PULSOID

USER MANUAL

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1 DEFINITIONS AND WRITING CONVENTIONS

This manual contains warnings and precautionary statements to help prevent personal injury and/or damage to the Pulsoid when properly followed. The safety and information symbols described in Table 1 are presented throughout the guide.

Table 1: Safety and Hazard Symbols

(lightbulb symbol)	This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations.
WARNING	Indicates a hazardous situation that could result in death or serious injury if not avoided. Do not proceed until all specified conditions are fully understood and met.
CAUTION	Indicates a hazardous situation that could result in minor or moderate injury if not avoided. Ensure all specified conditions are fully understood and met before proceeding.

Acronyms and definitions used in this manual are defined in Table 2.

Table 2: Terminology Used in this Manual

TERM	DEFINITION
Automatic Pressure System (APS)	A component of the Pulsoid that controls the applied pressure/vacuum.
Low Noise Amplifier (LNA)	A component of the Pulsoid that controls the voltage applied to the system in order to manipulate current.
Nanopore Pulse Sensing (NPS)	The technology used in the Pulsoid to measure nanoparticles.
Chip	Disposable component containing the pore through which particles translocate for measurement.
Fluid cell	Reusable component for sample loading and measurement.
Halo lighting	Circular lighting strip around the instrument lid.
Translocation	The movement of a particle through the chip.
P_{min}	The minimum pressure/vacuum that drives true translocations.
Recording	A single data collection run, typically lasting for ~500 blockades.

TERM	DEFINITION
Measurement	The group of recordings required to calculate at least one of particle size, zeta potential or sample concentration.
Measurement Electrolyte (ME)	Izon's recommended electrolyte, provided in the Reagent Kit.
Pressure Application Device (PAD)	A small suction cup used to clear blockages.
Pulsoid Control Suite (PCS)	Software used to operate the Pulsoid.
Izon Data Suite 2 (IDS2)	Software used to process data produced by the Pulsoid.
Root Mean Square (RMS) noise	A real-time measure of the background electrical noise of the system.

2 SAFETY AND HAZARDS

2.1 Safe Use Requirements and Specifications

Users must thoroughly review the complete User Manual before assembling, setting up, or operating the Pulsoid, and keep it readily accessible during operation. Operate the system strictly as outlined in the documentation to avoid potential hazards that could result in personal injury or equipment damage. Adhere to the safe use requirements specified in Table 3 below. Using the equipment in an unspecified manner may compromise the protection it provides.

Table 3: Safe Use Requirements and Specifications

SAFE USE REQUIREMENT		SPECIFICATION
Operating Temperature	**Indoor Use**	15-30 °C (recommended 18-23 °C)
Altitude		Up to 2000 meters above sea level
Relative Humidity		20-80% relative humidity
Power Consumption		12.75 W
Pollution Degree Rating		2
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

Do not attempt to run the Pulsoid outside of these conditions.

Liquid will be in contact with the following materials when it is analysed on the Pulsoid. Please ensure that your application is compatible with these materials:

- PMMA: Polymethyl methacrylate
- Silicon
- Nitrile

2.2 Hazards

The Pulsoid is a laboratory product. If biohazardous samples are present, adhere to current Good Laboratory Practices (cGLP) and comply with any local guidelines specific to your laboratory and location.

The Pulsoid system contains no potentially hazardous chemical materials.

The Pulsoid 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.

Table 4: List of Potential Hazards

	Stop operation if there is a smell of burning, if electricity leaks (e.g., buzzing when touched), if water ingresses into the instrument, if electrical parts show any signs of damage, or if the instrument fails to function as expected.
	Izon instruments must be operated with Izon supplied leads and power supplies only. Failure to use the correct power supply may result in invalid operation.
	Repair of the instrument must only be carried out by an Izon-approved technician. Maintenance must be carried out when the instrument is unplugged and under the guidance of Izon support (see Section 7: Maintenance and Troubleshooting).
	Keep cables away from liquids. Take particular care not to spill, spray or otherwise introduce liquids onto the power button.
	The Pulsoid contains a magnet. Individuals with pacemakers, ICDs, or other implanted medical devices should maintain a safe distance from the device, as magnets may interfere with the operation of such medical devices. Consult your physician before use if you have any concerns.
	The use of appropriate personal protective equipment (PPE), including lab coat, gloves and safety glasses, is recommended when operating and maintaining the instrument.
	Keep fingers and loose clothing clear of any moving parts and when opening/closing the lid.
	Dispose of biological samples, reagents, chemicals and contaminated consumables in accordance with laboratory local, regional and national regulations.

2.3 Unpacking

Exercise caution and use proper manual handling techniques when lifting or moving the instrument as it is heavy and may cause injury if incorrectly handled.

2.4 Transport

Before moving or shipping the Pulsoid, decontamination procedures must be performed. Always move or ship the Pulsoid with the supplied packaging materials, which will protect the instrument from damage. 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.

2.5 Disposal

The Pulsoid contains 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 PULSOID

3.1 Overview

The Pulsoid uses Nanopore Pulse Sensing (NPS) to deliver nanoparticle measurements on a particle-by-particle basis. It builds on Izon's proven Tunable Resistive Pulse Sensing (TRPS) platform to deliver a faster, simpler workflow, without compromising accuracy and precision.

The Pulsoid fluid cell consists of two chambers connected by a chip containing a nano-sized pore. By applying pressure and voltage across the chambers, particles are driven through the pore one at a time. As each particle passes through, it temporarily disrupts the electrical current. The magnitude, frequency, and duration of this disruption (or "blockade") are used to determine the sample's particle size, concentration, and zeta potential.

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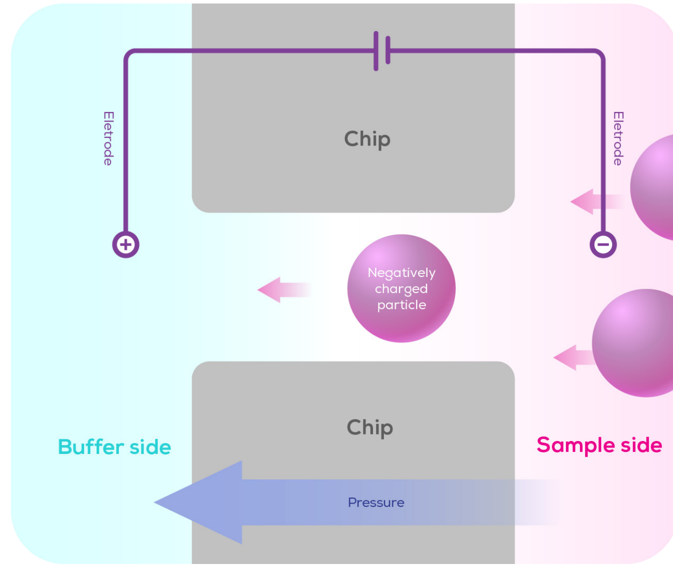

Figure 1. Overview of the how the Pulsoid works for negatively charged particles. For positively charged and neutral particles the polarity is reversed using the Pulsoid Control Software.

3.2 Intended Use

The Pulsoid is intended to measure size, concentration, and zeta potential of nanoparticles. The instrument has been designed exclusively for professional personnel and is not a medical device.

3.3 Pulsoid Workflow

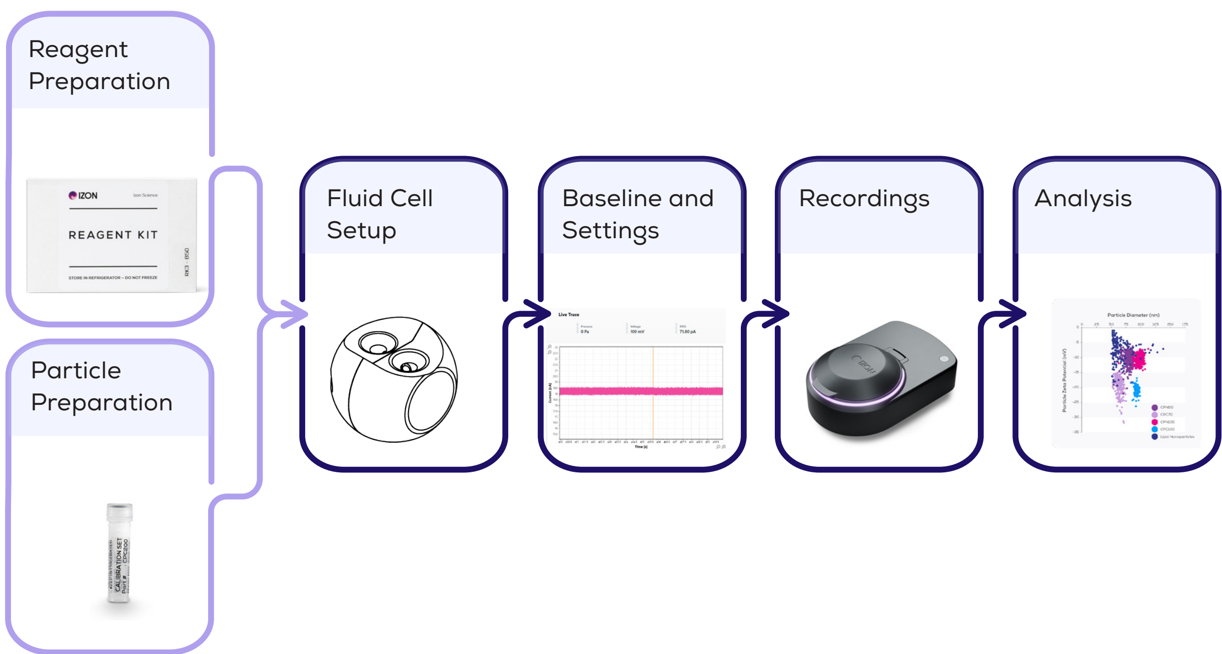

Figure 2. Overview of the Pulsoid workflow.

4 ASSEMBLY AND SETUP INSTRUCTIONS

4.1 System Components

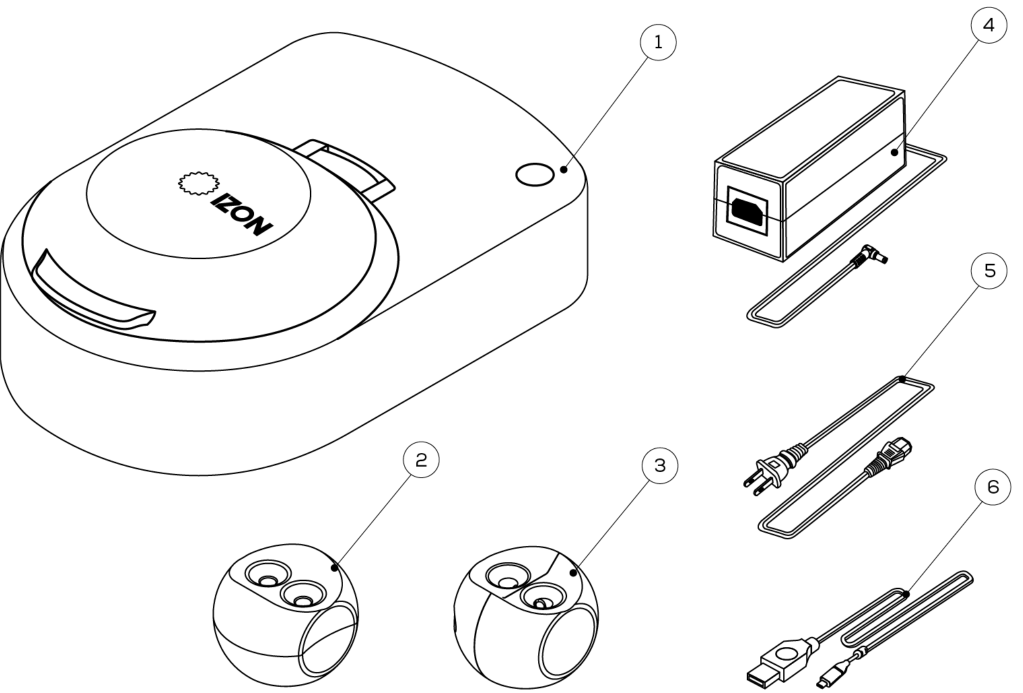

Figure 3. The Pulsoid and accompanying components.

The following components and quantities are provided:

1	Pulsoid Instrument	4	24 V Power Supply
2	Reference Cell	5	Power Lead
3	Fluid Cell (x2)	6	USB-A to USB-C 3.0 Cable

The NPS Training Kit, supplied with the Pulsoid, contains components that are required for operation and/or maintenance of the Pulsoid.

The NPS Training Kit contains the NPS Reagent Kit. Kit specifications, components, and preparation information can be found here, along with guidance on correct storage of Pulsoid chips:

support.izon.com/nps-kit-documents

Additional NPS Training Kits and NPS Reagent Kits can be purchased from store.izon.com.

The following components are required for operation and/or maintenance of the Pulsoid but are not provided:

- Calibrated pipettes (1 µL to 1 mL) and pipette tips
- Lint-free tissues
- Paper towels
- Powder-free disposable gloves
- 0.22 µm-filtered deionised (DI) water
- Vortex mixer
- 33 mm x 0.1 µm filters (optional)
- Sample(s) to be analysed

4.2 Minimum Computer Requirements

A laptop is provided with your Pulsoid. If you wish to use your own, you must use a PC at least meeting these minimum requirements for the Pulsoid software to work effectively:

- 16 GB RAM
- i7 processor
- 256 GB SSD
- Dedicated graphics processor (GPU) with at least 1 GB graphics memory
- Windows 10 Pro or above

4.3 Installing the Pulsoid Software

Before operating the Pulsoid, there are two software suites that must be installed:

- **Pulsoid Control Software (PCS):** Used to control the instrument and record data. Download the **non** 21 CFR Part 11 installation from support.izon.com/how-do-i-download-the-latest-pulsoid-control-suite-software
- **Izon Data Suite 2 (IDS2):** Required to review and analyse data collected using the PCS. Download the **non** 21 CFR Part 11 installation from support.izon.com/how-do-i-download-the-latest-version-of-the-izon-data-suite-2

4.4 Assembling and Installing the Pulsoid

The Pulsoid is to be used within the rated conditions noted in Section 2.1: Safe Use Requirements and Specifications.

1. Unpack the Pulsoid and box contents. Check whether all components are present so as not to inadvertently throw anything away.

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

If you choose not to keep the materials, please recycle them wherever cardboard recycling services are provided.

2. Place the Pulsoid onto a stable and level laboratory bench. Position the unit so it can be quickly and easily disconnected from the mains power.
3. Plug the provided power cable into a suitable wall socket.
4. Connect the cable from the 24 V power supply to the rear of the instrument.

To prevent heat build up do not cover the power supply.

Make sure the power supply is positioned away from fluids and any potential spills.

5. Plug the USB-C end of the provided cable into the back of the unit.
6. Plug the USB-A end of the cable into an appropriate computer (see specifications in Section 4.2: Minimum Computer Requirements).
7. Switch on the power socket at the wall (if applicable), then press and briefly hold the Pulsoid power button (located on the top of the instrument in the back right corner) until you see the halo lights turn on.
8. Go to the Start menu and click the software icon to launch the Pulsoid Control Software (PCS).
9. Check that the instrument status changes from 'Not Connected' to 'Connected' in the top left corner of the PCS (Figure 4). Once it has connected, the Automatic Pressure System (APS) will show as 'Calibrating'. Once the APS status changes back to 'Active', the Pulsoid is ready to use.

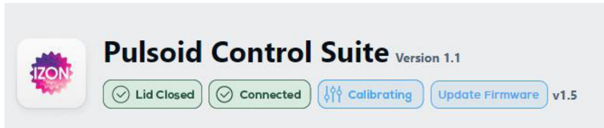

Figure 4. Instrument status reporting in the PCS.

5 OPERATING INSTRUCTIONS

5.1 Reagent Preparation

Prepare Measurement Electrolyte according to the protocol in the NPS Reagent Kit Specifications and Preparations Guide. Safety Data Sheets for Reagent Kit components and other relevant components can be found at support.izon.com/safety-data-sheets. The same electrolyte must be used for all sample and calibration recordings that will be analysed together.

5.2 Particle Preparation

Calibration Particles

All sample recordings will need an associated calibration recording using a calibration standard. CPN80s are used for calibrating concentration measurements and CPC100s are used for calibrating zeta potential measurements. Dilute calibration particles from concentrated stocks immediately before use, using Measurement Electrolyte (ME).

1. Homogenise the calibration particle stock by vortexing for 10 seconds.
2. Dilute your calibration particles in ME in a particle-free 1.5 mL tube. The optimal dilution will depend on the concentration of your calibration particles, which can be found on the label. Aim for a target concentration range of 1.5-2 x 10^{10} particles/mL for CPCs, and 3-5 x 10^{10} particles/mL for CPNs.
3. Vortex to mix.

If your sample is particularly dilute and requires high pressures to achieve an acceptable particle rate, you may need to further dilute the calibration particles to bring them in line with your sample.

Sample Particles

4. Dilute your sample in ME. The target concentration range is 2.5×10^9 to 2.5×10^{11}.
5. Depending on your sample type and application, your sample may benefit from filtering through a 0.1 µm filter to reduce blocking of the chip. Please be aware that filtering may change the composition of your sample.

If an initial approximate concentration of the sample is unknown, a series of samples may be prepared at different dilutions, e.g. 1:100, 1:10, 1:5.

5.3 Assembling the Fluid Cell

Ensure both halves of the fluid cell are clean and dry before assembling.

1. Place both halves of the fluid cell on the laboratory bench with the inside surfaces (the side containing the O-ring) facing upwards. One half will have an inset square depression as shown in Figure 5 below. This is the side to place the chip in.

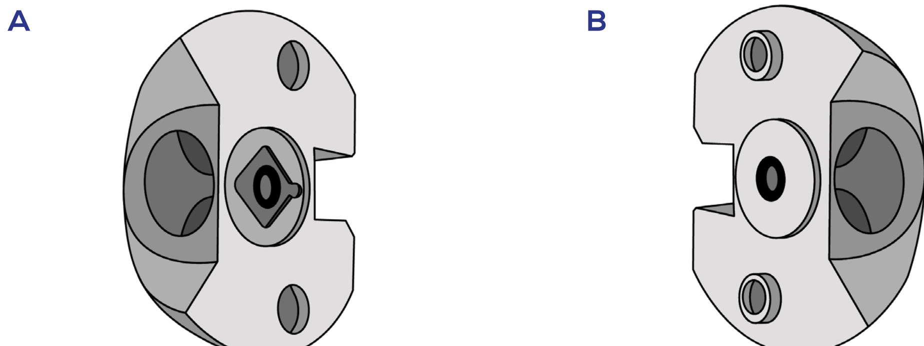

Figure 5. Fluid cell halves. A) Side with square depression for chip; B) Side that fits over the chip once it is in place.

2. Pick up the chip from its storage tray using tweezers. Carefully slide one tweezer tip under one side of the chip, away from the pore, and grasp it firmly but gently before lifting.

Chip edges may be sharp. Avoid squeezing the chip too tightly as it may crack. Cracked chip edges will be sharp.

Avoid placing the tweezers too close to the centre of the chip as they may damage the pore.

3. Identify the cavity side of the chip, shown in Figure 6 below. The cavity side has a visibly larger hole.

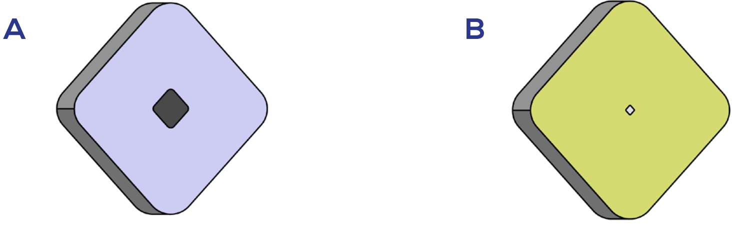

Figure 6. Chip faces. A) Cavity side; B) Aperture side.

4. Rinse the chip by squeezing DI water from a wash bottle gently across both faces, making sure the water flows across the pore.

5. **Very carefully** blot the chip by placing it on a lint-free tissue and then carefully flip it over to blot the other side. Avoid touching the area around the pore.

Do not rub the chip to dry it as this may damage the delicate membrane around the pore.

6. Place the chip with the cavity side facing down into the square depression in the fluid cell (Figure 7).

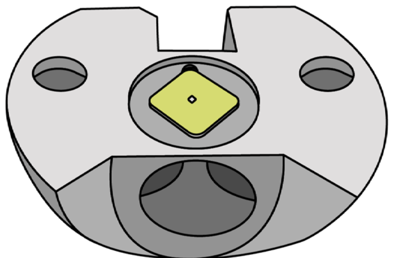

Figure 7. Fluid cell with chip in place.

7. If necessary, nudge the chip gently into position with the tweezers. The chip must fully cover the O-ring and the whole of the chip must be within the square depression.

Ensure the chip is contained entirely within the square depression in the fluid cell, otherwise it may be crushed when the fluid cell is screwed together.

Ensure the chip completely covers the O-ring, otherwise it may not form a seal when the fluid cell is screwed together and measurement will not be possible.

8. Place the second half of the fluid cell on top of the half containing the chip. The two halves must be oriented so the pogo pins are pointing in the same direction.

Do not slide the two halves of the fluid cell against each other as you may displace the chip. If you need to realign the two halves, lift them apart, check the chip position and replace them in the correct alignment.

9. Place a screw into the threadings on each side of the assembled fluid cell and screw the two halves together with the provided Allen key. Finger-tight is sufficient to achieve a seal between the O-rings and the chip.

Do not over tighten.

5.4 Filling the Fluid Cell

1. Identify the sample side of the fluid cell. The fluid cell will only fit onto the Pulsoid in one orientation. In this orientation the sample side will be on the right as you face the instrument (Figure 8).

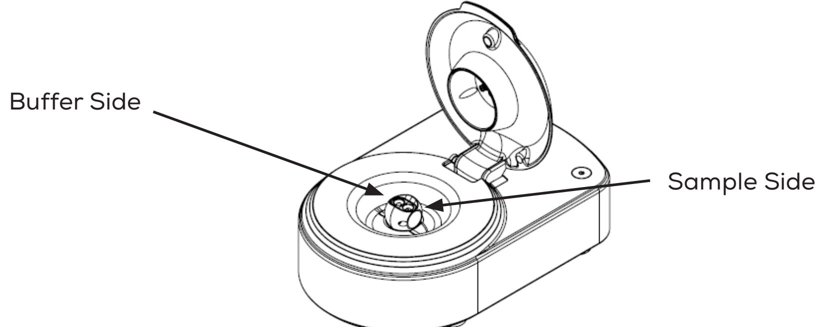

Figure 8. Sample and buffer sides of the fluid cell.

2. Set your pipette to 65 µL and carefully pipette measurement electrolyte into the very bottom of the sample side of the fluid cell. Try and avoid introducing any bubbles.
3. If a bubble forms within the fluid chamber (Figure 9), use the force of the fluid being ejected from the pipette to displace it. When the bubble detaches and reaches the surface of the chamber, it can be removed with a pipette tip.

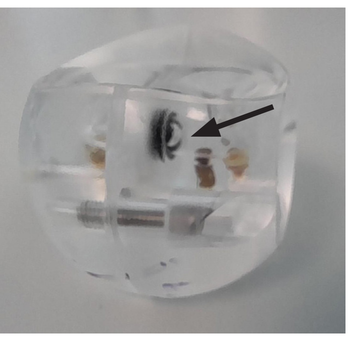

Figure 9. Bubble inside fluid cell assembly.

4. Repeat steps 2 and 3 for the opposite side (the buffer side) of the fluid cell.
5. Check that the fluid level covers the electrode but does not extend into the funnel at the top of the sample or buffer chamber (Figure 10). If any droplets are present on the funnel sides, blot them away with a lint-free tissue.

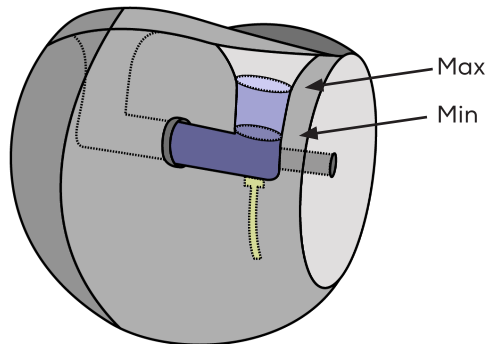

Figure 10. Minimum and maximum fill levels.

6. Add or remove measurement electrolyte as needed to optimise the fluid level on each side.

When both the sample and buffer sides are appropriately filled and free from bubbles, the fluid cell assembly is ready to use.

7. Mount the filled fluid cell onto the instrument and close the lid.

Do not force the fluid cell into position. If it is in the correct orientation it will slot into place easily.

It is essential that the lid is closed for operation as it provides shielding from electrical interference.

5.5 Setting System Parameters

System components, including the APS and LNA, are controlled through the Device Controls panel in the PCS (Figure 11).

Figure 11. Device Controls panel.

The function of each of these Device Controls is described in Table 5.

Table 5: Device Controls

FIELD	VALUE
Pressure	Input the desired applied pressure and press 'Apply'. Note that pressure is applied from the buffer side of the fluid cell. This means to increase particle rate you should apply a more negative pressure (vacuum).
Voltage	Input the desired applied voltage and press 'Apply'. Use a positive voltage to propel negatively charged particles from the sample side to the buffer side.
Sample Rate	The frequency with which the software records the state of the system. Lower rates will result in less accurate data while higher rates will result in larger data files and higher noise. The same rate must be used for all sample and matched calibration recordings.
Stopwatch	Displays the duration of the active recording.

Do not open the lid while pressure is applied. Set the pressure back to 0 Pa before opening the instrument.

5.6 Establishing a Baseline Current

1. In the PCS, apply a 100 mV voltage in the Voltage field of the Device Controls panel (Figure 11).
2. Check the Sample Rate is set to 250 ksps (Figure 11).
3. Monitor the baseline current in the Live Trace window (Figure 12). If the baseline is drifting up or down, wait for it to stabilise before proceeding.

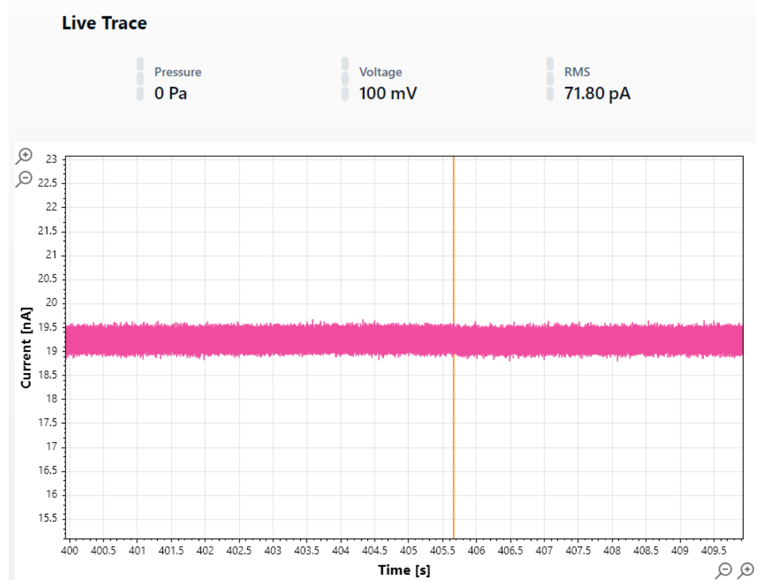

Figure 12. Live Trace window.

4. Check that the RMS noise is less than 100 pA at a sampling rate of 250 Ksps.
5. Make a note of the baseline current by reading the value on the y-axis corresponding to the centre of the pink trace line.
6. Set the voltage to 0 mV and note the new baseline current value.
7. Calculate the corrected baseline current using the following equation:

Corrected baseline current = baseline current (100 mV) - baseline current (0 mV)

The pore is considered wet when the corrected baseline current is stable and between 16 and 40 nA (note the exact value is chip dependent).

8. If any of the above checks do not pass, work through the troubleshooting methods listed below until the pore is successfully wetted:
   a) Use the PAD to cover the channel on the buffer side of the fluid cell (Figure 8). Press down gently then pull up sharply. Try to achieve a "pop" noise so you know pressure and vacuum forces are being applied to the chamber.
   b) Use the syringe to apply pressure to the buffer side. To do this, pull the plunger all the way up, apply the sealing cup to the buffer side, and push the plunger in. You will need to provide downwards force on the sealing cup to obtain an air seal. Sometimes a pumping motion can be beneficial.
   c) Use the syringe to apply a vacuum to the buffer side. To do this, depress the plunger all the way in, apply the sealing cup to the channel on the buffer side, and pull the plunger up.
   d) Repeat the above once more. If no or low current is still observed, remove the fluid cell from the fitting and tap hard on a padded surface at an angle such that the fluid channel is at a 45 degree angle from horizontal.
   e) If no current is observed after following all of the above steps, remove the chip and repeat step 4 onwards from Section 5.3: Assembling the Fluid Cell.
   f) Replace the chip.

5.7 Changing the Sample in the Fluid Cell

Once the baseline current is established, you can change to either calibration particles or your sample of interest. See Section 5.9: Sample Measurement for details of which to measure first, as it is dependent on your sample type and which characteristics you are measuring. Note that for the first changeover you can simply replace the measurement electrolyte on the sample side with the substance you wish to measure. For all subsequent changeovers you will need to fill the fluid cell with measurement electrolyte between samples to check it is clean, as described below:

1. Remove the fluid cell from the instrument.
2. Use a pipette to remove the fluid from both sides of the fluid cell. Tap the fluid cell gently on a padded surface to collect the last few droplets of liquid together before removing them with the pipette.
3. Fill both sides of the fluid cell with measurement electrolyte. Remove any bubbles by following step 3 in Section 5.4: Filling the Fluid Cell, and ensure the funnels leading into the fluid chambers are dry.
4. Place the fluid cell back on the Pulsoid and check that the residual Blockade Rate is less than 10 particles/minute.

If the residual Blockade Rate is > 10, you may have some particles left in the fluid cell. Pipette out all the fluid, replace it with clean measurement electrolyte and re-check the residual Blockade Rate. Repeat this rinsing until the Blockade Rate is less than 10. If you are having trouble achieving this, please refer to Section 7.2: Troubleshooting.

5. Once < 10 particles/min are detected, remove the fluid cell and replace the fluid in the sample side with the next sample to be measured and ensure there are no bubbles.
6. Replace the fluid cell on the instrument and close the lid.

If you are concerned about backflow of previously translocated particles from the buffer side into the new sample, you may wish to change the measurement electrolyte on both sides.

5.8 Entering Sample Details

The fields in the Recording Settings panel in the PCS must be filled out with information about the sample being run before you can make recordings (Figure 13).

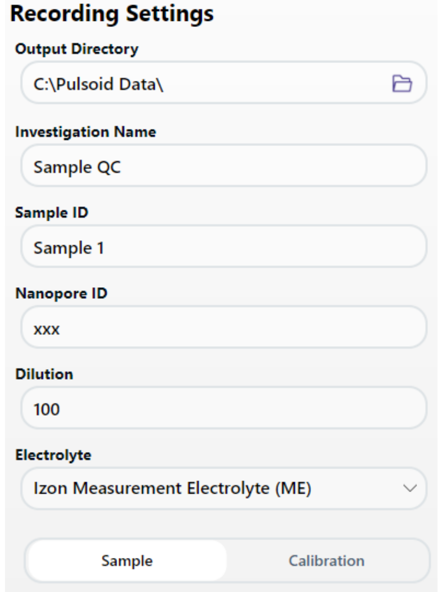

Figure 13. Recordings Settings panel.

1. Complete all required fields, as described in Table 6. Fields will have a red outline if they are empty, contain invalid characters, or if there is a space at the end of the field.

Avoid using symbols or non-standard characters in input fields (e.g., \ / : * ? " < > |).

Table 6: Recording Settings

FIELD	VALUE
Output directory	Use the folder icon to browse for the location on the PC where you want your investigation folders to be saved. Make sure to choose a location on the local hard drive.
Investigation name	This is what the folder that is used to store your recordings will be called.
Sample ID	This will be included in the name of the recording file. The field is limited to 18 characters.
Nanopore ID	The ID of the chip you are using e.g. Si15-H10.
Dilution	The dilution factor of your particles as either a number (E.g. 1000) or E notation (E.g. 1E3). Note that annotations such as '1:1000' or '1000x' are not valid.
Electrolyte	A dropdown menu for the measurement electrolyte being used. Downstream zeta analyses depend on using Izon's Measurement electrolyte (ME). If you are not using Izon ME then select 'Custom' and enter your electrolyte name in the free text field that appears. This is limited to 25 characters.
Sample/Calibration Toggle	Toggle to switch between creating sample or calibration recordings. When you select 'Calibration', the below fields will appear for the size and concentration of the calibration particles.
Mean Particle Size	The size of the calibration particles being used.
Raw Concentration	The concentration of the calibration particles prior to dilution.

5.9 Sample Measurement

Completing a measurement on the Pulsoid requires planning groups of recordings, as data analysis requires matched calibration recordings for each sample and different types of analysis require recordings at more than one pressure and/or voltage. The following must be kept constant for all recordings needed to complete a measurement:

- Instrument, fluid cell and chip
- Electrolyte
- Sampling frequency
- Sample dilution (if multiple recordings are needed at different pressures/voltages)
- Calibration dilution (note this can differ from the sample dilution), particle size and concentration

General points to note:

- Follow the instructions in Section 5.7: Changing the Sample in the Fluid Cell to change between samples.
- The same calibration recording(s) can be used for several samples, as long as system settings remain the same and the baseline is stable between each new sample that is measured.
- Try and keep the particle rate within 200-2500 particles/minute for all recordings. You may need to optimise your sample dilution in order to achieve this.

Figure 14. Screenshot of a live trace showing the measurement of calibration particles.

Particle rates of <100 particles/minute will trigger data quality warnings in IDS2.

- Remember to update the Recording Settings fields when you switch to a new sample.
- Record 500 blockades for sample recordings and 300 for calibration recordings. This ensures robust analyses. Fewer blockades may be sufficient to characterise monomodal samples while more may be desired for complex mixtures.

Particle counts of <300 will trigger data quality warnings in IDS2.

Size and Concentration Measurement

Concentration can be calculated from recordings using one, two or three different pressures. Using more pressures will generally increase the accuracy of your concentration estimate. However, using pressures that are too high may result in loss of particles at the lower end of the detectable size range. A recommended workflow using two pressures for sample and calibration recordings is detailed below.

The recommended calibration particles for size and concentration measurements are CPN80s. The recommended settings for running CPN80s are a voltage of 300 mV and pressures of -500 and -800 Pa.

1. Load your particles and set the initial voltage according to Table 7. You may need to iterate through several voltage/pressure combinations to optimise your particle rate.

Recordings made with a voltage of 0 mV cannot be analysed in IDS2.

Table 7: Setup Requirements by Particle Type

SAMPLE	VOLTAGE
Negatively-charged particles (≤ -5 mV)	300 mV
Neutral or positively-charged particles (> -5 mV)	-300 mV

2. Decrease the pressure in -100 Pa increments until blockades are visible, evenly spaced and at least 1 nA in size. If the particles being run are calibration particles, blockade sizes should be consistent.

Occasionally blockade events visible in the trace may not be true particle translocations. To obtain accurate measurements, it is necessary to ensure that blockades represent true translocations.

3. Check for the following indicators of erroneous blockades:

- Abnormally small blockades (note this could also indicate the particle size is at the lower resolution limit of the chip)
- Blockades occurring in bursts (Figure 15).

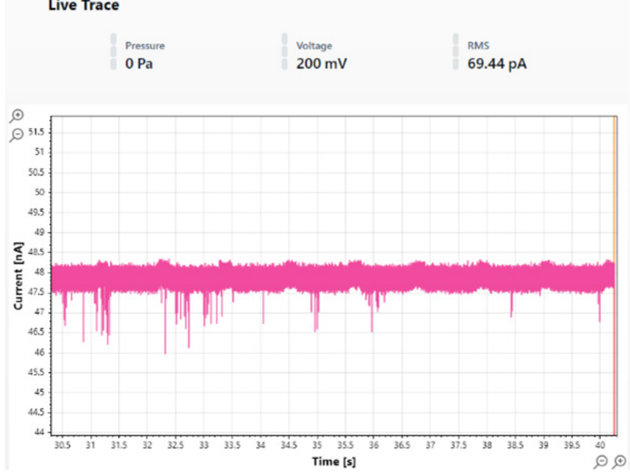

Figure 15. Blockade bursts.

4. If either of the above issues are present, continue decreasing the pressure in -100 Pa increments until you see a substantial improvement in the blockade pattern. There should be no change in the baseline during this process.

Note that in some cases samples will transition from no blockades to erroneous blockades before consistent blockades are reached.

5. The pressure at which true blockades first become visible (i.e. the pressure required to drive particle translocations through the pore) is known as P_{min}. Make a note of this value.
6. If you cannot achieve a suitable blockade profile, please refer to Section 7.2: Troubleshooting. If issues persist, you may need to try a different chip.
7. Set the pressure to 100 Pa less than P_{min} (i.e. apply a stronger vacuum). For example, if P_{min} was determined to be -300 Pa then apply a pressure of -400 Pa.
8. Start the recording by clicking 'Start Recording', in the top right hand corner of the PCS window (Figure 15).

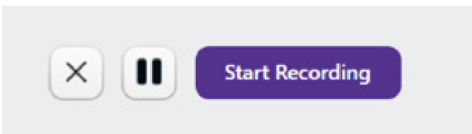

Figure 16. Recording controls.

9. Monitor the baseline during recording; if stepping, drifting, or noise is observed then the recording should be either paused and then resumed once the baseline is re-established, or cancelled and a new recording started once the issue is resolved (Figure 17).

For troubleshooting baseline changes during measurement, follow the methods outlined in step 8 of Section 5.6: Establishing a Baseline Current. Do not change the Device Controls settings.

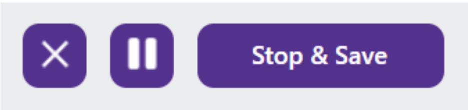

Figure 17. Buttons to cancel, pause or finish the recording.

10. When you have recorded the required number of blockades, click 'Stop & Save' (Figure 17).

Long recordings result in large data files that are slow to load in IDS2.

11. Change the pressure to 700 Pa less than P_{min} (i.e. apply a much stronger vacuum) and record the appropriate number of blockades.
12. Change the particles in the fluid cell to whichever of sample of interest/calibration you are running second.
13. Repeat the process to obtain suitable recordings for these particles.
14. We recommend that you check your data quality in IDS2 as you go and repeat any recordings that fail data quality checks (Section 6.2: Data Checking).

Size and Zeta Potential Measurement

Calculating zeta potential requires a single sample recording and multiple calibration recordings at different voltages and pressures. The measurement group for calculating size and zeta potential consists of five recordings.

The recommended calibration particles for size and zeta potential measurements are CPC100s.

You must use Izon's Measurement Electrolyte for zeta recordings. If you would like to measure zeta potential using your own buffer, please reach out to the support team to discuss custom solutions.

1. Load your sample particles and set the initial voltage to 200 mV for negatively-charged particles (≤ -5 mV) and -200 mV for neutral or positively-charged particles (> -5 mV). You may need to iterate through several voltage/pressure combinations to optimise your sample rate. Make a note of the voltage you use for your sample (referred to as V1) as you will need it to calculate the voltages for your calibration recordings.
2. Find P_{min} as described in steps 2 to 6 in the section on Size and Concentration Measurement above.
3. Set the pressure to 100 Pa less than P_{min} (i.e. apply a stronger vacuum).
4. Record 500 blockades as described in steps 8 to 10 in the section on Size and Concentration Measurement above.
5. Change the particles in the fluid cell to calibration particles.
6. Set the pressure to 0 Pa. If your sample voltage was 200 mV or -200 mV then record at least 300 blockades at each of 120 mV, 150 mV, and 200 mV for the calibration particles.
7. Change the pressure to -300 Pa and the voltage to 200 mV and record at least 300 blockades.
8. For other sample voltages, calculate the corresponding settings for calibration recordings using Table 8. For calibration particles other than CPC100s, the pressure for Calibration 1 may require optimisation but it must be at least 200 Pa different from 0 Pa.

Table 8: Voltage and Pressure Requirements for Zeta Measurements

RECORDING	RECOMMENDED VOLTAGE	PRESSURE
Sample	V1	P_{min} - 100 Pa
Calibration 1	±V1	-300 Pa
Calibration 2	Same as Calibration 1	0 Pa
Calibration 3*	0.75 x Calibration 1	0 Pa
Calibration 4*	0.6 x Calibration 1	0 Pa

*Acceptable values for the Calibration 3 voltage are >0.65 to ≤0.85 x Calibration 1. Acceptable values for Calibration 4 voltage are ≥0.4 to ≤0.65 x Calibration 1.

5.10 Shutting Down the Pulsoid

1. Remove the fluid cell from the instrument and replace the fluid on both sides with measurement electrolyte. Check the residual Blockade Rate is less than 10 particles/minute.
2. Remove the fluid cell from the instrument and replace the fluid on both sides with DI water.
3. Replace the fluid cell on the instrument and check there is no current, to confirm the fluid cell is completely clean.
4. Remove the fluid cell from the instrument and disassemble it. Tap it gently onto paper towel to remove any residual fluid.

For best results, use a new Pulsoid chip at the beginning of each session (from Pulsoid activation to shutdown). Reuse may be possible in some cases; refer to support.izon.com/how-should-i-store-pulsoid-chips for guidance.

5. Dry the faces of the fluid cell halves with a lint-free tissue.
6. You may wish to reassemble the fluid cell without the chip before storing so as not to lose the small parts.
7. Close the PCS.
8. We recommend switching the instrument off when not in use. Press and hold the power button until the instrument shuts down.

6 IZON DATA SUITE 2

6.1 Importing Data

1. Launch the IDS2 software.
2. Click on the icon of a folder with the plus sign to create a new project (Figure 18). This will open a File Explorer window.

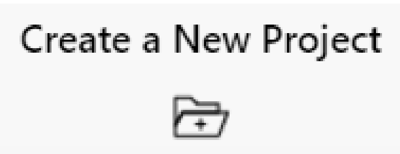

Figure 18. Create project icon.

3. Navigate to where you wish to create your project. You can either select the output data folder containing the raw data produced by the Pulsoid or create a new folder.

You can only create one project per folder, so if your Pulsoid data folder contains data from more than one project you may wish to create new folders for each analysis.

4. To create a new folder for analysis, right click in the file display area and select "New › Folder".
5. Once you have selected the folder for your project, click 'Select Folder'. A project will be created with the same name as the selected folder.

Once a project has been created, you can open it next time you launch IDS2 by clicking on the plain folder icon under the heading 'Open Existing Project'.

6. Once the project has been created, it is ready to import recordings from a Pulsoid output folder. Start by clicking on the 'Import Recordings' icon (Figure 19).

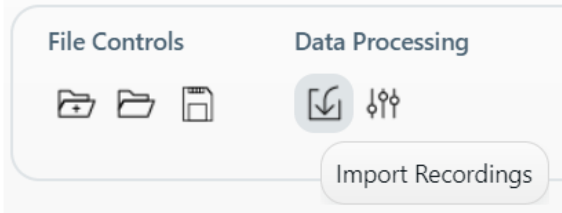

Figure 19. Import Recordings icon in IDS2.

7. Navigate to the required Pulsoid data folder and select the .idf2 file.
8. Click 'Open' and wait while the recordings are processed. The time this takes will vary depending on the size and number of recordings. A simple size and concentration measurement will take approximately a minute.

6.2 Data Checking

Data can be reviewed in the IDS2 software (Figure 20) as you go, to check it is suitable for analysis and identify any recordings that need repeating.

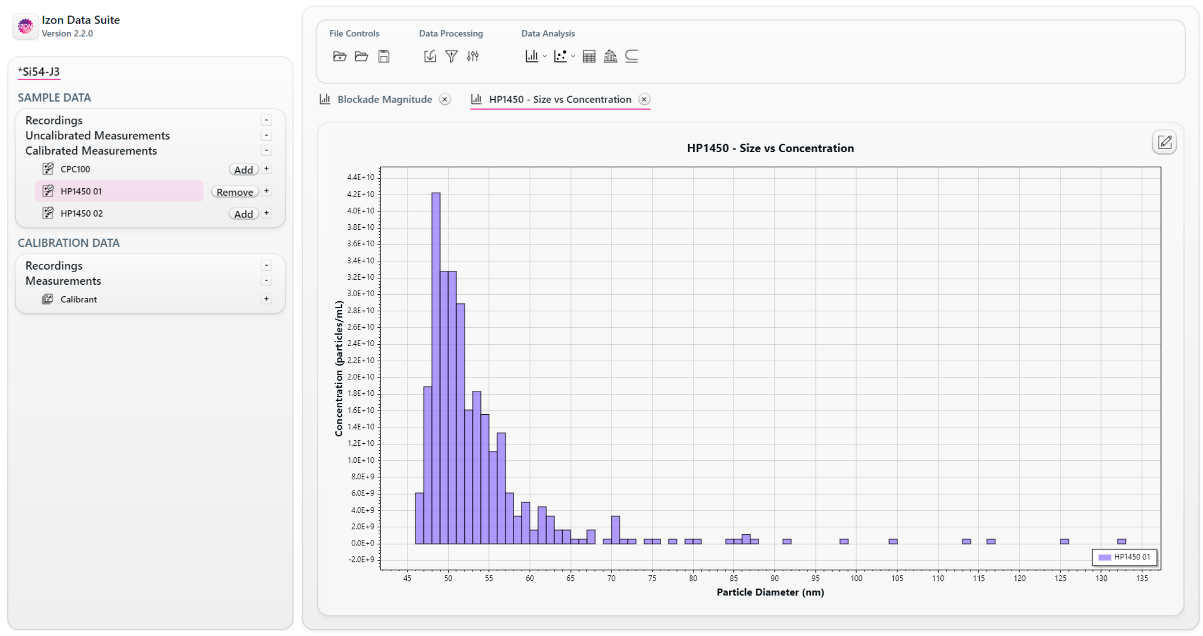

Figure 20. Key features in IDS2.

1. Select a recording.
2. Click on the scatter plot icon under Data Analysis and choose 'Blockade Count vs Blockade Start Time Scatter Plot'.
3. Use the 'Add' buttons in the Recordings list to plot all the recordings for a measurement group.
4. Check that the particle counts are linear over time, do not overlap and that the higher pressures and larger magnitude voltages have a higher particle rate, as shown in Figure 21.

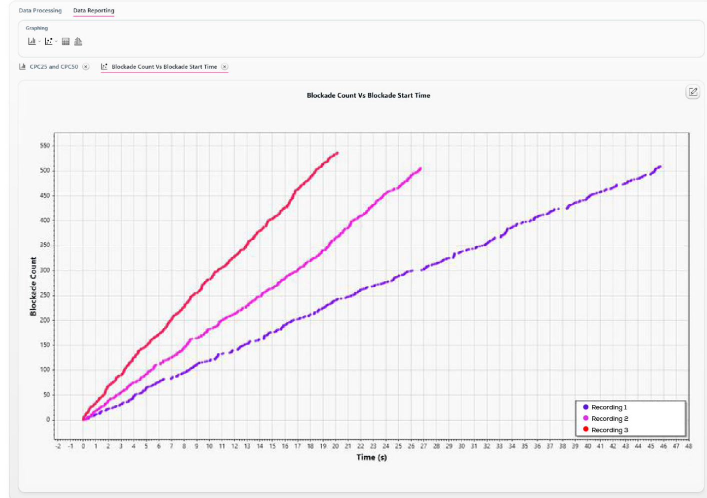

Figure 21. A good particle rate plot for a size and concentration measurement group, showing particle rate (gradient) increasing with pressure.

5. Repeat this check for each measurement group.
6. If any rate lines overlap or are in the wrong order, the recordings will need to be repeated (Figure 22).

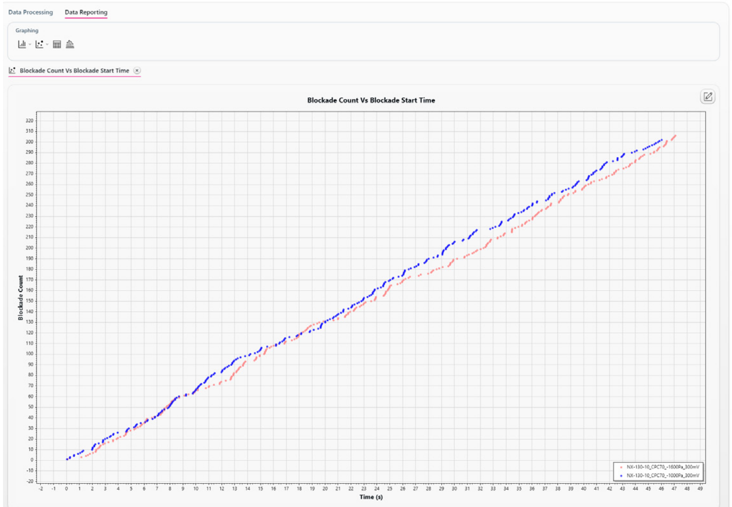

Figure 22. An unacceptable particle rate plot.

6.3 Calibrating Measurements

Once a suitable set of recordings has been collected, the sample(s) of interest can be calibrated. During this step the software uses the known characteristics of the calibration sample to calculate the properties of the test sample.

1. Click on the 'Calibrate Measurement' icon in the Data Processing menu bar.
2. Select the type of calibration to perform – either size and zeta or size and concentration.
3. Enter a name for the calibrated measurement.
4. When creating a calibration measurement group for size and zeta analysis you will have to enter the zeta potential of your calibration particle in your measurement electrolyte. Values for Izon calibration particles in Izon's Measurement Electrolyte are printed on the vial.
5. Click on the names of the sample and calibration recordings you wish to include. You must ensure that recordings are suitable based on the instructions for generating data in Section 5.9: Sample Measurement and the data quality control described in Section 6.2: Data Checking.

Select the recording(s) for only one sample per calibrated measurement. If you have measured more than one sample, repeat this process for each sample individually – selecting one sample recording and its corresponding calibration recording(s) each time.

6. When creating a calibration measurement group for size and zeta analysis you will have to enter the zeta potential of the chip, unless you are using Izon's ME and entered the electrolyte name into the Pulsoid Control Software exactly as specified in Table 6. If you used ME but it is being detected by IDS2 as a custom electrolyte, you can edit the recording to update the electrolyte. Alternatively you can enter a Nanopore Zeta value of -11 mV when creating your calibration measurement.

7. Once all required fields are completed and both sample and calibration recordings are selected, click 'Check Selection'. Warning symbols will appear if there are problems with any of the selected recordings, or with the combination of recordings. Hover over each warning symbol to see a list of the issue(s) that triggered the warning. Orange warnings will allow you to proceed with calibrating the measurement. Red warnings will prevent you from calibrating the measurement until they are resolved. Change your selection and re-check if needed.
8. Click 'Confirm' to create the calibated measurement. It will appear in the Sample Data section of left hand side menu bar. Summary statistics, blockade data and various graphs are available under Data Analysis.
9. Repeat for any additional samples. Once you have created one calibrated measurement, the group of calibration recordings will be available as a measurement group that can be selected instead of the individual recordings.

6.4 Data Export

1. Calibrated datasets can be exported in CSV format by clicking into the Blockade Summary Data table in the Data Reporting tab and clicking 'Export CSV' in the bottom right corner.
2. Graphs can be exported by right clicking in the graph window and selecting 'Save Image'.

6.5 Closing the Software

Once you have finished analysing your project you can either close the software or start a new analysis. If you do wish to start work on a different project, we recommend closing all plots and tables for the current project before opening or creating the next project. Otherwise follow the steps below to save your work and exit IDS2.

1. If you have unsaved changes, click the 'Save Project' icon in the File Controls section of the menu bar (Figure 23).

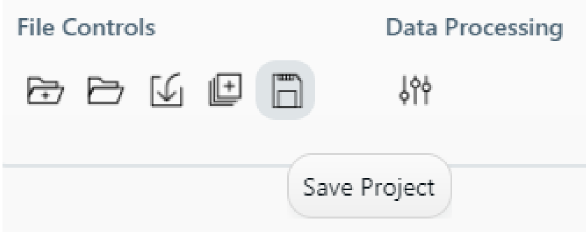

Figure 23. Save Project icon in IDS2.

2. Click the cross in the top right hand corner to close the software. If you forget to save first, you will be prompted to do so before the software exits.

7 MAINTENANCE AND TROUBLESHOOTING

7.1 Maintenance

Device Cleaning

Ensure the instrument is switched off and unplugged at the wall prior to cleaning.

Any splashes on the exterior surfaces of the Pulsoid should be wiped off immediately with a dry paper towel.

If decontamination is required, the instrument and disassembled fluid cell may be exposed to UV light for the minimum time necessary to destroy biologicals.

Ensure that no moisture gets on to the electrical connections or the power button during operation or cleaning.

Fluid Cell Maintenance

Before each use check the O-rings for any visible signs of distortion or damage. Spare O-rings are provided and can be used as needed. For any other issues, or for replacement fluid cells, please contact Izon Support.

Repair and Servicing

There are no user-serviceable parts in the Pulsoid. Please contact Izon Support to return equipment to Izon for service and repair.

Firmware updates

New firmware versions will be made available through the Izon Support site.

7.2 Troubleshooting

OBSERVATION	FAULT	SOLUTION
Recording will not start	Recording Settings fields incomplete or contain invalid characters	Fill out the required Recording Settings fields (outlined in red) and remove any invalid characters (e.g., \ / : * ? " < > | or trailing whitespace).
Pressure not holding at set point	APS leaking	Power cycle the unit. If the issue persists, contact Izon Support.

OBSERVATION	FAULT	SOLUTION
No response when trying to set pressure	APS not working	Power cycle the unit and check the APS status shows as calibrating when it turns on. This should take approximately 70 seconds. If the status does not update to calibrating, there is no noise from the APS while it is calibrating, or calibration lasts longer than expected, contact Izon Support.
Railed current	Broken chip Short circuit	Replace the chip. Check the fluid cell contacts and pogo pins for signs of corrosion. Check the O-rings in the fluid cell are fully sealing and no liquid is seeping out. This requires disassembly of the fluid cell. If there is still railed current when reassembled, the chip may be damaged. Try a new chip and if the issue persists with the new chip, contact Izon Support.
Step change decreases in baseline current, unstable baseline	Blocked pore	Use the orange suction cup to cover the channel on the buffer side of the fluid cell. Press down gently then pull up sharply. Use the syringe to apply pressure to the buffer side. Pull the plunger up, apply the sealing cup to the buffer side, and push the plunger in. Sometimes a pumping motion can be beneficial. Use the syringe to apply a vacuum to the buffer side. To do this, depress the plunger all the way in, apply the sealing cup to the channel on the buffer side, and pull the plunger up. Remove the fluid cell from the fitting and tap hard on a padded surface at an angle such that the fluid channel is at a 45 degree angle from horizontal with the cavity side of the chip facing slightly upwards. Replace the chip.
Baseline current stable but much higher or lower than expected	Chip or fluid cell issue	Follow the solution given above for railed current. If the issue persists, contact Izon Support.

OBSERVATION	FAULT	SOLUTION
Blockades present when using blank measurement electrolyte	Previous sample not fully removed from fluid cell	Remove all fluid from both sides of the fluid cell, tapping it gently to collect any droplets that are stuck at the edges. Replace with fresh measurement electrolyte, making up a new stock if contamination of the old stock is suspected.
	Measurement electrolyte contaminated with particles	Ensure the consumables you use to make up stocks are free from particulates.
	Contaminated O-ring	Rinse the O-ring with 70% ethanol and let it dry. Replace the O-ring if necessary.
Symmetrical and/or regularly spaced noise in the baseline	Electrical noise	Remove the fluid cell and put it back on. Check there are no sources of interference, such as mobile phones, nearby. Check there are no sources of vibrations, such as centrifuges, on the bench. If the issue persists, contact Izon Support.
Lid is closed but PCS displays 'Lid Open' warning	Lid Open sensor not working	Check the magnet is in place underneath the lid on the back right by the hinge. If the issue persists, contact Izon Support.
Software doesn't launch but displays a 'No device detected' error message	Connection issue	Ensure that all cables are securely plugged in and then power on the instrument before launching the software. Power cycle the unit if the connection issues persist.
Instrument shows as Disconnected in PCS interface	Connection issue	Check the USB cable is securely plugged in and the instrument is powered on. Power cycle the unit if the connection issues persist.
Live Trace flashing	Software issue	Close and reopen software.
Live Trace window shows solid pink	Software issue	Change a value in one of the fields under Device Controls, click Apply, then revert the value back to desired settings.
Error during firmware update	Update failure	Power cycle the unit and it will revert to the previous firmware version.

How to Power Cycle the Pulsoid

1. Press and hold the power button until the lights turn off.
2. Wait 10 seconds.
3. Press and hold the power button until the lights turn back on.

8 CONTACT US

Additional support material is available at support.izon.com.

If you have any questions that are not answered on the support portal, or your instrument requires repairs/maintenance, please contact our support staff via the online support portal by raising a support ticket or by emailing support@izon.com.

When reporting Pulsoid issues to Izon support, please provide the serial number of the instrument, which can be found on the rear, as shown in Figure 24.

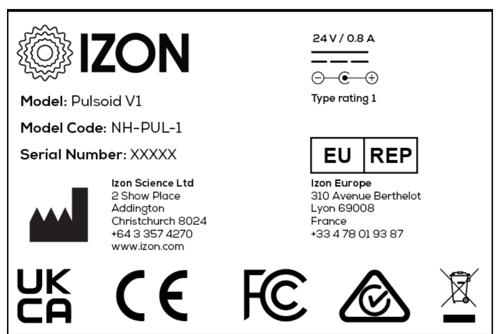

Figure 24. The Pulsoid Information label.

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