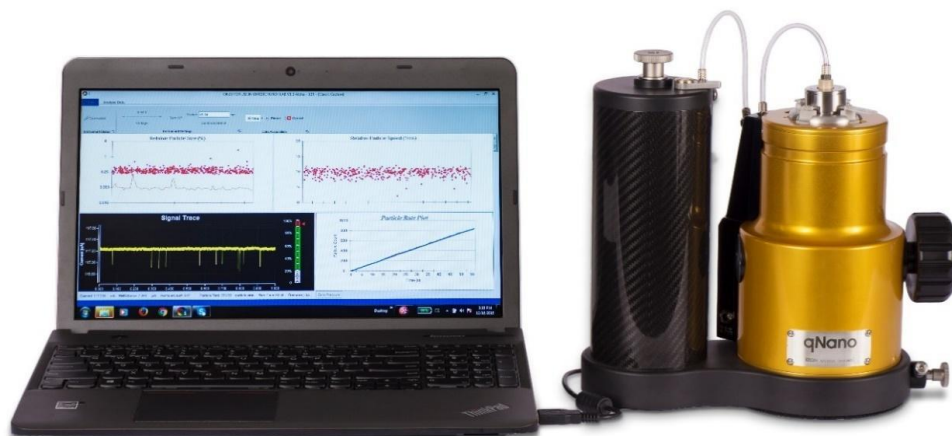


Izon TRPS Training Programme



TRAINING PROGRAMME OVERVIEW

Before starting this training programme, make sure you have:

- Read through and understood the qNano User Manual and the Reagent Kit Technical Note.

There are two parts to the training programme:

1. Practical assessment section 1: basic size and concentration measurement
2. Practical assessment section 2: bimodal size and concentration measurement

Recording vs Measurement

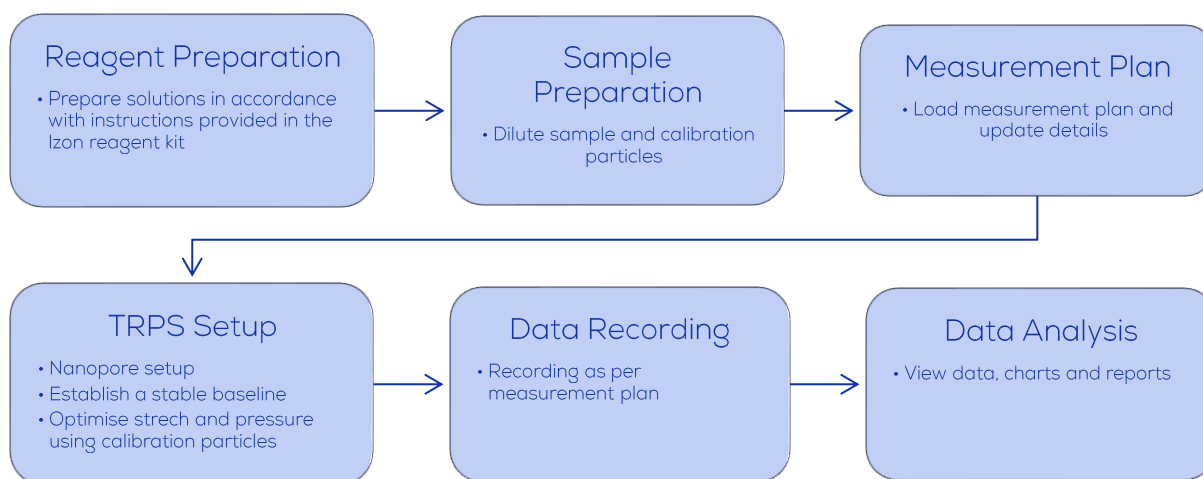
In order to understand this document properly it is important to know these two key definitions:

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

1. PRACTICAL ASSESSMENT SECTION 1

Overview

- Expected time: 2-2.5 hours
- Complete a pre-defined measurement plan
- Edit sample details
- Complete a basic size and concentration measurement
- Self-assess your results (guidance provided) and repeat if required



Reference Material

The USB drive in the Izon Training Kit contains additional reference and support material including videos. There is also reference material available by selecting the “Home” tile on the home screen of the Izon Control Suite Software (CSS).

Prepare Reagents

Use the reagent kit tech note to prepare measurement electrolyte (ME), deionised water, and coating solution.

Sample Preparation

Prepare Solution S (Sample) and TKP200 (Calibration) at a dilution factor of 51. To do this put 20 µL of the particles using **forward pipetting** into 1000 µL of ME in a 1.5 mL Eppendorf tube. Remember to label your vials accordingly.

Loading A Measurement Plan

- Open the software before connecting the USB cable, and then the power supply.
- Select “Custom Planner” -> “Next” -> “Load Measurement Plan.”
- Locate the measurement plan on the USB stick following this file path:

Training Module>Measurement Plan>“TK-Section 1.idfs”
- Enter the Nanopore ID and click “Next.”
- Fill out the sample and calibration details by selecting the “Edit Sample”/“Edit Calibration” and filling in the relevant information.
- Click “Next” until prompted asking if you are using a reagent kit, select “Yes.”

TRPS Setup

Follow the on-screen instructions to prepare the fluid cell, calibrate the nanopore stretch, wet the nanopore*, coat the nanopore, establish a stable baseline, insert the calibration particles, optimise the stretch and pressure.


*After wetting the nanopore, and before coating the nanopore, verify that the nanopore is properly wetted by exchanging the wetting solution for ME in the top and bottom fluid cell. Look for a stable baseline with an RMS noise <15 pA, and a current of >20 nA which does not drift.

Data Recording

You can adjust the signal trace zoom at any stage using the controls in the top left-hand corner of the signal trace window.

During recording monitor the baseline current closely, if it steps down or becomes noisy then the recording should be cancelled and restarted. The baseline current should not differ by more than 5% between data recordings. The particle rate plot also requires monitoring and should follow the linear guidance line, with a particle rate between 200-1500 particles per minute.

There are three recording features:

 Stop – Stops the recording and saves it before the required particle count or runtime target has been reached.

 Pause – Pauses the recording to recover from a blockage.

 Play – Resumes the recording once a blockage has been removed while the recording was paused.

 Cancel – Cancels the recording and prompts the user to either repeat the recording or exit the assistant.

Assess recording quality

To confirm a good quality recording for calibration particles, there must be one population of particles and a linear particle rate plot (A linear rate plot applies for all recordings, not just calibration).

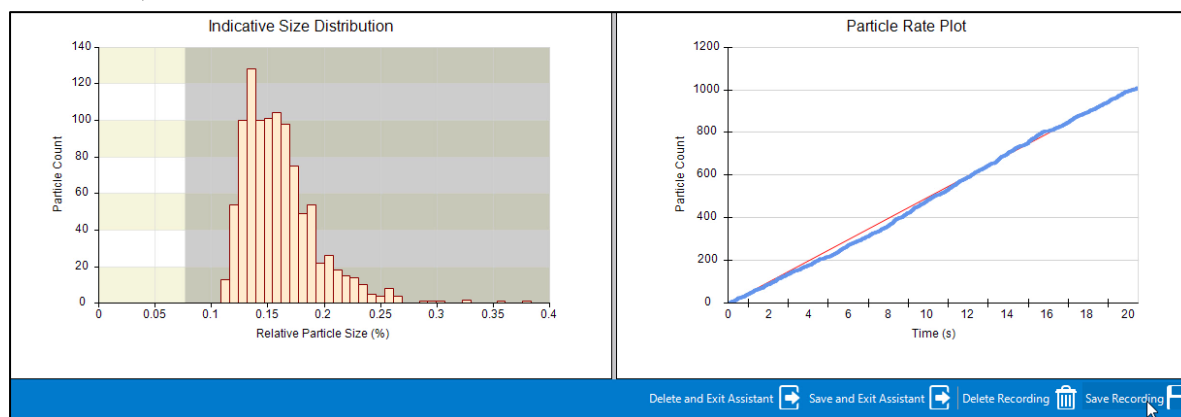


Figure 1. An example of a good recording with only one particle population and a linear particle rate plot.

Depending on the quality of your data you have four options:

- **Save Recording** and continue with the measurement plan
- **Delete Recording** and repeat if the quality is poor
- **Save the recording and Exit** the software
- **Delete the recording and Exit** the software

Assess measurement quality

Once the measurement is complete, the Izon CSS will output a particle rate vs pressure plot. Follow the onscreen instructions for the acceptability criteria. If the criteria are not met, delete the measurement and start the recordings again.

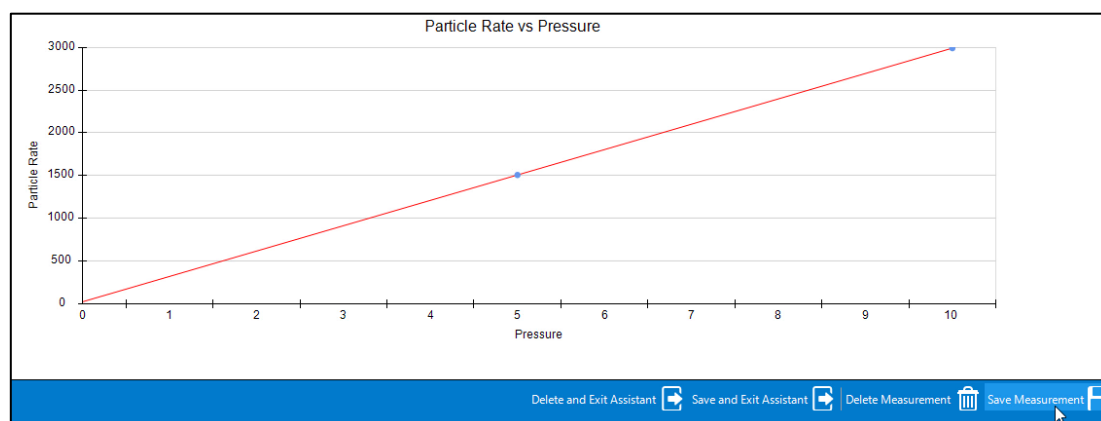


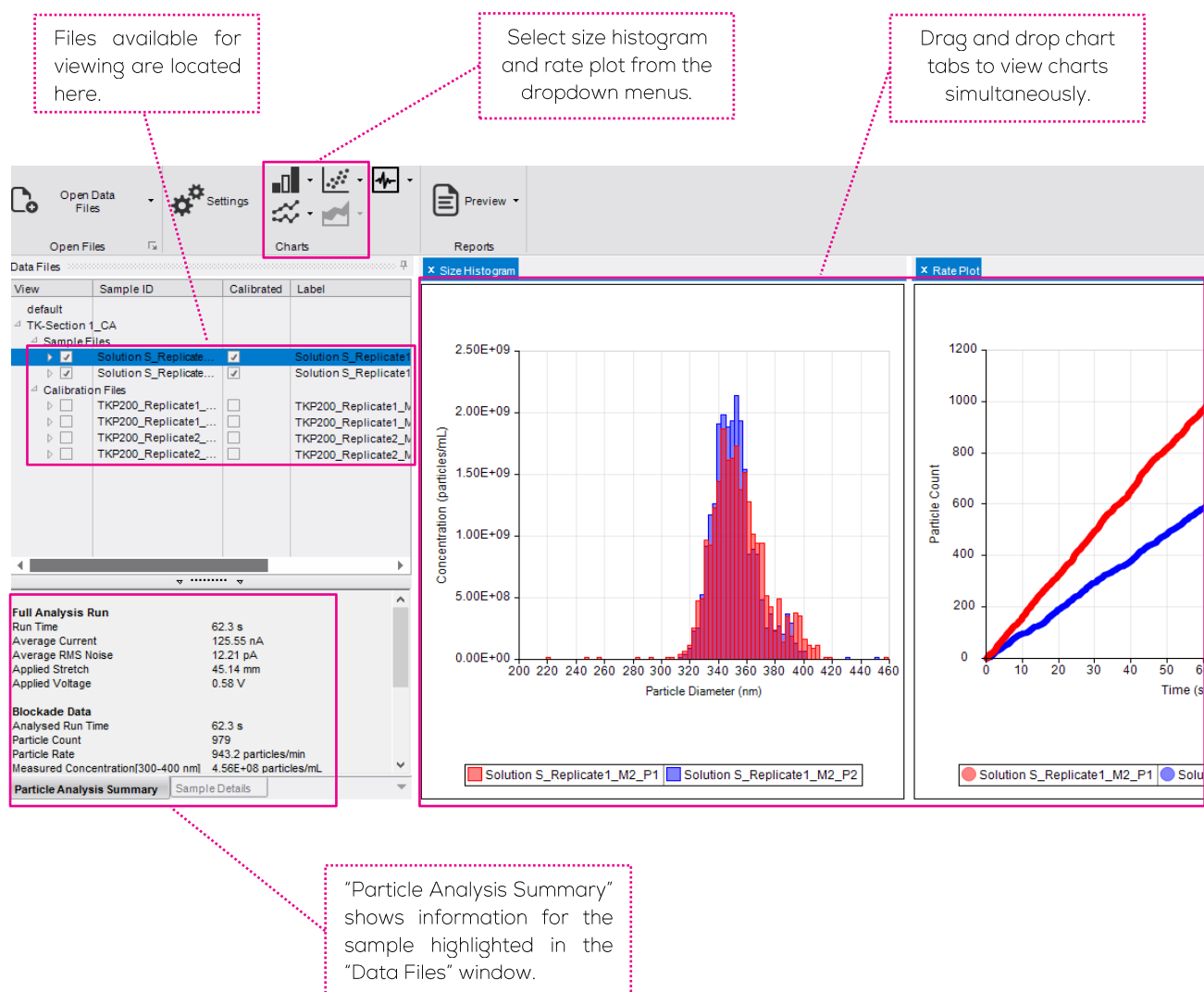
Figure 2. An example of a good recording with particle rate increasing with pressure.

Once a good quality measurement has been achieved and saved, the CSS will guide you through sample changeover and further measurements.

Data Analysis

If you will be proceeding to section 2 of this training module, **DO NOT** remove the nanopore as prompted by the Izon CSS. Leave it with ME in the top and bottom while you look at the data from section 1.

Below is a guide to useful functions in the CSS data view. "Settings" will allow you to change the bin size and axis scales among other things. You can check mark multiple recordings to display more than one on the same chart at one time.



Assess the quality of the data:

- Is there any contamination present? *
- Is there one distinct peak observed in the size histogram?
- Is the average baseline current similar $\pm 5\%$?
- Are the rate plots linear with the higher pressure having a higher particle rate?

*Using a filter

If there is contamination present, you can filter this out by hovering over the grey bar in the very bottom left corner. This will open the information for all the individual data points, at which point you can select the funnel icon, which will add a line for you to enter your filters e.g. if you have contamination less than 300 nm diameter than put >300 as the filter. This will *include* all data points larger than 300 nm and will exclude all others. You can also “deselect” individual data points by unticking the checkbox to the left under the “Include” header. 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.

Open data point details.

Enter filter requirements here.

Open filter panel.

Full Analysis Run

Run Time	74.2 s
Average Current	124.41 nA
Average RMS Noise	7.02 pA
Applied Stretch	46.99 mm
Applied Voltage	0.52 V

Blockade Data

Analysed Run Time	74.2 s
Particle Count	563
Particle Rate	455.0 particles/min
Blockade Magnitude Mean	0.345 nA (SD=0.106)
Blockade Magnitude Mode	0.311 nA ±0.014
Blockade Baseline Duration Mean	5.20 ms (SD=2.62)
Blockade Baseline Duration Mode	3.71 ms ±0.29
Blockade FWHM Duration Mean	0.69 ms (SD=0.61)
Blockade FWHM Duration Mode	0.91 ms ±0.07

Blockade Summary - A62771 mix200 p0

Include	Blockade Start Time (s)	Baseline Current (nA)	RMS Noise (pA)	Voltage (V)	Stretch (mm)	Applied Pressure	Blockade Magnitude (nA)	Blockade Duration (ms)	Blockade Fwhm Duration (ms)
<input checked="" type="checkbox"/>	0.093	124.823	16.29	0.52	46.99	0.00	0.289	5.06	0.83
<input type="checkbox"/>	0.208	124.827	8.11	0.52	46.99	0.00	0.262	6.16	0.85
<input type="checkbox"/>	0.233	124.792	21.33	0.52	46.99	0.00	0.408	3.72	0.57
<input checked="" type="checkbox"/>	0.527	124.806	13.70	0.52	46.99	0.00	0.856	12.48	0.33

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Apply filters to all viewed records by default Clear All Filters

NOTE: Blockade Magnitude (nA) will change to Particle Diameter (nm) in a calibrated data set.

Once you are happy with your data, select “preview” to generate a measurement report which can be saved as a PDF. Check the acceptance criteria:

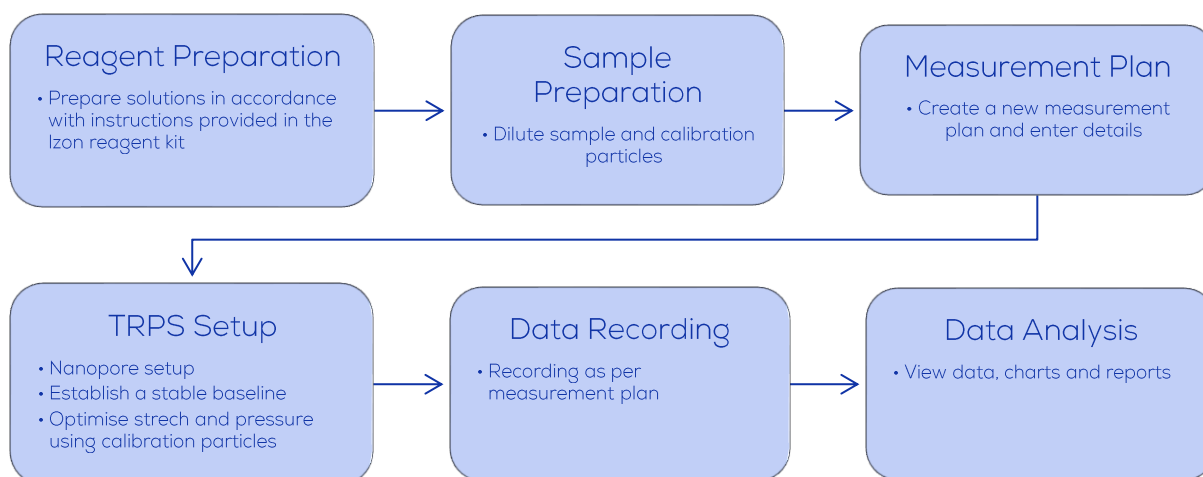
Mean Measured Particle Size = Labelled Particle Size ± 5%
Measured Raw Concentration = Labelled Concentration ± 20%

If the acceptance criteria are met, proceed immediately to section 2. If not, repeat section 1 (contact Izon Support for advice).

2. PRACTICAL ASSESSMENT SECTION 2

Overview

- Expected time: 1-2 hours
- Create and run a new measurement plan
- Apply filters to measured data
- Calculate size and concentration for both populations in a bimodal sample
- Work with grouped data
- Self-assess your results (guidance provided) and repeat if required



Creating a new measurement plan

In the Custom Planner, populate the measurement settings as follows:



Investigation (Experiment name. This is also the folder name)	TK-Section2
Nanopore ID	Nanopore Serial Number
Nanopore Type	NP250
Resolution Range	140 nm (smallest particles)
Electrolyte	Measurement Electrolyte
Data File	(Leave as default)
Notes	Additional notes, if necessary

Enter sample details by selecting “Add Sample” and entering the relevant details in the window that pops up.

Add details of the samples and calibration :

Samples

Sample ID
Bimodal


 Add Sample
  Edit Sample

Sample ID:

Dilution:

Batch:

Resolution Range: 120-600 nm

 Analysis Size Range:
 Min: nm
 Max: nm

qEV:




Notes:

Enter calibration details by selecting “Add Calibration” and entering the relevant details printed on the TKP200 vial. In the window that pops up.

Add details of the samples and calibration set to be used in the measurements.




Samples

Sample ID	Electrolyte	Dilution	Batch
Bimodal			

 Add Sample
  Edit Sample
 

Calibration Set

Sample ID

 Add Calibration
  Edit Calibration
  Delete Calibration

Sample ID:

calibration size must be between 190 and 410 nm

Mean Diameter:

Concentration:

Dilution:

Batch:

Notes:

Populate the measurement plan by selecting “Add Measurement” and editing the sample measurement conditions as follows:

Sample	Bimodal	Notes
Calibration sample	Calibration 2 The software will automatically pair any sample measurement with the calibration recording following it.	You can choose the calibration file to use for each sample recording from the drop down.
Target Particle Count	1000	Software will stop once these conditions are met
Min Run Time (secs)	30	
Max Run Time (secs)	120	
No. of Pressure Steps	3	If one pressure step is selected, the concentration value is an estimate.

Select “OK” followed by the “Next” button. Click “Yes” for are you using a reagent kit?

Sample Preparation

Prepare Solution S (Sample) and TKP200 (Calibration) at a dilution factor of 51, or if you have any left over from Section 1 then you can use these. Take 200 µL of each and mix them together in a clean 1.5 mL centrifuge tube. Remember to label your vials accordingly.

Running the Measurement Plan

Follow the on-screen instructions to perform your measurements in the same way as in Section 1, remembering to look out for key indications of measurement quality such as stable baseline, low RMS noise, and a linear rate plot, among other things. Once a good quality measurement has been achieved and saved, leave the nanopore running with electrolyte in the upper and lower fluid cell while you process the data. This is so that if you need to run the measurements again, the pore is still set up and ready to go.

Data Analysis

Pro Tip: Click on the “Instructions” button in the Analyse Data tab to open up a guide that details all aspects of data processing. Alternatively, go to our Support Portal to search for articles related to data processing.

Assess the quality of the data:

- Is there any contamination present? *
- Is there one distinct peak observed in the size histogram?
- Is the average baseline current similar $\pm 5\%$?
- Are the rate plots linear with the higher pressure having a higher particle rate?

Filter Data for the Small Population

1. Edit filter settings:
 - Keep all three sample files ticked for viewing.
 - Enter a filter in the particle diameter column (should be similar to 150–280 nm).
 - Select “Apply filters to all viewed records.”
 - Close the filter pane.
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 for submission.
 - Right click on the group name.
 - Save group as “Small Population”.
 - Right click and select “Group Report.”
 - Save as PDF.

Filter Data for the Large Population

Follow the steps for filtering for a small population, except the size filter should be about 280–400 nm. Save this group as “Large Population” before producing the group report.

Prepare Group File for Assessment

Remove all the filters and create a compressed copy of the data files which were used to produce the group report. Navigate to the Custom Assistant folder in C:\Izon Data and find your compressed copy. Send this, along with the two group report PDFs to support@izon.com for evaluation.

Please use **TK-Section 2 Assessment_(NAME)_(INSTITUTE)** as the subject line.

Packing Up

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

Nanopore Cleaning Procedure

Clean the Nanopore by stretching it to 47 mm and running it with electrolyte in the upper and lower fluid cells at full pressure until there are no particles present, followed by running it with Nanopore Washout Solution (100 mL of deionised water and 100 μ L of Wetting Solution Concentrate) in the upper and lower fluid cells at full pressure until the current is ± 2 nA from the usual current offset (The usual current offset of the system can be found by simply taking the fluid cell cap and upper fluid cell off and reading the current value).

Pro Tip: Wash the upper fluid cell with DI water and dry with compressed gas between these steps. This will speed up the cleaning process as there will be no residual liquids in the upper fluid cell.

Remove the nanopore, rinse it with DI water and dry it with compressed gas before storing it back inside its ziplock bag. It is a good idea to write on the ziplock bag what the pore has been used for and how long it was in use, this way if someone wishes to re-use the pore then they know the pore's history.

Fluid Cell Cleaning and System Shutdown Procedure

Rinse the fluid cell cap, the upper fluid cell, and the lower fluid cell liquid contact areas with DI water and blow dry with compressed gas. Reassemble the fluid cell in the top of the qNano, don't forget to screw in the SMA cable so that the next person to use the system does not try use the system without the fluid cell being connected. Dry the PM2 nozzle with a kimwipe and connect it to the fluid cell. Leaving the vent valve open, push the VPM plunger all the way in so that it is out of the way. See Izon online resources for more rigorous system maintenance.

Proceeding with unknown samples

Once a TRPS certificate has been received from Izon, then use the Izon support portal at support.izon.com for more information on how to process your experimental samples.