

What does it take to accurately measure concentration of nanoparticles in colloids

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VP Engineering

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Counts

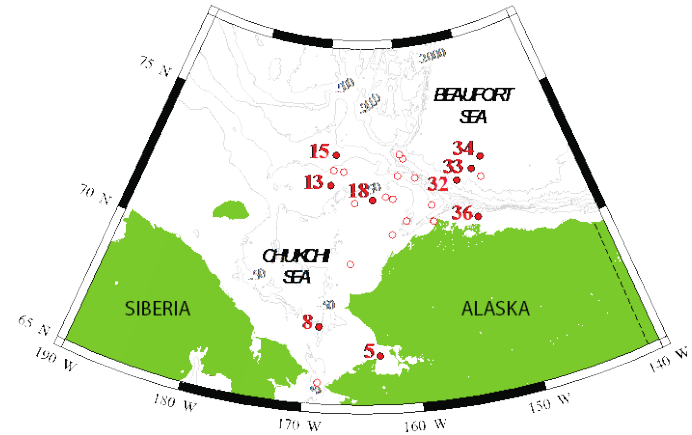
Volume

in chemistry typically mass [mg/mL] or volume [μ L/mL] concentrations

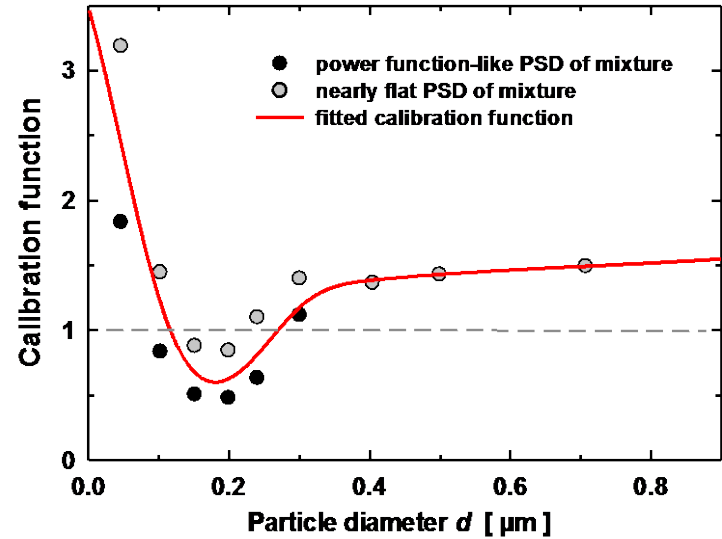
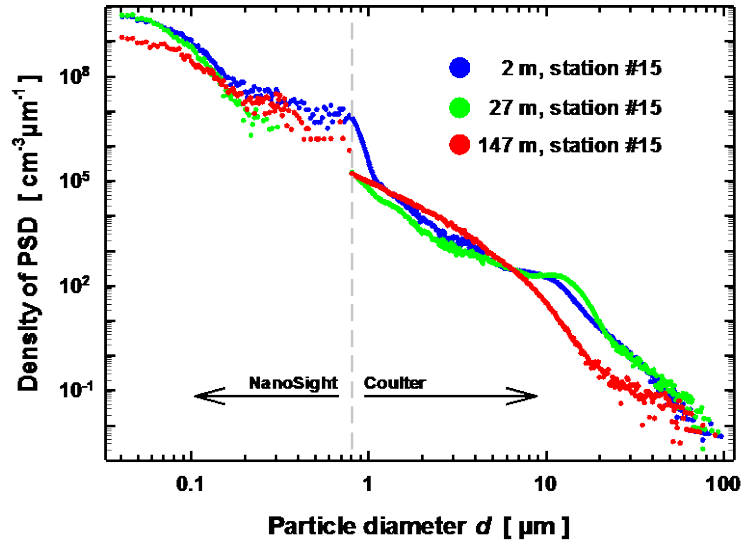
Beginnings



NASA sponsored ICESCAPE Project
two cruises on USCGC *Healy*
2010 - 2011



Results



J. J. Tatarikiewicz, R. A. Reynolds, and D. Stramski *Counting and sizing of colloidal particles in the Arctic Ocean* 2012 Ocean Sci Meeting A0412

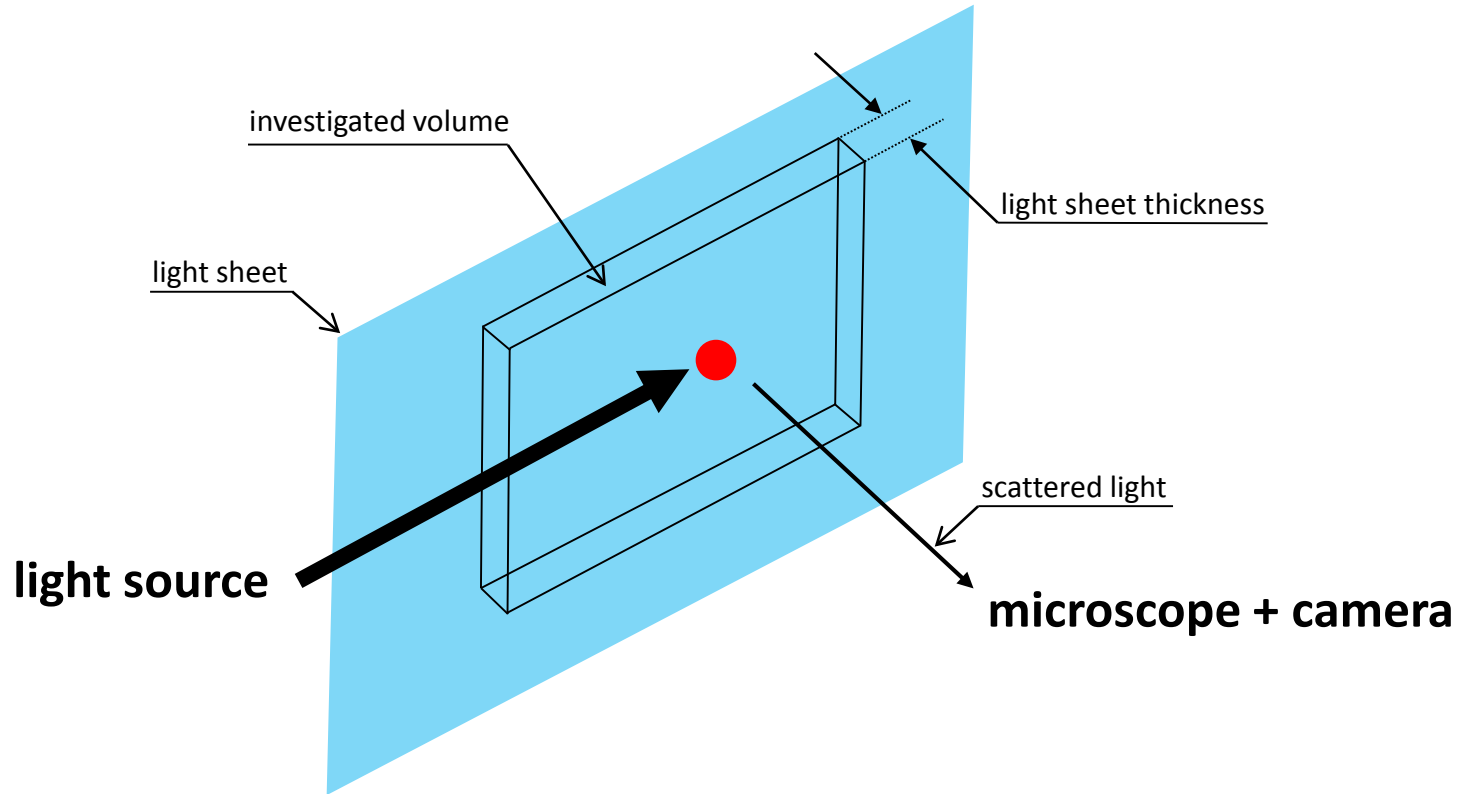
This is a very strange function...

2nd generation NTA

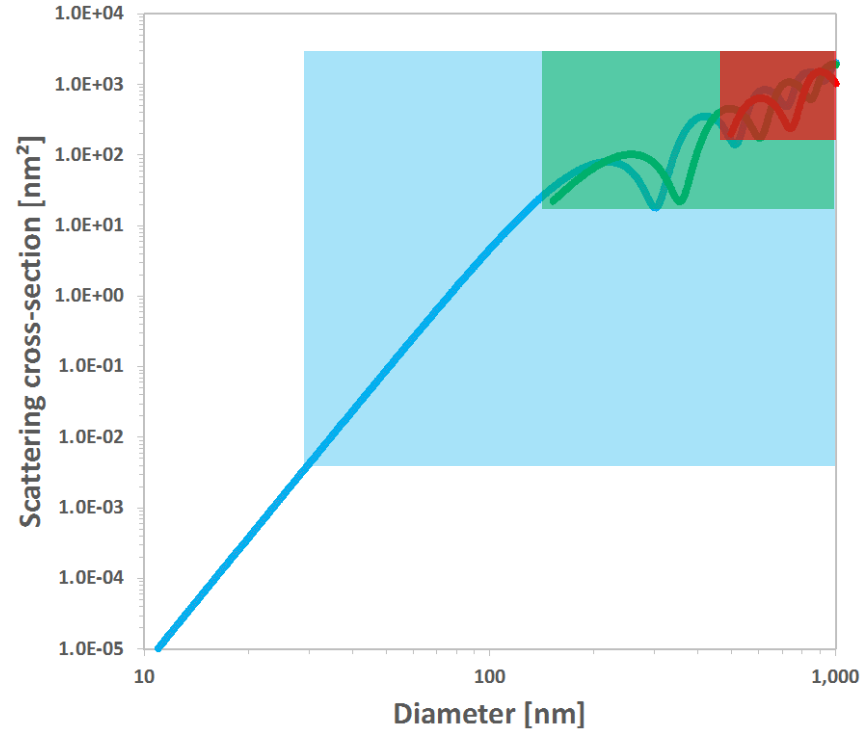


- **M**ultispectral **A**dvanced **N**anoparticle **T**racking **A**nalysis
- NSF grant for MRI #1126870, 2012-2014
- MANTA Instruments, Inc. founded in 2014
- US patents granted up to now:
 - 9541490, 9645070, 9857283, 9909972

Visualization



MANTA



Mie calculations for 445 nm, 520 nm and 635 nm polarized laser beams scattering on PSL in water ($n=1.337$), objective NA=0.28, $80^\circ\div 100^\circ$ integration

Sizes



- Mean Squared Distance MSD (2D, N frames track, n frames lag*):

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

- Diffusion coefficient D (least-squares fit of MSD as a function of n):

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

- Hence hydrodynamic diameter: $d_h = \frac{k_B T}{3\pi\eta D}$

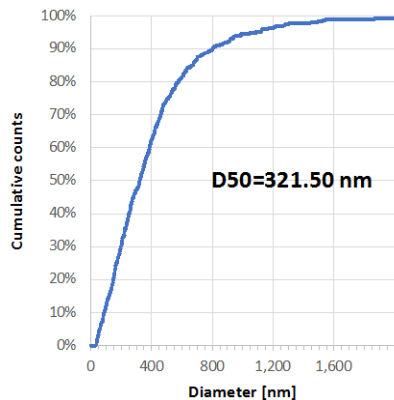
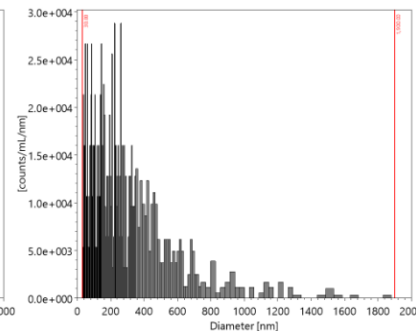
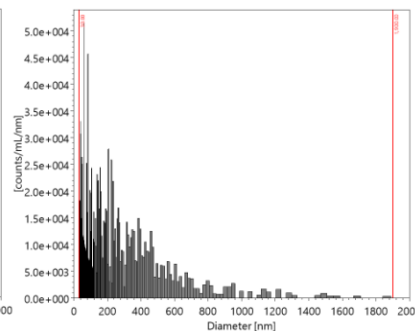
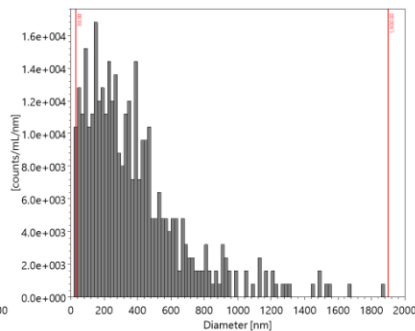
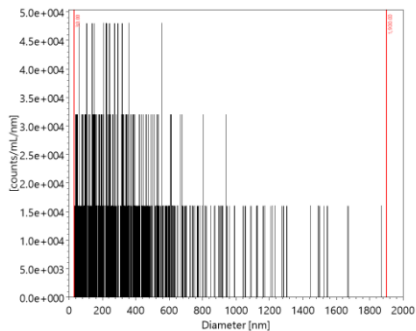
* ergodicity: assembly average \equiv time average

Statistics

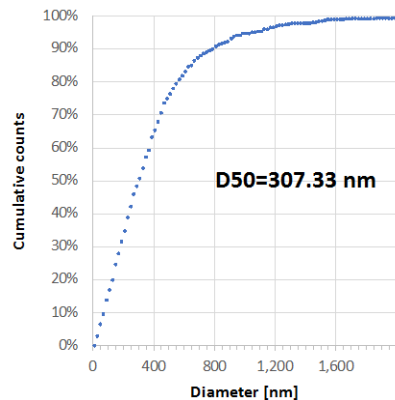


- Cramér-Rao statistics decides length of each track used for optimal MSD fitting
 - X. Michalet and A.J. Berglund *Optimal diffusion coefficient estimation in SPT*, Phys Rev **E85**, 061916 (2012)
- Binning diameters with different schemes (like equal or logarithmic widths) into density of particle-size distribution (PSD) with **variable investigated volume** (*explained later*)
- Statistical parameters of PSD (average size, standard deviation)

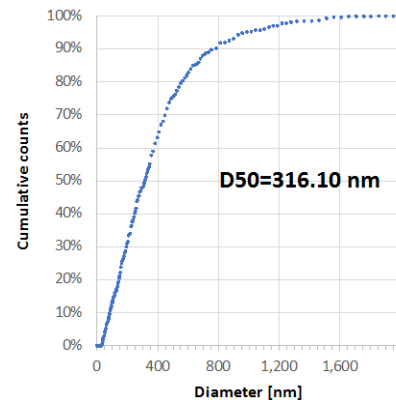
Mode, D50?



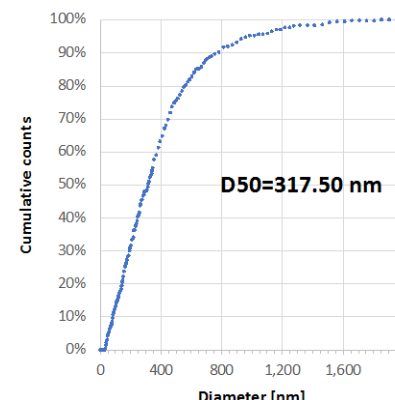
narrow equal bins



wide equal bins



logarithmic bins

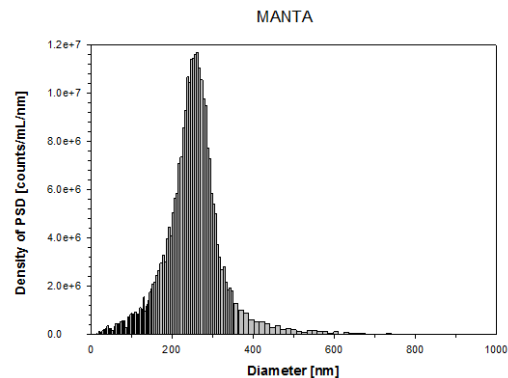
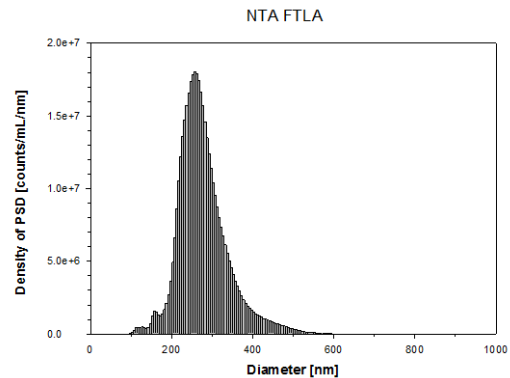
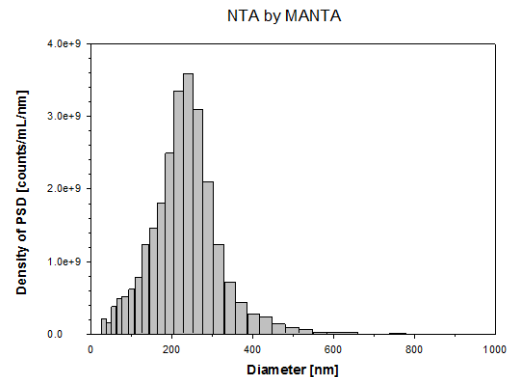
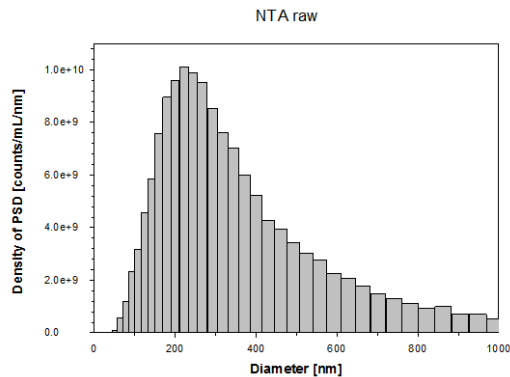


variable bins

Processing



240 nm PSL in water



Counts



- 25 (*or more*) short videos (*300 frames each*) recorded*
- Track and count particles detected on 1st frame of each video
- Mixing sample between videos to get different aliquots (magnetic stirrer)
 - external fluidics for magnetic materials and low concentration samples – do **not** use sample flow during recording
- Proper PSD binning (*bin widths*) for polydispersity

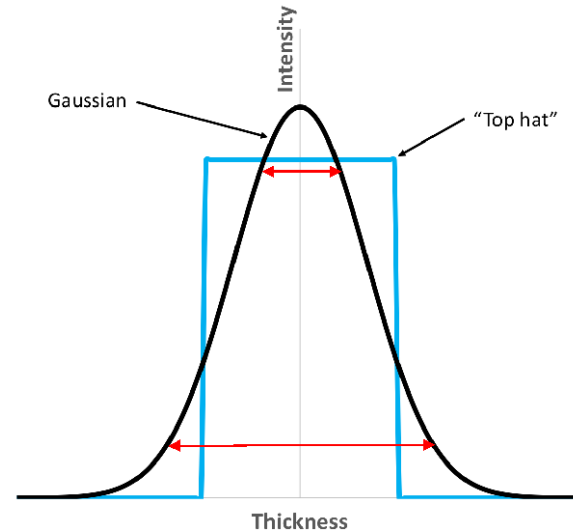
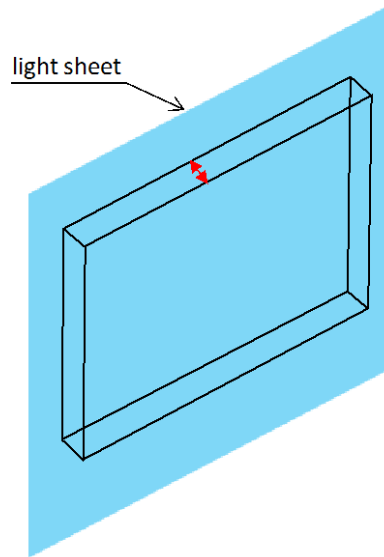
* typically between 100 and 150 particles tracked per video

Thickness



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

$$V_0 \approx 2.5 \text{ nL}$$



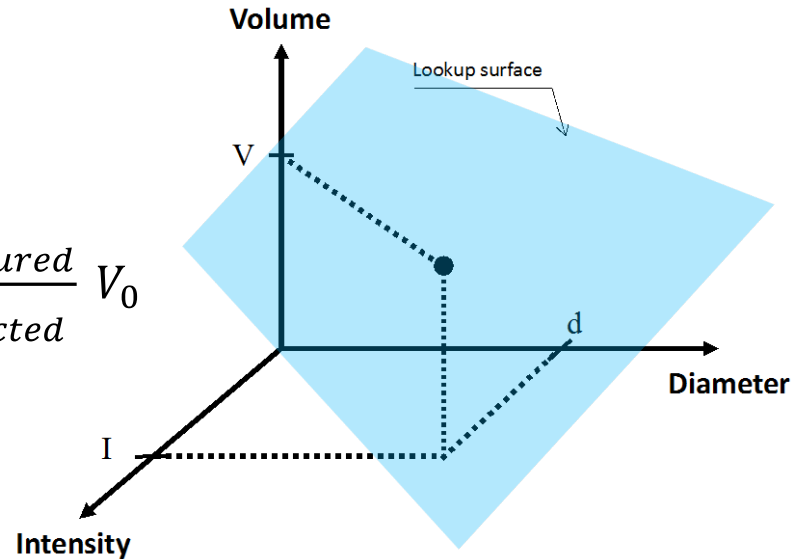
Volume



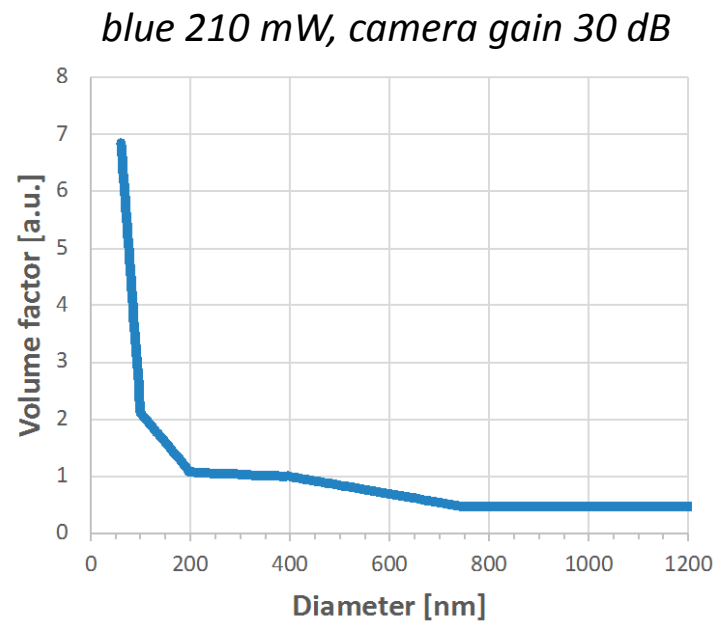
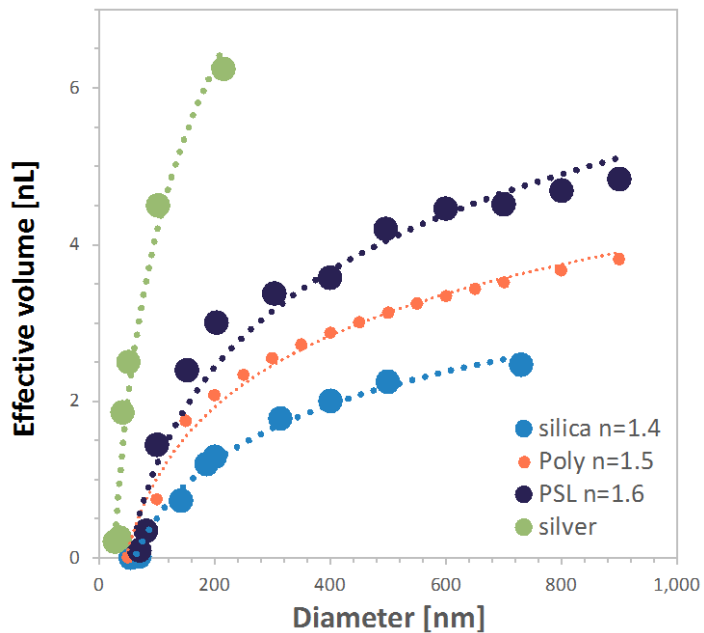
How to calibrate volume:

- Measure concentrations for standards of different sizes and made out of different materials (various RIs)
- Determine effective volumes
- Create look-up surface of volume
- Extrapolate by using intensity of individual tracks and applying Mie scattering cross-section formula

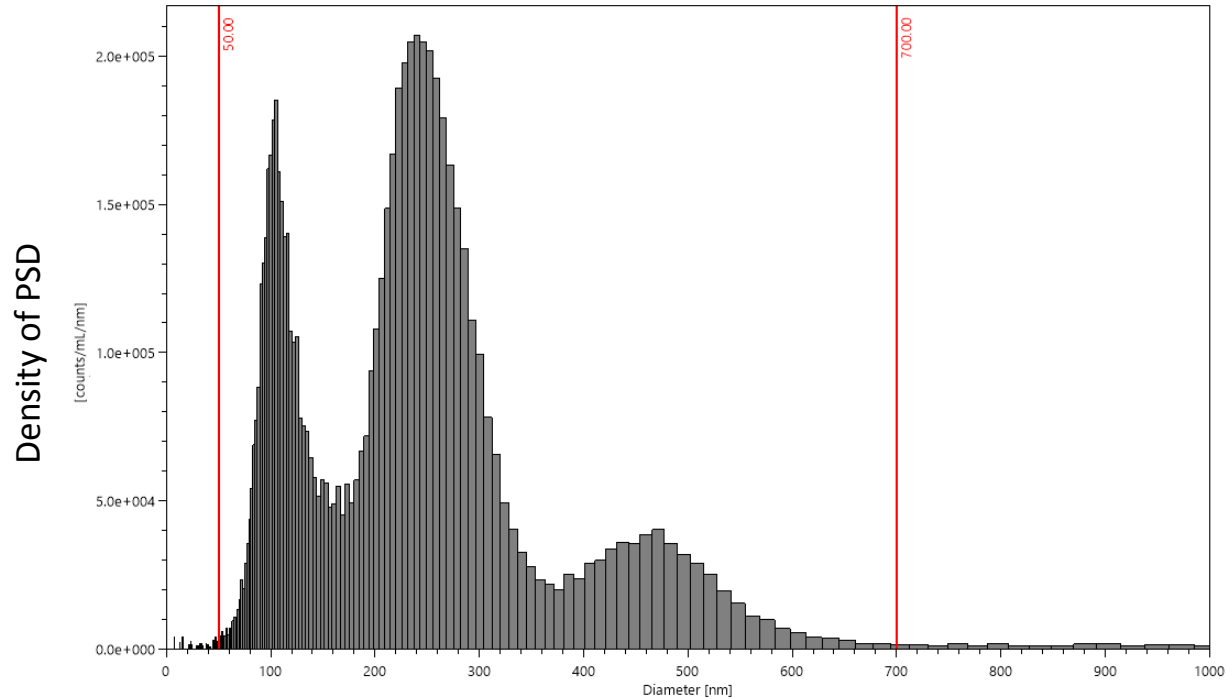
$$V = \frac{N_{measured}}{N_{expected}} V_0$$



Simplified method



Histogram



Concentration from 50 nm to 700 nm = area of density of PSD histogram

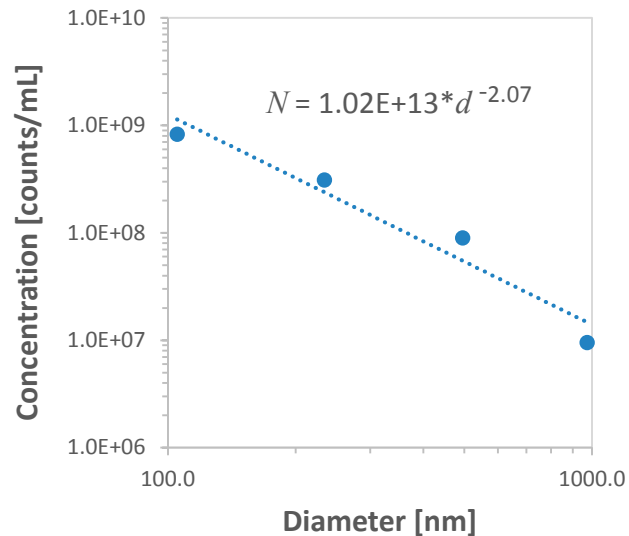
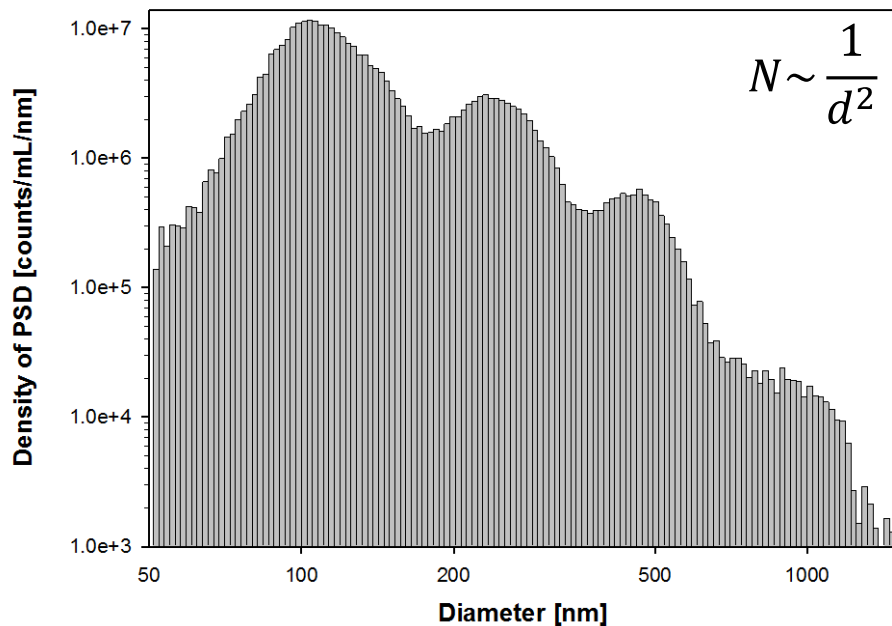
$$\delta_i = \sum_m \frac{1}{b_i \cdot V_m}$$
$$C_{kl} = \sum_{i=k}^l \delta_i \cdot b_i = \sum_{i=k}^l \sum_m \frac{1}{V_m}$$

Concentration



- Integrate density of PSD ($counts/mL/nm$) across sizes of interest, for example from 50 to 700 nm, to get concentration ($counts/mL$)
- Instruments are calibrated for optics scaling (nm/pix) and for laser(s) power (mW) (*manufacturing variability of active elements*)
- For unknown materials, extrapolate investigated volumes by using Mie scattering cross-sections of known test materials
- Use measured data with statistically significant number of counts, do **not** use fitted distributions (*PSD is **not** an invariant*)

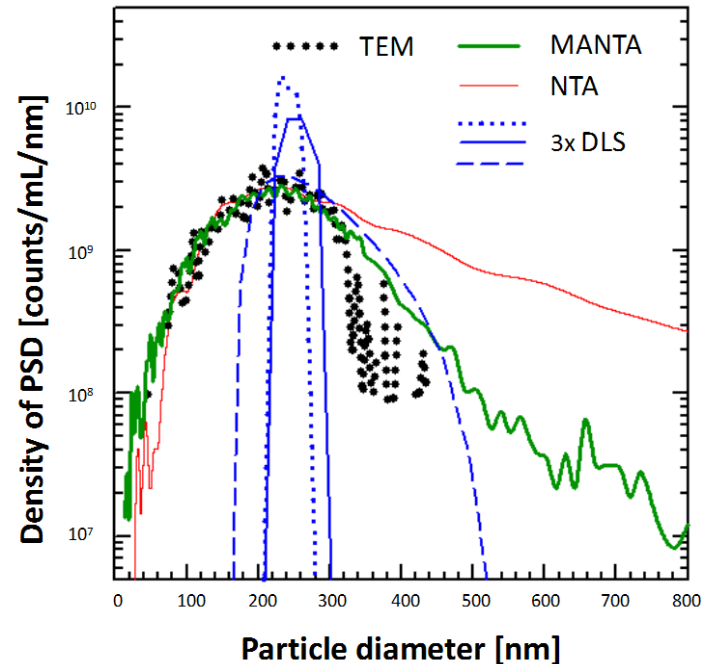
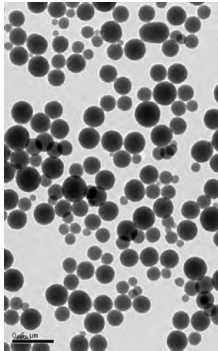
NIST exploratory mix



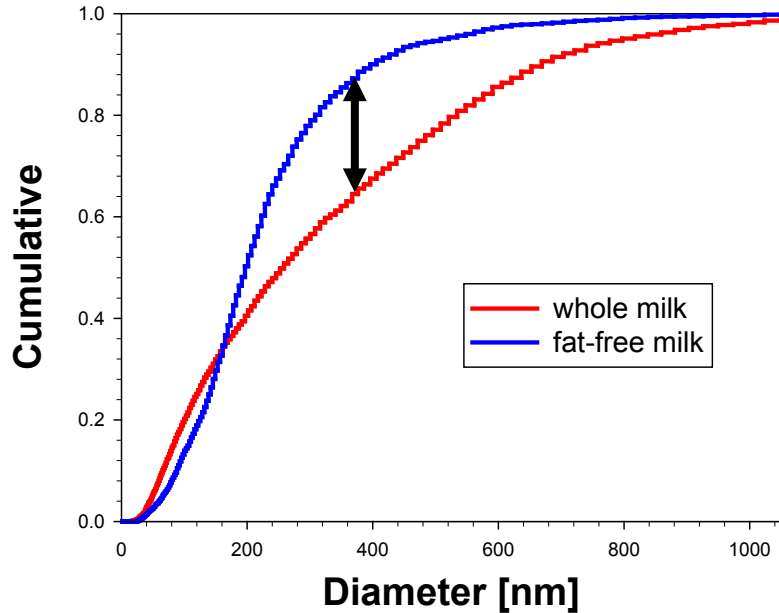
TEM, DLS & NTA vs. MANTA



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



Comparing PSDs



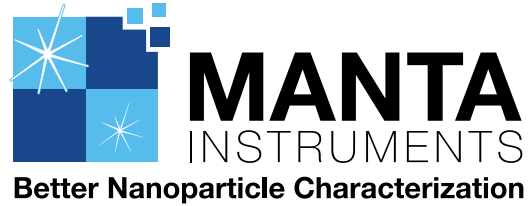
$d_{av}=256$ nm, $SD=145$ nm, $CV=0.57$

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

see Anscombe's quartet of descriptive statistics

Non-parametric tests like
Kolmogorov-Smirnov statistics:

$D_{A,B}$	α	$D_{A,B,\alpha}$	Reject?
0.2335	0.05	0.0338	yes



Thank you

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