Workshop Training Series

NanoSight NS300 for Nanoparticle Characterization

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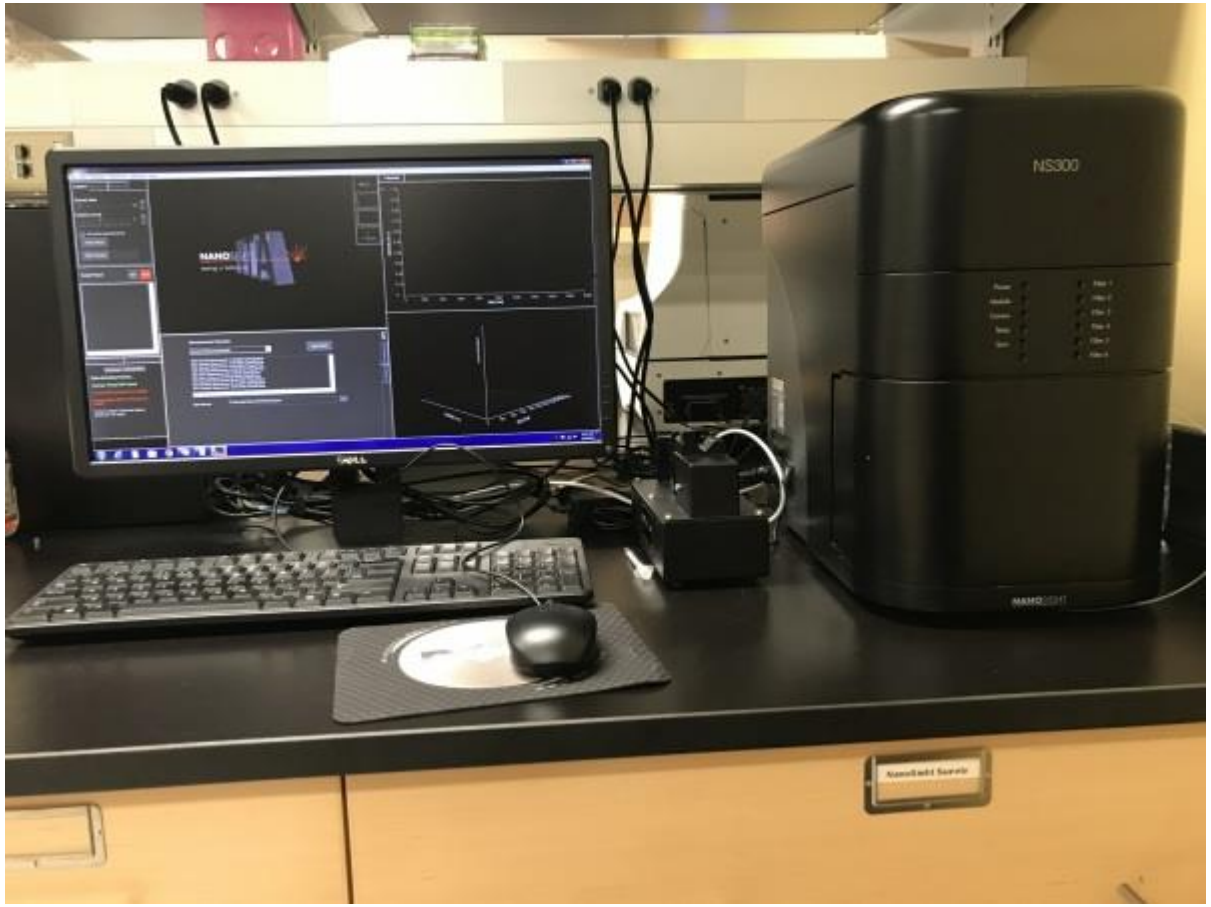
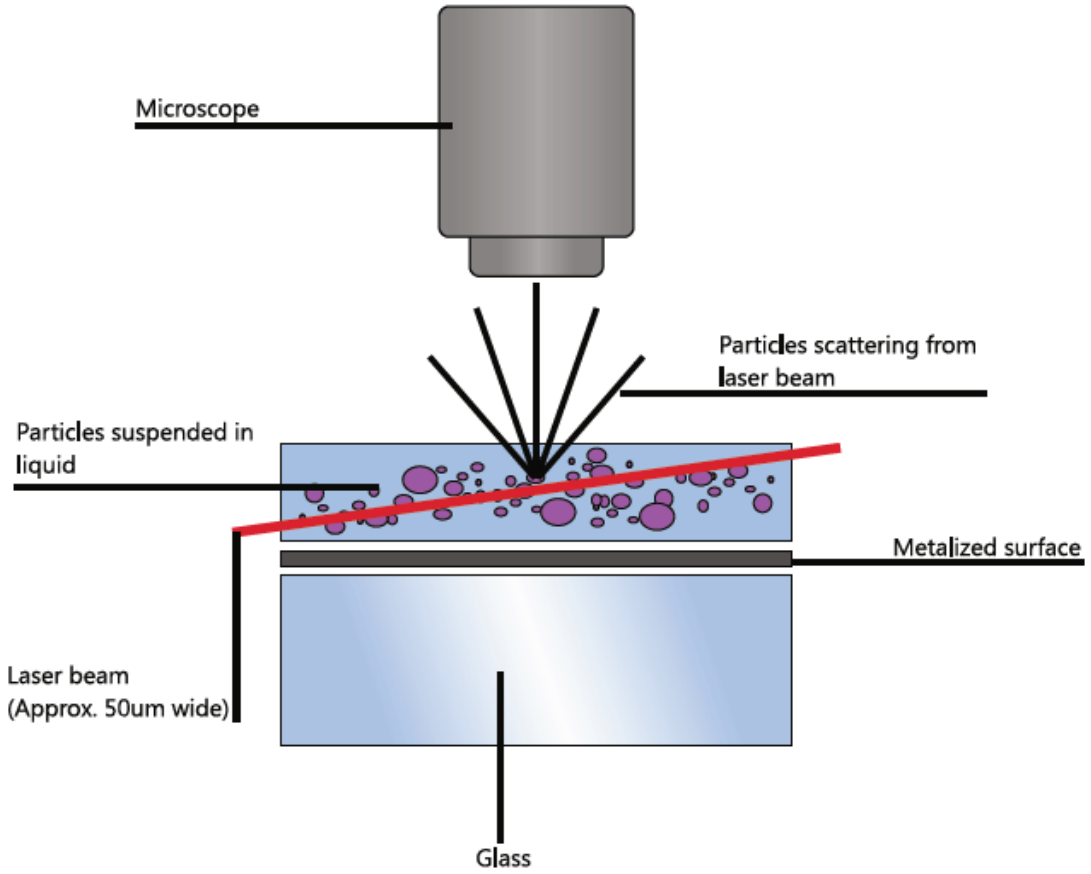
What's NanoSight300



The NanoSight series of instruments utilize Nanoparticle Tracking Analysis (NTA) to characterize nanoparticles in solution

This particle-by-particle methodology produces high resolution results for particle size distribution and particle concentration.

Schematic of laser sample chamber



Nanoparticle Tracking Analysis (NTA) utilizes the properties of both light scattering and Brownian motion in order to obtain the particle size distribution of samples in liquid suspension.

How to determine the size of the particles

$$msd(\tau) = \langle \Delta r(\tau)^2 \rangle = \langle [r(t+\tau) - r(t)]^2 \rangle = 2dD\tau \text{ (Einstein 1905)}$$

msd is mean-squared displacement.
r(t) is the position of the particle at time t, and τ is the lag time between the two positions taken by the particle used to calculate the displacement $\Delta r(\tau) = r(t+\tau) - r(t)$.
The average <...> designates a time-average over t and/or an ensemble-average over several trajectories.
The d is the dimension of the particle movement (here is 2)
This equation is used to calculate D, the diffusion constant that can be used to calculate the particle diameter d_H.

$$d_H = \frac{kT}{3\pi\eta D}$$

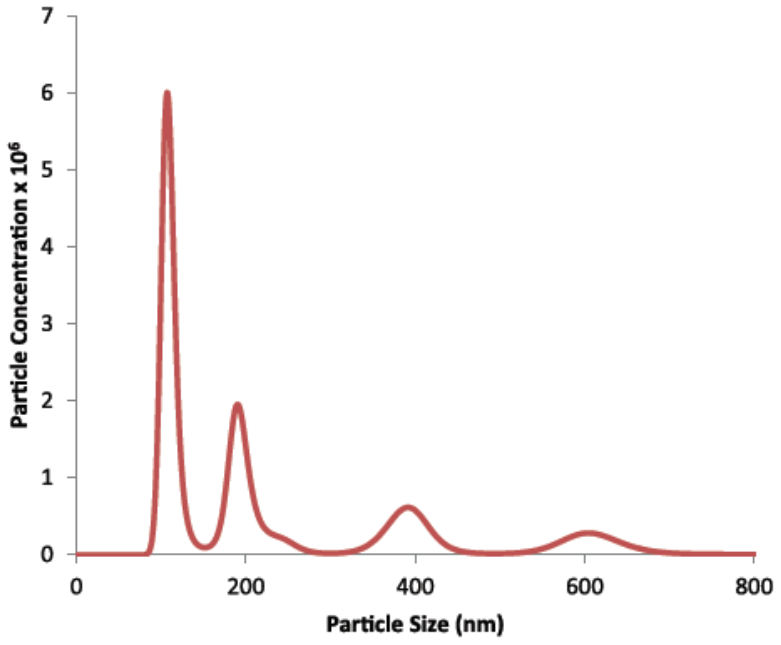
Stokes–Einstein equation

D is the diffusion constant;
η is the viscosity of the medium
k is Boltzmann's constant;
T is the absolute temperature.

How to determine the distribution of the particles

- A particle size distribution can, in principle, be formed simply by forming a histogram of the number of occurrences of a large number of individual size measurements.

- NTA 3.0 employs an improved and high-resolution finite track length adjustment (FTLA) particle size distribution algorithm which provides a better separation of different particle size populations



Graph of a sample with distinct peaks at 100 nm, 200 nm, 400 nm and 600 nm.

How NTA works?

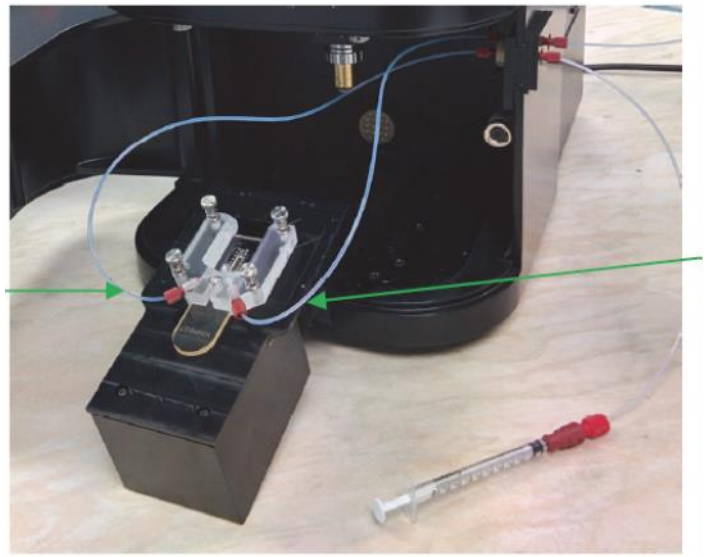
- Brownian motion of each particle is recorded in real-time via video camera.
- Video analysis software measures **mean square displacement** in two dimensions =diffusion coefficient (D)
- **Particle diameter** (sphere equivalent hydrodynamic) d_H is then obtained from the Stokes-Einstein equation
- **Finite track length adjustment (FTLA)** particle size distribution algorithm is used for distribution analysis

http://web.mit.edu/savin/Public/.Tutorial_v1.2/images/tracking.html

How to Use NanoSight300?

The process to use NanoSight300 can be generalized as four steps:

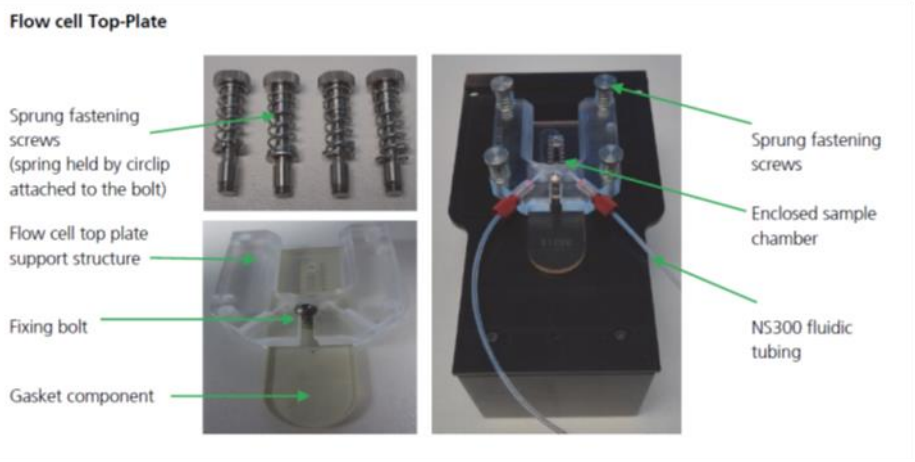
- Load samples
- Optimize the image
- Take measurements
- Data Analysis



https://cehs.unl.edu/borc/

Laser Module

- The major hardware part you will operate is the Laser Module with a flow-cell top-plate into which the samples are loaded.
- The laser module is very delicate and need be taken very carefully. Please follow the instruction strictly in the manual.
- Please read operational manual 2.2.4 carefully about the Assembling and Cleaning the Laser Module.



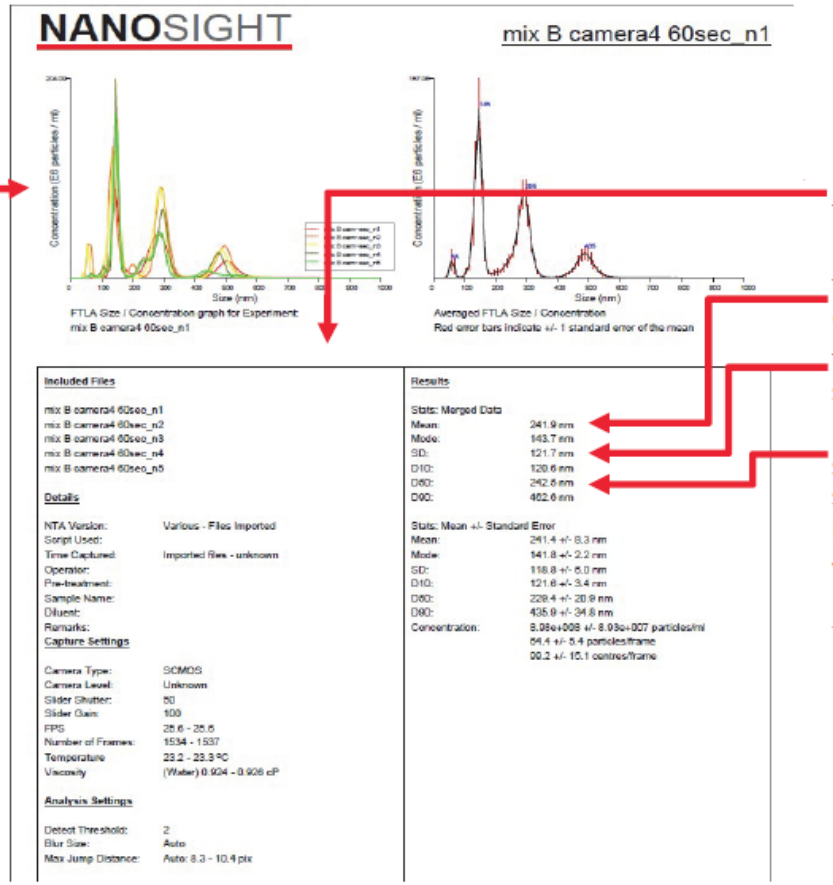
What Do The Data Look Like?

PDF Data Export

Example of exported PDF report for n=5 data from one sample;

The size distribution profile data are shown over-plotted

Mean ± SEM data are shown, with the size of the key peaks annotated.



The lower panels include data on the settings used along with a results summary.

Typically the modal particle size is used to describe the sample.

The SD is a measure of the width (spread) of the size distribution profile.

D10, D50 and D90 values indicate percent under-size, for example 50% particles are 229nm or smaller, giving another indication of the spread of particle sizes within the sample.

When vibration has been detected during the measurement, the size reported will be smaller than the true size of the particles in the sample.

Major Parameters of NanoSight300

Sample volume	300 ul
Solvent	Noncorrosive solvent + water
Size range	10-2000nm
Concentration	10^7-10^9 particles/ml (20-100 field view)

Applications in Biomedical Research

- **Protein aggregation studies**
- **Fluorescently labeled particles**
- **Exosomes and microvesicles**
- **Pharmaceutical nanoparticles-liposomes**
- **Drug delivery vehicles**
- **Viruses and Virus-like particles (VLPs)**

Rules and Precautions

- Use glass beaker for water. Do NOT use plastic tubes, and Do NOT use a syringe to get water directly from a squeeze bottle.
- Only use 1 ml syringe on the system. Be extremely gentle when pushing.
- Use the pump to push the samples.
- Wash with 5% EtOH one time, then wash with ddH2O several times before and after usage.
- Do NOT overtight the screws! which might damage the gasket and cause leaking.
- Do NOT leave the machine on if you need to leave more than 10 min.