MANTA
INSTRUMENTS

Better Nanoparticle Characterization

Improved Visualization, Counting and Sizing of Polydisperse Nanoparticle Colloids using ViewSizer® 3000

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VP Engineering

MANTA Instruments, Inc.

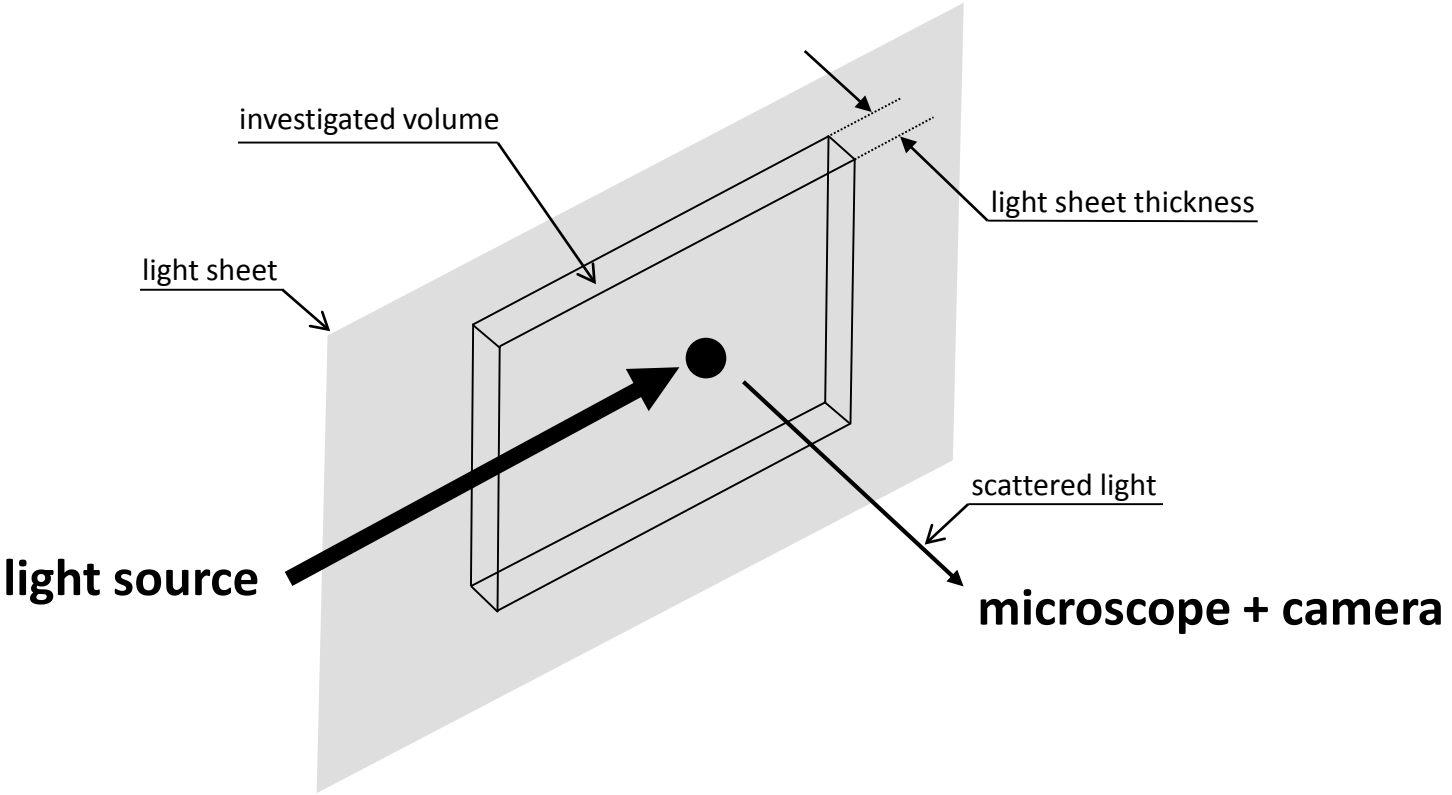
Established technologies

- Flow Cytometry (FC)
- Transmission Electron Microscopy (TEM)
- Static Light Scattering (SLS)
- Dynamic Light Scattering (DLS)
- conventional Nanoparticle Tracking Analysis (cNTA)

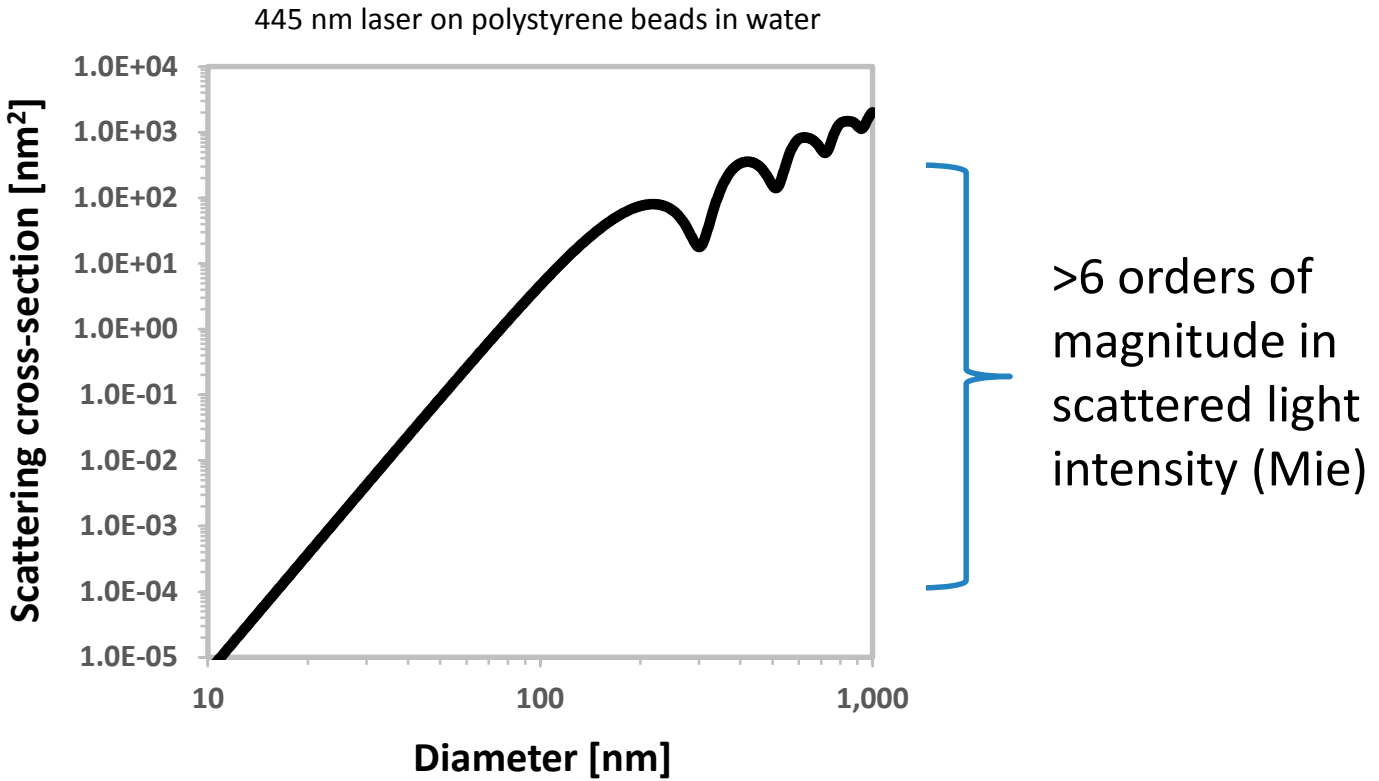
Unmet needs

- Accurate & reproducible measurement of:
  - particle number concentration
  - particle size distribution
  - particle kinetic processes
- and visualization of highly polydispersed colloids

Visualization of Brownian motion



Problem


445 nm laser on polystyrene beads in water
Scattering cross-section [nm²]
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
10
100
1,000
Diameter [nm]
>6 orders of magnitude in scattered light intensity (Mie)

Six orders of magnitude

- DLS – large particles skew results
  (small ones not detected)
- DLS – experimental complications that users overlook
  (concentration-dependent results)
- cNTA – different sized particles can’t be seen simultaneously
  (highly irregular images for large particles, dim for smallest)
- cNTA – interrogated volume depends on particles sizes and their refractive indices (similar to FC problem when sizing)

Problem is well known

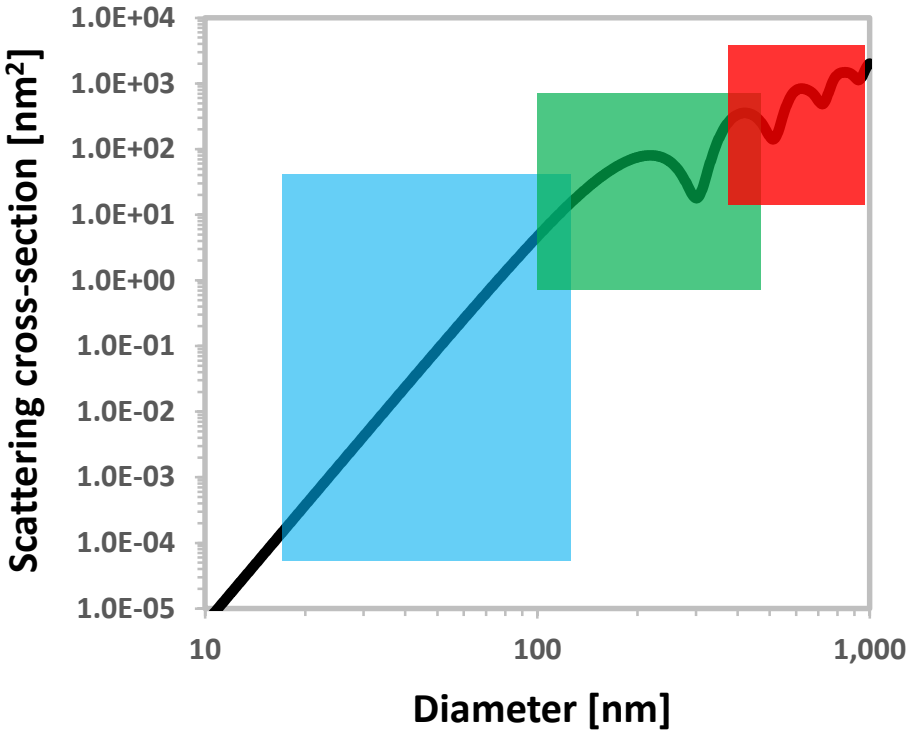
INTERNATIONAL STANDARD **ISO 19430**

Particle size analysis — Particle tracking analysis (PTA) method

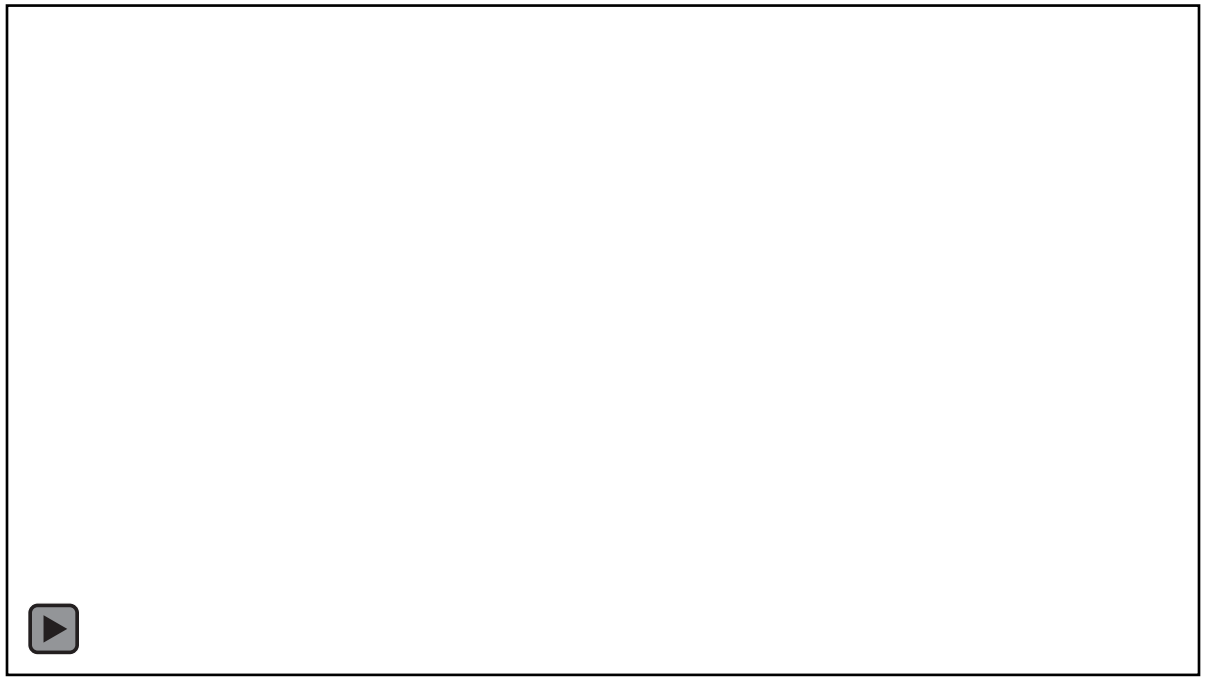
"Sample polydispersity affects the ability to track and therefore analyze different size fractions in the particle number-size distribution. […]
In a polydisperse sample large particles scatter a lot more than small particles making it difficult to detect or track small size particles."

MANTA solution *US patent 9645070*

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



Sample of three color video



Size determination (*Einstein 1905, Langevin 1908*)

- Mean Squared Distance (MSD in 2D, N frames, jumps of n):

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

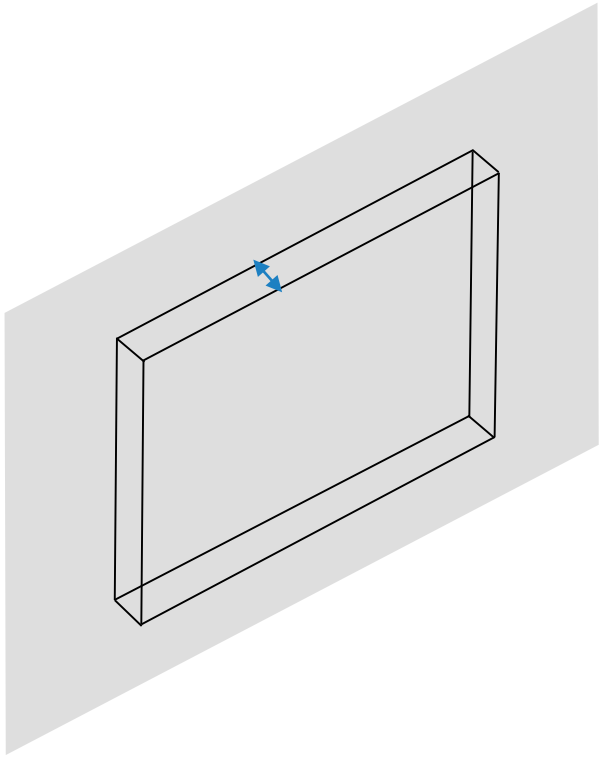
- Diffusion coefficient D (optimized least-square fit of MSD vs. n):

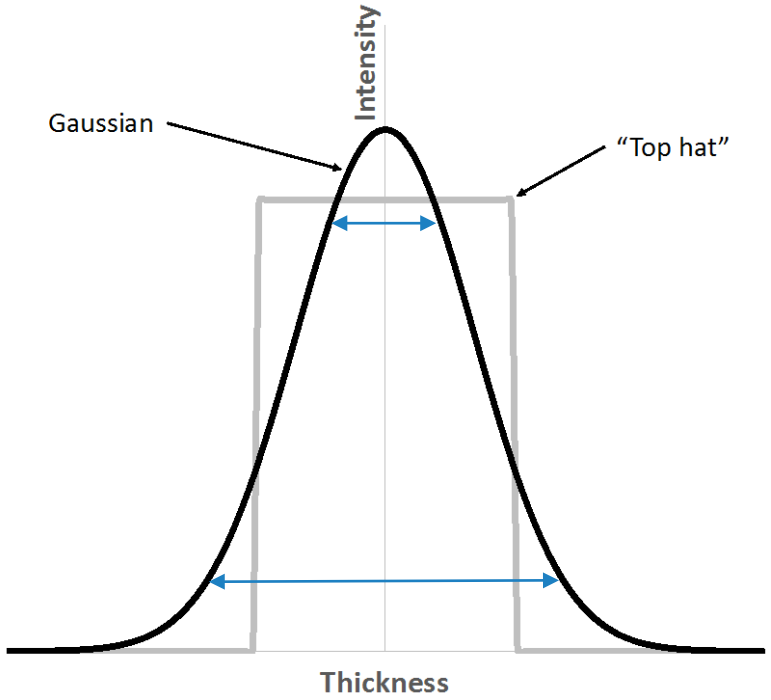
$$MSD(n) = (4 \cdot \Delta t \cdot D) \cdot n$$

- Hence diameter:

$$d = \frac{k_B T}{3\pi\eta D}$$

Light sheet thickness

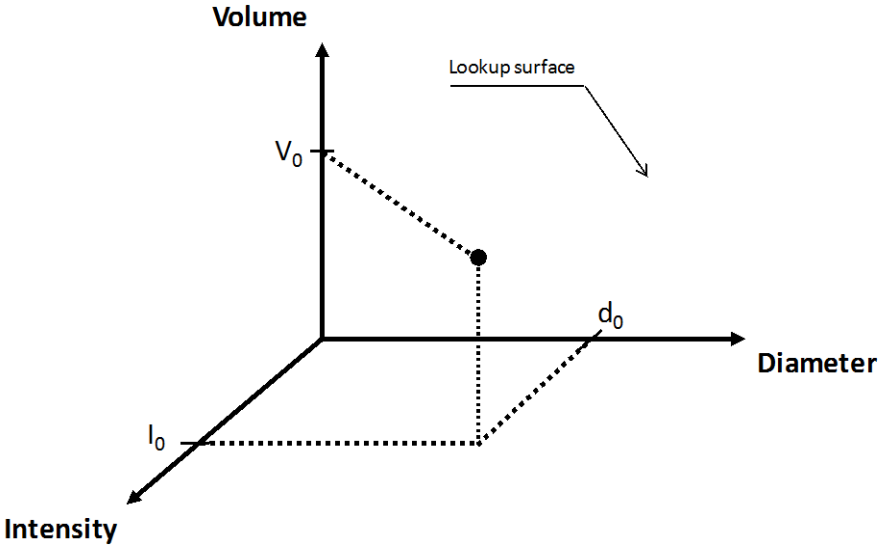


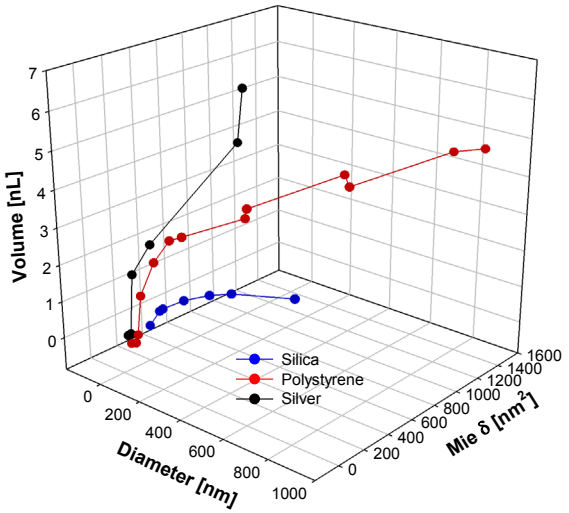


Concentration (*counts per volume*)

- 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 exposure & gain into account*)
- Density of particle size distribution (PSD) is calculated with variable volume factor for each size bin

Volume factor *US patent 9857283*





ViewSizer® 3000

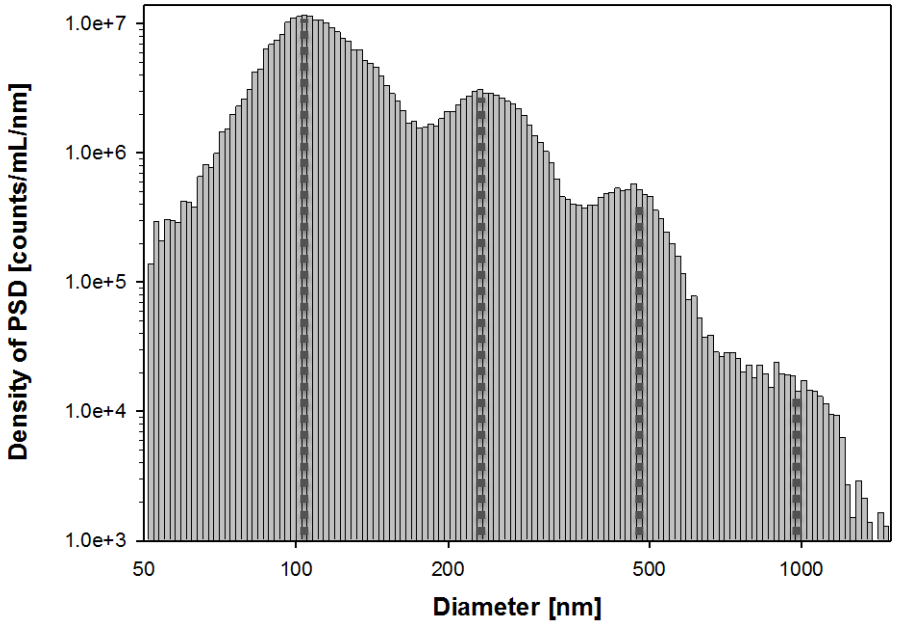
cuvette w/insert
US patent 9541490

Specifications

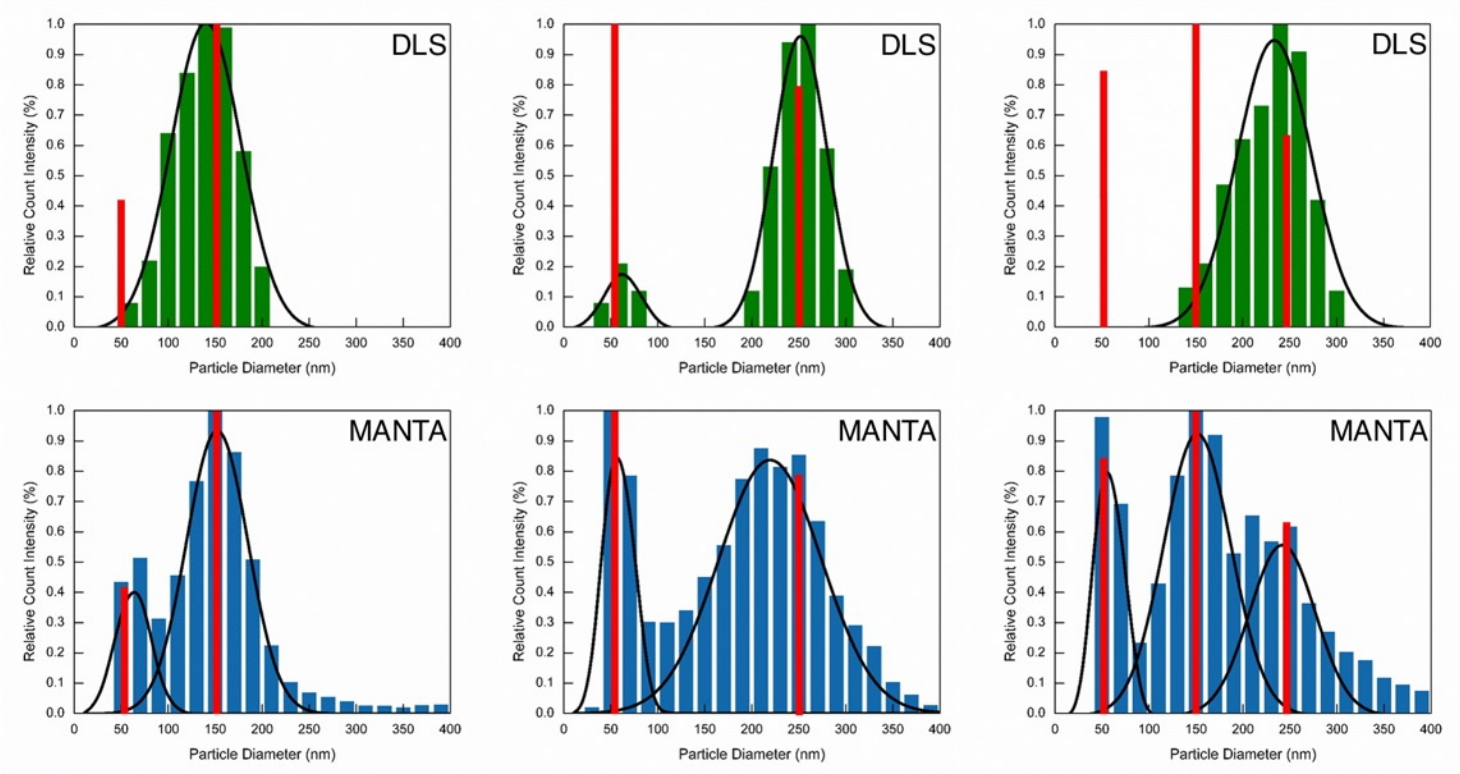
Range of particle sizes measured*	10 nm to 15 µm
Minimum sample volume	0.4 mL
Typical sample concentration	5 x 10^6 to 1 x 10^8 particles/mL
Sample temperature range (controlled)	10 °C to 50 °C, ± 0.1 °C (-15 °C to 110 °C available)
Dimensions	55 cm W x 66 cm D x 35 cm H
Weight	27 kg
Operational Environment	15 °C to 30 °C with < 85% RH

**Sample material dependent*

NIST exploratory poly-standard

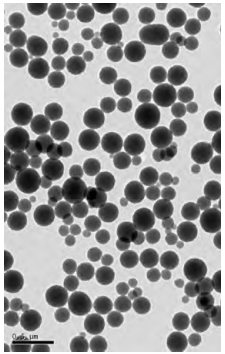


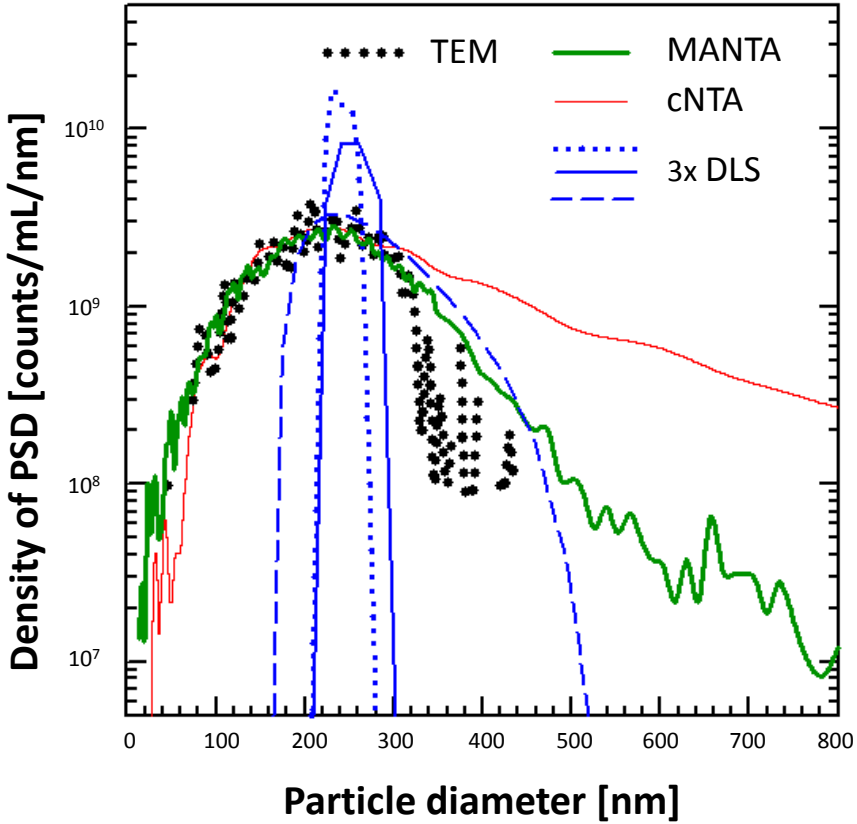
Gold mixes: DLS vs. MANTA



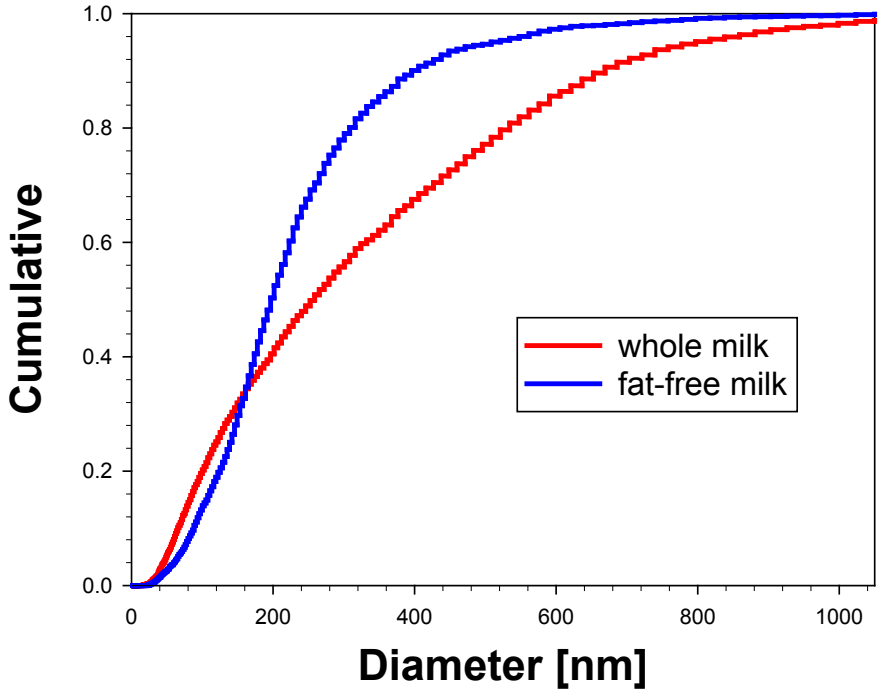
TEM, DLS & cNTA vs. MANTA

α-lactalbumin nanoparticles made as per Arroyo-Maya et al. *J. Dairy Sci.* (2012) **95**, 6204-6214





Whole vs. fat-free milk



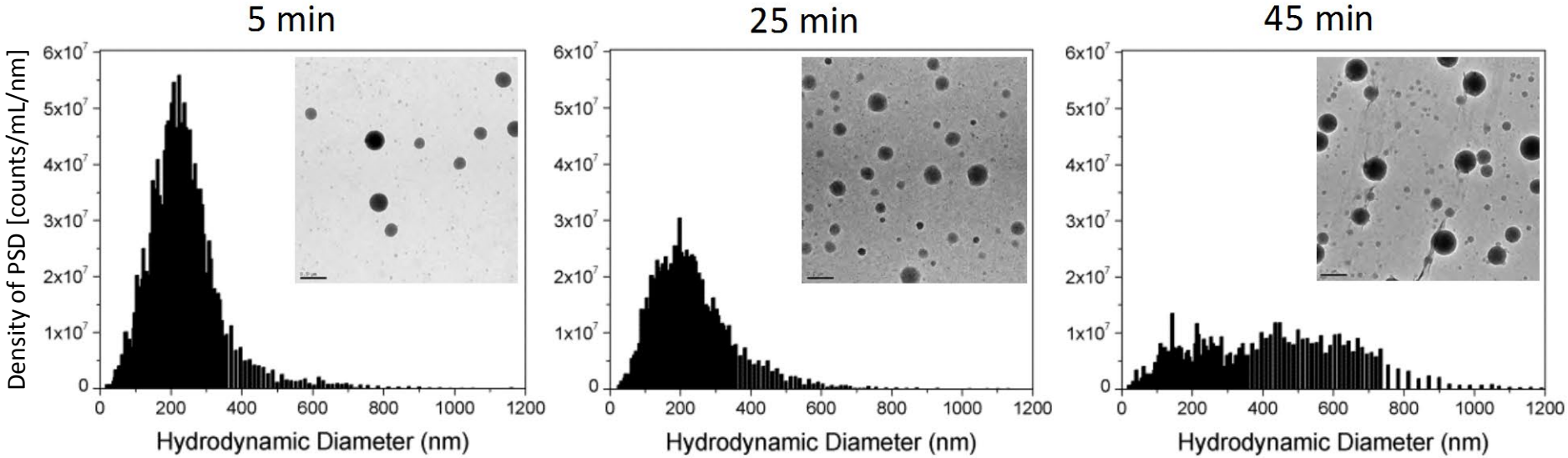
How to compare two distributions with unknown shapes (no theory)? Use the so called nonparametric tests like **Kolmogorov-Smirnow statistics**:

$D_{A,B}$	*alpha*	$D_{A,B,\alpha}$	*Reject?*
0.2335	0.050	0.0338	**yes**

d_{av}=256 nm, SD=145 nm, CV=0.57

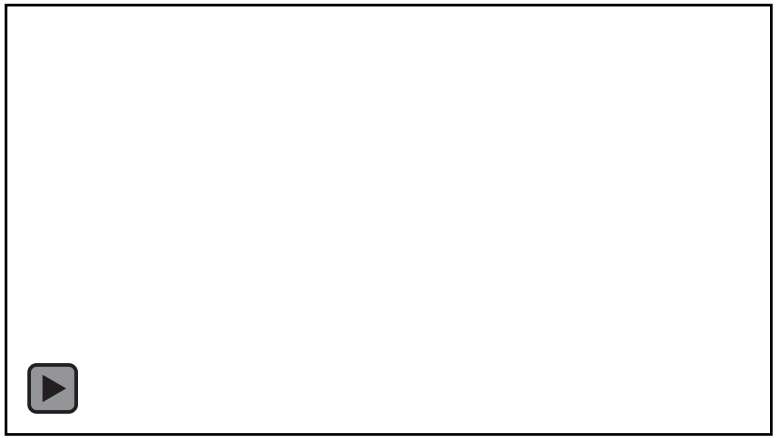
d_{av}=163 nm, SD=68 nm, CV=0.42

Micellisation of a polymer

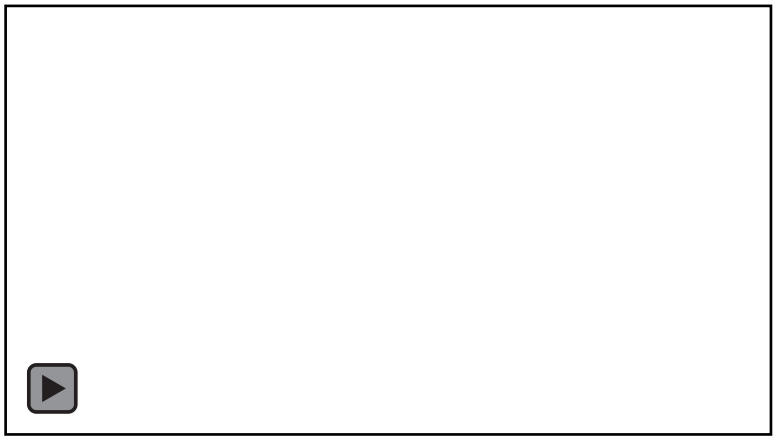


If number of particles retained, diameter/volume increases -> growth by adding polymer

Neat proteins



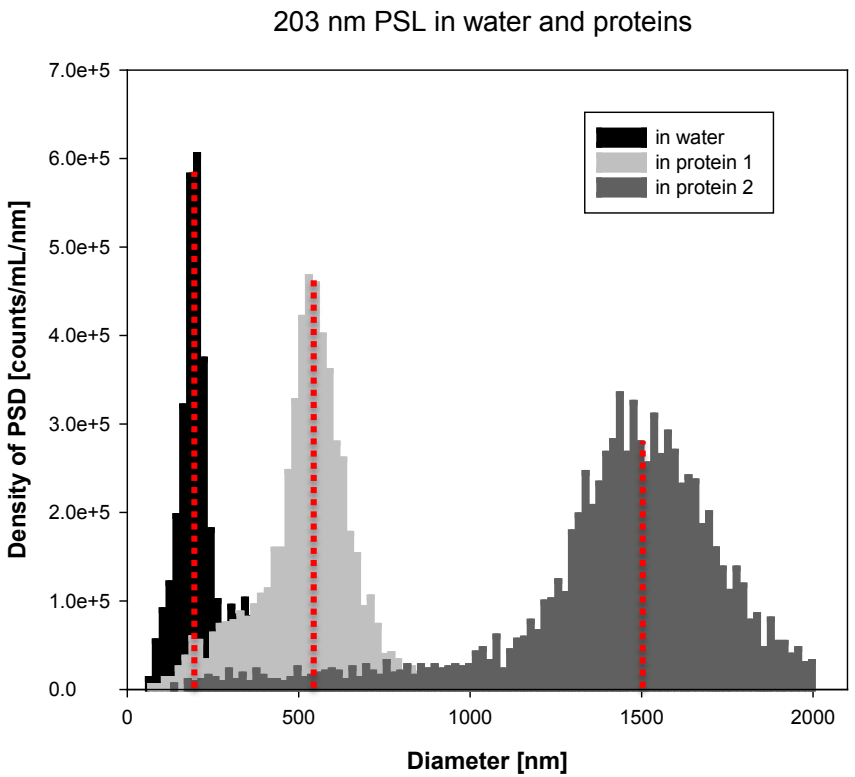
Sample 1



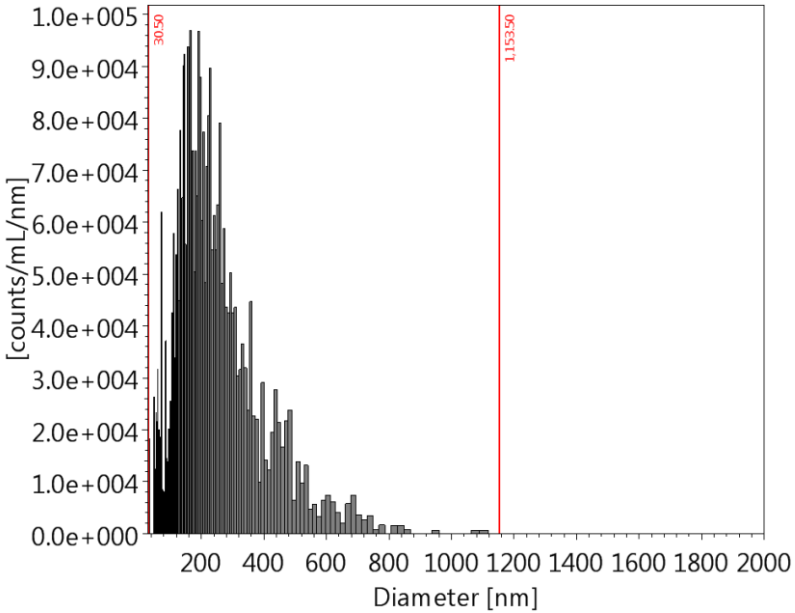
Sample 2

Viscosity of proteins

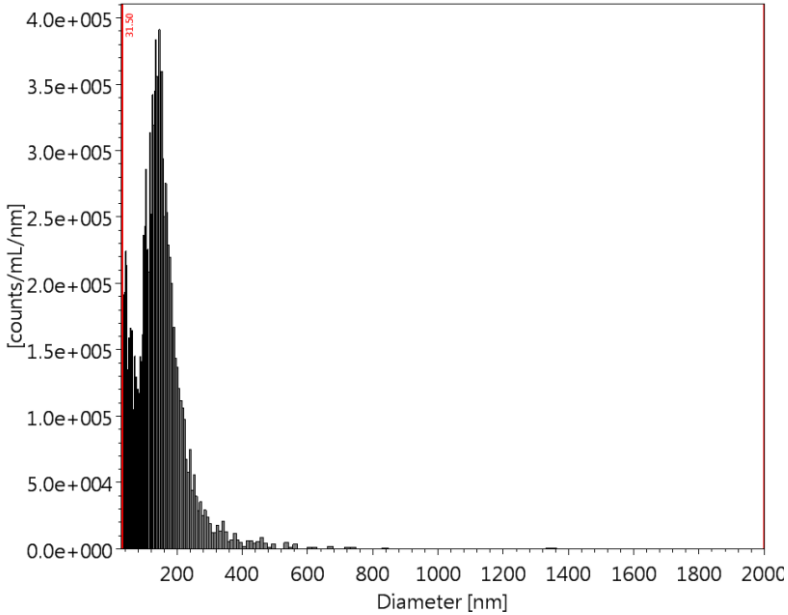
$$\eta_{protein} = \frac{d_{in\ protein}}{d_{in\ water}} * \eta_{water}$$



Neat proteins PSD



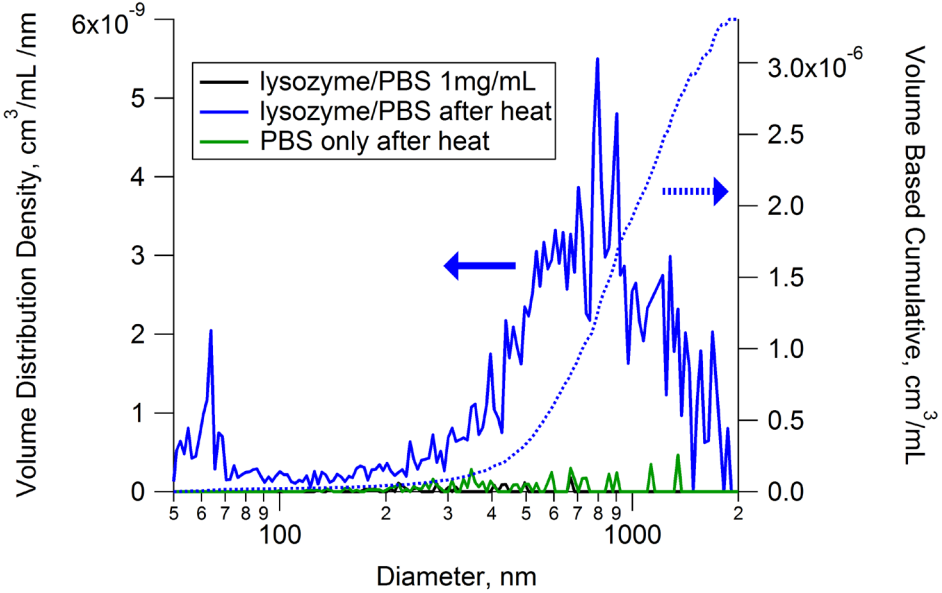
Sample 1

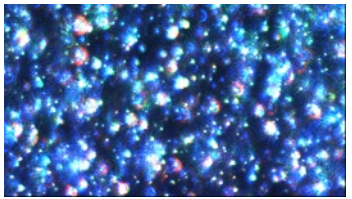


Sample 2

Lysozyme heated to 60 °C

before

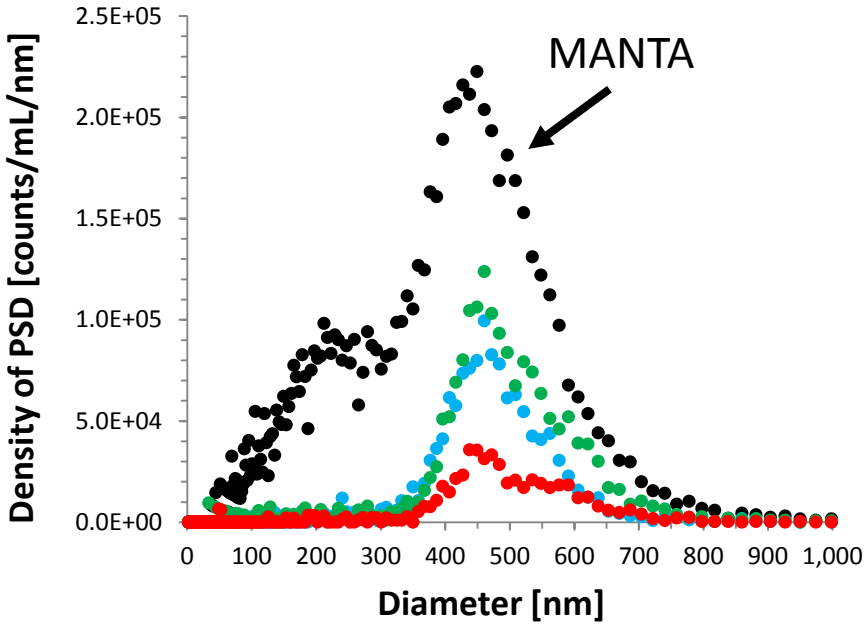




after

Fluorescence

Mix of three types of carboxylate fluorescent beads (all nominally 500 nm diameter, stained with Fluoresbrite®)



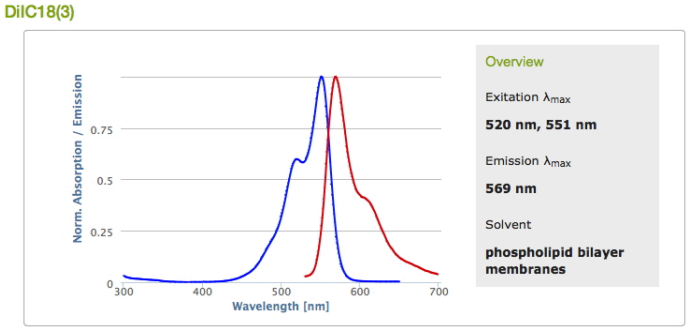
Gravimetric mix:
B 44%
G 33%
R 23%

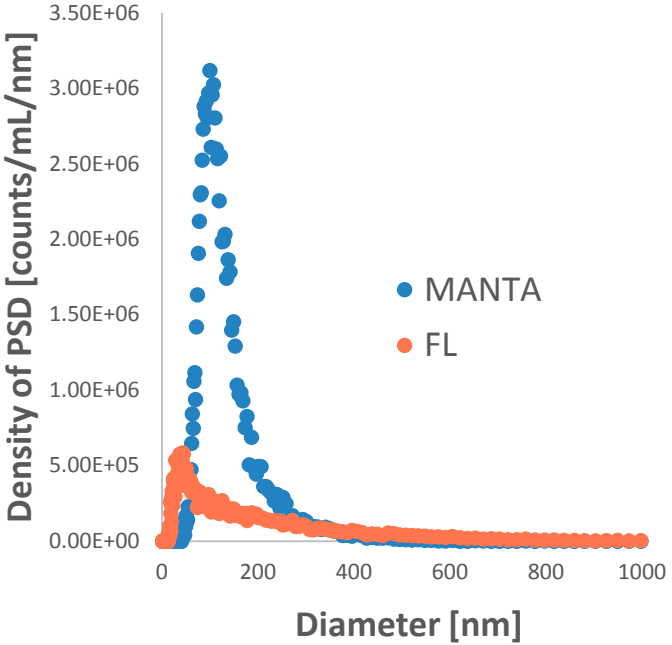
FL measured:
B 36%
G 49%
R 15%

Vesicles stained with fluorophore

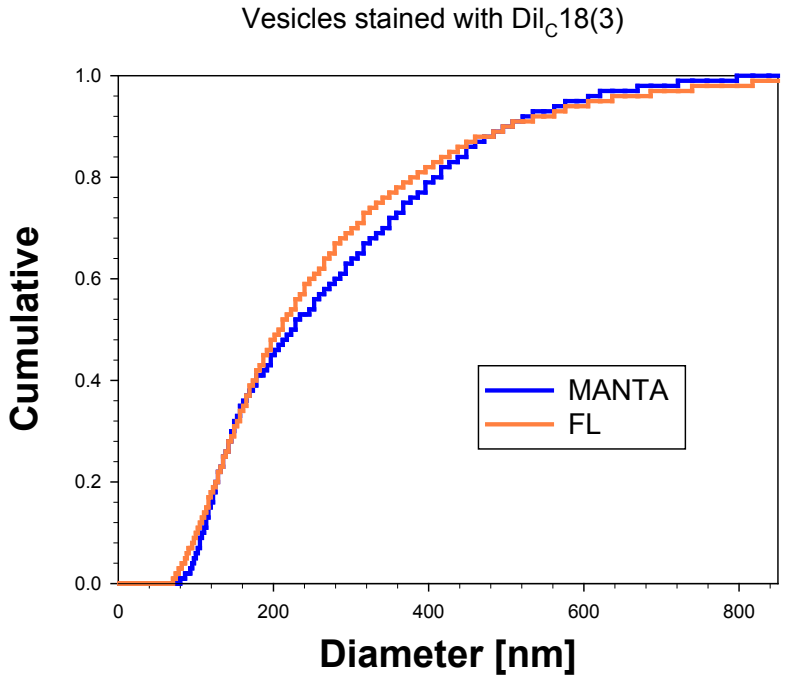
Fluorophore DilC$_{18}$(3) E_x=520÷551 nm, E_m=569÷620 nm

1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine-5,5'-Disulfonic Acid





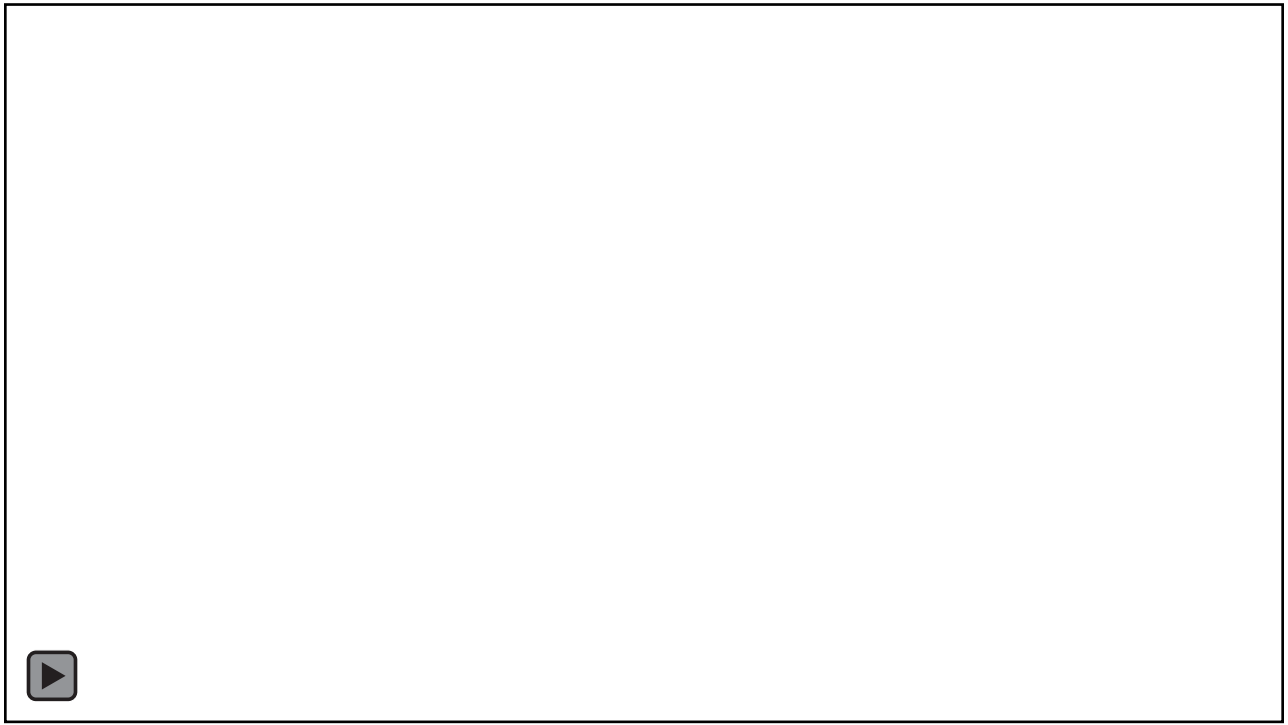
Vesicles processed differently



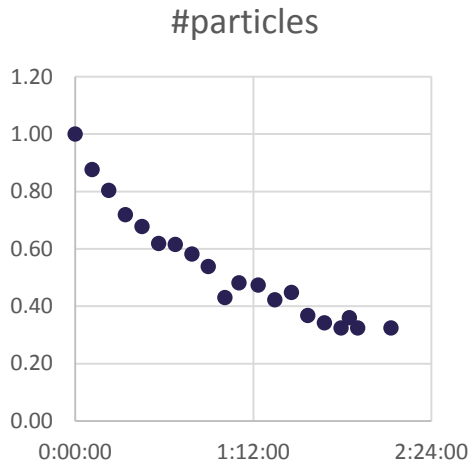
$D_{A,B}$	*alpha*	$D_{A,B,\alpha}$	*Reject?*
0.0647	0.050	0.0297	**yes**

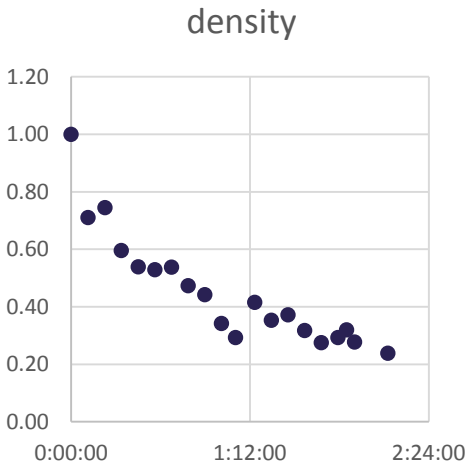
d_{av}=278 nm, SD=166 nm, CV=0.60
d_{av}=264 nm, SD=169 nm, CV=0.64

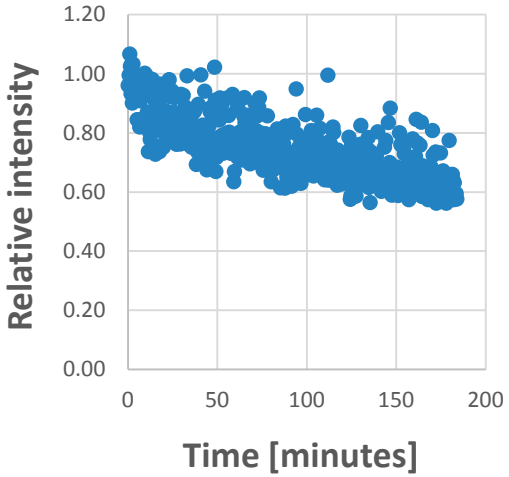
Nanoparticles dissolution rate



Nanoparticles dissolution rate II

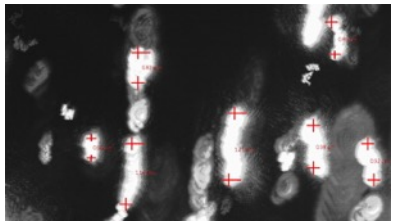




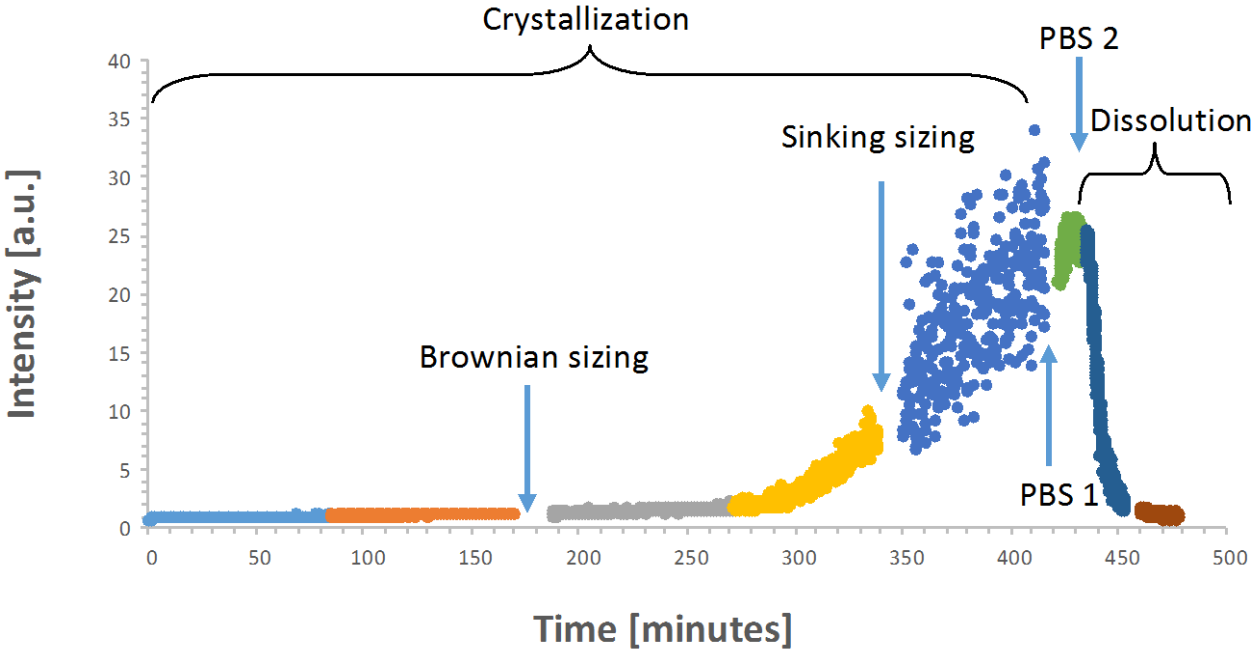


Settling rate for large particles

$$d = \sqrt{\frac{18 * v * \eta}{g * (\rho - \rho_0)}}$$



Protein crystallization rate



*Wittbold & Tatarkiewicz 2017, BioProcess International **15** (3)*

Successfully tested samples

polystyrene, also with PEG coating, silica, silver, gold, 316L stainless, sand/dirt, clay, CaO, YAG, SiO_2, carbon, PMMA, LiMnO

sea water, fresh water, rain water, tap water, acetone, wine, urine, blood plasma, milk, ammonia, jet A-1 fuel

small molecule APIs, protein aggregates, silicon oil, protein crystals, liposomes, exosomes, vesicles, micelles, α-lactalbumin, rolled DNA, RNA, viruses, bacteriophages, emulsions, polymeric API carriers, self-adjuvanted proteins

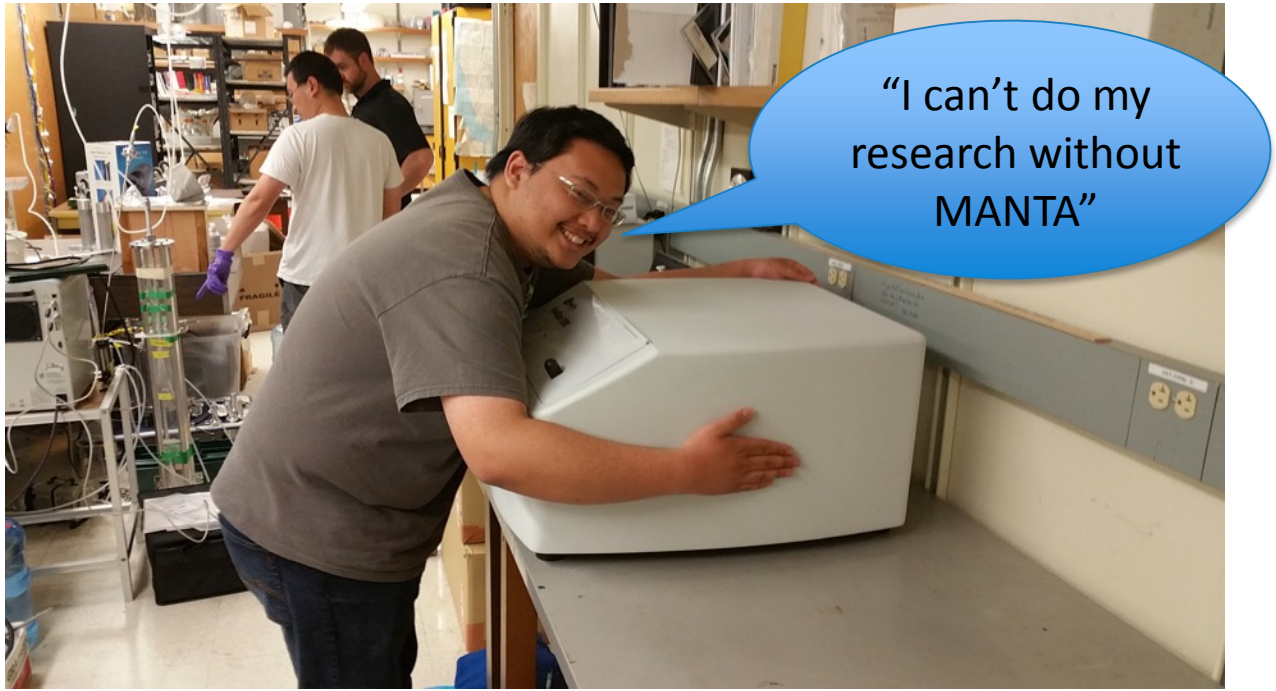
Key benefits of MANTA

- Individual particle method, not ensemble average
- Accurate density of PSD for polydisperse samples
- Concentration measured, not estimated
- Absolute method (no calibration w/standards needed)
- Particles and processes visualization

Other benefits

- Kinetic processes (time constants)
- Temperature range and ramp rates
- Real time agitation
- Real time reagent addition
- Multiple tests w/o changing sample

Customers love ViewSizer® 3000



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Better Nanoparticle Characterization

Thank you

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VP Engineering

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