NanoSight NTA Concentration Measurement Upgrade

PARTICLE CONCENTRATION

Introduction

Nanoparticle tracking analysis (NTA) provides single particle size and concentration measurements. The growing popularity of the technique has driven the requirement to reduce user input, reduce variability and provide interlaboratory consistency in size and concentration measurements. The NTA concentration measurement has been modified to provide improved accuracy, precision and reproducibility of concentration measurements on a range of nanoparticle types, covering a broad size and concentration range. Concentration measurement repeatability and reproducibility have been greatly increased whilst removing measurement sensitivity to user settings over the recommended concentration range for NTA analysis.

Nanoparticle Tracking Analysis (NTA) Overview

NTA utilizes the properties of both light scattering and Brownian motion in order to obtain the particle size distribution of samples in liquid suspension. A laser beam is passed through the sample chamber, and the particles in suspension in the path of this beam scatter light in such a manner that they can easily be visualized via a 20x magnification microscope onto which is mounted a camera. The camera, which operates at approximately 30 frames per second (fps), captures a video file of the particles moving under Brownian motion within the field of view of the camera (Figure 1).

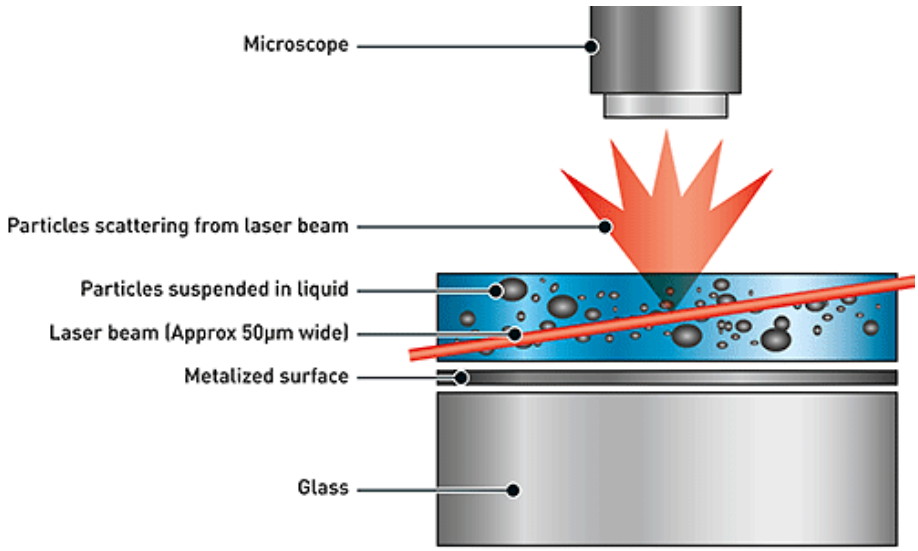

Figure 1: Schematic of the optical configuration used in NTA.

The movement of the particles is captured on a frame-by-frame basis. The proprietary NTA software simultaneously identifies and tracks the center of each of the observed particles, and determines the average distance moved by each particle in the x and y planes. This value allows the particle diffusion coefficient (Dt) to be determined from which, if the sample temperature T and solvent viscosity η are known, the sphere-equivalent hydrodynamic diameter, d, of the particles can be identified using the Stokes-Einstein equation (Equation 1).

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

Equation 1

where KB is Boltzmann's constant.

NTA is not an ensemble technique interrogating a very large number of particles, but rather each particle is sized individually, irrespective of the others. An example of the size distribution profile generated by NTA is shown in Figure 2.

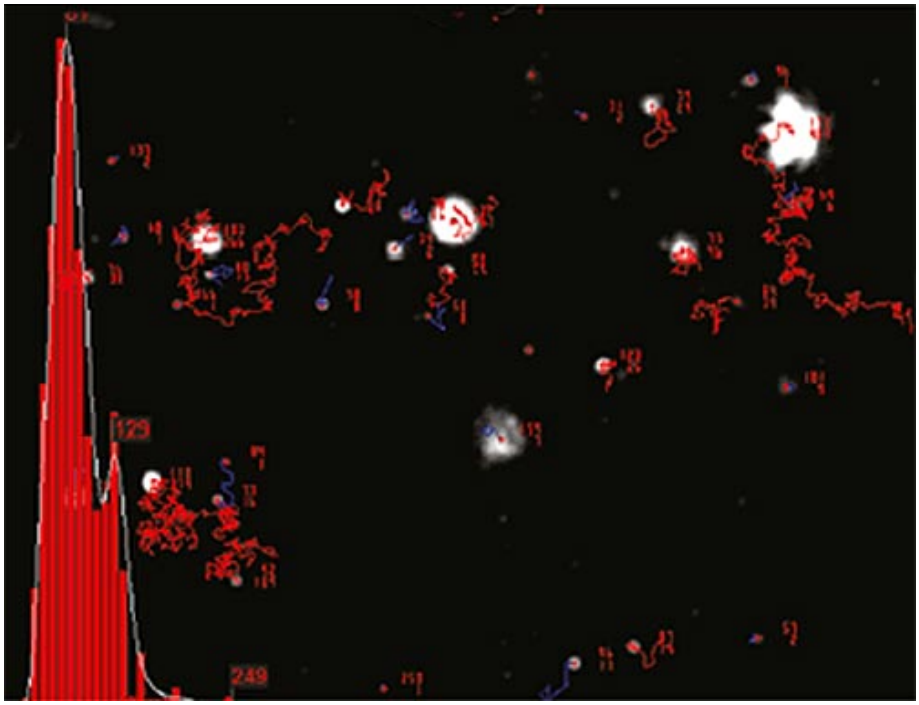

Figure 2: An example of the size distribution profile generated by NTA. The modal size for this sample is found to be approximately 70 nm, with larger sized particles also present.

In addition, because NTA can measure particle by particle the number of particles within the field of view is known. Whilst standard NTA measurements use a fixed field of view (approximately 100 µm by 80 µm) illuminated by a beam approximately 10 µm in depth allowing a scattering volume of the sample to be estimated. The new NTA concentration measurement upgrade reduces the influence of both capture and analysis settings on the concentration result, giving more accurate, precise and repeatable concentration values.

Results

Concentration measurement accuracy has been shown to increase significantly when using the NTA Concentration Measurement Upgrade, as has inter-laboratory precision (Figure 3). Identical samples were analysed at user selected settings on two different NS500 instruments in 2 different laboratories. Before the NTA concentration measurement upgrade was applied results from the two laboratories varied significantly, especially at higher concentration levels. After the upgrade is applied the reproducibility between the two labs is greatly improved and the accuracy of the measurement to the sample concentration is also significantly improved.

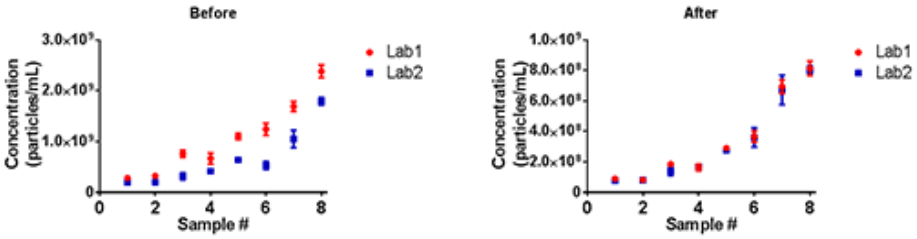

Figure 3: Interlaboratory precision on a range of sample types and concentrations before and after the NTA Concentration Measurement Upgrade.

The use of the NTA syringe pump has also been shown to help drive down variation between measurements whilst improving both sizing and concentration repeatability. An example of the improvements seen with the use of the NTA syringe pump is shown in Figure 4 where a sample of 100nm polystyrene standards was analysed using either static (no syringe pump) or flow (with syringe pump) measurements with the same capture and analysis settings.

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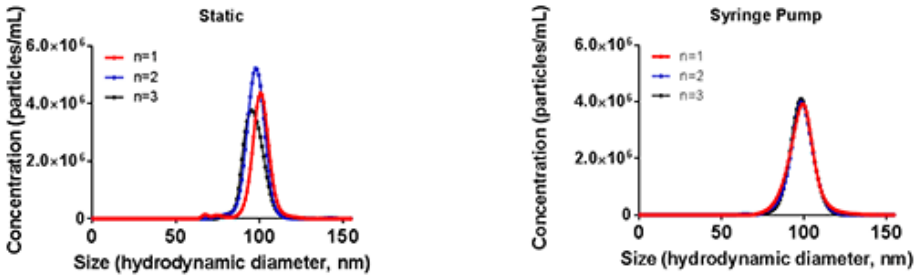

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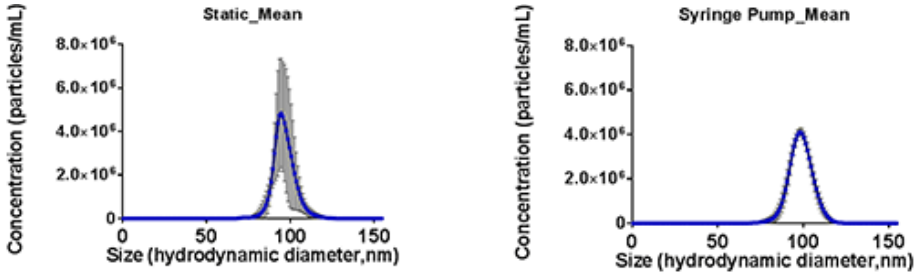

Figure 4: Improvements in sizing and concentration measurement repeatability when using the NTA syringe pump, compared to static measurements, in conjunction with the NTA concentration measurement upgrade. Samples (100nm polystyrene standards) were analysed as 5 x 60 second videos at the same camera levels and detection thresholds with 3 repeats being compared (a). The variation between 5 individual 60 second measurement is shown for static and flow conditions (b).

A protocol for sample delivery and measurement has been developed which has contributed to improvements in precision. These combined steps provide repeatability values as low as 3 % (cv). Measurements of dilution linearity can

provide accurate results with an error as low as 13 % across a concentration range of 1x106 – 1x109 particles per ml (subject to particle size and refractive index).

The NTA Concentration Measurement Upgrade reduces the susceptibility of the concentration measurement to user settings (camera level and detection threshold) as shown in Figure 5. Samples recorded at different camera levels were analysed over a range of detection thresholds. Whereas before the NTA concentration measurement upgrade concentration results varied with chosen detection threshold, after the upgrade the results are comparable over the range of detection thresholds tested. Results recorded at different camera levels but analysed at the same detection threshold again show variation in concentration before the upgrade is applied but this variation is greatly reduced with the application of the upgrade.

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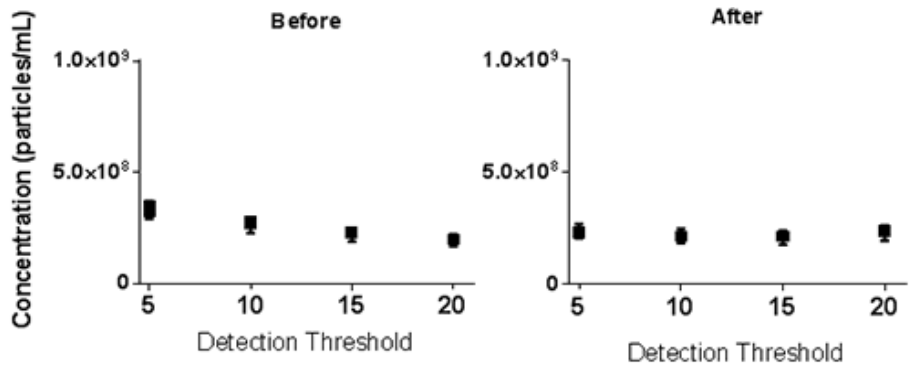

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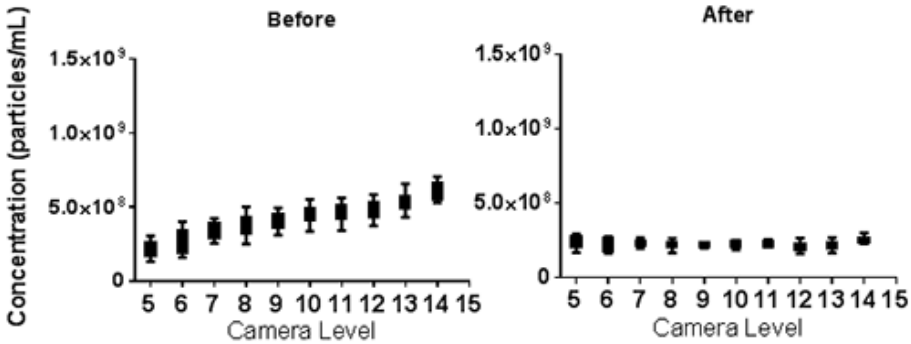

Figure 5: Concentration data showing the influence of camera level and detection threshold setting before and after Concentration Measurement Upgrade. a) 100nm PSL sample recorded at a single camera level then analysed at different detection thresholds and b) 100nm PSL sample recorded at varying camera levels and analysed at a single detection threshold.

Whilst total particle concentration has been shown to vary (Figure 5) as a result of user settings before the upgrade is applied the overall distribution also shows

reduced variation upon upgrade application (Figure 6). This reduction has been seen over a range of particle sizes, concentrations and materials.

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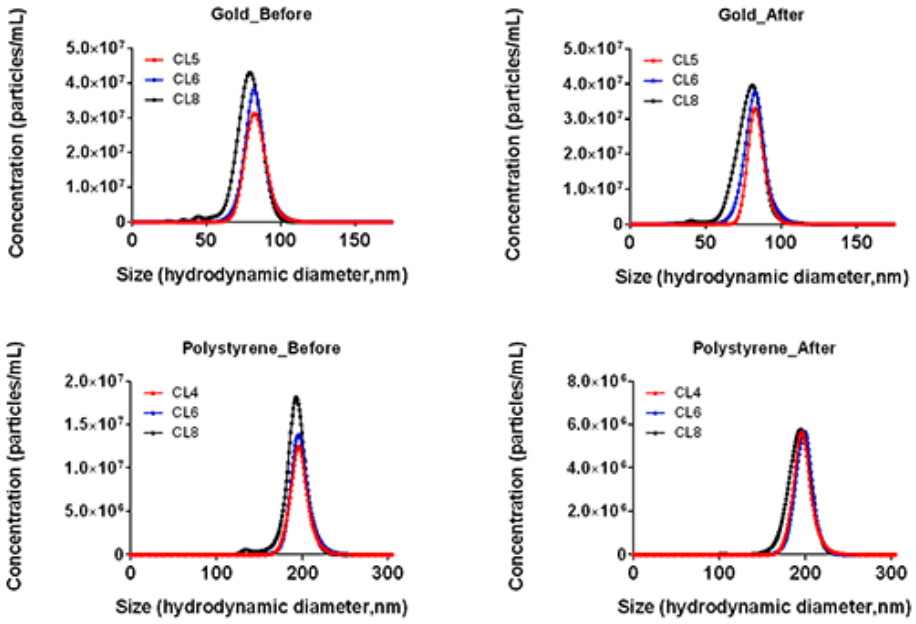

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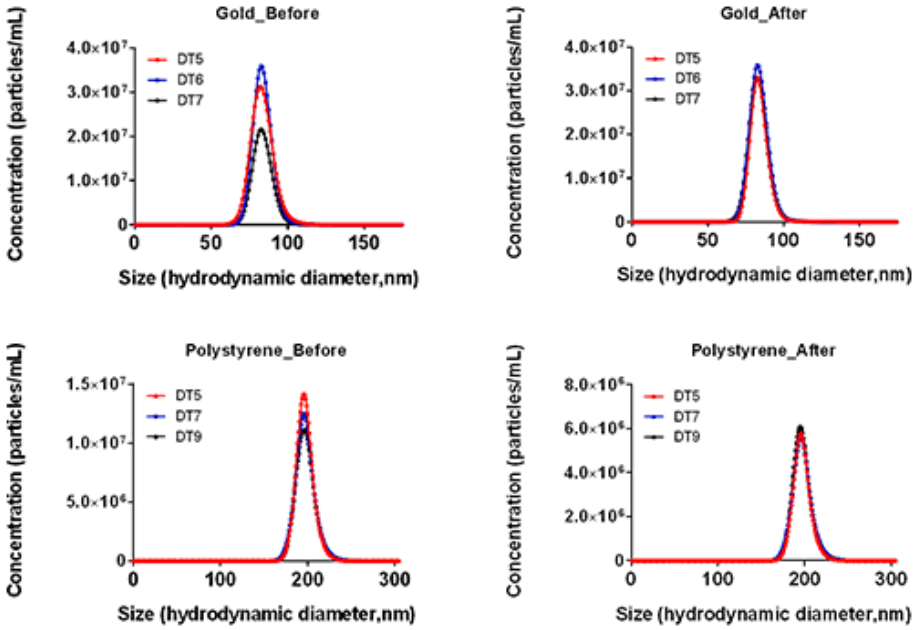

Figure 6: Influence of user settings on NTA concentration measurements and distributions, with and without the NTA concentration measurement upgrade applied, over a range of (a) camera levels and (b) detection thresholds for both 80nm gold and 200nm polystyrene particles.

The study of extracellular vesicles is an area that has become the subject of intense study and research in recent years. These vesicles are apparently

ubiquitous in a broad range of prokaryotic and eukaryotic organisms and it is believed they have a wide role to play in many physiological and pathological processes. They are typically described either as exosomes which are produced from the cell endosome or microvesicles, produced by cell membrane budding. Their cellular origin, structure, functions and characterization are still the subject of much debate. Also debated by the research community are the size of such vesicles though exosomes are agreed to be smaller in size (typically 100 nm in diameter or smaller) whilst micro vesicles are larger (typically described as being up to 1 µm in diameter).

The NTA Concentration Measurement Upgrade has also been shown to improve the accuracy and precision of concentration measurements on non-standard samples including exosomes and microvesicles (Figure 7). In this exosome example, different users typically selected camera level 14, 15 or 16. The application of the upgrade has been shown to increase the repeatability of measurement at different camera levels whilst reducing setting induced differences.

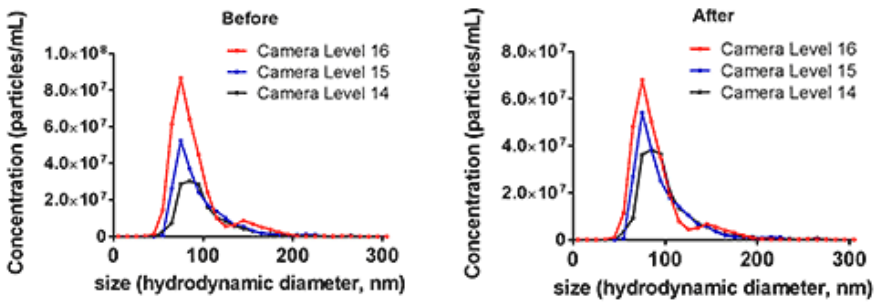

Figure 7: Exosome concentration data showing improved accuracy and precision after the NTA Concentration Measurement Upgrade at three different camera levels.

Conclusion

The NTA concentration measurement upgrade has been shown to improve both the accuracy and precision of concentration measurements over a wide range of sample sizes, concentrations and materials. The inter- and intra- laboratory repeatability and reproducibility are greatly improved with the application of the upgrade, which has also been shown to reduce the influence of user selected capture and analysis settings on measurements. The use of a NTA syringe pump significantly increases the accuracy, precision and repeatability of both size and concentration measurement and the upgrade has also been shown to positively benefit concentration measurements of non-standard sample types such as exosomes and microvesicles.

Parameter	Specification
Repeatability	3% (15% before upgrade)
Reproducibility	10% (44% before upgrade)
Dilution Linearity error	13%
Concentration Range	$1x10^6$-$1x10^9$ particles per ml

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