INTRODUCING EXOID

THE NEW GENERATION OF TRPS

IZON

www.izon.com

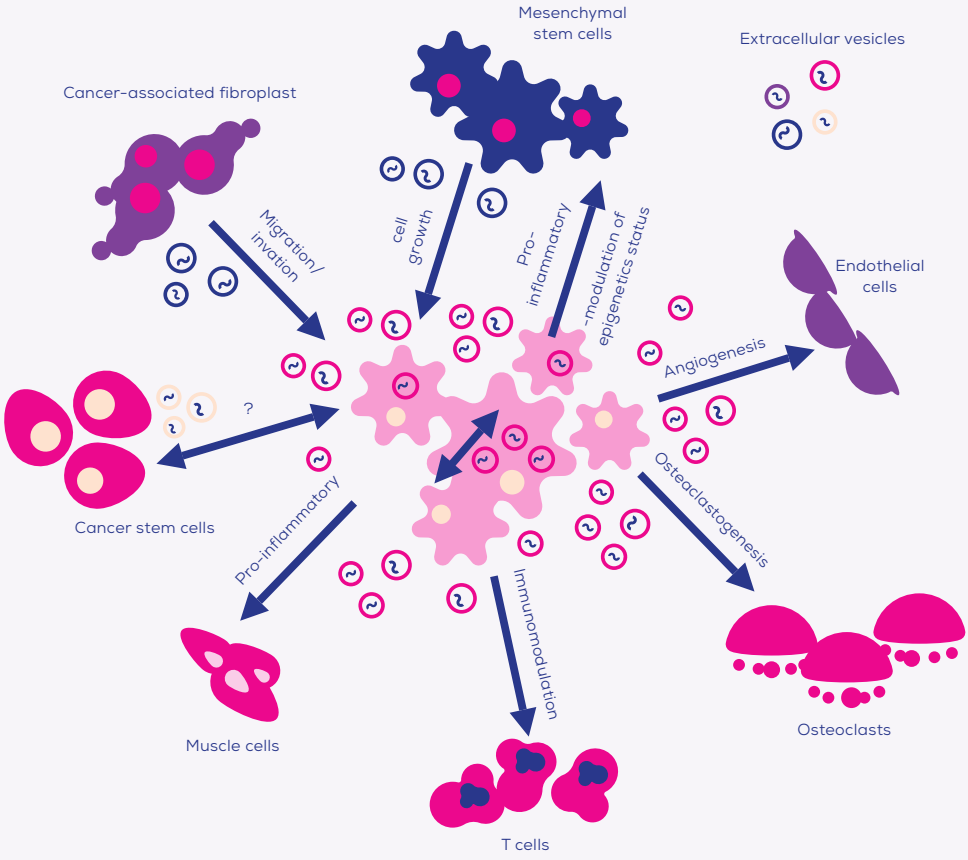
This is the era of **biological nanoparticle analysis**

The existence and importance of biological nanoparticles (bio-nanoparticles) is becoming recognised. With increased interest in naturally occurring nanoparticles such as lipoprotein particles, viruses and virus-like particles, magnetosomes, and ferrihydrite, accurate and reliable characterisation of these particles has become a critical aspect of research. In recent decades, discovery of the ubiquitous existence of extracellular vesicles (EVs) and their contribution to almost all aspects of cell function has led to their recognition as one of the most important factors of human health. In order for the huge potential of these vesicles in fundamental research, diagnostics, and therapeutics to be realised, accurate and repeatable methods of quantification and characterisation are urgently required.

The importance of **multi-parameter analysis**

Size is often considered the most important parameter of bio-nanoparticles; however, growing evidence of the influence of other properties—such as surface charge and particle morphology—on their behaviour in solution, in vivo, and in formulations means that reliable, multi-parameter measurement techniques are essential. As the potential of bio-nanoparticle-based therapeutics becomes increasingly recognised, regulatory bodies are demanding that reliable, reproducible, and comparable multi-parameter characterisation data be provided for such particles.

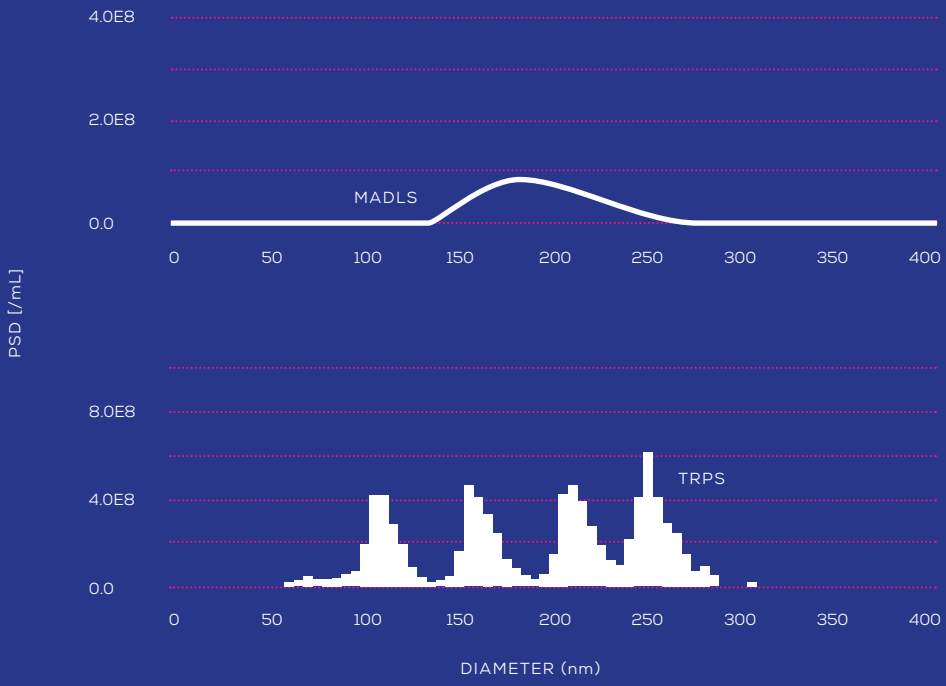
- Standardisable methods are required for appropriate regulation as well as quality control of bio-nanoparticle products
- Reliable and accurate data enable inter- and intra-laboratory comparisons, which are critical for progress in both fundamental research and therapeutic development
- Regulatory agencies are likely to mandate the use of validated, reliable methods for approval of bio-nanoparticle-based products in the near future


Mesenchymal stem cells
Extracellular vesicles
Cancer-associated fibroplast
Migration/ invation
cell growth
Pro- inflammatory
-modulation of epigenetics status
Endothelial cells
Angiogenesis
?
Cancer stem cells
Pro-inflammatory
Osteaclastogenesis
Immunomodulation
Muscle cells
Osteoclasts
T cells

The current state of nanoparticle measurement

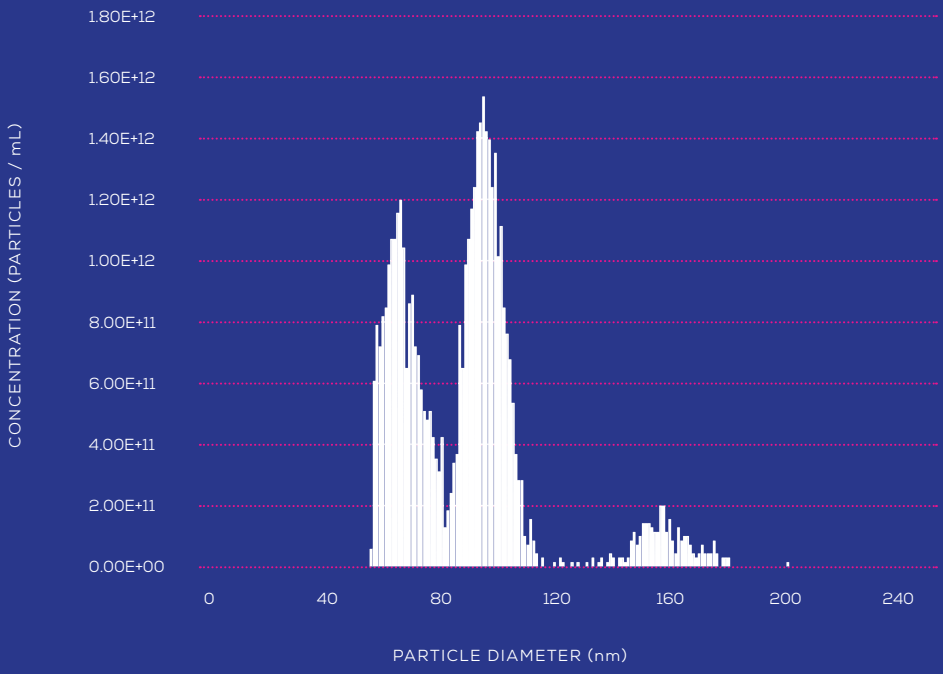
European Commission (EC) guidelines and sector-specific regulations for the measurement of bio-nanoparticles indicate that validated methods with sufficient resolution to measure the entire nano range (1–1000 nm) and able to determine number-based size distribution are urgently needed. Despite huge advances in recent years, accurate analysis of bio-nanoparticles remains challenging due to their size, tendency to aggregate, and heterogenous nature. Progression in the fields of nanomedicine and bioanalysis is limited by the lack of reliable, universally accepted, and validated methods of particle analysis.

While several techniques are currently available for characterising bio-nanoparticles, the field is limited by a reliance on ensemble techniques, such as dynamic light scattering (DLS), which provides data with insufficient resolution for analysis and quality control or regulatory pathways (Figure 2). Furthermore, increased awareness of the existence and importance of smaller (<50 nm) nanoparticles means that techniques capable of providing accurate, single-particle data in this size range are becoming essential. A number of single-particle techniques are currently available, including nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM), each with specific strengths and limitations (Table 1). However, the results obtained are often influenced by the analytical method selected, the settings of the instrument, or user input. Obtaining reproducible and comparable data can, therefore, be challenging.


4.0E8
2.0E8
0.0
MADLS
0
50
100
150
200
250
300
350
400
PSD [/mL]
8.0E8
4.0E8
0.0
TRPS
0
50
100
150
200
250
300
350
400
DIAMETER (nm)

Obtain reliable and reproducible results with **Tuneable Resistive Pulse Sensing**

The precision, resolution, and accuracy of TRPS nanoparticle measurements is well established, and TRPS is the only technique to provide data of sufficient quality for bio-nanoparticle analysis. Using this method, you can be sure of the quality of your data and reproducibility of your results. Inter- and intra-laboratory comparisons can thus be made in confidence. Calibration with NIST-traceable particle standards guarantees the collection of accurate data which is suitable for regulatory purposes (Figure 3). The simplicity of Izon's tailor-made bio-analytical system means that even inexperienced users can collect reliable data, greatly increasing your laboratory's productivity.


1.80E+12
1.60E+12
1.40E+12
1.20E+12
1.00E+12
8.00E+11
6.00E+11
4.00E+11
2.00E+11
0.00E+00
CONCENTRATION (PARTICLES / mL)
0
40
80
120
160
200
240
PARTICLE DIAMETER (nm)

Overview of **commonly used methods of nanoparticle analysis.**

Technique	Strengths	Limitations
DLS **Type of technique** – Ensemble (composite) **Possible parameters** – Size, size distribution. **Size range** – 1 nm–3 µm	Simple protocols. Sample can be recovered.	Accurate knowledge of the optical properties of particles and solute is required. Subpopulations cannot be resolved. Concentration, charge, and other particle properties may affect accuracy. Limited buffer compatibility (very low salt tolerance). Unsuitable for raw biofluids Ensemble nature limits the accuracy and precision of results. Provides an intensity- (not number-) weighted distribution. Presence of large particles skew the distribution. Reports hydrodynamic radius, which may not accurately reflect accurate particle size. Requires large volume and dilute sample. Cannot measure particle concentration.
NTA **Type of technique** – Single particle **Possible parameters** – Size, size distribution, concentration. **Size range** – 30–600 nm (sample dependent)	Simple protocols. Provides number-weighted distribution. Can measure additional parameters such as fluorescence. May provide higher resolution than DLS for multimodal samples.	Requires advanced data analysis. Reports hydrodynamic radius, which may not accurately reflect accurate particle size. Accurate knowledge of optical properties of particles and dispersant is required. Large sample volume required (600 µl). Measurements depend on instrument settings and user input. Reproducibility is limited. Concentration measurements are only accurate on a log scale, making comparisons complex.

Technique	Strengths	Limitations
Optical methods **Type of technique** – Single particle **Possible parameters** – Size, size distribution, morphology, oligomeric state **Size range** – <1 nm–no upper limit	Direct measurement.	Time consuming and laborious. Very low throughput. Prone to artefacts.
TRPS **Type of technique** – Single particle **Possible parameters** – Size, size distribution, zeta potential, concentration, oligomeric state **Size range** – 40 nm–20 µm	Fast, simple, reliable. High throughput. Accurate measurement of true size distribution. Able to resolve up to four (possibly six) subpopulations. Direct measurement. Single-particle technique. Very small sample volume (35 µl) required. Simple, semi-automated protocols. Unmatched accuracy and precision Provides number-based size distribution. Able to measure multiple parameters simultaneously. Accurate measurement of particle concentration on a linear scale.	

Overview of **Tuneable Resistive Pulse Sensing (TRPS)**

The basis of TRPS is measuring nanoparticles that are suspended in an electrolyte solution as they pass through a nanopore. Each particle is characterised individually, providing an accurate and precise picture of your sample, a result which is unachievable by ensemble techniques such as light-based methods. When you begin using TRPS for bio-analysis, you will see an impressive improvement on the resolution of multimodal samples compared with previously used methods. Up to four subpopulations can be resolved, possibly more for some heterogenous samples. Currently used in over 45 countries, with results reported in over 400 publications, TRPS is fast becoming an essential aspect of nanoparticle analysis and will soon prove to be indispensable in your research laboratory.

Carry out multi-parameter measurements

Individual particle size, concentration, and zeta potential are calculated rapidly and simultaneously during TRPS analysis, enabling you to carry out complex, high-throughput measurements with ease. Furthermore, comparison of individual particle data with NIST-traceable calibration particles ensures that you can be confident of the accuracy and reproducibility of your data.

Obtain precise and reliable particle size data

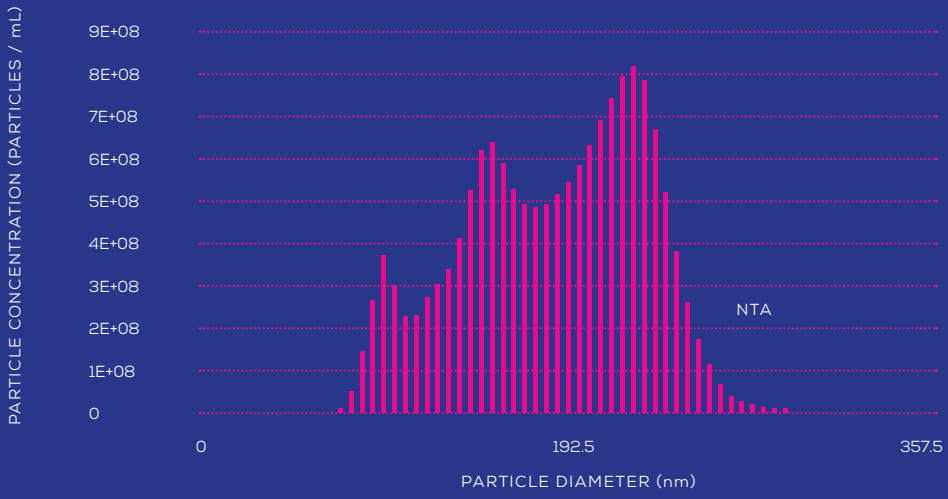
The transient block in current flow that occurs as a particle traverses the pore is directly proportional to particle volume. As such, particle size calculations are not affected by optical properties, buffer composition, or other external factors and you can therefore be certain of obtaining precise size measurements for your particles. A calibration-based method allows for buffer composition and viscosity, for example, to be accounted for when measuring your sample. Automated calibration means that results are delivered in real-time, giving quick insight into the nature of your sample or enabling evaluation of reaction progression or aggregation.

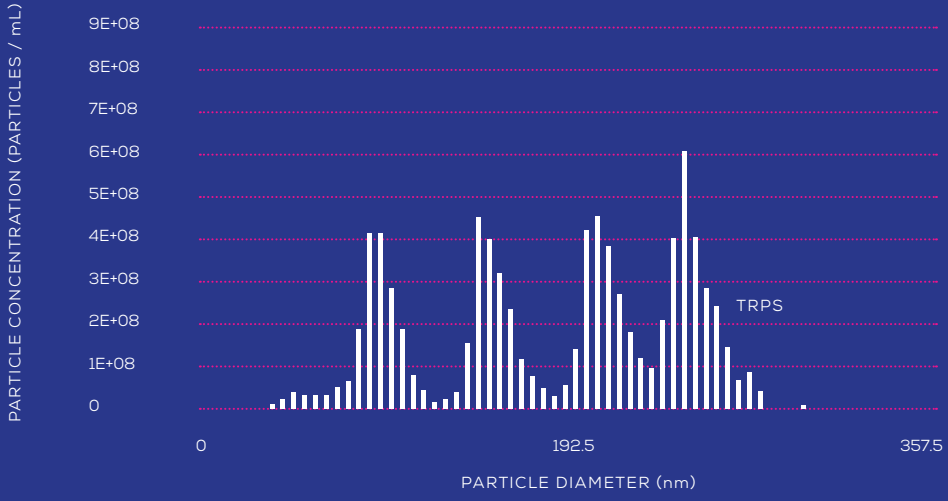
Measure particle concentration parameters with ease

Particle concentration is calculated from the frequency of particles traversing the pore per unit time meaning that concentration measurements are accurate on a linear scale. This is superior to many other techniques, which are only accurate on a log scale, and thus will enable you to easily compare results between samples or replicates. Furthermore, total particle concentration as well as concentration in a particular size range can be analysed, providing a level detail unobtainable by other methods without the need for complex protocols.

Determine zeta potential with high accuracy

Blockade duration changes with the velocity that a particle traverses the pore. As this velocity is affected by particle charge, the blockade duration can be used to determine the zeta potential of each particle. Blockade magnitude, duration, and frequency data are converted into particle size, zeta potential, and concentration by the sophisticated software which incorporates data from calibration recordings of particles of known size, concentration, and surface charge to guarantee that you can be certain of the accuracy of your measurements.


PARTICLE CONCENTRATION (PARTICLES / mL)
9E+08
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0
NTA
0
192.5
357.5
PARTICLE DIAMETER (nm)


PARTICLE CONCENTRATION (PARTICLES / mL)
9E+08
8E+08
7E+08
6E+08
5E+08
4E+08
3E+08
2E+08
1E+08
0
TRPS
0
192.5
357.5
PARTICLE DIAMETER (nm)

Obtain accurate data with next-generation TRPS analysis

User feedback has indicated that, despite the excellent data obtained, operation of early TRPS instruments required a high level of familiarity and skill, as well as ongoing training.

The latest-generation TRPS instrument, the Exoid, is the solution for complex analyses of bio-nanoparticles. Incorporating the unparalleled accuracy of TRPS into a semi-automated machine with improved sensitivity and usability.

The Exoid will provide you with a simple approach to characterise particles as small as 40 nm in diameter with minimal sample preparation. Impedance across the nanopore is sampled 64,000 times per second, ensuring every particle that passes through the pore is detected.

This high-resolution, single-particle data obtained provide a true insight into the nature of biological samples.

Increase research productivity

The sophisticated software of the Exoid provides a "press and go" system in which measurement parameters and protocols can be pre-programmed. Your research will be streamlined by the ability to carry out complex experiments involving multi-parameter measurements with minimal intervention. Troubleshooting, re-calibration (when required), and data-quality are monitored automatically and instrument settings adjusted accordingly. With colour-coded lighting displays, you can check experimental progress at a glance and run multiple instruments simultaneously with ease.

New features of the Exoid

- Automation of pressure, voltage, and pore size increases measurement consistency and minimises the potential for user error.
- Reduced noise levels mean smaller particles can be measured with minimal sample preparation.
- Continual monitoring of key parameters enables the instrument to automatically optimise and troubleshoot protocols, as well as monitor data quality.
- Increased sensitivity enables more heterogenous samples to be analysed, minimising required sample preparation. qEV Isolation followed by Exoid measurements provide a complete and simple bio-analytical system.

The Exoid is essential for bioanalysis and quality control

TRPS is well established in the fields of bioanalysis and nanomedicine as the most precise and accurate method of particle measurement. Individual particle size and zeta potential can be measured as well as the concentration of particles in defined size ranges to provide a true representation of the nature of the sample. Because the technology does not rely on knowledge or input of the optical properties of particles or solutions, TRPS has the unique ability to determine the zeta potential of individual particles with ease and accuracy. TRPS is fast becoming essential for nanomedicine research and development.

Accurate knowledge of particle size and concentration is required in order to predict the in vivo behaviour of particles, an essential aspect of the stringent quality control and safety checks involved in the production, monitoring, and regulation of nanomedicines. Currently, progress in the field is limited by the lack of standardised methods for particle measurement. Specific regulatory procedures and requirements are likely to come into play soon; therefore, reliable methods of measuring nanoparticles with single-particle resolution will be indispensable. As the only technique with the capability to provide such measurements, TRPS is rapidly becoming crucial in many areas of nanomedicine.

The Exoid system is at the forefront of bio-nanoparticle analysis, don't be left behind.

Analysis Range (nm)	40-10,000 nm
Concentration Range (p/ml)	1 E5 – 1 E11 (size dependant)
Applied Pressure	± 2500 Pa
Weight	10.80 kg
Height	246 mm
Footprint	70582.2mm2

IZON

www.izon.com