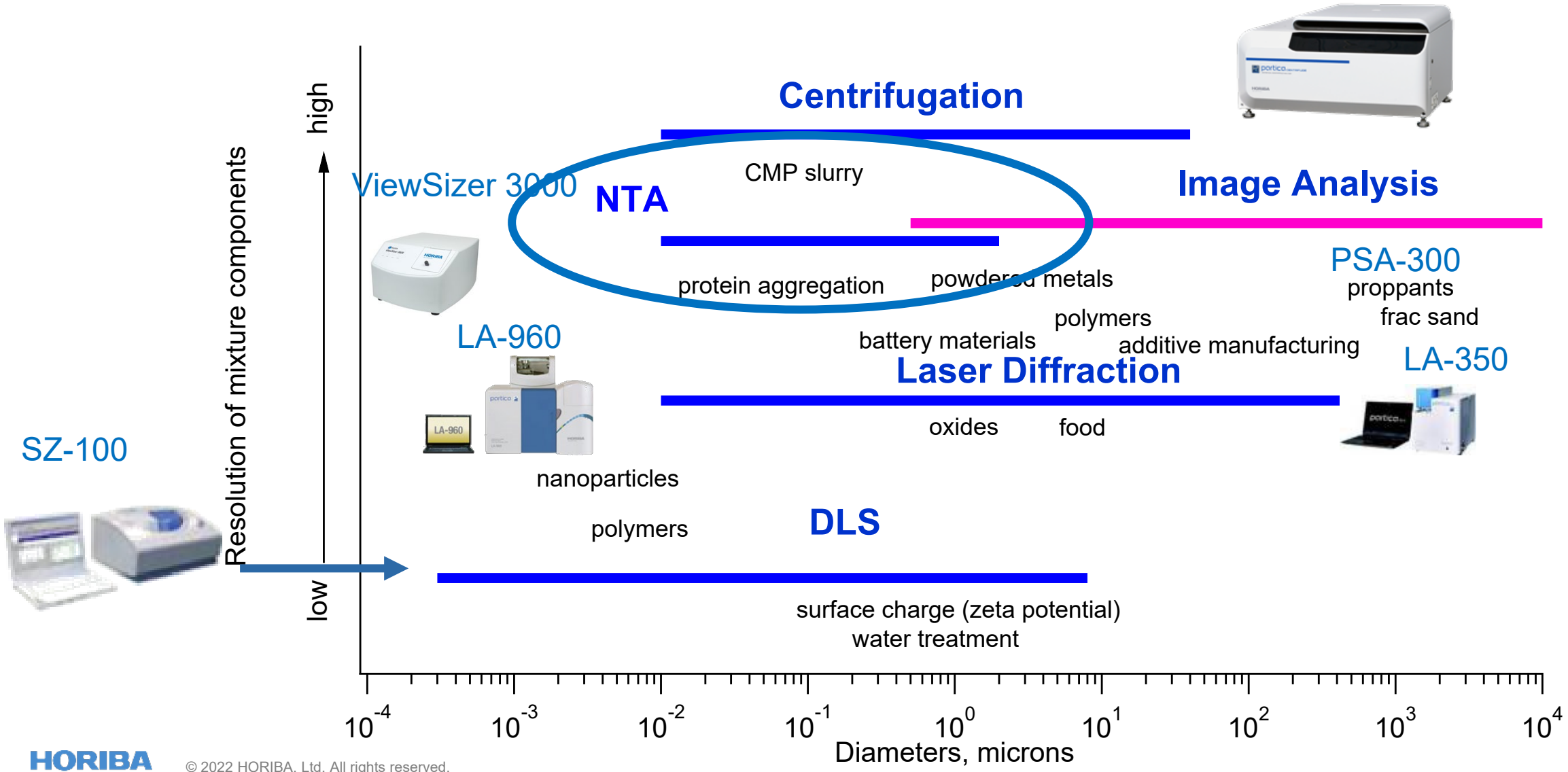
HORIBA Scientific
Particle Characterization

Introduction to Nanoparticle Tracking Analysis (NTA)

Jeffrey Bodycomb, Ph.D.

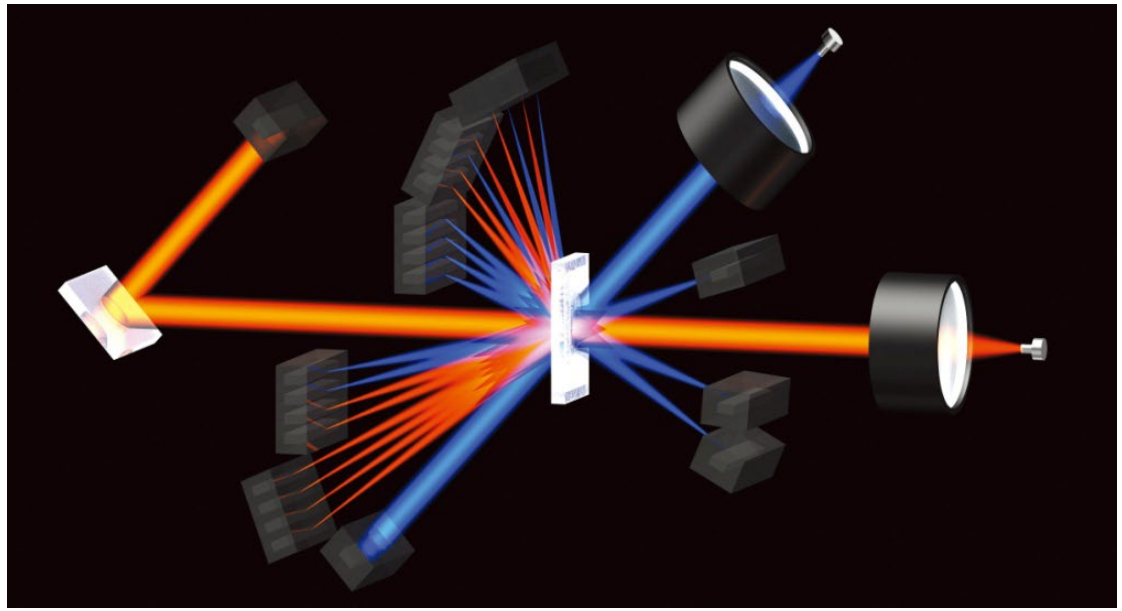
November 17, 2022

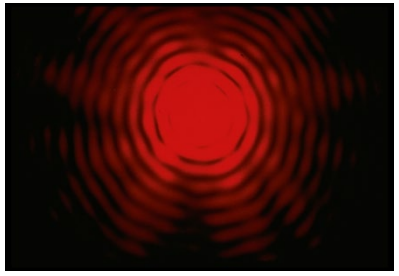
Analysis techniques



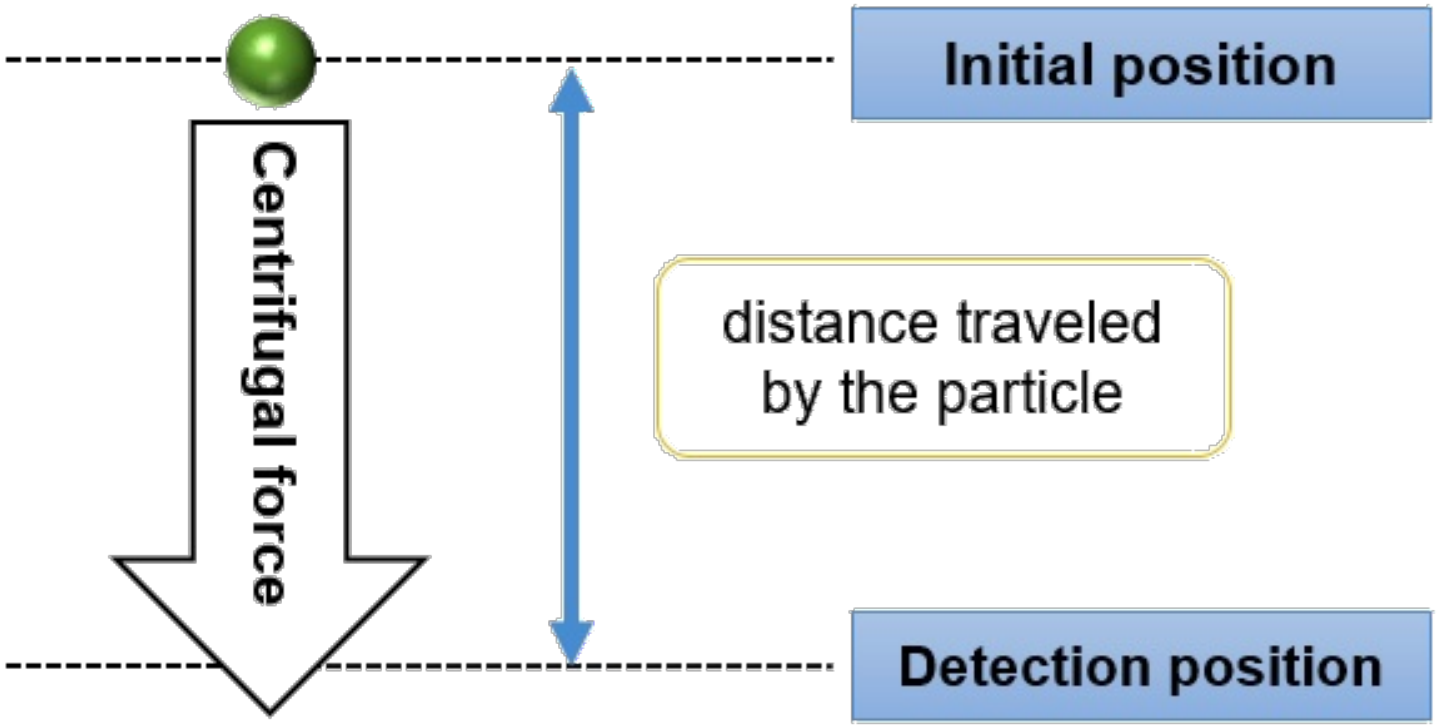
Laser diffraction

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





Centrifugation



Settling velocity | Particle density | Particle size

$$V = \frac{1}{18} \times \frac{(\rho - \rho_0)g}{\eta_0} \times D^2$$

acceleration

Solvent Viscosity | Solvent density

Dynamic light scattering

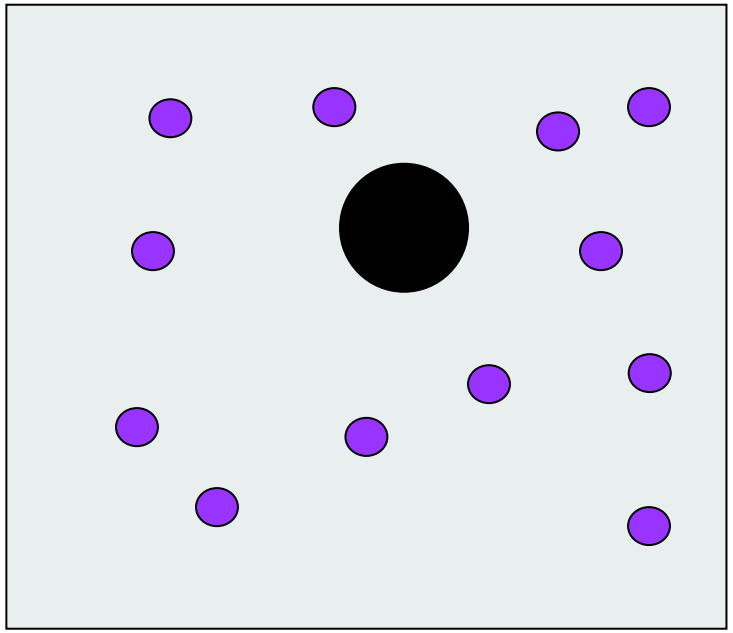
Dynamic light scattering (DLS) refers to measurement and interpretation of light scattering data on a microsecond time scale.

Determine

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

Particle motion

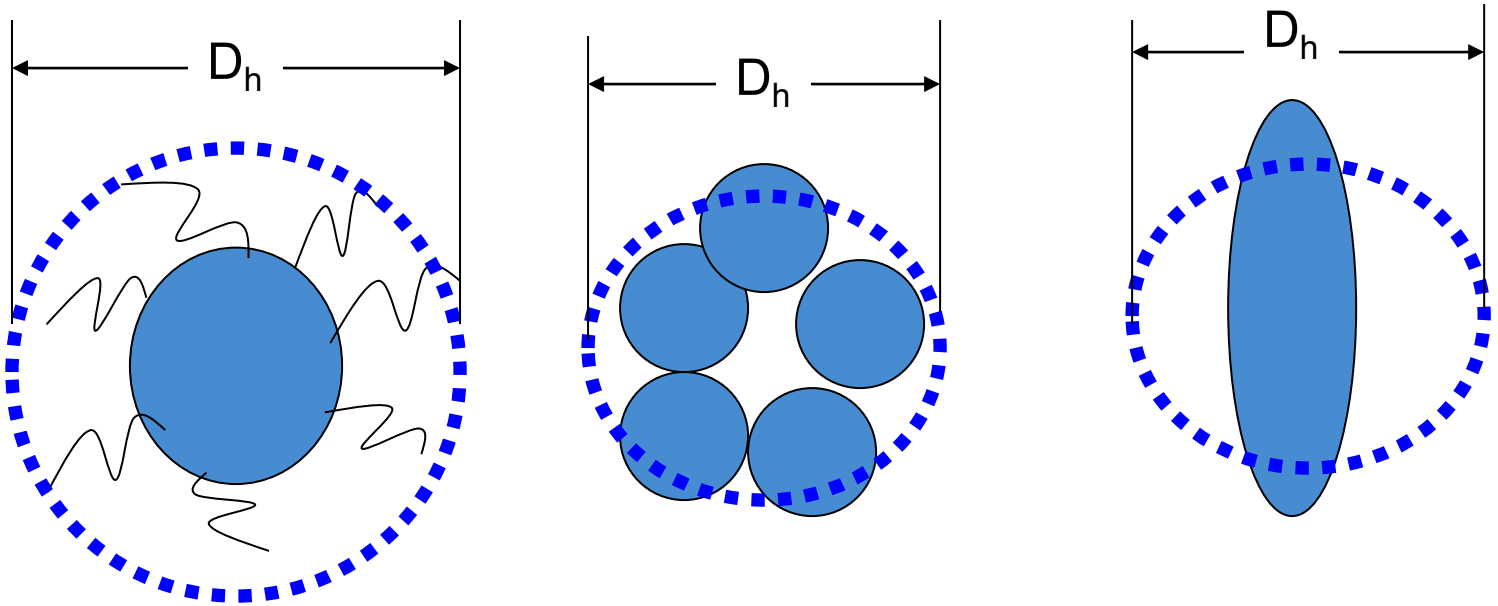
Particles in suspension undergo
Brownian motion (random thermal motion).



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

What is hydrodynamic size?

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

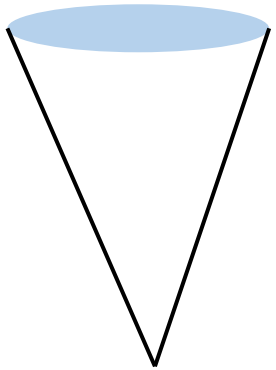


Rayleigh criteria?

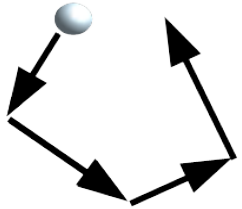
Resolution limit of a “classic” microscope is set by lens size/magnification through numerical aperture and wavelength of light.

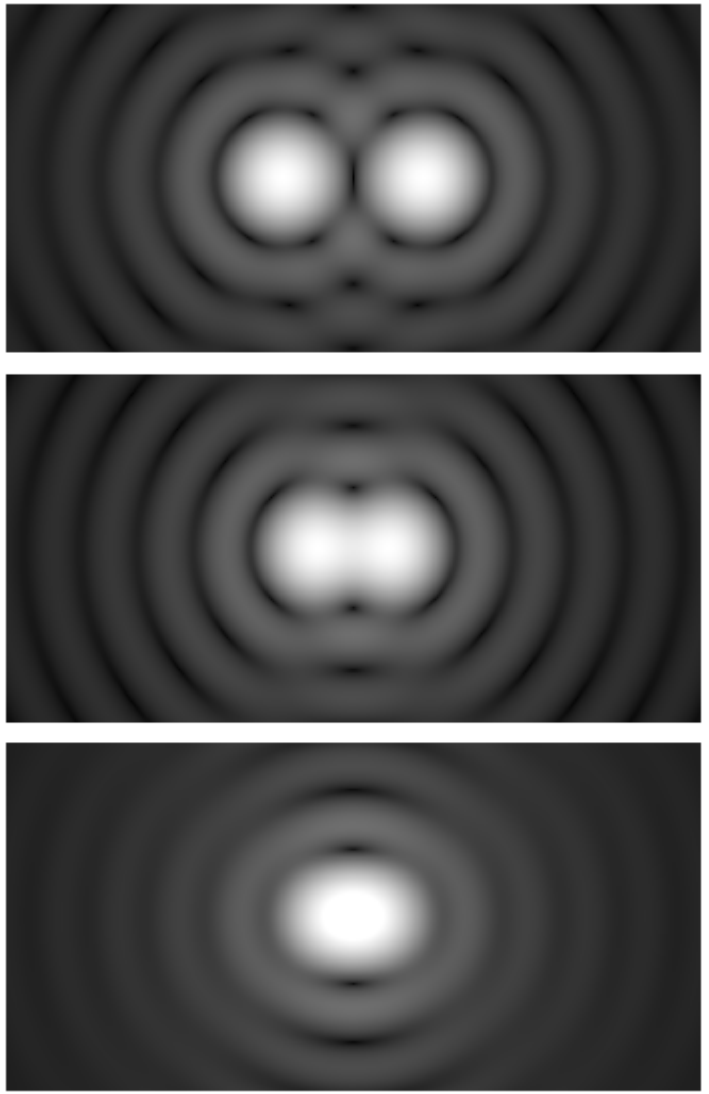
Oil immersion objective, can get to 261 nm in theory.

We don’t measure particle image…we measure motion.



Tiny particles, but big steps…





Public Domain Image, Wikimedia Commons
https://commons.wikimedia.org/wiki/File:Airy_disk_spacing_near_Rayleigh_criterion.png

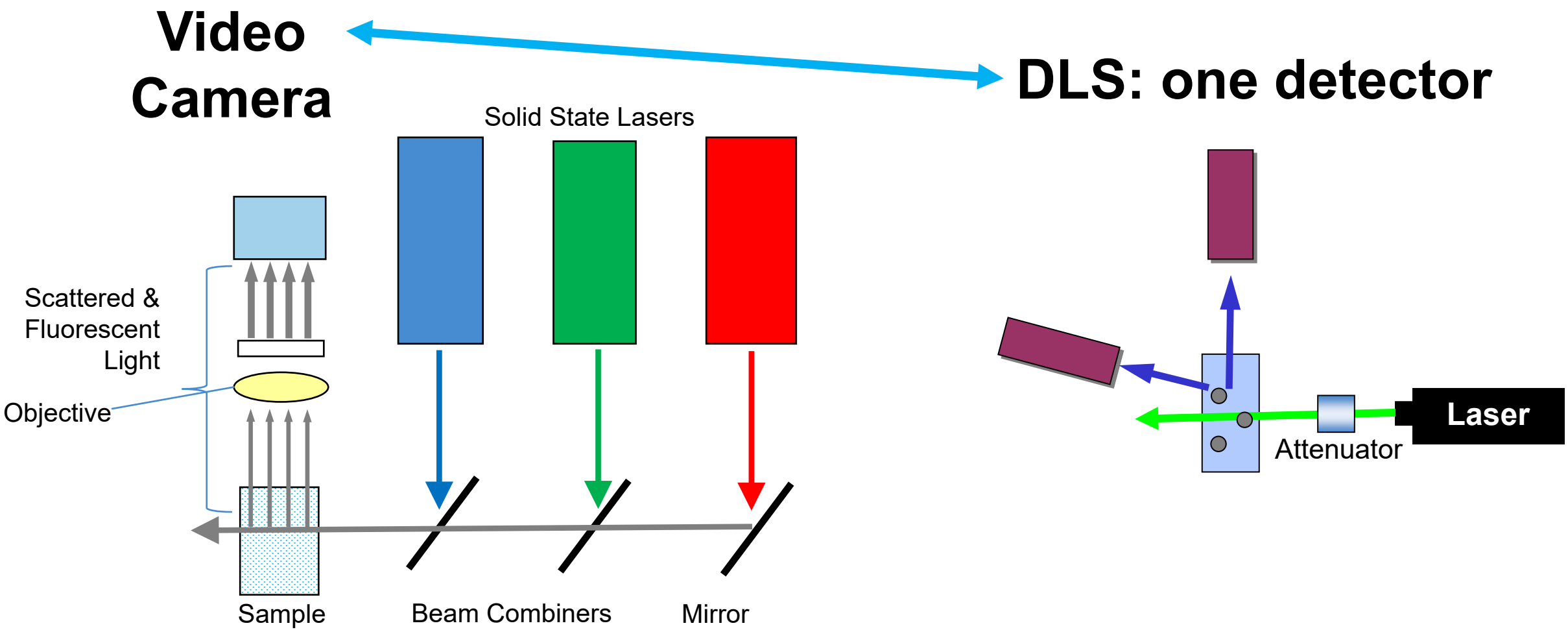
Nanoparticle Tracking

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

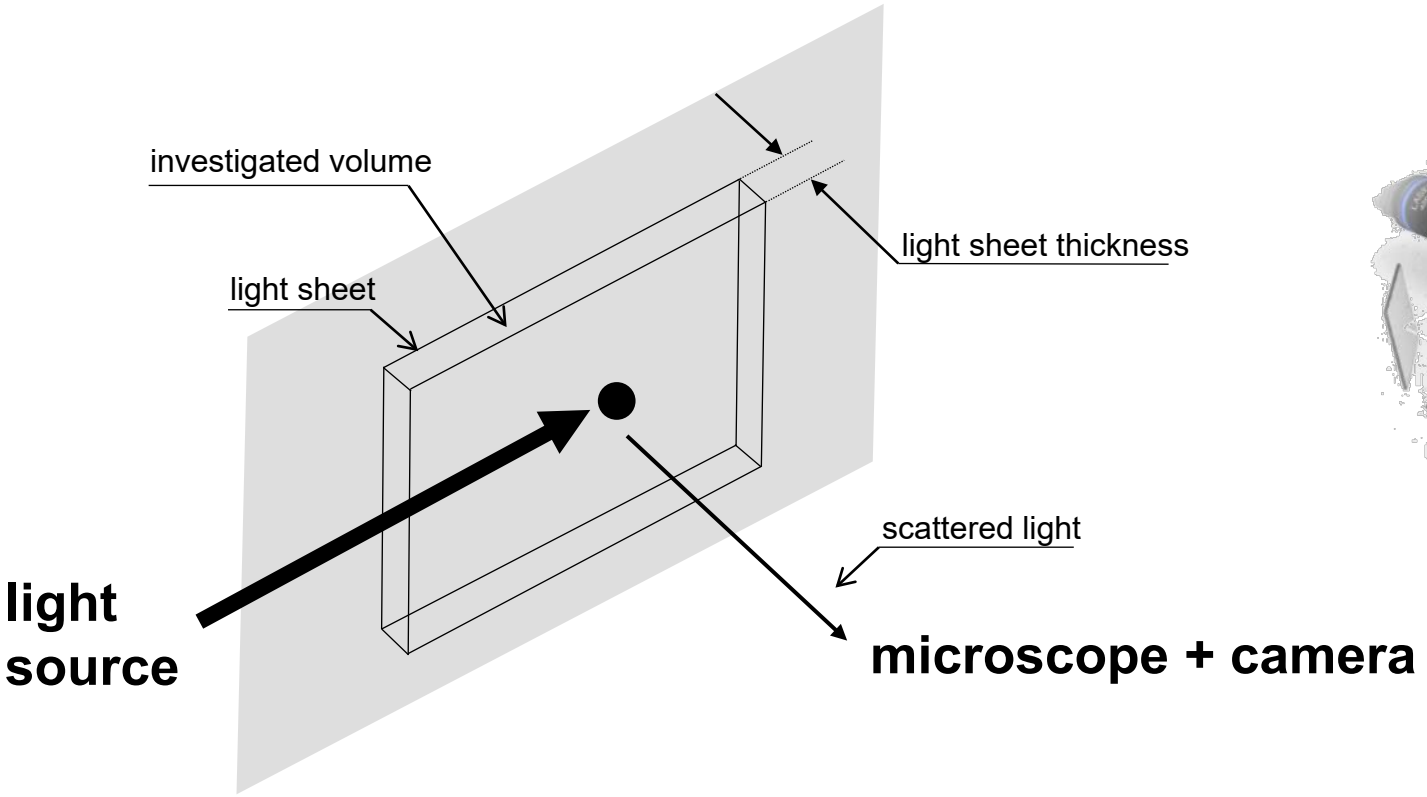
What you get:

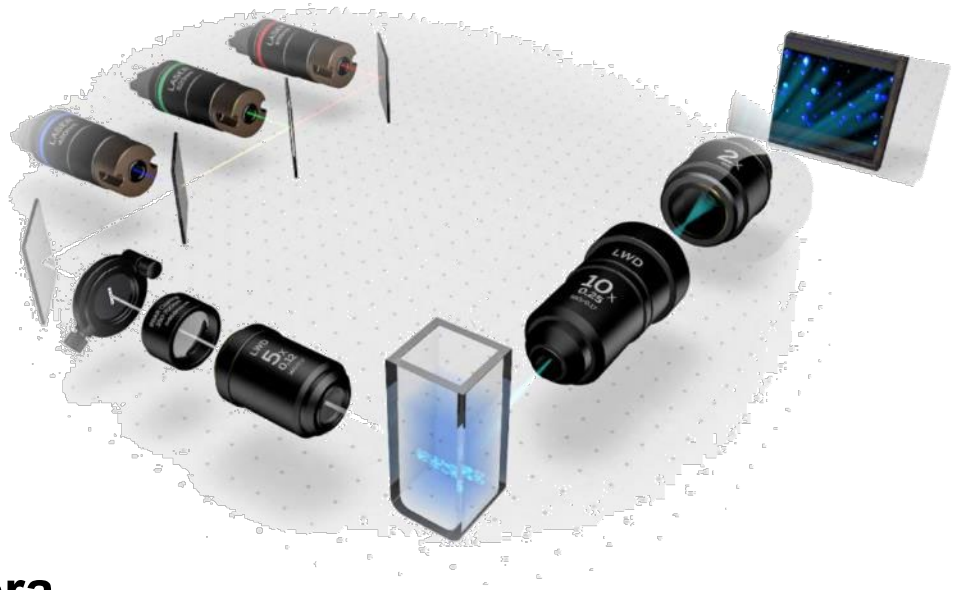
- Visualization of polydisperse particles
- Accurate & reproducible measurement of:
  - Particle number concentration
  - Particle size distribution

Top view of multi-laser NTA optics



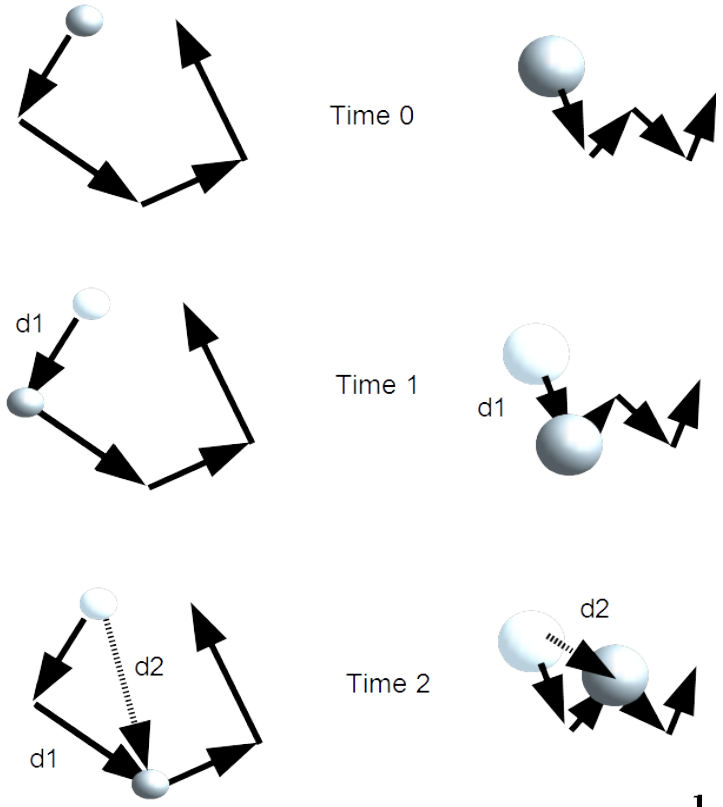
Visualization of Brownian motion



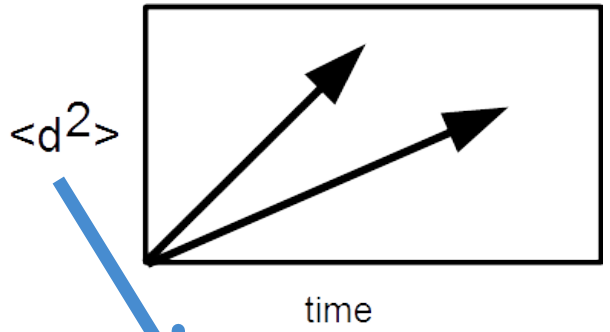


Nanoparticle Tracking (NTA) Data

Video (megapixel detector) data over time.



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

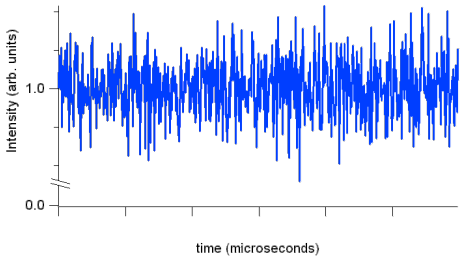


$$MSD(n) = (4\cdot\Delta t\cdot D)\cdot n$$

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

DLS signal

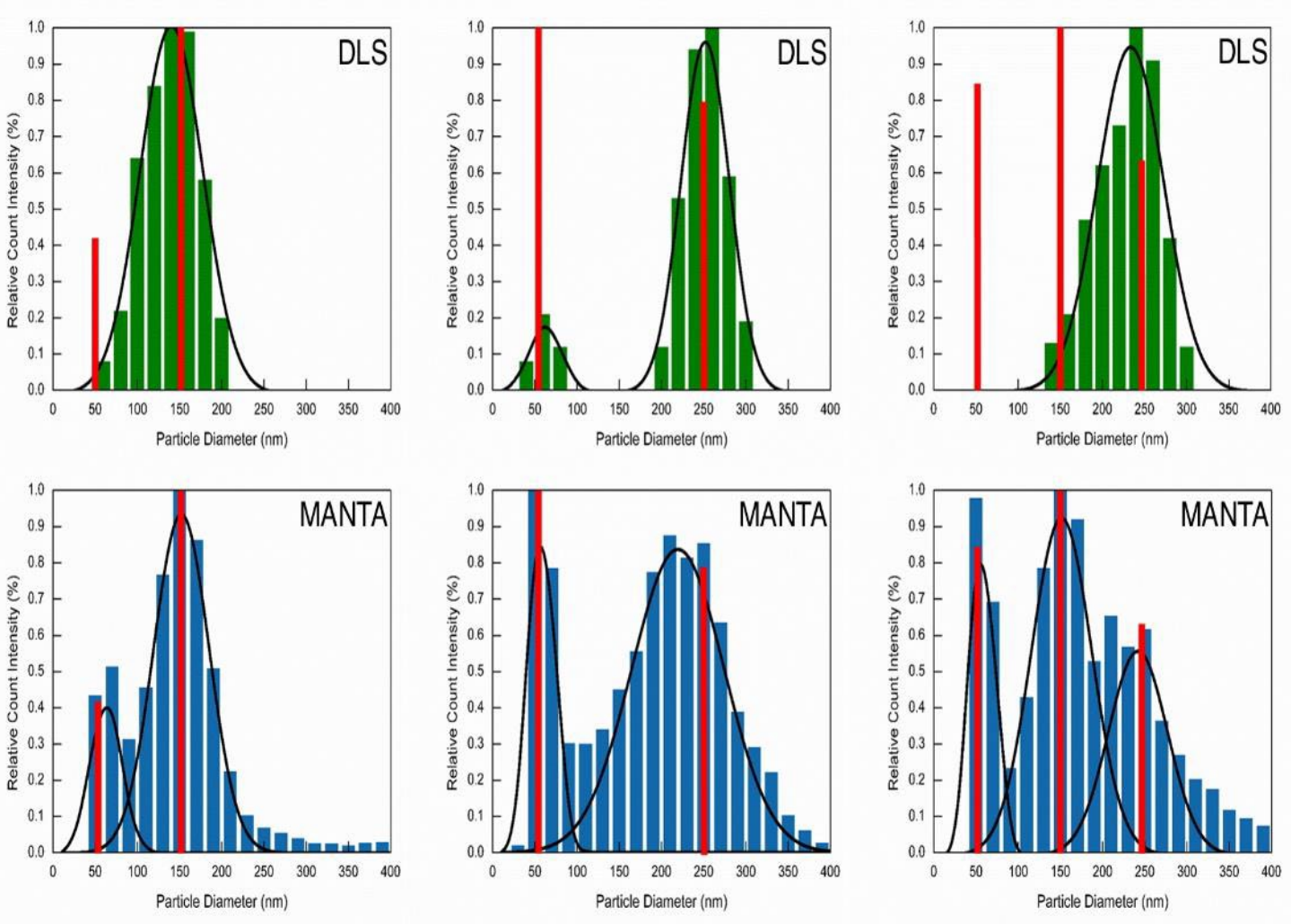
Single detector data over time.



Gold mixes: DLS vs NTA

DLS is much faster: ~1 min vs ~10 min.

But, m-NTA gets you the detailed distribution means m-NTA.



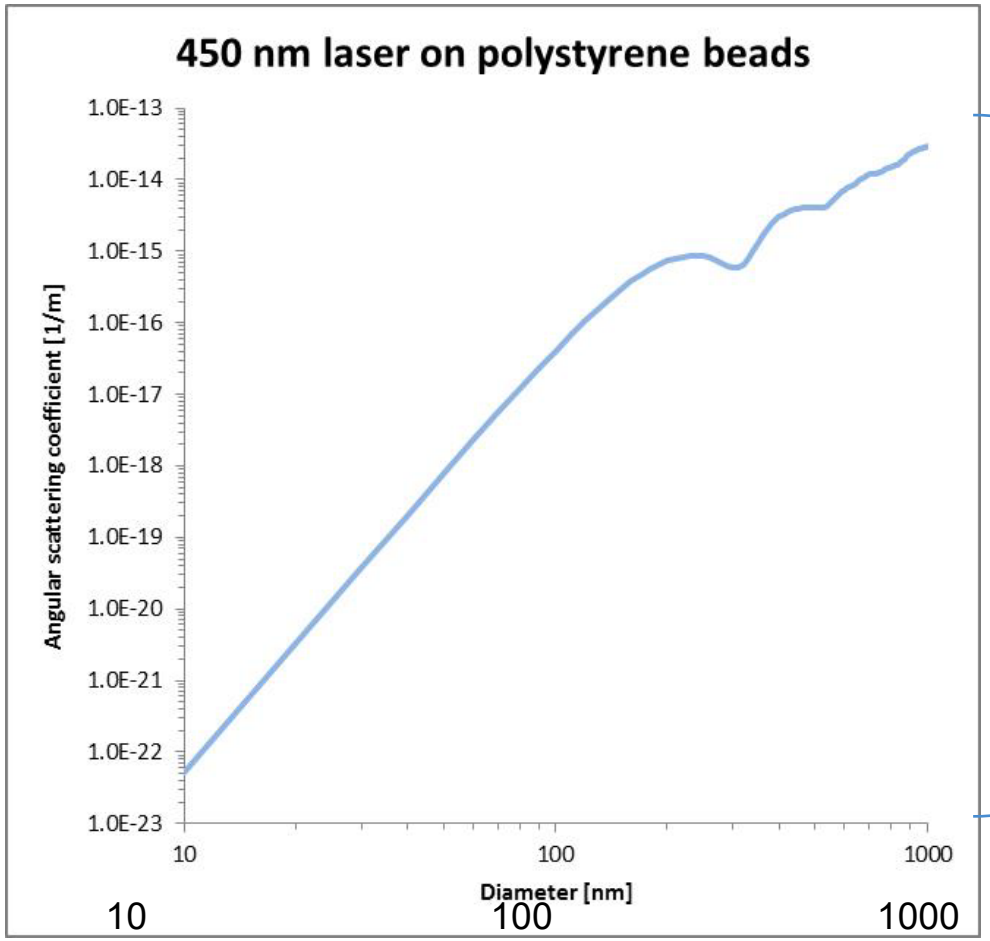
Problem: Intensity vs size

450 nm laser on polystyrene beads

Angular scattering coefficient [1/m]

1E-13

1E-23

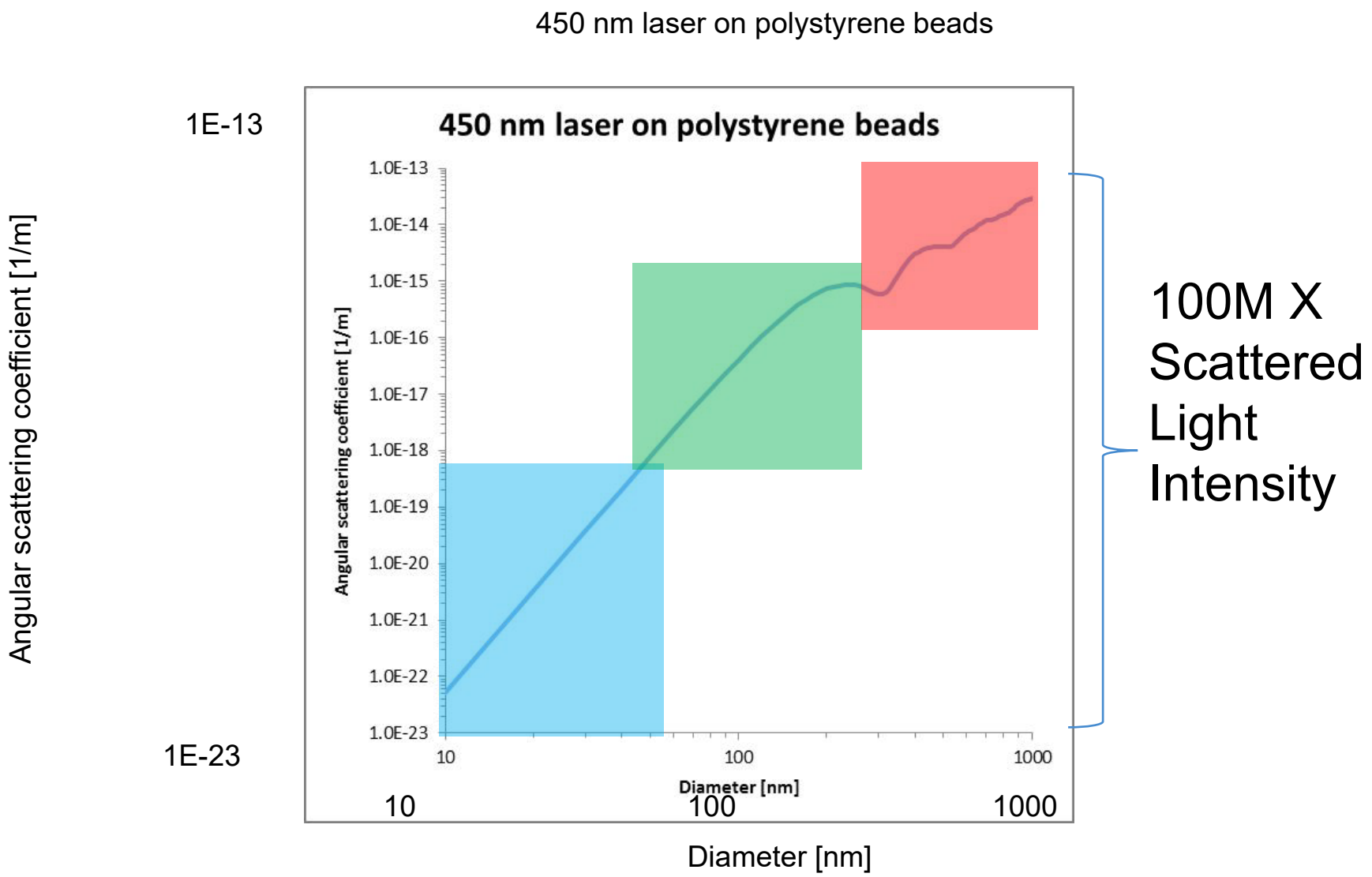


10 100 1000

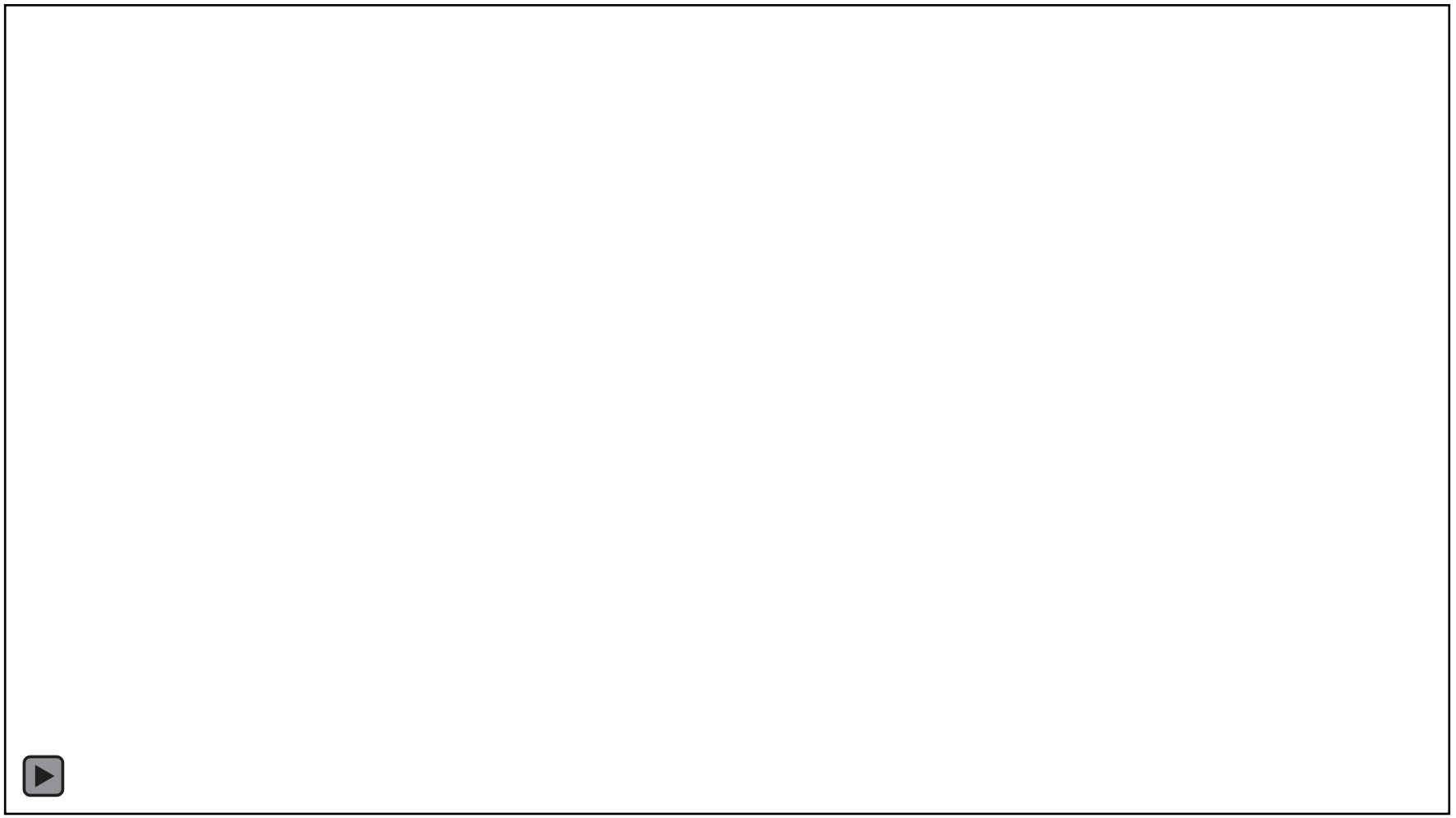
Diameter [nm]

100M X Scattered Light Intensity

Solution: Intensity vs size



Why three colors?



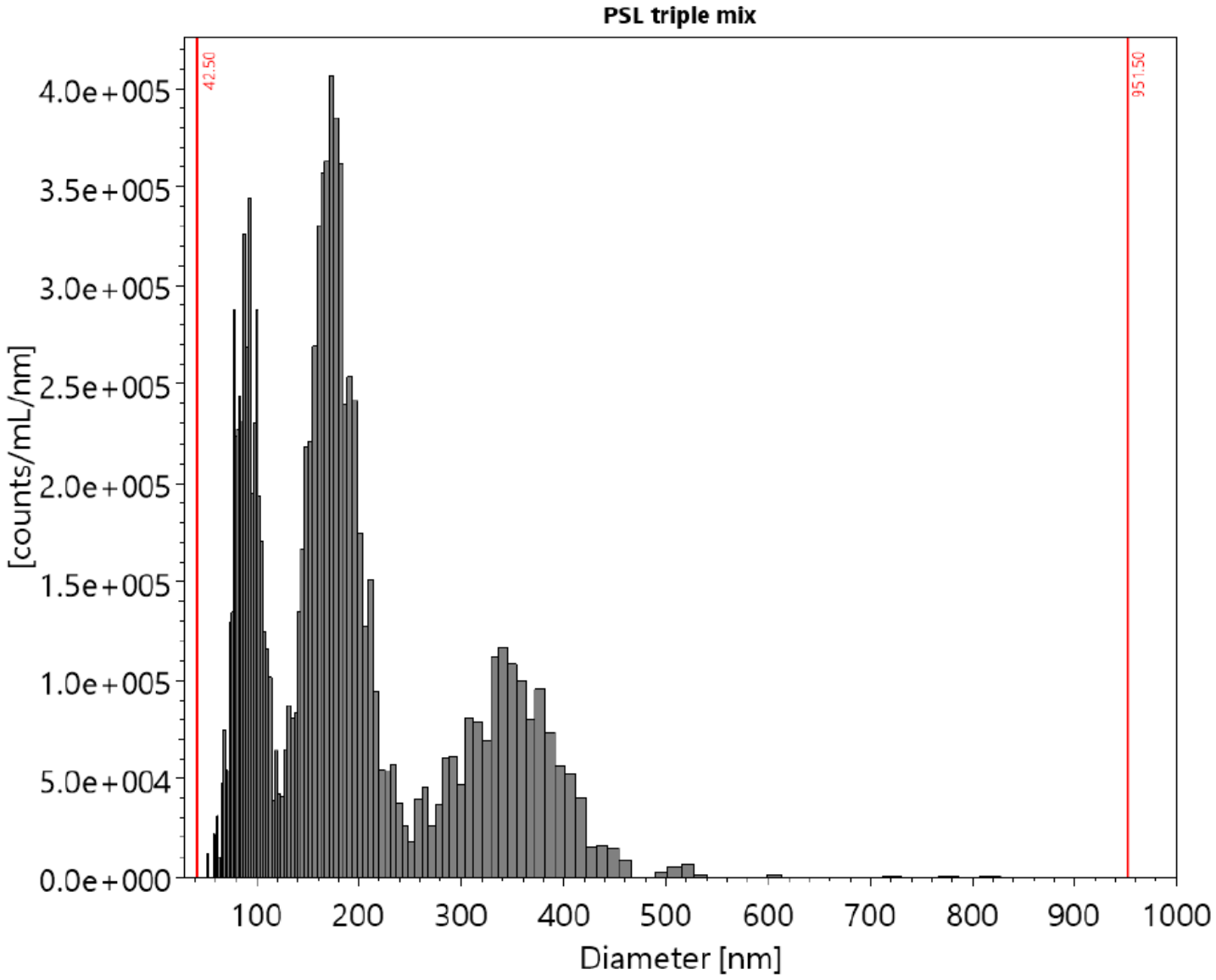
But what can it do for me?

Where can you use details on distribution and concentration?

Latex mixtures for coatings

Analyze mixture of latex particles to find details of distribution that is not available from DLS.

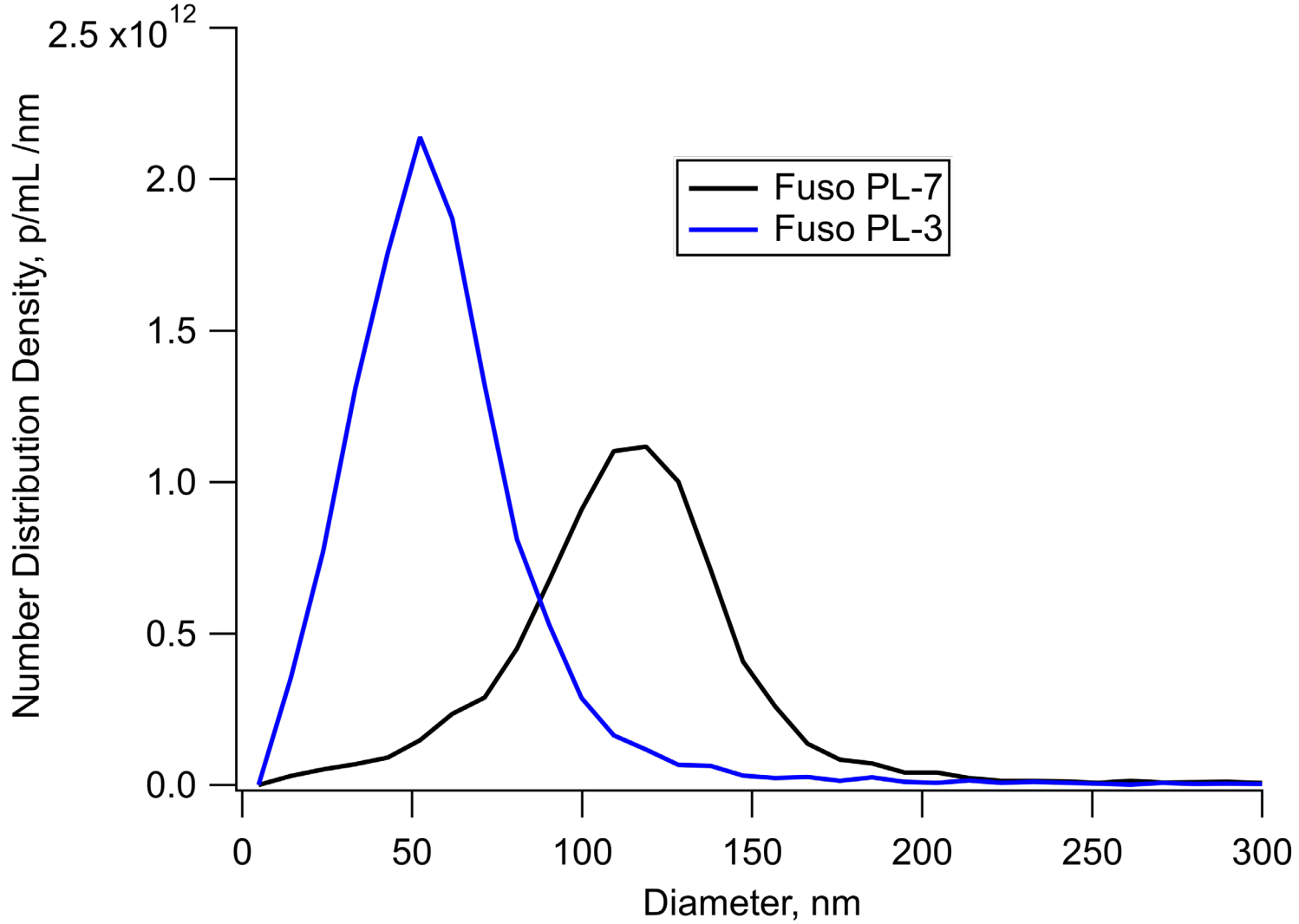
Useful for advanced coatings.



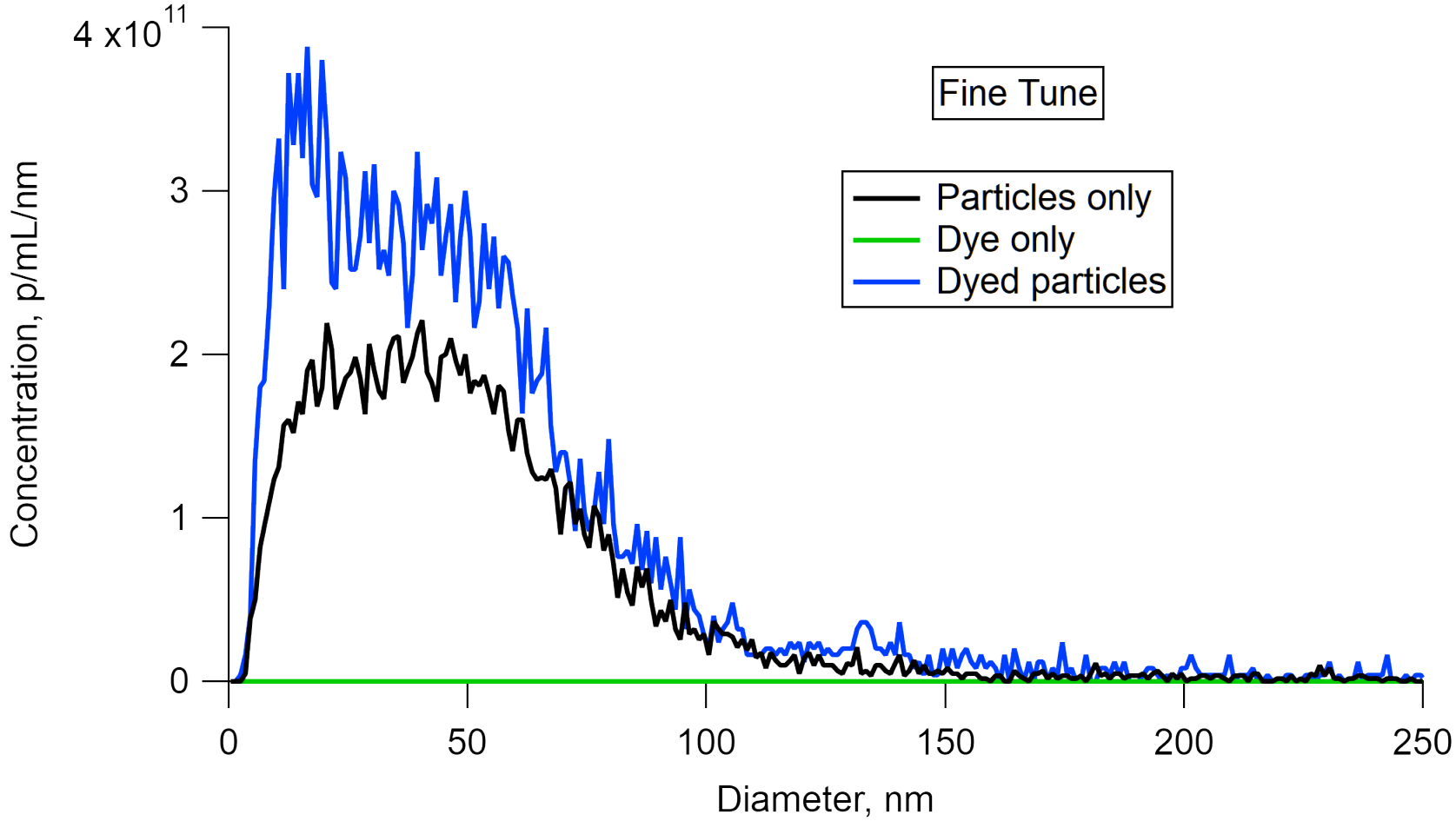
CMP slurry: silica particles

Number, not volume based distribution.

Particle concentration!



Dying silica to increase sensitivity

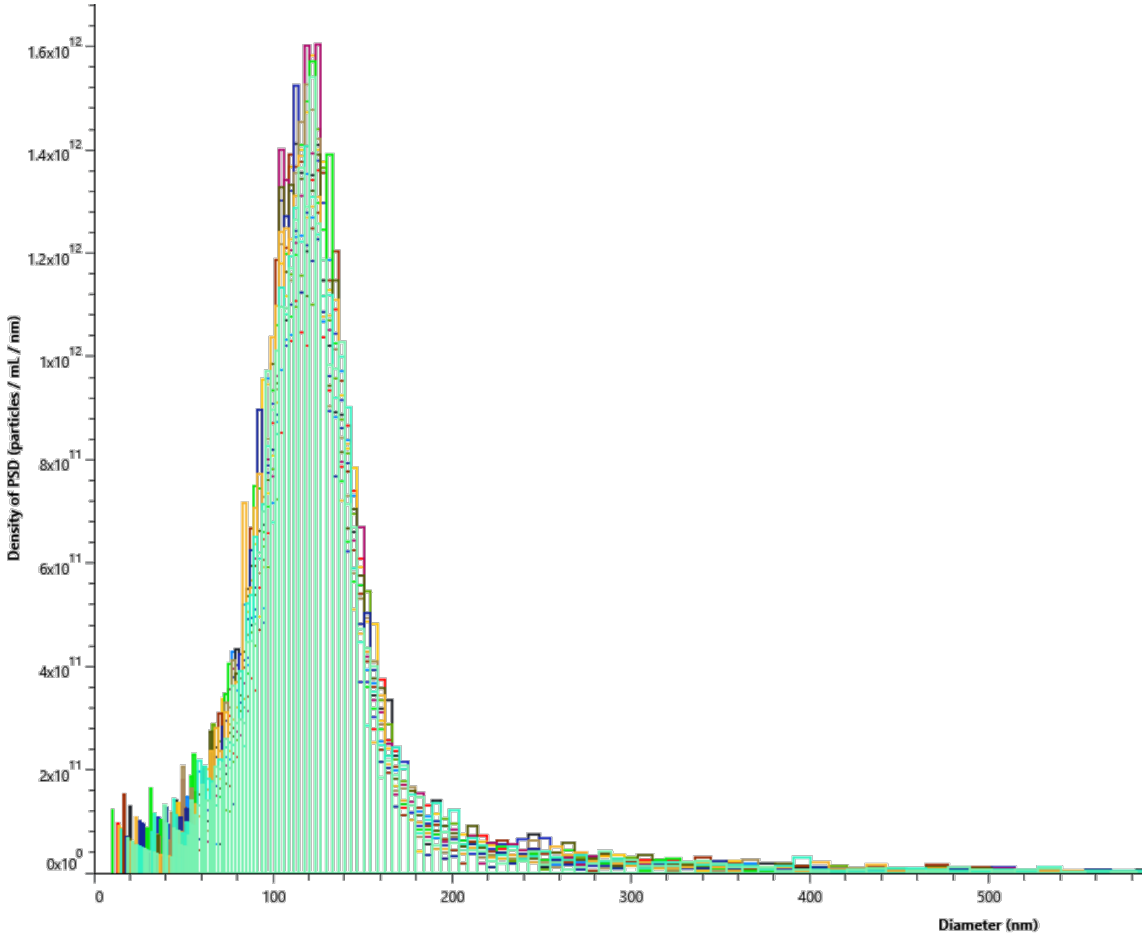


Silica Repeatability

Dilute and run PL-7 15 times

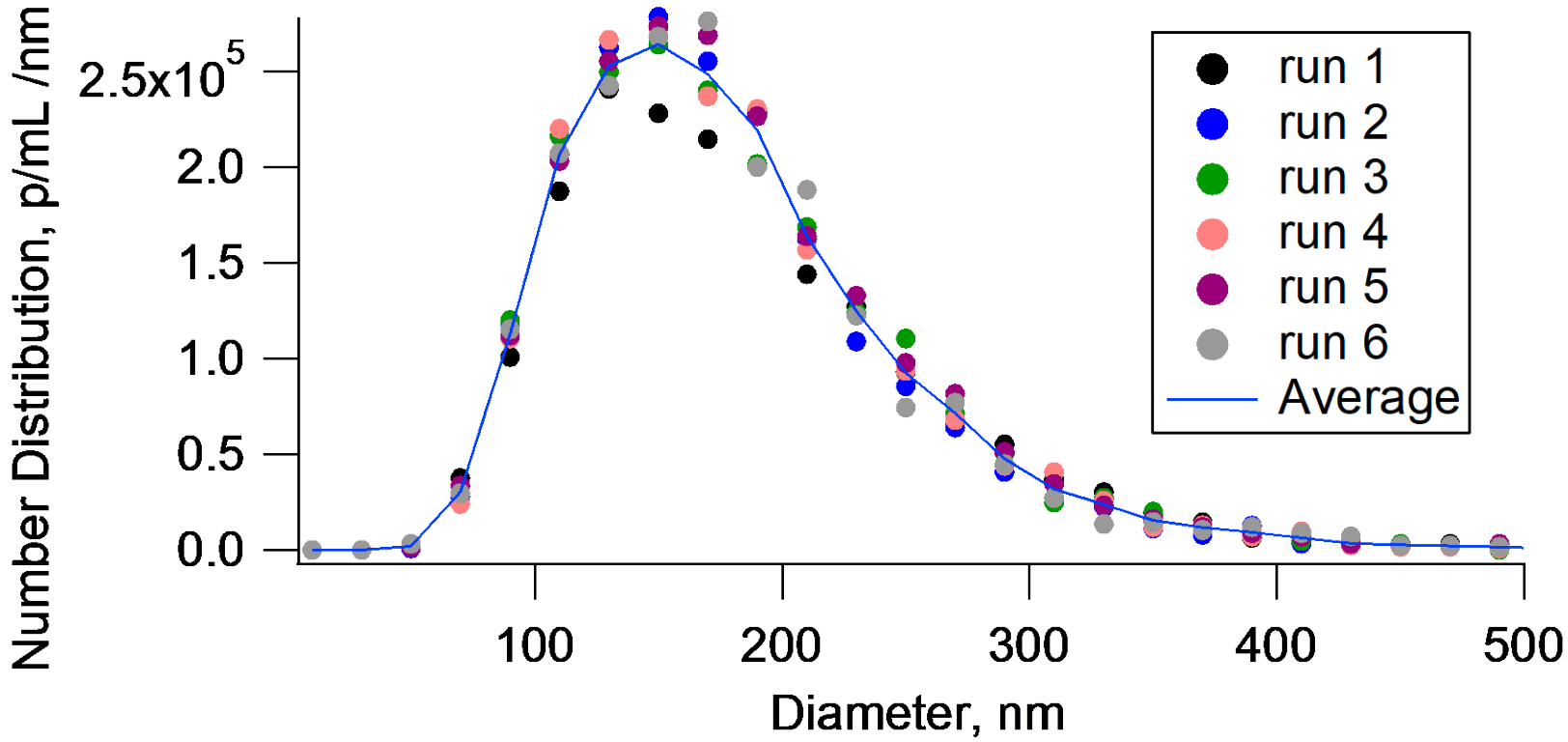
Concentration: 8.48E+13 p/mL
CoV (SD/mean): 3.54%

Median Size: 120.93 nm
CoV (SD/mean): 1.04%

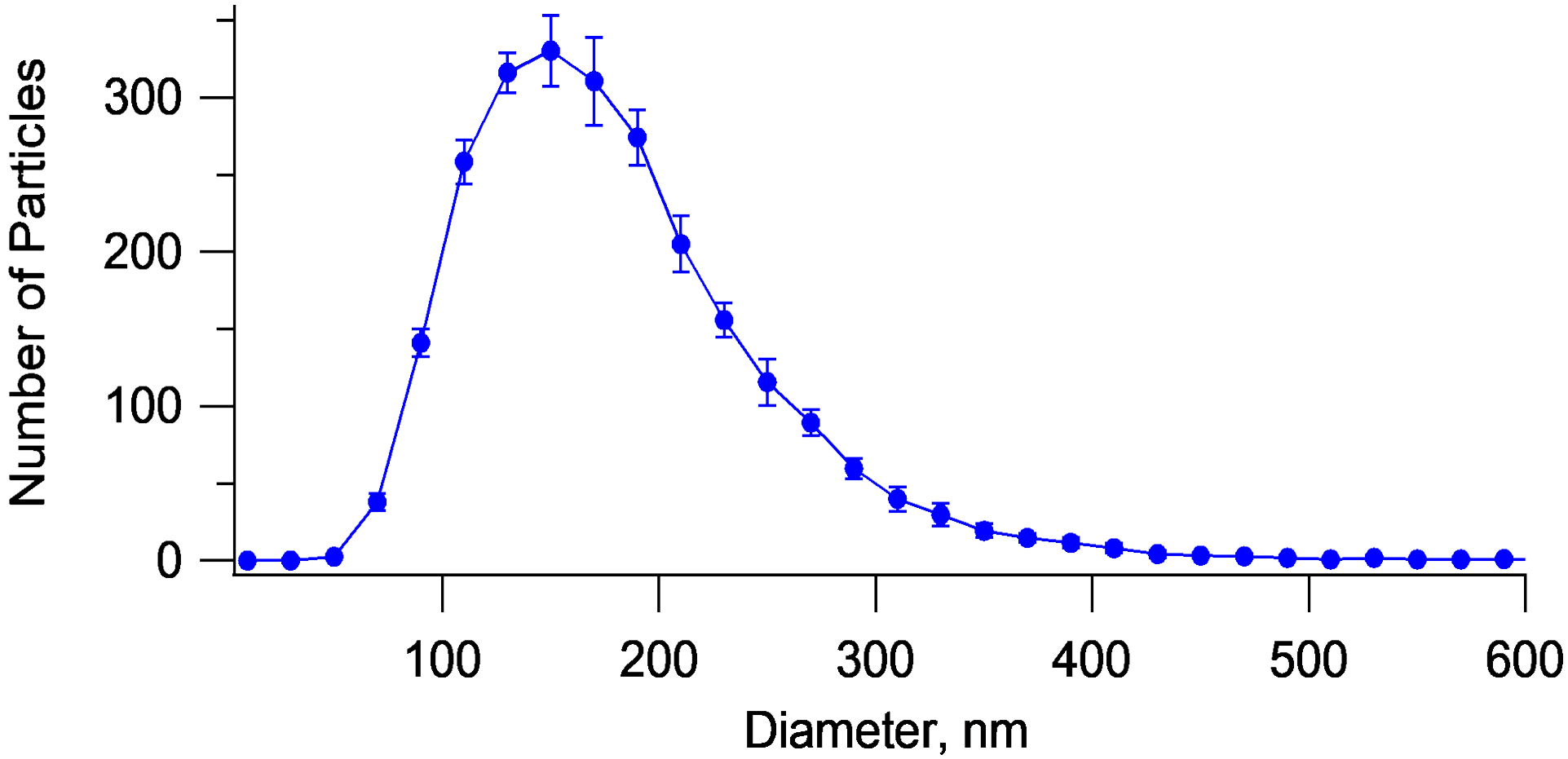


Ceramics: Zirconia (and some statistics)

Do we follow counting (Poisson Statistics)?
Zirconia, run six times as repeats

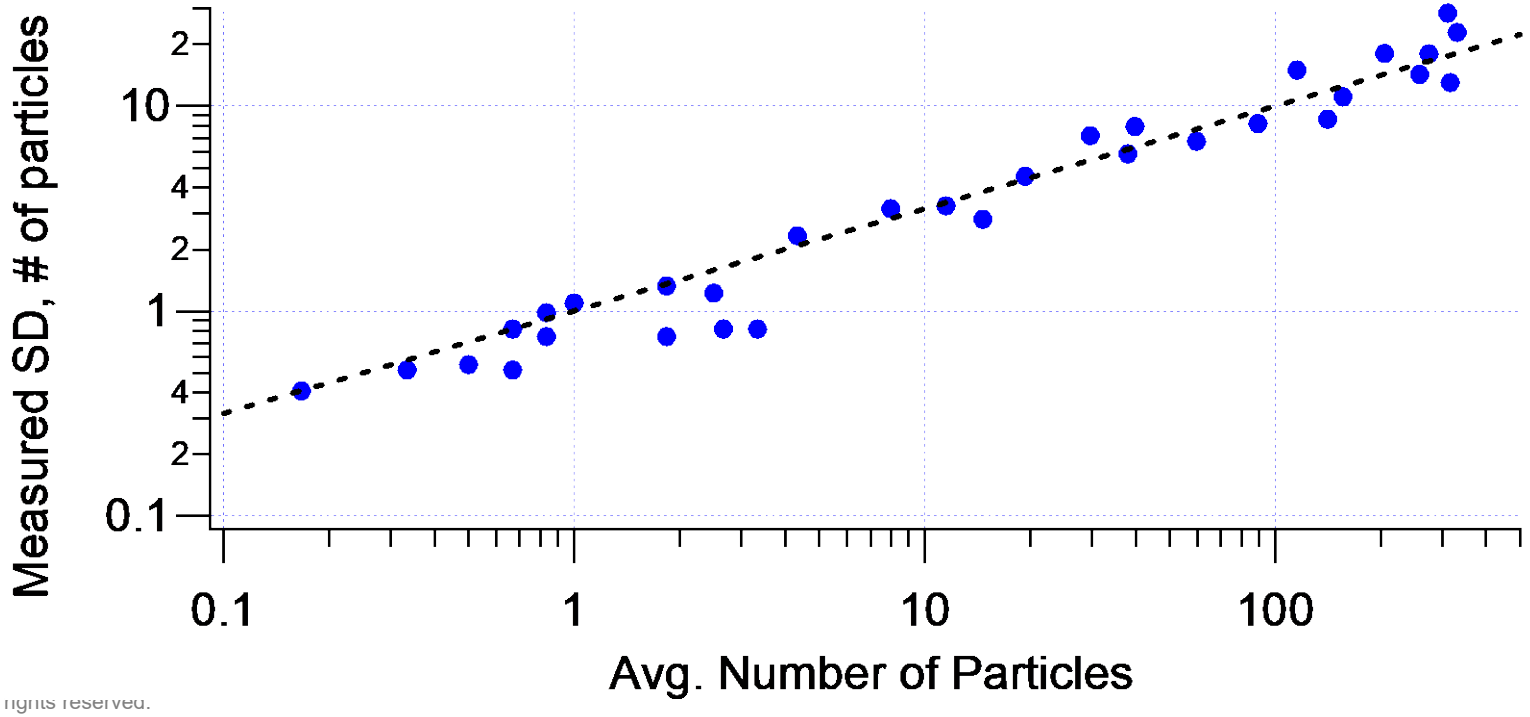


Error bar is standard deviation of 6 repeats



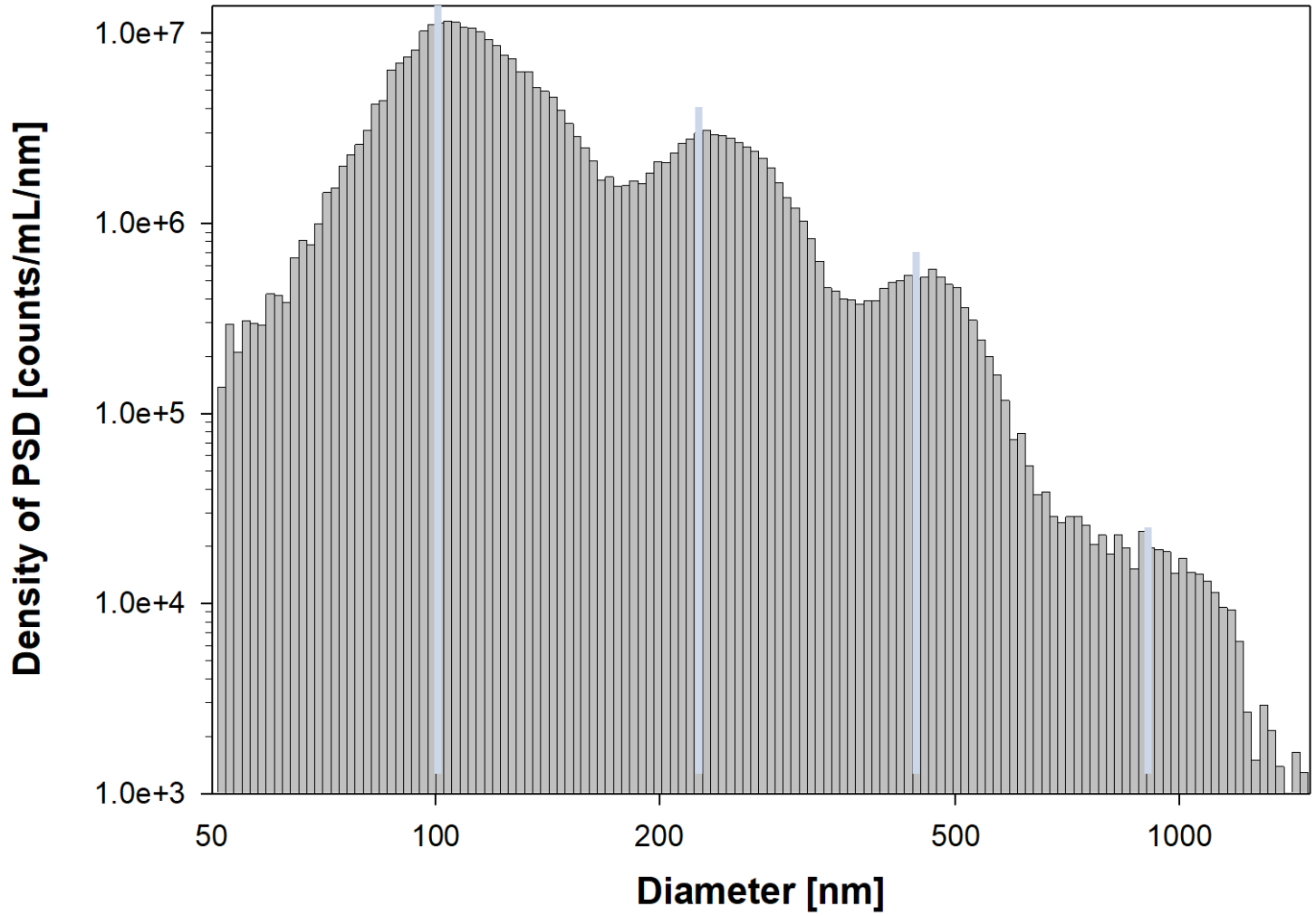
Uncertainty in bins follows Poisson statistics

Plot standard deviation as a function of number of particles in each bin.
Dashed line is sqrt(num particles) and theoretical.
This means
1) mixing is correct (random sampling)
2) uncertainty can be estimated by looking at particle count.



Aggregation: NIST exploratory material

Concentration values
AND detailed distribution
means m-NTA

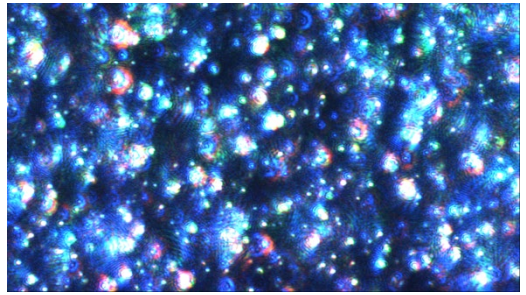


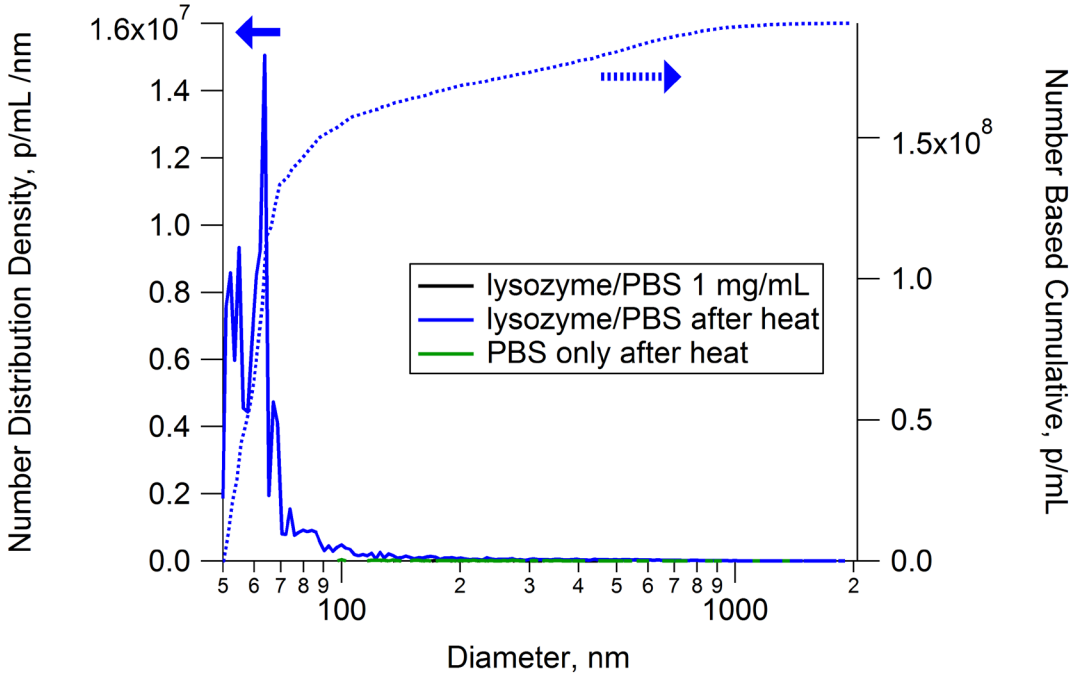
Proteins: Lysozyme heated to 60 C

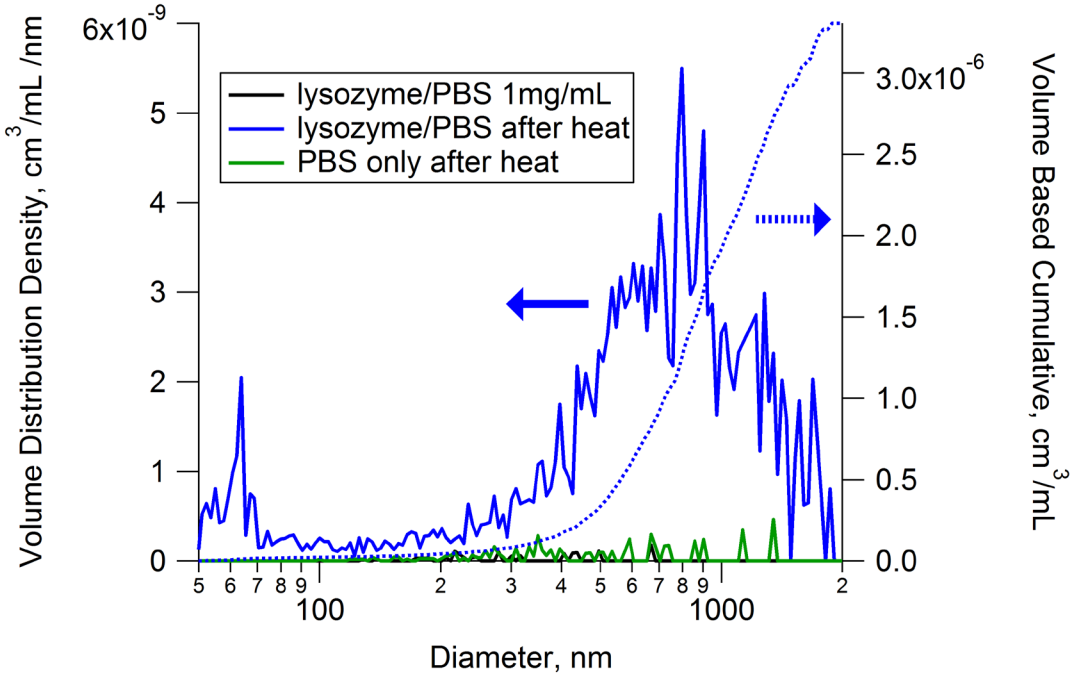
PBS after heating

Lysozyme before heating

Lysozyme after heating

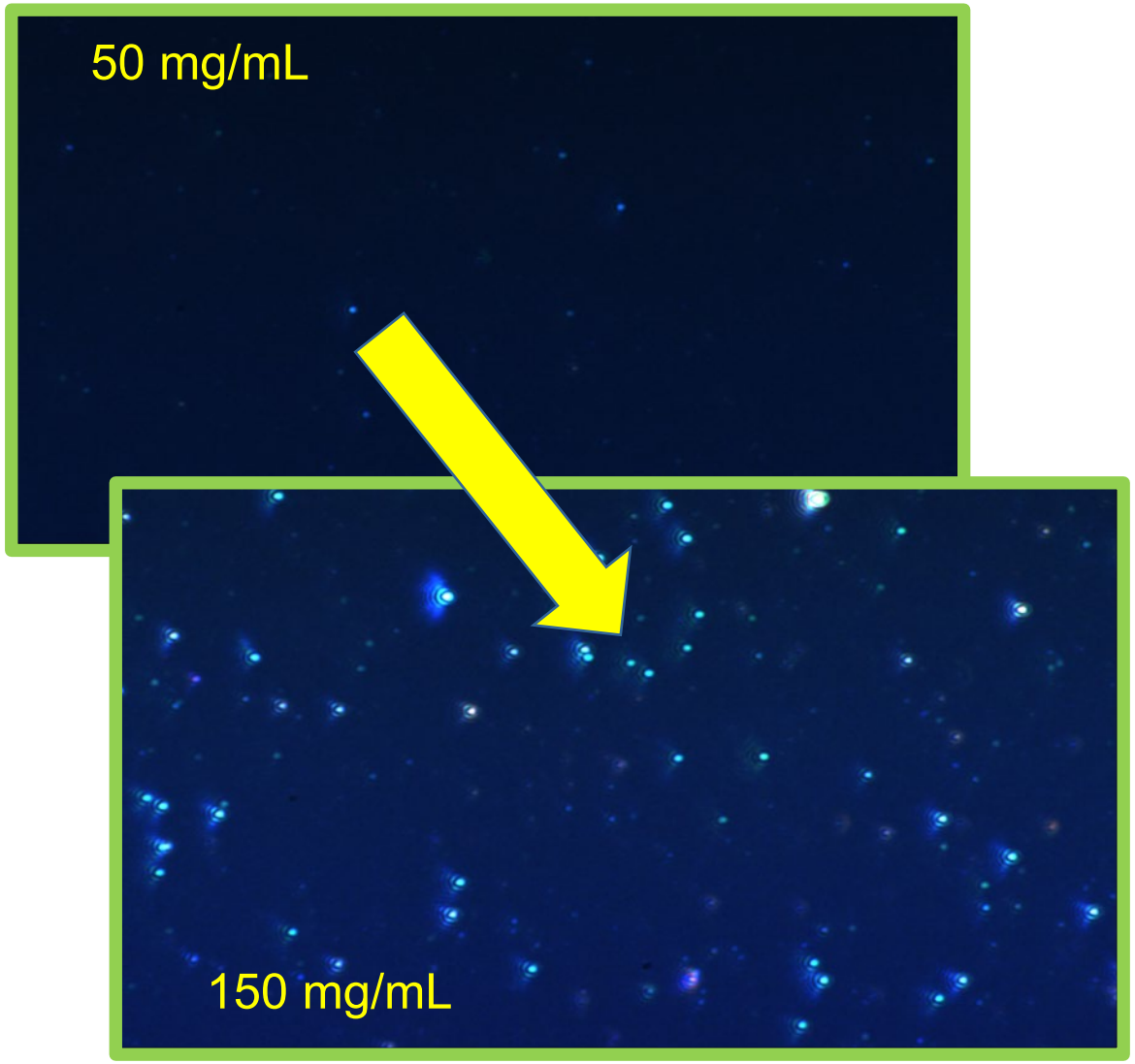


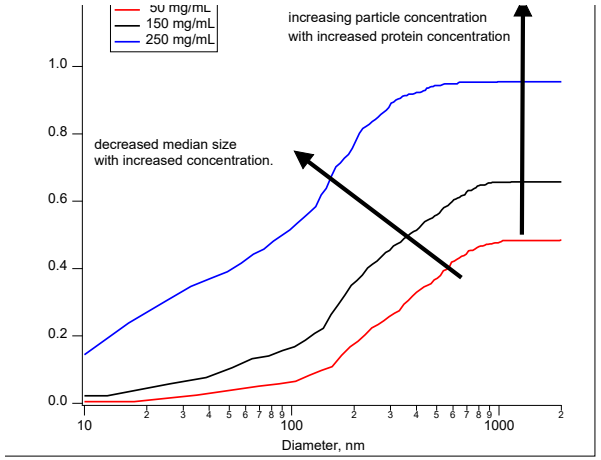




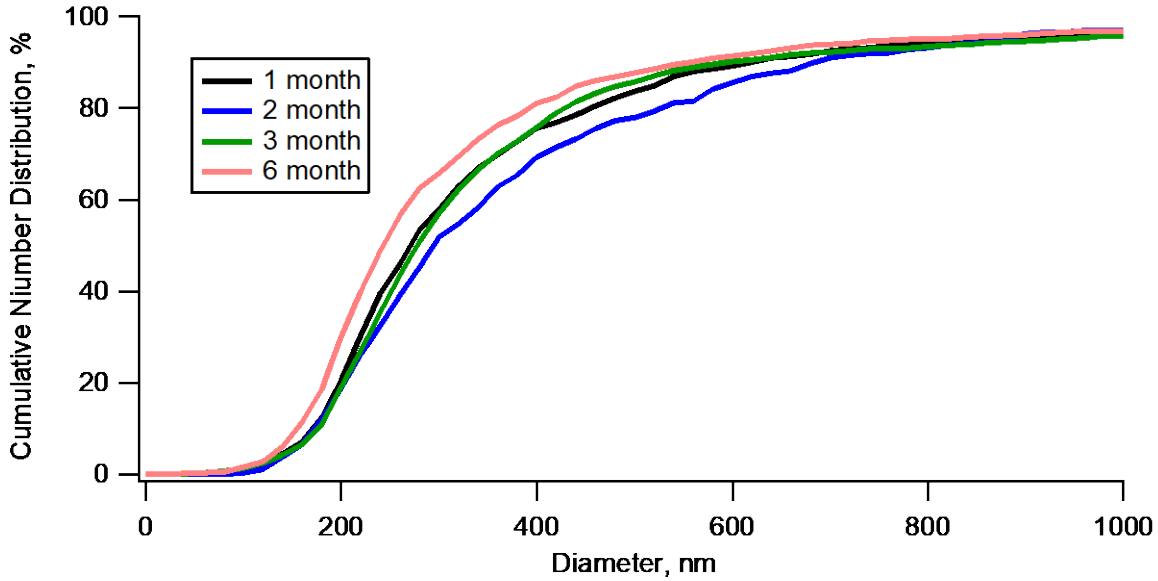
HORIBA Scientific

Proteins: BSA

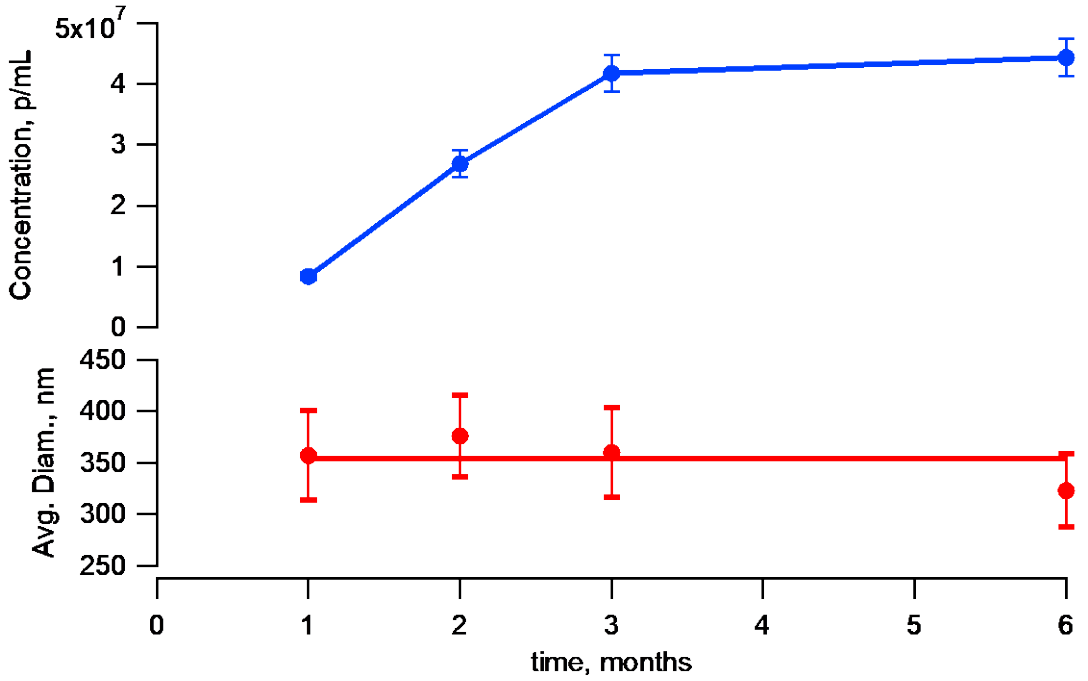




Particulate formation in protein drug



Proprietary protein drug w/ 0.25 polysorbate 80



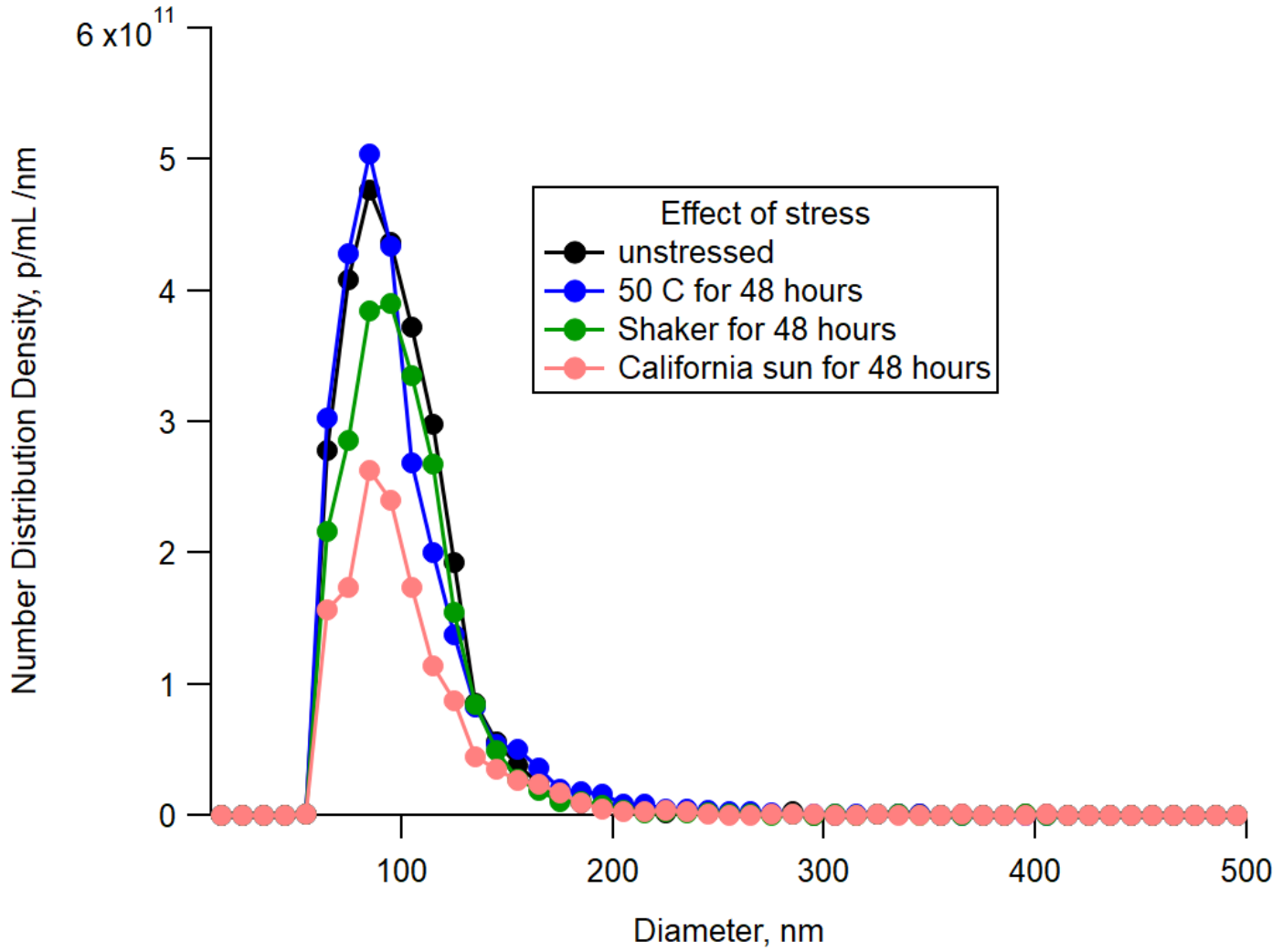
Size shift is small compared to concentration. Nucleation is important.

Vaccine: effect of stress

Commercial canine vaccine formulation (Canine Spectra 6)

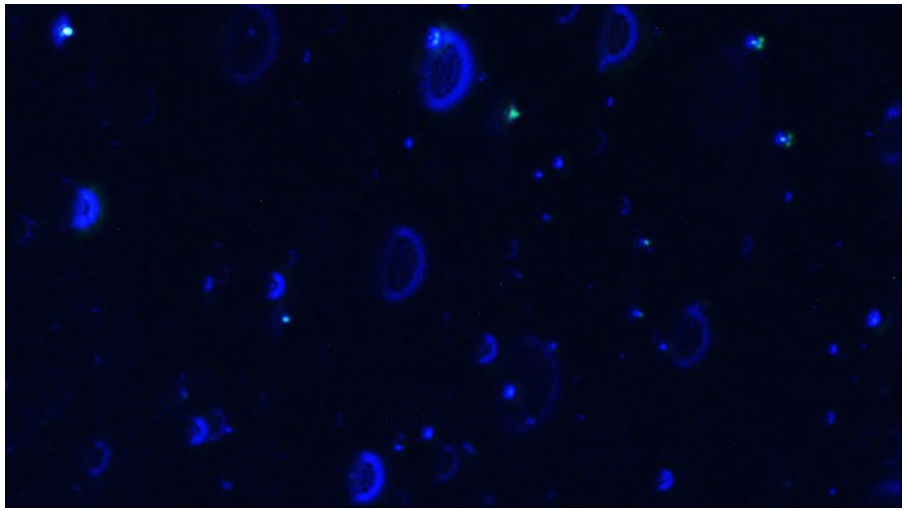
Label clearly states refrigeration required.

Conclusion: don't leave it in the California sun!

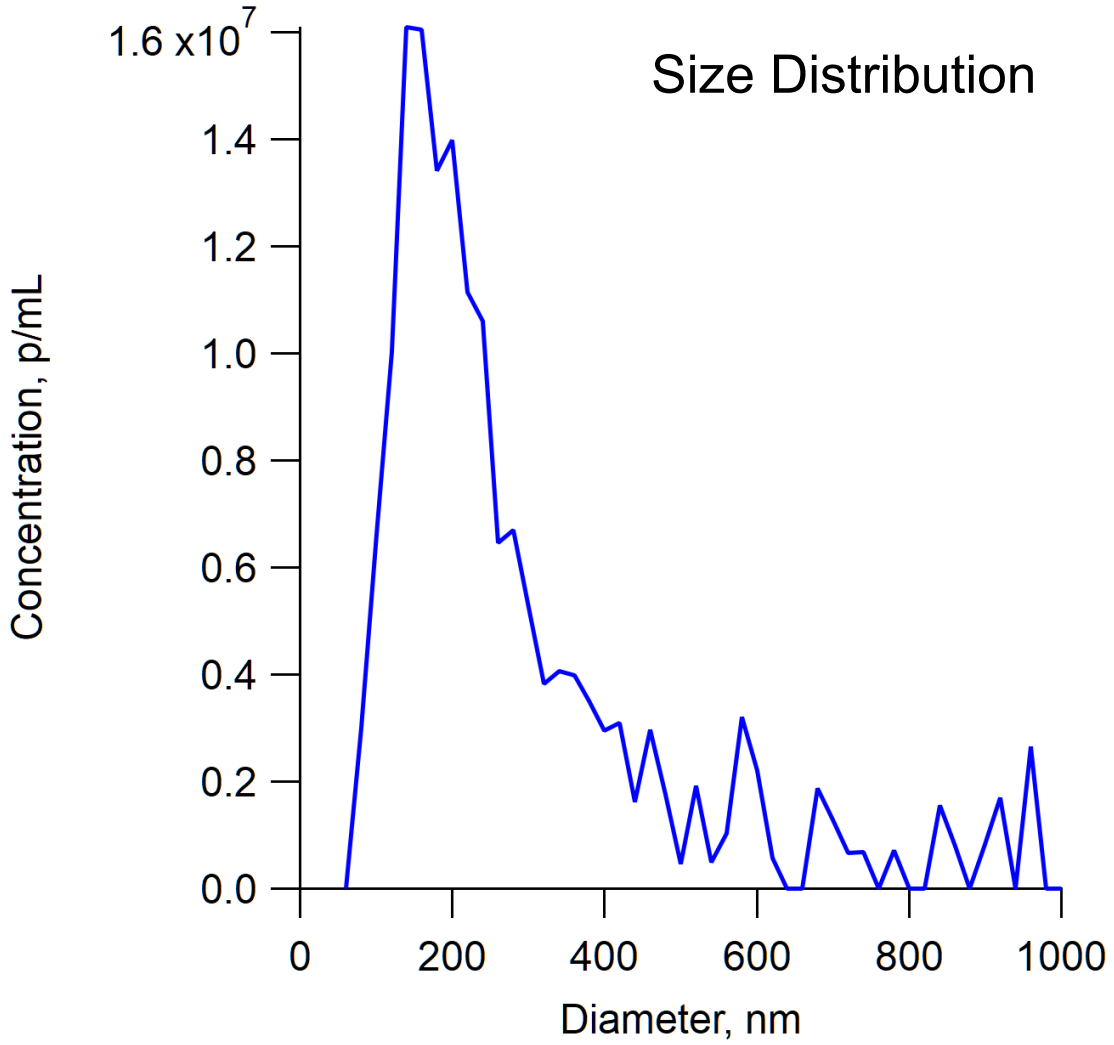


Plant virus data

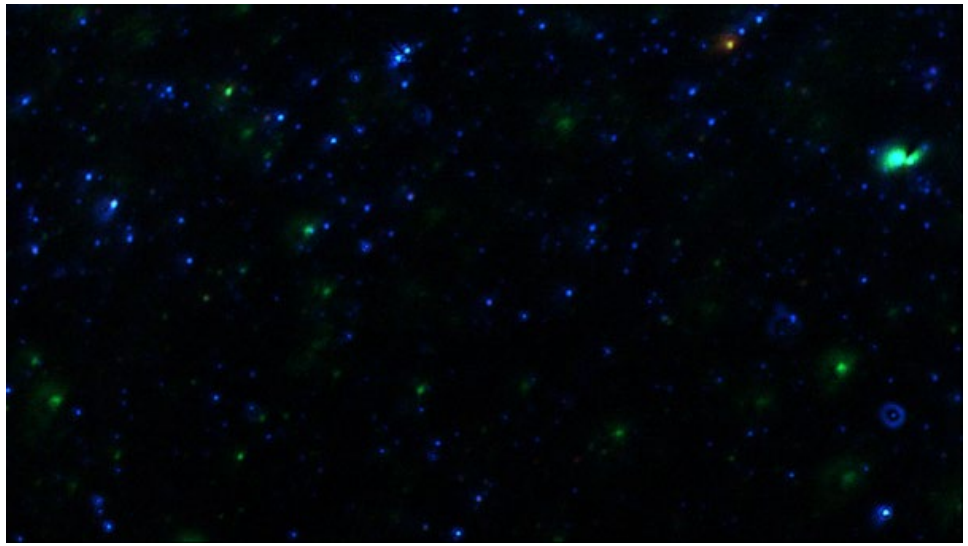
Tobacco Mosaic Virus (TMV)



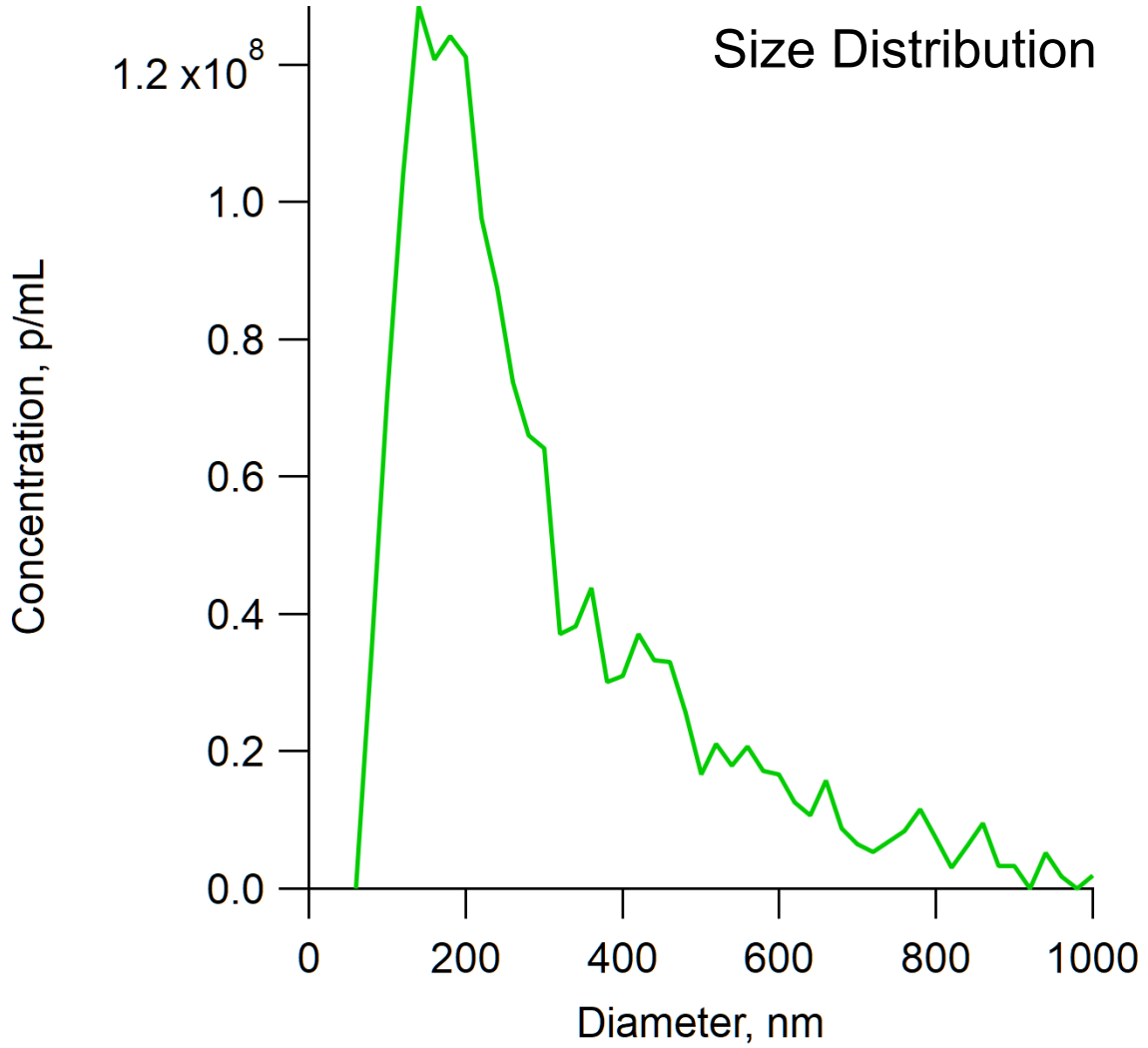
Single frame from video. Points correspond to scattering from individual virus particles and aggregates.



Livestock virus data

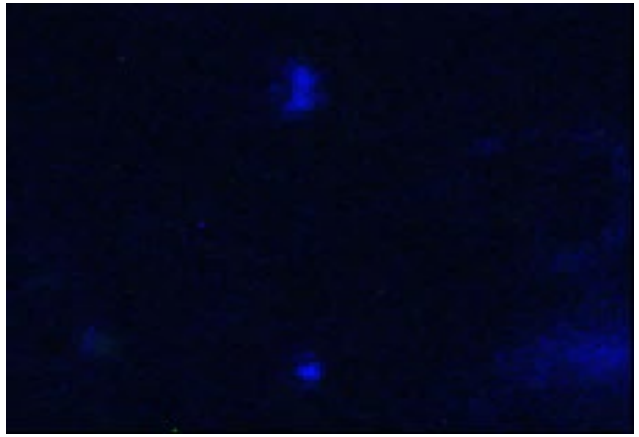


Single frame from video. Points correspond to scattering from individual virus particles and aggregates.

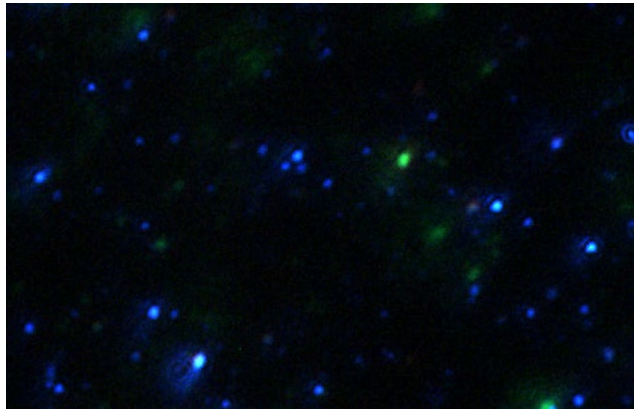


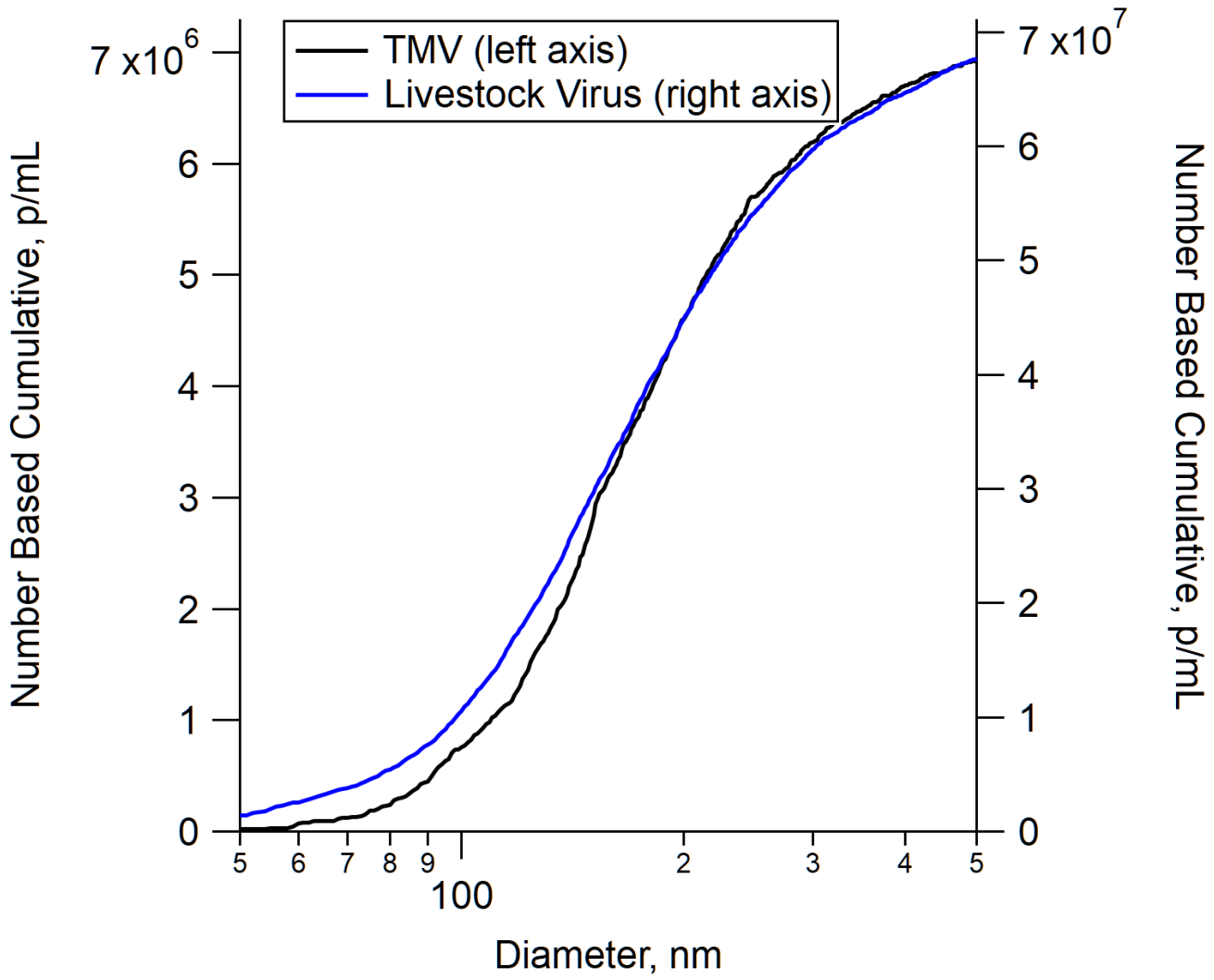
Comparison

TMV

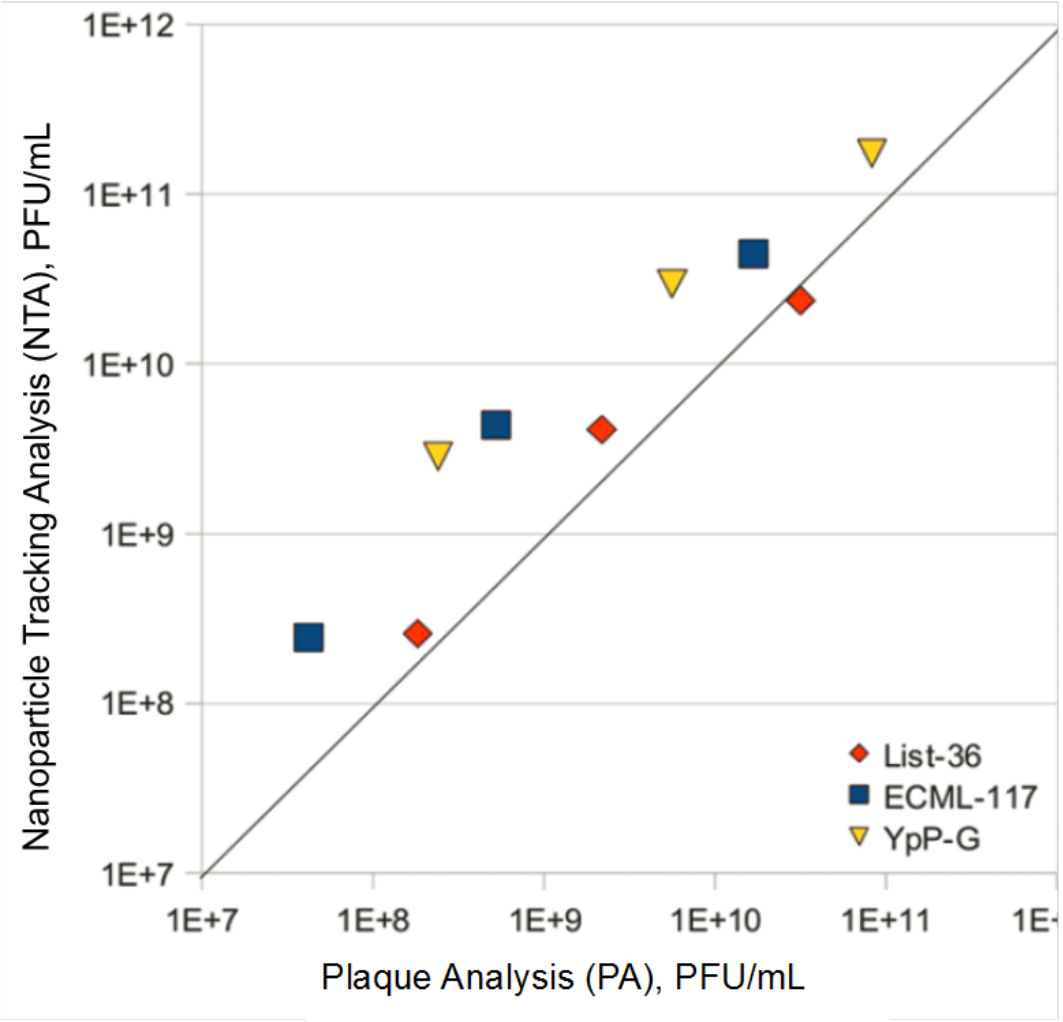


Livestock





Phage Analysis: correlate with infectious titer



Bacteriophages:

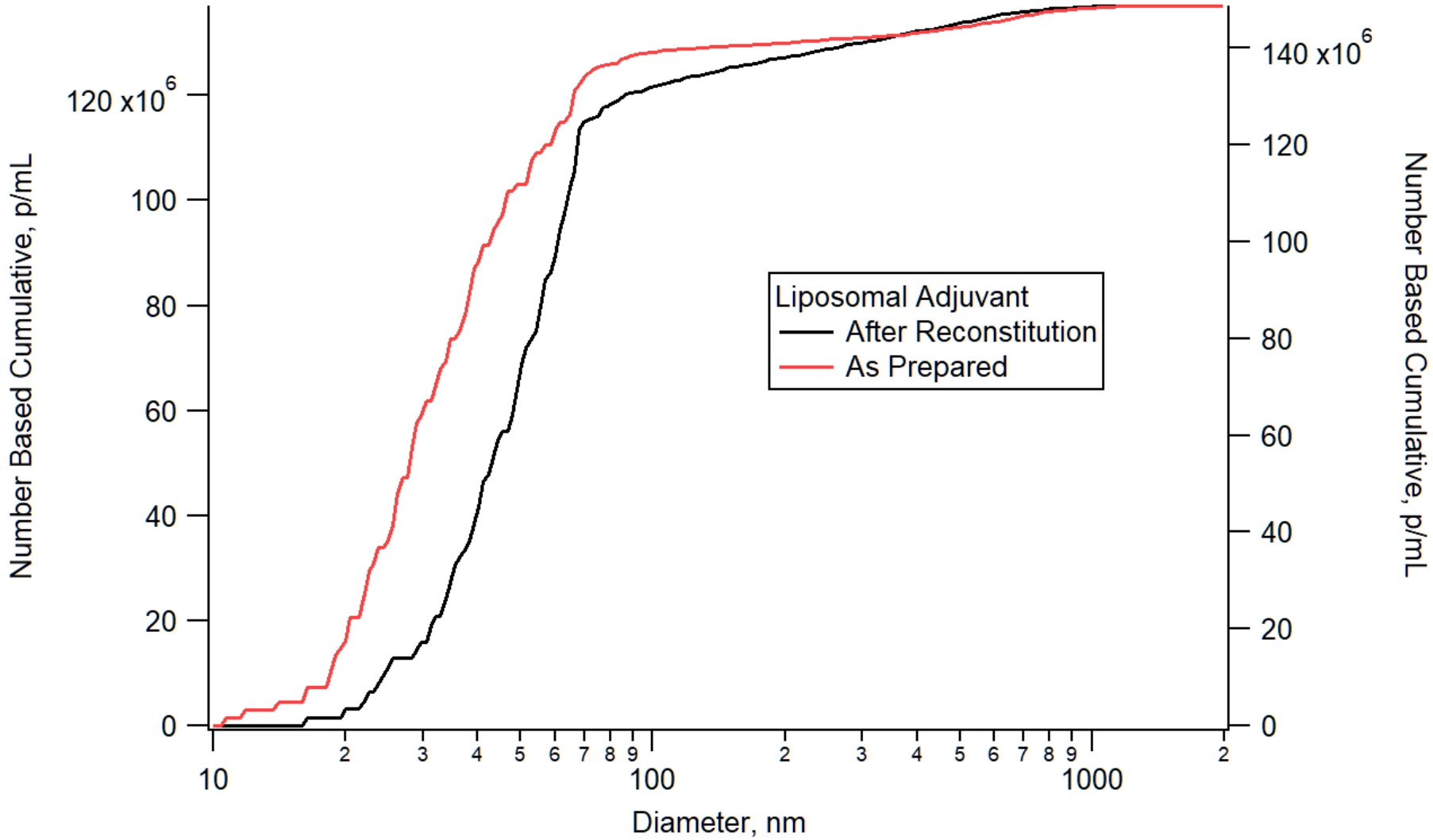
List-36: Listeria monocytogenes

ECML-117: Escherichia phage

YpP-G: Yersinia phage

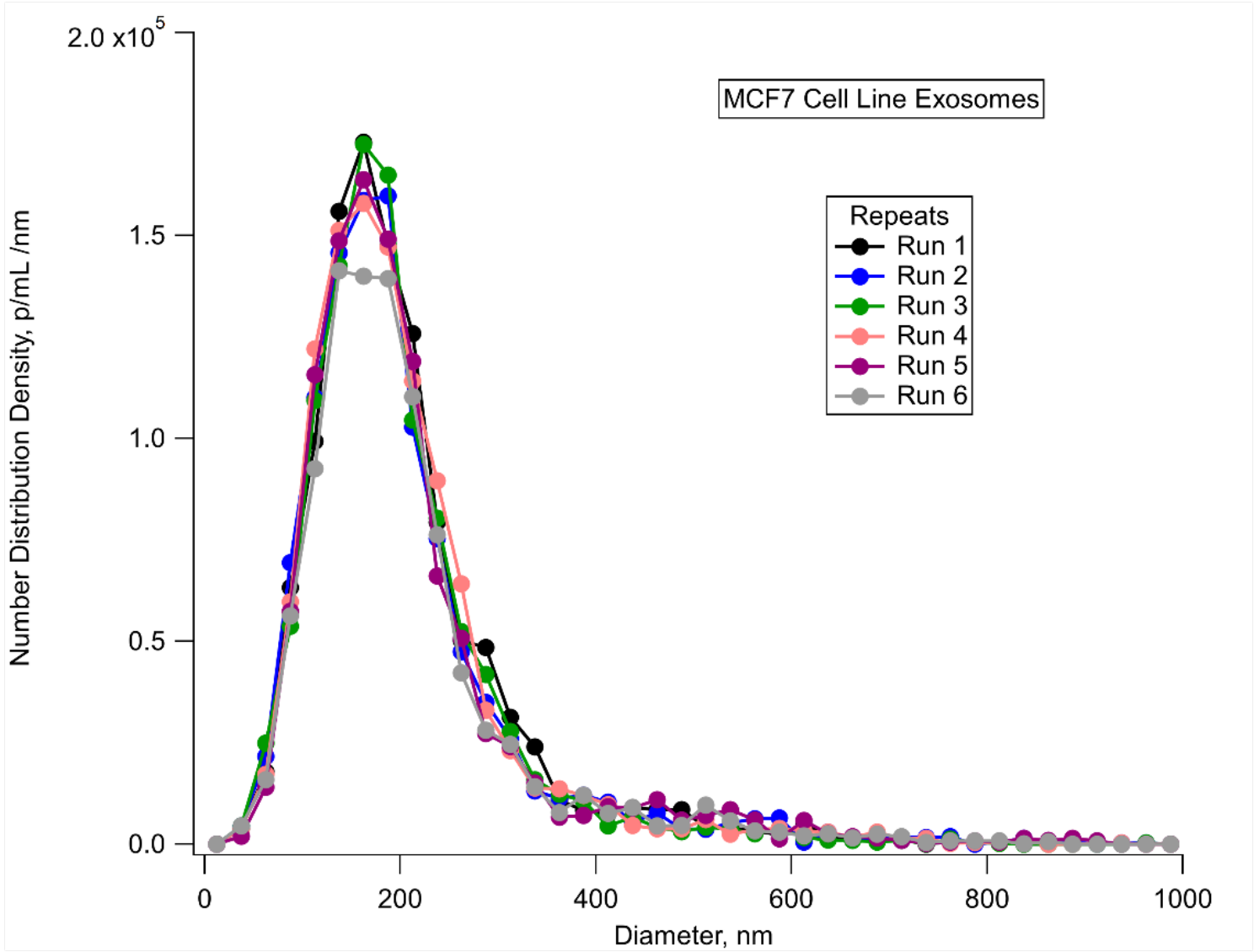
Bacteriophage. 2011 Mar-Apr; 1(2): 86–93

Liposomal Adjuvant Formulation



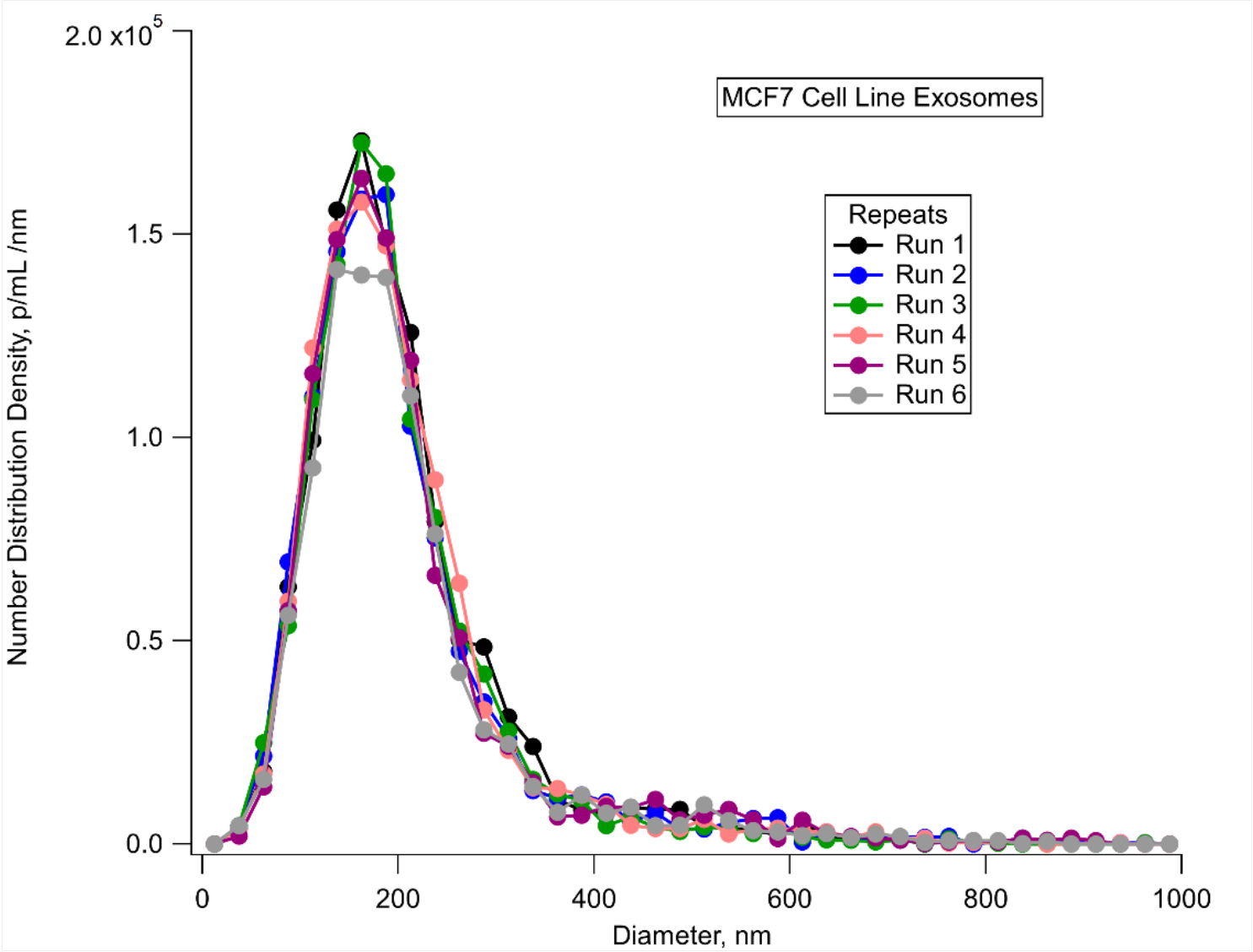
Repeatability of Exosome Measurements

Do we follow counting (Poisson Statistics)? Abcam exosomes (breast cancer), run six times as repeats



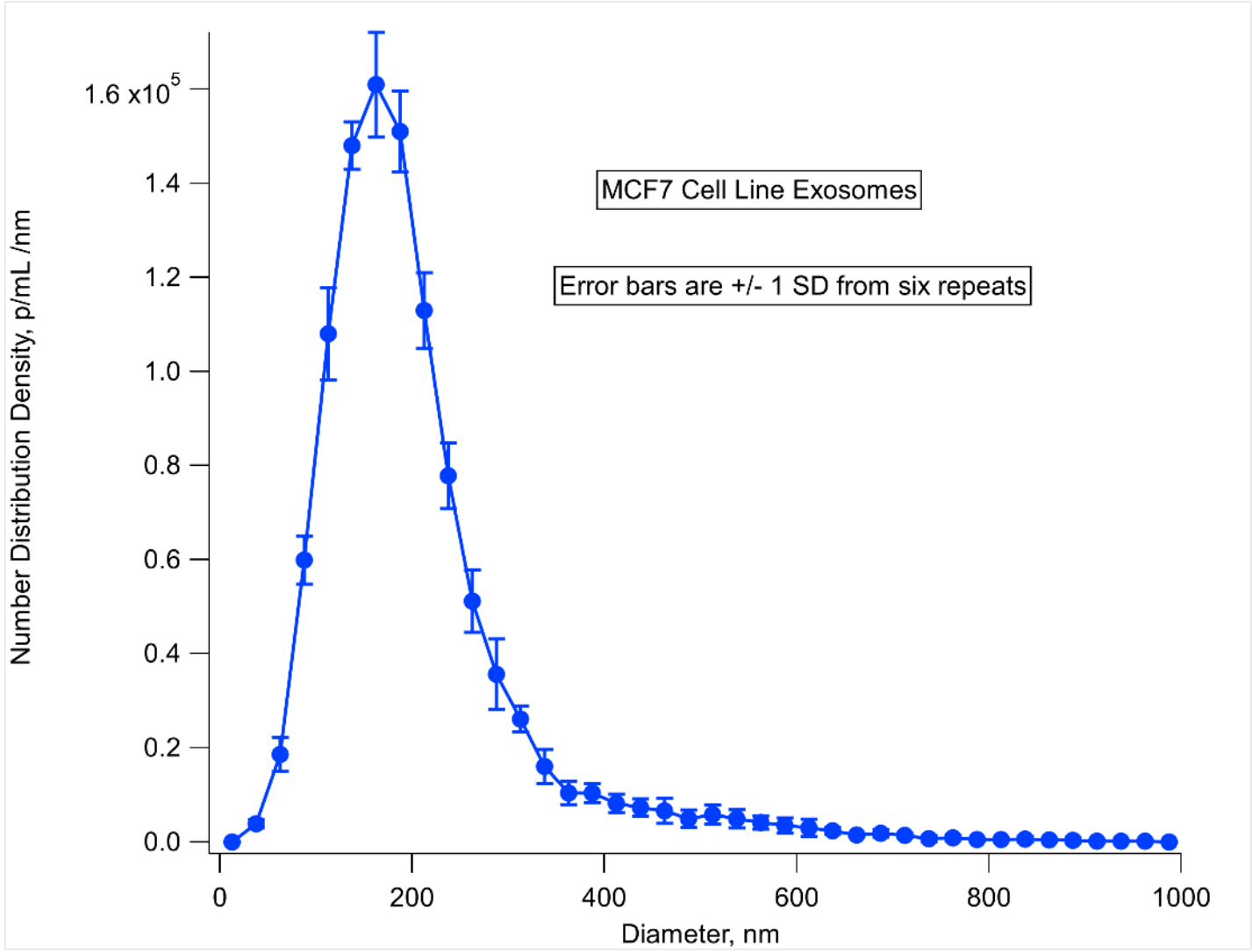
Repeatability of Exosome Measurements

Do we follow counting (Poisson Statistics)? Abcam exosomes (breast cancer), run six times as repeats



Error bar is standard deviation of 6 repeats

These are the error bars estimated from the repeated measurements.

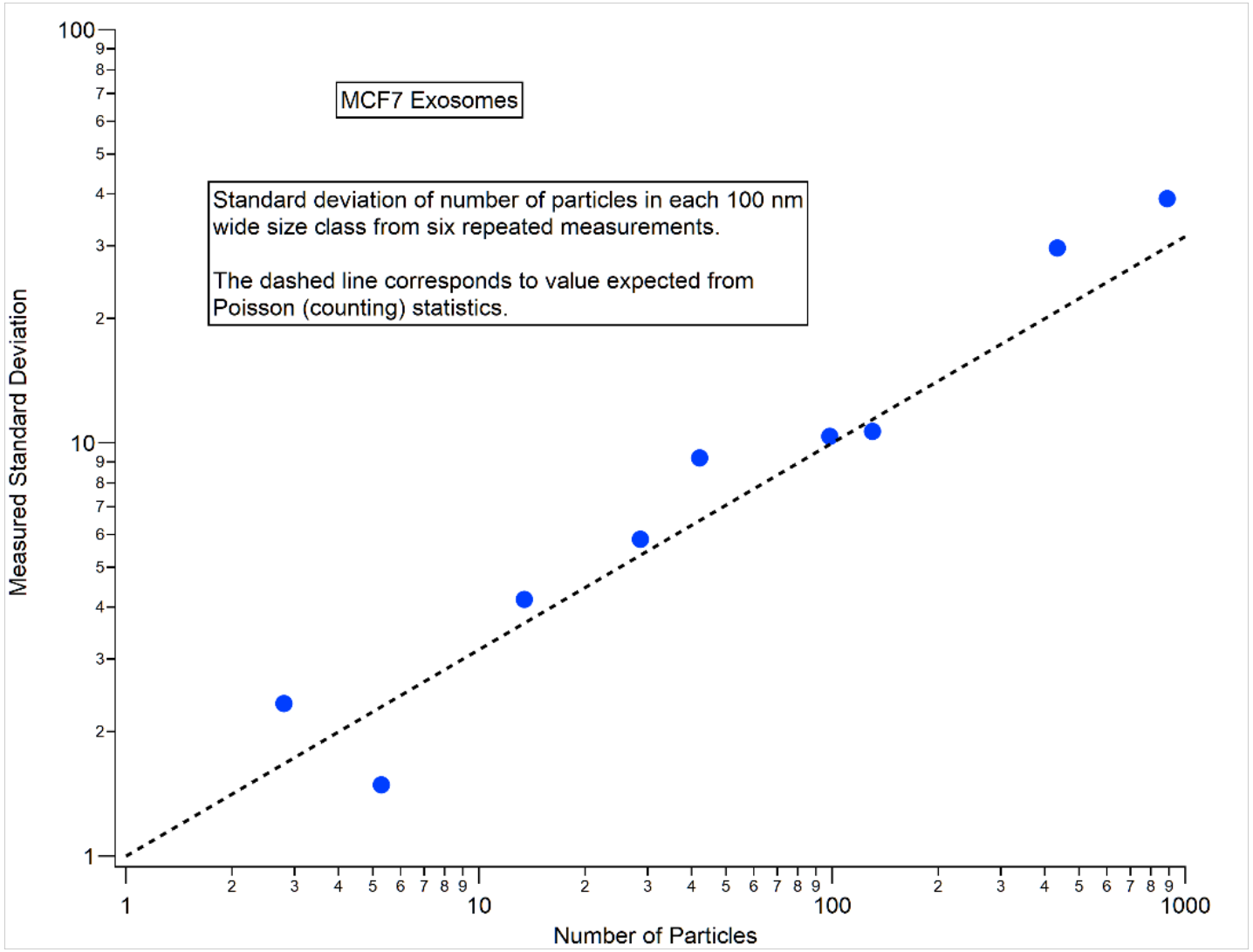


Uncertainty estimation

Plot standard deviation as a function of number of particles in each bin. Dashed line is sqrt(num particles) and theoretical. This means

1) mixing is correct (random sampling)

2) uncertainty can be estimated by looking at particle count.



Overall results

Overall ~1640 particles.

Repeatability is sqrt(1640) = ~40.

Expected CoV (std. dev./avg) = 2.4%

We obtain 4.3% (and 2.05 if you ignore last run…). Close to expectation.

	Dn50, nm	DnAvg, nm	DnSD, nm	Measured Concentration, p/mL	Particles
1	179.8	162.1	45.1	2.75E+07	1718
2	178.3	159.5	46.0	2.64E+07	1652
3	178.1	161.6	44.9	2.64E+07	1653
4	178.9	161.2	46.3	2.70E+07	1685
5	178.3	160.7	43.7	2.61E+07	1632
6	179.4	162.2	45.8	2.42E+07	1511
Avg.	178.8	161.2	45.3	2.63E+07	1642
Std. Dev.	0.68	1.03	0.94	1.13E+06	71
CoV	0.38 %	0.64%	2.08%	4.32%	4.32%

What drives repeatability?

Sample Stability (drift in results with time)

Cell cleanliness (background particle counts do not have good repeatability)

Measurement Duration: Particle counting -> std. deviation = number of particles

# of particles is ~2000 for NTA

Life Science Measurements

Often performed in a buffer like PBS (osmotic pressure is a thing). You need to ensure your buffers are free of particles. Filtration sterilization is a great help. Some premade buffer is very clean. Somehow buffer gets dirty if bottle is open for a couple of days.

If the buffer is not clean, you can filter it.

Sample concentrations are poorly known in advance. You will end up preparing multiple dilutions and checking to see if concentration is right by eye.

DLS concentration for comparison

We can plot counts as a function of concentration to make a calibration curve from 3E-3 to 0.1 mg/mL, r^2=0.999

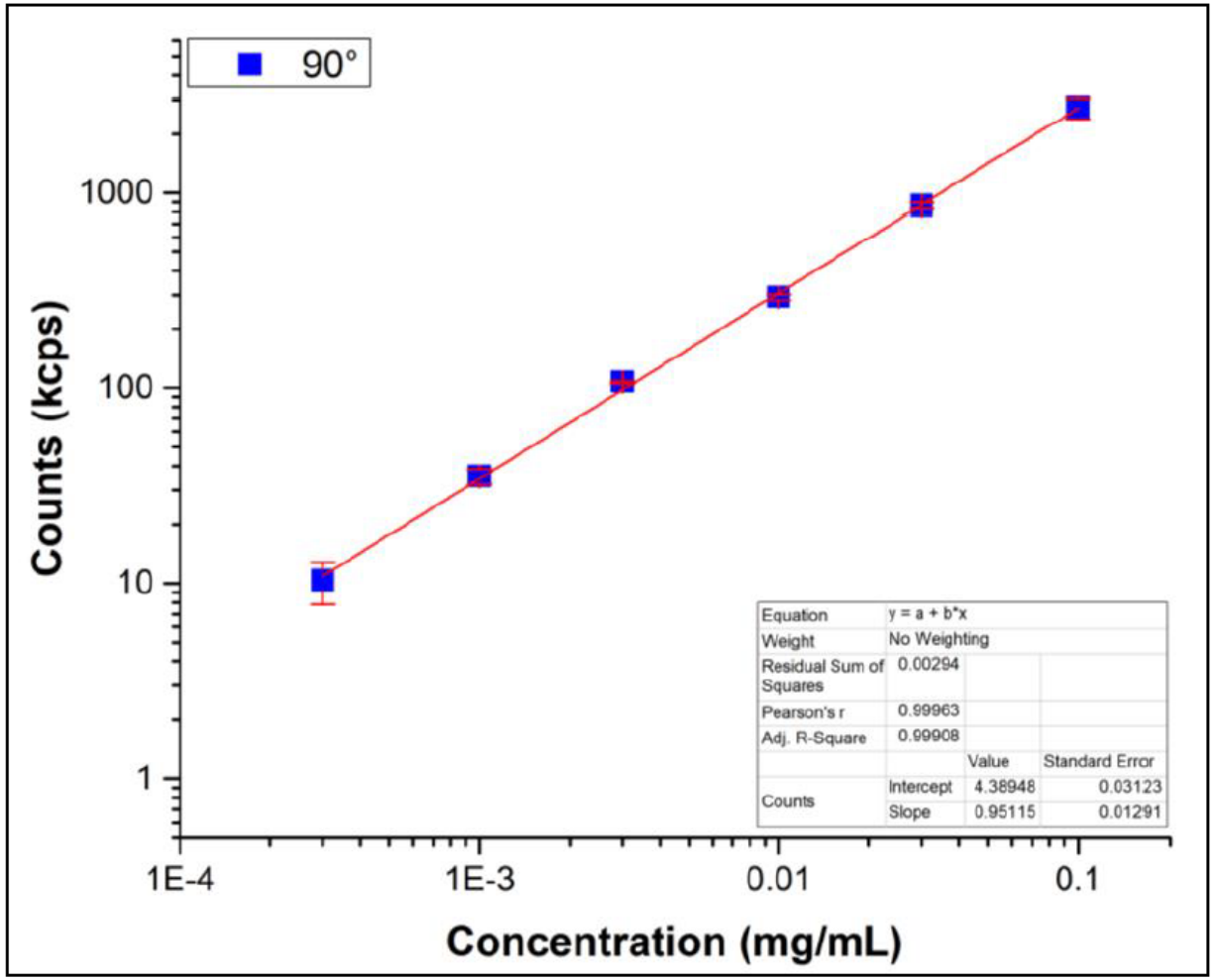


Figure 2: 100 PSL calibration curve on 90° detector from 0.0003 to 0.1 mg/mL.

Concentration from DLS data?

Total scattering from particles can be determined in concert with DLS data.

Concentration can be determined as

$$I_{ex} = C_{par} I_{s,\,par}$$

where

I_{ex} = excess scattering (measured)

C_{par} = Number concentration of particles (desired)

$I_{s,\,par}$ = Scattering from a single particle (calculated)

DLS conc. error due to particle diameter

$I_{s,\,par}$ = Scattering from a single particle

Varies as

d_s^6

where

d_s is particle diameter for <u>diffraction</u> (static light scattering), not DLS

Scattering goes by d^6, so a 10% error in particle diameter gives a 60% error in S_{par}.

d_s is NOT the diameter from DLS. For irregular particles, d_s is related to the particle moment of inertia, NOT the hydrodynamic size determined by DLS. The required size average is not the average determined by DLS.

For a mixture, you will need to know the intensity weighted “static light scattering size” confusingly called z-average, but from a completely different kind of measurement.

Concentration from DLS data?

Total scattering from particles can be determined in concert with DLS data.
Concentration can be determined as

$$I_{ex} = C_{par} I_{s,\,par}$$

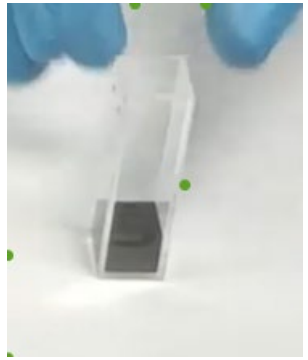
Large errors in $I_{s,\,par}$ mean close to 60% errors in determined concentration with optimistic assumptions about determining size distribution.

Operation

insert

+

cuvette
+
stir bar

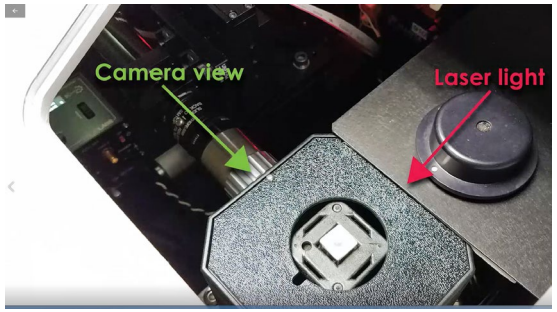


ready

Load sample.

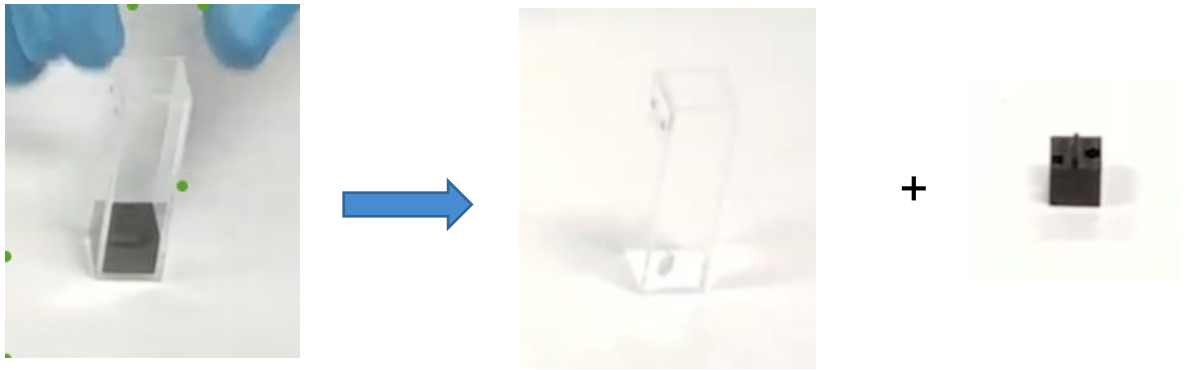
Components are readily separated for easy and thorough cleaning.

Operation



Place in analyzer.

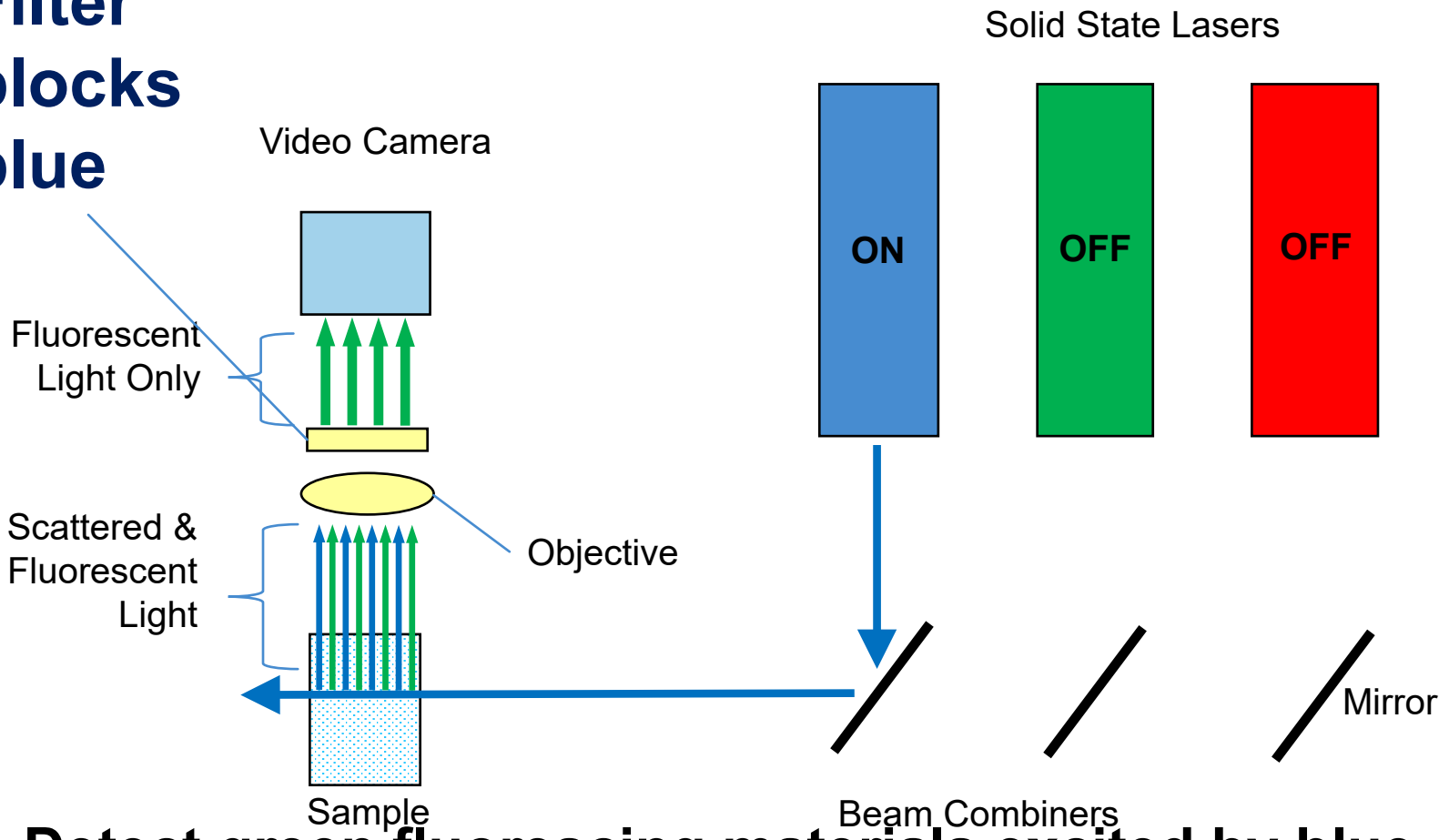
Press record



To prevent cross contamination, separate for thorough cleaning.

Fluorescence Analysis

Filter blocks blue



Detect green fluorescing materials excited by blue laser

Fluorescence - challenges

Photobleaching (reduced emission intensity from overexposure)

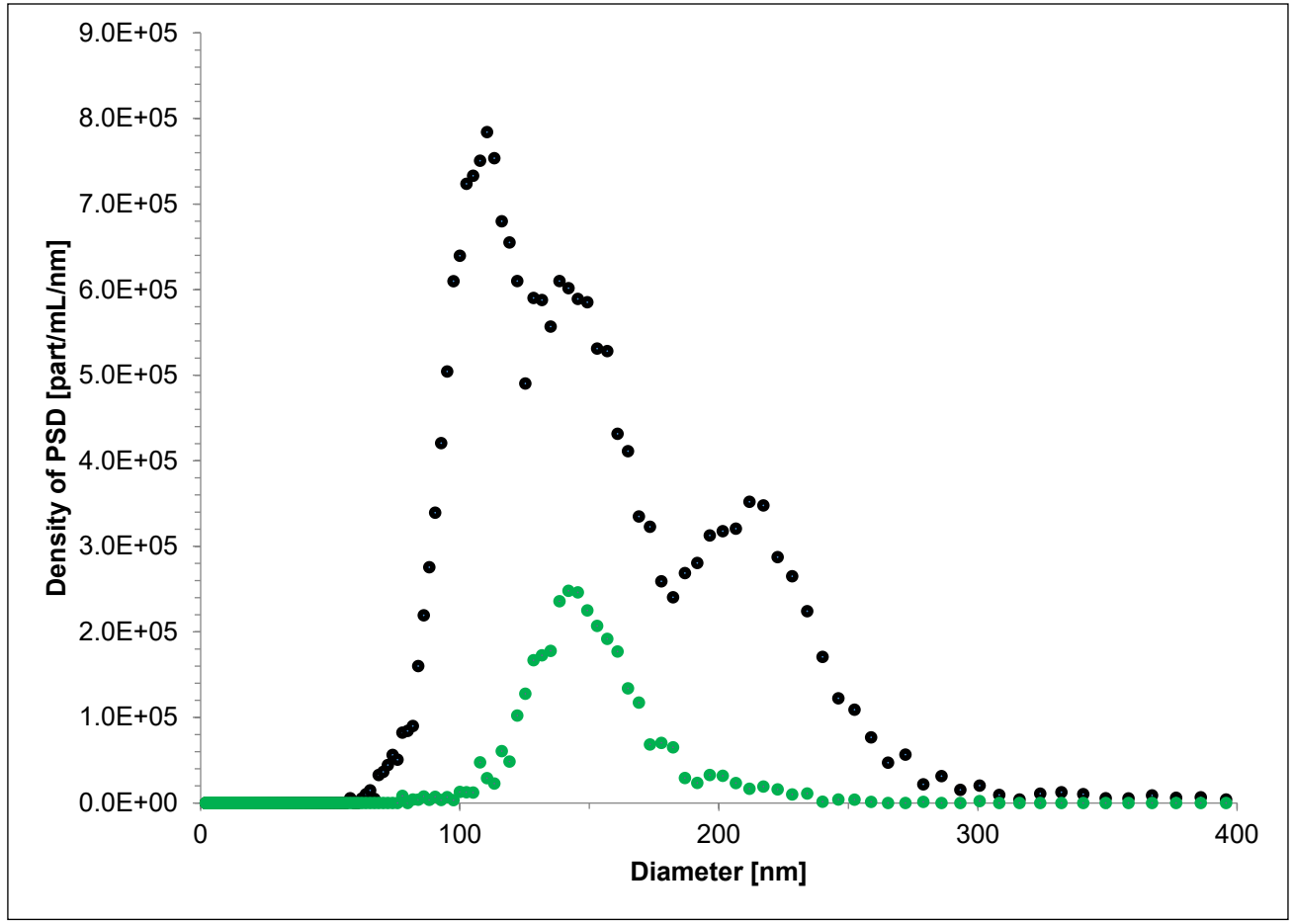
- Lasers pulsed in synch with camera shutter minimizes excitation energy
- Laser power levels can be adjusted individually
- Sample stirring introduces fresh aliquots of unbleached sample
- Measure concentrations only if applicable (size measurements take longer)

Detection limits

- Primarily a question of particle size
- How much fluorescent material can be attached to, or included in, a nanoparticle?
- Very much application specific (fluorophore to sample optimization)

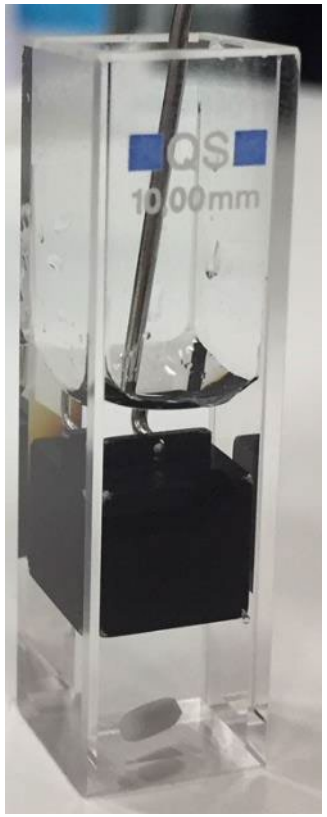
Analyzing a mixture

Mix of Fluoro-Max beads 140 nm dia with 102 nm and 203 nm dia PSL



HORIBA Scientific

ViewSizer 3000



cuvette w/insert

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

Closing Comparison

Issue	Laser Diffraction	DLS	Centrifuge	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
THANK YOU
Grazie
ขอบคุณครับ
Большое спасибо
Obrigado
ありがとうございました
Cảm ơn
Merci
감사합니다
Dziękuję