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# ZetaView®
# Nanoparticle Tracking Analyzer


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# ZetaView® Nanoparticle Tracking Analyzer

## Measure, what you see

With ZetaView®, individual particle tracking, classical micro-electrophoresis and Brownian motion are presented in a modern analysis tool. Auto-alignment and auto-focusing make the principle of "Seeing is Believing" user friendly. By sub-volume scanning, robust results of zeta potential and size histograms are derived from thousands of particles. In addition, particle concentrations can be determined by video frame assessment counting. Sample cell handling is reduced to a handling.

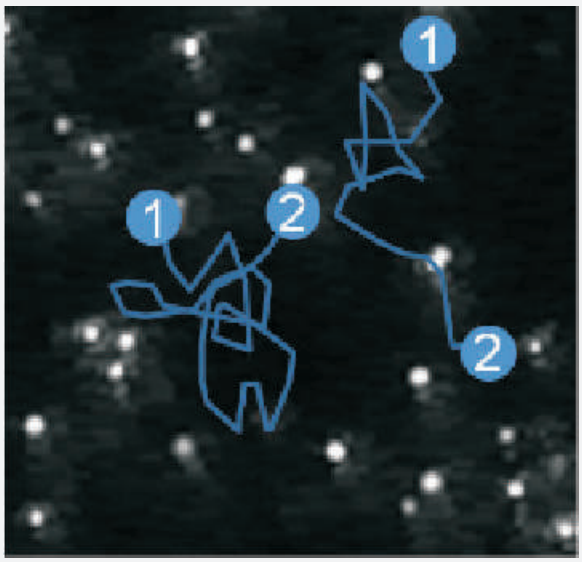


Fig. 1: Following the movement of individual particles

## Advantages of using ZetaView®

**Automation and Passive Stability** - the "Auto-Alignment" procedure keeps the system in focus for days, even after cell removal. The anti-vibration design enhances the video image stability. By scanning multiple sub-volumes and averaging the measurements, statistically robust results are guaranteed. Three measurement modes are available: Size, Zeta Potential and Concentration. Asymmetrical cell coating by sedimentation is avoided by the orientation of the cell walls (Fig. 2). The cell channel is integrated in a „slide-in" cassette (Fig. 3). This is provided with temperature control and snap-in couplings.

**Established Theory** - The translational diffusion constant is calculated from the direct observation of Brownian motion to calculate size. From the measurement of the electrophoretic mobility, zeta potential is calculated.

**Fast Results** - The main difference between DLS and NTA is the handling of concentration range. When the concentration is too low for DLS, the ZetaView Nanoparticle Tracking Analyzer will perform the task brilliantly and fast.
Versatility - Conversely, the NanoFlex®180° DLS system is ideal for analysis of high concentration samples.

**Broad Measurement Range** - Depending on the sample and the instrument model, the direct tracking of particles is possible over a size range starting at 10 nm for gold nanoparticles (correspondingly higher for particles with less scattering power). Provided the sample is stable and does not sediment or float, the zeta potential upper size limit can be 20 µm while for particle sizing, it is 3 µm.

**Particle Counts from Video Frame Assessment** - The particle concentration is derived from analyzing the video frames to observe particle numbers. It is normalized to the scattering volume per relevant size class. A minimum concentration of 105 particles /cm³ can be detected while the maximum is 1010 /cm³. In volume concentration, up to 1000 ppm of 200 nm sized particles can be analyzed.


- Autoscan over up to 100 subvolumes
- Autofocus
- instrument fits into a pilot case
- anti-vibration design
- Lasers from UV to red
- Slide-in cell cassette

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**Excellent Accuracy and Precision** - For zeta potential, the accuracy is 5 mV, precision 4 mV and instrument-to-instrument repeatability is 5 mV. For size determination of a 100 nm standard latex suspension, the accuracy is 6 nm for number calculations, precision is 4 nm and instrument-to-instrument repeatability 4 nm. For 100 nm particles at a concentration of 10 million particles / mL, accuracy is 0.8 Mio / mL and precision 0.5 Mio / mL. Instrument-to-instrument repeatability is 1 Mio / mL. All given data are valid providing correct camera settings and sample preparation are adhered to.

**Sensitivity** - The ZetaView® laser light scattering microscope is sensitive to nanoparticles 100 times below the diffraction limit of ~ 1 µm (Fig. 2).

**Flexibility** - The instrument is compact and can be carried in a pilot case. Setup is in minutes. It can be operated in a network or stand-alone.

## The Method

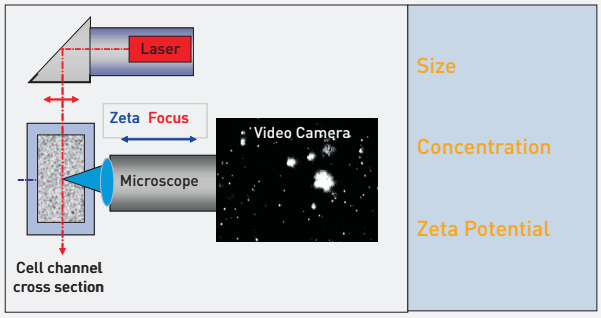


**Fig. 2:** Optical Layout of the automated particle tracking ZetaView® Laser Light Scattering unit having "ZetaFocus" as synchronous control of the laser and microscope focus. No change of microscope optics is necessary.

The image of the particles is focused onto the video camera. From measuring the particle velocity and direction under an applied electric field, the electrophoretic mobility and polarity are determined. With no field taken into account, only Brownian motion is detected. Electric field, temperature and conductivity are also monitored at each experiment. By scanning the cell cross section and sequencing sub-volumes, excellent statistical results can be obtained.

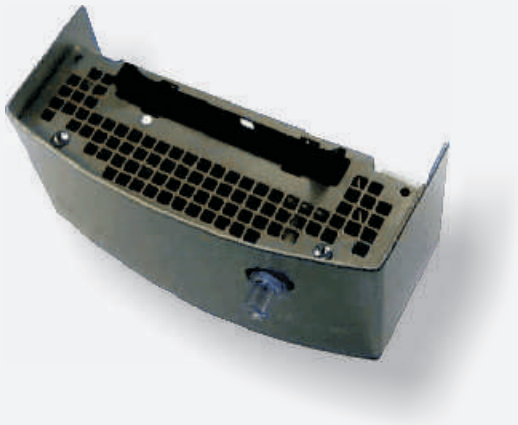

**Fig. 3:**
Slide-in cell cassette with sample cell carrier on top.

## ZetaView® for zeta potential AND quality checking

The zeta potential distribution is calculated from the electrophoretic mobility results at the two stationary layers in the cell (ζ-layer" / "ZP", Fig.4) or from an electrokinetic velocity profile obtained by scanning throughout the cell. In addition to the particle zeta potential result, the curvature of a profile delivers extra information on the polarity and amount of ionic coating on the cell walls. While wall coating is always present when dealing with ultrafine particles, this does not change the correctness of the electrophoresis zeta potential determination as this is not influenced by the wall coating.

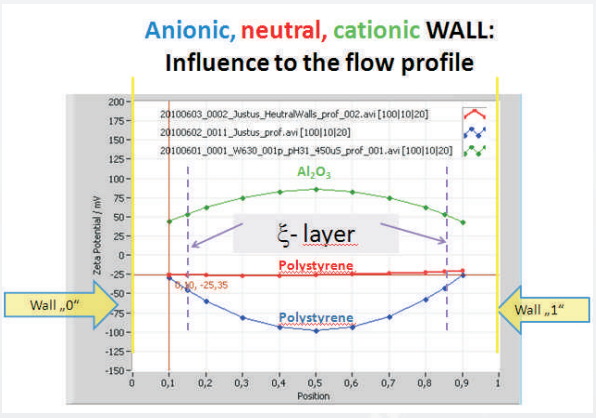


**Fig. 4:**
Green: ZP of $Al_2O_3$ is +50 mV, walls are cationically coated. Red: -25 mV ZP anionic polysterene, -; the walls are neutral. Blue: polystyrene -40 mV ZP, cell walls are anionic as uncoated glass usually is.

**Zeta potential distributions** - A few examples highlight the capability of the ZetaView in measuring zeta potential distributions. A 60 nm gold nanoparticle dispersion was studied for size and zeta potential distribution (Fig. 5). The sample is slightly bi-modal. Curve A (red) represents gold particles in a 2m KCl environment while curve B (blue) is of the same sample dispersed in distilled water. The zeta potential distribution of curve B shows a peak near 0 mV, which is making the suspension unstable. The majority of the 60 nm particles are therefore agglomerated which is clearly seen in the size distribution of curve B.

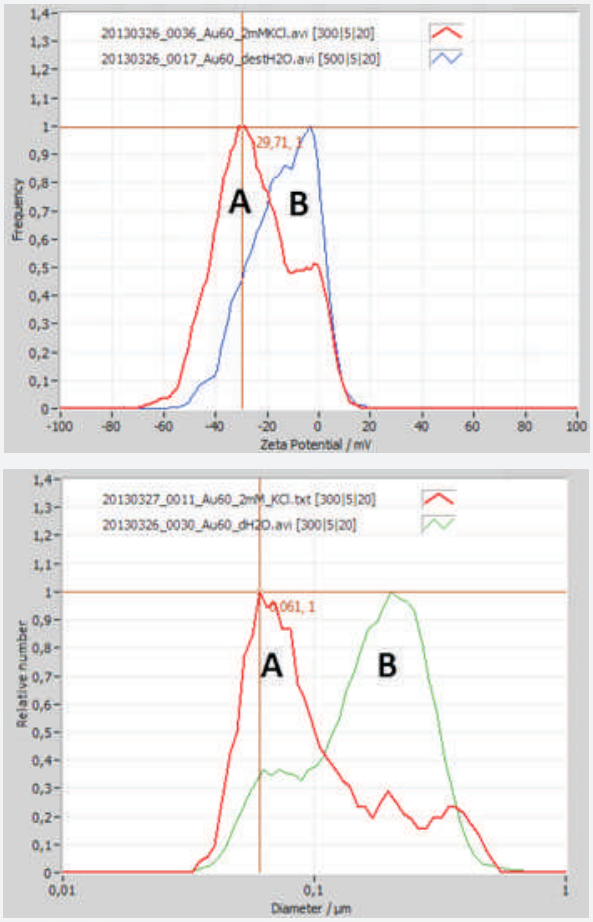


**Fig. 5:** Above: Zeta potential distribution of 60 nm gold particles. A: dispersed in 2 mM KCl solution (stable). B: dispersed in distilled water (unstable). Below: Correspondingly, the less stable sample B showing coagulated objects.

## Translational diffusion size distribution

The PMX 110 ZetaView has a lower detectable limit of 20 nm LOQ (Fig. 6). For substances with lower scattering efficiency, the lower size limit is correspondingly higher.

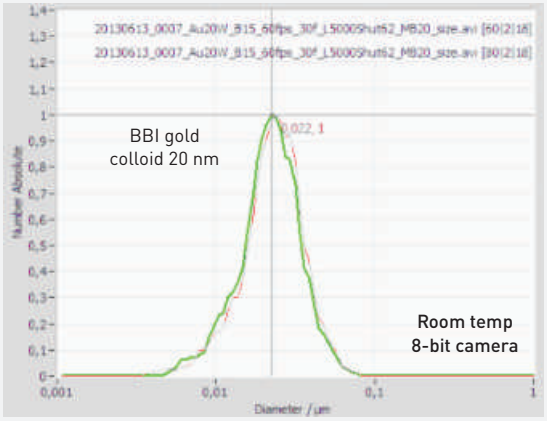


**Fig. 6:** Size distribution (LOQ) of 20 nm BBI gold particles in distilled water.

In an example of cell-derived exosomes in a pH 7 Sørensen phosphate buffer, the interdependence between conductivity, zeta potential and size distribution is demonstrated (Fig. 7). The conductivity is automatically monitored during the data acquisition.

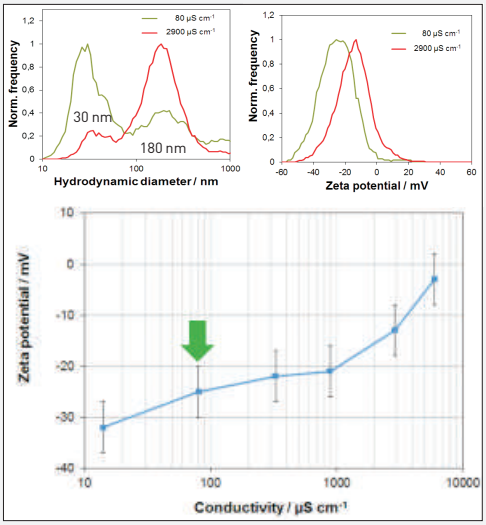


**Fig. 7:** The interdependence between conductivity, zeta potential and particle size distribution, demonstrated with exosomes* (see text).

## Choice of lasers

Lasers can be selected from UV to red. Contact Particle Metrix about the laser you require.

## With one fill of 500 µL ...

You can obtain a simultaneous multi-sub-volume and multi-parameter result with concentration, size, zeta potential, conductivity and temperature.

Foot notes:
[1]C. Helmbrecht, K. deMiroschedji, A.-K. Ludwig, B. Giebel and H. Wachernig:, zeta potential, size distribution and concentration analysis of extracelluar vesicles, imaginenano conference , biomed section, Bilbao 2013.

# NANO-flex® - 180° DLS

## Extending the size and concentration range with combination of DLS

### NANO-flex®

The NANO-flex® 180° DLS System is designed to measure size distributions in the range between 0.8 and 6500 nm. It is suitable to analyze samples of broad size distributions with high resolution. Depending on the sample, it is possible to perform analyses from very low concentrations up to as high as 40%.

The NANO-flex® design offers a flexible measuring probe with 8 mm diameter offering many applications possibilities.

### The 180° DLS–Method

The laser (class 1) is focused onto the sample via an optical fiber and a sapphire window. The scattered light from the particles is measured at 180° to the beam. It passes through the fiber to the detector where it is optically amplified by the laser which is partially reflected at the window. Both beams interfere at the detector providing an amplified signal of the scattered light. See Fig. 8.

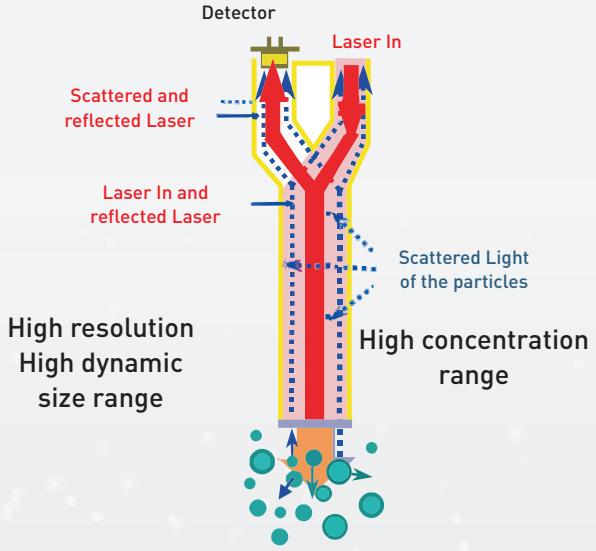


**Figure 8**: The described 180° DLS-method.

### Why use 180° DLS

- Shortest light path in the sample means:
  - o there is no multiple light scattering
  - o samples from transparent to opaque may be studied
- up to 40% concentration of particles may be analyzed
- the size result does not change over decades of sample concentration
- high dynamic range and high resolution is obtained in ONE measurement

### Applications

DLS applications with the NANO-flex® module are almost unlimited provided the viscosity of the sample is in the Newtonian range. This is a condition for the free Brownian movement in the fluid. The medium of the particles can be of organic or aqueous nature. Examples are shown in Figs. 9-11.

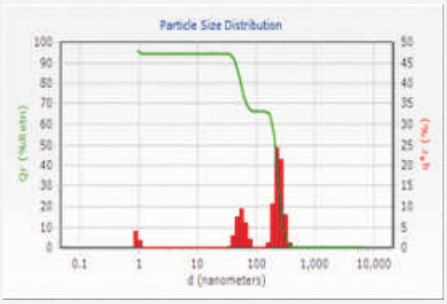


**Fig. 9**: Multiple peak size distribution of filtered beer

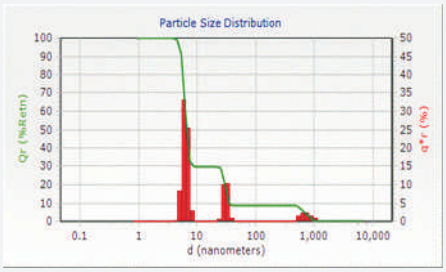


**Fig. 10:** Protein cristallisation after 3 hours

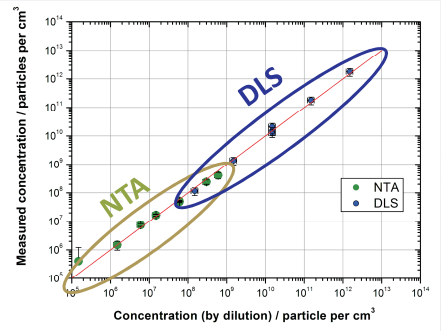


**Fig. 11**: The combination of DLS and NTA offers a superior concentration range from $10^5$ up to $10^{12}$ particles per cm³.


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