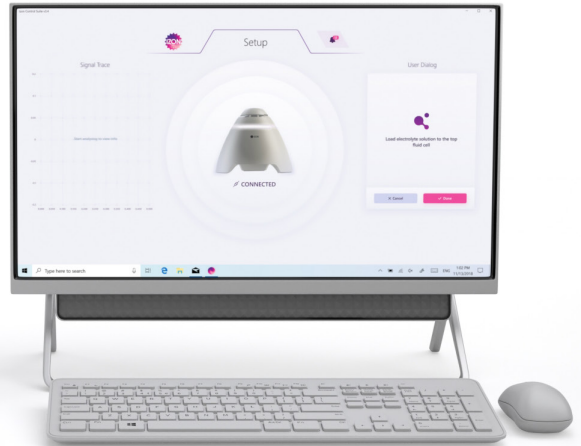


EXOID
USER MANUAL



www.izon.com



Increased output with minimal intervention. Incorporating the unparalleled accuracy of tunable resistive pulse sensing (TRPS) into a semi-automated machine with improved sensitivity and usability, the Exoid represents a reliable method of measuring individual particles. At Izon Science, we are committed to helping you get an accurate measurement of your particles, here we will help guide you through this process to get the best possible data from your Izon Exoid.

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1 / DEFINITIONS AND WRITING CONVENTIONS

Make sure to follow the precautionary statements presented in this guide. Safety and other special notifications will appear in boxes and include the symbols detailed in Table 1.

Table 1: Safety and Hazard Symbols



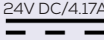
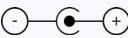

| SYMBOL | MEANING |
|---|--|
|  | This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations. |
|  | This symbol indicates where special care should be taken. |
|  | Direct Current (DC) |
|  | Voltage polarity of the jack |
|  | Crushing Hazard |

Table 2: Terminology used in this manual

| TERM | DEFINITION |
|---------------------------|--|
| Baseline drift | The amount of current (nA) that the current moves by over a period of time. |
| Blockade duration | A measurement of the velocity of the particle, critical for Zeta analysis. |
| Blockade frequency | Directly proportional to the particle concentration, higher concentrations will have a higher particle rate. |
| Blockade magnitude | Directly proportional to the particle size. The amount of current that a particle blocks as it goes through the nanopore. |
| Calibration | Aliquot of diluted calibration particles of a known size. |
| Exoid buttons | The buttons near the top of the Exoid, to control the stretch. |
| Electrolyte | A liquid which contains ions and can transmit a current when voltage is applied. |
| Exoid Control Suite (ECS) | The software used to operate the Exoid. |
| Exoid lighting | Lighting halo located around the top of the Exoid. |
| Load position | When the stretcher unit is at the position which enables a nanopore to be clipped on to the teeth. |
| Measurement | The complete sample analysis, made up of multiple recordings. |
| Nanopore | Flexible polyurethane membrane with a nanoscale size hole in the centre. |
| Nanopore type | Nanopore size classification see section 3. |
| Recording | One part of a whole measurement, particles (sample or calibration) run at one set of parameters. |
| RMS noise | Background electrical noise of the system. |
| Sample | The unknown particles to be analysed using the Exoid system. |
| Shielding lid | Hinge-able cap on the top face of the Exoid which opens to reveal the fluid cell and nanopore working area. Closing this will reduce noise from external sources such as large machinery and fluorescent lighting. |
| Stretcher unit | A set of jaws with teeth which the nanopore can clip on to. |

2 / SAFETY AND HAZARDS

Safe Use Requirements and Specifications

Make sure to adhere to the safe use requirements as specified in the table below. In the event that the equipment is used in a manner not specified, the protection provided by the equipment may be impaired.

| SAFE USE REQUIREMENT | | SPECIFICATION |
|--|------------|--|
| Operating Temperature | Indoor Use | Ambient temperature of 5–40 °C is safe for the instrument, however Izon recommends operating at 5–25 °C. |
| Altitude | | Up to 2,000 meters above sea level |
| Relative Humidity | | 20–80% relative humidity |
| Power Consumption | | 80 W |
| Pollution Degree Rating | | 2 |
| Ingress Protection Rating | | IP30 / Type 1 - UL50E |
| Power Supply Unit (TRH100A240-21E11 + CCCVI) | Input AC | 100–240 V 1.5 A 47/63 Hz |
| | Output DC | 24 V nom 4.17 A max current 100.08 W max power |

Hazards

The Exoid is a laboratory product, however if any biohazardous samples are present then adhere to current good lab practices (cGLP) and comply with any local guidelines specific to your laboratory and location.

Fire or Electrical Hazard

The Exoid poses no uncommon electrical or fire hazard to operators if installed and operated properly without physical modification and connected to a power source of correct specification.

Chemical Hazards

The Exoid system contains no potentially hazardous chemical materials.

Mechanical Hazards



The Exoid stretcher unit contains automatically moving parts. Keep fingers and loose clothing clear while automatic processes are in operation.

Transport

Before moving or shipping the Exoid, decontamination procedures must be performed. Always move or ship the Exoid with the supplied packaging materials which will protect the instrument from damage, and use appropriate heavy object lifting techniques so as to avoid injury. If appropriate packing materials cannot be obtained then contact your local Izon Science office.

Disposal

The Exoid system contains electrical or electrical materials; it should be disposed of as unsorted waste and must be collected separately, according to the European Union Directive: Waste Electrical and Electronic Equipment. The user is fully responsible for ensuring that the obsolete Equipment and/or Consumables are recycled or disposed of in accordance with this and/or any other relevant laws and regulations in the countries where the instrument is being recycled or disposed of. Contact your local Izon Science representative for more information.

3 / INTRODUCTION TO THE EXOID

Overview

Characterisation of particles using Tunable Resistive Pulse Sensing (TRPS) provides a reliable and accurate method of measuring size, concentration, and surface charge. It is the only technology that can deliver the concentration of particles in fluid as a number per volume across a specified detectable size range, as well as calculate effective surface charge of individual nanoparticles. The Exoid eliminates many of the inaccuracies associated with nanoparticle analysis with its advanced technology and automated systems.

TRPS technology uses the Coulter particle counting principle on the nanoscale. Voltage is applied across the fluid cell via the silver/silver chloride electrodes, therefore creating a baseline current due to the flow of electrolytic ions. A temporary decrease in current is detected as particles pass through the nanopore, which allows for the sizing and counting of particles suspended in electrolyte.

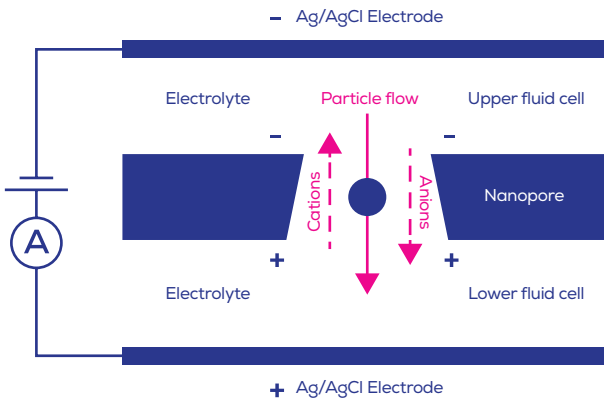
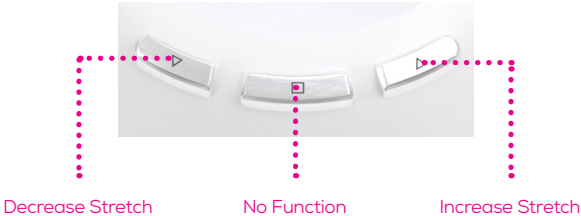


Figure 1. A schematic representation of how TRPS works within the Izon system.

The Exoid uses automated systems to improve the speed and accuracy of the system as a whole. The automatic stretcher unit (also referred to as Delta) can stretch the nanopore cruciform up and down faster than mechanically winding by hand, saving time in a high throughput environment. The buttons near the top of the Exoid can control the stretcher unit in the event that access to the manual software control is no longer available.



The Automatic Pressure System (APS) applies a pressure far more accurate than that of the previous Variable Pressure Module (VPM) system as it can read and adjust itself to the operator entered value without any further input from the operator. The APS is also capable of applying pressures (positive and negative) 50% greater than that of the VPM.

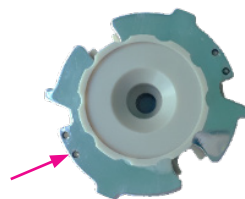
Visual indications of the state of the Exoid using the Exoid lighting allow the user to immediately assess how the system is functioning.

| HALO COLOUR | EXOID STATUS |
|-------------|------------------------------------|
| White | Connected, idle. |
| Red | Disconnected, see troubleshooting. |
| Blue | System requires user input. |
| Purple | System performing an operation. |

Fluid Cell V2

The Exoid system comes with a new and improved fluid cell design. A dedicated return path built into the electronics of the fluid cell and improved shielding mean that the entire system is more resilient to external noise interference. This new design is also less susceptible to the “short circuits” that plagued the original fluid cell, making for easier troubleshooting.

The new design has two pogo pins on the lower fluid cell which fit into sockets on the underneath of the upper fluid cell when twisted into place correctly. To assist with this, the upper fluid cell has two position indicators on each side which, when installed correctly, will be half obscured by the lower fluid cell.



Intended Use

The Exoid is used to automate nanoparticle analysis of samples, to give size, concentration, and surface charge data. The instrument is intended to be used in research laboratories by professional personnel. The Exoid is not intended for diagnostic purposes and should not be used to make treatment decisions.

For verification of the entire system, it is recommended that Good Laboratory Practices (GLP) be followed to ensure reliable analyses.

Important Information

Pipetting

While using the Exoid it is important to employ correct pipetting practices. For all particle and sample dilutions the user should use forward pipetting, reverse pipetting should be used when pipetting into the upper fluid cell. For more information on these two pipetting techniques visit support.izon.com/pipetting-techniques

Choosing a Nanopore

Choosing the right nanopore size will depend on the target particle size of the sample to be measured. The table below provides some guidance on how to select a nanopore.

| | | | |
|--------------|---|---------------|---|
| NP80 | <ul style="list-style-type: none">- 40-255 nm- Calibration: CPC70, 100- Target Conc. /mL: 1×10^{10} | NP400 | <ul style="list-style-type: none">- 185-1100 nm- Calibration: CPC400, 800- Target Conc. /mL: 5×10^8 |
| NP100 | <ul style="list-style-type: none">- 50-330 nm- Calibration: CPC100, 200- Target Conc. /mL: 5×10^{10} | NP600 | <ul style="list-style-type: none">- 275-1570 nm- Calibration: CPC800, 1000- Target Conc. /mL: 5×10^8 |
| NP150 | <ul style="list-style-type: none">- 70-420 nm- Calibration: CPC100, 200- Target Conc. /mL: 5×10^9 | NP800 | <ul style="list-style-type: none">- 385-2050 nm- Calibration: CPC800, 1000- Target Conc. /mL: 1×10^9 |
| NP200 | <ul style="list-style-type: none">- 85-500 nm- Calibration: CPC200, 400- Target Conc. /mL: 2×10^9 | NP1000 | <ul style="list-style-type: none">- 490-2900 nm- Calibration: CPC1000, 2000- Target Conc. /mL: 5×10^7 |
| NP250 | <ul style="list-style-type: none">- 110-630 nm- Calibration: CPC200, 400- Target Conc. /mL: 2×10^9 | NP2000 | <ul style="list-style-type: none">- 935-5700 nm- Calibration: CPC2000, 4000- Target Conc. /mL: 5×10^6 |
| NP300 | <ul style="list-style-type: none">- 150-900 nm- Calibration: CPC200, 400, 800- Target Conc. /mL: 1×10^9 | NP4000 | <ul style="list-style-type: none">- 1990-11300 nm- Calibration: CPC4000- Target Conc. /mL: 5×10^5 |

4 / ASSEMBLY AND SETUP INSTRUCTIONS

Instrument power and general operation

- ✓ Make sure the power supply box is positioned away from fluids
- ✓ To prevent heat build up do not cover the power supply box
- ✓ Position the unit so it can be quickly and easily disconnected from the mains power.
- ✓ Check the local supply meets the AC input requirement given in the specification.



Izon instruments are only to be operated with Izon supplied leads and power supplies. Failure to use the correct power supply may result in invalid operation.

Make sure the power supply is placed away from the instrument and to the rear of the instrument to avoid coming into contact with any spills or fluid.

General Operating Procedures

- ✓ For indoor use only and to be used within the rated conditions noted in system specifications.
- ✓ Take care not to spill fluids on electrical parts during operation.

Assembling and Installing the Exoid

1. Unpack the Exoid and box contents



We recommend that you save the box and packaging materials in case the instrument needs to be returned for servicing.



The Exoid must be installed into Earth Grounded Protected Outlets ONLY. Position equipment away from electrical switching gear and interfering equipment or noise may increase under sample running conditions.

2. Place the Exoid onto a stable and level laboratory bench.
3. Ensure that the buttons near the top of the Exoid are facing the user.
4. Connect the power lead to the 24 V power supply.
5. Make sure the power is off at the wall and at the Exoid before plugging the power lead into a wall socket and connecting the cable from the 24 V power supply to the rear of the instrument.
6. Connect the USB cable to the instrument and the computer.
7. Turn the instrument on first using the power switch at the wall socket and then the power switch on the Exoid.

Connecting to a computer



Devices that can be connected to the equipment should be compliant with a relevant safety standard such as IEC 60950-1 for IT equipment or IEC 61010-1 for laboratory equipment and should provide double or reinforced insulation from hazardous voltage sources. Always use Izon supplied USB cables to connect to the instrument.

Computer Minimum specifications

- ✓ Windows (7 onwards) Professional Edition (64-bit)
- ✓ i7 processor
- ✓ 16 GB RAM
- ✓ Dedicated graphics processor and memory (1 GB)
- ✓ Hard drive with at least 50 GB free space for software installation and data
- ✓ One free USB port

Windows Home Edition is not suitable for the installation of the ECS. Ensure that the computer is installed with Windows Professional Edition.



On board graphics is not suitable for the operation of the Exoid. Ensure that the connected computer has a dedicated graphics processor with at least 1 GB of memory. Please contact Izon Support if this is an issue.

To operate the Exoid, there are two software suites that must be installed. The Exoid Control Suite (ECS) is used to control the Exoid and gather data. The Izon Data Suite (IDS) is a separate software required to analyse the data collected in the ECS. Both software packages for **non** 21 CFR Part 11 installations can be downloaded from the link below.

support.izon.com/how-can-i-get-the-latest-exoid-control-suite-software-release

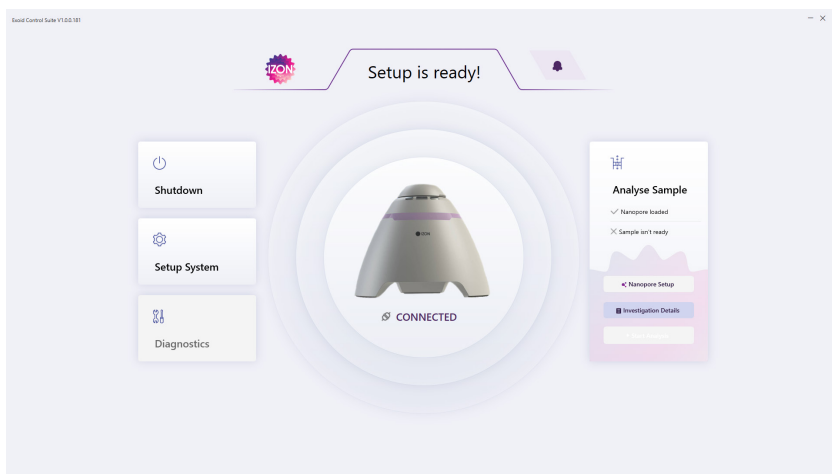
5 / OPERATING INSTRUCTIONS

Initial Connection



If using a laptop to operate the Exoid, ensure that the laptop charging cable is plugged in and providing power at all times during operation of the instrument.

Once the instrument is connected to the computer according to the earlier instructions, open the ECS. A welcome splash screen followed by the home screen will be displayed, showing a render of the instrument with the word “Connected” beneath it. There are several internal mechanics of the Exoid which need to have completed their start-up process before this will occur. A successful connection of all components will also be indicated by activation of the Exoid lighting. If either the lights or the “connected” are not displaying, please refer to the troubleshooting section at the end of this document. Notifications about system updates appear at the top right of the screen.



Investigation Details

This is where the user will populate the name of the investigation they will be performing that day, as well as allow the user to load a "Calibration File" from the computer if they are using a nanopore which has already completed the setup process.

Nanopore Setup

This section guides the user through all the appropriate procedures required to prepare the nanopore for sample analysis. This includes loading, wetting, coating (if applicable) and characterisation. This is recommended for all nanopores and will save a "Calibration File" to the computer which can be loaded into the ECS in the "Investigation Details" screen if the nanopore is used again after the software has been closed for any reason.

Nanopore Loading

Follow the on-screen instructions for loading the nanopore. A correctly loaded nanopore is one where the serial number is facing upwards, and all arms are fitted snugly onto the stretcher unit teeth. At this stage, the user will have the option to "resume previous setup" if for some reason the software has been closed before nanopore setup has been completed.

Nanopore Wetting

The user will also be guided through wetting the nanopore as part of the nanopore loading procedure. User dialogue will appear on the right-hand side of the screen, with prompts on what actions to take next and what information to fill in. At most steps there will be the option to cancel and go back at any time. As part of the wetting process, the nanopore will also go through a "soak" phase. This phase allows the baseline to stabilise after it has wetted, meaning that baseline drift will be at a minimum for the characterisation of the nanopore. The Exoid will do this automatically once the user has followed the setup prompts, if there are any issues during this process then the ECS will notify the user that intervention is required. The user will then be required to inform the system whether or not their sample is of a biological nature. If yes then they will proceed to coating, if no they will proceed to nanopore characterisation.

Nanopore Coating

The user will be prompted to perform nanopore coating in the event that the analysis is being performed on samples of a biological nature. The user will inform the system if this is the case and then will be guided through the protocol via the on-screen instructions.

Izon coating solution prevents non-specific binding of biological nanoparticles to the nanopore membrane, skipping this step will increase chance of blockages and decrease the life of the nanopore. Izon coating solution powder is available for purchase with the Izon Reagent Kit, which will also provide all other reagents needed for nanoparticle analysis on the Exoid.

Nanopore Characterisation

The Exoid will then automatically characterise the nanopore based on fitting an analysis of the current at different stretches to a pre-determined model. After this step, the user will be asked to load calibration particles of a certain size in order to calibrate the nanopore.

Once this has been achieved, the user will be informed which particles that particular nanopore is suitable for measuring. Once this step is complete then the user will be taken back to the home screen where they can then start their analysis.

Sample analysis

If there is no nanopore information currently stored in the system then the user will be asked to confirm that their nanopore has been set up correctly upon initiation of sample analysis, if there is already nanopore information stored in the system then this step will automatically be skipped. The next step in sample analysis is for the user to choose which type of analysis they wish to perform. Upon selection of analysis type, the user will be asked to enter their sample details, as well as choosing the target analysis size range with the slider bar on the next prompt screen. Data collection is started by selecting "Start Analysis," post system optimisation. During data collection the user should not have to interact with the system at all, providing the conditions are stable. The ECS will automatically stop recordings at 500 particle count before moving on to the next pressure step.

The ECS will guide the user through analysing the sample, the first step being to run a calibration for the system.





Calibration is an absolute requirement to obtaining meaningful size and concentration data. The calibration collection will happen in much the same way as the sample collection, just follow the on-screen instructions and the ECS will do everything else automatically.

While a recording is in progress the user will have access to three options, “Pause Analysis,” “Cancel” and “Advance.” The pause option allows the user to pause the collection of data. The advance option allows the user to manually finish the recording and save the data before the automatic recording length has been reached. The cancel button allows the user to return to the previous step.

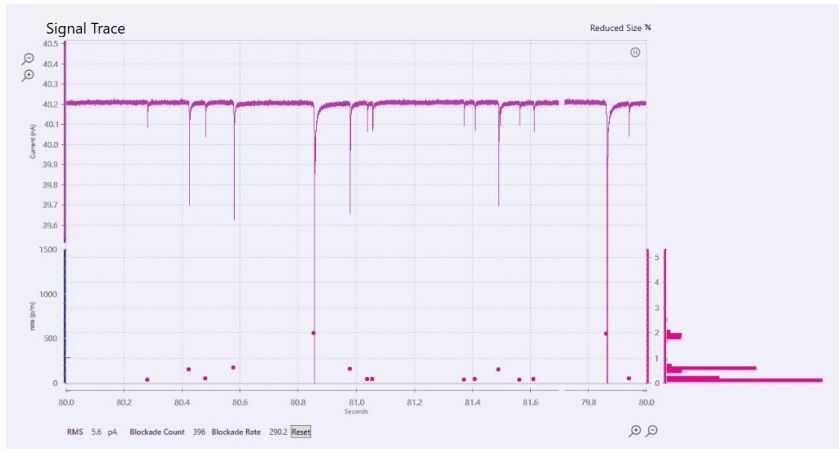
While a recording is paused, the user is able to “Resume Analysis,” and “Restart Run.” Resuming will begin data collection from the point it was paused at, while restarting will begin data collection from the start of that particular pressure measurement, discarding any data that was already collected for that pressure only.

Guide to real time data

Trace and particle size plot

This is the visual representation of baseline current, RMS noise, and blockade magnitude. The user is able to alter the scale of both the X and the Y axis by pressing either the + or – magnifying symbols next to the appropriate axis. Zooming in on the Y axis will allow the user to see more detail in the variability of the baseline trace, but will make it more difficult to visualise if the baseline is stable or not, or distinguish the particles from the noise by eye. Zooming out will give a much better visual for the particle blockades, however zooming out too far will make it extremely difficult to detect an increase in RMS noise quickly.

Zooming in or out on the X axis will change the time period displayed over the course of the plot. Zooming in will mean that the trace moves much faster as the time period is shorter, while zooming out will mean that the trace moves much slower as the time period is longer.



Below the trace plot is the particle size plot. This displays each significant blockade event as a dot, plotted against a relative particle size scale which will display in nano amps (nA). This is an important visualisation as blockades that are too small run the risk of not being fully detected by the ECS. Here the particles visualised as dots will line up with their corresponding blockade event. Also displayed here is RMS noise, total particle count, and particle rate.

Both plots can be "popped out" to be bigger by selecting the **Full Size** button in the top right of the signal trace plot. Similarly, once it has been popped out, it can be popped back in by pressing the **Reduced Size** button.

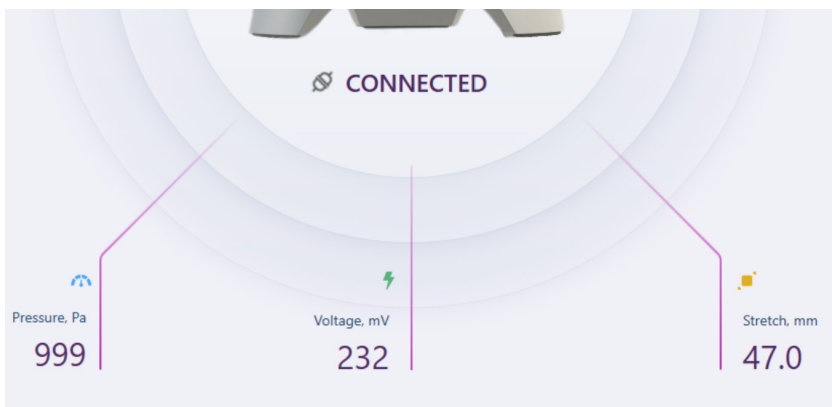
Particle size distribution histogram

This graph is located to the right of the particle size plot when the trace is popped out, it will display the size distribution of the unknown particle being analysed on the Exoid in real time.

Guide to real time system metrics

Analysis and Device Summary Panels

This displays basic information and running conditions for the current recording, the user will be able to change pressure, voltage, and stretch parameters here by clicking on the displayed value.



Sample Changeover

The user will be prompted to choose whether or not they wish to run another sample once the previous one has completed; this will require a sample changeover process to avoid cross contamination of the samples. Sample changeover is again guided by the ECS, where the user will exchange their sample for electrolyte and the system will automatically flush any residual sample particles through. The system is then ready for a new sample to be loaded.

Nanopore and System Cleaning

If the user does not elect to continue with further samples then they will be guided through a nanopore cleaning process. The ECS will guide the user through removing the nanopore and cleaning the necessary hardware appropriately. Instructions can be found at <https://support.izon.com/what-is-the-best-practice-for-cleaning-the-nanopore>

6 / TYPES OF ANALYSIS

Size and concentration measurements

When a size and concentration analysis has been selected, the user will have the option to change the stretch of the nanopore using a slider bar which will give them information about what particles can be analysed at that particular stretch. Once an appropriate stretch has been chosen, follow the on-screen instructions for performing calibration and sample analysis. Running parameters should be chosen by the user when prompted on screen.

Sample particles are driven through the nanopore by applying a combination of voltage and pressure, and each particle translocation event causes a resistive pulse or “blockade” signal that is detected and measured by the application software as displayed in [Figure 2](#). By comparing unknown sample particles to known calibration standards, it is possible to obtain highly accurate size and concentration data.

Size and zeta measurements



For zeta measurements there are specific electrolytes which must be used, outlined in the ECS, please contact Izon Support if this requirement is an issue for the sample or there are problems obtaining suitable materials.

Not all nanopores are suitable for zeta analysis, to help with this the user can specifically request zeta capable nanopores by contacting Izon Support. Once a zeta capable nanopore has been detected by the system then the user will be guided by the on-screen instructions to complete the measurement. The process is almost identical to size and concentration measurements, following a matched calibration-sample protocol, however the process will differ in that four calibration recordings with varying voltages and pressures need to be taken while only one recording needs to be taken for the sample.

Signal trace with resistive pulses

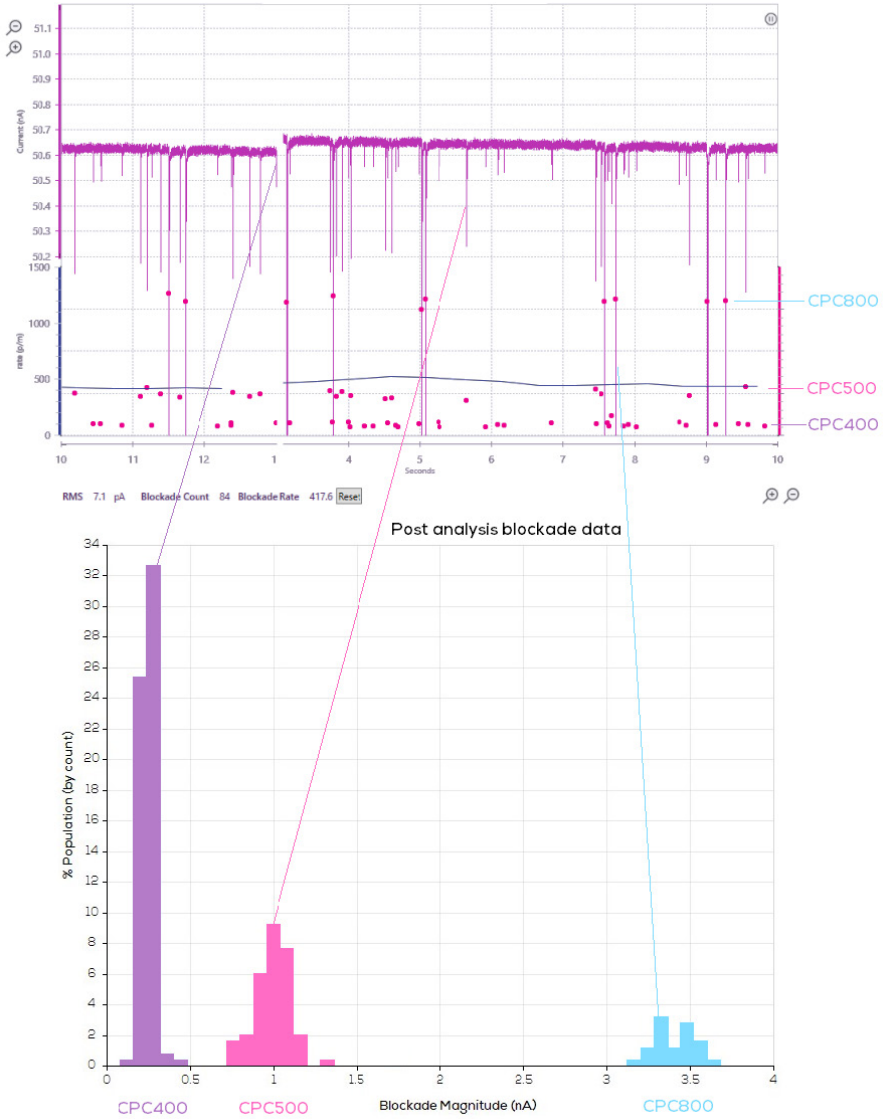


Figure 2. A correlation of real time data visualised during data collection paired with post processing blockade data.

For conducting zeta analysis the blockade duration, Full Width Half Maximum (FWHM) changes with the velocity of the particle and can be used to calculate the relative surface charge of each particle.

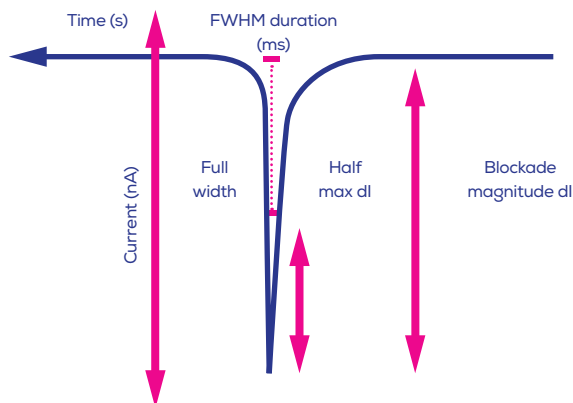


Figure 3. Blockade duration values are converted into particle zeta potential values by calibrating with particles of known size and surface charge.

The FWHM is shown as the horizontal pink bar in the centre of the blockade above.

A sample with different speed signals implies that the suspension contains particles with different surface charges. A particle with a larger negative surface charge will experience a greater attraction towards the positively charged electrode in the lower fluid cell and will travel through the nanopore at a greater speed than a more neutral particle.

7 / MANUALLY CONTROLLING SYSTEM PARAMETERS

In some cases the user may wish to control aspects of the system manually when the manual controls are available. This section will outline what effect each parameter will have on the recording and why it may be useful. For accurate and precise results, the sample and calibration particles must be recorded under identical system settings, which includes the electrolyte characteristics, which affect the baseline current and blockade magnitude. Adjusting any of the parameters between the sample and calibration recordings will affect blockade magnitude and particle rate, invalidating the sample-calibration pairing.

Stretch

Altering the stretch of the system essentially changes the size of the nanopore hole, this is useful if the user's blockades are too close to the noise or are so large that they have the potential to block the nanopore. Decreasing the stretch will increase the blockade magnitude and increasing the stretch will decrease the blockade magnitude. If the stretch has been manually adjusted, manual adjustment of the applied voltage may also be necessary if the current is no longer falling inside the recommended range of 100-140 nA. If the stretch has been decreased, the voltage will need to be increased and vice versa.

| STRETCH (MM) | | | | | | | |
|--------------|----|---------------------------|----|----|----|---|------|
| 43.5 | 44 | 45 | 46 | 47 | 48 | 49 | 49.5 |
| * | | Optimal operating stretch | | | * | Very stable recordings, but nanopore is at risk of being stretched out of shape | |

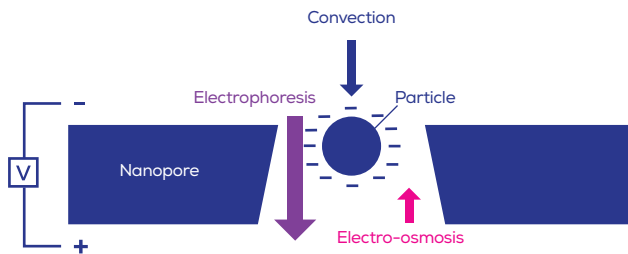
*Operating at a stretch of 43-45 mm is possible but at low cruciform tension the nanopore may exhibit less baseline stability and may be more prone to blockages. Operating at a stretch of 48 mm and greater may work well but the nanopore may become problematic for further use at lower stretches. This is because the cruciform has undergone some structural changes at the high stretch. On some occasions, excessive stretch can cause a rupture of the nanopore.

The Exoid has built in limit switches to prevent the stretcher being moved to a position that will damage the winding mechanism, these are located approximately at 41 and 51 mm. This means that the user will not be able to stretch beyond these values.

Applied Pressure

The pressure applied to the system will have an affect on particle rate and duration. An increase in pressure will result in an increase in particle rate. It is recommended to aim for particle rates of 200-2000 particles per minute. This is because if the particle rate is too high, some data points may be missed if too many are being detected at the same time and if the particle rate is too low then the recording will take a long time therefore increasing the chance of needing to troubleshooting mid recording.

The applied pressure with also have an effect on the relative particle speed as it is pushing the nanoparticles through the nanopore faster, therefore the FWHM will be decreased as the particle spends less time inside the hole. It is for this reason that the user should only use minimal pressure, if any, for zeta measurements, as the convective force from the APS will be much greater than any differences in speed due to particle surface charge and electrophoresis.



Convection – There is always a static pressure head due to gravitational force on the fluid. An additional pressure or vacuum can be applied to the system using the APS.

Electrophoresis – Electrophoretic mobility relates to the movement of charged nanoparticles through an electrolyte solution towards an oppositely charged electrode. It is proportional to particle surface charge (ζ -potential) and the applied voltage

Electro-osmosis - Electro-osmosis relates to the fluid flow caused by currents of solvated ions moving along the surface of the nanopore. It is proportional to the nanopore surface charge (ζ -potential) and the applied voltage

8 / TROUBLESHOOTING

Manual Troubleshooting

Before initiating manual troubleshooting measures that require removal of the pressure nozzle make sure that the applied pressure is set to zero. This is not detrimental if left out, however the user will have to wait some time for the APS to detect a system leak, attempt to hold the current pressure setting and reset itself to its zero position.

Unstable baseline with only Electrolyte



As preventative measures, use the reverse pipetting technique when introducing fluid into the upper fluid cell to avoid placing an air bubble directly on top of the nanopore and replace fluid in the lower fluid cell periodically (once every half-hour) to prevent bubbles from gathering around the nanopore.

| ISSUE | ACTIONS THAT MAY BE NEEDED |
|-----------------------------------|---|
| Cannot establish baseline current | <ul style="list-style-type: none">- Ensure that a voltage is applied to the system and that the nanopore is stretched.- Use reverse pipetting to prevent bubbles forming on top of the nanopore and blocking the fluid flow. |
| Unstable baseline current | <ul style="list-style-type: none">- Gently mix the fluid in the upper fluid cell without introducing any bubbles.- If consistently destabilising, wash and dry the upper fluid cell, and gently replace the fluid in the lower fluid cell to remove any bubbles.- There may be many invisible bubbles beneath the nanopore. Put a droplet of electrolyte on top of the nanopore, take the nanopore off the Exoid and wipe the bottom gently with a lint free tissue before remounting on the Exoid and applying the same stretch as before. Re-establish your baseline current. |

Running biological sample particles

| ISSUE | ACTIONS THAT MAY BE NEEDED |
|--|--|
| <p>Stable baseline current, but very few particles</p> | <ul style="list-style-type: none"> - Nanopore is partially blocked by a bubble or an aggregate. Unblock nanopores by using the Izon Pressure Application Device (PAD). - Mix sample with pipettes or by gently inverting/swirling samples by hand, vortexing or sonicating can damage biological nanoparticles. - Make sure you have coated the nanopore if working with biological samples, as non-specific binding of biological macromolecules to the aperture surface can cause partial blockages and instability. - Increase the sample concentration. Use low pressures until you have a stable system, otherwise you risk blocking the nanopore. - Nanopore is no longer the predicted size, try a different calibration standard or nanopore. |
| <p>Sample is blocking the nanopore (unstable baseline current)</p> | <ul style="list-style-type: none"> - Run highly-polydisperse samples on two different sized nanopores e.g. first run the sample unfiltered through an NP250 or NP300, and then filter the sample through a 0.22 µm filter and run on an NP100. Once calibrated, the results from both nanopores can be displayed on the same plot. - If only interested in exosomes from polydisperse EV samples, filter the sample through a 0.22 µm filter and run with a suitable calibration on a NP100. - All biological samples should be run through a qEV SEC column before TRPS analysis, to exclude proteins that are likely to interact with and block nanopores. - Nanopore is partially blocked by a bubble or an aggregate. Unblock nanopores by using the Izon Pressure Application Device (PAD). |

Running Synthetic or Inorganic Particles

| ISSUE | ACTIONS THAT MAY BE NEEDED |
|---|---|
| Stable baseline current, but very few particles | <ul style="list-style-type: none">- Increase the sample concentration. Use low pressures until you have a stable system, otherwise you risk blocking the nanopore.- Consult the sample preparation guide and enhance suspension stability.- If the sample particles have positively charged surface/terminal groups, they are likely to stick to and block the nanopore instead of going through. Contact Izon Support if this may be the case. |

Short-circuits

Electrolyte can creep in between metal connection points inside or around the fluid cell, causing significant noise fluctuations. Troubleshoot this by pipetting out the liquid in the upper fluid cell and observing the current. If the current does not change from pre-liquid removal then there is liquid somewhere causing a short circuit. Remove the upper fluid cell, then wash and dry it, making sure all the metal parts of the fluid cell are dry. Then re-establish the baseline current.

Always ensure that you do not pipette more than 35 μL into the upper fluid cell, or more than 75 μL into the lower fluid cell, or the fluid may leak between connection points and cause the noise to gradually increase.

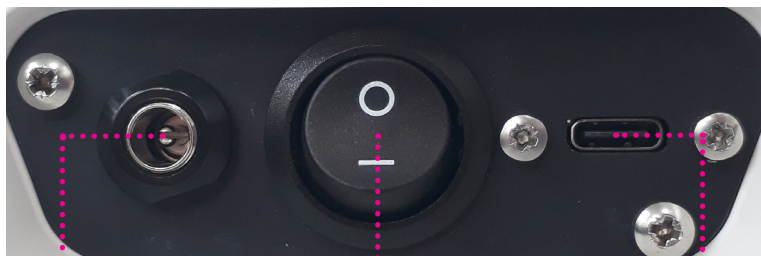
Instrument Connection

If some time has passed and instrument connection is still not complete, proceed to support.izon.com/why-are-none-of-the-exoid-modules-connected to resolve the issue.

Power Cycling

Follow these instructions carefully when the need to power cycle the instrument arises.

1. Turn off the switch on the Exoid, the off position is where the full "O" symbol is pressed down as indicated in the image below.
2. Unplug the USB and power cables from the Exoid.
3. Wait 5 full seconds.
4. Plug the USB and power cables back in to the appropriate sockets.
5. Turn the power switch on the Exoid on.



Power Socket

Power Switch

USB Socket

9 / FURTHER SUPPORT

Additional support material is available at support.izon.com

If you have any questions that are not answered on the support portal, please contact our support staff via the online support portal, or directly by email (support@izon.com).

Device Cleaning

When cleaning the exterior of the instrument do not use solvent-based cleaners or UV as this can affect the integrity of the powder coating, instead use warm soapy water. The fluid cell must be disassembled before cleaning can take place. First remove the fluid cell cap and upper fluid cell. Then remove the lower fluid cell by pulling directly up on the "arms" firmly until it releases from the lower fitting.



DO NOT GET ANY SUBSTANCE INSIDE THE PIN HOLES IN THE LOWER FITTING.

This will cause major functional issues and should be remedied immediately after occurrence by blow drying extremely well with compressed air.

The components of the fluid cell which have now been removed (the fluid cell cap, the upper fluid cell, and the lower fluid cell) can be cleaned carefully with 70% Ethanol Absolute. Make sure to dry everything thoroughly, with compressed air if available, before re-assembling the fluid cell. Any liquid that remains on the silver plated pins underneath the lower fluid cells will cause major issues when the pins are pushed back into the lower fitting. **DO NOT** soak any part of the fluid cell.

Repair and Servicing

There are no user-serviceable parts. Return equipment to Izon for service.

All replacement parts, cables, mains lead, power supply, Nanopores, Fluid Cells must be obtained via Izon Science Ltd or from an authorised distributor.

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FCC Declaration of Conformance:

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions:

- (1) This device may not cause harmful interference

- (2) This device must not accept any interference received, including interference that may cause undesired operation. Changes or modification not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

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