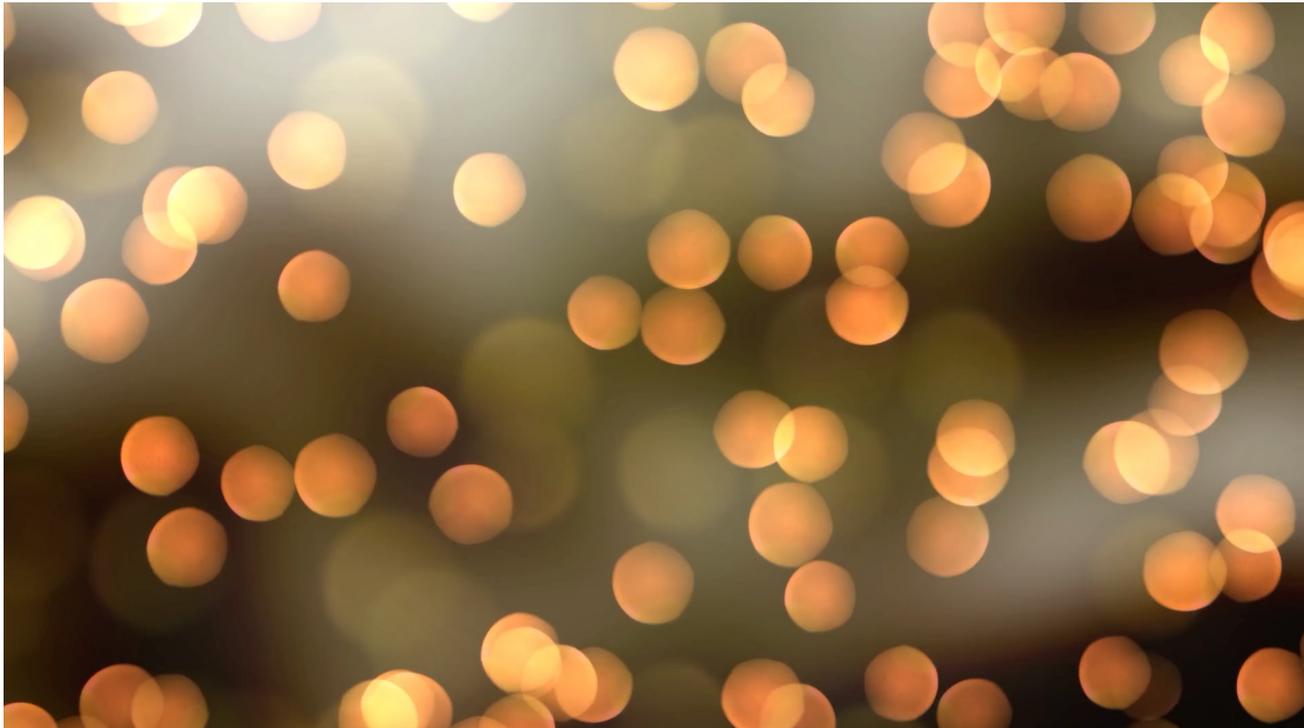


# Dynamic Light Scattering Training

## Achieving reliable nano particle sizing

Bruno GRASSL



# 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”



<https://youtu.be/OXoKZPLb6Qo>

# Rayleigh Theory

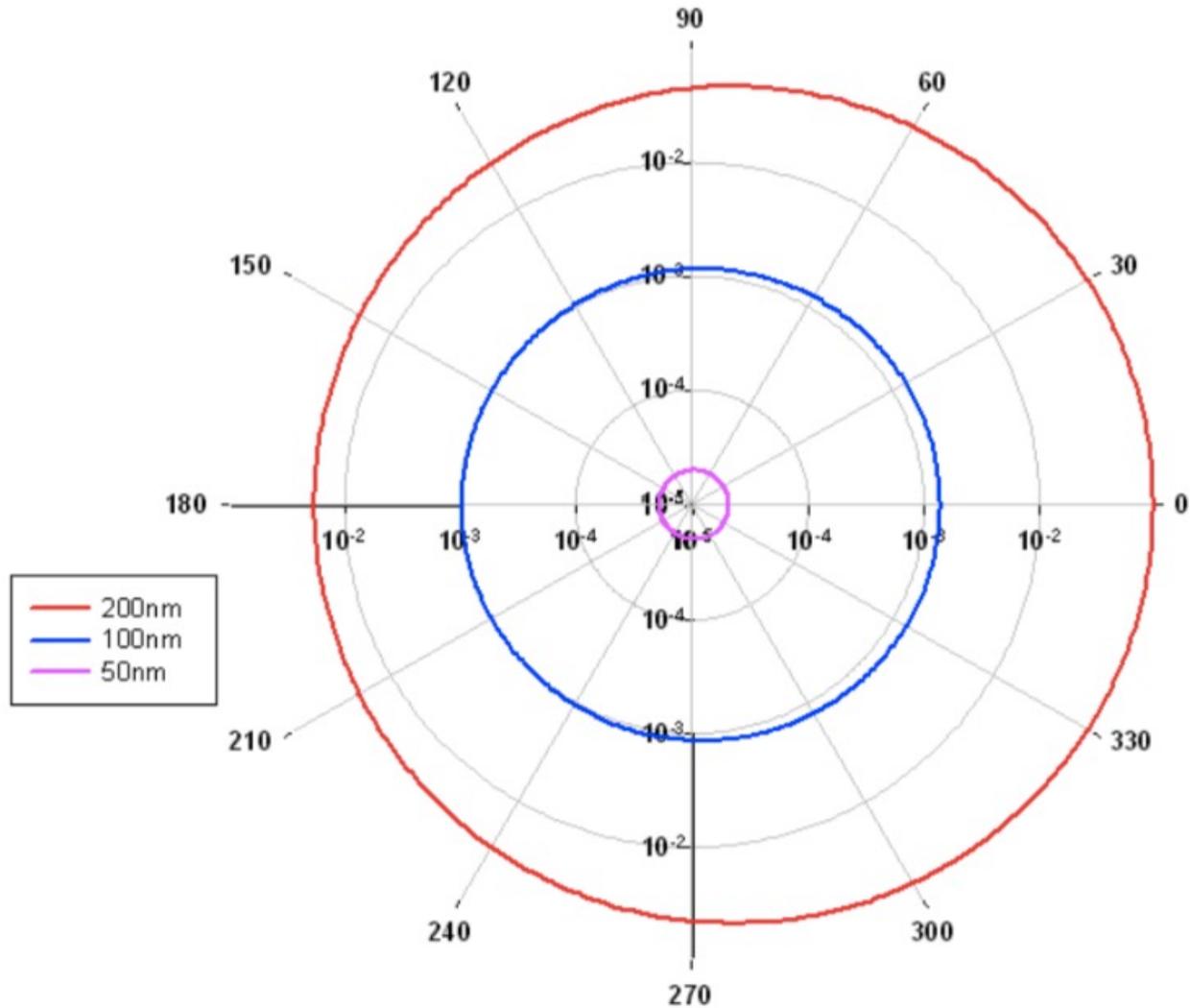
- › Rayleigh theory is applicable for small particles and molecules whose diameters are less than  $1/10^{\text{th}}$  of the laser wavelength ( $\lambda$ )
- › For the He-Ne laser ( $\lambda = 633\text{nm}$ ) used in the instrument, this equates to particles less than about 60nm
- › The scattering produced 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

$$I \propto d^6$$

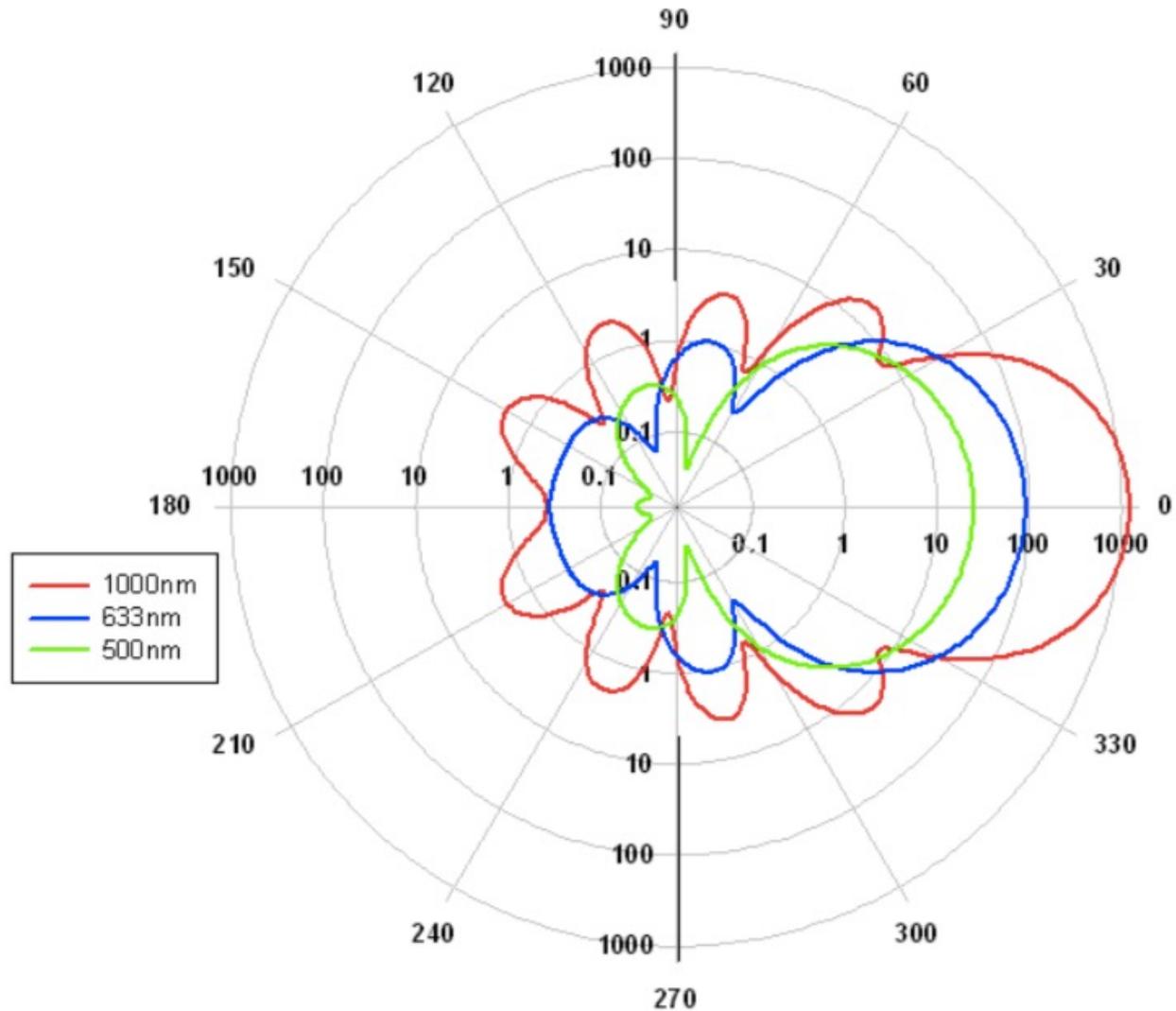
# Mie Theory

- 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 correctly explains the maxima and minima

# 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

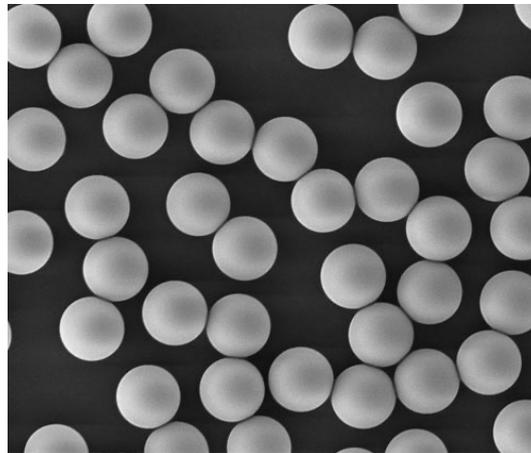
# Particle Size

- › 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

# Spheres : usual radius or diameter

**MALS: multi-angle light scattering**

- **Gyration radius**
- **Geometric radius**



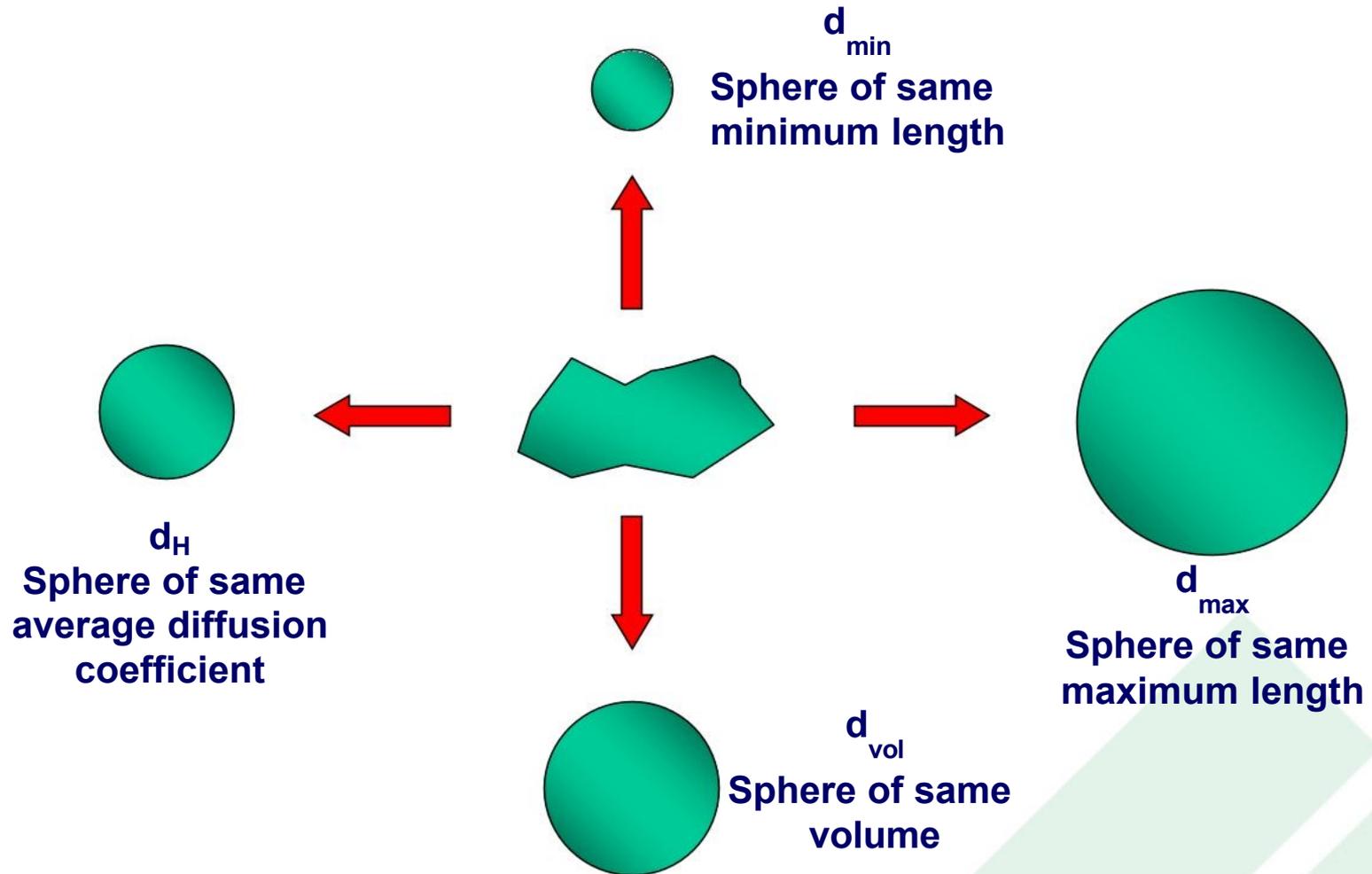
**Microscopy**

- **Geometric radius**

**Dynamic light scattering**  
**Nanoparticle Tracking Analysis**

- **hydrodynamic radius**

# Equivalent Spheres



# Dynamic Light Scattering and Brownian Motion

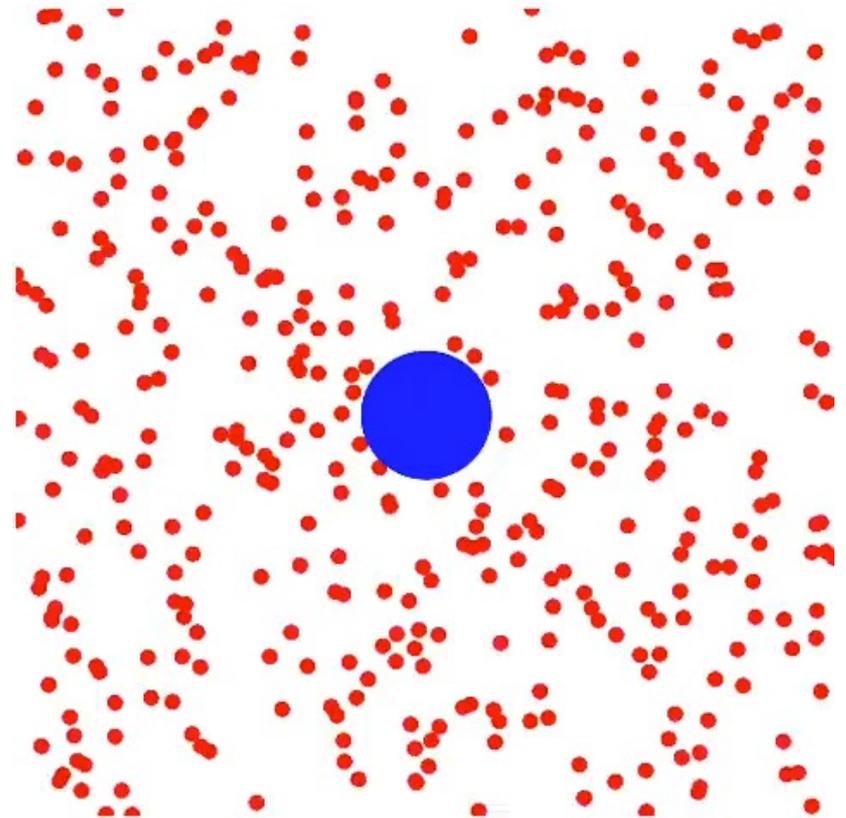
- › Dynamic light scattering is a non-invasive technique for measuring the size of particles and molecules in suspension
- › Brownian motion is the **random** movement of particles due to collisions caused by bombardment by the solvent molecules that surround them
- › The technique of dynamic light scattering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

$\eta$  = viscosity

$D$  = diffusion coefficient

# Hydrodynamic Size

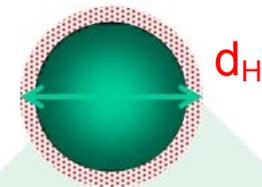
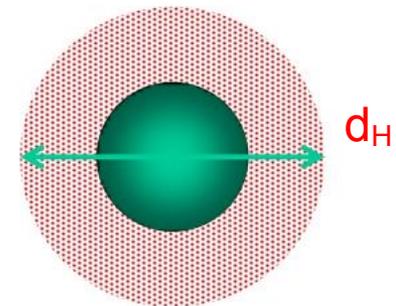
## › Definition of Hydrodynamic Diameter ( $d_H$ ):

The diameter of a hard sphere that diffuses at the same speed, *i.e.* the same diffusion coefficient, as the particle or macromolecule 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

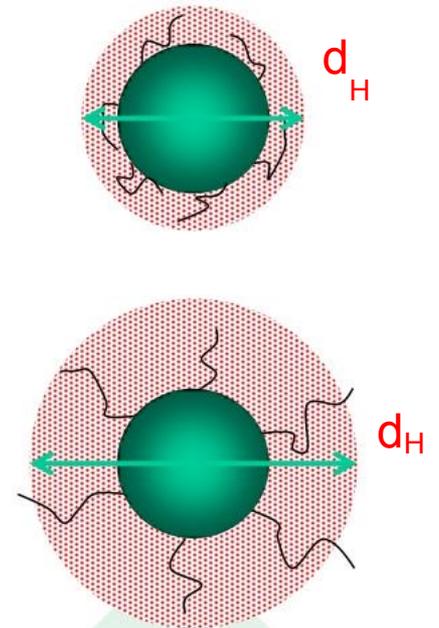
# Hydrodynamic Size: Effect of Ionic Strength

- › 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 ( $\kappa^{-1}$ )
- › 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



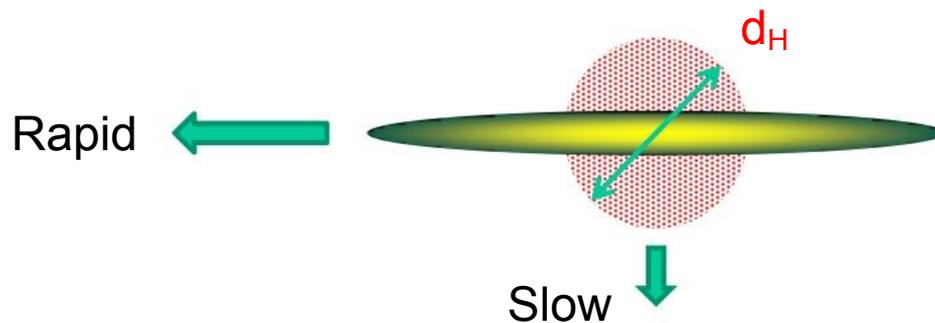
# Hydrodynamic Size: Surface Structure

- 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

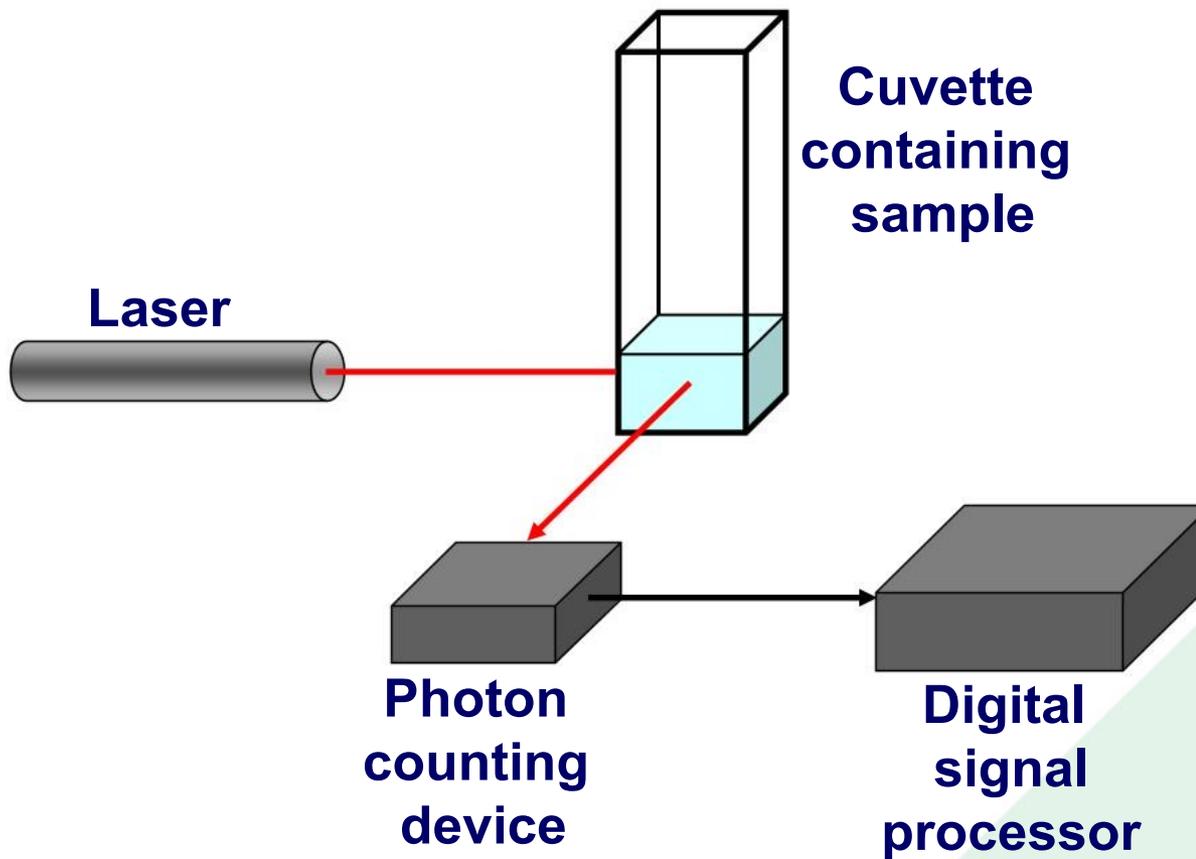


# DLS Theory

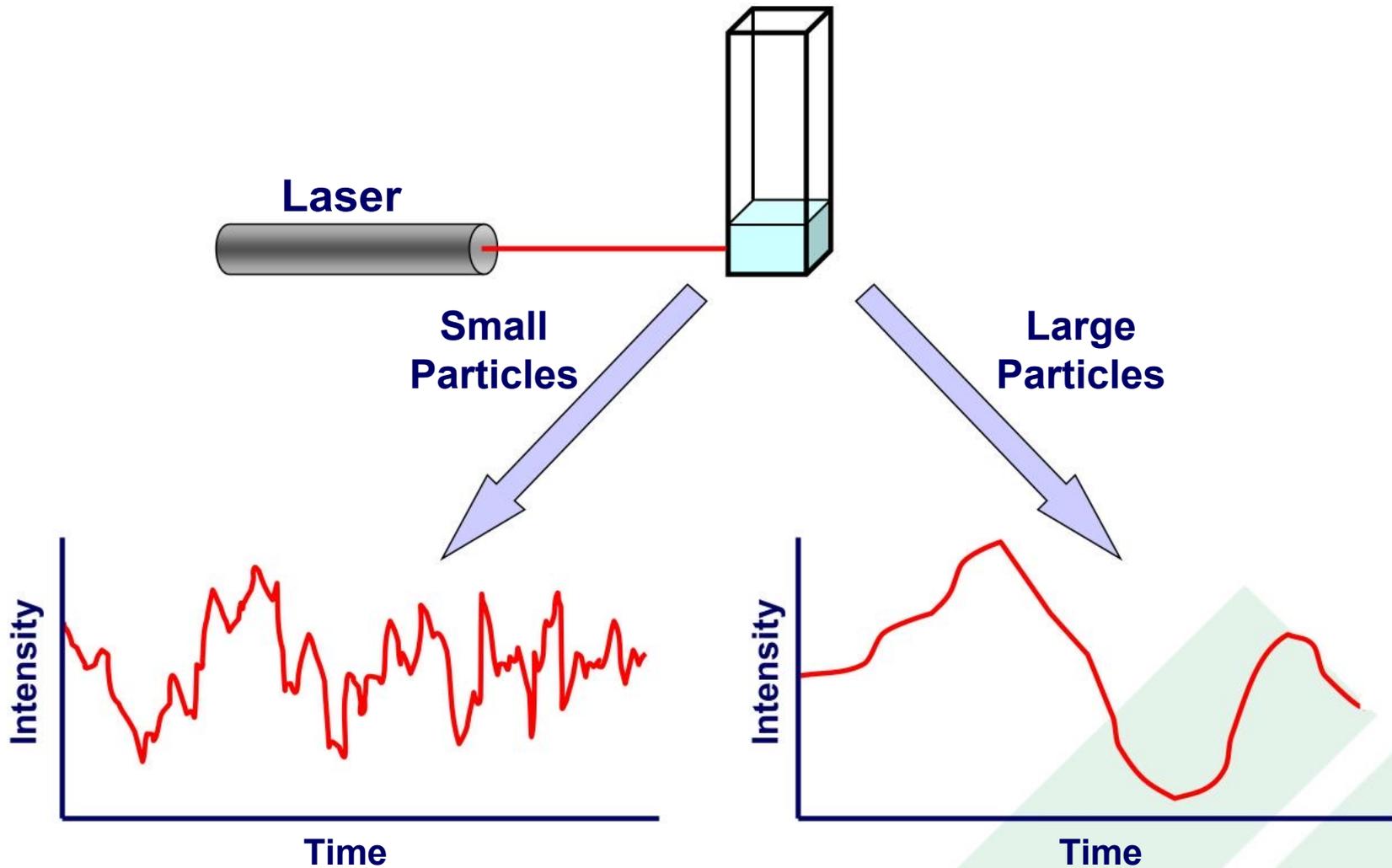
## Aims of this Section.....

- 1 Understand what components make up a 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 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  $g_2(\tau)$  of the scattered intensity according to

$$g_2(\tau) \equiv \langle \Delta I(t) \Delta I(t + \tau) \rangle / \langle \Delta I^2 \rangle$$

where

$I$  = intensity,

$t$  is the time and

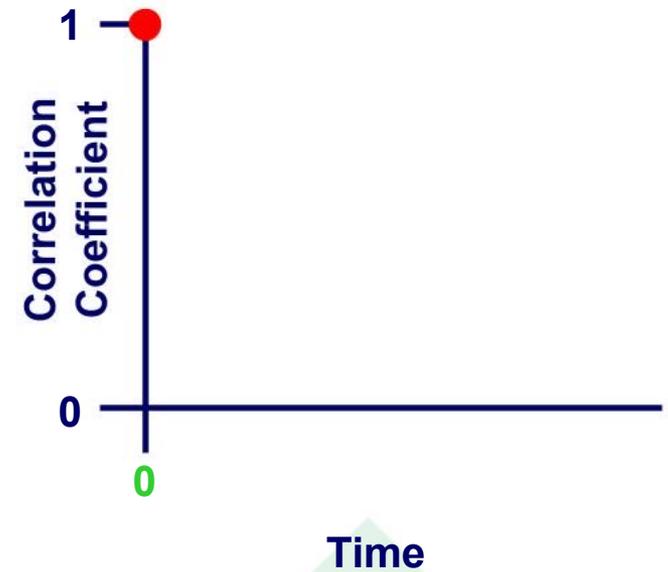
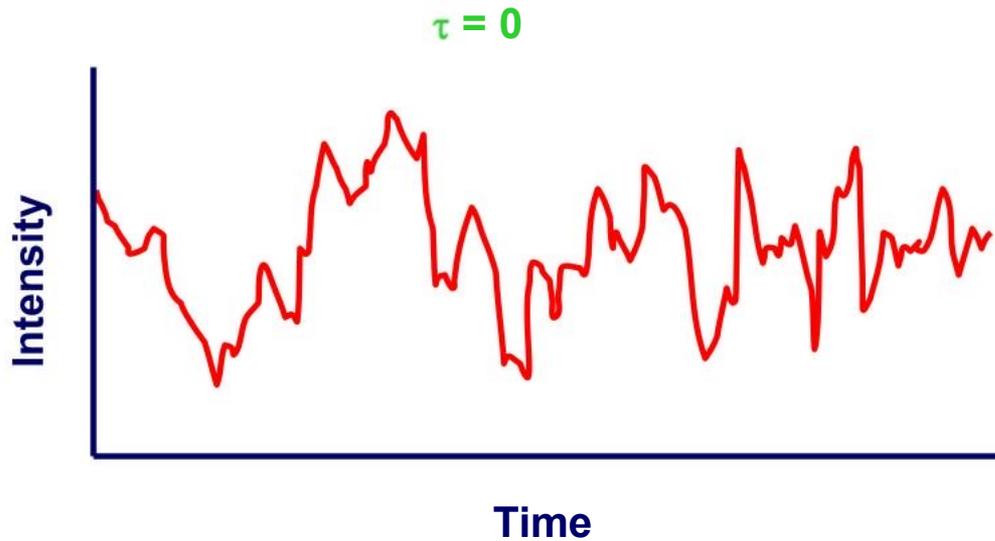
$\tau$  = the delay time

# Correlation in Dynamic Light Scattering

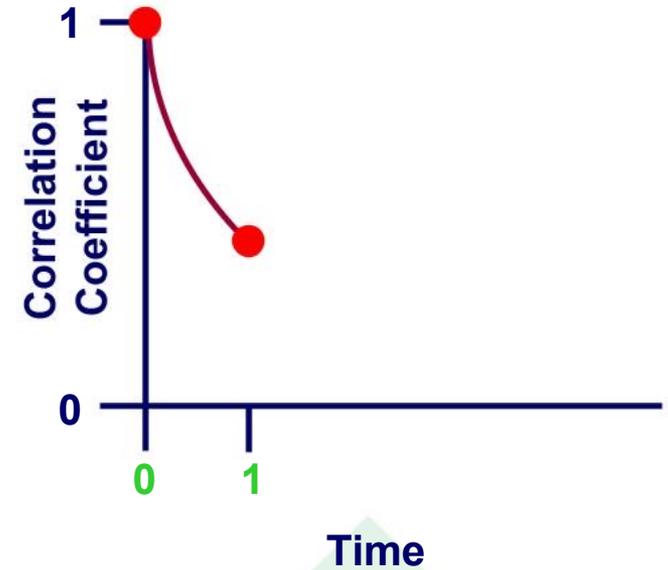
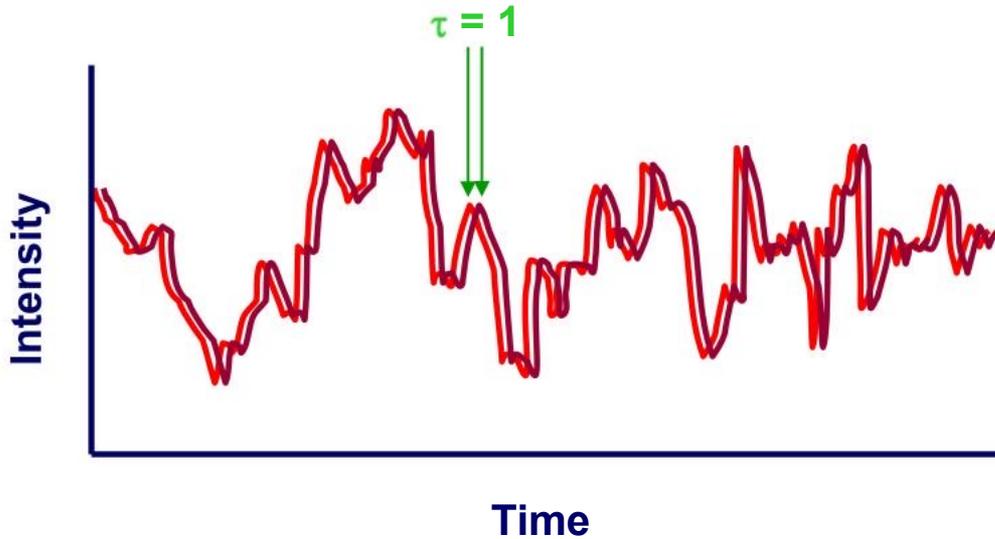


**Similitude with exposure time in photography:  
distinction of the water jets decreases with the time of exposure**

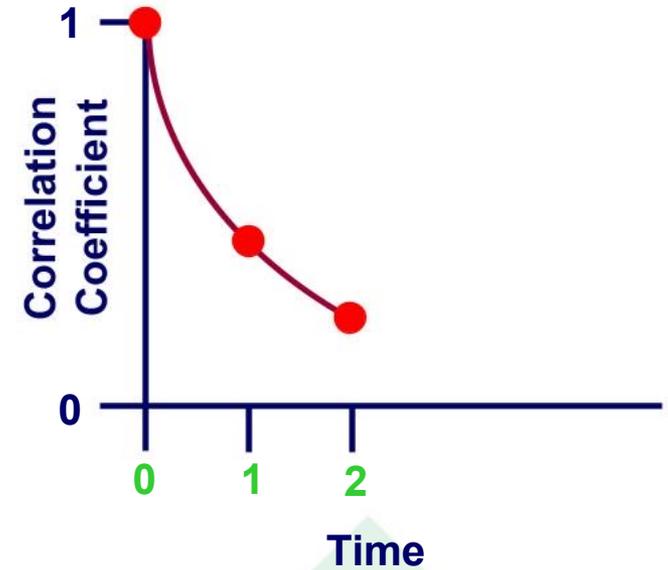
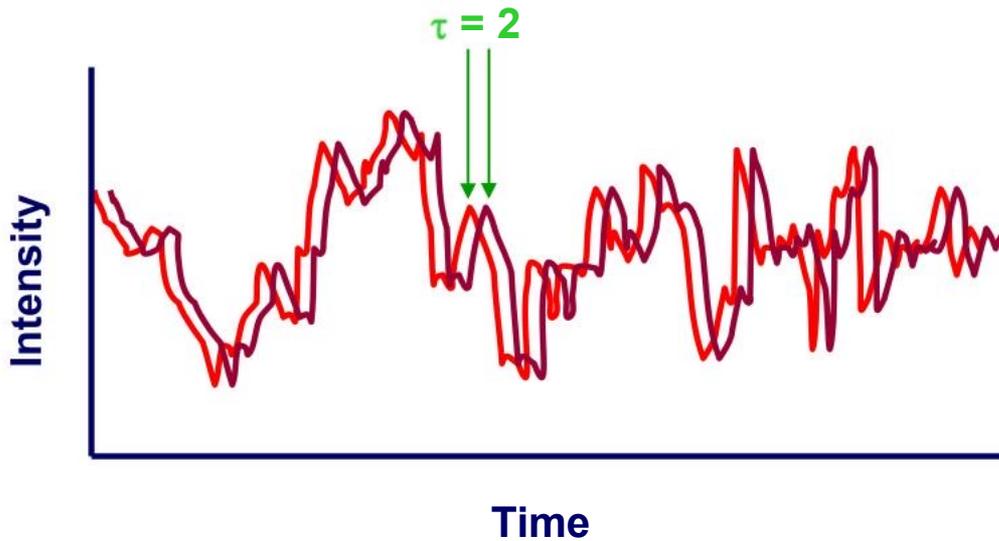
# Correlation: Small Particles, Rapid Fluctuations



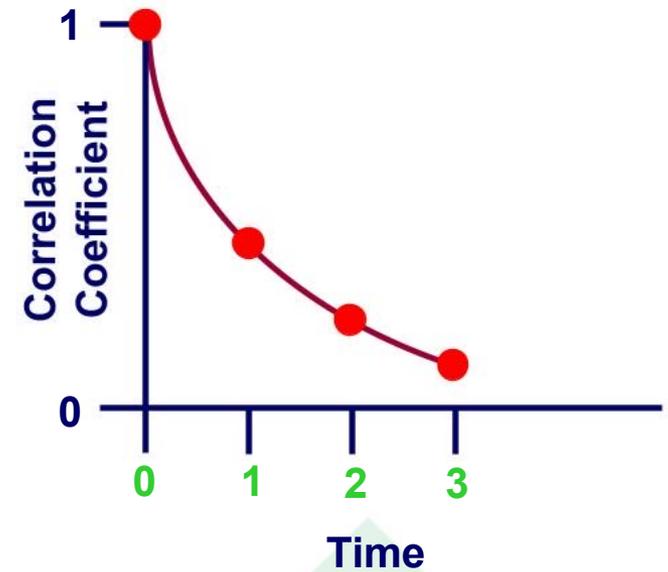
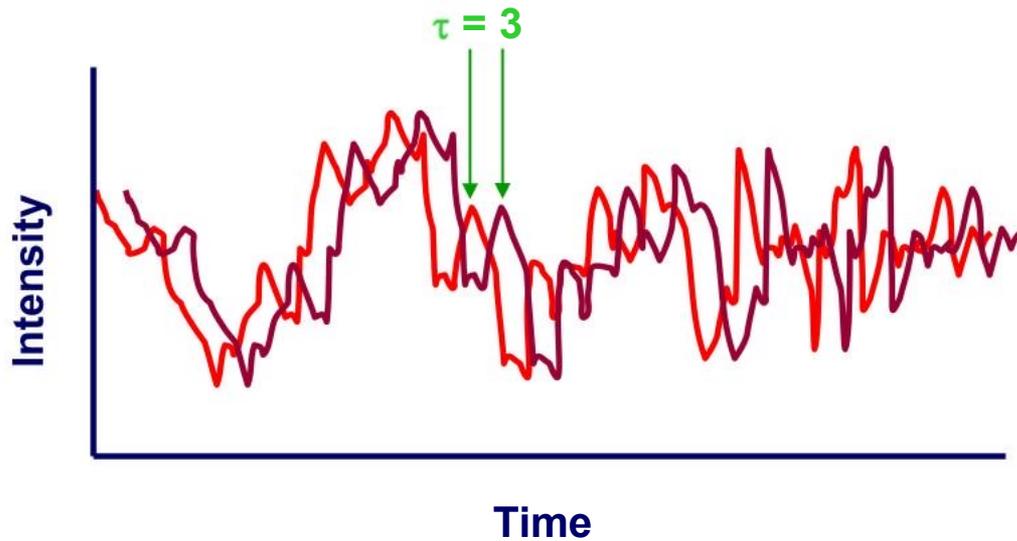
# Correlation: Small Particles, Rapid Fluctuations



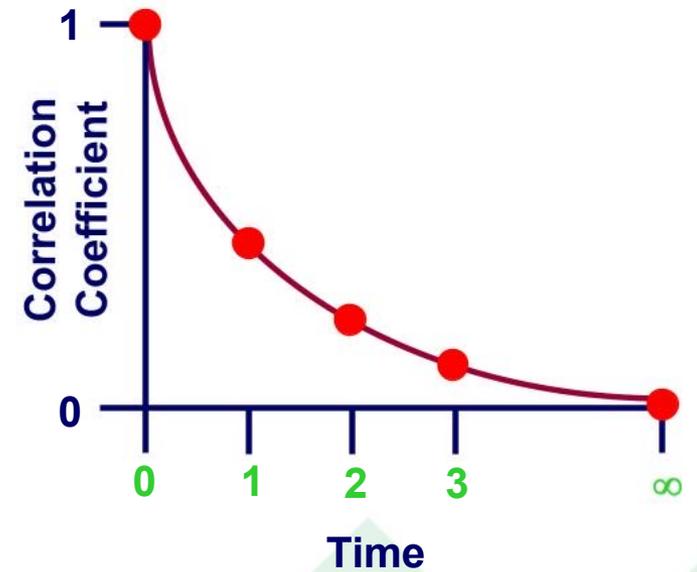
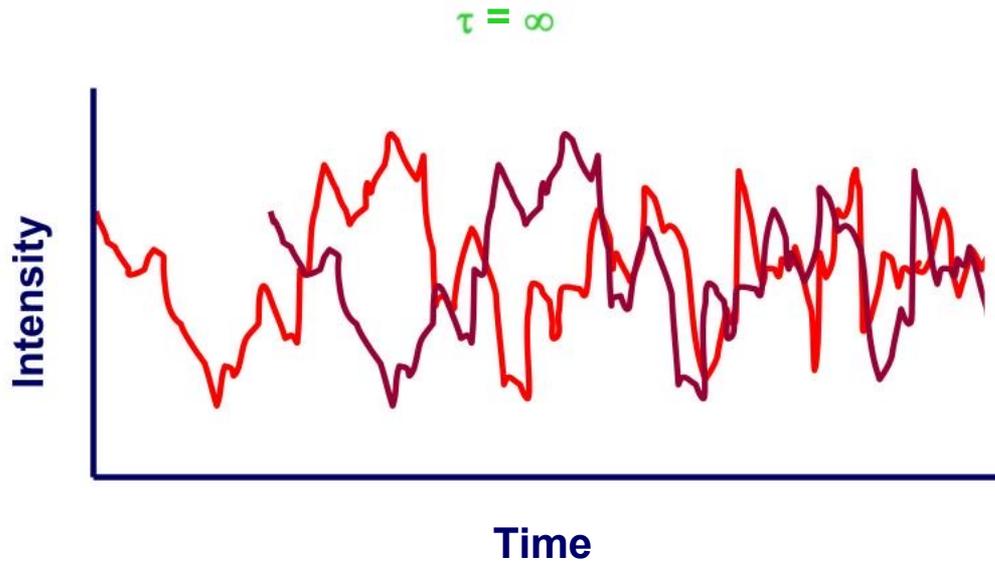
# Correlation: Small Particles, Rapid Fluctuations



# Correlation: Small Particles, Rapid Fluctuations

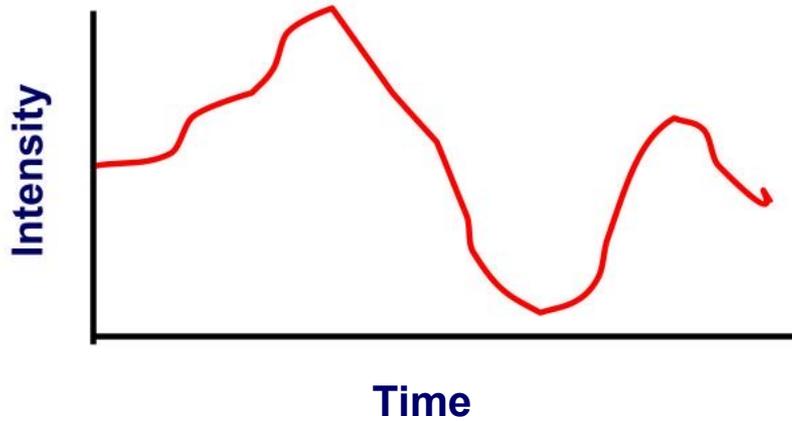


# Correlation: Small Particles, Rapid Fluctuations

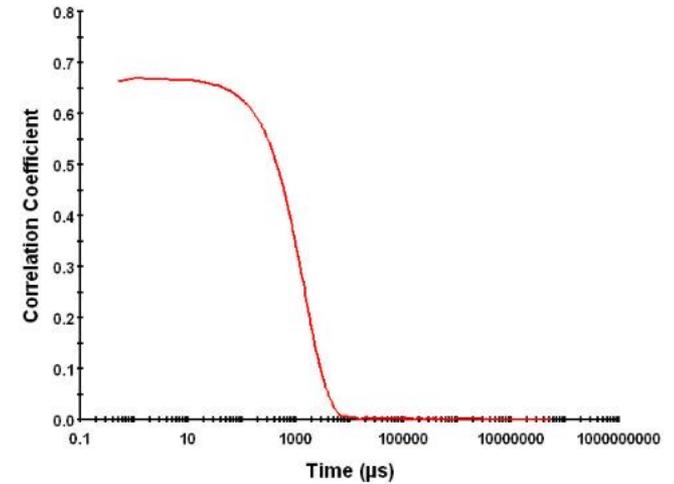


# Correlation Functions

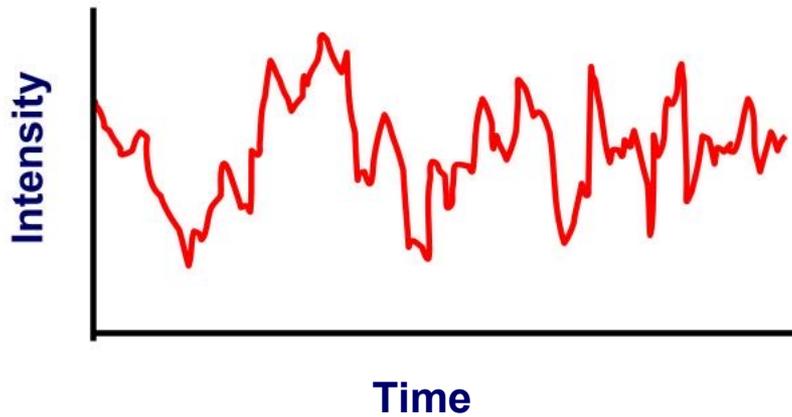
Large Particles



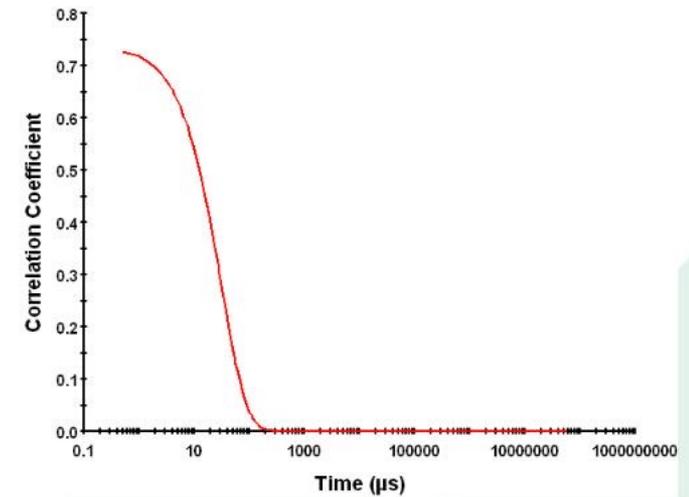
Correlate



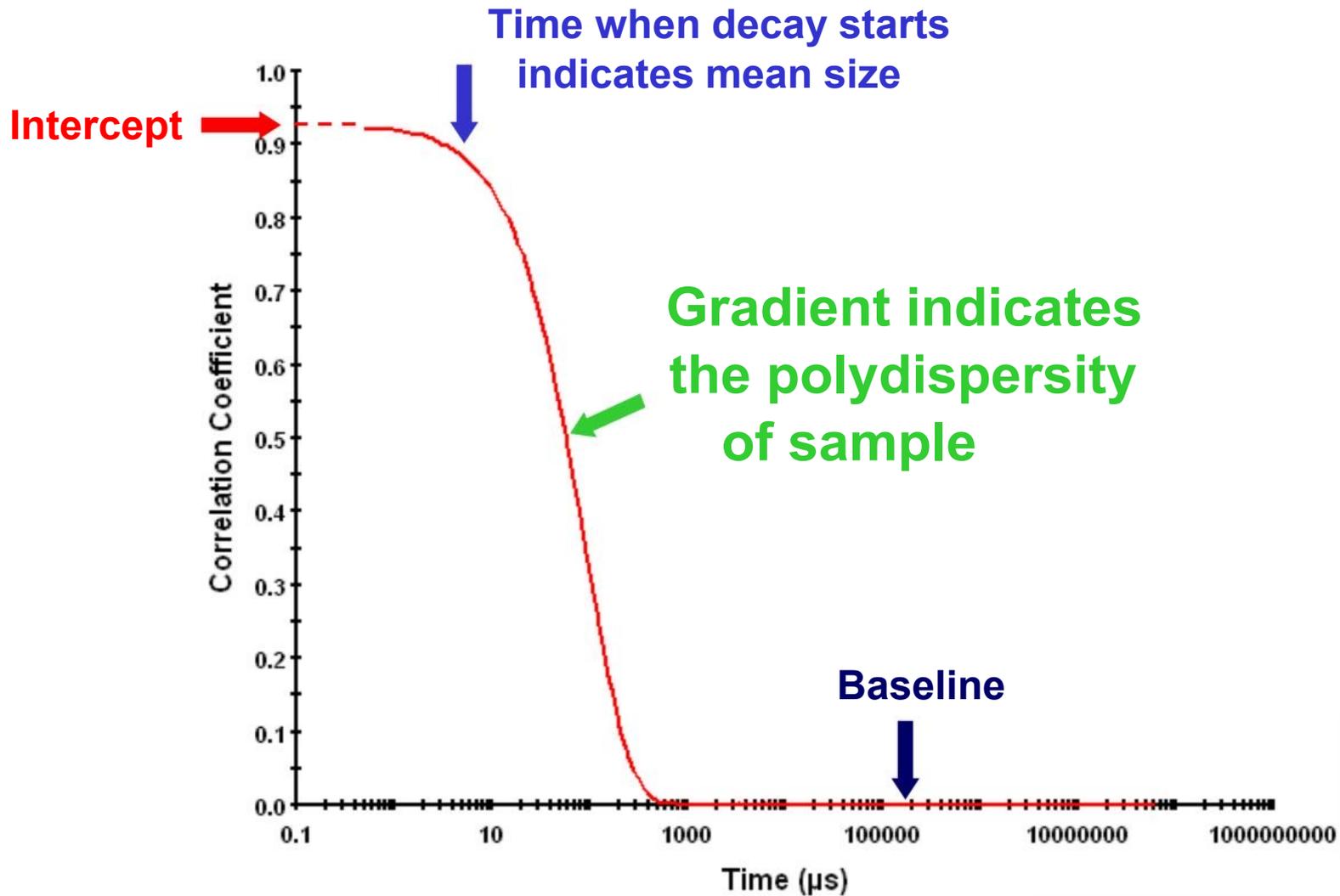
Small Particles



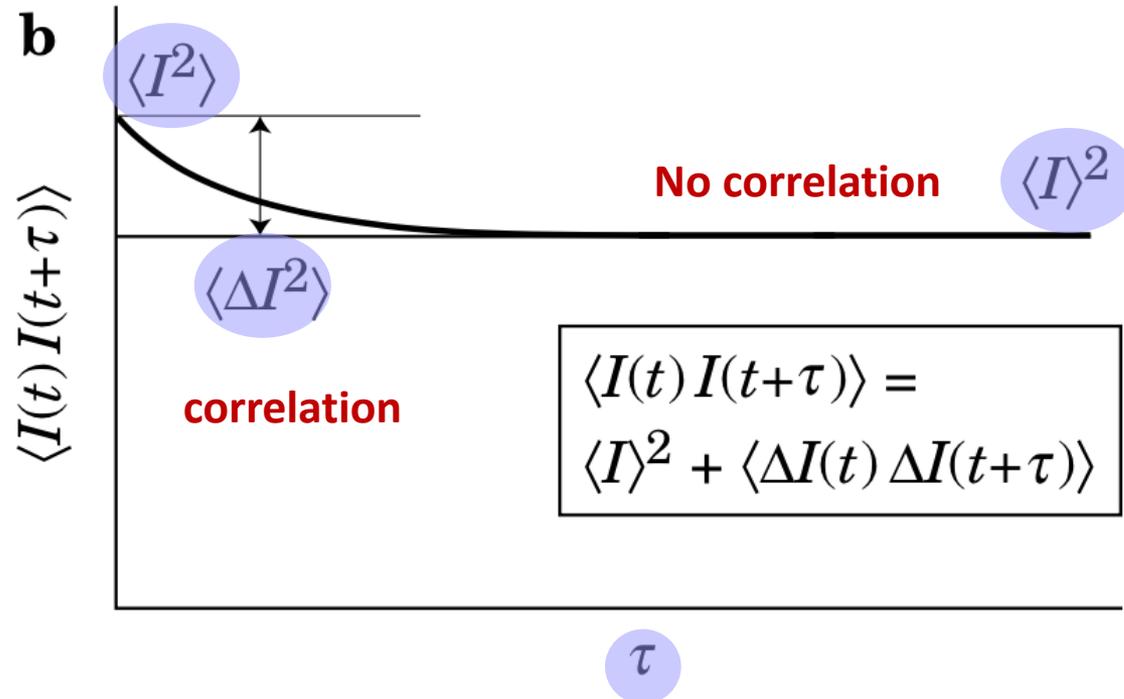
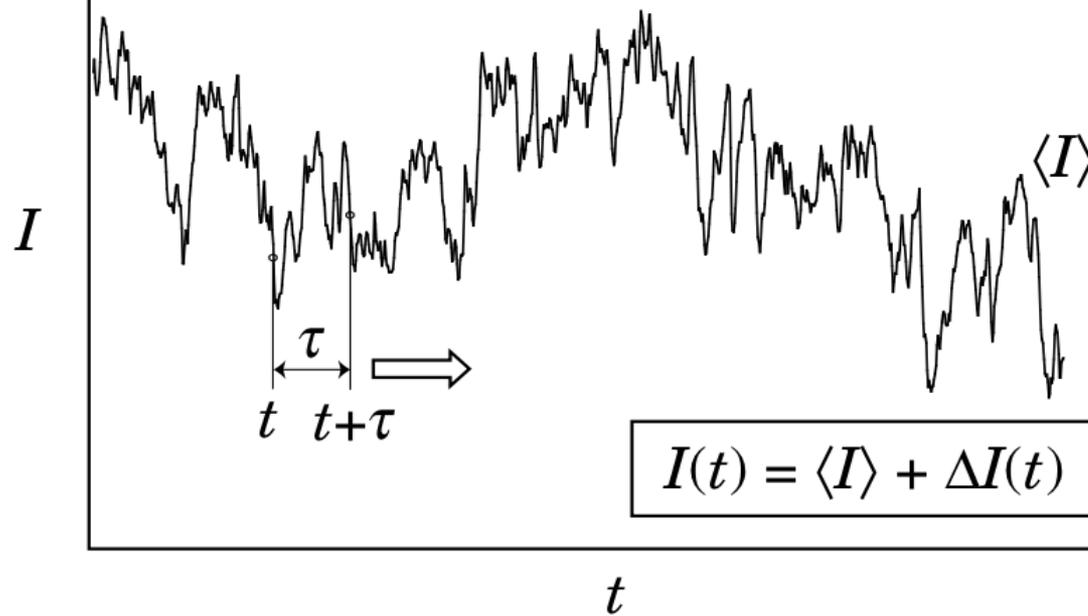
Correlate



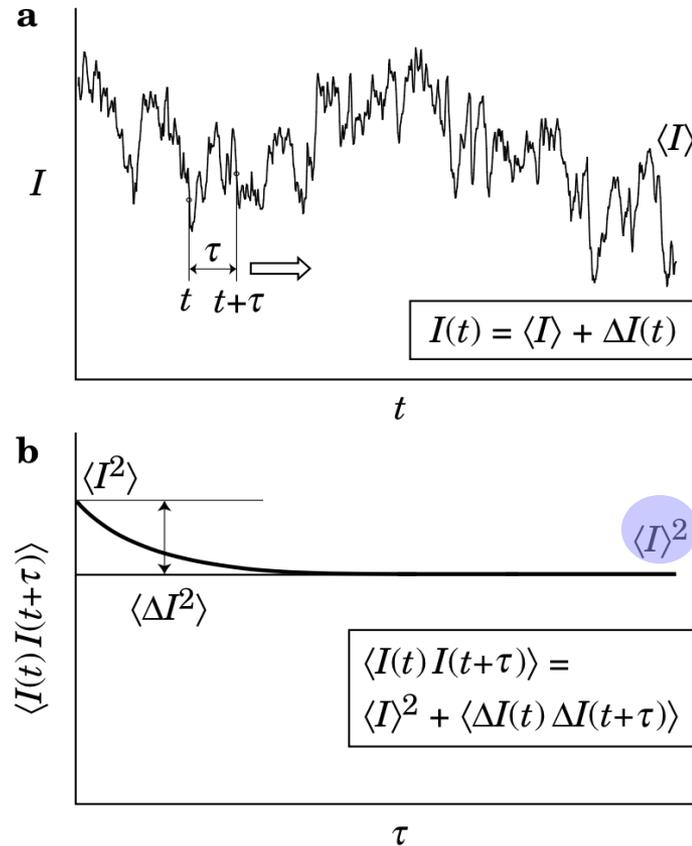
# Correlation Functions



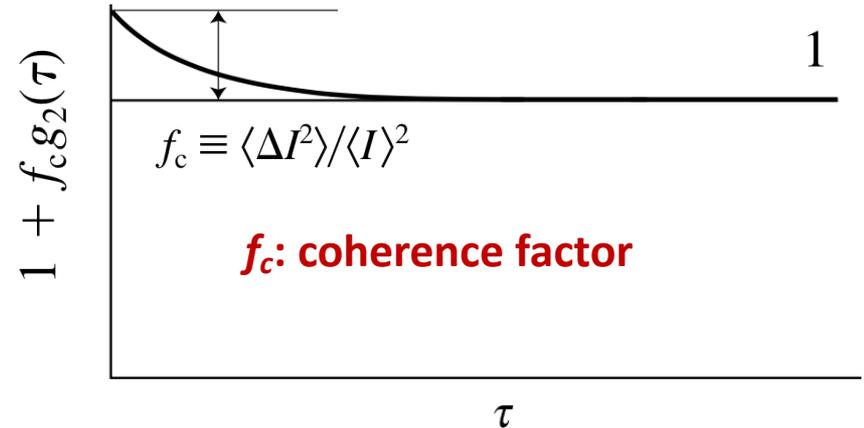
# a Correlation Functions: mathematical approach



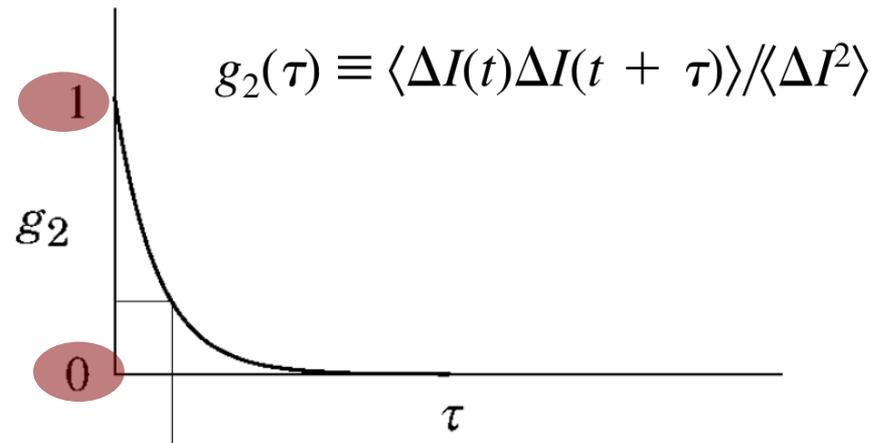
# Normalization and baseline correction



$$\begin{aligned} \langle I(t)I(t + \tau) \rangle / \langle I \rangle^2 &= 1 + \langle \Delta I(t) \Delta I(t + \tau) \rangle / \langle I \rangle^2 \\ &= 1 + f_c g_2(\tau) \end{aligned}$$



**$g_2(\tau)$ : autocorrelation function, corrected for baseline (-1) and normalized to  $f_c$ :**



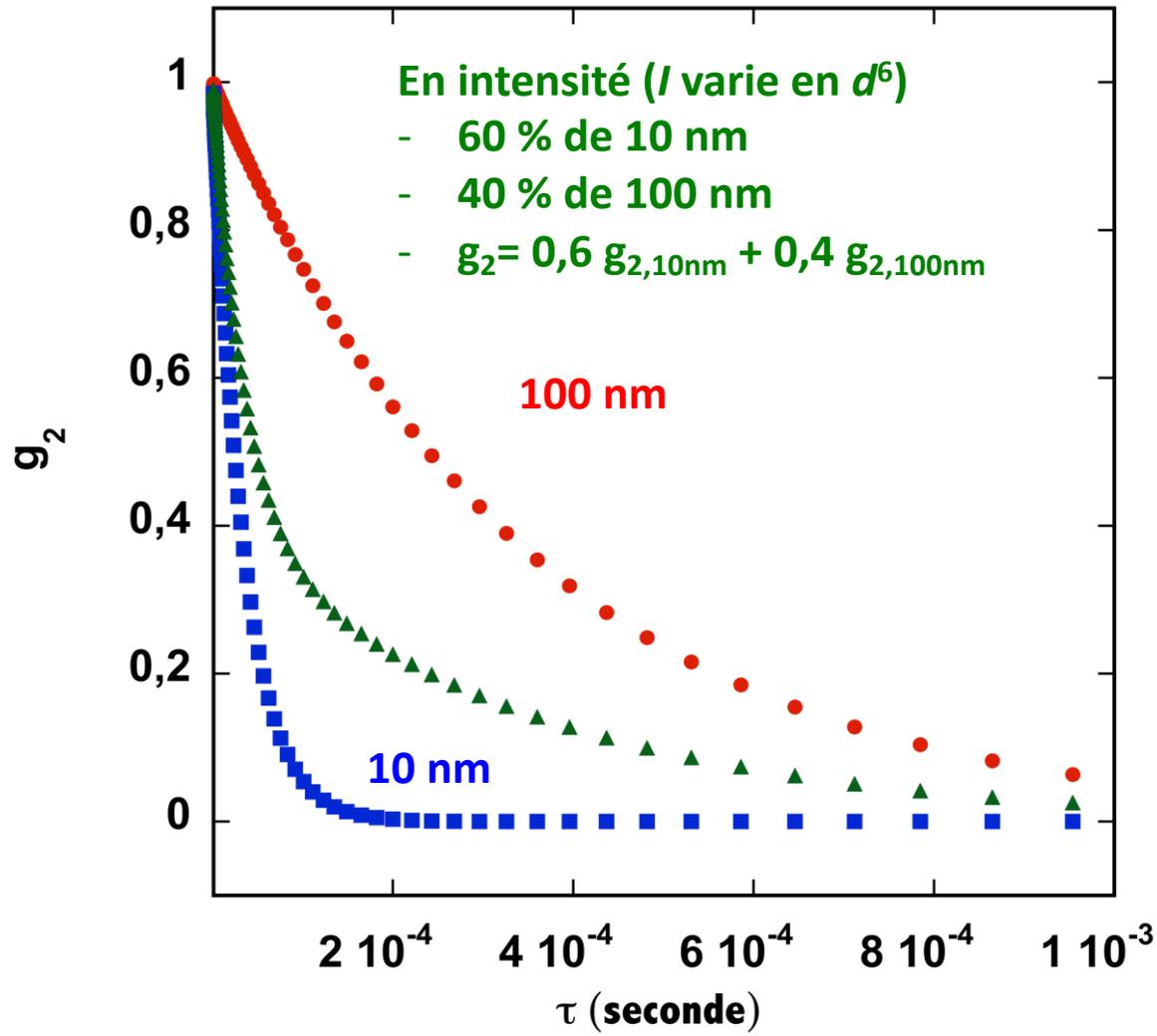
# Correlation Functions

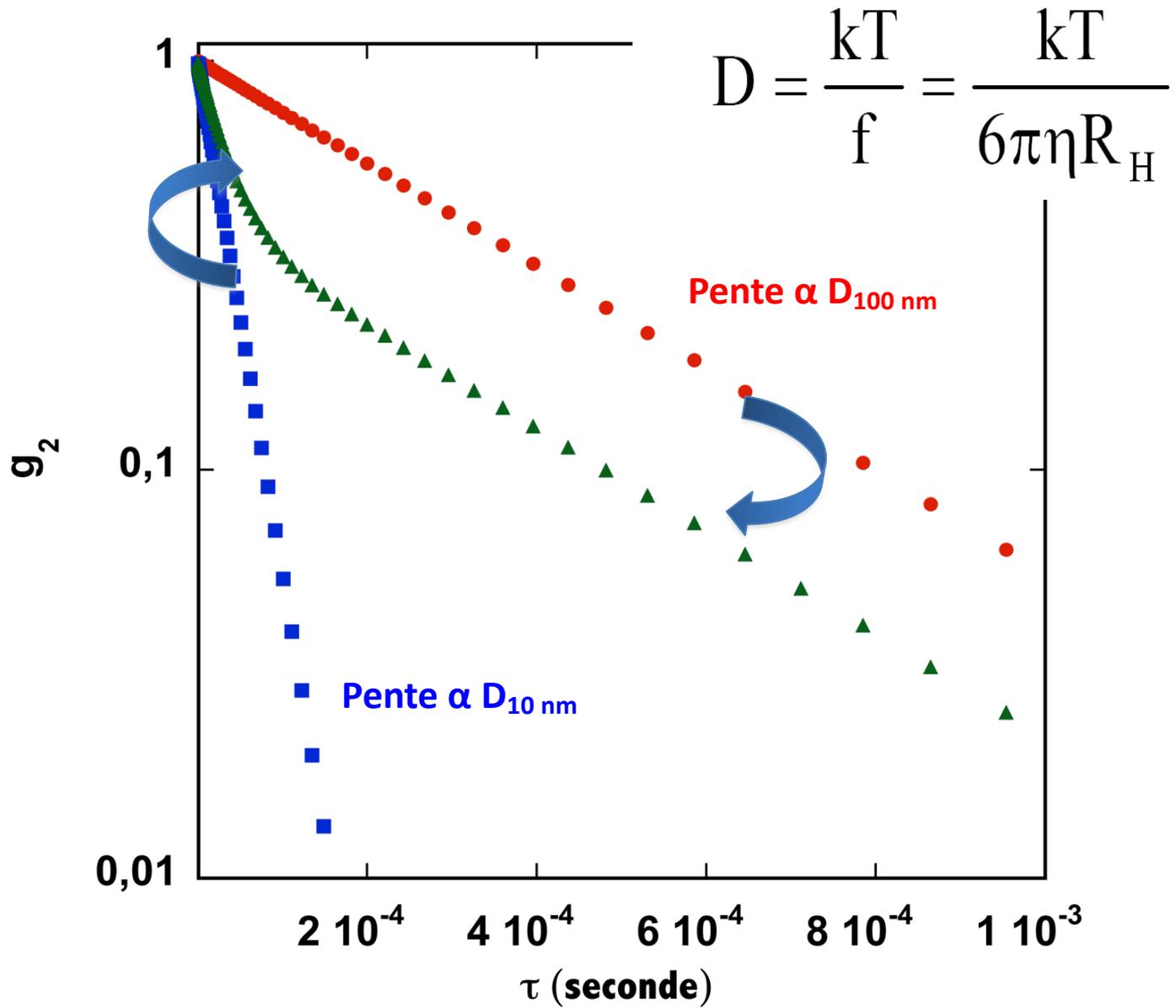
- › The correlation function can be modelled with an exponential expression such as:

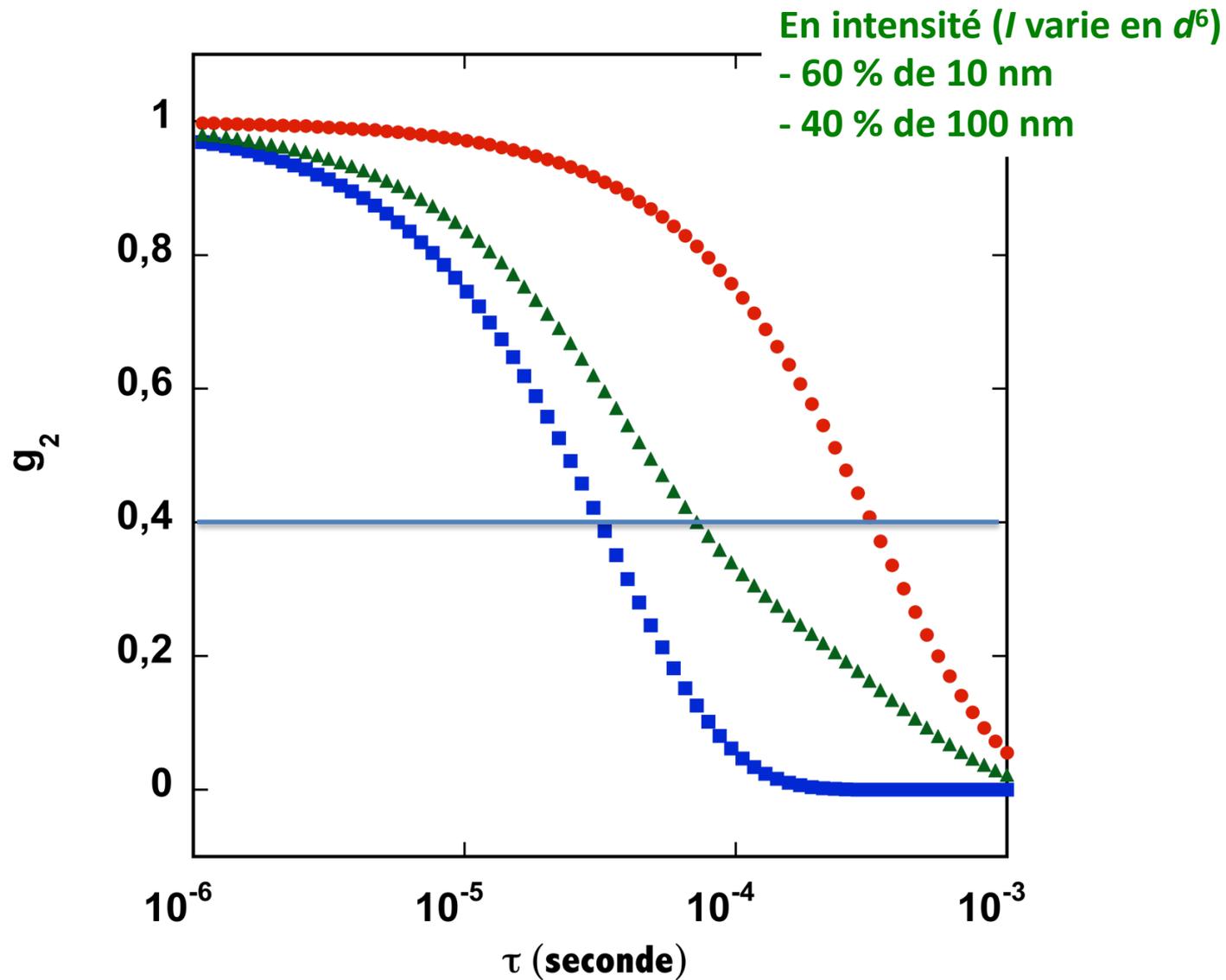
$$g_2(\tau) = \sum A_i \exp(-2D_i q^2 \tau)$$

Where

- **A = amplitude**
- **q = scattering vector =  $(4\pi n/\lambda_0) \sin(\theta/2)$** 
  - where  $n$  = dispersant refractive index,
  - $\lambda_0$  = laser wavelength and
  - $\theta$  = detection angle,
- **D = diffusion coefficient and**
- **$\tau$  is the correlator delay time**

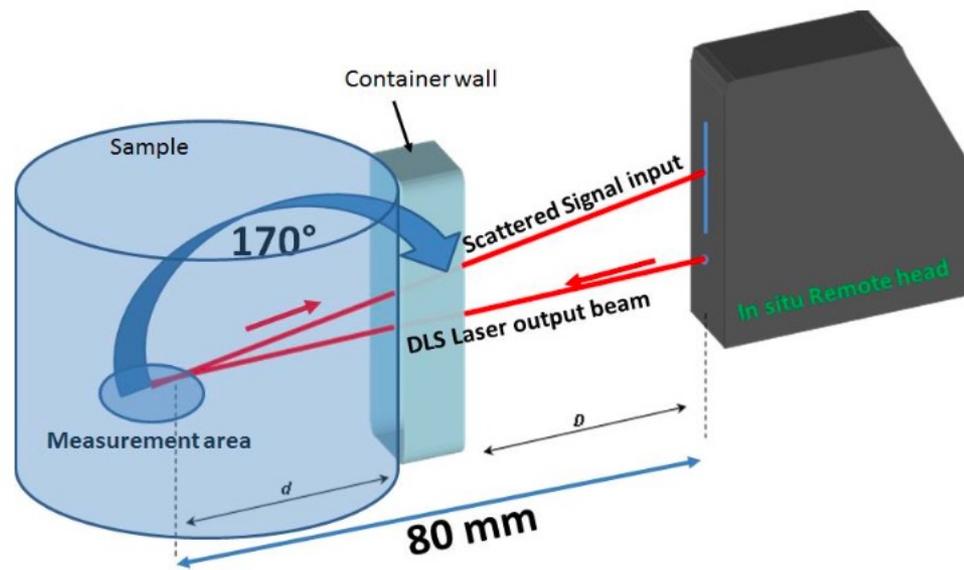






# Analysing The Correlation Function

- › 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



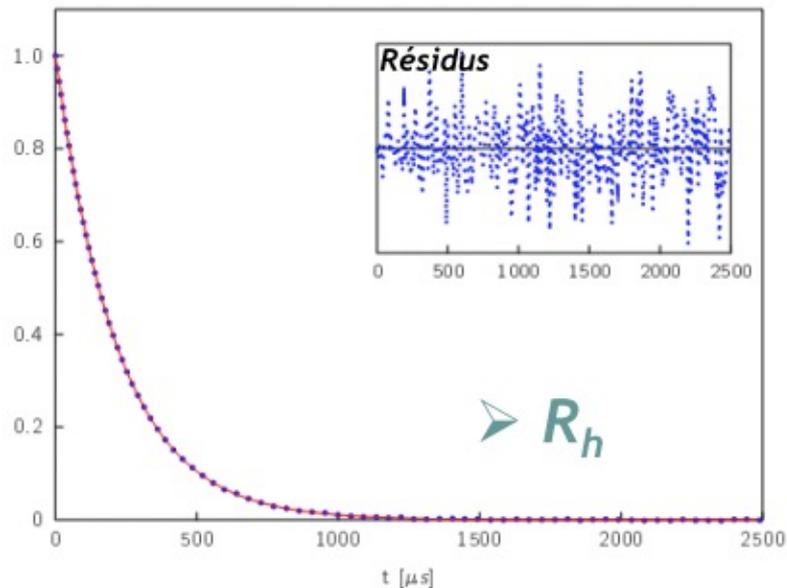
# Analysing The Correlation Function

## Fonction d'auto-correlation

Systeme monodisperse

$$g_2(\tau) \propto \exp(-2Dq^2\tau)$$

➤ Algorithme des cumulants

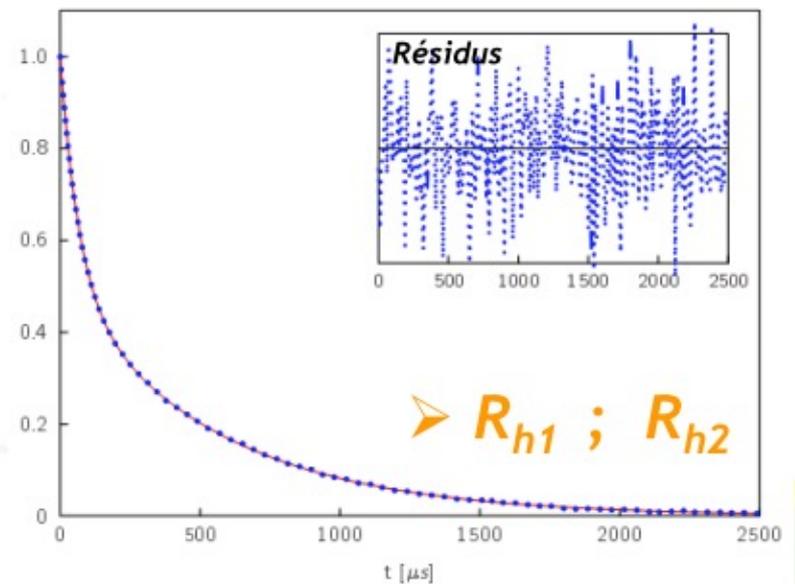


Experimental data and cumulant's fit

Systeme polydisperse

$$g_2(\tau) \propto \sum A_i \exp(-2D_i q^2 \tau)$$

➤ Algorithme de Padé-Laplace



Experimental data and Padé-Laplace fit

# 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**)

**$d_z$  = z-average**

**Pdl = polydispersity index**

- › Only the dispersant refractive index and viscosity are required for this analysis

# The z-Average Diameter

## › Definition of the z-Average Diameter ( $d_z$ ):

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 (Pdl)

## › Definition of the Polydispersity Index (Pdl):

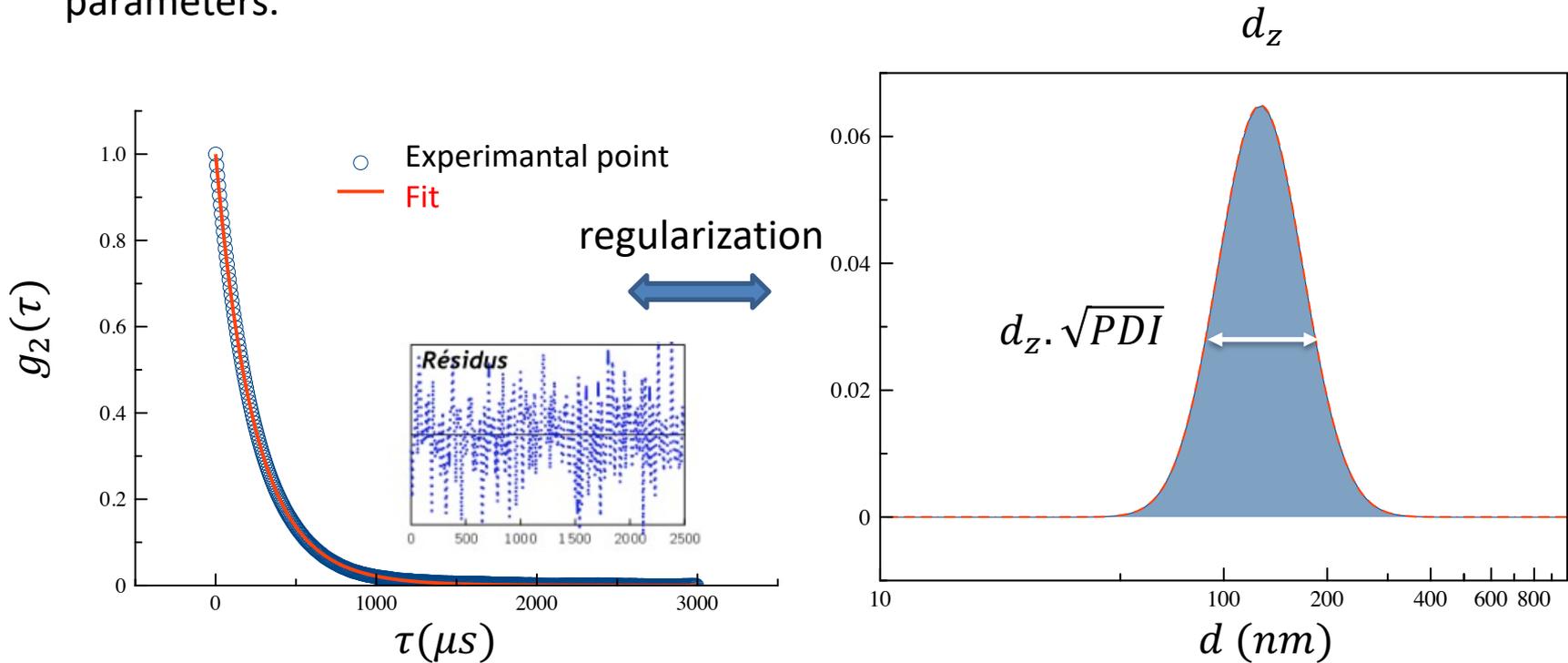
A dimensionless measure of the broadness of the size distribution calculated from the cumulants analysis

› In the 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

# Cumulants Analysis: dz and Pdl by regularization technique

regularization techniques correspond to imposing Gaussian distributions on model parameters.



$$g_2(\tau) = \sum \exp(-2Dq^2\tau) \quad \begin{array}{l} \text{regularization} \\ \longleftrightarrow \text{ Gaussian distribution} \\ \text{Residues criteria minimization} \end{array}$$

# Polydispersity Index (PDI)

## 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 PDI. It is the range over which the distribution algorithms best operate over

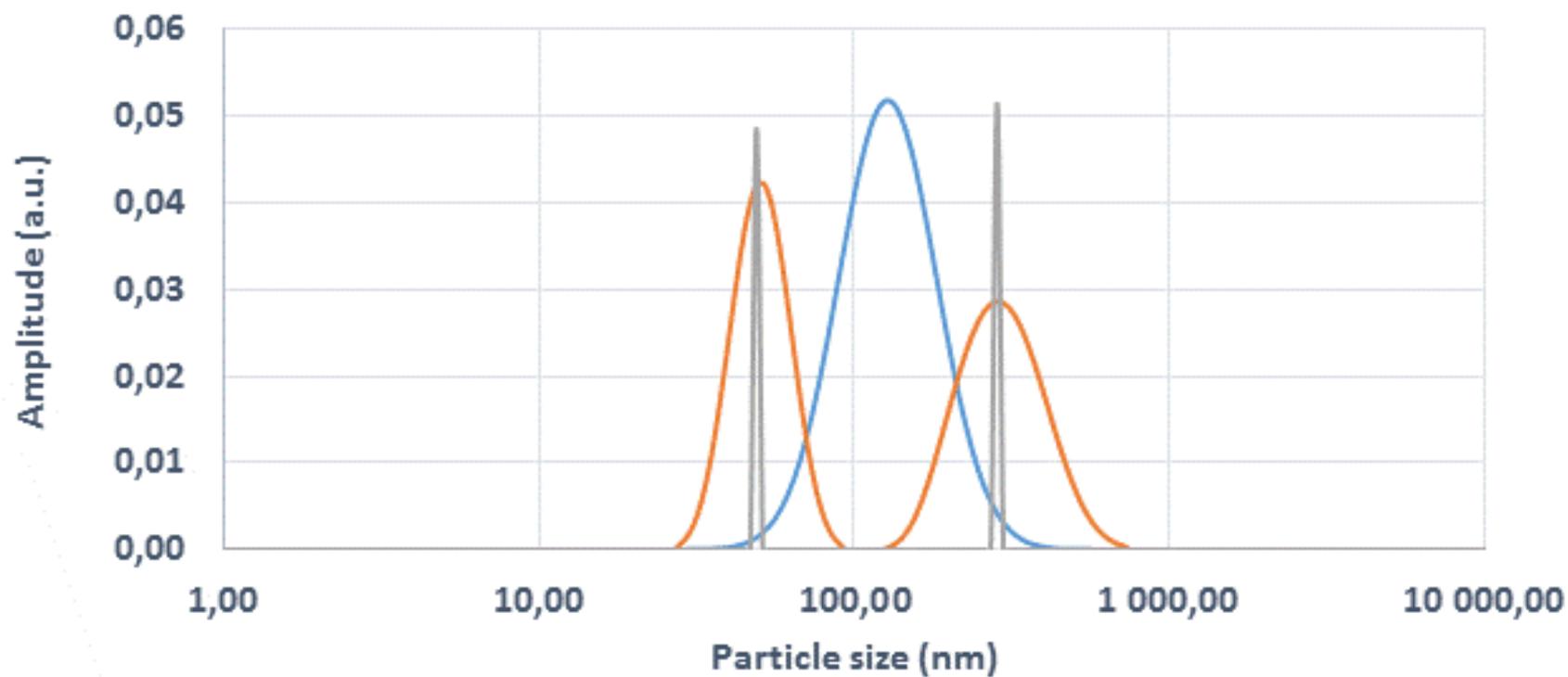
>0.7

Indicates a very broad distribution of particle sizes

# Distribution Analysis

- › Default algorithms present in the Cordouan software for calculating the size distribution are:
  - **Cumulant** is assuming that there is only one main size of NPs with a specific Gaussian distribution,
  - **Padé-Laplace** is assuming that there is a discrete number of different sizes
  - **SBL (L-curve)** is looking for multi-modal and multi dispersity size distribution.
- › The difference between these algorithms is the regularizer used

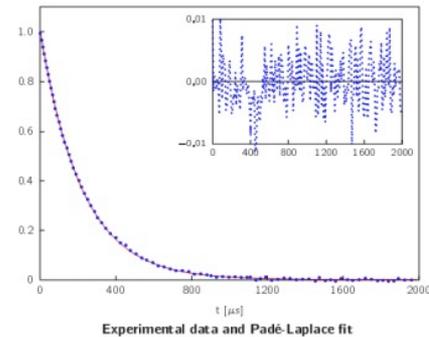
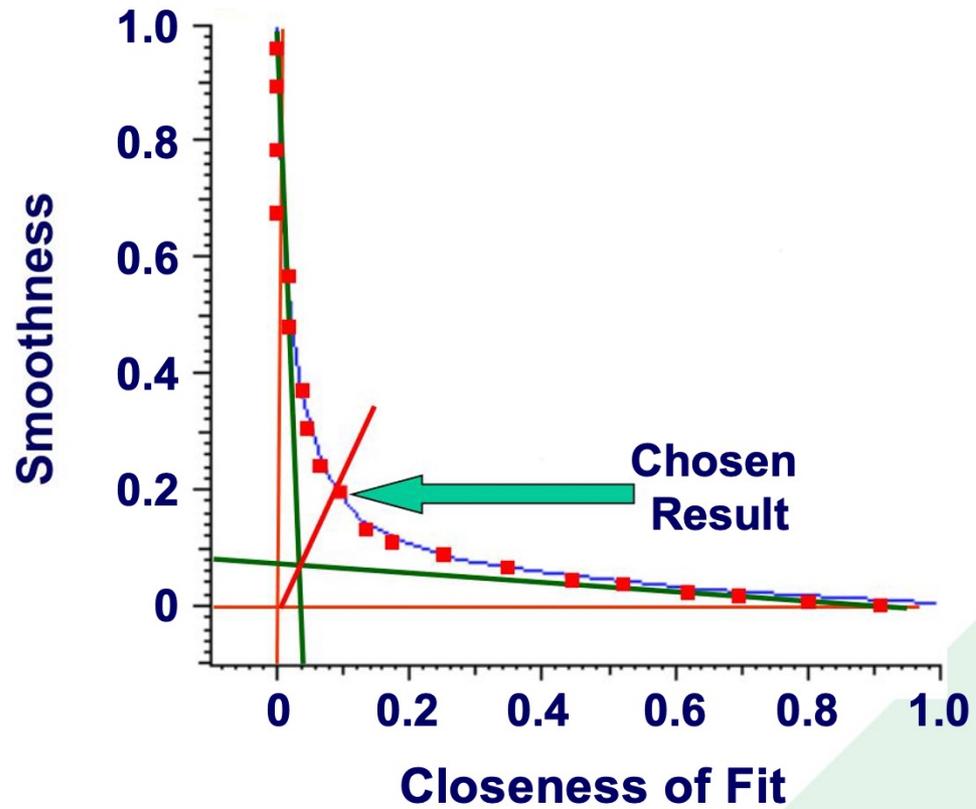
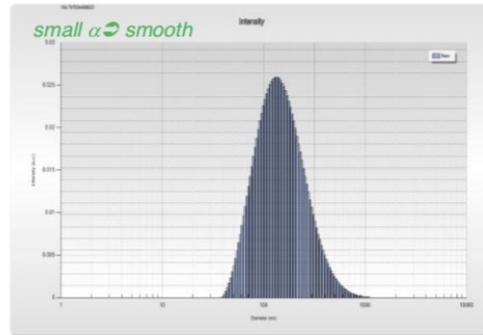
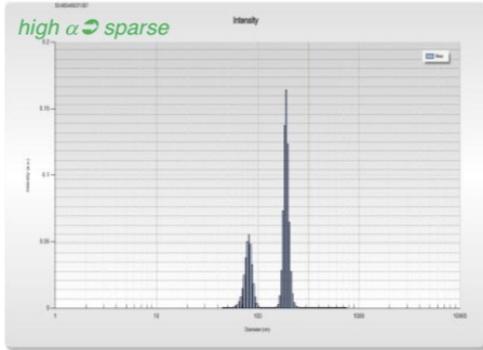
### Cumulant vs Pade Laplace vs SBL



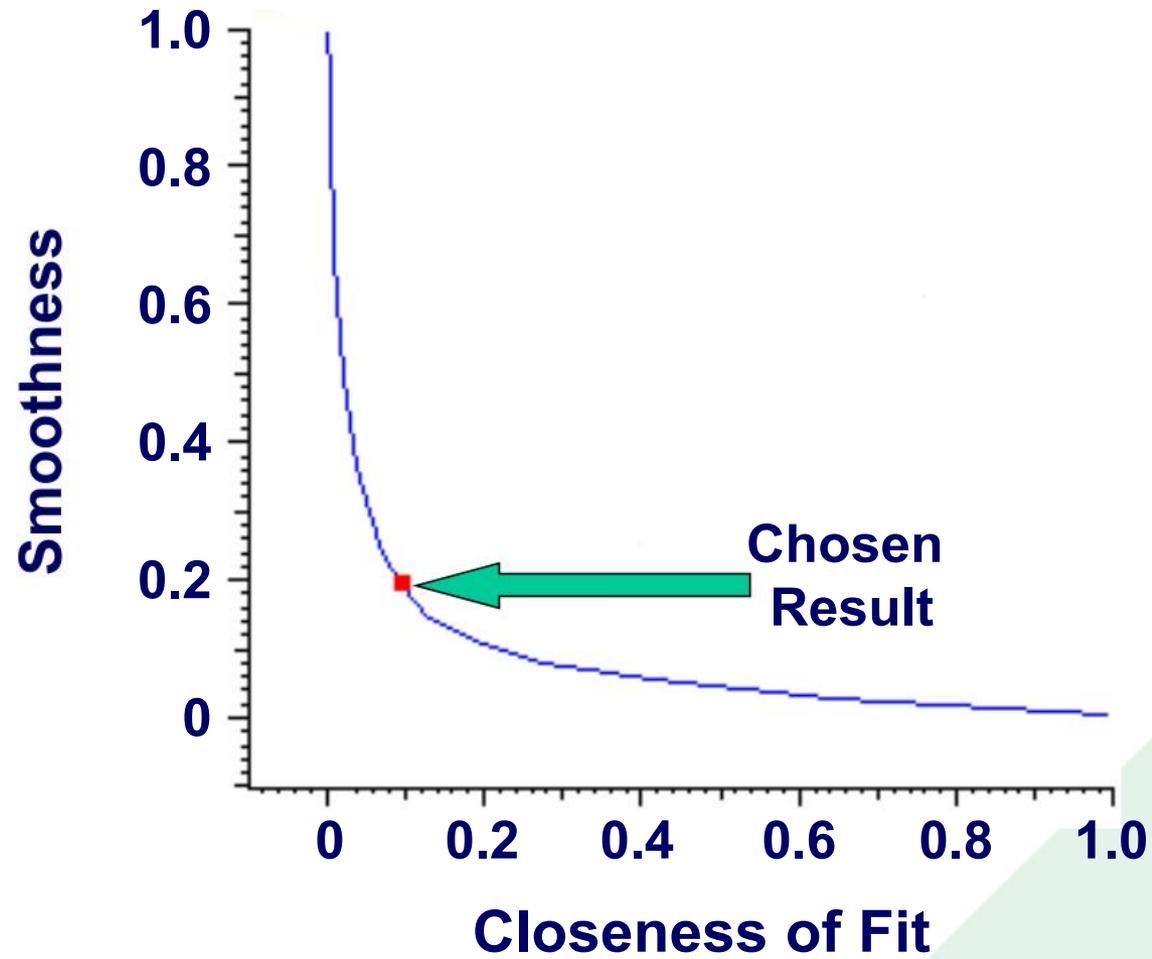
## **SBL (L-curve): Sparse Bayesian Learning**

- › 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

# SBL (L-curve)

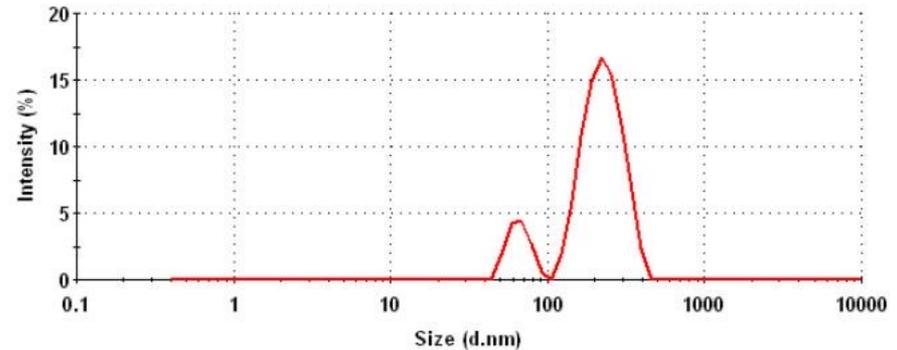


# SBL (L-curve)



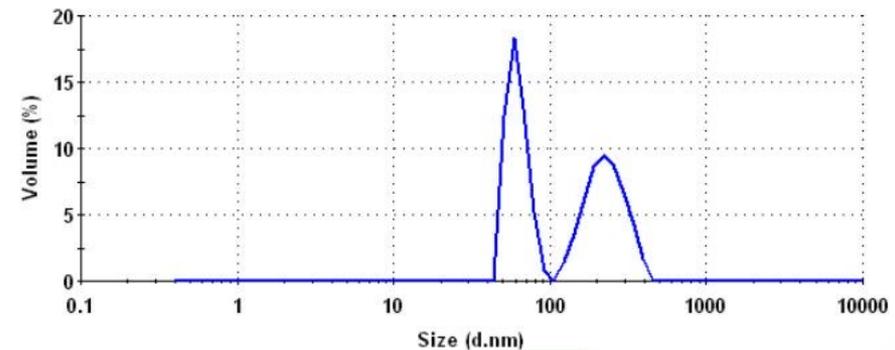
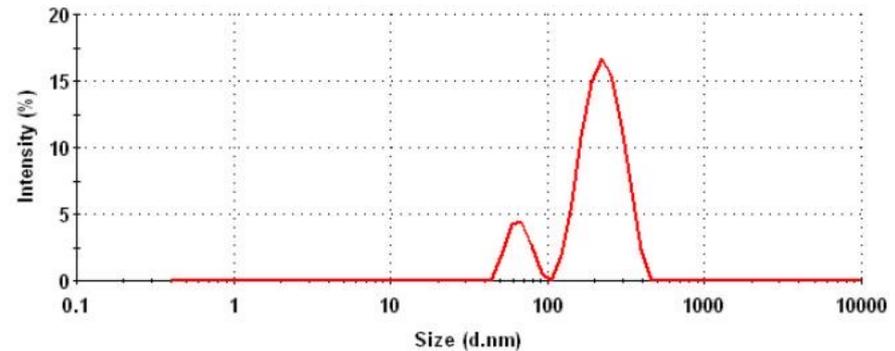
# Intensity Size Distributions

- 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

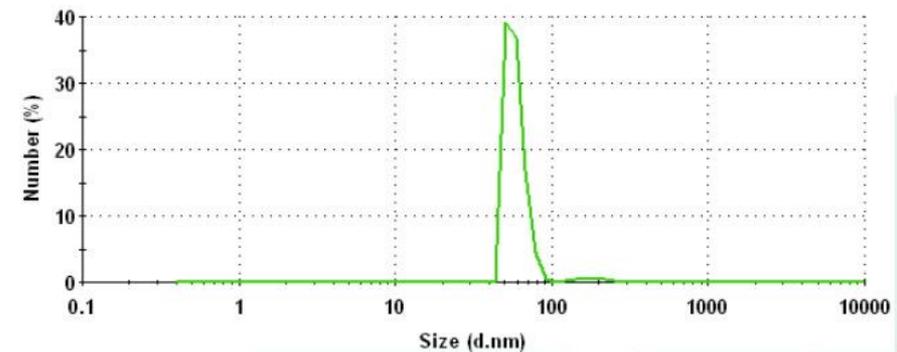
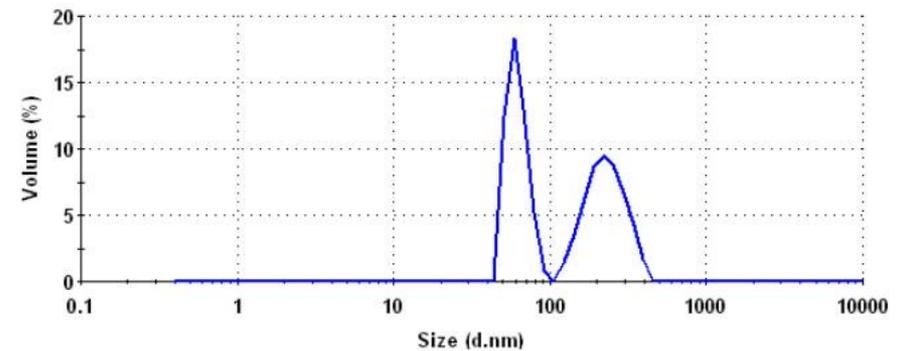
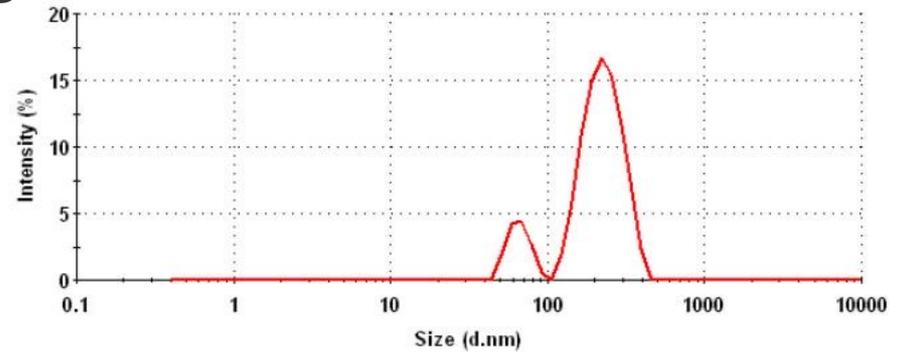
- › Derived from the intensity distribution using Mie theory
- › Equivalent to the mass or weight distribution
- › The optical properties of the particles are required to make this transformation
  - Particle refractive index
  - Particle absorption



# Number Size Distributions

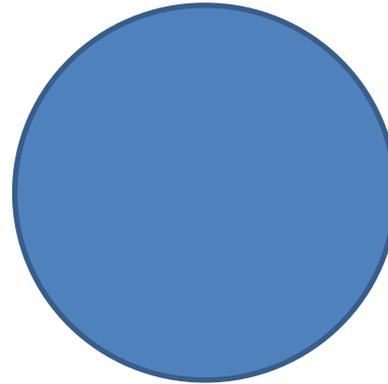
- Derived from the intensity distribution using Mie theory
- The optical properties of the particles are required to make this transformation
  - Particle refractive index
  - Particle absorption

**no requirement for a size below 200 nm**

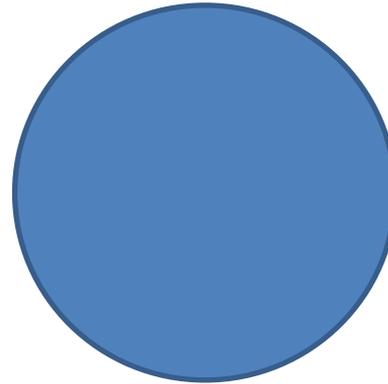


# Size Distributions From DLS

- 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



Size (nm)	10	100
Intensity $\propto d^6$	1	1
<b>Intensity %</b>	<b>50%</b>	<b>50%</b>
Volume ( $d^3$ ) $\propto I/d^3$	$1/10^3$	$1/100^3$
V proportion	1000	1
<b>Volume%</b>	<b>99,9%</b>	<b>0,1%</b>
Number $\propto V/V_{\text{particle}} \propto V/d^3$	$1000/10^3$	$1/100^3$
N proportion	$10^6$	1
<b>Number %</b>	<b>100%</b>	<b>0 %</b>



Size (nm)	10	100
Number proportion	1	1
<b>Number %</b>	<b>50%</b>	<b>50%</b>
Volume $\propto N \cdot d^3$	$10^3$	$100^3$
Volume proportion	1	1000
<b>Volume %</b>	<b>0.1%</b>	<b>99.9%</b>
Intensity $\propto 10^6$	$10^6$	$100^6$
Intensity proportion	1	$10^6$
<b>Intensity %</b>	<b>0%</b>	<b>100%</b>

# 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**

# 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**

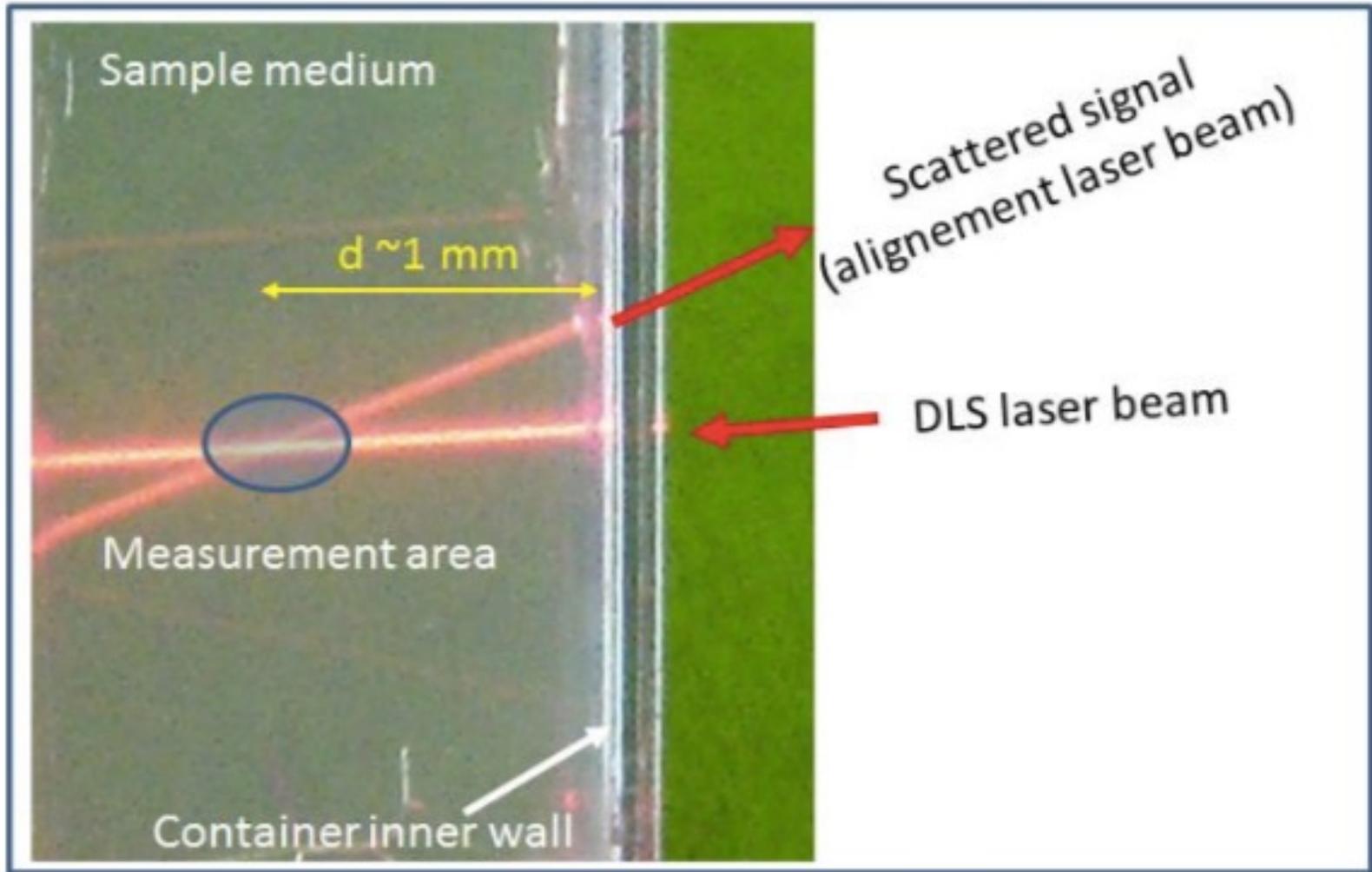
## Upper Size Limit of DLS: Sedimentation

- 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
- look for stable count rate over multiple measurements on same sample (decreasing count rate indicates loss of particles due to sedimentation).
- **sedimentation is not aggregation** (increasing in size over multiple measurement indicates aggregation)

# 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

# Upper Size Limit of DLS: Number Fluctuations



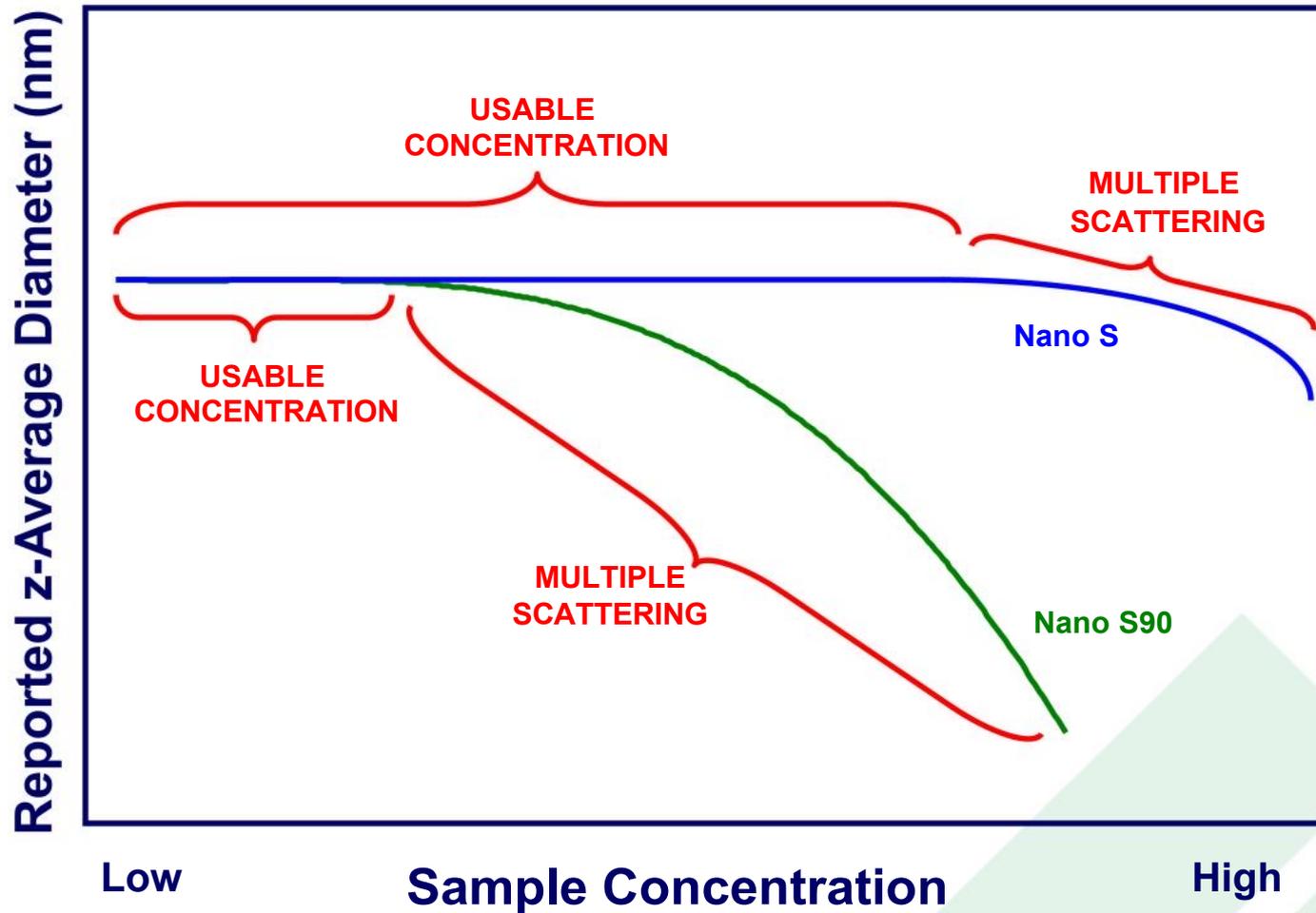
› **Upper Concentration Limit** is sample dependent  
and is defined by

- **Multiple Scattering**
- **Restricted Diffusion**
- **Particle Interactions**

## Upper Concentration Limit: Multiple Scattering

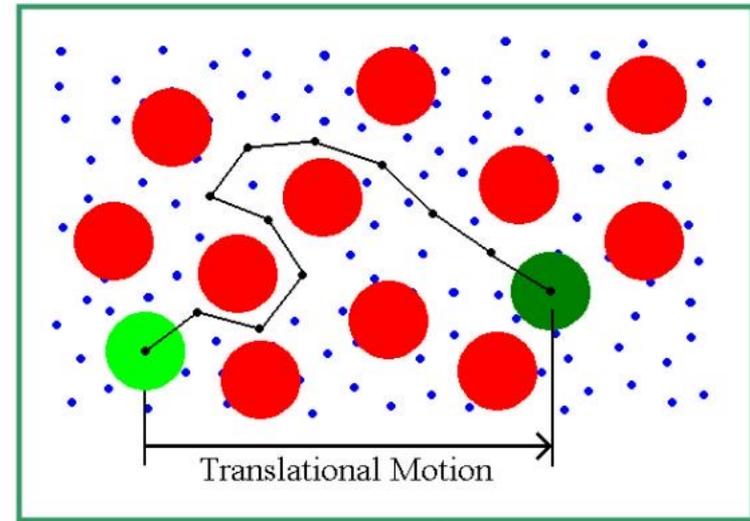
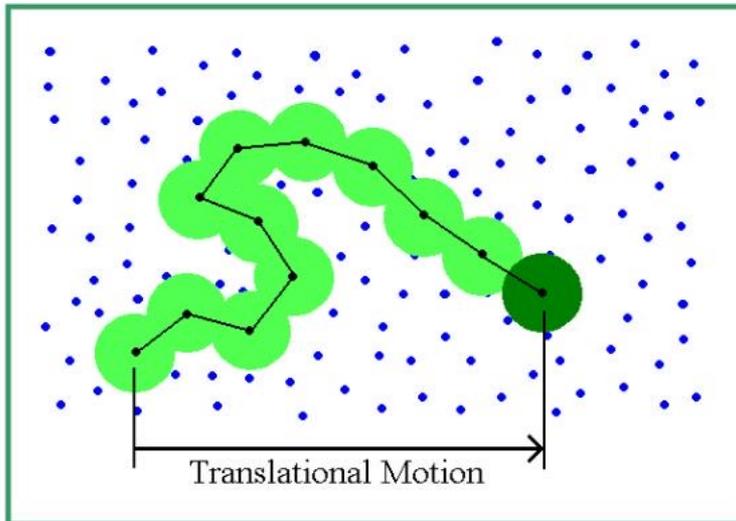
- The ideal situation in a DLS measurement is to have singly scattered light
- This means that every photon which reaches the detector was scattered by only one particle
- This will be the case for samples at low concentrations
- However, as the sample concentration is increased, the probability of the 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

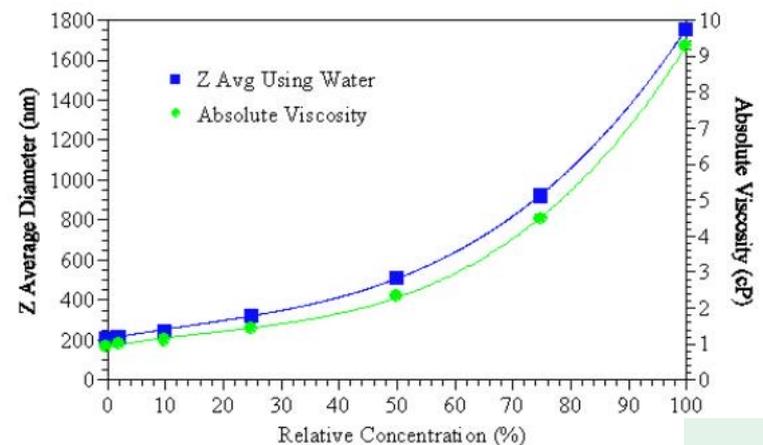
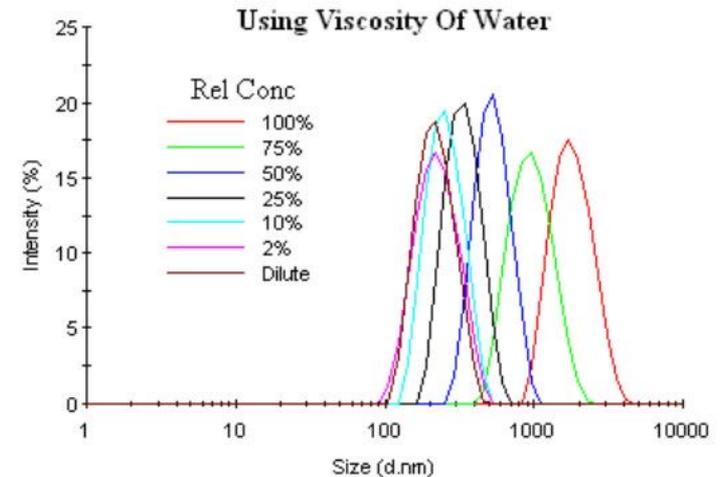
- Restricted diffusion describes the phenomenon where the presence of other particles hinders free particle diffusion



**Same distance - longer time**

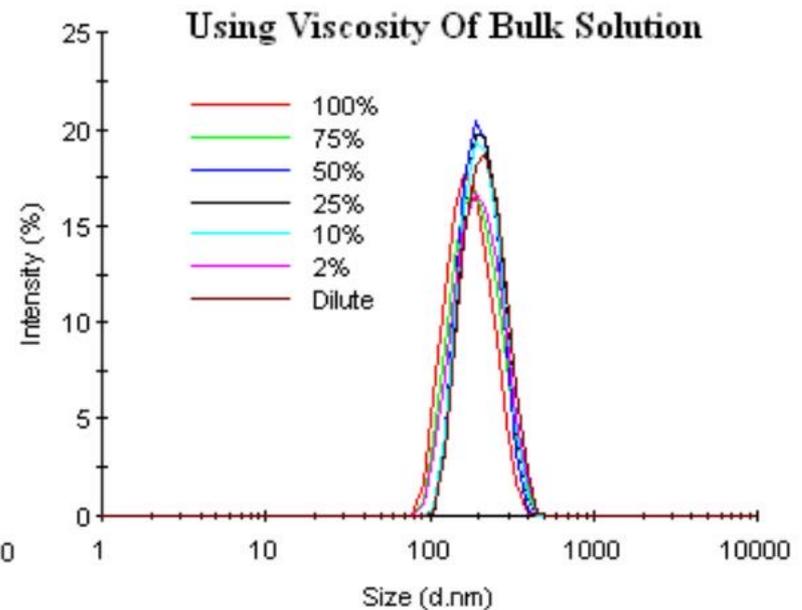
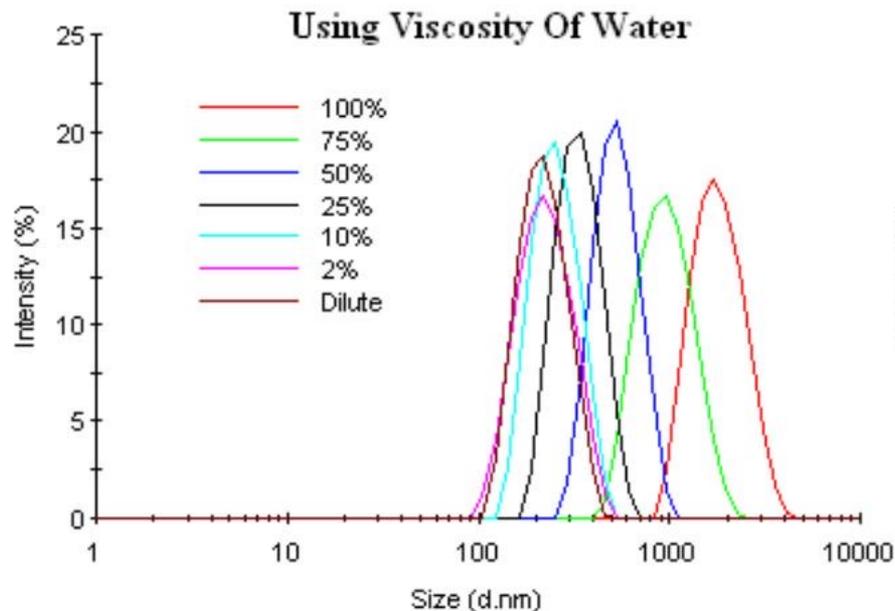
# Upper Concentration Limit: Restricted Diffusion

- **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



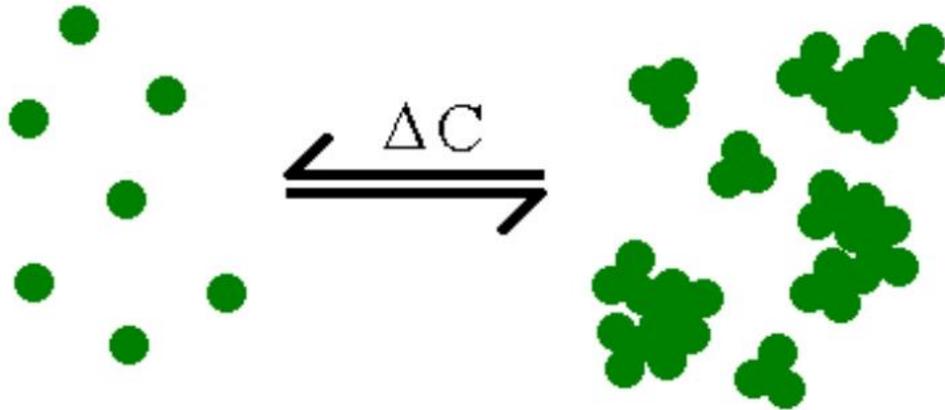
# Upper Concentration Limit: Restricted Diffusion

- 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

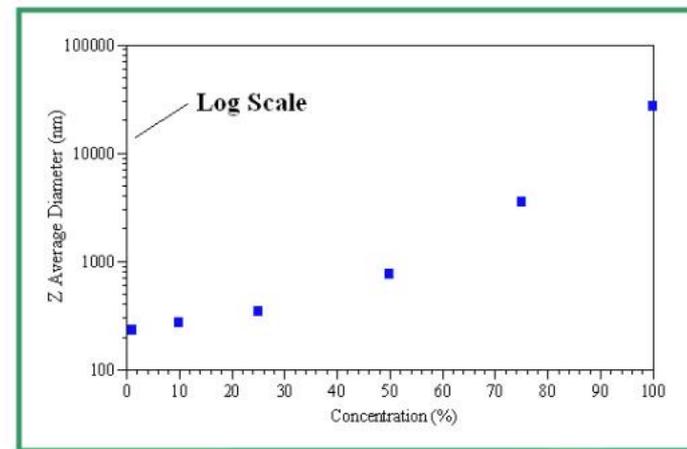
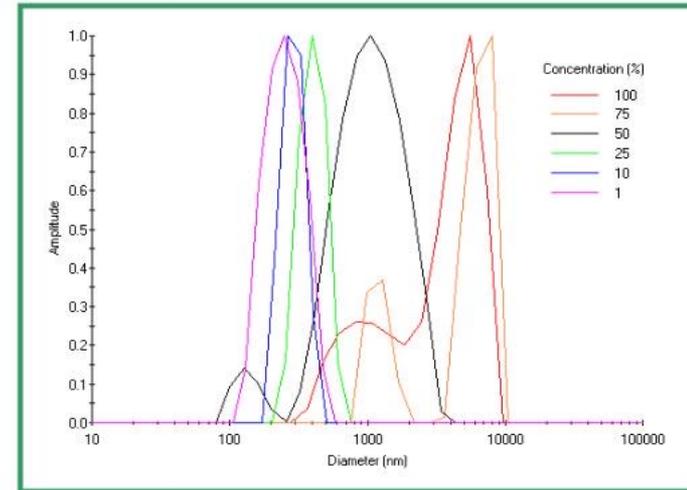
- › At higher concentrations, particle interactions can modify the free diffusion of particles and this can lead to non-specific aggregation that modifies the size distribution obtained



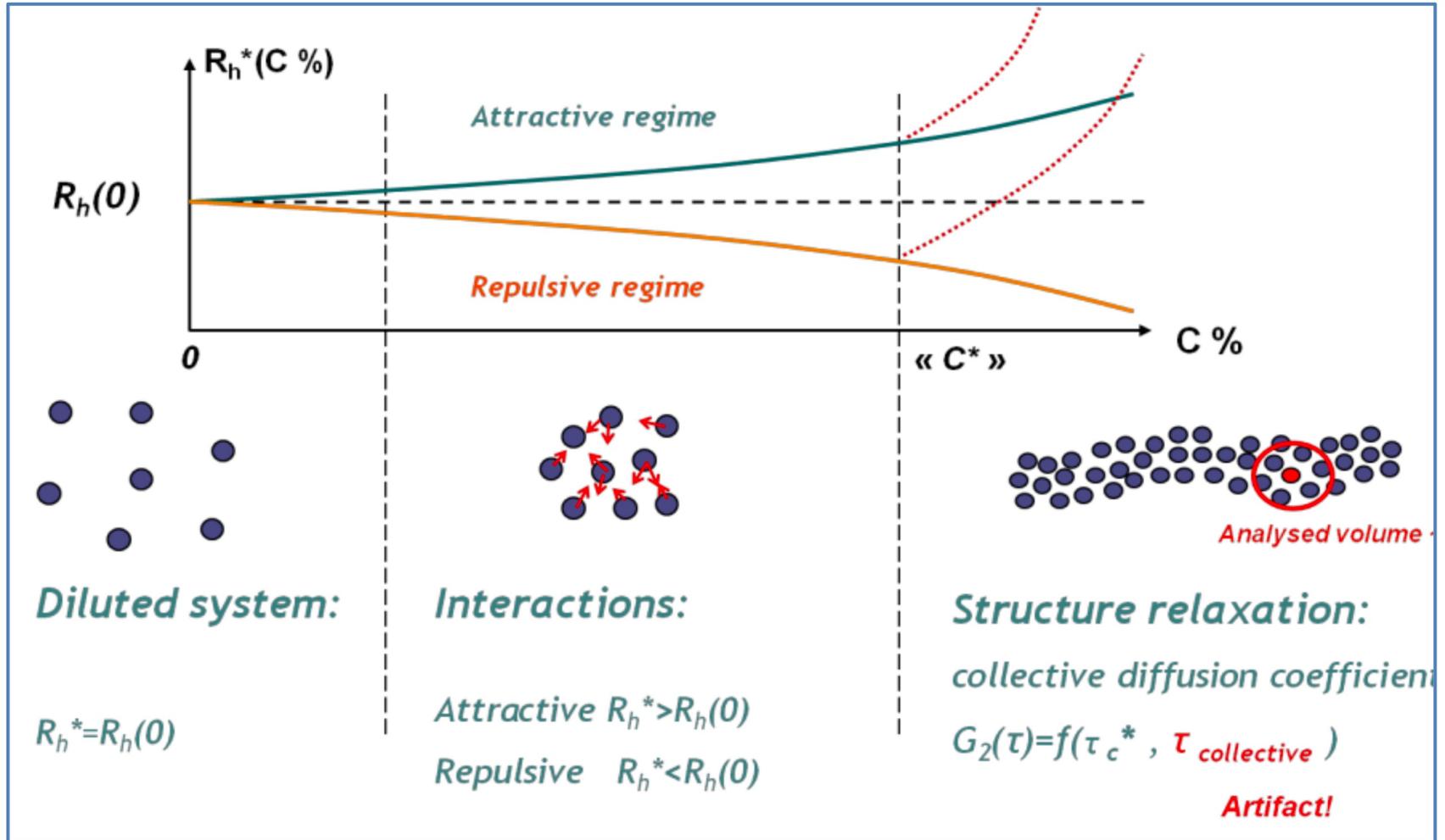
# Upper Concentration Limit: Particle Interactions

## ➤ 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**



# Upper Concentration Limit: Particle Interactions



# 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 and multiple experiments**

# 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  $\mu\text{m}$  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



# Cell Cleaning

- › Non-disposable cuvettes 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](http://www.hellma-worldwide.de))
    - Recommend 2% v/v concentration
    - Soak cuvettes for 20 minutes
    - Ultrasonication aids cleaning action

# Exemple of application

## Aims of this Section.....

- 1 Détermination of the CCC by Time-resolved DLS:  
application to nanoplastic in sea water

# Effect of the Surface Hydrophobicity–Morphology–Functionality of Nanoplastics on Their Homoaggregation in Seawater

Cloé Veclin, Cloé Desmet, Alice Pradel, Andrea Valsesia, Jessica Ponti, Hind El Hadri, Thomas Maupas, Virginie Pellerin, Julien Gigault, Bruno Grassl,\* and Stéphanie Reynaud



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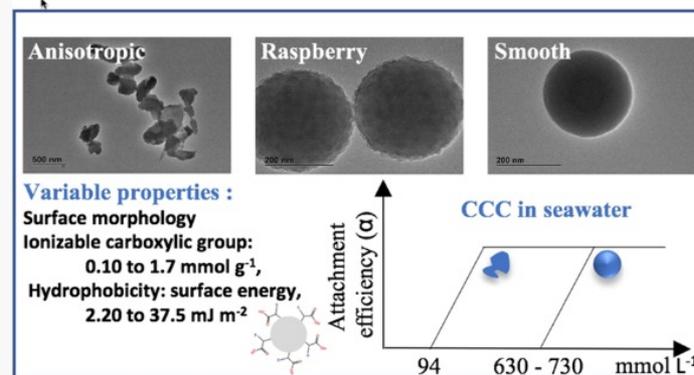
Article Recommendations



Supporting Information

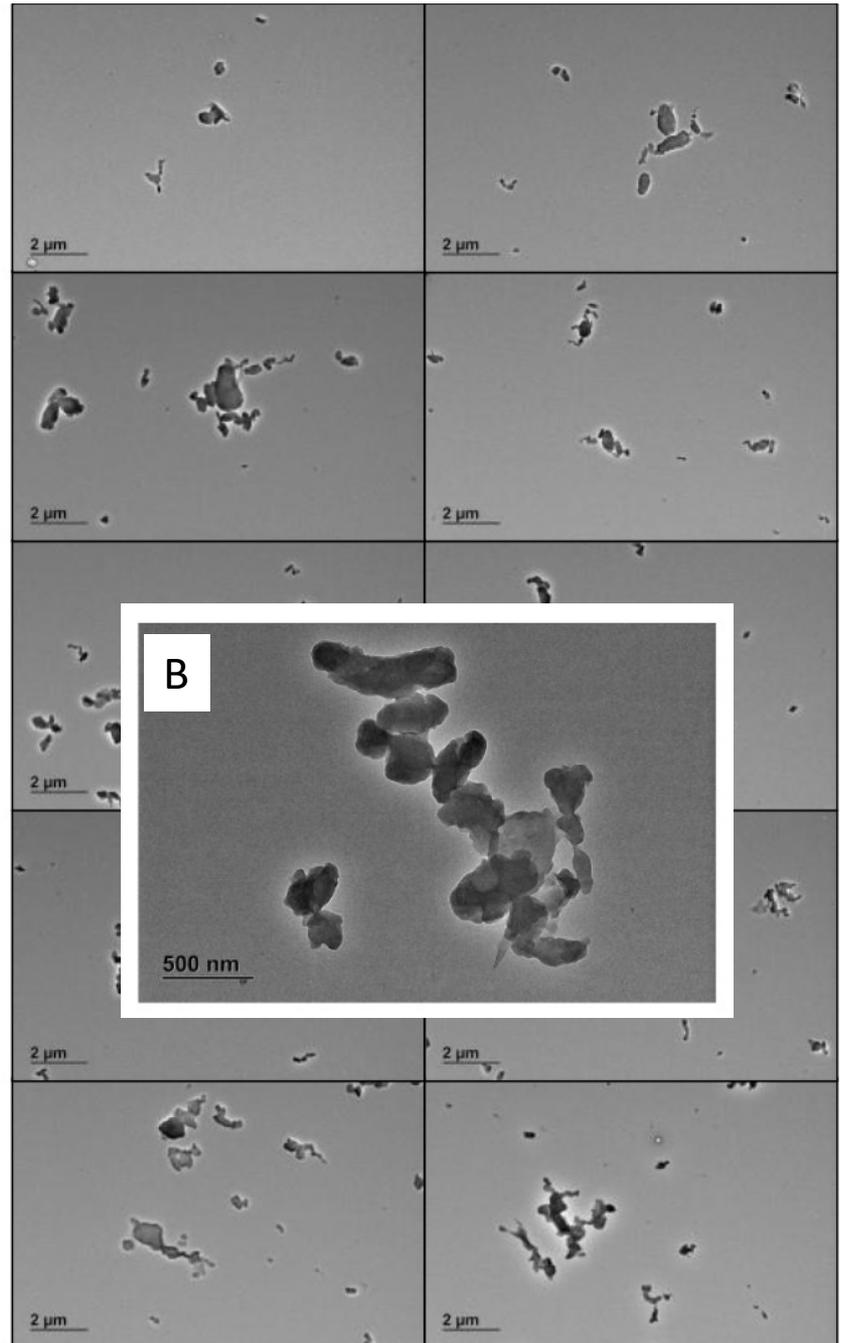
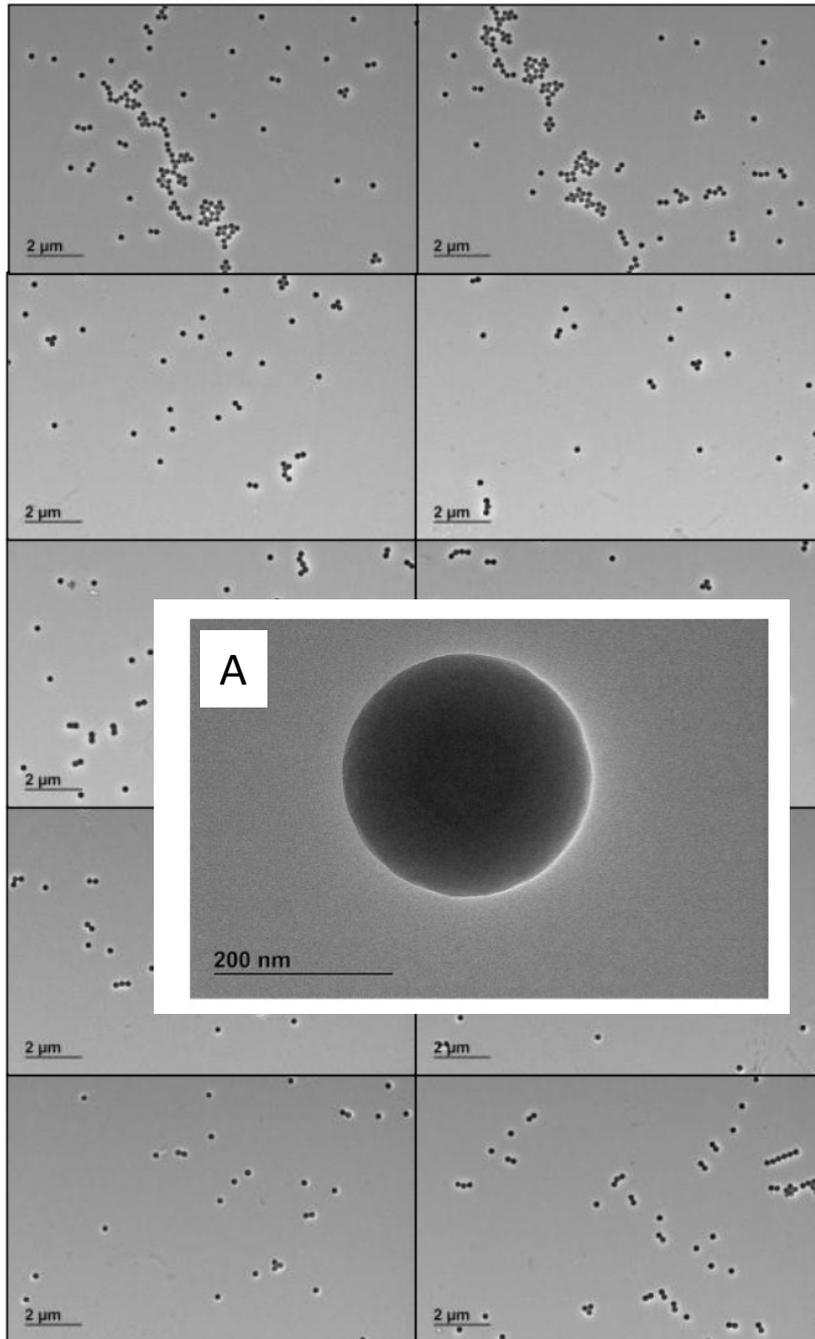
**ABSTRACT:** The way nanoplastics aggregate in the environment is one of the key properties that control their final fate and impact on the environment. In the present work, to better predict their transportation pathways, nanoplastic homoaggregation was studied in saltwater to predict the behavior in seawater. We designed nanoplastic models that are free of additives with a chemical control of the surface to model surface weathering. The samples present a wide distribution of relevant surface properties such as functionality (ionizable carboxylic group, 0.10 to 1.7 mmol g<sup>-1</sup>), hydrophobicity (surface energy, 2.20 to 37.5 mJ m<sup>-2</sup>), surface morphology (smooth or “raspberry-textured,”) zeta potential (−31 to −21 mV), and anisotropy in shape. The critical coagulation concentration (CCC) measurements demonstrate that spherical nanoparticles are more stable in seawater (CCC > 600 mmol L<sup>-1</sup>) than anisotropic nanoplastics (CCC ~ 100 mmol L<sup>-1</sup>). The results highlight the importance of considering the surface properties and shape when assessing the behavior of nanoplastics in the environment.

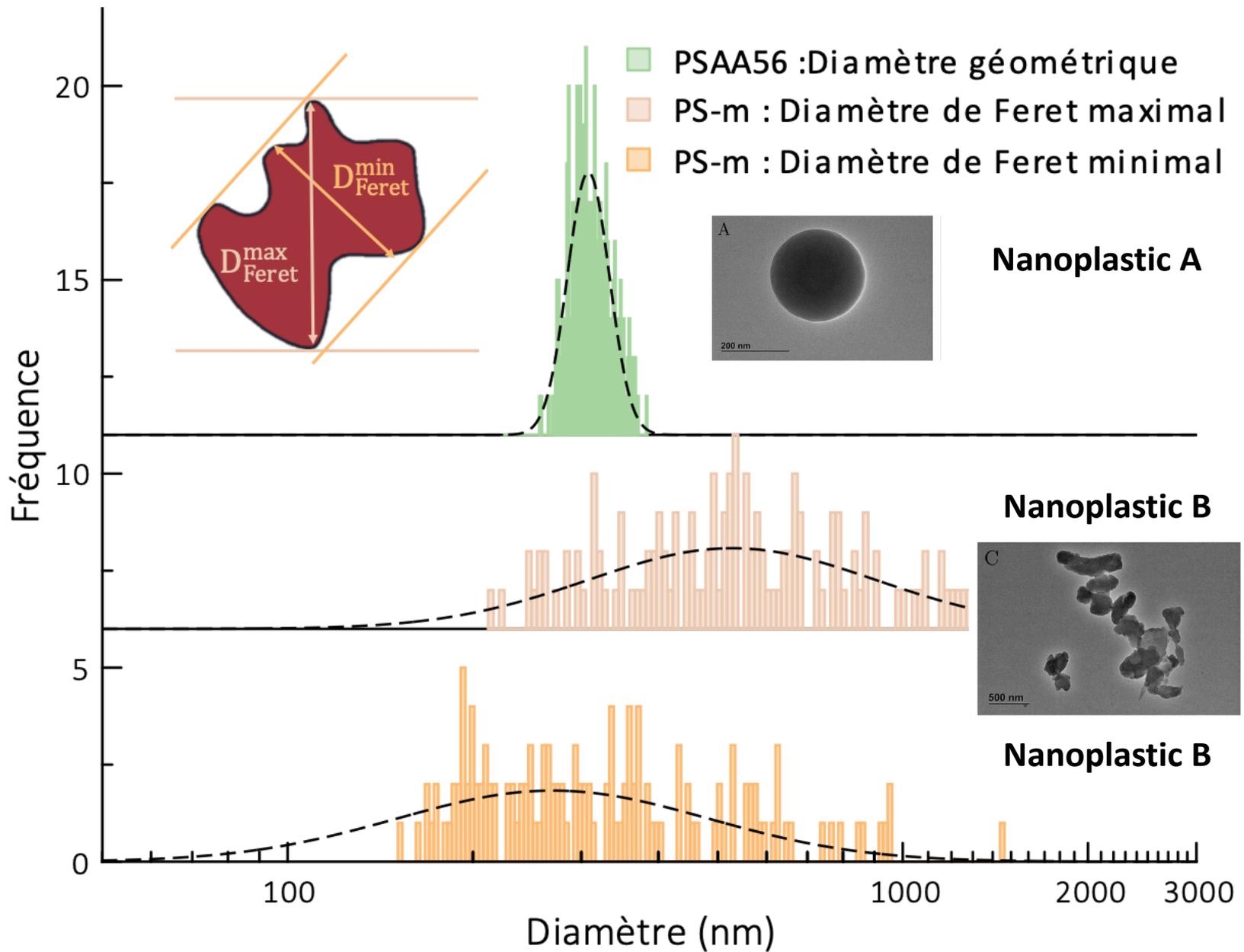
**KEYWORDS:** nanoplastic, aggregation, seawater, colloid, stability

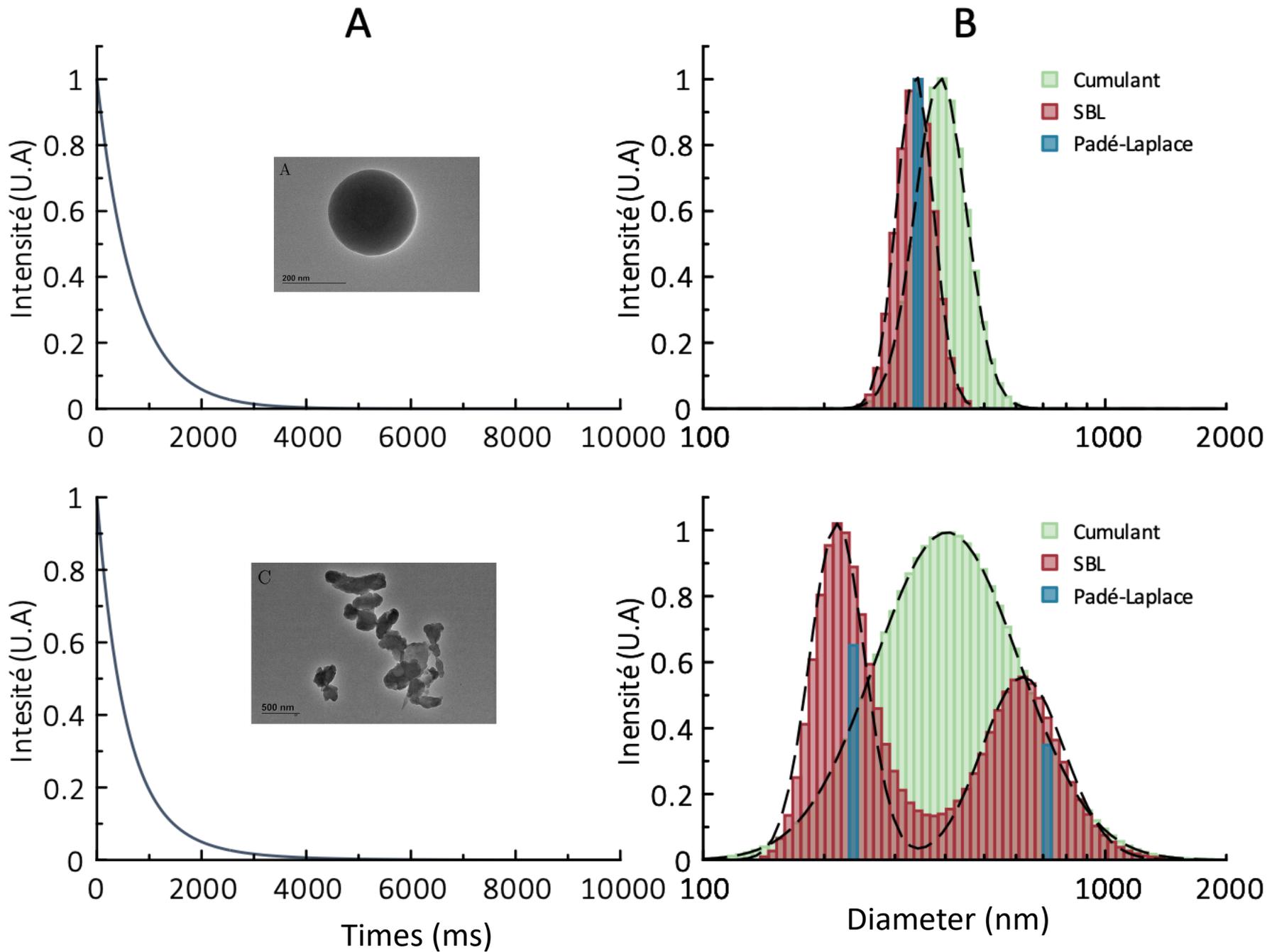


# Nanoplastic A

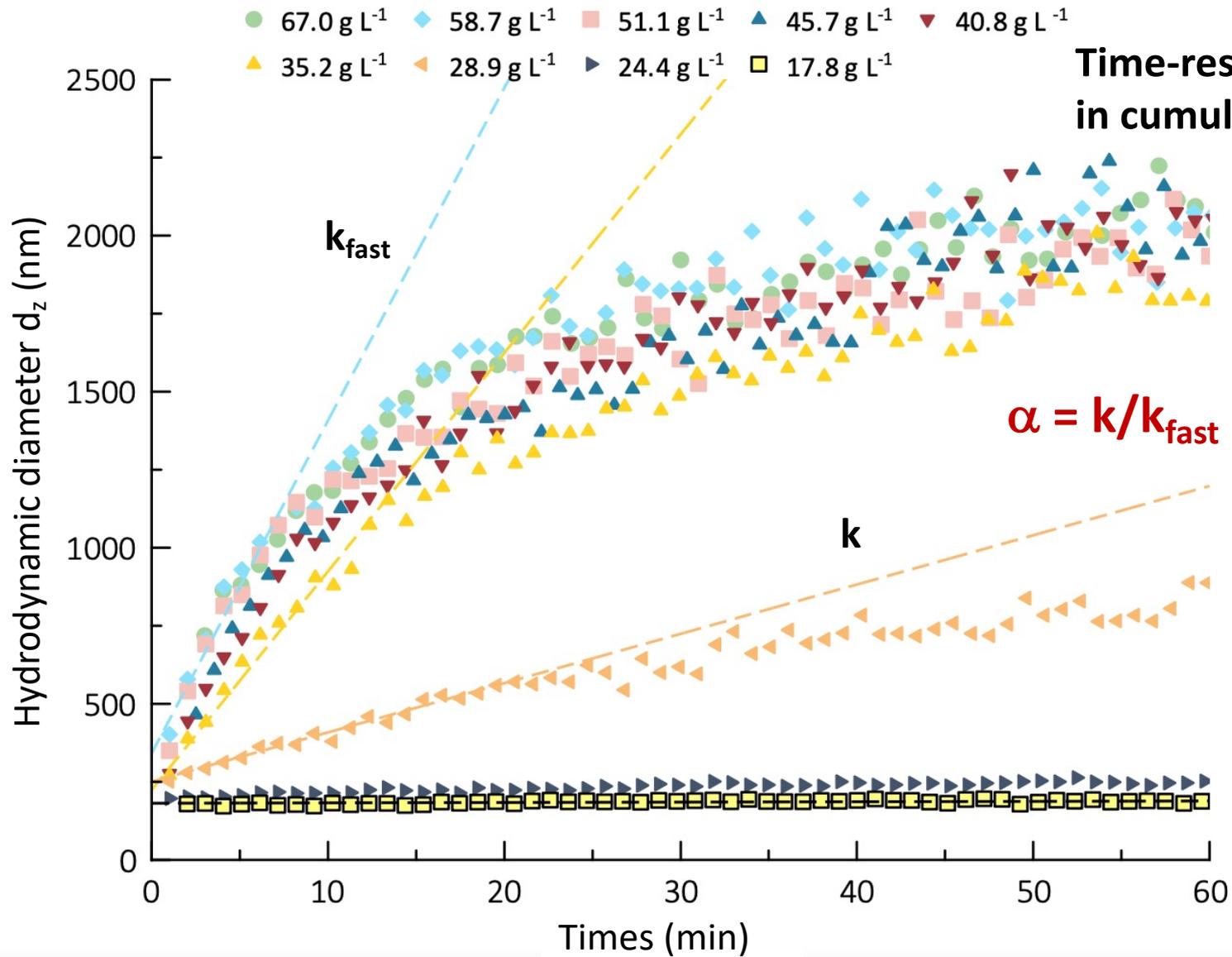
# Nanoplastic B







# Time-resolved DLS in cumulant mode



The aggregation kinetics of nanoplastics are characterized by the **attachment efficiency**  $\alpha$  that is determined by normalizing the **aggregation rate constant**  $k$  for a solution to the rate constant under the fastest aggregation condition  $k_{\text{fast}}$

