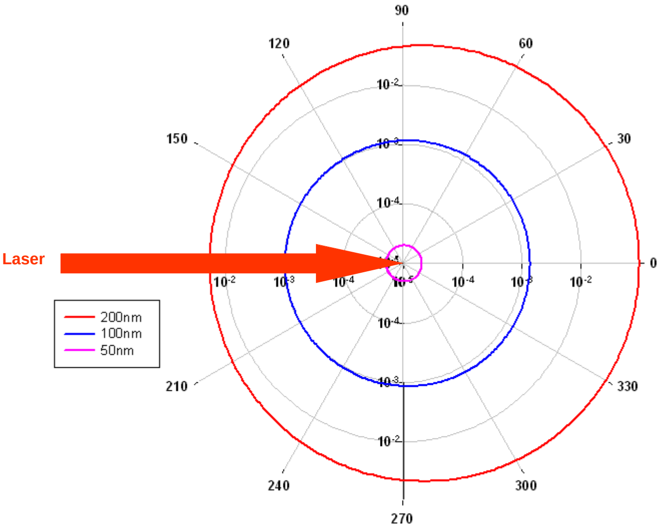
Rayleigh Theory

- Applicable for small particles and molecules whose diameters are less than 1/10th of the laser wavelength (λ)
- He-Ne laser (λ = 633nm) $\approx$ < 60nm
- Isotropic scattering i.e. equal in all directions
- **Intensity = d^6**

Mie Theory

- Exact description of how spherical particles of all sizes and optical properties scatter light
- Particles > λ/10, scattering distorts in the forward scattering direction
- Particle size => λ, scattering is a complex function of maxima and minima with respect to angle which is correctly explained by Mie theory

Mie Theory: Polar Plots



Mie Theory: Polar Plots

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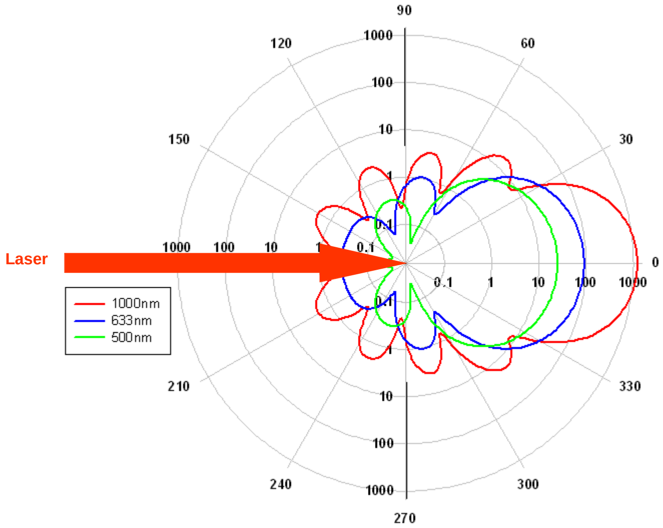

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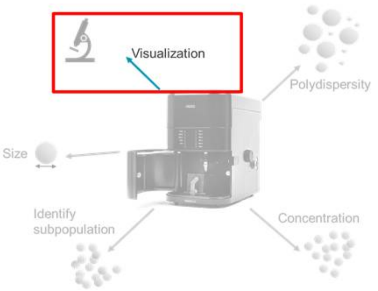
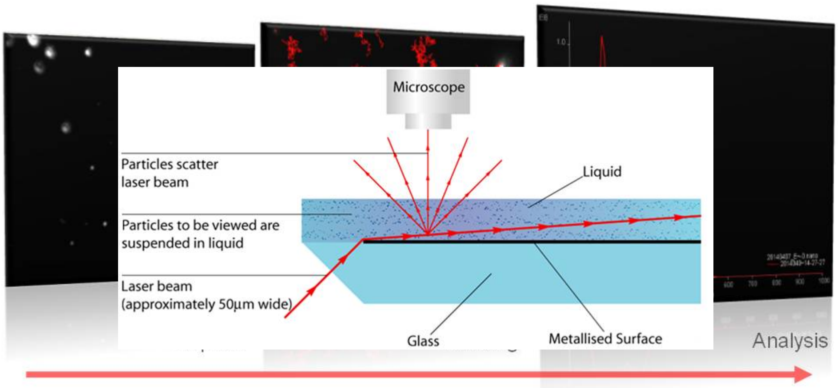
Visualization

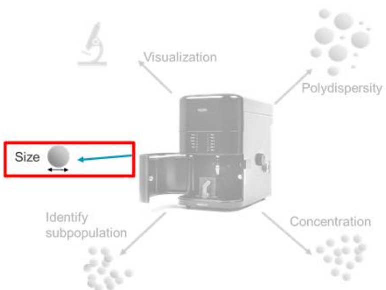
Nanoparticle Tracking Analysis (NTA)





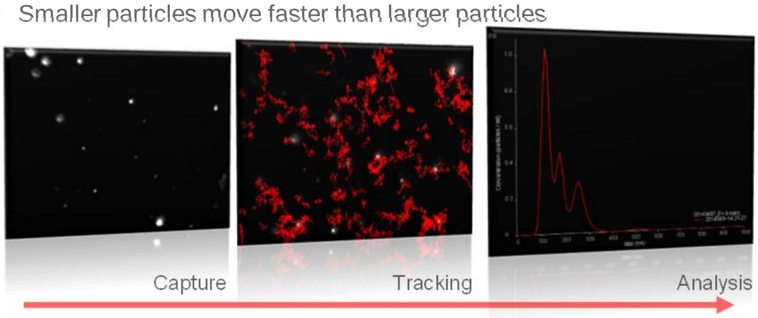
No need to know sample refractive index or density

Size Measurement



- NanoSight tracks nanoparticles moving under Brownian Motion
- Diffusion coefficients determined by tracking the particles and used to calculate size (Stokes-Einstein equation)
- Smaller particles move faster than larger particles

ISO19430: Particle Tracking Analysis (PTA) method describes limitations, quantification parameters as well as instructions on how to operate the equipment in a certified manner.



Polydisperse Samples

Sample distribution examples

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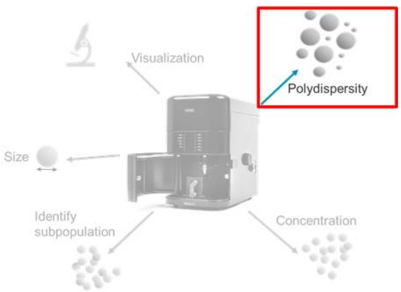
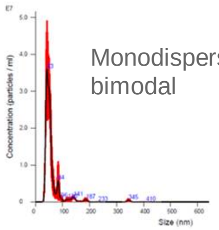
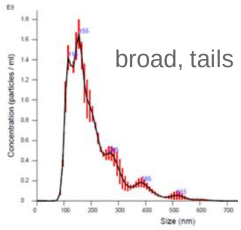
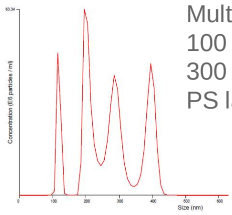




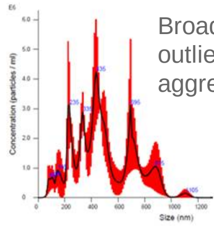
Monodisperse / bimodal



broad, tails



Multimodal:
100 nm, 200 nm, 300 nm, 400 nm
PS latex



Broad, tails, outliers, aggregation…

15 Nanoparticle Tracking Analysis (NTA)

Concentration

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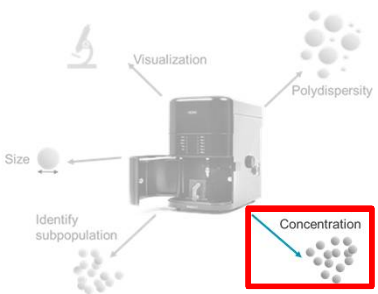
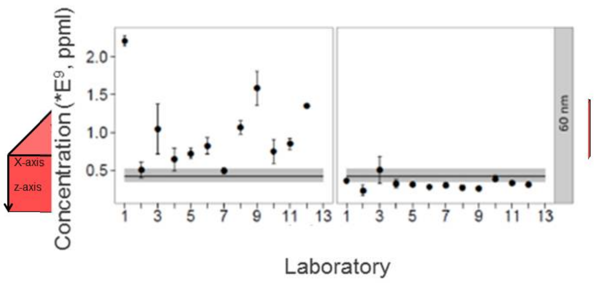


- NanoSight tracks individual particles, allowing their exact number to be determined
- The volume is determined by the field of view (X and Y) and from the laser beam profile (Z)



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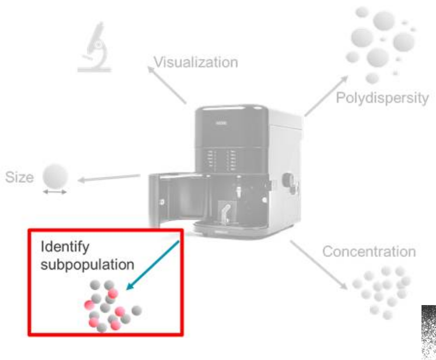
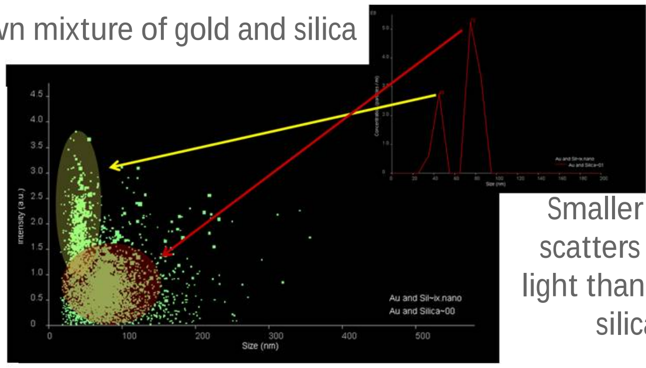
Identify Subpopulation

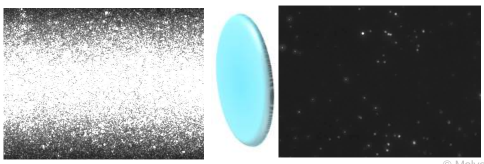
Relative Refractive Index or Fluorescence

Unknown mixture of gold and silica





Smaller gold scatters more light than bigger silica



Different laser wavelengths for different fluorophores
1.405 (430) nm
2.488 (500) nm
3.532 (565) nm
4.642 (650) nm

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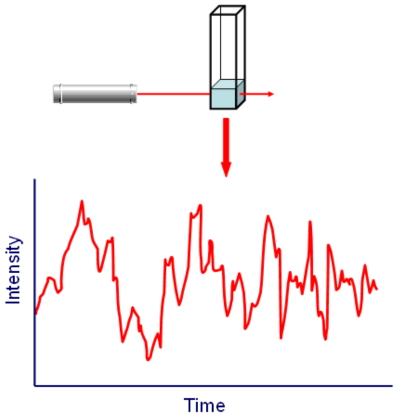
Dynamic Light Scattering

Particle and Molecular Size Measurements

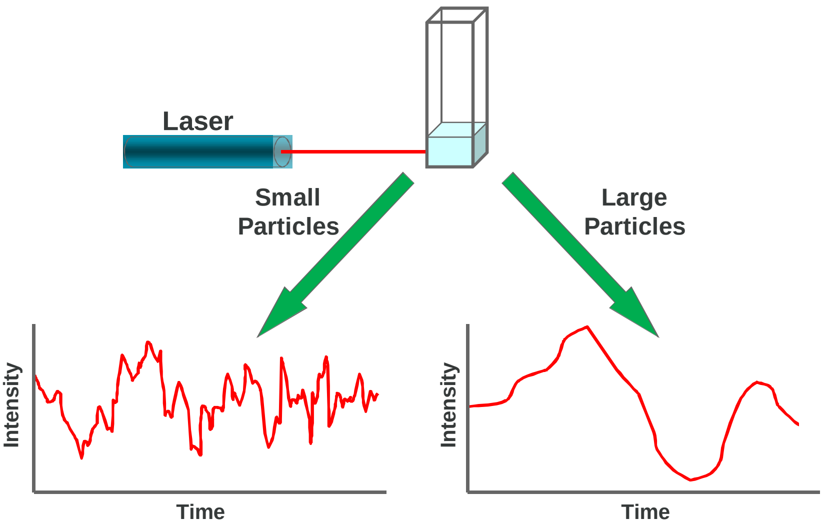


Time-dependent fluctuations in the scattering intensity to determine the **translational diffusion coefficient (D),** and subsequently the **hydrodynamic diameter (D_H)** (from the Stokes-Einstein equation)

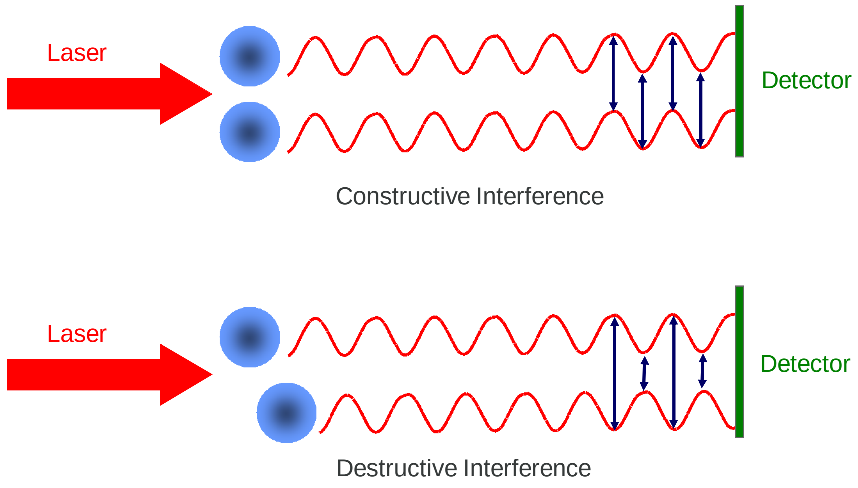
$$D = \frac{kT}{3\pi\eta D_H}$$

Where k = Boltzmann's constant,
T = absolute temperature
η = viscosity

Brownian Motion and Intensity Fluctuations



Constructive and Destructive Interference



Correlation in Dynamic Light Scattering

- Technique for extracting the time dependence of a signal in the presence of "noise"
- Time analysis carried out with a correlator
- Constructs the time autocorrelation function G(τ) of the scattered intensity according to

$$G(\tau) = \langle \frac{I(t_0) * I(t_0 + \tau)}{I(t_\infty)^2} \rangle$$

where I = intensity, t is the time and τ = the delay time

Correlation

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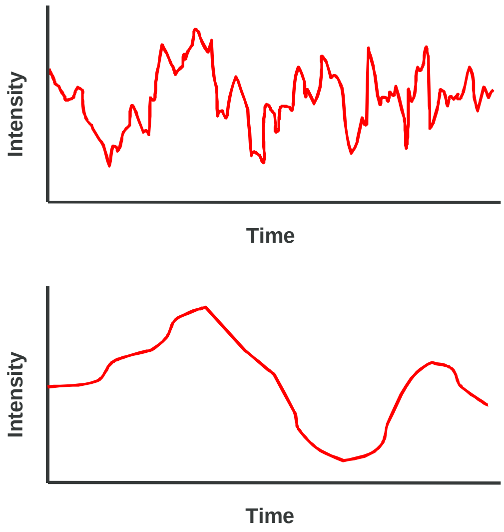

Correlation

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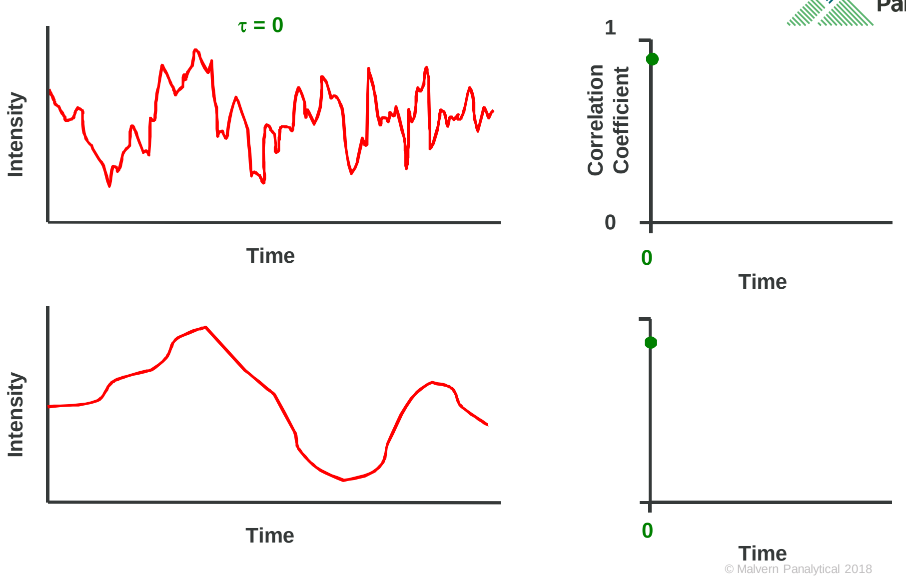

Correlation

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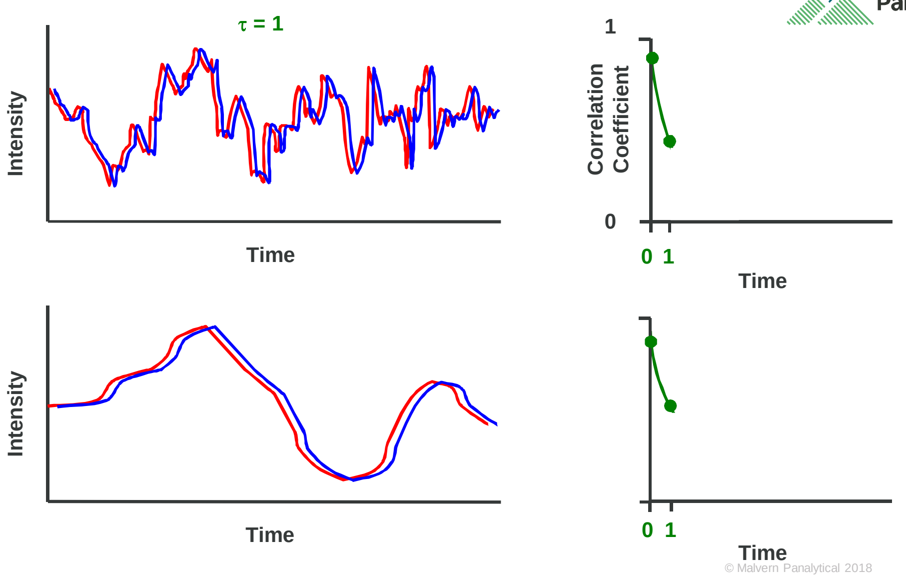

Correlation

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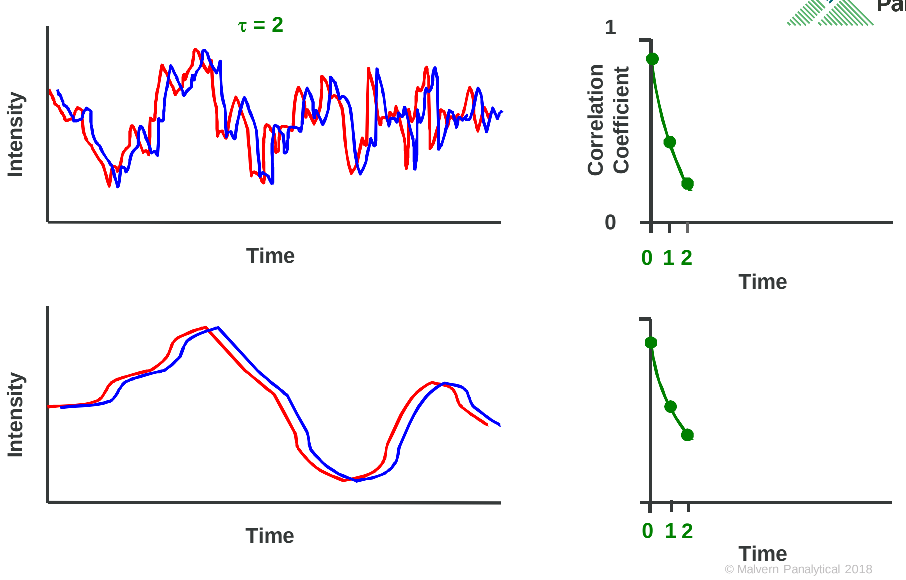
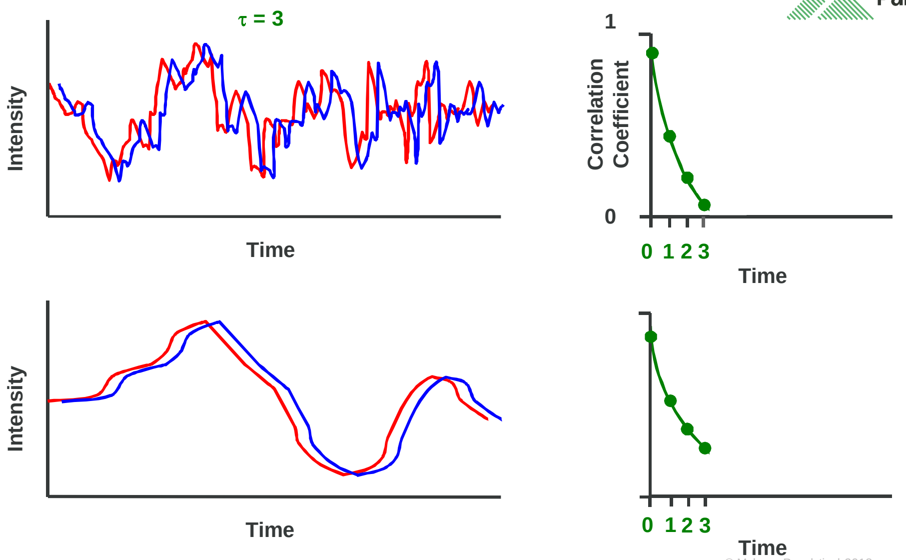


Correlation



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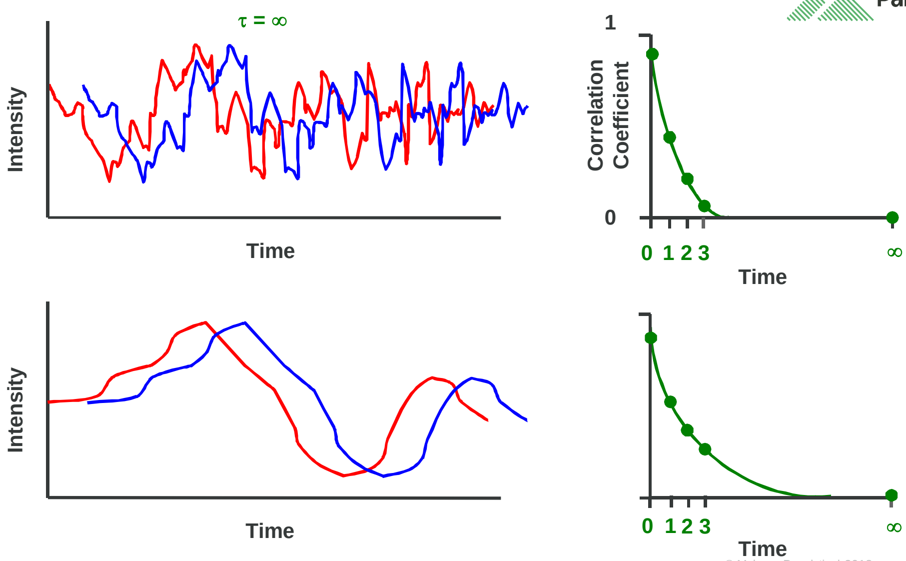
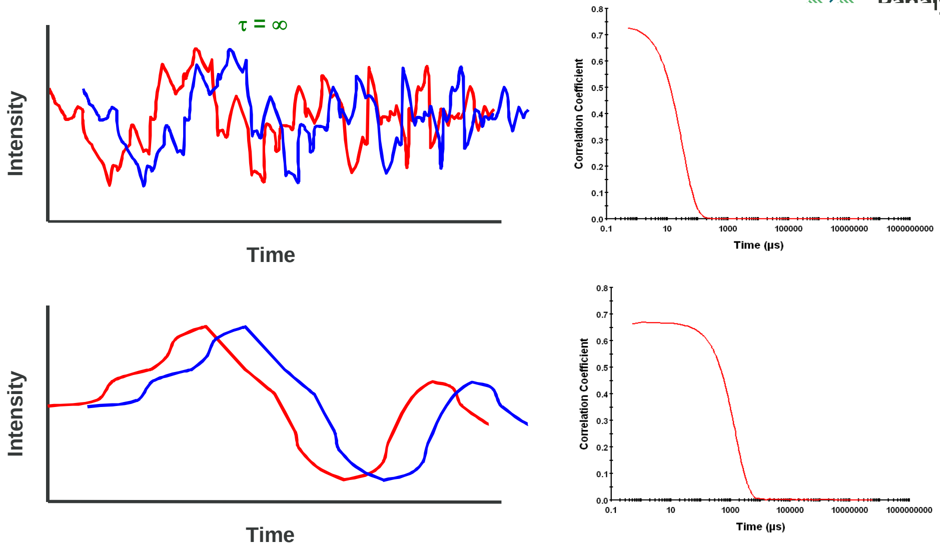
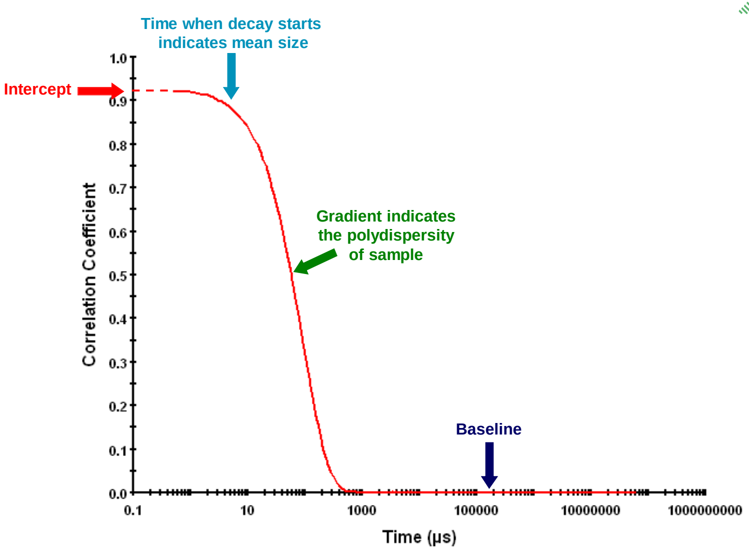


Correlation



Correlation Functions



Analysing The Correlation Function

- Correlation function contains the diffusion coefficient information required to be entered into the Stokes-Einstein equation
- The diffusion coefficients are obtained by fitting the correlation function with a suitable algorithm

Analysing The Correlation Function

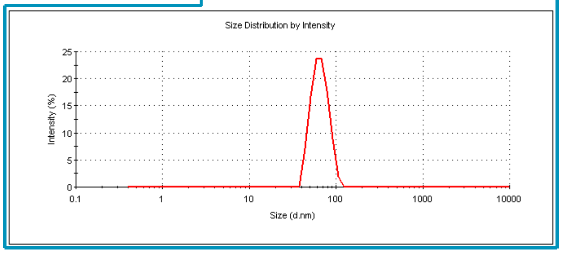
Two different analyses are performed:

Cumulants analysis
- Mean size (z-average)
- Polydispersity index

Distribution analysis
- Distribution of sizes

Z-Average (d.nm):	63.13
PdI:	0.029
Intercept:	0.965
Result quality :	Good

	Diam. (nm)	% Intensity	Width (nm)
Peak 1:	65.94	100.0	14.35
Peak 2:	0.000	0.0	0.000
Peak 3:	0.000	0.0	0.000



Cumulants Analysis

- Defined in the International Standards ISO22412 (2017)
- Only gives a mean particle size (z-average) and an estimate of the width of the distribution (polydispersity index)
- Only the dispersant refractive index and viscosity are required for this analysis

z-Average Diameter

- Definition of the z-Average Diameter (Z_D):

The intensity-weighted mean diameter derived from the cumulants analysis

- Specific to light scattering
- Very sensitive to the presence of aggregates or large contaminants due to the inherent intensity weighting

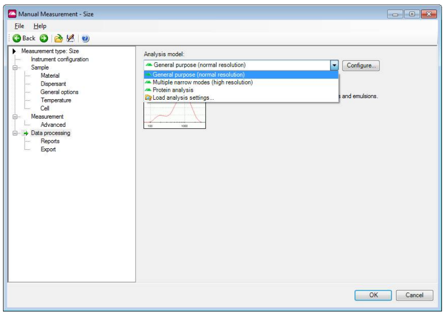
Polydispersity Index

- Definition of the Polydispersity Index (PdI):

A dimensionless measure of the broadness of the size distribution calculated from the cumulants analysis

- Ranges from 0 to 1 in the Zetasizer software
- Values > 1 indicate that the distribution is so polydisperse, the sample may not be suitable for measurement by DLS

Distribution Algorithms



- **General Purpose** (non-negative least squares (NNLS))
- **Multiple Narrow Modes** (non-negative least squares (NNLS))
- **Protein Analysis** (L-curve)

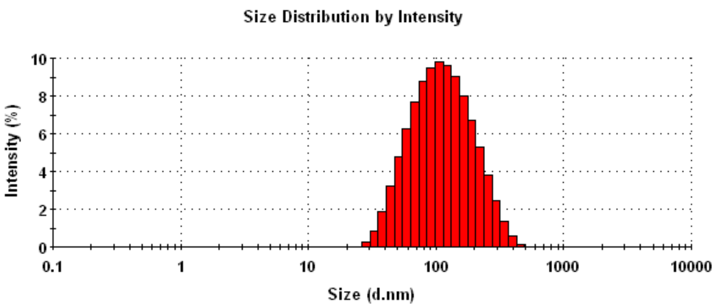
- The difference between these algorithms is their resolution capability

Zetasizer Distribution Algorithms

- **General Purpose**
  - Suitable for the majority of samples where no knowledge of the distribution is available
  - Will give broad, smooth distributions
- **Multiple Narrow Modes**
  - Suitable for samples suspected to contain discrete populations
  - Will give narrow peaks
- **Protein Analysis**
  - Best suited for protein samples – will give narrow peaks
  - Automatically picks the optimal distribution

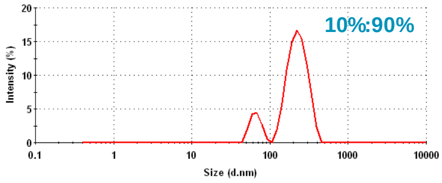
Size Distributions in the Zetasizer Software

- Primary size distribution is **intensity**-weighted
- A plot of the **relative intensity of light scattered** by particles (on the Y axis) versus various **size classes** (on the X axis) which are logarithmically spaced



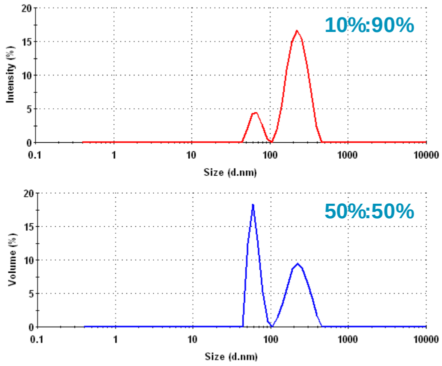
Intensity Size Distributions

- Primary result
- Based upon the intensity of light scattered by particles
- Sensitive to the presence of large particles/aggregates /dust
- Only the dispersant viscosity and refractive index values are required



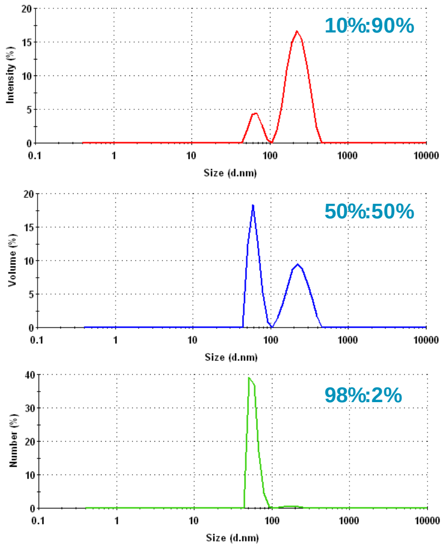
Volume Size Distributions

- Derived from the intensity distribution using Mie theory
- Equivalent to the mass or weight distribution
- Particle optical properties required to make this transformation
  - Particle refractive index
  - Particle absorption



Number Size Distributions

- Derived from the intensity distribution using Mie theory
- Particle optical properties required to make this transformation
  - Particle refractive index
  - Particle absorption



Size Distributions From DLS

- Transformation from intensity to volume or number makes the following assumptions:
  - All particles are spherical
  - All particles have an homogenous and equivalent density
  - The optical properties are known (refractive index and absorbance)
  - There is no error in the intensity distribution

Size Distributions From DLS

- DLS technique tends to overestimate the width of the peaks in the distribution
- This effect is magnified in the transformations to volume and number

- The volume and number size distributions should only be used for estimating the **relative amounts** of material in separate peaks as the means and particularly the widths are less reliable

Volume/Number Distributions

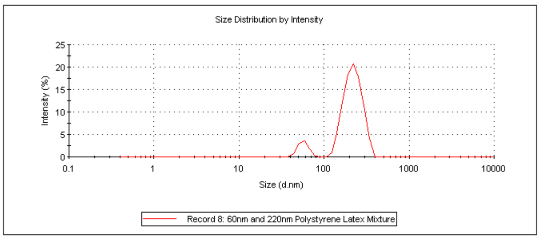
Recommended Use

- Use the **Intensity PSD** for reporting the size of each peak in the distribution
- Use the **Volume** or **Number PSD** for reporting the relative amounts of each peak in the distribution

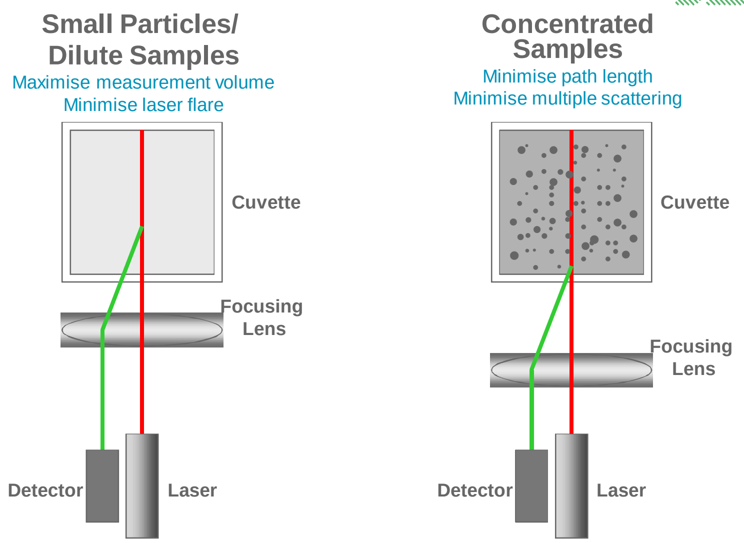
(Modal Size Report)

Z-Average (d.nm):	175.1
PdI:	0.191
Intercept:	0.866
Result quality :	Good

	Diam. (nm)	Width (nm)	% Intensity	Volume	Number
Peak 1:	222.9	51.67	90.8	52.3	2.0
Peak 2:	57.07	7.544	9.2	47.7	98.0
Peak 3:	0.000	0.000	0.0	0.0	0.0



NIBS: Variable Measurement Position



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Electrokinetic Effects

- Charged particles exhibit certain effects under the influence of an applied electric field
- Collectively defined as **electrokinetic effects**

- **Electrophoresis** = movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field
- **Electro-osmosis** = movement of a liquid relative to a stationary charged surface under the influence of an electric field

Electrophoretic Light Scattering

Zeta Potential Measurements

- Combination of **electrophoresis** and **light scattering**
- **Electrophoresis** = movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field

Electrophoretic Light Scattering
Zeta Potential Measurements

- Combination of **electrophoresis** and **light scattering**
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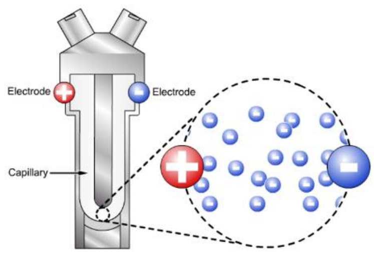
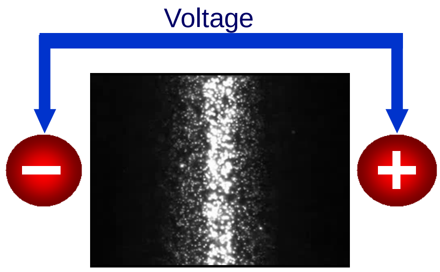
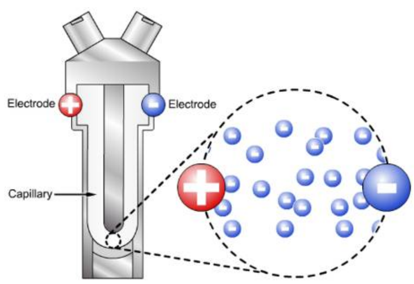




Electrophoretic Light Scattering
Zeta Potential Measurements



- Particles velocity dependent on:
  - **Zeta potential**
  - Field strength
  - Dielectric constant of medium
  - Viscosity of the medium

Electroosmosis

- **Electroosmosis:** the movement of a liquid relative to a stationary charged surface under the influence of an electric field

Electrophoresis in a
Closed Capillary Cell

Measurement Technique M3

Mixed Mode Measurement

- The Zetasizer Nano uses a combination of fast field reversal (**FFR**) and slow field reversal (**SFR**)
- **FFR** measures the **mean zeta potential value** due to electrophoresis only
- **SFR** allows a **zeta potential distribution** to be determined

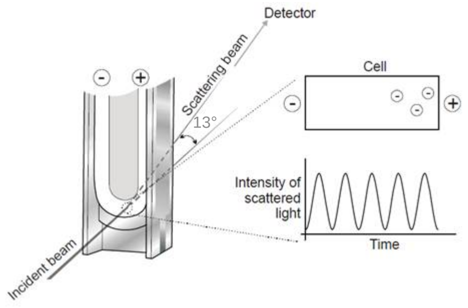
Electrophoretic Light Scattering

Zeta Potential Measurements

- Scattered light is frequency shifted
- Frequency shift

  $$\Delta f = 2\nu \sin(\theta/2)/\lambda$$

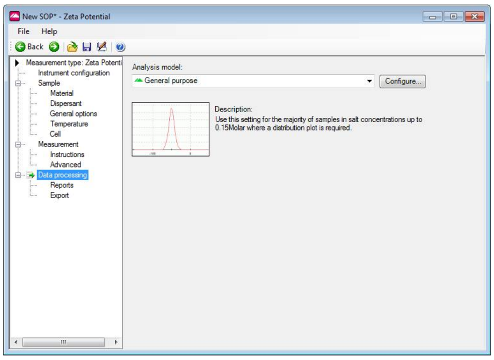
  ν = the particle velocity
  λ = laser wavelength
  θ = scattering angle
- Frequency shifts determined by **Fourier transformation** and **phase analysis light scattering**
- Measured **electrophoretic mobility** converted into **zeta potential** using **Henry's equation**



- Combination of PALS with M3 (**M3-PALS**) is patented

General Purpose

- General Purpose = FFR + SFR
- FFR = zeta potential mean (electrophoresis only)
- SFR = zeta potential distribution (electrophoresis and electro-osmosis)



General Purpose: Voltage Plot

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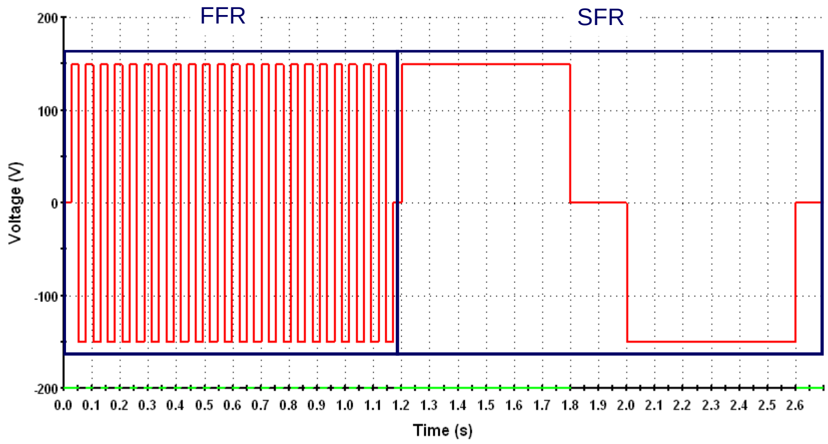

Monomodal

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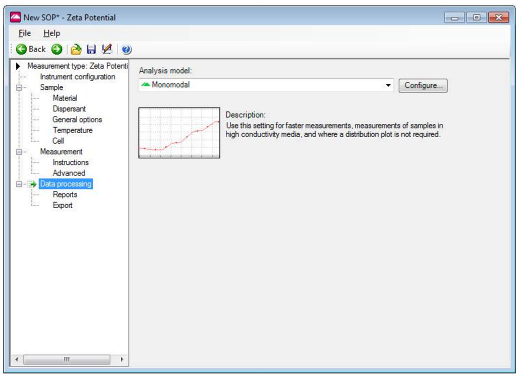
- Monomodal = FFR only
- Zeta potential mean only
- No zeta potential distribution is determined



Monomodal: Voltage Plot

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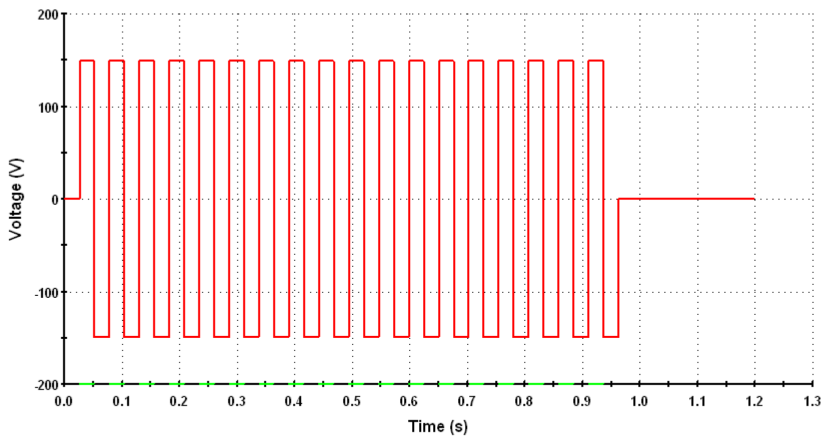

Auto Mode

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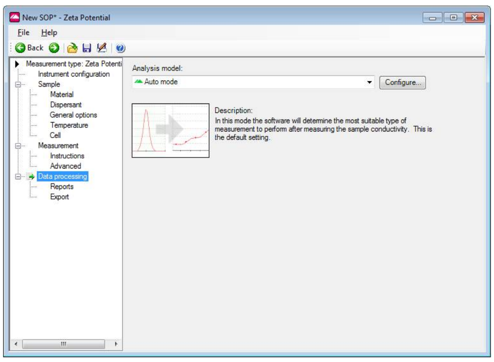
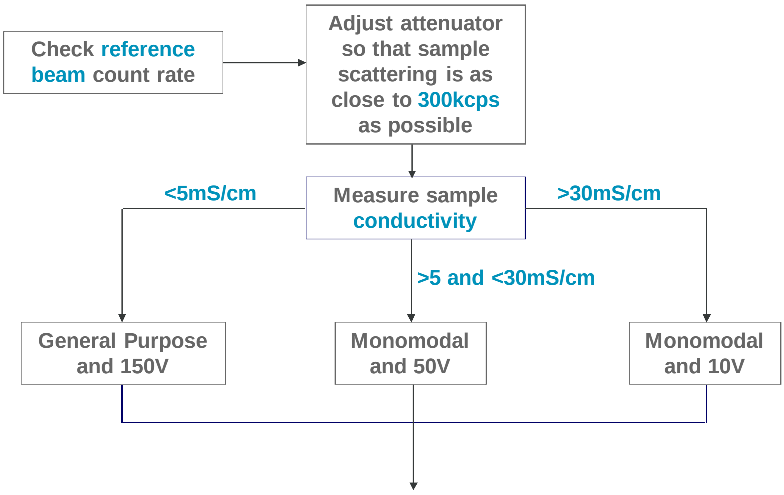
- Default measurement option
- Software determines the most suitable type of measurement to perform after measuring the sample conductivity



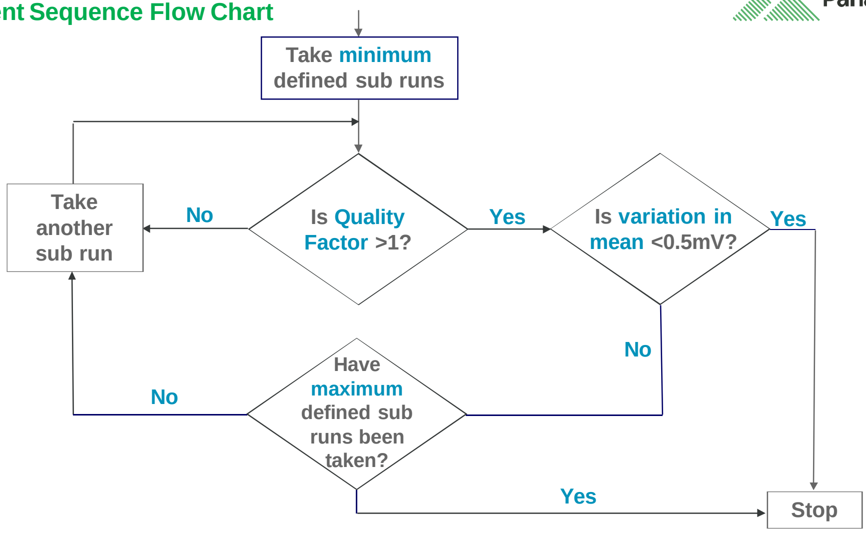
Auto Mode

Measurement Sequence Flow Chart



Auto Mode

Measurement Sequence Flow Chart



Contents

- Instruments using light
- Light scattering
- Nanoparticle Tracking Analysis
- Dynamic light scattering (DLS)
- Electrophoretic light scattering (ELS)
- **Static light scattering (SLS)**

Static Light Scattering

Absolute Molecular Weight

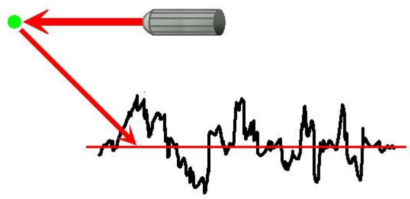
- $I \alpha (M_W)(C)$
  - I = intensity of scattered light
  - M_w = weight-averaged molecular weight
  - C = concentration
- Zetasizer measures the intensity of scattered light of various known concentrations of sample at one angle
- Called a **Debye plot** and allows for the determination of
  - Weight-averaged Molecular Weight
  - 2nd Virial Coefficient

2nd Virial Coefficient (A_2)

- A thermodynamic property describing the interaction strength between the molecule and the solvent
- For samples where **$A_2 > 0$**, the molecules tend to stay in solution
- When **$A_2 = 0$**, the molecule-solvent interaction strength is equivalent to the molecule-molecule interaction strength – the solvent is described as being a theta solvent
- When **$A_2<0$**, the molecule will tend to crystallise or aggregate

Static Light Scattering

$I \propto$ (MWt) (C)



$$\frac{KC}{R_\theta} = \left(\frac{1}{M_W} + 2A_2C\right)P_\theta$$ **(Rayleigh Equation)**

K = Optical constant
M_W = Molecular weight
A_2 = 2nd Virial coefficient
C = Concentration
R_θ = Rayleigh Ratio of the sample
P(θ) = Shape factor

Static Light Scattering

$$\frac{KC}{R_\theta} = \left(\frac{1}{M_W} + 2A_2C\right)P_\theta$$

$$K = \frac{4\pi^2}{\lambda_o^4 N_A}\left(n_o \frac{dn}{dc}\right)^2$$

λ_o = laser wavelength
N_A = Avogadro's number
n_o = Solvent RI
dn/dc = differential refractive increment

$$P_\theta = 1 + \frac{16\pi^2 n_o^2 R_g^2}{3\lambda_o^2}\sin^2\left(\frac{\theta}{2}\right)$$

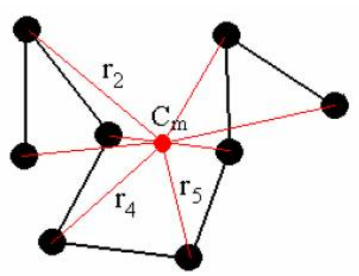
R_g = Radius of gyration
θ = Measurement angle

$$R_\theta = \frac{I_A n_o^2}{I_T n_T^2} R_T$$

I_A = Intensity of analyte (sample I – solvent I)
n_o = Solvent RI
I_T = Intensity of standard (toluene)
n_T = Standard (toluene) RI
R_T = Rayleigh ratio of standard (toluene)

Radius of Gyration (R_g)

Defined as the mass weighted average distance from the centre of mass to each mass element in a macromolecule



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

Static Light Scattering

$$\frac{KC}{R_\theta} = \left(\frac{1}{Mw} + 2A_2C\right)P_\theta$$

For Rayleigh scatterers, P(θ) = 1 and the equation is simplified to

$$\frac{KC}{R_\theta} = \left(\frac{1}{Mw} + 2A_2C\right) \quad \text{(y = mx + c)}$$

A plot of KC/R_θ versus C should give a straight line whose intercept at zero concentration will be 1/M and whose gradient will be A_2

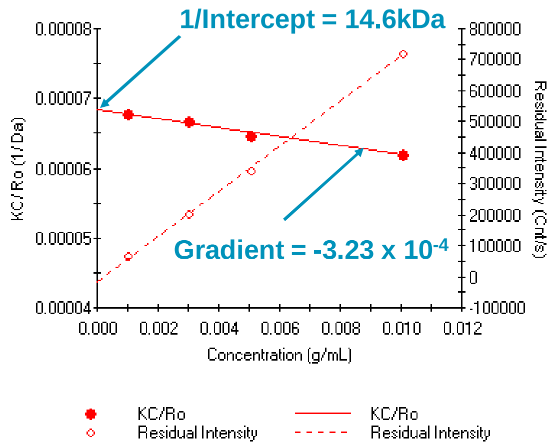
Lysozyme in PBS Example

$$\frac{dn}{dc} = 0.185(ml/g)$$

I_{tol} = 192630 (counts/sec)
I_{sol} = 21870 (counts/sec)

Lysozyme Conc^n (mg/ml)	Measured Intensity (counts/sec)	Intensity of Analyte (counts/sec)	KC/R_θ (1/Da)
1.006	87,830	65,960	6.1994×10^{-5}
3.018	222,900	201,030	6.4765×10^{-5}
5.029	366,770	344,900	6.6682×10^{-5}
10.059	742,570	720,700	6.7743×10^{-5}

Lysozyme in PBS Example



Zetasizer Nano SLS M_W Specifications

For single angle M_W measurements with Zetasizer Nano instruments:

- Globular proteins
  - Up to 32 nm diameter
  - Up to 20,000,000 Da (g/mol)
- Random coil polymers
  - Up to 42 nm diameter
  - Up to 500,000 Da (g/mol)

Thank you for your attention
Any questions?

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