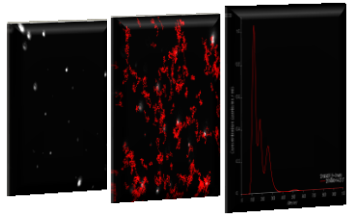
Malvern Panalytical

Advances in particle concentration measurements

Dr Hanna Jankevics Jones – Principal Applications Scientist

Outline

Advances in particle concentration from Malvern Panalytical

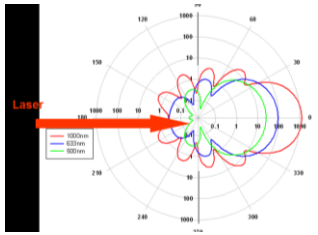


Capture Tracking Analysis

Nanoparticle tracking analysis – quick overview

New!

NanoSight Sample Assistant – increase throughput and remove operator variation

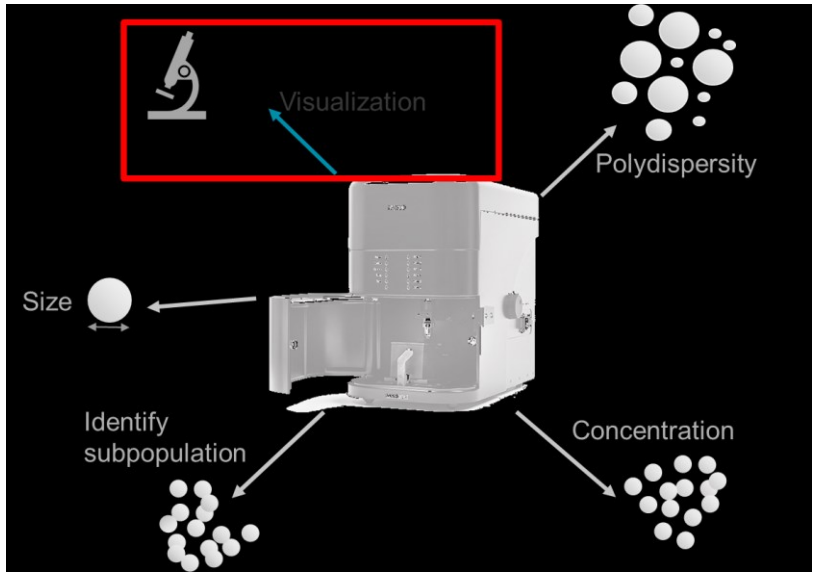


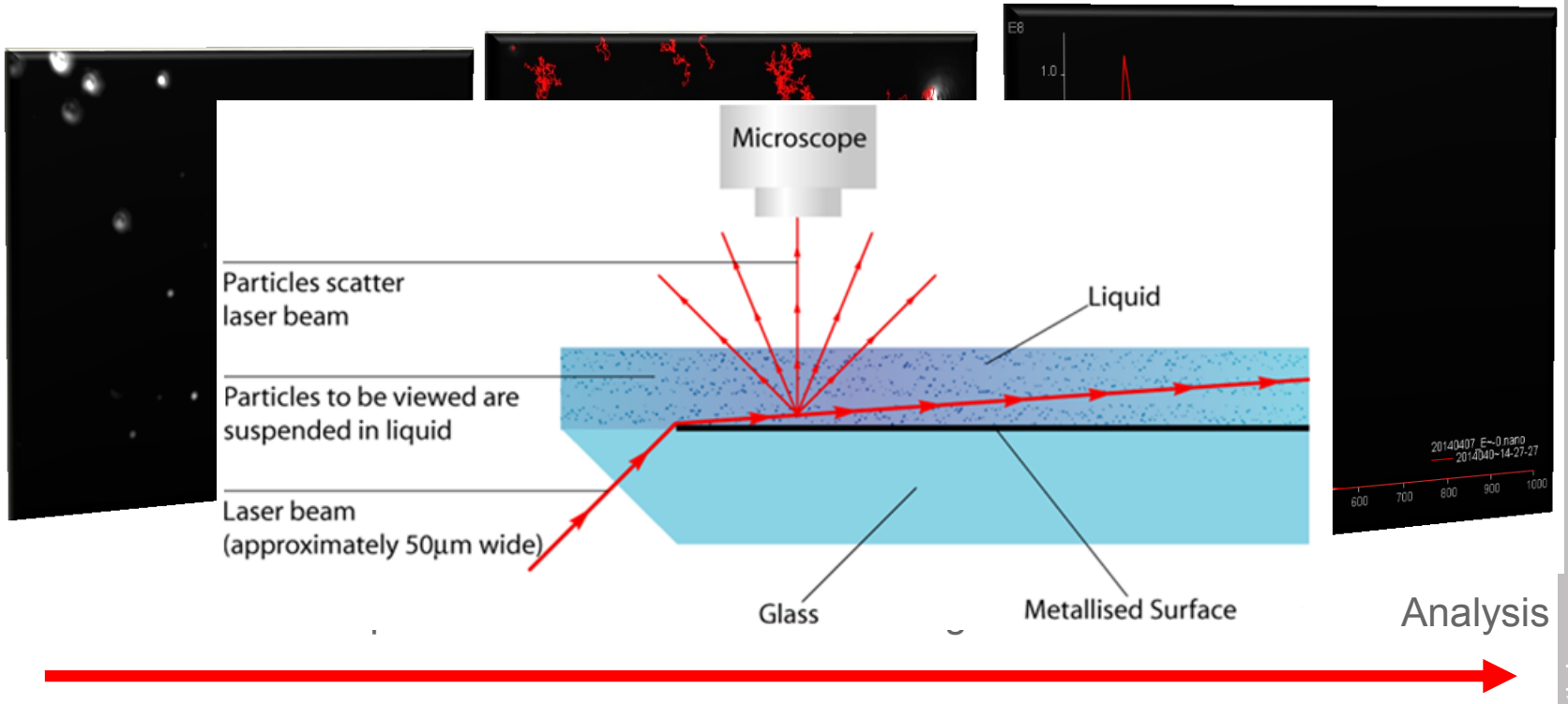
New!

Multi-angle dynamic light scattering gives higher resolution and individual particle populations' concentration

Visualisation

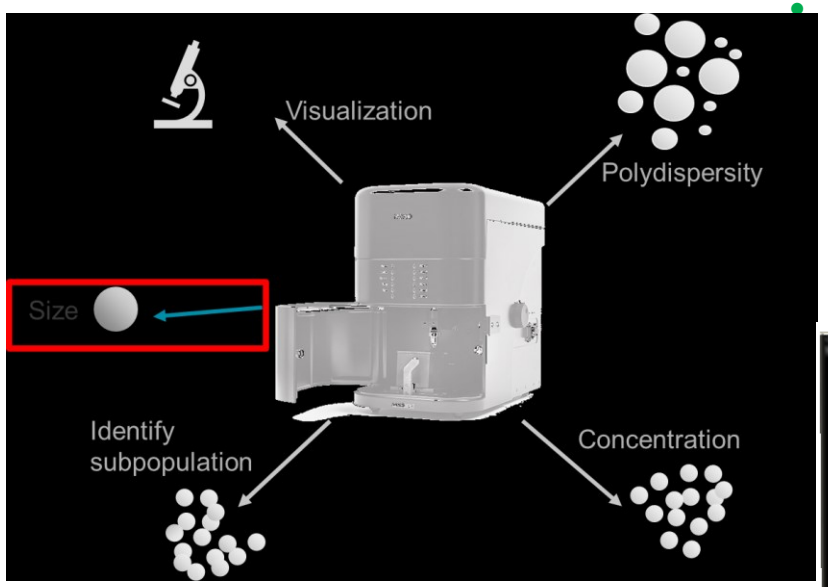
Nanoparticle tracking analysis (NTA)



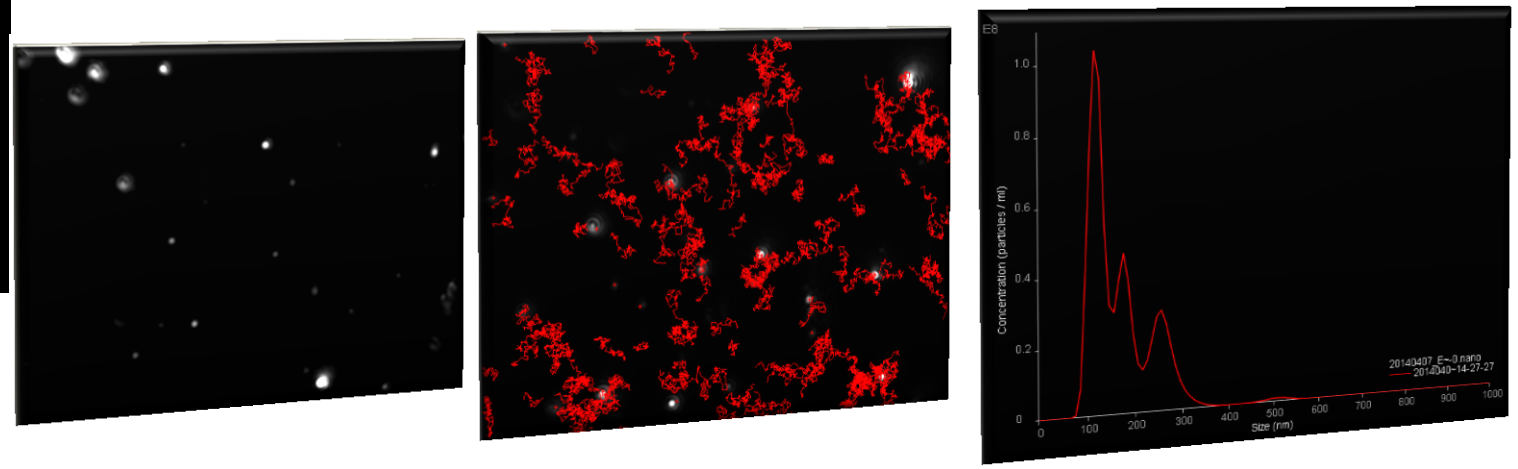


No need to know sample refractive index or density

Size Measurement



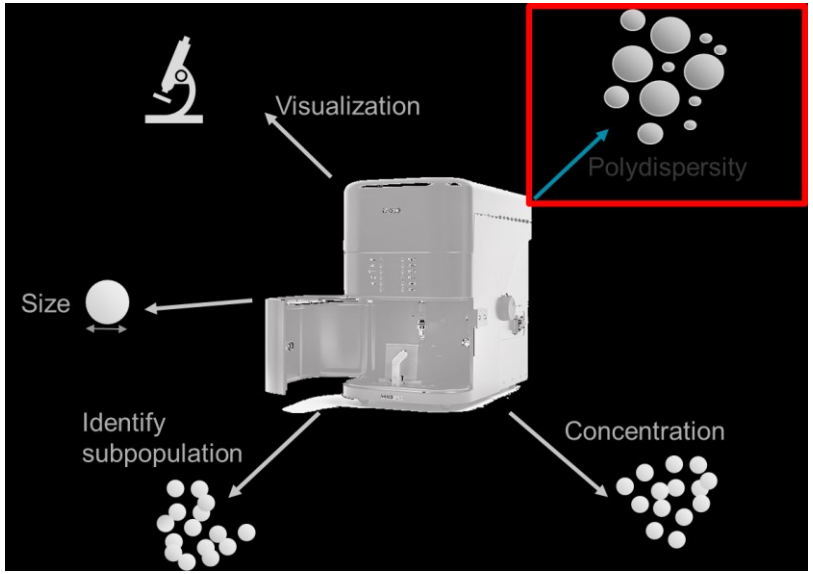
- NanoSight tracks nanoparticles moving under Brownian Motion
- By tracking the particles, we can determine the diffusion coefficient and use it to calculate the size (Stokes-Einstein equation)
- Smaller particles move faster than larger particles

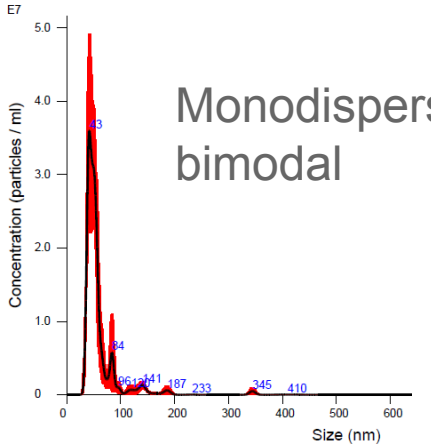


ISO19430: Particle Tracking Analysis (PTA) method describes limitations, quantification parameters as well as instructions on how to operate the equipment in a certified manner.

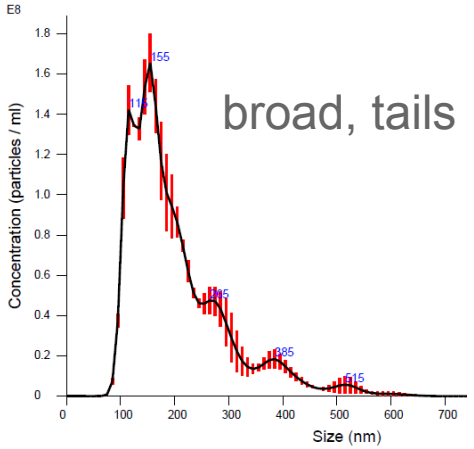
Polydisperse Samples

Sample distribution examples

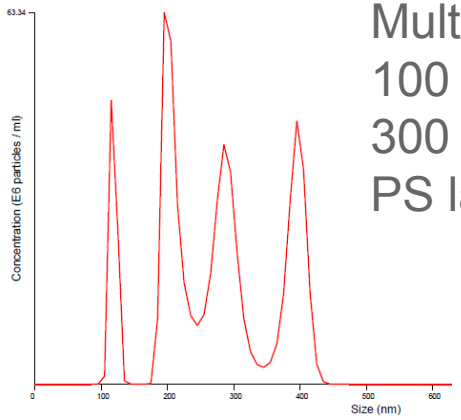




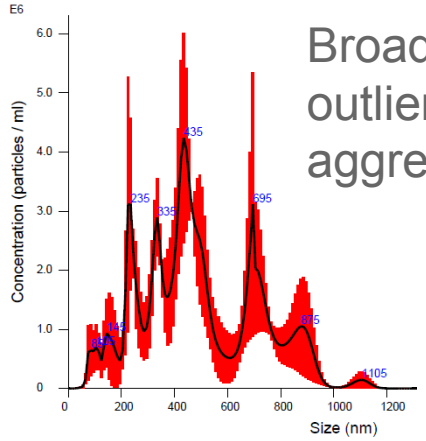
Monodisperse / bimodal



broad, tails



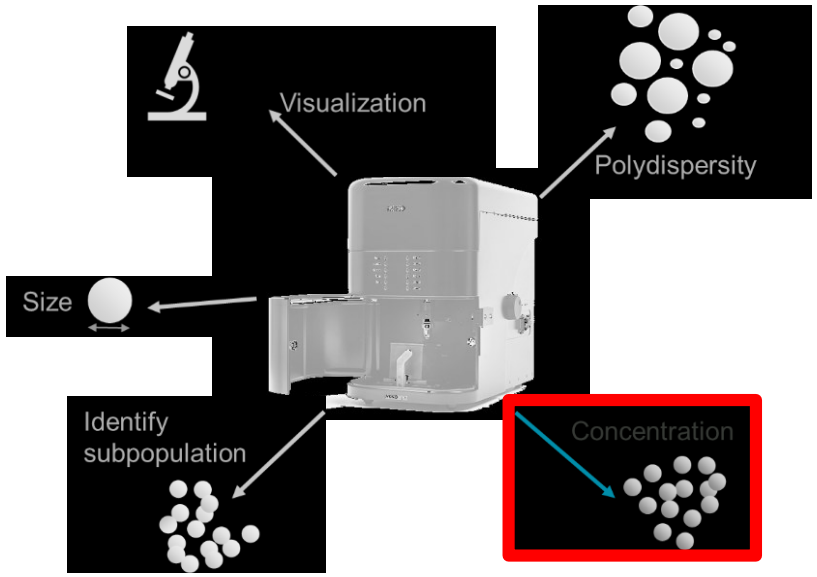
Multimodal:
100 nm, 200 nm,
300 nm, 400 nm
PS latex



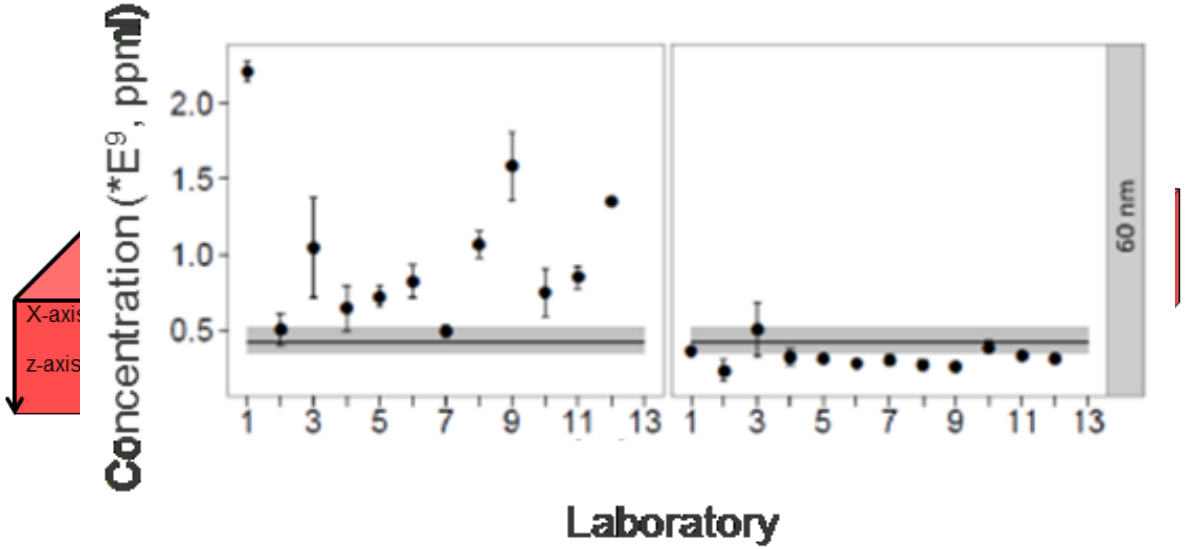
Broad, tails, outliers, aggregation…

Concentration

Concentration upgrade - can be done by the user in their lab



- NanoSight track individual particles, allowing to have their exact number
- The volume is determined by the field of view (X and Y) and from the laser beam profile (Z)



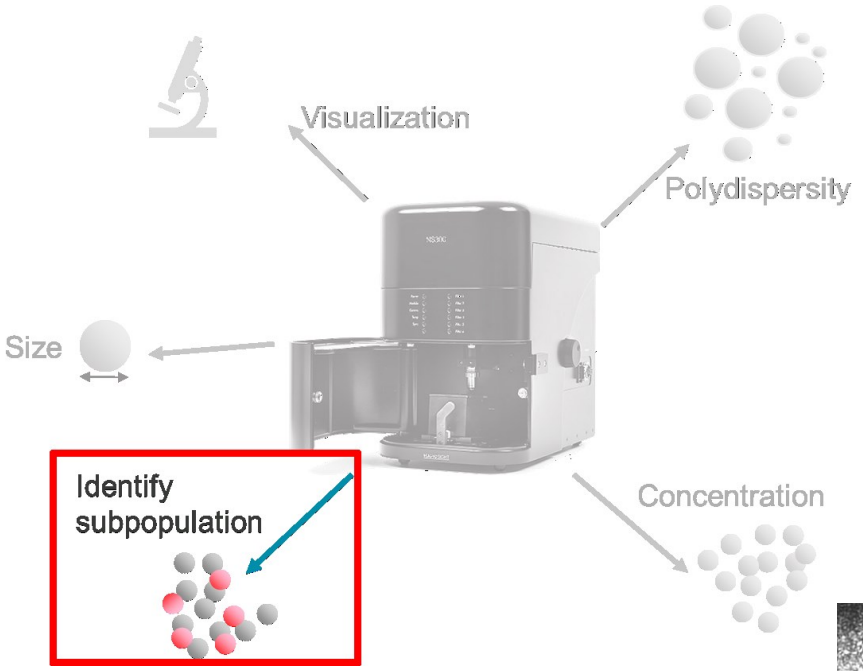
Concentration upgrade for NS300 and NS500, *requires syringe pump

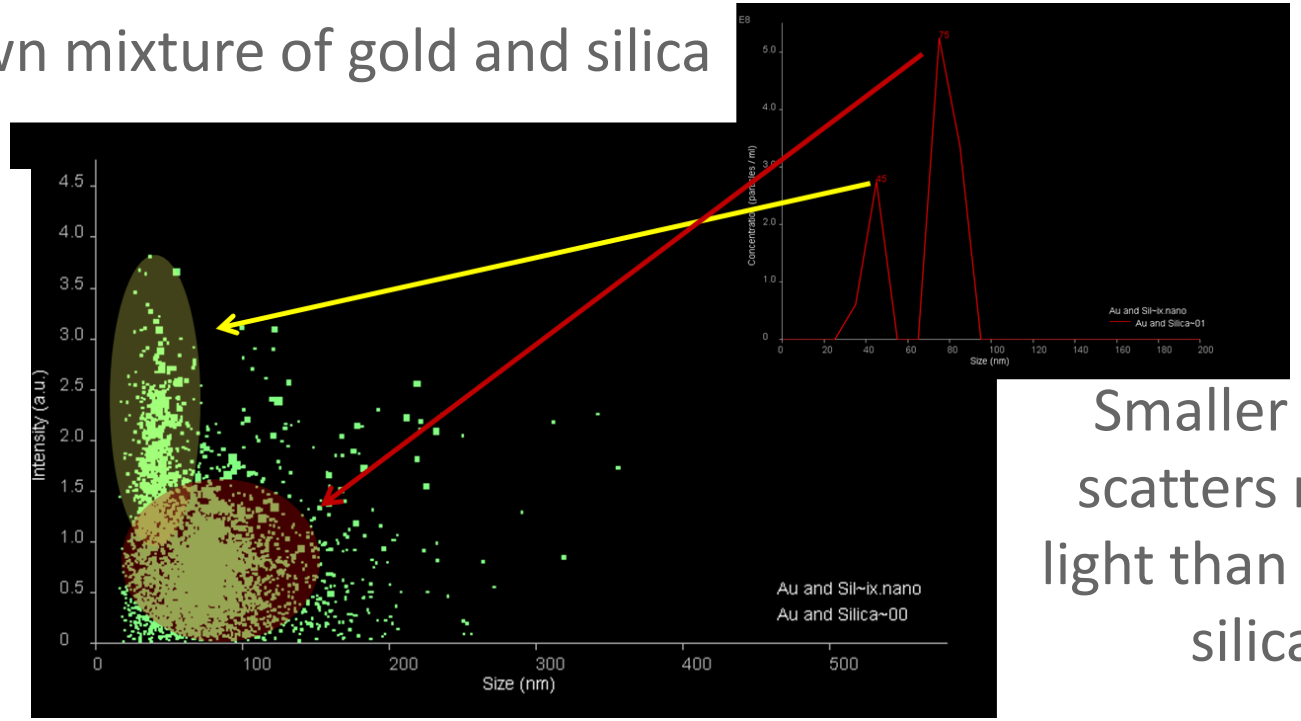
Journal of Micro and Nano Manufacturing, December 2017

Identify Subpopulation

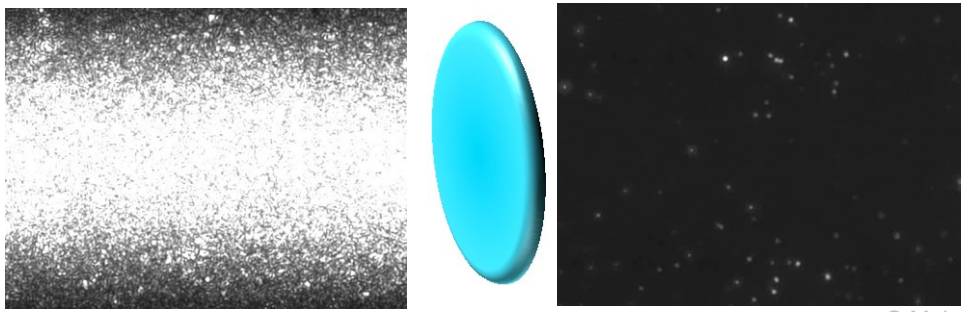
Relative refractive index or fluorescence

Unknown mixture of gold and silica





Smaller gold scatters more light than bigger silica



Different laser wavelengths for different fluorophores

1.405 (430) nm

2.488 (500) nm

3.532 (565) nm

4.642 (650) nm

NanoSight Sample Assistant

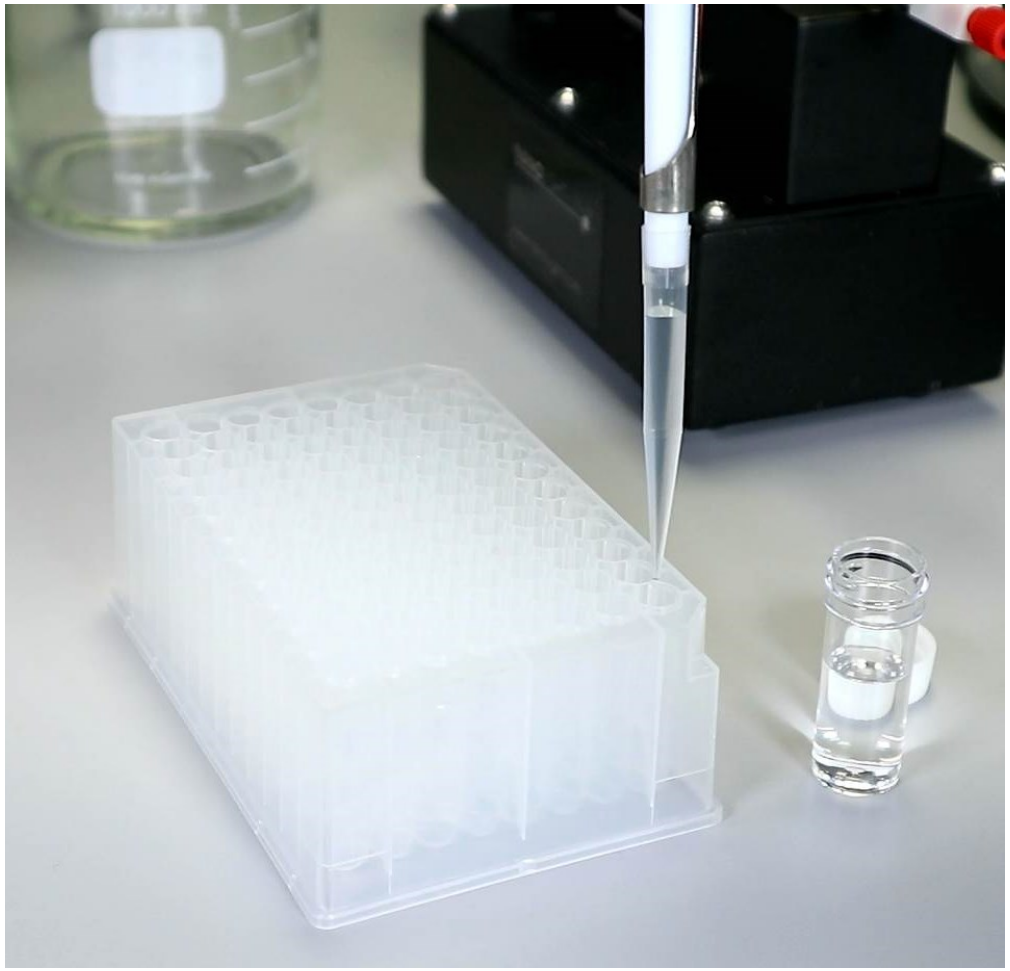
Samples

Requirements and Compatibility

- 1ml per sample, using deep well plate
- Sample in water or aqueous buffer , using low volume flow cell (LVFC)

Examples:

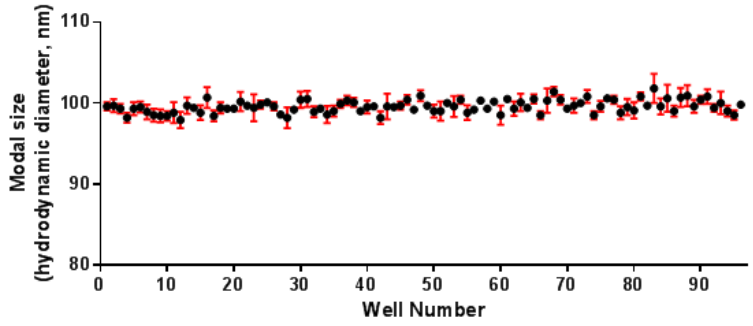
- Exosomes
- Viruses
- Low concentration proteins
- Drug delivery nanoparticles
- Metal nanoparticles
- Water treatment samples

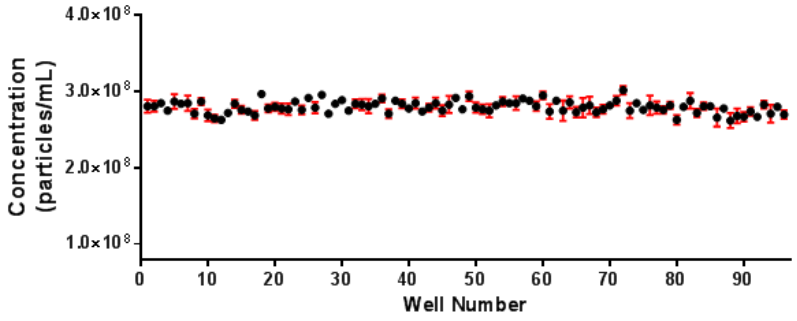


Data repeatability

100nm polystyrene latex

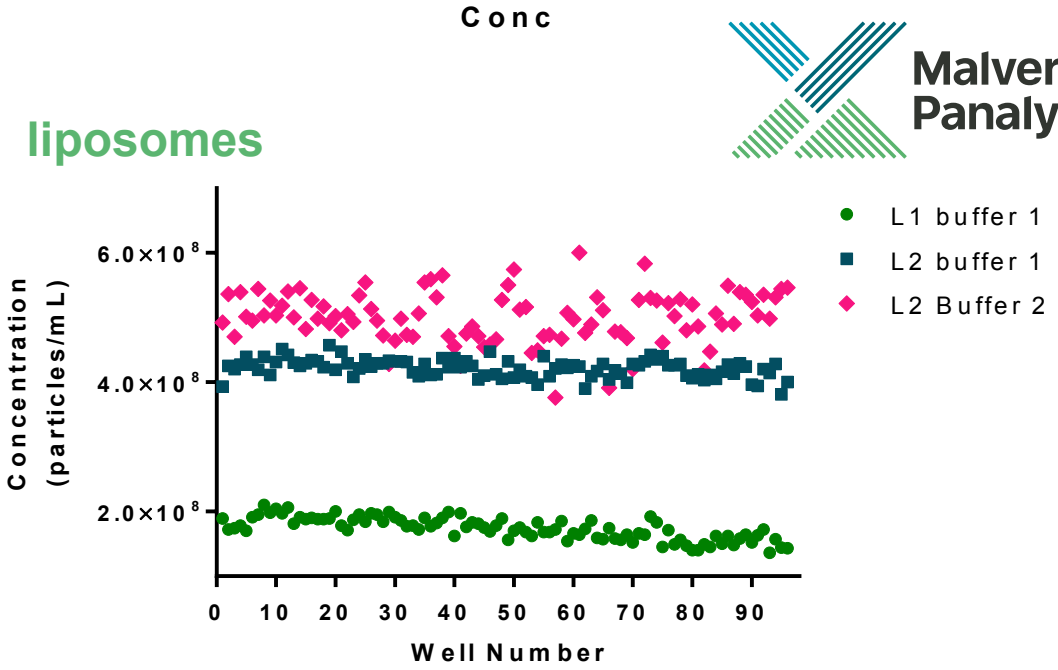
96 well plate 3x60 second captures in flow-mode, data generated in 24 hours

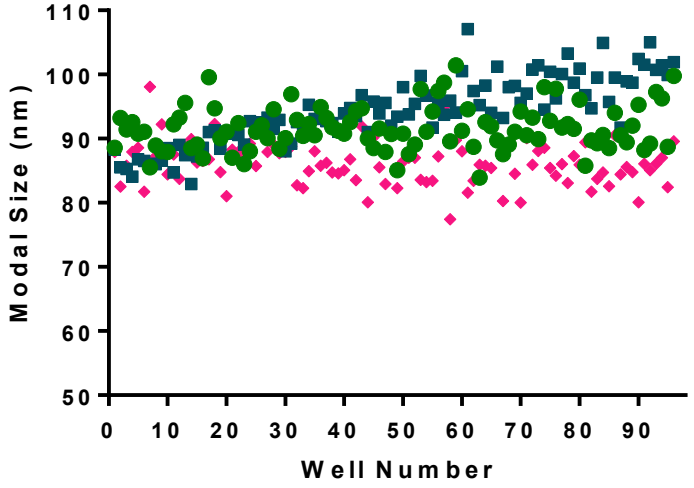




% Coefficient of Variation	Size (Mode, nm)	Concentration (particles/mL)
Expert user	1.24	7.75
Sample Assistant	0.57	2.35

liposomes



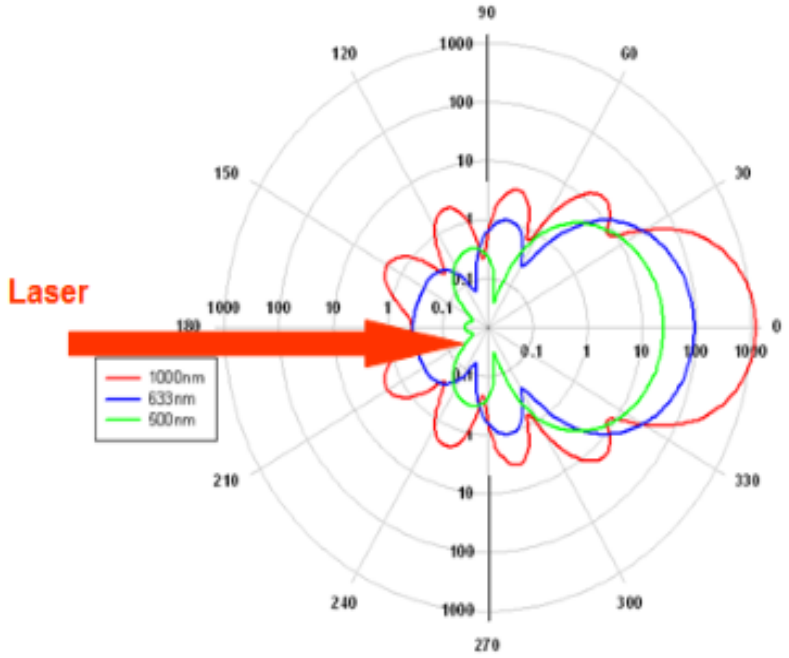


Size and concentration monitored over 15hours.

Most stable condition for these liposomes identified as L2 Buffer 2.

Summary

NanoSight Sample Assistant Specifications	
Sample capacity	96 max (1 x 96 Deep well plates)
System set-up time	Under 30 minutes
Sample measurement time	Under 10 min/sample for a 3x60s measurement including clean and analysis
Cross contamination	<0.1%
Required sample volume per sample	1000uL
Sample compatibility	Aqueous (Low Volume Flow Cell)
Availability	With new NS300 orders or as an After Sales accessory



Concentration and higher resolution size distribution from multi-angle dynamic light scattering

Malvern Panalytical

10 May 2018

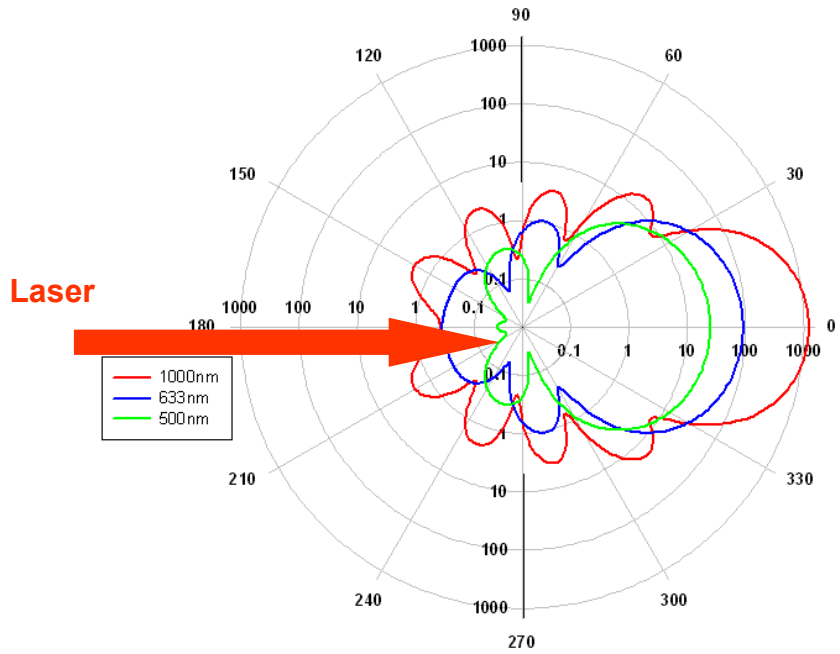
Higher resolution sizing and particle concentration per population

by multi-angle dynamic light scattering

- What does it do?
  - Combines scattering from multiple angles into one result giving sample size distribution and concentration

- What do we need?
  - Scattering data from multiple angles
  - Buffer scattering background data
  - Material and dispersant absorption and refractive index
  - System scattering sensitivity (one off reference sample measurement, normalising the attenuators)

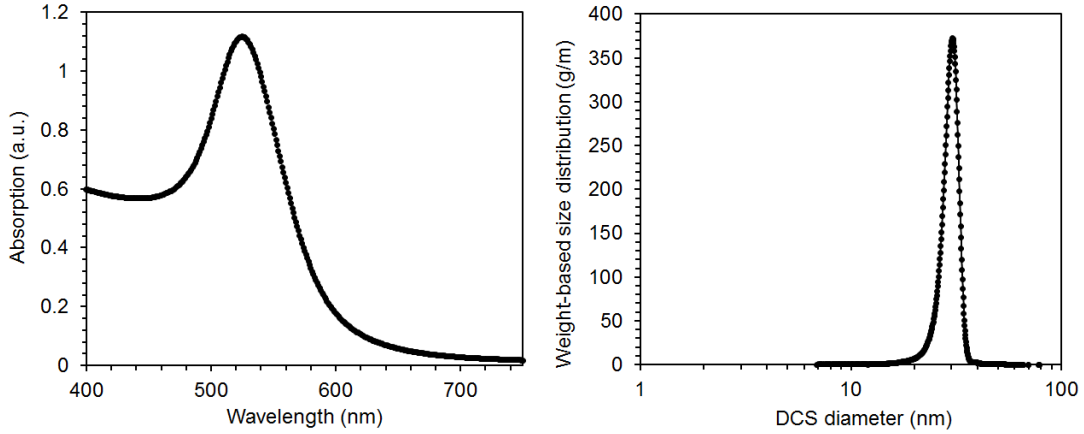
- What does it give you?
  - Cuvette based measurements of size and concentration for a range of materials: liposomes, gold, silica, protein, virus etc
  - increased size resolution – down to 1:2 for some systems* (e.g 150nm vs 300nm PS latex)
  - Particle concentration (#/mL) for each resolvable particle population

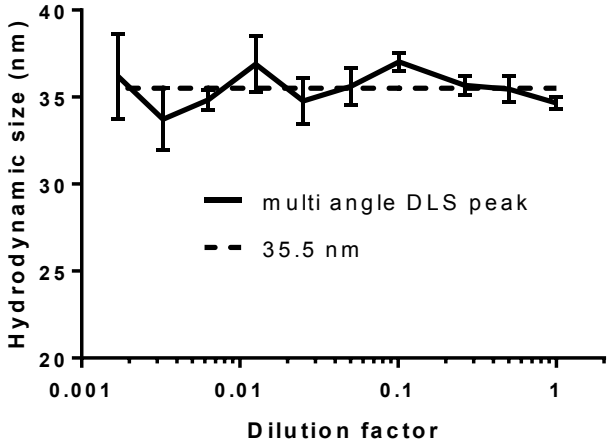


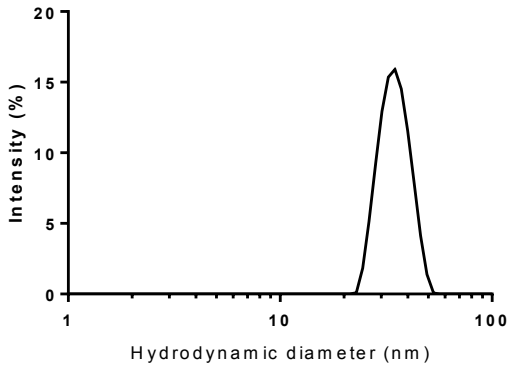
Concentration measurements of 30nm gold particles

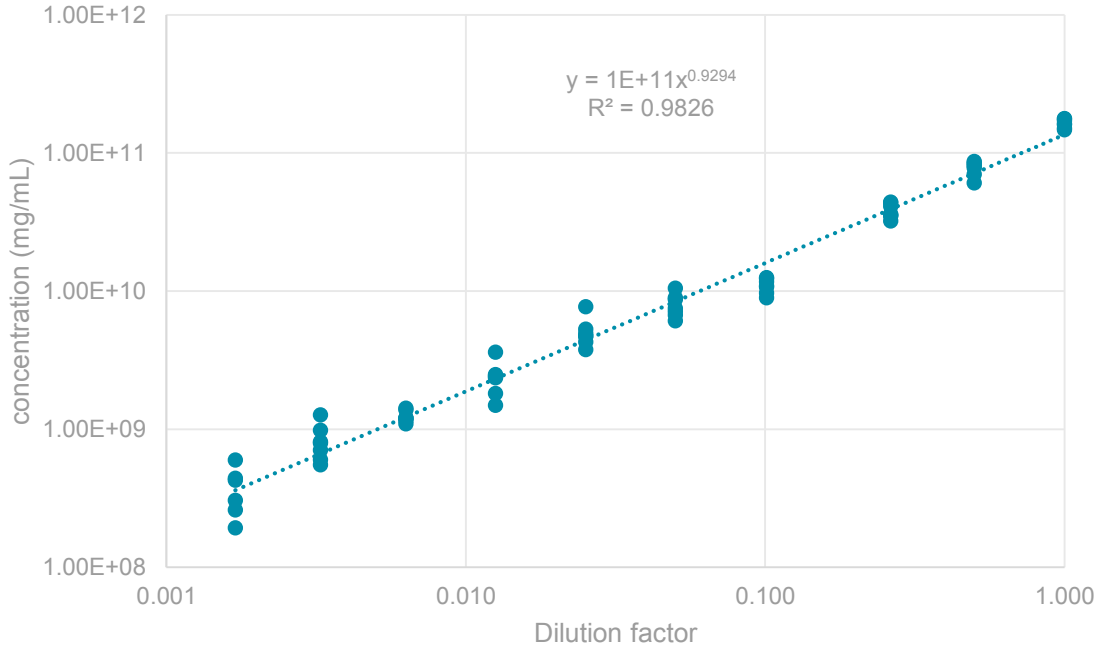
collaboration with Caterina Minelli and Magdalena Wywijas

- 30 nm gold particles from BBI solutions



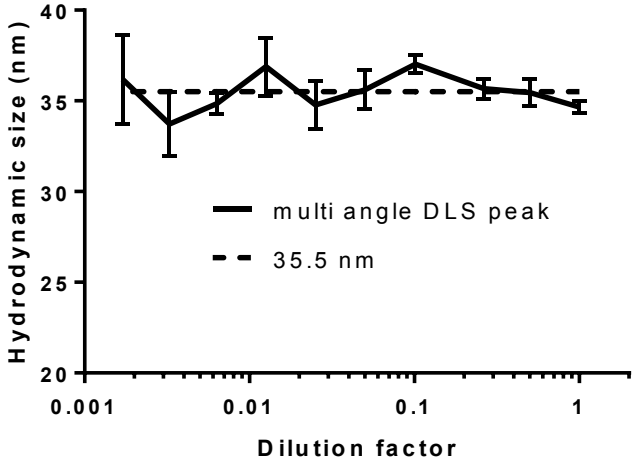


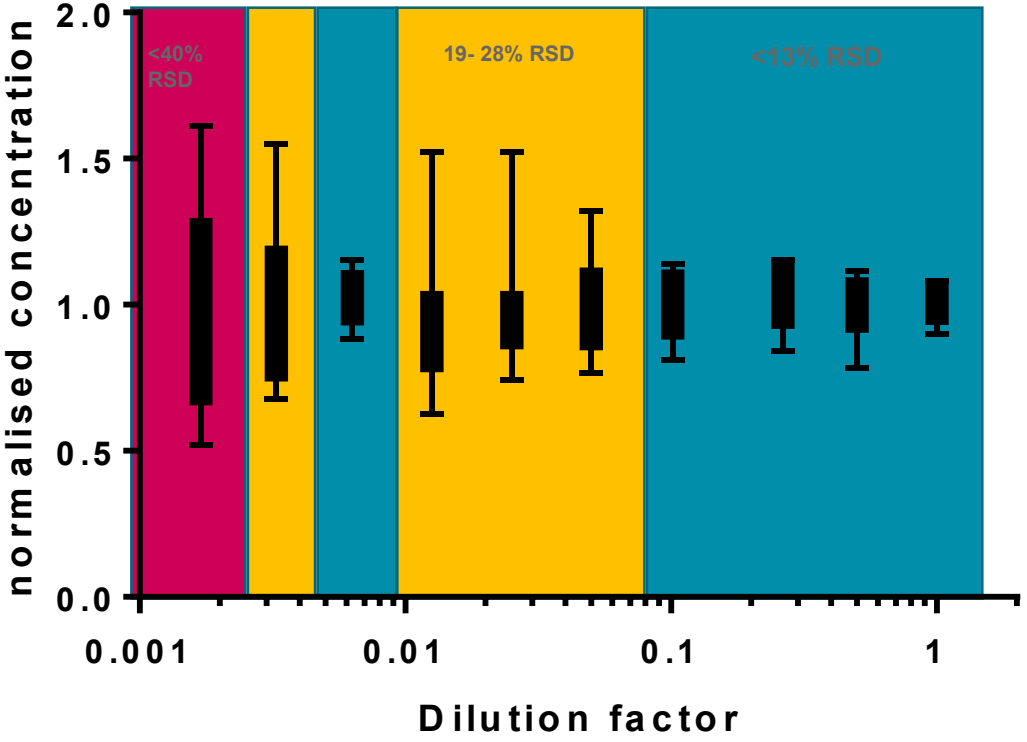


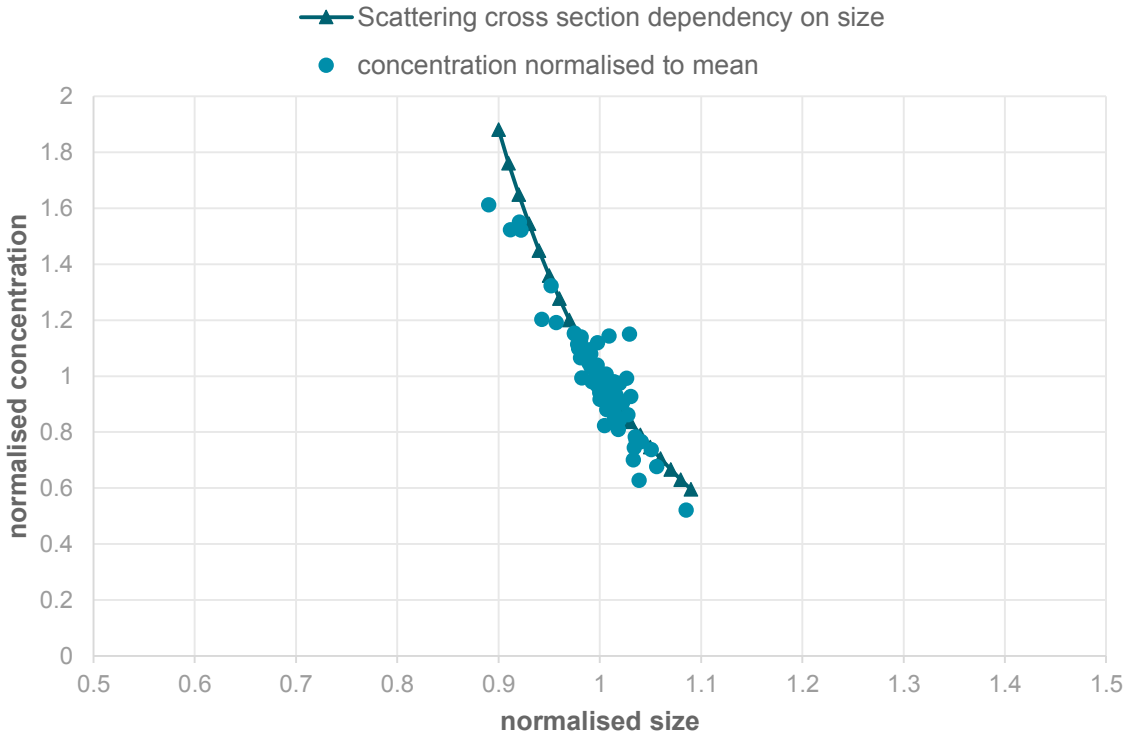


Concentration repeatability

Dependency on size measurement…



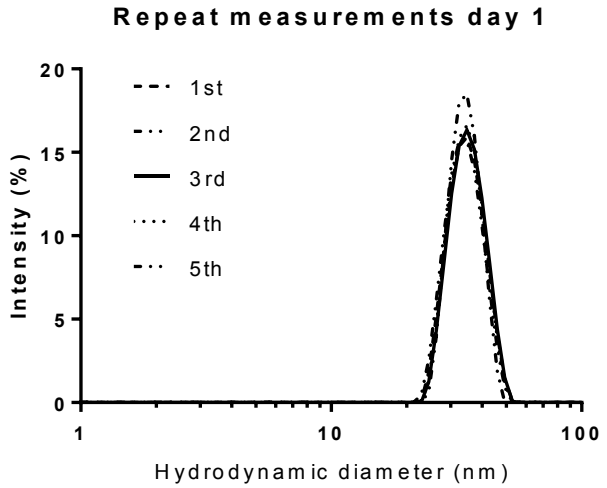


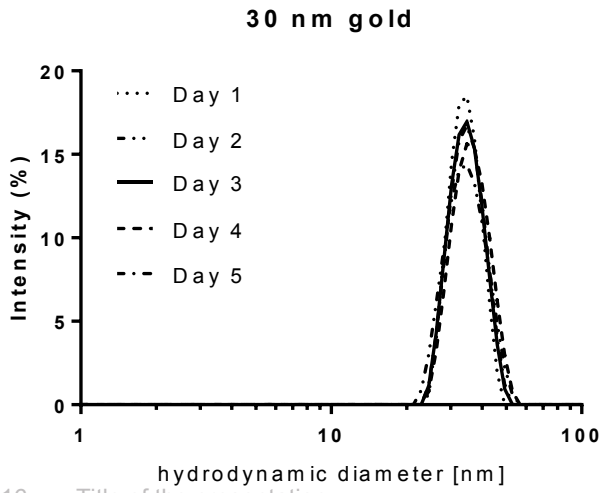


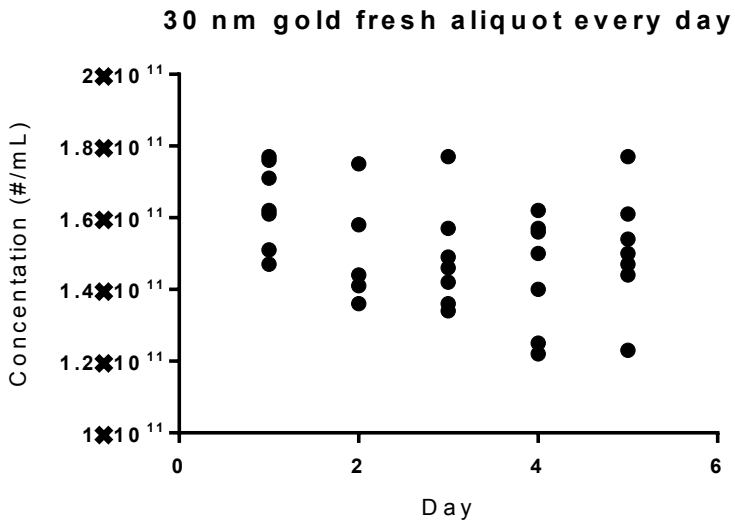
How accurate and repeatable are the concentrations?

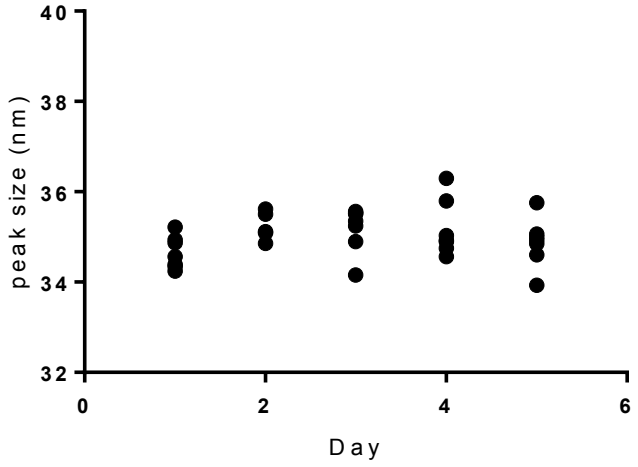
collaboration with Caterina Minelli and Magdalena Wywijas

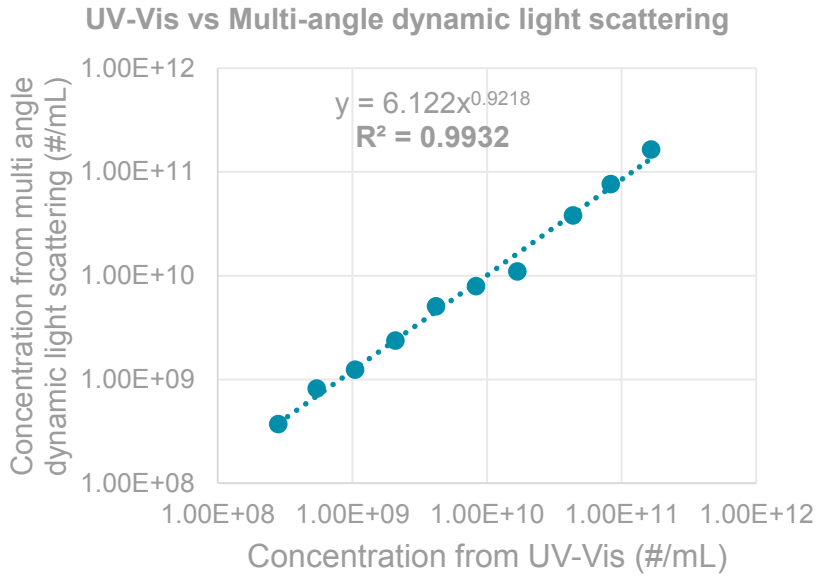
- Fresh aliquot every morning from the same stock solution (kept in fridge)





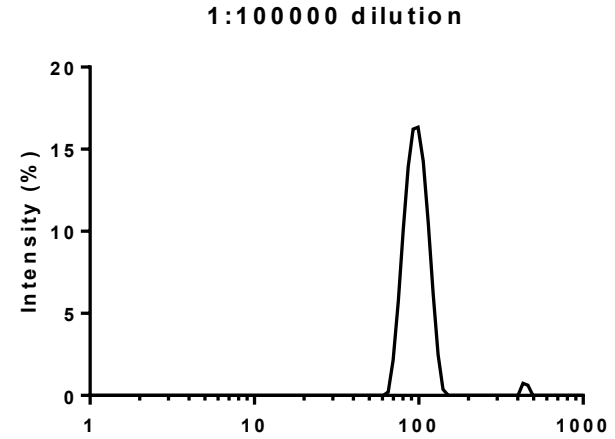


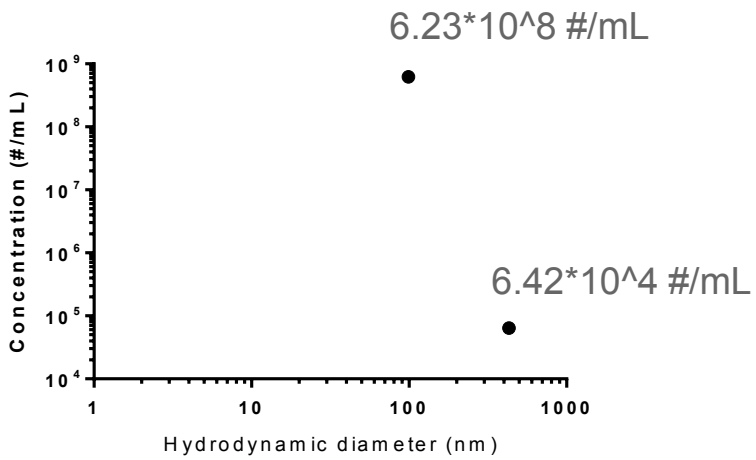


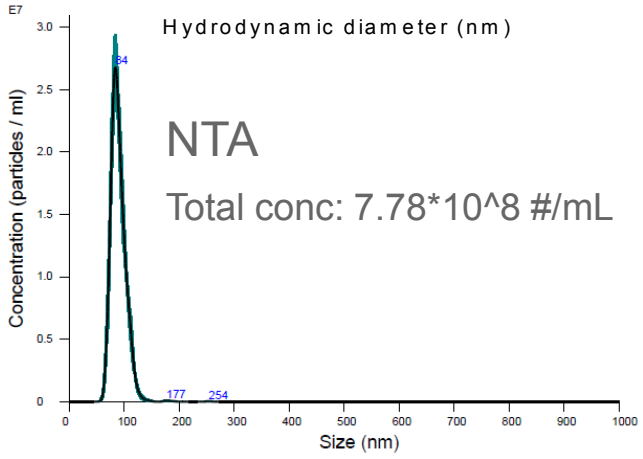


Liposome size and concentration measurements

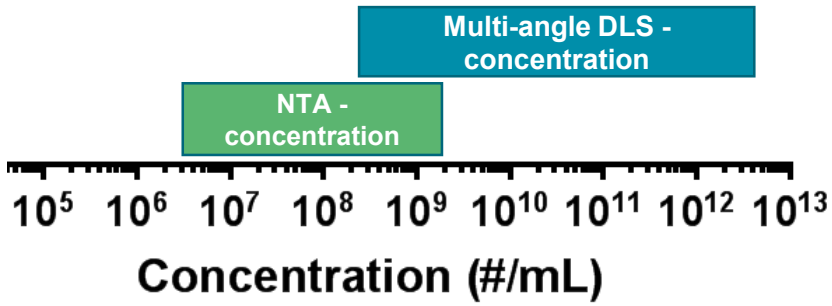
Examples from a dilution series from stock (36.5mg/mL HSPC/CHOL 55:45 Liposomes) down to 1:10,000,000

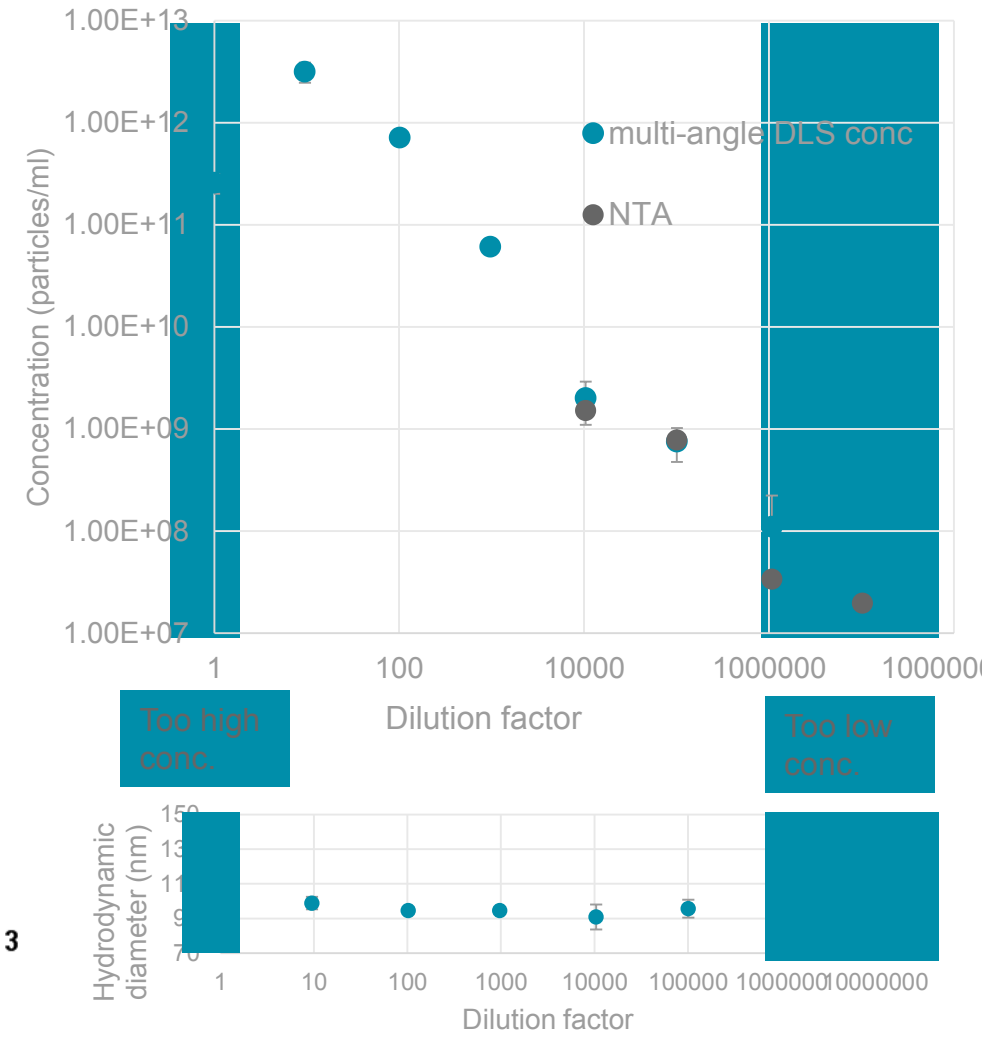






Averaged FTLA Concentration / Size for Experiment:
100,000x dilution 2018-04-20 10-39-00
Error bars indicate + / -1 standard error of the mean





Summary

Advances in particle concentration measurements

- **NTA and Sample Assistant** provides higher throughput NTA measurement, with reduced variation
- Cleaning protocols ensure reliable sampling and minimises cross-contamination
- Sizing, polydispersity, sub-population separation and concentration all available with the Sample Assistant
- Flexible software to set up multiple different measurements across a plate, to reduce operator time spent on measurements and analysis
- **Multi-angle dynamic light scattering** provides a higher resolution and concentration per population in a quick cuvette based measurement without the need of calibration or separation
- It can go down to 1:2 in resolution, e.g. 150 vs 300nm latex
- It can measure a wide range of sample materials such as gold, silica, liposomes etc
- The concentration range is across approx. 4-7 orders of magnitude depending on particle size and particle material

Thank you for your attention
Questions?

www.malvernpanalytical.com