There are particular solutions
for every problem.
We reduce them to the max.

ZetaView®
Particle Tracking Analyzer

Particle Metrix.
share our view

individual particle processing
size – concentration – zeta potential
> 20 nm size distribution
10^4 to 10^{10} particles/mL concentration
zeta potential stability parameter
patented image sharpness stability

ZetaView® Particle Tracking Analyzer

Measure, what you see

With ZetaView® individual particle tracking, classical micro-electrophoresis and Brownian motion are presented as modern analysis tools. Auto-alignment and auto-focusing make the "Seeing is Believing" principle 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 few handholds.

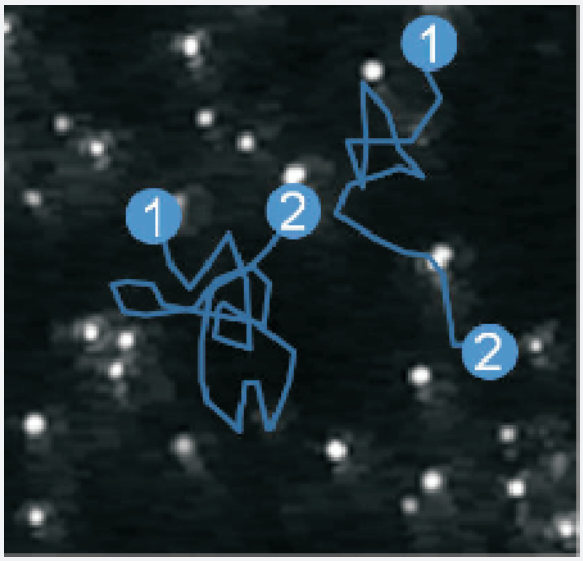

Fig. 1: Following the movement of individual particles

Features of the ZetaView®

Automation and Passive Stability - The "AutoAlignment" procedure keeps in focus for days, even after a 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 selectable: Size, Zeta Potential and Concentration. Unsymmetrical cell coating by sedimentation is avoided by the orientation of the cell walls (figure 2). The cell channel is integrated in a „slide-in" cassette (Photo), which is provided with temperature control and snap-in couplings.

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. Both methods are described in the Theory Annex.

Particle Tracking Analyis (PTA) and Dynamic Light Scattering (DLS) – All light scattering instruments – particle tracking included – suffer from the rapidly decreasing sensitivity below 100 nm. The lowest size level for DLS Dynamic Light Scattering is ~ 0.5 nm, for particle tracking analysis ~ 10 nm. In general the main relevant difference between DLS and PTA is the concentration range, when the concentration is too low for DLS, the ZetaView Particle Tracking Analyzer will perform the task brilliantly and fast. Conversely, the 180° DLS system is ideal for analysis of high concentration samples.

Measurement range - Depending on the sample and the instrument model, the direct tracking of particles is possible in a size range starting at 10 nm for gold and 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 50 µm, and for particle sizing 3 µm.

Particle counts from video frame assessment - The particle concentration is derived from analyzing the video frames for observed particle numbers. It is normalized to the scattering volume per relevant size class. A minimum concentration of 10^5 particles per cm^3 can be detected, the maximum is 10^{10} p/cm^3. 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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Trueness and precision - For zeta potential, the trueness is 5 mV, precision 4 mV and , instrument to instrument repeatability 5 mV. For size determination of a 100 nm standard latex suspension the trueness 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.

The Method

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

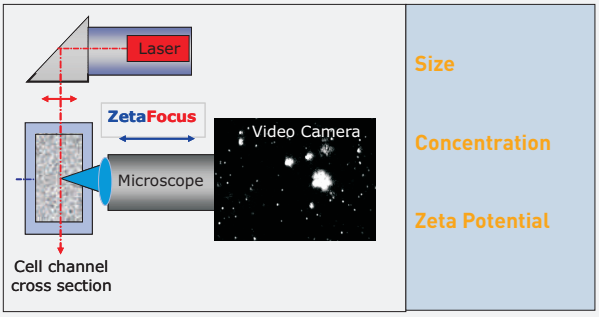


Fig. 2: Optical Layout of the automated particle tracking ZetaView® Laser Light Scattering unit having "ZetaFocus" as synchronous control of the laser and microscope focus. No change of microscope optics is necessary..

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.

Quality of measurements – The quality of data may be affected due to bubbles or dirt in the cell. Therefore a statistical evaluation of the image quality is performed. Adequate documentation identifies these positions.

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 subvolumes excellent statistical results can be obtained.

Photo: 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 2 stationary layers in the cell (ζ-layer" / "ZP", fig.3) or from an electrokinetic velocity profile obtained by scanning throughout the cell. The zeta potential is calculated from the Smoluchowski or Henry equation (see "Theory"). In addition to the particle zeta potential result, the curvature of a profile delivers free information on the polarity and amount of ionic coating on the cell walls. WALL COATING IS ALWAYS PRESENT when dealing with ultrafine particles. The correctness of the electrophoresis zeta potential determination is not influenced by the wall coating.

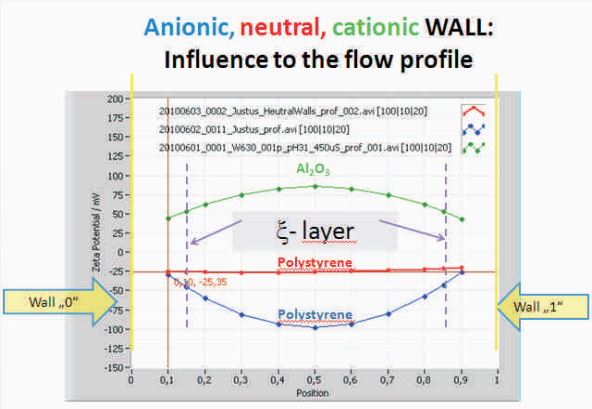


Fig. 3:
Green: ZP of Al_2O_3 is +50 mV, walls are cationically coated. Red: -25 mV ZP anionic polysterene, -; the walls are neutral. Blue: polystyrene -40 mV ZP, cell walls are anionic as uncoated glass usually is.

Zeta potential distributions - A few examples shall highlight the capability of the ZetaView in measuring zeta potential distributions. A 60 nm gold dispersion was studied for size and zeta potential distribution (fig. 4). The sample is slightly bi-modal. Curve A (red) represents gold particles in a 2m KCl environment, curve B (blue) the same sample but 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 is therefore agglomerated, which is clearly seen in the size distribution of curve B.

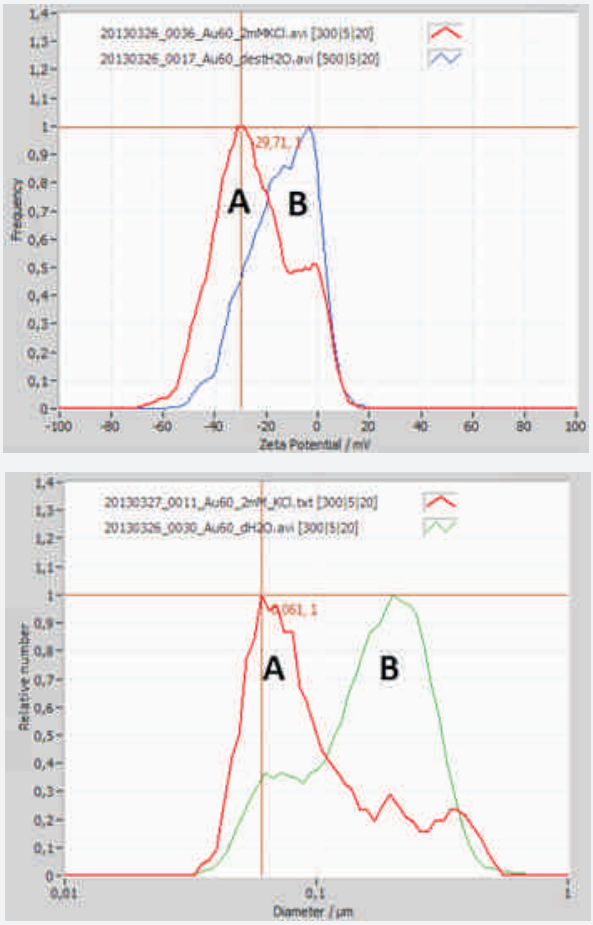


Fig. 4: Above: Zeta potential distribution of 60 nm gold particles. A: dispersed in 2 mM KCl solution (stable). B: dispersed in distilled water (unstable). Below: Correspondingly, the less stable sample B showing coagulated objects.

Translational diffusion size distribution

The PMX 110 ZetaView has a lower detectable limit of 20 nm LOQ (fig. 5). For substances with lower scattering efficiency, the lower size limit is correspondingly higher.

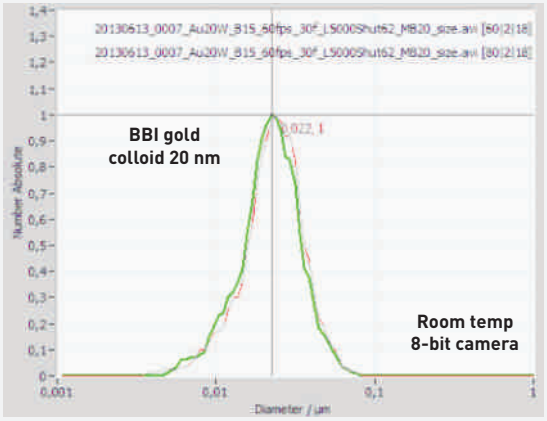


Fig. 5: Size distribution (LOQ) of 20 nm BBI gold particles in distilled water.

In an example of BMBO cell derived exosomes in a pH 7 Soerensen buffer, the interdependence between conductivity, zeta potential and size distribution is demonstrated (fig.6). The conductivity is automatically monitored during the data acquisition.

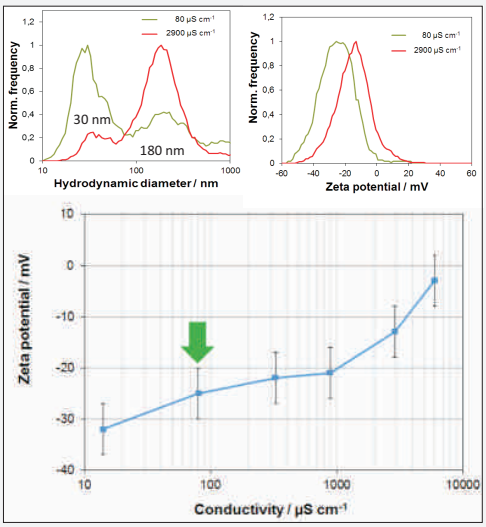


Fig. 6: The interdependence between conductivity, zeta potential and particle size distribution, demonstrated with exosomes* (see text).

Choice of lasers

Lasers can be selected from UV to red. The price of the system depends on the laser type.

With one fill of 500 µL ...

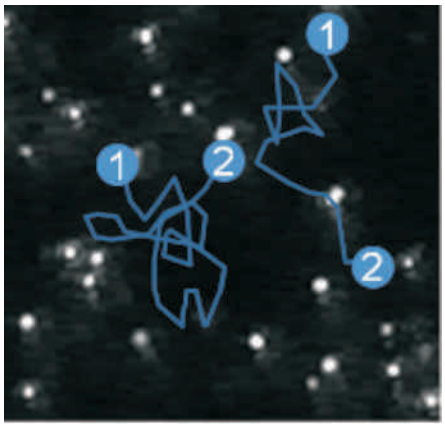
you obtain a multi-subvolume and a multi-parameter result with concentration, size, zeta potential, conductivity and temperature.

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.

Theory

Particle size by Brownian motion: Nano Tracking Analysis (NTA)



AuNP 40 nm

Quantification of average mean square displacement per time interval

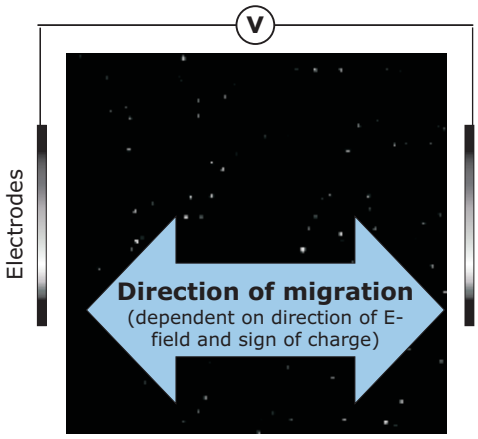
$$Dt = \frac{<\overline{x,y}^2>}{4}$$

Stokes - Einstein equation

$$D = \frac{k_B T}{6\pi\eta r}$$

D = Diffusion coefficient
$<\overline{x,y}^2>$ = Mean square displacement
k_B = Boltzmann constant
T = Temperature
η = Viscosity
r = Particle radius

Principle of zeta potential determination



PS 300 nm

Electrophoretic migration is captured for each single particle

$$\mu_e = \frac{v}{E}$$

Zeta potential ζ is obtained from **Smoluchowski** (f=1), **Henry** (f=1.5, depending on size and conductivity)

$$\zeta = \frac{4\pi\eta}{\varepsilon} f(\kappa a) \cdot \mu_e$$

ε = Dielectric constant
η = Viscosity of medium
$f(\kappa a)$ = Debye function
ζ = Zeta potential
μ_e=Electrophoretic mobility
v = Velocity of particle in E-field
E = Electrical field

ZetaView® PMX 110 Technical Data

Measurement principles	Micro-electrophoresis zeta potential and Brownian diffusion size, particle concentration by video frame assessment
Optical layout	Laser scattering video microscope with individual particle tracking. Auto-alignment and auto-focusing design
Measurement cell and cassette	Fused silica channel, slide-in cassette fitting onto 2 fluidic ports for rinsing and subvolume transport
Applied cell voltage	– 24 V, + 24 V for zeta potential, 0 V for size
Optical System	Microscope objective x 10 and digital camera, 640x480 px, 30 and 60 fps Laser type depending on application
Zeta potential range	- 200 to + 200 mV
Range of detectable particle size	0.02 - 50 µm for zeta potential determination 0.02 - 1 µm for particle size determination Lower and upper limits dependant on sample and laser
pH-range	1 – 13
Temperature range **Temperature control**	5 – 45°C outside temperature RT -5°C, up to 45°C
Conductivity range	0 - 4 mS/cm
Internal control - outputs	Temperature, conductivity, electric field, drift
Trueness of measurement	± 4 mV in zeta potential; ± 6 nm for a 100 nm PS Latex
Reproducibility	± 4 mV in zeta potential; ± 5 nm for a 100 nm PS Latex
Sample	Aqueous and polar media based dispersions, minimum 500 µL
Sample concentration range	10^6 - 10^8 particles/mL for size measurements, 10^6 - 10^{10} particles/mL for zeta potential measurements
Test standards	Auto-alignment and daily check suspensions to dilute
Electrical supply	90 – 240 V, 47 - 63 Hz, 50 VA
Laser safety	Instrument protection to safety class I. Laser inside housing: safety class 3B, switched off for access to the measurement cell.
External dimensions	20 (W) x 25 (H) x 30 (D) cm
Weight	Main unit 7 kg, PC extra
Measurement software	System control, auto-alignment. Tracking of individual particles. Zeta potential distributions, size distributions, particle counting. Profile and averaging functions. Export and report functions.
Theory	Conversion of measured electrophoretic mobility into zeta potential according to Smoluchowski equation. Size distribution following Stokes Einstein formula
Material parameters	Viscosity and dielectric constant, for water tabulated
Data management	Video files, txt files, pdf reporting, single and overlay output

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