# TRPS

## Tunable Resistive Pulse Sensing

TRPS is the most powerful nanoparticle characterisation system available. It is an essential tool for nanoparticle research, development and quality assurance, particularly in fields like nanomedicine and extracellular vesicles . In this context the term nanoparticle includes a wide range of synthetic and biological particles in the nano and sub-micron range.

Recent developments mean that TRPS is now rapid and convenient, with a suite of additional tools available to make life easier for the TRPS user.

TRPS measures the main particle parameters with very high accuracy and repeatability. Measurements are all calibrated and objectively derived, ie within the agreed tolerances they do vary from user to user or instrument to instrument. These main parameters are:

- Particle concentration, being the number of particles/ml for a specified size range
- Particle size and accurate number based size distribution, derived on a real particle by particle basis
- Particle charge and number based charge distribution, also derived on a real particle by particle basis


Accurate and reliable measurement of these main parameters allows for many other techniques to be applied to particle sets to obtain further detailed information such as biological or biochemical analysis.

With the availability of TRPS and subsequent ease of obtaining high quality data, the particle measurement field needs better definitions in order for the field to keep up with technical developments.

These new definitions are gradually being introduced to and adopted by the various particle communities.

### Principles

TRPS is an impedance based system. No lasers are involved. A voltage is applied across a pore that is filled with electrolyte, resulting in an ionic current. As particles cross the pore they briefly increase electrical resistance, creating a resistive pulse, which is precisely proportional to particle volume.

The core principles of Resistive Pulse Sensing (RPS) were developed over 60 years ago for cell counting and measurement. More recently the technique has been applied to high throughput DNA sequencing.

Izon Science added to the existing state of the art and created Tunable Resistive Pulse Sensing, by firstly developing a size tunable pore, then by developing tools and measurement concepts using tunable driving forces, primarily pressure and voltage. While molecular sizes can be measured, TRPS is presently aimed at particles in the range of 40nm to 10 microns, with the submicron range being of particular interest.

The actual measurement of each particle crossing the pore is achieved through the use of calibration particles that have been accurately calibrated for size and concentration.

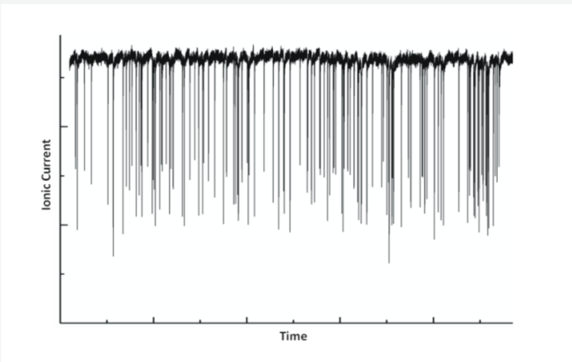
The rate of flow of particles is proportional to particle concentration, so particle number can accurately obtained at the same time as individual particle sizes.
A core principle of TRPS is that the particle number and accurate particle size distribution need to be provided together.

The number of particles in each size band is given.
This is considered to be essential information for modern nanoparticle work. Older techniques that can only provide theoretical, low resolution percentages of particles vs size are not adequate for the demands of fields like nanomedicine or extracellular vesicles.

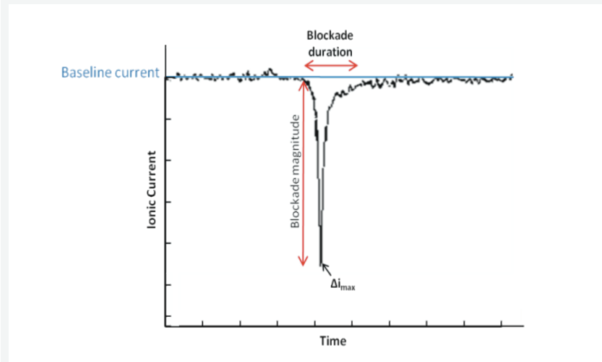
As particles traverse the pore they do so with a velocity that can be controlled and measured. While the depth (magnitude) of the resistive pulse is proportional to particle volume, the duration (pulse width) is a function of particle length and particle velocity.

Varying pressure and voltage in a controlled way and analysing the effect on the particle velocities enable the electrophoeretic mobility of each particle crossing the pore to be derived.
This is a very powerful nanomeasurement tool with many valuable applications. Now that is available, its use in nanoparticle research, development and QA is considered to become mandatory for journal publications and for any biomedical applications.

### Ionic current resistive pulses as seen in TRPS




Ionic current "pulses" as seen in real time, generated by individual particles passing through the pore




The close up view of a single current pulse shows the characteristic pulse shape

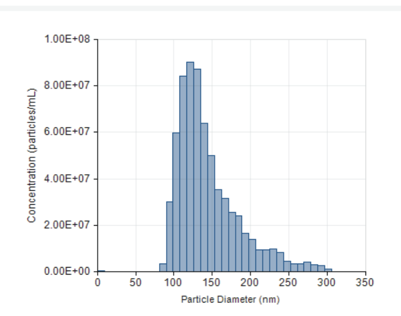
## Instruments Using TRPS

The most common way to access TRPS measurement is via the qNano instrument manufactured by Izon Science, which has now been upgraded to qNano Gold. The qNano Gold suite encompasses a range of complementary products including the instrument, nanopore membranes, pore coating reagents, analytical software, user training modules and the newly released qEV sample preparation products.

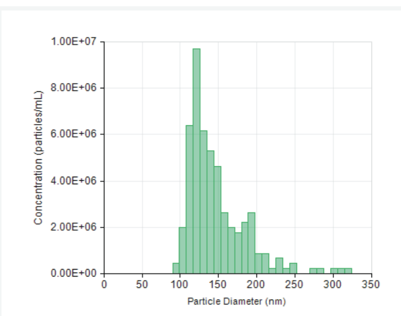
## Size measurement

TRPS can be readily used to measure the real size distribution of nanoparticles and EVs in a number of different sources.
Note the important point that the vertical axis on each graph below represents the actual number of particles in each size band, not percentages. TRPS is the only particle measurement method able to do that robustly and reliably. Any credible particle research or development project needs both the level of detail and the certainty. Any particles subject to regulation will also need that detail and certainty. The easy availability of data of this quality is expected to result in rapid advancement in nanoparticle development.

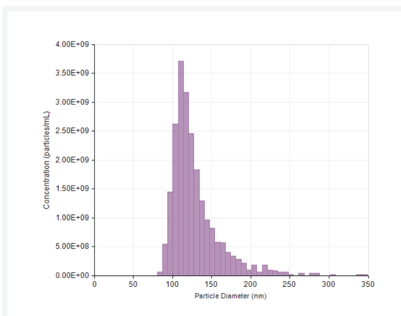
### Urine




### CSF



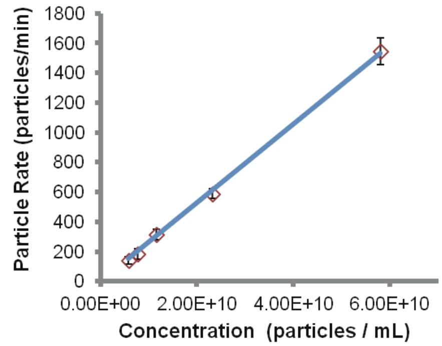

### Plasma



## Concentration measurement

TRPS produces high accuracy concentration data which particle rate is directly proportional to concentration: This correlation is independent of particle composition therefore the concentration of any particle within a particular size range – Cmin –Cmax can be calculated by comparing to calibration standards of precisely known concentration.
For a complete picture, particle concentration data will be provided together with a size histogram but there a number of applications where and simpler form of data presentation is appropriate.

TRPS provides the most accurate and reliable concentration data, in a simple and easily verifiable way.



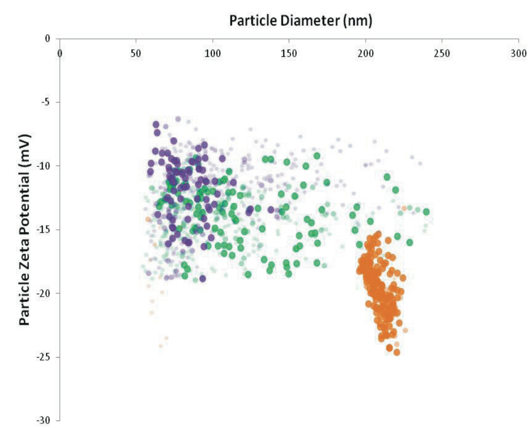

## Particle charge measurement, including zeta potential

TRPS measures individual particle surface charge by analyzing the durations of the resistive pulses under varying driving forces and by using reference particles calibrated for size, surface charge and concentration. Detailed analysis of each pulse width, which varies as the particle traverses the conical pore, enables each particle's velocity to be measured. Velocity enables the electrophoretic mobility of each particle to be derived, meaning that simultaneous size and charge measurements can be easily carried out. Note that electrophoretic mobility is routinely converted into zeta potential by most charge techniques.

TRPS instruments will provide particle zeta potential values however the electrophoretic mobilities be the preferred property. In either case, the easy availability of particle by particle charge distribution and charge vs size data enables a very thorough understanding of the particle properties to be developed. Given the easy availability of this data it is no longer credible to rely on low quality data from outmoded techniques.

One useful application of particle by particle charge is to check how surface functionalisation of nanparticles has occurred. Another is to use charge measurement as a bioanalytical tool by using aptamers, which change the charge of the target particle if a reaction has occurred.

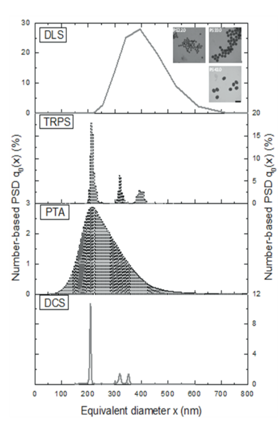
The chart below shows size vs charge for EVs derived from different CSF samples, indicating that detailed charge analysis of EVs is a useful research tool.




Particles from healthy samples (green dots) have a mean zeta potential of -17 mV, whereas particles from diseased samples (purple dots) have a mean zeta potential of -15mV in PBS buffer. (Calibration particles are shown in orange).

## Accuracy and Resolution

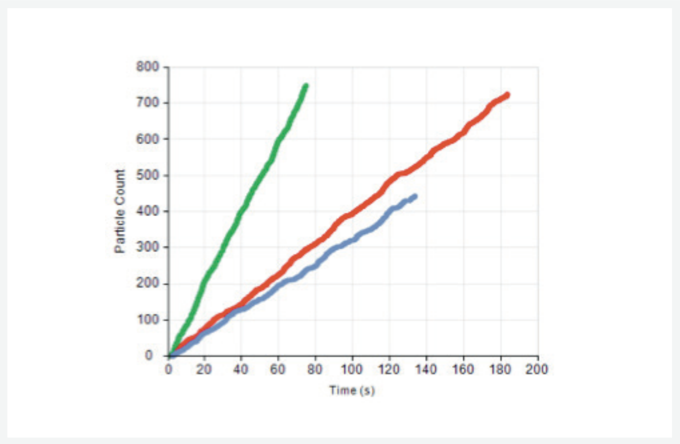
Real world particles sets are usually polydisperse and heterogeneous. One needs the measurement data to infer what the particle set consists of, so any system that doesn't offer that is essentially useless. There is no point in using any measurement technique that can only measure monosized particles unless the particles are known to be monosized. The charts below are the results of measuring an artificially created and known trimodal mixture of standard particles. Of the four main particle measurement techniques (DLS, TRPS, PTA/NTA, DCS) only TRPS was able to provide data to show particles were present and in what proportion in proportion. In addition TRPS also provides the number/ml of each size and is the only technique that can do that too.



## Sample Preparation

Homogeneous vesicles such as liposomes resolve typically with excellent stability on TRPS equipment. Inhomogeneous samples like extracellular vesicles (EVs) or virus preparations will benefit from sample preparation to clean up the particles enabling faster and more precise measurement. Izon Science has developed a product named qEV specifically for that purpose. Introduction of the complementary particle preparation products, along with other new developments such as pore coating reagents have made TRPS into a highly practical option, with dramatically improved time to result.

For the EV field for instance, the complete cycle of sample preparation, measurement and analysis of EVs from blood plasma using the qEV and TRPS is now by far the fastest option as well as the most reliable and accurate.



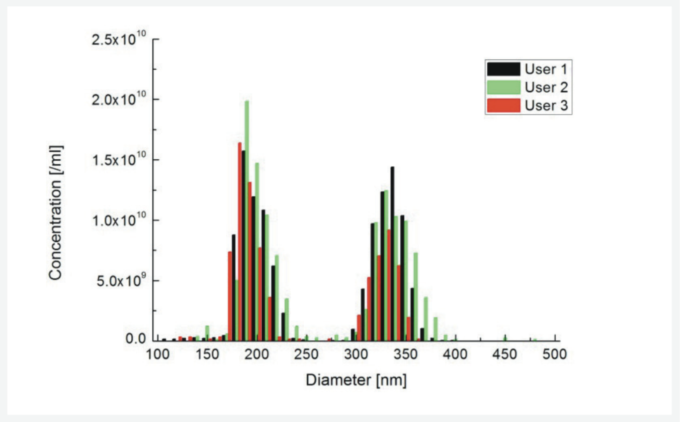

**This diagram shows EVs purified by qEV columns**
After qEV purification, three elution fractions were analysed for EV content. The EVs present in these fractions ran with consistent particle rates, indicating a stable measurement was achieved.

## Reproducibility

A major issue of alternative techniques for nanoparticle characterisation is the influence of user-defined settings, and requirement of considerable prior knowledge of the sample composition. This means that the results obtained will differ between users and between instruments, and are therefore not comparable with each other. Without resolving that issue the EV field would never be able to translate all of the research into clinical use. Fortunately TRPS does resolve that issue very well and as a result the EV field should experience continuing growth and acceptance by the medical community.

As every TRPS measurement is calibrated to a known standard, the accuracy of the measurement is guaranteed and can be directly compared.

Repeat measurements of the same sample show high reproducibility, the same bimodal sample was distributed to and measured by three users in different groups across the world, without prior communication between the groups. The size distributions overlay very well, indicating the reliability of the data.




## About IZON Science

Izon provides customers with complete solutions, primarily for accurate nano-particle size, charge and concentration characterisation including precision instrumentation, consumables and reagents. Izon Science has undertaken extensive research and development in partnership with users to ensure that its instruments can deliver accurate, reproducible and reliable data to support your research. Izon's products are regarded as essential equipment in a wide range of organisations including research institutes, universities and scientific companies.


WWW.IZON.COM

**EUROPE**
**The Oxford Science Park**
Magdalen Centre, 1 Robert Robinson Ave,
Oxford OX4 4GA,
United Kingdom

**Tel:** +44-1865-784-630
**Fax:** +44-1865-784-631
**Email:** uk-info@izon.com

**NORTH AMERICA**
**85 Bolton Street**
Cambridge,
MA 02140
United States

**Tel:** +1-617-945-5936
**Fax:** +1-857-259-6623
**Email:** usa-info@izon.com

**ASIA PACIFIC**
**8C Homersham Place**,
PO Box 39168, Burnside,
Christchurch 8053,
New Zealand

**Tel:** +64 3 357 4270
**Fax:** +64 3 357 4273
**Email:** info@izon.com