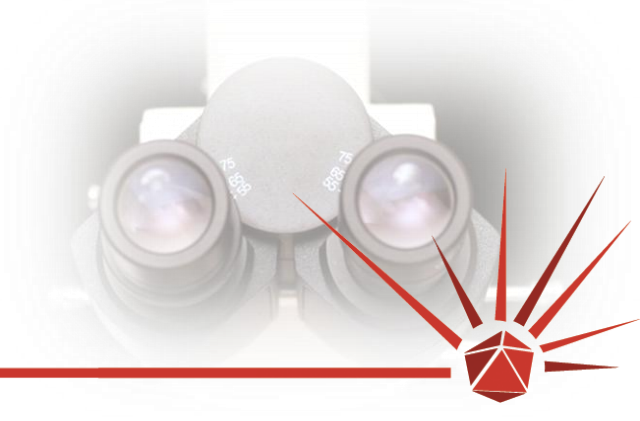
NANOSIGHT

Malaga (Spain) · March 23>26
NANOSPAINCONF2010

Nanoparticle Tracking Analysis;

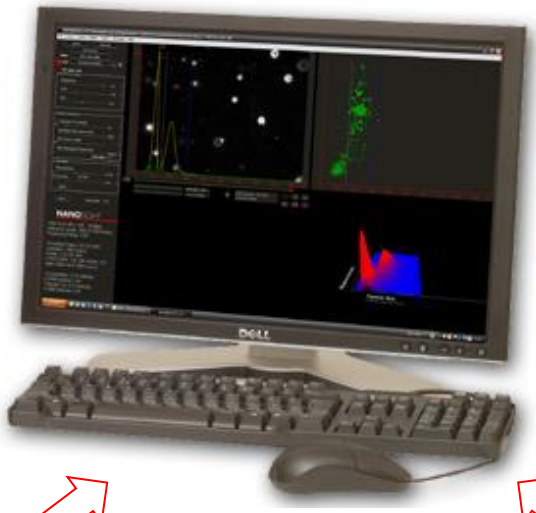
Sizing, Counting and Visualizing of Nanoparticles

Dr Bob Carr, Founder and CTO NanoSight Ltd



The Instrument Range

See us at schaefer



LM10 Series

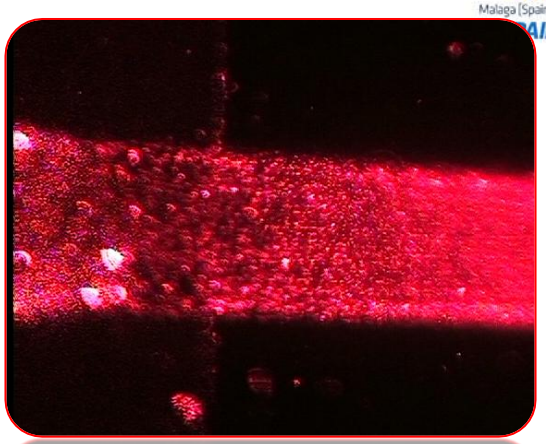
LM20

NS500

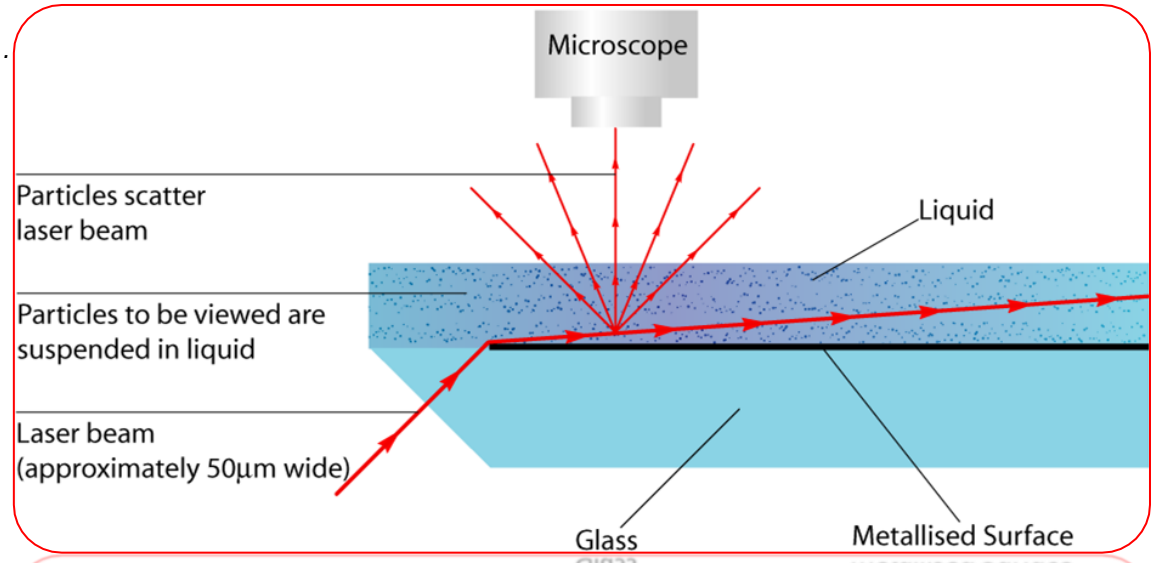
NanoSight's Technology

As the schematic below shows, the NanoSight technology comprises:

- a proprietary optical element
- illuminated by specially configured laser beam

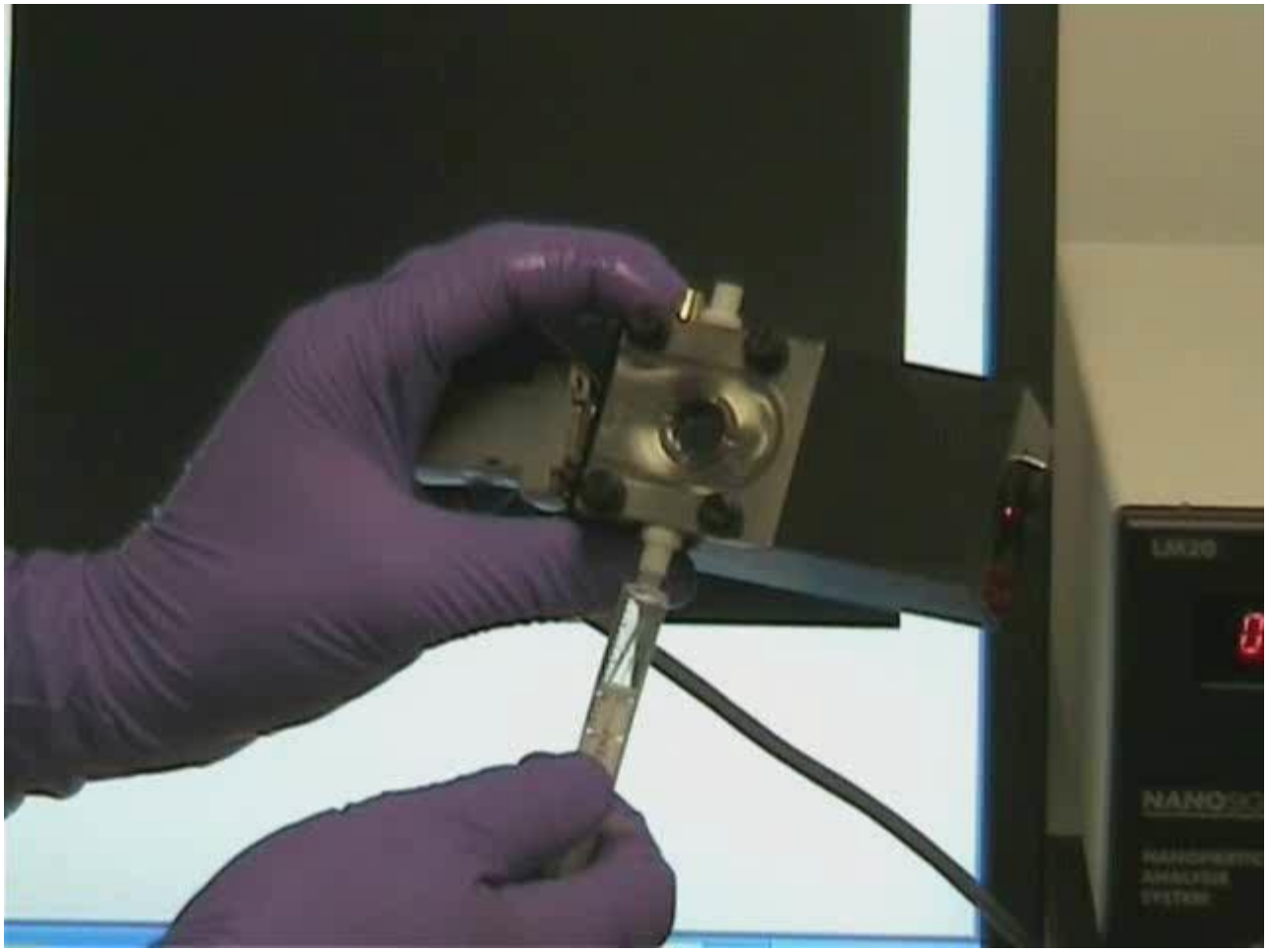


Above: the laser as seen at low magnification



Below: the NanoSight Viewing Cell

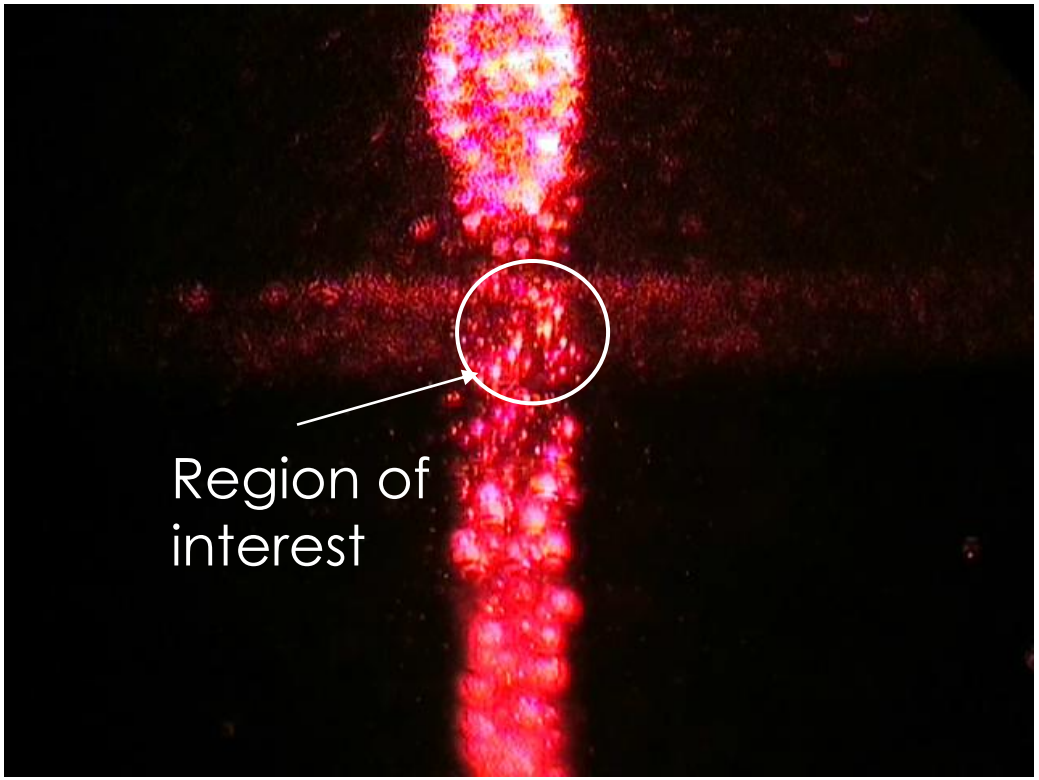
NanoSight LM20 in Practice



Load sample **Insert unit** **Observe nanoparticles!**

The NanoSight System in Action

View of beam passing through sample and zooming into desired field of view.

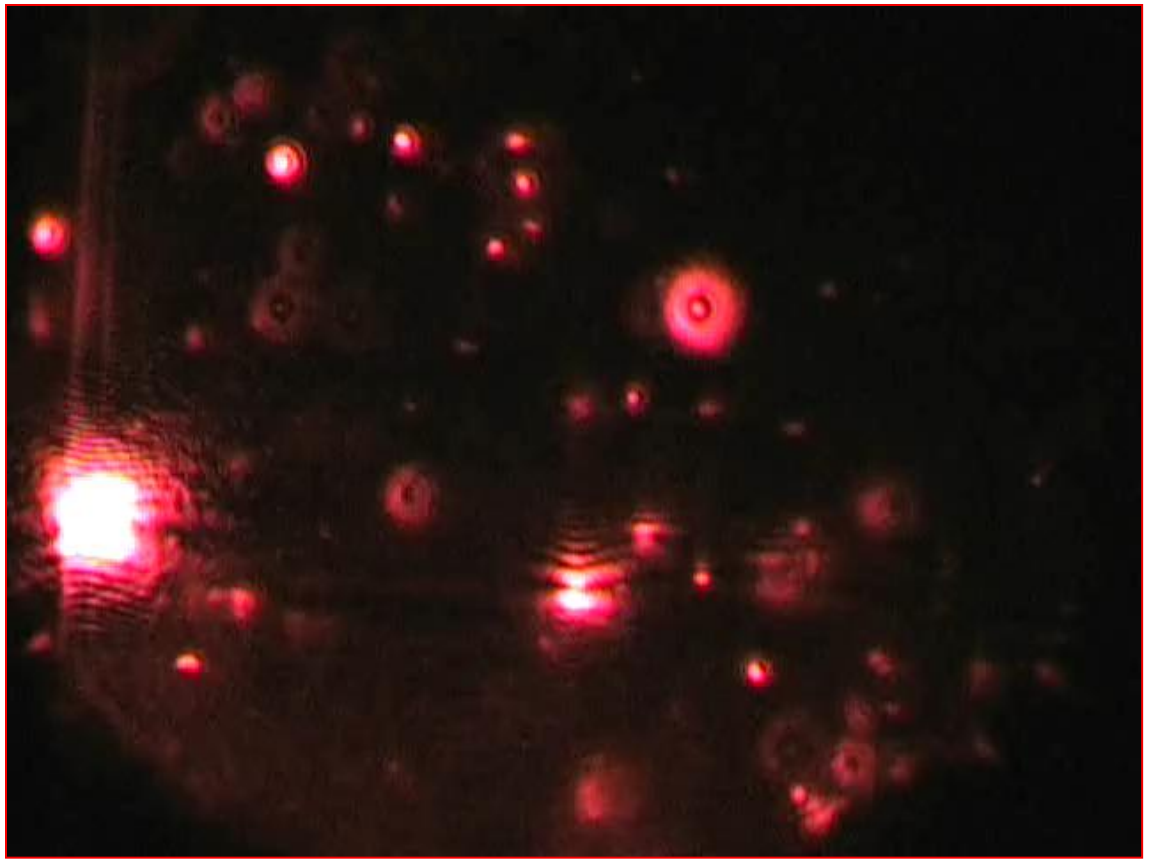


Region at which illumination beam emerges into sample

Border of sensing zone

Optimal viewing region

An immediate Visualisation of Particles



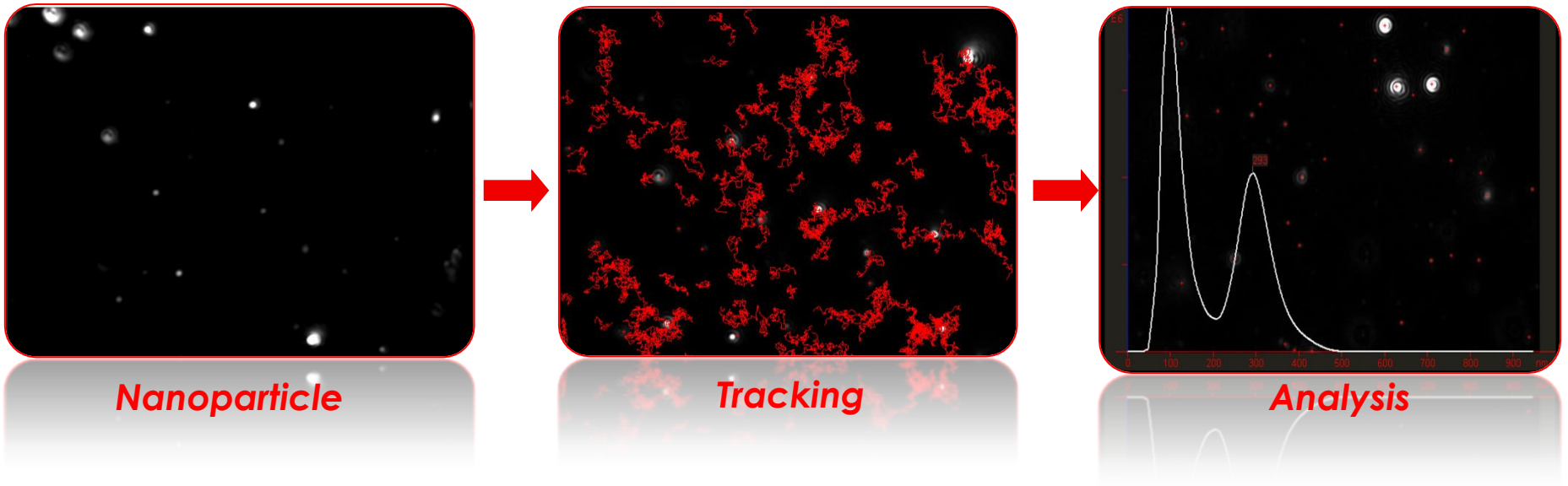
Titanium Dioxide (in water)

- An idea of the presence, size, polydispersity and concentration of nanoparticles can be obtained *immediately* on loading of sample
- This TiO2 sample shows clear polydispersity
- The particles sizes are approximately 60 nm to 800 nm

Nanoparticle Tracking Analysis

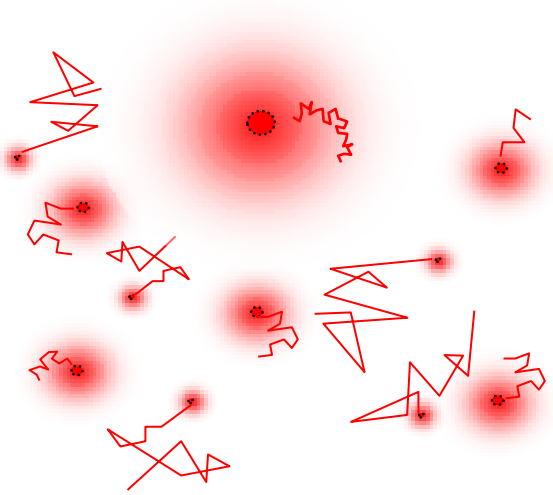
Nanoparticle Tracking Analysis (**NTA**) measures particle size by video tracking, simultaneously, many individual particles.

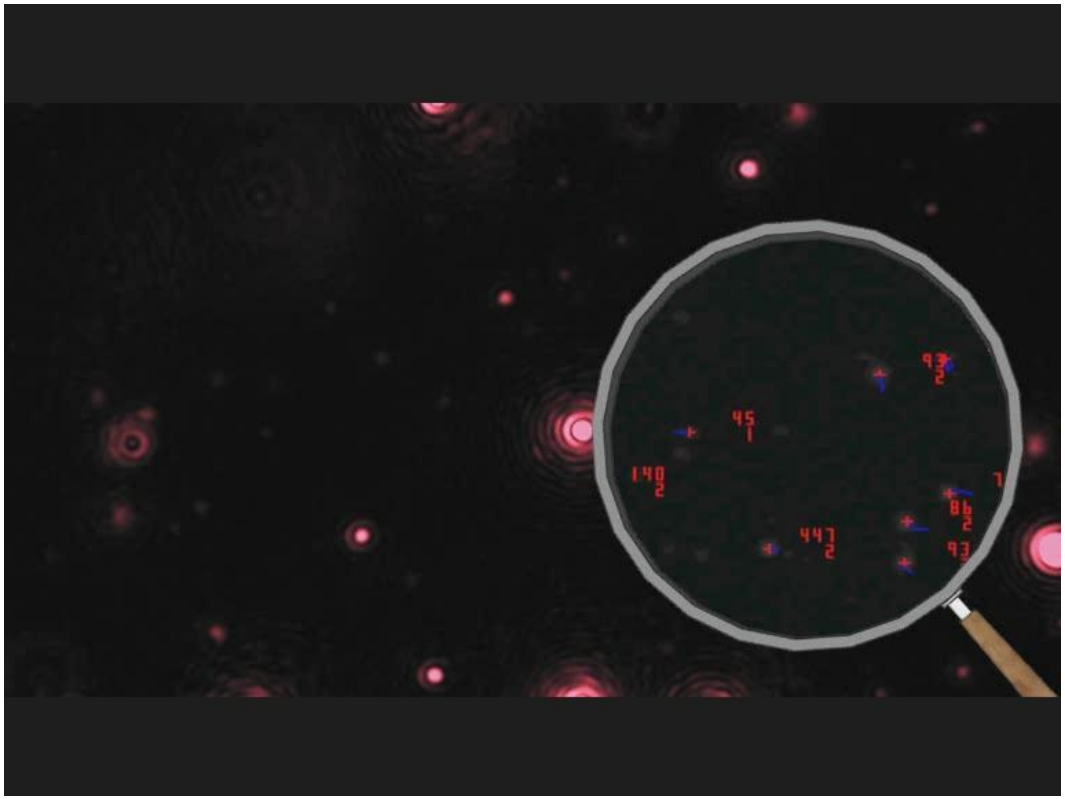
This results in a particle size distribution of high resolution, particle concentration and an ability to include additional particle characteristics such as relative light scattering intensity or fluorescence.



Principle of Measurement

- Nanoparticles move under Brownian motion
- Small particles move faster than larger particles
- Diffusion Coefficient can be calculated by tracking and analysing the movement of each particle separately but simultaneously.
- Through application of the Stokes-Einstein equation, particle size can be calculated
- Scattering or fluorescence properties of particles are also measured
- Particle concentration/number can be estimated





NTA Sizing… is an Absolute Method

- Brownian motion of each particle is followed in real-time via video
- Video analysis software measures mean square displacement in two dimensions = diffusion coefficient (D_t)

$$\frac{\overline{x, y}^2}{4} = Dt$$

- Particle diameter (sphere equivalent hydrodynamic) *d* is then obtained from the Stokes Einstein equation

$$Dt = \frac{TK_B}{3\pi\eta d}$$

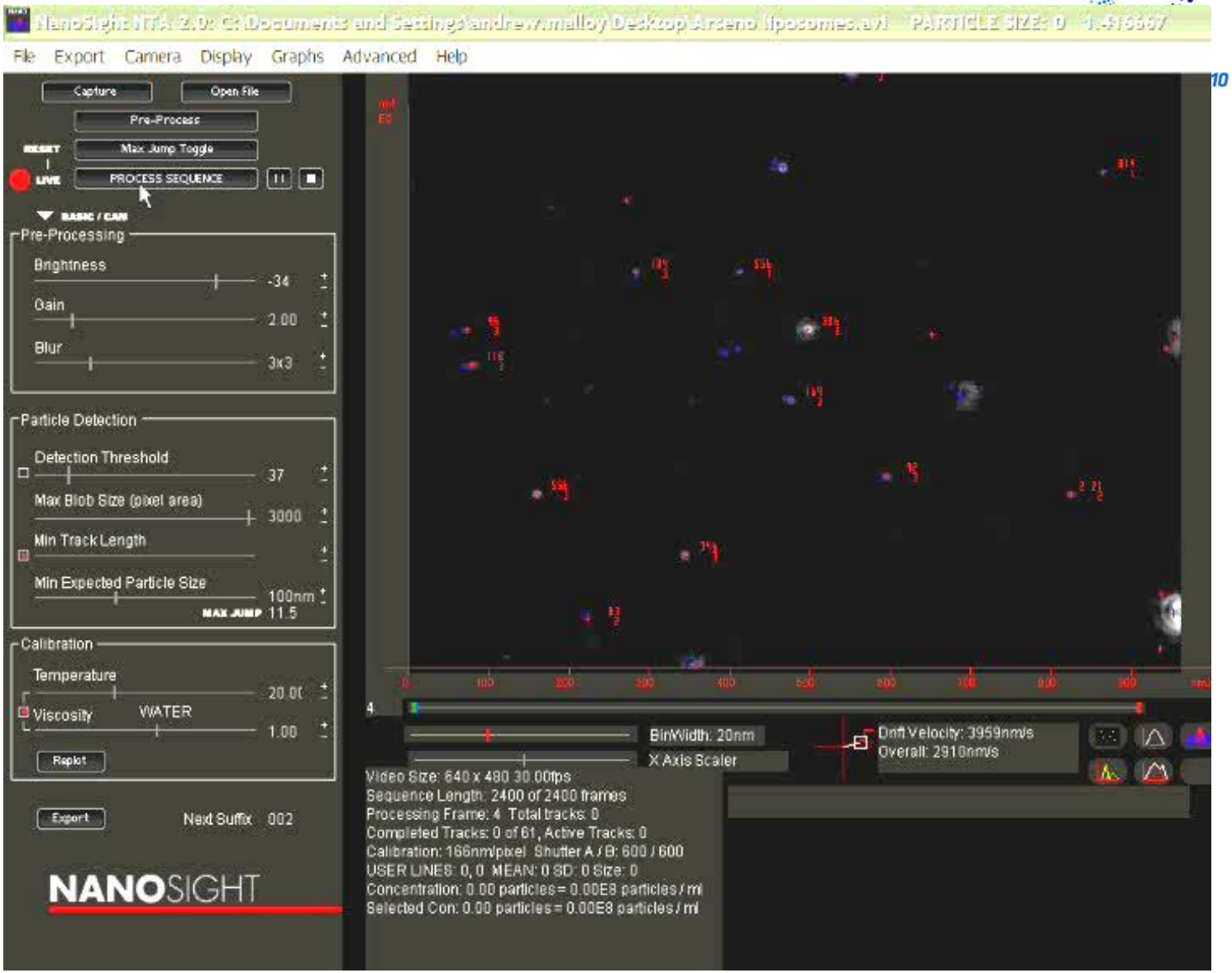
K_B = Boltzmann Constant
η= viscosity
T = Temperature

- NTA is an absolute method therefore no user calibration is required

Particle Sizing in Action

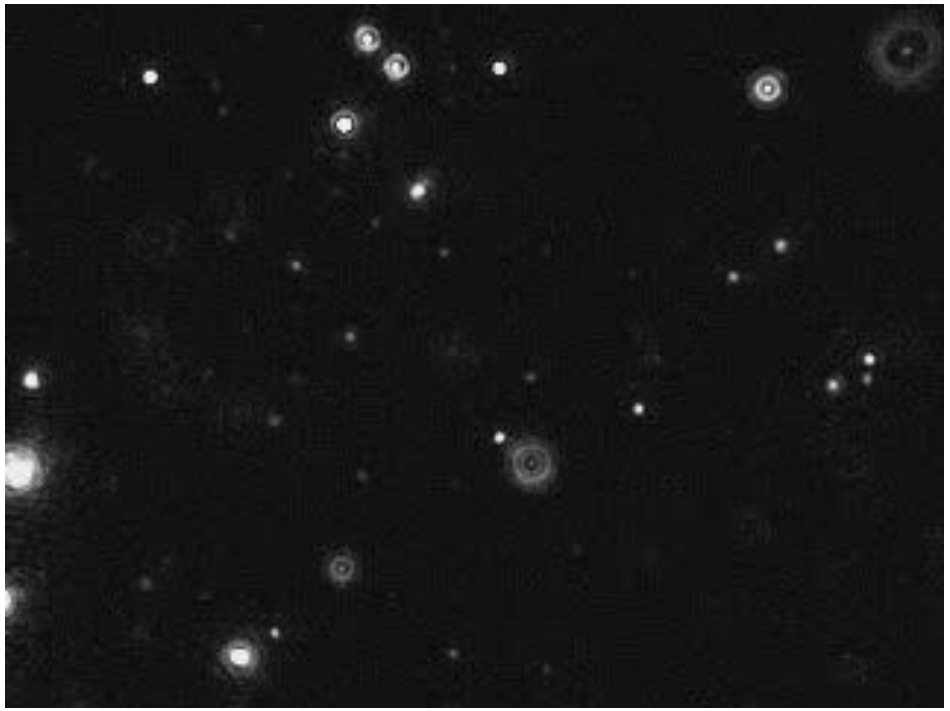
An immediate idea of sample concentration and size is gained in seconds

A live view of progress ensures optimal analysis is maintained until stable size distribution profile is obtained



125 nm Liposomes

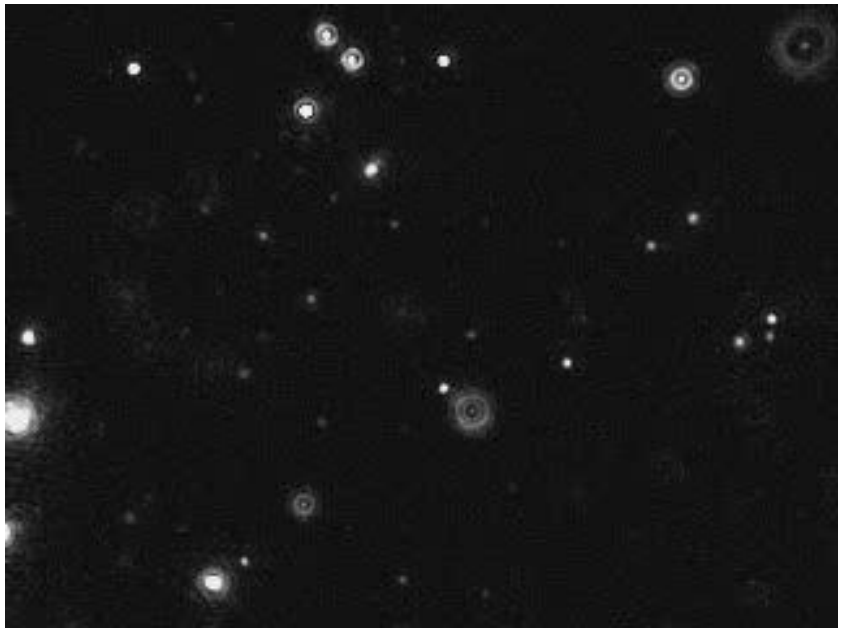
NanoSight provides a visualisation of the light scattered by nanoparticles - this is not a resolved *Image* of the Particles



100+200nm polystyrene in water

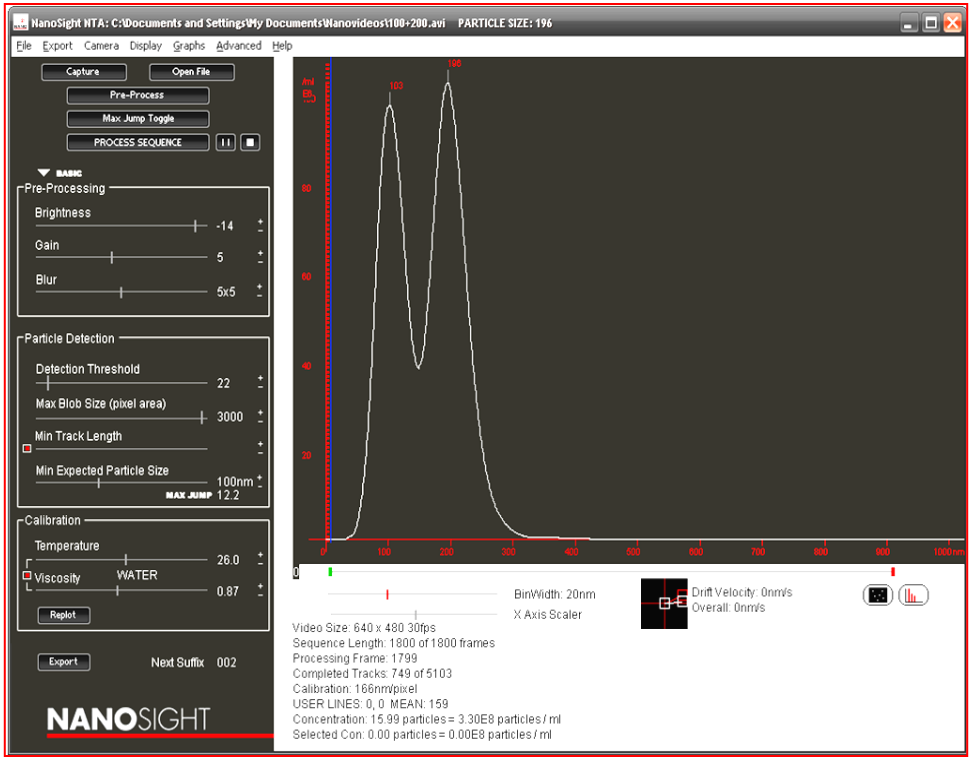
- Particles are too small to be imaged by microscope
- Small particles are visualised as point scatterers moving under Brownian motion
- Larger particles scatter significantly more light

Particles are Counted and Concentration is measured



- Mixture of 100 nm and 200 nm latex microspheres dispersed in water in 1:1 ratio

- Particle distribution displays a number count vs. particle size*
- The particle concentration by user-selectable size class is provided



*(Size is not intensity weighted, as in Dynamic Light Scattering)

NTA Detection Limits

Size

Minimum Size limit is related to:

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

- **10 - 40nm**

Maximum Size limit is related to:

- Limited Brownian motion

- **1000-2000nm**

Concentration

Minimum concentration is related to:

- Poor statistics (Requiring longer analysis time)

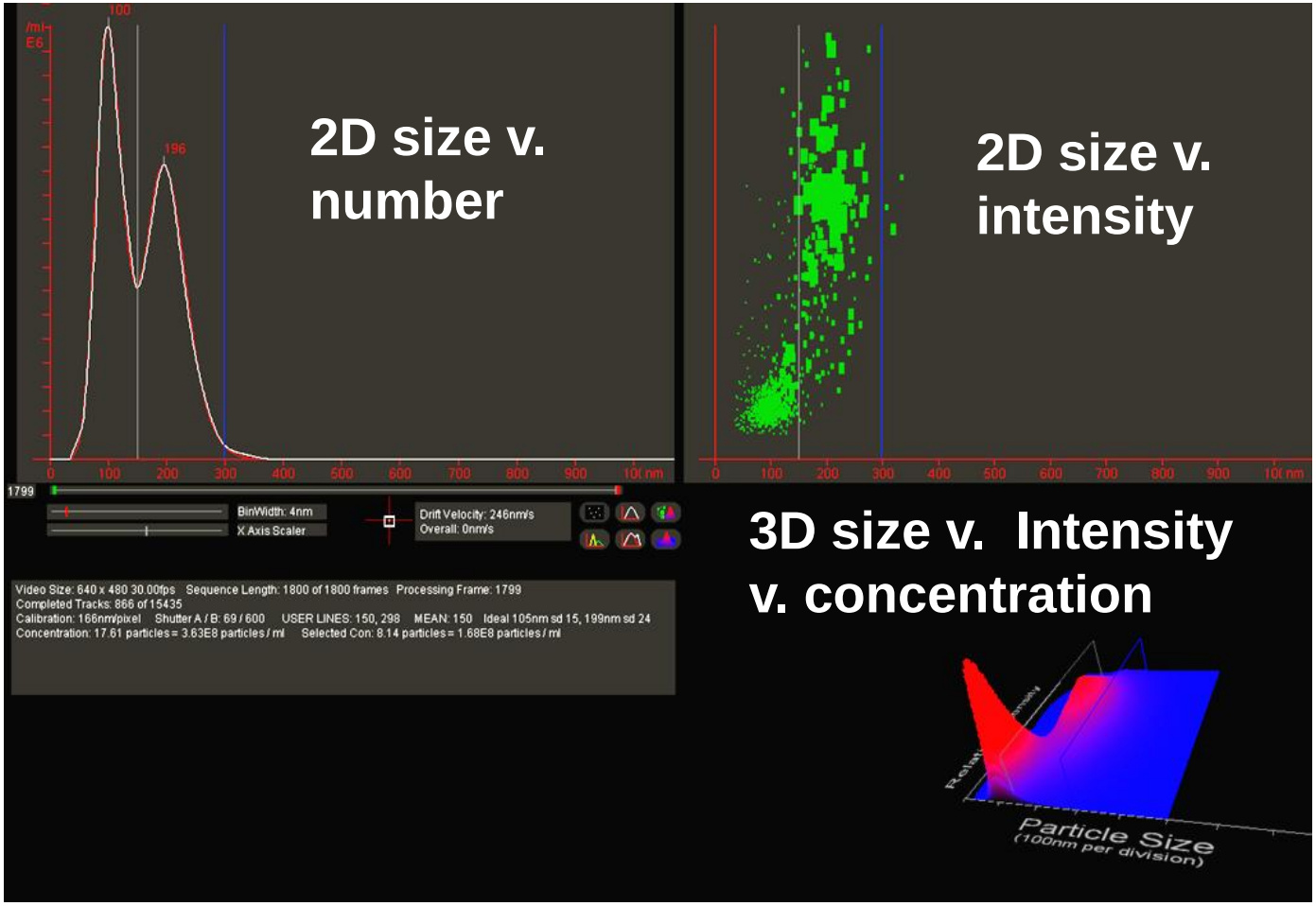
- **approx 10^6/ml**

Maximum concentration is related to:

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

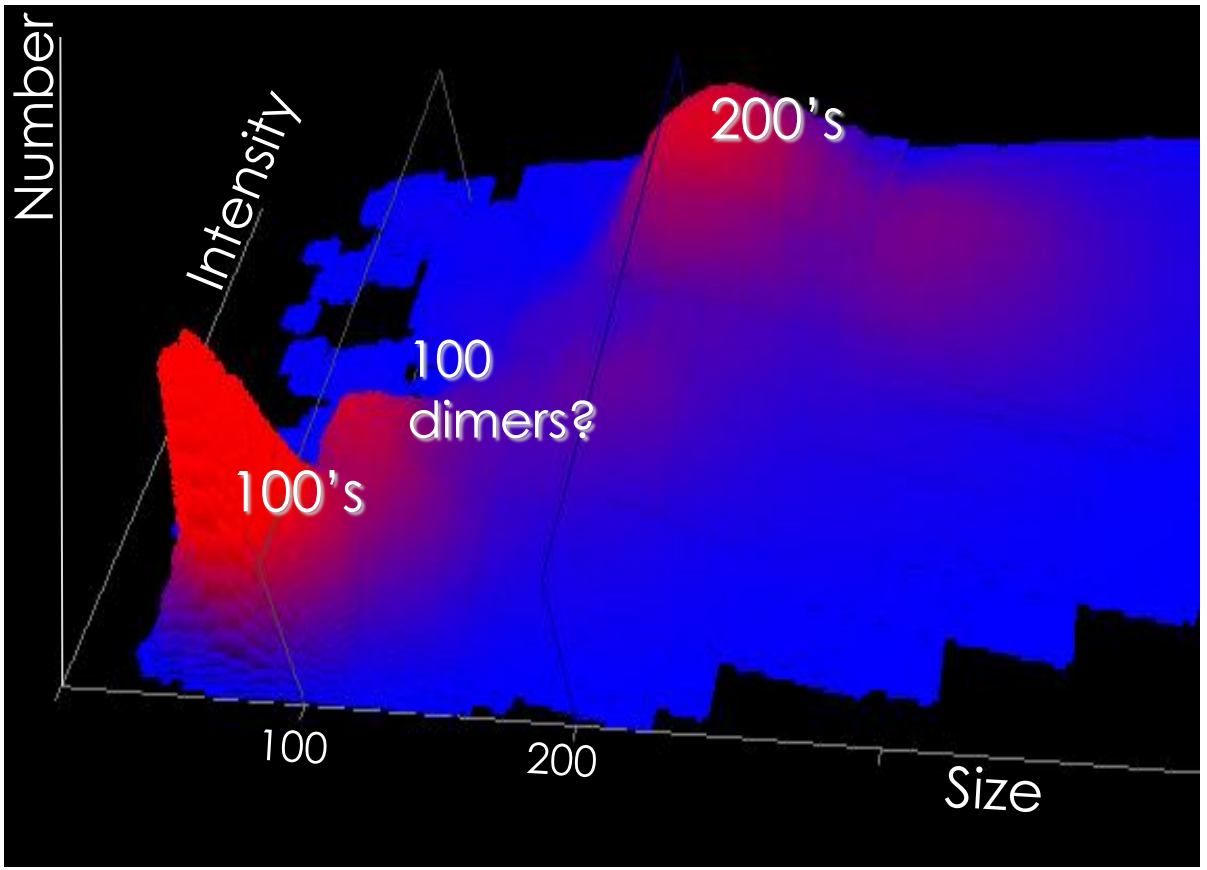
- **approx 10^{10}/ml**

3D Plots (size v. intensity v. number)



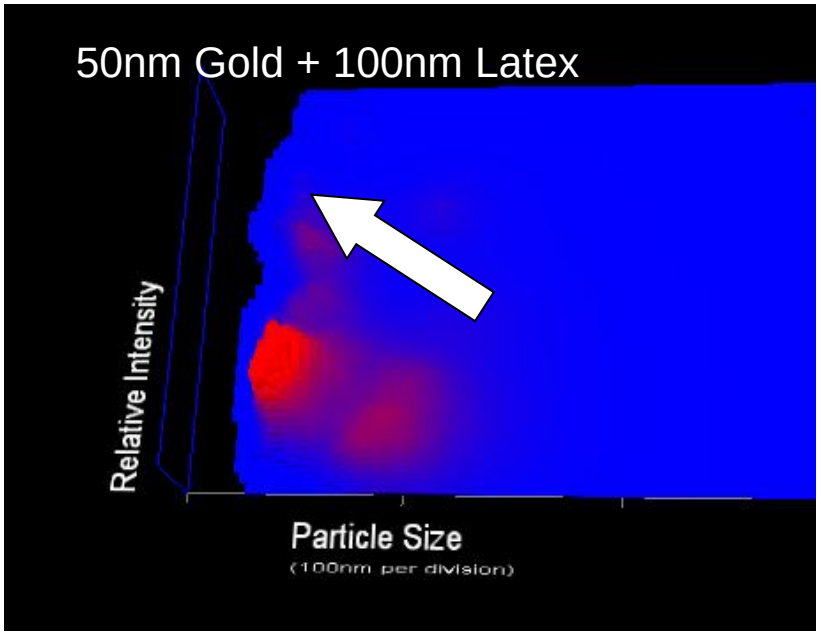
NanoSight has the unique ability to plot each particle's size as a function of its scattered intensity

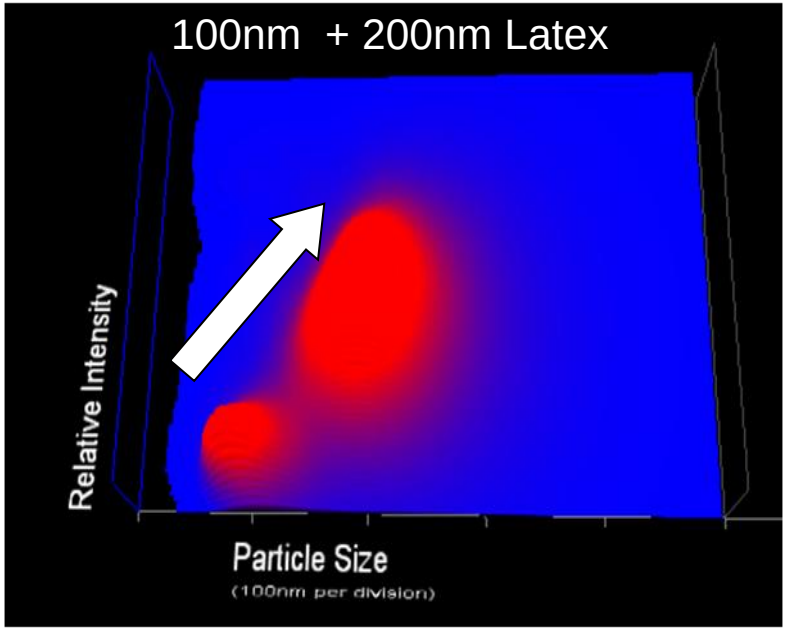
Scattered Intensity – An Additional Variable



- The ability of NanoSight to plot nanoparticle size against I_{scat} allows high resolution plots to be obtained from samples in which such information may be lost in a single plot of particle size distribution only.

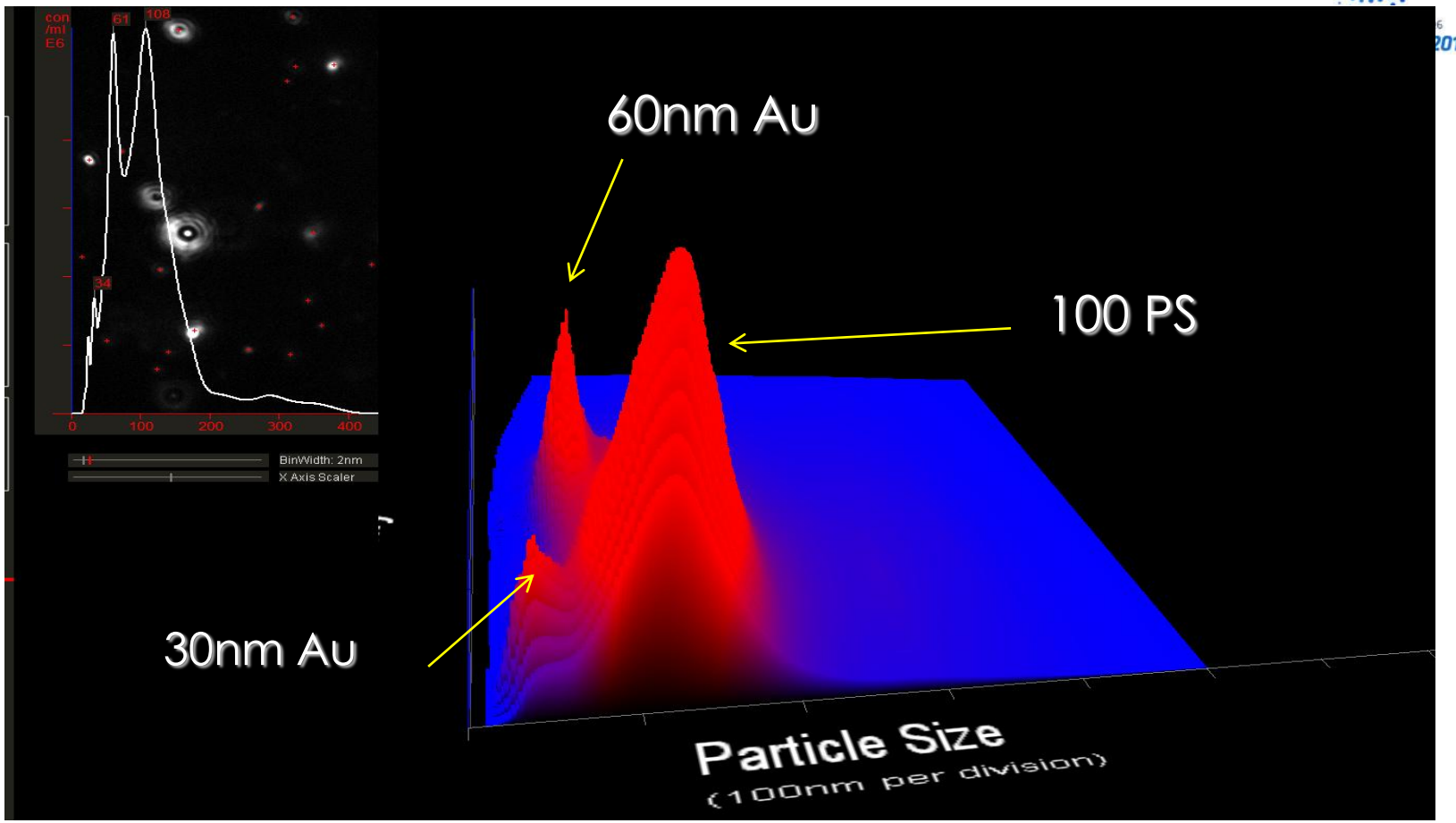
Information about type of particle





Plotting size v relative scattering intensity (≡ particle R_i) allows differences in particle composition to be explored. Note the higher scattering but smaller size of gold v. polystyrene (left) compared to the scaling of size to intensity for two sizes of polystyrene (right)

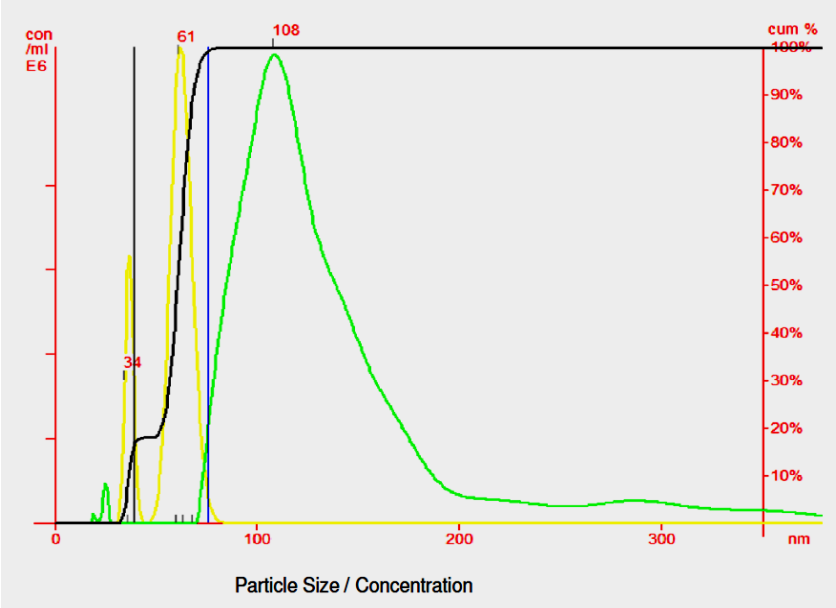
Resolving mixtures of different particle types and sizes.



In this mixture of 30nm and 60nm gold nanoparticles mixed with 100nm polystyrene, the three particle types can be clearly seen in the 3D plot confirming indications of a tri-modal given in the normal particle size distribution plot. Despite their smaller size, the 60nm Au can be seen to scatter more than the 100nm PS.

Auto-generated Report contains:

1. User and sample details
2. Capture settings
3. Analysis settings
4. Histogram data
5. Graphs
6. Tables
7. Excel compatible raw data files



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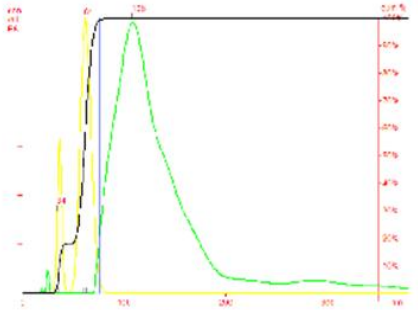
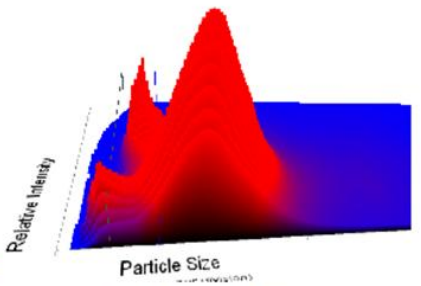
SAMPLE REPORT

Nanoparticle Tracking Analysis (NTA) Version 2.0 Test Version Build 0252

Sample: Unknown Au nanoparticle mix
Video File: NMIAu.avi; Analysis no. 003
Date/Time of Capture: 24 November 2009 02:01
Operator: Bob Carr, NanoSight
Comments: Initially unknown, this sample of Au nanoparticles contained, in fact, a mixture of 30nm and 60nm Au particles. Prior to this analysis, a sample of 100nm polystyrene latex had been analysed. Either there was a degree of cross contamination from this nprevious sample or, as is possible, the operator inadvertantly diluted the Au sample with a dilute sample of the previous 100nm PS sample. Either way, the results show that the 30nm and 60nm peakas are clearly visible on the three dimensional plot and the 60nm high refractive index (i.e. highly scattering) particle peak is not interfered with by the larger but lower intensity 100nm PS peak.



Particle Size / Concentration



Particle Size / Relative Intensity 3D plot

Bin Centre (nm)	Concentration E6 particles / ml	Percentile Undersize
10	0.318	0.00%
30	21.387	0.00%
50	56.001	18.42%
70	86.193	93.68%
90	82.709	100.00%
110	107.042	100.00%
130	71.027	100.00%
150	44.211	100.00%
170	24.800	100.00%
190	10.594	100.00%
210	6.016	100.00%
230	5.058	100.00%
250	4.144	100.00%
270	4.663	100.00%
290	5.260	100.00%
310	4.302	100.00%
330	3.388	100.00%
350	3.046	100.00%
370	2.343	100.00%
390	1.257	100.00%
410	0.442	100.00%
430	0.096	100.00%
450	0.011	100.00%
470	0.000	100.00%
490	0.000	100.00%
510	0.000	100.00%

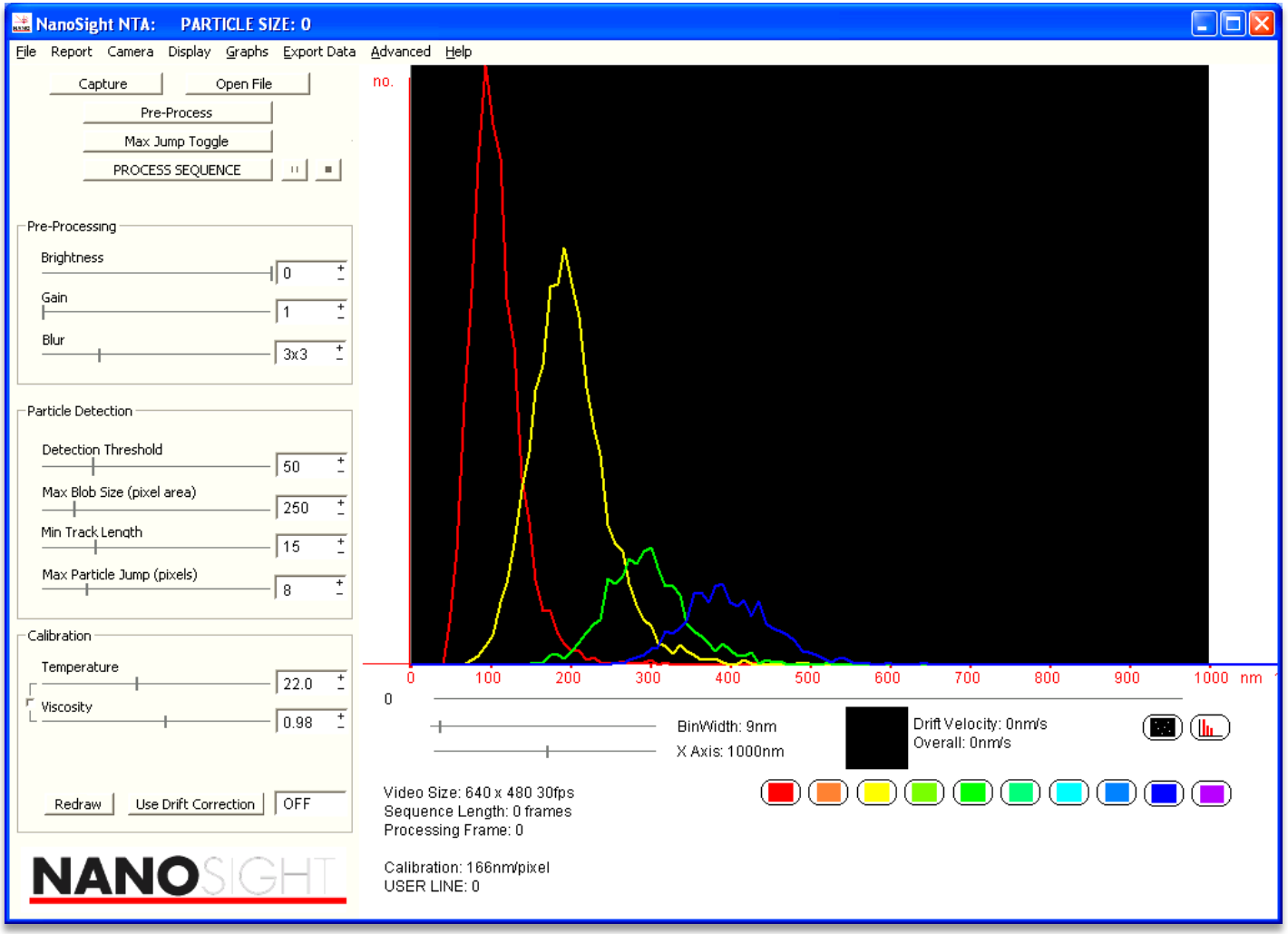
Bin Centre (nm)	Concentration E6 particles / ml	Percentile Undersize
530	0.000	100.00%
550	0.000	100.00%
570	0.000	100.00%
590	0.000	100.00%
610	0.000	100.00%
630	0.000	100.00%
650	0.000	100.00%
670	0.000	100.00%
690	0.000	100.00%
710	0.000	100.00%
730	0.000	100.00%
750	0.000	100.00%
770	0.000	100.00%
790	0.000	100.00%
810	0.000	100.00%
830	0.000	100.00%
850	0.000	100.00%
870	0.000	100.00%
890	0.000	100.00%
910	0.000	100.00%
930	0.000	100.00%
950	0.000	100.00%
970	0.000	100.00%
990	0.000	100.00%
1000-2000	0.000	100.00%

Results
Mean: 113 nm
Mode:106 nm
SD: 61 nm
D10: 36 nm
D50: 60 nm
D90: 68 nm
User Lines: 39, 76 nm
Concentration: 5.44 x 10^8 particles/ml

Measurement Conditions
Temperature: 22.00 °C
Viscosity: 0.95 cP
Frames Per Second: 30.00
Measurement Time: 31 of 31 seconds
Drift Velocity: 337 nm/s

Analysis Conditions
Brightness: -9
Gain: 2.67
Blur: 5x5
Detection Threshold: 13
Max Blob Size (pixel area): 3000
Min Track Length: Auto
Min Expected Size: 50 nm

User-programmable Batch Measurement for Time-Based Study



- Particle size distribution at hourly intervals follows aggregation process

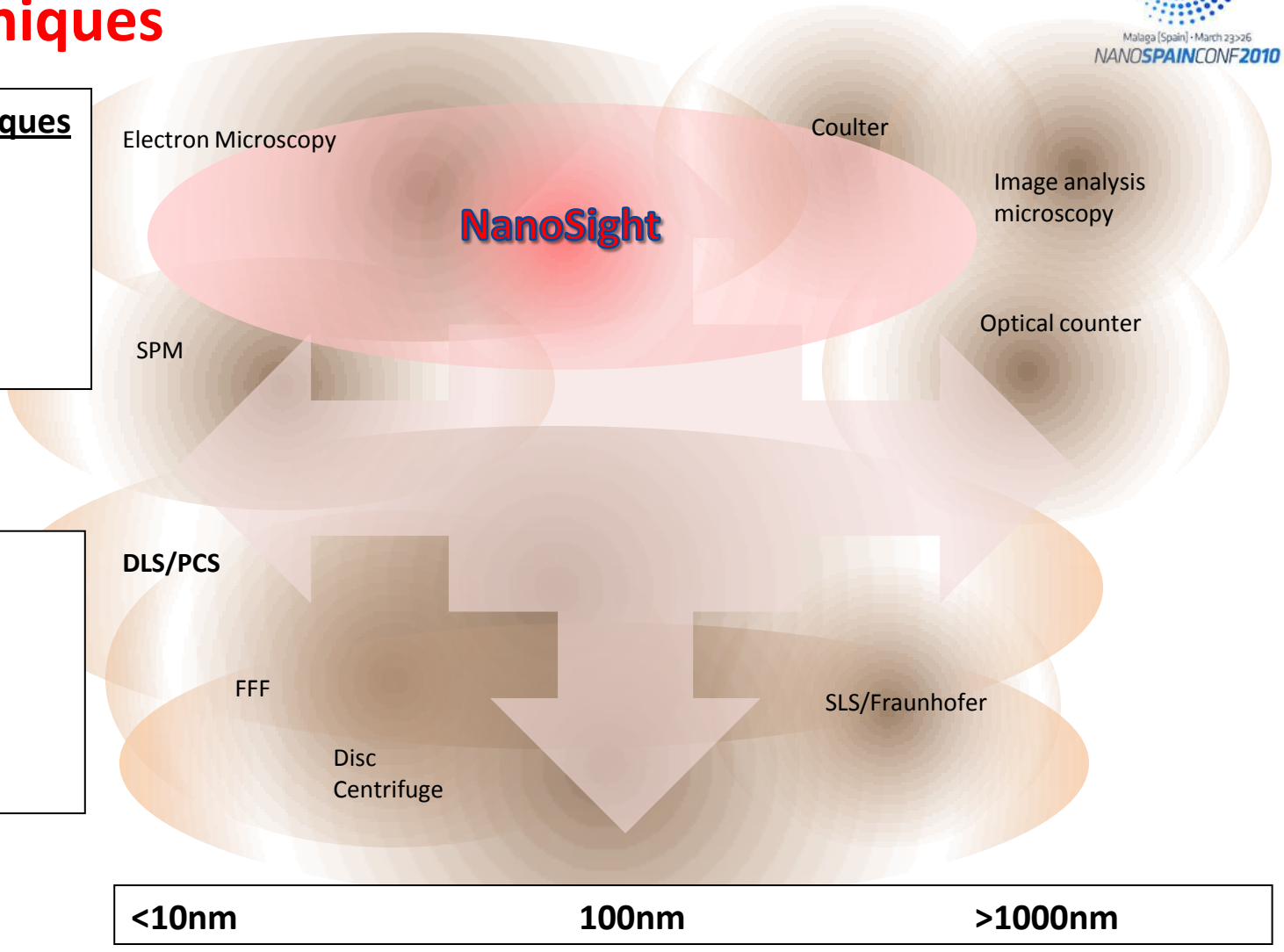
Where NanoSight sits in relation to other particle sizing techniques

Single particle techniques

- High resolution
- Multi-parameter per particle
- Concentration

Ensemble techniques

- Low resolution
- Single parameter of population
- No count



Comparison to Dynamic Light Scattering (DLS)

- DLS (alternatively known as Photon Correlation Spectroscopy (PCS)) is deservedly an industry standard technique and widely used for 40 years...
    - BUT
- In poly-dispersed samples only an average particle size is produced which is intensity biased towards the larger particles.
- Particle size distribution analysis is inherently limited to resolving only >3:1 diameter ratios.
- No concentration information
- Black-box approach

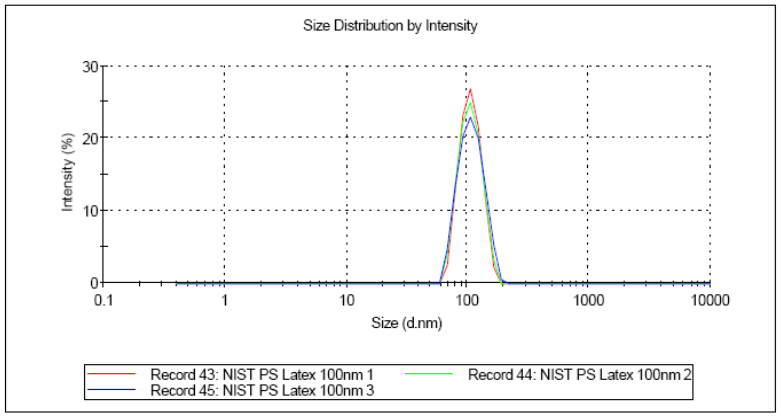
NTA vs. DLS – Monodisperse Result

DLS Analysis

Results

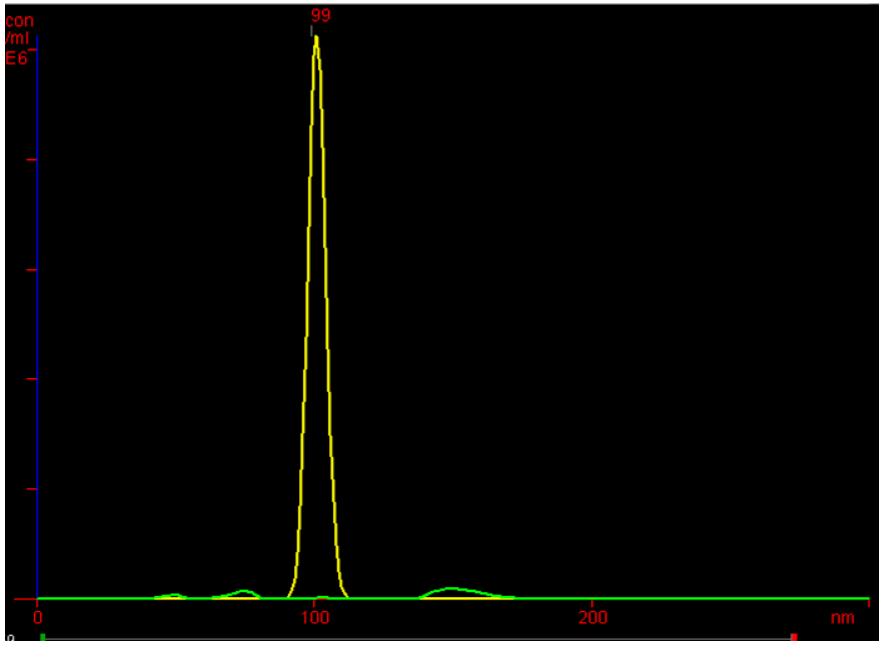
		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 104.1	Peak 1:	109.0	100.0	25.34
PdI: 0.020	Peak 2:	0.000	0.0	0.000
Intercept: 0.935	Peak 3:	0.000	0.0	0.000

Result quality : Good



100 nm polystyrene reference particles in water

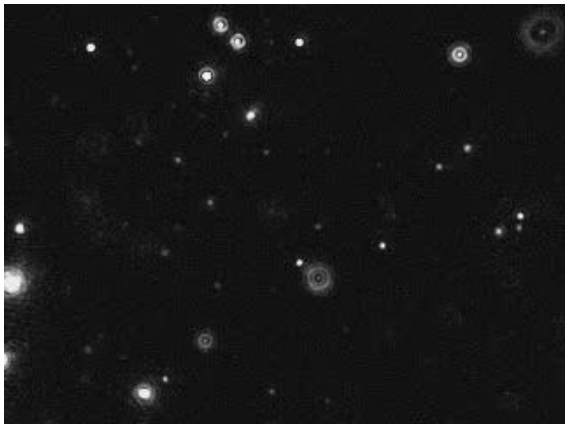
Similar NanoSight Result



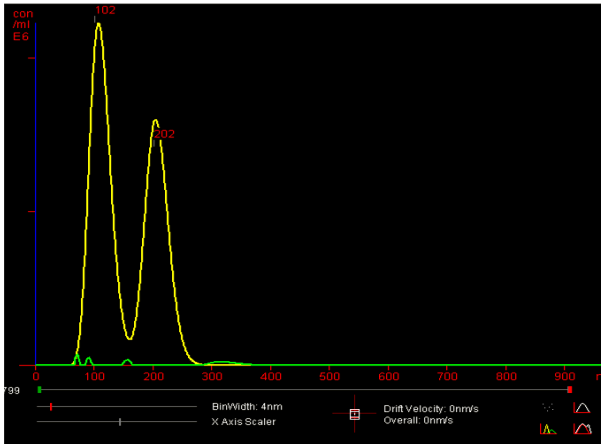
Note: linear scale over 0-300nm (compared to wide range logarithmic scale of DLS)

DLS (Dynamic Light Scattering) also know as Photon Correlation Spectroscopy (PCS).

NTA vs DLS – Bimodal Sample



Polystyrene reference spheres in water (100 nm and 200 nm)

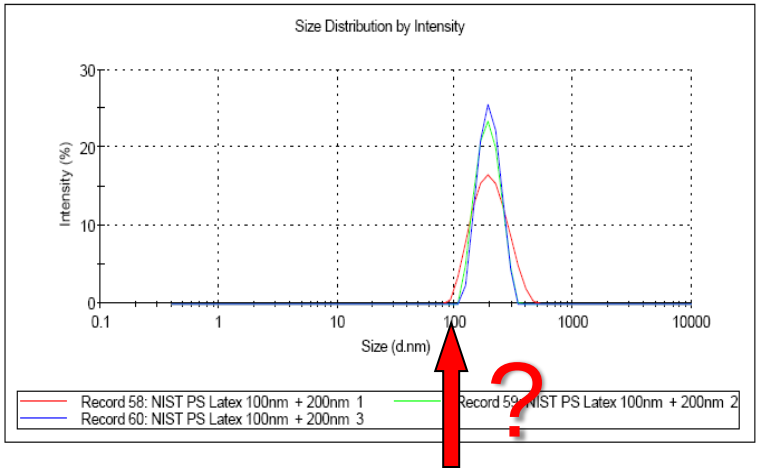


With NTA, the analysis clearly shows both 100nm and 200nm peaks

….Can get down to 1:1.25 ratio

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	190.4	Peak 1:	197.6	100.0	41.23
PdI:	0.008	Peak 2:	0.000	0.0	0.000
Intercept:	0.944	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

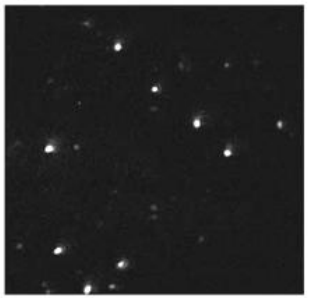


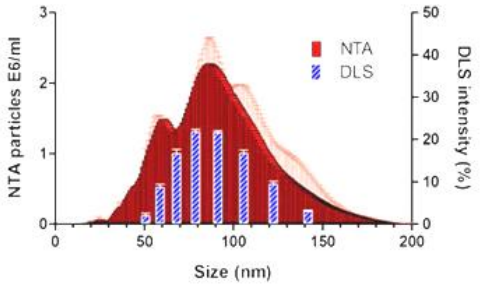
In DLS, 100 nm particles are not detected as their presence is masked by the higher scatter signal of the 200 nm particles

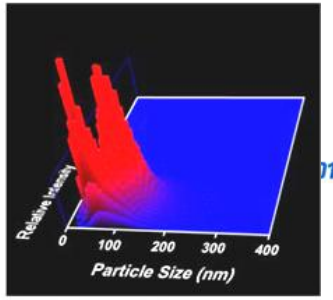
NTA v. DLS

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

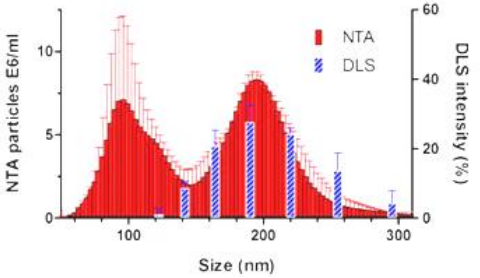
a) 60 and 100 nm beads

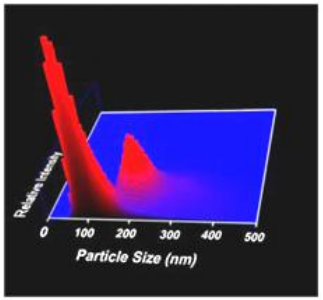




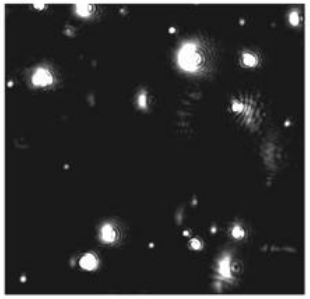


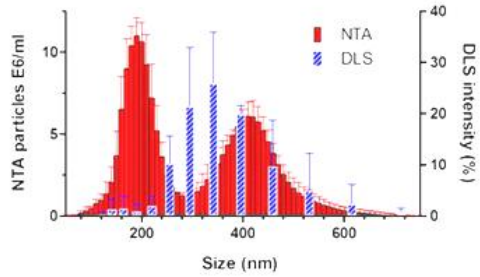
b) 100 and 200 nm beads

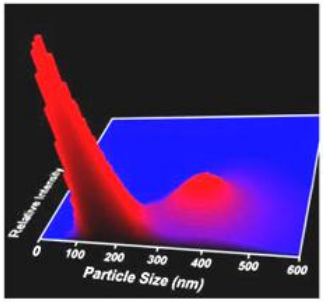




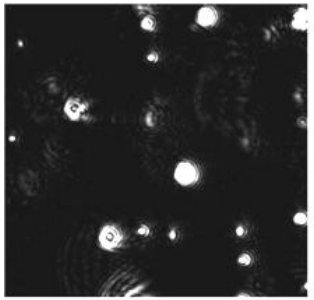
c) 200 and 400 nm beads

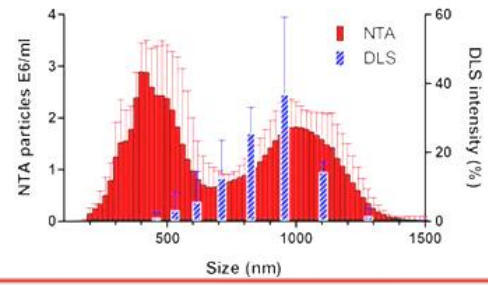


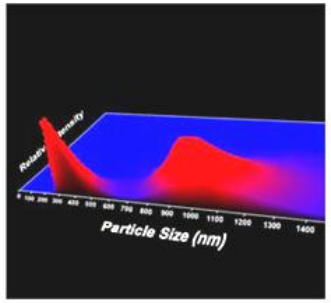




d) 400 and 1000 nm beads







Data reproduced from **Filipe, Hawe and Jiskoot (2010) "Critical Evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the Measurement of Nanoparticles and Protein Aggregates"**, ***Pharmaceutical Research, DOI: 10.1007/s11095-010-0073-2***

NanoSight's Advantages vs. DLS/PCS

- NTA is not intensity-weighted towards larger particles . DLS is.
- While DLS is an ensemble technique, NTA operates particle-by-particle
- NTA has much higher resolving power with respect to multimodal and polydisperse samples and heterogenous/mixed sample types
- NTA requires no information about collection angle, wavelength or solvent refractive index. DLS does.
- NTA provides particle concentration information. DLS doesn't.
- Unique view from NanoSight shows the sample and validates particle size distribution data
- Number vs. Intensity vs. Size is provided for each particle size class in NTA.
- Now multi-parameter - per particle

The NanoSight system is widely applicable

- ✓ Liposomes and other drug delivery vehicles
- ✓ Virus samples
- ✓ Protein aggregation
- ✓ Ink jet inks and pigment particles
- ✓ Magnetic Nanoparticles
- ✓ Multi-walled Carbon nanotubes
- ✓ Cosmetics
- ✓ Foodstuffs
- ✓ Ceramics
- ✓ Fuel additives
- ✓ Metal oxides in magnetic storage media
- ✓ Precursor chemicals for wafer fabrication.
- ✓ Quantum dots
- ✓ Polymers and colloids
- ✓ CMP Slurries
- ✓ Nanobubbles

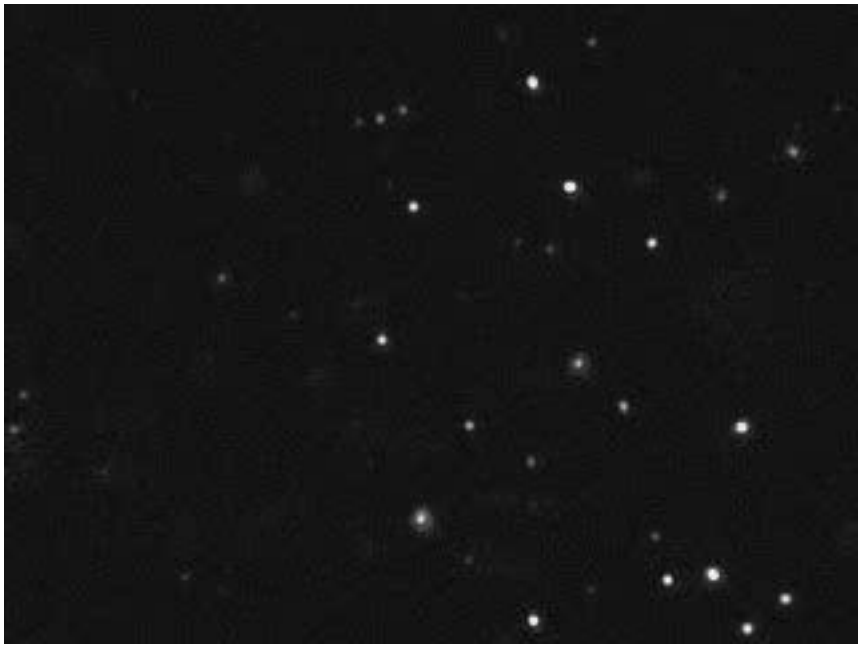
Selected Users

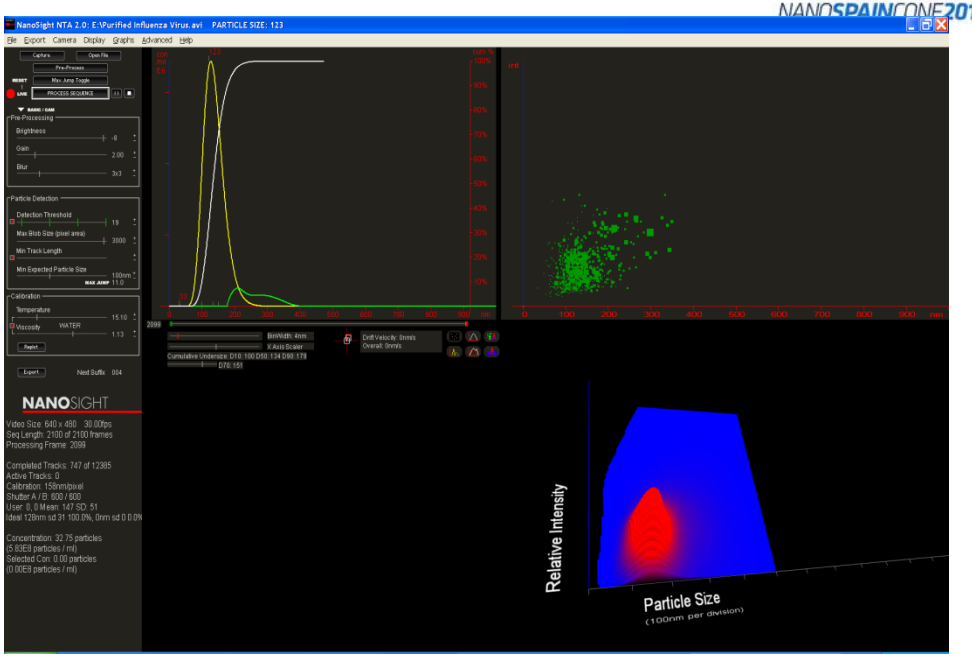
- BASF, Europe
- BP Castrol, Europe
- DuPont, USA
- Epson, Japan
- Exxon Mobile, USA
- GlaxoSmithKline, Europe
- Merck, USA
- Medimmune
- Nestle, Europe
- NIST, USA
- Novartis, Europe
- Proctor & Gamble, USA
- Roche, Europe
- Smith & Nephew, Europe
- Solvay, Europe
- Toshiba, Japan
- Unilever, Europe
- US EPA
- US Forces
- Wyeth Biopharma, USA

.....and over 100 major universities and academic institutions worldwide.

200+ units sold world-wide

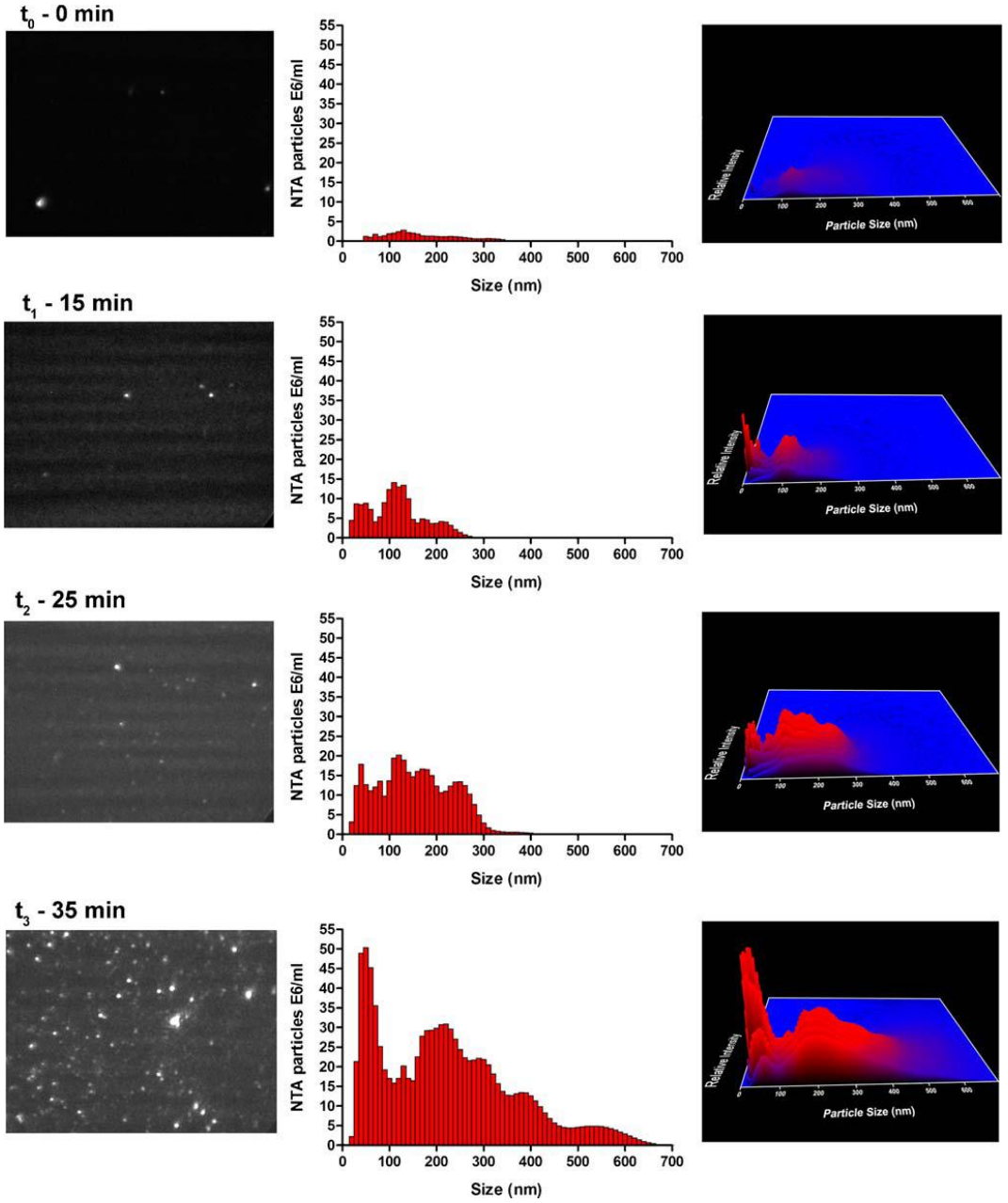
Example 1: Purified Influenza Virus





- The ability to count viruses in liquid suspension is essential for those working in vaccine development
- Current methodologies for counting such, as plaque assay, only count infectious particles which often represent a small component in attenuated vaccines i.e. perhaps only 1% of product remains infective. NanoSight analyses all particles.
- Particle aggregation and yield quality are factors which need to be understood when developing these viral vaccines

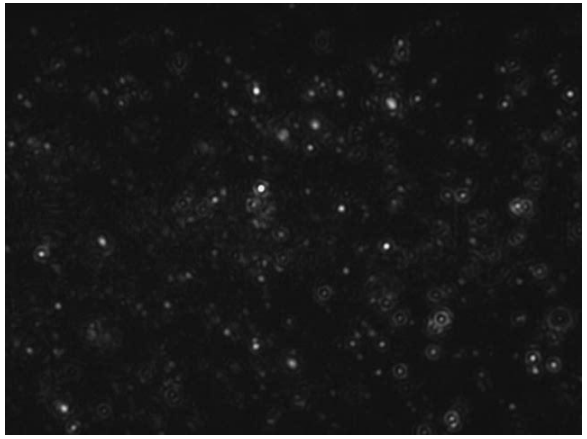
Example 2 – Protein Aggregation at 50°C



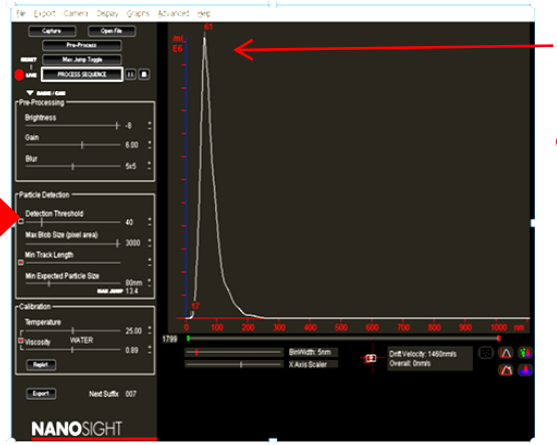
- NanoSight technology has a unique application in the detection of early stage aggregation in protein therapeutics
- Protein monomer is too small to be individually resolved by this technique, but early stage aggregates are readily detected
- Protein monomer at high concentration causes high background 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.

Data reproduced from Filipe et al (2010), Pharmaceutical Research, DOI: 10.1007/s11095-010-0073-2

Example 3: Particle Aggregation 60nm Gold

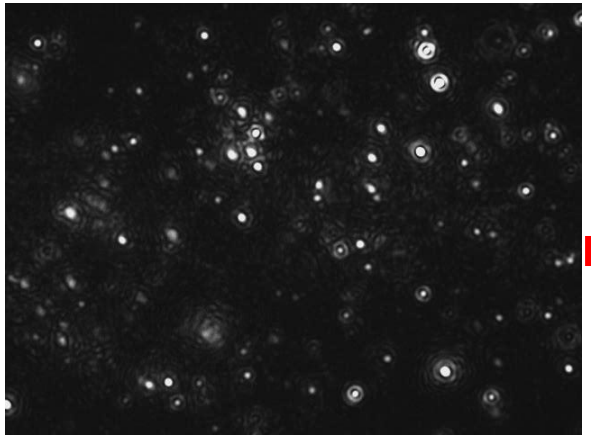


Prior to aggregation

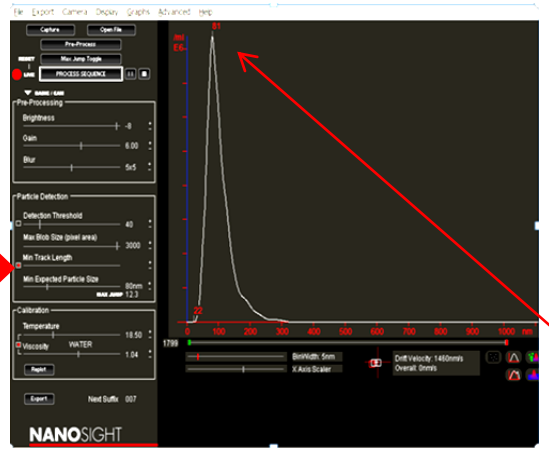


61 nm

- DNA mediated aggregation. Potentially provides an alternative to fluorescence based assays or signal amplification procedures such as PCR, in nucleic acid diagnostics



Following addition of DNA



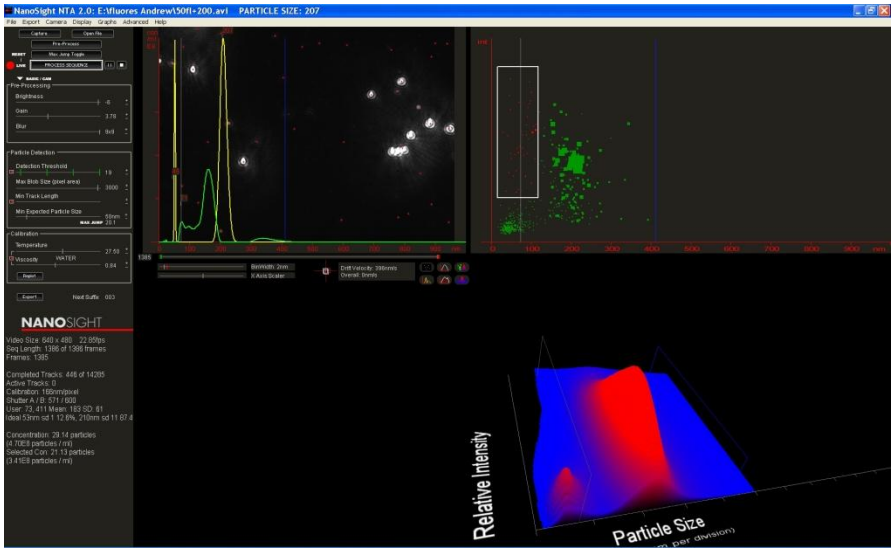
- In the second video the aggregates are brighter and slower moving. Their increased hydrodynamic diameter is detected

81 nm

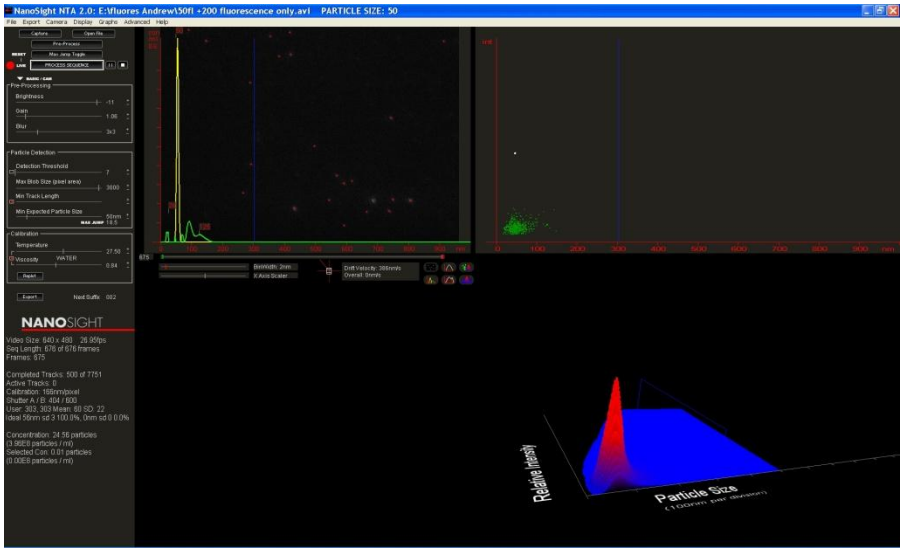
Fluorescent option now available...

- NanoSight LM10 system can now be (retro) fitted with a blue (**405nm**) or green (**532nm**) laser diode capable of exciting fluorophores and quantum dots
- Filters allow specific nanoparticles to be tracked in high backgrounds
- Applications in:
  - Nanoparticle toxicity studies
  - Nano-rheology
  - Bio-diagnostics

Example 5 - Detection of fluorescent particles in mixture



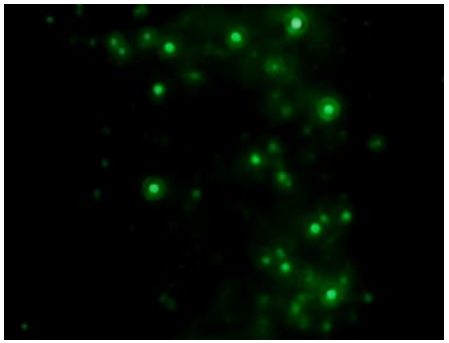
A mixture of 50nm fluorescent particles and 200nm non-fluorescent particles analysed under light scatter mode



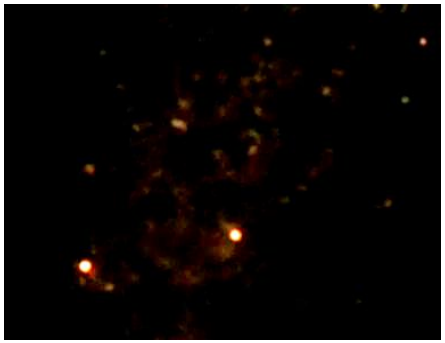
Same mixture when scatter removed by fluorescence filter

Example 6 - Fluorescence detection in biological fluids

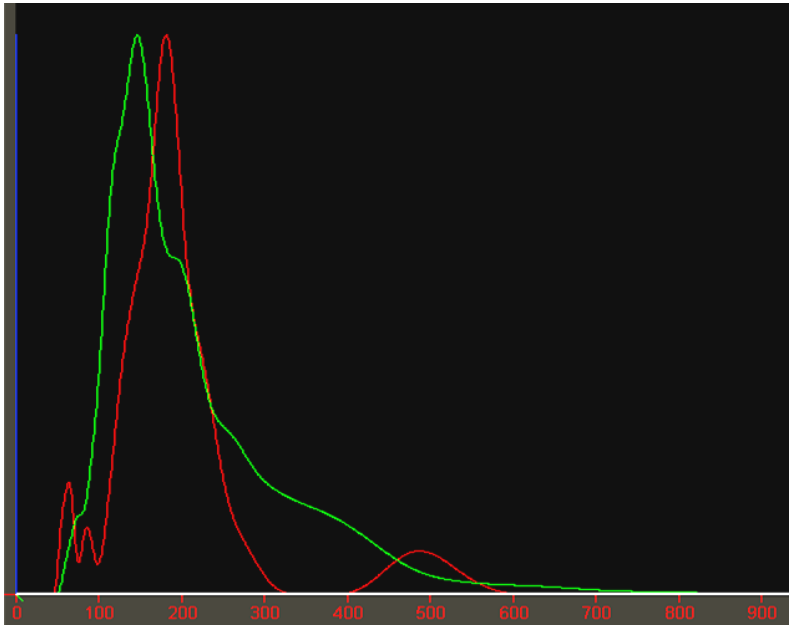
Example of analysis of cellular micro- and nano-vesicles labelled with an appropriate fluorescent antibody.



Light scatter

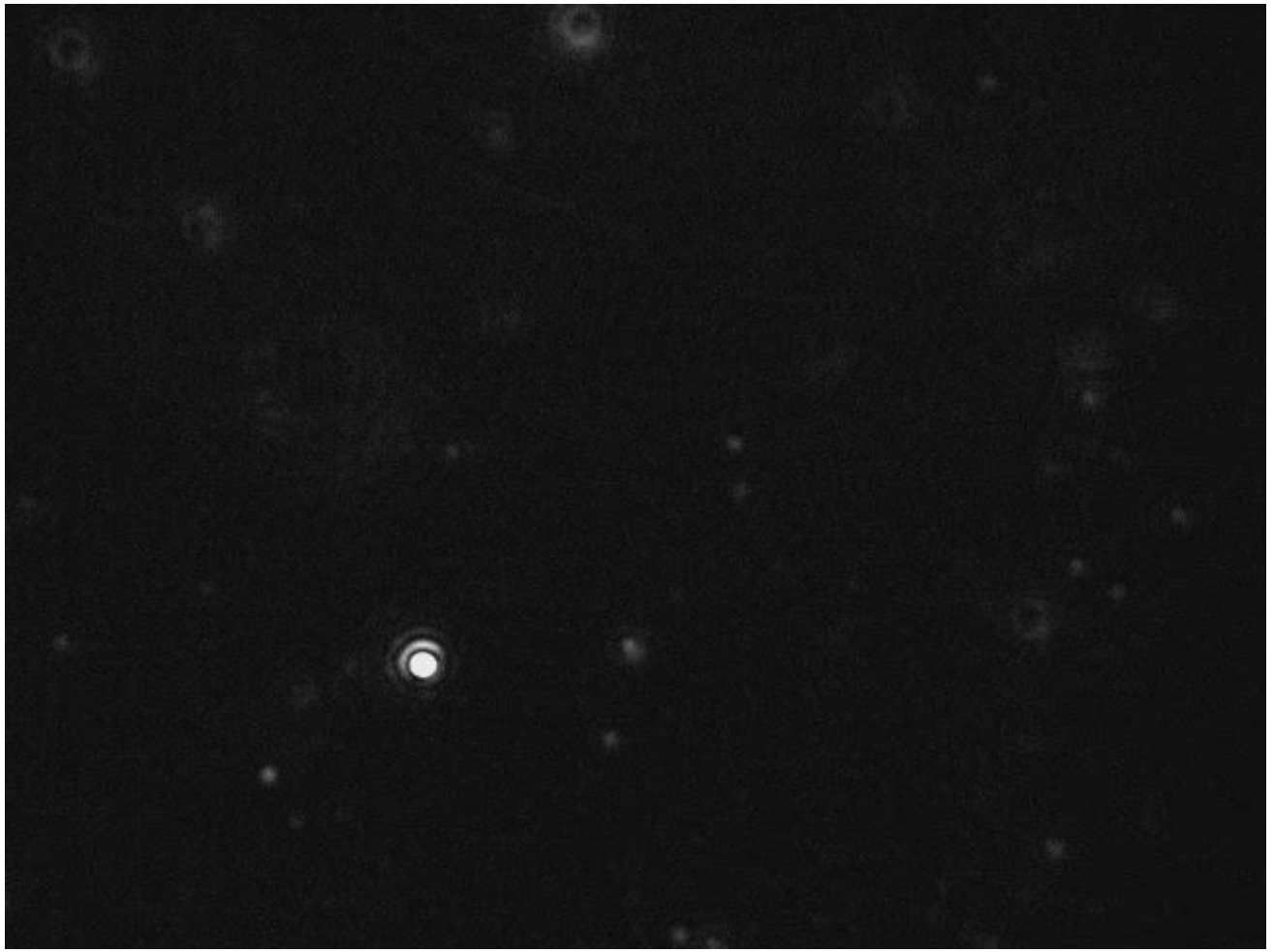


Antibody Fluorescence



Particle size distribution profile (normalised) of sample under light scatter (green line) and fluorescent (red line) analysis.

Example 7 – Particle asymmetry



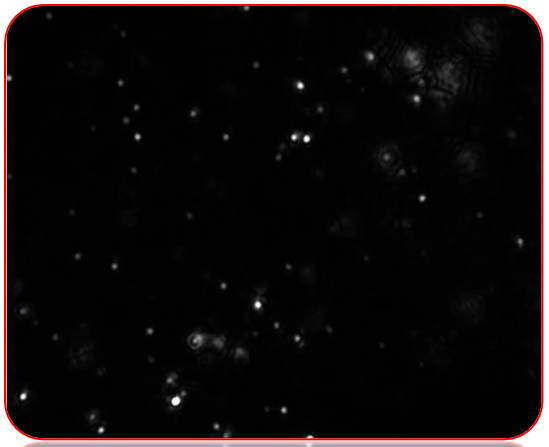
Nano-Mica.

Highly asymmetric plates which flash on and off due to their rotation.

Flashing is ALWAYS and ONLY seen with very non-spherical particles

NanoSight in Summary

- ✓ **High resolution particle size distribution through single particle detection and analysis**
- ✓ **Visualisation of particles down to 10 nm, dependant on material**
- ✓ **Count and concentration measurement**
- ✓ **Simultaneous scattered light and fluorescence measurement**
- ✓ **Minimal sample preparation**
- ✓ **Real time information**
- ✓ **Rapid results**
- ✓ **Low Cost**



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Malaga (Spain) · March 23>26
NANOSPAINCONF2010

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...for latest updates and
Distributor locations

NanoSight Ltd
Minton Park,
London Road, Amesbury,
Wiltshire, SP4 7RT,
UK
T: +44 (0)1980 676060
F: +44 (0)1980 624703
admin@nanosight.com

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