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Particle Analysis

Jeff Bodycomb, Ph.D.

Sizing and Counting Viruses and Virus-like Particles

Outline

Background

Technology overview

Results

Fluorescence

Concluding Comments

A word from our sponsor

Did you sign up for our e-mail newsletter?

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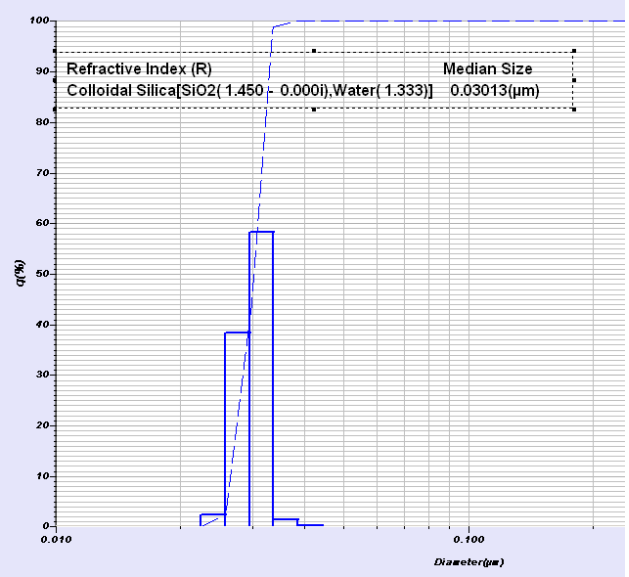
Background

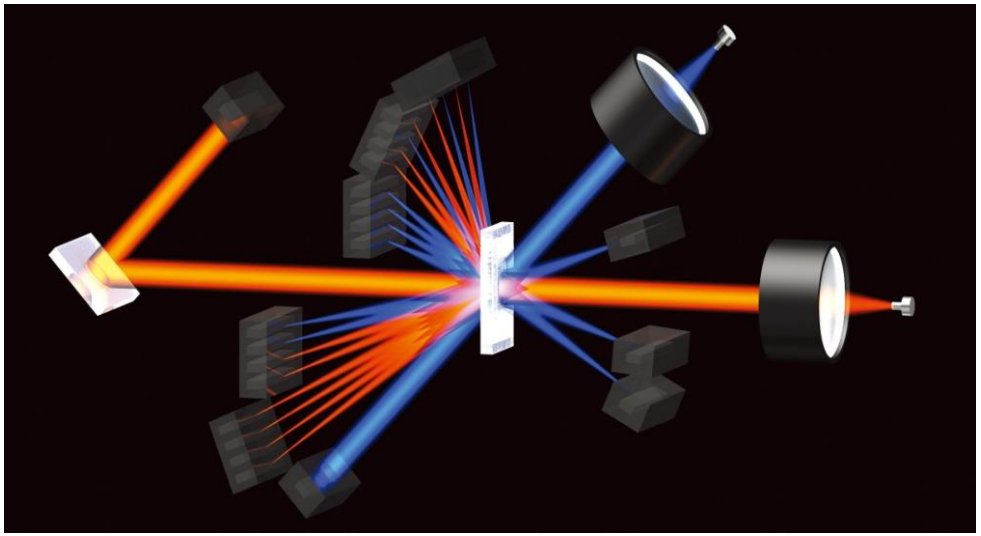
Established technologies

1. Dynamic Light Scattering (DLS)
2. Laser Diffraction
3. Nanoparticle Tracking Analysis (NTA)
4. Transmission Electron Microscopy (TEM)

Laser diffraction

Silica
~ 30 nm





Laser Diffraction

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

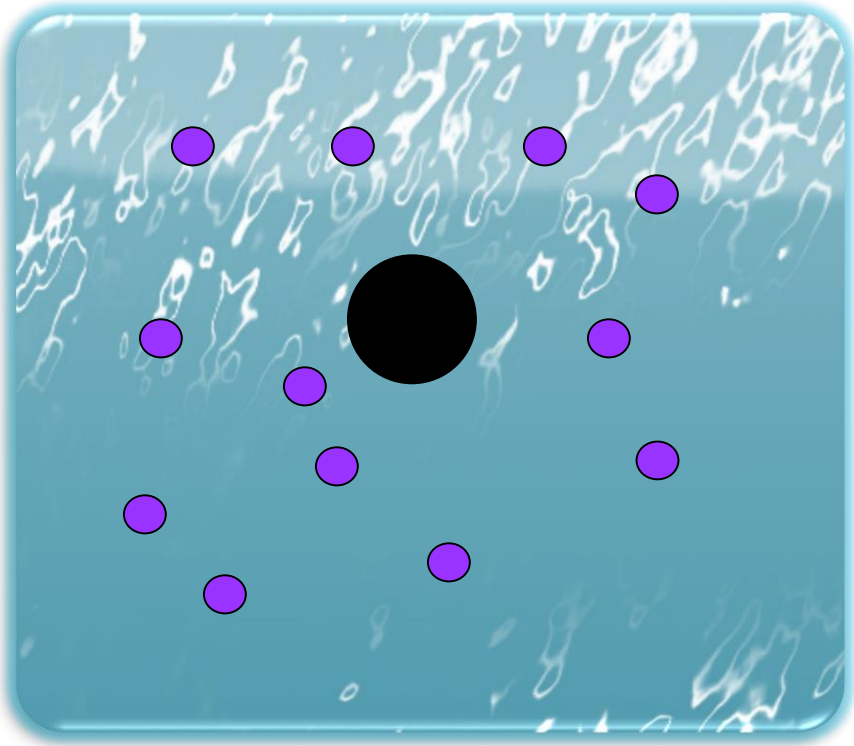
Unmet needs

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

Technology

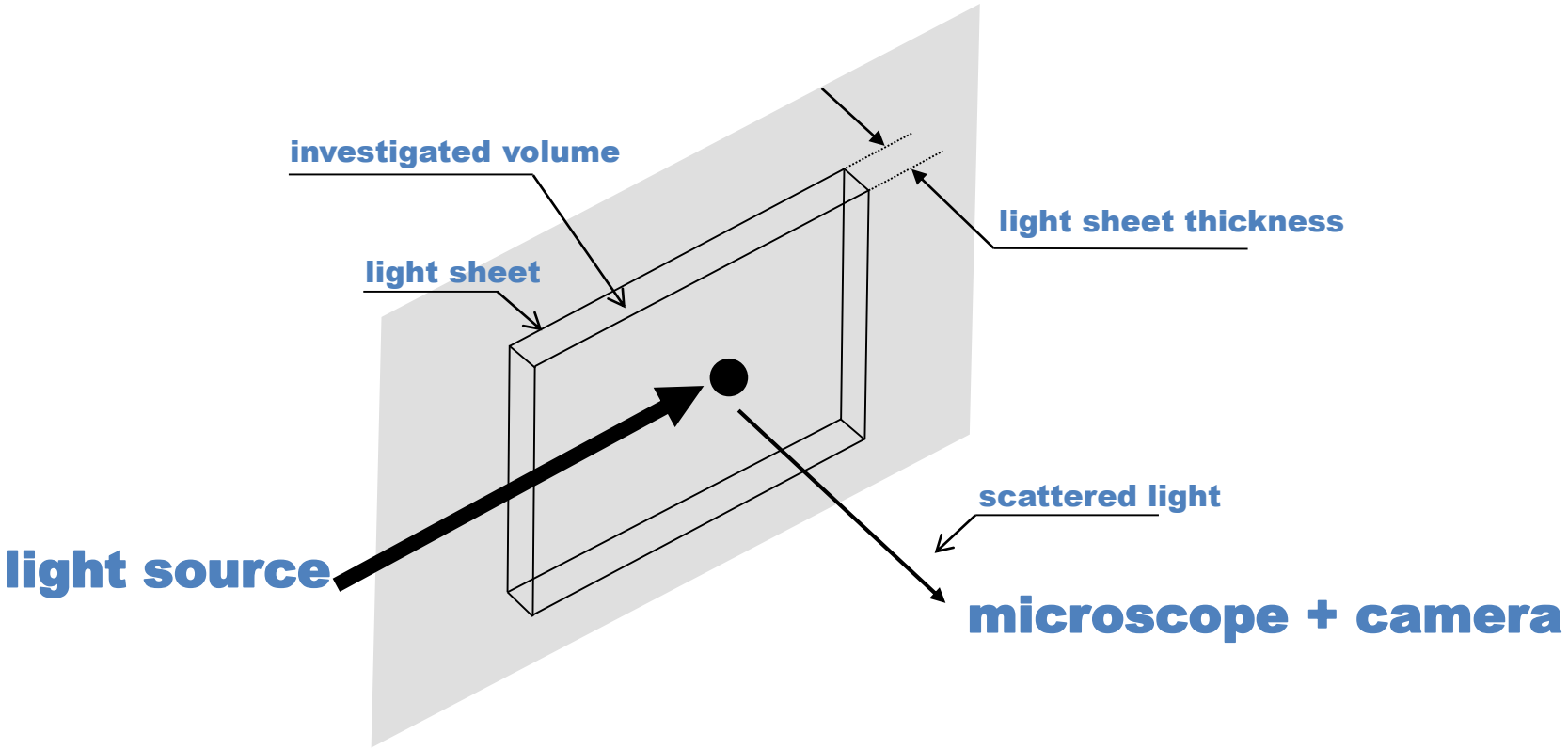
Brownian motion

Particles in suspension undergo Brownian motion due to solvent molecule bombardment in random thermal motion

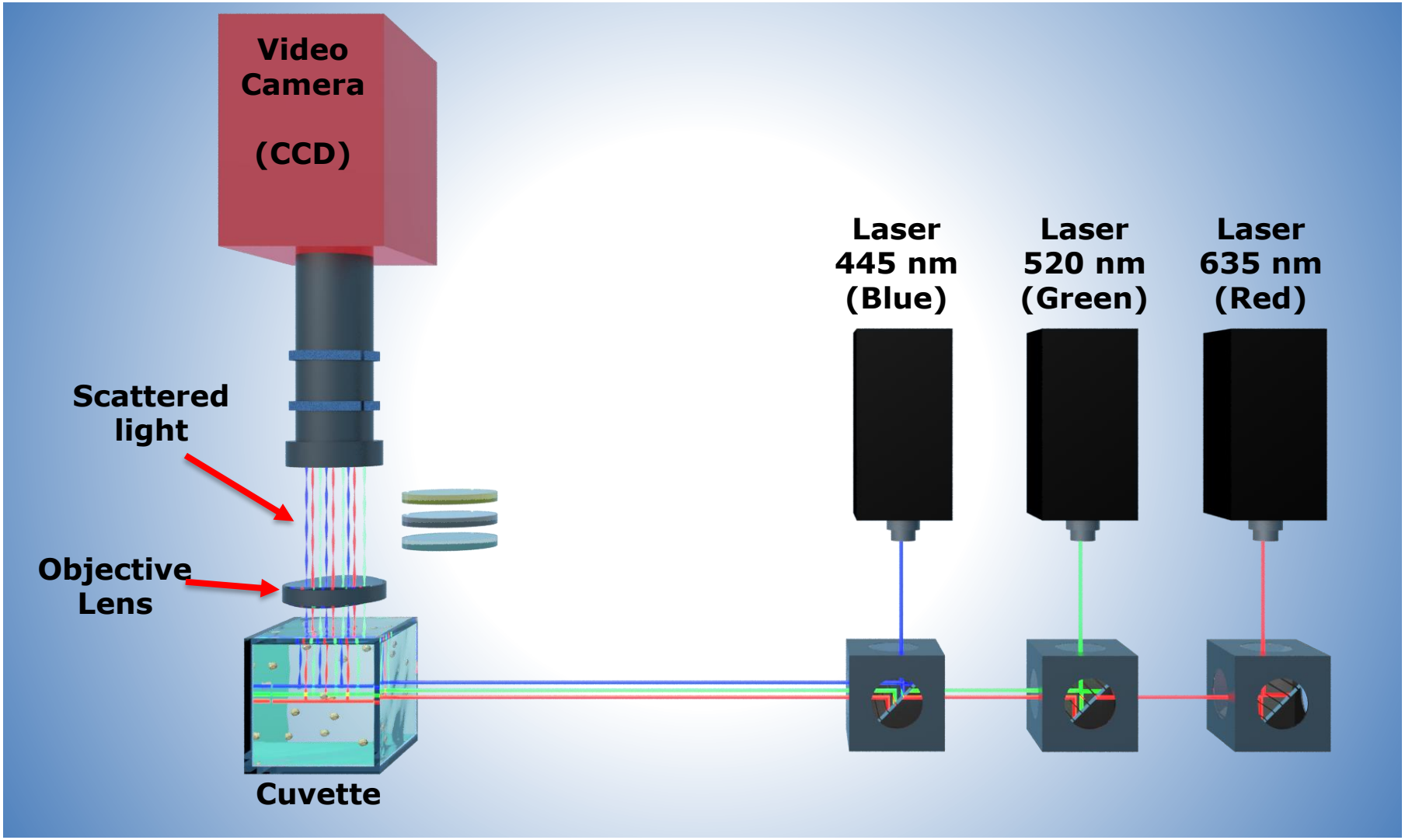


- Brownian Motion
  - Random
  - Related to Size
  - Related to viscosity
  - Related to temperature

Visualization of Brownian motion

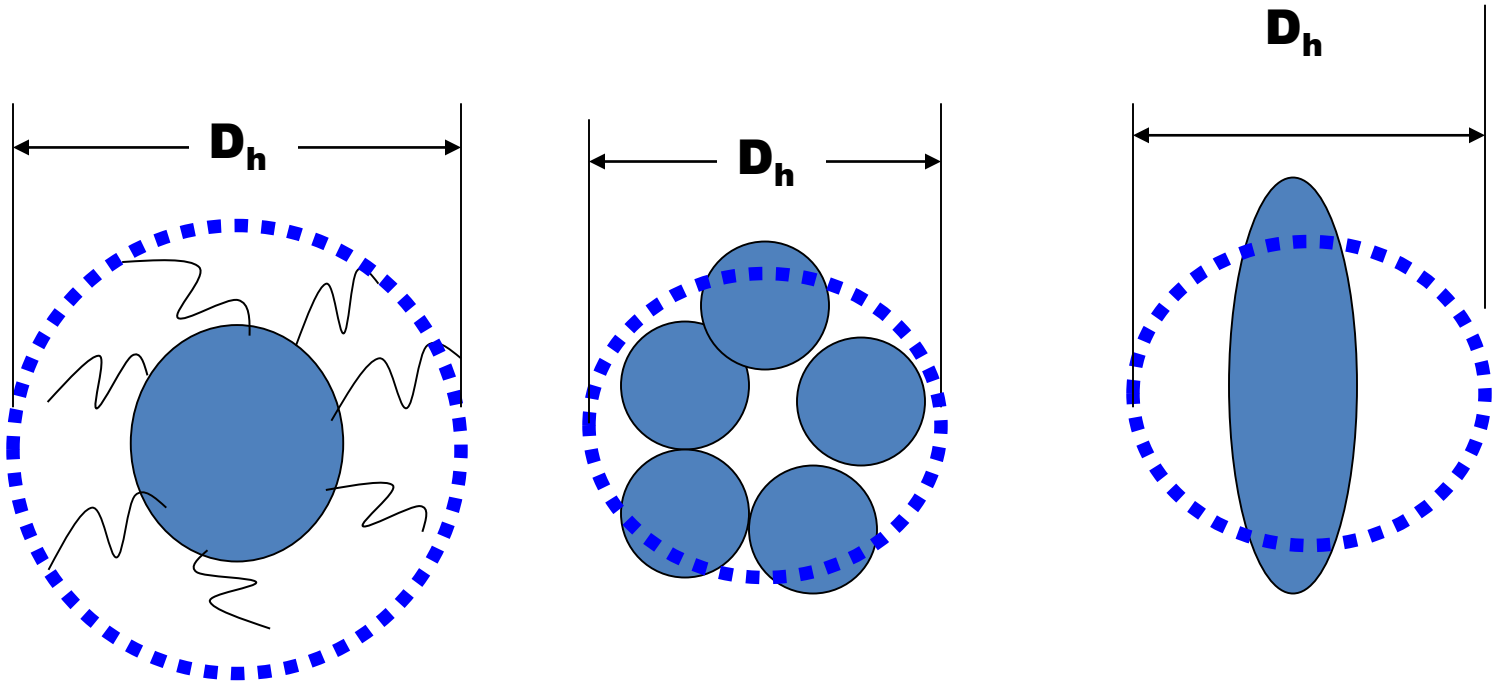


Instrument Schematic



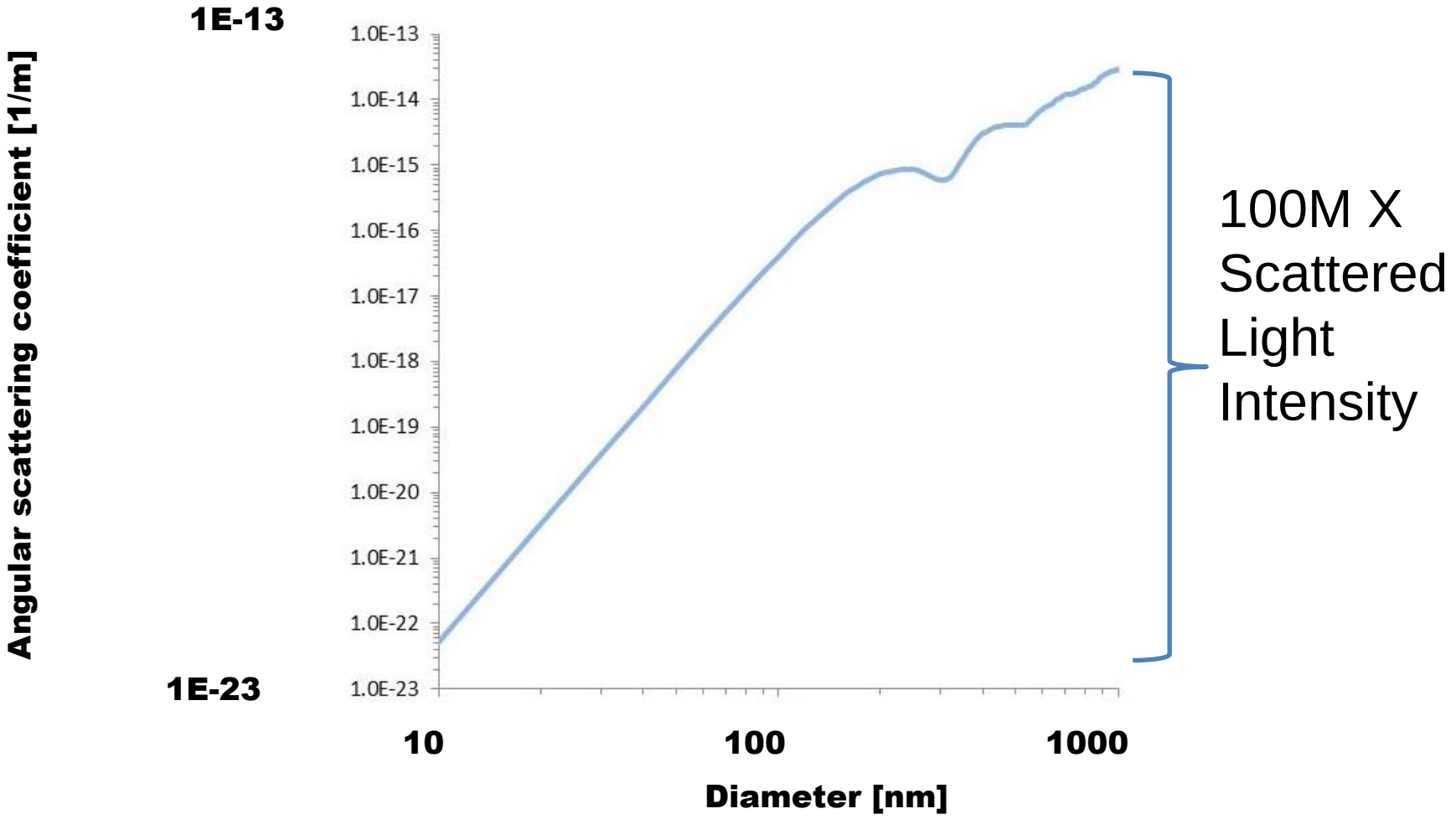
Hydrodynamic size

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



Eight orders of magnitude

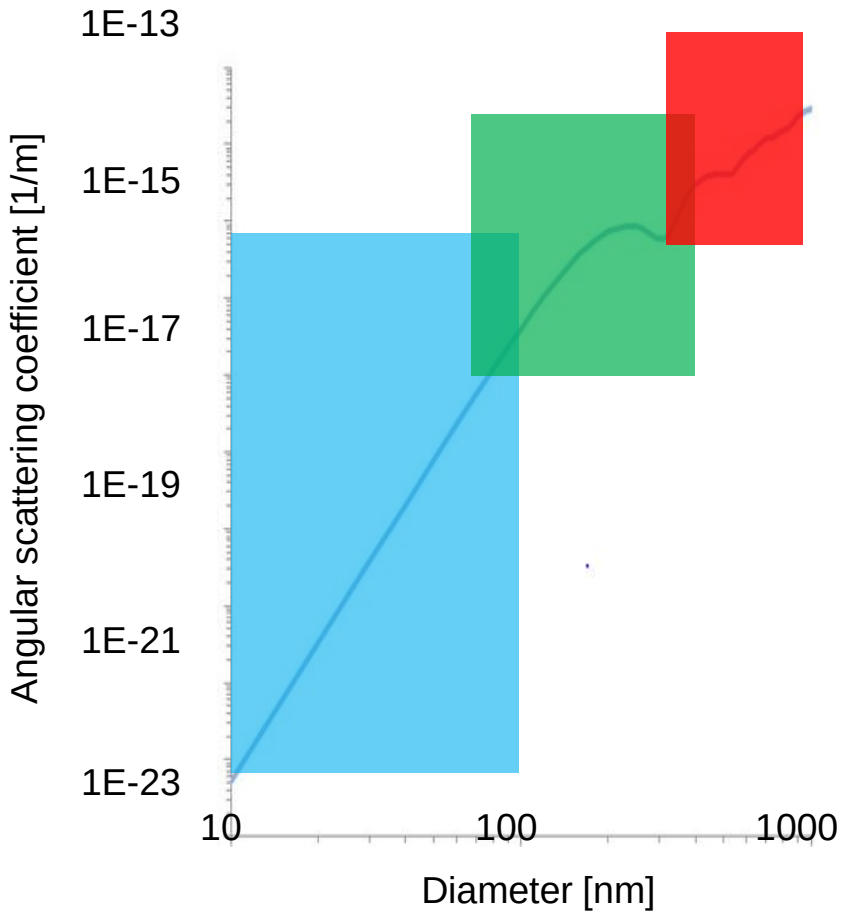
- DLS – large particles skew results (small not detected) or mask small particles
- cNTA – different sized particles can’t be seen simultaneously (highly irregular images for large particles, dim for smallest)
- cNTA – interrogated volume depends on particles size and their refractive index (similar to FC problem when sizing)

Problem is well known

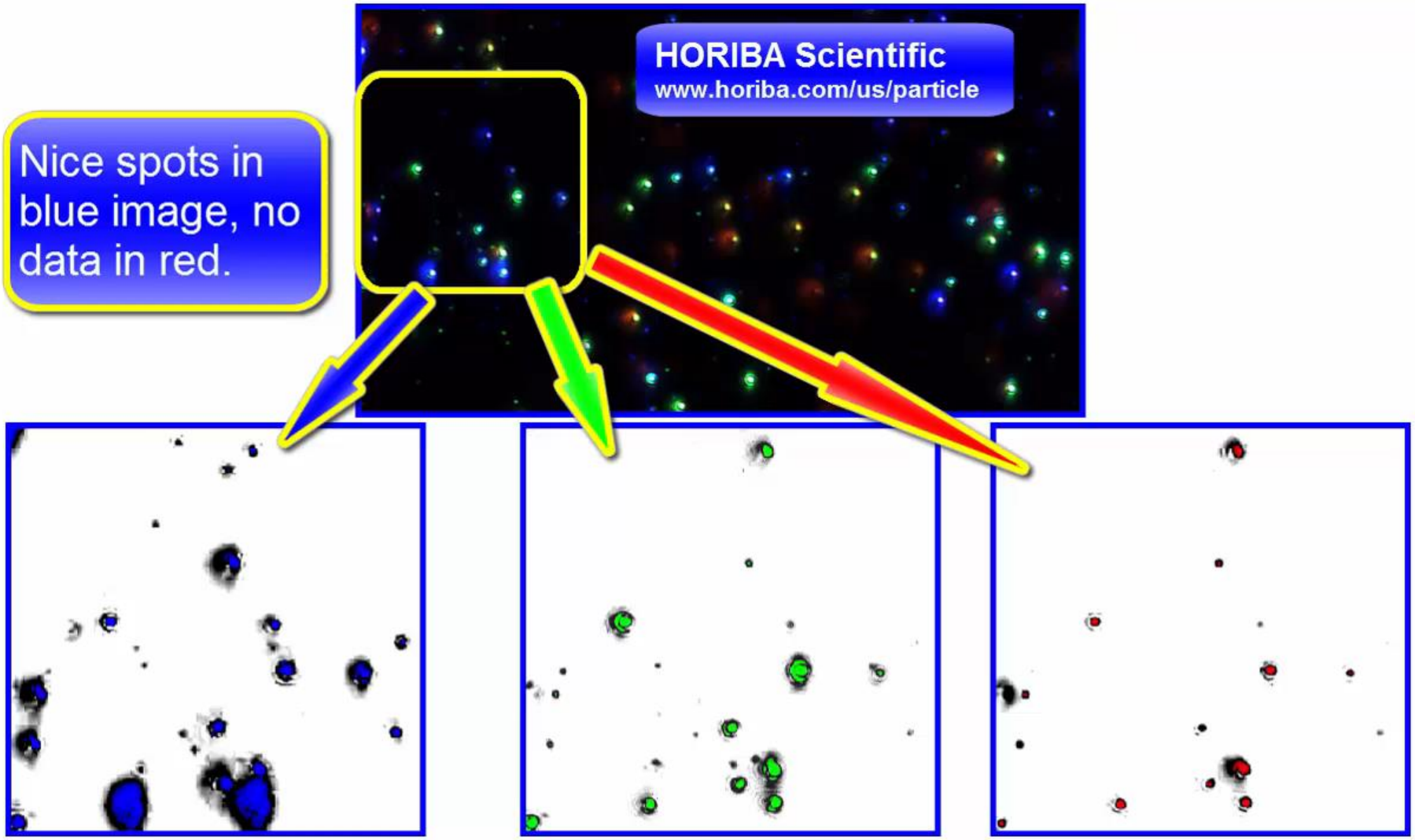
"Sample polydispersity affects the ability to track and therefore analyze different size fractions in the particle number-size distribution.
[…]
In a polydisperse sample large particles scatter a lot more than small particles making it difficult to detect or track small size particles."

ViewSizer solution

(***M**ultispectral **A**dvanced **N**anoparticle **T**racking **A**nalysis*)



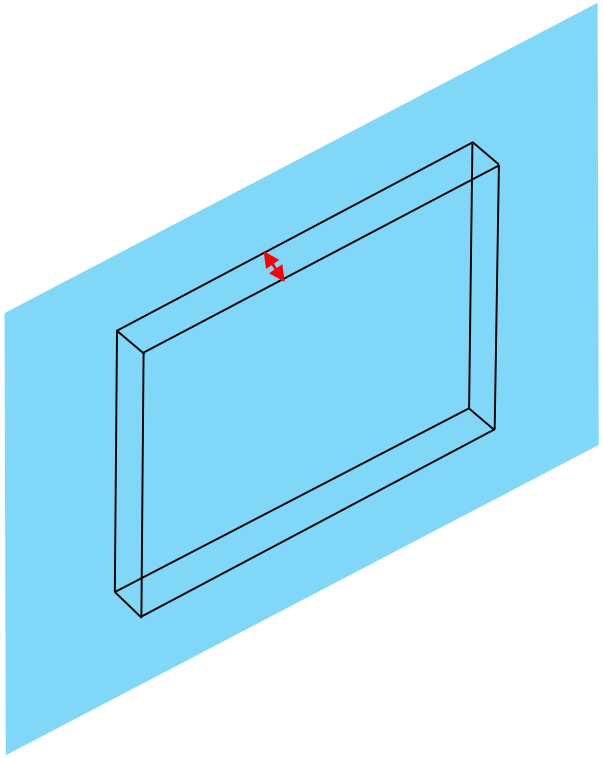
Why three colors?

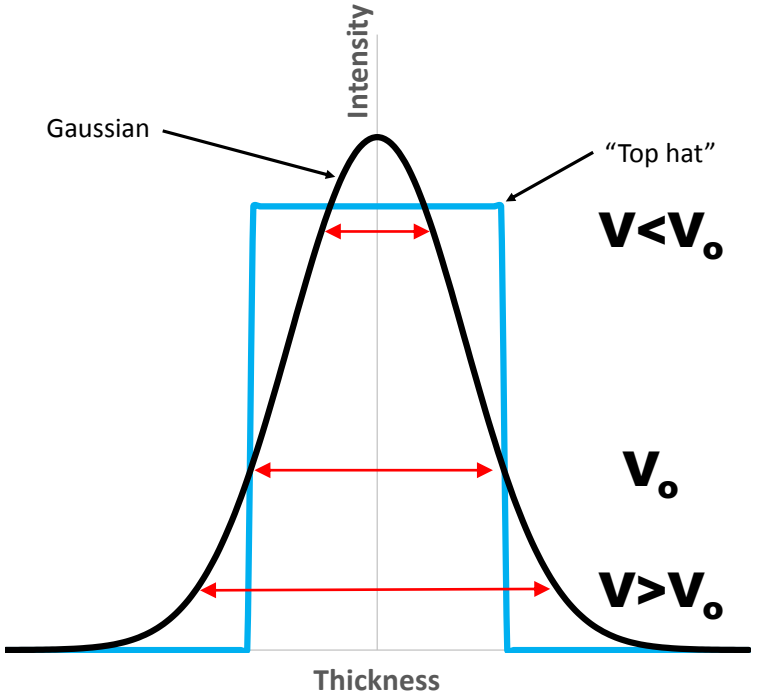


Concentration (*counts per volume*)

- Observed volume depends on intensity of scattered light
- Calibration of interrogated volume is done using standards (various sizes and refractive indices) → lookup table
- Volume factor is calculated from average intensity of scattered light for each tracked particle (takes laser power & camera gain into account)
- Particle size distribution is calculated with variable volume factor for each size bin

Light sheet thickness

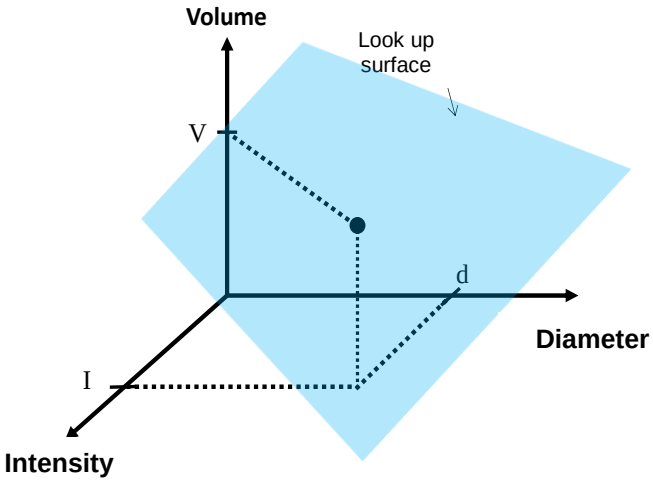


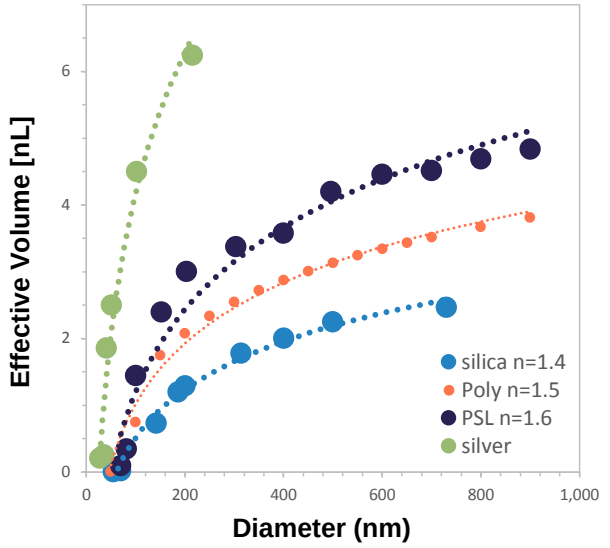


$$C = \frac{N}{V}\left[\frac{counts}{mL}\right]$$

$$V_0 \approx 2.5\ nL$$

Volume calibration

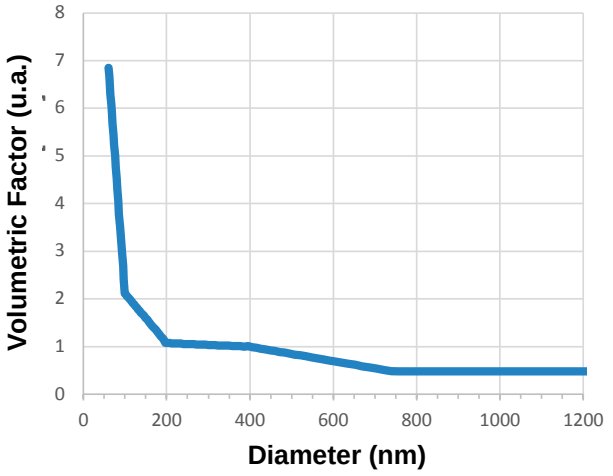




Silica - Blue laser (210 mW), Camera gain 30 dB

How volume calibration is performed:

- Measure concentrations for patterns of different sizes and made of different materials (various refractive indices)
- Determine the effective volumes
- Create volume look up surface
- Extrapolate using intensity of individual tracks and applying the Mie dispersion formula



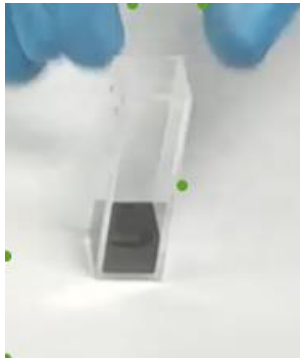
US patent 9,857,283

Operation

insert

+

cuvette
+
stir bar

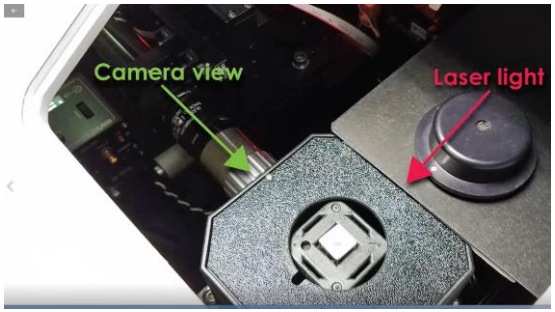


ready

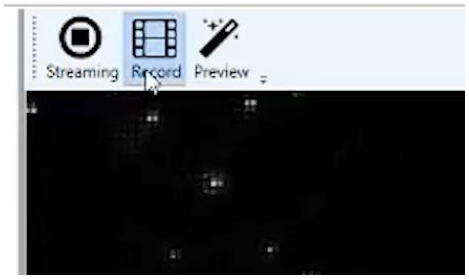
Load sample.

Components are readily separated for easy and thorough cleaning.

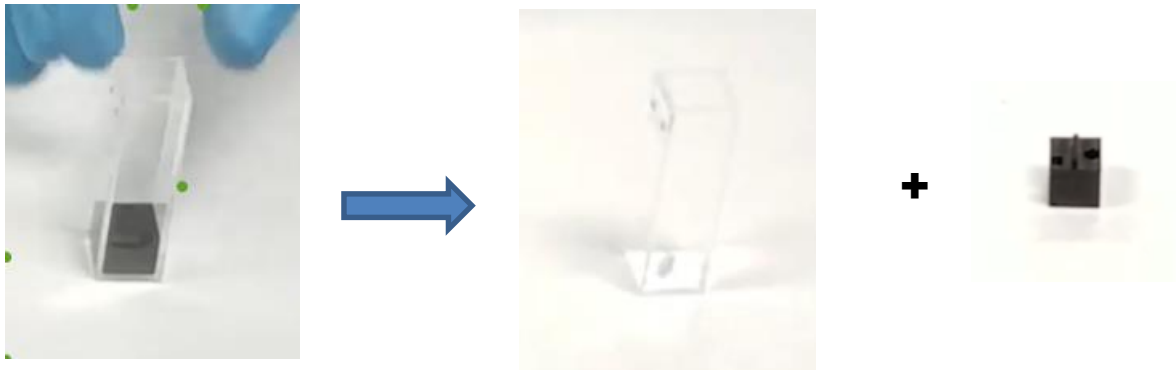
Operation



Place in analyzer.



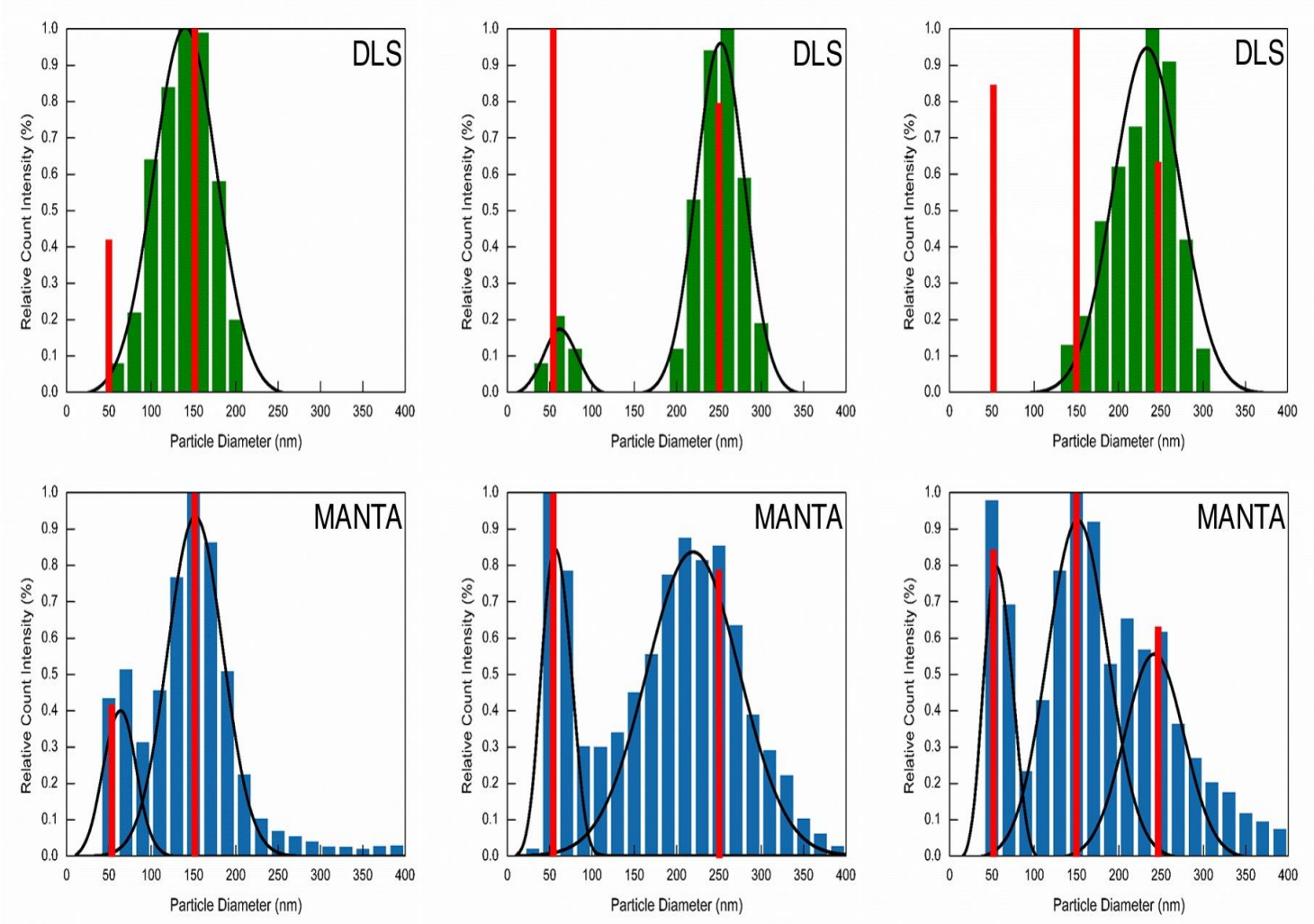
Press record



To prevent cross contamination, separate for thorough cleaning.

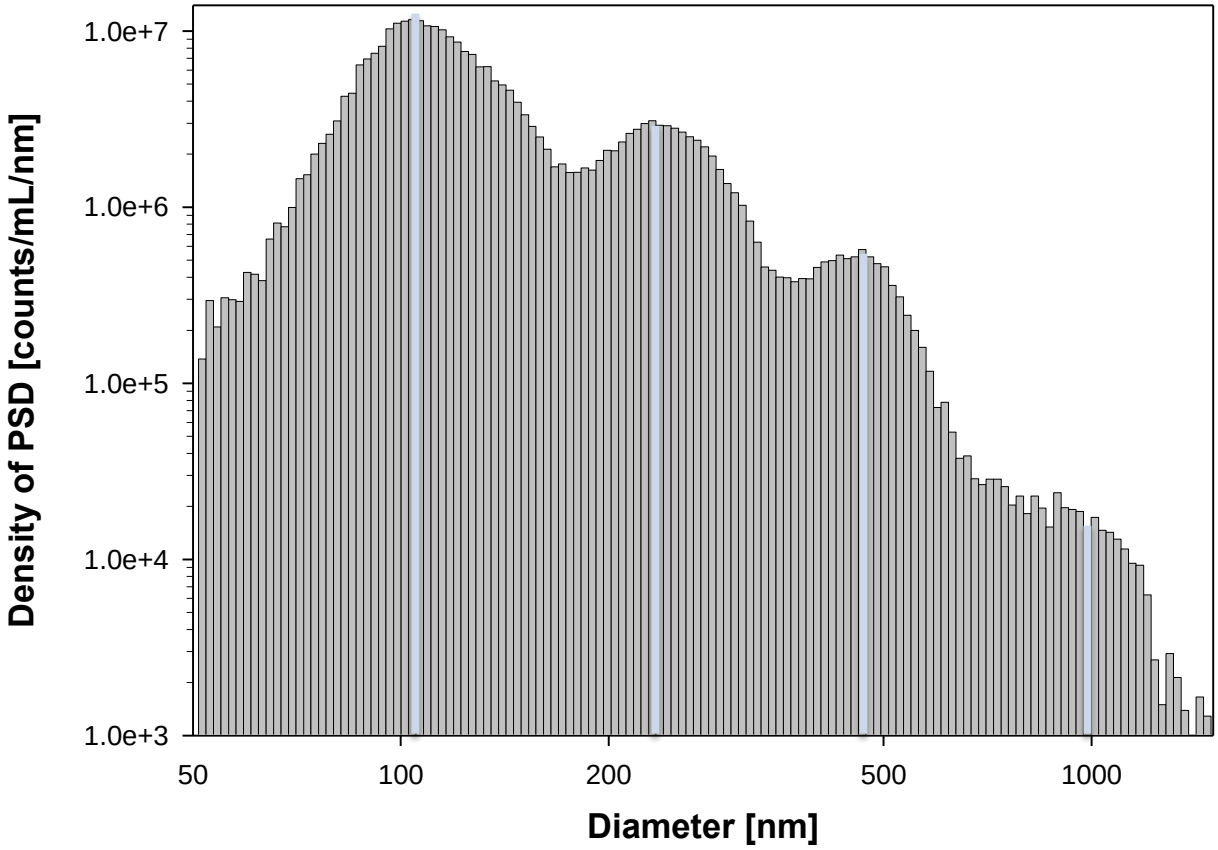
Results

Gold mixes: DLS vs. MANTA



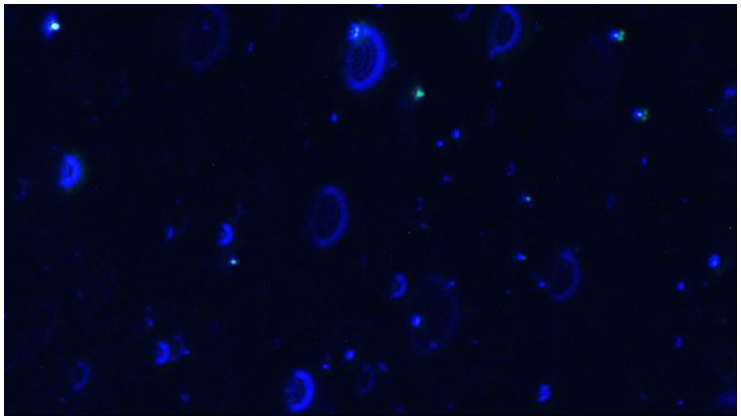
NIST exploratory sample

Initial effort to simulate protein aggregate mixture.



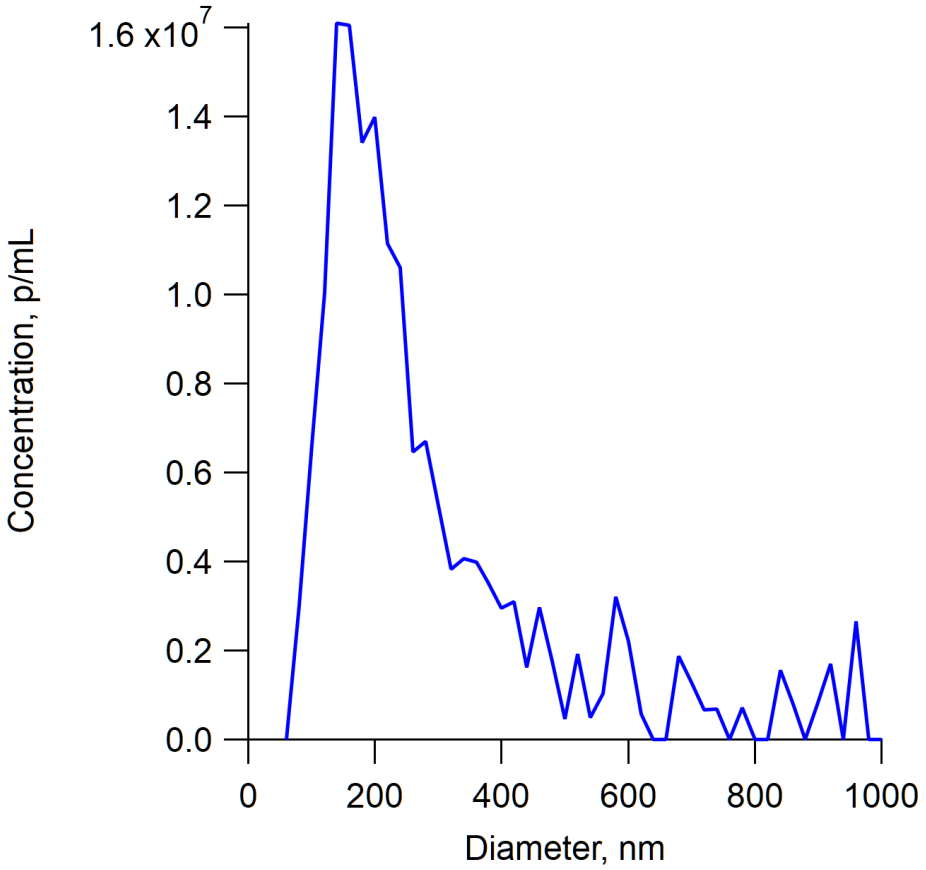
Plant virus data

Tobacco Mosaic Virus (TMV)

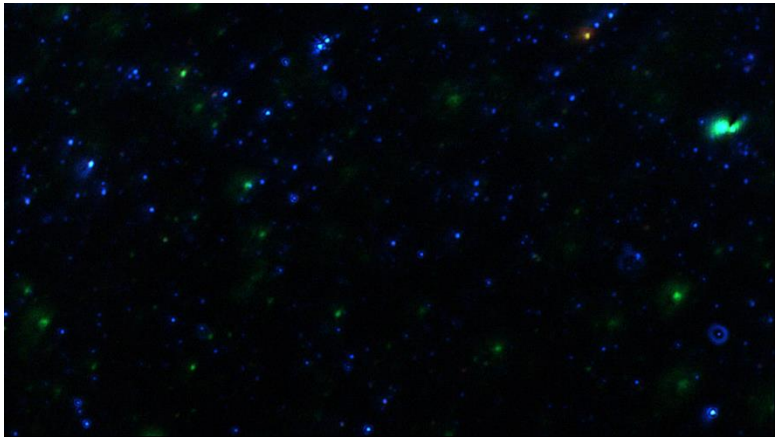


Single frame from video.
Points correspond to scattering from individual virus particles and aggregates.

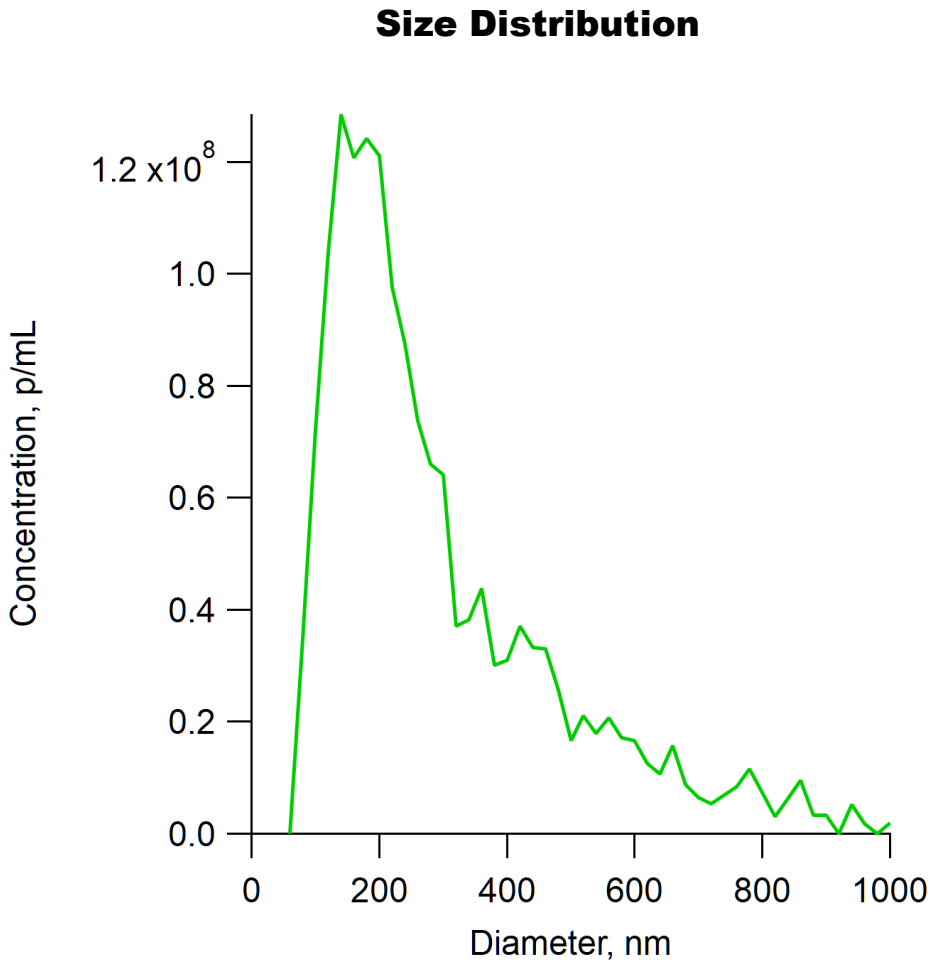
Size Distribution



Livestock virus data

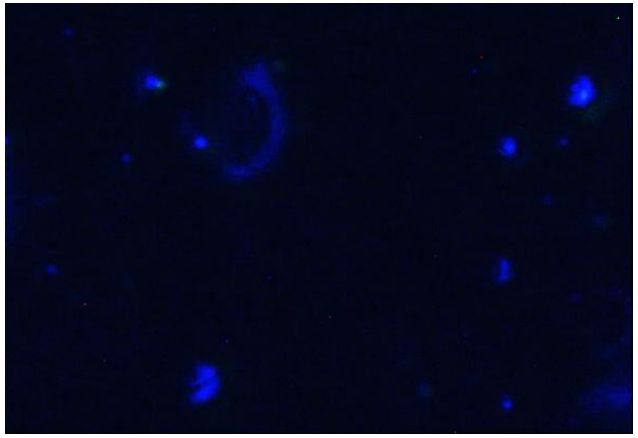


Single frame from video.
Points correspond to scattering from individual virus particles and aggregates.

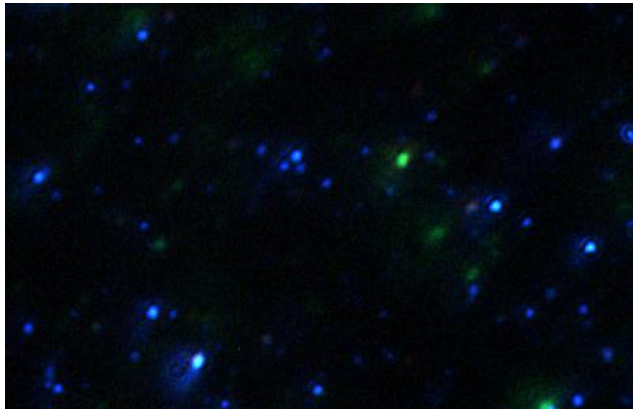


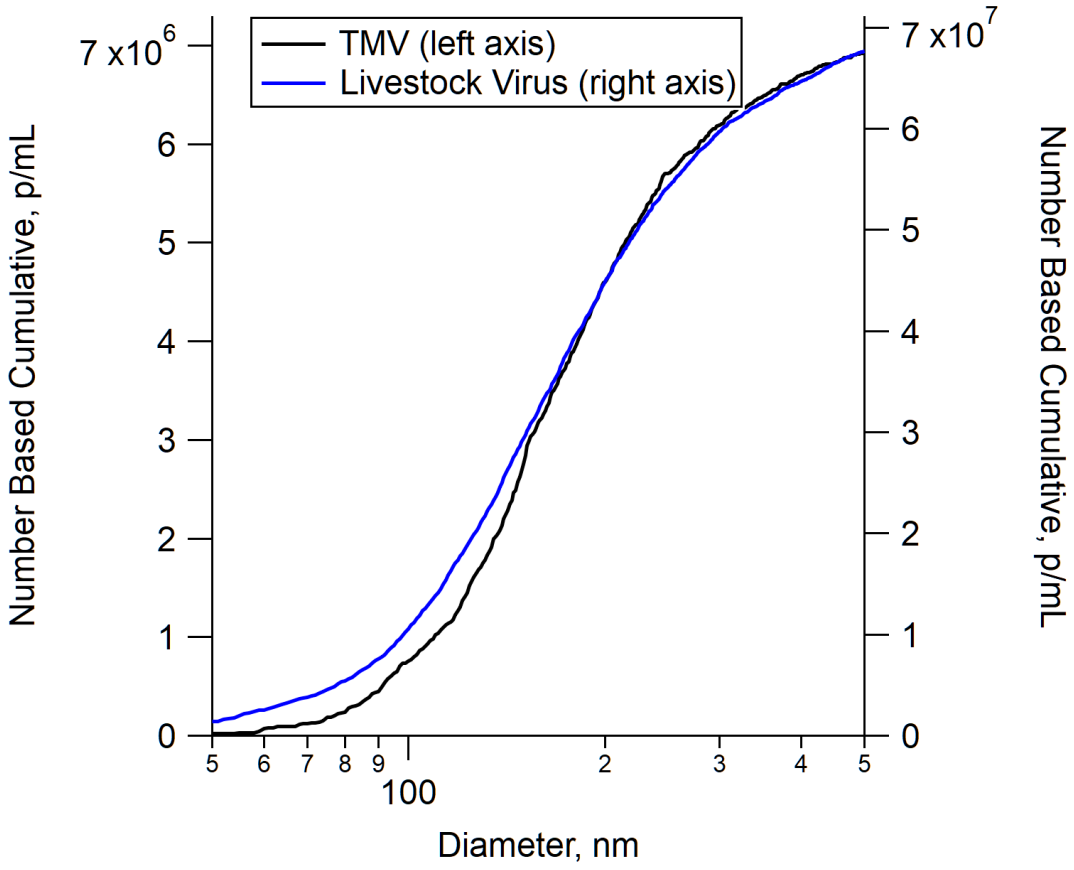
Comparison

TMV

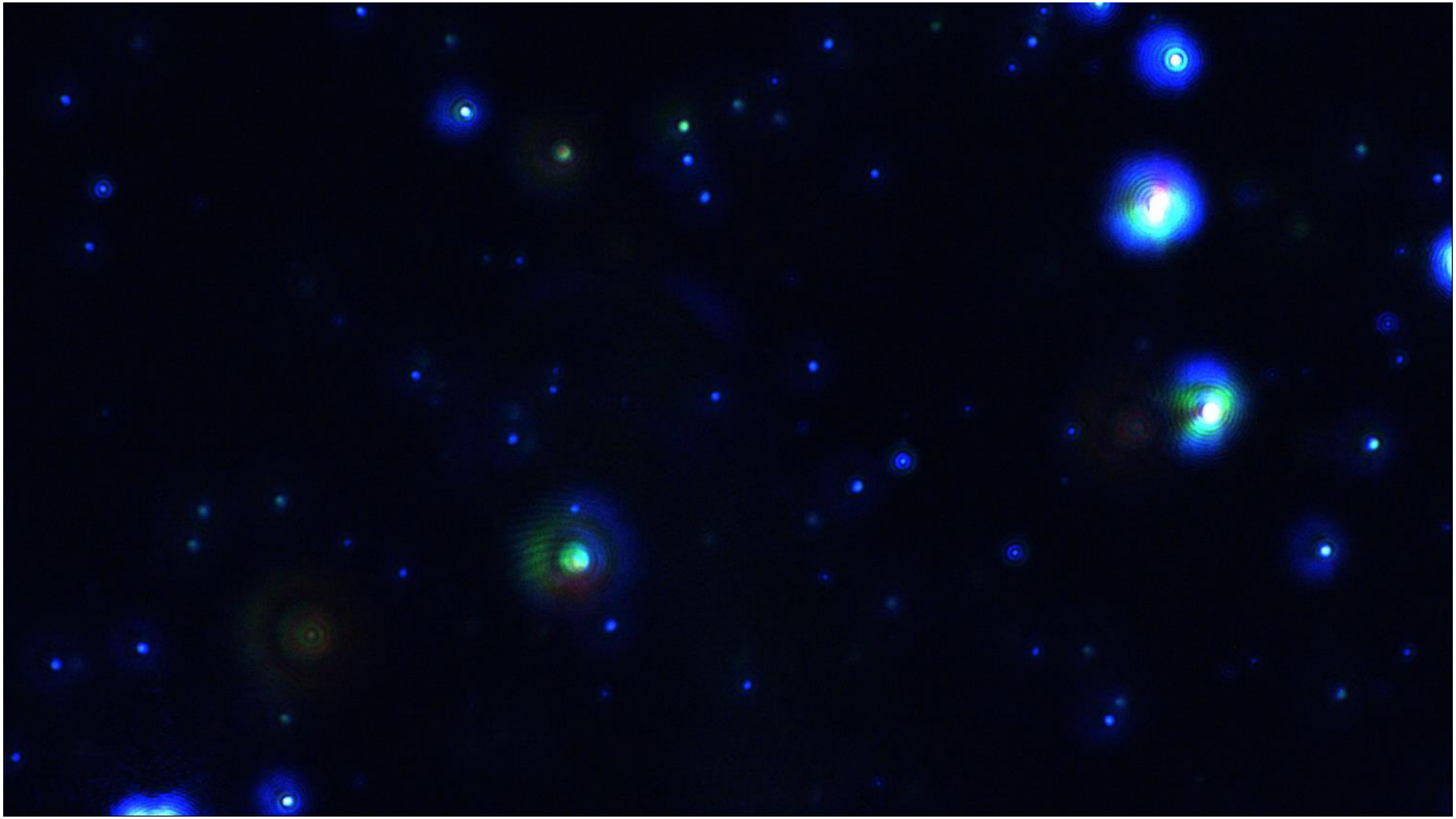


Livestock





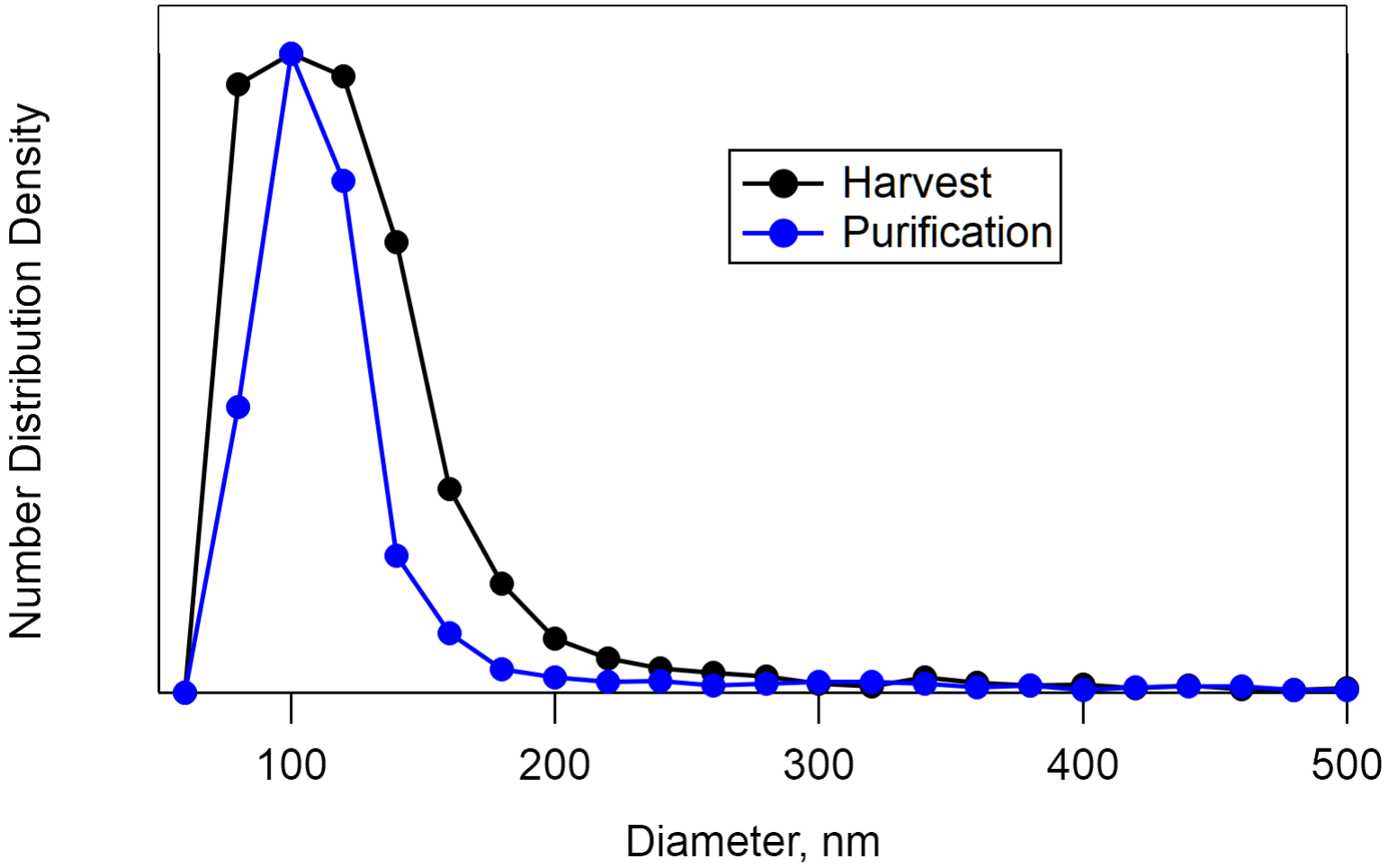
Human viral vector



Screen shot of purified viral vector
(aka Jeff's favorite photo from Europe last year)

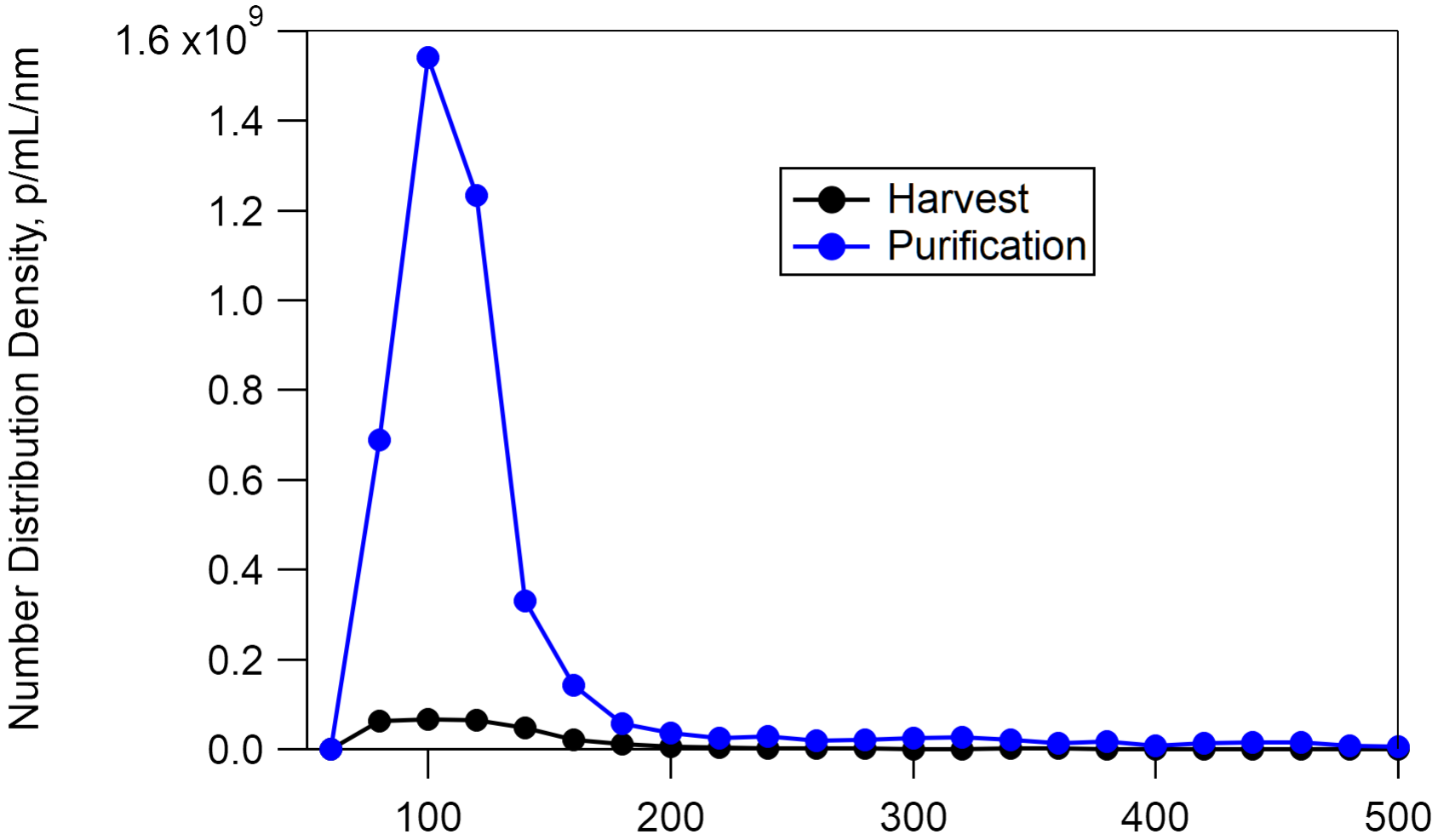
Human viral vector

Compare size distribution at harvest and after a purification step.



Human viral vector

Compare concentration. Purification also concentrates virus for next step.

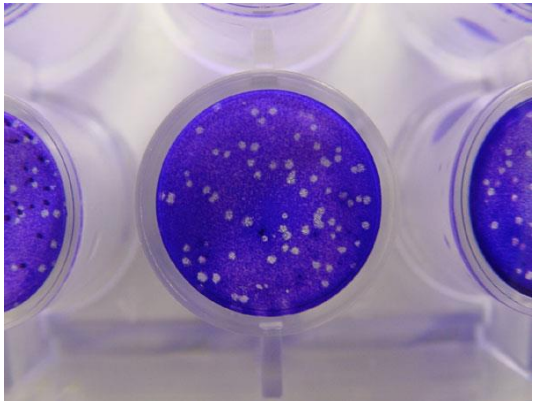


Infectious Titer Methodologies

- Viral Plaque Assay – Time-tested Gold Standard of Virology
  - Inexpensive, effective, time-consuming, user-dependent results (manual counting)
- Quantitative Polymerase Chain Reaction (qPCR)
  - Expensive, accurate, requires virus specific primers
  - Tracks DNA or RNA, not entire virus particles

Viral Plaque Assay

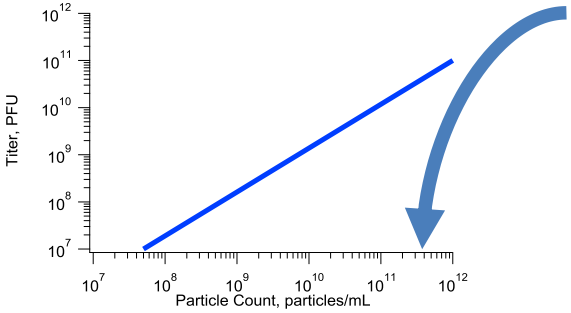
- Viral Plaque Assay
  - Serially dilute viral preparations, infect plates of confluent cells
    - –Incubate (that is, wait)
    - –Apply agar gel slow diffusion of virus
    - –Count the number of plaques (groups of dead cells) for each serial dilution
  - Pretty simple but there are some drawbacks
    - –**User to user variability**
    - –Not all viruses cause drastic disruptions to cell morphology or cell death
    - –**TIME**



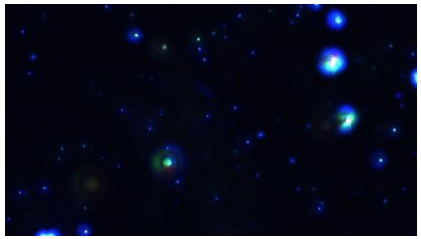
Credit: Wikimedia user Y tambe

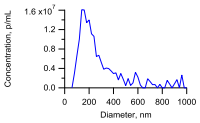
Virus with ViewSizer

Takes days



Correlation means saving time and money during virus manufacture for gene therapy.





ViewSizer takes 15 minutes

ViewSizer tracks all particles, including empty virus particles and infectious aggregates.
There is correlation between particle count and titer…

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

Virus life cycle

1. Enter cell

2. Use cell to reproduce

3. Shed

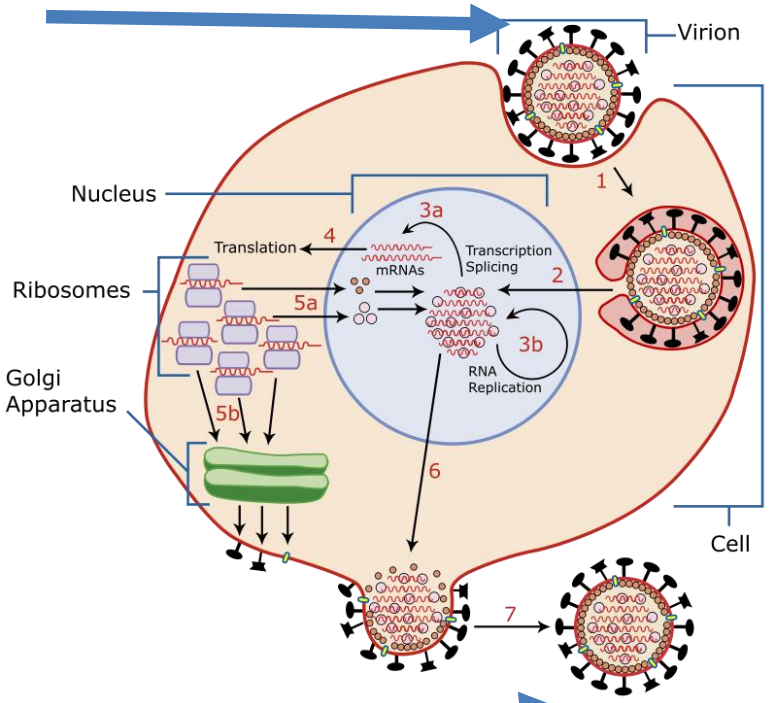


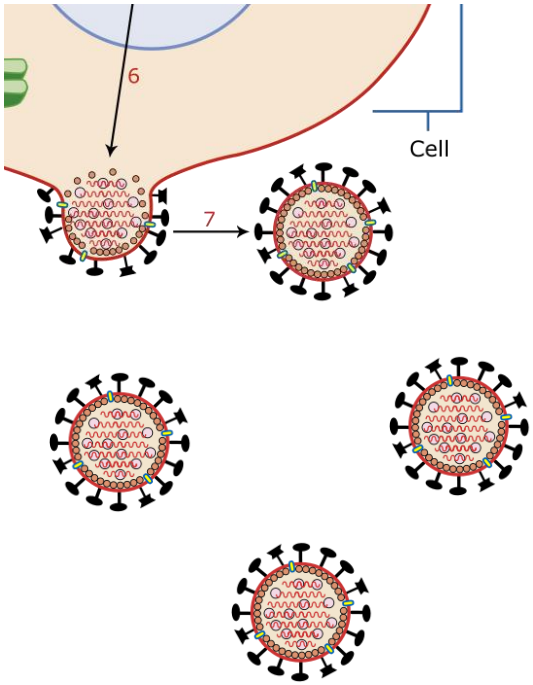
Image: Wikipedia user YKTimes

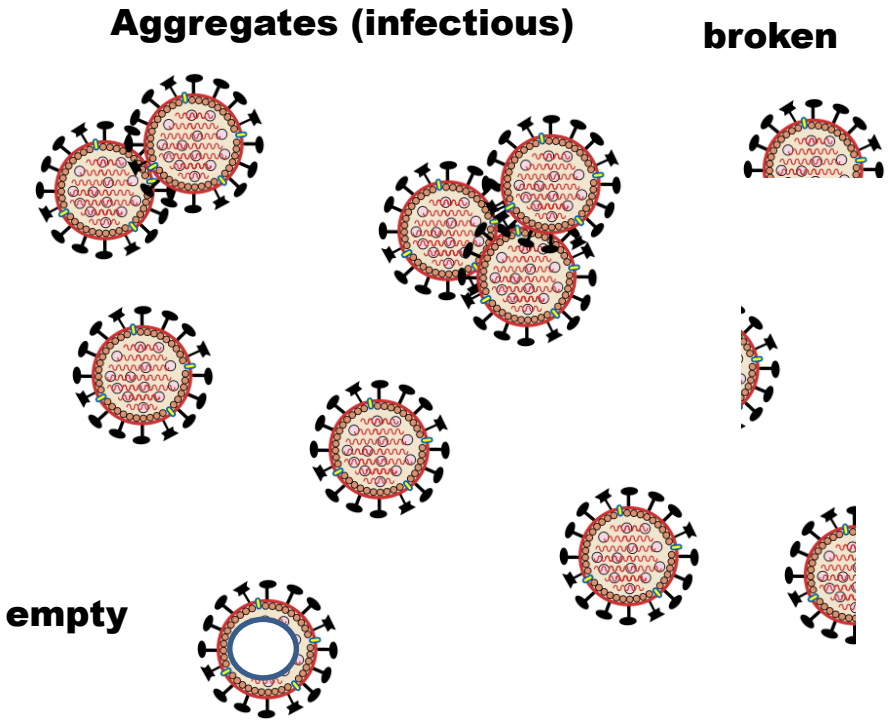
BUT… The result is very poor. There are lots of aggregates, partially formed, and empty virus particles.

To get correct titer correlation, you need to look at a range of sizes properly.

Single laser won't do…

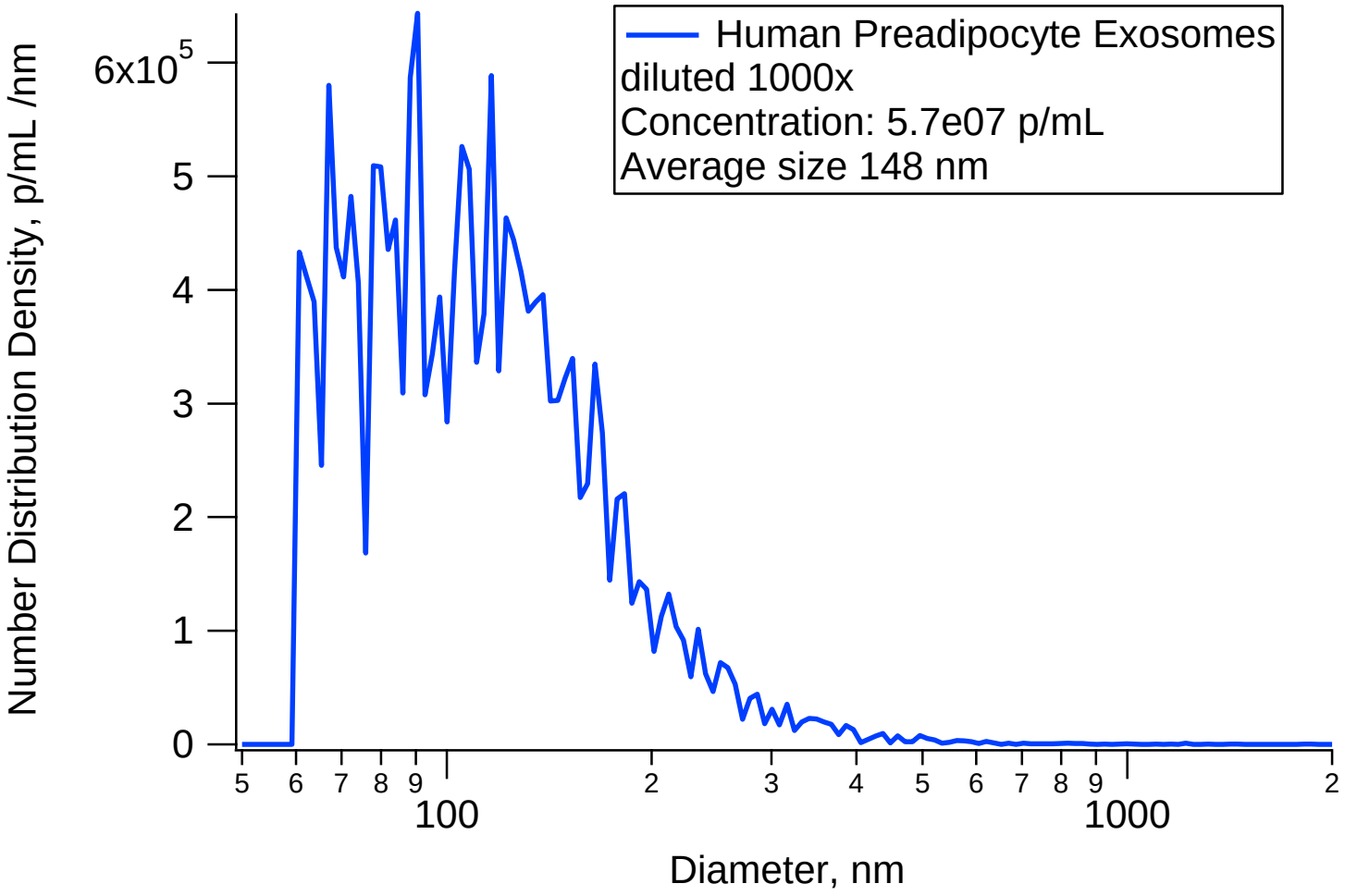
Virus Life Cycle





Wide size range means you need 3 lasers to properly characterize all the virus particles.

Exosomes

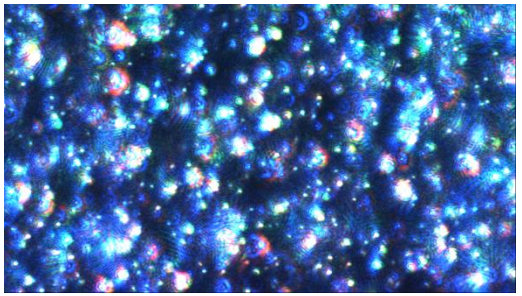


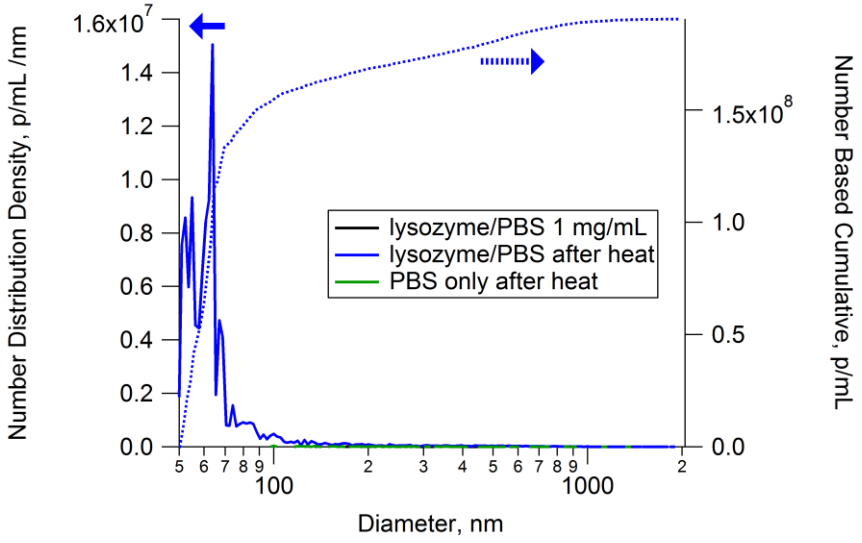
Lysozyme heated to 60 C

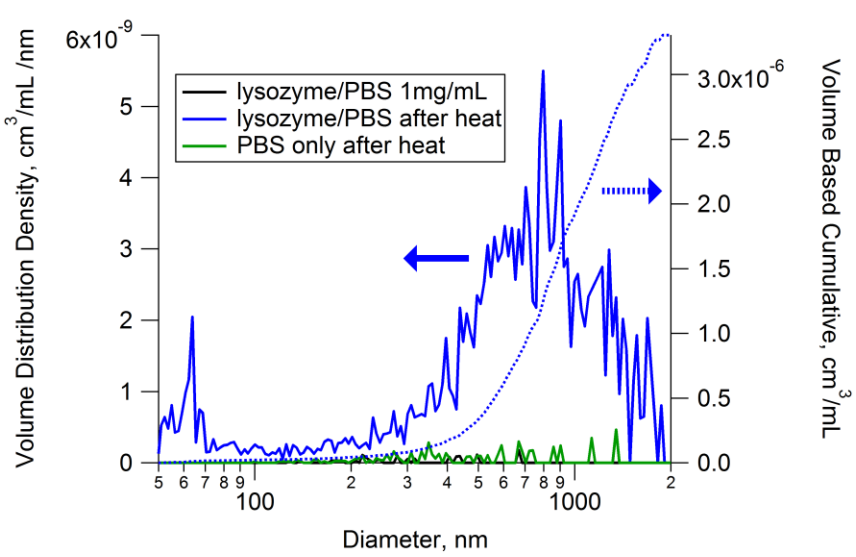
PBS after heating

Lysozyme before heating

Lysozyme after heating

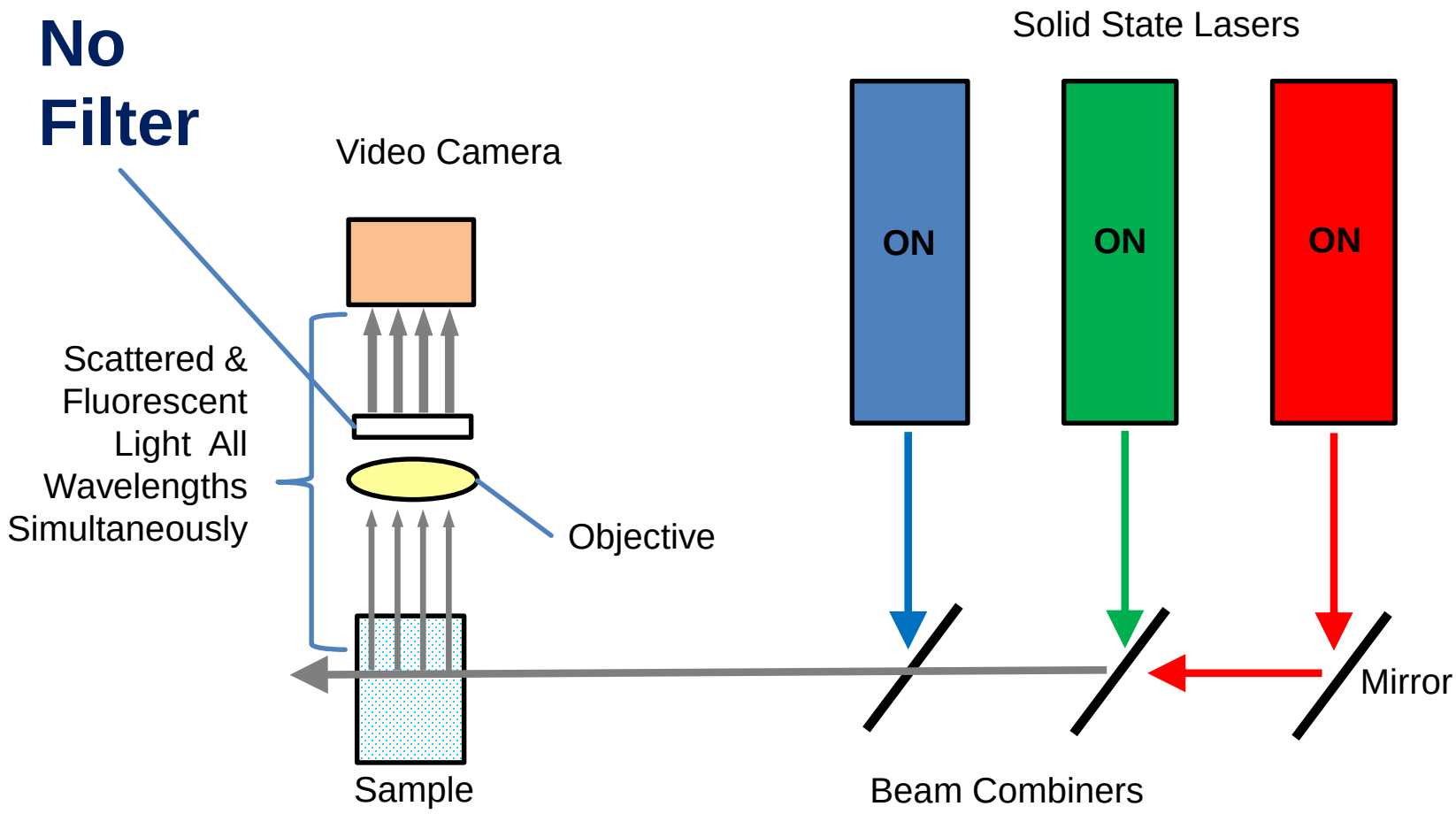






Fluorescence

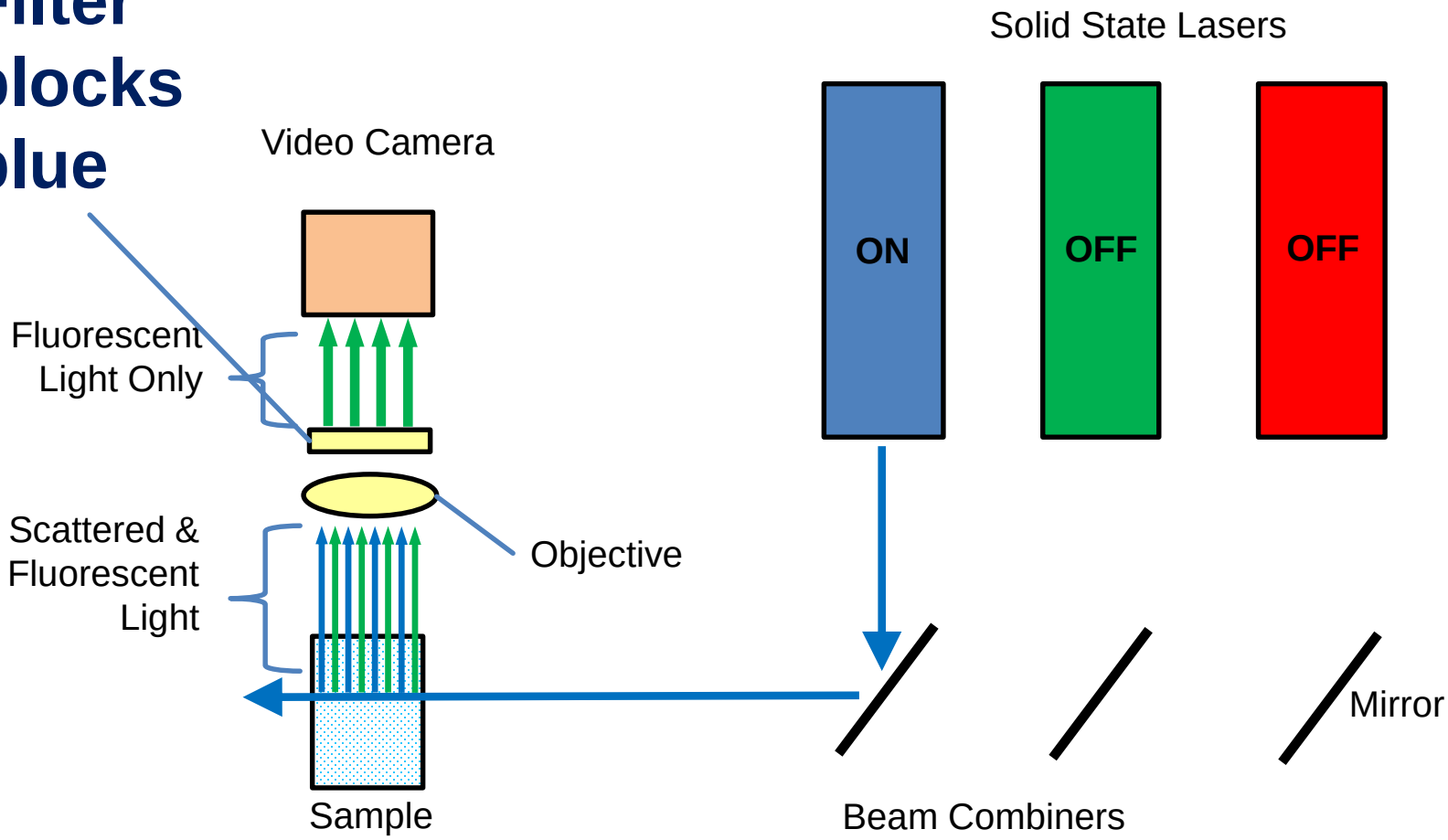
Normal mode: no filter



Use normal MANTA method to measure all particles

Use one laser and filter

Filter blocks blue

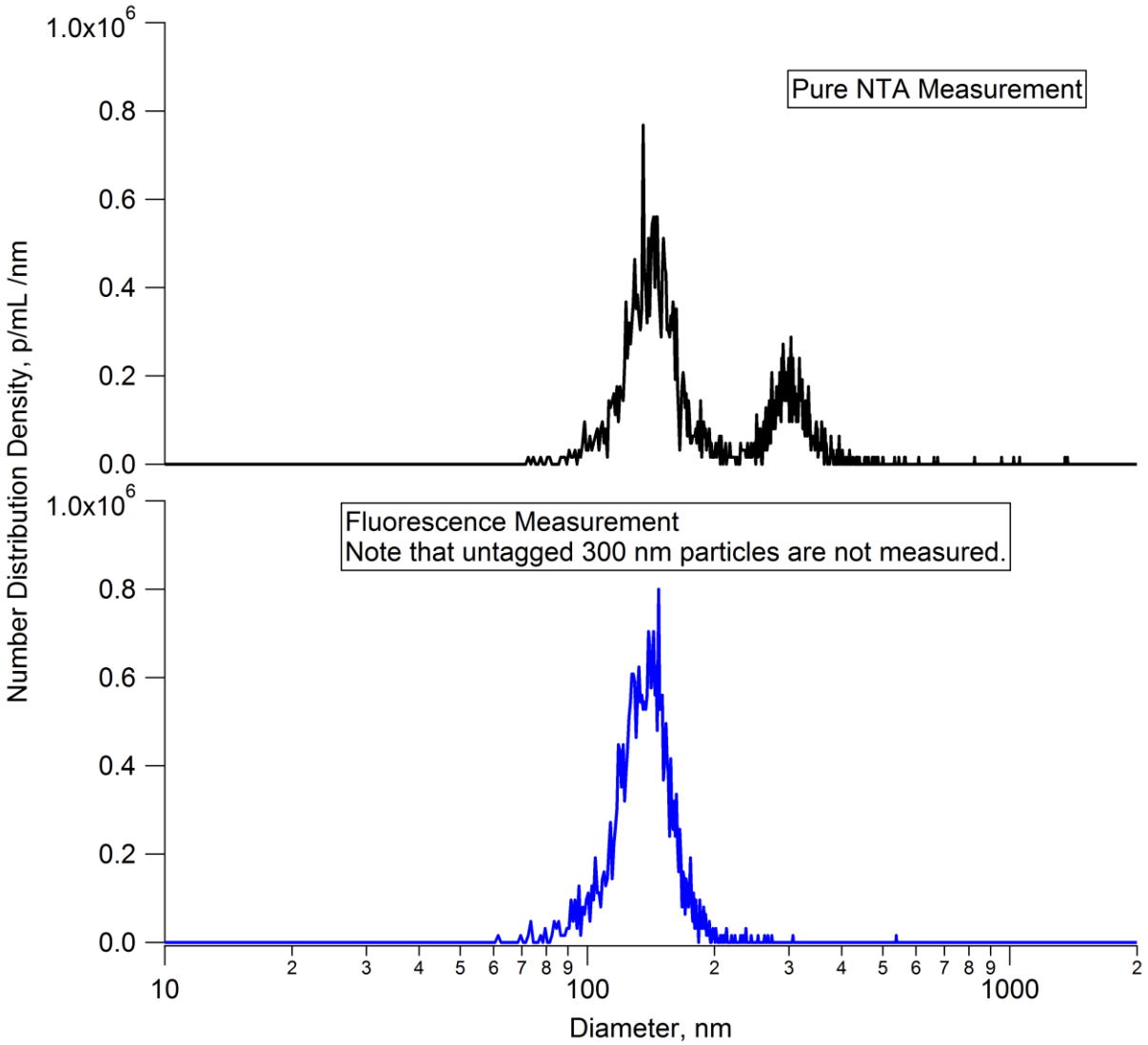


Detect green fluorescing materials excited by blue laser.

Fluorescence - challenges

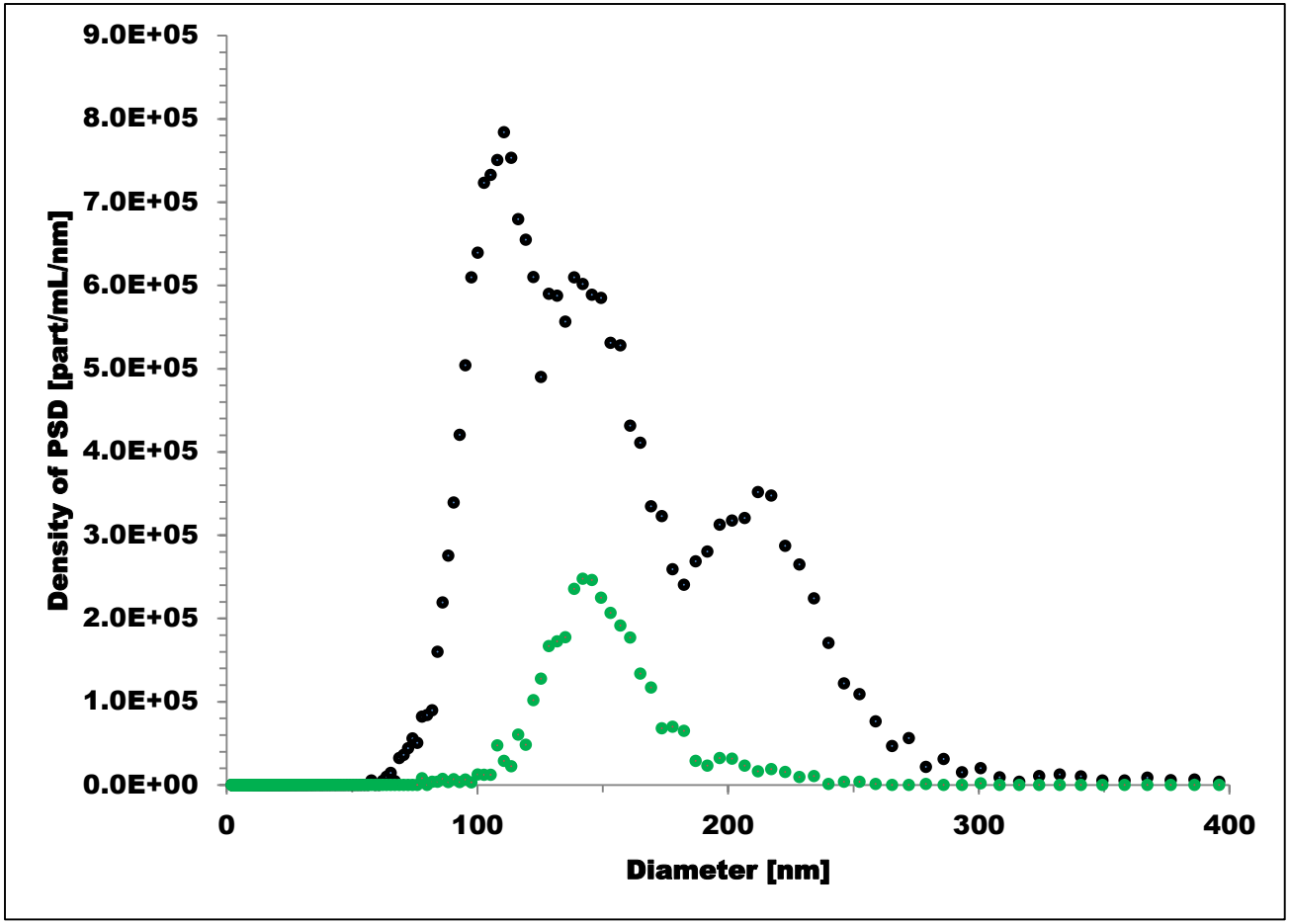
- Photobleaching (reduced emission intensity from overexposure)
  - Lasers pulsed in synch with camera shutter minimizes excitation energy
  - Laser power levels can be adjusted individually
  - Sample stirring introduces fresh aliquots of unbleached sample
  - Measure concentrations only if applicable (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



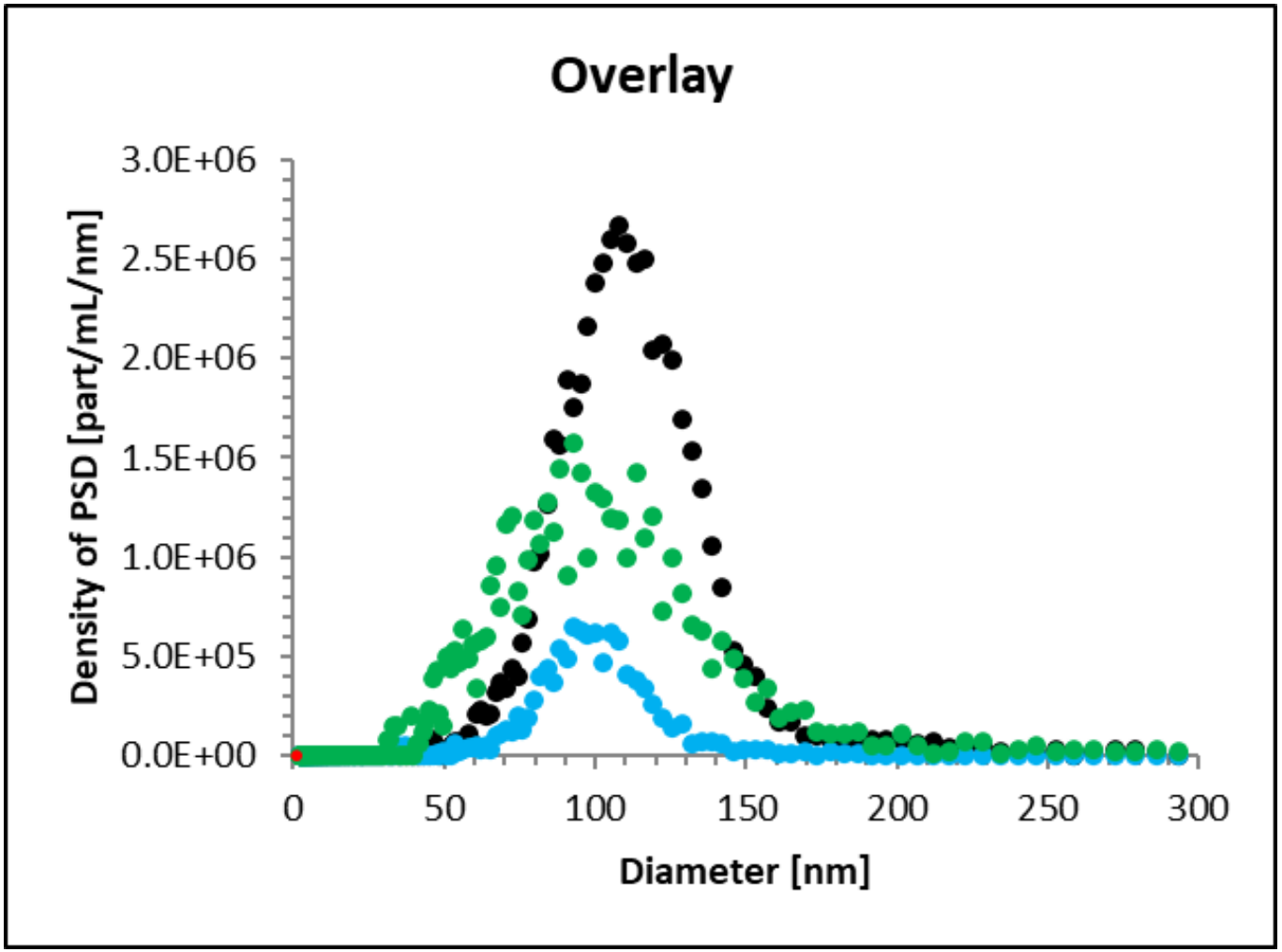
More mixtures

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



Multiple fluorophores

Mix of Two Carboxylate Fluorescent Beads (both nominally 100 nm dia)



Concluding comments

NTA - Disadvantages

Samples: Suspensions

Range: 10 nm – 40 µm

- Slow (≈15 min)
- Does not perform morphology analysis
- Method development (dilution)

NTA - Advantages

Samples: Suspensions

Range: 10 nm – 40 µm

- Particle concentration
- Good representation of particles throughout the whole measurement range
- Particle counter
- Determination of kinetic processes
- Fluorescence analysis
- Absolute method (no calibration required)
- Visualization

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

Specifications

Range of Particle Sizes Measured *	10 nm to 2 µm, 15 µm with settling
Minimum Sample Volume	0.4 mL
Number of Lasers	3: red-635 nm, green-520 nm, blue-450 nm
Camera Type	Scientific color CCD
Typical Sample Concentration	5 x 10^6 to 2 x 10^8 particles/mL
Sample Temperature Range (Controlled)	5 °C to 50 °C, +/- 0.1 °C (-15 °C to 110 °C available)
Dimensions	55 cm W x 66 cm D x 35 cm H
Weight	27 kg
Operational Environment	15 °C to 30 °C with < 85% RH

* Sample dependent

ViewSizer 3000

cuvette w/insert

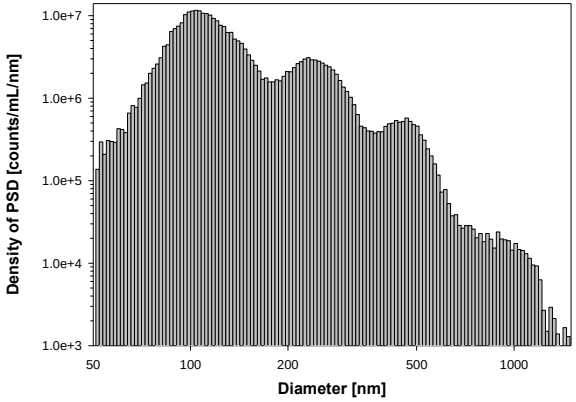
Summary

Breakthrough technology

New & better particle characterization

High resolution distribution

Particle concentration/count



HORIBA Scientific

Thank you

Did you sign up for our newsletter?

Use the chat box or e-mail us at labinfo@horiba.com

Thank you

Omoshiro-okashiku

Joy and Fun

감사합니다

Cảm ơn

ありがとうございました

Dziękuję

धन्यवाद

Grazie

Merci

谢谢

நன்ற

ขอบคุณครับ

Obrigado

Σας ευχαριστούμε

شُكْرا

Tack ska ni ha

Большое спасибо

Danke

Gracias