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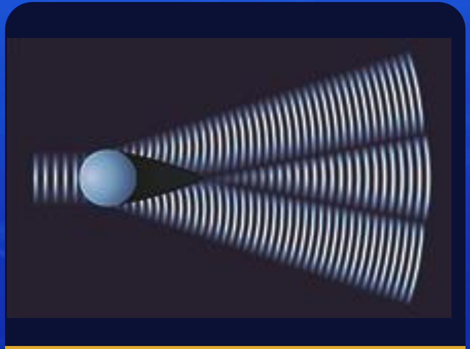
Explore the future

Modern Particle Characterization Techniques Series

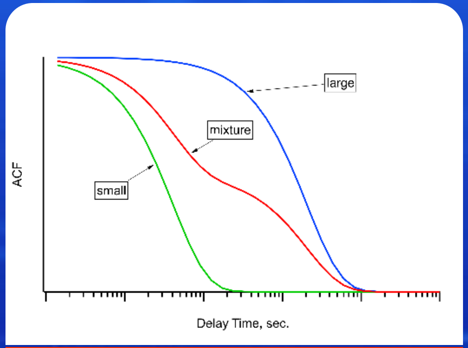
Dr. Mike Pohl

I: Introduction



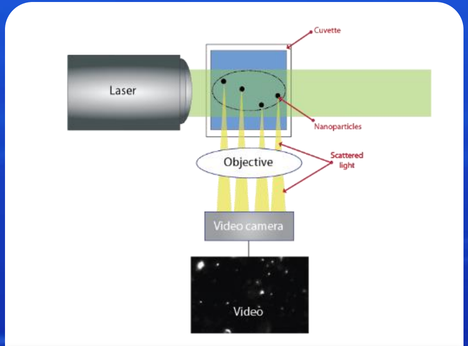
Julie Chen Nguyen

II: Laser Diffraction



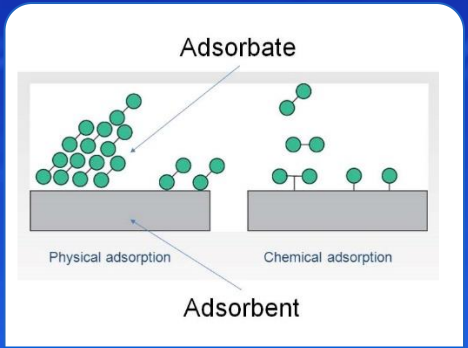
Dr. Jeff Bodycomb

III: DLS



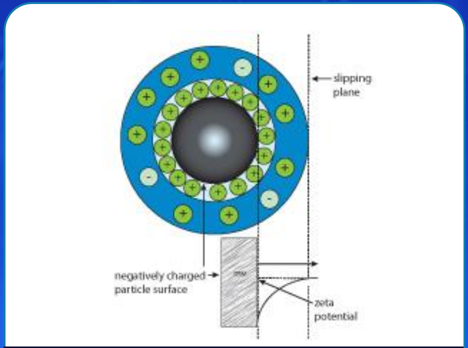
Dr. Sean Travers

IV: Multi-laser NTA



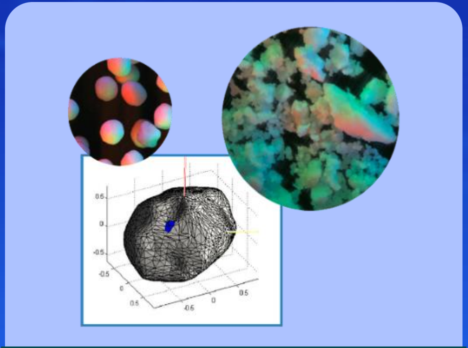
Carl Lundstedt

Series V: BET



Dr. David Fairhurst

VI: Zeta Potential



Darren McHugh

VII: Image Analysis

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Particle Characterization

Jeffrey Bodycomb, Ph.D.

Modern Particle Characterization Techniques Series III: Dynamic Light Scattering (DLS)

July 8, 2020

Outline

- Introduction
- What is DLS and what, exactly, does it measure?
- Method Development

A word from our sponsor

Did you sign up for our newsletter?

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Send us a chat or e-mail your desire to labinfo@horiba.com

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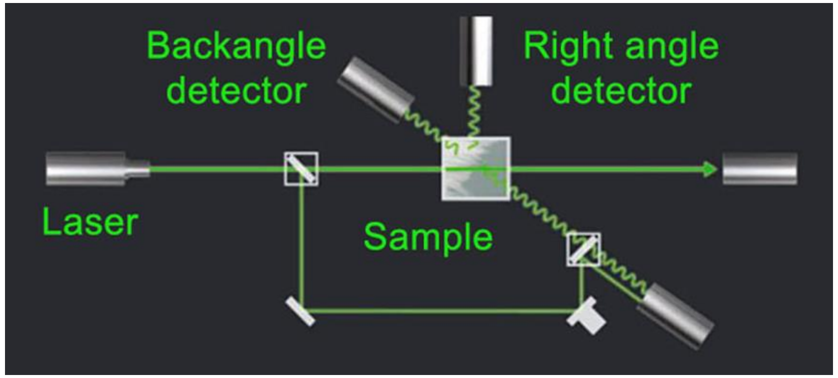
What is dynamic light scattering?

- 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



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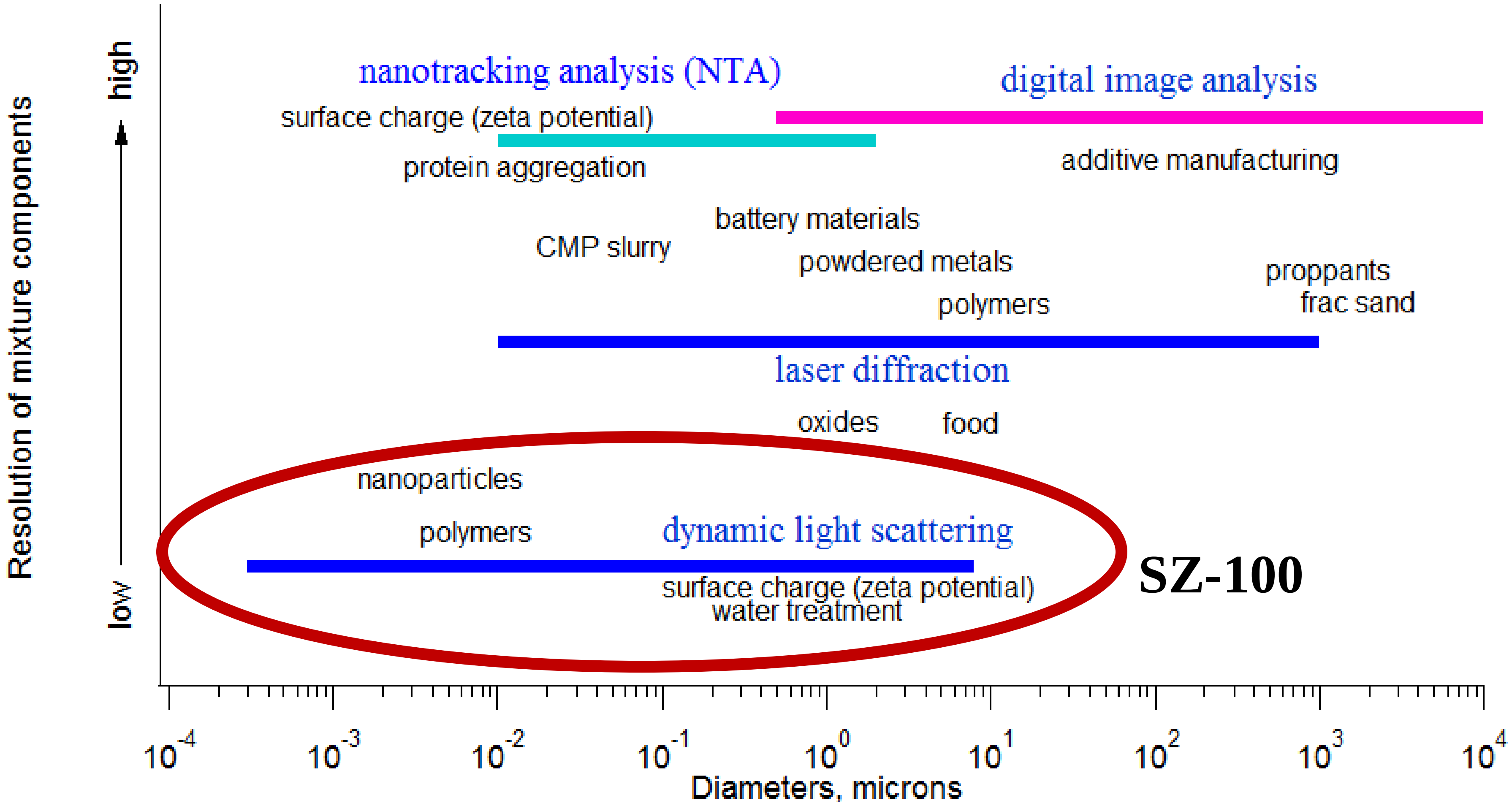
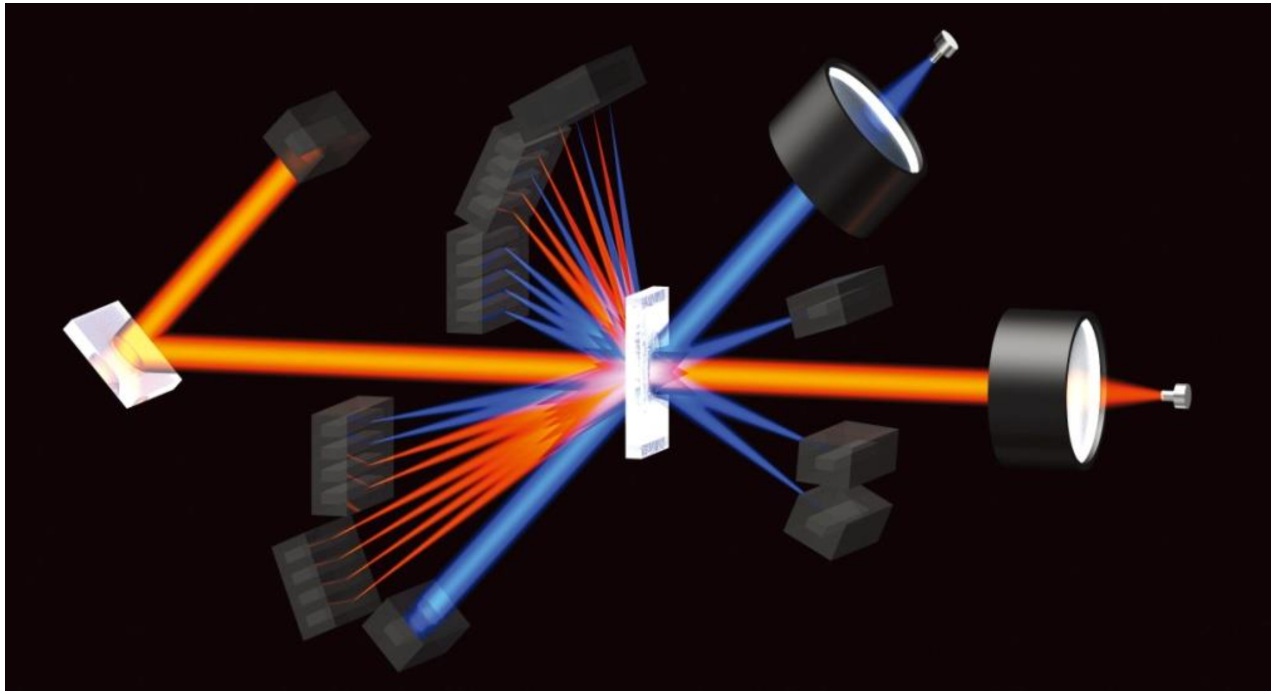
Other light scattering techniques

- 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



Laser diffraction



Laser Diffraction

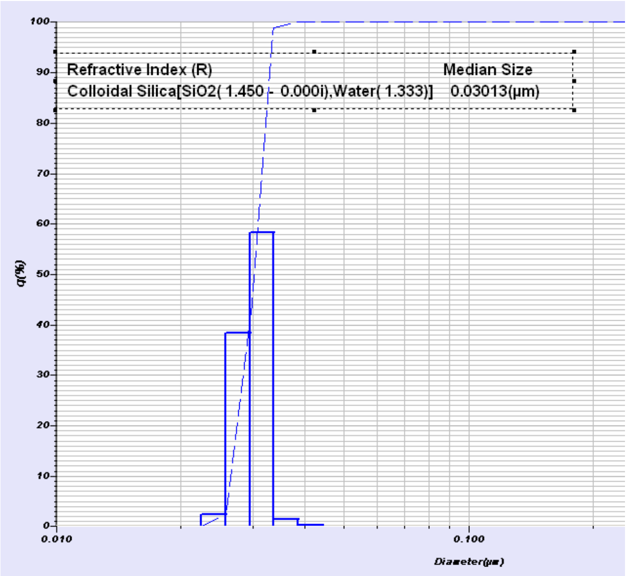
- Particle size 0.01 – 3000 µm
- Converts scattered light to particle size distribution
- Quick, repeatable
- Most common technique
- Suspensions & powders

Laser diffraction

Suspension

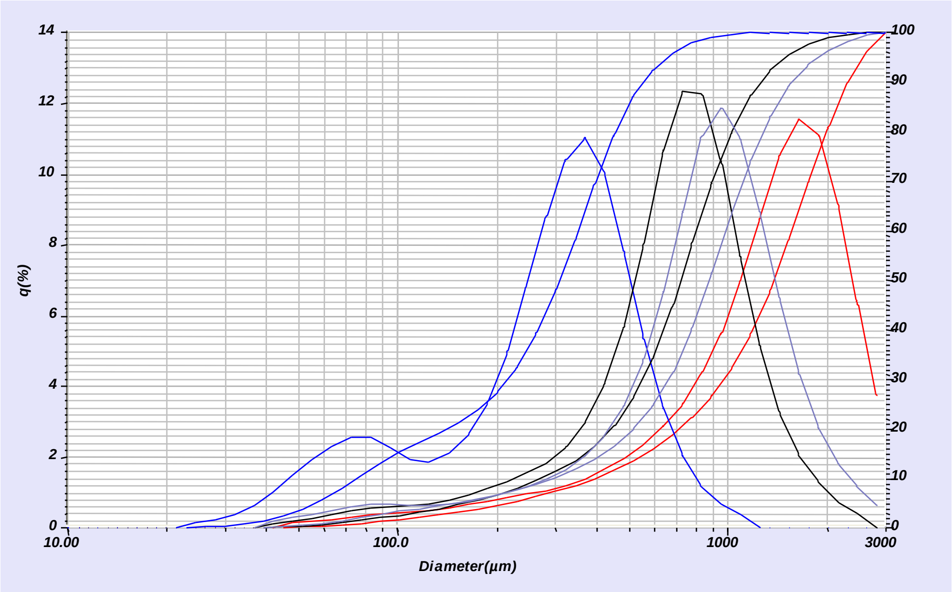
Silica
~ 30 nm

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

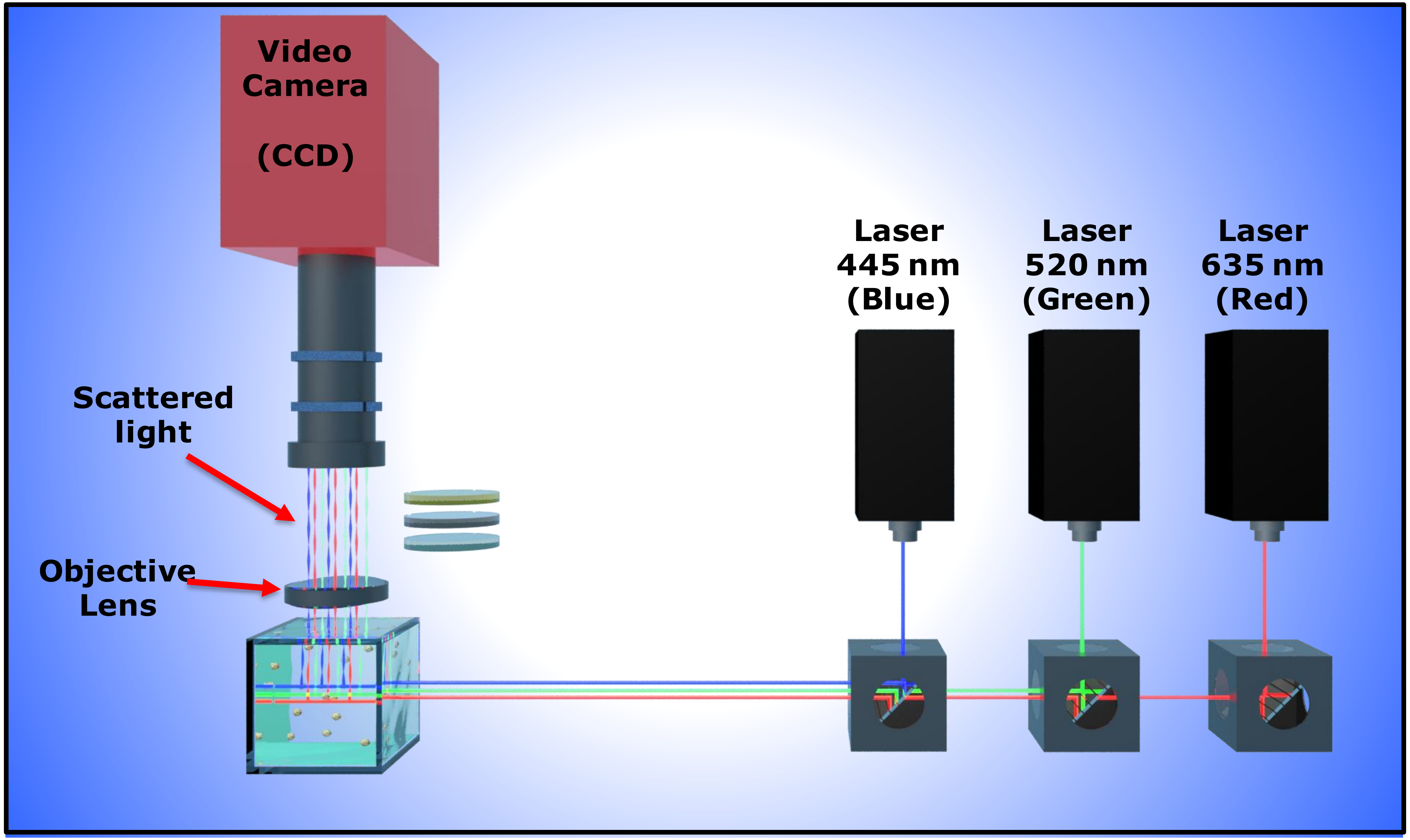


Powders

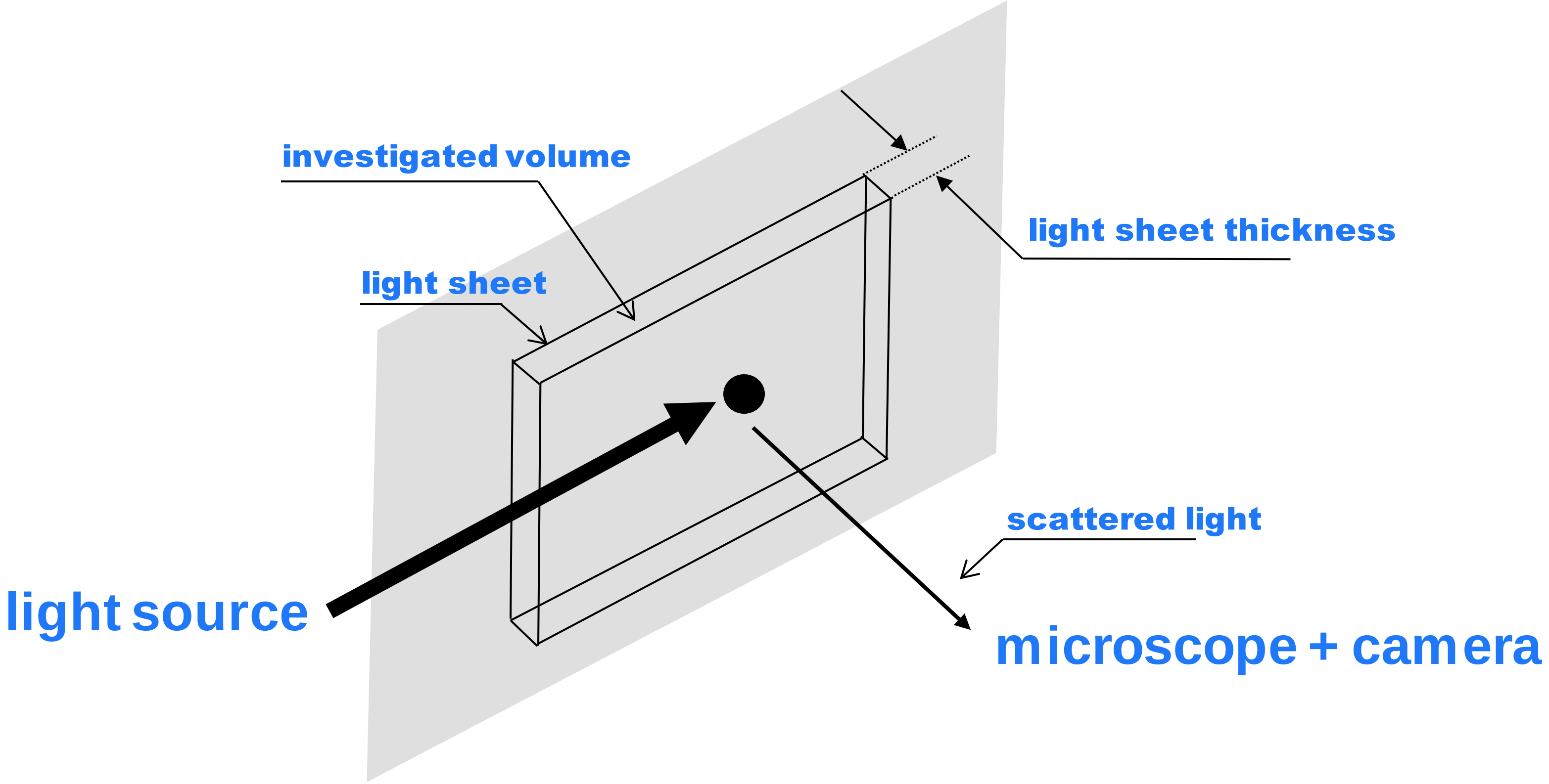
Coffee Results
0.3 – 1 mm



Nanoparticle tracking analysis (NTA)



Nanoparticle tracking analysis (NTA)



Outline

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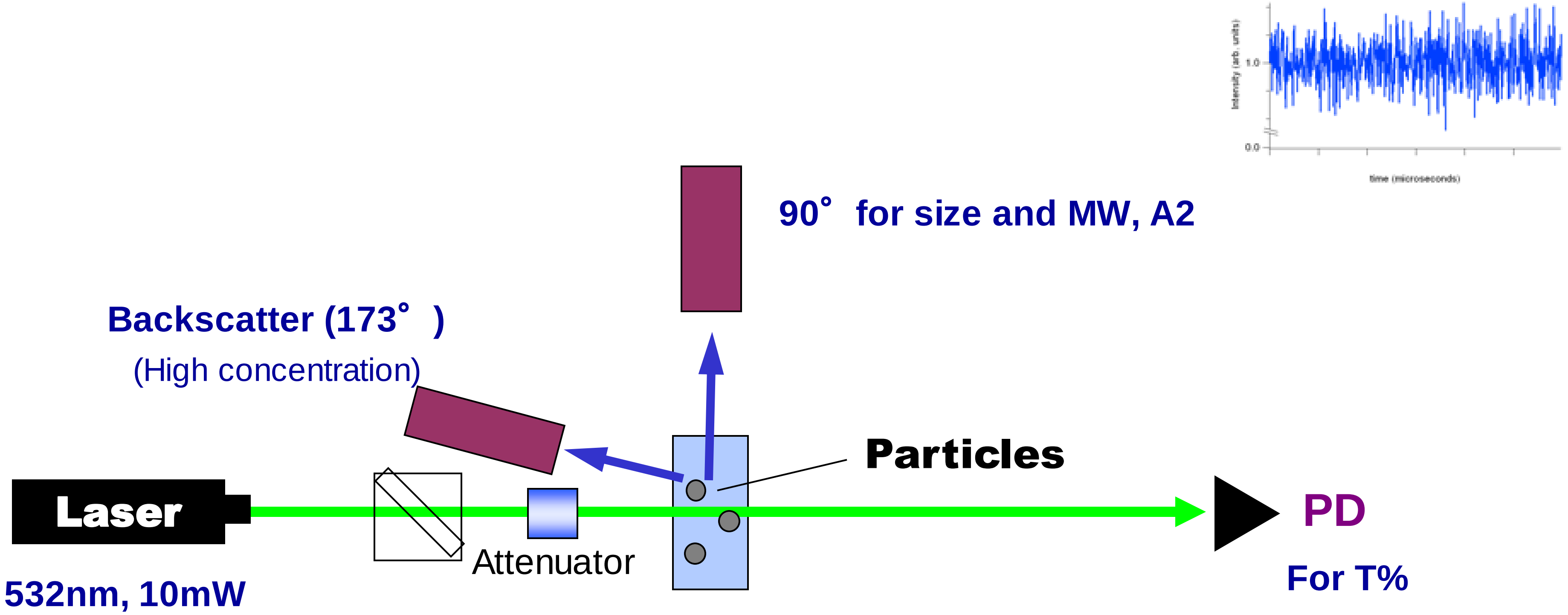
- What is DLS and what, exactly, does it measure?
- Method Development

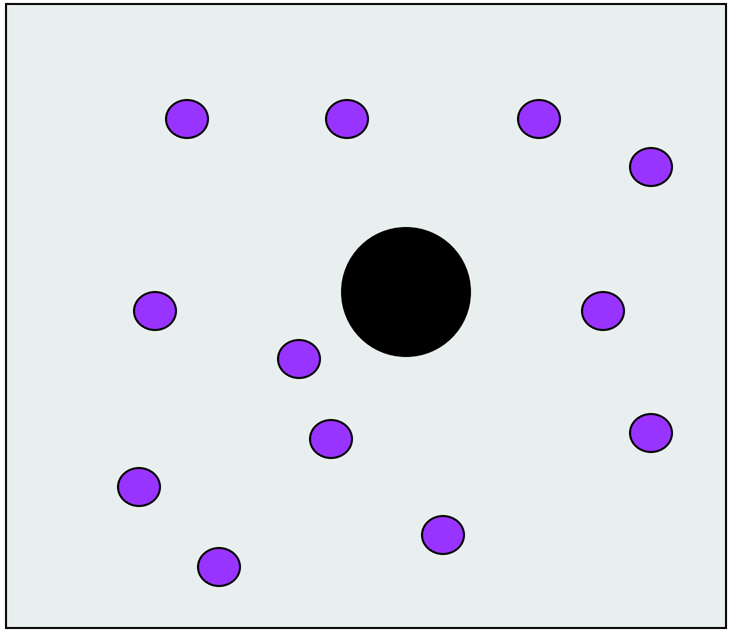
DLS optics



Particles moving
due to Brownian
motion

Brownian motion

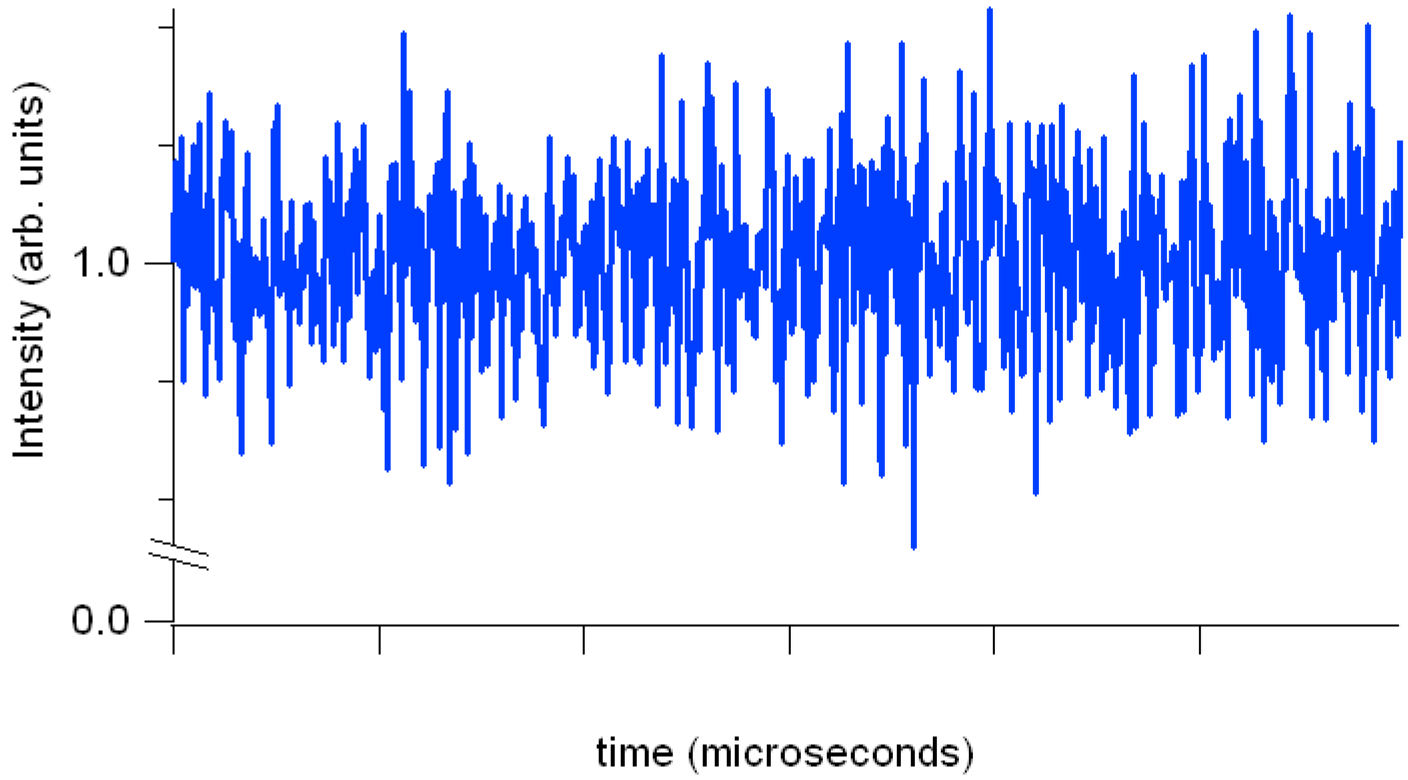
Particles in suspension undergo Brownian motion (random thermal motion).



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

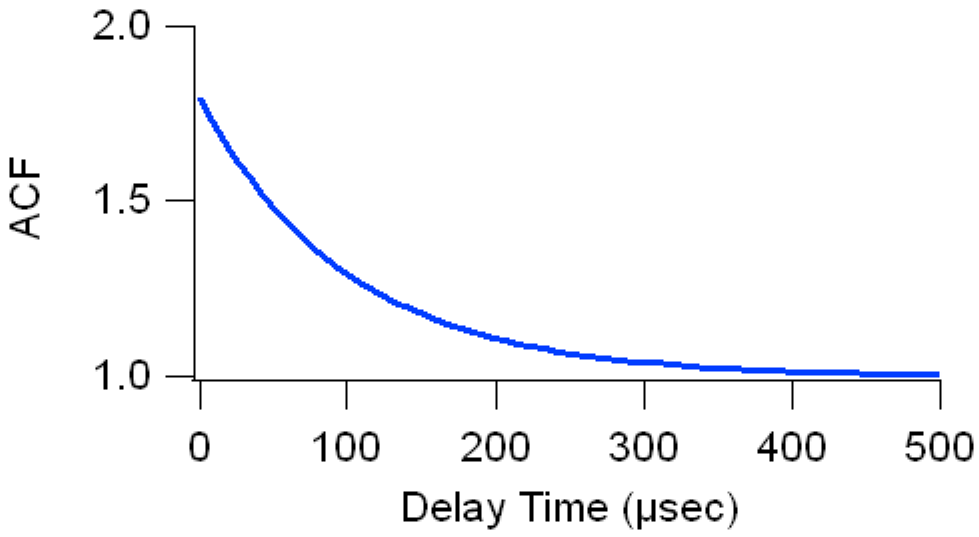
DLS signal

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).

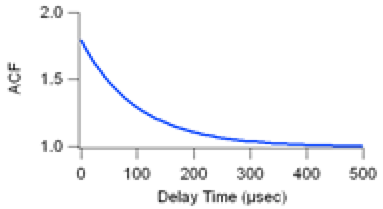


$$C(\tau) = \frac{\int_0^T I(t)I(t+\tau)dt}{\langle I(t)I(t)\rangle}$$

↓

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

Gamma to size



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

$$\Gamma = D_m q^2$$

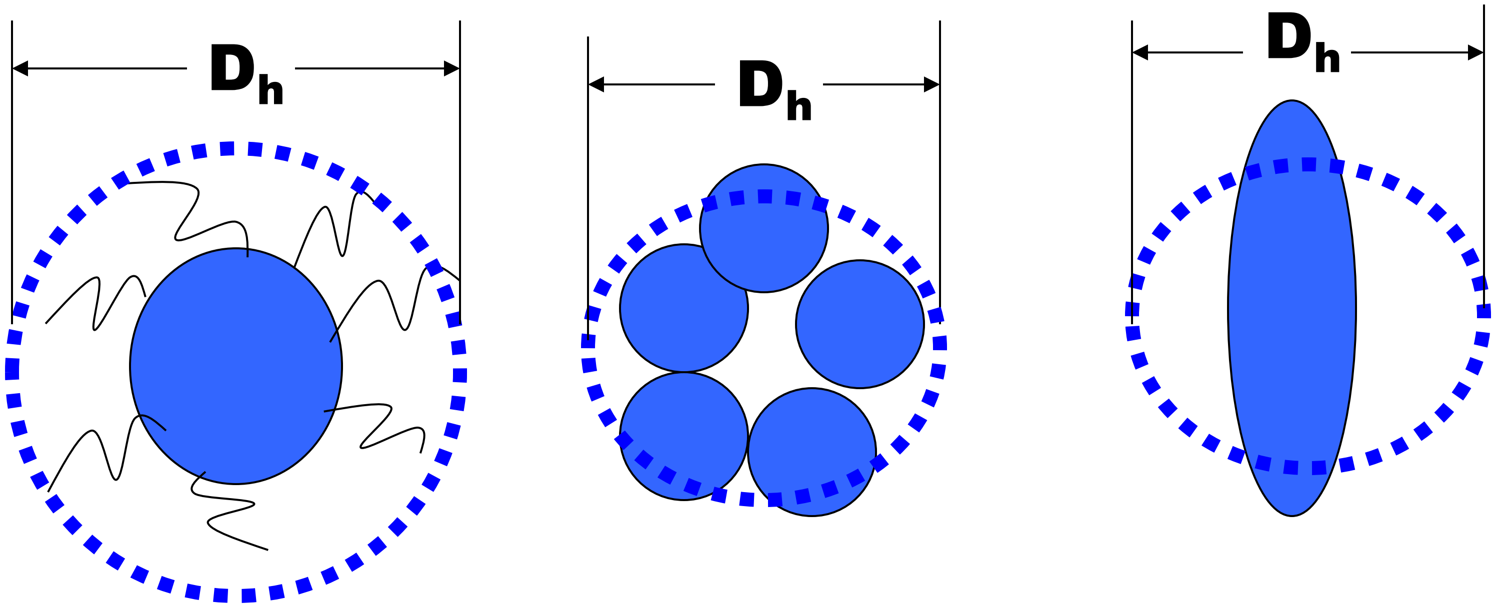
$$D_h = \frac{k_B T}{3\pi\eta(T) D_m}$$

Note effect of temperature!

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

What is hydrodynamic size?

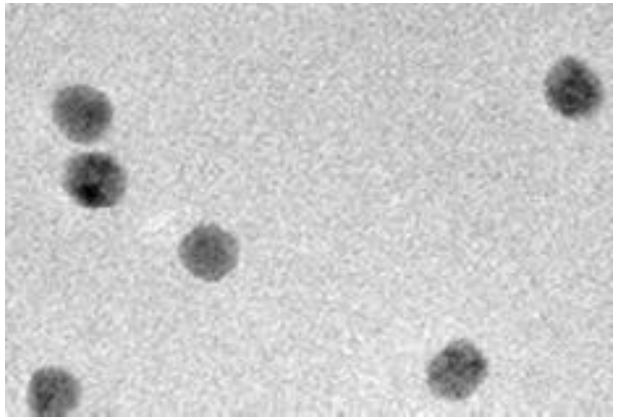
DLS gives the diameter of a sphere that moves (diffuses) the same way as your sample.

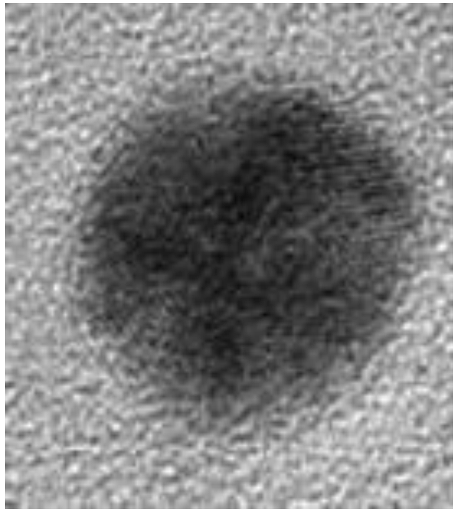


Hydrodynamic size

Gold Colloids

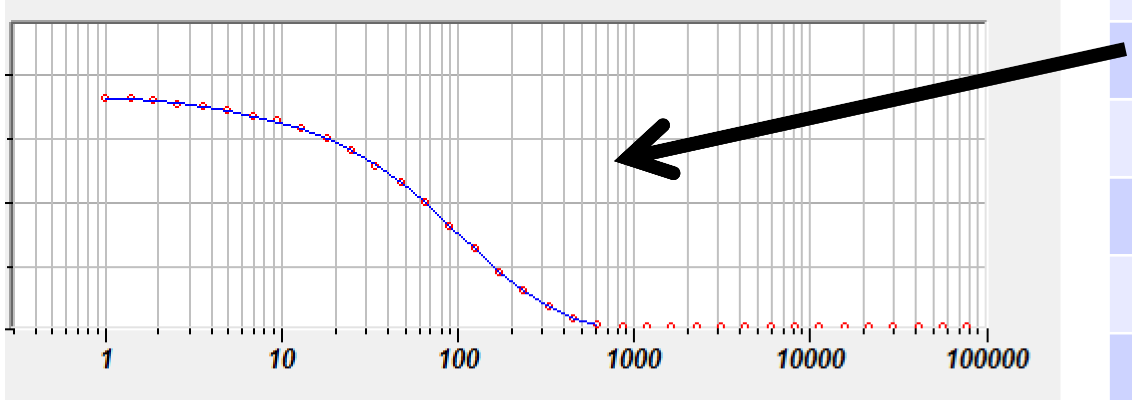
Technique	Size nm
Atomic Force Microscopy	8.5 ± 0.3
Scanning Electron Microscopy	9.9 ± 0.1
Transmission Electron Microscopy	8.9 ± 0.1
Dynamic Light Scattering	13.5 ± 0.1





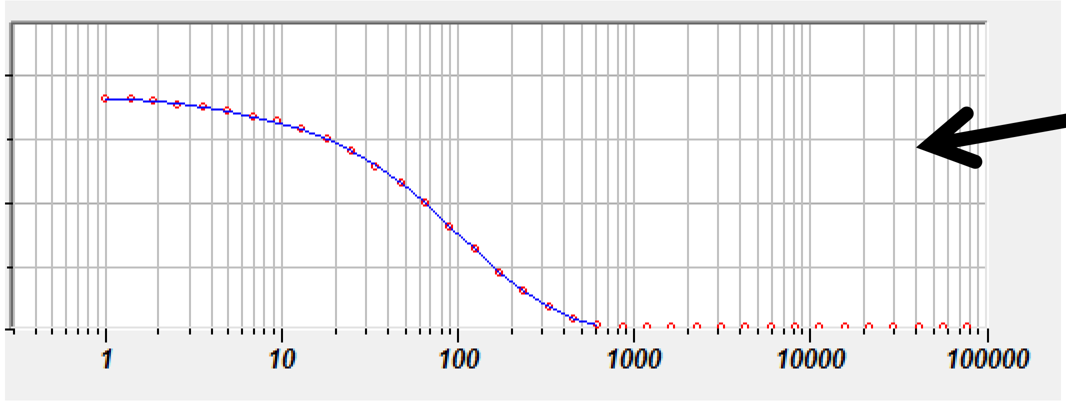
SEM (above) and TEM (below) images for NIST RM 8011

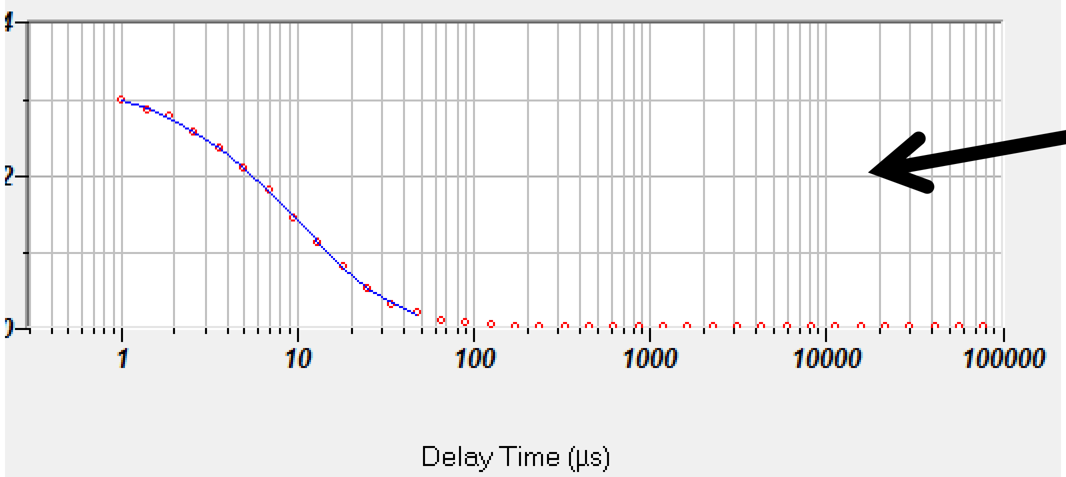
Nanogold data



	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 %

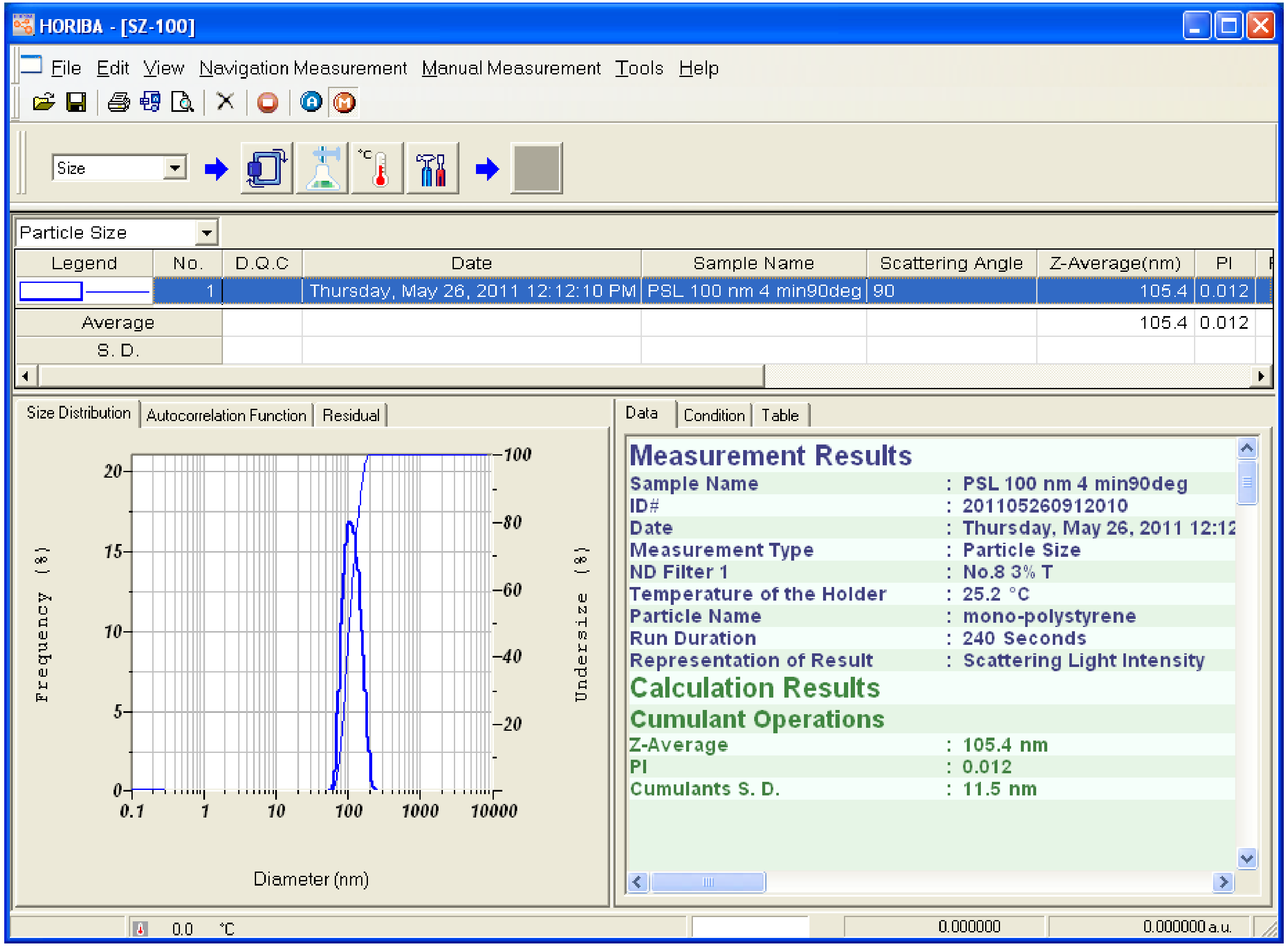
	Z-average Diameter, nm
Run 1	10.5
Run 2	10.6
Run 3	10.2
Run 4	10.5
Run 5	10.3
Avg.	10.4
St. Dev.	0.2
COV	1.9 %

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



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

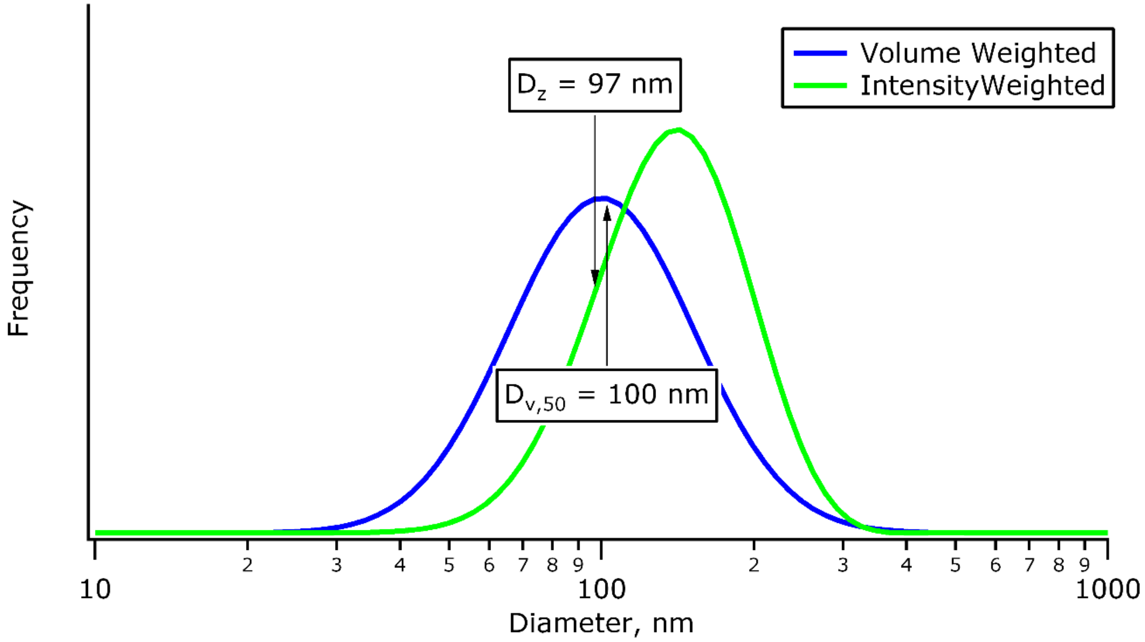
Size determined from intensity weight diffusion coefficient ~1/D

Intensity weighted harmonic mean size

$$^{1}/_{D_z} = {}^{\sum D_i S_i}/_{\sum S_i}$$

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

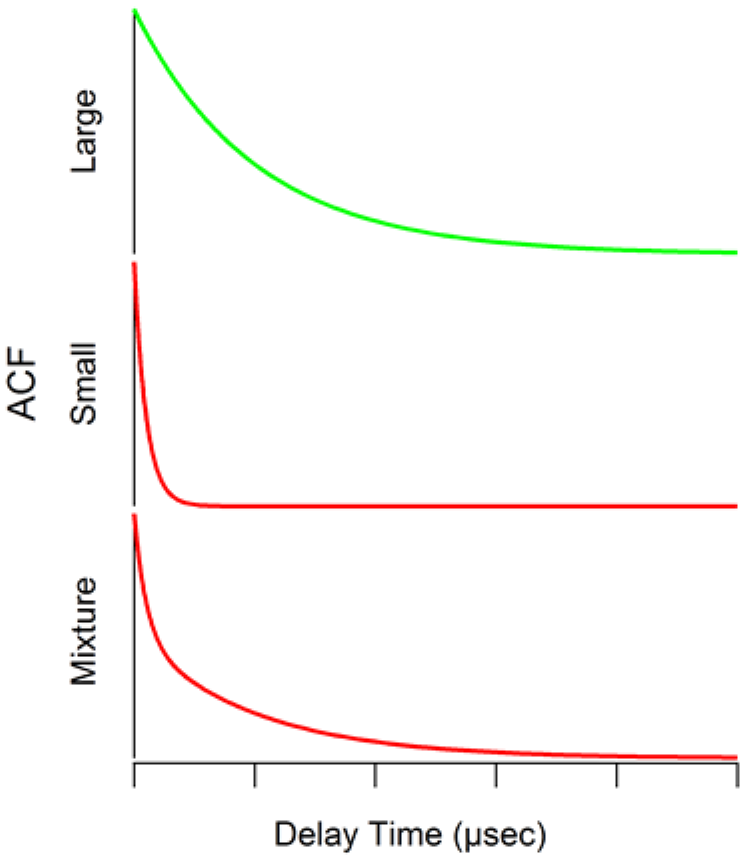
As size goes up, so does D_z.

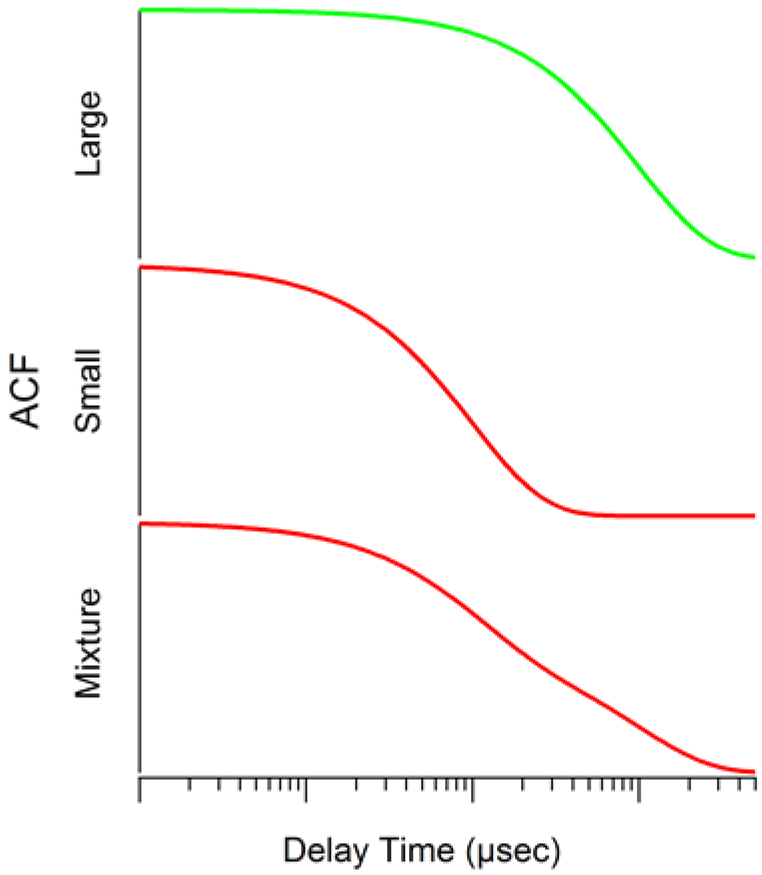


SiO_2

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

Mixtures of particles





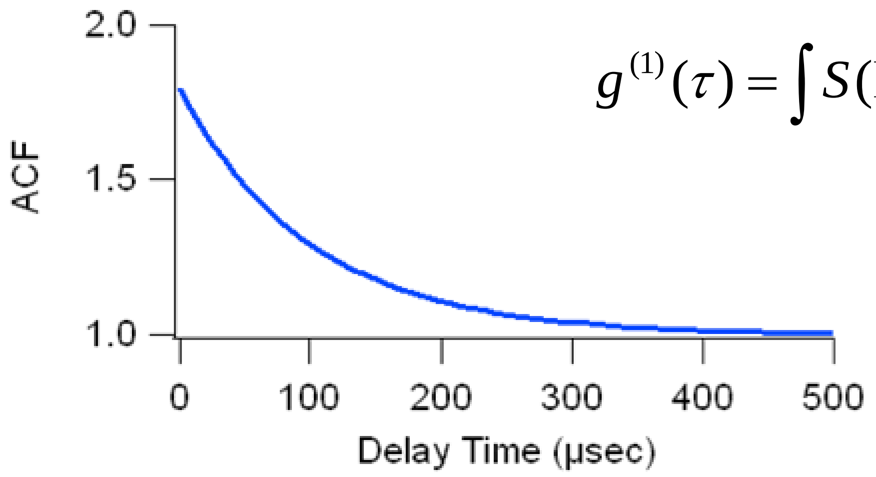
Sum the autocorrelation functions

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.

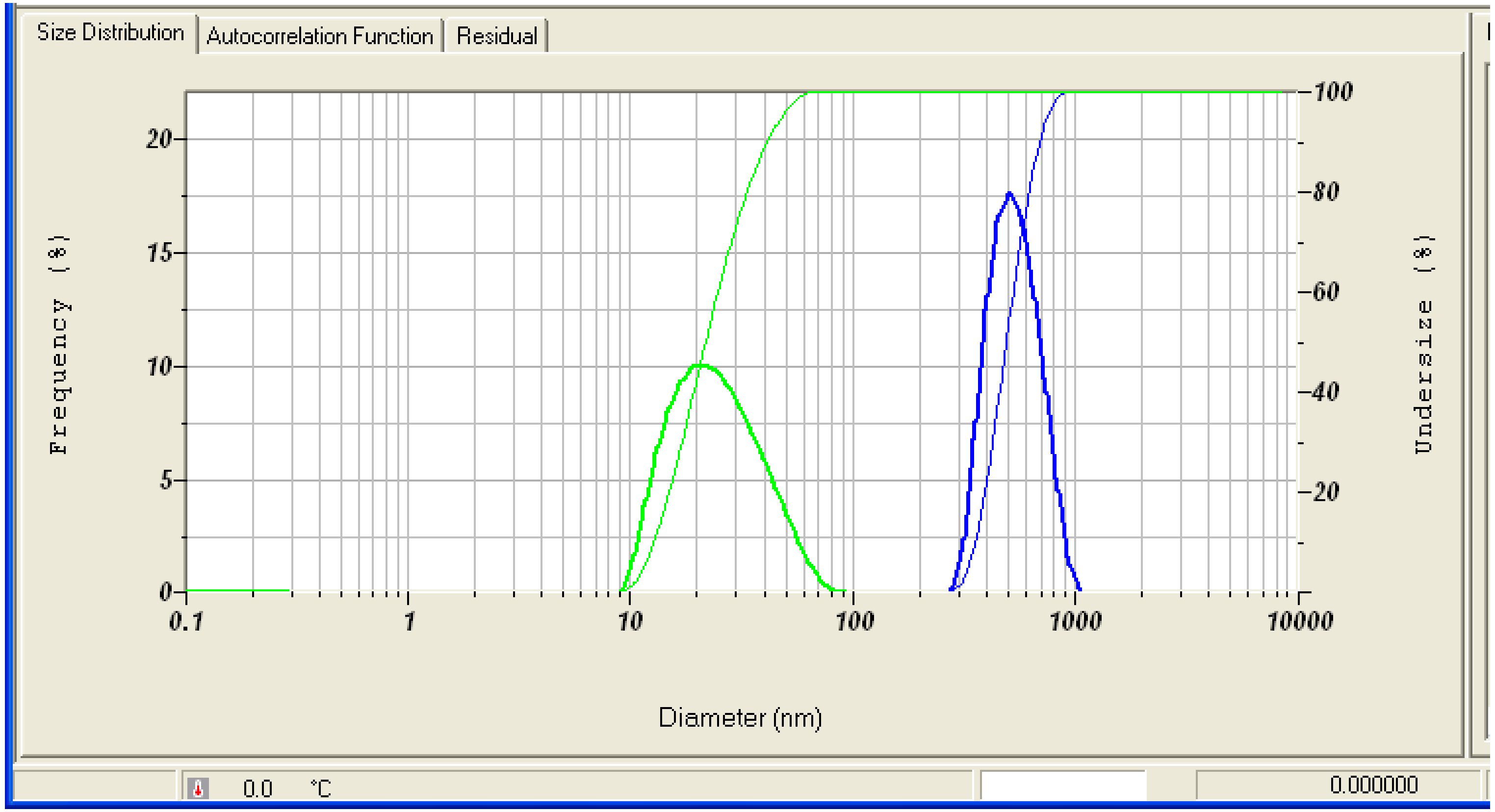
$$C(\tau) = 1 + \beta \left| g^{(1)}(\tau) \right|^2$$

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



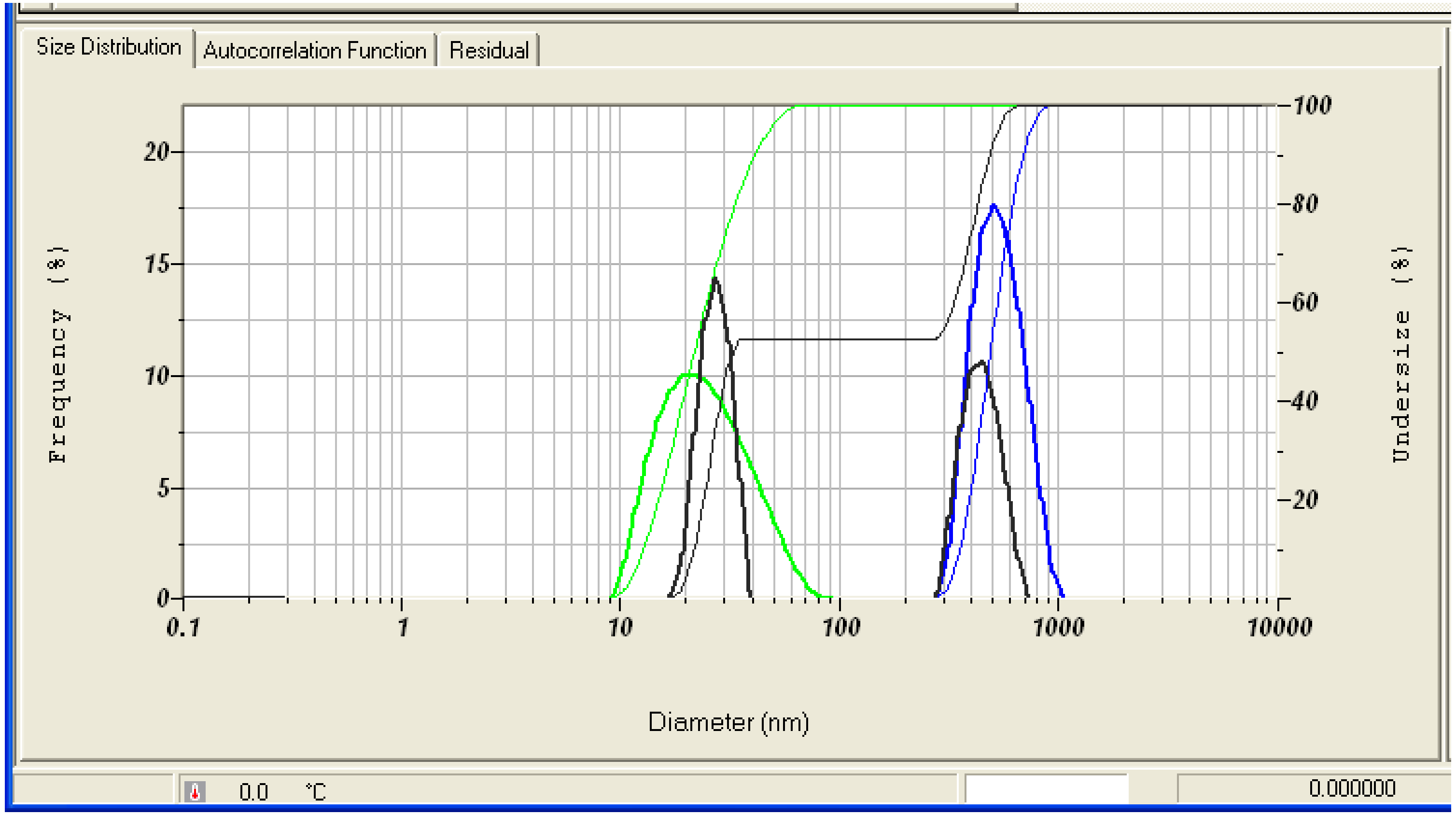
Bimodal sample

Nominal 20 nm and 500 nm latex run individually



Bimodal sample

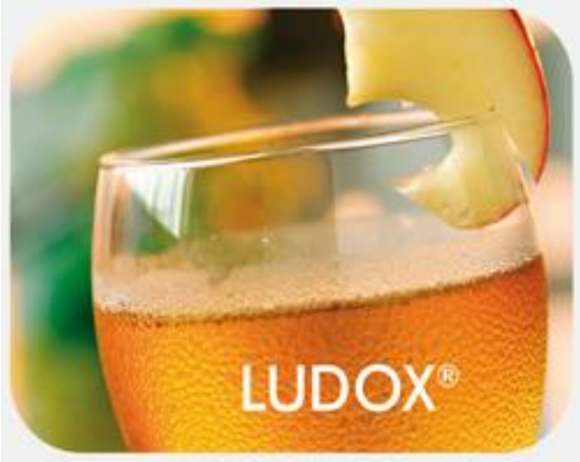
Mixed sample (in black)

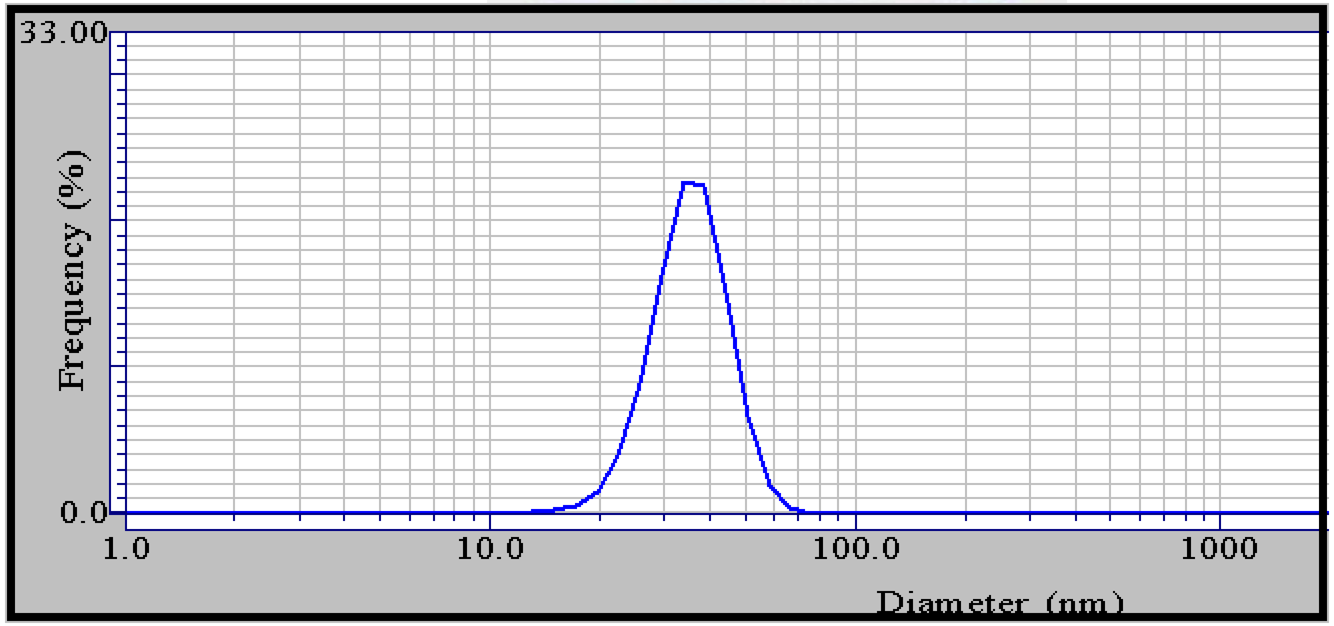


Colloidal Silica

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

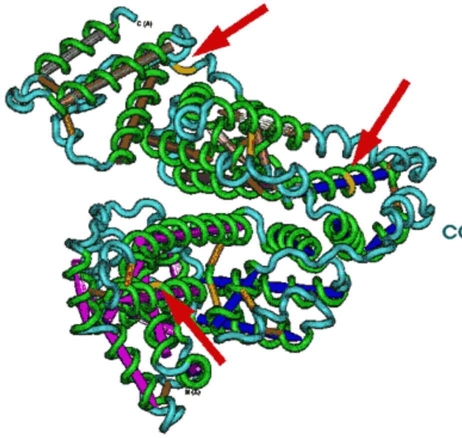
- Matches data from the LA-960 (laser diffraction)

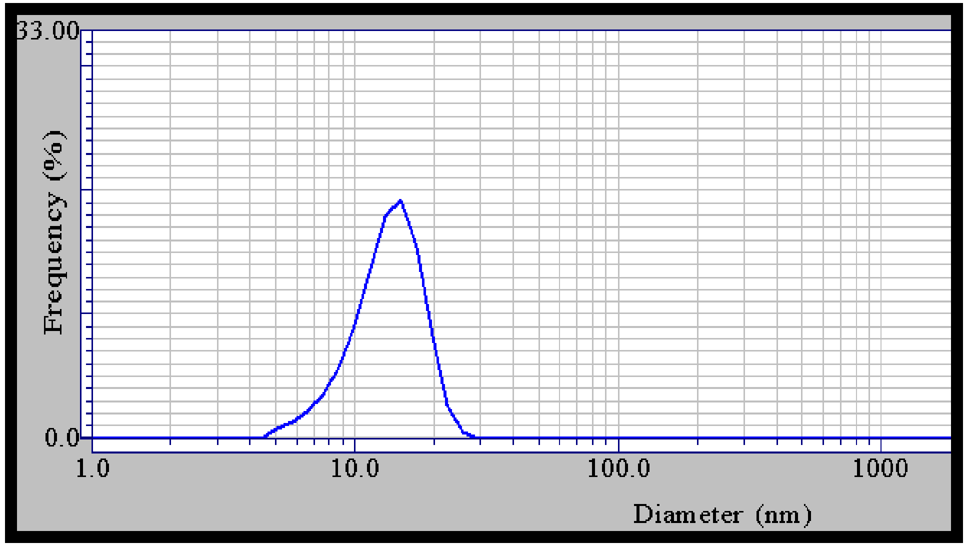




BSA

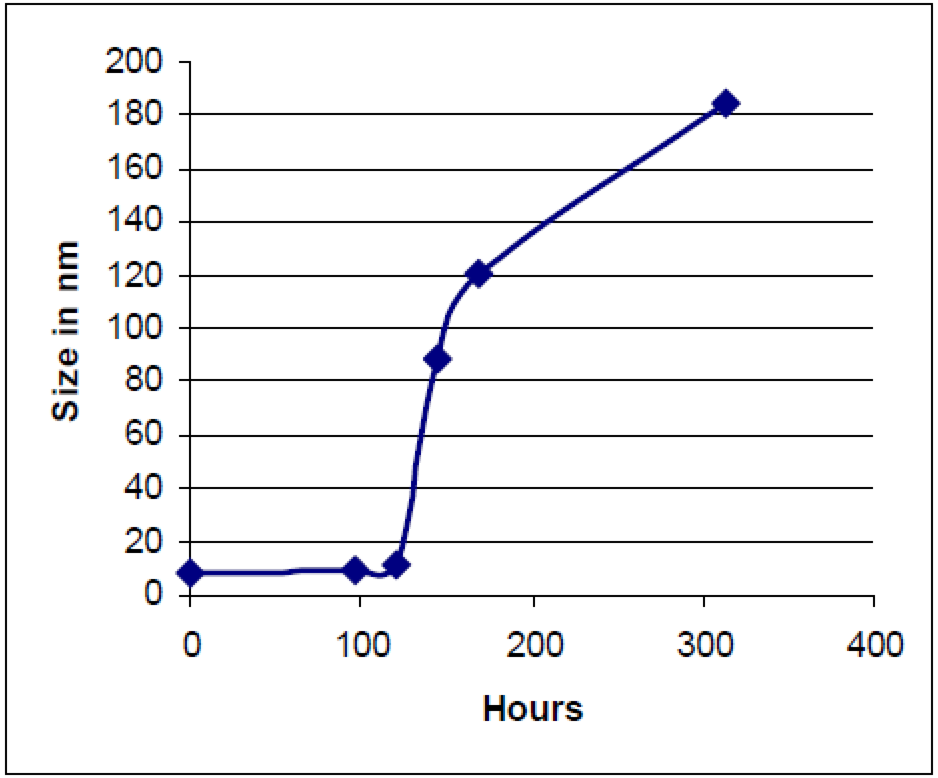
- BSA- well characterized protein
- DLS – Can be used to determine the aggregation state of the protein





Protein aggregation

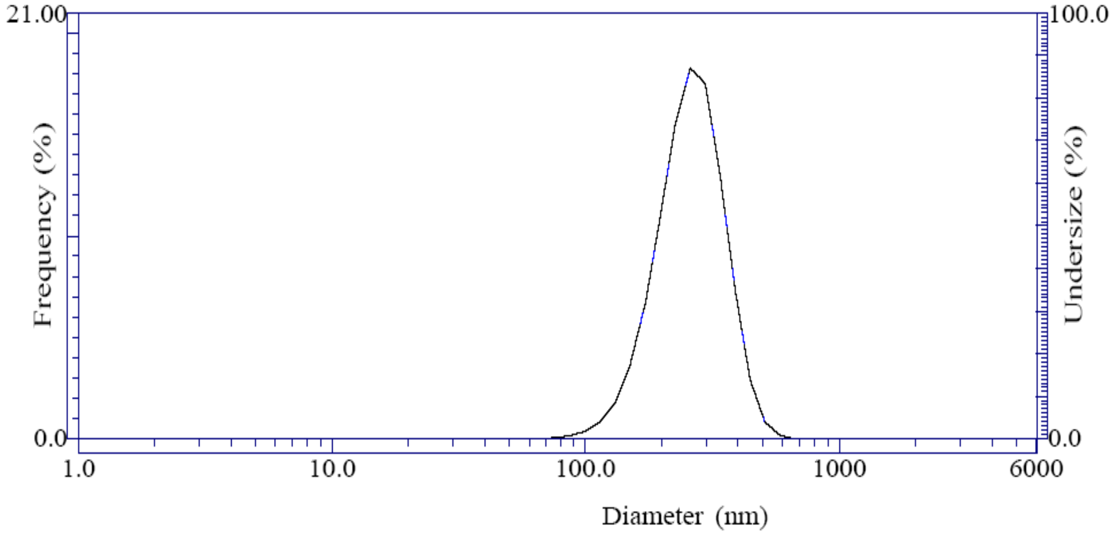
- Unstabilized 10mg/ml lysozyme at pH 2
- Lisa Cole and Ben Burnett at the Florida Institute of Technology
- Can also be done with ViewSizer (NTA)



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



Outline

- Introduction
- What is DLS and what, exactly, does it measure?
- Method Development

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 in sizes 20nm 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

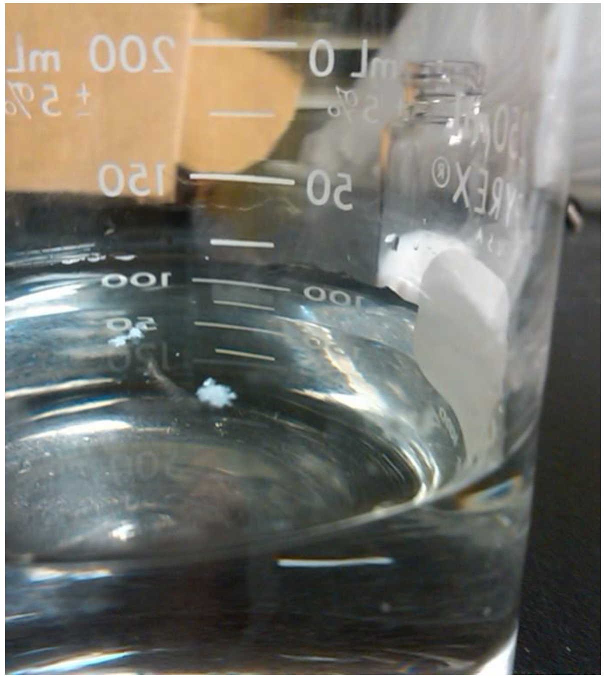
Surfactants

- 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

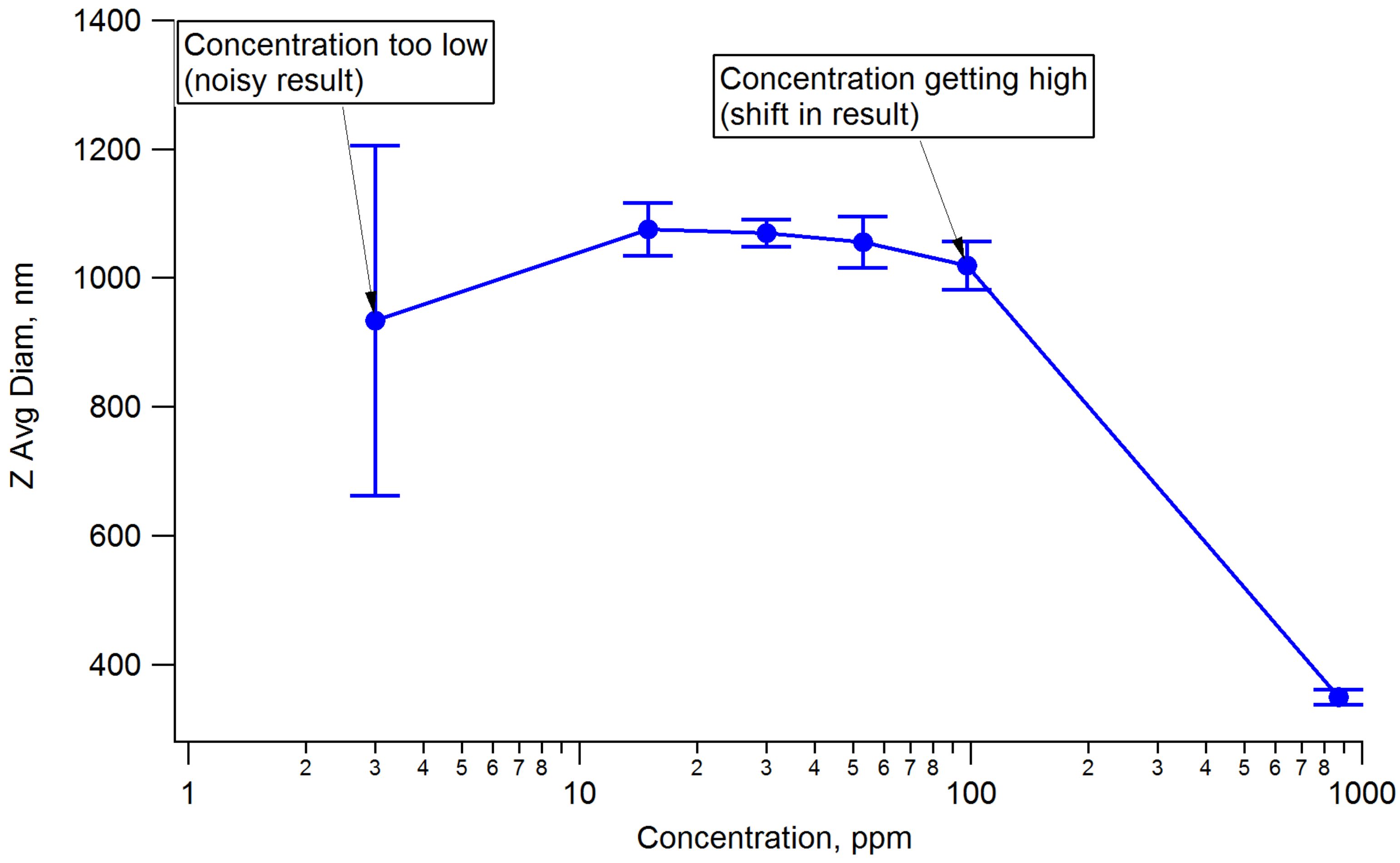
- Working with aqueous systems is usually easier for many reasons.
- But don’t forget to try a less polar solvent such as isopropyl alcohol.
- And, don’t forget that organic solvents are more difficult to handle due to fire and health hazards.

Try a series of options

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

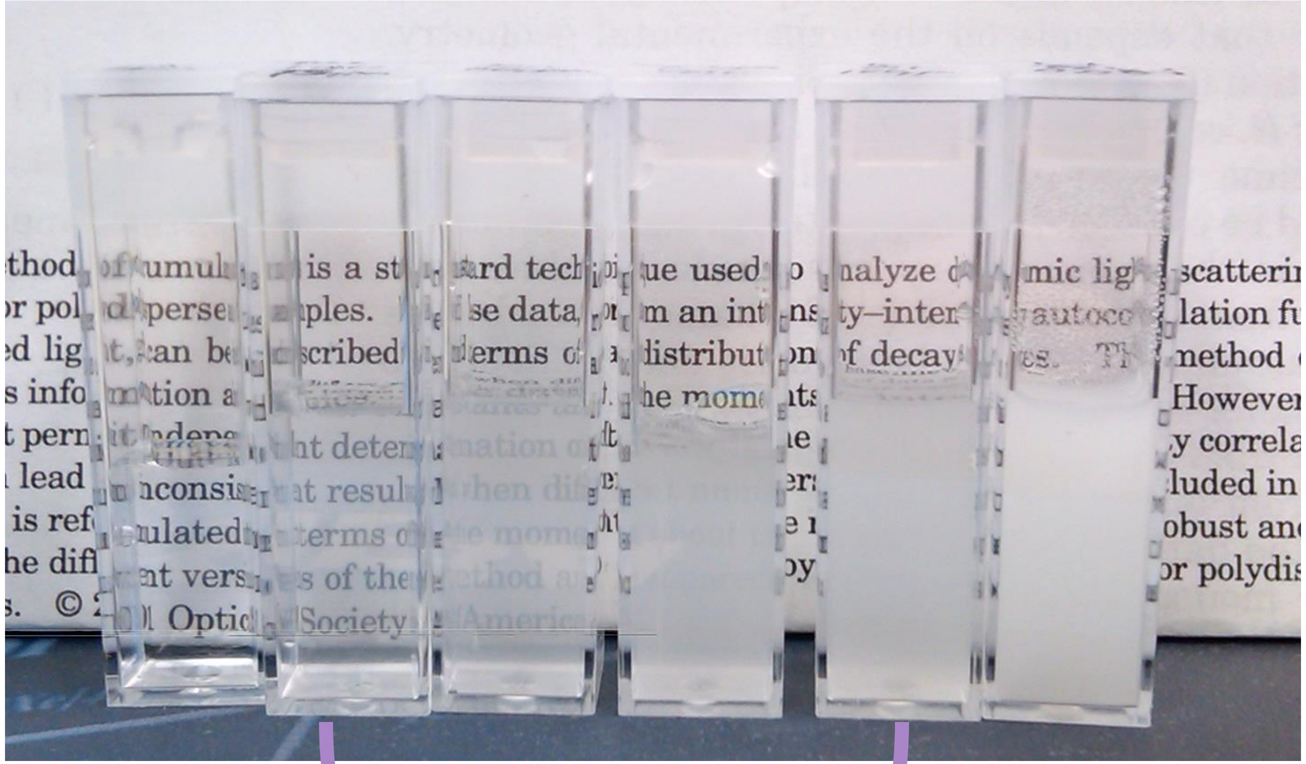
Concentration

Make a plot like this to learn range of concentrations for your sample



Estimate concentration by eye

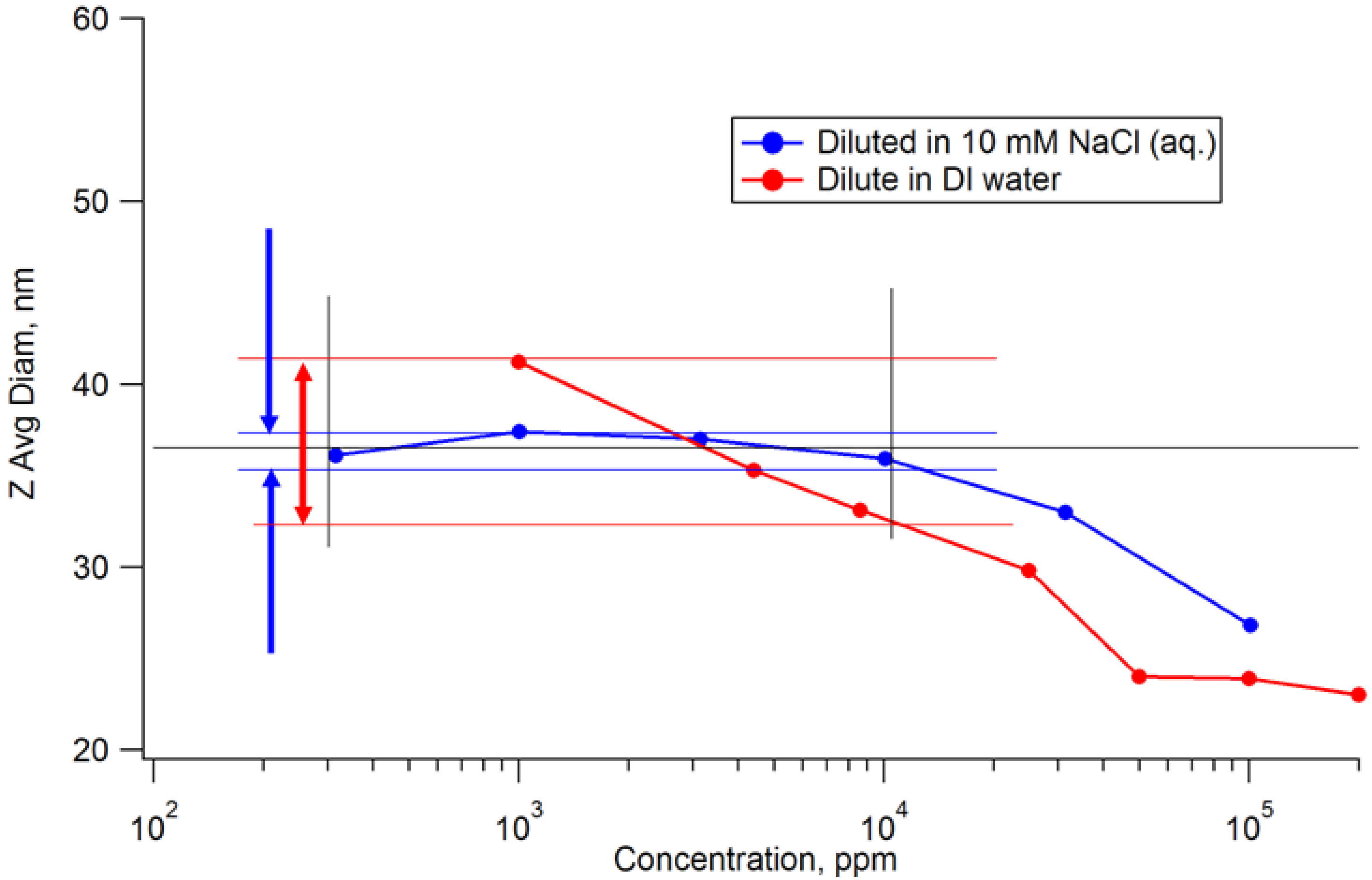
Read a newspaper through it



Best range for this material

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

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Settling and DLS

Not all motion is Brownian motion ☹

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

The Natural limit for Dynamic Light Scattering: Gravitational Settling

Gravitational Settling occurs at about 1-3μm

DLS disadvantages

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

DLS Advantages

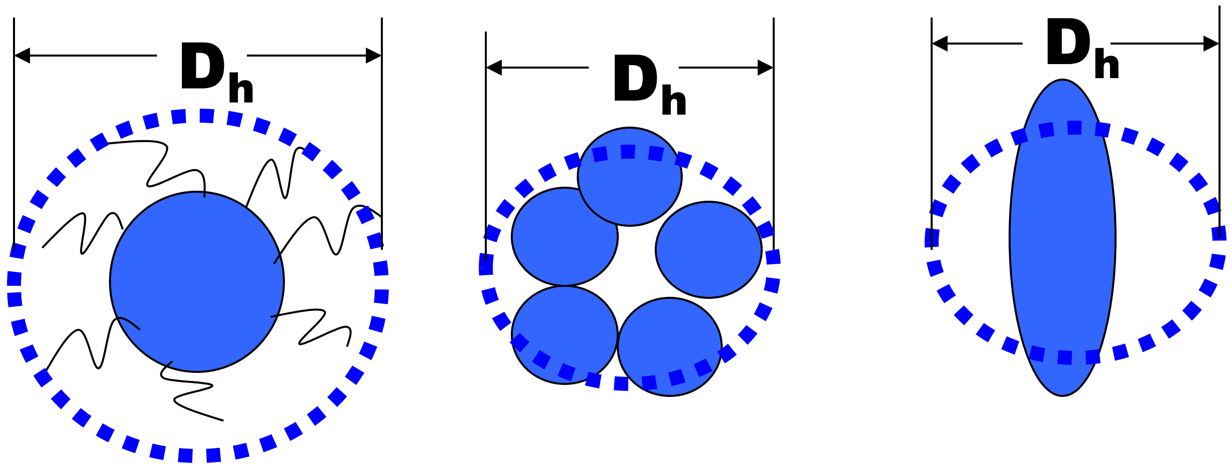
- Noninvasive
- Requires only small quantities of sample
- Good for detecting trace amounts of aggregate
- Good for macromolecular sizing
- Reaches smallest particle sizes

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:



Thank you

HORIBA Scientific

Omoshiro-okashiku
Joy and Fun

Terima kasih
நன்றி
Gracias
谢谢
Danke
Tack ska du ha
Σας ευχαριστώ πάρα πολύ
धन्यवाद
Grazie
THANK YOU
شُكْرًا
ขอบคุณครับ
Obrigado
Большое спасибо
ありがとうございました
Cảm ơn
Merci
감사합니다
Dziękuję