PULSOID BETA

USER MANUAL

IZON

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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

	This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations.
	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.
	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.
Pressure Application Device (PAD)	A small suction cup used to clear blockages.
Pulsoid Control Suite (PCS)	Software used to operate the Pulsoid.
Pulsoid Data Suite Beta (PDSB)	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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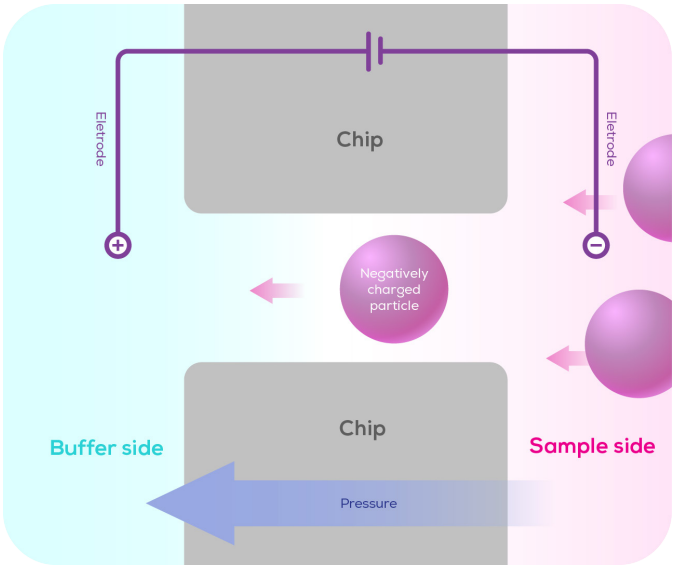

Figure 1. Overview of the how the Pulsoid works for negatively charged particles. For positively charged and neutral particles the polarity is reversed using the Pulsoid Control Software.

3.2 Intended Use

The Pusloid is used to measure size, concentration, and zeta potential of nanoparticles. The instrument is intended for Research Use Only. The Pulsoid 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 cGLPs are followed to ensure reliable analyses.

3.3 Pulsoid Workflow

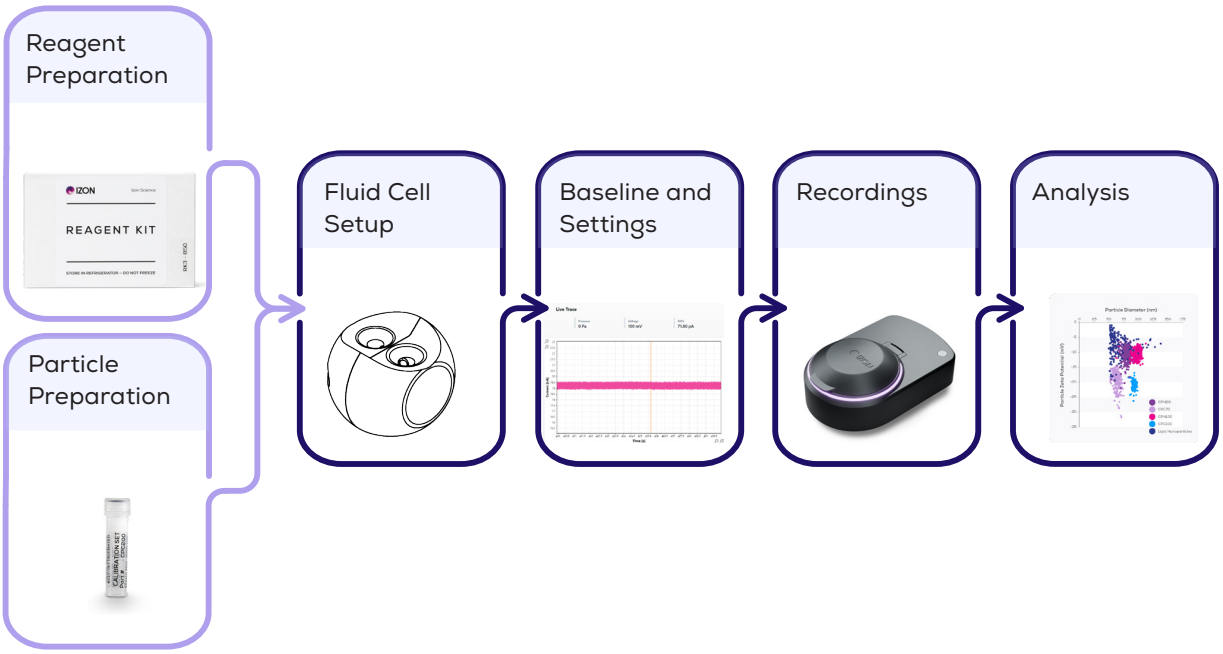

Figure 2. Overview of the Pulsoid workflow.

4 ASSEMBLY AND SETUP INSTRUCTIONS

4.1 System Components

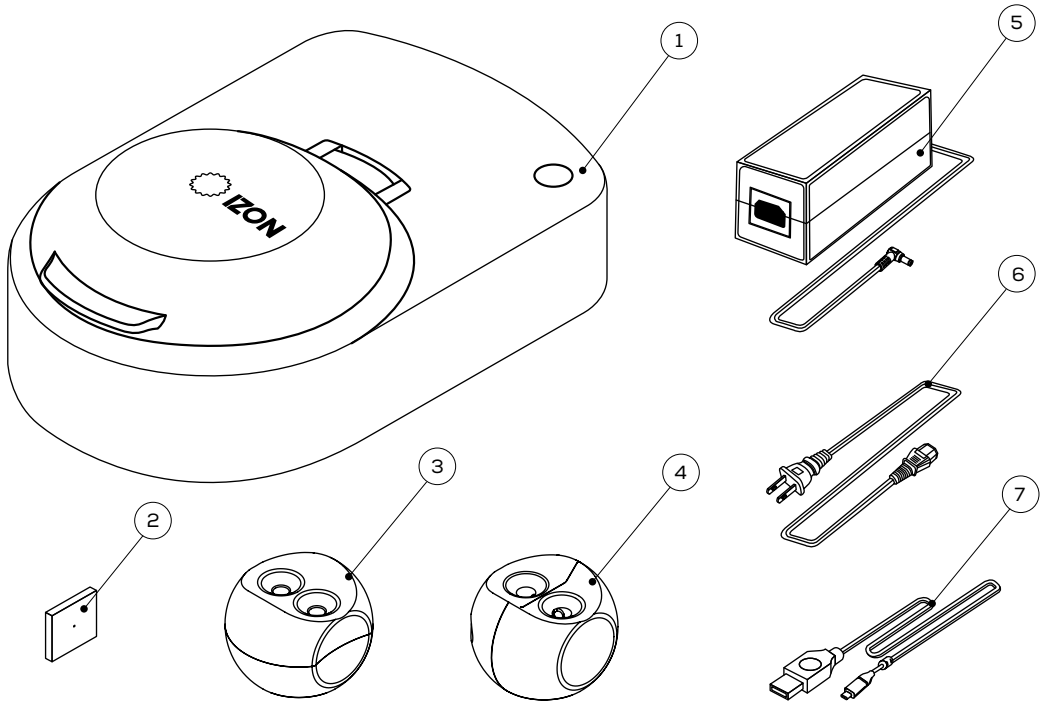

Figure 3. The Pulsoid and accompanying components.

The following components and quantities are provided:

1	Pulsoid Instrument	5	24 V Power Supply
2	SC80 Chip (x20)*	6	Power Lead
3	Reference Cell	7	USB-A to USB-C 3.0 Cable
4	Fluid Cell (x2)		

*On arrival chips should be stored at 18-28 °C and 20-30% humidity until used.

The following components are required for operation and/or maintenance of the Pulsoid and are provided in the NPS Training Kit supplied with the Pulsoid:

- 1x CPC100 vial
- 1x CPN80 vial
- 1x Pressure application device (PAD)
- 2x Spare fluid cell screws (M3x10 cap hex)
- 4x Spare fluid cell O-rings (3x1)
- 1x Allen key (2.5 mm)
- 1x Dual force syringe
- 3x 10 mL syringes
- 10 x 1.5 mL Axygen tubes
- 4 x 15 mL tubes
- 1x NPS Reagent Kit

Additional Training Kits can be purchased from store.izon.com.

The following components are required for operation and/or maintenance of the Pulsoid and are provided in the NPS Reagent Kit:

- 4x PBS tablets
- 1x Wetting solution concentrate
- 20x Syringe filters (13 mm x 0.22 µm)

Additional 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
- Pointed tweezers
- 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
- **Pulsoid Data Suite Beta (PDSB):** 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-pulsoid-data-suite-beta

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 shows as 'Connected' in the top left corner of the PCS (Figure 4).

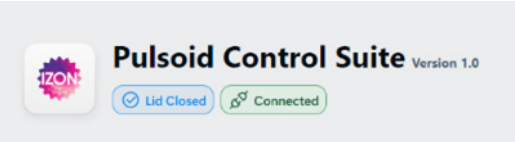

Figure 4. Instrument status reporting in the PCS.

The Pulsoid is now successfully installed.

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.

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.

1. Homogenise the calibration particle stock by vortexing for 10 seconds.
2. For CPC100s add 1 µL calibration particles to 999 µL Measurement Electrolyte in a particle-free 1.5 mL tube and vortex to mix.
3. For CPN80s add 1 µL calibration particles to 299 µL Measurement Electrolyte in a particle-free 1.5 mL tube and 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 Measurement Electrolyte. The target concentration range is $2.5x10^9$ to $2.5x10^{11}$.
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.

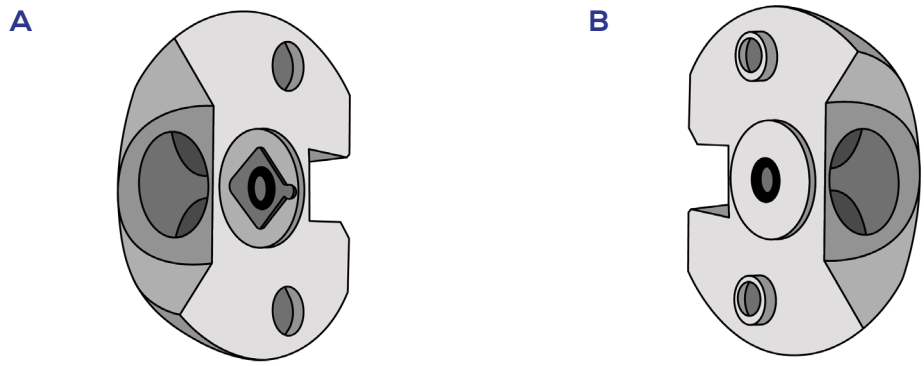

Figure 5. Fluid cell halves. A) Side with square depression for chip; B) Side that fits over the chip once it is in place.

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.

CAUTION Avoid squeezing the chip too tightly as it may crack.

CAUTION 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.

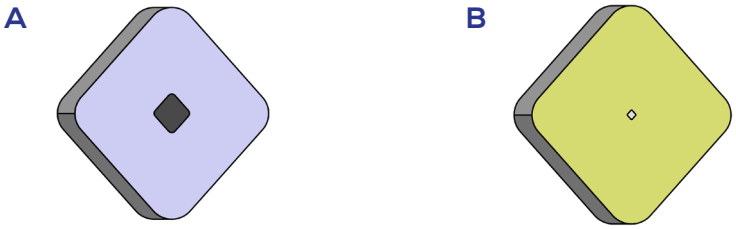

Figure 6. Chip faces. A) Cavity side; B) Aperture side.

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.

CAUTION 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).

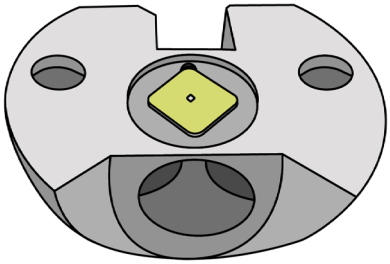

Figure 7. Fluid cell with chip in place.

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.

CAUTION 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.

CAUTION 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.

CAUTION 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.

CAUTION 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).

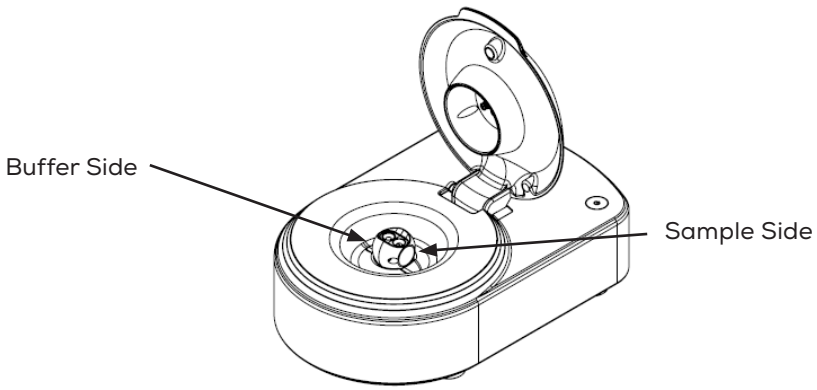

Figure 8. Sample and buffer sides of the fluid cell.

2. Set your pipette to 75 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), tilt the fluid cell 45° so the bubble is pointing up towards the sample entry channel and tap the fluid cell gently on a padded surface, such as a lab bench covered with several layers of paper towel, until the bubble detaches and reaches the surface of the chamber. It can then be removed with a pipette tip.

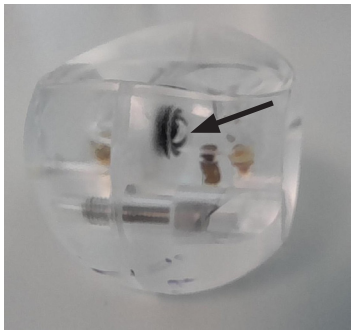

Figure 9. Bubble inside fluid cell assembly.

CAUTION Do not hit the fluid cell too hard, or directly onto a hard surface, as you may damage the fluid cell and/or spill the measurement electrolyte.

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.

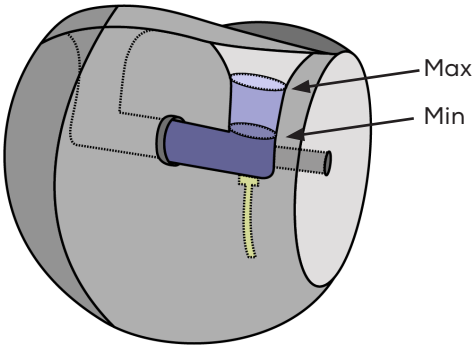

Figure 10. Minimum and maximum fill levels.

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.

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

CAUTION

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).

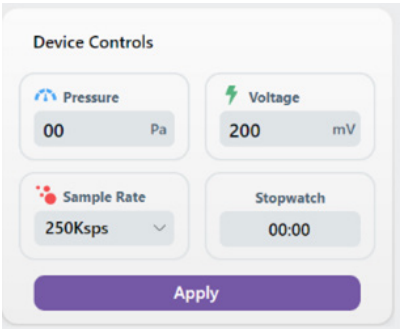

Figure 11. Device Controls panel.

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.
Stopwatch	Displays the duration of the active recording.

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.

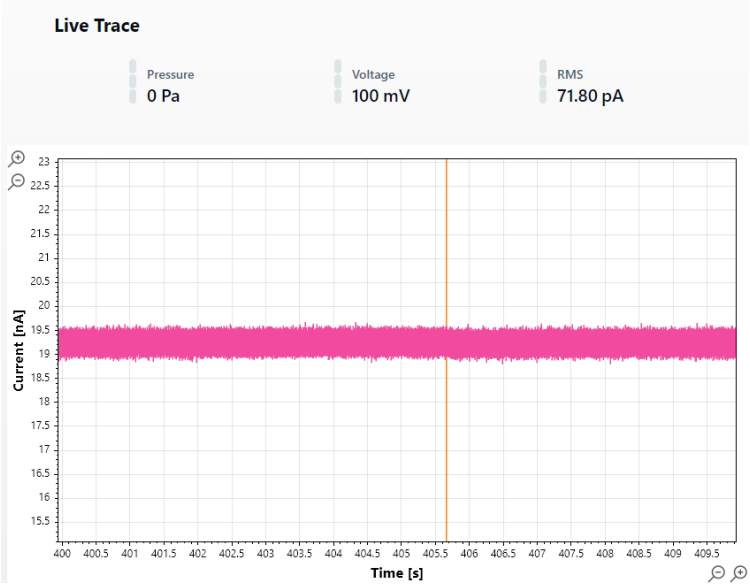

Figure 12. Live Trace window.

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 with the cavity side of the chip facing slightly upwards.

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).

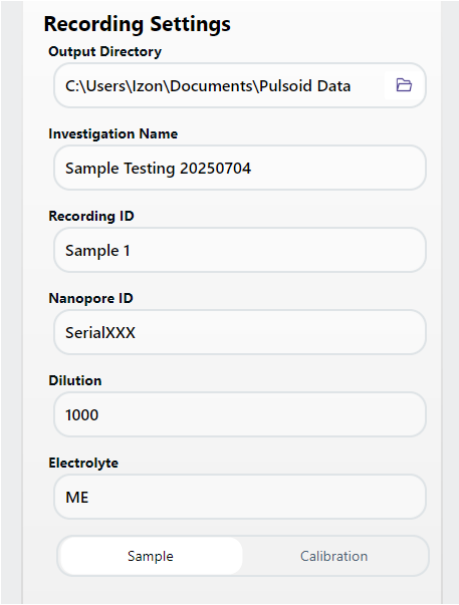

Figure 13. Recordings Settings panel.

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.

CAUTION 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.
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 and will be displayed in the Sample ID field in the PDSB analysis software.
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.
Buffer	The measurement electrolyte being used.

FIELD	VALUE
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 concentration analysis requires recordings at more than one pressure.

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 200-1500 particles/minute for all recordings. You may need to optimise your sample dilution in order to achieve this.
- 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.

Size and Concentration Measurement

Calculating concentration requires a pair of recordings at two different pressures. The same pair of pressures must be used for both the sample of interest and the calibration particles. The smallest possible measurement group if you wish to measure concentration is therefore four recordings—two calibration particle recordings at the same voltage but different pressures, and two sample recordings at the same system settings as those used for calibration.

The recommended calibration particles for size and concentration measurements are CPN80s.

1. Load your particles and set the voltage according to Table 7.

Table 7: Setup Requirements by Particle Type

SAMPLE	RUN ORDER	VOLTAGE
Negatively-charged particles (≤ -5 mV)	Sample first	200 mV
Neutral or positively-charged particles (> -5 mV)	Calibration first	-200 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 14)

Figure 14. 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).

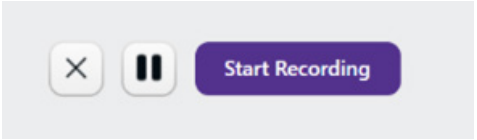

Figure 15. Recording controls.

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 16).

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.

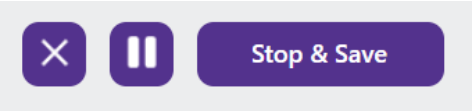

Figure 16. Buttons to cancel, pause or finish the recording.

10. When you have recorded the required number of blockades, click 'Stop & Save' (Figure 16).

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

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.

You may wish to update the Sample ID field between recordings to include the pressure value as part of the Sample ID. This allows you to distinguish between recordings when they are opened in PDSB.

12. Change the particles in the fluid cell to whichever of sample of interest/calibration you are running second.
13. Make two recordings at the same two pressures as used in steps 7 and 11 above.
14. We recommend that you check your data quality in PDSB as you go and repeat any recordings that fail data quality checks (Section 6: Data Analysis).

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.

1. Load your sample particles and set the voltage to 200 mV for negatively-charged particles (≤ -5 mV) and -200 mV for neutral or positively-charged particles (> -5 mV).
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 and record 300 blockades at each of 100 mV, 150 mV, and 200 mV.
7. Change the pressure to -300 Pa and the voltage to 200 mV and record 300 blockades.

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.
5. If you wish to keep the chip, gently blot it dry, taking care not to rub the area around the pore on the aperture side. Store in a clean 1.5 mL tube submerged in DI water.
6. Dry the faces of the fluid cell halves with a lint-free tissue.
7. You may wish to reassemble the fluid cell without the chip before storing so as not to lose the small parts.
8. Close the PCS.
9. We recommend switching the instrument off when not in use. Press and hold the power button until the instrument shuts down.

6 DATA ANALYSIS

6.1 Data Checking

Size and concentration data can be checked and analysed in the PDSB software (Figure 17).

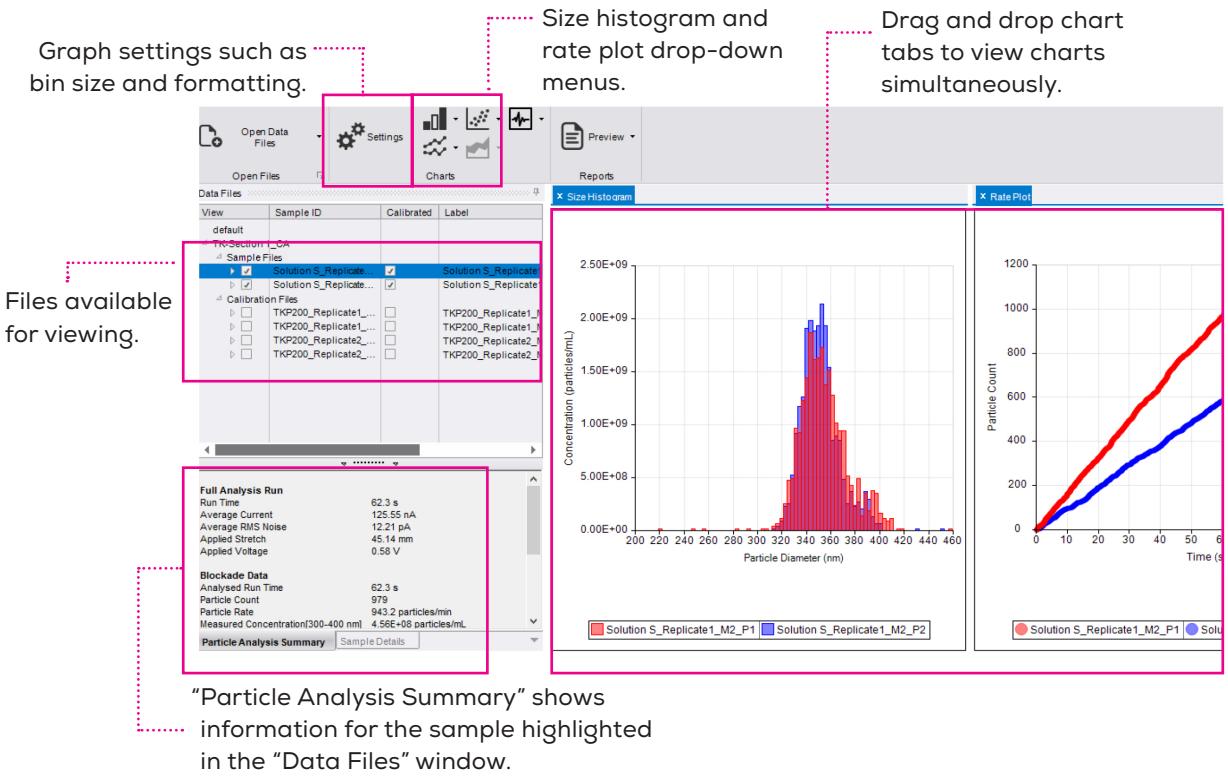

Figure 17. Key features in PDSB.

1. Launch PDSB and select the 'Open Data Files' button in the top left corner. Navigate to the filepath and folder name that you specified in the Recording Settings for the run you wish to analyse. Select the file(s) you wish to load and click 'Open'.
2. The files will initially load as unprocessed data. Right click on the heading 'Unprocessed Data' and click 'Process'.
3. For multi-pressure recordings, ensure the checkbox in the 'View' column is checked for each pressure and open the Particle Rate Plot (Figure 18).

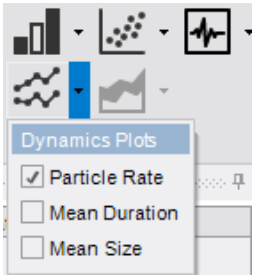

Figure 18. Drop-down menu containing Particle Rate Plot.

4. Check that the particle counts are linear over time, do not overlap and that the higher pressures have a higher particle rate, as shown in Figure 19.

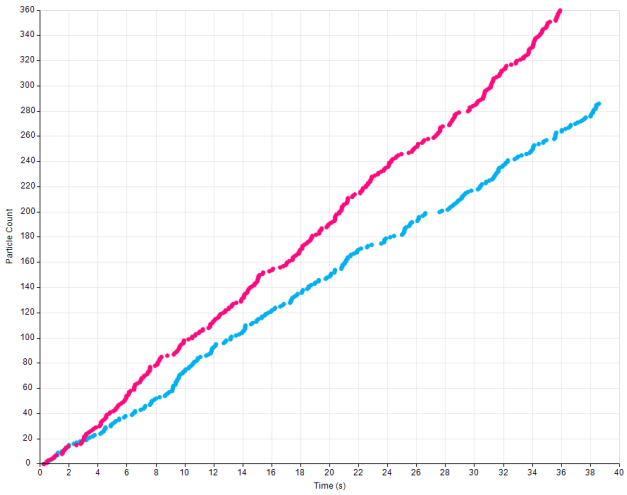

Figure 19. An acceptable particle rate plot. The pink line shows the rate at the higher pressure, while the blue line shows the rate at the lower pressure.

5. Repeat this check for the calibration recordings.
6. If any rate lines overlap or are in the wrong order, the recordings will need to be repeated (Figure 20).

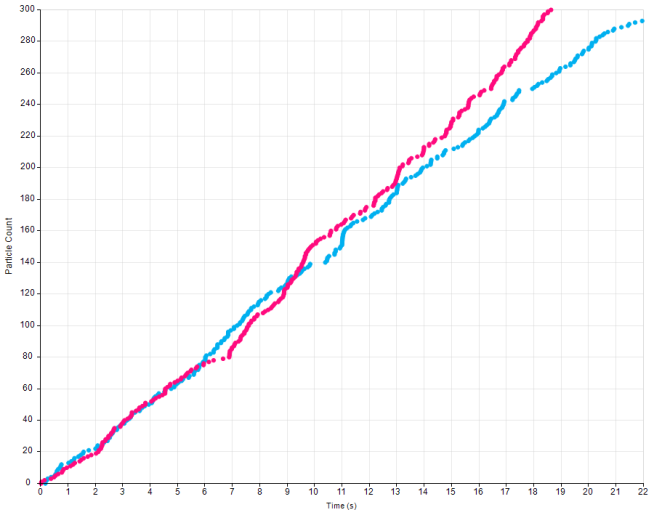

Figure 20. An unacceptable particle rate plot, with no clear separation of rates between different pressures.

6.2 Calibration

1. Once you are satisfied with your sample and calibration recording(s), check the calibration box in the 'Calibrated' column to the right of any sample file to open the calibration window.

In the calibration window there may be multiple types of calibration options available, depending on which files are present. For example, if you recorded a multi-pressure calibration you will have the option to perform a multi-pressure calibration or a single-pressure calibration.

2. Select which sample files to calibrate, calibration files with matching parameters will be displayed automatically below.
3. For each sample file select one calibration file with matching parameters. If only one is available in the dataset, it will be automatically selected.
4. Click 'OK'. The sample size histograms will now display in nm instead of nA.

6.3 Data Export and Reporting

1. Analysed data can be exported as a PDF report using the Reports options in the top menu bar.
2. Datasets can be exported in CSV format by clicking on the Save Data Table icon in the Blockade Summary table in the bottom left of the IDS window.
3. If you wish to calculate zeta potential, you will need to export the blockade duration datasets for each zeta calibration recording plus the sample recording(s) you wish to analyse. To do this, right click on each file name and click 'Export Blockade Duration Data Button', as shown in Figure 21.

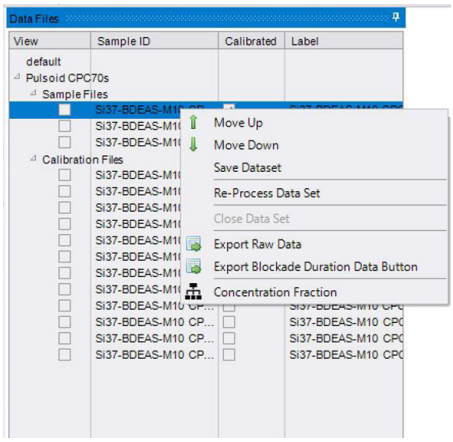

Figure 21. Menu and export option for blockade duration data.

Separate instructions on how to calculate zeta potential for the Pulsoid Beta are available from Izon Support.

More information on PDSB can be found at support.izon.com/data-analysis, as it has the same functionality as the Izon Data Suite (IDS).

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.

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.
No response when trying to set pressure	APS not working	Power cycle the unit and listen for the APS calibrating when it turns on. If there is no noise, or the noise lasts longer than 1 minute, contact Izon Support.

OBSERVATION	FAULT	SOLUTION
Railed current	Short circuit	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.
Blockades present when using blank measurement electrolyte	Previous sample not fully removed from fluid cell Measurement electrolyte contaminated with particles Contaminated O-ring	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. Ensure the consumables you use to make up stocks are free from particulates. Replace the O-ring if it is dirty.

OBSERVATION	FAULT	SOLUTION
Symmetrical and/or regularly spaced noise in the baseline	Electrical noise	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 and then reset the Sample Rate

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 22.

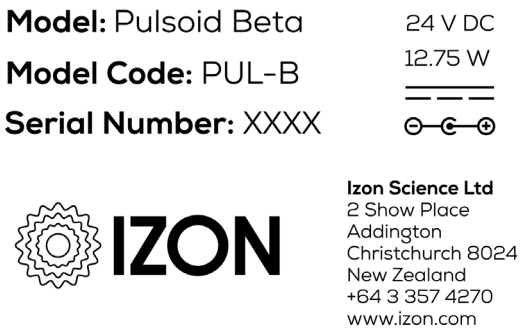

Figure 22. The Pulsoid Information label.


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