Characterisation of Nanoparticles using Nanoparticle Tracking Analysis.

NanoSight Limited
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## The NanoSight System is Widely Applicable

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NanoSight’s Technology

As the schematic shows, the NanoSight technology comprises:

- A metallised optical element
- Illuminated by laser beam
Principle of Measurement

• Nanoparticles move under Brownian movement due the random movement of water molecules (red molecules in movie) surrounding them.

• Small particle move faster than larger particles.

• Diffusion Coefficient can be calculated by tracking the movement of each particle and then through application of the Stokes-Einstein equation particle size can be calculated.
NanoSight Sizing... is an absolute method

- Brownian motion of each particle is followed in real-time via video.
- Tracking software establishes mean square displacement and diffusion coefficient ($D_t$).
- Then from Stokes Einstein can be obtained particle (sphere equivalent hydrodynamic) diameter, $d_h$

\[
D_t = \frac{K_B T}{3\pi \eta d_h}
\]

**Stokes-Einstein equation**

- $K_B$ = Boltzmann Constant
- $\eta$ = viscosity
Particle Example – Titanium Dioxide

Titanium Dioxide (in water)

This TiO2 sample shows clear polydispersity.
Nanoparticle Tracking Analysis (NTA) is the gathering of unique information and comes from assessment of *individual* particles, rather than averaging over a bulk sample.

This provides a distinct advantage in determining particle size.
Particles are Visualised, not Imaged

Particles are too small to be imaged by the microscope.

Particles seen as point scatterers moving under Brownian motion.

Larger particles scatter significantly more light.

Speed of particles varies strongly with particle size.

Microvesicles purified from serum by ultracentrifugation
The NanoSight System is Easy to Use

Loading of 200nm latex standard.
The NanoSight NTA 2.3 (nanoparticle tracking) analysis suite allows for captured video footage to be simultaneously tracked and analysed...

100+200 nm nanoparticles being tracked and analysed by NanoSight NTA 2.3
Real-Time Applications

The capability to individually visualise every particle allows real-time characterisation of:

- Aggregation and Flocculation
- Dissolution
- Dispersion efficiency
- Microemulsion stability/breakdown
- ……and other time-related events
Characterisation of Nanoparticles using NTA

NanoSight technology has a unique application in the detection of early stage aggregation in protein therapeutics.

- Protein monomer is too small to be individually resolved by this technique, but early stage aggregates are readily detected.
- Protein monomer at high concentration causes high background noise in image, with the aggregate forming the resolvable particles.
- Both size and number of aggregates can be calculated and studied, providing insight into product stability.

Example – Protein Aggregation at 50°C

Data reproduced from Filipe et al (2010), Pharmaceutical Research, DOI: 10.1007/s11095-010-0073-2
Currently particles are distinguished by their Brownian motion.
Whilst the size of a particles influences the amount of light scattered, the particle composition also has an influence.
By characterising the amount of light scattered from a particle it is possible to distinguish between particles of similar size but differing refractive indices.
Concentration Measurement

- Defined focus volume
- Reliable calculation of the concentration
- 1-2 minute analysis time

Above image shows effective scattering volume in which particles are detected and counted.
NTA Vs. DLS

NTA (red profiles) Vs. DLS (blue bars) for mixtures of polystyrene of different sizes

**Protein Aggregation**

DLS analysis of aggregated protein sample generally produces a bimodal analysis.

The protein monomer and the very large aggregates get picked up through DLS as these are the regions which scatter most light.

The monomer scatters a lot of light by virtue of its high concentration and the larger particles by virtue of their size. Clearly a protein sample cannot aggregate from monomer to large micron sized aggregates with nothing in between. Whilst NanoSight cannot measure the monomer it can provide valuable information in the 30nm and above range which is typically the region which is poorly served by alternative techniques.
NanoSight technology has a unique application in the detection of early stage aggregation in protein therapeutics. Protein monomer is too small too by individually resolved by this technique, but early stage aggregates can be resolved.

Protein monomer at high concentration causes high background noise in image, with the aggregate forming the resolvable particles. Both size and number of aggregates can be calculated and studied, this is essential when understanding product stability.
Fluorescent Labelling

Videos shows a mixture of fluorescently labelled 50nm latex particles with 200nm unlabelled latex particles. The start of the video shows the sample being viewed under light scatter mode. Half way through the video a filter is applied to remove the signal from the 200nm unlabelled particles to leave only the signal from the 50nm fluorescent particles.
Measuring Zeta Potential
NTA Detection Limits

**Size**
Minimum Size limit is related to:

- Material type (eg. gold or protein)
- Difference of refractive index particle to medium
- Wavelength and power of laser
- Sensitivity of the camera
  - $10 – 30 \text{ nm}$

Maximum Size limit is related to:

- Limited Brownian motion
  - $1500-2000 \text{ nm}$

**Concentration**
Minimum concentration is related to:

- Poor statistics (Requiring longer analysis time)
  - approx $10^7/\text{ml}$

Maximum concentration is related to:

- Inability to resolve neighboring particles
- Tracks too short before crossing occurs
  - approx $10^{10}/\text{ml}$

Optimum $10^8-10^9/\text{ml}$
NanoSight NTA in Summary

NanoSight’s NTA technology offers:

- Single particle detection and analysis
- Visualisation of particles down to 10 nm
- Minimal sample preparation (ca. 300 µl)
- Real time information
- Particle-particle interaction analysis
- Rapid results (ca. 1 min)
- ASTM international standard E2834
Summary of Applications

- Concentration
- Polydispersity – true PSD
- Size
- "Refractive index"
- Charge or zeta potential
- Mikrorheology (Viscosity)
- Fluorescence
Product Range

Software NTA2.3

Basic System: 30 k€

Basic System: 45 k€

Basic System: 41 k€
Optional Addons

Autosampler (NS500)
- up to 144 x 1.5 ml Eppis
- up to 4x 96 well plates
- measurement time ca. 3 min/sample

Syringe Pump
Thank you for your attention!

Any questions?