HORIBA Scientific
Particle Characterization

Introduction to Dynamic Light Scattering (DLS)

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March 23, 2023

Outline

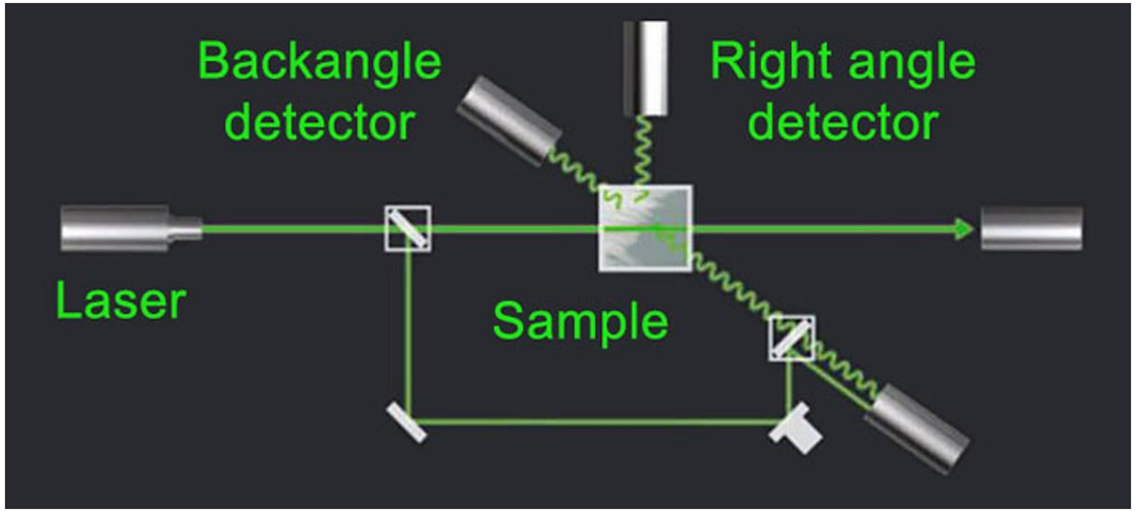
- Introduction
- What is DLS and what does it measure?
- Method development

What is dynamic light scattering?

Dynamic light scattering refers to measurement and interpretation of light scattering data on a <u>microsecond</u> time scale.

Dynamic light scattering can be used to determine

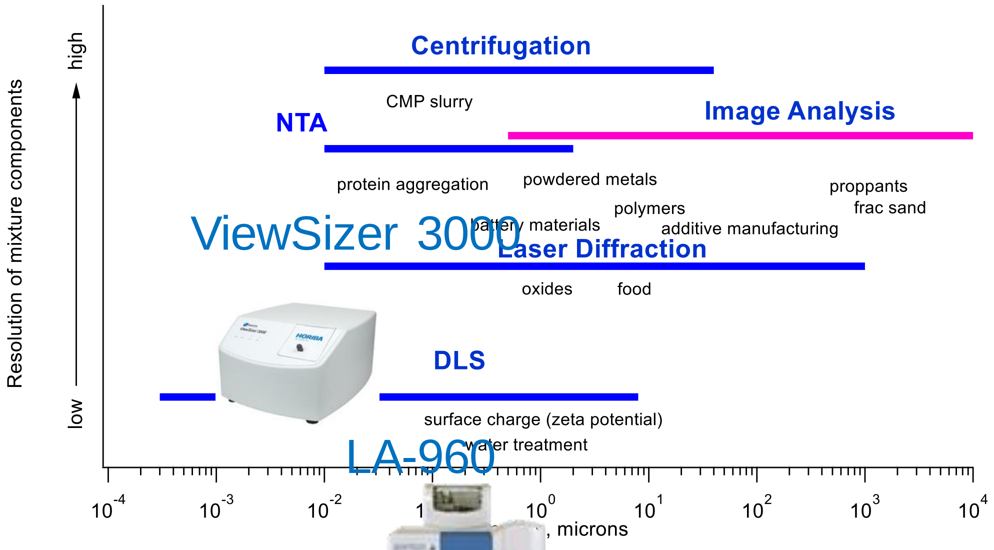
- Particle/molecular size
- Size distribution
- Relaxations in complex fluids



Other light scattering techniques

- **Static Light Scattering**: over a duration of ~1 second. Used for determining particle size (diameters greater than 10 nm), polymer molecular weight, 2nd virial coefficient, R_g.
- **Electrophoretic Light Scattering**: use Doppler shift in scattered light to probe motion of particles due to an applied electric field. Used for determining electrophoretic mobility, zeta potential.
- **Nanoparticle Tracking Analysis (NTA)**: use scattering to track particle location as a function of time, that is, particle motion. Use motion to determine particle size.

Sizing techniques



ViewSizer 3000

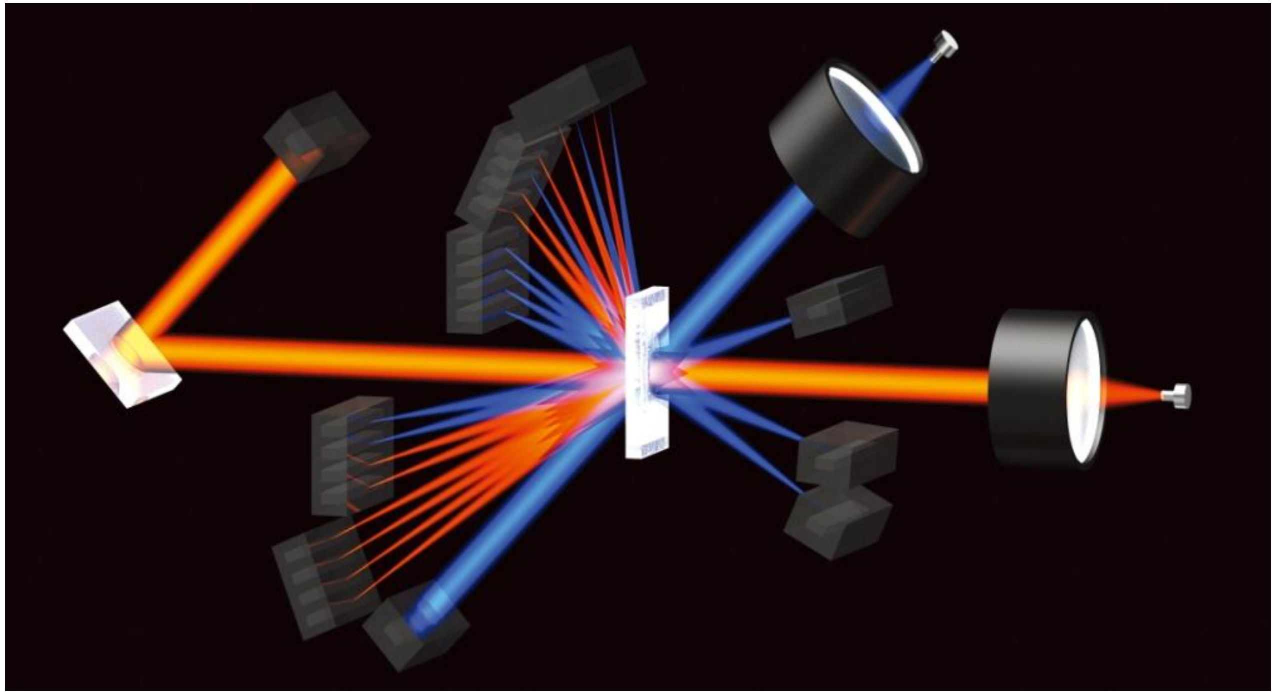
LA-960

PSA-300

LA-350

SZ-100

Laser diffraction



Laser Diffraction

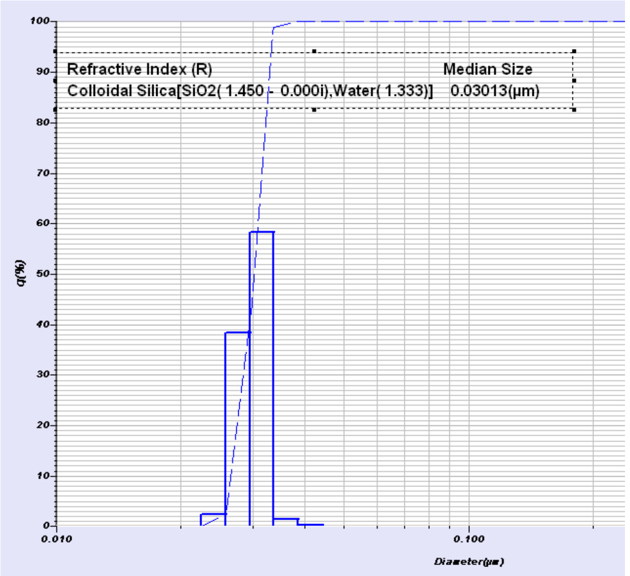
- Particle size 0.01 – 5000 µm
- Converts scattered light to particle size distribution
- Quick, repeatable
- Most common technique
- Suspensions & powders

Laser diffraction

Suspension

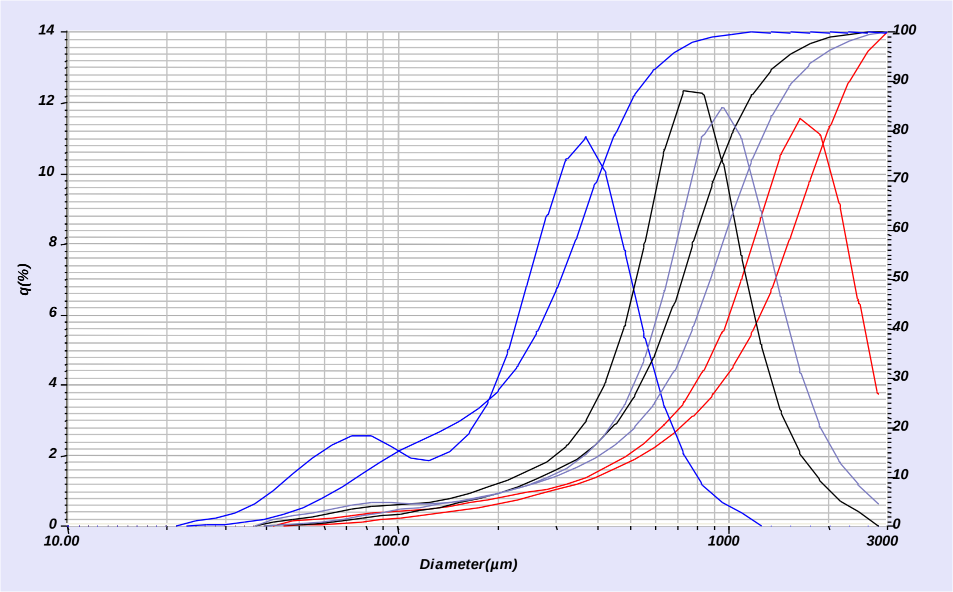
Silica
~ 30 nm

S.P.Area	:	2.0183E+6(cm²/cm³)
Mean Size	:	0.02990(µm)
Variance	:	5.0313E-6(µm²)
Median Size	:	0.03013(µm)
Mode Size	:	0.0302(µm)
Skewness	:	-0.2901



Powders

Coffee Results
0.3 – 1 mm



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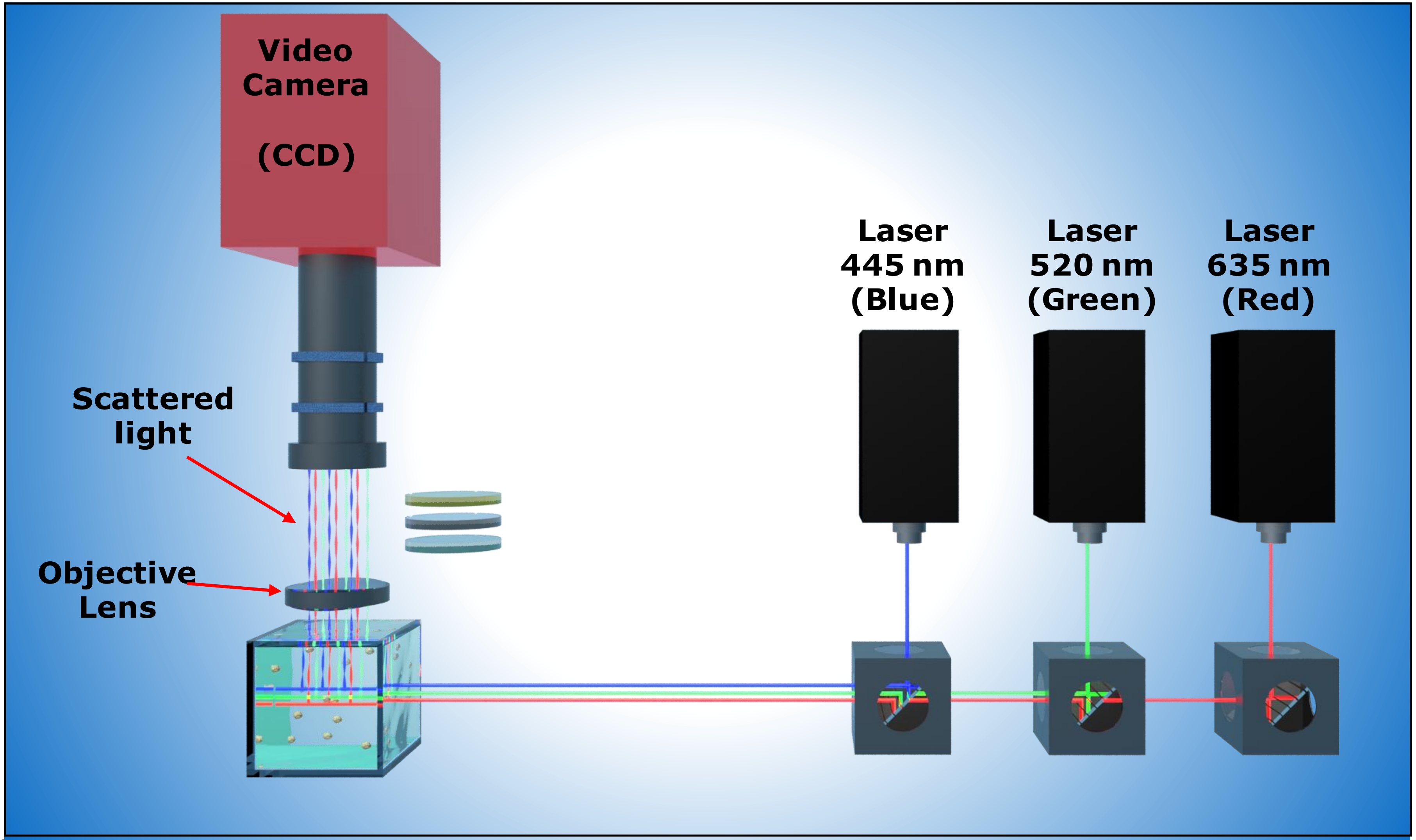
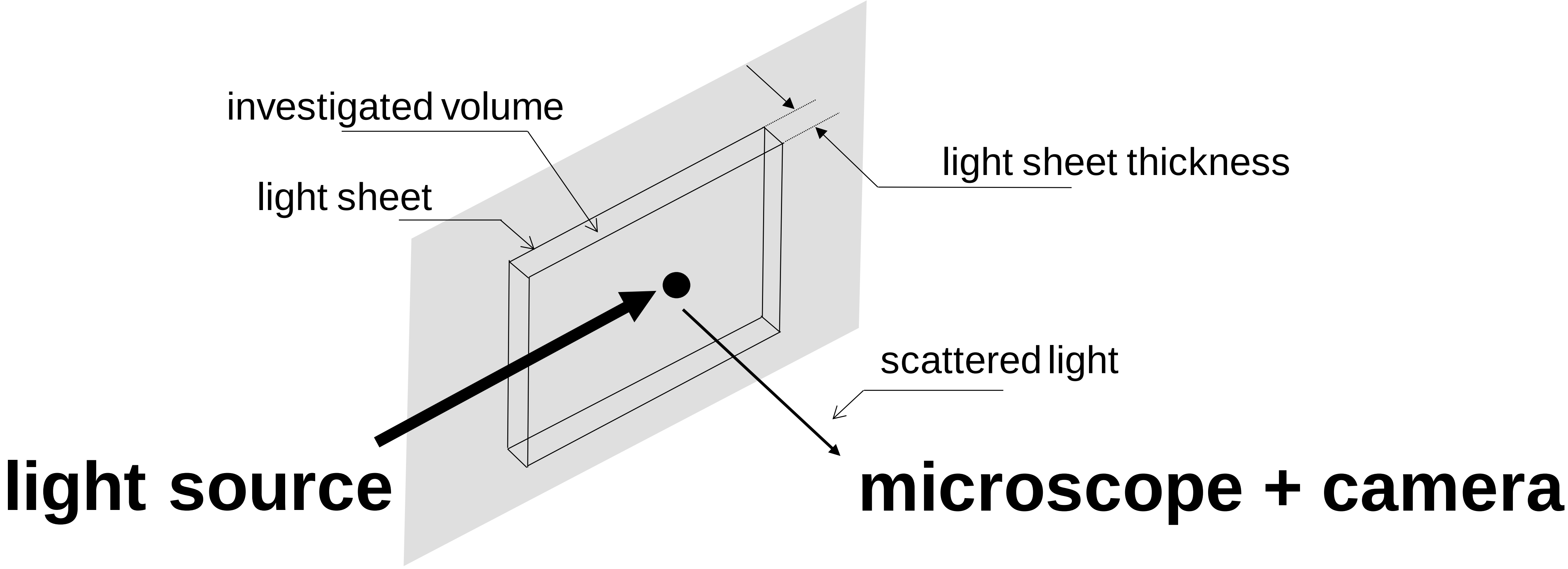
Nanoparticle tracking analysis (NTA)



Nanoparticle tracking analysis (NTA)



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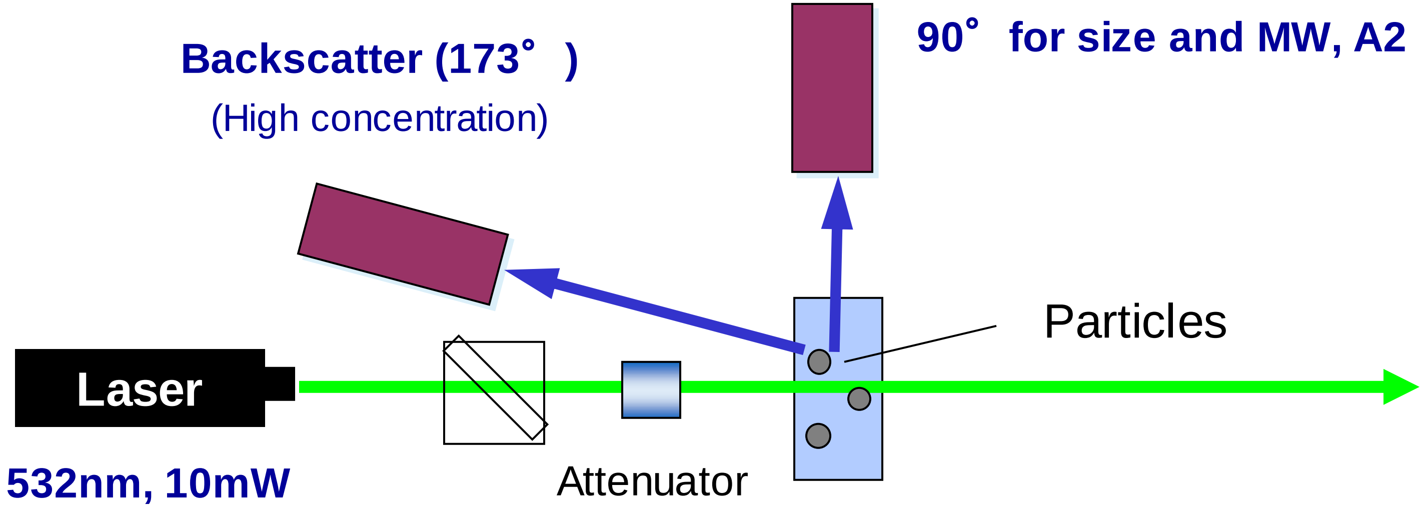
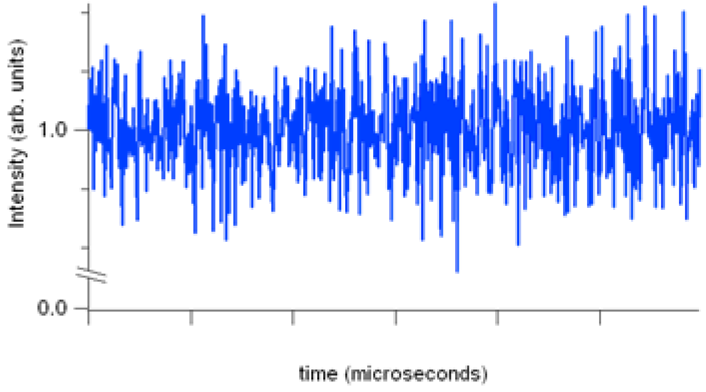
DLS optics

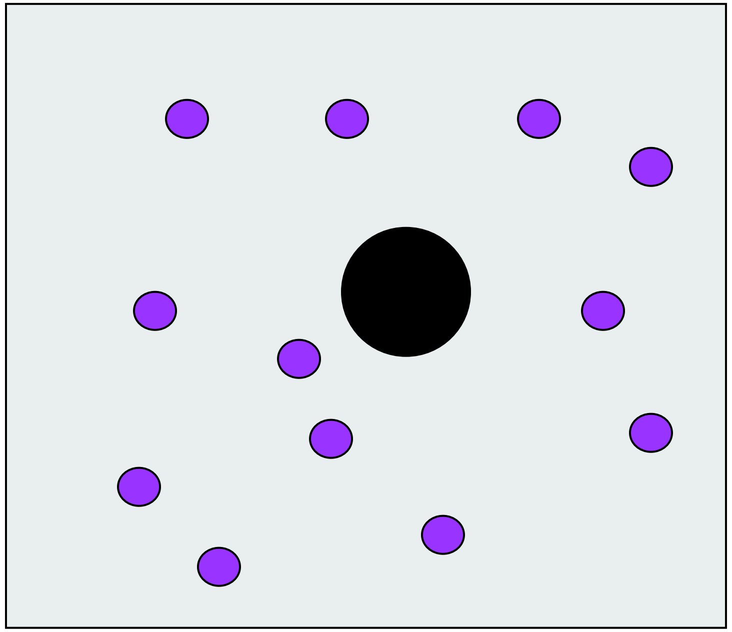




Particles moving due to Brownian motion

Brownian motion

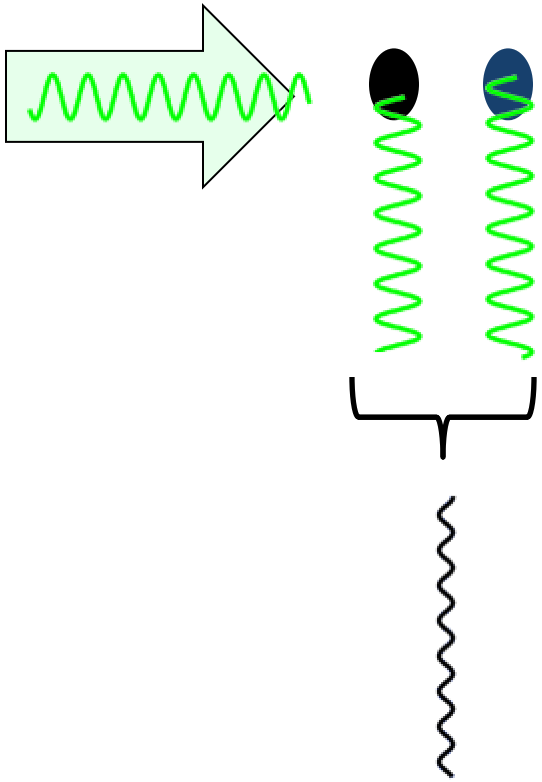
Particles in suspension undergo Brownian motion (random thermal motion).

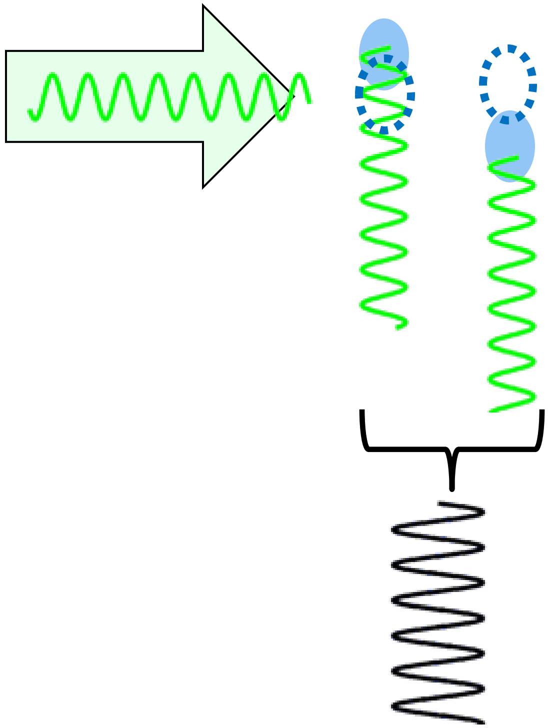


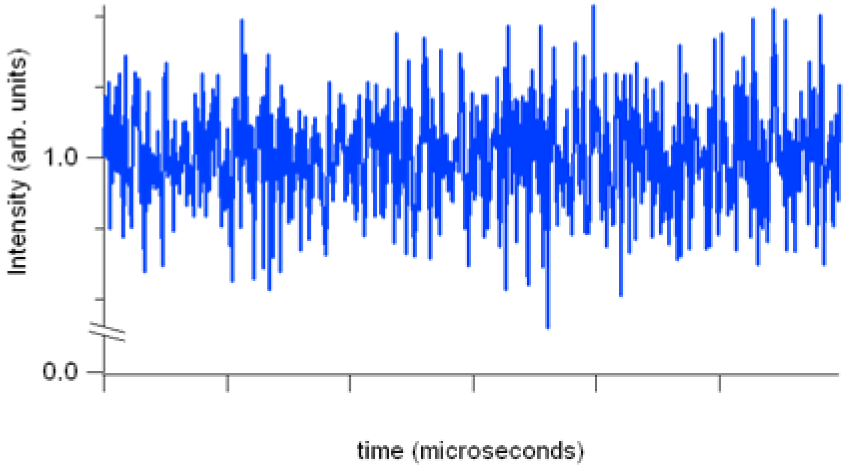
- Brownian Motion
  - Random
  - Related to Size
  - Related to viscosity
  - Related to temperature

DLS signal

Random motion of particles leads to random fluctuations in signal (due to changing constructive/destructive interference of scattered light).

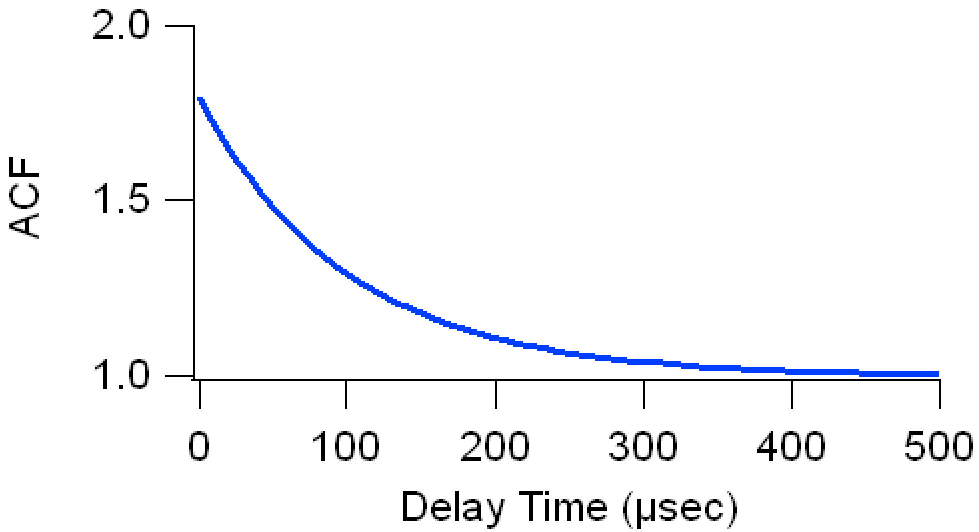






Correlation function

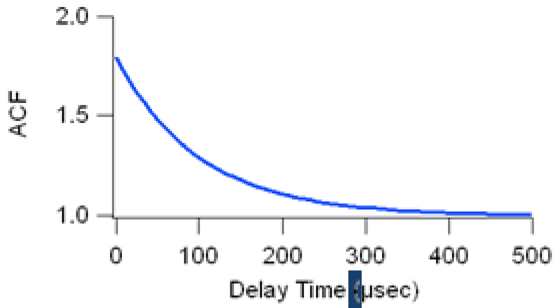
Random fluctuations are interpreted in terms of the autocorrelation function (ACF), C(τ).



$$C(\tau) = \frac{\int_0^T I(t)I(t+\tau)dt}{\langle I(t)I(t)\rangle}$$

$$C(\tau) = 1 + \beta \exp(-2\Gamma\tau)$$

Gamma to size



$$q = \frac{4\pi n}{\lambda} \sin\left(\theta/2\right)$$

$$\Gamma = D_m q^2$$

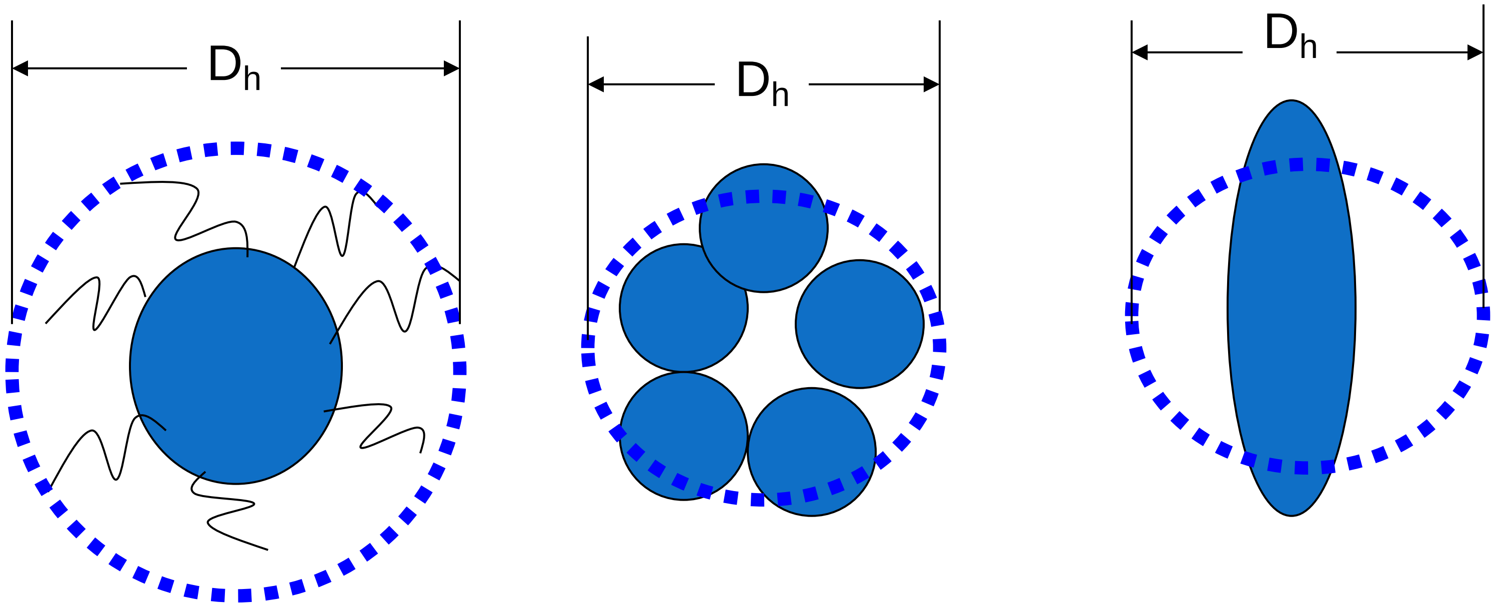
$$D_h = \frac{k_B T}{3\pi \eta(T) D_m}$$

Γ decay constant
D_m diffusion coefficient
q scattering vector
n refractive index
λ wavelength
θ scattering angle
D_h hydrodynamic diameter
η viscosity
k_B Boltzman's constant

Note effect of temperature!

What is hydrodynamic size?

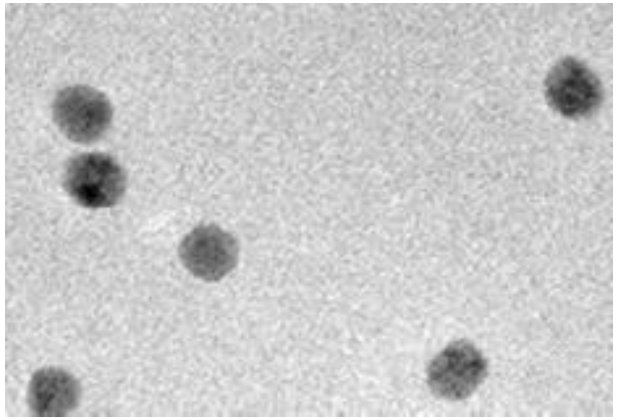
DLS gives the diameter of a sphere that moves (diffuses) the same way as your sample.

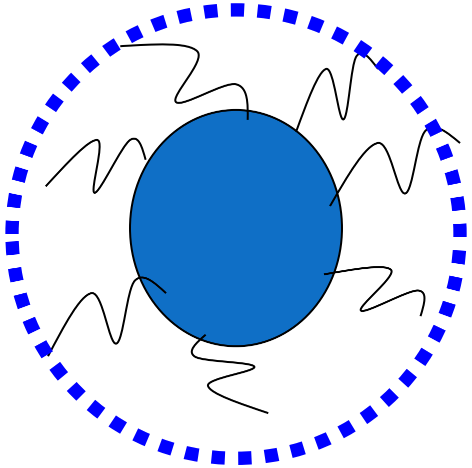


Hydrodynamic size

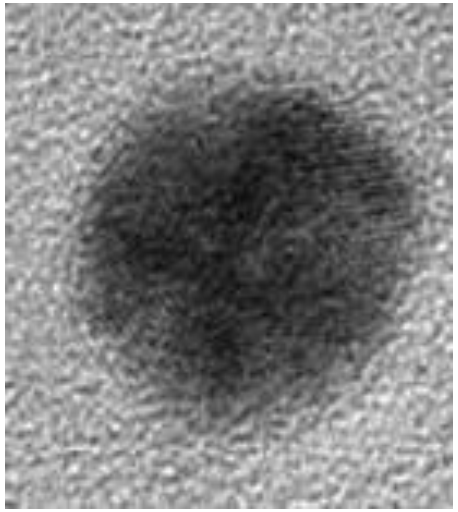
Gold Colloids

Technique	Size nm
Atomic Force Microscopy	8.5 ± 0.3
Scanning Electron Microscopy	9.9 ± 0.1
Transmission Electron Microscopy	8.9 ± 0.1
Dynamic Light Scattering	13.5 ± 0.1





SEM (above) and TEM (below) images for NIST RM 8011



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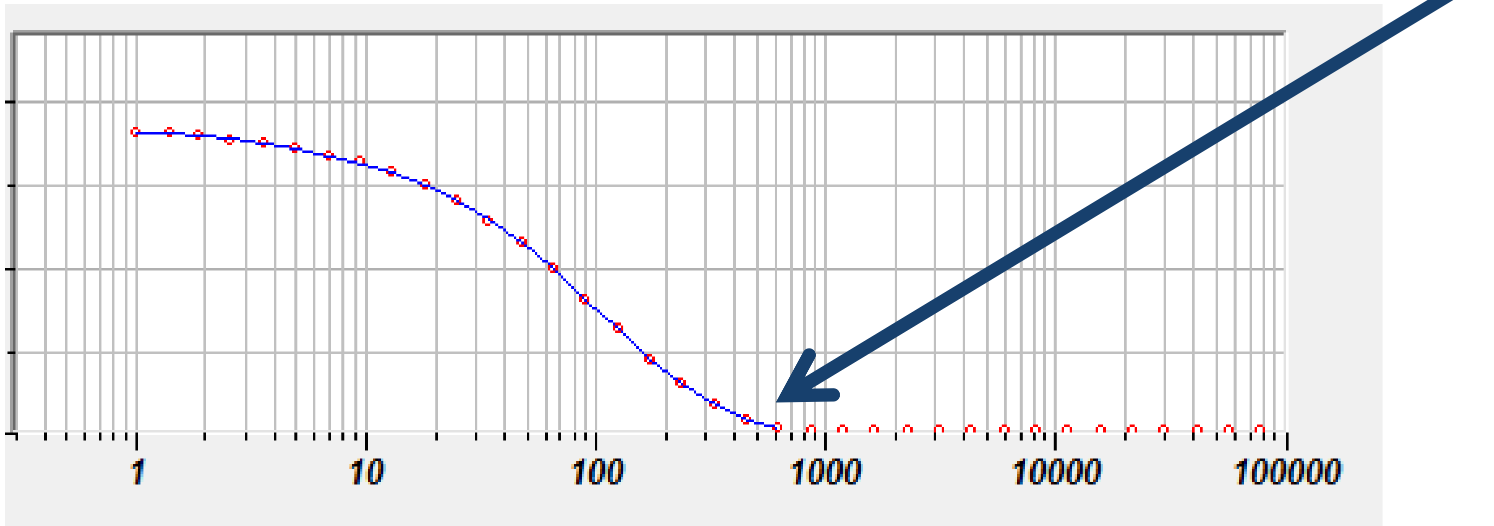
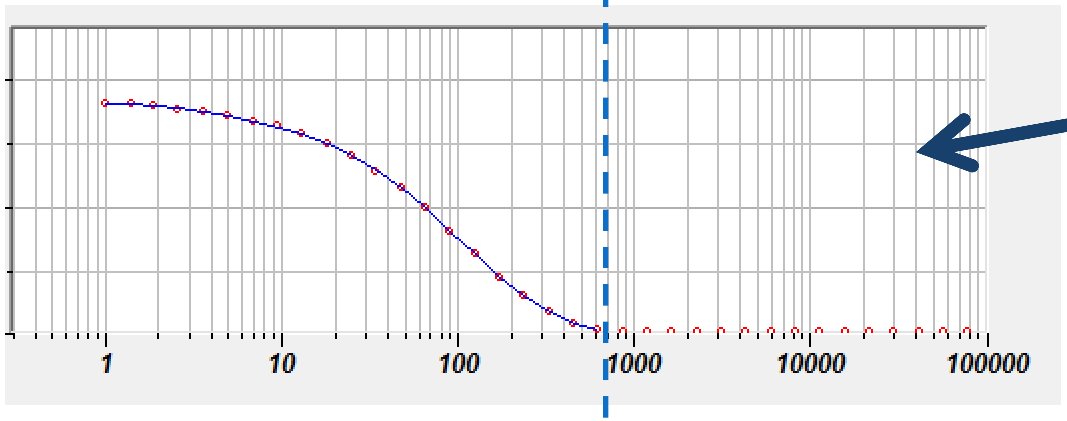
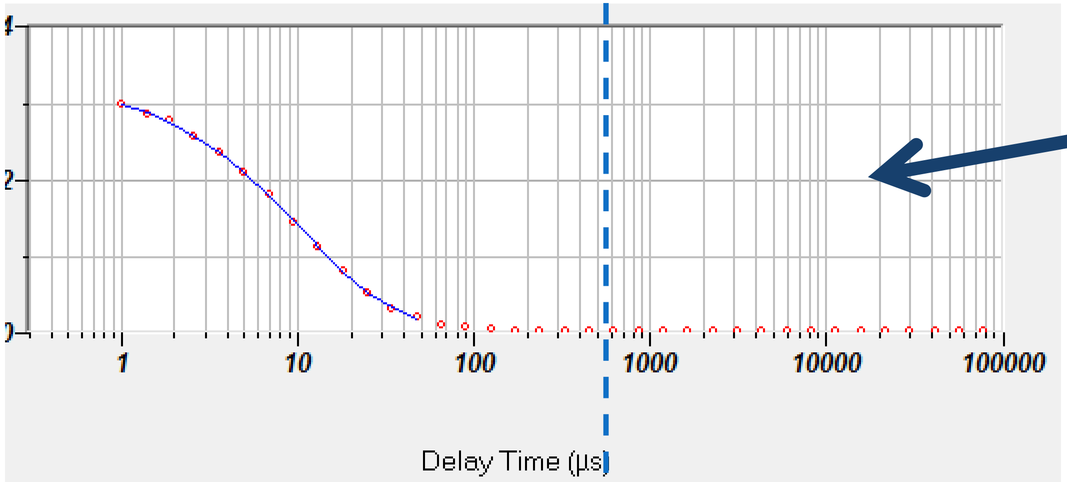
Nanogold data



	Z-average Diameter, nm
Run 1	50.5
Run 2	51.1
Run 3	49.2
Run 4	51.5
Run 5	49.7
Run 6	50.9
Avg.	50.5
St. Dev.	0.9
COV	1.7 %

Nanogold data





	Z-average Diameter, nm
Avg.	50.5
St. Dev.	0.9
COV	1.7 %

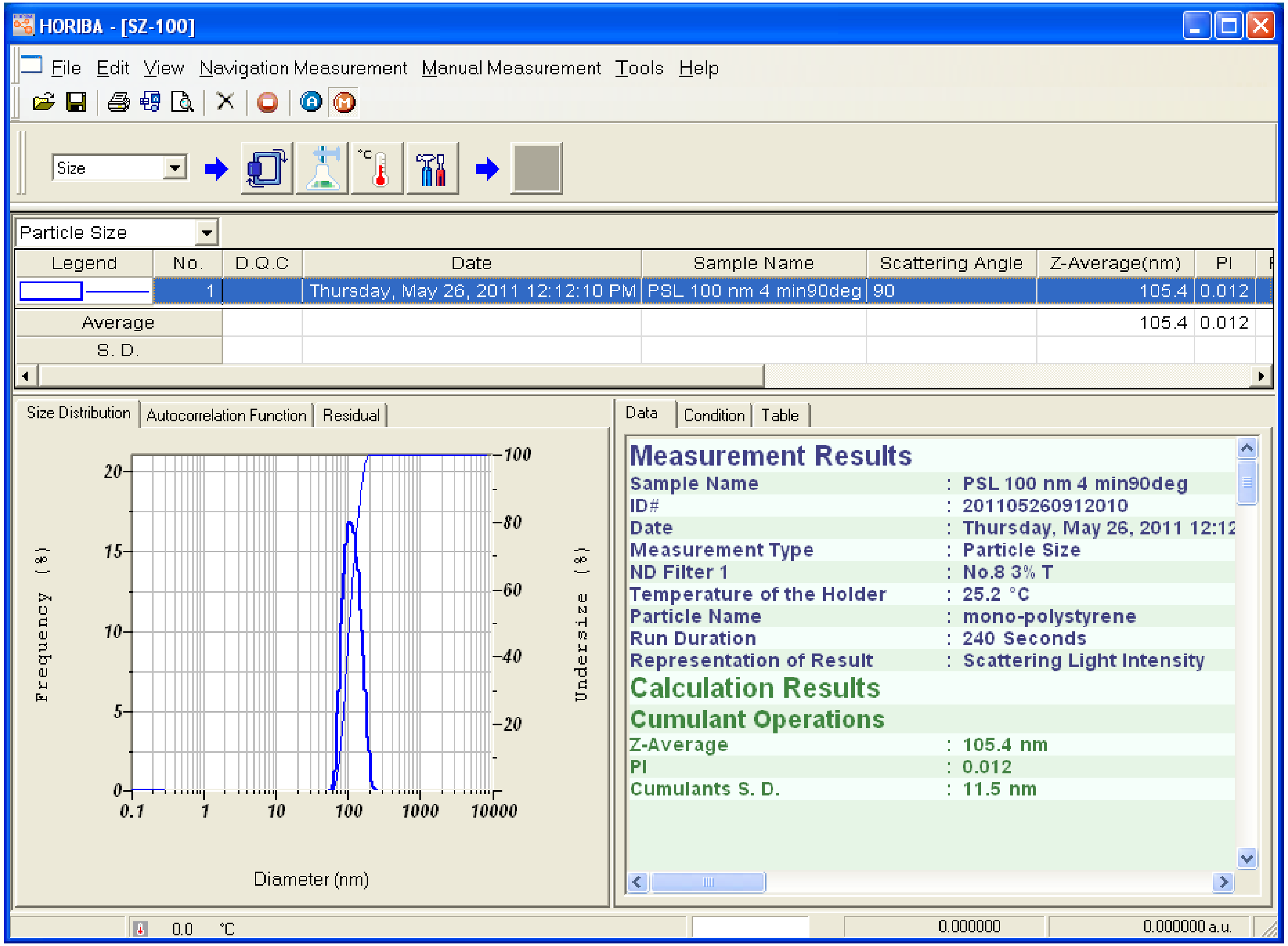
	Z-average Diameter, nm
Run 1	10.5
Run 2	10.6
Run 3	10.2
Run 4	10.5
Run 5	10.3
Avg.	10.4
St. Dev.	0.2
COV	1.9 %

Lab to lab comparison

Colloidal Silica

	Mean determined Z-average size (nm)	COV (%)
Dynamic Light Scattering with SZ-100, laboratory 1	34.4	0.7
Dynamic Light Scattering with SZ-100, laboratory 2	34.6	0.3

Polystyrene latex



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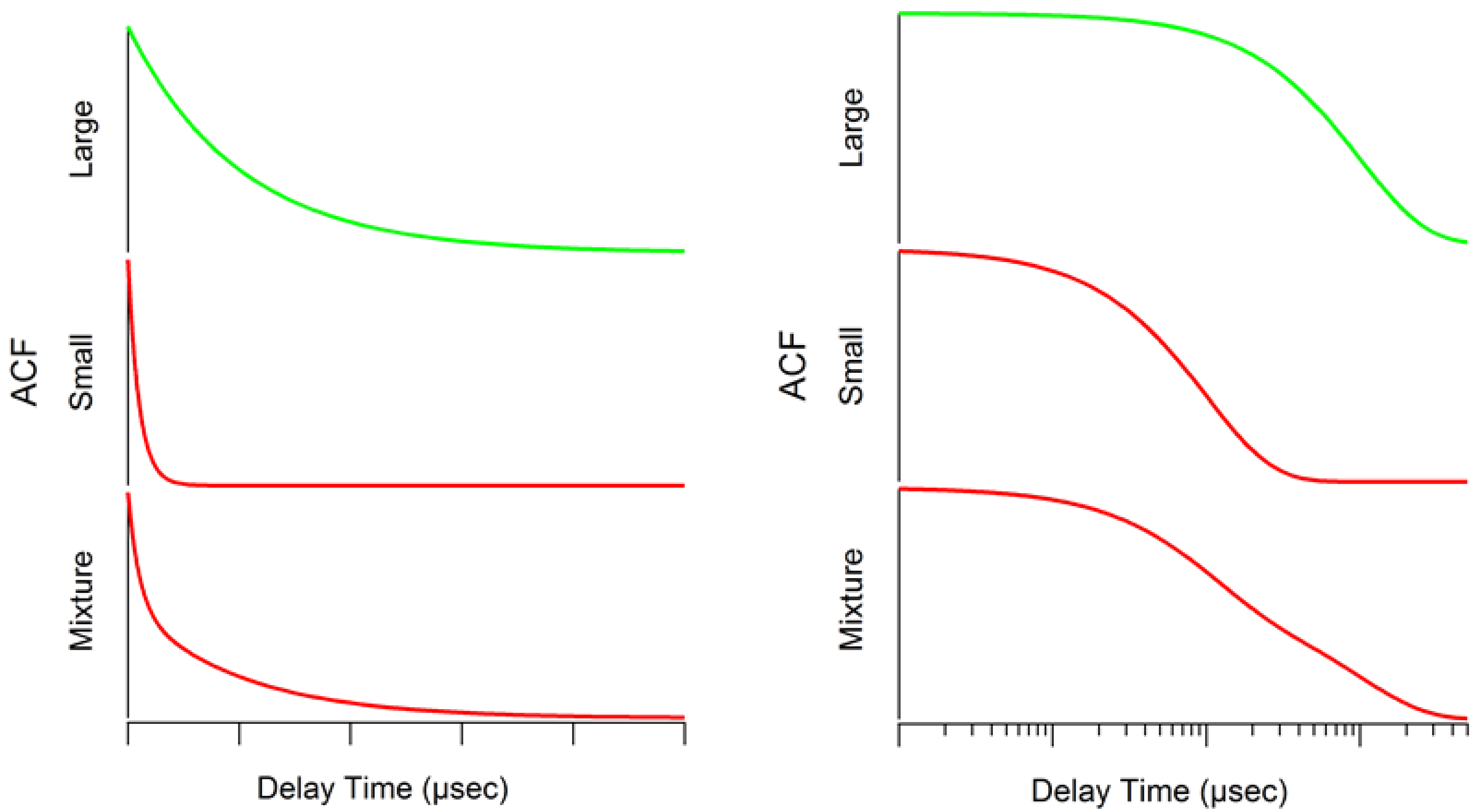
Mixtures of particles



Sum the autocorrelation functions

Polydisperse samples: cumulants

For a mixture of sizes, the autocorrelation function can be interpreted in terms of cumulants. This is the most robust method of analyzing DLS data.

$$C(\tau) = 1 + \beta \exp(-2\Gamma\tau)$$

$$C(\tau) = 1 + \beta \exp\left[2\left(-\bar{\Gamma}\tau + \left(\mu_2 / 2!\right)\tau^2 - \cdots\right)\right]$$

$$\bar{\Gamma} = \overline{D_m} q^2$$

"z-average size"

$$D_{z,h} = \frac{k_B T}{3\pi\eta(T)\overline{D_m}}$$

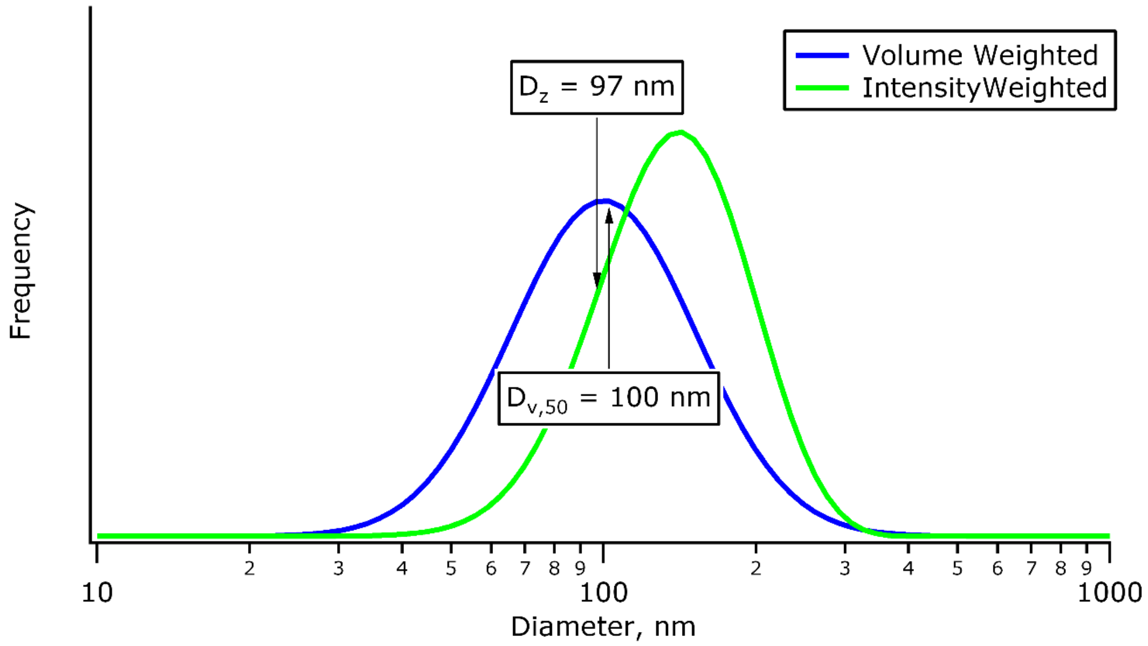
$$Polydispersity = \mu_2 / \bar{\Gamma}^2$$

Z-average

Size determined from intensity weight diffusion coefficient ~1/D

Intensity weighted harmonic mean size

$$1/D_z = \frac{\sum D_i S_i}{\sum S_i}$$



D_z = z-average
S_i = total scattering from all of species i
D_i = Diameter of species all

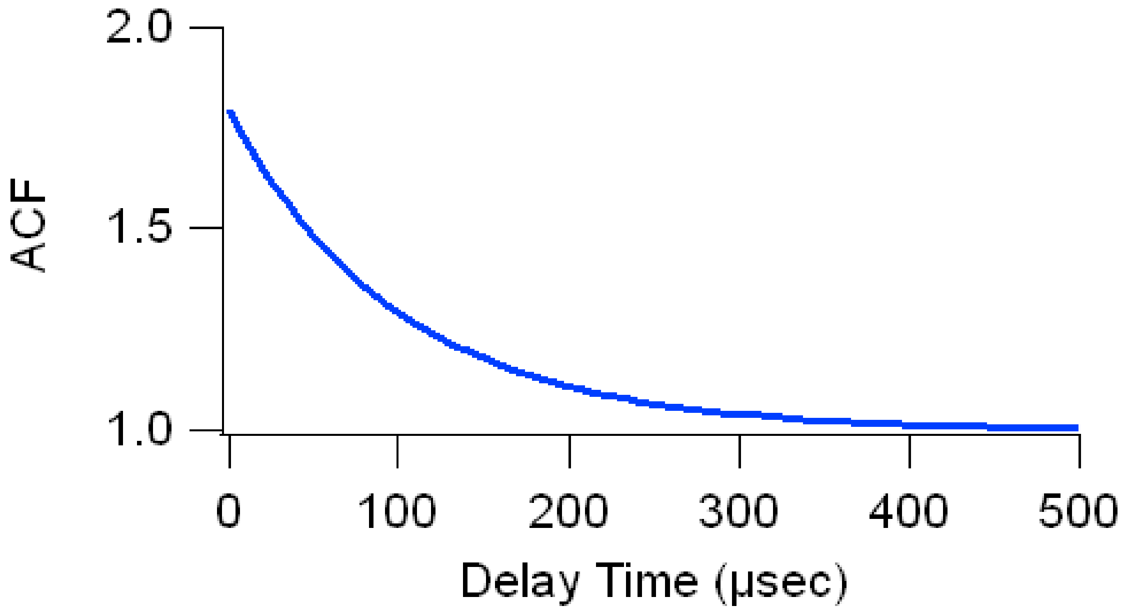
As size goes up, so does D_z.

SiO_2

Run	Z-average Diameter (nm)	Polydispersity Index
1	473.2	0.127
2	479.5	0.066
3	478.8	0.077
4	487.7	0.039
Avg.	479.8	0.077

Polydisperse sample: ILT

A more general relationship can be given between the autocorrelation function and the size distribution. Let each size have a relation constant Γ. The scattering from each population is then given by S(Γ). Now we have an integral equation. Solving for S(Γ) gives us size distribution.

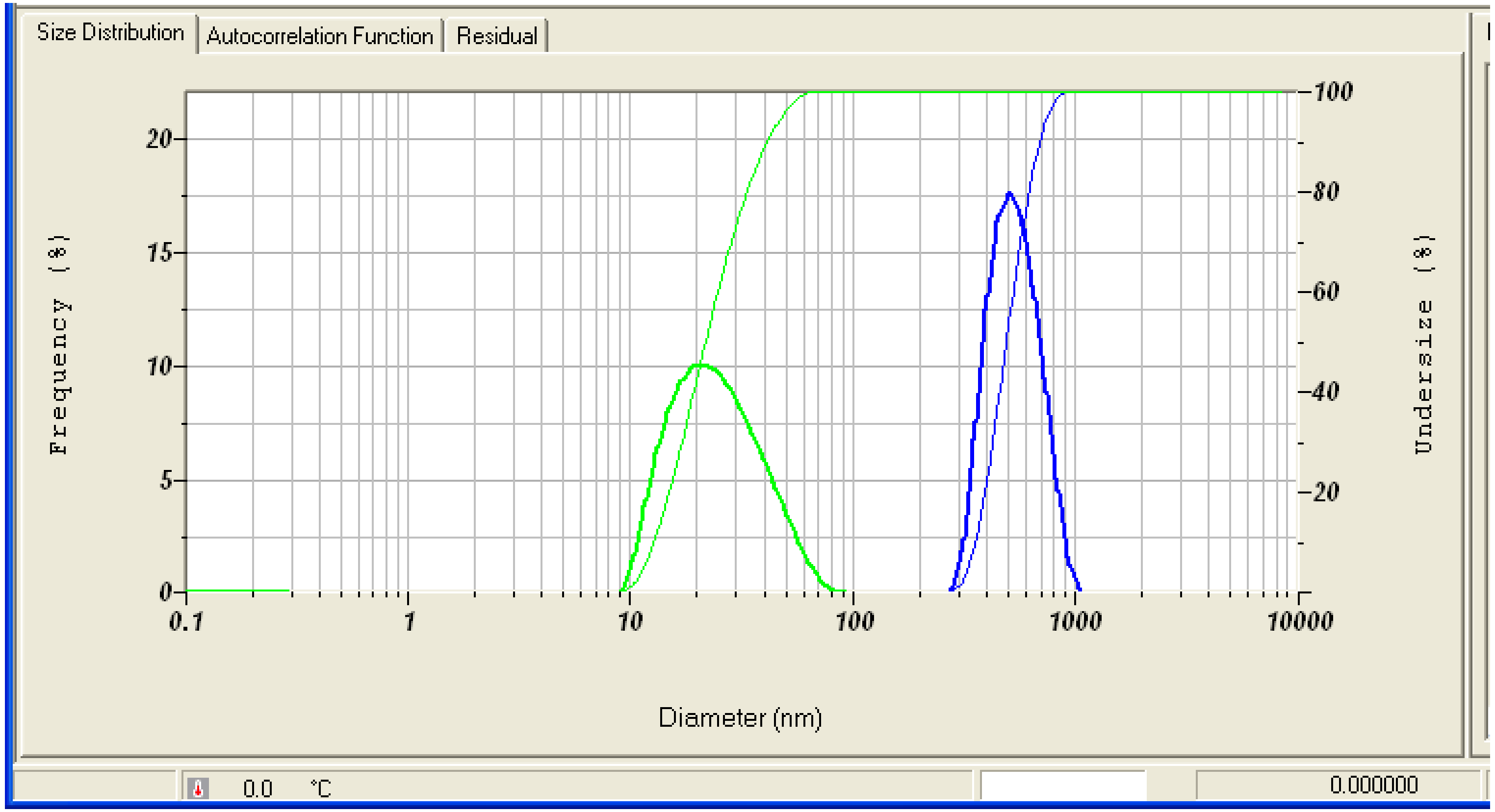


$$C(\tau) = 1 + \beta \left| g^{(1)}(\tau) \right|^2$$

$$g^{(1)}(\tau) = \int S(\Gamma)\exp(-\Gamma\tau)d\Gamma$$

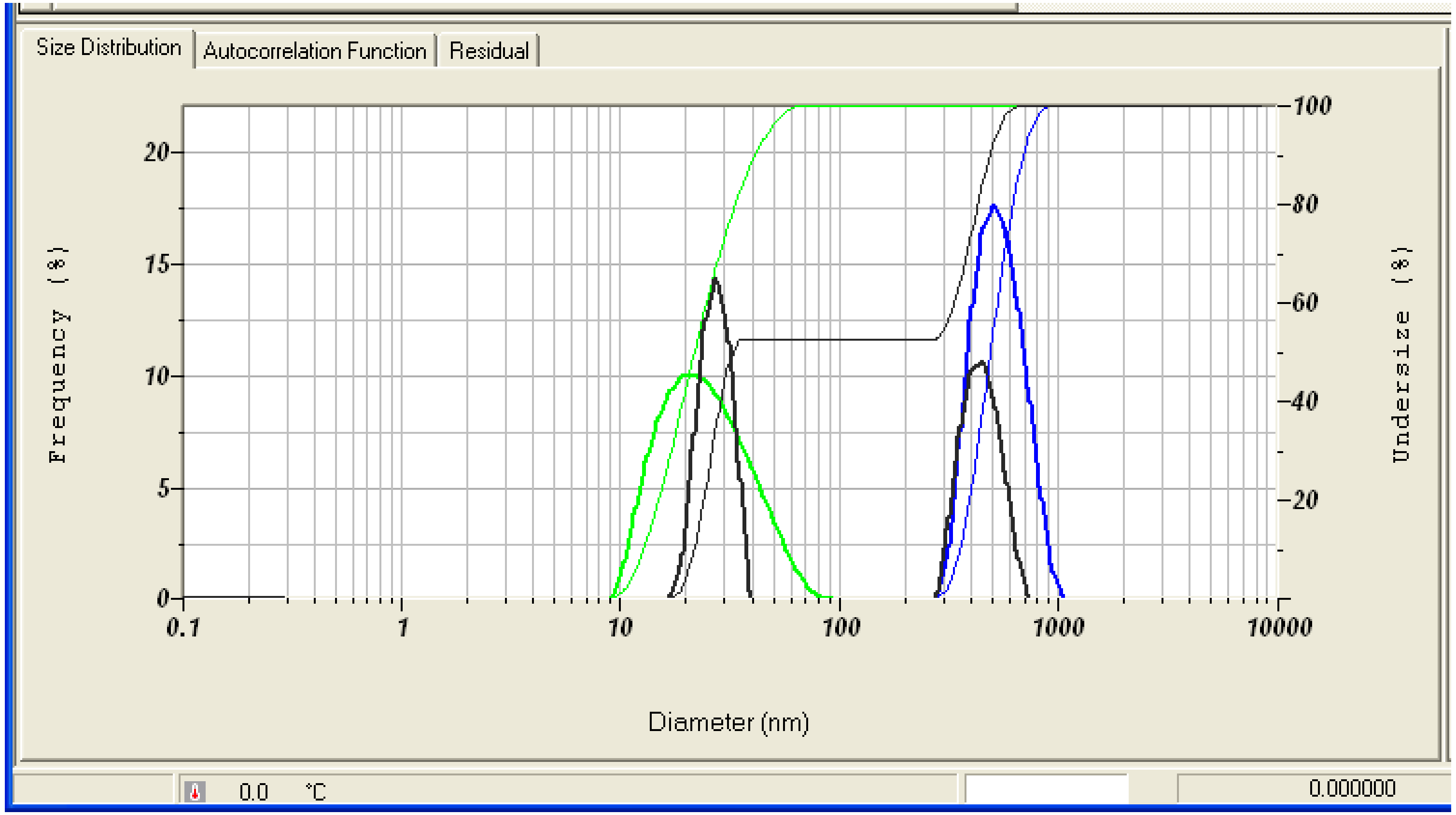
Bimodal sample

Nominal 20 nm and 500 nm latex run individually



Bimodal sample

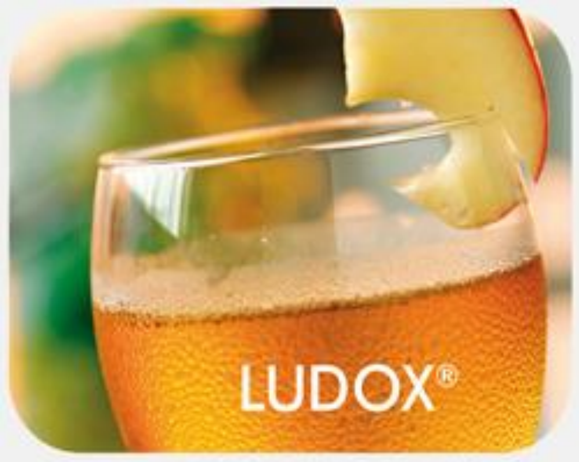
Mixed sample (in black)

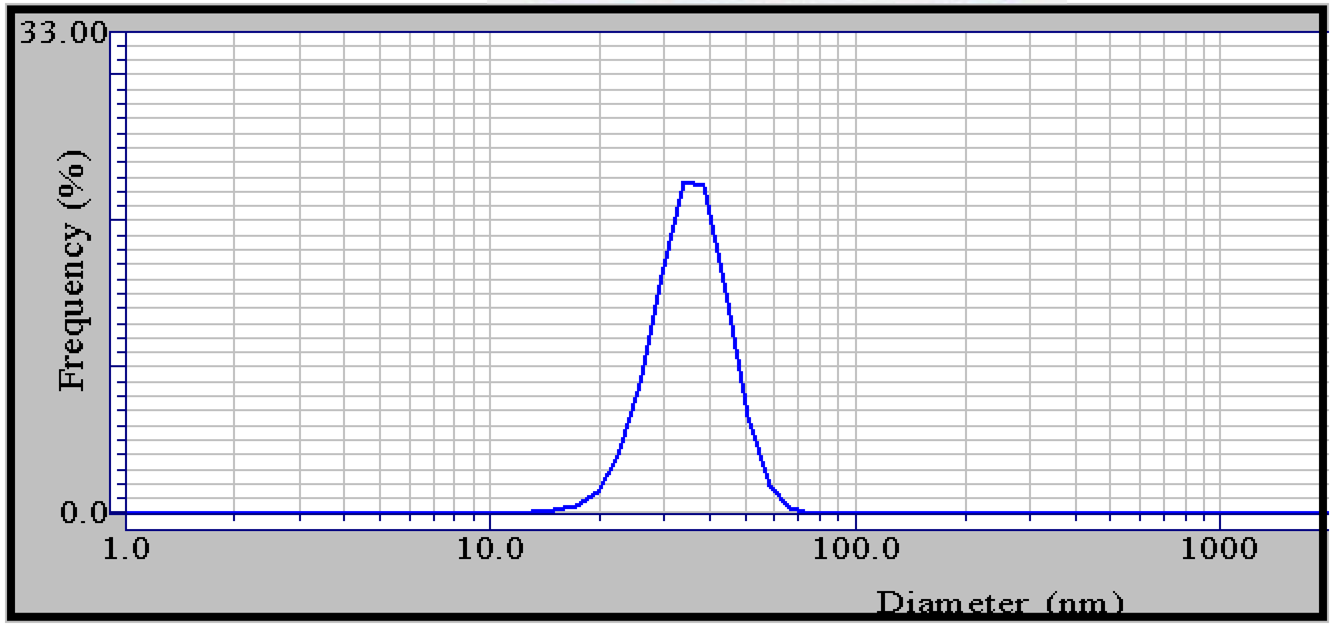


Colloidal Silica

- Standard off-the-shelf Ludox
  - Colloidal Silica
  - Used to clarify beer, wine, and juice

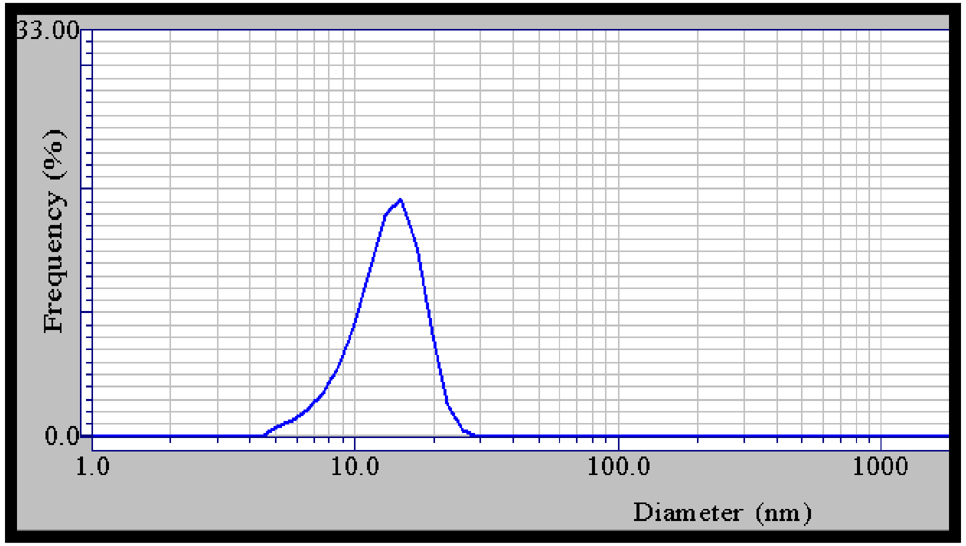
- Matches data from the LA-960 (laser diffraction)

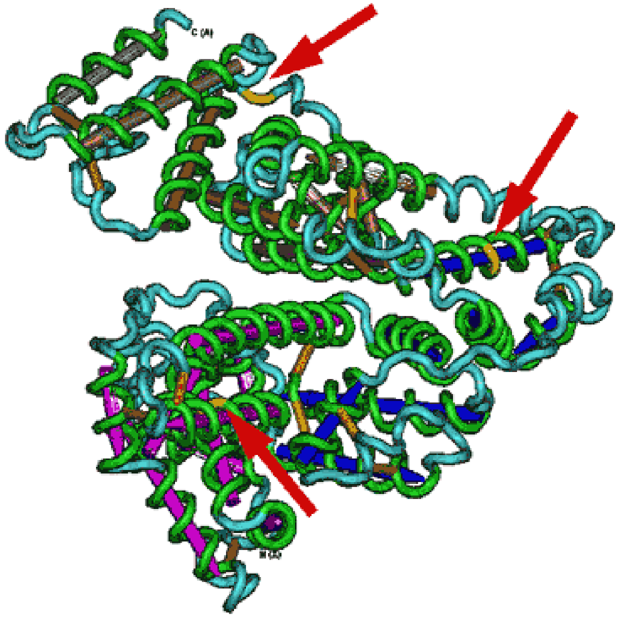




BSA

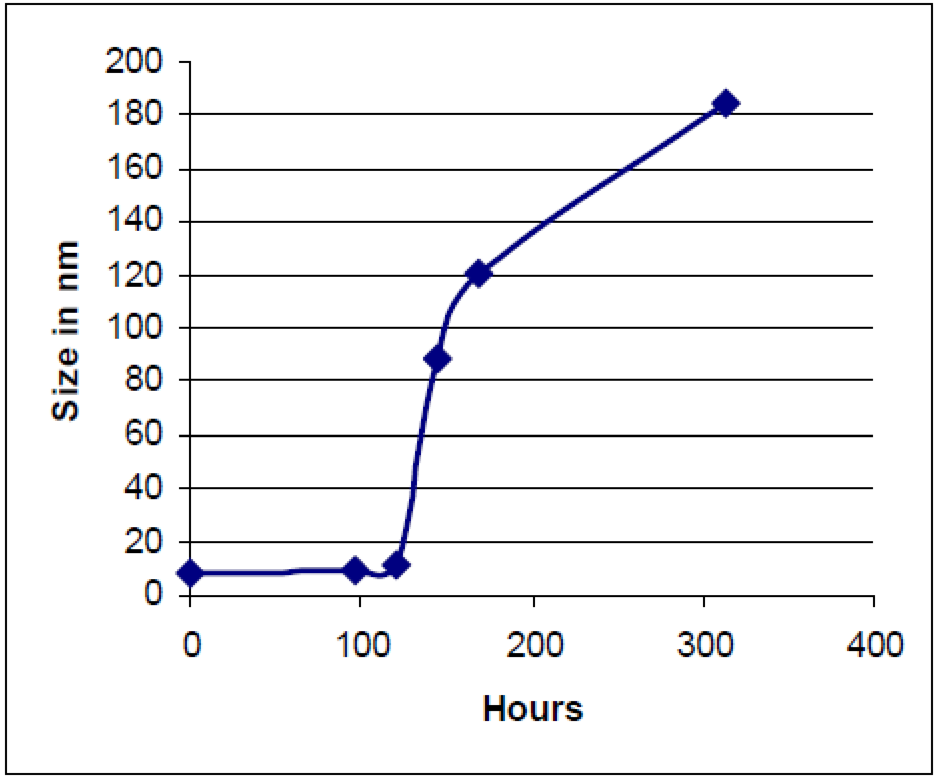
- BSA- well characterized protein
- DLS – Can be used to determine the aggregation state of the protein





Protein aggregation

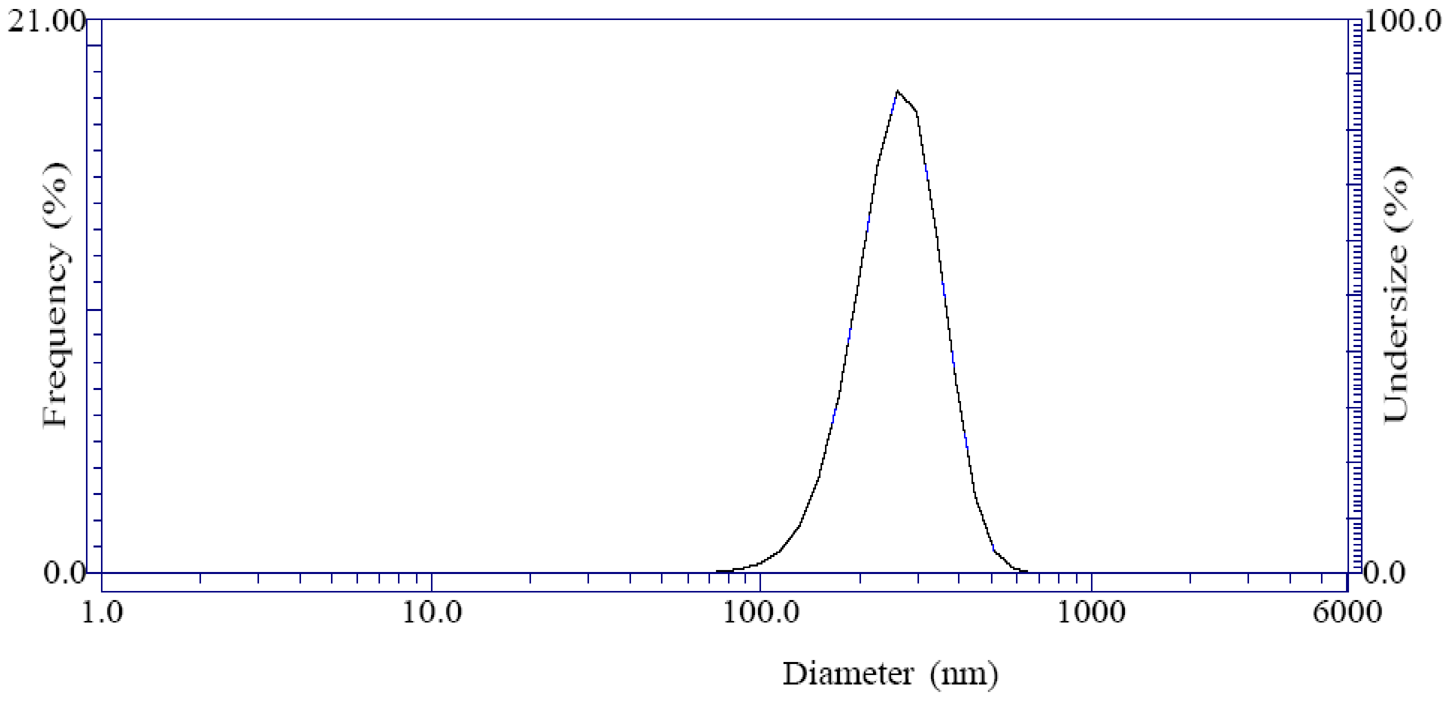
- Unstabilized 10mg/ml lysozyme at pH 2
- Lisa Cole and Ben Burnett at the Florida Institute of Technology
- Can also be done with ViewSizer (NTA)



Protein size in nm vs. time in hours

Liposomes

- Liposomes to target tumor growth
- Size is critical to how the liposome
  - Encapsulates protein
  - Functions within body
  - Remains stable over time
  - Delivers the protein



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Dust

- Dust: large, rare particles in the sample
- Generally not really part of the sample
- Since they are rare cannot get good statistics

Filters are your friend

- Filter to remove dust. If particles are too large (D >50 nm for 0.1 μm filter), at least filter diluent.
- Filters available with pore sizes from 20 nm to 2μm.
- We can also centrifuge the sample and extract the supernatant.

Suspension liquid

- **Choose a liquid that**
  - does not dissolve the particles
  - prevents loose agglomerates
- **Add energy to break up loose agglomerates**
  - stirring
  - ultrasound

Surfactants

Enable wetting

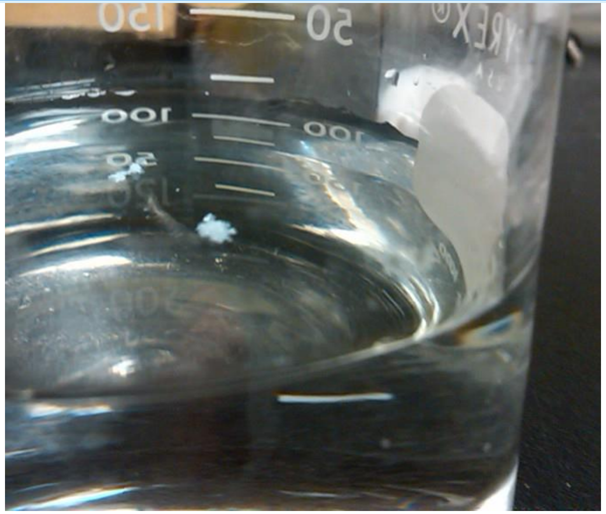
Prevent agglomeration

- Common concentration: 0.01-0.1%
- example:
  - Tetrasodium pyrophosphate (TSPP)
  - Triton X

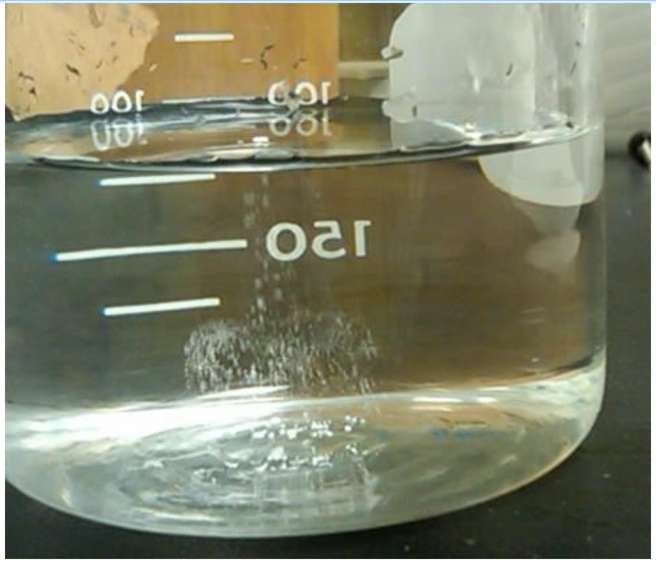
Wetting

- Many dry particle samples will never form a nanoparticle suspension without significant effort.
- Sprinkle particles on top of target dispersant. If the particles float on top and do not penetrate the water surface, they are not wetted. This is usually a bad sign.
- If the particles break through surface and sink, they are a) wetted or b) so big that gravity is more important than surface tension. If it is case (a), you are in luck.

Particles floating on top



Plume of sinking particles



Solvents

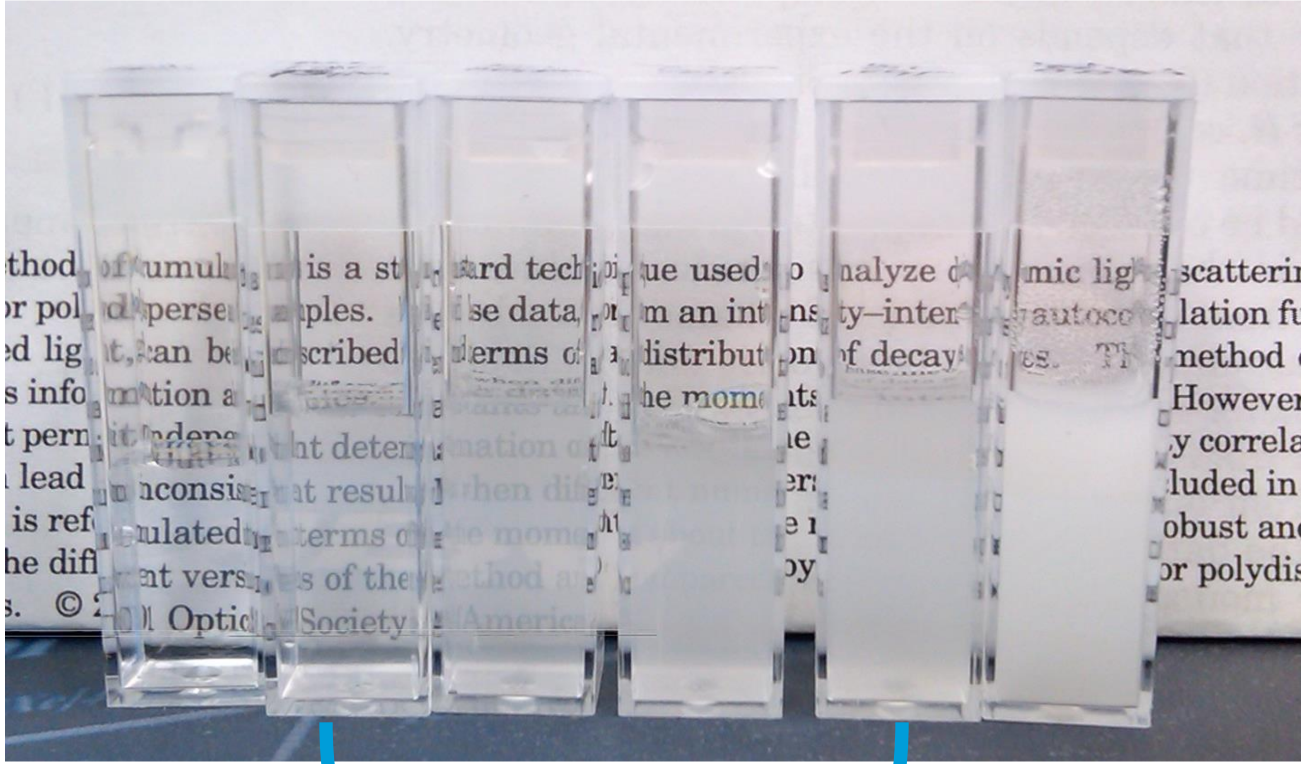
- Working with aqueous systems is usually easier for many reasons.
- But don’t forget to try a less polar solvent such as isopropyl alcohol.
- Don’t forget that organic solvents are more difficult to handle due to fire and health hazards.

Try a series of options

Make a series of suspensions and check them by eye, then measure.

Estimate concentration by eye

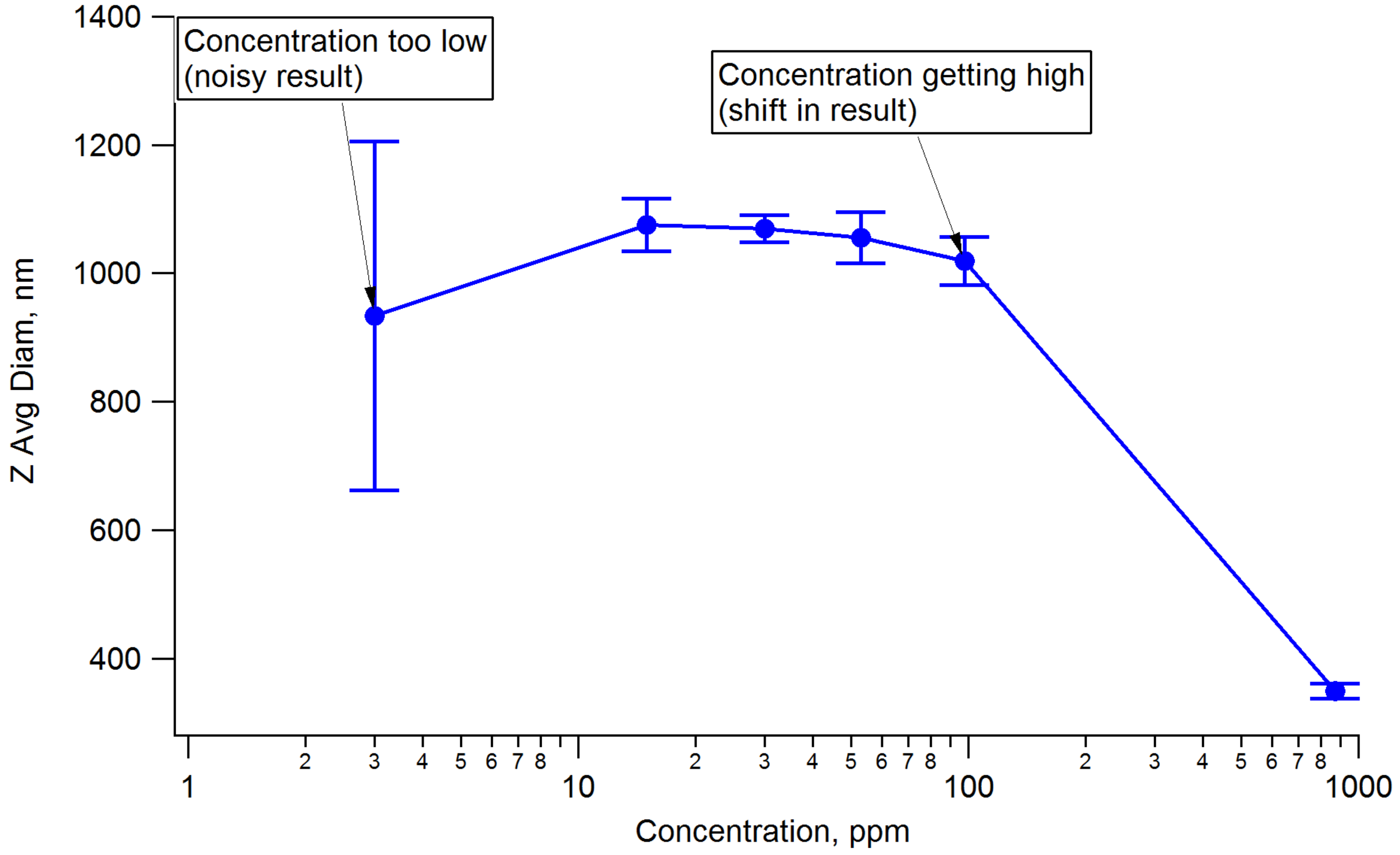
Read a newspaper through it



Best range for this material

Concentration

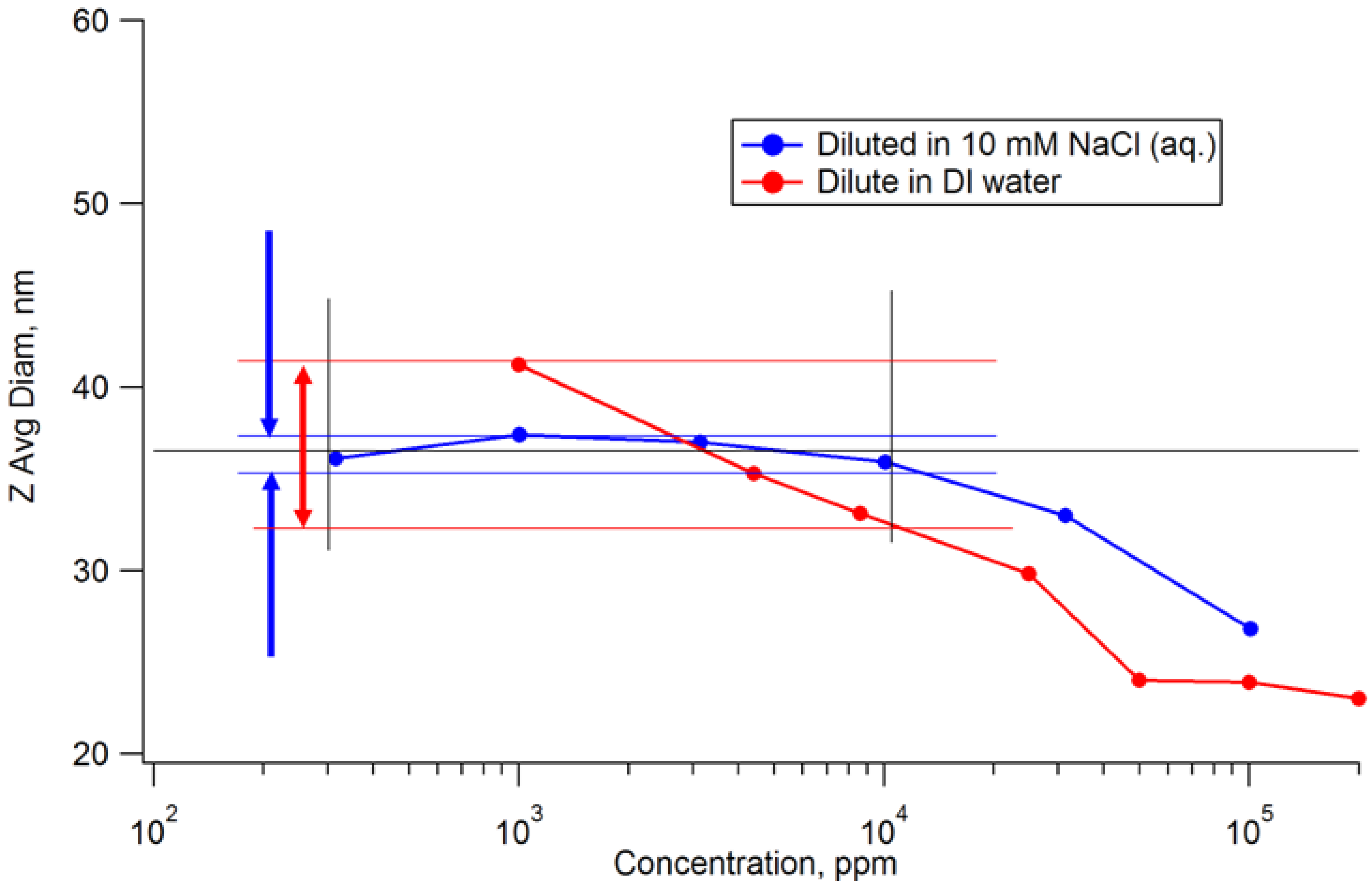
Plot measured size vs. concentration or dilution to determine concentration range. Note log scale.



Effect of salt concentration

- Note that when we suppress effect of charges by adding salt, the effect of concentration is suppressed.

- Concentration effects are due to changes in particle motion, not just multiple scattering.



Hints Summary

- **Web search**
- **Consider solvent and surfactant**
- **Consider ultrasound**
- **Expect to filter**
- **Choose largest cell you can**
- **Optimize concentration**

Settling and DLS

Not all motion is Brownian motion ☹

The Natural limit for Dynamic Light Scattering: Gravitational Settling

Gravitational Settling occurs at about 1-3μm

Particle Diameter (μm)	Movement due to Brownian Motion		Movement due to Gravitational Settling
0.01	2.36	>>	0.005
0.25	1.49	>	0.0346
0.50	1.052	>	0.1384
1.0	0.745	~	0.554
2.5	0.334	<	13.84
10.0	0.236	<<	55.4

DLS disadvantages

- **Sensitive to large particles**
- **Poor resolution of distribution**
- **Not appropriate where settling is significant (use laser diffraction)**

DLS Advantages

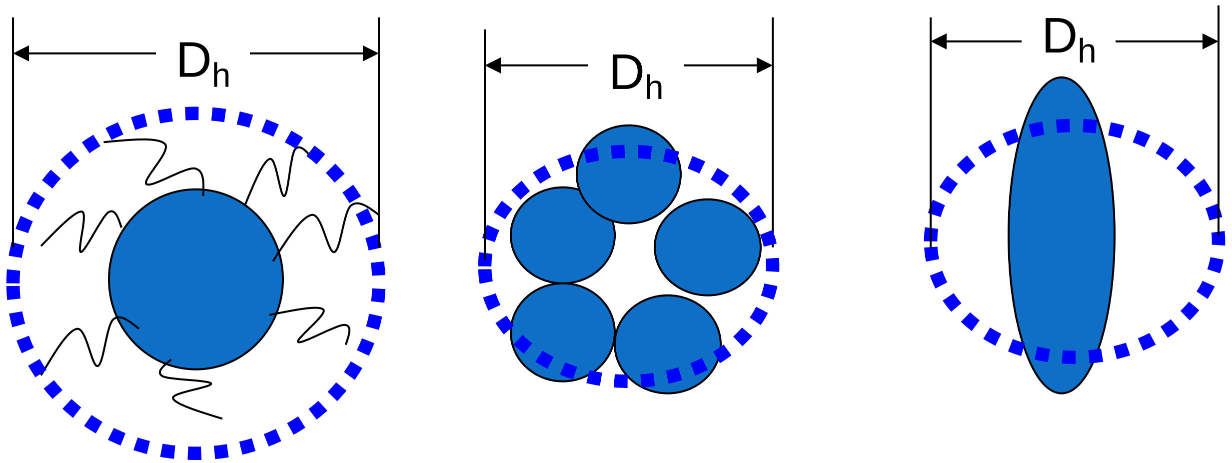
- Fast
- Repeatable
- Noninvasive
- Requires only small quantities of sample
- Good for detecting trace amounts of aggregate
- Good for macromolecular sizing
- Reaches smallest particle sizes

SZ-100

Single compact unit that performs size, zeta potential, and molecular weight measurements.

Summary

- **Fast, repeatable nanoparticle sizing**
- **Think about suspension chemistry in method development**
- **Reports hydrodynamic size:**



Omoshiro-okashiku
Joy and Fun

Terima kasih

நன்றி

Gracias

谢谢

Danke

Tack ska du ha

Σας ευχαριστώ πάρα πολύ

धन्यवाद

Grazie

THANK YOU

شكرًا

ขอบคุณครับ

Большое спасибо

ありがとうございました

Obrigado

Cảm ơn

Merci

감사합니다

Dziękuję