HORIBA Scientific
Particle Characterization

Fresh Insights into Nanoparticle Characterization: DLS, Diffraction, or NTA

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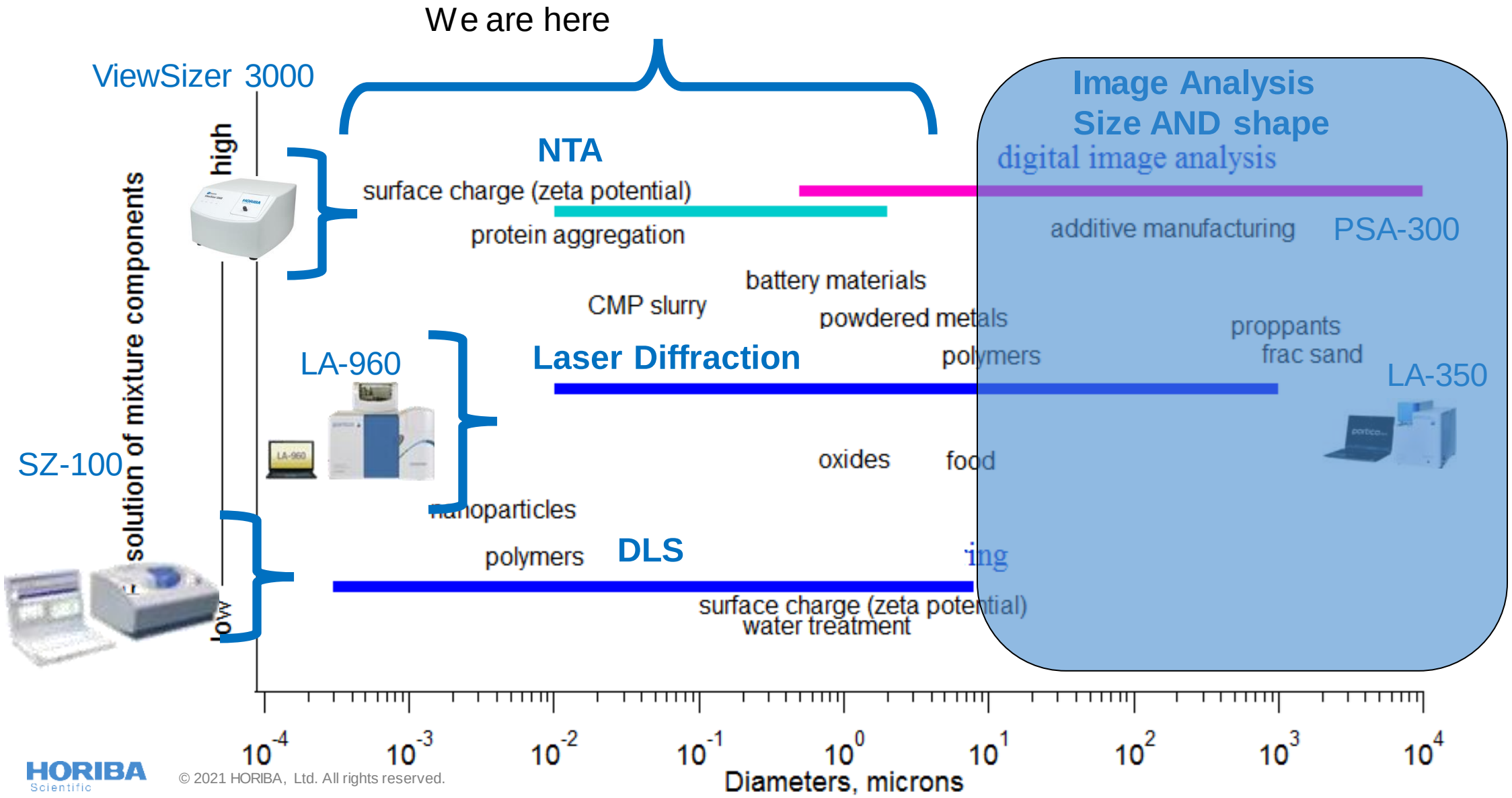
Outline

Overview

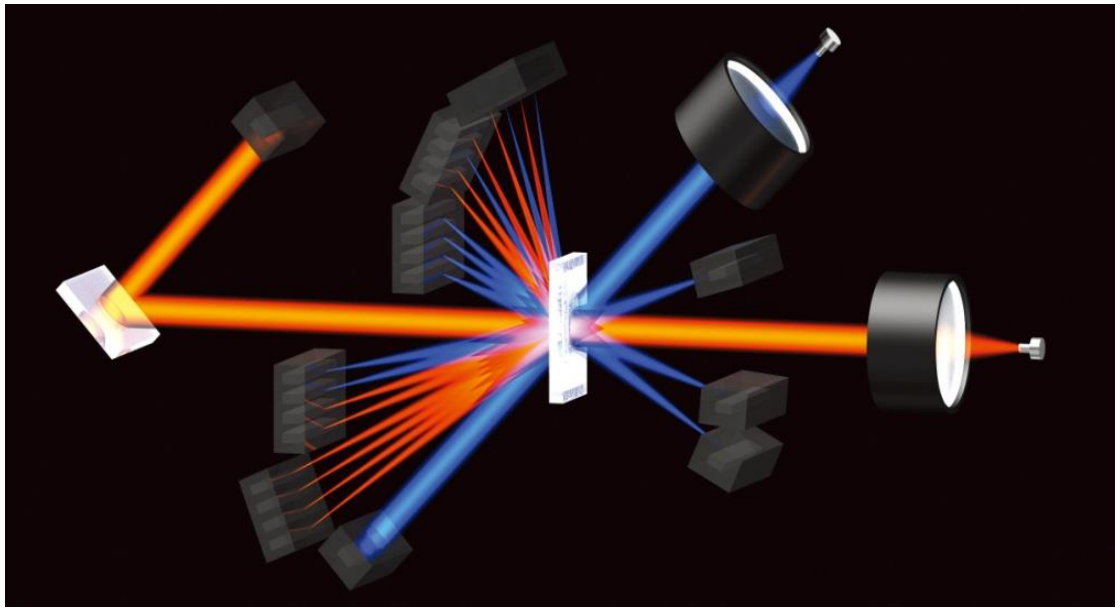
- **Laser Diffraction**
- **Dynamic Light Scattering**
- **Nanoparticle Tracking Analysis**

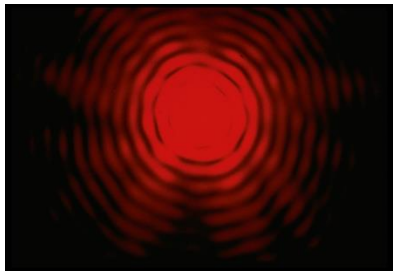
Analysis Techniques



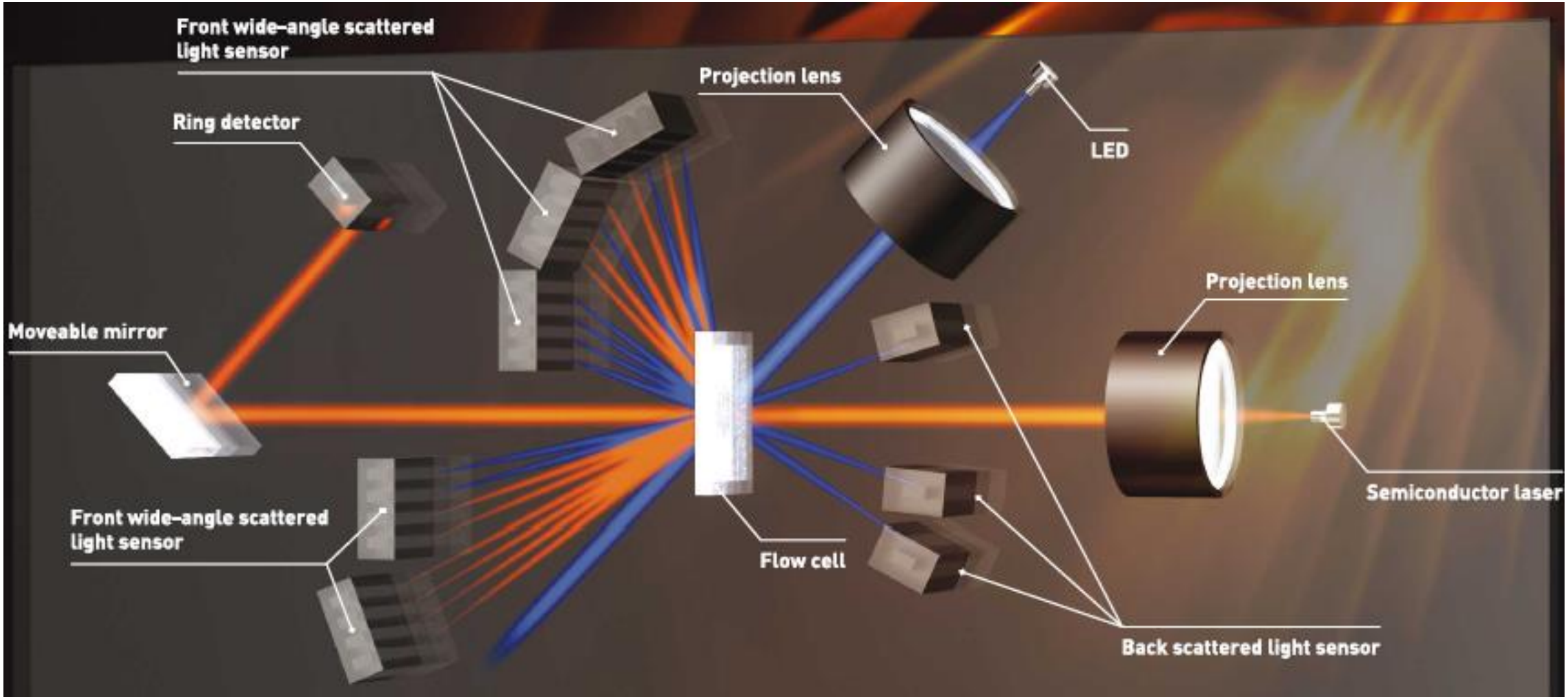
Laser Diffraction

- Converts scattered light to particle size distribution
- Quick, repeatable
- Powders, suspensions
- Most common technique



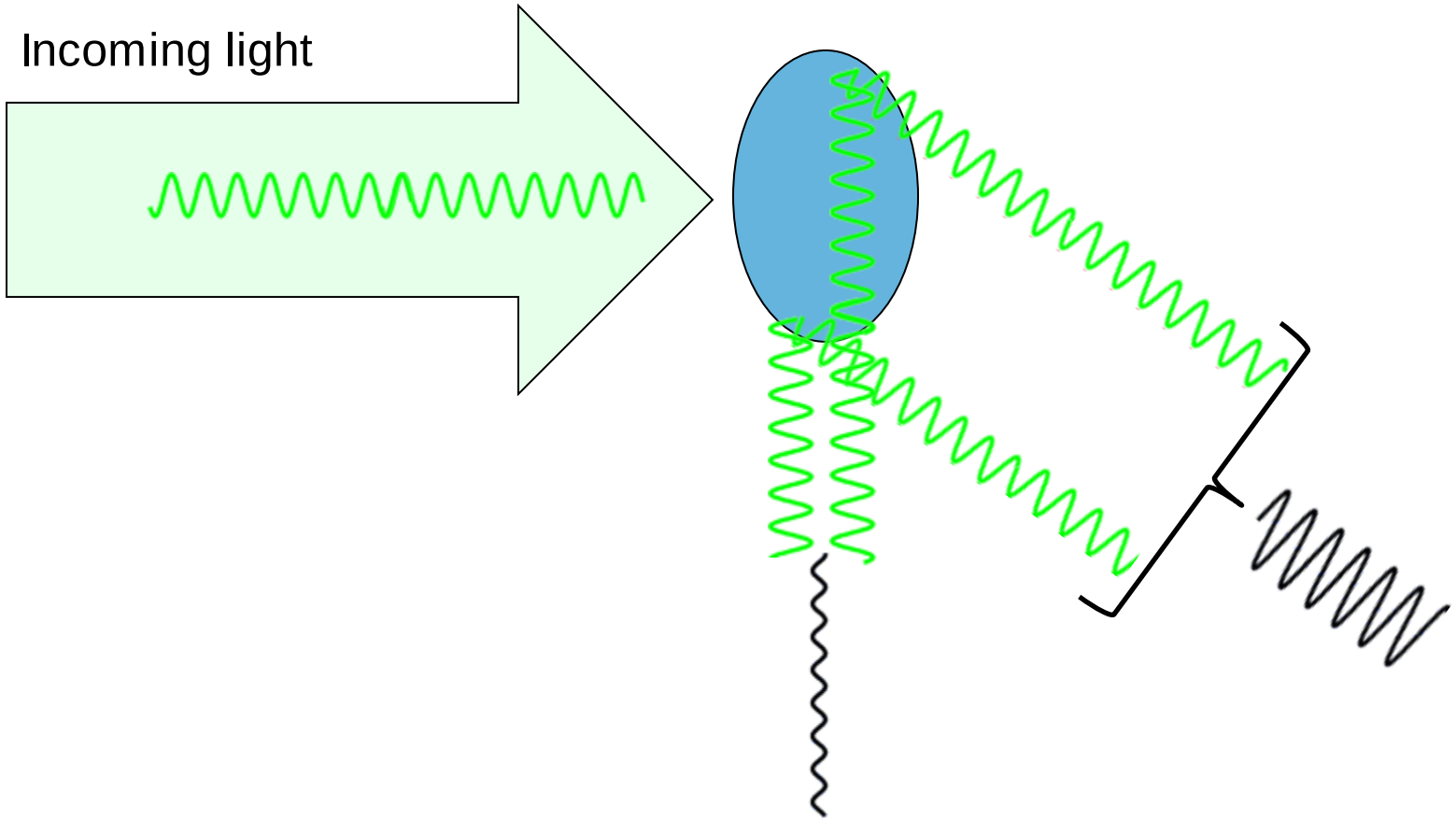


LA-960 Optics



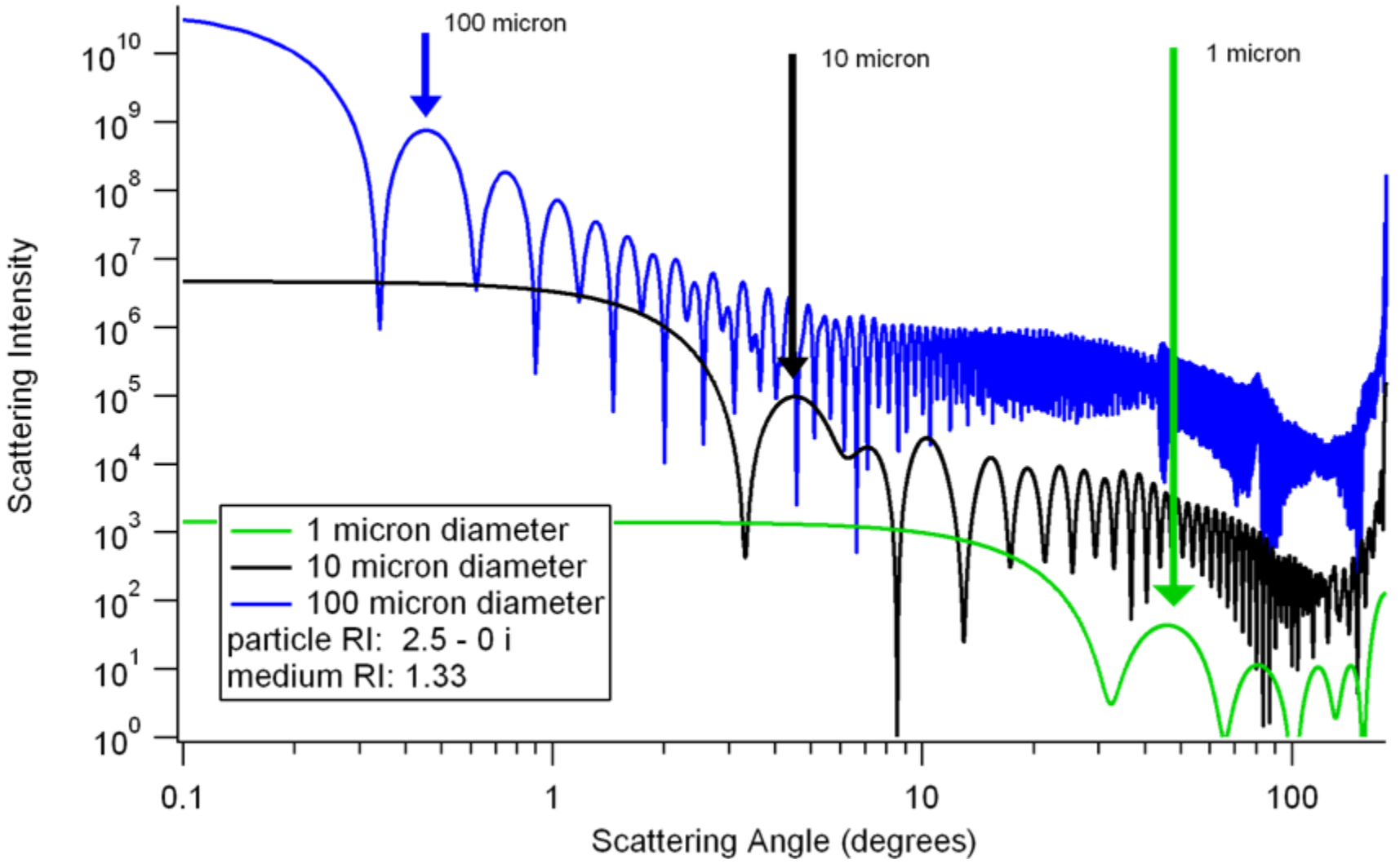
Path Length Difference

Diffraction effects arise due to scattering from various points in the particle (and, in the large particle limit only the edges)



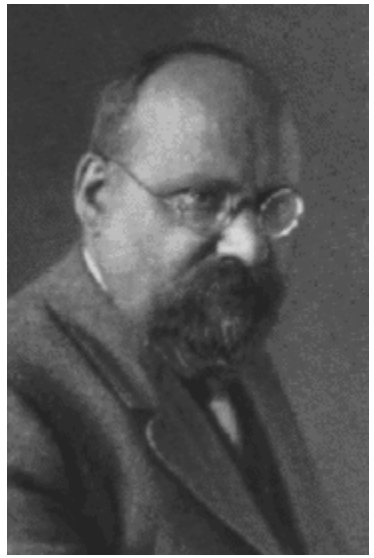
Effect of Size

As diameter increases, intensity (per particle) increases and location of first peak shifts to smaller angle.



Interpreting Scattering Data

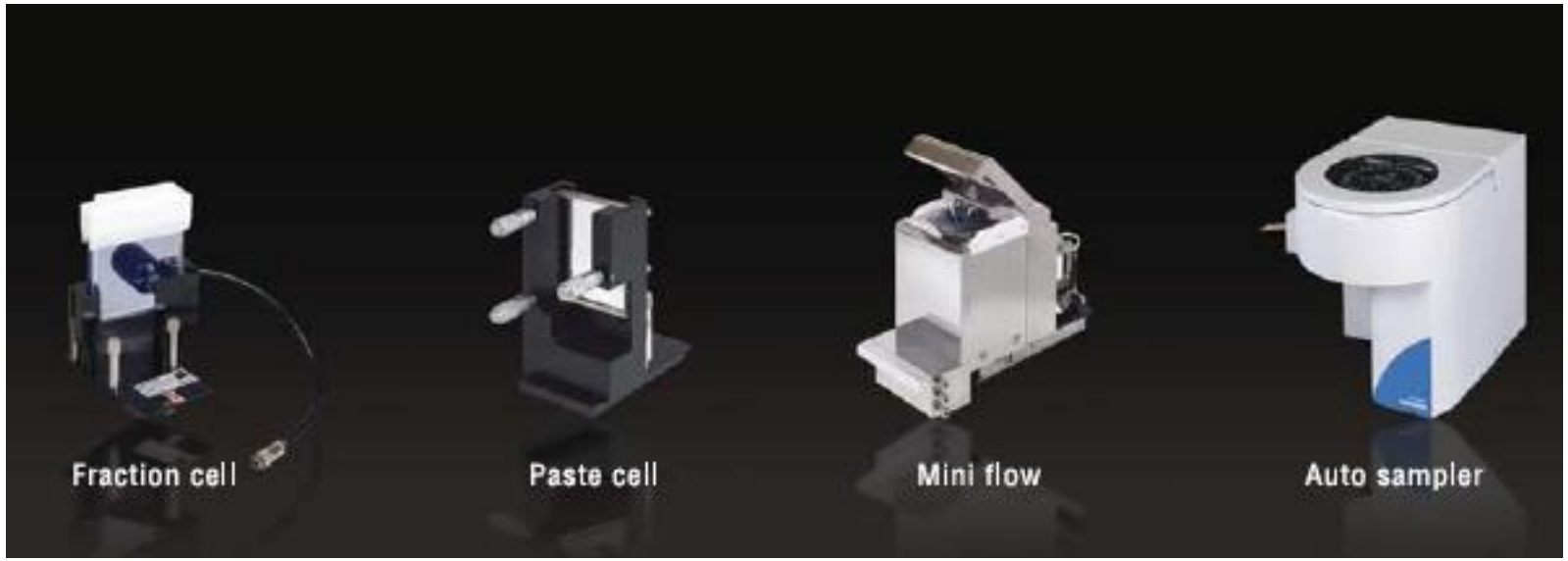
- Scattering data typically cannot be inverted to find particle shape.
- We use optical models to interpret data and understand our experiments.
- Modern systems use particle refractive index in a 3-D calculation (Mie Theory) that includes behavior of light inside of particles.



Gustave Mie

Image courtesy
ThomasHB4 at English Wikipedia

Flexible Sample Handlers



5-10 ml | 35 ml | 200 ml | powders

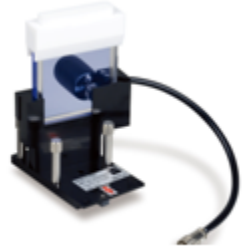
- Wide range of sample cells depending on application
- High sensitivity keeps sample requirements at minimum
- Technology has advanced to remove trade-offs

How much sample (wet)?

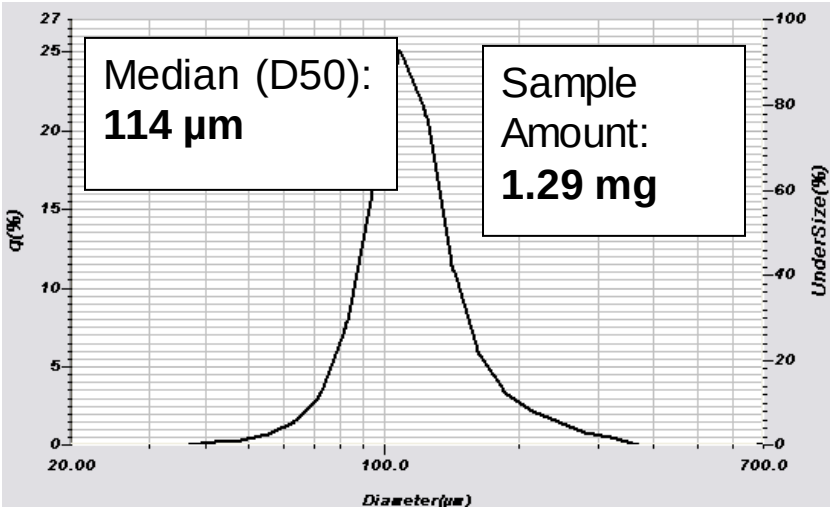
It depends on sample, but here are some examples

Larger, broad distributions require larger sample volume

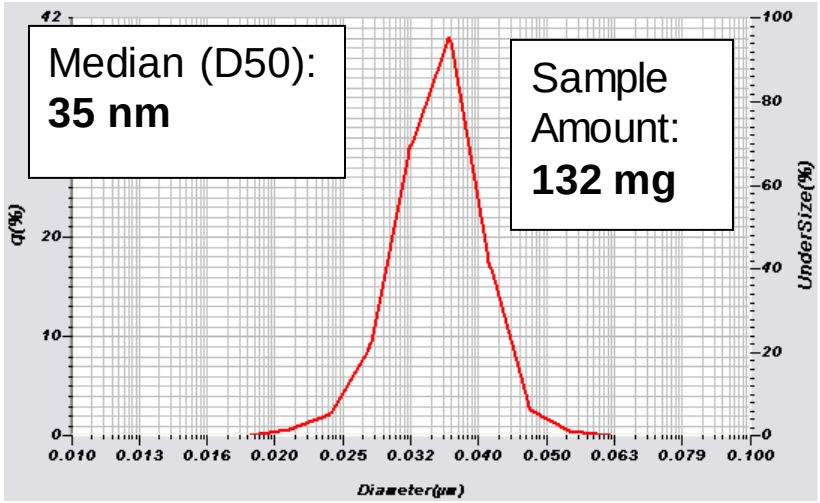
Lower volume samplers for precious materials or solvents



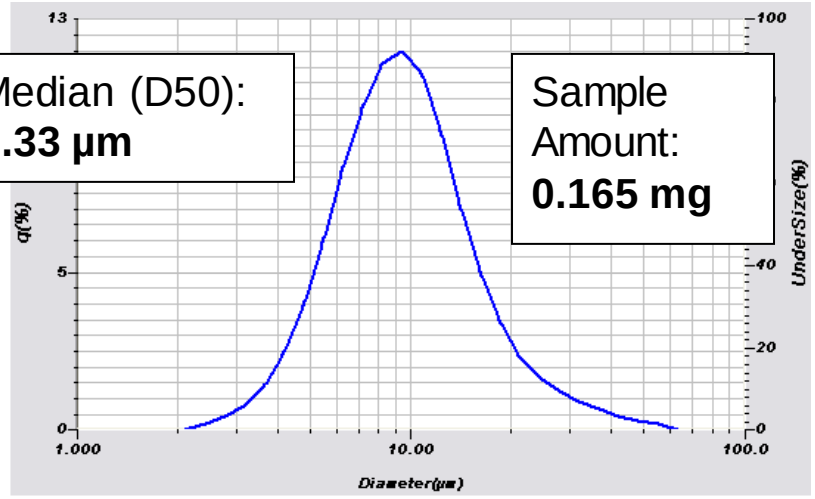
Sample Handlers	Volume (mL)
Aqua/SolvoFlow	**180 - 330**
MiniFlow	**35 - 50**
Fraction Cell	**15**
Small Volume Fraction Cell	**10**



Bio polymer



Colloidal silica



Magnesium stearate

Instrument to instrument variation

4 instruments (real sample)

	Dmean	D5	D10	D50	D90	D95
Average (nm)	155	112	119	152	193	208
Std. Dev. (nm)	0.8	0.8	0.7	1.0	1.1	0.7
CV (%)	0.5	0.7	0.6	0.6	0.6	0.3

Figure 8: Instrument to instrument variation across four LA-950 systems for Formulation 1.

	Dmean	D5	D10	D50	D90	D95
Average (nm)	193	136	147	187	247	264
Std. Dev (nm)	1.5	0.5	0.4	0.6	0.4	1.1
CV (%)	0.8	0.4	0.3	0.3	0.2	0.4

Figure 9: Instrument to instrument variation across four LA-950 systems for Formulation 2.

Laser Diffraction Benefits

Wide size range
Most advanced analyzer measures from 10 *nano* to 5 *milli*
Important when quantifying larger particles in sample

Flexible sample handlers
Powders, suspensions, emulsions, pastes, creams

Very fast
Allows for high throughput, 100's of samples/day

Easy to use
Many instruments are highly automated with self-guided software

Good design = Excellent precision
Reduces unnecessary investigation/downtime

First principle measurement
No calibration necessary

Massive global install base/history

What is Dynamic Light Scattering?

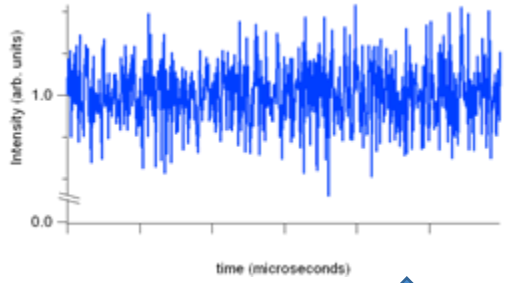
Dynamic light scattering (DLS) refers to measurement and interpretation of light scattering data on a microsecond time scale.

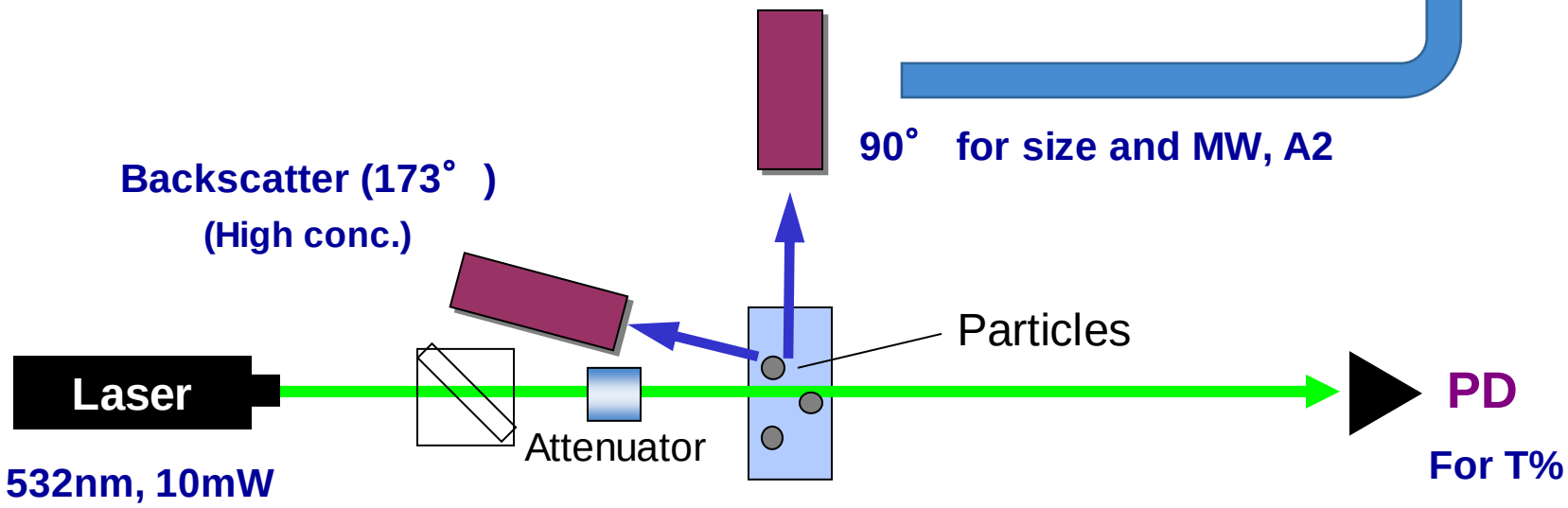
Dynamic light scattering can be used to determine

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

DLS Optics

Use scattering as a function of time to determine size and size distribution

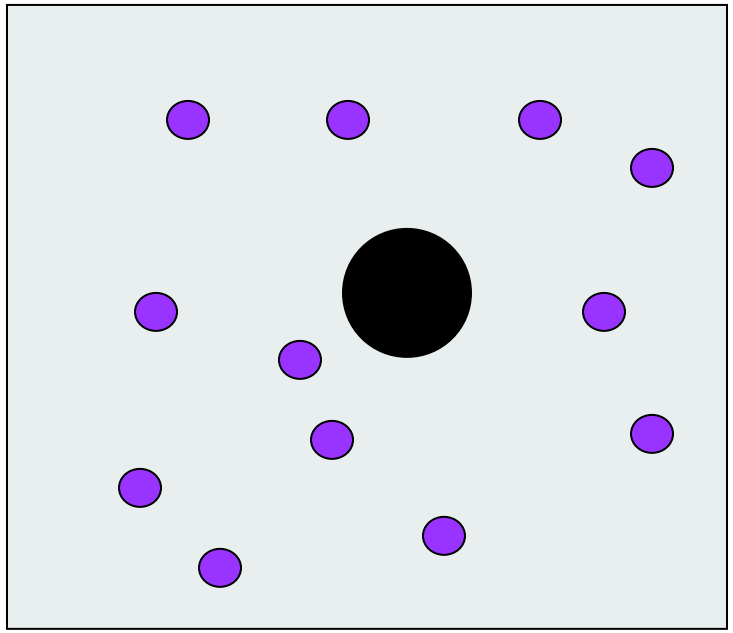




Particles moving due to Brownian motion

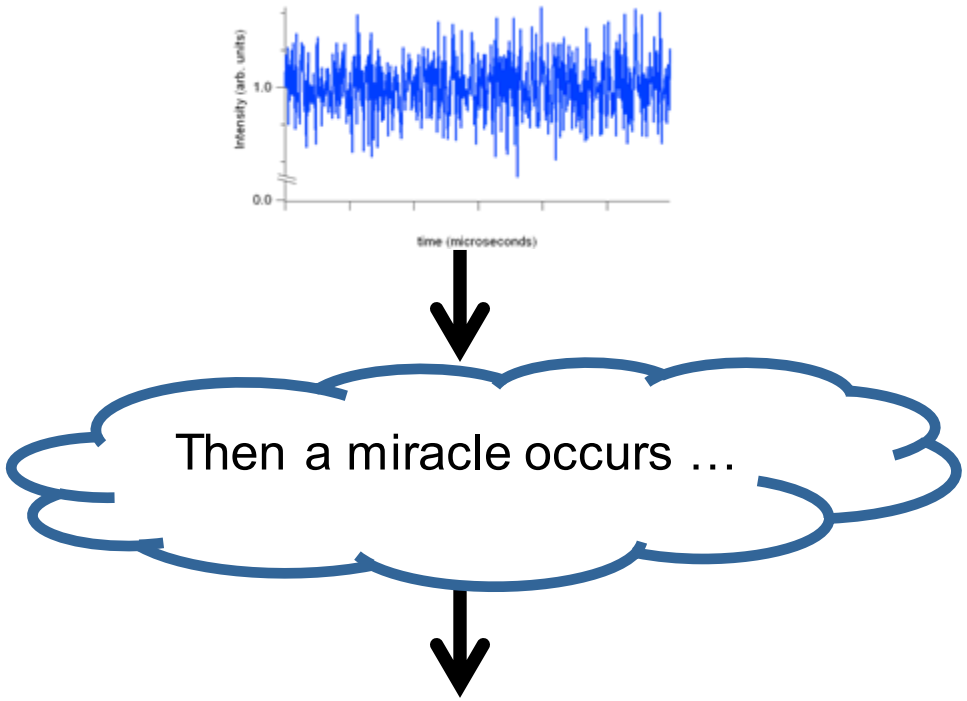
Brownian Motion

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



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

Hydrodynamic Diameter

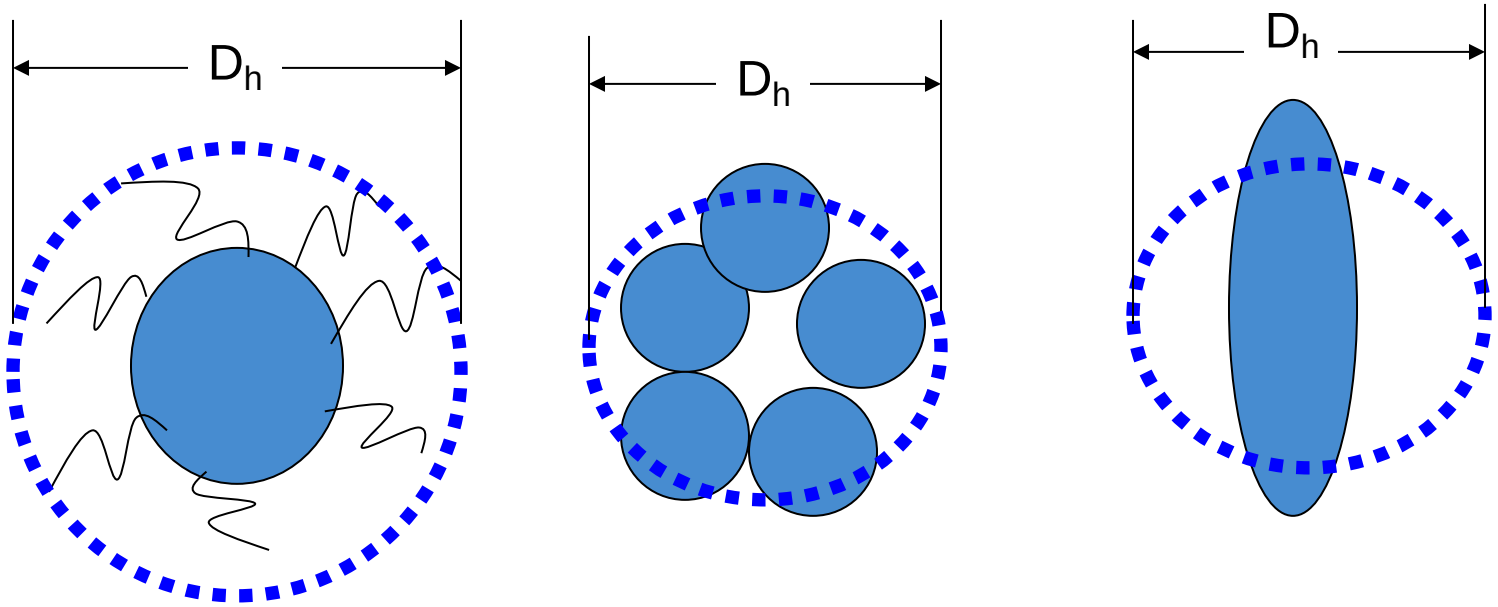


$$D_h = \frac{k_B T}{3\pi\eta(T)D_m}$$

D_m diffusion coefficient
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.



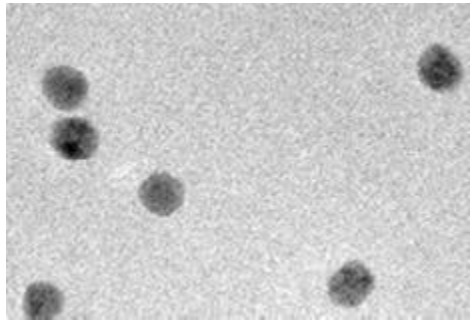
Hydrodynamic Size

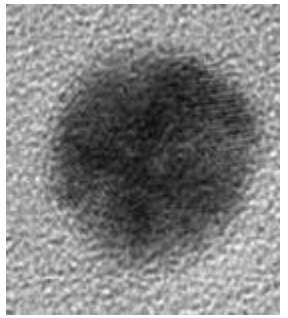
The instrument reports the size of sphere that moves (diffuses) like your particle.

This size will include any stabilizers bound to the molecule (even if they are not seen by TEM).

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 RM 8011

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

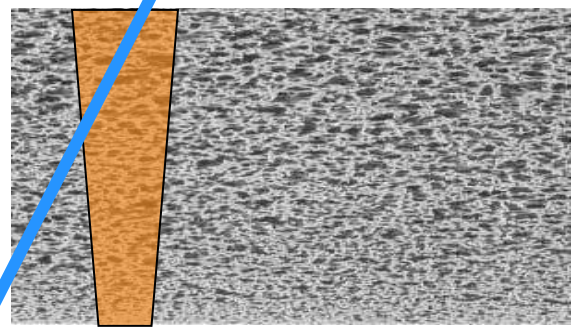
Why DLS?

- Non-invasive measurement
- Requires only small quantities of sample
- Good for detecting trace amounts of aggregate
- Good technique for macro-molecular sizing

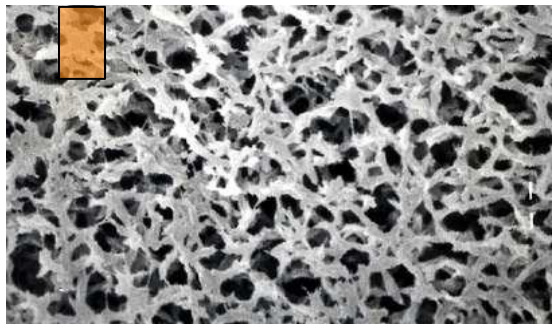
DLS vs. Diffraction: Nanoemulsion

Nanoemulsion production challenges:

- Stability
  - The presence of particles over 1 micron may destabilize the emulsion through Ostwald-ripening
- Sterilization
  - Removal of bacteria by filtering though a 0.22 micron rated filter
  - Preferred sterilization method in vaccine adjuvants, cancer drugs (injectables)
  - Most particles should be below 0.22 microns so they do not plug the filter



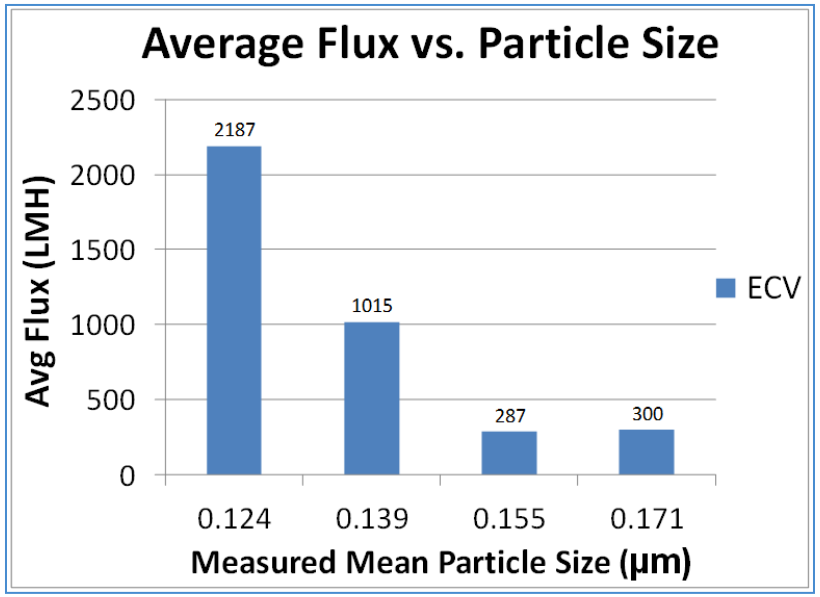
Asymmetric

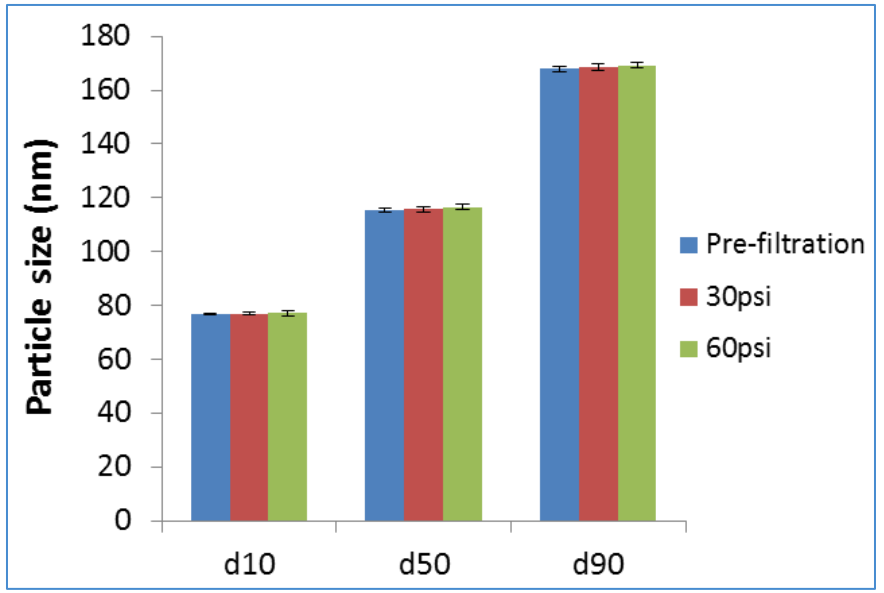


Symmetric

NEED effective measurements of particles over 1 micron.
Recommend laser diffraction

Nanoemulsion – Vaccine Adjuvant





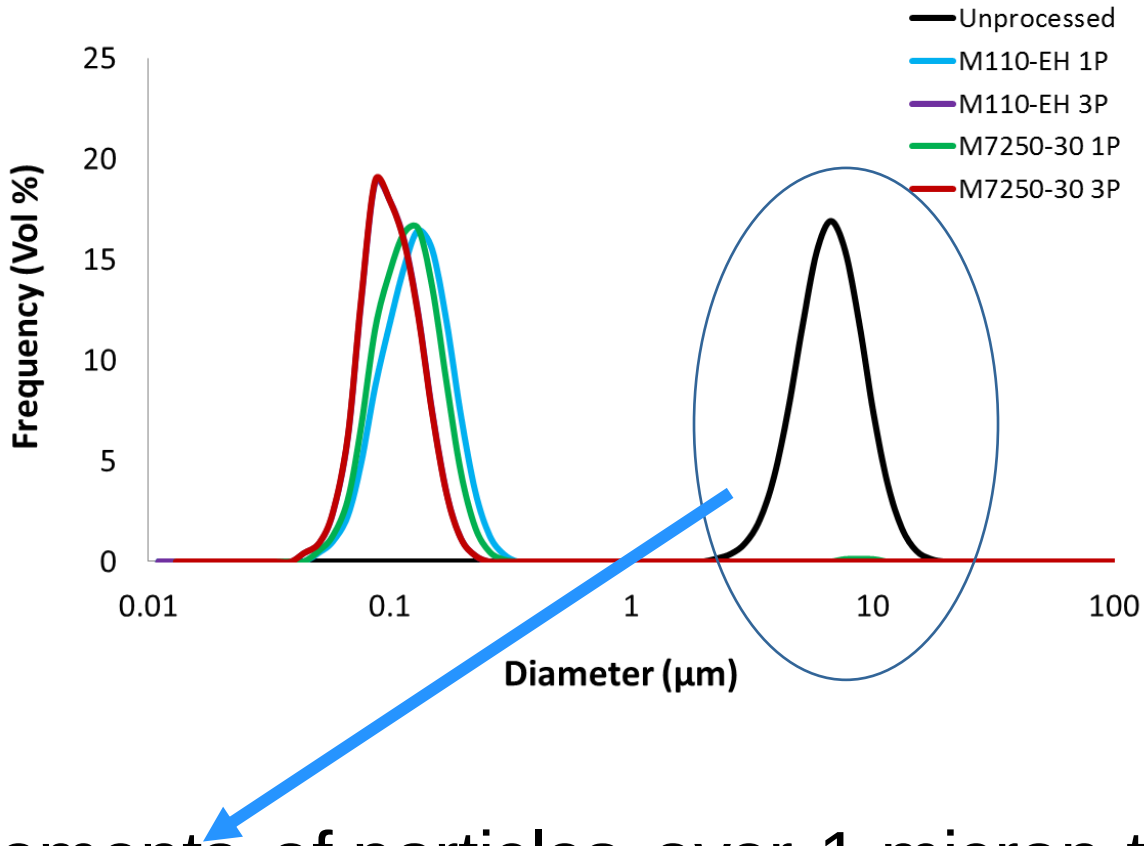
Collaboration with Pall Life Sciences

PALL Pall Corporation

Smaller particles lead to better flow through filter.
You want to follow your size reduction process to help the downstream steps.

Nanoemulsion – Vaccine Adjuvant

- **Squalane Emulsion processed with Microfluidizer**



Effective measurements of particles over 1 micron to give consistent results from start to finish: Recommend laser diffraction

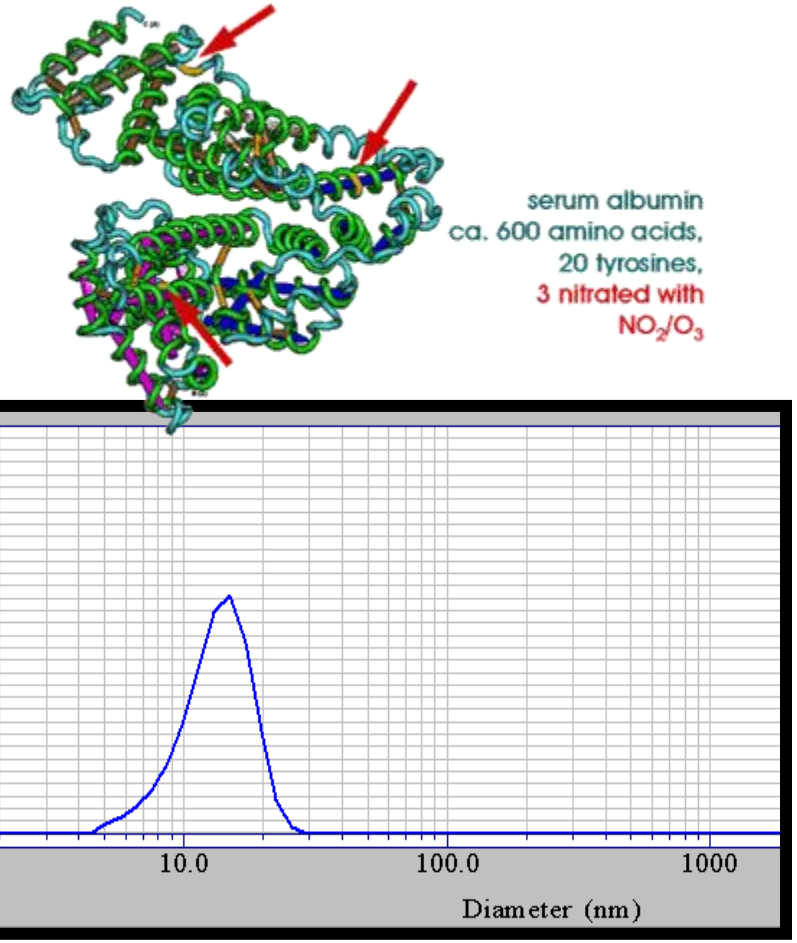
DLS vs Diffraction BSA

BSA- well characterized protein (and likes to aggregate!)

DLS – Can be used to determine the aggregation state of the protein

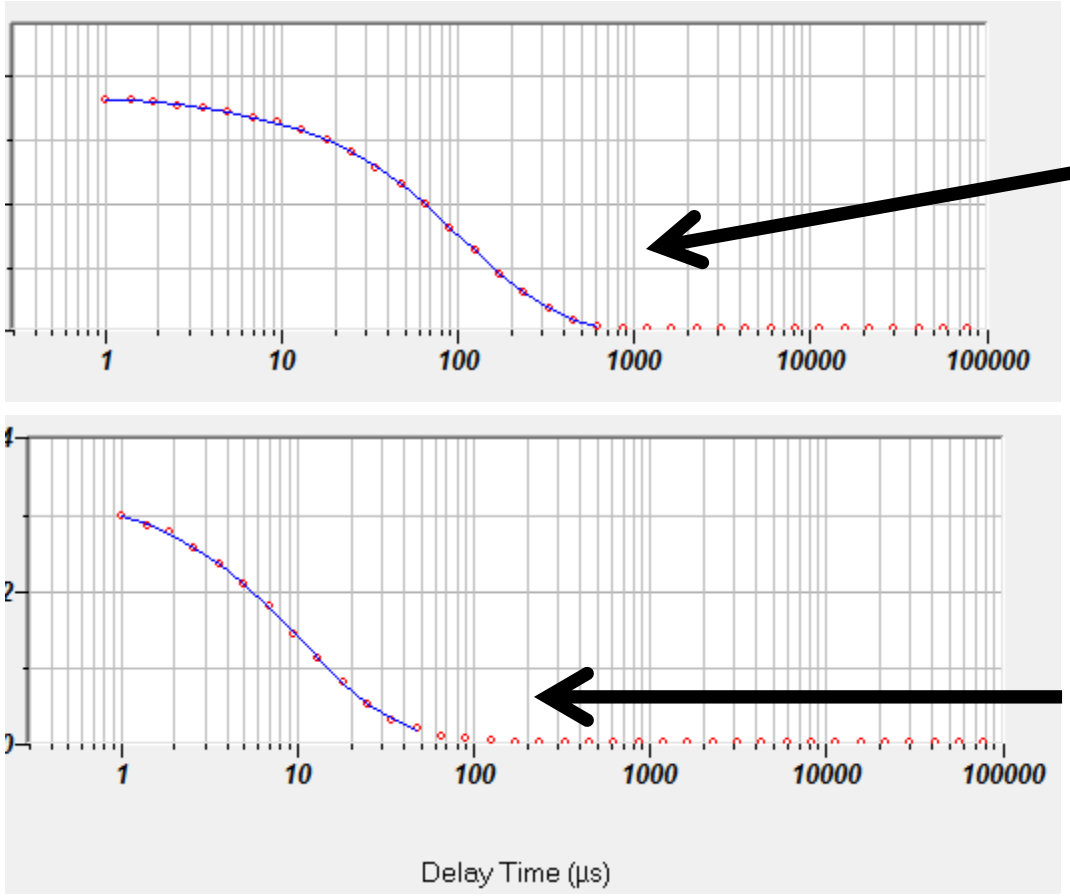
Example on right is aggregated BSA with a lot of dimer.

DLS: does well at small particle size, tiny sample volume (10 microliters



Should be just over~ 7 nm

Nanogold Data



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

	Z-avg. 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 %

10 nm is lower limit of laser diffraction.
DLS is much more comfortable.

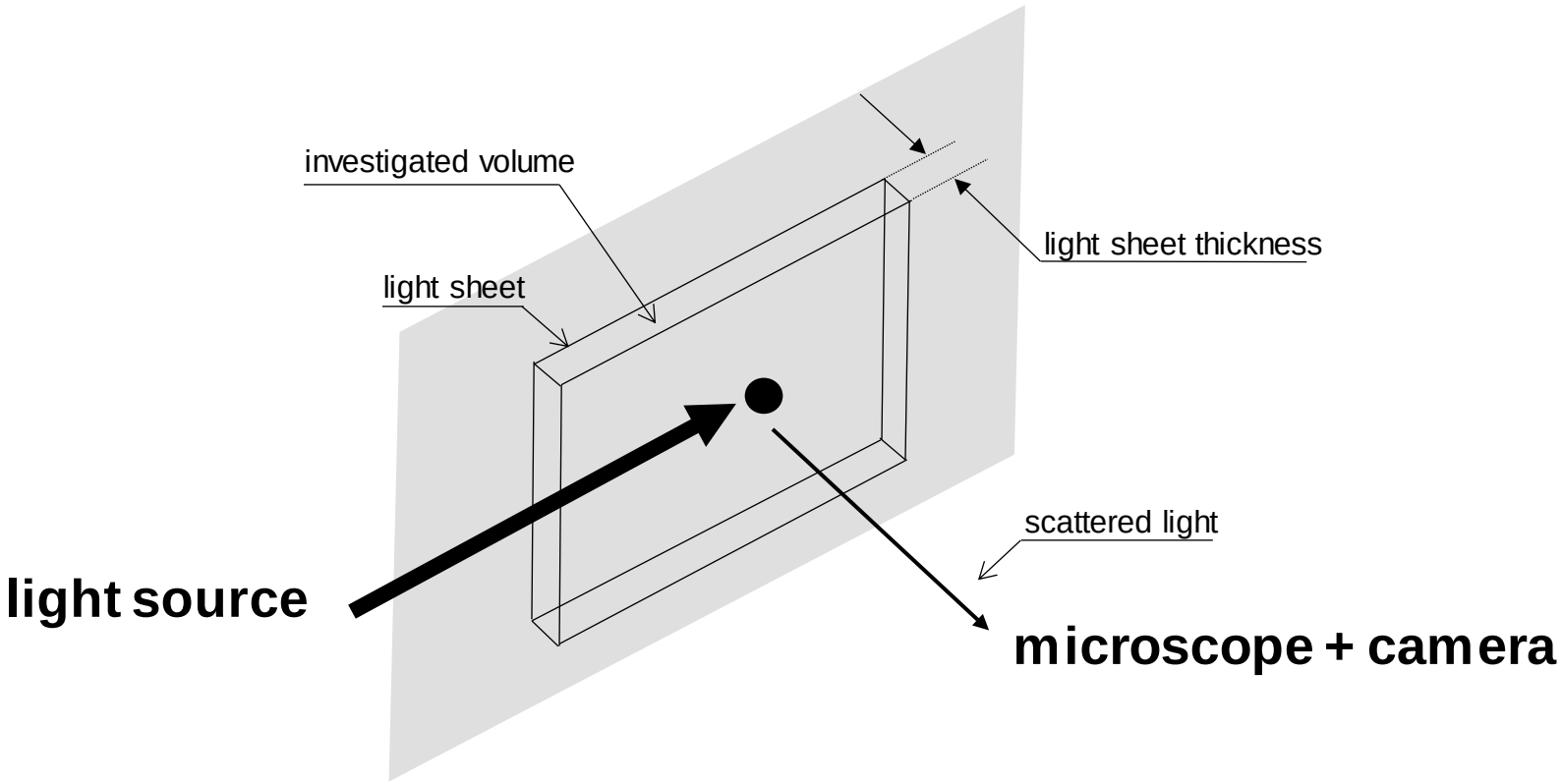
Nanoparticle Tracking

Use scattering to track positions of particles over time to extract size of each particle.

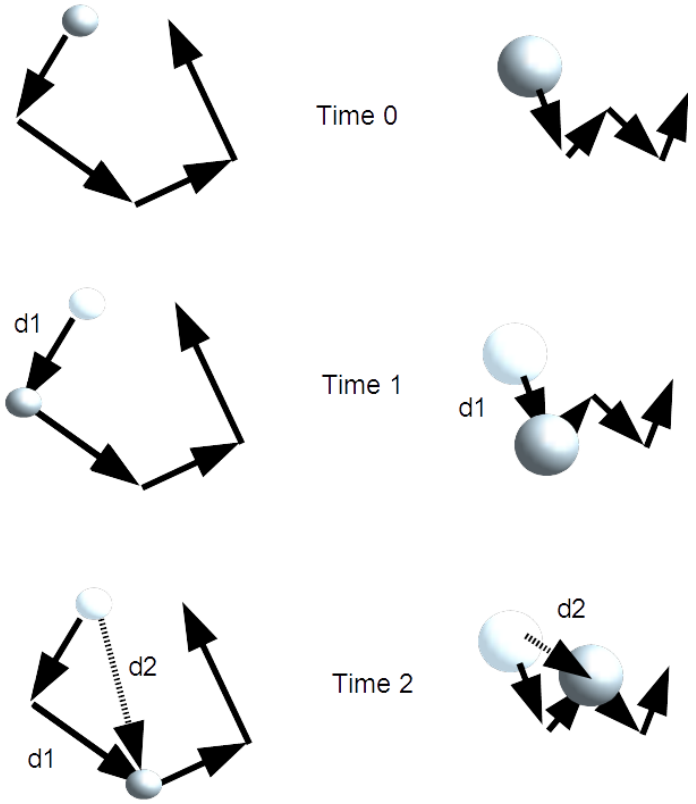
Missing so far…

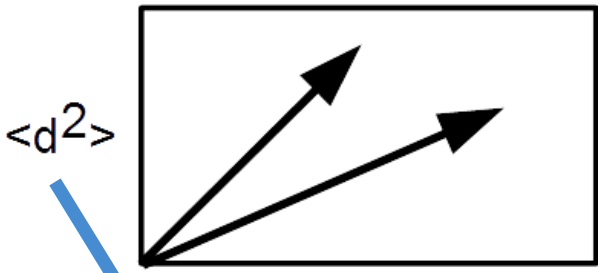
- **Visualization of polydisperse particles**
- **Accurate & reproducible measurement of:**
  - Particle number concentration
  - Particle size distribution
  - Particle kinetic processes

Visualization of Brownian motion



What is nanoparticle tracking?





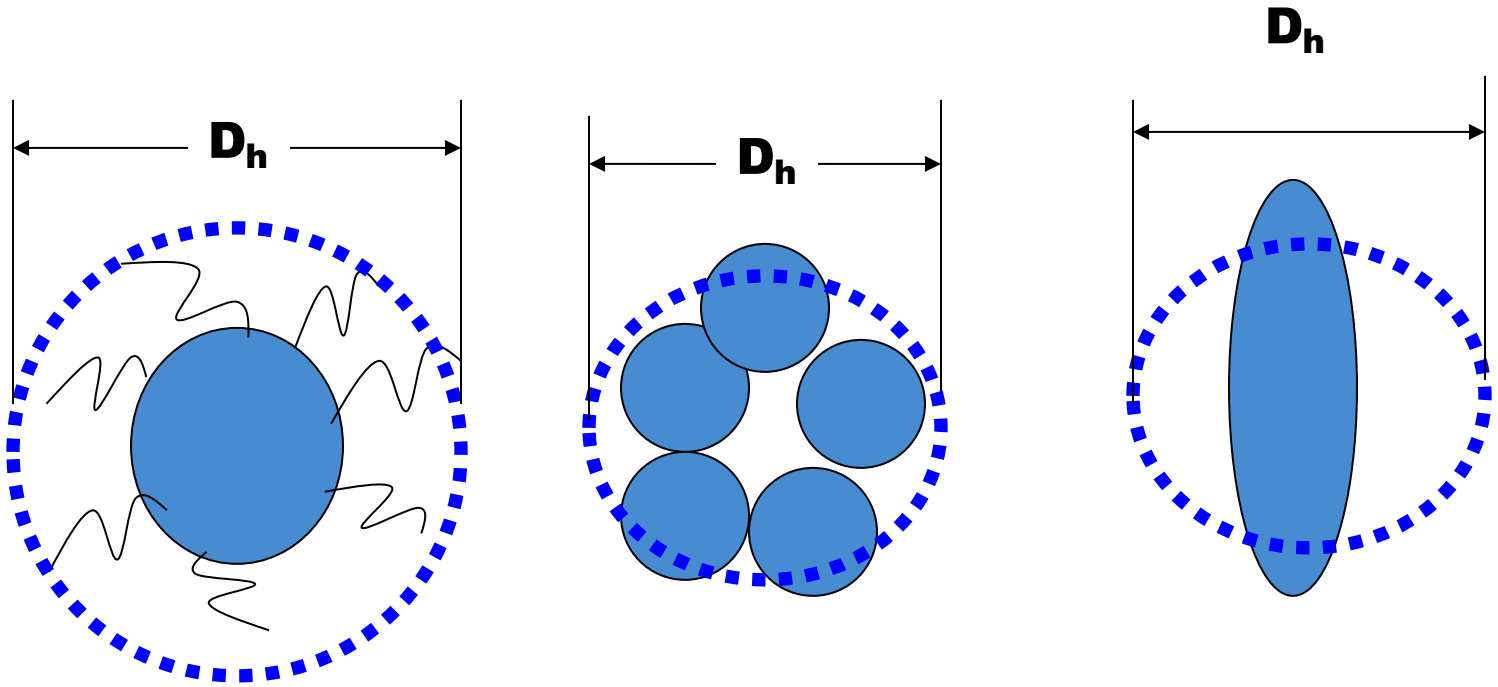
$$MSD(n) = (4 \cdot Dt \cdot D) \cdot n$$

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

$$MSD(n) = \frac{1}{N-n} \sum_{i=1}^{N-n} (x_{i+n} - x_i)^2 + (y_{i+n} - y_i)^2$$

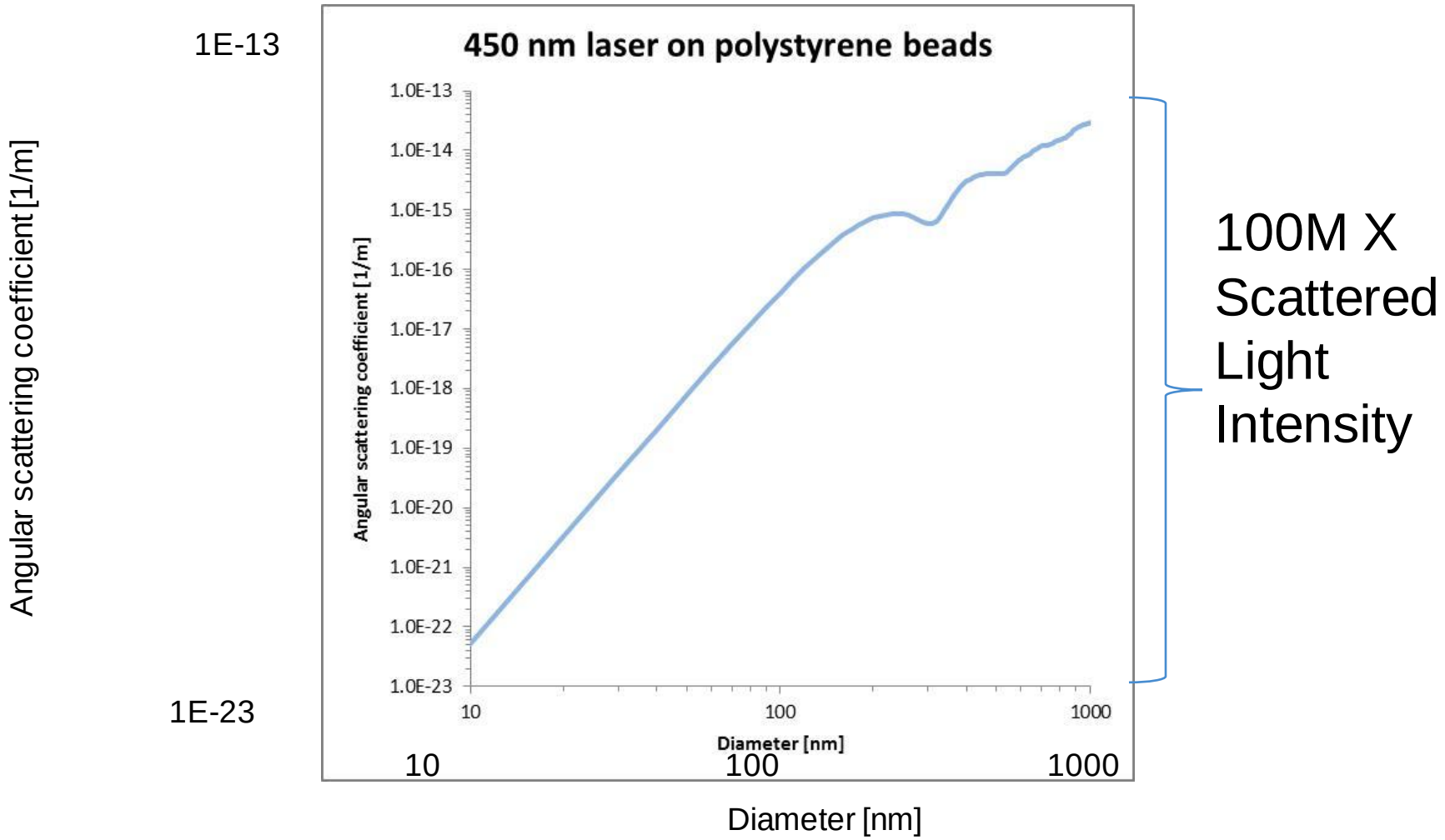
Hydrodynamic size

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



Problem: Intensity vs size

450 nm laser on polystyrene beads



Intensity issues:

DLS – large particles skew results (small not detected) or mask small particles

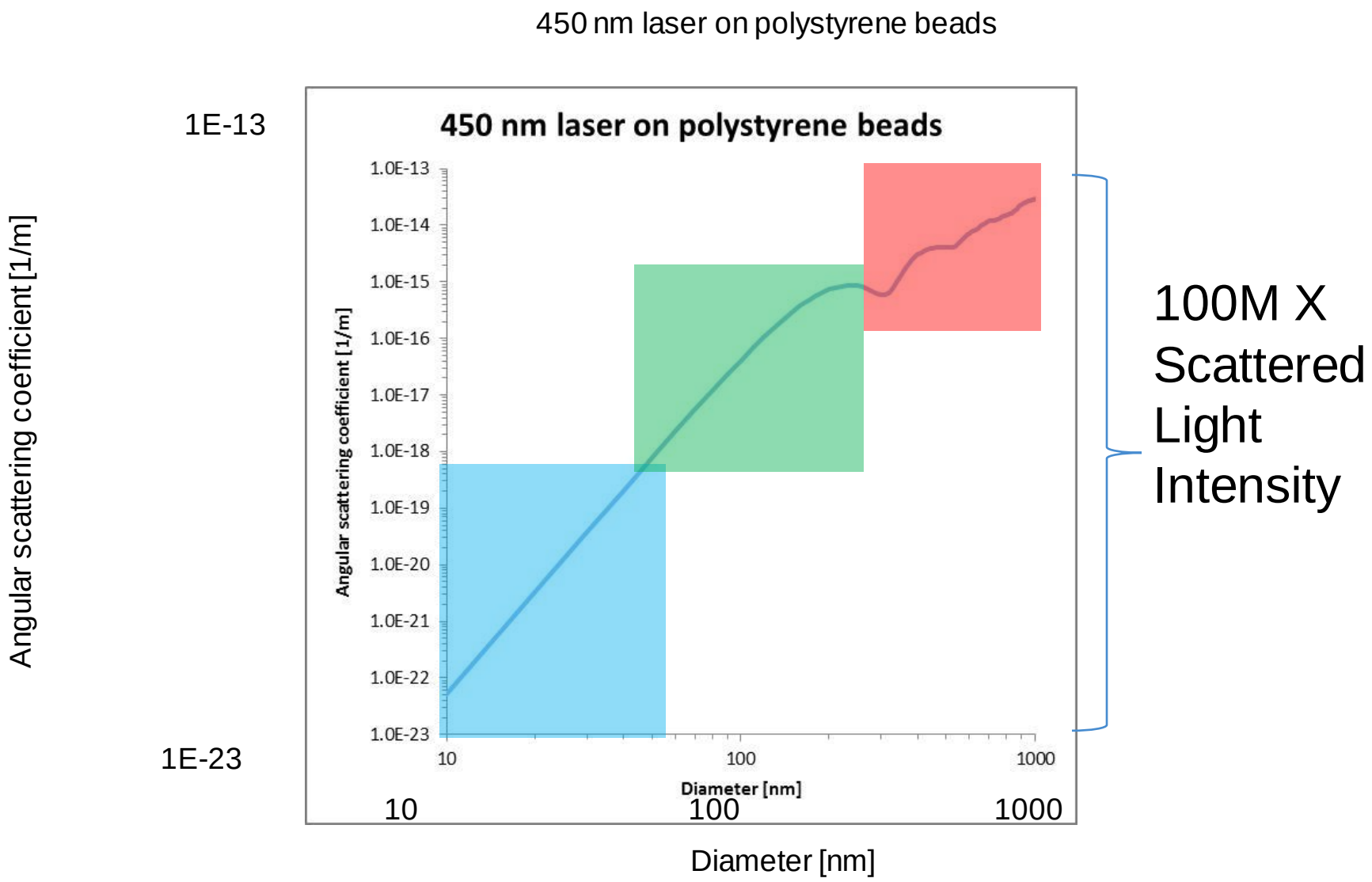
cNTA – different sized particles can't be seen simultaneously (highly irregular images for large particles, dim for smallest)

cNTA – interrogated volume depends on particles size and their refractive index (similar to FC problem when sizing)

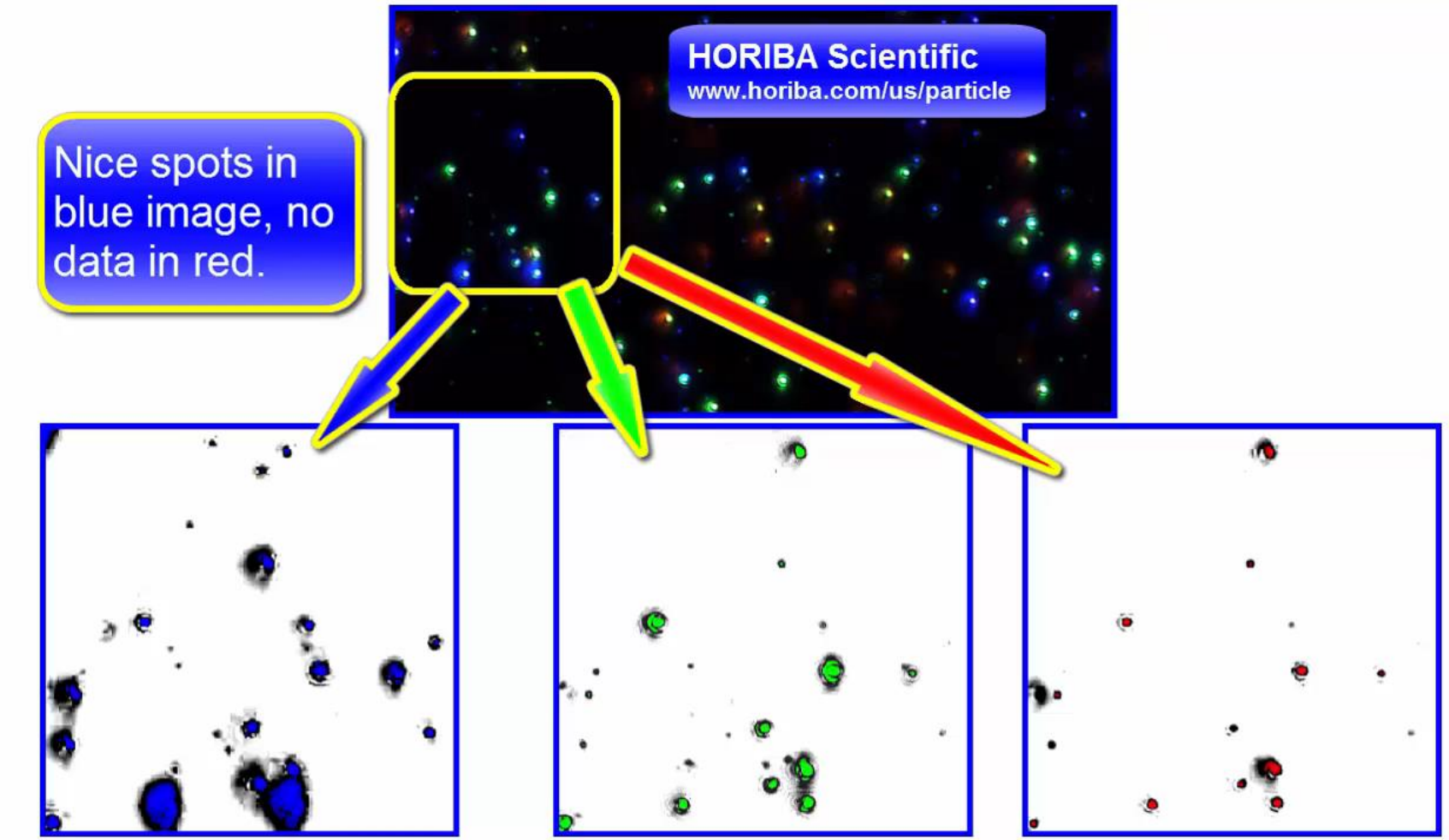
Well known problem:

"Sample polydispersity affects the ability to track and therefore analyze different size fractions in the particle number-size distribution.
[…]
In a polydisperse sample large particles scatter a lot more than small particles making it difficult to detect or track small size particles."

Solution: Intensity vs size



Why three colors?

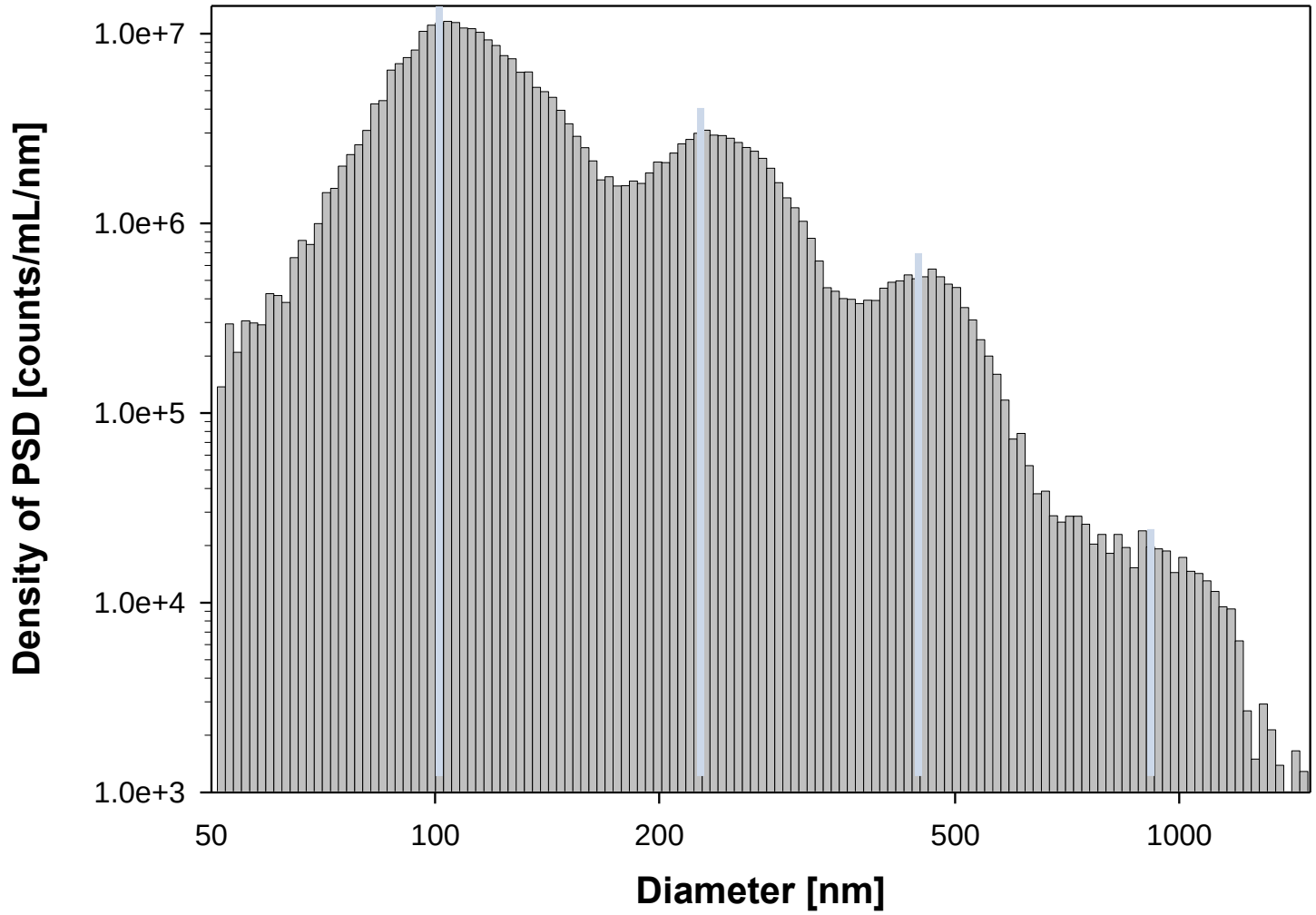


Key benefits of ViewSizer

- Individual particle method, not ensemble average
- Accurate PSD for polydisperse samples
- Concentration measured, not estimated
- Absolute method (no calibration needed)
- Particle visualization

NIST exploratory poly-standard

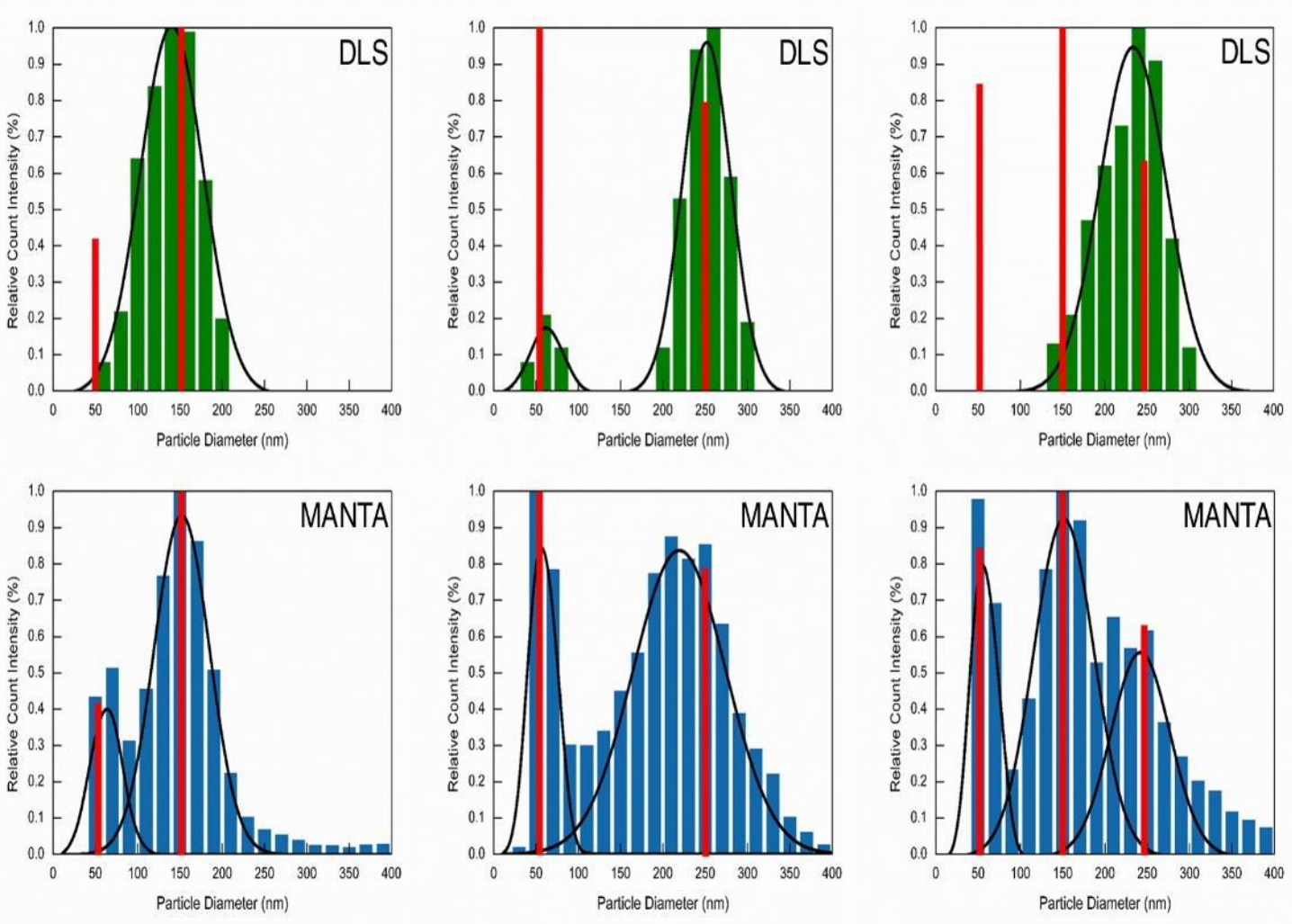
Concentration values AND detailed distribution means m-NTA



Gold mixes: DLS vs. MANTA

Detailed distribution means m-NTA.

BUT DLS is much faster: ~1 min vs ~13 min.

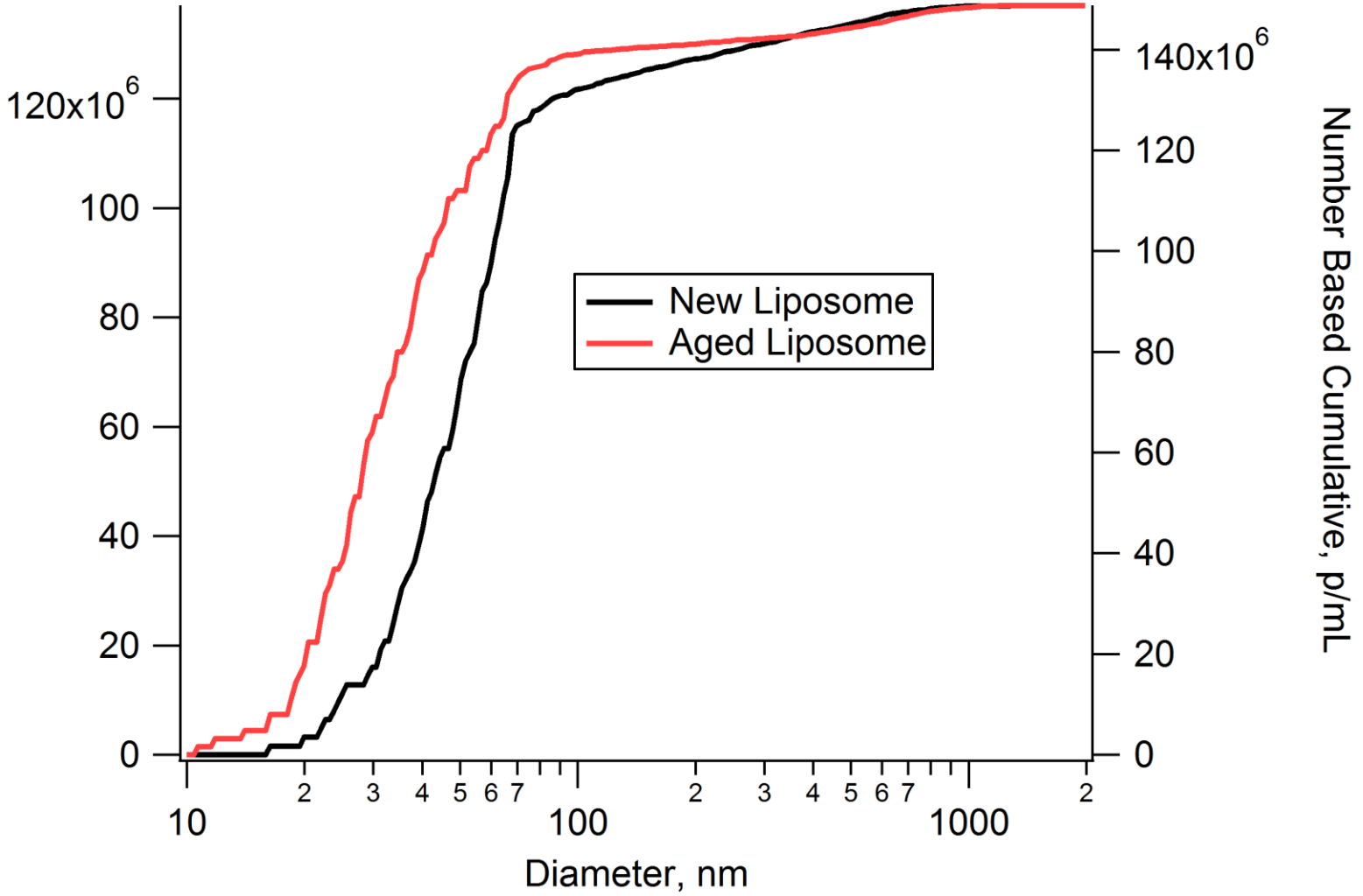


Liposomal Formulation

Concentration values AND detailed distribution means m-NTA

Again, you pay in speed.

Laser diffraction needs more sample.



Lysozyme heated to 60 C

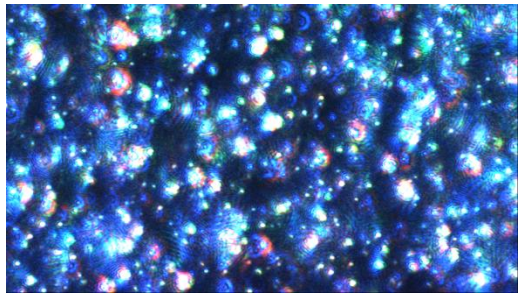
Concentration values AND detailed distribution means m-NTA

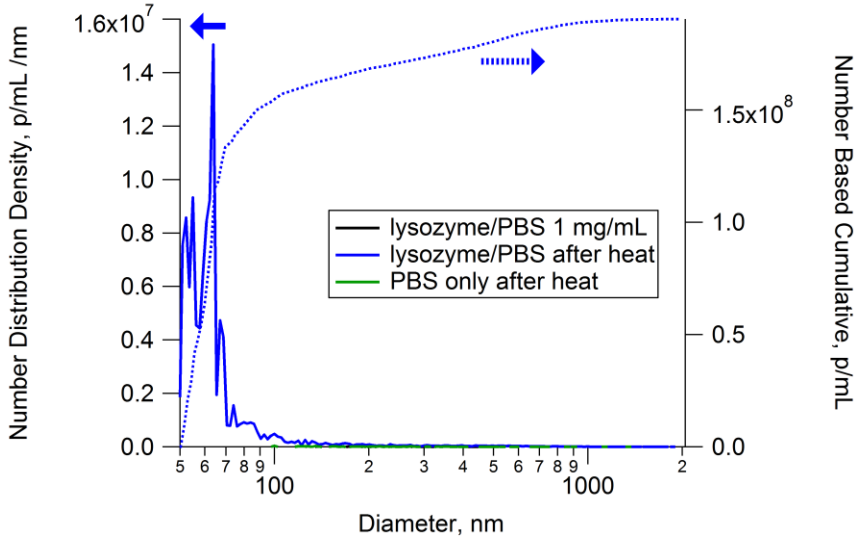
But you don't see monomer-dimer like you would with DLS.

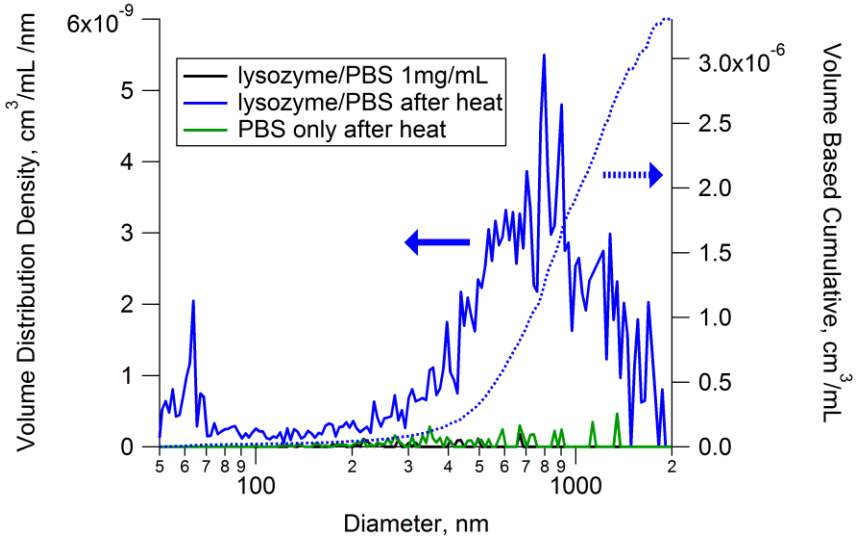
PBS after heating

Lysozyme before heating

Lysozyme after heating

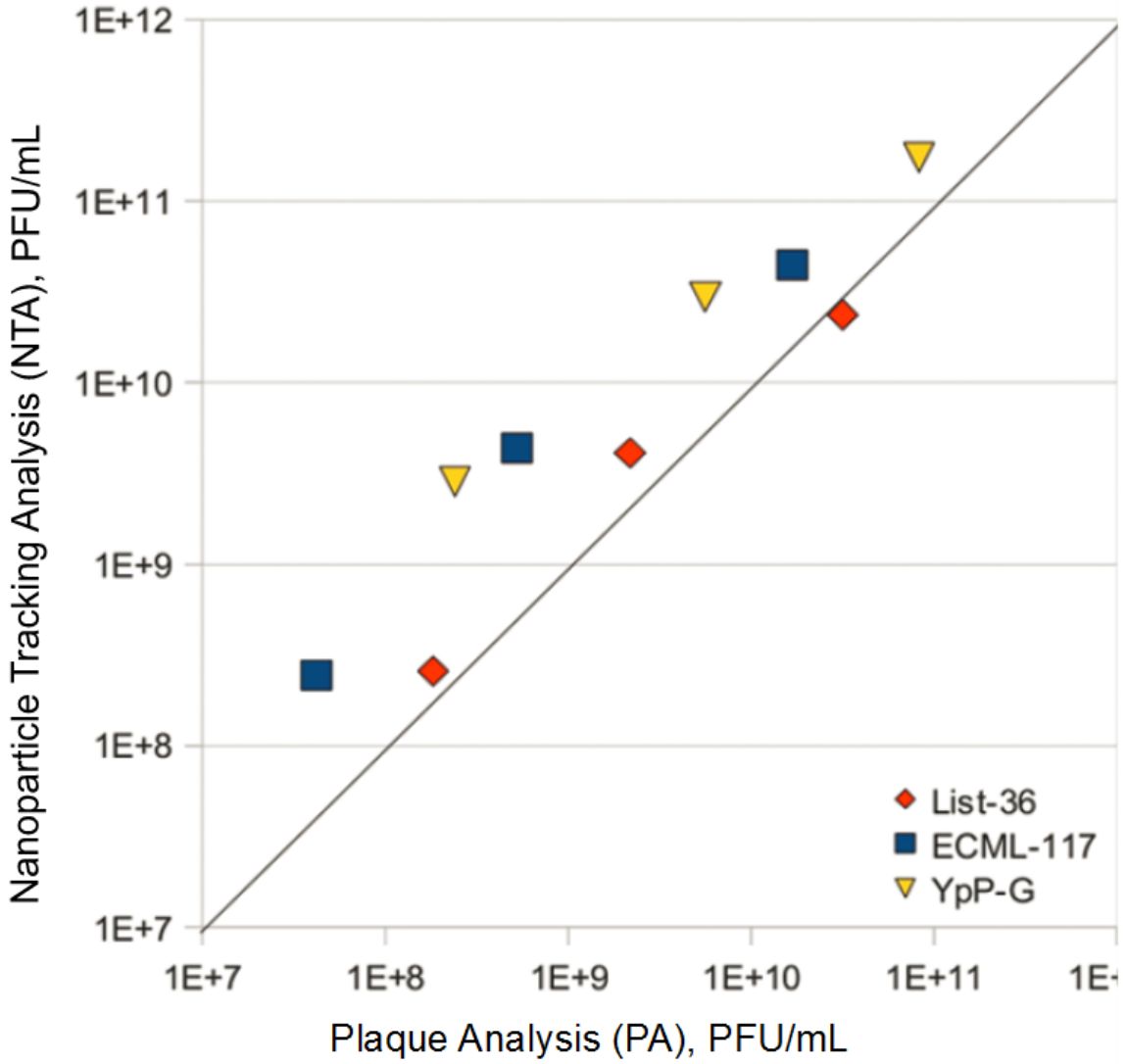






Phage Analysis

Concentration values means m-NTA



Bacteriophages:

List-36: Listeria monocytogenes

ECML-117: Escherichia phage

YpP-G: Yersinia phage

Bacteriophage. 2011 Mar-Apr; 1(2): 86–93

Closing Comparison

Issue	Laser Diffraction	DLS	Multi-laser nanoparticle tracking
Large (>1 micron) particles in sample that need to be analyzed	++	--	-
Small quantity of sample	-	+	+
Smallest particles (<10~50 nm)	-	++	-
Speed	++	+	-
Nanoparticle Distribution	-	-	+
Analyze only tagged particles	--	--	+
Nanoparticle Concentration	--	--	+

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