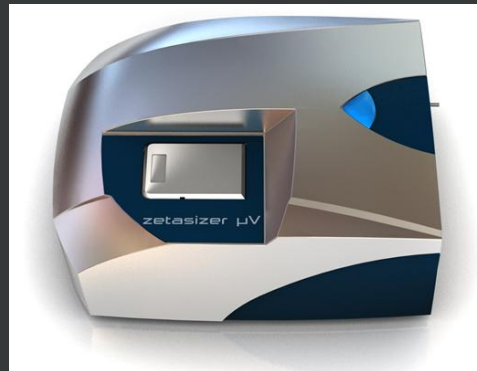


Dynamic Light Scattering Training

Achieving reliable nano particle sizing



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Light Scattering Theory

Aims of this Section.....

- 1 Understand what light scattering is
- 2 Understand what Rayleigh scattering is
- 3 Understand what Mie scattering is

What is Light Scattering?

- Light scattering is a consequence of the interaction of light with the electric field of a small particle or molecule
- An incident photon induces an oscillating dipole in the electron cloud
- As the dipole changes, energy is radiated in all directions
- This radiated energy is called “scattered light”

Rayleigh Theory

- › Rayleigh theory is applicable for small particles and molecules whose diameters are less than $1/10^{\text{th}}$ of the laser wavelength (λ)
- › For the He-Ne laser ($\lambda = 633\text{nm}$) used in the Zetasizer, this equates to particles less than about 60nm
- › The scattering produced by such small particles is isotropic i.e. equal in all directions
- › The intensity of light they produce is proportional to d^6 where d is the particle diameter (ie. $I \propto d^6$)

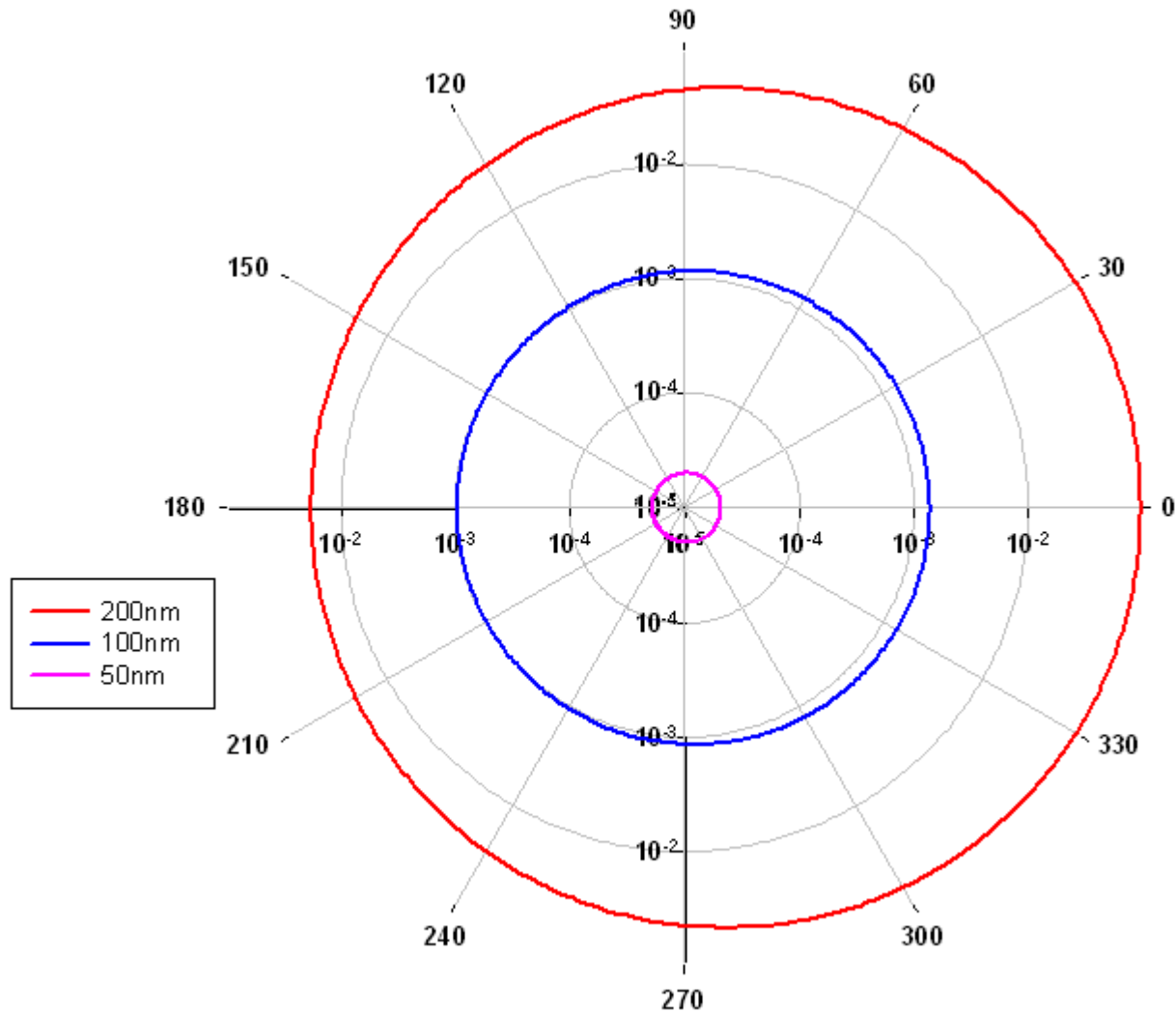
Mie Theory

- › Mie theory is an exact description of how spherical particles of all sizes and optical properties scatter light
- › When particles become larger than $\lambda/10$, the scattering changes from being isotropic to a distortion in the forward scattering direction
- › When the size of the particles becomes equivalent to or greater than the wavelength of the laser, the scattering becomes a complex function with maxima and minima with respect to angle

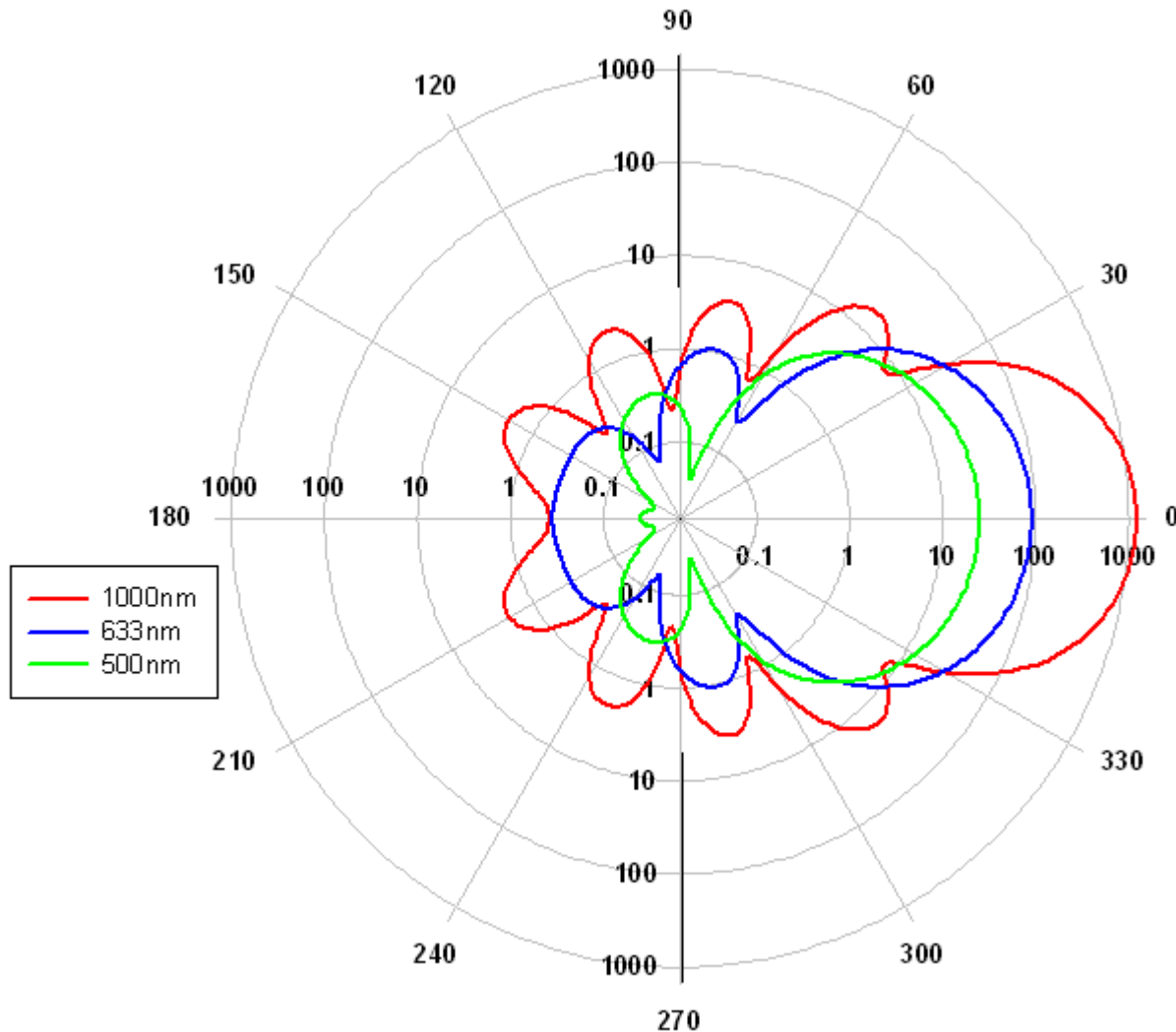
Mie Theory

- › Mie theory correctly explains the maxima and minima in the plot of intensity with angle
- › The Zetasizer Nano uses Mie theory by default to convert the intensity size distributions into volume and number for all sizes of particles

Mie Theory: Polar Plots



Mie Theory: Polar Plots



Particle Size Concepts

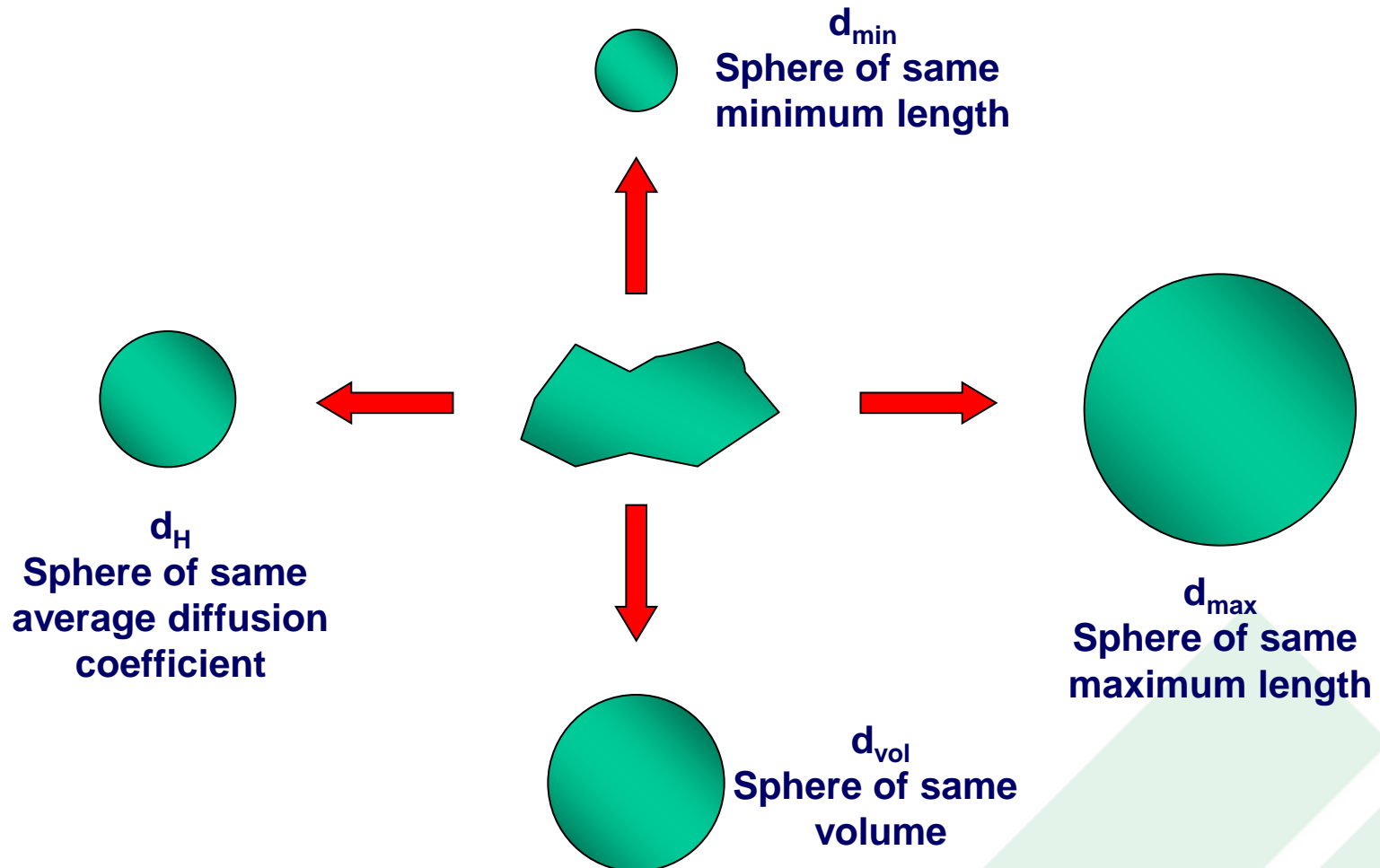
Aims of this Section.....

- 1 Understand what is being measured in a DLS measurement
- 2 Understand the Stokes-Einstein equation
- 3 Understand what a hydrodynamic size is
- 4 Understand what influences the hydrodynamic size

Particle Size

- › How can a 3-dimensional object be described with one number?
 - **Most techniques use an equivalent spherical diameter**
- › All particle size analysis techniques measure some property of a particle and report results as the equivalent spherical diameter based on this measured parameter
- › Different measurement techniques often give different sizes for the same sample

Equivalent Spheres



Dynamic Light Scattering and Brownian Motion

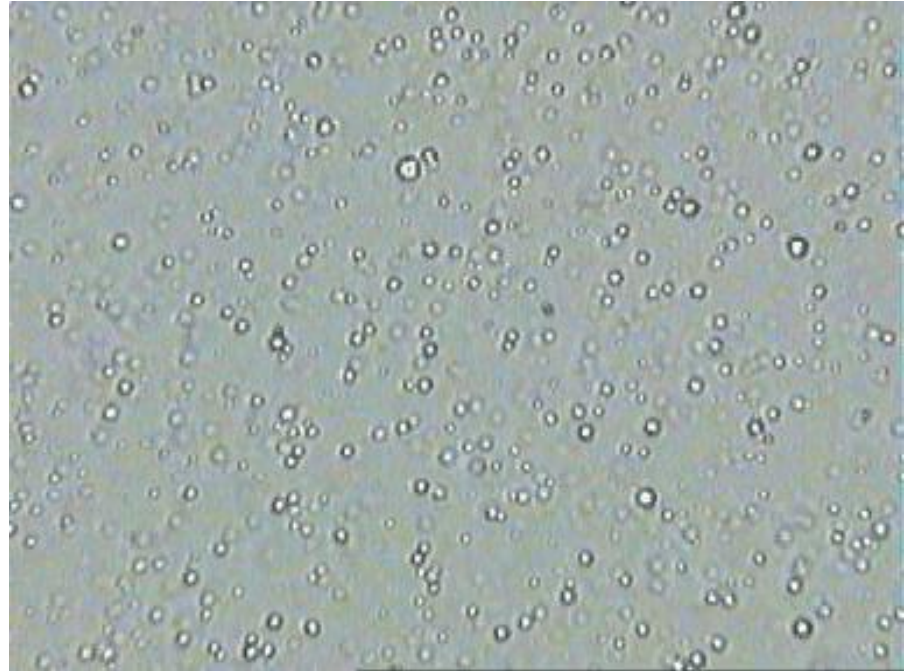
- › Dynamic light scattering is a non-invasive technique for measuring the size of particles and molecules in suspension
- › Brownian motion is the **random** movement of particles due to collisions caused by bombardment by the solvent molecules that surround them
- › The technique of dynamic light scattering measures the speed of particles undergoing Brownian motion

Dynamic Light Scattering and Brownian Motion

- The speed of the Brownian motion is influenced by
 - **Particle size**
 - **Sample viscosity**
 - **Temperature**
- Viscosity is important in determining the speed of Brownian motion.
 - **The temperature must be accurately known**
 - **Automatically read back by the software**
- The temperature needs to be stable during a measurement.
 - **Convection currents in the sample cause non-random movements which prevents accurate size interpretation**

Brownian Motion and Particle Size

- The smaller the particle is, the more rapid the Brownian motion becomes
- The larger the particle is, the slower the Brownian motion becomes
- The higher the temperature the more rapid the Brownian motion



Brownian Motion

- › Velocity of the Brownian motion is defined by the translational diffusion coefficient (D)
- › The translational diffusion coefficient can be converted into a particle size using the **Stokes-Einstein** equation

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

Where :

d_H = hydrodynamic diameter,

k = Boltzmann's constant,

T = absolute temperature,

η = viscosity and

D = diffusion coefficient

Hydrodynamic Size

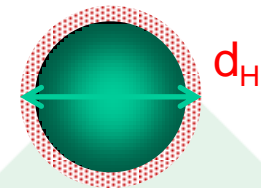
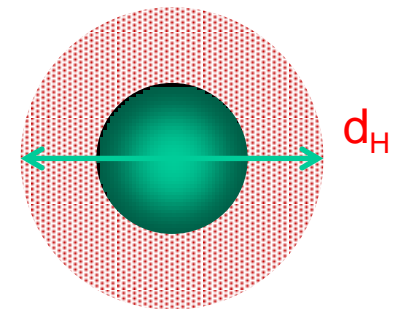
› Definition of Hydrodynamic Diameter (d_H):

The diameter of a hard sphere that diffuses at the same speed as the particle or molecule being measured

- › The hydrodynamic diameter will depend not only on the size of the particle “core”, but also on any surface structure, as well as the type and concentration of any ions in the medium

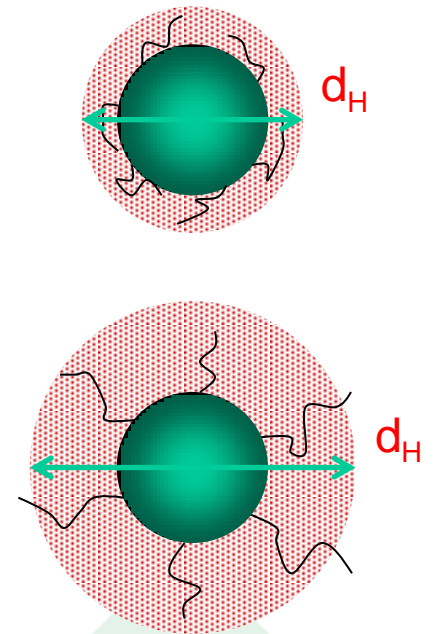
Hydrodynamic Size: Effect of Ionic Strength

- › The ions in the medium and the total ionic concentration may affect the particle diffusion speed by changing the thickness of the electric double layer called the Debye length (κ^{-1})
- › A low concentration ionic medium will produce an extended double layer of ions around the particle, reducing the diffusion speed and resulting in a larger, apparent hydrodynamic diameter
- › Higher ionic concentration media ($\geq 10\text{mM}$) will compress the electrical double layer and reduce the measured hydrodynamic diameter



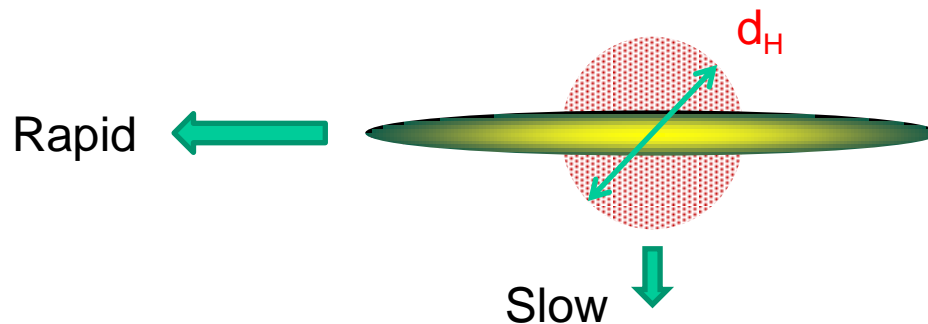
Hydrodynamic Size: Surface Structure

- Any change to the surface of a particle that affects the diffusion speed will change the apparent size of the particle
- An adsorbed polymer layer projecting out into the medium will reduce the diffusion speed more than if the polymer is lying flat on the surface
- The nature of the surface and the polymer, as well as the ionic concentration of the medium can affect the polymer conformation, which in turn can change the apparent size by several nanometres



Hydrodynamic Size: Non-spherical particles

- › A sphere is the only object whose size can be unambiguously described by a single number
- › For a non-spherical particle, DLS will give the diameter of a sphere that has the same **average** translational diffusion coefficient as the particle being measured

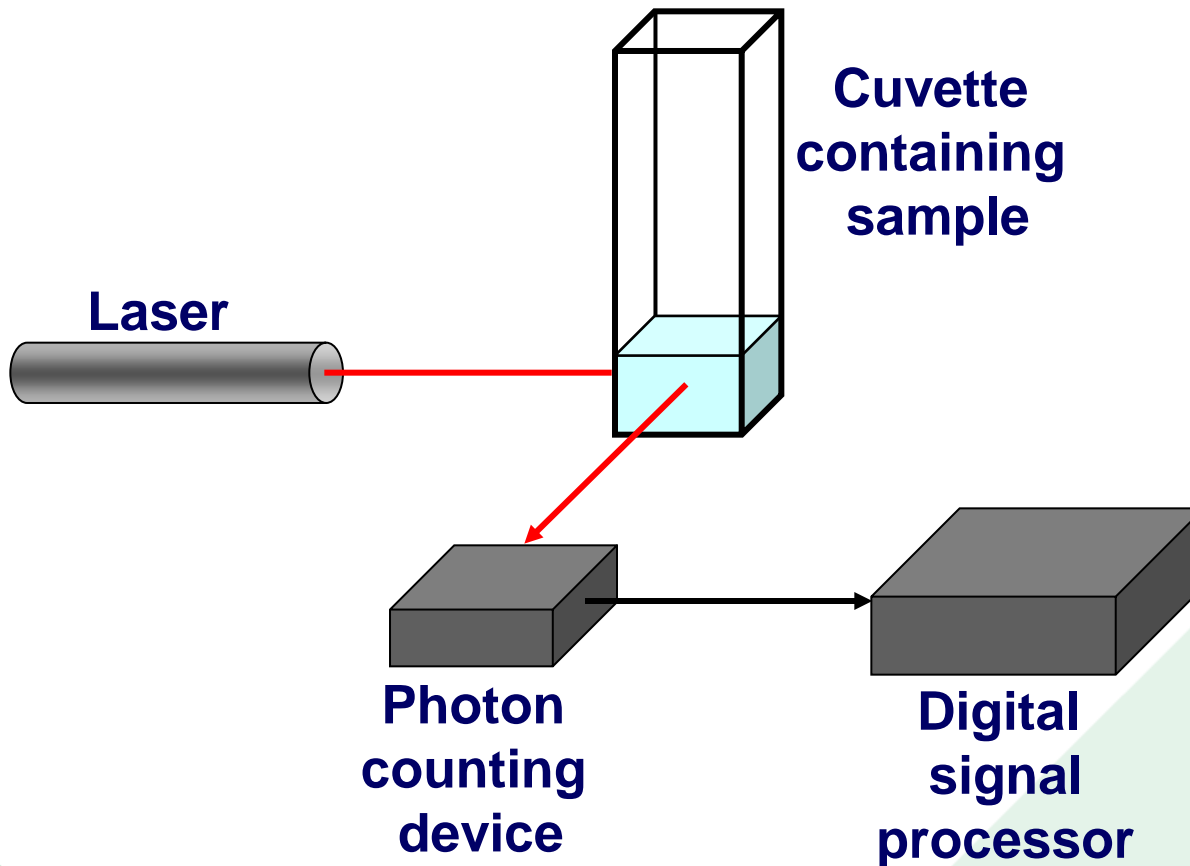


DLS Theory

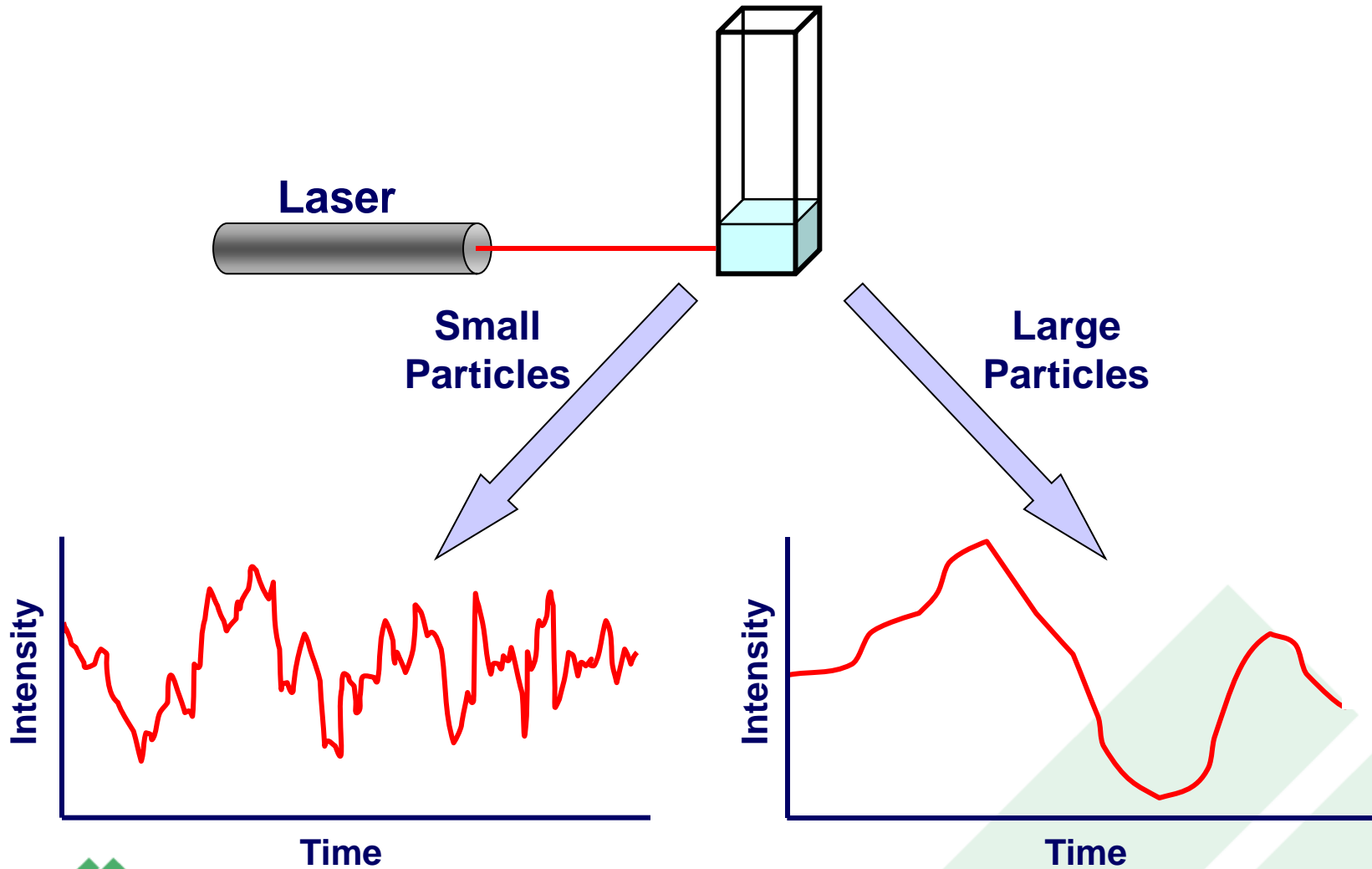
Aims of this Section.....

- 1 Understand what components make up a Malvern DLS instrument
- 2 Understand the relationship between Brownian motion intensity fluctuations
- 3 Understand correlation
- 4 Understand what a correlation function is
- 5 Understand what analysis options for the correlation function are available

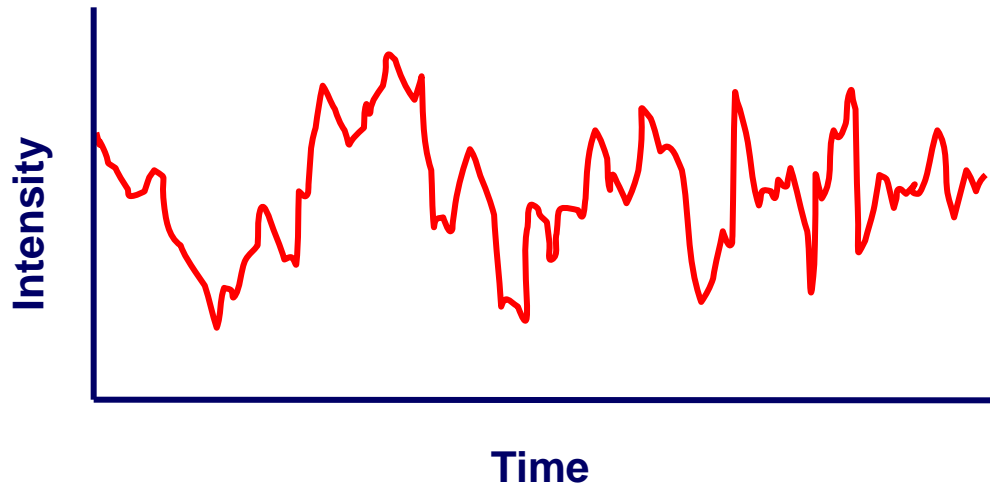
Components of a Dynamic Light Scattering Instrument



Intensity Fluctuations and Brownian Motion



Correlation: Small Particles, Rapid Fluctuations



Correlation in Dynamic Light Scattering

- › Correlation in DLS is a technique for extracting the time dependence of a signal in the presence of “noise”
- › In a DLS measurement, the time analysis is carried out with a correlator which constructs the time autocorrelation function $G(\tau)$ of the scattered intensity according to

$$G(\tau) = \left\langle \frac{I(t) * I(t + \tau)}{I^2(t)} \right\rangle$$

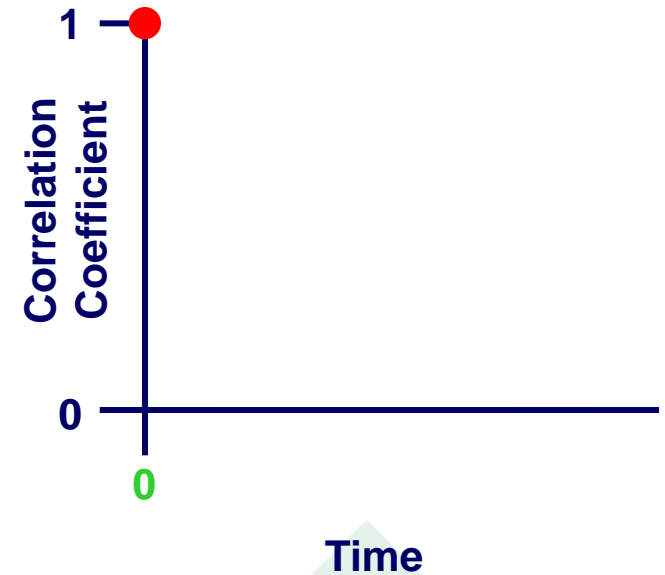
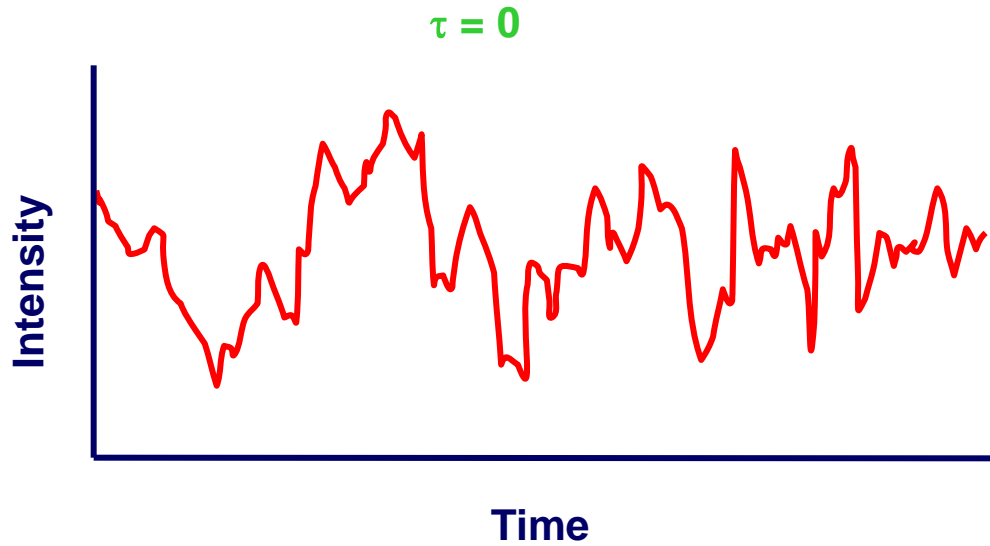
where

I = intensity,

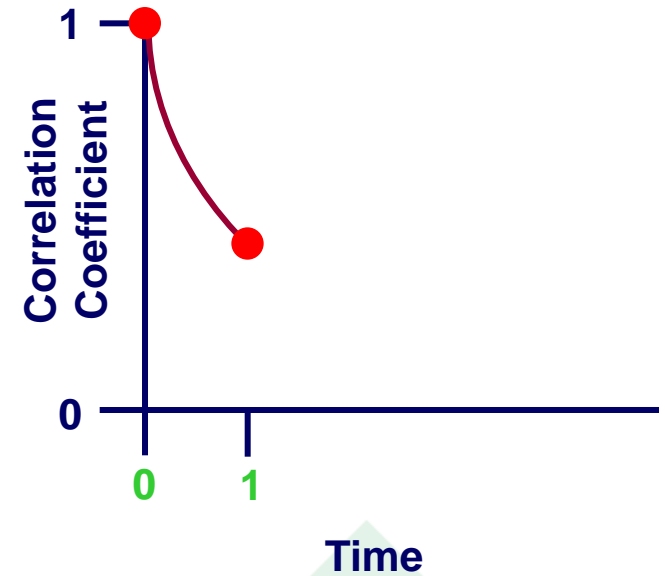
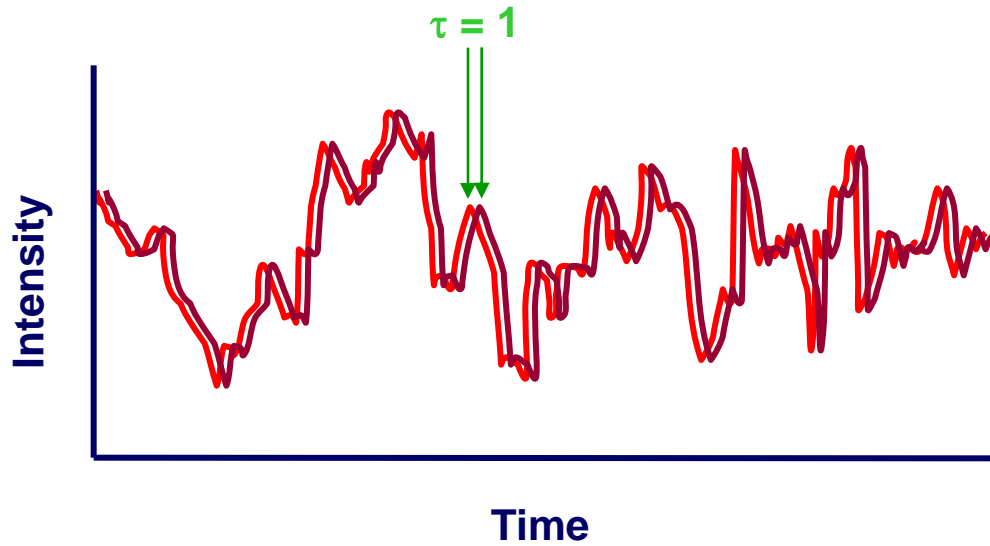
t is the time and

τ = the delay time

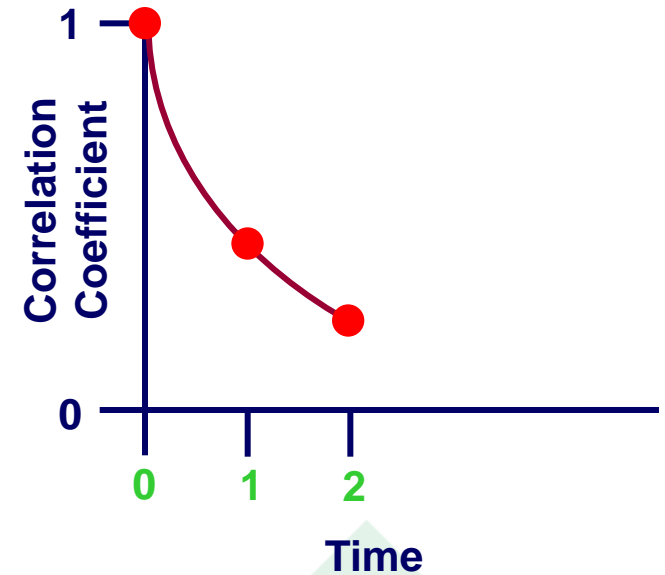
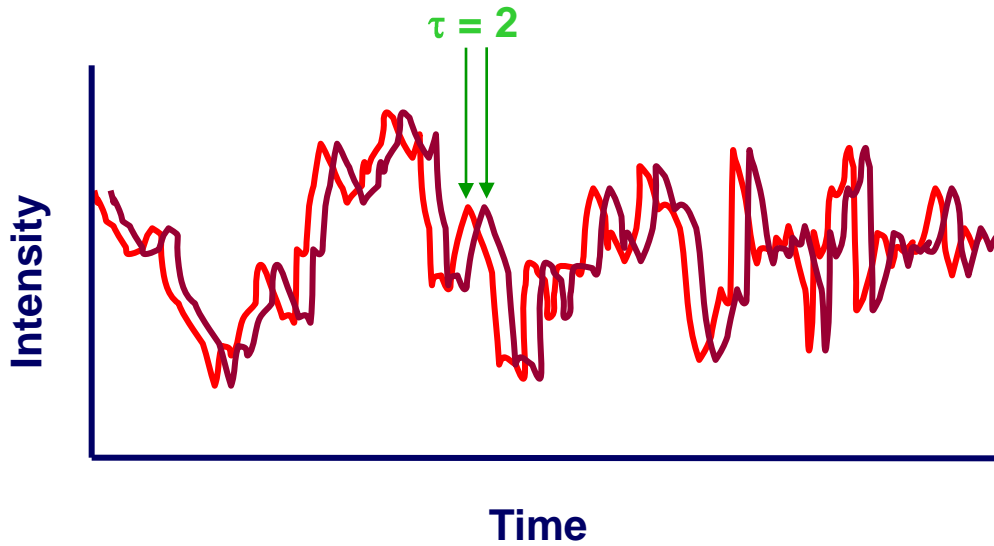
Correlation: Small Particles, Rapid Fluctuations



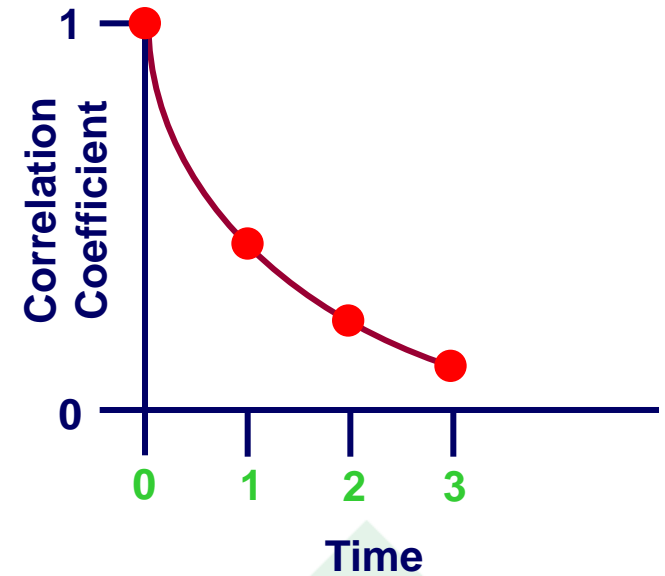
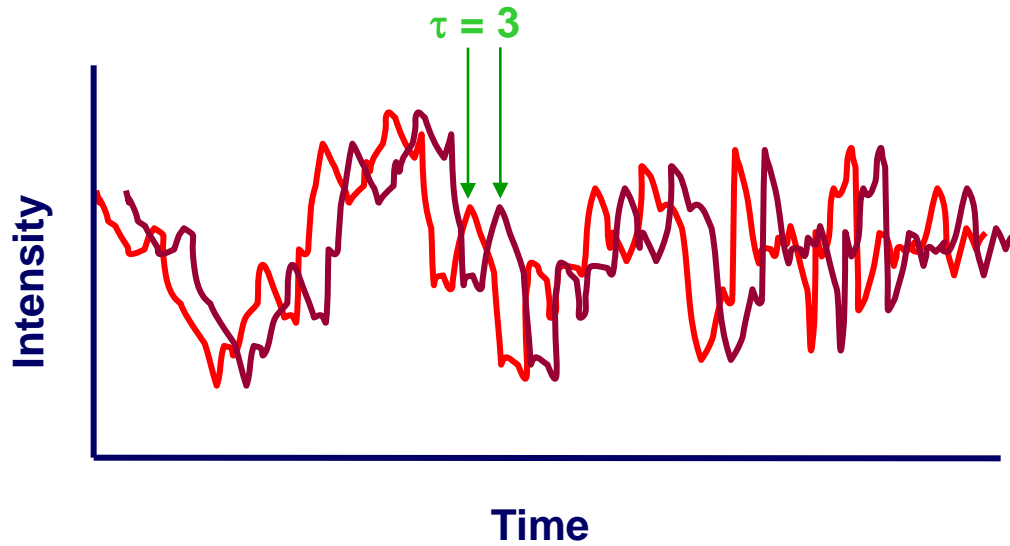
Correlation: Small Particles, Rapid Fluctuations



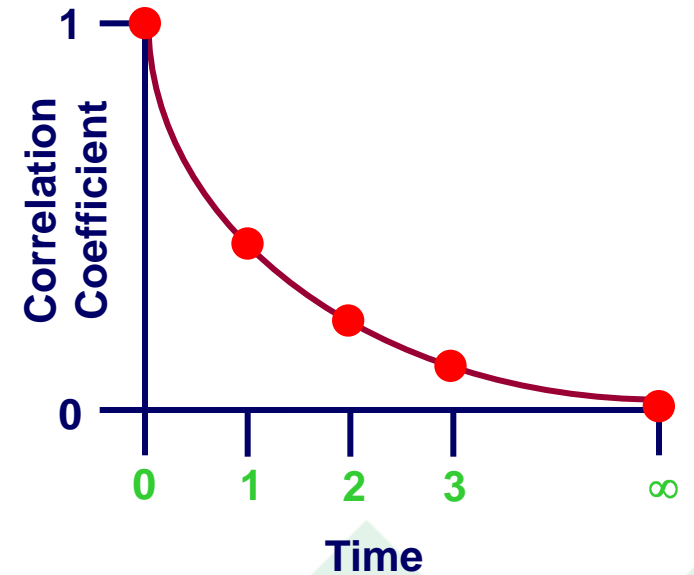
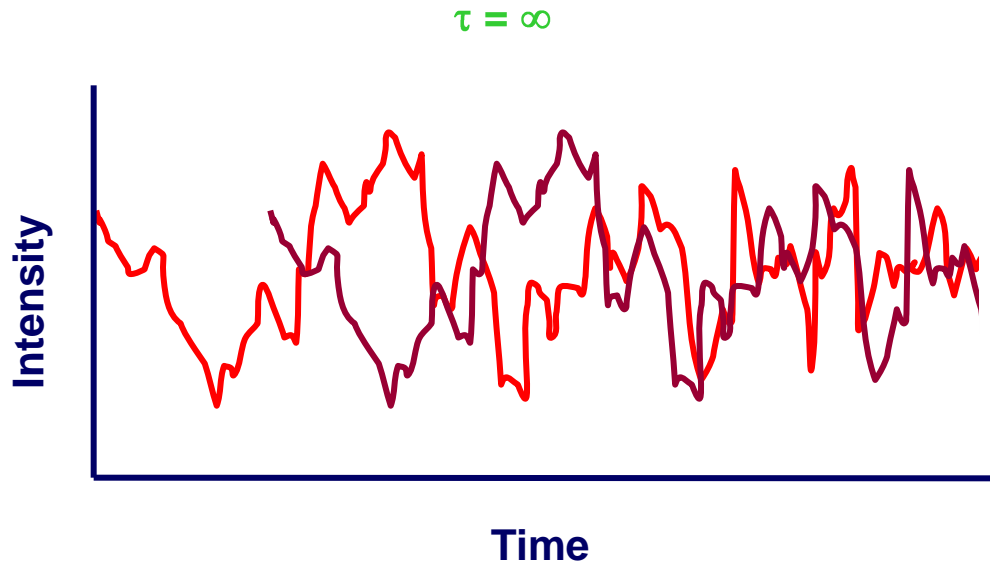
Correlation: Small Particles, Rapid Fluctuations



Correlation: Small Particles, Rapid Fluctuations

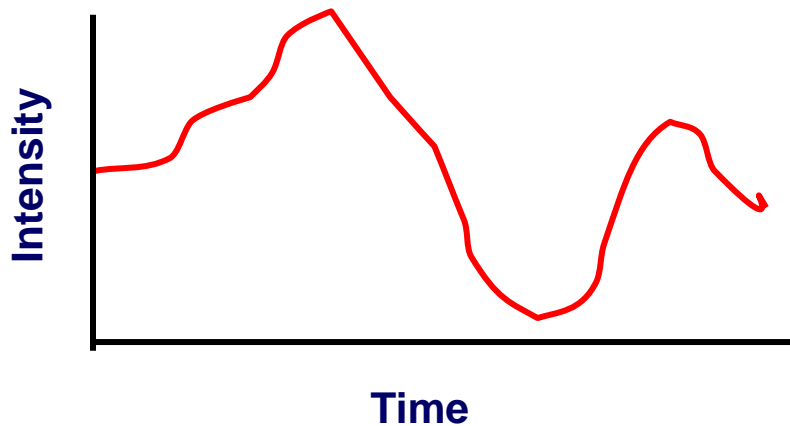


Correlation: Small Particles, Rapid Fluctuations

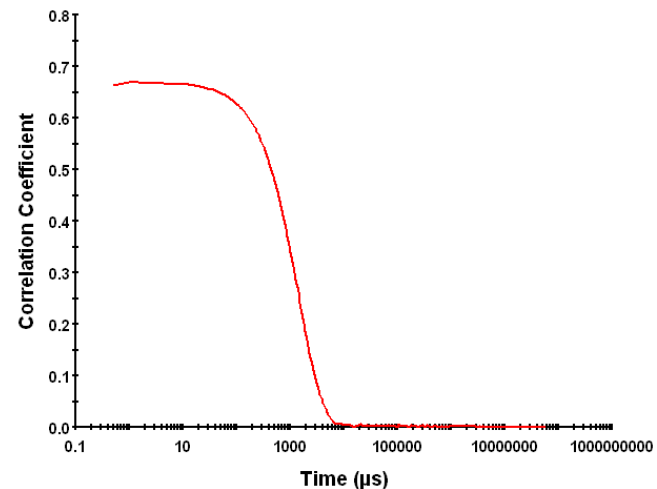


Correlation Functions

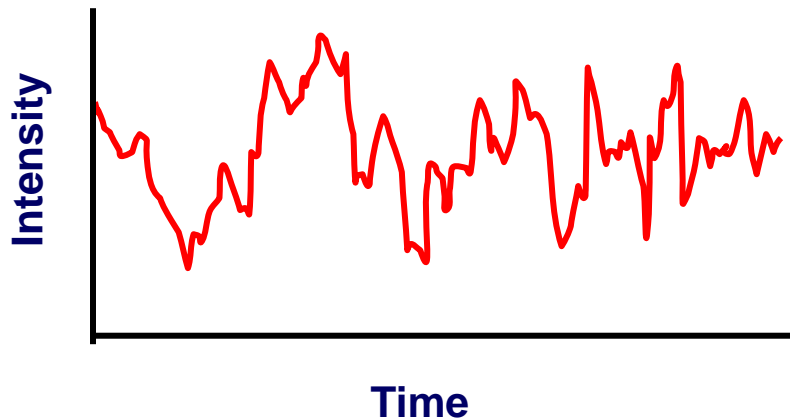
Large Particles



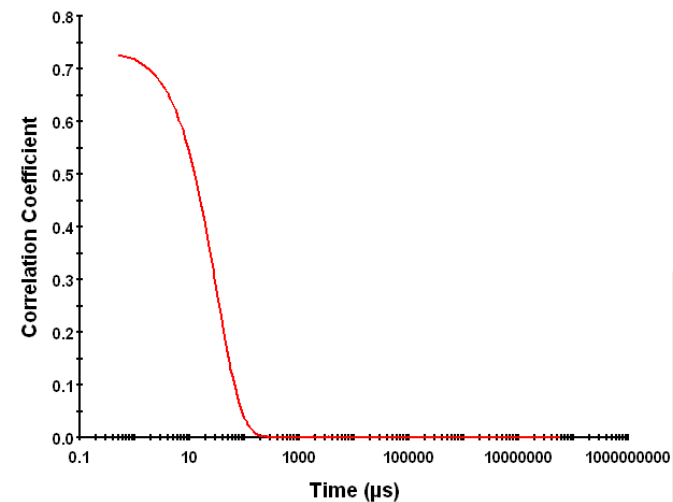
Correlate



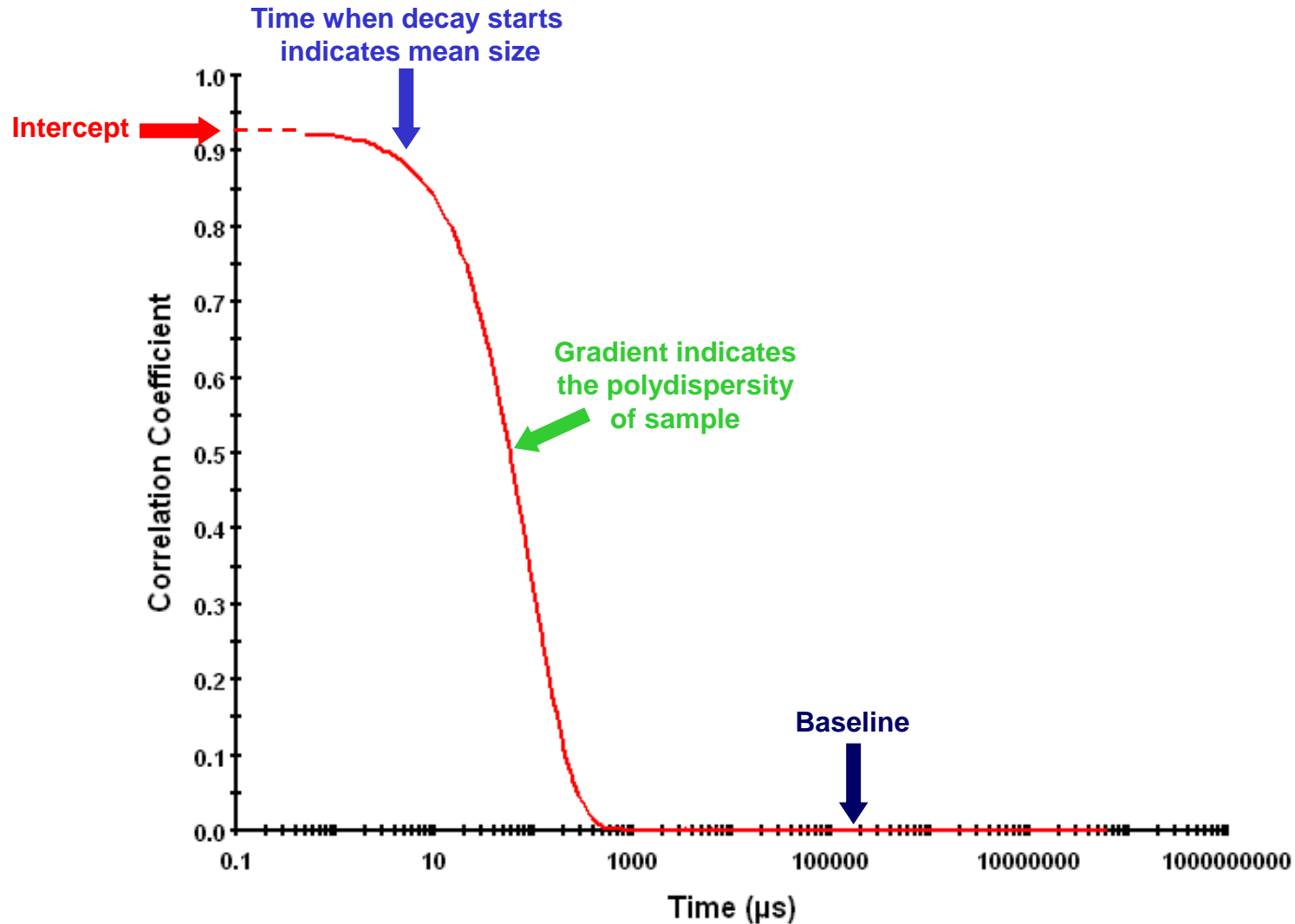
Small Particles



Correlate



Correlation Functions



Correlation Functions

- › The correlation function can be modelled with an exponential expression such as:

$$G(\tau) = B + A \sum e^{-2q^2 D \tau}$$

Where

- **B = baseline at infinite time,**
- **A = amplitude (or intercept),**
- **q = scattering vector = $(4\pi n/\lambda_0) \sin(\theta/2)$**
 - where n = dispersant refractive index,
 - λ_0 = laser wavelength and
 - θ = detection angle,
- **D = diffusion coefficient and**
- **τ is the correlator delay time**

Analysing The Correlation Function

- › The correlation function contains the diffusion coefficient information required to be entered into the Stokes-Einstein equation
- › These diffusion coefficients are obtained by fitting the correlation function with a suitable algorithm
- › Two methods of analysis are used
 - **Cumulants analysis**
 - Determines a mean size and polydispersity index
 - **Distribution analysis**
 - Determines actual size distribution from suitable data

Cumulants Analysis

- The cumulants analysis is defined in the International Standard on Dynamic Light Scattering ISO13321 (1996) and ISO22412 (2008)
- This analysis 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

The z-Average Diameter

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

The intensity-weighted mean diameter derived from the cumulants analysis

- › This mean is specific to light scattering
- › It is very sensitive to the presence of aggregates or large contaminants due to the inherent intensity weighting

Polydispersity Index

- › **Definition of the Polydispersity Index (Pdl):**

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

- › In the Zetasizer software it **ranges from 0 to 1**

- › Values greater than 1 indicate that the distribution is so polydisperse the sample may not be suitable for measurement by DLS

Polydispersity Index

Polydispersity Index Value	Comments
<0.05	Only normally encountered with latex standards or particles made to be monodisperse
<0.08	Nearly monodisperse sample. Normally, DLS can only give a monomodal distribution within this range
0.08 to 0.7	Mid-range value of Pdl. It is the range over which the distribution algorithms best operate over
>0.7	Indicates a very broad distribution of particle sizes

Distribution Analysis

- › Default algorithms present in the Zetasizer software for calculating the size distribution are:
 - **General Purpose** (non-negative least squares (NNLS) analysis)
 - **Multiple Narrow Modes** (non-negative least squares (NNLS) analysis)
 - **Protein Analysis** (non-negative least squares (NNLS) analysis followed by (L-curve))
- › The difference between these algorithms is the regularizer used

Regularizer

- A small amount of noise in the correlation function can generate a large number of distributions
- The regularizer can be thought of as an estimator of the noise contained in the correlogram
- It controls the acceptable degree of “spikiness” in the size distribution obtained
 - **Large regularizer values produce smooth distributions**
 - **Small regularizer values produce spiky distributions**
- There is no ideal regularizer value; the appropriate value depends on the sample being measured

Available Algorithms and Associated Regularizer

Algorithm	Regularizer
General Purpose	0.01
Multiple Narrow Modes	0.001
Protein Analysis	Variable (appropriate value automatically determined)

Gen. Purpose and Multiple Narrow Modes

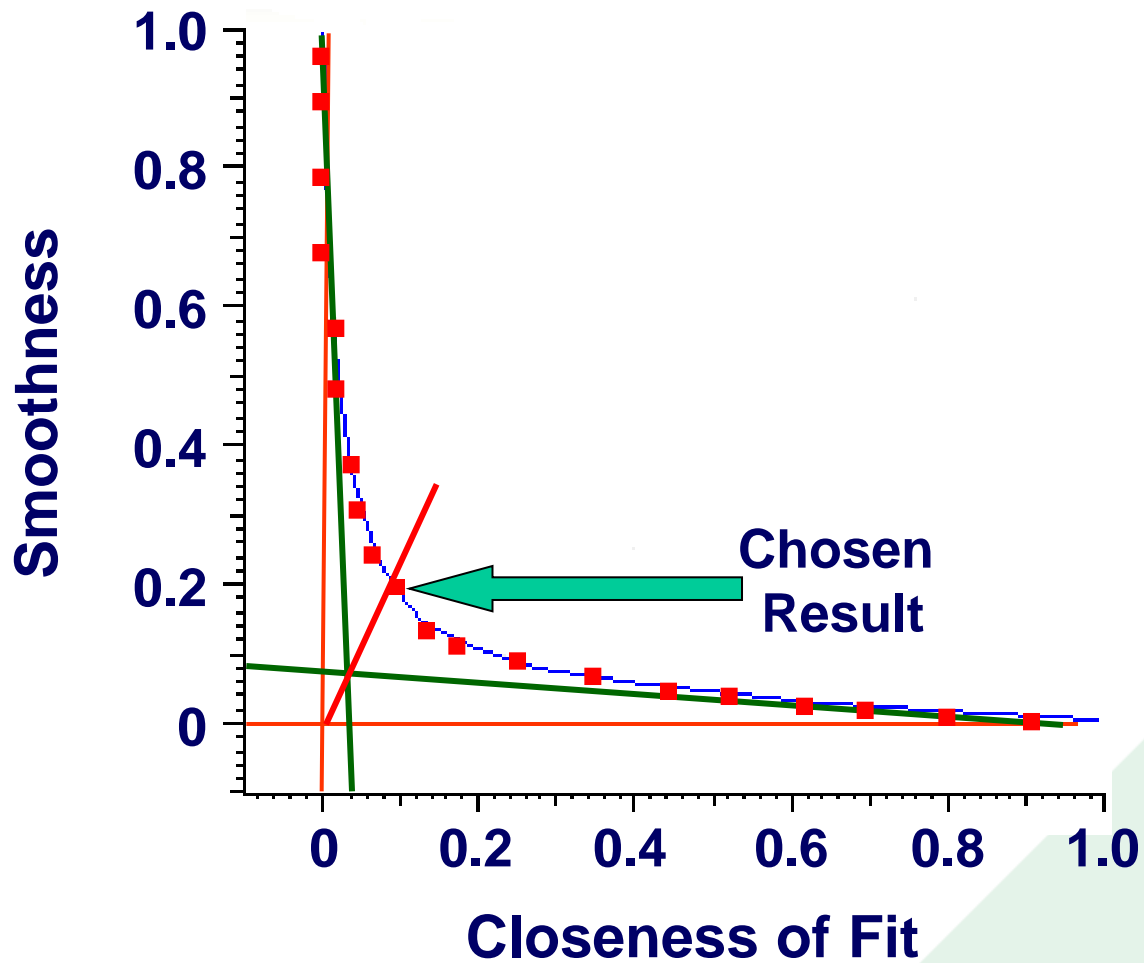
- › **General purpose** suitable for the majority of samples where no knowledge of the distribution is available
 - **Regulariser (fixed to 0.01)**

- › **Multiple narrow mode** suitable for samples suspected to contain discrete populations
 - **Regulariser (fixed to 0.001)**

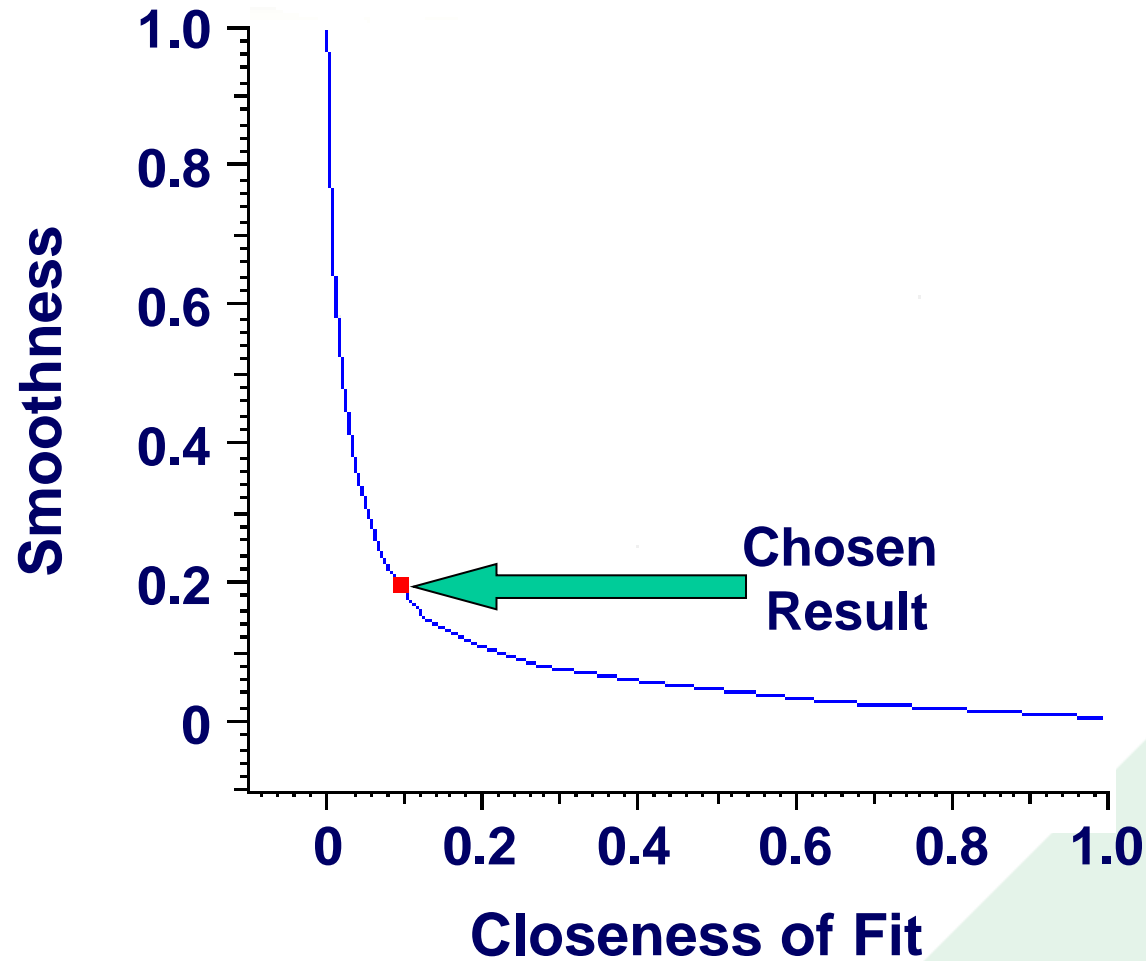
Protein Analysis (L Curve)

- › Best suited for protein samples – will give narrow peaks
- › Automatically picks the optimal solution (distribution)
- › Calculates 20 possible distributions and the closeness of fit (X-axis) is plotted as a function of smoothness (Y-axis)
- › Tangents are fitted and the intersection between the two is extrapolated to the origin
- › The distribution closest to the intersection is the chosen one

Protein Analysis (L Curve)



Protein Analysis (L Curve)

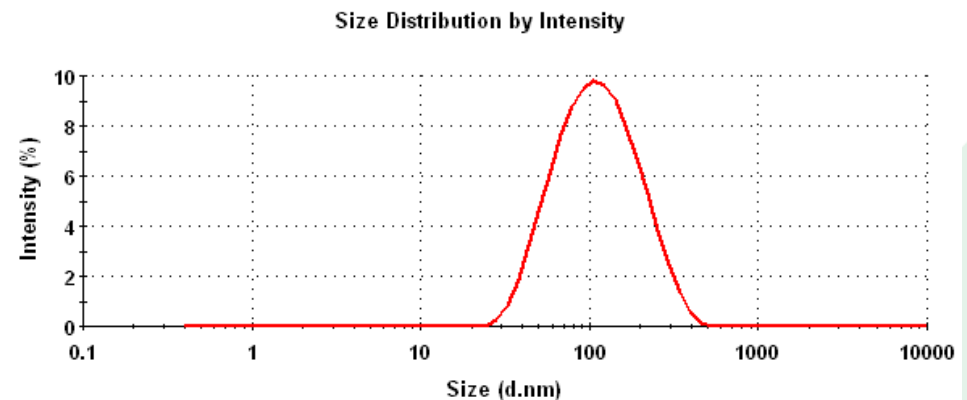
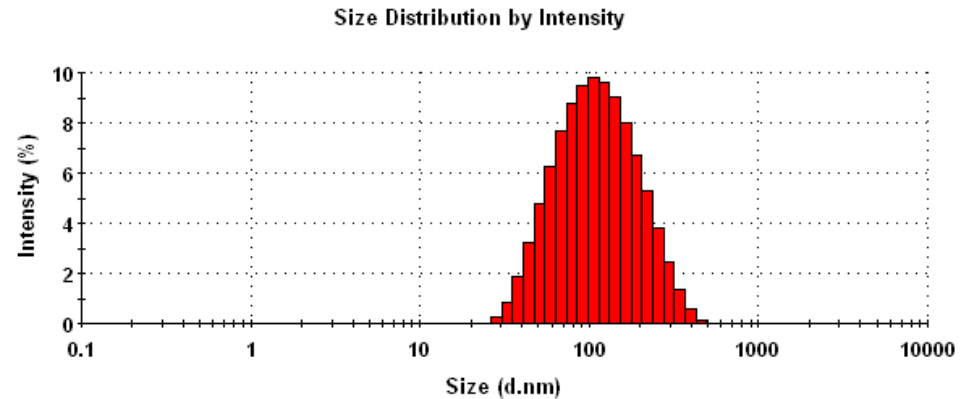


Size Distributions in the Zetasizer Software

- › The primary size distribution obtained from a DLS measurement is the intensity-weighted distribution obtained from the chosen analysis
- › This size distribution is displayed as 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
- › General Purpose and Multiple Narrow Modes use 70 size classes
- › Protein Analysis uses 300 size classes

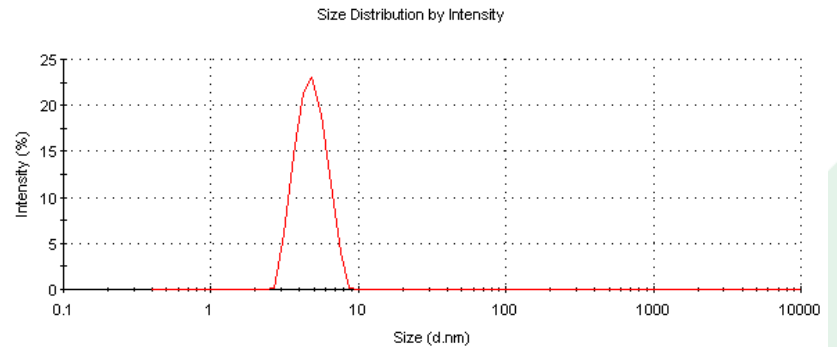
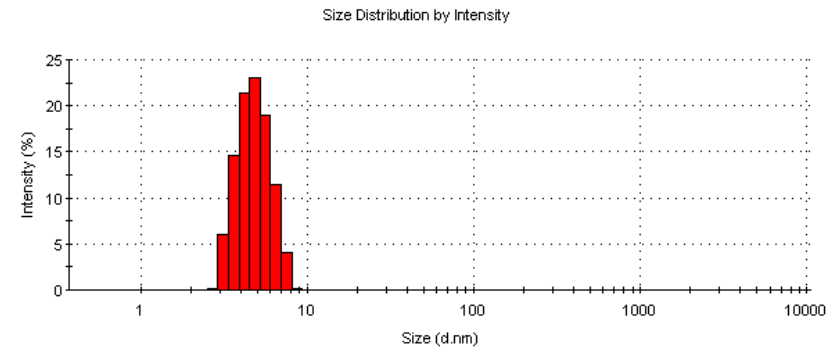
Size Distributions with General Purpose and Multiple Narrow Modes: 70 Classes

Size d.nm	Intensity %	Size d.nm	Intensity %	Size d.nm	Intensity %
0.4000	0.0	13.54	0.0	458.7	5.1
0.4632	0.0	15.69	0.0	531.2	3.1
0.5365	0.0	18.17	0.0	615.1	1.4
0.6213	0.0	21.04	0.0	712.4	0.4
0.7195	0.0	24.36	0.0	825.0	0.0
0.8332	0.0	28.21	0.0	955.4	0.0
0.9649	0.0	32.67	0.0	1106	0.0
1.117	0.0	37.84	0.0	1281	0.0
1.294	0.0	43.82	0.0	1484	0.0
1.499	0.0	50.75	0.0	1718	0.0
1.736	0.0	58.77	0.0	1990	0.0
2.010	0.0	68.06	0.0	2305	0.0
2.328	0.0	78.82	0.1	2669	0.0
2.696	0.0	91.28	1.0	3091	0.0
3.122	0.0	105.7	2.7	3580	0.0
3.615	0.0	122.4	5.1	4145	0.0
4.187	0.0	141.8	7.6	4801	0.0
4.849	0.0	164.2	9.8	5560	0.0
5.615	0.0	190.1	11.4	6439	0.0
6.503	0.0	220.2	12.2	7456	0.0
7.531	0.0	255.0	12.1	8635	0.0
8.721	0.0	295.3	11.2	1.000e4	0.0
10.10	0.0	342.0	9.5		
11.70	0.0	396.1	7.4		



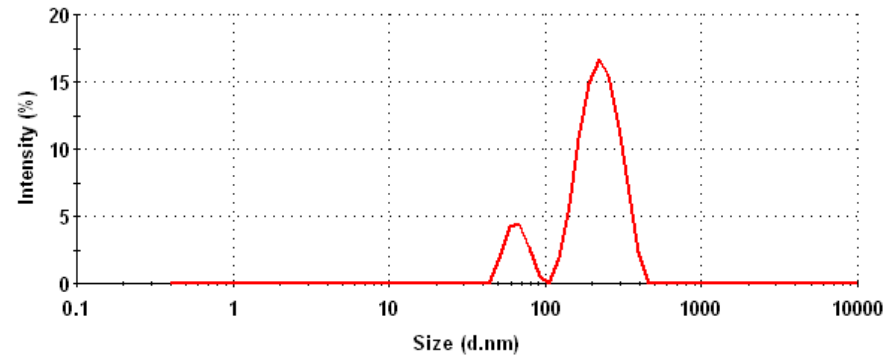
Size Distributions with Protein Analysis: 300 Classes

Size d.nm	Intensity %	Size d.nm	Intensity %	Size d.nm	Intensity %	Size d.nm	Intensity %	Size d.nm	Intensity %	Size d.nm	Intensity %	Size d.nm	Intensity %
0.01000	0.0	0.07292	0.0	0.5318	0.0	3.878	0.0	28.28	0.0	206.2	0.0	1504	0.0
0.01047	0.0	0.07637	0.0	0.5570	0.0	4.062	0.0	29.62	0.0	216.0	0.0	1575	0.0
0.01097	0.0	0.07999	0.0	0.5833	0.0	4.254	0.0	31.02	0.0	226.2	0.0	1650	0.0
0.01149	0.0	0.08377	0.0	0.6109	0.0	4.455	0.0	32.49	0.0	236.9	0.0	1728	0.0
0.01203	0.0	0.08773	0.0	0.6398	0.0	4.665	0.0	34.02	0.0	248.1	0.0	1809	0.0
0.01259	0.0	0.09188	0.0	0.6700	0.0	4.886	0.0	35.63	0.0	259.8	0.0	1895	0.0
0.01319	0.0	0.09622	0.0	0.7017	0.0	5.117	0.0	37.32	0.0	272.1	0.0	1985	0.0
0.01382	0.0	0.1008	0.0	0.7349	0.0	5.359	0.0	39.08	0.0	285.0	0.0	2078	0.0
0.01447	0.0	0.1055	0.0	0.7696	0.0	5.613	0.0	40.93	0.0	298.5	0.0	2177	0.0
0.01516	0.0	0.1105	0.0	0.8060	0.0	5.878	0.0	42.87	0.0	312.6	0.1	2280	0.0
0.01587	0.0	0.1158	0.0	0.8442	0.0	6.156	0.0	44.89	0.0	327.4	0.3	2387	0.0
0.01662	0.0	0.1212	0.0	0.8841	0.0	6.447	0.0	47.02	0.0	342.9	0.5	2500	0.0
0.01741	0.0	0.1270	0.0	0.9259	0.0	6.752	0.0	49.24	0.0	359.1	0.6	2619	0.0
0.01823	0.0	0.1330	0.0	0.9697	0.0	7.071	0.0	51.57	0.0	376.1	0.7	2742	0.0
0.01910	0.0	0.1393	0.0	1.016	0.0	7.405	0.0	54.01	0.0	393.8	0.6	2872	0.0
0.02000	0.0	0.1458	0.0	1.064	0.0	7.756	0.0	56.56	0.0	412.5	0.5	3008	0.0
0.02094	0.0	0.1527	0.0	1.114	0.0	8.123	0.0	59.23	0.0	432.0	0.4	3150	0.0
0.02193	0.0	0.1600	0.0	1.167	0.0	8.507	0.0	62.04	0.0	452.4	0.2	3299	0.0
0.02297	0.0	0.1675	0.0	1.222	0.0	8.909	0.0	64.97	0.0	473.8	0.1	3455	0.0
0.02406	0.0	0.1754	0.0	1.279	0.0	9.330	0.0	68.04	0.0	496.2	0.0	3618	0.0
0.02520	0.0	0.1837	0.0	1.340	0.0	9.772	0.0	71.26	0.0	519.7	0.0	3790	0.0
0.02639	0.0	0.1924	0.0	1.403	0.0	10.23	0.0	74.63	0.0	544.2	0.0	3969	0.0
0.02764	0.0	0.2015	0.0	1.470	0.0	10.72	0.0	78.16	0.0	570.0	0.0	4157	0.0
0.02894	0.0	0.2111	0.0	1.539	0.0	11.22	0.0	81.85	0.0	596.9	0.0	4353	0.0
0.03031	0.0	0.2210	0.0	1.612	0.0	11.76	0.0	85.73	0.0	625.2	0.0	4559	0.0
0.03174	0.0	0.2315	0.0	1.688	0.0	12.31	0.0	89.78	0.0	654.7	0.0	4775	0.0
0.03325	0.0	0.2424	0.0	1.768	0.0	12.89	0.0	94.03	0.0	685.7	0.0	5000	0.0
0.03482	0.0	0.2539	0.0	1.852	0.0	13.50	0.0	98.47	0.0	718.1	0.0	5237	0.0
0.03646	0.0	0.2659	0.0	1.939	0.0	14.14	0.0	103.1	0.0	752.1	0.0	5484	0.0
0.03819	0.0	0.2785	0.7	2.031	0.0	14.81	0.0	108.0	0.0	787.6	0.0	5744	0.0
0.04000	0.0	0.2917	1.9	2.127	0.5	15.51	0.0	113.1	0.0	824.9	0.0	6015	0.0
0.04189	0.0	0.3055	2.8	2.228	2.4	16.24	0.0	118.5	0.0	863.9	0.0	6300	0.0
0.04387	0.0	0.3199	2.8	2.333	5.1	17.01	0.0	124.1	0.0	904.7	0.0	6598	0.0
0.04594	0.0	0.3350	2.1	2.443	7.8	17.82	0.0	129.9	0.0	947.5	0.0	6910	0.0
0.04811	0.0	0.3509	0.9	2.559	10.0	18.66	0.0	136.1	0.0	992.3	0.0	7237	0.0
0.05039	0.0	0.3675	0.0	2.680	11.4	19.54	0.0	142.5	0.0	1039	0.0	7579	0.0
0.05277	0.0	0.3848	0.0	2.806	11.9	20.47	0.0	149.2	0.0	1088	0.0	7937	0.0
0.05527	0.0	0.4030	0.0	2.939	11.3	21.43	0.0	156.3	0.0	1140	0.0	8313	0.0
0.05788	0.0	0.4221	0.0	3.078	9.7	22.45	0.0	163.7	0.0	1194	0.0	8706	0.0
0.06062	0.0	0.4421	0.0	3.224	7.4	23.51	0.0	171.4	0.0	1250	0.0	9117	0.0
0.06349	0.0	0.4630	0.0	3.376	4.7	24.62	0.0	179.5	0.0	1309	0.0	9548	0.0
0.06649	0.0	0.4849	0.0	3.536	2.2	25.79	0.0	188.0	0.0	1371	0.0	1.000e 4	0.0
0.06963	0.0	0.5078	0.0	3.703	0.4	27.00	0.0	196.9	0.0	1436	0.0		



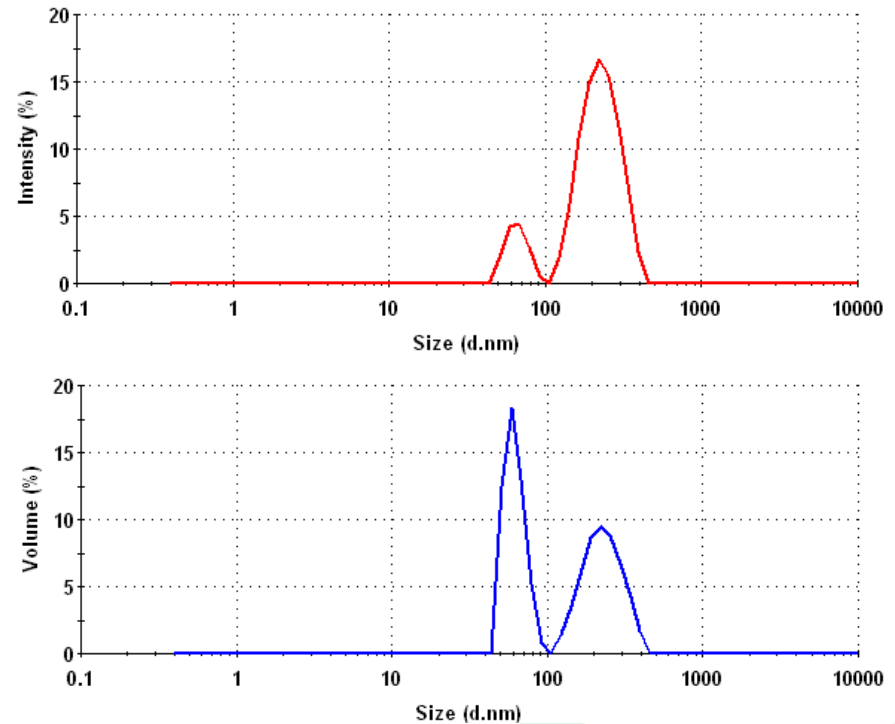
Intensity Size Distributions

- › Primary result obtained from a DLS measurement
- › Based upon the intensity of light scattered by particles
- › Sensitive to the presence of large particles/aggregates /dust
- › The only sample properties required are the dispersant viscosity and refractive index



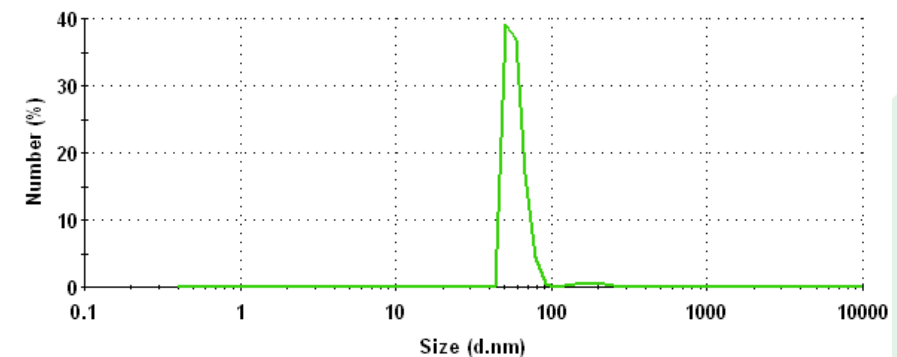
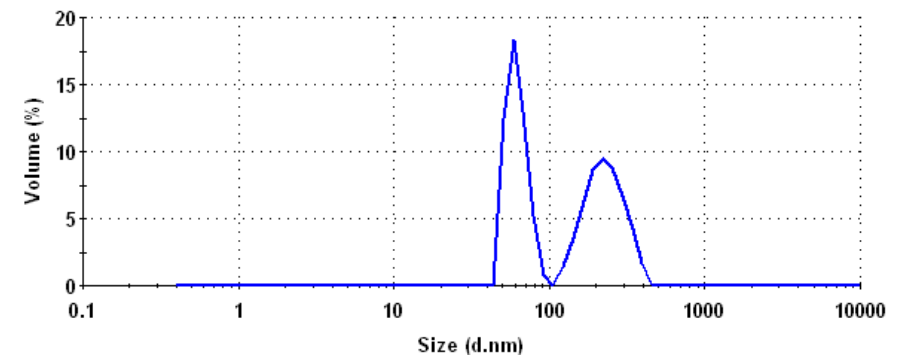
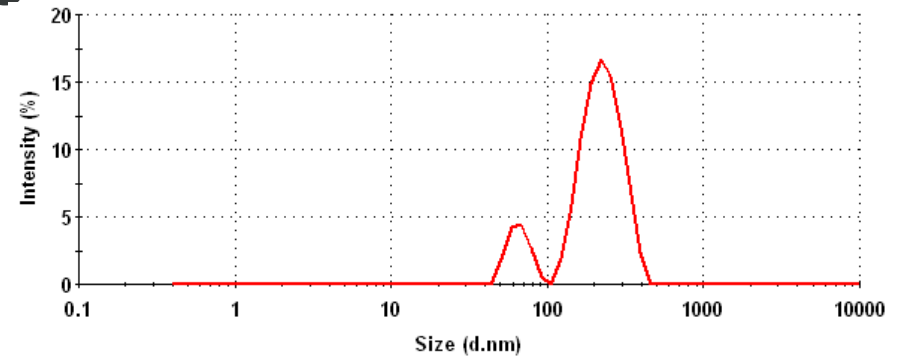
Volume Size Distributions

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



Number Size Distributions

- Derived from the intensity distribution using Mie theory
- The optical properties of the particles are 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 & equivalent density**
 - **The optical properties are known (RI & Abs)**
- DLS tends to overestimate the width of the peaks in the distribution and this effect can be 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

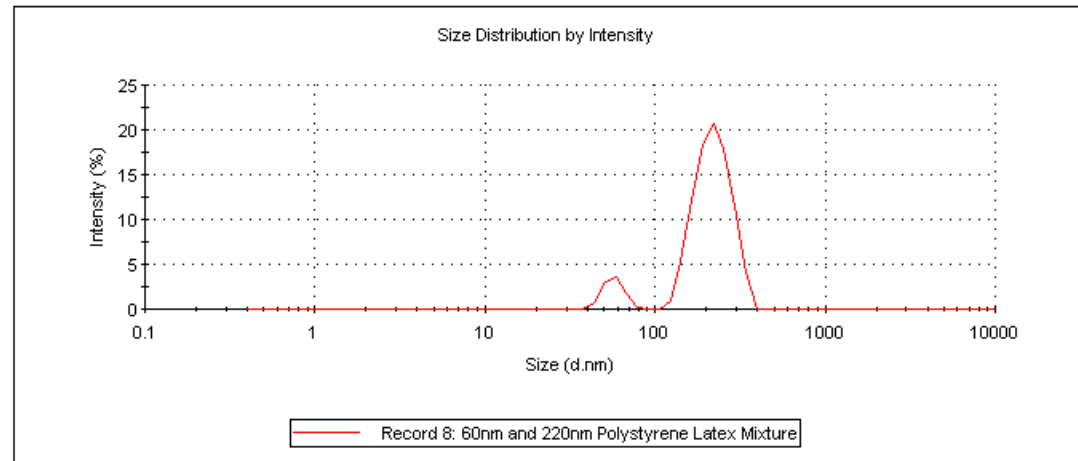
Volume/Number Distributions: Recommended Use

- › 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



Sample Requirements

Aims of this Section.....

- 1 Understand the sample requirements for DLS measurements
- 2 Understand the dispersant requirements for DLS measurements
- 3 Understand the lower and upper size limits for DLS
- 4 Understand the lower and upper concentration limits for DLS

Sample Requirements

- › The sample should consist of a dispersion of particles in a liquid medium
- › The **dispersant** should meet the following requirements:
 - **It should be transparent**
 - **Refractive Index should be different from particles'**
 - **RI & Viscosity should be known with accuracy better than 0.5%**
 - **Should be compatible with the particles (i.e. not cause swelling, dissolution or aggregation)**
 - **It should be clean and filterable**

International Standard ISO 13321 (1996)

Lower Size Limit of DLS

Depends on:

- › The amount of scattered light from the particles
 - **Relative refractive index**
 - **Sample concentration**

- › Instrument sensitivity
 - **laser power and/or wavelength**
 - **detector sensitivity**
 - **optical configuration of the instrument**

λ 2nm

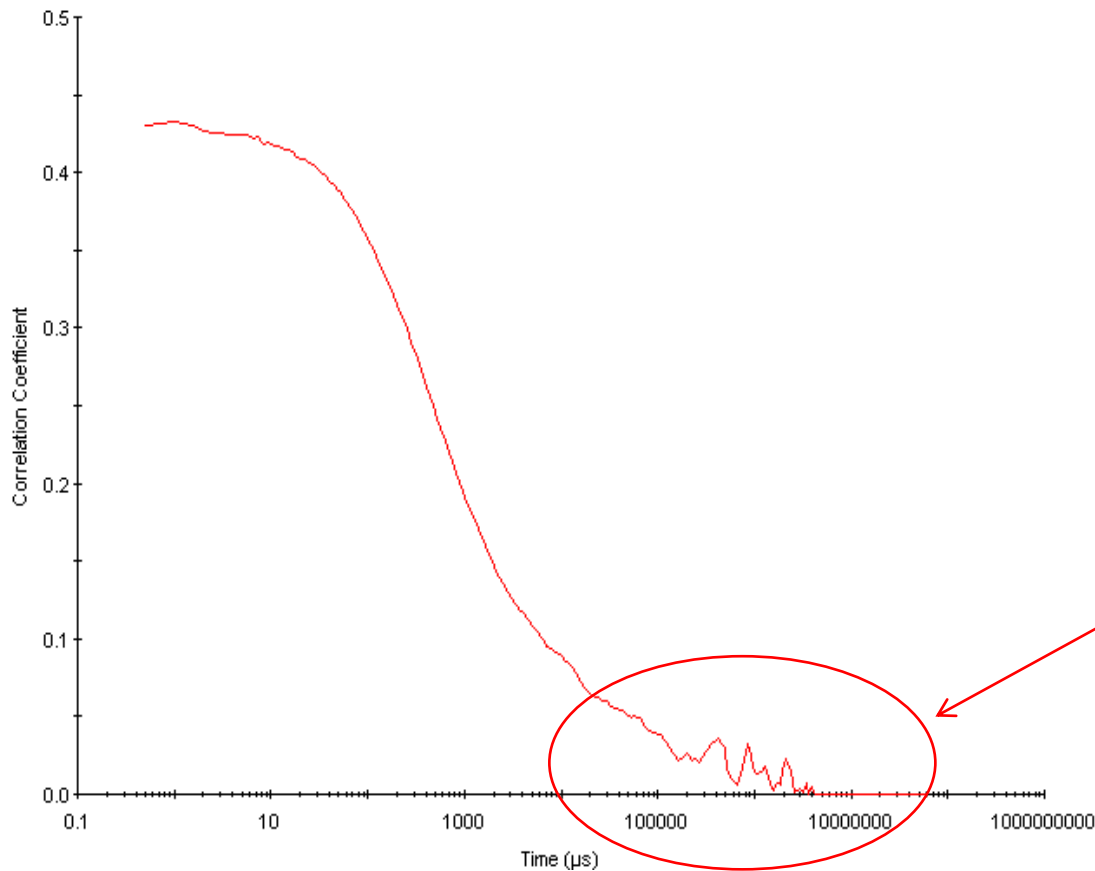
Upper Size Limit of DLS

- DLS measures the random movement of particles undergoing Brownian motion and will not be applicable when the particle motion is not random
- The upper size limit is sample dependent and is defined by
 - **Onset of sedimentation**
 - **Number fluctuations**

Upper Size Limit of DLS: Sedimentation

- All particles will sediment.
- The rate of sedimentation will depend on particle size as well as relative densities of the particles and suspending medium
- For DLS measurements to be successful, the rate of sedimentation should be much slower than the rate of diffusion
- Evidence of sedimentation can be seen in correolgram
- Also look for stable count rate over multiple measurements on same sample (decreasing count rate indicates loss of particles due to sedimentation).

Upper Size Limit of DLS: Sedimentation



For random Brownian motion, correlation curve should always decay.

Increases in correlation at high delay times indicates non-random movement (ie. Sedimentation)

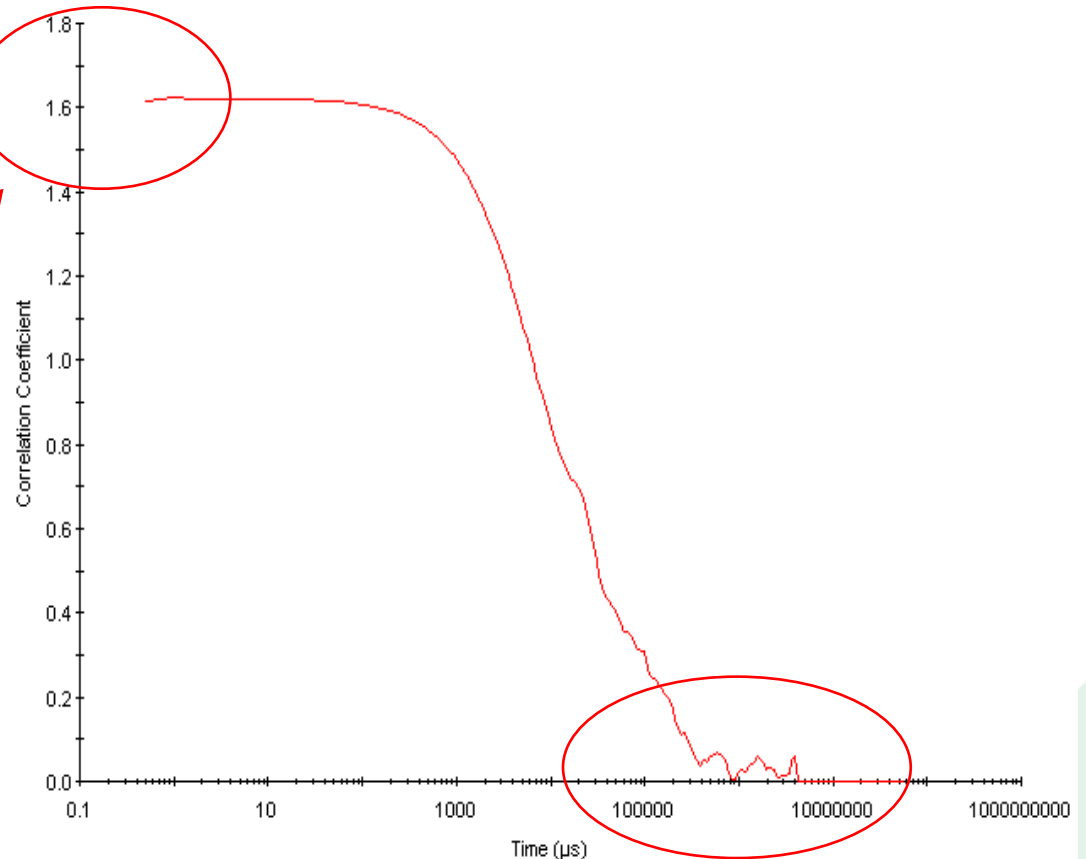
Upper Size Limit of DLS: Number Fluctuations

- Another factor to consider when measuring large particles is the number of particles present in the measurement volume
- The intensity of light scattered by large particles may be sufficient to make successful measurements
- However, if the number of particles are too low, we see severe fluctuations in the momentary number of particles in the measurement volume will (i.e. number fluctuations)
- Result is large fluctuations in the scattered intensity which masks those due to Brownian motion
- Evidence of this can be seen in the correlogram

Upper Size Limit of DLS: Number Fluctuations

Number fluctuations can also cause elevated baselines.

When number fluctuations are large it makes definition of baseline difficult and can lead to intercept values > 1 .



Sample Concentration Overview

- › Results obtained from a DLS measurement should be independent of sample concentration (ISO 13321)
- › If sample concentration is too low, there may not be enough light scattered to make a measurement
- › If sample concentration is too high, the result may not be independent of sample concentration
- › During method development, determining the correct sample concentration may involve several size measurements at different concentrations

Lower Concentration Limit

Dependent upon

- › The amount of scattered light from the particles
 - **Relative refractive index**
 - **Particle size**

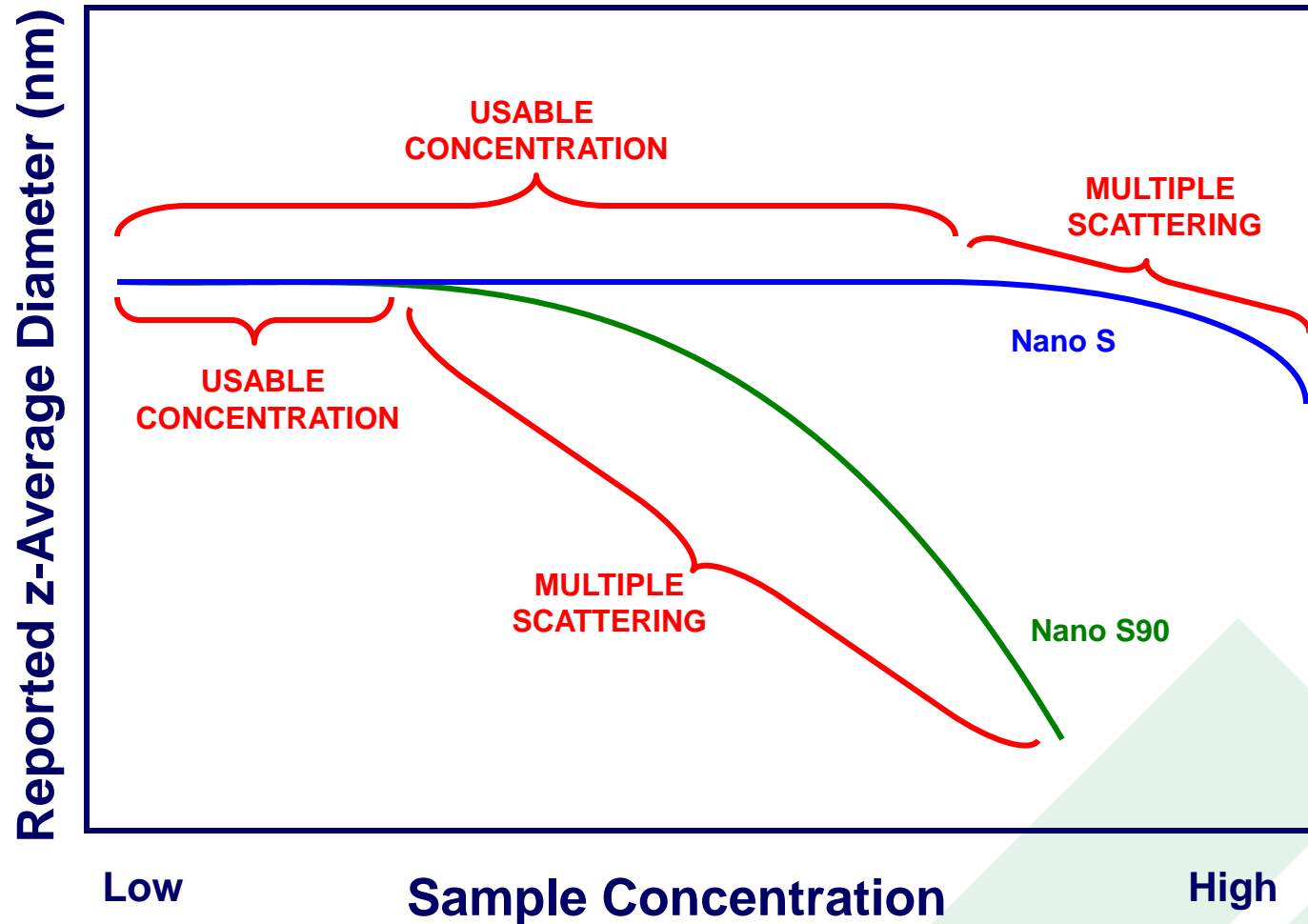
- › Instrument sensitivity
 - **Laser power and/or wavelength**
 - **Detector sensitivity**
 - **Optical configuration of the instrument**

λ 2nm

Upper Concentration Limit: Multiple Scattering

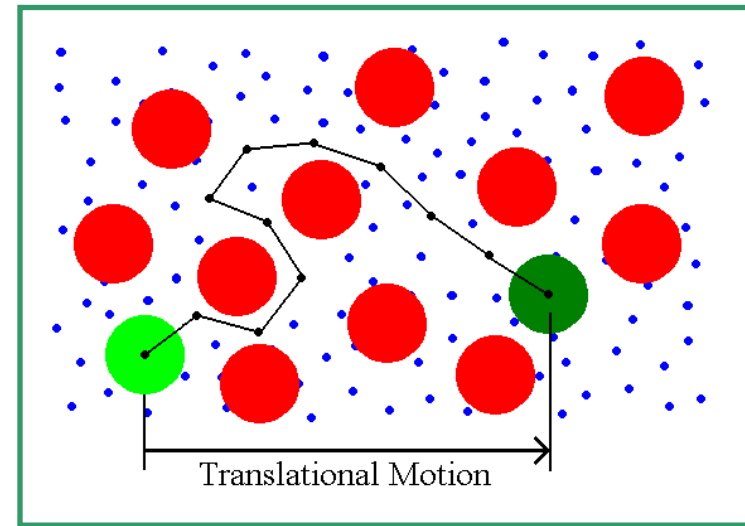
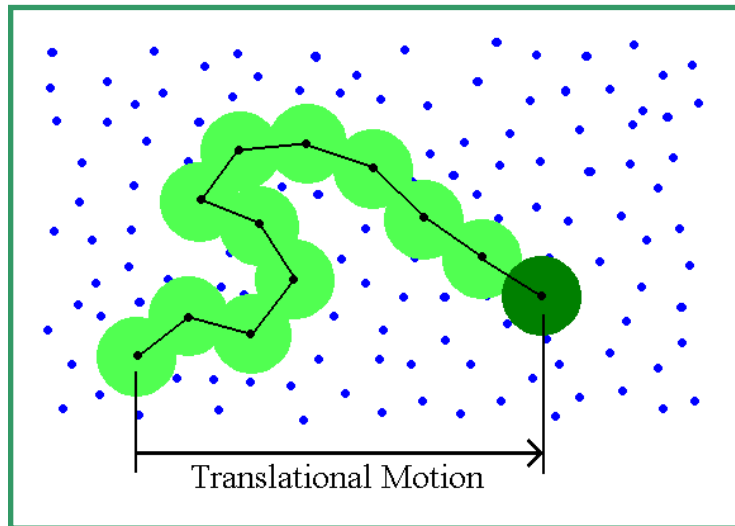
- The ideal situation in a DLS measurement is to have singly scattered light
- This means that every photon which reaches the detector was scattered by only one particle
- This will be the case for samples at low concentrations
- However, as the sample concentration is increased, the probability of the scattered photon being "re-scattered" by other particles increases
- This phenomenon is **multiple scattering**
- The presence of multiple scattering during a DLS measurement will reduce the measured size

Upper Concentration Limit: Multiple Scattering



Upper Concentration Limit: Restricted Diffusion

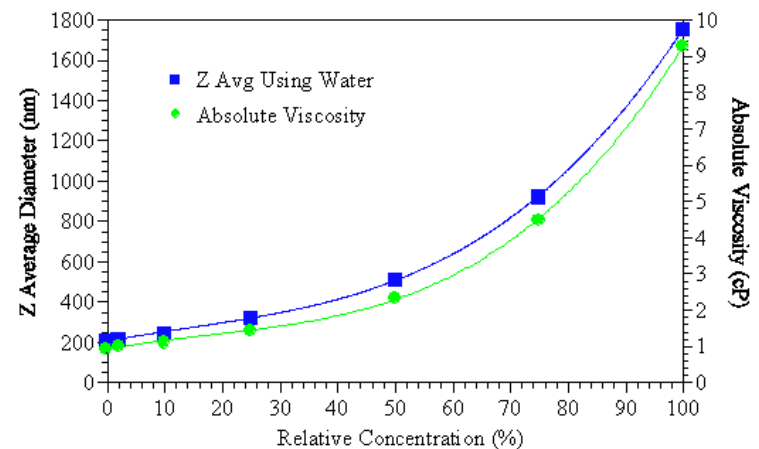
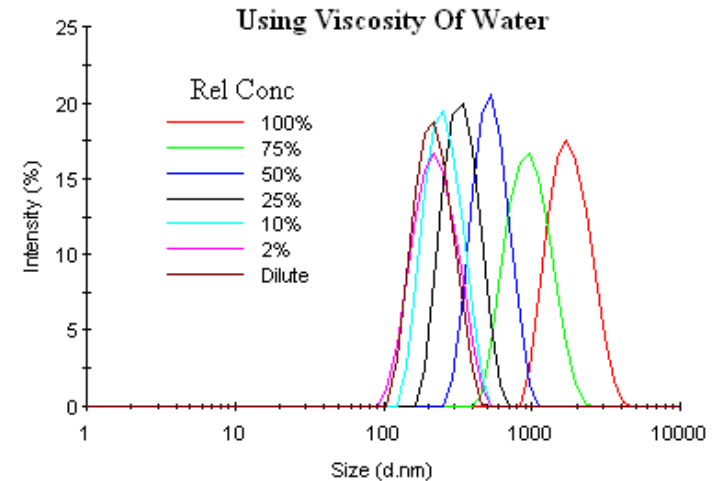
- Restricted diffusion describes the phenomenon where the presence of other particles hinders free particle diffusion



Same distance - longer time

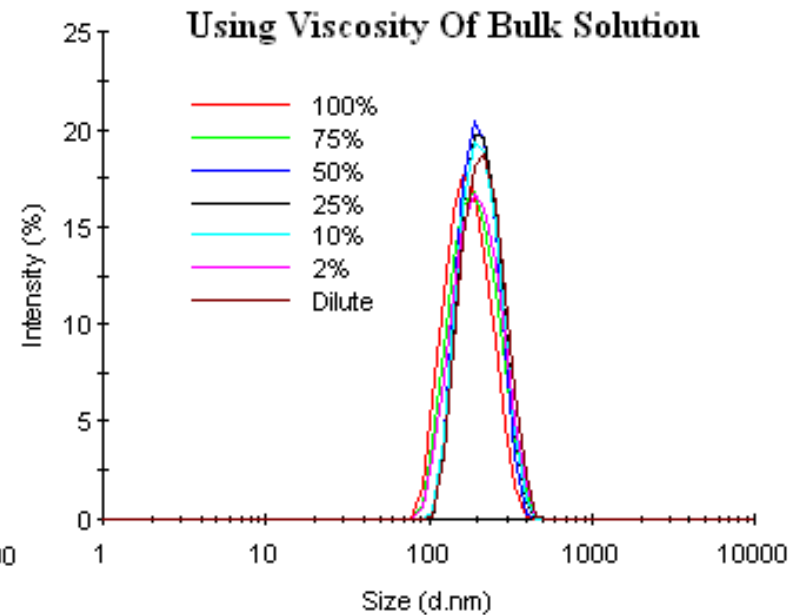
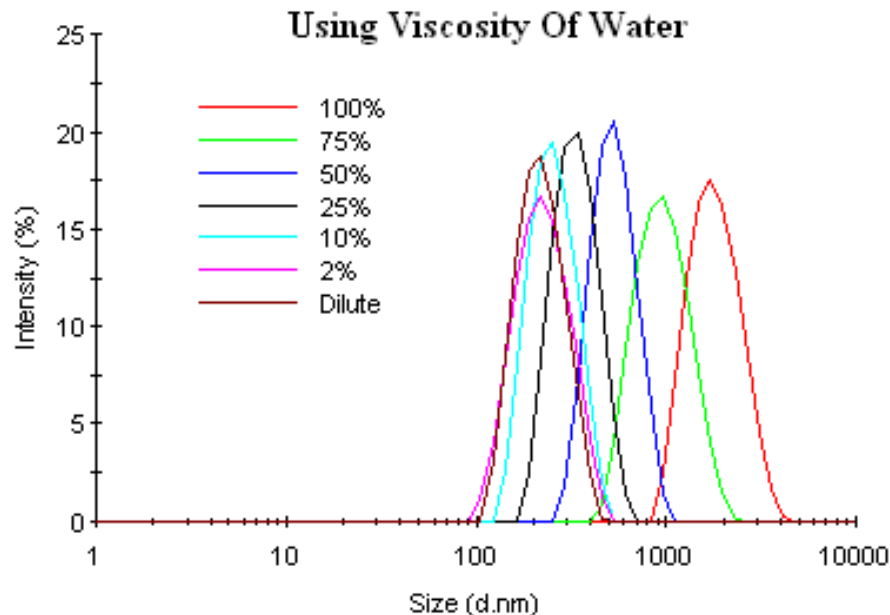
Upper Concentration Limit: Restricted Diffusion

- **Symptoms of restricted diffusion effects include:**
 - A shift in size, with no change to modality or polydispersity, to larger sizes when the solvent viscosity is used for size calculations at high sample concentrations
 - A concentration dependence of the z-average which parallels that of the bulk viscosity of the sample



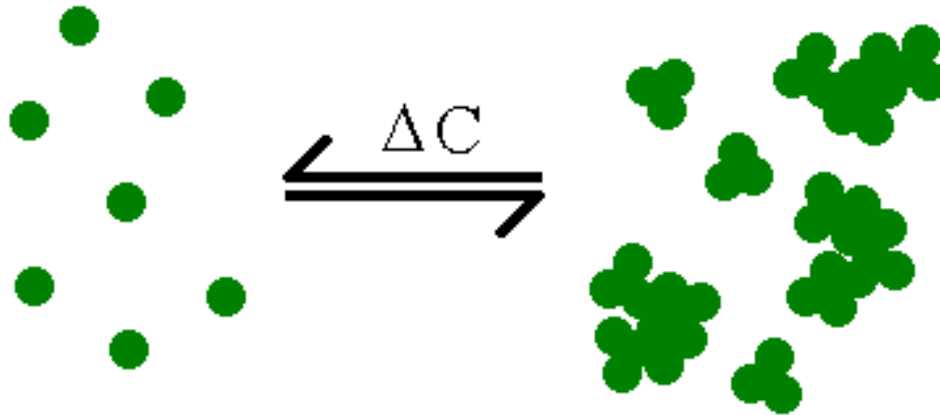
Upper Concentration Limit: Restricted Diffusion

- As a general rule of thumb, effects of restricted diffusion can be corrected for by using the bulk, rather than the solvent, viscosity for the size calculations



Upper Concentration Limit: Particle Interactions

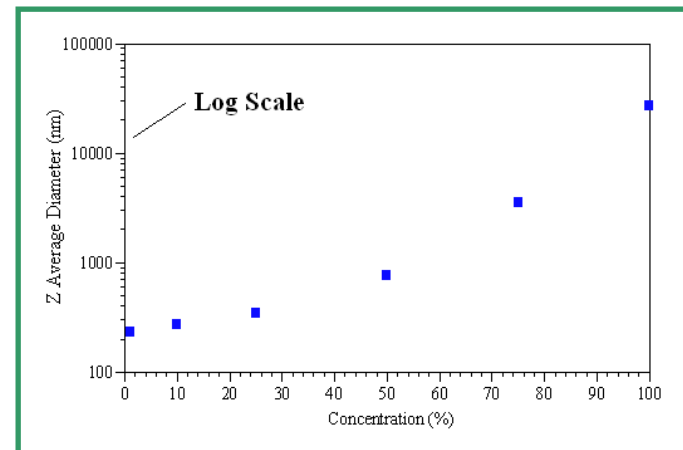
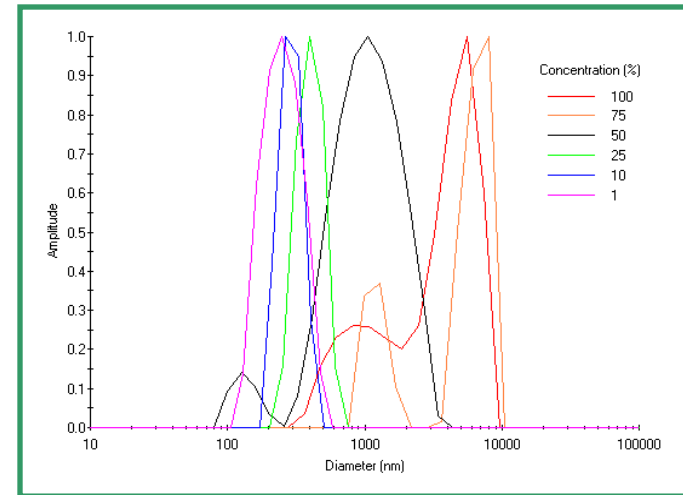
- At higher concentrations, particle interactions can modify the free diffusion of particles and this can lead to non-specific aggregation that modifies the size distribution obtained



Upper Concentration Limit: Particle Interactions

› Symptoms of particle interaction effects include:

- Increase in the distribution modality
- Increase in the sample polydispersity
- Z average diameter and viscosity concentration dependence are uncorrelated
- **Cannot be corrected**



Sample Preparation

Aims of this Section.....

- 1 Understand how to correctly prepare a sample for a DLS measurement

Sample Preparation Overview

- › A backscatter instrument can measure the size of any sample in which the particles are mobile
- › Therefore high concentrations can be measured
- › However, some samples may require dilution to ensure concentration is within ideal range
- › This should be determined experimentally, through a series of dilutions to ensure size obtained is independent of concentration

Sample Dilution

- › Sample dilution needs to be performed carefully to ensure the equilibrium of any absorbed species between the particle surface and bulk solution is preserved
- › The diluent should be the same as the continuous phase of the original sample
- › The diluent could be obtained by:
 - Filtering or centrifuging the original sample to obtain a clear supernatant
 - Making up a continuous phase as close as possible to that of the sample

Diluent Filtration

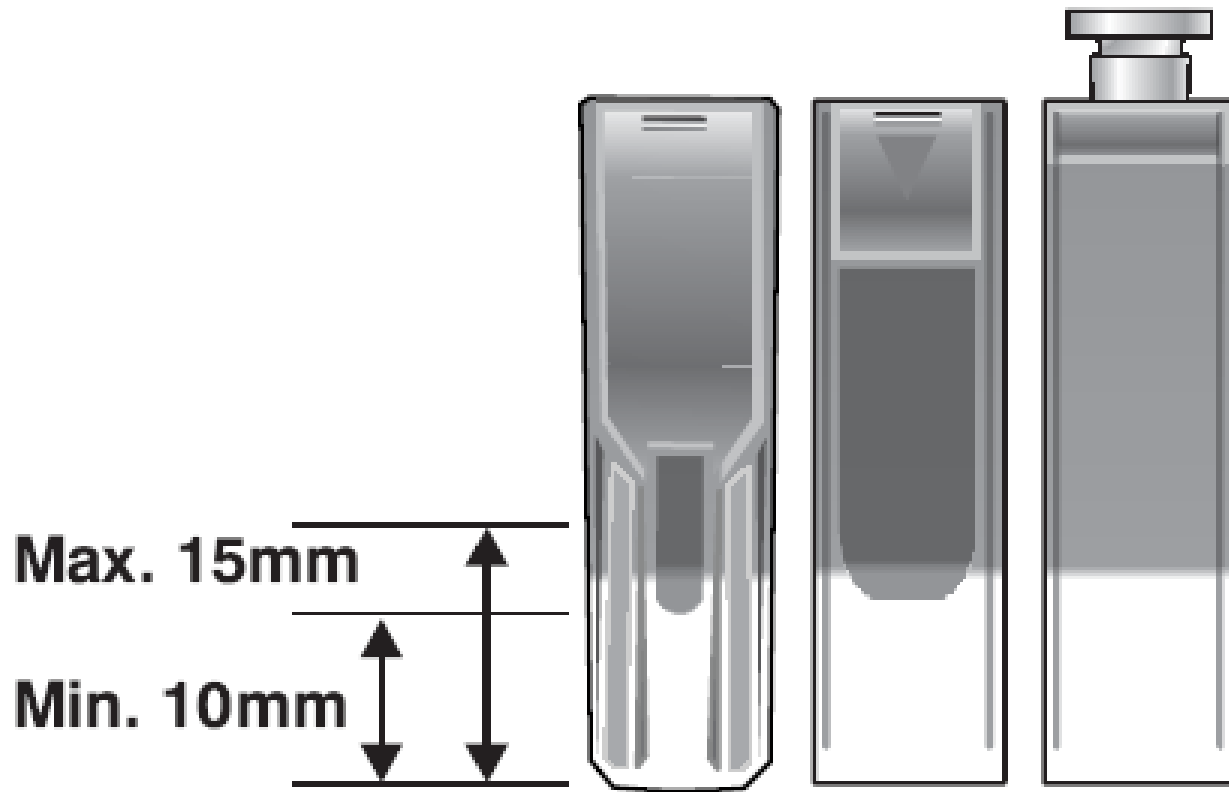
- › Dust is one of the major problems (source of contamination) in DLS measurements and may bias the results obtained if present
- › To avoid any possible dust contamination during dilution, the dispersing medium should be filtered
- › Commercial syringe filters are available for use, with pore sizes ranging from 1 μm down to 20nm

Filling the Cell

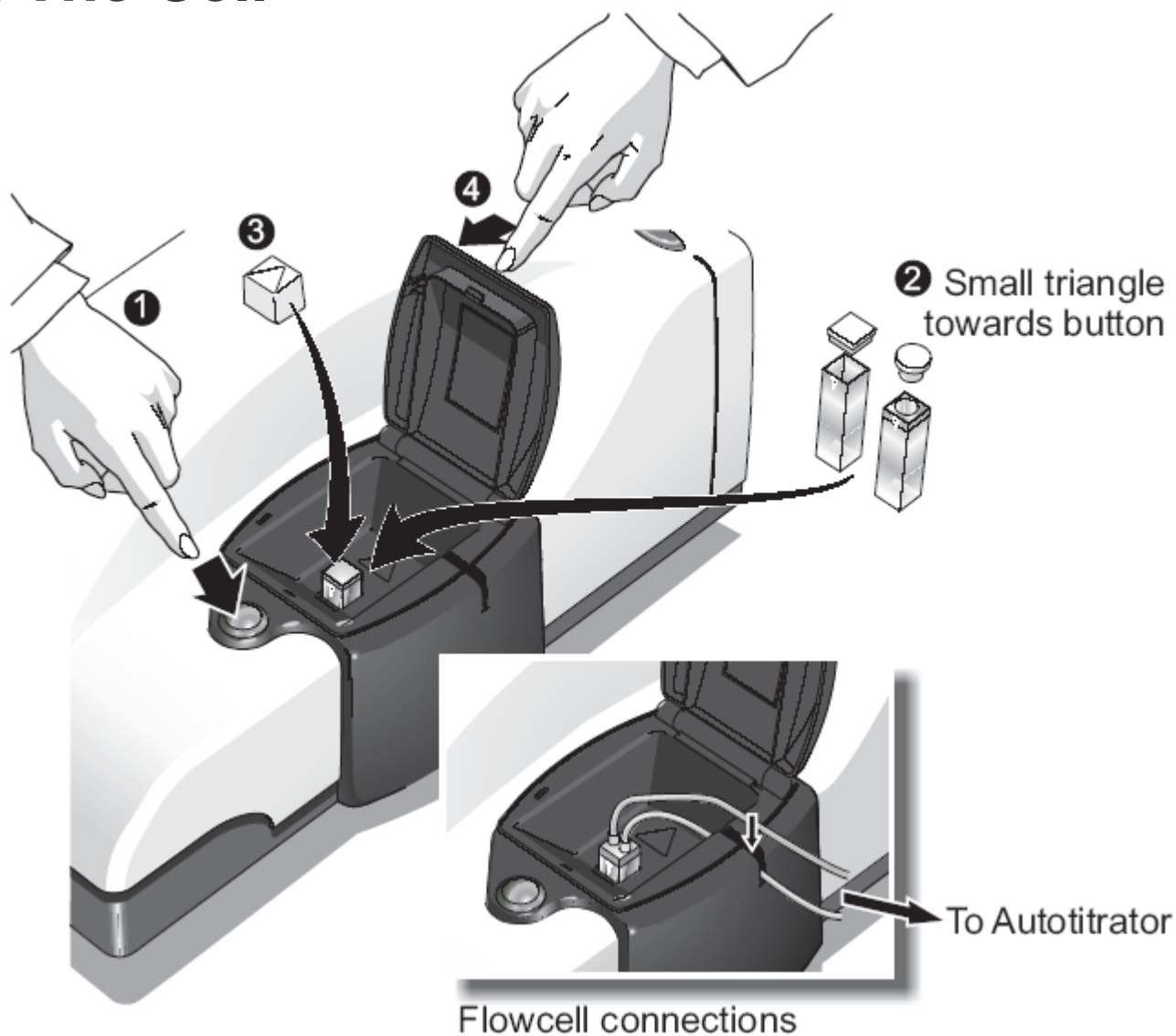
- Only clean cells should be used
 - **Size cells should be rinsed/cleaned with filtered dispersant before use**
- Fill the cell slowly to avoid air bubbles being created
 - **Using a pipette and tilting the cell at an angle will aid with this**
- If using syringe filters for the dispersant, discard the first few drops in case of any residual dust particles in the filter that may contaminate the dispersant



Filling The Cell



Inserting The Cell



Cell Cleaning

- Non-disposable cuvettes (e.g. PCS8501) should be cleaned after each sample has been measured
- This could be done in one of several ways
 - Rinsing in clean dispersant
 - Ultrasonication in clean dispersant
 - Hellmanex[®] II (available from www.hellma-worldwide.de)
 - Recommend 2% v/v concentration
 - Soak cuvettes for 20 minutes
 - Ultrasonication aids cleaning action

Measurement Sequence

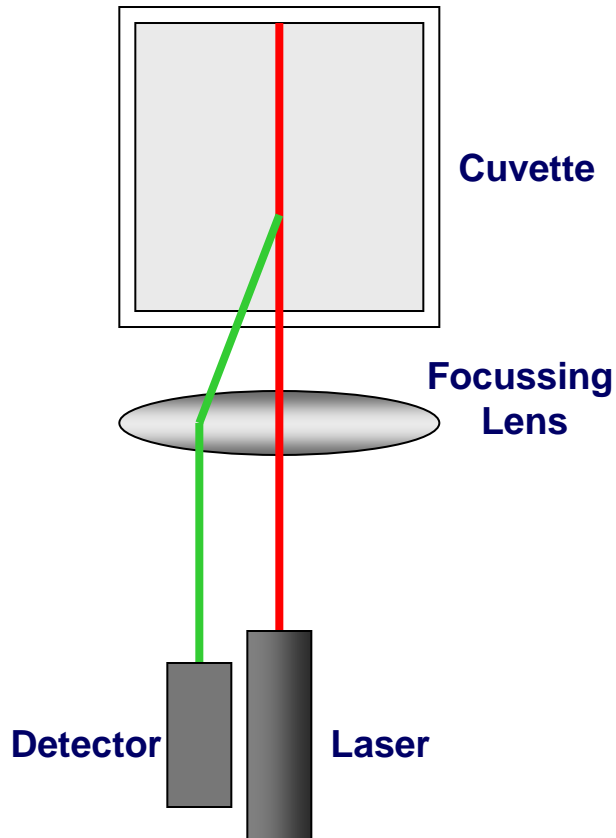
Aims of this Section.....

- 1 Understand how the measurement position is optimised in a backscatter instrument (the cell positioning factor)
- 2 Understand the protocol for optimizing the measurement position in a backscatter instrument
- 3 Understand the measurement sequence in the Zetasizer-Nano
- 4 Understand how the measurement duration is determined
- 5 Understand how to manually set up the measurement in terms of measurement position, attenuator setting and duration

NIBS: Variable Measurement Position

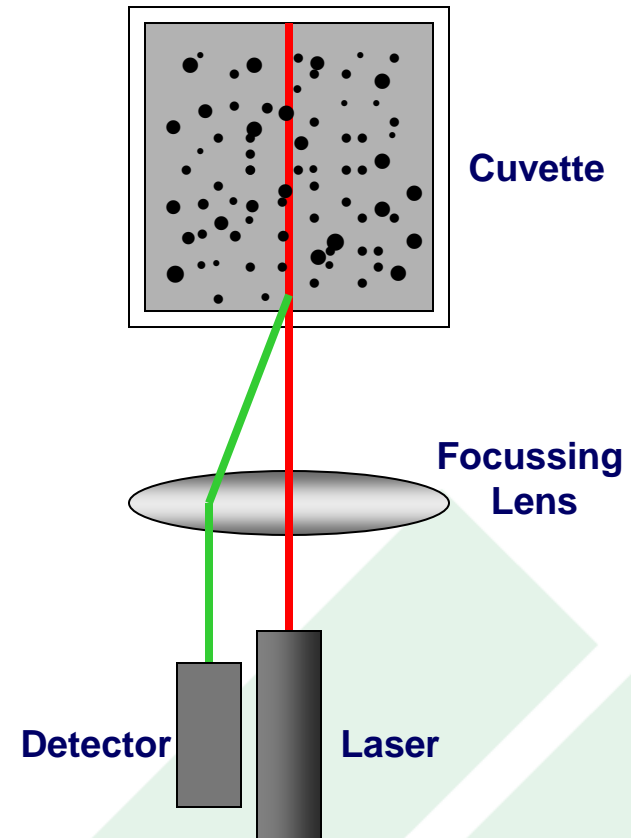
Small Particles/ Dilute Samples

Maximise measurement volume
Minimise laser flare

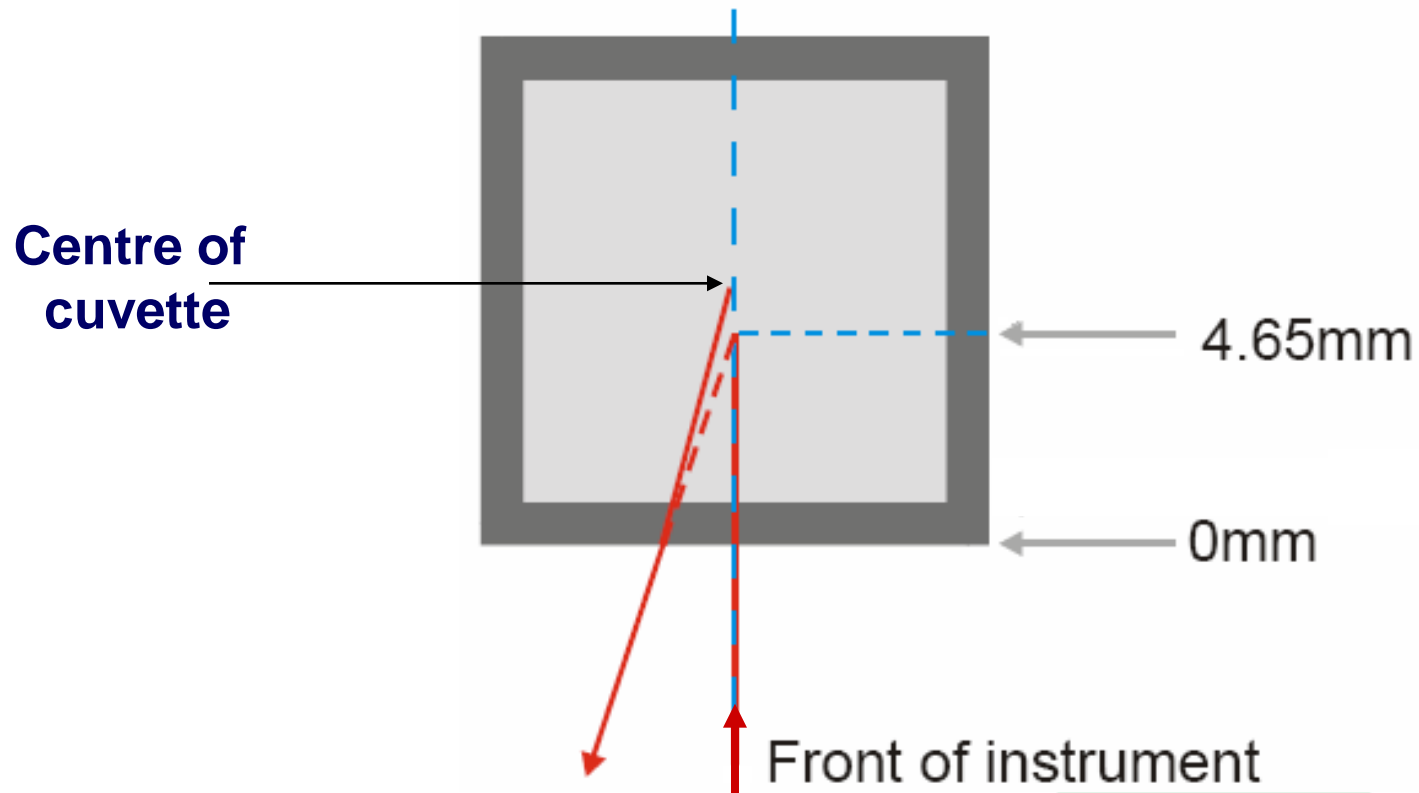


Concentrated Samples

Minimise path length
Minimise multiple scattering



The Measurement Position



Measurement Duration

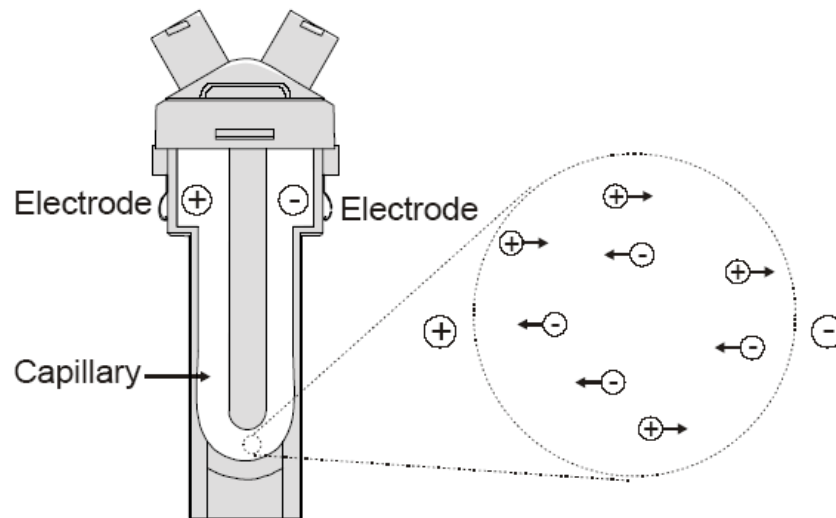
- The duration of a measurement is automatically determined from the count rate detected
- The lower the count rate, the longer the duration will be
- Each measurement is split into a series of 10 second sub runs to minimize the effects of any dust which may be present in the sample
- By default, 50% of the sub runs with the lowest mean count rates are used in the analysis
- In Automatic settings, a clean/easy sample will typically use 11-15 sub runs
- If the software chooses a high number of sub-runs, it may indicate a dirty/difficult sample

Measuring Zeta Potential

› **Electrophoresis** is the movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field

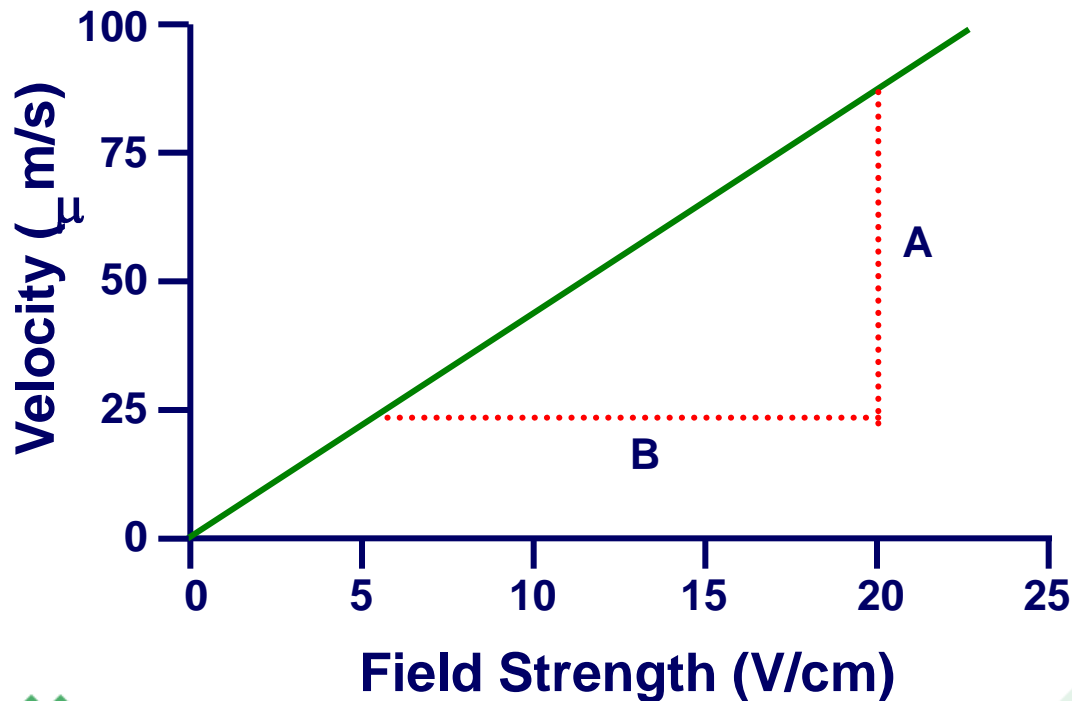
▶ The particles move with a velocity which is dependent on:

- Zeta potential
- Field strength
- Dielectric constant of medium
- Viscosity of the medium



Electrophoretic Mobility

- › Velocity = distance/time = m/s
- › Field strength = volts/distance = V/cm
- › Electrophoretic Mobility Units = $\text{m}^2/\text{Vs} \times 10^{-8}$ or $\mu\text{mcm}/\text{Vs}$



$$\frac{A}{B} = \text{Electrophoretic Mobility}$$

Electrophoresis

ZETA POTENTIAL is related to the
ELECTROPHORETIC MOBILITY
by the **HENRY EQUATION**

$$U_E = \frac{2 \varepsilon z F(k a)}{3 \eta}$$

U_E = electrophoretic mobility

z = zeta potential

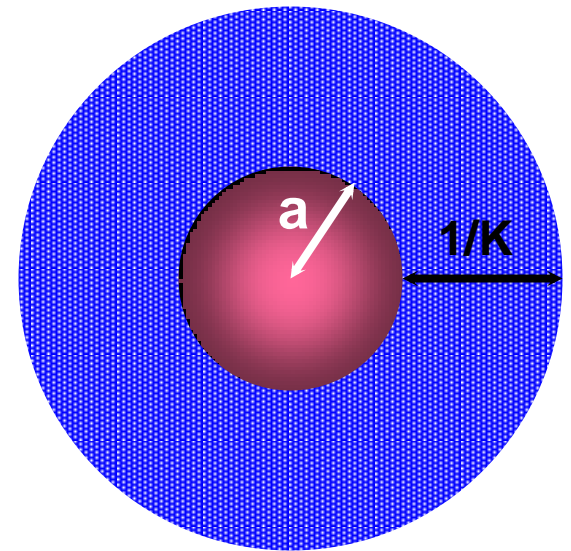
ε = dielectric constant

η = viscosity

$F(k a)$ = Henry's function

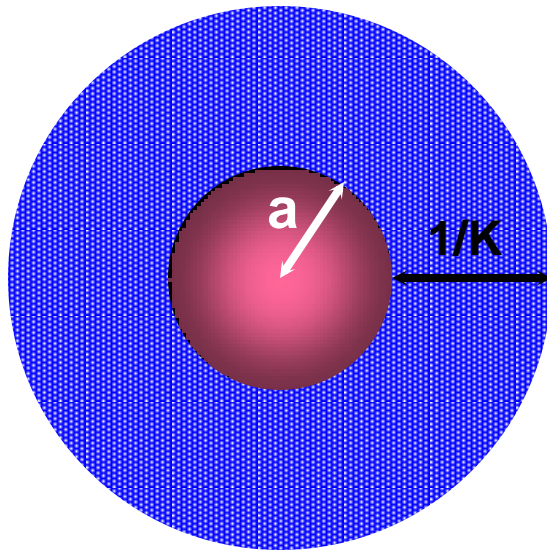
Henry's Function $F(ka)$

- › k = electrical double layer thickness (Debye length) with the dimensions of inverse length (k^{-1} or $1/k$)
- › a = the particle radius
- › Therefore $ka = 1/k \times a =$ the ratio of particle radius to double layer thickness (a/k)



Henry's Function $F(ka)$

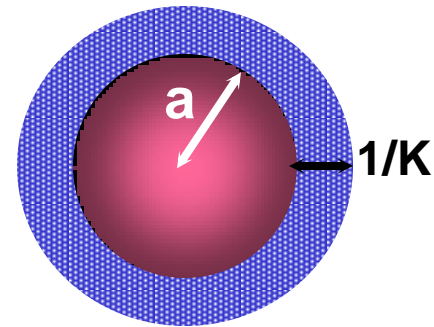
Non-polar
Media



Huckel
approximation

$$F(ka) = 1.0$$

Polar
Media



Smoluchowski
approximation

$$F(ka) = 1.5$$

Calculation of the Debye Length (if required)

More exact values of Henrys function can be calculated and entered into the Zetasizer software. The Debye Length can be calculated from the following equation:

$$\kappa^{-1} = ((\epsilon_0 \epsilon_r k_B T) / (2000 e^2 I N))^{0.5}$$

where;

ϵ_0 = permittivity of free space (= $8.854 \times 10^{-12} \text{ Fm}^{-1}$)

ϵ_r = relative permittivity of liquid (or dielectric constant)

k_B = Boltzmann's constant (= $1.38 \times 10^{-23} \text{ JK}^{-1}$)

T = temperature in Kelvin

e = electronic charge in Coulombs (= $1.6022 \times 10^{-19} \text{ C}$)

N = Avogadro's number (= $6.022 \times 10^{23} \text{ mol}^{-1}$)

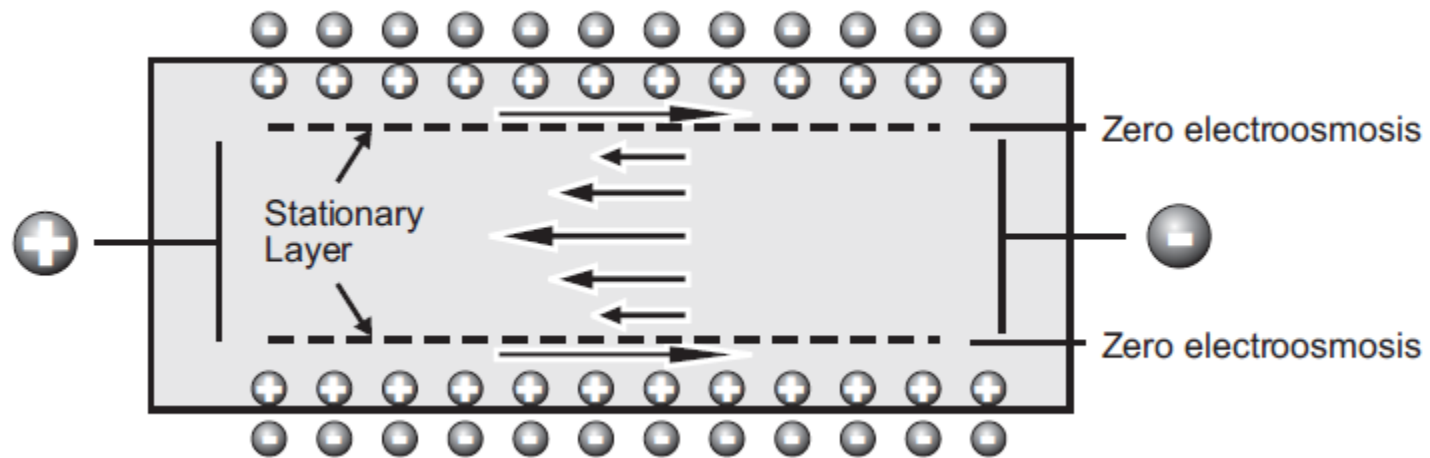
I = ionic concentration (in mol/L)

Relationship of Ka to F(Ka)

Ka	F(Ka)
0	1.000
1	1.027
2	1.066
3	1.101
4	1.133
5	1.160
10	1.239
25	1.370
100	1.460
∞	1.50

Electroosmosis

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



Measurement Technique

- › The Zetasizer Nano uses a combination of fast field reversal (FFR) and slow field reversal (SFR)
- › FFR allows measurement of the true particle mobility before electroosmosis starts
- › SFR allows a distribution of zeta potentials to be determined

Laser Doppler Electrophoresis

- › The Zetasizer-Nano measures Electrophoresis using the Doppler Effect
- › A laser beam is passed through a sample undergoing electrophoresis and the scattered light from the moving particles is frequency shifted
- › The frequency shift Δf is equal to:

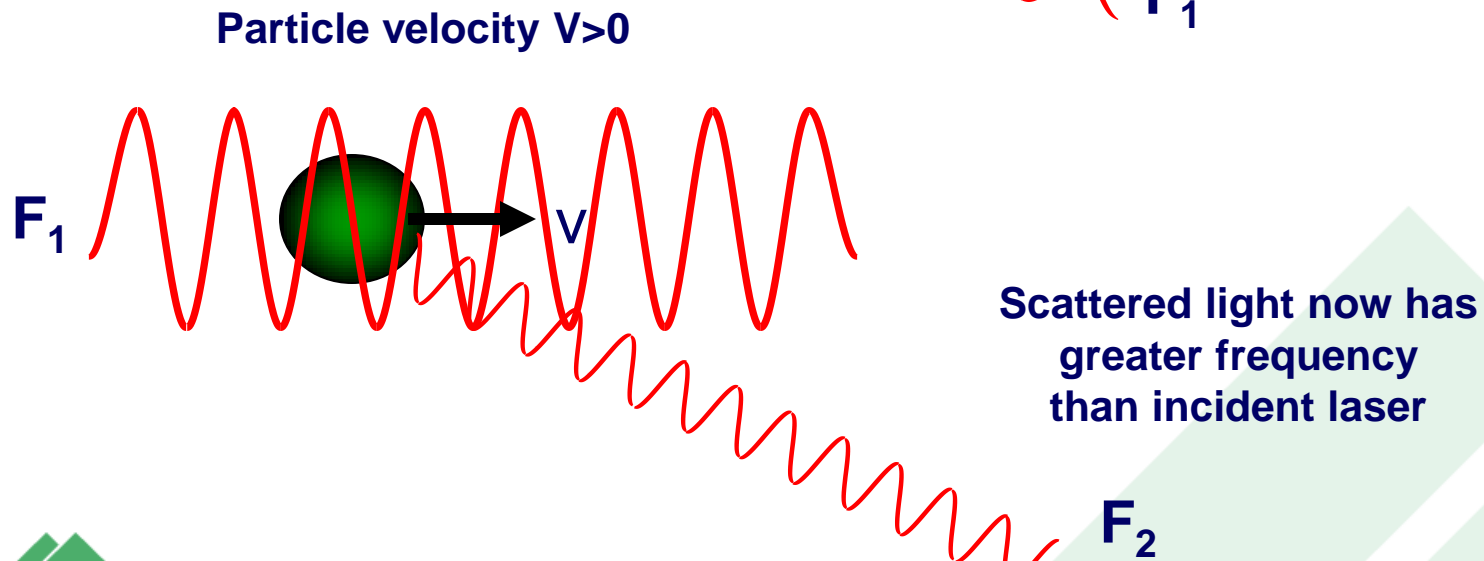
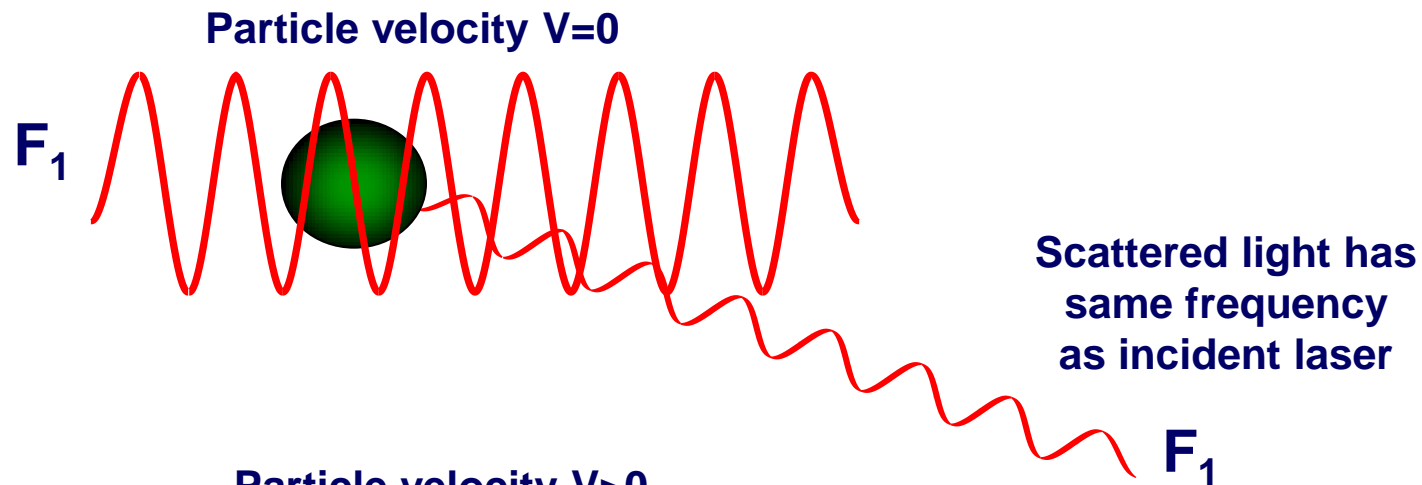
$$\Delta f = 2v \sin(\theta/2)/\lambda$$

v = the particle velocity

λ = laser wavelength

θ = scattering angle

Laser Doppler Electrophoresis



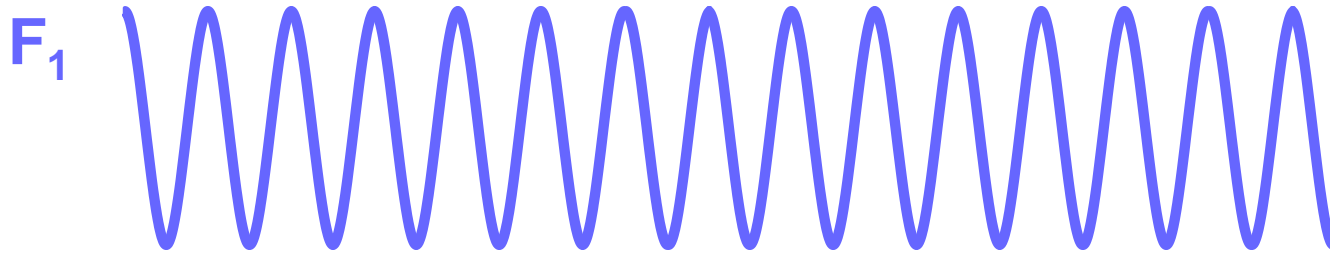
Laser Doppler Electrophoresis

- Since the frequency of light is so high (10^{14} Hz), the shift in frequency can only be measured by an optical mixing or interferometric technique
- This is done by using a pair of mutually coherent laser beams derived from a single source
 - One of these beams must pass through the sample (this is called the **scattering** beam)
 - The other beam (called the **reference** beam) is routed around the cell
- The scattered light from the particles is combined with the reference beam to create intensity variations

How Do These Intensity Variations Arise?

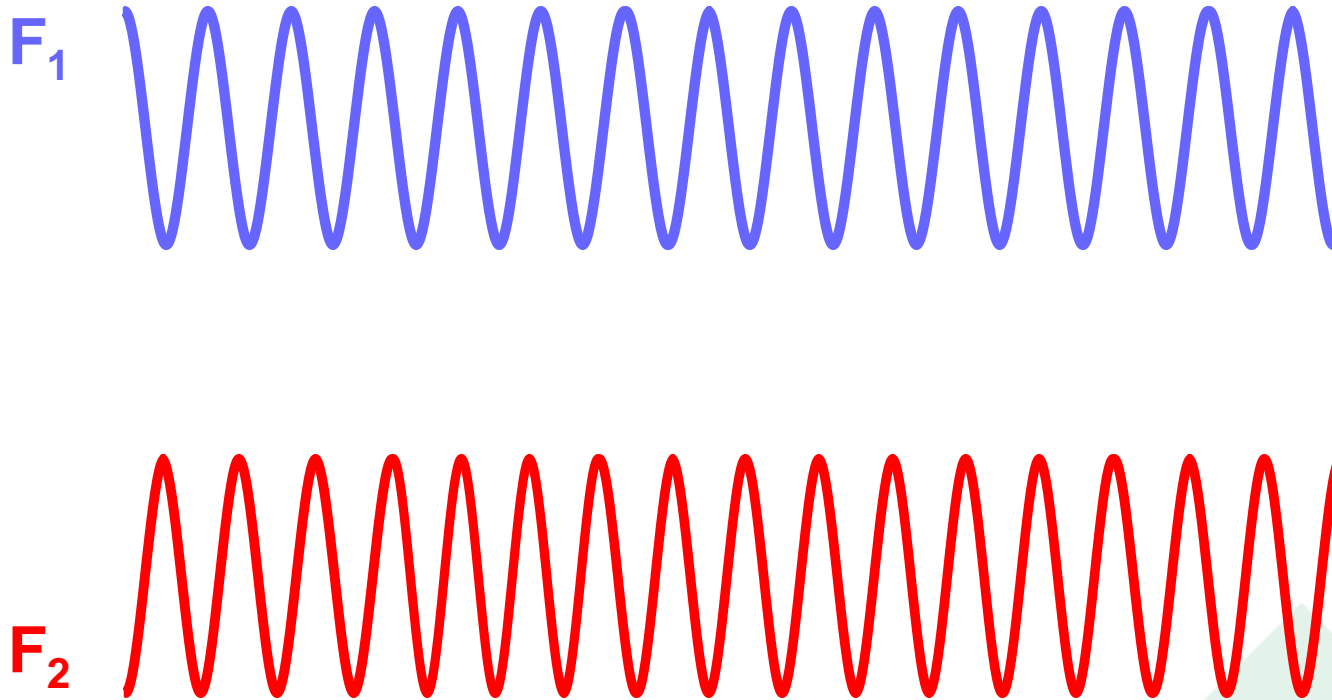
How Do These Intensity Variations Arise?

Reference beam F_1



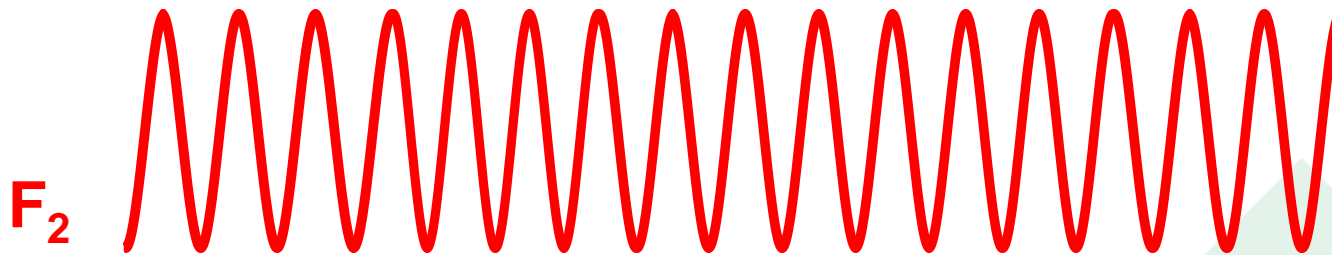
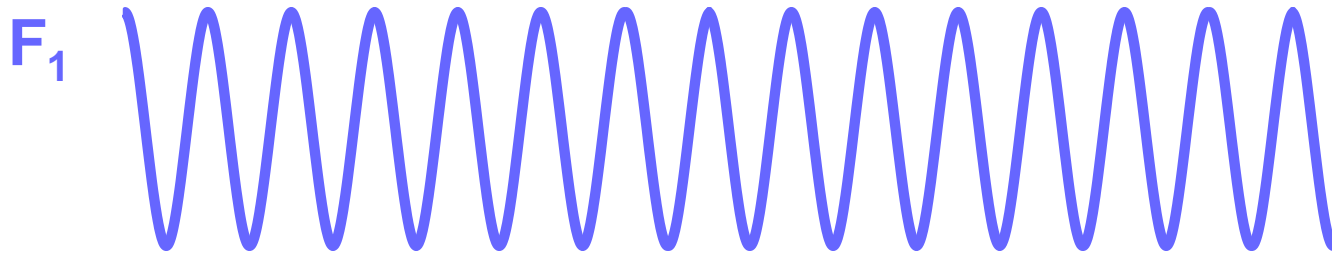
How Do These Intensity Variations Arise?

Reference beam F_1 and scattered beam F_2



How Do These Intensity Variations Arise?

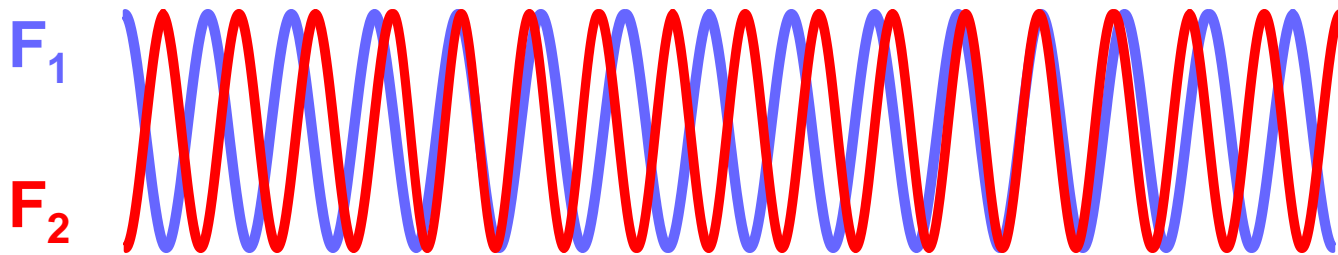
Reference beam F_1 and scattered beam F_2



Lets combine them

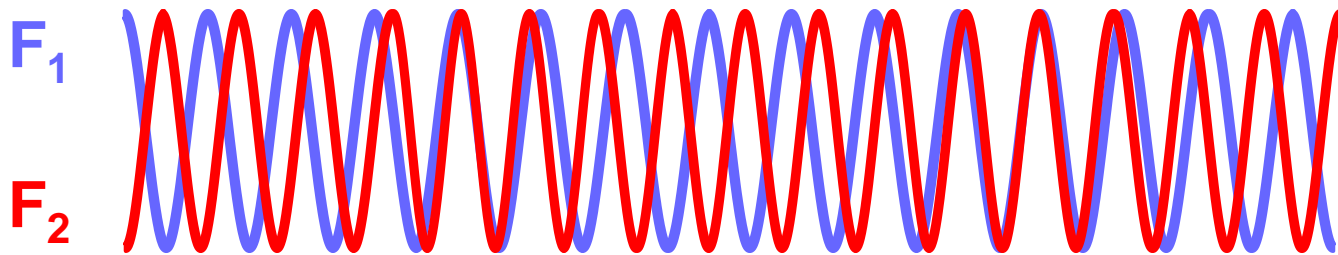
How Do These Intensity Variations Arise?

Reference beam F_1 and scattered beam F_2



How Do These Intensity Variations Arise?

Reference beam F_1 and scattered beam F_2

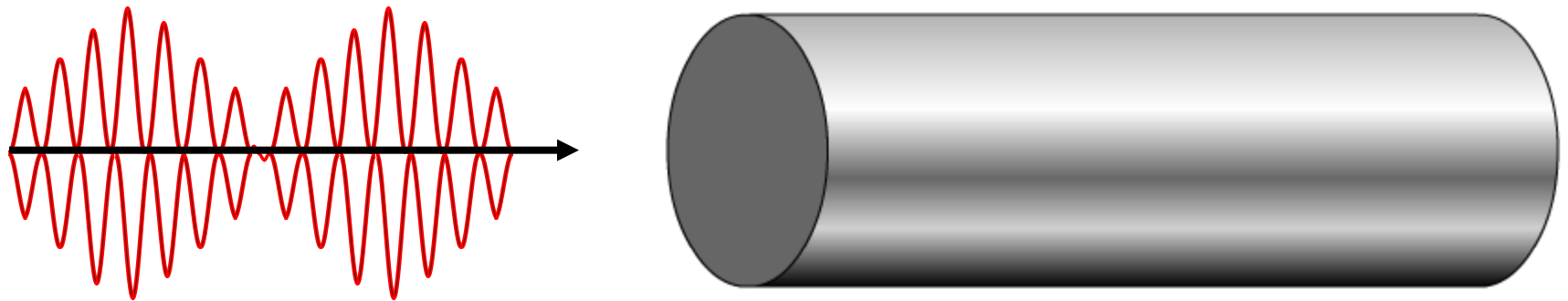


The two waves interfere constructively at A and destructively at B

$$F_1 - F_2 = \Delta f$$

The interference produces a modulated beam having a much smaller frequency equal to difference of F_1 and F_2

The Beat Frequency Is Focussed Onto The Detector



It is the intensity variations of the “beat” frequency which are detected

Determining the Sign of the Doppler Shift

- › Determined by comparing the **beat frequency** with that of a **reference frequency**
- › **Reference frequency** produced by **modulating** one of the laser beams with an oscillating mirror (320 Hz)
- › The mobility of the particles in an applied field produces a frequency shift away from that of the modulator frequency (320 Hz)
- › This gives an unequivocal measure of the sign of the zeta potential

Determining the Sign of the Doppler Shift

Zeta Potential (mV)	Reference Frequency (Hz)	Measured Frequency (Hz)
0	320	320
+50	320	370
-50	320	270

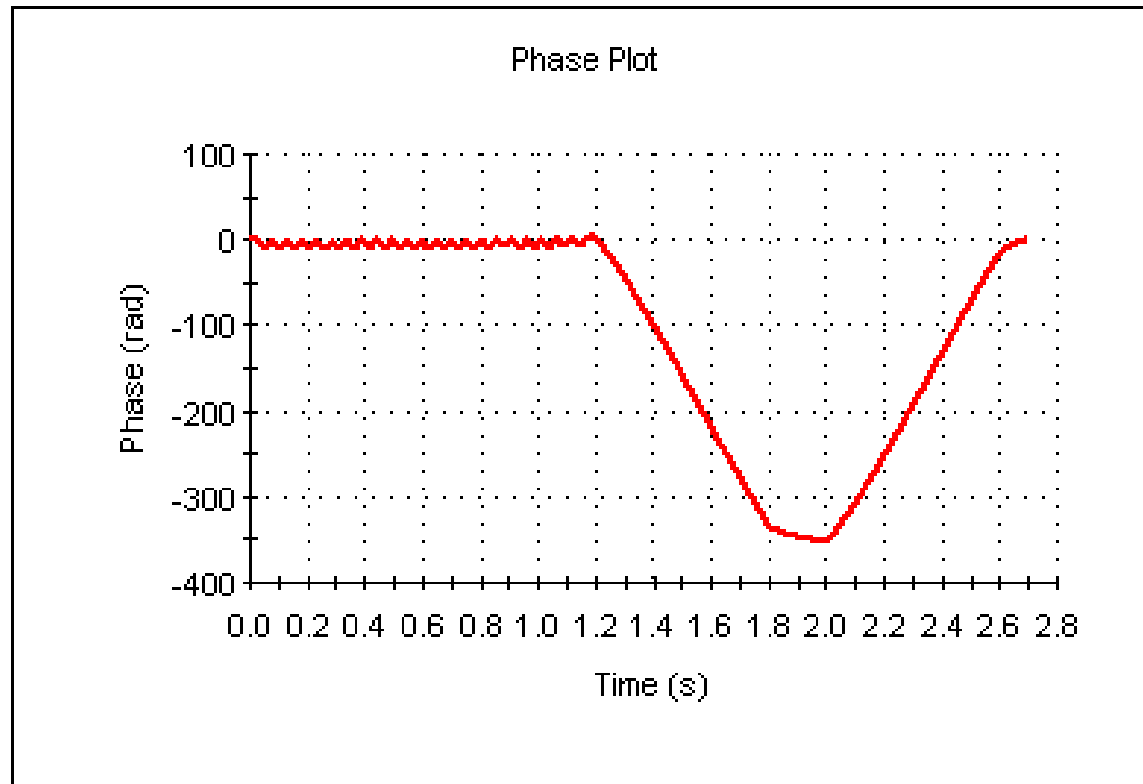
Phase Analysis Light Scattering (PALS)

Aims of this Section.....

- 1 Understand the technique of phase analysis light scattering
- 2 Understand what a phase plot is

Phase Plots

- › The phase plot shows the **difference in phase** between the measured **beat frequency** and a **reference frequency** as a function of time

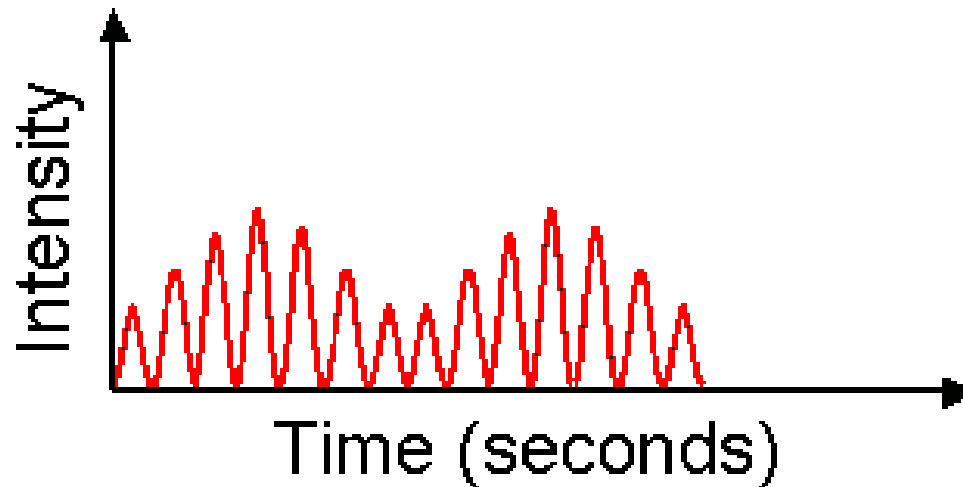


Phase Analysis Light Scattering

- › Very accurate determination of a frequency shift
- › In the presence of experimental noise and particle random motions (Brownian motion and non-random thermal drift), it is at least >100 times that obtained by standard Fourier Transform (FT)
- › PALS gives the ability to accurately measure samples that have low particle mobilities, Eg.
 - **high conductivity samples**
 - **high viscosity**
 - **non-aqueous applications**

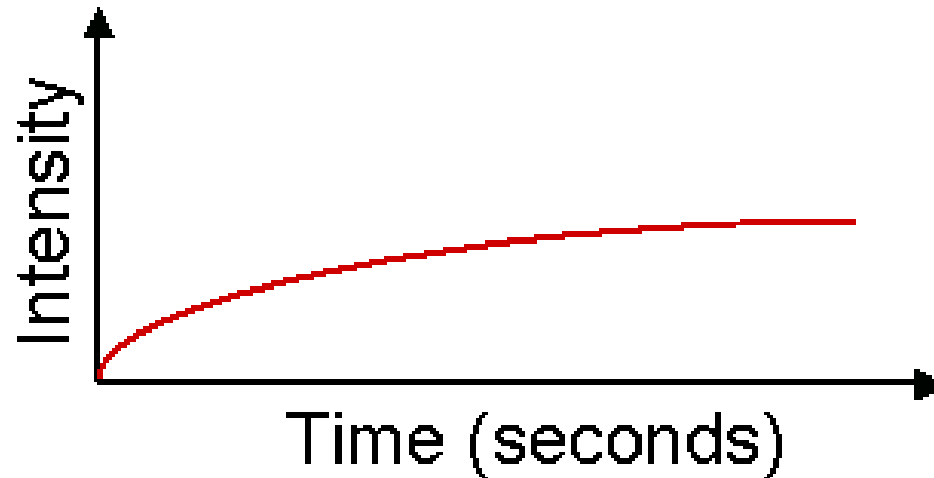
Phase Analysis Light Scattering

- › High mobility particles – many oscillations in a short space of time
- › Easy to measure



Phase Analysis Light Scattering

- › Low mobility particles – only a fraction of an oscillation may have been completed within measurement time
- › Impossible to measure using Fourier Transform
- › PALS able to measure very small phase shifts

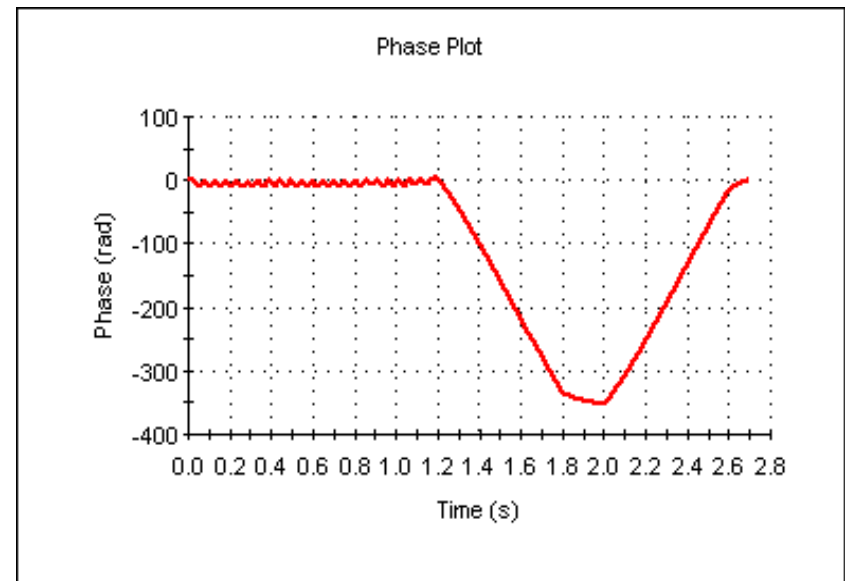


Phase Analysis Light Scattering

- › The measured phase change is proportional to the mobility of the particles
- › $\text{Phase} = \text{Frequency} \times \text{Time}$
- › $\text{Phase}/\text{Time} = \text{Frequency}$

Phase Analysis Light Scattering

- › The mobility of the particle is determined by measuring the **phase difference** between the **beat frequency** and a **reference frequency**
- › The reference frequency used in the Zetasizer Nano is that of the **optical modulator (320Hz)**
- › The **mean phase shift with time** measures the electrophoretic mobility of the particle



Data Interpretation

Aims of this Section.....

- 1 Understand which parameters and reports best aid data interpretation
- 2 Understand what the zeta quality report is and what information it contains
- 3 Understand what the expert advice system is
- 4 Understand where the expert advice system can be found in the software
- 5 Understand what information the expert advice system contains

Data Interpretation Overview

- › The quality of the data obtained from the measurement is essential in determining how repeatable the answers will be
- › To aid with interpretation of data, a number of report pages and record view parameters can be viewed

Data Interpretation: Recommended Reports

	Report Name	Description
1	Zeta Potential	The zeta potential result obtained from the measurement
2	Zeta Quality Report	Incorporates 7 tests on a selected record
3	Expert Advice	Quality checks on a single record and trends for 3 or more records
4	Phase	The phase difference between the measured beat frequency and the reference frequency plotted as a function of time
5	Frequency	The frequency spectrum obtained from the SFR part of the measurement
6	Voltage and Current	The voltage applied and the current detected in the cell over the duration of the measurement

Data Interpretation: Recommended Parameters (1)

	Parameter Name	Description
1	Zeta Potential Mean	The mean zeta potential value
2	Zeta Potential Width	The standard deviation of the zeta potential distribution
3	Conductivity	The conductivity of the sample determined from the measurement

Data Interpretation: Recommended Parameters (2)

	Parameter Name	Description
1	Attenuator	The attenuator position used during the measurement
2	Quality Factor	A signal to noise based parameter derived from a phase analysis during the FFR stage of the measurement
3	SFR Spectral Quality	A signal to noise based parameter that is derived from the frequency analysis during the SFR stage of the measurement
4	Effective Voltage	The voltage requested during the measurement
5	Zeta Runs	The number of sub runs used in the measurement

Report Tabs: Zeta Potential (M)

Records View Zeta potentia...

1

Sample Name: dts50
SOP Name: zeta standard.sop
File Name: Version 4 Example results.dts
Record Number: 3
Date and Time: 24 June 2004 16:13:44
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 79.0

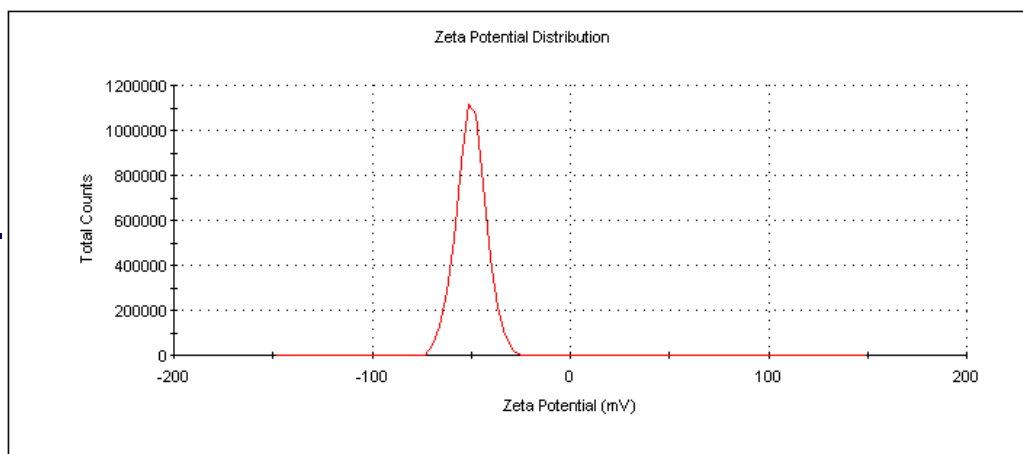
2

Temperature (°C): 25.0
Count Rate (kcps): 2683.0
Cell Description: Green disposable zeta cell
Zeta Runs: 30
Measurement Position (mm): 2.00
Attenuator: 9

3

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -50.0	Peak 1: -50.0	100.0	7.51
Zeta Deviation (mV): 7.51	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.318	Peak 3: 0.00	0.0	0.00

4



Zeta Quality Report Overview

- › The zeta quality report incorporates a number of tests on any selected record
- › If any of the tests fall outside specified limits, a warning message is displayed together with advice of possible reasons for the warning
- › If none of the tests fail, a “**Result Meets Quality Criteria**” message is displayed

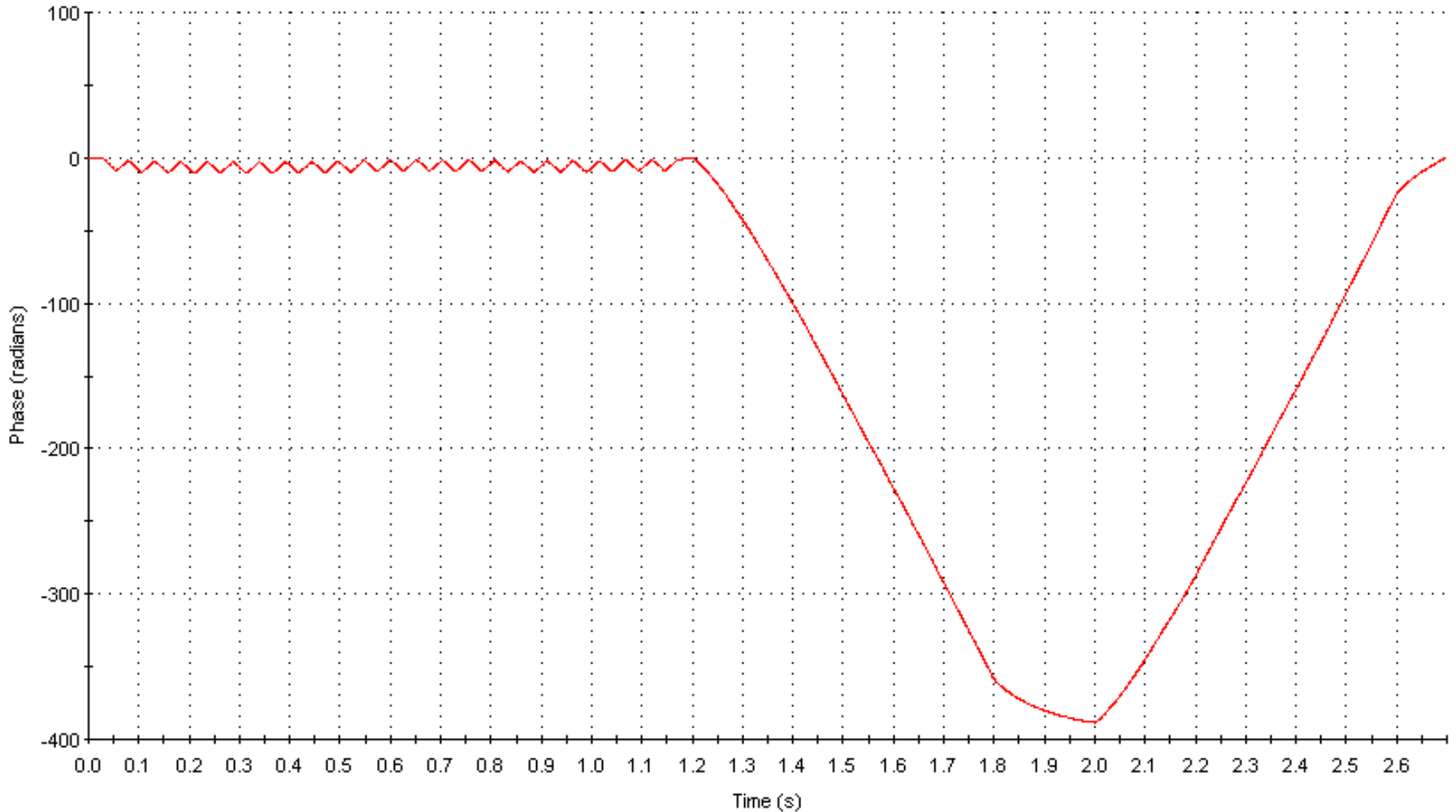
Zeta Quality Report

Test Number	Test Description	Warning Message	Possible Reasons For Warning Message	Possible Actions
1	Check the intensity of the reference beam (Is Reference Beam < 500 kcps?)	Reference beam count rate too low. If you see this warning for a number of zeta potential results then please contact your nearest Malvern representative	Misalignment of the reference beam	Contact your nearest Malvern representative

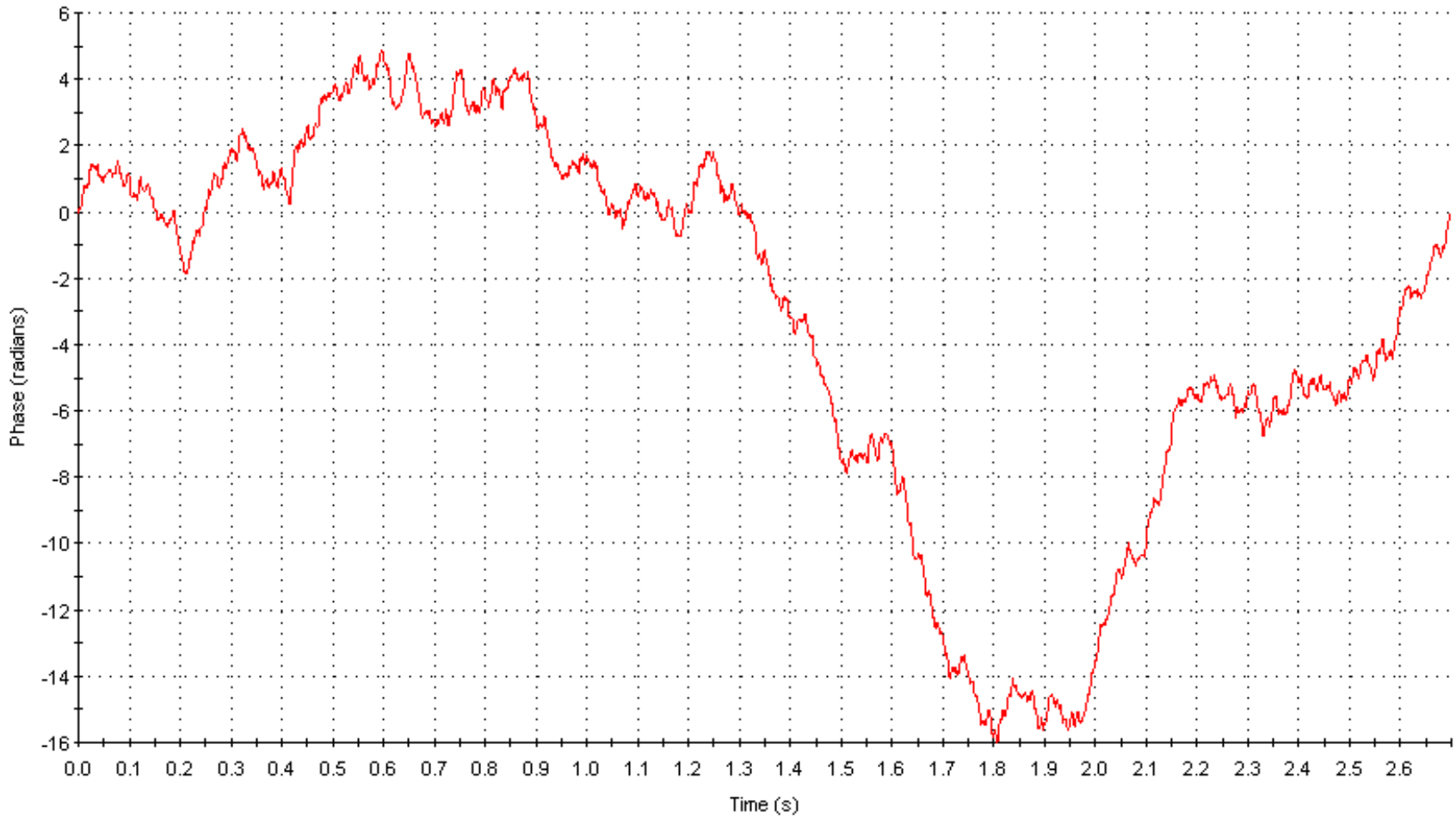
Zeta Quality Report

Test Number	Test Description	Warning Message	Possible Reasons For Warning Message	Possible Actions
2	Check the quality of the phase plot data (Is Quality Factor < 1?)	Phase data poor – signal to noise ratio low	Sample concentration too low	Increase number of sub-runs per measurement
				Increase sample concentration and re-measure
			Sample concentration too high	Increase number of sub-runs per measurement
				Dilute sample and re-measure
			High conductivity causing sample/electrode degradation	Ensure that the Monomodal analysis is used
	Manually reduce voltage			
			Low zeta potential – close to neutral	None

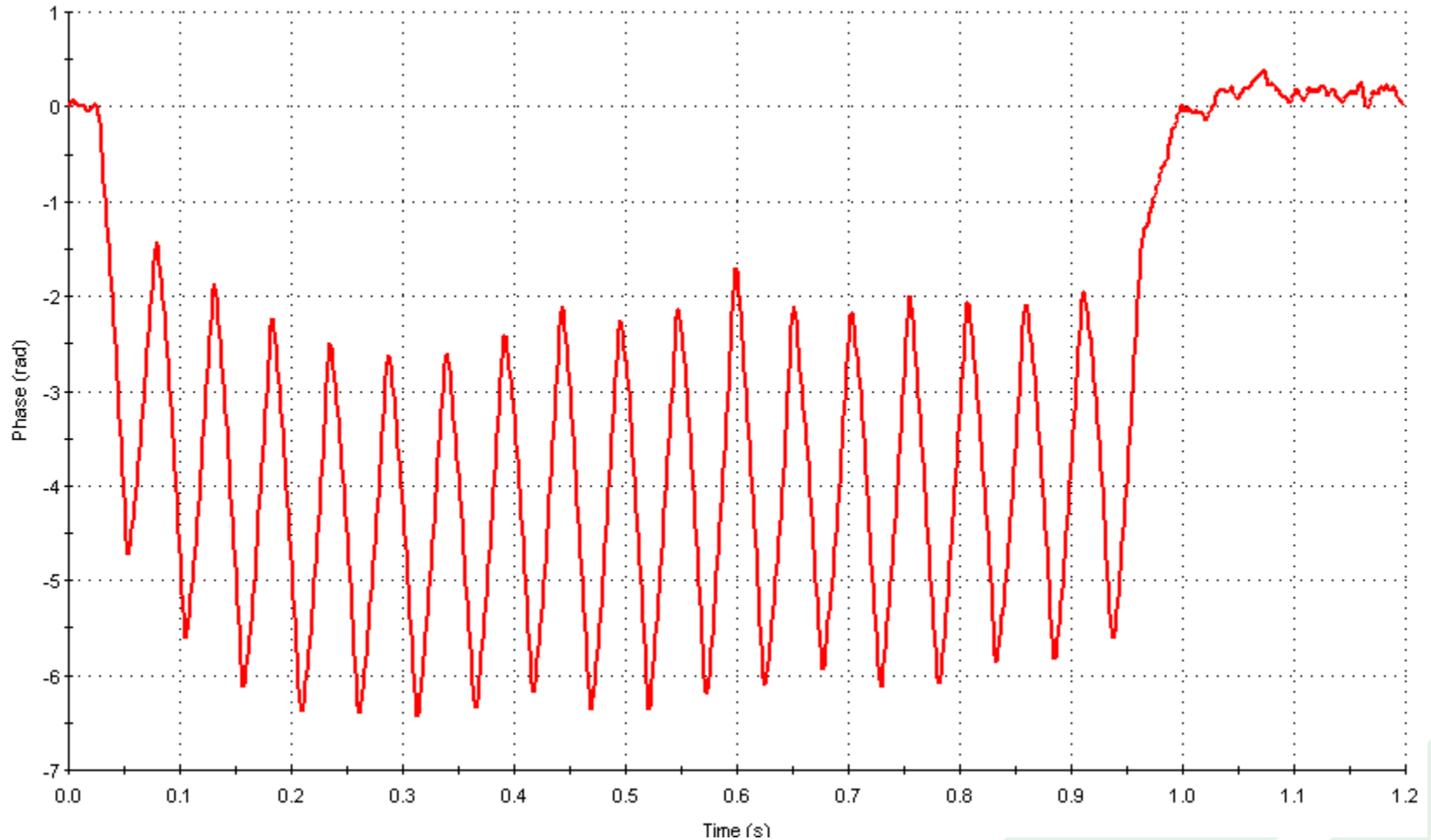
Phase Plot: **Good** Quality General Purpose



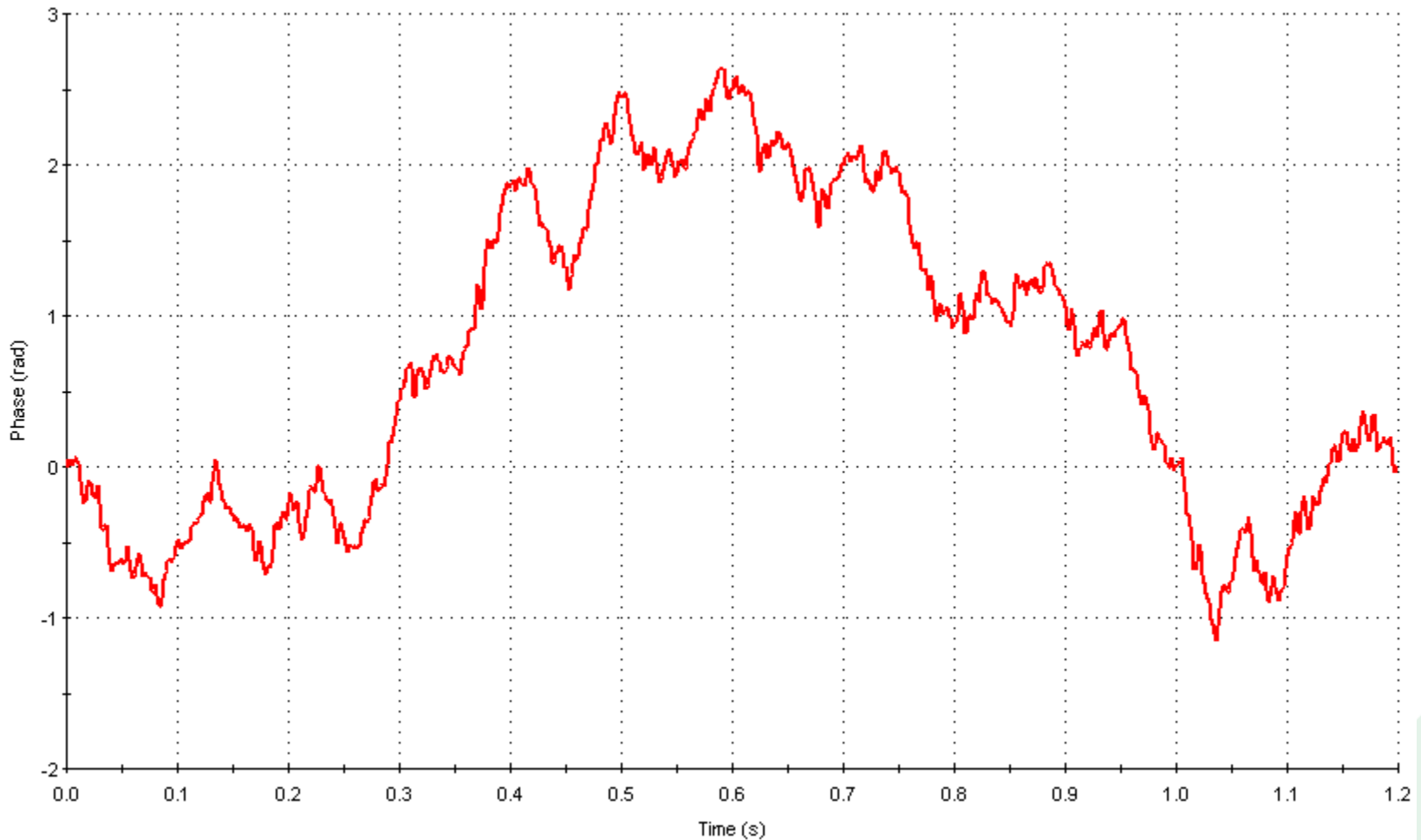
Phase Plot: **Poor** Quality General Purpose



Phase Plot: **Good** Quality Monomodal



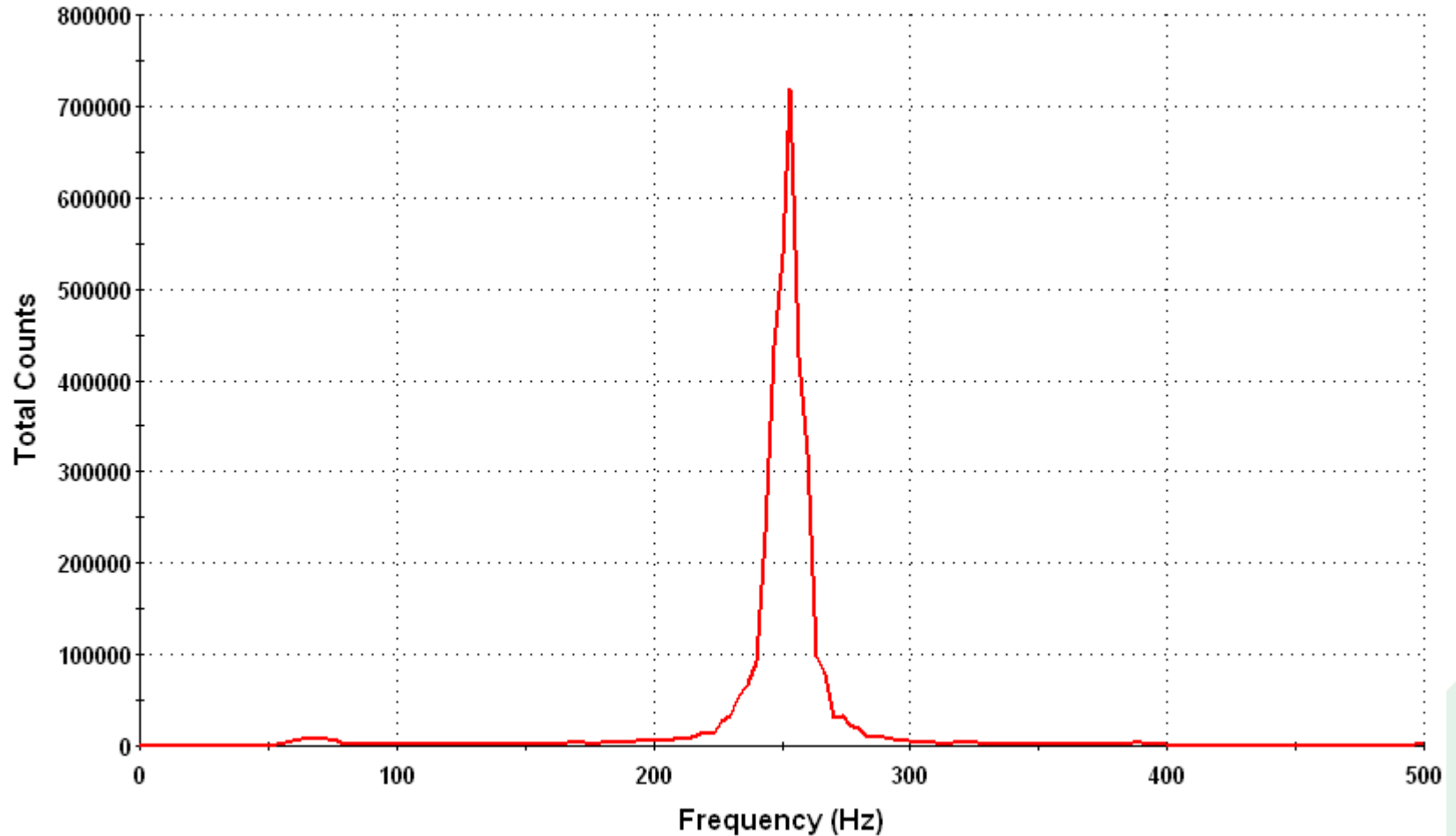
Phase Plot: **Poor** Quality Monomodal



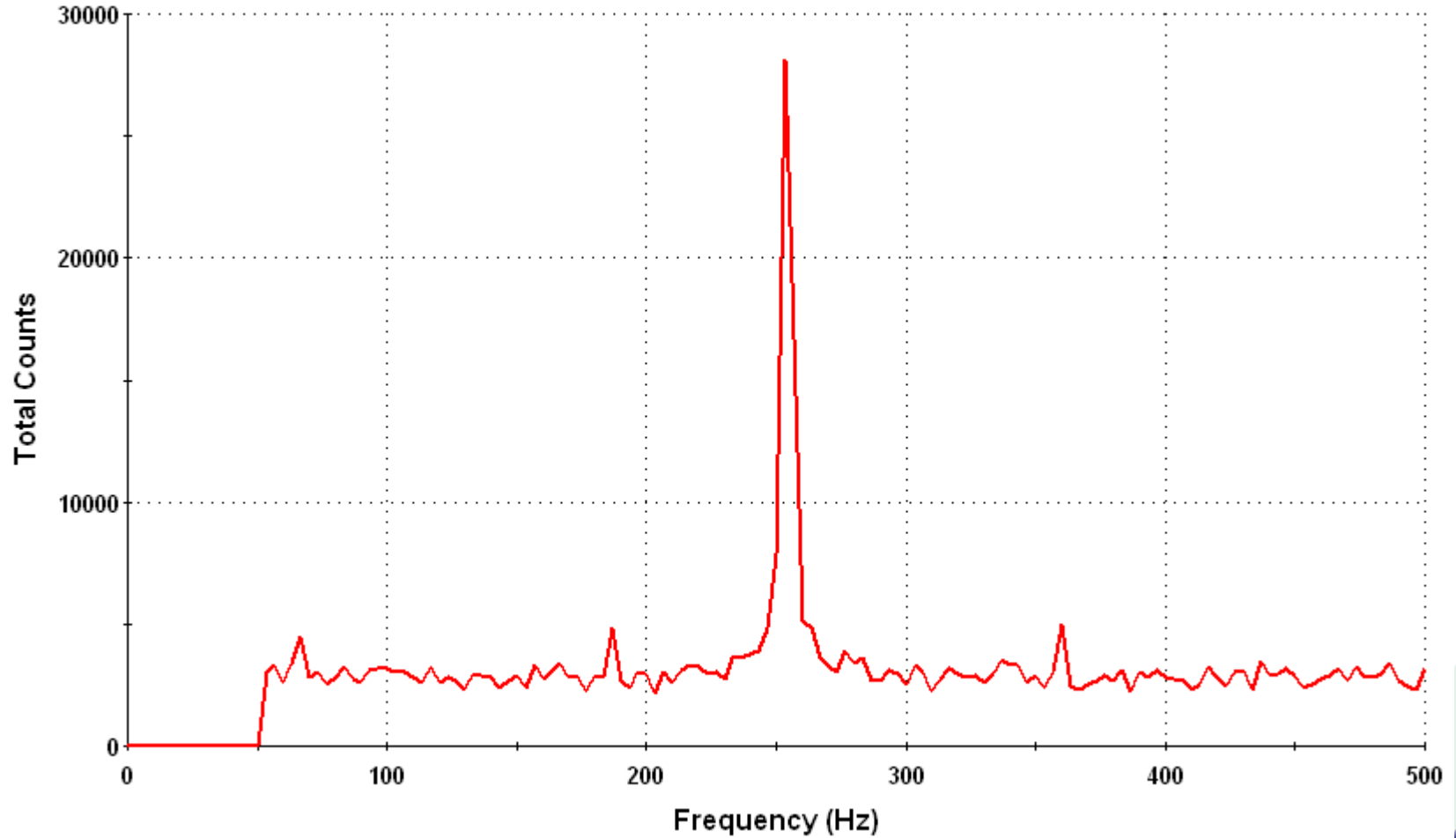
Zeta Quality Report

Test Number	Test Description	Warning Message	Possible Reasons For Warning Message	Possible Actions
3	Check the quality of the distribution plot data (Is SFR Spectral Quality < 1?)	Distribution data poor	Sample concentration too low	Increase number of sub-runs per measurement
				Increase sample concentration and re-measure
			Sample concentration too high	Increase number of sub-runs per measurement
				Dilute sample and re-measure
			High conductivity causing sample/electrode degradation	Ensure that the Monomodal analysis is used
				Manually reduce voltage

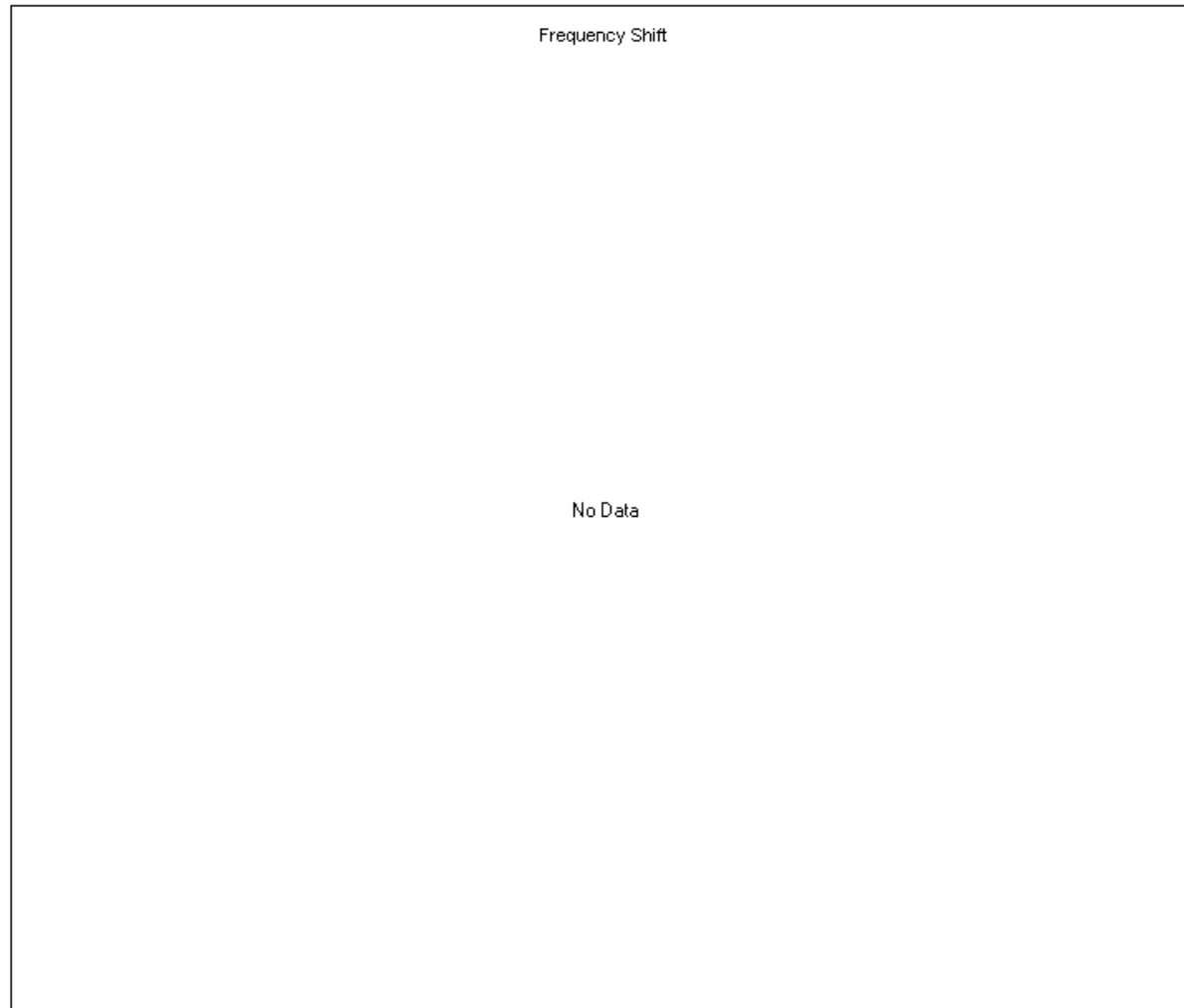
Frequency Plots: **Good Data**



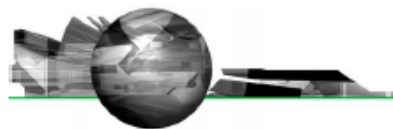
Frequency Plots: **Poor** Data



Frequency Plot: Monomodal



Further Information



technical
note

Zeta potential quality report for the Zetasizer Nano



Introduction

A quality report has been developed for the Zetasizer Nano range of instruments to simplify the interpretation of the data obtained from a zeta potential measurement.

The zeta potential quality report is displayed as default as one of the reports in the zeta potential workspace in software version 4.10 or later.

Several tests are performed on a selected record and if any of the tests fall outside specified limits, a warning message is displayed together with advice of how to improve the result. If none of the tests fail, a "Result Meets Quality Criteria" message is displayed. The Zeta Potential Quality Report incorporates 6 tests on any selected record.

Table 1 is a summary of the tests incorporated and lists descriptions of the tests, the warning message and actions that might remedy it.

This technical note uses example results to illustrate the warning messages and provides details of possible actions of how to improve the results.

Table 1: Summary of the tests incorporated into the zeta potential quality report

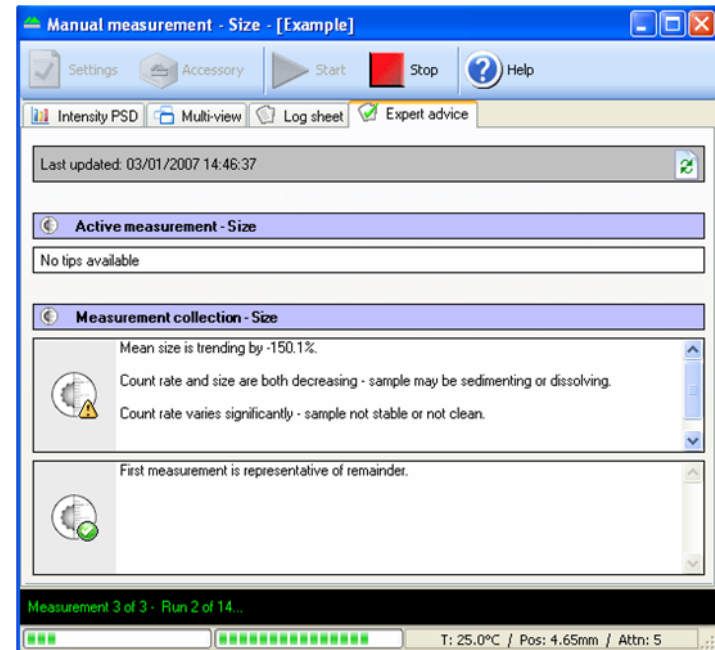
Test Number	Test Description	Warning Message	Possible Reasons for Warning Message	Possible Actions
1	Check the quality of the phase plot data	Phase data poor – signal to noise ratio low	Sample concentration too low	Manually increase number of sub-runs per measurement Increase sample concentration and re-measure
			Sample concentration too high (sample looks turbid)	Manually increase number of sub-runs per measurement Dilute sample and re-measure
			High conductivity causing sample/electrode degradation	Ensure that the Monomodal analysis is used Manually reduce voltage
			Measurement duration manually set	Set measurement duration to automatic and re-measure Manually increase number of sub-runs per measurement and re-measure

The Expert Advice System

- › The Expert Advice System provides information on the quality of the data and results obtained from both **live** and **stored** measurements
- › **Live** measurements
 - Data quality from a single measurement
 - Information on whether repeat measurements (3 or more) display trending tendencies
- › **Stored** measurements
 - Information on the quality of the measurements
 - Information on variability in the results from changes in the sample or where the first measurement is different to the others

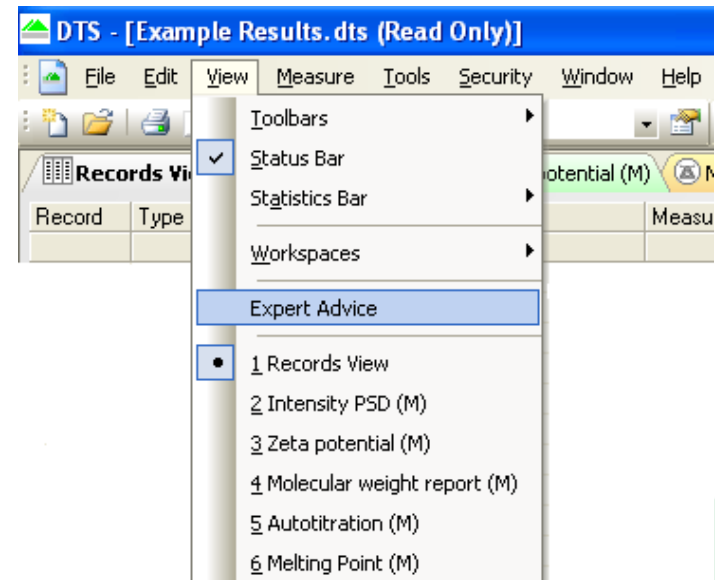
Expert Advice System Location

- › The **Expert Advice Tab** available during a **live** measurement in the Measurement Display Window



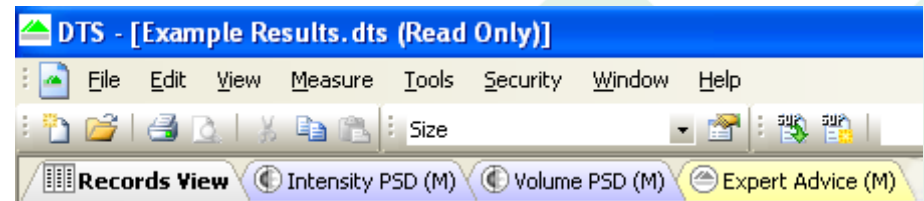
Expert Advice System Location

- › The **Expert Advice Tab** available during a **live** measurement in the Measurement Display Window
- › The **Expert Advice System** accessed **stored** measurement by right mouse clicking on the records, or from Select View, Expert System



Expert Advice System Location

- › The **Expert Advice Tab** available during a **live** measurement in the Measurement Display Window
- › The **Expert Advice System** accessed **stored** measurement by right mouse clicking on the records, or from Select View, Expert System
- › The **Expert Advice Report** available **stored** measurement in the workspace



Expert Advice: Single Measurements

- › 7 tests found in the **Zeta Quality Report** are used
- › During a measurement, if any of the tests fall outside specified limits, warning messages and possible reasons for failure are displayed in the **Expert Advice Tab** in the Live Measurement Window
- › If none of the tests fail, a “**Data Meets Quality Criteria**” message is displayed in the **Expert Advice Tab**