

# Characterisation of Subvisible Particles and Nanomaterials with Nanoparticle Tracking Analysis and Dynamic Light Scattering



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# Overview



**Introduction into Nanoparticle Tracking Analysis**

**Dynamic Light Scattering**

**Application Example NTA/DLS**

# Nanosight

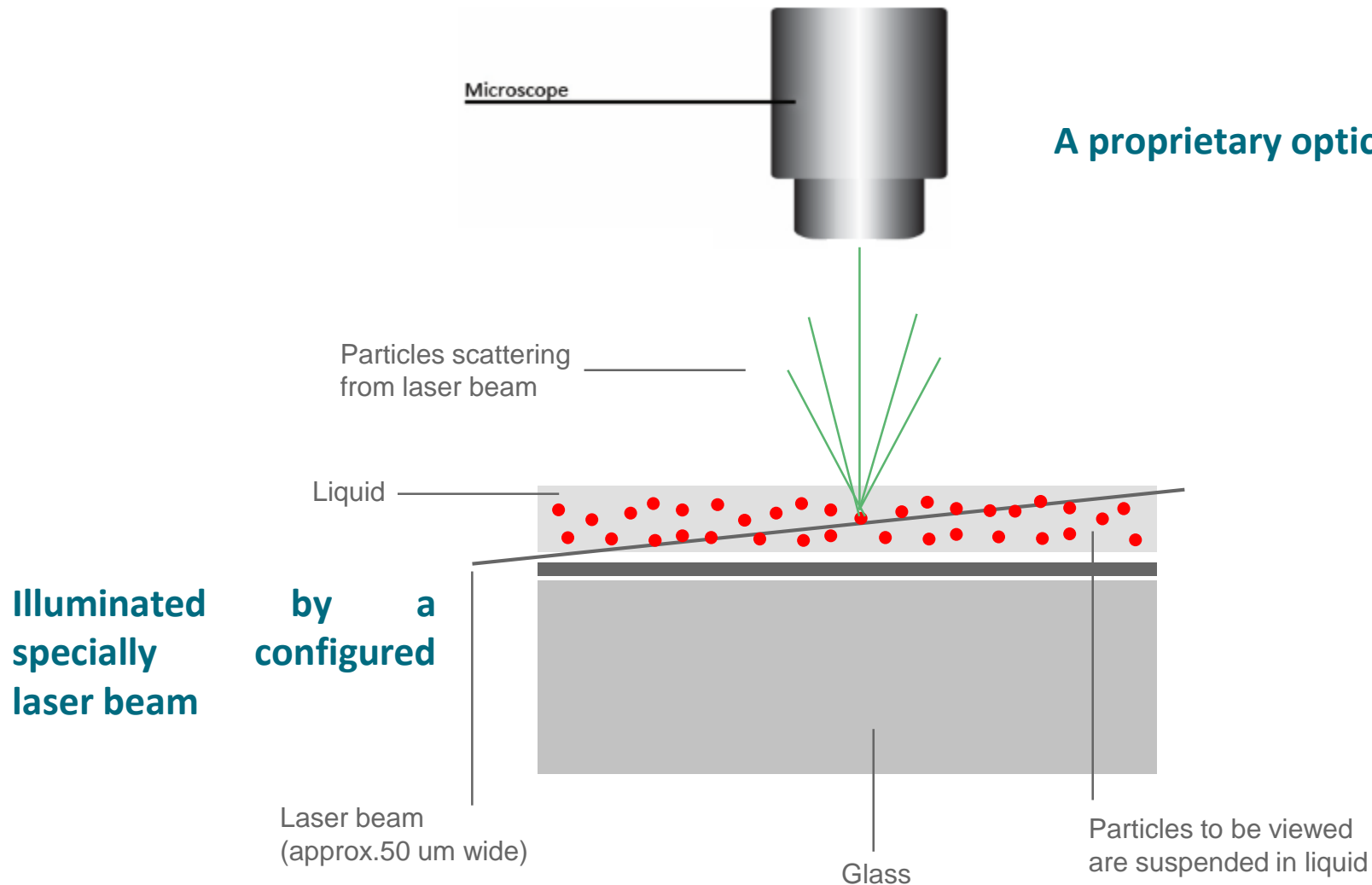
## nanoparticle tracking analysis



## Principle and Set up



# NANOSIGHT TECHNOLOGY



A proprietary optical element

## Minimum Size limit is related to:

- Material type
- Wavelength and power of illumination source
- Sensitivity of the camera

**10 – 40 nm**

## Maximum Size limit is related to:

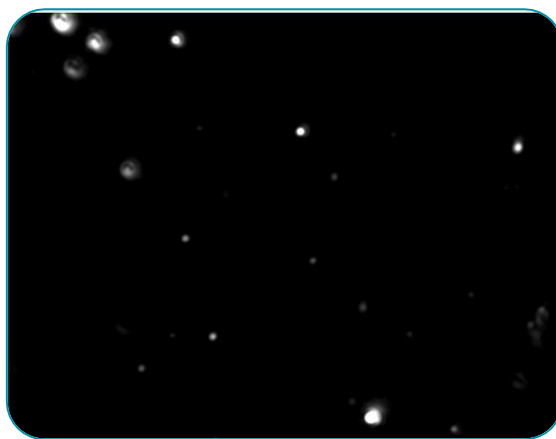
- Limited Brownian motion

**1000-2000 nm**

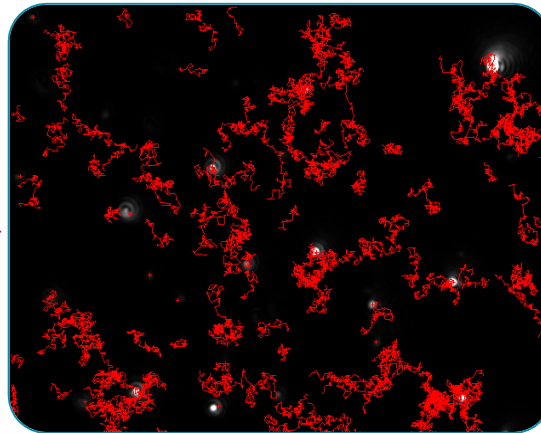
Schematic of laser sample chamber

# NTA Experimental Protocol

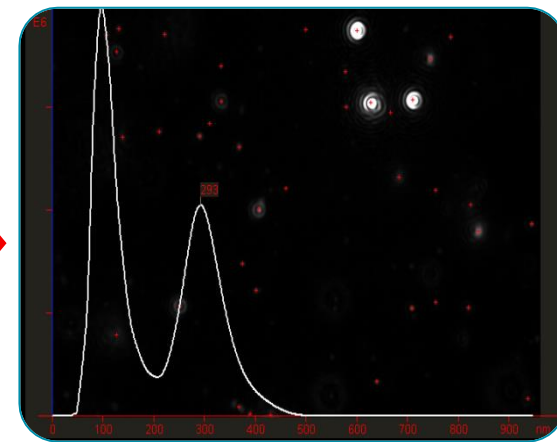
1. Capture: The software captures a movie file of the particles moving under Brownian motion
2. Tracking: The software locates each particle and tracks the motion of each particle independently, yielding mean square displacement.
3. Analysis: Application of the Stokes Einstein equation converts mean square displacement to particle size. The distribution is an accumulation of all the single particle measurements.



**Capture (~60 sec)**



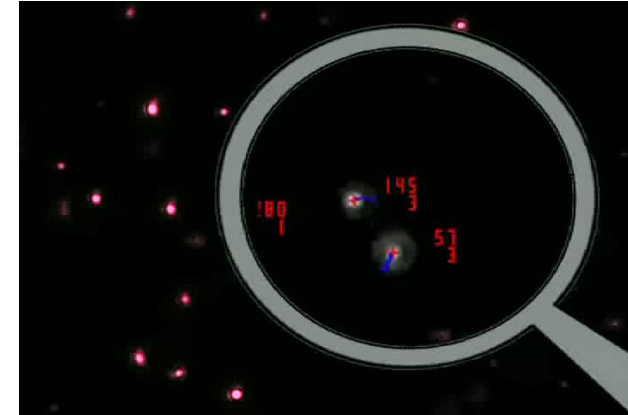
**Tracking**



**Analysis**

# Sizing: Stokes-Einstein

- Measure particles' mean square displacement (MSD) due to Brownian motion
- Calculated parameter is particle's sphere equivalent hydrodynamic diameter.
- Temperature measured and appropriate viscosity used.
- Absolute method – no calibration required
- Independent of refractive index, density or mass.



$$\langle \text{MSD} \rangle = \frac{4K_B T t_s}{3\pi\eta d_h}$$

*Stokes-Einstein equation*

$K_B$  = Boltzmann Constant

$T$  = temperature

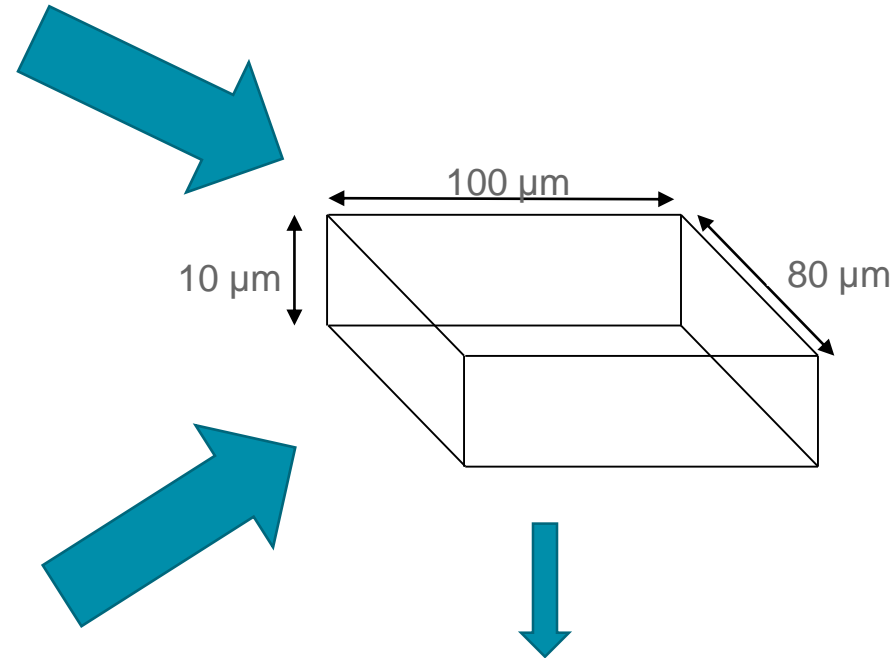
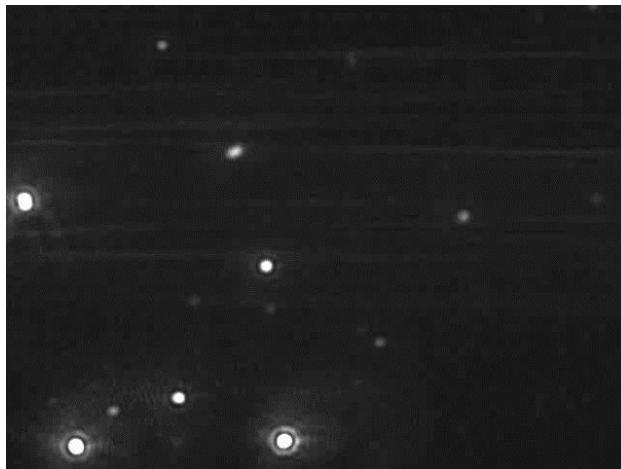
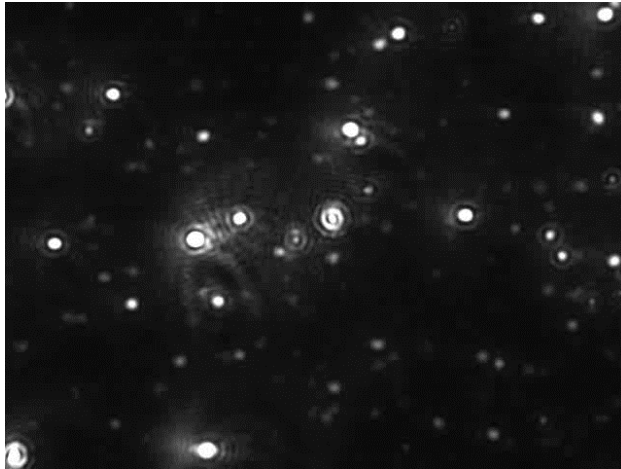
$t_s$  = sampling time

$\eta$  = viscosity

$d_h$  = hydrodynamic diameter

# Concentration: Quantify particles in known volume

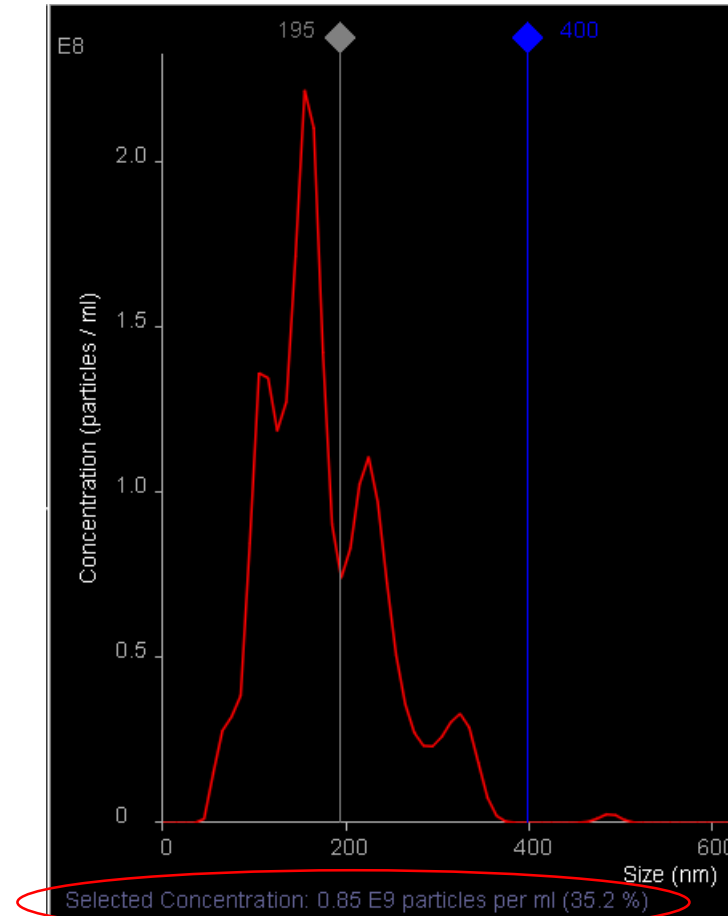
(total viral/particle titer)



**Absolute number concentration**

# Concentration of selected range

- Concentration can be reported for any subset
- Data is accumulation of individual particle measurements, so any statistical measure can be applied.



Concentration info:  
Particles per frame : 122.2  
Particles per ml : 2.4 x 10e9



# NTA Specifications: Size and Concentration Limits



## Size



**Minimum** size limit is related to:

- › Material type
- › Camera Sensitivity
- › Laser wavelength

**Maximum** Size limit is related to:

- › Limited Brownian motion
- › Viscosity of solvent

## Concentration



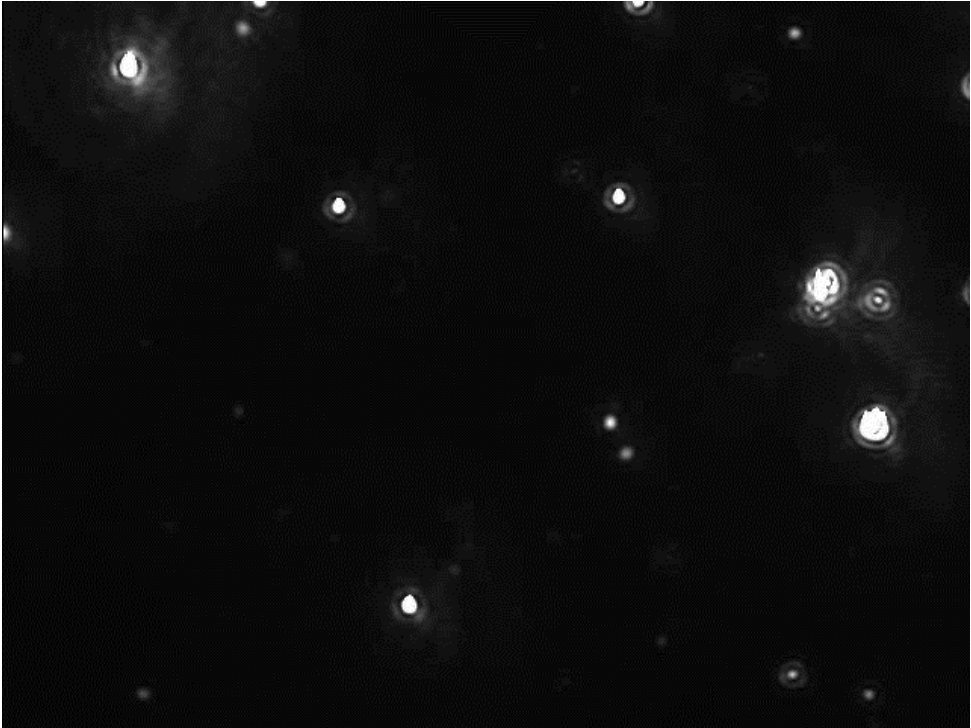
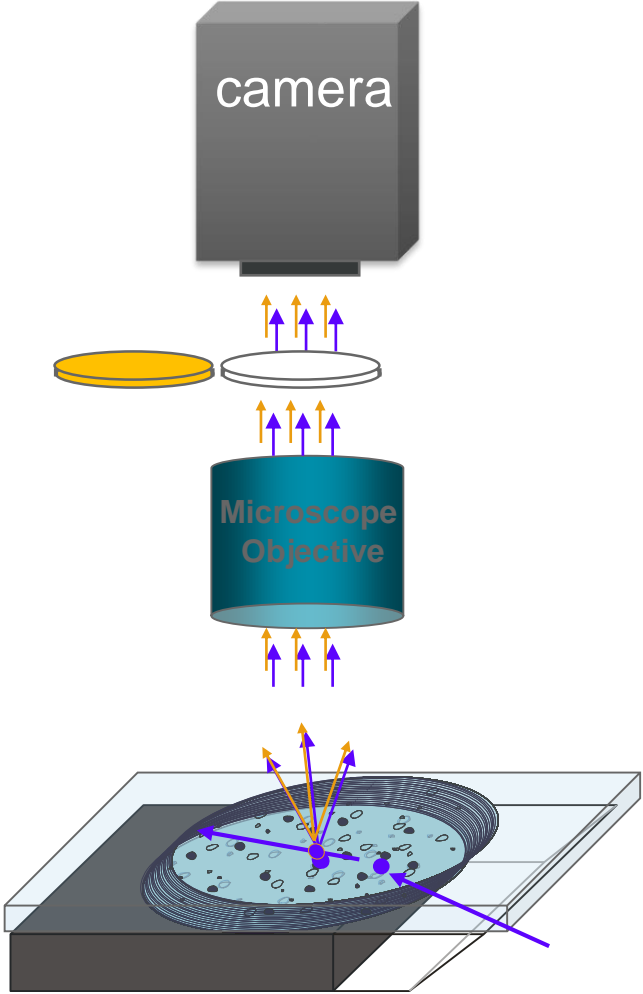
**Minimum** is related to:

- › Poor statistics (Requiring longer analysis time)

**Maximum** is related to:

- › Inability to resolve neighboring particles
- › Tracks too short before crossing occurs

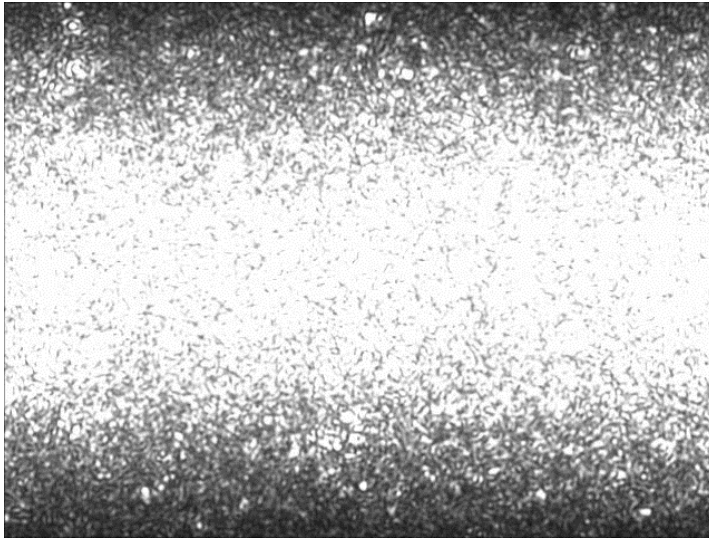
# Fluorescence measurement (optional)



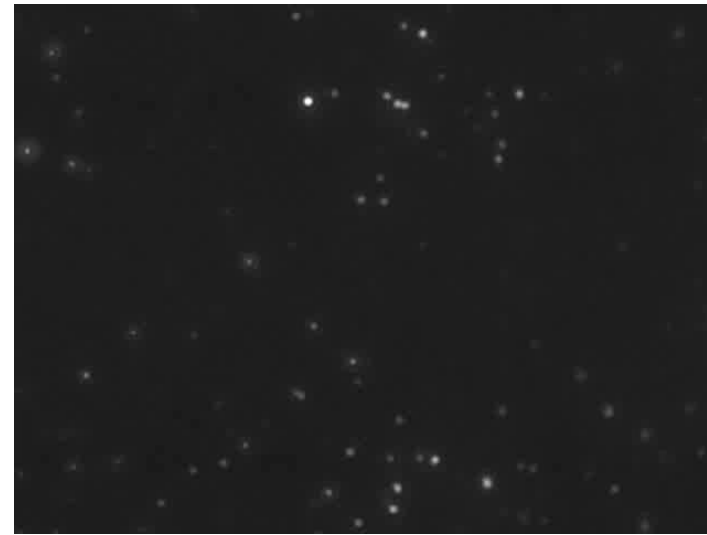
Video of fluorescence process

# Analysis in Complex Biological Media

- 100nm fluorescent particles in FBS



Scatter Mode  
(all particles)



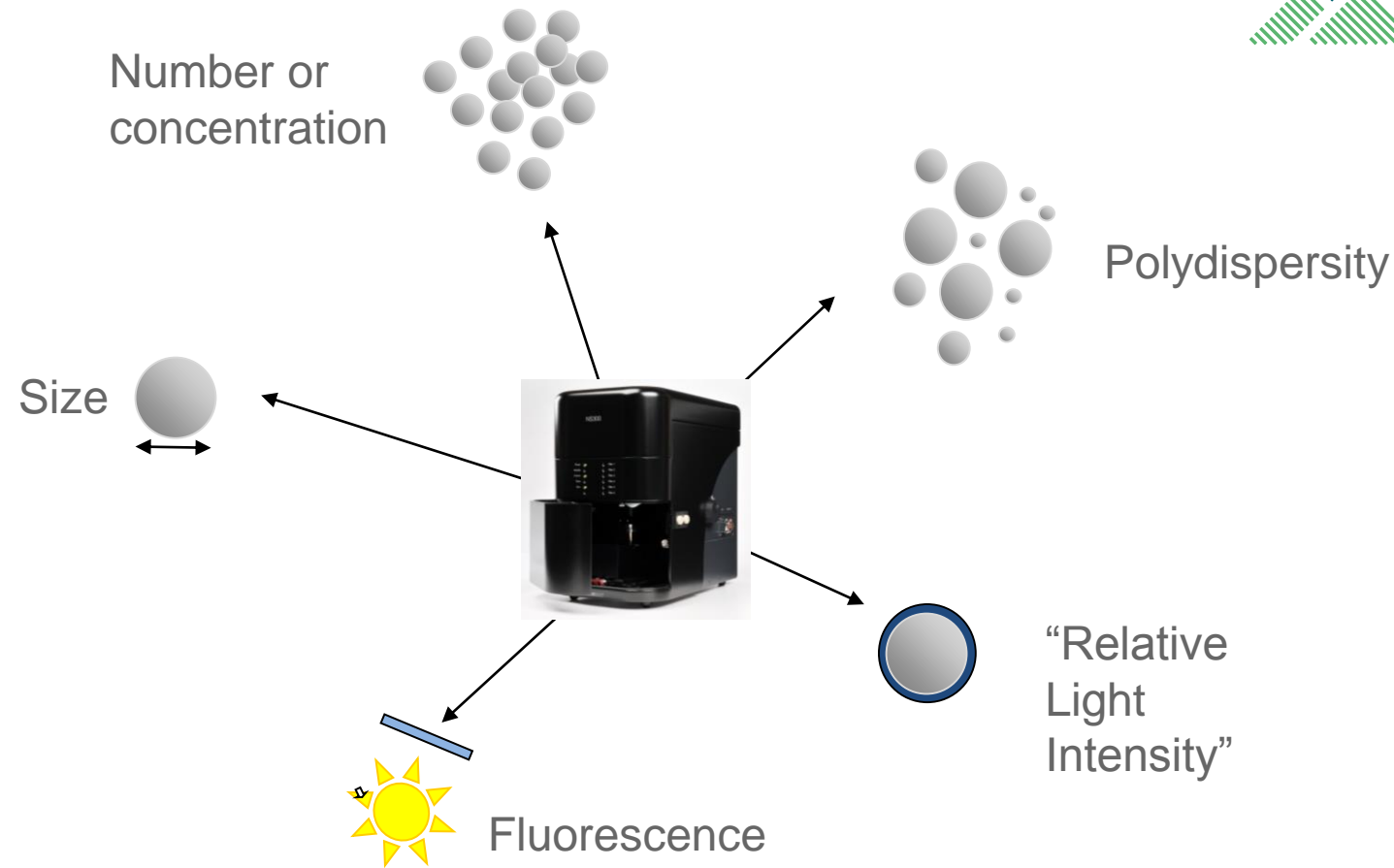
Fluorescence Mode  
(only labelled particles)

# Available options for fluorescence



Available laser sources (nm)	Standard filter provided (nm)	Fluorophores known to work based on customer feedback	
Violet 405	430 long pass	-QD605 -QD625 -QD705	
Blue 488	500 long pass	-AlexaFluor 488 -PE -E-GFP / GFP -DiO -FITC	-PerCP -FM1-43 -Anti-HA (H5N1) -PKH67
Green 532	565 long pass	-PE -Nile-Red -Spiro-Red -Cy3	-Rhodamine-PE -Alexa 546 -Dil -PKH26
Red 638	650 long pass	-AlexaFluor 647	

# Summary Parameters measured by NTA



...simultaneously, 'real time', particle-by-particle  
.....with visual validation



## News: Zetasizer Ultra advanced features

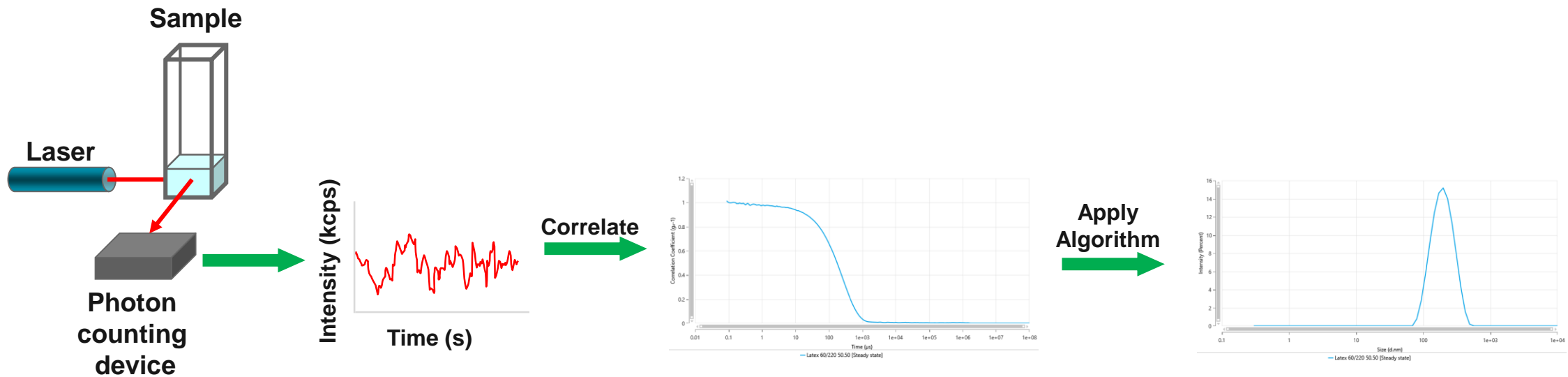
# Dynamic Light Scattering

## What is it and How does it work?

- Non-invasive technique for measuring the size of **particles** and **molecules** dispersions
- Analyses the **time-dependence** in the **intensity of the scattered light (auto correlation)** to determine their **diffusion speed (Brownian motion)** and subsequently their **hydrodynamic size**

*(A. Einstein (1926) Investigations on the theory of the Brownian movement.*

*In: Fürth R., ed. Cowper, A.D., translator. Methuen, London, 124)*



# Multi Angle Dynamic Light Scattering

## How it Works

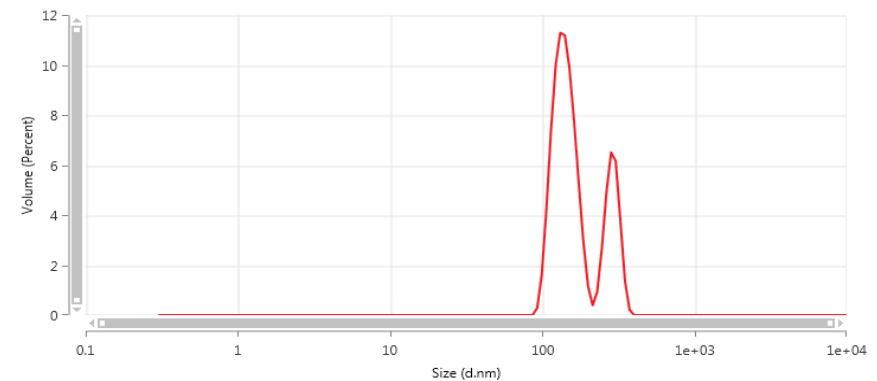
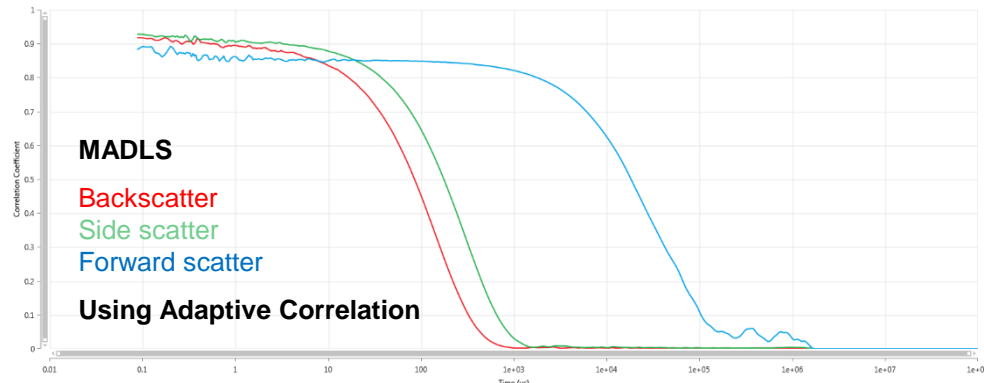
Merge  
autocorrelation data  
from all angles

Calculate predicted  
autocorrelation  
functions for 3  
angles

Apply weighting  
based on Mie theory  
& known optical  
properties

Minimize residual  
between measured  
and calculated  
correlograms

Extract PSD as  
intensity, volume or  
number weighted





# Particle Concentration



- Particle concentration measurements are an extension of **MADLS**, and require no calibration
- The **total scattering** detected from the sample is converted into **number of particles per mL** by:
  - Using the **PSD** obtained from MADLS to determine how much each **individual population is scattering**
  - Using the PSD to calculate the scattering cross-section and amount of **scattering per particle**
- Instrument **detection efficiency** is normalized using toluene (known **Rayleigh ratio**)

Complementary  
technologies:

Zetasizer Ultra and NTA



# Comparing DLS and NTA



- Understanding what each instrument does well, what are their limitations is the basis of deciding which instrument to select and when both may provide additional information.

## › DLS Strengths:

- **Dynamic Size Range**
- **Concentration Dynamic Range (NIBS)**
- **Ease of Use**
- **Routine Zeta potential measurement**
- **Recognized technology.**
- **Very good trend spotter. Microrheology**

## › NTA Strengths:

- **Higher resolving power (peak-to-peak) due to single particle measurement.**
- **Measurement of particle concentration.**
- **Fluorescence mode speciation**
- **Less bias towards aggregates in sample.**
- **Image of particles provides valuable information.**
- **Measures particle diffusion coefficient.**

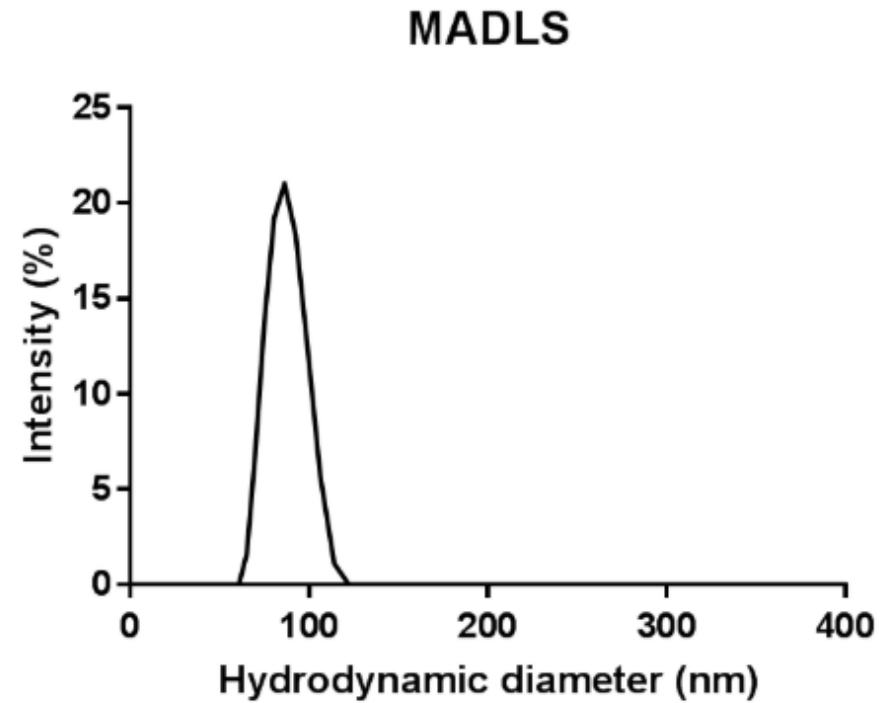
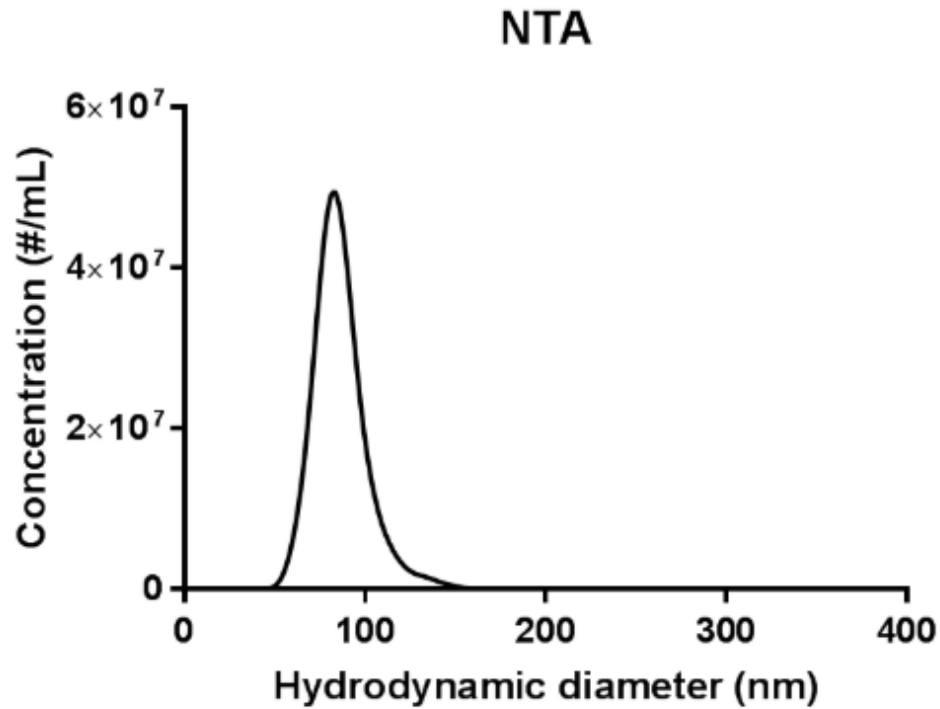
# Why use both?

- Limitations of one addressed by the other
- Complementary data
- How can I trust my data?
- Is the data affected in any way by the measurement technology?
- Or by the user; through sample preparation or analysis parameters employed?
- Together = **Comprehensive suite of measurement parameters**



# Particle Distribution

## DLS and NTA 1:10000 dilution Liposomes



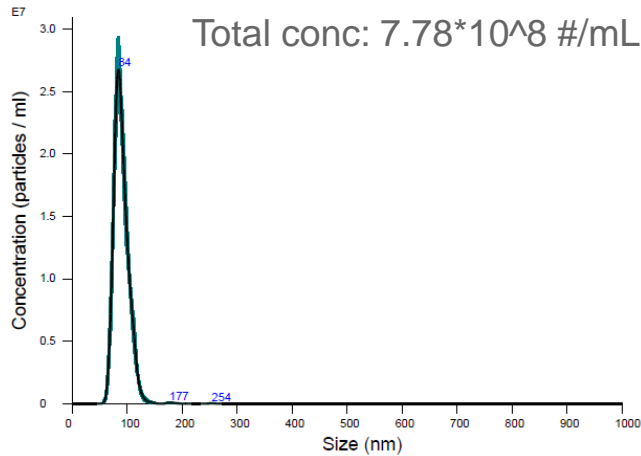
# Particle Concentration

Liposomes (36.5mg/mL HSPC/CHOL 55:45 Liposomes) down to 1:10,000,000



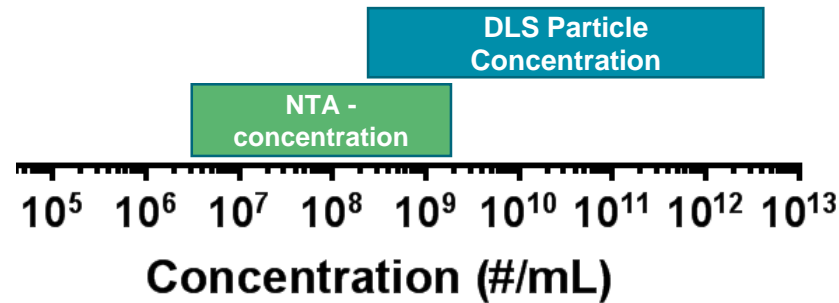
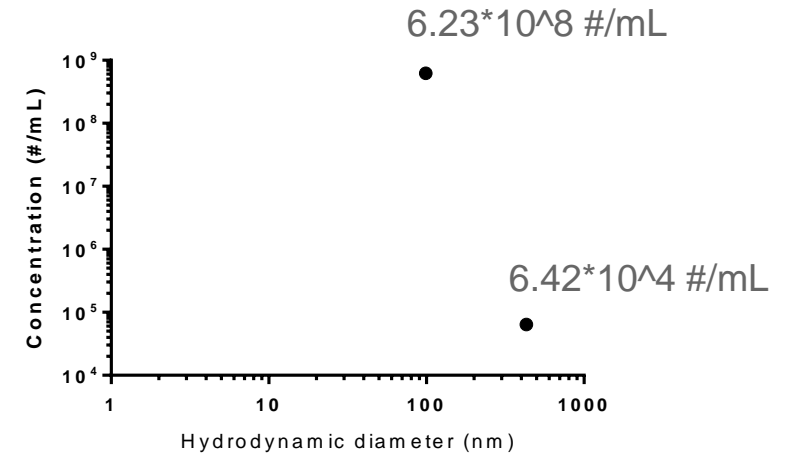
NTA

Total conc:  $7.78 \times 10^8$  #/mL



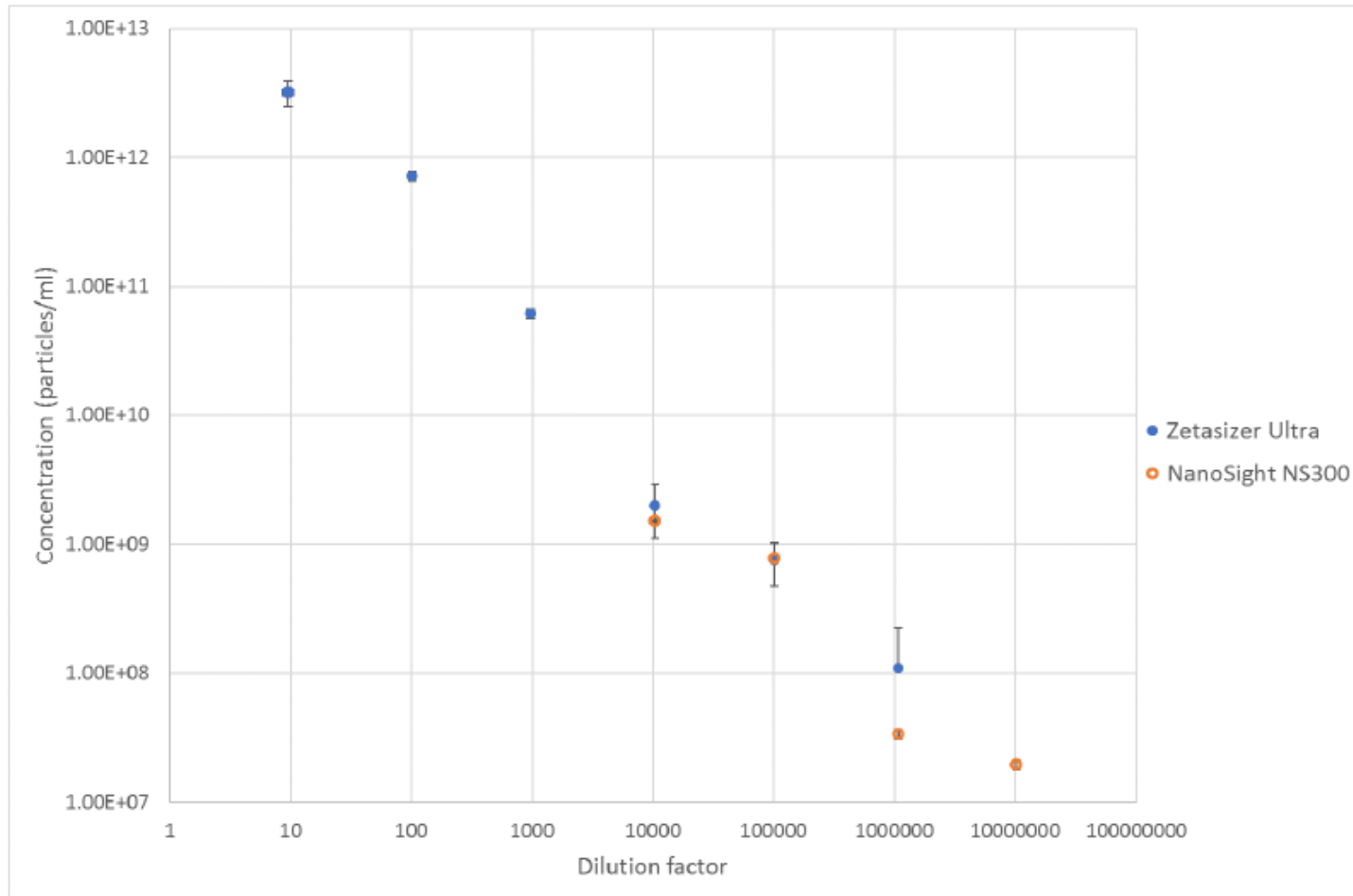
Averaged FTLA Concentration / Size for Experiment:  
 100,000x dilution 2018-04-20 10-39-00  
 Error bars indicate + / -1 standard error of the mean

DLS Particle Concentration



# Particle Concentration

## Liposomes measured with NTA and DLS



Measured with DLS and NTA

Application Note AN180519

*Figure 3 Concentration results from measurements of liposomes on a Zetasizer Ultra and Nanosight NS300.*

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