

# Zeta Potential Analysis using Z-NTA

## Summary

Zeta Potential Nanoparticle Tracking Analysis (Z-NTA) adds measurements of electrostatic potential to simultaneous reporting of nanoparticle size, light scattering intensity, fluorescence and count, and does so particle-by-particle. Individual particle analysis produces number-weighted, not intensity-weighted data, avoiding any bias towards larger particles. Polydisperse and complex suspensions of both positively and negatively charged particles are readily characterised, and the results are verified by real-time observation of particles moving under both electrophoresis and Brownian motion, without the need for any particle labelling. Changes in the zeta potential distribution with pH, concentration, temperature and particle size can be studied, and aggregation can be measured quantitatively in real-time. No other methodology comes close to providing such simultaneous, multiparameter nanoparticle characterisation.

## Introduction

The zeta potential of a system is a measure of charge stability and controls all particle-particle interactions within a suspension. Understanding zeta potential is of critical importance in controlling dispersion and determining the stability of a nanoparticle suspension, i.e. to what degree aggregation will occur over time. The zeta potential is the measure of the electric potential at the slip plane between the bound layer of diluent molecules surrounding the particle, and the bulk solution. This can be closely linked to the particle's surface charge in simple systems but is also heavily dependent on the properties of the diluent solution. A higher level of zeta potential results in greater electro-static repulsion between the particles, minimising aggregation/flocculation

Samples with zeta potentials of between -30mV and +30mV typically tend to aggregate, although the precise stability threshold will vary according to particle type. Determining the stability of a sample, either to minimise aggregation for drug delivery and pharmaceutical applications (high zeta potential), or to facilitate the removal of particles too small to filter out for water treatment applications (low zeta potential) is of great importance in nanoparticle research.



Figure 1. The NanoSight NS500 instrument, fitted with the ZetaSight module, incorporating a customised Zeta Potential sample chamber and integrated electronics.

The ZetaSight system, an upgrade module for the NanoSight NS500, allows the zeta potential of individual particles in aqueous suspension to be determined. This provides a detailed view of the particle distribution in terms of electrical potential and related stability.

## Measuring Zeta Potential with NanoSight

The ZetaSight system allows the zeta potential of nanoparticles in aqueous suspension to be measured on a particle-by-particle-basis. The customised zeta potential sample chamber is fitted with platinum electrodes, which allow a variable electric field to be applied to a sample of nanoparticles suspended in aqueous solution.

The electric field causes motion of both the sample particles, (electro-phoresis), and the aqueous diluent, (electro-osmosis). The NanoSight technique records the apparent drift velocity for each tracked particle, which will be a superposition of these two motions. By observing the total velocity at different depths within the sample chamber, it is possible to separate these components and obtain a measurement of the electro-phoretic velocity (due to the force impinged directly on the particles), and hence the zeta potential of the particles.



## A Self-Calibrating Technique

### Correcting for Electro-osmosis

With the application of an electric field near to the glass sample chamber surfaces, electro-osmosis will contribute to the apparent particle velocities observed by the NanoSight Z-NTA (Zeta Potential Nanoparticle Tracking Analysis) technique, and must be corrected for. Glass has an inherent negative surface charge, which for a polar liquid like water, causes a charge imbalance of diluent molecules near the glass boundary. Viscous forces carry the resulting fluid flow through the chamber, causing a parabolic flow profile for a closed system, as shown in Figure 2.

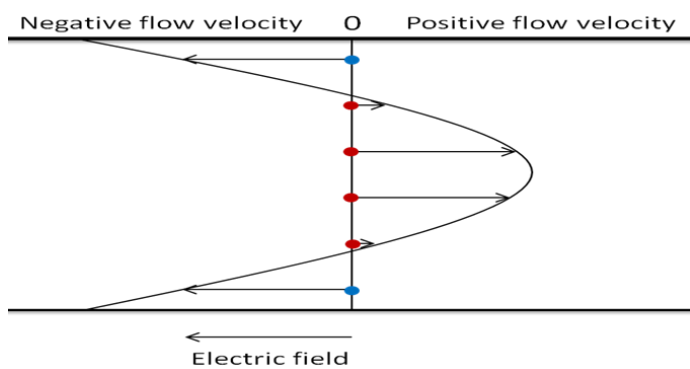


Figure 2. Electro-osmotic velocity profile for water in a closed sample chamber under the influence of an applied electric field

Accurate characterisation of the particle velocity profile within the NS500 sample chamber depends critically on the depths at which particles are tracked. The depth of each capture position with respect to the top and bottom surface of the channel is standard for all systems, and is programmed into the NS500 control software. The NS500 instrument, which incorporates motorised stage control, can then scan through a sequence of 6 fixed positions, as shown in Figure 3, in order to determine the velocity profile, which can then be used to compensate for the effect of electro-osmosis.

In a closed system the electro-osmotic velocity component will always have an overall value of zero when summed over the entire channel depth. Any offset which causes the total measured velocity profile to not sum to zero represents the average electrophoretic velocity of all particles tracked.

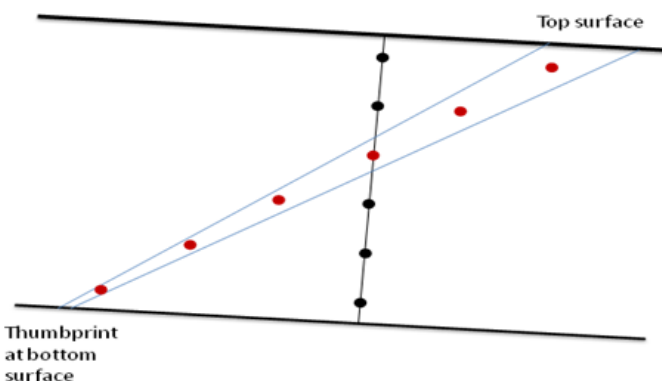


Figure 3. Diagram showing profile map positions where 6 standardised depth locations intersect the laser beam.

By tracking particles at depths throughout the channel and subtracting this offset it is possible to obtain a measurement of the electro-osmotic profile of the diluent within the channel, as shown in Figure 4.

The electro-osmotic contribution to the total observed velocity can then be found for any of 6 channel depths at which particles are tracked, and removed from the total observed drift velocity. This electro-osmotic profile is measured for each experimental run, providing data sets which automatically account for the electro-osmotic effect, without the need to assume that the diluent flow profile or the chamber surface chemistry remains constant.

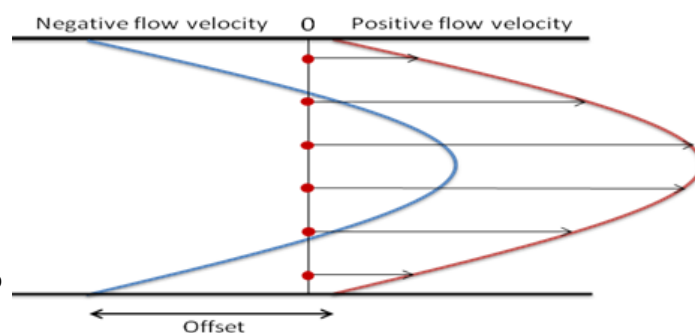


Figure 4. Total velocity profile measured using NTA (red) and electro-osmotic velocity profile (blue) inferred by subtraction of a contact depth offset to obtain a velocity profile that sums to zero over the channel depth



## Correcting for Thermal Effects

As well as electro-osmotic motion, any other effect which causes particles tracked by NTA to move must be accounted for and removed in order to obtain a measurement of the true electro-phoretic particle velocity. Thermal effects due to laser heating or joule heating from the electric current passing through the sample between the electrodes, can cause convection flows in the diluent. By reversing the voltage polarity, any velocity component which is not dependent on the electric field direction can be characterised. For each capture position, particles are tracked under positive and negative polarity electric field, and any bias is removed from the raw data.

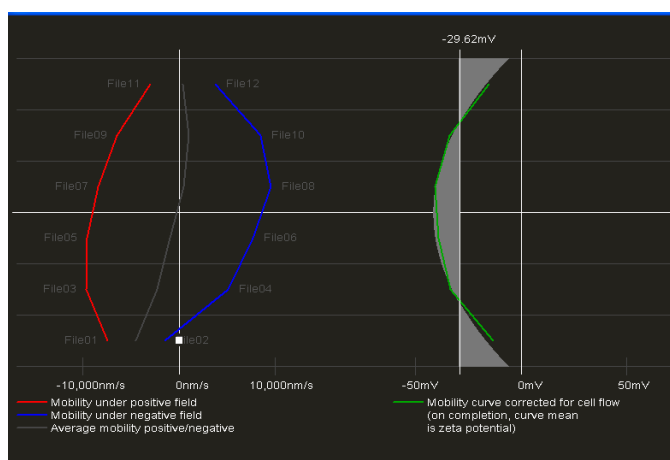


Figure 5. Velocity profile for a filled sample chamber displaying significant convection flows near the glass interface where the laser enters the sample.

Figure 5 shows the velocity profile in sample chamber where particles have become attached to the bottom glass surface. This causes increased heating near the interface where the laser enters the sample, resulting in a convection flow. Using the voltage reversal technique, this flow can be subtracted from the measured flow profile (shown in red and blue for positive and negative field polarity respectively) to give a corrected flow profile (shown in yellow)

## Calculating Zeta Potential on a Particle-By-Particle Basis

Once the velocity components due to electro-osmosis and thermal convection have been removed, the corrected drift velocities, calculated on a particle-by-particle basis, then provide a measure of the electro-phoretic velocity of each particle in the sample.

The electric field strength (E) within the flow cell is determined using the Voltage (V) applied through the sample by the electrodes, and the distance between the electrode surfaces (d).

$$E = \frac{V}{d}$$

The particle velocities can then be converted into electro-phoretic mobilities (velocity divided by electric field strength).

By application of the Henry equation using the Smoluchowski approximation (appropriate for aqueous diluent media with moderate electrolyte concentration) the zeta potential (ZP) for each particle can be calculated:

$$ZP = \frac{\mu \eta}{\epsilon_0 \epsilon_r}$$

where  $\mu$  is the electrophoretic mobility of the particle,  $\epsilon_0$  is the permittivity of free space,  $\epsilon_r$  is the relative sample solution permittivity and  $\eta$  is the sample solution viscosity.

## Zeta Potential of NIST standards

Monodisperse single particle populations, such as NIST calibration quality polystyrene size standards, are often only described in terms of an average zeta potential value. In reality however, particles will always have a range of values and knowledge of the full zeta potential distribution can provide much more information. This is especially critical where small changes in the zeta potential distribution have a large effect on the behaviour of a nanoparticle product, or for the detailed comparison of samples which may all be close to the stability threshold.

## 100nm polystyrene microspheres in deionised water

The ZetaSight technique was used to analyse NIST 100nm polystyrene size standards from Duke Scientific, diluted in deionised water to a concentration of  $4 \times 10^8$  particles/ml. The measured zeta potential distribution is shown in Figure 6, with a modal peak at a value of  $-48$ mV.



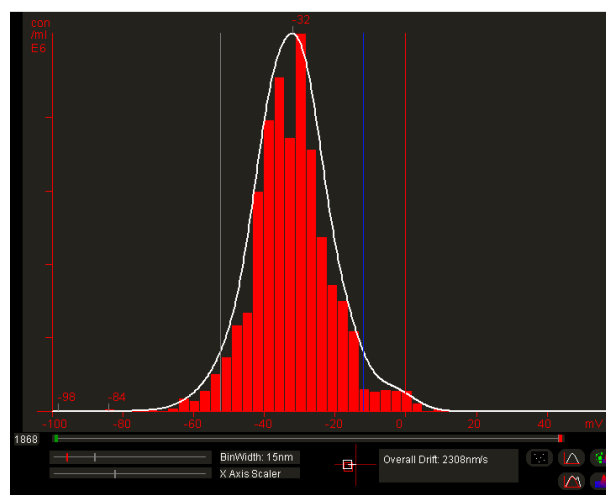
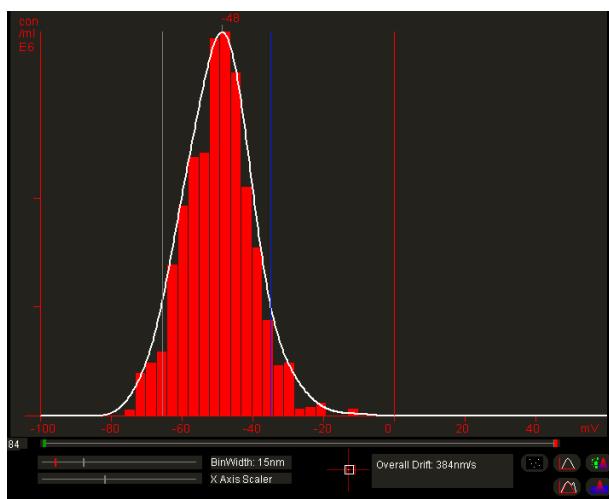


Figure 6. Zeta potential distribution for 100nm polystyrene microspheres diluted in deionised water. Top plot shows number concentration as a function of zeta potential. Bottom plot shows a scatter graph of zeta potential against particle size.

Figure 7. Zeta potential distribution for 100nm polystyrene microspheres diluted in laboratory tap water, showing the shift in zeta potential compared with Figure 6.

The distribution shows a spread in the zeta potential, with most particles lying within the range from  $-35\text{mV}$  to  $-65\text{mV}$ , as shown on the scatter plot. This confirms the high stability of polystyrene particle standards when diluted in clean de-ionised water. The particle size, measured simultaneously, is also shown on the horizontal axis in the bottom panel of Figure 6. The measured Brownian motion is corrected for any net drift velocity, so that the calculated size is reported correctly at 100nm, even when the particles are also moving under the influence of an electric field.

### 100nm polystyrene microspheres in tap water

The same analysis run on 100nm polystyrene diluted to the same concentration in laboratory tap water shows a peak in the distribution at a much lower zeta potential.

This demonstrates how the zeta potential of a sample is heavily dependant on the diluent used, as well as the properties of the solid particles.

The results displayed in Figure 7 show that polystyrene standards diluted in tap water are near the limit of stability in terms of the zeta potential, with a modal peak in the distribution at  $-32\text{mV}$ . The distribution indicates significant numbers of particles with a zeta potential between  $-30\text{mV}$  and  $+30\text{mV}$ . These particles will have a tendency to aggregate over time. Contaminating aggregates are seen in standards diluted in tap water over a period of several days, which is in agreement with these results.



## Zeta Potential Transfer Standard

The Z-NTA technique was used to analyse the zeta potential transfer standard (DTS1230), manufactured by Malvern Instruments Ltd. The standard comes in a form too concentrated for the NanoSight technique and was therefore diluted down to optimal concentration using filtered samples of the supplied buffer. The results are shown in Figure 8.

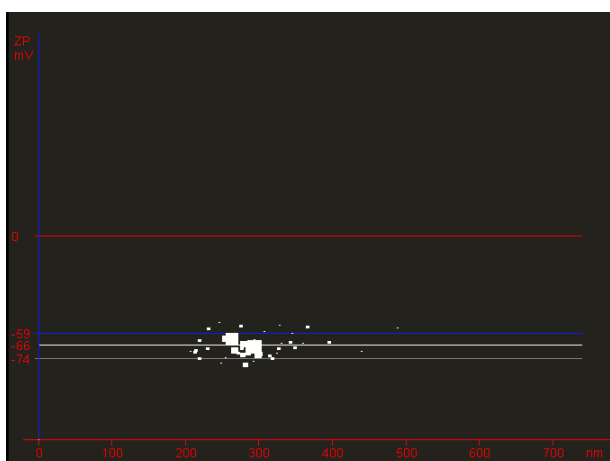
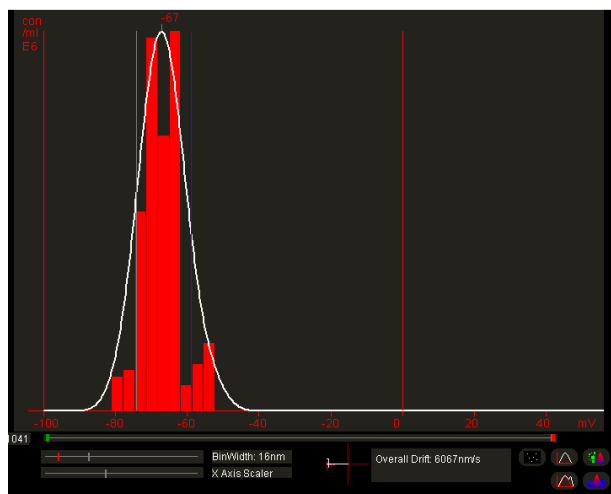


Figure 8. Zeta potential distribution for the transfer standard DTS1230, diluted in the supplied buffer.

The results show a modal peak at  $-67\text{mV}$ , in accordance with the stated value of  $-68 \pm 7\text{mV}$ . The distribution shows a much narrower range in zeta potential than for the polystyrene samples, as expected for a suspension designed as a standard for this parameter. In contrast the size distribution is much broader, with particle size ranging from 250 to 350nm.

## Simultaneous Measurement of Size, Zeta Potential and Light Scattering Intensity

The ZetaSight technique allows the simultaneous measurement of size, zeta potential and light scattering intensity for individual nanoparticles in solution. This allows particle populations to be separated in terms of any one of these parameters, and for the relationship between parameters, for example the dependence of zeta potential on particle size, to be studied.

Figure 10 shows 2-dimensional slices from a 3-dimensional plot, taken from the NanoSight Z-NTA software display, that demonstrates the analysis of two separate particle populations. Results for the 100nm NIST polystyrene size standard in tap water are shown in blue. The white scatter points are for the zeta potential transfer standard (DTS1230), with a size of approximately 300nm.

The top panel shows the relationship between light scattering intensity and size, the bottom panel shows the plot rotated to display the relationship between zeta potential and size.

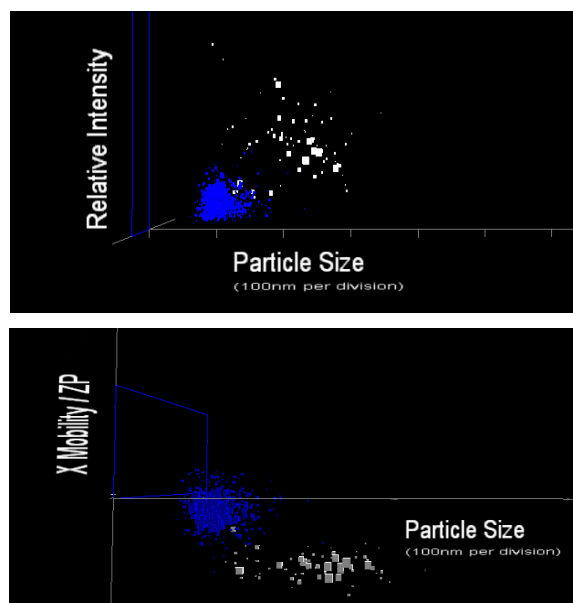


Figure 9. Multiparameter plots showing the simultaneous measurement of light scattering intensity, size and zeta potential for two analysed samples

## Contact Details

For further information contact NanoSight or your local distributor, listed at [www.nanosight.com](http://www.nanosight.com)

