

THE EXOID TRAINING PRACTICAL MODULE

SIZE AND CONCENTRATION



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GETTING STARTED

Before starting this practical module, make sure you have:

- ▶ Read through and understood the Fundamentals of Tunable Resistive Pulse Sensing (TRPS) Theory Manual, The Exoid User Manual and Reagent Kit Technical Note.
- ▶ Set up a computer with the correct specifications and installed the Exoid Control Suite (ECS) and Izon Data Suite (IDS).
- ▶ Signed up to academy.izon.com and registered for the Exoid Beginner Training course. This manual is designed to be used as the practical component of the online course.

There are two sample types run in this practical module:

1. Monomodal: Uniform, simple sample.
2. Bimodal: A more complex sample comprised of two populations.

Recording vs Measurement

In order to understand this document properly it is important to know these two key definitions:

- ▶ A recording is defined as an individual data recording.
- ▶ A measurement is a collection of multiple recordings. A complete size and concentration measurement includes both sample and calibration recordings.

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1 / OVERVIEW

Required Materials

In addition to the Exoid and the control computer, the following materials are required to complete this practical module. Some of these are provided in the TRPS Training Kit that can be requested with the instrument, indicated by an asterisk (*).

- ▶ Calibrated micropipettes: 1 μ L to 1 mL
- ▶ Pipette tips
- ▶ Lint-free tissues for drying
- ▶ Powder-free disposable gloves
- ▶ NP250 Nanopore*
- ▶ 15 mL tubes*
- ▶ 10 mL luer lock syringes*
- ▶ 1.5 mL tubes*
- ▶ Freshly prepared Wetting Solution
- ▶ Freshly prepared Measurement Electrolyte
- ▶ Freshly prepared DI water
- ▶ TKP200 stock particles*
- ▶ Solution S stock particles*

Table 1: Overview of practical module

Reagent and Sample Preparation	<ul style="list-style-type: none"> - Prepare solutions in accordance with instructions provided in the TRPS Reagent Kit. - Dilute sample and calibration particles.
Nanopore Setup	<ul style="list-style-type: none"> - Wet the nanopore. - Establish a stable baseline. - Characterise nanopore.
Monomodal Data Recording	<ul style="list-style-type: none"> - Optimise stretch, voltage and pressure using calibration particles. - Record calibration. - Record sample.
Monomodal Data Check	<p>Check data:</p> <ul style="list-style-type: none"> - Assess particle rate. - Assess size histogram.
Bimodal Data Recording	<ul style="list-style-type: none"> - Optimise stretch, voltage and pressure using calibration particles. - Record calibration. - Record sample.
Bimodal Data Check	<p>Check data:</p> <ul style="list-style-type: none"> - Assess particle rate. - Assess size histogram.
Data Analysis	<ul style="list-style-type: none"> - Calibrate Data. - Apply filters as necessary. - Assess data for: <ul style="list-style-type: none"> - Size. - Concentration.
Shut Down System	<ul style="list-style-type: none"> - Clean the nanopore. - Clean the fluid cell.
Izon Academy	<ul style="list-style-type: none"> - Package the files into a ZIP folder. - Upload to the Izon Academy. - Complete Exoid Beginner Exam.

2 / PRACTICAL

Prepare the System

Load 75 μL of Measurement Electrolyte (ME) onto the lower fluid cell channel and leave for ~15 minutes while completing subsequent preparation steps. This is to wet the silver/silver chloride (Ag/AgCl) electrode and reduce the formation of bubbles, which can become trapped underneath the loaded nanopore.

Connect the power and USB cable and switch the unit on before opening the Exoid Control Suite (ECS).

Pipetting Techniques

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

Prepare Reagents

Prepare reagents (excluding Coating Solution) following the protocol in the Reagent Kit Technical Note. Coating Solution is only required for biological particles, therefore it is not required for this training module.

Sample Preparation

Prepare Solution S (Sample) and TKP200 (Calibration) at a dilution factor of 51 by adding 20 μL of the particles into 1000 μL of ME in a 1.5 mL centrifuge tube. To prepare a bimodal sample, take 200 μL of each of your diluted TKP200 and Solution S and mix them together in a clean 1.5 mL centrifuge tube. Remember to label your tubes accordingly.

Investigation Details

Before you can begin, the Investigation Details tab needs to be filled out. Populate all three fields appropriately; the Investigation ID should be something distinctive that you can identify later as this will be the name of the folder the data saves in. Alternatively, you can upload a previously saved nanopore calibration file. This can be useful if for any reason you need to restart the software after nanopore setup has been completed.

2.1 Nanopore Setup

This process guides the user through all of the appropriate procedures required to prepare the nanopore for sample analysis, including loading, wetting, coating (if applicable) and characterisation. Nanopore setup is recommended to ensure the nanopore is set up correctly for training; if this process is not completed the user will be asked to confirm their nanopore is set up correctly before proceeding with sample analysis. Upon completion, a nanopore calibration file will be saved to the computer. If the software is closed between measurements (using the same nanopore) the relevant file can be loaded later in the Investigation Details tab.

Nanopore Loading

The Exoid will move to the loading position of 41 mm stretch and ask for the nanopore to be loaded. A correctly loaded nanopore has the serial number facing upwards and all the arms fitted snugly onto the teeth of the stretcher unit.

Nanopore Wetting

The wetting process replaces the air trapped inside the nanopore with the surrounding electrolyte solution to bridge the upper and lower electrodes. Wetting Solution is used for this step as it contains a higher percentage of surfactant to aid in wetting the nanopore.

Following the on-screen instructions, the baseline current should increase from 0 nA to at least 7 nA, indicating the air in the nanopore has been displaced and current is now flowing. To check that the air has been fully displaced first increase the voltage to achieve a current of 100-140 nA, and check if the baseline is stable. When the baseline appears stable (see definition below), tap the top of the pressure nozzle with the back of the PAD gently. If the baseline current increases by >0.1 nA when hit, keep gently tapping until the current is consistent.



A stable baseline current is one that is 100-140 nA, drifts by less than 0.5 nA in 60 seconds and has an RMS noise of <15 pA. An unstable baseline current will produce unreliable measurements and should not be used under any circumstances.

If you are having difficulty establishing a stable baseline, follow the steps below to achieve a baseline that meets the criteria. It is not necessary to continue on to subsequent steps once the baseline is stabilised.

Table 2: Establishing a stable baseline

Apply a Positive Pressure	<ul style="list-style-type: none">- Apply a positive pressure of 2500 Pa and apply a sharp impact by tapping the top of the pressure nozzle firmly using the back of the PAD.
Apply a Negative Pressure	<ul style="list-style-type: none">- Apply a negative pressure of -2500 Pa and apply a sharp impact by tapping the top of the pressure nozzle firmly using the back of the PAD.
Stretch and Positive Pressure	<ul style="list-style-type: none">- Stretch the nanopore up to 49 mm and apply a full positive pressure of 2500 Pa.- Apply a sharp impact by tapping the top of the pressure nozzle firmly using the back of the PAD.
Stretch and Negative Pressure	<ul style="list-style-type: none">- Leave the nanopore at 49 mm and apply a full negative pressure of -2500 Pa.- Apply a sharp impact by tapping the top of the pressure nozzle firmly using the back of the PAD.
Apply the PAD	<ul style="list-style-type: none">- Set the pressure to 0 Pa and remove the pressure nozzle.- Using the plunging end of the PAD, apply pulses of pressure to the upper fluid cell.
Re-setup the System	<ul style="list-style-type: none">- Set the pressure to 0 Pa and remove the pressure nozzle.- Reduce the stretch and remove the nanopore.- Dry the nanopore, fluid cell and pressure nozzle and re-assemble the system.

Once a stable baseline current has been established, select **OK** and the software will soak the nanopore at 100 mV using 2500 Pa pressure to check the stability of the current.

At the end of the nanopore wetting step, the software will ask if your sample is of a biological nature to determine if coating needs to be completed; select **No** to skip coating for the purposes of this training.

Nanopore Characterisation and Calibration



If the baseline current de-stabilises at any point during characterisation and calibration, pause the process and perform troubleshooting steps to re-establish a stable baseline before resuming. Note that some level of change in the current is acceptable while the stretch or voltage is being altered, however the baseline should be stable between these times.

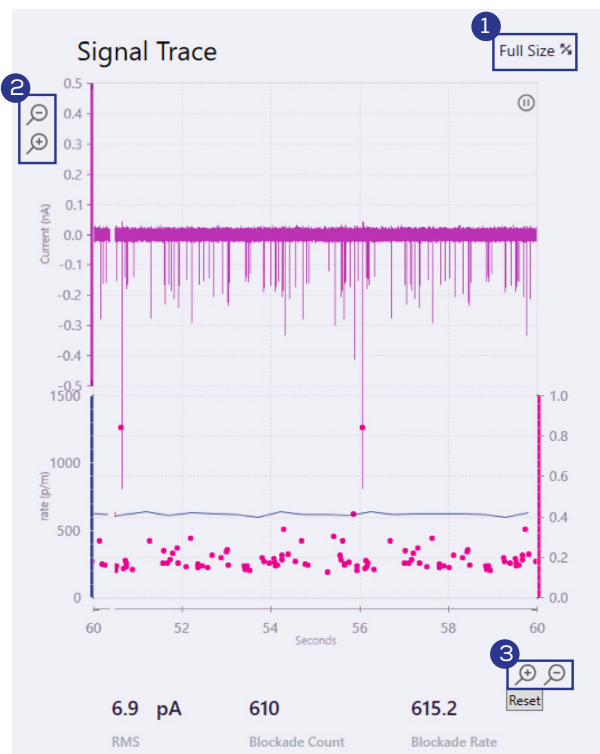
After completing wetting (or coating), the software will evaluate the nanopore by fitting an analysis of the current at different stretches and voltages to a pre-determined model. Following successful characterisation, the software will ask for diluted calibration particles to be loaded into the upper fluid cell. For training, the diluted TKP200 calibration particles are used for this step. Once the calibration particles have been measured by the system, nanopore setup is complete and the ECS will output a display message announcing the resolvable particle range with that particular nanopore. If this range does not cover 200 and 350 nm then set up a new nanopore as it will not resolve the training particles.

2.2 The Signal Trace

The signal trace is where the user can observe the baseline current and any particle blockades, as well as real-time values for RMS noise, blockade count and particle rate.

The user can adjust the signal trace baseline zoom at any stage using the controls in the top left-hand corner of the signal trace window, while the time period zoom can be changed on the bottom right. The user can also expand (or collapse if already expanded) the signal trace window by pressing the symbol in the top right.

The baseline should be monitored closely; if stepping down, drifting, or noise is observed then the recording should first be paused and recovered, or cancelled and restarted if recovery is not possible. The baseline current should not differ by more than 10% between data recordings. The particle rate also requires monitoring and should have a particle rate between 500-1500 particles per minute. The rate should also remain constant over the course of a recording.



1. Expand the signal trace window here.
2. Y-axis zoom allows for magnification of the baseline. If you are too zoomed in, it will be difficult to distinguish blockades from the noise. If you are too zoomed out, it will be difficult to visualise blockades and baseline instabilities.
3. X-axis zoom changes the time period displayed over the course of the plot.

Figure 1: A screen capture from the Exoid Control Suite, showing the signal trace and some interactable components available to the user.

2.3 Monomodal Size and Concentration Analysis

Select Analysis Type

Select **Start Analysis** and then **Size and Concentration**. The baseline current established earlier during nanopore setup should be visible. Enter the sample details, using the batch ID of Solution S as the sample name, making sure that the dilution factor entered is correct before proceeding.

Use the slider bar to select a stretch that encapsulates 200 nm and 350 nm particles. Select **Yes** for "Would you like to run a calibration at these settings?" Enter the calibration details using the batch ID of TKP200 in the Calibration ID, making sure the size, raw concentration and dilution factor are entered correctly before proceeding. Select **Yes** for "Does the upper fluid cell contain calibration particles?".

Precalibration System Optimisation

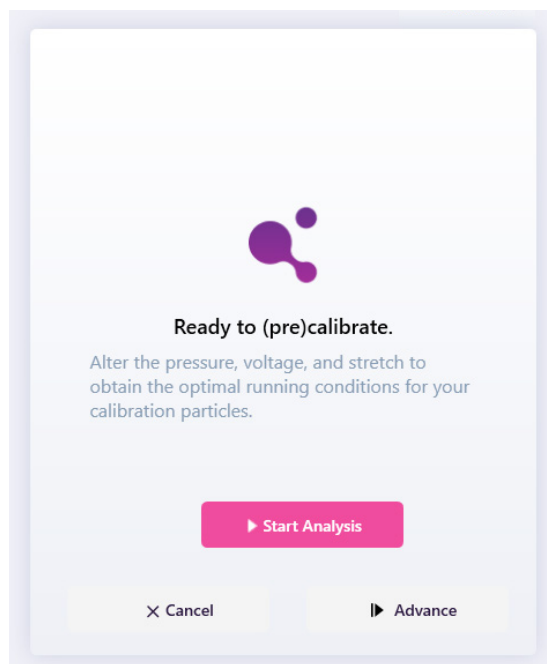
After the system has entered the "Ready to (pre)calibrate" screen (**Figure 2**), edit the pressure and voltage values to optimise the system. Apply a voltage that gives a current of 100-140 nA, and choose a pressure (at least 300 Pa) that will give a particle rate of 500-1500 particles/min. Check that the blockades are at least 0.2 nA in size. If the nanopore setup was completed successfully then an adjustment shouldn't be required. However, sometimes the nanopore can change due to the nature of the material. If this is the case, increase blockade size by adjusting the stretch, voltage and pressure values accordingly.



Parameters **MUST** be set before any data recording commences, otherwise the conditions will not be optimal for producing a measurement. If the user does not wish to run a precalibration then parameters must be set in the "Great! You're good to go." screen.

- ▶ Decreasing stretch will increase blockade size, but will also decrease current.
- ▶ Increasing voltage will increase current.
- ▶ Increasing pressure will increase particle rate, but will also decrease blockade size.

Data Recording



Write down the applied pressure, voltage, stretch, and current for reference once you are done with optimisation and ready to proceed.



See section 4.1 for more detailed information on manually controlling system parameters.

Figure 2: A screen capture from the Exoid Control Suite, showing that the user is prompted to optimise their settings.

Select **Start Analysis** to begin recording calibration data. The system will measure 500 blockades at three pressure settings; your chosen pressure, 200 Pa below the chosen pressure, and 400 Pa above the chosen pressure. Providing the system remains stable during this process, no interaction is required.

During data recording there are four recording features available to the user, as well as the option to cancel.



If the baseline destabilises at any point during data recording, QUICKLY press the **Pause** button, perform troubleshooting procedures, and then resume or restart the recording once the baseline has been re-established and is stable.

▶ Advance

Saves the recording and advances to the next pressure step before the required particle count has been reached.

▶ Resume Analysis

Resumes the recording once system troubleshooting is complete.

|| Pause

Pauses the recording so that the user can manually troubleshoot the system.

🔄 Restart Run

Restarts the current pressure recording.

✕ Exit

Cancels the recording and allows the user to return to the previous step without saving the current data set.

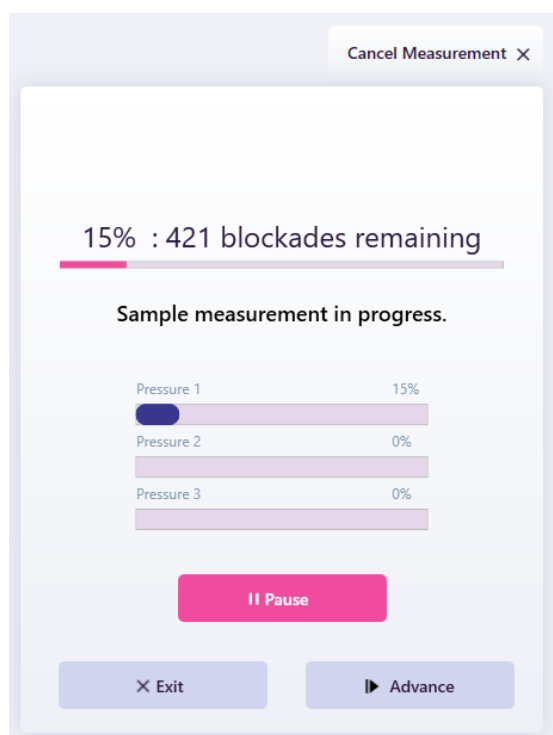


Figure 3: A screen capture from the Exoid Control Suite, showing the unpaused data collection screen with the Advance, Pause, and Exit buttons available.

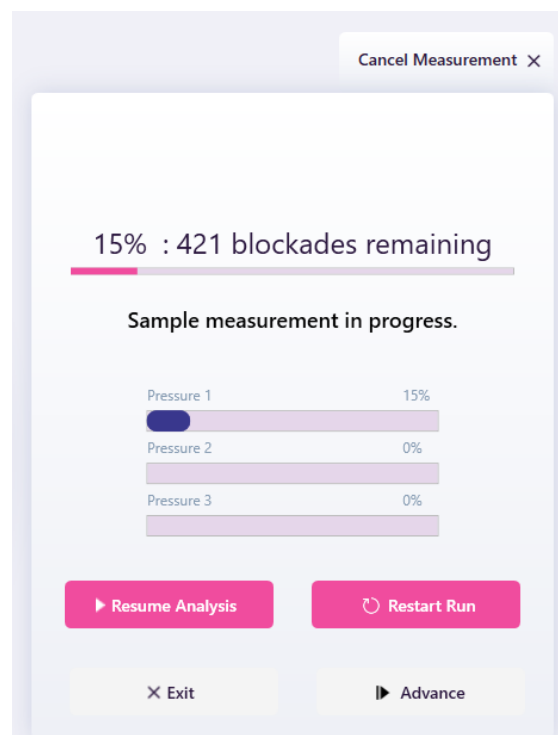


Figure 4: A screen capture from the Exoid Control Suite, showing the paused data collection screen with the Resume Analysis and Restart Run buttons available.

Cleaning

After calibration, follow the on-screen instructions to clean residual particles out of the system before proceeding to sample recording. The ECS will automatically assess whether or not the nanopore is clean, and request further cleaning if required. If manual cleaning is required, follow the cleaning steps on-screen, then press the **Reset** button and wait for one minute. If the particle count is >10, the nanopore is not clean enough to proceed and manual cleaning should be repeated.

Sample Recording

Load diluted Solution S when prompted by the ECS and proceed through data recording. The ECS will prompt the user with a warning if the stretch, voltage, or pressure is different from the parameters used to take the calibration recordings. It is up to the user to determine whether or not the baseline is in an appropriate condition to allow for accurate calibration sample pairing post processing. As with calibration, if the baseline destabilises at any point during data recording, QUICKLY press **Pause**, perform troubleshooting procedures, and then **Resume Analysis** or **Restart Run** to start the recording once the baseline has been re-established.



An appropriate baseline current is one that is within 10% of the original current used to measure calibration particles, drifts by less than 0.5 nA in 60 seconds and has an RMS noise of <15 pA.

Once the measurement is complete you will be prompted to clean the system again in the same manner as before. After particle cleaning is complete, select **No** when asked if you want to run a calibration and leave the nanopore set up while you perform data analysis. Monitor the stability of the baseline while processing your data as nanopores are likely to block if they are left in an unstable state for too long.

2.4 Check Data

Open the Izon Data Suite (IDS) and use the "Open Data Files" button to open the measurement; usually files will be found in the pinned "Exoid Data" location at the top of the open data files window. Check mark multiple recordings to display more than one on the same chart at one time.

- ▶ Assess the quality of both the sample and calibration data:
- ▶ Is there any contamination present?
- ▶ Is the average current similar between recordings $\pm 10\%$?
- ▶ Are the rate plots linear, with the higher pressure(s) having a higher particle rate?

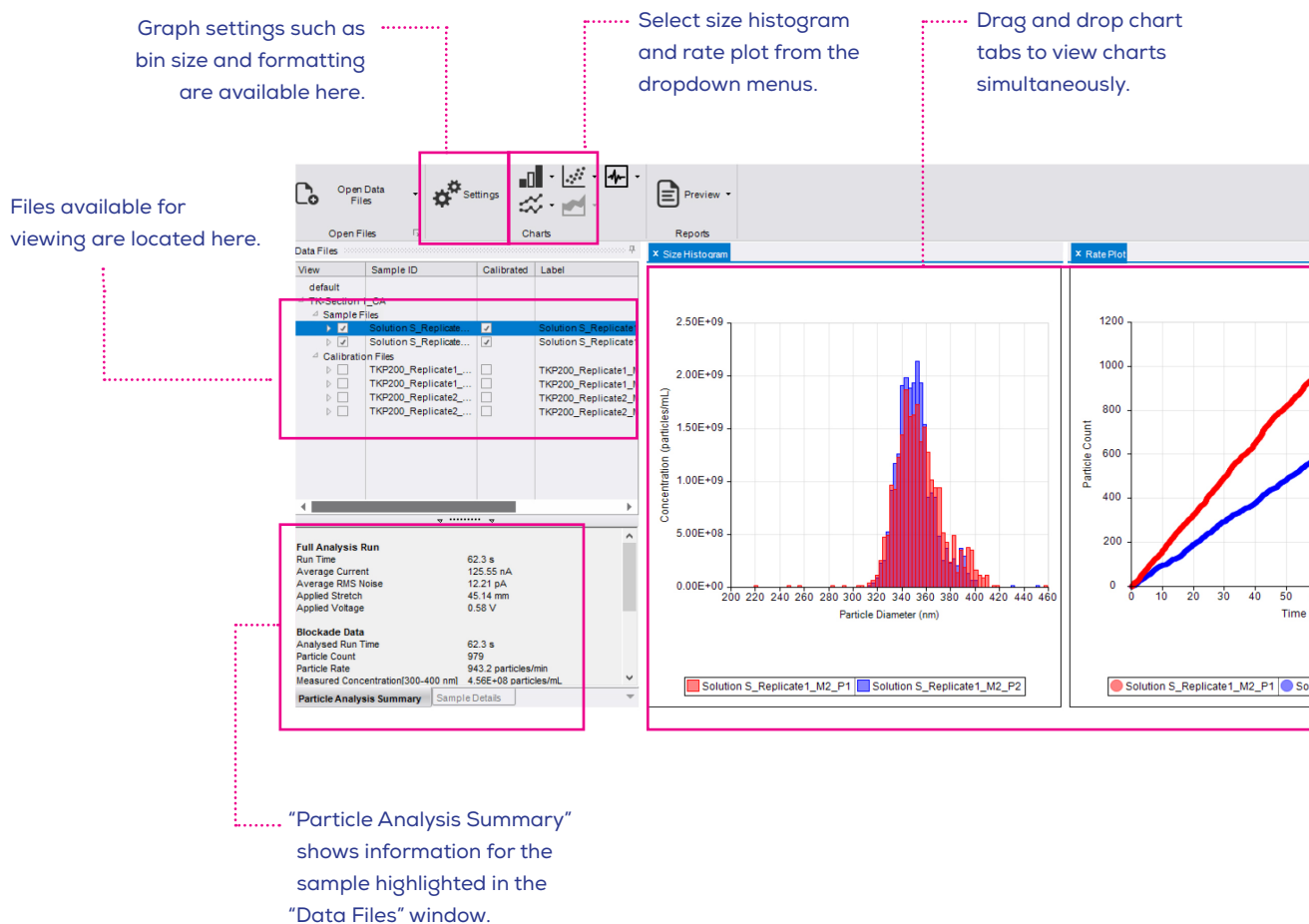


Figure 5: A screen capture from the Izon Data Suite, showing key features available to the user with a short explanation of the purpose of each feature.

Rate Plots

Check the box to the left of your three calibration files and select the rate plot from the dropdown menu. The recording marked P1 should have the lowest particle rate, P3 the highest particle rate, and P2 should fall in between the two, but closer to P1 than P3 (**Figure 6**). If any rate lines overlap or are in the wrong order the concentration data will be inaccurate and the recordings will need to be redone. Do the same for all three sample files and assess using the same criteria.

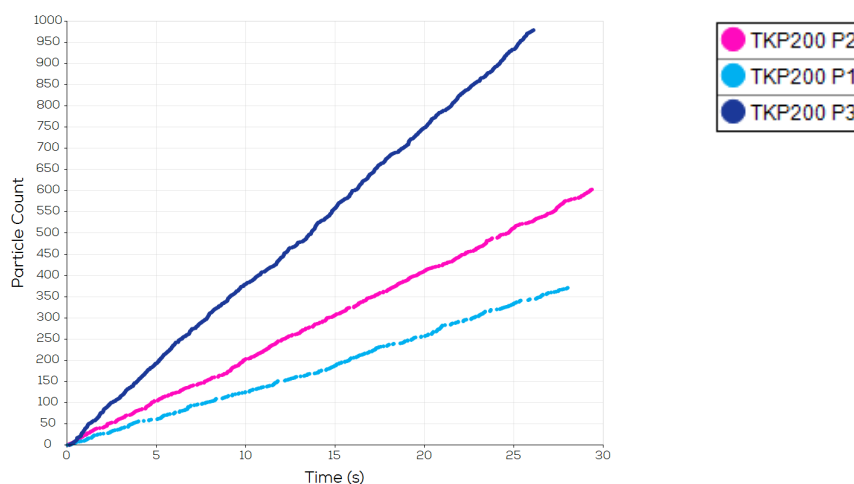


Figure 6: A screen capture from the IZON Data Suite, showing the particle rate for three pressure recordings for TKP200 calibration particles. This is an example of a good recording set which can be used for further analysis and does not need to be repeated.

Size Histograms

Select the calibration files for viewing and open the size histogram from the dropdown menu. All three size distributions should follow a normal distribution with no skewing, and they should almost fully overlap with a slight downwards shift correlated to an increase in pressure (**Figure 7**). If any distributions are not normal the size data will be inaccurate and the recordings will need to be redone. Do the same for all three sample files and assess using the same criteria.

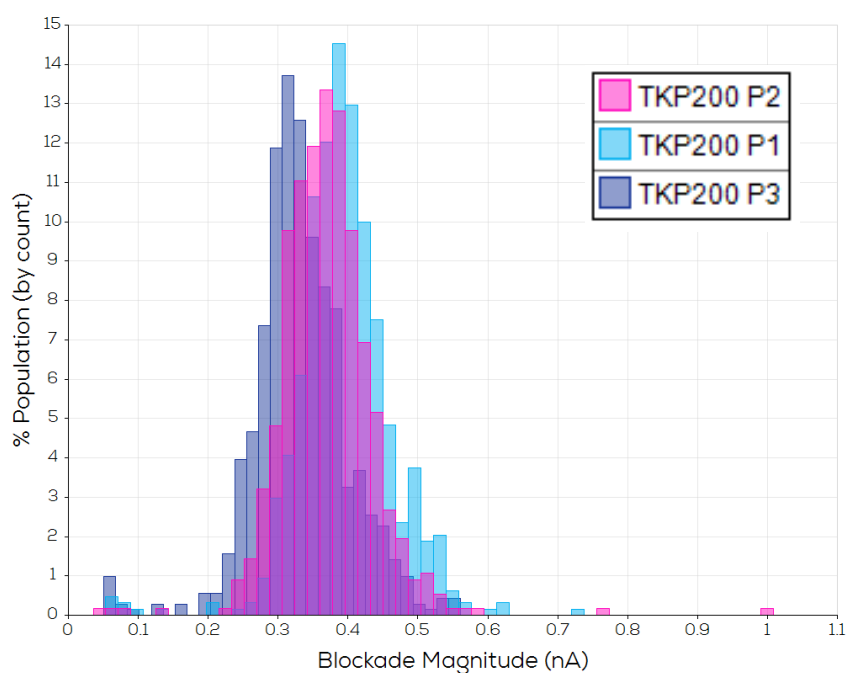


Figure 7: A screen capture from the Izon Data Suite, showing the size distribution histogram for three pressure recordings for TKP200 calibration particles. This is an example of a good recording set which can be used for further analysis and does not need to be repeated.

2.5 Bimodal Size and Concentration Analysis

Select Analysis Type

Return to the ECS and select **One More** and then **Size and Concentration**. The baseline current established should be visible. If the baseline is unstable at this stage then it should be re-stabilised immediately to prolong the life expectancy of the nanopore. Enter the sample details, making sure that the dilution factor entered is correct before proceeding.

Use the slider bar to select a stretch that encapsulates 200 nm and 350 nm particles. Select **Yes** for "Would you like to run a calibration at these settings?" Enter the calibration details, making sure the name is different to the previous calibration, and that size, raw concentration and dilution factor are entered correctly before proceeding. Select **No** for "Does the upper fluid cell contain calibration particles?" and follow the on-screen instructions.

Pre-calibration System Optimisation

After the system has entered the "Ready to (pre)calibrate" screen* (**Figure 2**), edit the pressure and voltage values to optimise the system. Apply a voltage that gives a current of 100-140 nA, and choose a pressure (at least 300 Pa) that will give a particle rate of 500-1500 particles/min. Check that the blockades are at least 0.2 nA in size. If the current is comparable ($\pm 10\%$) to your monomodal recording, then an adjustment shouldn't need to be made. However, sometimes nanopores can change over time resulting in a different current at the same settings. If this is the case, increase blockade size by adjusting the stretch, voltage and pressure values accordingly.

Data Recording

Select **Start Analysis** to begin calibration data recording. Proceed through calibration data recording, nanopore cleaning, sample data recording using the bimodal sample prepared earlier, and another round of cleaning.

Check Data

Open and check the bimodal sample and calibration files using the same criteria used for the monomodal sample in terms of particle rates and size distribution. If the files do not appear, you may have to re-process the group by right-clicking on the group name and selecting "Re-Process Group." The size distribution histogram for the bimodal sample should have two distinct populations, make sure that the smaller of the two populations is not truncated on the lower end.

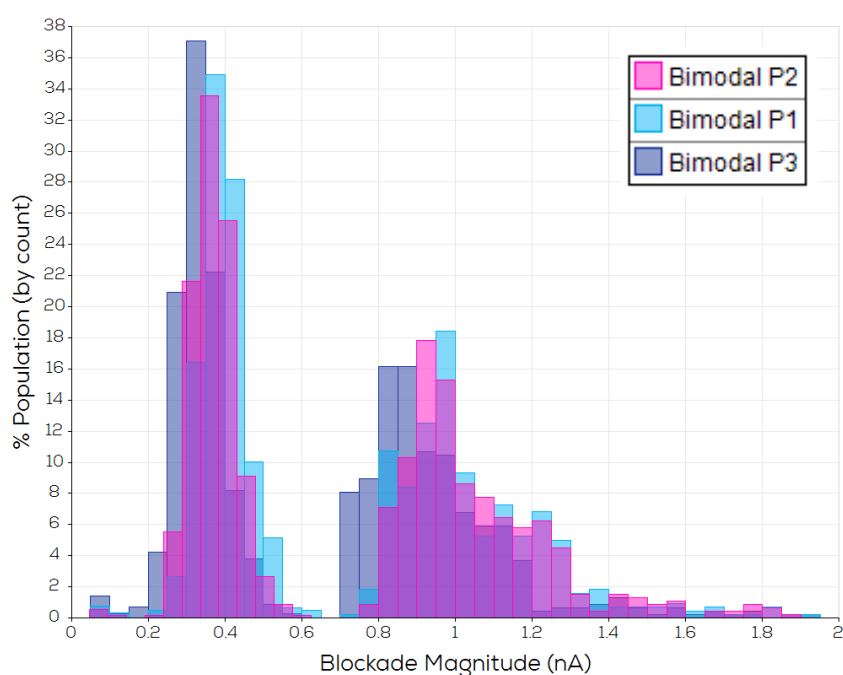


Figure 8: A screen capture from the Izon Data Suite, showing the size distribution histogram for three pressure recordings for bimodal sample particles. This is an example of a good recording set which can be used for further analysis and does not need to be repeated.

2.6 Data Analysis

Calibrating Data

Once you have determined that no recordings need to be redone, check the calibration box in the "Calibrated" column to the right of any of your three sample files to open the calibration window. In the Calibration window there will be multiple types of calibration options available depending on which files are present. If the software determines that the files open are insufficient, i.e., they were run with non-matching parameters, then the calibration required for those files will not be available. Make sure you match the correct calibration with the correct sample if there are multiple recordings that match the automatically assessed parameters.

Single-Pressure Calibration

- ▶ Used to calibrate for size only in nanopores NP300 and smaller, and in some cases for size and concentration measurements for nanopores NP400 and larger.
- ▶ Match individual sample files to individual calibration files with the same parameters.

Multi-Pressure Calibration

- ▶ Used to calibrate for size and concentration for nanopores NP300 and smaller.
- ▶ Select which sample files to calibrate, calibration files with matching parameters will be displayed automatically below.
- ▶ Each sample file in the set needs one calibration file with matching parameters. If only one is available in the dataset it will be automatically selected.

Select **OK**.

Once calibrated, the sample size histograms will now display in nm instead of nA.



If an uncalibrated data file is selected then all graphs will be displayed in nA. Select only calibrated files to view multiple datasets in nm.

Assess Monomodal Sample and Save a PDF Report

Select all three calibrated sample files from the monomodal measurement for viewing and generate a group report using the "Preview" dropdown in the tools ribbon. Compare your results with the values on the Solution S stock bottle label, and check that:

Mean Measured Particle Size = Labelled Particle Size \pm 5%

Measured Raw Concentration = Labelled Concentration \pm 20%

If the criteria passes, save the group report as a PDF called "Monomodal_SOLUTION S BATCH ID_YOUR NAME_YOUR COMPANY", replacing the capital letters with your own details as appropriate. If it does not pass the recordings will have to be repeated.

Repeating Recordings

If the data is of insufficient quality, repeat the experiment. Think carefully about what issues the first set of recordings had and how they can be resolved or mitigated. Re-attempt the experiment at least once more before contacting Izon Support for help. If you are having issues with running the monomodal sample, do not proceed to the bimodal sample before requesting assistance.

Using Filters

The use of data filters may be of interest if there is contamination present, or if you are interested in a specific size range. To find the filter function, hover over the grey bar in the very bottom left corner of the IDS (**Figure 9**). This will open the information for all individual data points, at which point you can select the funnel icon. A line will become available for you to enter your filters. To exclude particles less than 300 nm in diameter, for example, then enter >300 as the filter. This will include all data points larger than 300 nm and will exclude all others. Alternatively, you can select a range of values by using the "-" symbol; e.g. 280-400 will display all data points between 280 and 400 nm. It is also possible to deselect individual data points by unticking the check-box to the left in the "Include" column. The units of the filter parameters will depend on whether or not a dataset is calibrated (calibrated datasets: nm, uncalibrated: nA). If you change the calibration status while a filter is in place, then the filter values (and units) will update automatically.



If you apply a filter while assessing your dataset (except size) post calibration, you must re-calibrate your dataset by unticking and re-ticking the calibration box.

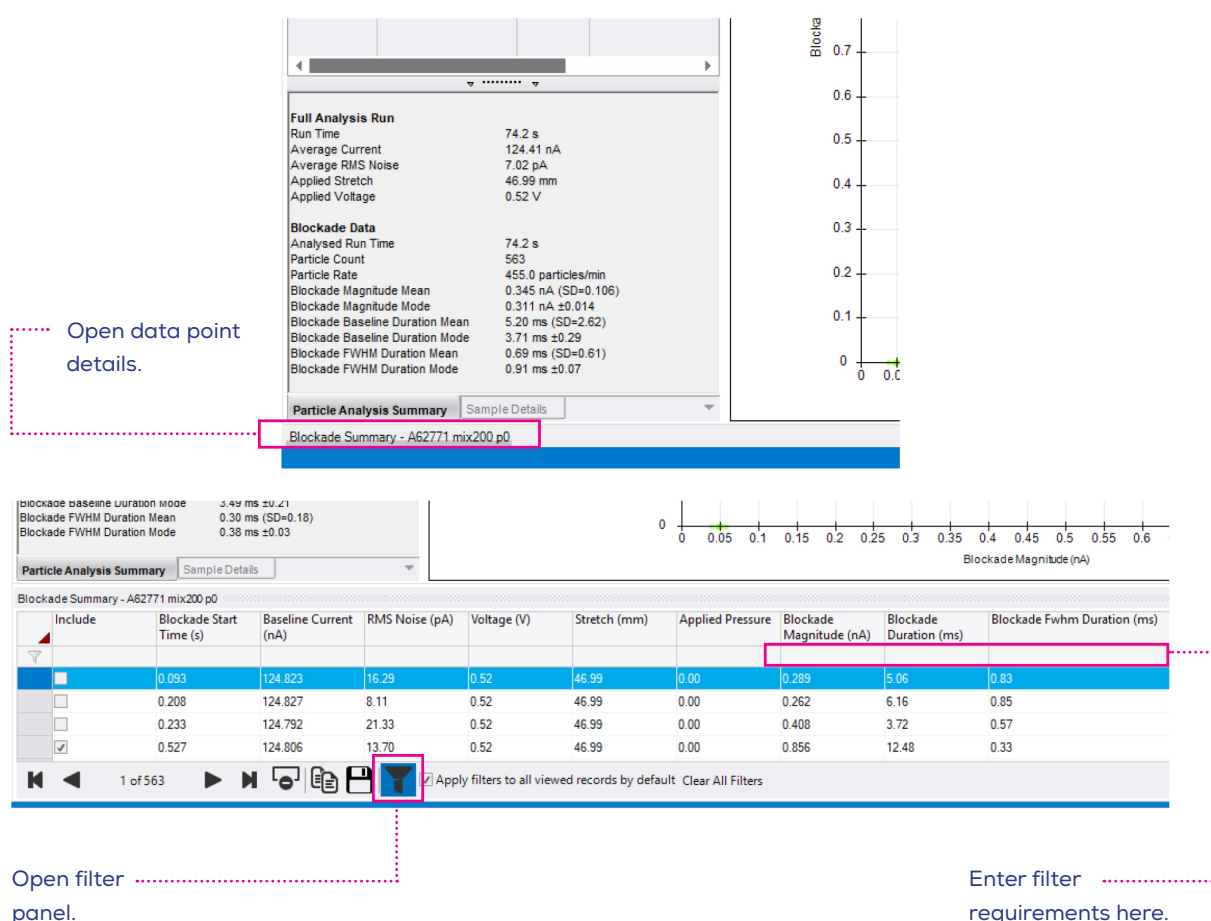


Figure 9: A screen capture from the IZON Data Suite, showing the Particle Analysis Summary and the location to open the Blockade Summary table, as well as the Blockade Summary table once opened with the filter panel open. NOTE: Blockade Magnitude (nA) will change to Particle Diameter (nm) in a calibrated data set.

Filter Data for the Bimodal Sample

Small Population

1. Edit filter settings
 - ▶ Select all three sample files for viewing
 - ▶ Enter a filter in the particle diameter column that encapsulates the entire small population
 - ▶ Select "Apply filters to all viewed records"
 - ▶ Close the filter table
2. Particle concentration will no longer be displayed
3. Edit dilution factor
 - ▶ Click on "Sample Details"
 - ▶ Change the dilution to 102 for all three sample files
4. Recalibrate sample to update concentration
 - ▶ Re-tick the box in the "calibrated" column
 - ▶ Go to the "Multi-Pressure Calibration" tab
 - ▶ Select all three sample files, all calibration files associated with the sample files will be populated
 - ▶ Select all three calibration files to calibrate with
5. Produce a group report for this population and save as a PDF
 - ▶ Generate a group report
 - ▶ Compare your results with the values on the TKP200 stock bottle, and check that:

Mean Measured Particle Size = Labelled Particle Size \pm 5%

Measured Raw Concentration = Labelled Concentration \pm 20%

If the criteria passes, save the group report as a PDF called "Bimodal_Small_YOUR NAME_YOUR COMPANY," replacing the capital letters with your own details as appropriate. If it does not pass, the recordings will have to be repeated.

Large Population

Follow the steps for filtering for a small population, as above, except the size filter will be different and the results should be compared against the Solution S stock bottle. Save this group report as "Bimodal_Large_YOUR NAME_YOUR COMPANY," replacing the capital letters with your own details as appropriate.

2.7 Packing Up

Once this section is complete and a good quality data set has been sent to Izon, the system can be shut down for the day.

Select **Finish** in the ECS and follow the on-screen instructions for further cleaning of the nanopore. Remove the nanopore, rinse it with deionised (DI) water and dry it with a lint-free tissue before storing it back inside its ziplock bag. It is a good idea to write on the ziplock bag what the nanopore has been used for and how long it was in use, this way if someone wishes to re-use the nanopore then they know the nanopore use history.

Fluid Cell Cleaning and System Shutdown Procedure

Rinse the upper fluid cell and the lower fluid cell liquid contact areas with DI water, and blow dry with compressed gas if available. Reassemble the fluid cell in the top of the Exoid. Dry the APS nozzle with a kimwipe and connect it to the fluid cell. See Izon online resources for more rigorous system maintenance.

3 / COMPLETE THE EXOID TRAINING MODULE

Prepare Group Files for Assessment

Right-click on any data file and select "Save Dataset" before closing the IDS. Navigate to your investigation folder using the file explorer, usually located in C:/Exoid Data, and move the three group reports generated earlier into that location. Zip the investigation folder by right-clicking and selecting "Send to > Compressed (zipped) folder." Submit the ZIP file via the Izon Academy at the appropriate point for submission, remember to include your Solution S and TKP200 batch numbers when you submit your results.

ZIP file checklist:

- ▶ Raw data file
- ▶ Monomodal group report PDF
- ▶ Bimodal small population group report PDF
- ▶ Bimodal large population group report PDF

The results for your measurements will be assessed by an Izon Academy instructor, this will typically be done within 24-48 hours.

Once you have finished measurements and submitted your files, you should also complete the Exoid Exam in the Exoid Beginner Training module. A score of 80% is required to pass and you have unlimited attempts. Some questions may require the help of articles located at support.izon.com to answer, but most of the answers have been covered in the online training programme. Full completion of the online course along with graded measurements will grant you a certificate that you have been trained on the Exoid.

4 / PROCEEDING WITH UNKNOWN SAMPLES

Once a TRPS certificate has been received from Izon, follow the "TRPS Experimental Design" training module on the Izon Academy for advice on working with experimental samples.

4.1 Manually Controlling System Parameters

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

Stretch (mm)

Choosing a suitable operating stretch is critical, as this will have an impact on the stability of the nanopore as well as the resolution. Altering the stretch of the system essentially changes the size of the nanopore aperture. This is useful if the user's blockades are too close to the noise or are so large that they have the potential to block the nanopore. Blockade magnitude is inversely proportional to stretch, therefore decreasing the stretch will increase the blockade magnitude and vice versa. If the stretch has been manually adjusted, manual adjustment of the applied voltage may also be necessary if the current is no longer falling inside the recommended range of 100-140 nA. If the stretch has been decreased, the voltage will need to be increased and vice versa.

Table 3: Nanopore operating stretch guidelines

STRETCH (MM)								
43.5	44	45	46	47	48	49	50	
*		Optimal operating stretch					*	Stable recordings, but nanopore is at risk of rupturing.

*Operating at a stretch of 43.5-45 mm is possible but at low cruciform tension the nanopore may exhibit less baseline stability and may be more prone to blockages. Operating at a stretch of 49 mm and greater may work well but the nanopore may become problematic for further use at lower stretches. This is because the cruciform may undergo structural changes at high stretch. On some occasions, excessive stretch can cause a rupture of the nanopore.

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

Applied Pressure (Pa)

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

Applied Voltage (mV)

The voltage applied to the system will have an affect on the baseline current. Baseline current is directly proportional to applied voltage, therefore an increase in voltage will result in an increase in current and vice versa.

5 / TROUBLESHOOTING

5.1 Nanopore Unblocking Guide

Follow the steps outlined to re-stabilise the baseline current in the event of a nanopore blockage. A blockage is indicated by an increase in RMS noise and/or a decrease in the expected current value for the applied voltage. Once the baseline stabilises, there is no need to continue to subsequent troubleshooting steps. If the nanopore blocks again, then follow this unblocking guide from the beginning.



Reminder: a stable baseline current is one that is 100-140 nA, drifts by less than 0.5 nA in 60 seconds and has an RMS noise of <15 pA. An unstable baseline current will produce unreliable measurements and should not be used under any circumstances.

Table 4: Troubleshooting steps for unblocking a nanopore

Apply a Negative Pressure	<ul style="list-style-type: none">- Make sure the pressure nozzle is inserted and apply a negative pressure of -2500 Pa for 10-30 seconds.
Tap the Pressure Nozzle	<ul style="list-style-type: none">- Without changing the pressure, use the back of the Pressure Application Device (PAD) to firmly tap the top of the pressure nozzle.
Apply the PAD	<ul style="list-style-type: none">- Set the pressure to 0 Pa and remove the pressure nozzle.- Use the plunging end of the PAD to apply pulses of pressure to the upper fluid cell.
Pipette Mix Contents	<ul style="list-style-type: none">- Using a further 35 μL of fluid (ME, sample or calibration particles, depending on what you are working with), mix the fluid in the upper fluid cell by pipetting up and down.- After mixing remove the extra 35 μL.
Stretch and Positive Pressure	<ul style="list-style-type: none">- Increase the nanopore stretch to 50 mm and apply a full positive pressure of 2500 Pa for 10-30 seconds. You may need to reduce the voltage to avoid current saturation.
Stretch and Negative Pressure	<ul style="list-style-type: none">- Increase the nanopore stretch to 50 mm and apply a full negative pressure of -2500 Pa for 10-30 seconds.
Reset the System	<ul style="list-style-type: none">- Set the pressure to 0 Pa and remove the pressure nozzle.- Reduce the stretch and remove the nanopore.- Rinse with DI water and dry the nanopore, upper fluid cell and pressure nozzle.
Reassemble the System	<ul style="list-style-type: none">- Reassemble the system with Measurement Electrolyte in the upper and lower fluid cells and stretch to 47 mm, or the stretch used previously (prior to the blockage).
Flush the System	<ul style="list-style-type: none">- Replace all the fluids with DI water and apply maximum pressure of 2500 Pa until the current is <5 nA. You may need to flush the system multiple times to meet this criteria.

5.2 Troubleshooting Steps



As a preventative measure, use the reverse pipetting technique when introducing fluid into the upper fluid cell to avoid placing an air bubble directly on top of the nanopore.

Table 5: Troubleshooting strategies

ISSUE	ACTIONS THAT MAY BE NEEDED
Baseline current increases sharply	<ul style="list-style-type: none"> - Reset the system, if the nanopore still has a much higher current at the same setting used, the nanopore is likely ruptured and has become permanently larger. This may require using a new nanopore for measurements if a suitable blockade magnitude (>0.2 nA) can no longer be achieved even at lower stretches.
Cannot establish baseline current	<ul style="list-style-type: none"> - Ensure that a voltage is applied to the system and that the nanopore is stretched. - Use reverse pipetting to prevent bubbles forming on top of the nanopore and blocking the fluid flow.
Current changes when pressure nozzle is inserted	<ul style="list-style-type: none"> - Wipe liquid off the end of the pressure nozzle before inserting.
Observing blockades when no sample is loaded	<ul style="list-style-type: none"> - Clean the nanopore surface, upper fluid cell, and pressure nozzle with 70% EtOH solution and dry thoroughly. - Filter fresh PBS solution (or your electrolyte) to check for contamination. - Replace the pressure nozzle o-ring.
Trace freezes or disappears	<ul style="list-style-type: none"> - Click on the connection button, disconnect the VCA, wait for 10 seconds then reconnect the VCA.
Trace is a flat line at 178 nA	<ul style="list-style-type: none"> - Reduce the voltage to bring the current back within the detectable range of the instrument. - Check the outside of the nanopore for rogue liquid creating a short circuit. - Wipe up liquid that overflows out of the fluid channel during pipetting.
Stable baseline current, but very few particles	<ul style="list-style-type: none"> - Increase the pressure. - Increase the sample concentration. Use low pressures until you have a stable system, otherwise you risk blocking the nanopore. - Increase or decrease the size of the particles being measured.
System detects a leak when trying to apply pressure	<ul style="list-style-type: none"> - Check pressure nozzle is fully inserted into the upper fluid cell. - Check o-ring for damage, replace if required. - Cover pressure nozzle with a thumb and apply pressure, if leak persists check tubing is connected properly and bayonet fixtures are screwed in tight.
Unstable baseline current	<ul style="list-style-type: none"> - Gently mix the fluid in the upper fluid cell without introducing any bubbles. - If consistently destabilising, wash and dry the upper fluid cell, and gently replace the fluid in the lower fluid cell to remove any bubbles. - There may be many invisible bubbles beneath the nanopore. Put a droplet of electrolyte on top of the nanopore, take the nanopore off the Exoid and wipe the bottom gently with a lint-free tissue before remounting on the Exoid and applying the same stretch as before. Re-establish your baseline current.



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