Instruction Manual for the NanoBrook Series

Particle/Protein Size and Zeta Potential Analyzer

PLEASE READ

This is your instruction manual for the Brookhaven NanoBrook, particle/protein size and zeta potential analyzer. Please read it carefully before making measurements. Familiarize yourself with the software by running the program with some of the data sets that are provided for this purpose. If you have any questions, comments or suggestions, please contact Brookhaven.

This guide has been written to include all of the features present in the NanoBrook Omni, instrument configuration. Features presented may not be applicable if a different configuration of the NanoBrook is being used.

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The BIC warranty extends for a period not exceeding the warranty period of the Original Equipment Manufacturer where applicable. The typical warranty period on printers and computer peripherals is 90 days. Please contact BIC for copies of applicable OEM warranties.

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Installing the Instrument and Software

Installation of the NanoBrook is surprisingly simple.

1. Unpack the instrument from the box

2. Place the instrument on a flat, sturdy, surface

3. Install the Particle Solutions software

4. To install the program, you must run the setup program provided on the flash drive or CD. You may also need to install some additional runtime support software, depending on the configuration of the computer being used.

- Setup process for CD is as follows:
  Insert the CD in the drive. The setup program should start automatically.
  a. If the program does not start (perhaps AutoStart is disabled on your computer), use Windows Explorer to run Setup.exe directly from the CD.
  If you get a message that begins with "Some of the prerequisite files required to run this application are missing…"
  b. Click "OK" to exit the setup program
  c. Run the DotNET Runtime Setup.exe program
  d. Run Setup.exe again
  The final screen of the setup program will ask if you want to run the Particle Solutions program. Also, a shortcut will be created for Particle Solutions in the "Brookhaven Instruments" section in the Start Menu.
- Setup process for flash drive is as follows:
  a. Insert flash drive. Choose to view files on the drive.
  b. One folder is titled, "Particle_Solutions". Double click to open. There is an Application named "PS_version number". Double click to run. Click through the software installation wizard to begin installation.
  c. Once installed, allow the software to run.
  d. Once the software is running, it will ask about a license key. The flash drive has two versions if the order has been paid in full. One license key that is a temporary 60 day license, and one that is a full license unique to the serial number of the NanoBrook instrument. If the full license is present on the flash drive, open that document and copy and paste it into the license key box in Particle Solutions (this can be accessed by selecting File→License….
  e. If only the 60 day license is available, copy and paste that into the same location. This will allow the full use of the software for 60 days. After that, measurements

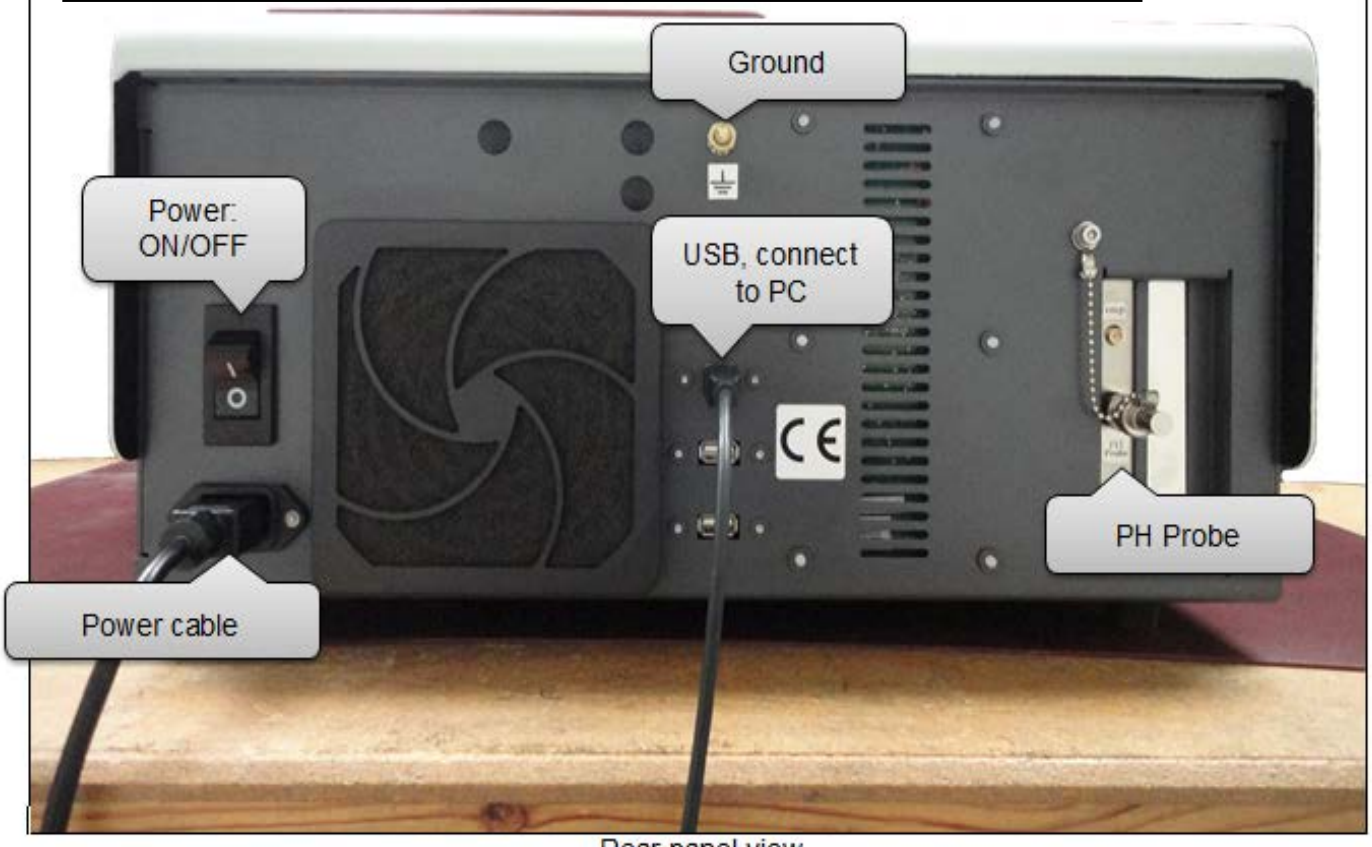
cannot be made with the instrument and software. Go to brookhaveninstruments.com to request a full license.

5. Plug the instrument in to the computer via the included USB cable. The instrument drivers will be automatically installed

6. The NanoBrook is now be ready to use.

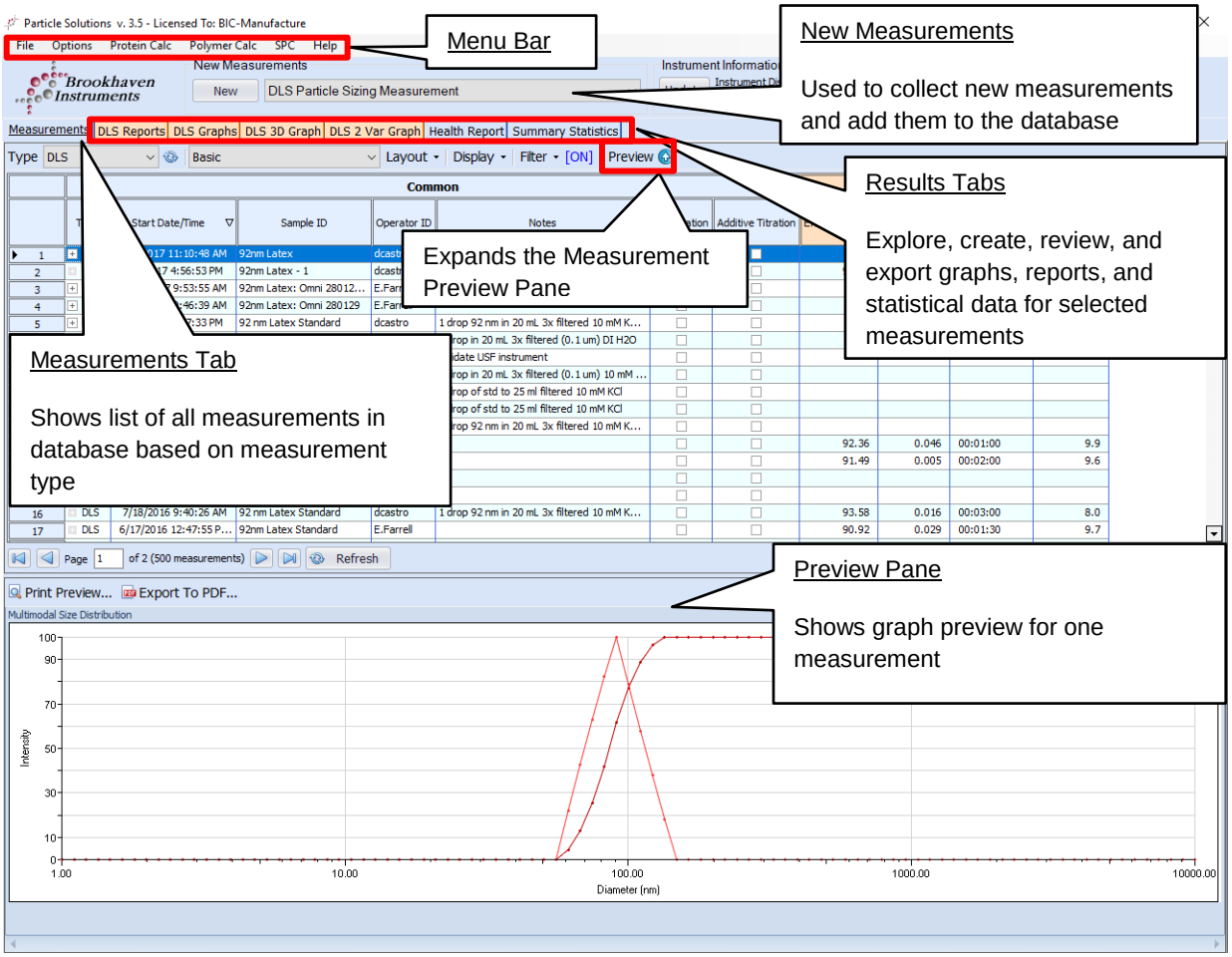
Please Note

Due to the nature of the measurements performed by the instrument, its precision electronics could be susceptible to electrical fast transients/bursts. The user should make sure that the instrument is properly grounded and no electrical equipment capable of causing electrical transients is connected to the same power circuit. In case if the power line is noisy (e.g. in industrial environment) and there is no possibility to connect the instrument to a stable power line – the use of an external UPS is recommended.



Rear panel view

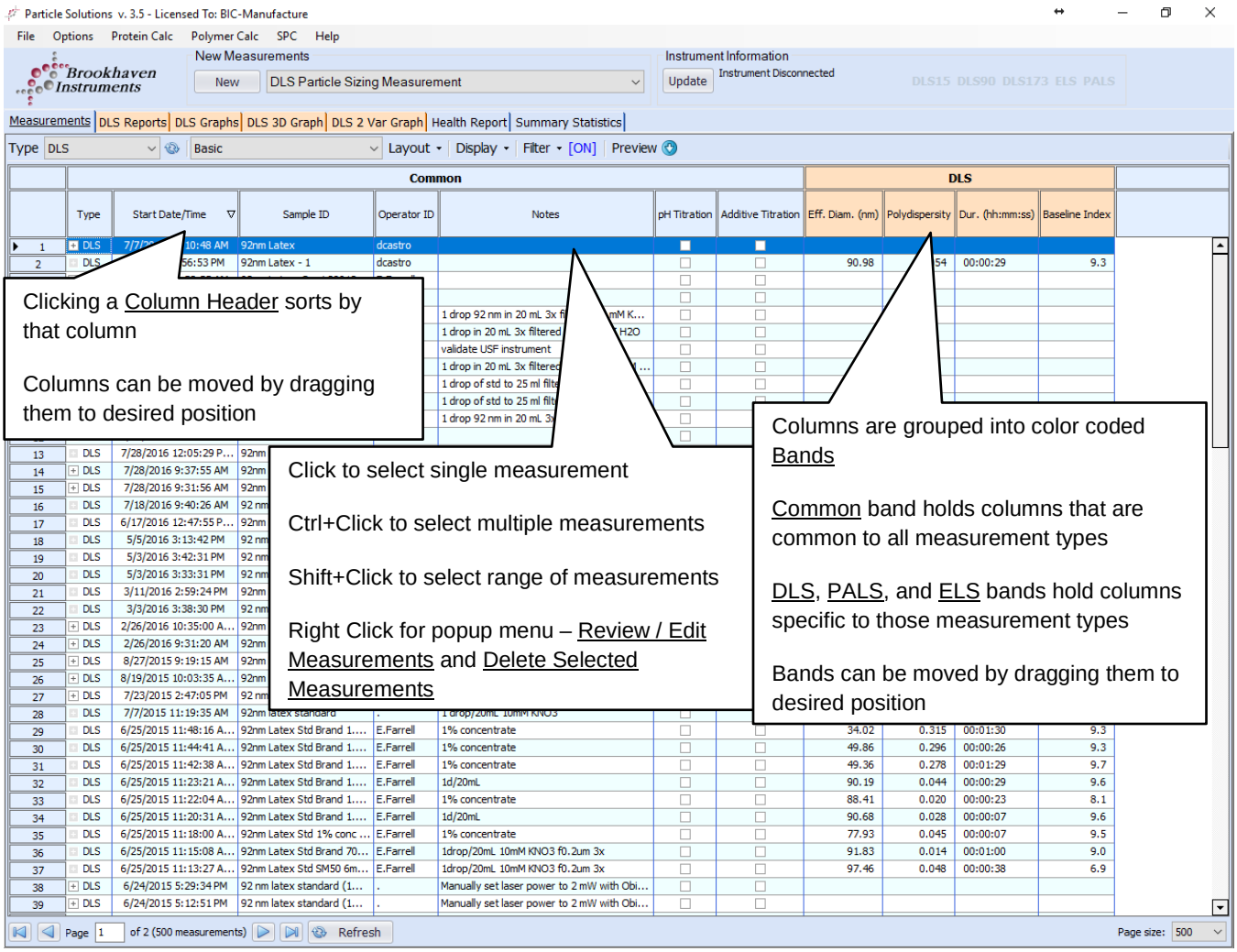
Overview of Main Software Window



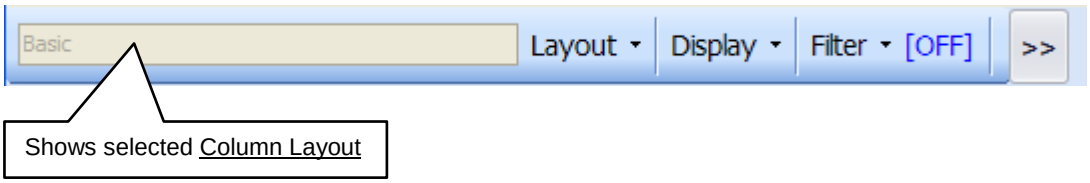
Measurements Pane

Overview

Note: Preview Pane is hidden for clarity



Menus

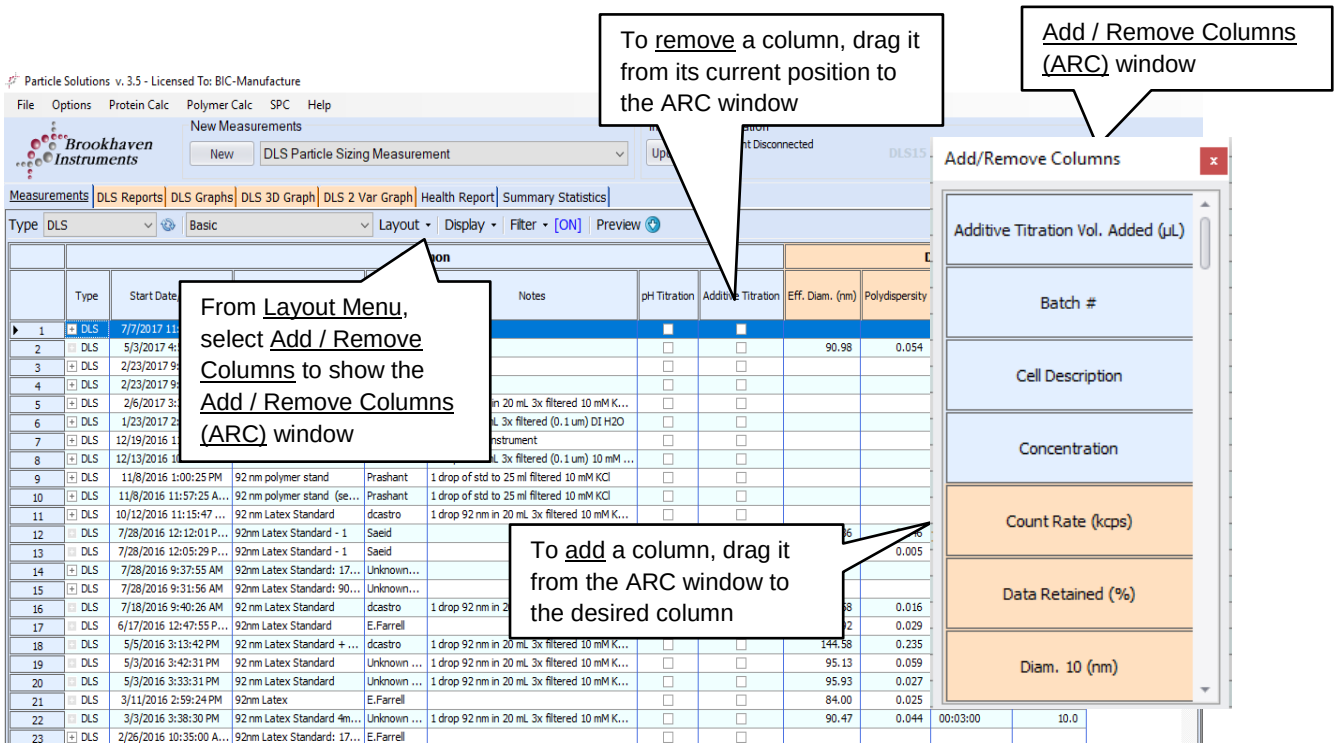


Layout Menu

The Layout Menu is used to manage column layouts in the Measurements Pane.

Load…	Loads a predefined Column Layout file. Predefined files are Basic, Advanced, All Columns, etc.
Save As…	Saves the current column layout to a file
Add / Remove Columns…	Shows the Add / Remove Columns (ARC) window.
Show All Columns	Shows all the columns defined for selected measurements

Adding / Removing Columns



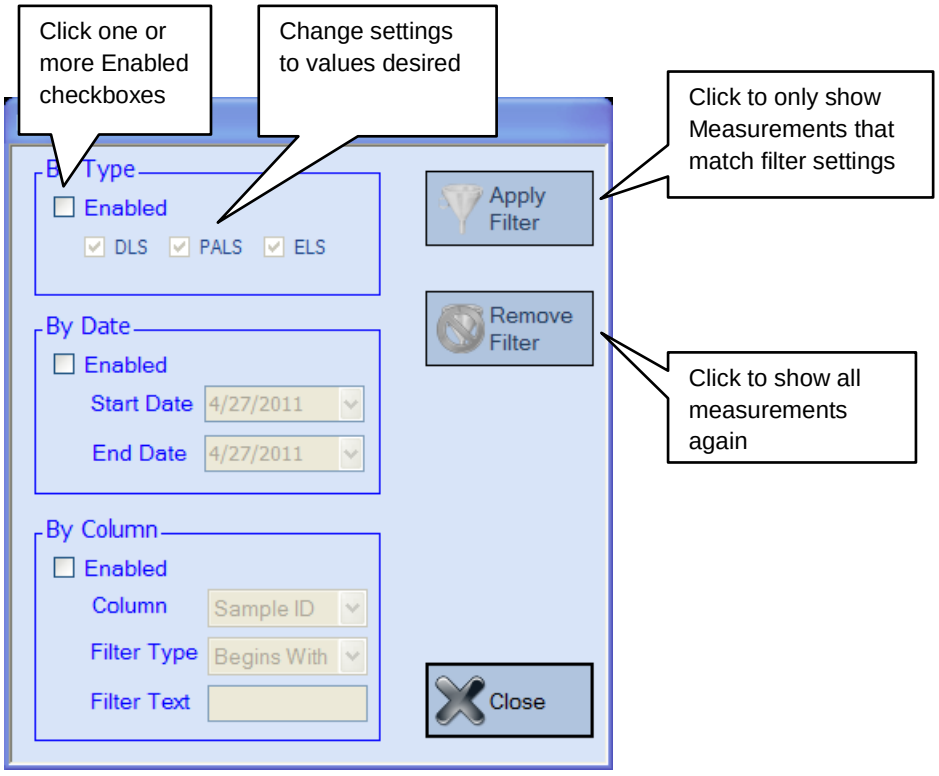
Display Menu

All Measurements	Displays all measurements in the database
Selected Measurements Only	Only displays measurements that were previously selected by the user

Filter Menu

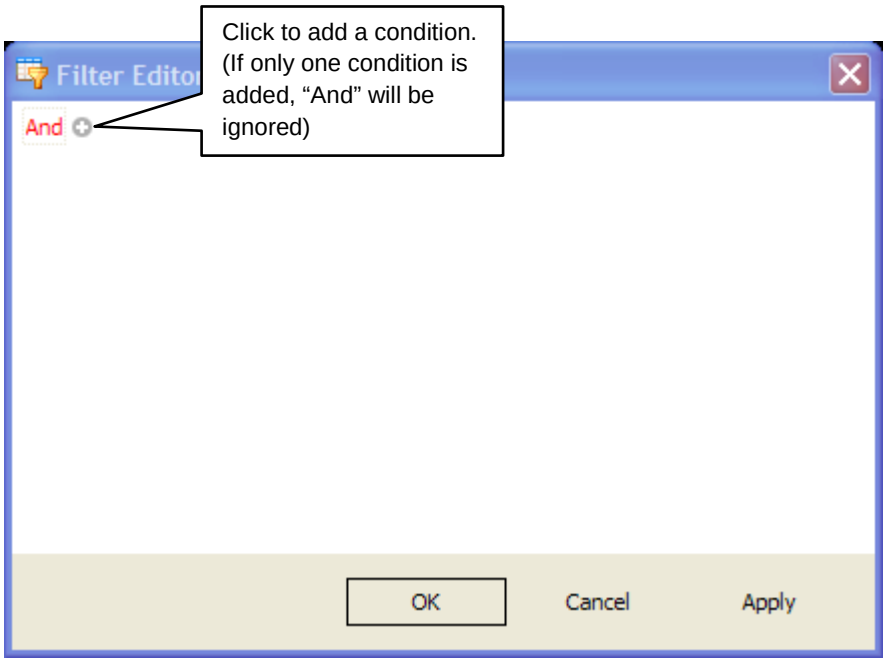
Basic Filter	Shows a filter window designed for basic filtering of measurements – by type, by date, and by column
Advanced Filter	Shows a filter window designed for advanced filtering of measurements. Complex filters can be created using any columns, ranges of column values, and logical operators
Remove	Removes all filtering – both basic and advanced

Basic Filter

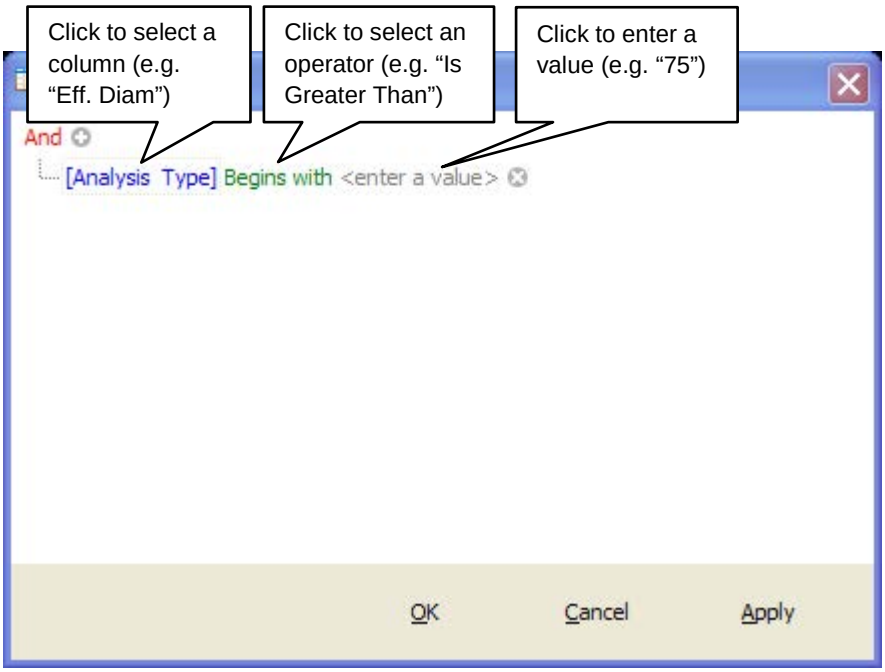


Advanced Filter

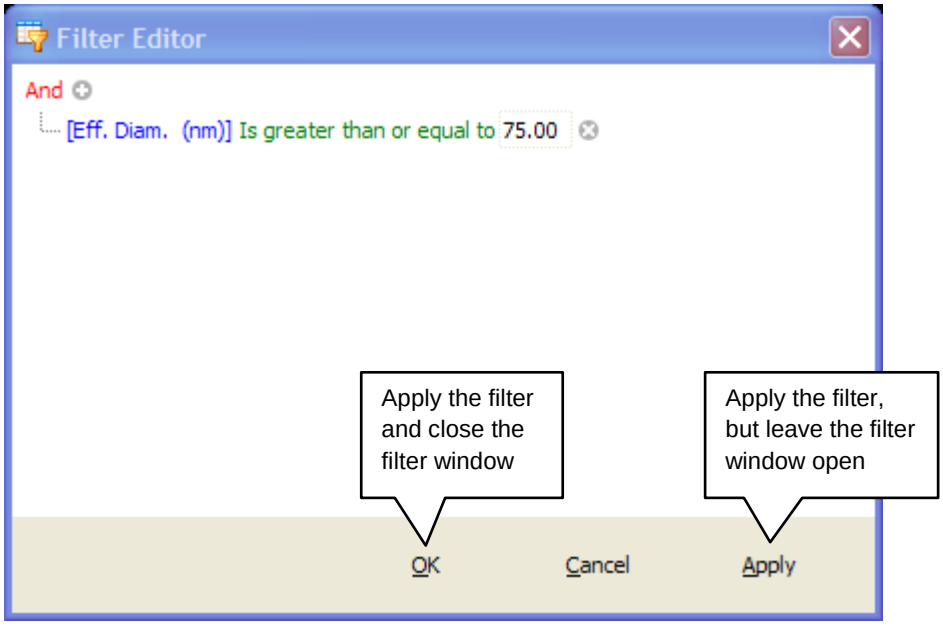
Step 1



Step 2



Step 3


Filter Editor
And
[Eff. Diam. (nm)] Is greater than or equal to 75.00
Apply the filter and close the filter window
Apply the filter, but leave the filter window open
OK
Cancel
Apply

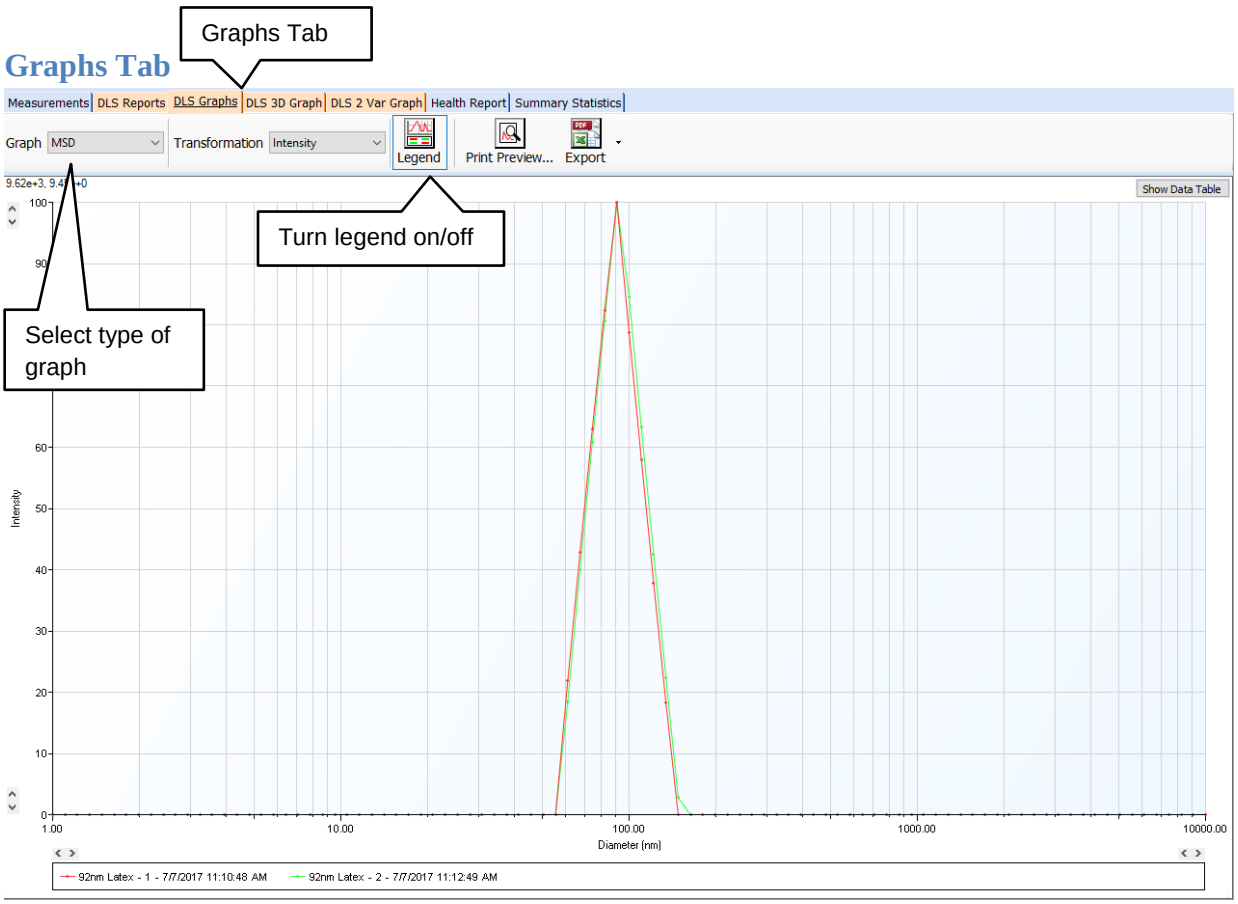
Results

Overview

Reports Tab Menu

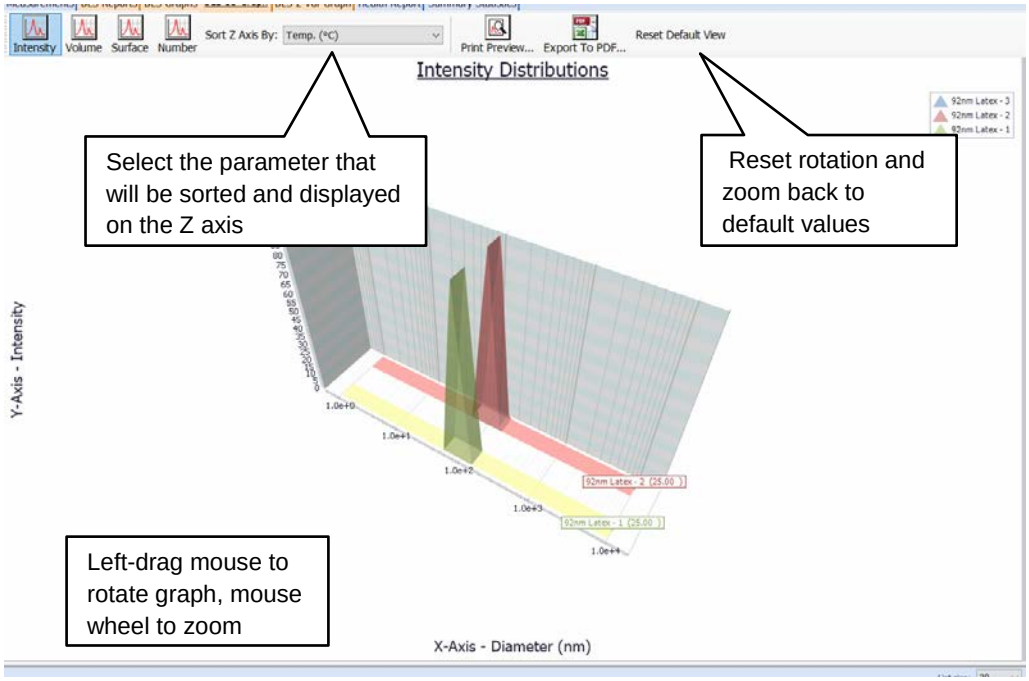
Print Preview…	Opens the Print Preview window. Reports can then be printed from this window
Export to PDF…	Exports the reports to a file, in PDF format
Report Designer…	Opens the Report Desgner window

Graphs Tab



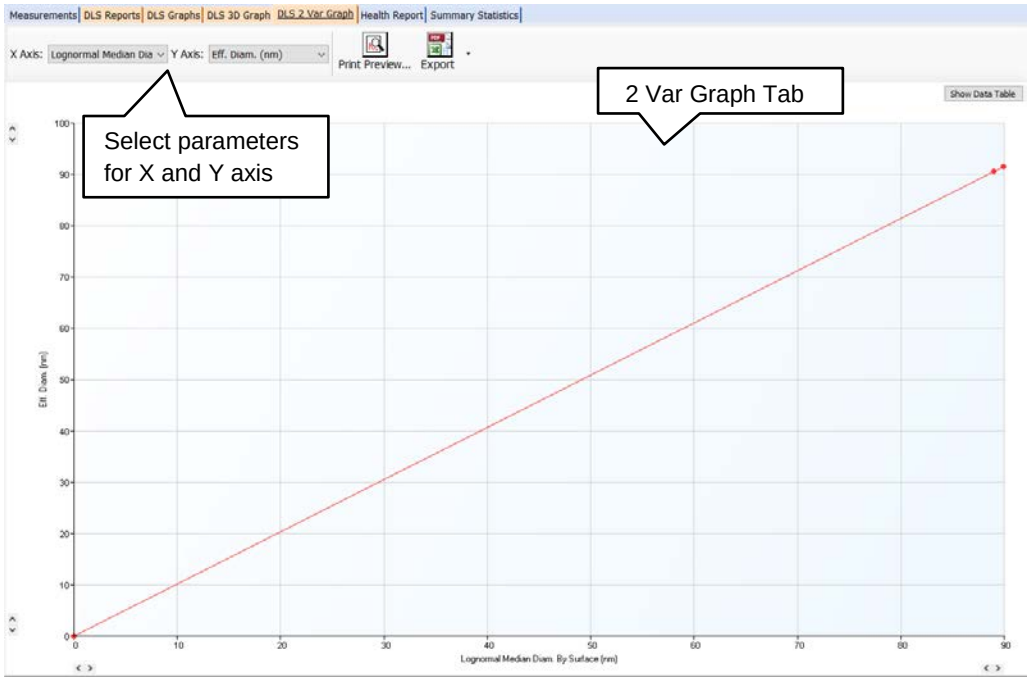
DLS 3D Graph Tab

Note: Only shown when multiple DLS measurements selected



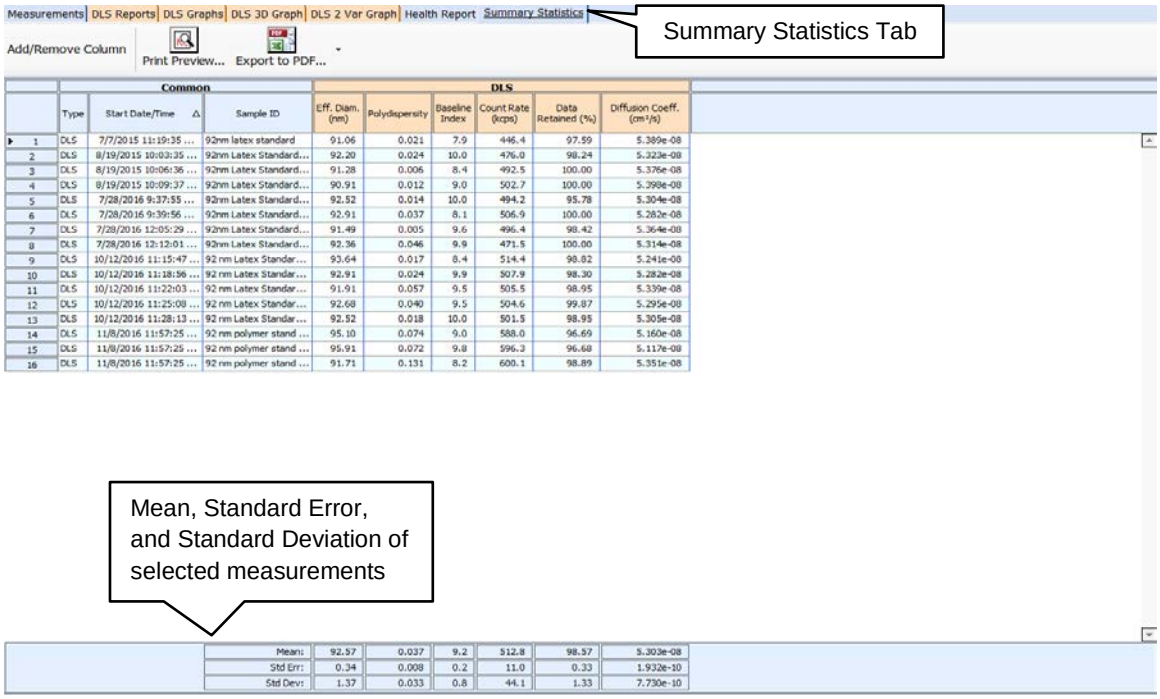
2 Var Graph Tab

Note: Only shown when multiple measurements are selected



Summary Statistics Tab

Note: Only shown when multiple measurements are selected

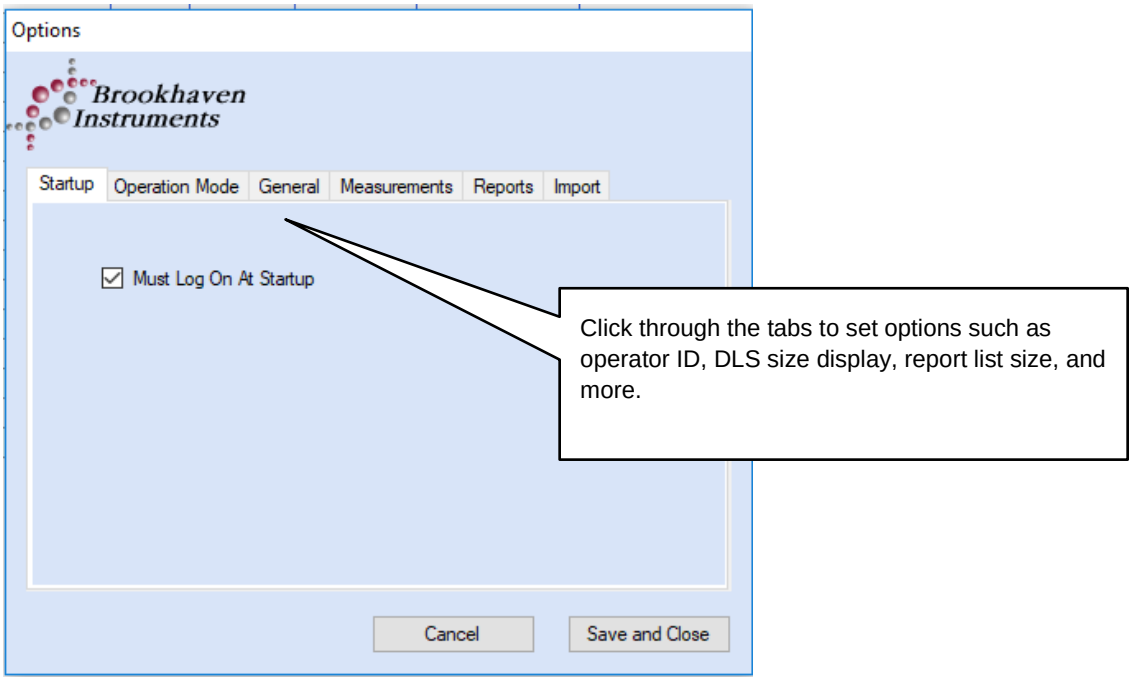


Menu Bar

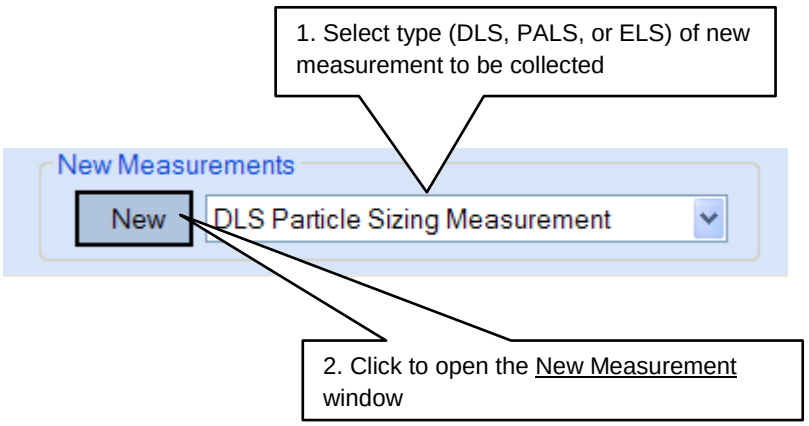
File Menu

Import Legacy Database…	Imports measurements made with earlier versions of Brookhaven software into the Particle Solutions database
Export	Exports the selected measurements to Excel or CSV text files
Save Archive File…	Saves selected measurements to an archive file, using a proprietary binary format
Load Archive File…	Load a saved archive file, and add it's measurements to the database
Rebuild Database…	rebuild and re-index the Particle Solutions database
Options…	Show the Options window

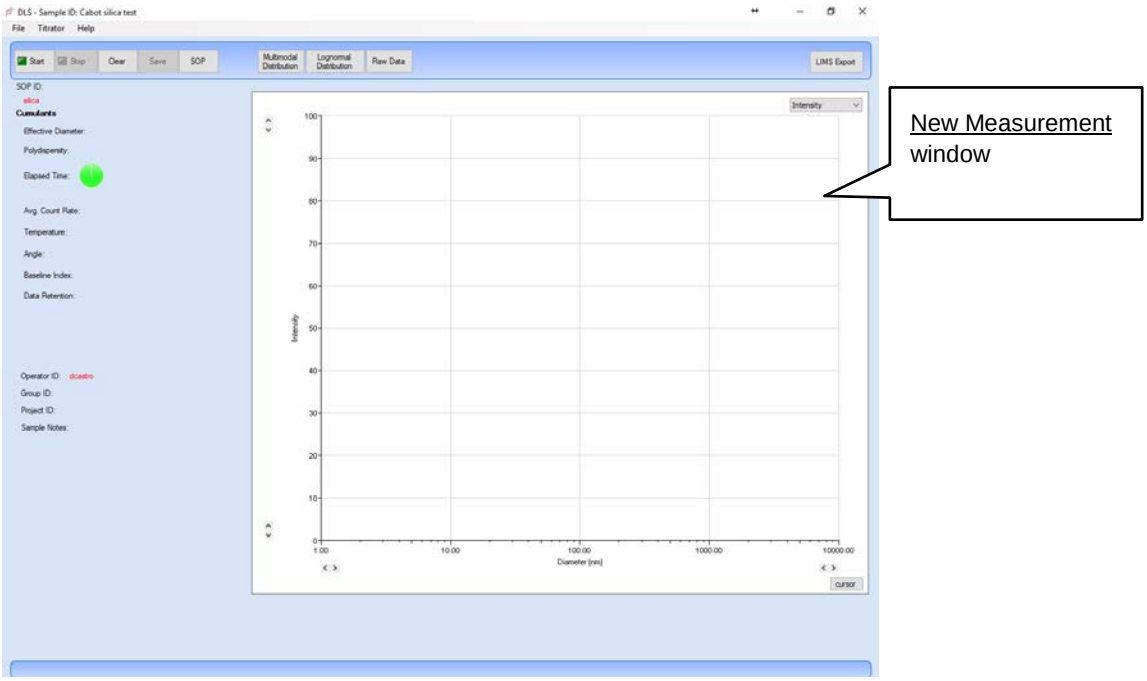
Options



New Measurements



New Measurement Window



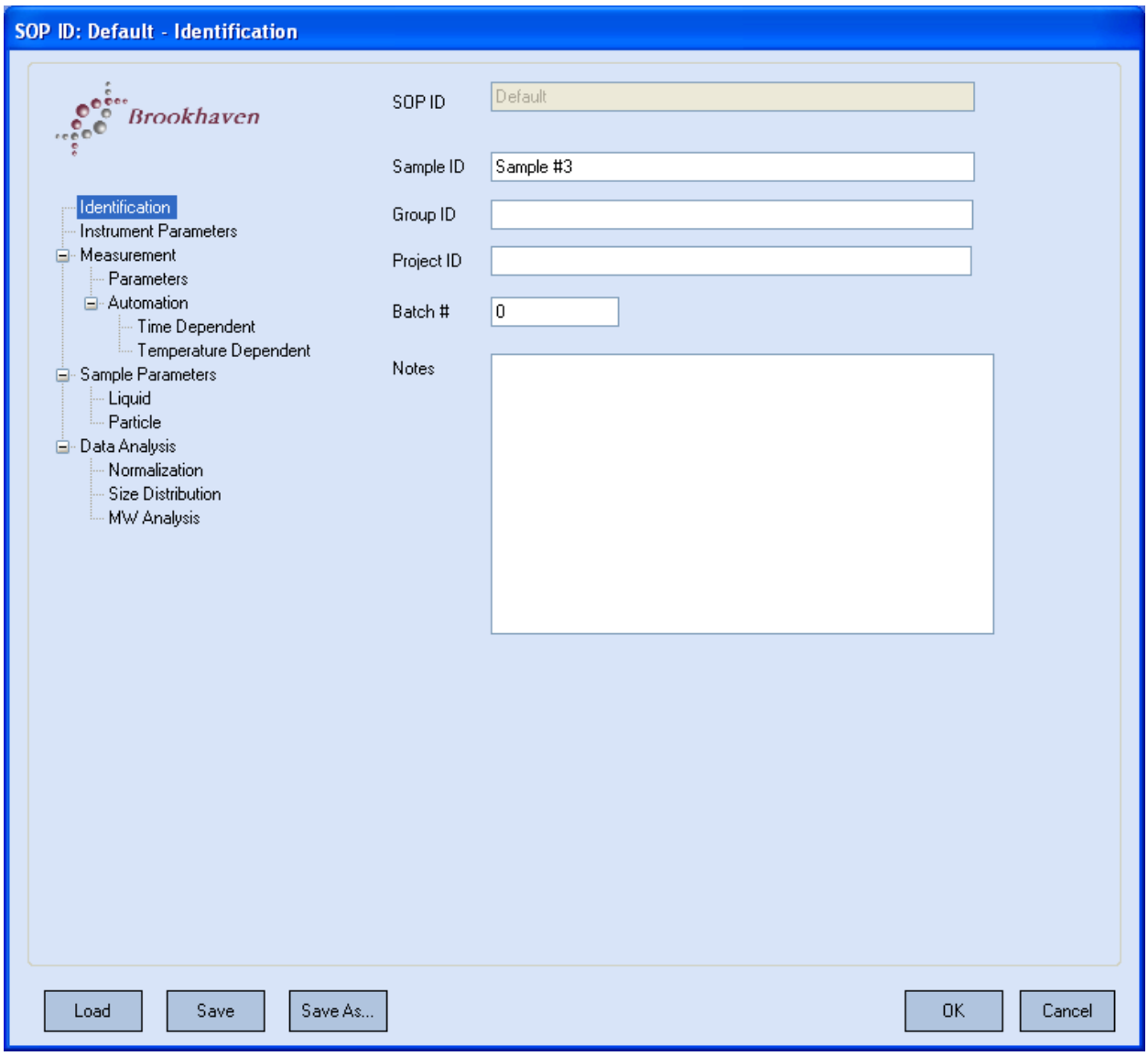
The DLS measurement window is used to collect new measurements.

The Start and Stop buttons are used to initiate and pause a measurement's progress. When Start is pressed, a sequence of one or more measurements will be collected as defined in the measurement SOP.

The Clear button can be used to clear the current measurement. Once a measurement sequence is completed, the final measurement from the sequence will be visible. It must be cleared before an operator can begin a new measurement sequence.

You may choose to view either size distribution results or raw correlation function data using the buttons on the right side of the window.

DLS SOP Identification Window

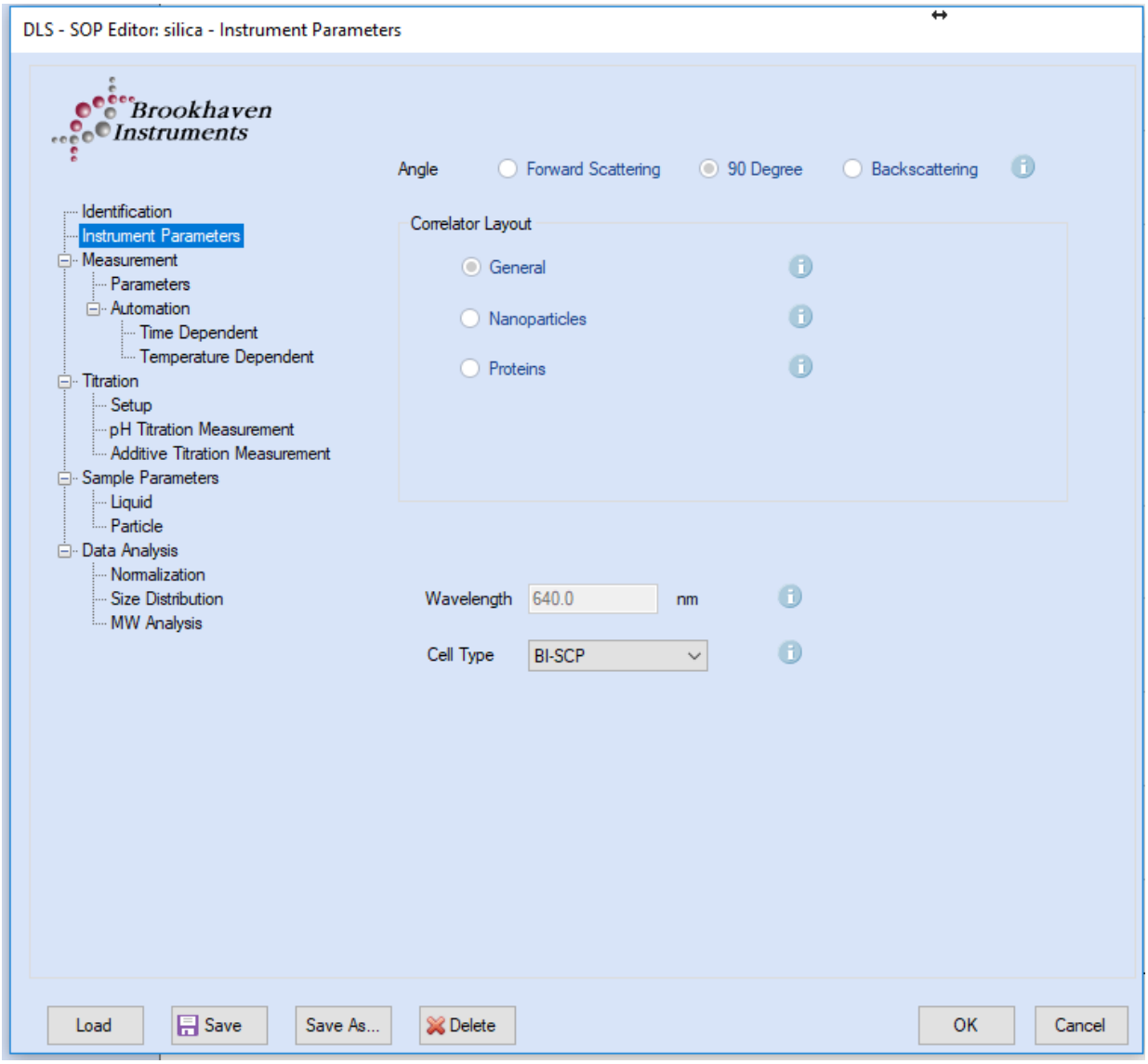


Throughout the SOP, there are information icons next to every parameter. Click on these to see more details about the field and how to make a choice. When in doubt, click the “i”!

Measurement Identification parameters are indicated the identification window.

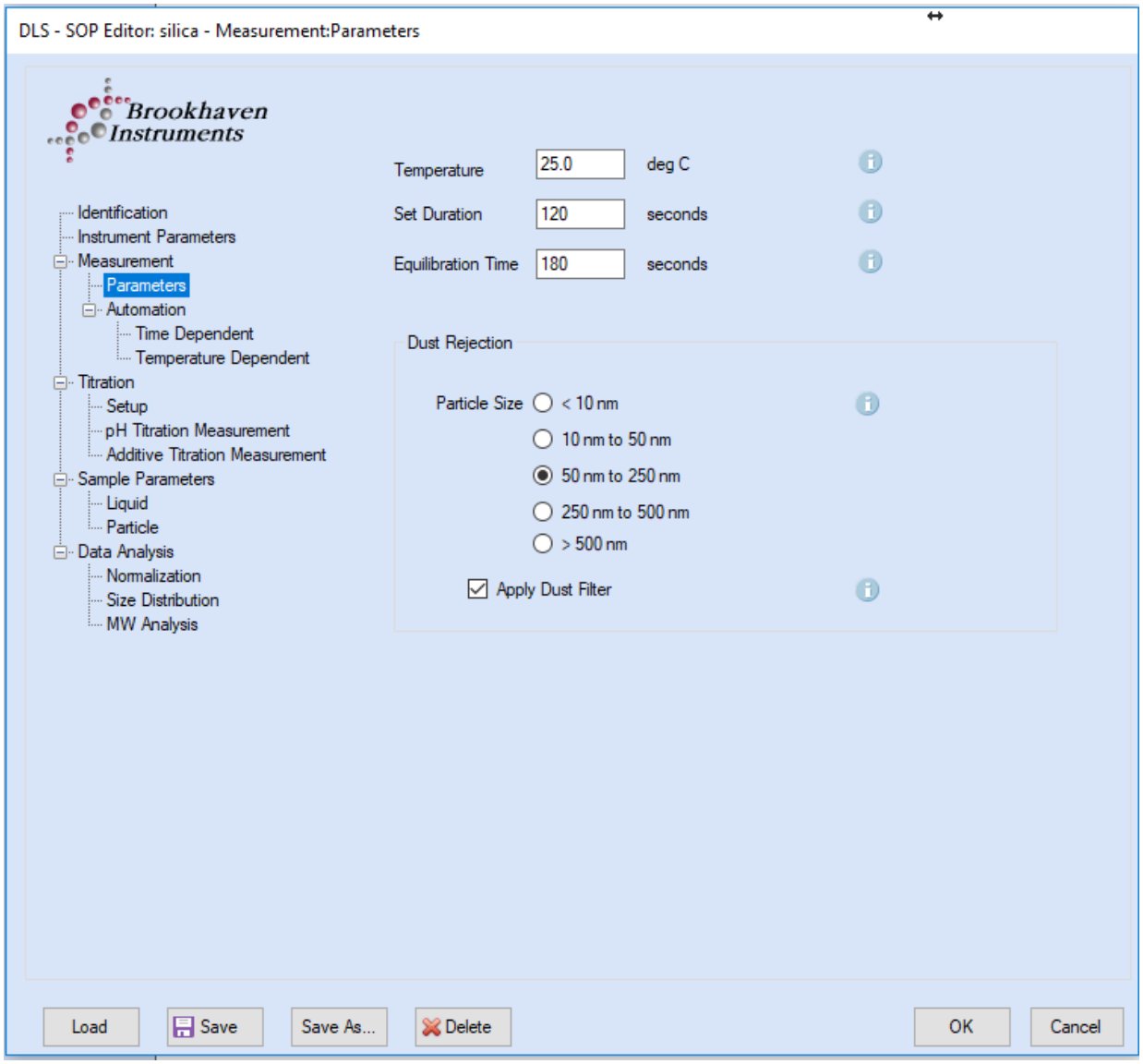
The SOP ID is the name of the SOP file that is currently in effect. You may load and save SOP files as needed before a measurement sequence is started. Once started, you may not change the SOP to a different file.

DLS SOP Instrument Parameters



Instrument parameters are indicated in this window. This is where the scattering angle and correlator layout can be chosen.

DLS SOP Measurement : Parameters Window



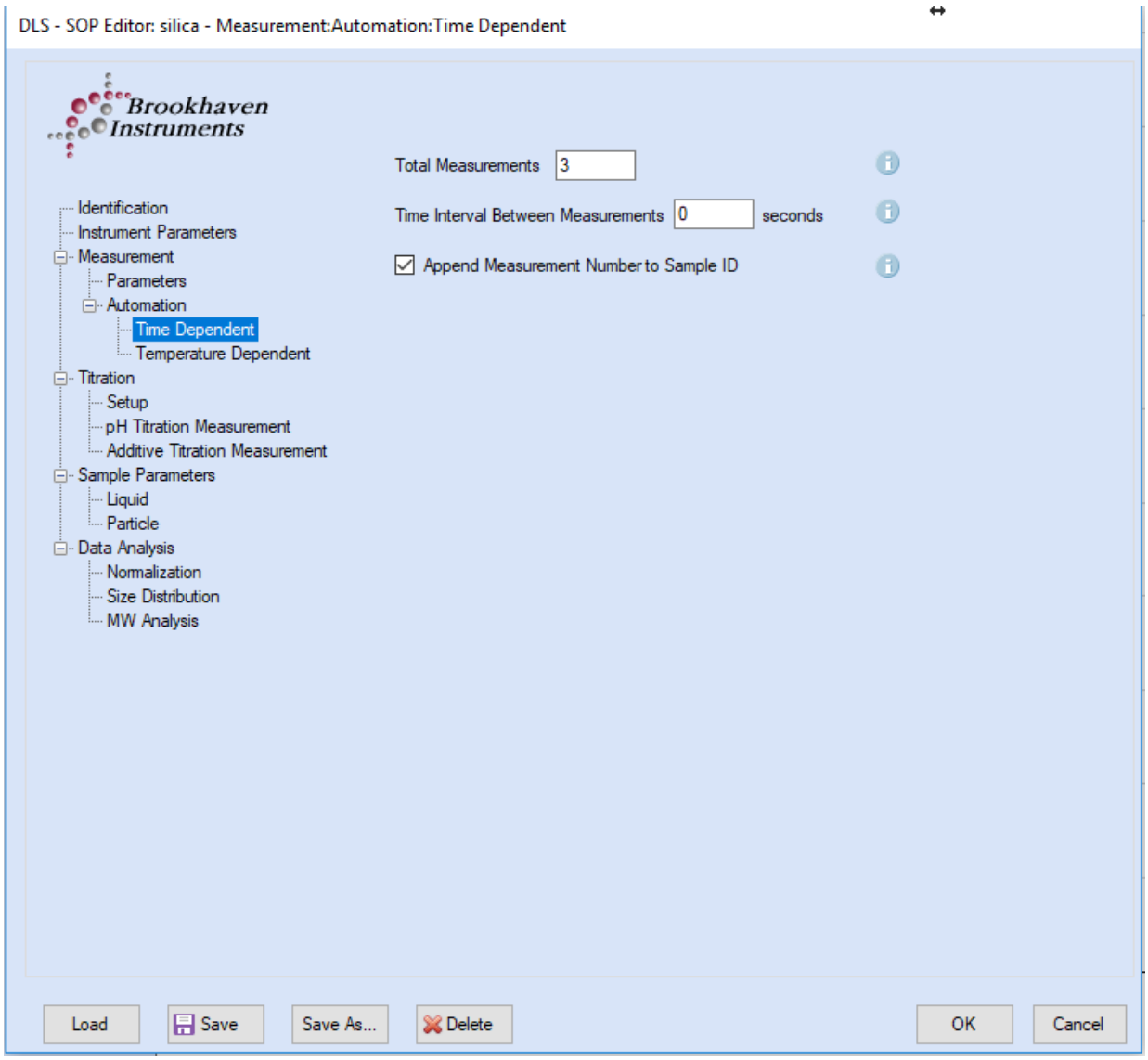
Use Set Duration to indicate the measurement duration.

The Equilibration Time may be used to invoke a delay after the temperature has been stabilized, before the actual measurement data acquisition begins.

The dust rejection filter is used to eliminate any signal fluctuations that are characteristic of dust, based on the expected size range of the particles being measured.

Once a measurement sequence is started, many of the SOP parameters, such as measurement duration, cannot be changed and are used for the entire measurement sequence.

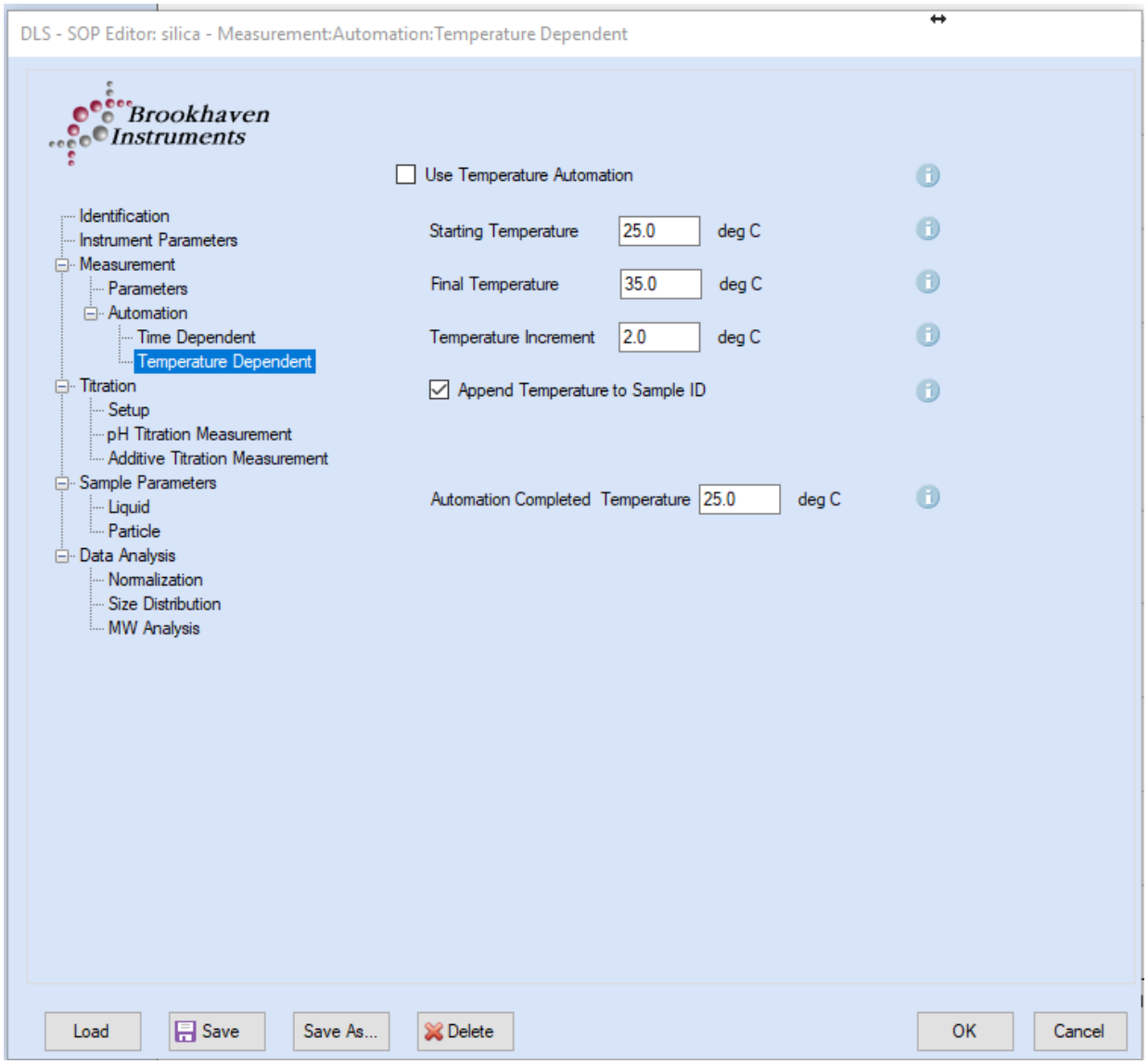
DLS SOP Measurement : Automation : Time Dependent Window



In this window, you may indicate the number of measurements to make using Total Measurements.

You may also indicate a time interval between measurements. The interval is the number of seconds between the end of one measurement and the start of a new measurement.

DLS SOP Measurement : Automation : Temperature Dependent Window



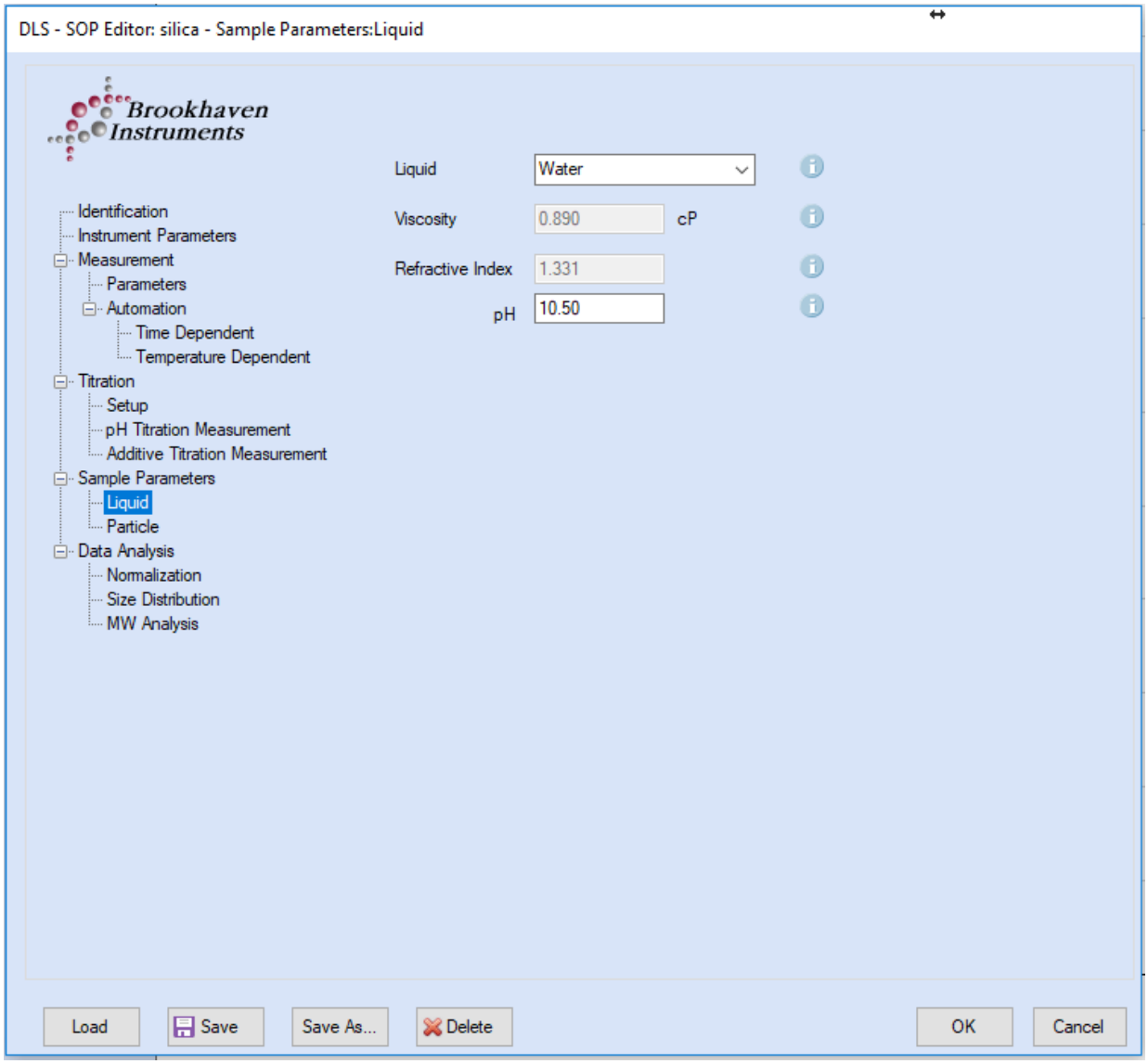
In this window, you may indicate a temperature automation sequence. You must indicate a start, and final temperature, and a temperature increment.

The Use temperature automation checkbox must also be checked in order to use the temperature automation settings in the measurement sequence.

Note that the number of measurements requested in the Measurement Automation : Time Dependent Window will be made for each temperature in the temperature automation sequence.

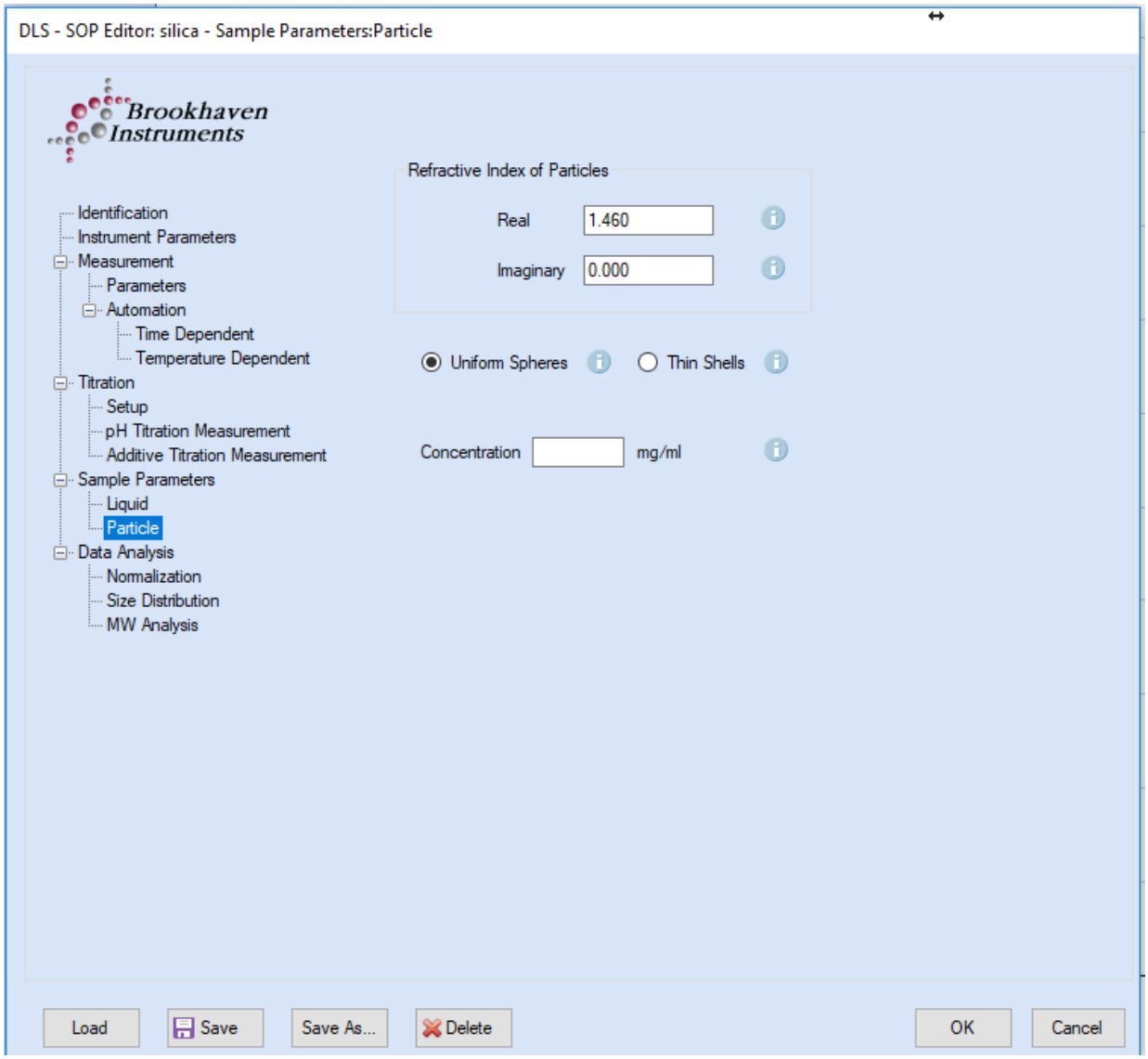
The final temperature that the instrument should return to at the end of the measurement sequence is also indicated on this page.

DLS SOP Sample Parameters : Liquid Window



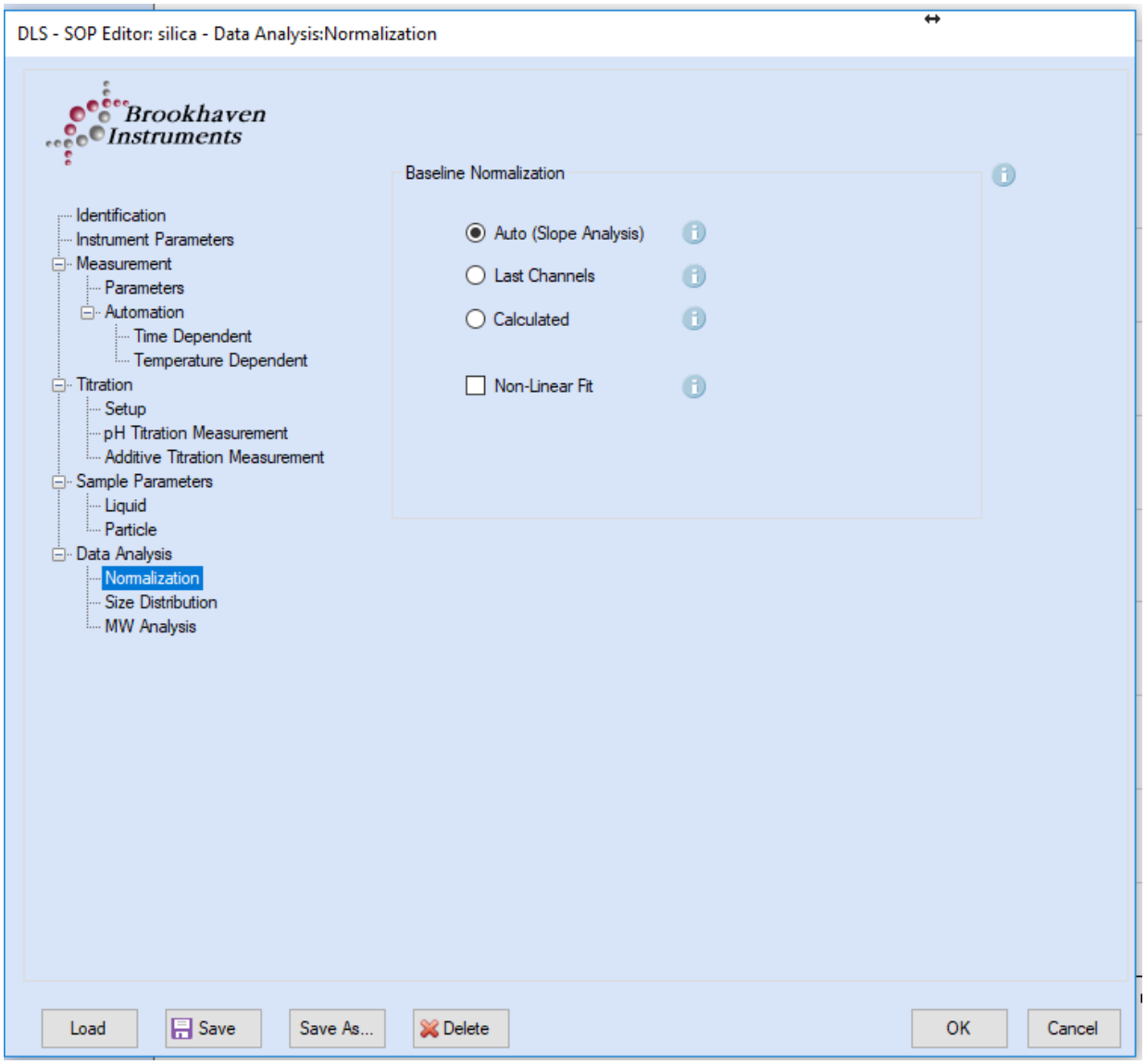
In this window the sample liquid, viscosity and refractive index are indicated. Here, liquids are preloaded with their parameters, or one can type in their own liquid and provide the respective constants manually.

DLS SOP Sample Parameters : Particle Window



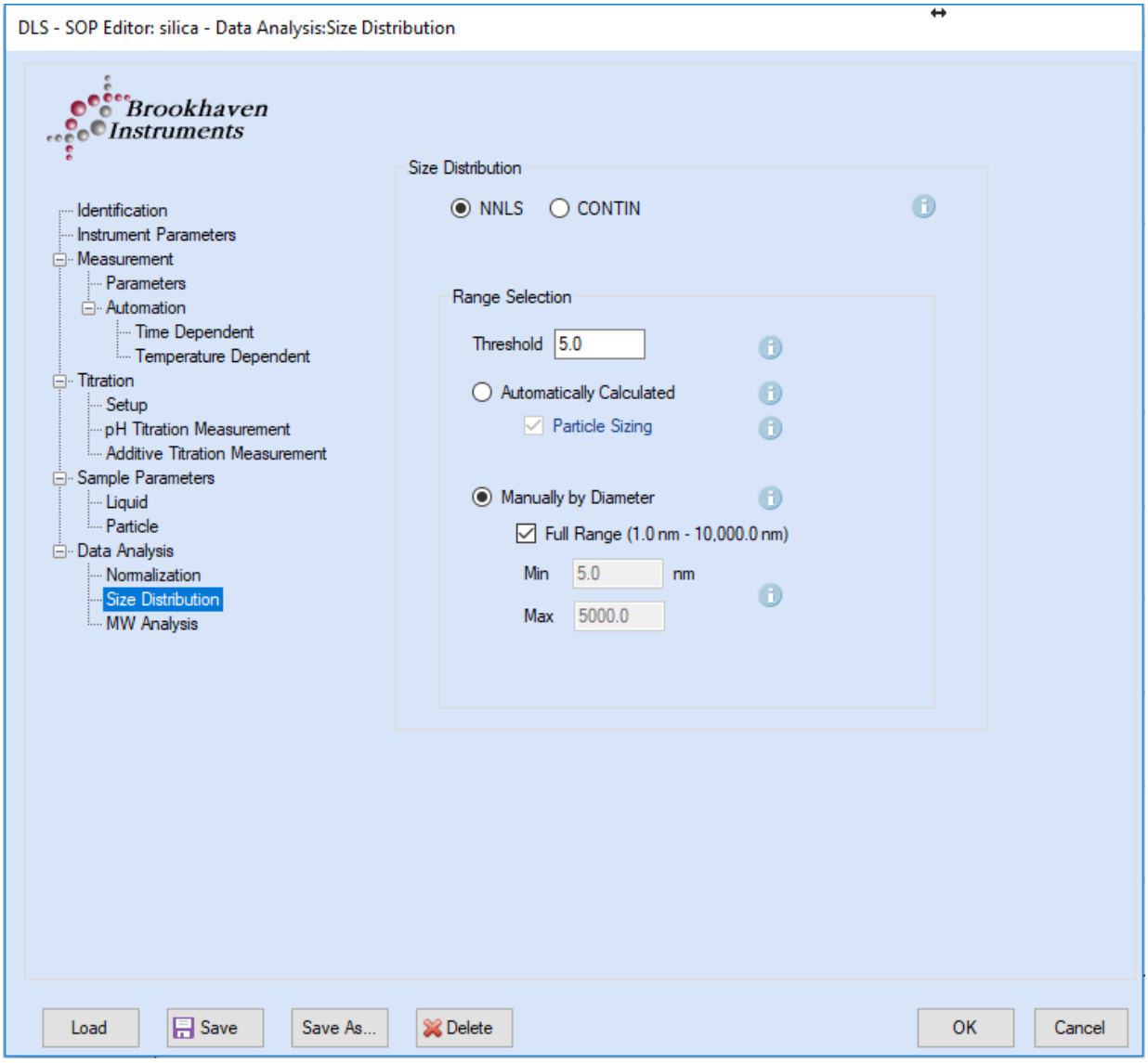
In this window, particle characteristics are defined.

DLS SOP Data Analysis : Normalization



In this window the correlation function baseline normalization selection is indicated.

DLS SOP Data Analysis : Size Distribution

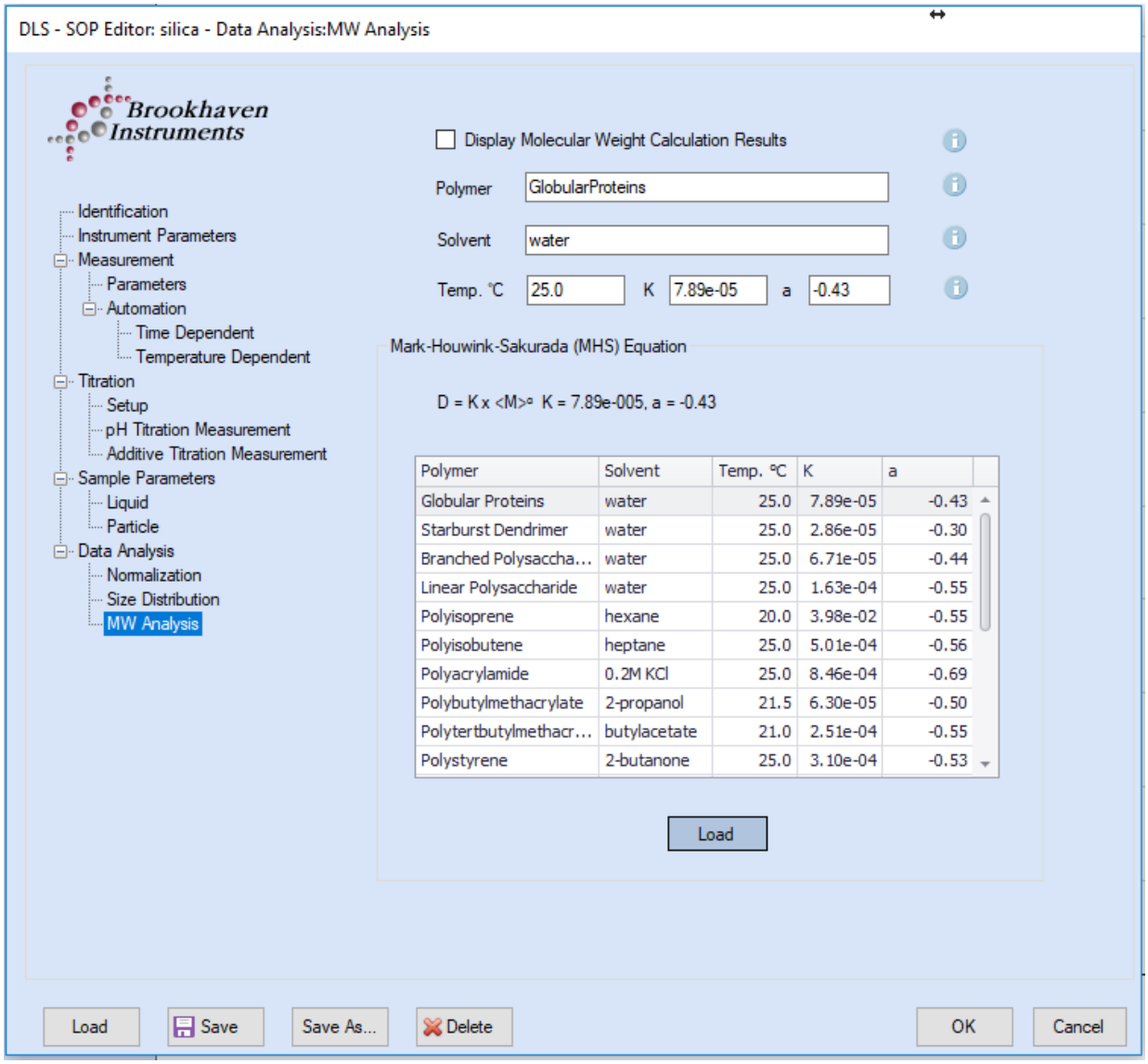


In this window the size distribution settings are indicated. You may choose between NNLS or CONTIN algorithms.

The threshold can be used to indicate the minimum acceptable peak height, expressed as percentage of the maximum peak.

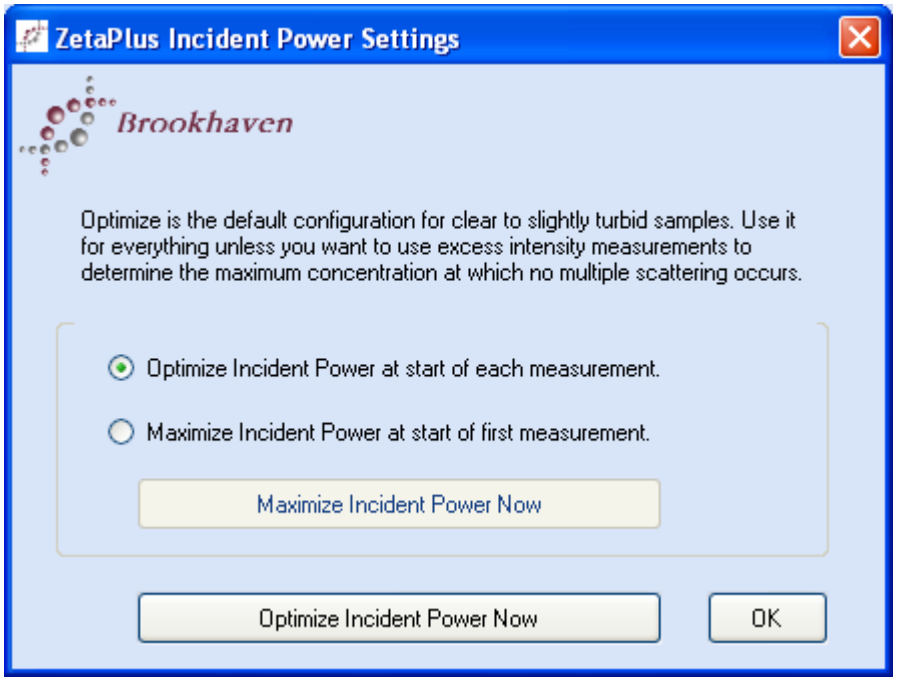
Automatic range selection can be used to invoke multiple calls to the distribution algorithm until an appropriate size range for the sample distribution is determined. Alternatively, a manual range for the distribution calculations may be used.

DLS SOP Data Analysis : Mw Analysis



In this window you may request the display of a calculated molecular weight.

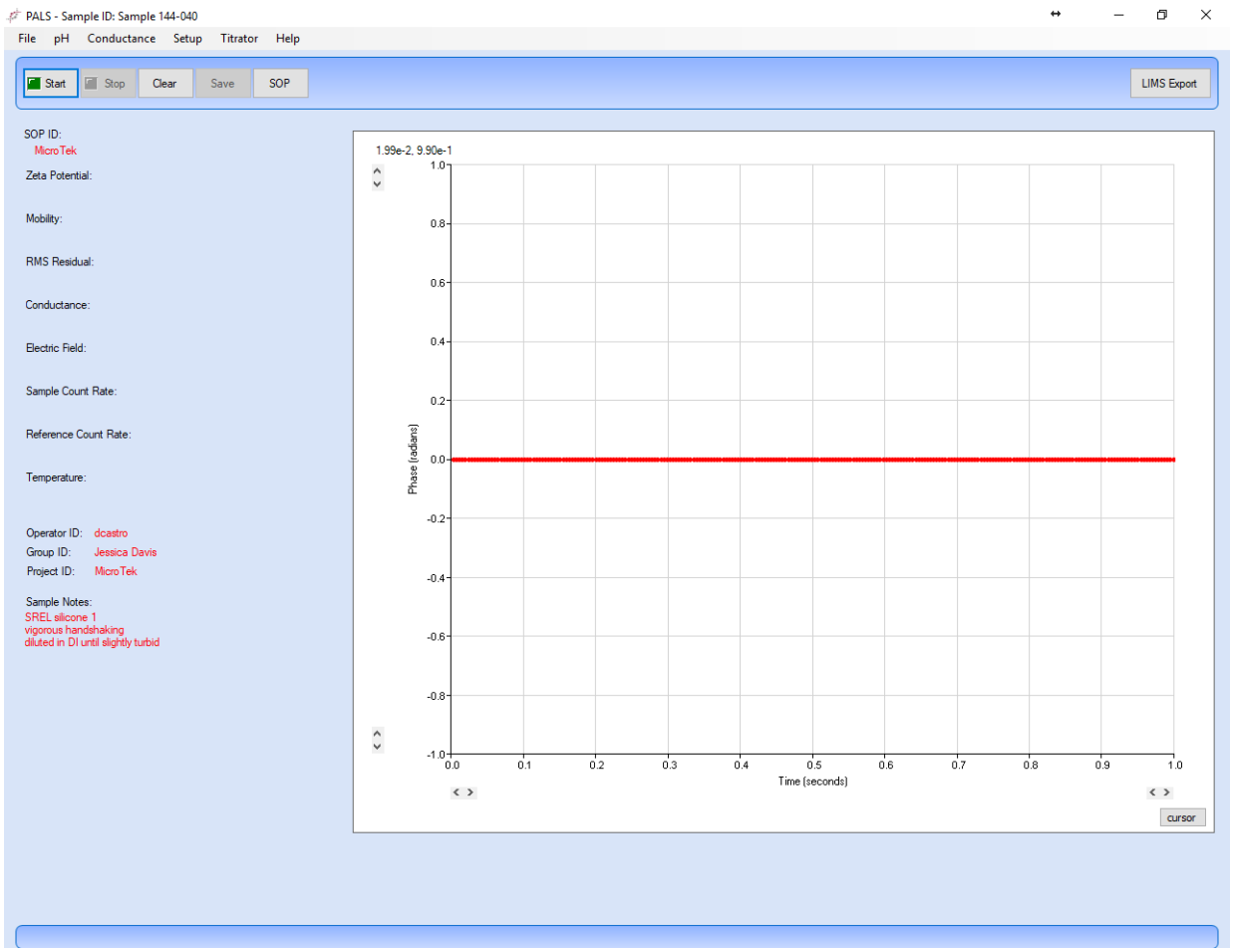
DLS New Measurement Window : Setup : Incident Power Settings



For DLS measurements, the Incident Power Settings for new measurements are indicated in this window.

This window can be accessed from the applications main window by selecting Setup\Incident Power Settings from the DLS measurement window's main menu.

PALS New Measurement Window



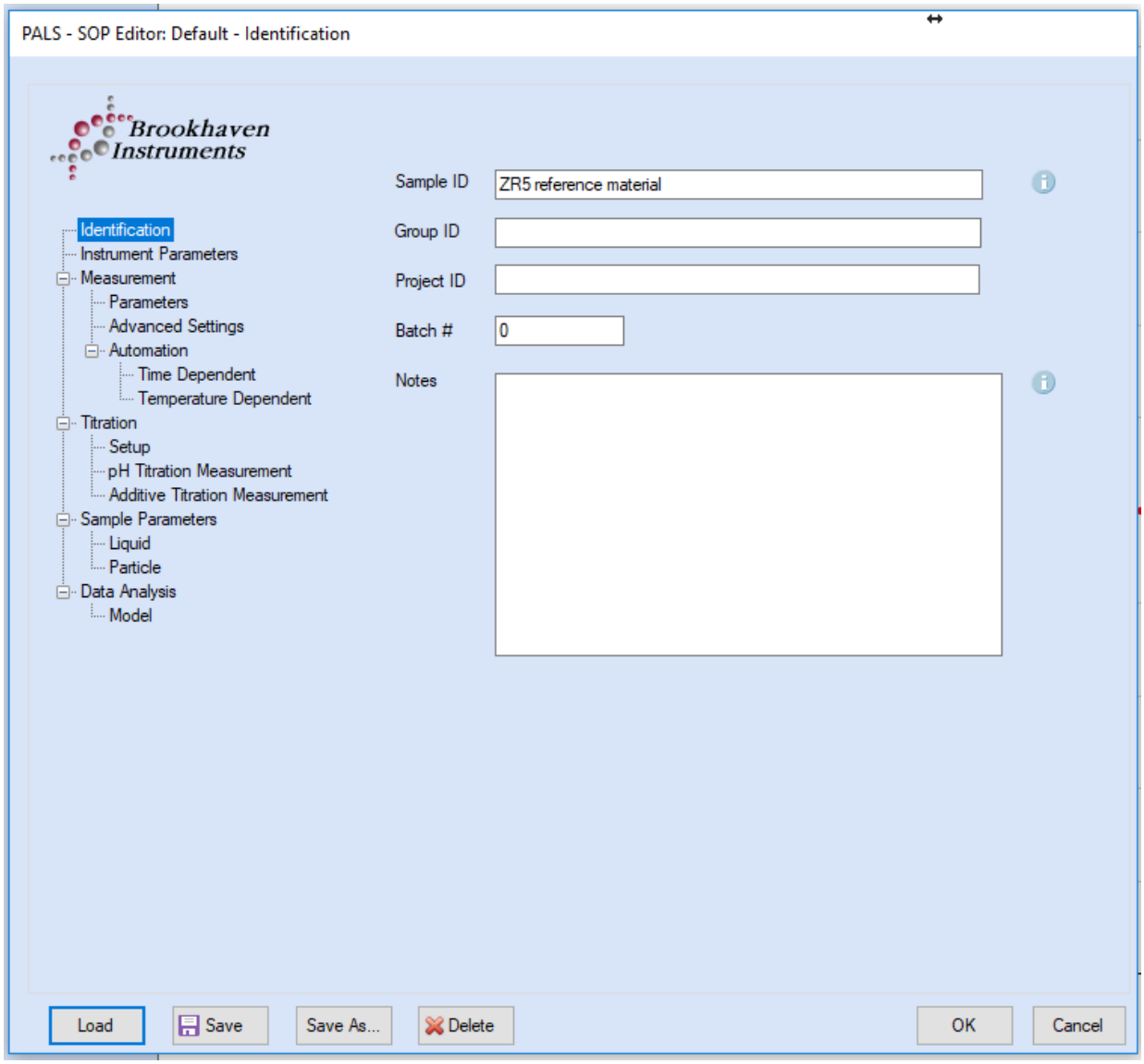
The PALS measurement window is used to collect new measurements.

The Start and Stop buttons are used to initiate and pause a measurement's progress. When Start is pressed, a sequence of one or more measurements will be collected as defined in the measurement SOP.

The Clear button can be used to clear the current measurement. Once a measurement sequence is completed, the final measurement from the sequence will be visible. It must be cleared before an operator can begin a new measurement sequence.

You may choose to view either size distribution results or raw correlation function data on the right side of the window.

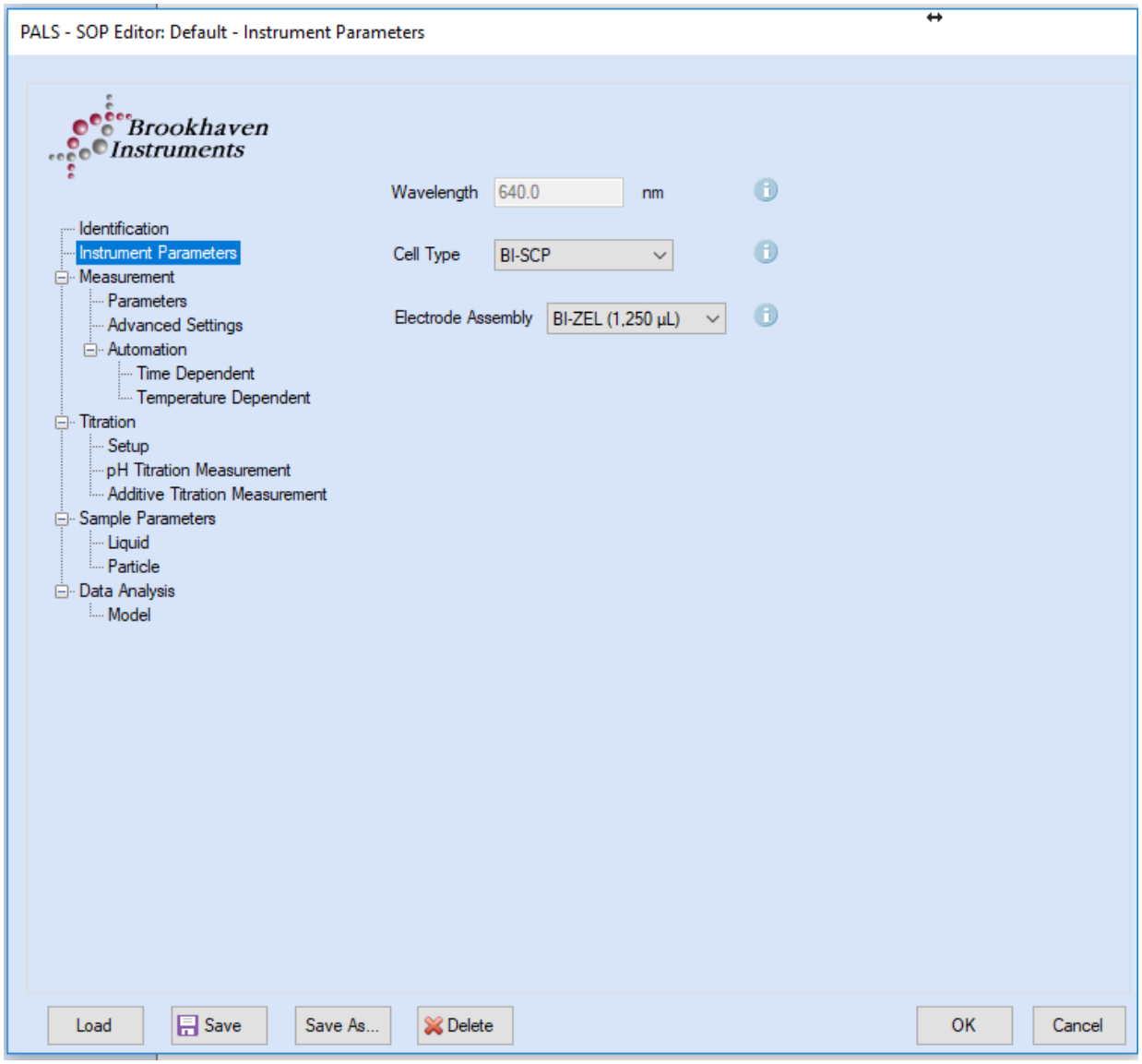
PALS SOP Identification Window



Measurement Identification parameters are indicated in the identification window.

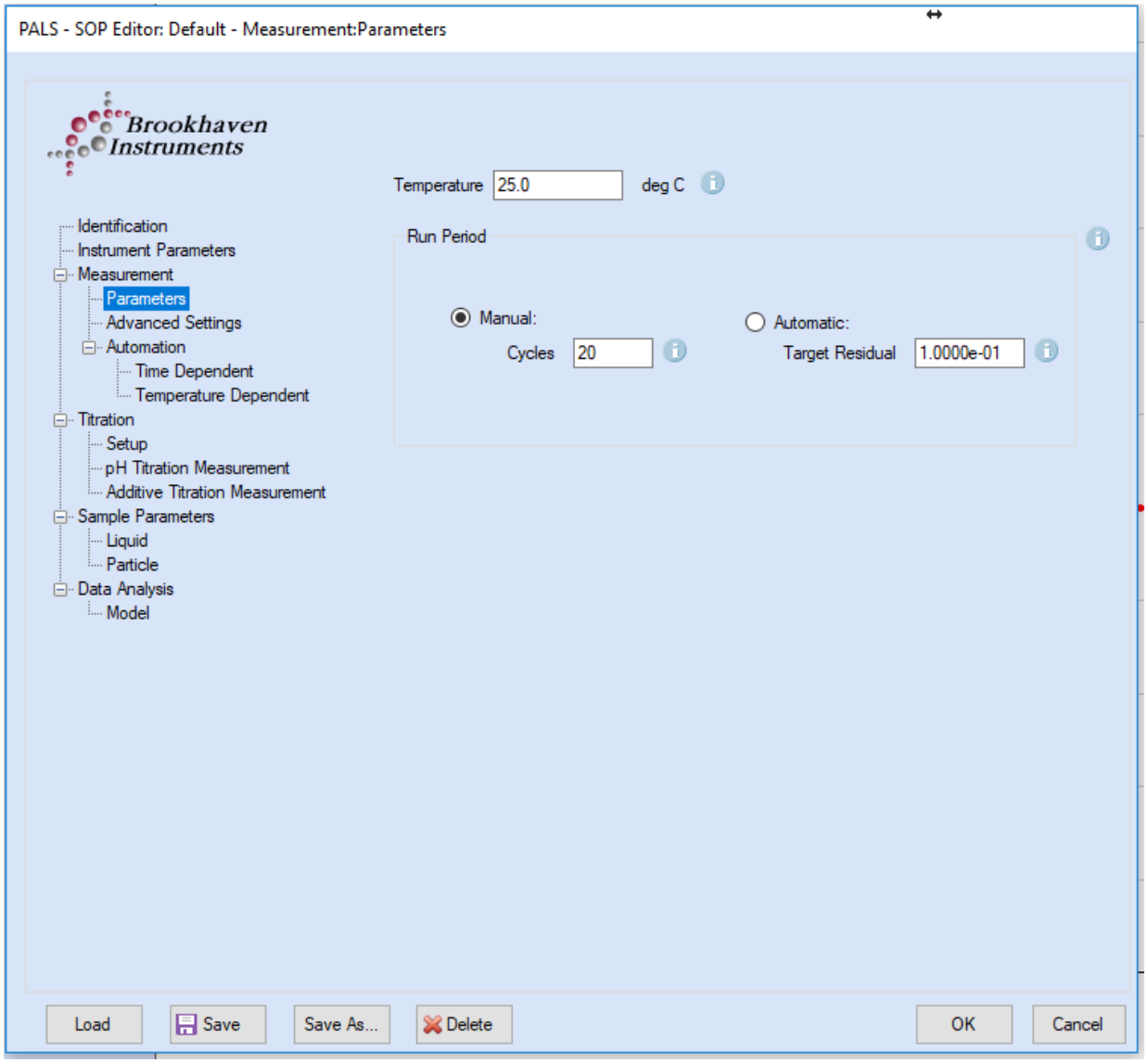
The SOP ID is the name of the SOP file that is currently in effect. You may load and save SOP files as needed before a measurement sequence is started. Once started, you may not change the SOP to a different file.

PALS SOP Instrument Parameters



Instrument parameters are indicated in this window.

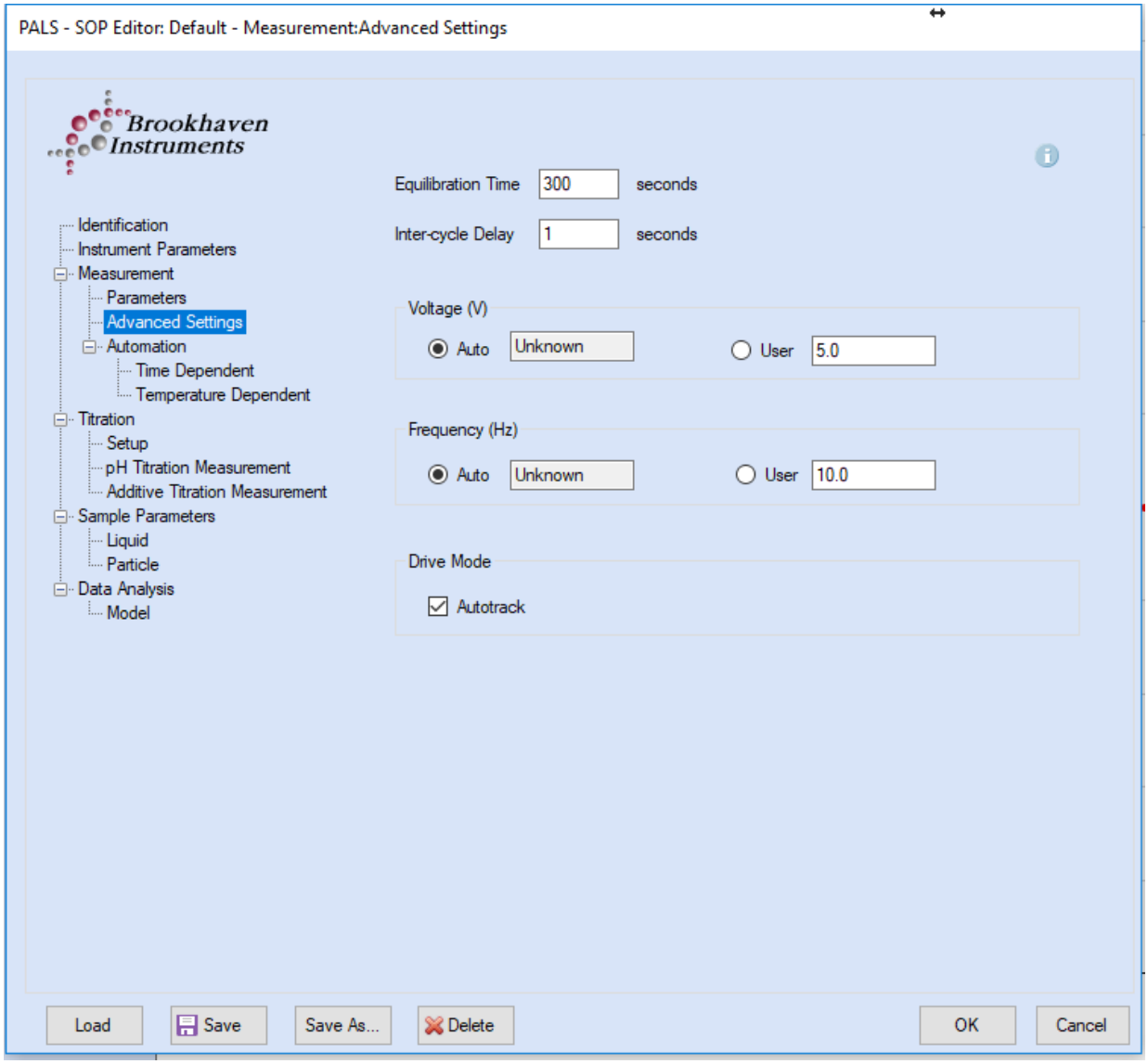
PALS SOP Measurement : Parameters Window



Use the Run Period settings to control the duration of the measurement.

Once a measurement sequence is started, many of the SOP parameters, such as measurement run period, cannot be changed and are used for the entire measurement sequence.

PALS SOP Measurement : Advanced Settings Window

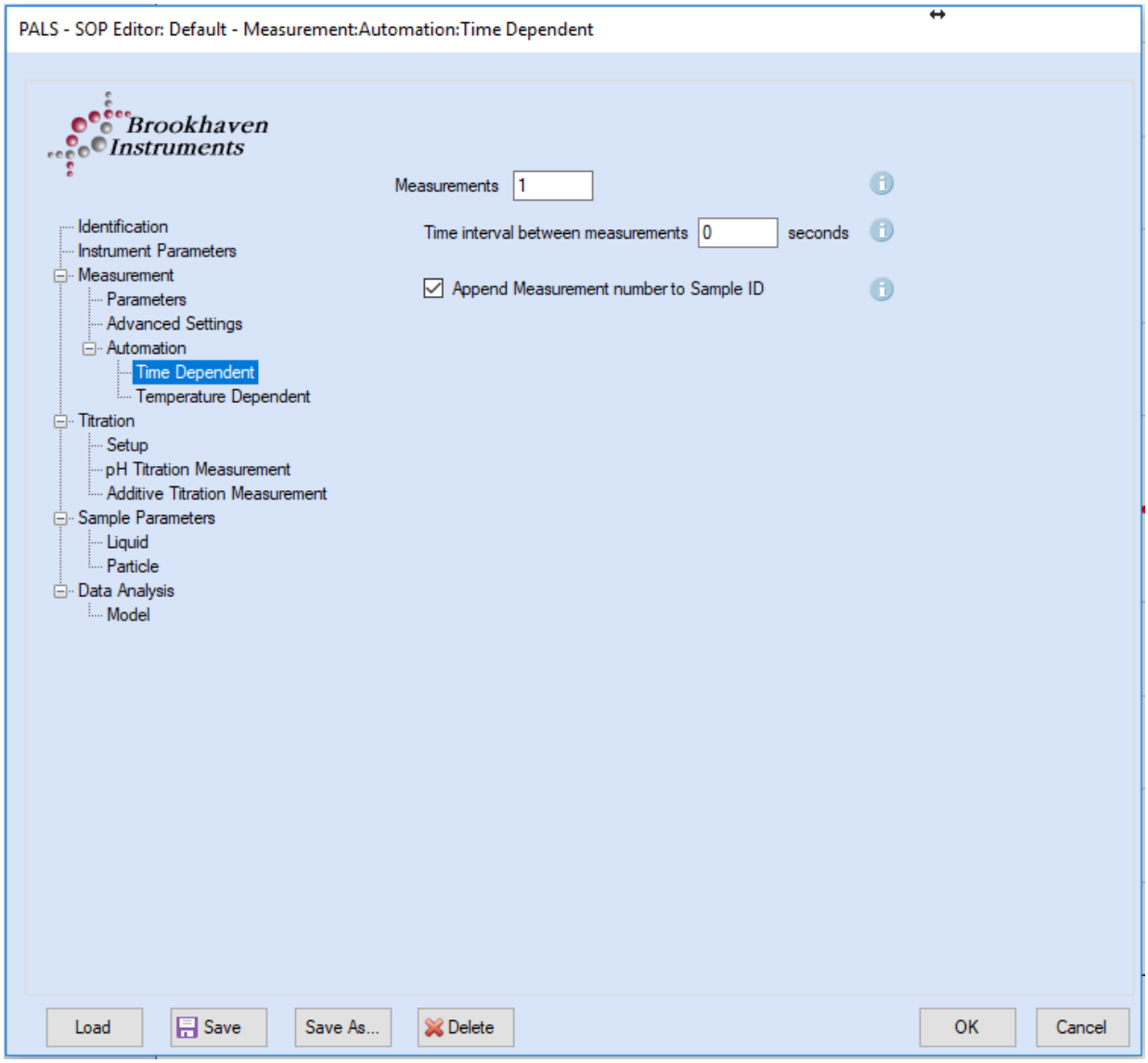


You may use the Advanced Settings Widow to alter the measurement conditions.

The Equilibration Time may be used to invoke a delay after the temperature has been stabilized, before the actual measurement data acquisition begins.

The Inter-cycle Delay may be used to invoke a delay between the application of the electric field during cycle acquisition.

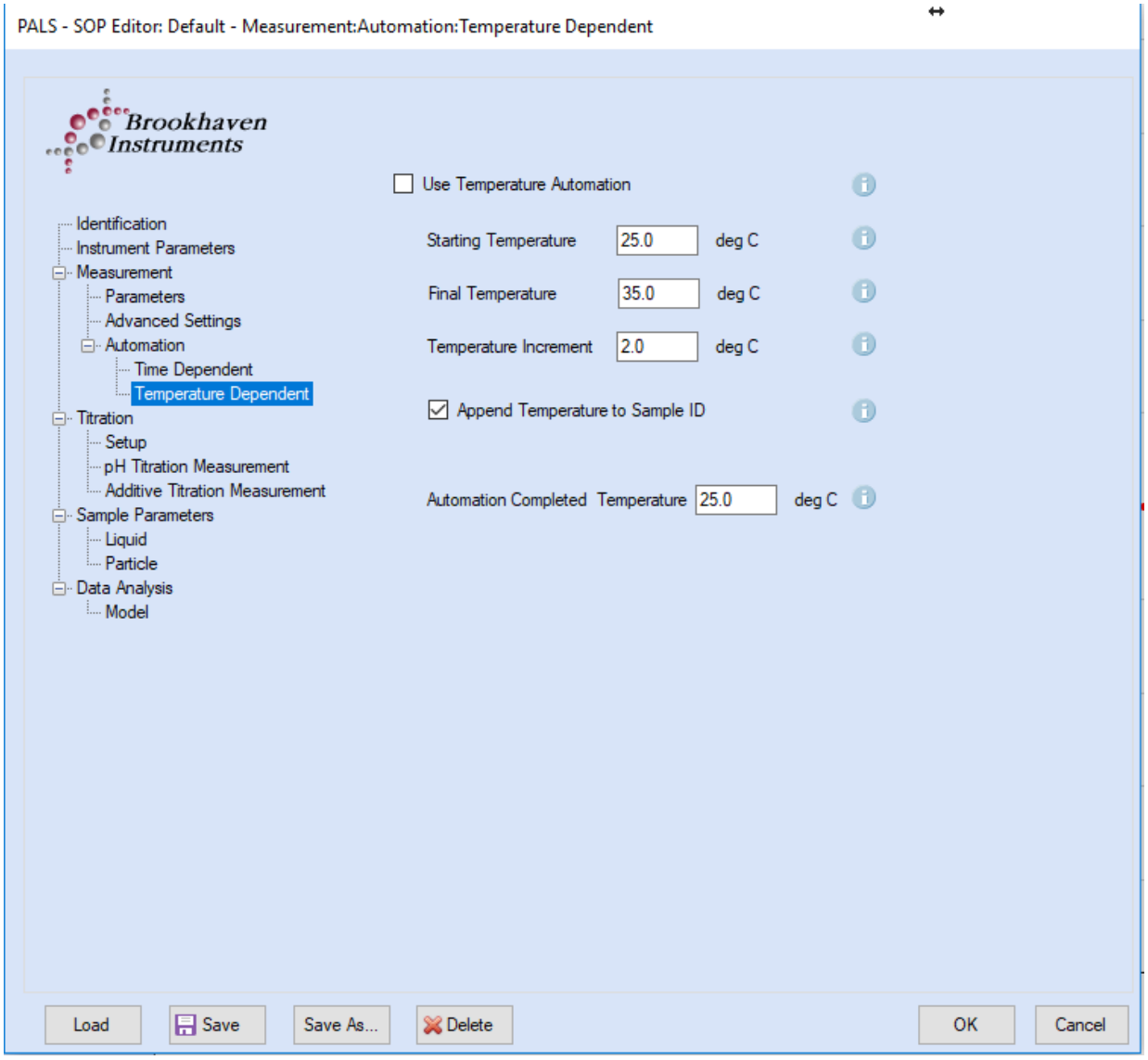
PALS SOP Measurement : Automation : Time Dependent Window



In this window, you may indicate the number of measurements to make using Total Measurements.

You may also indicate a time interval between measurements. The interval will set the delay between the start of a new measurement and the end of the prior measurement.

PALS SOP Measurement : Automation : Temperature Dependent Window

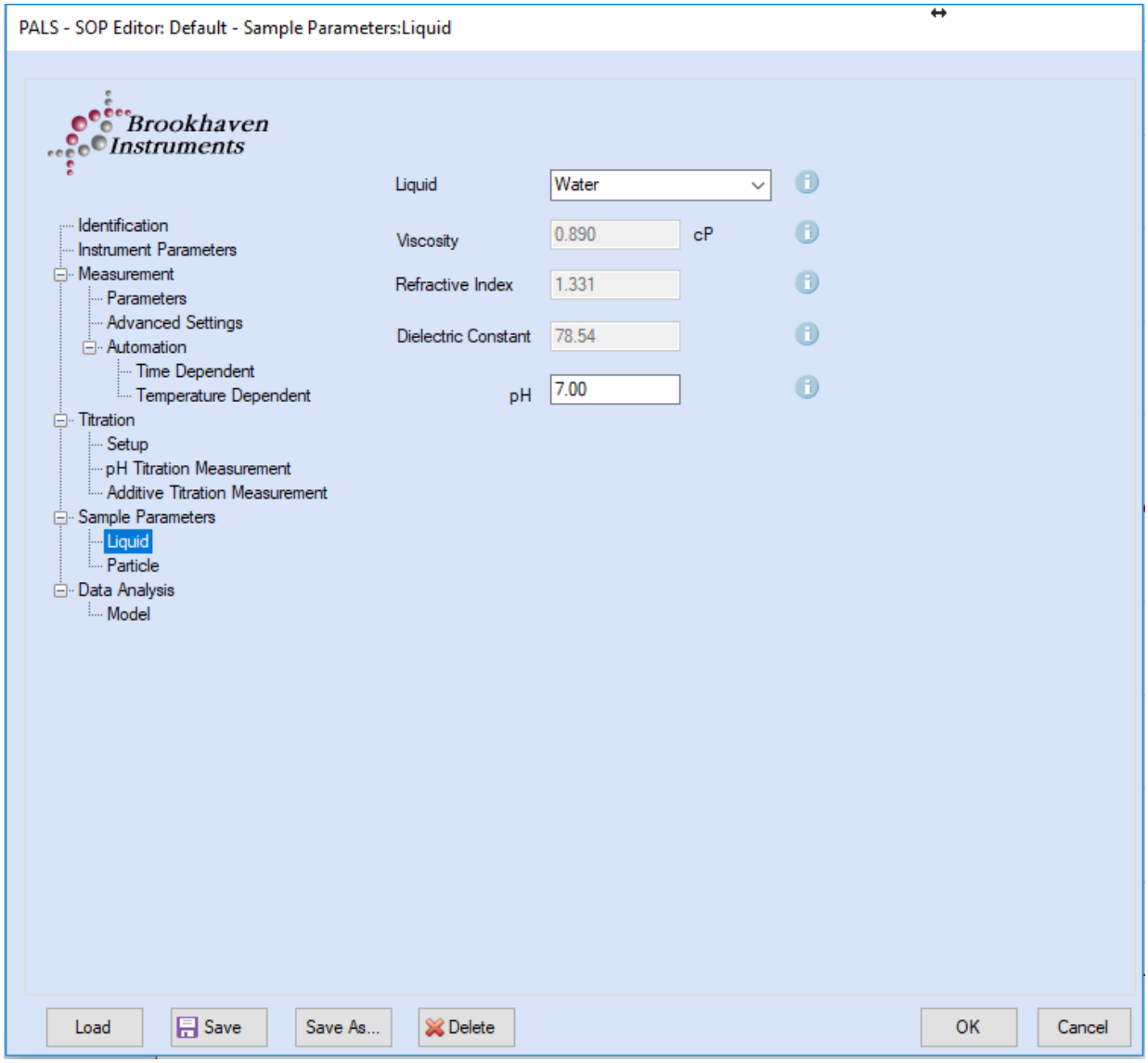


In this window, you may indicate a temperature automation sequence. You must indicate a start, final temperature and a temperature increment. The Use temperature automation checkbox must also be checked in order to use the temperature automation settings in the measurement sequence.

Note that the number of measurements requested in the Measurement Automation : Time Dependent Window will be made for each temperature in the temperature automation sequence.

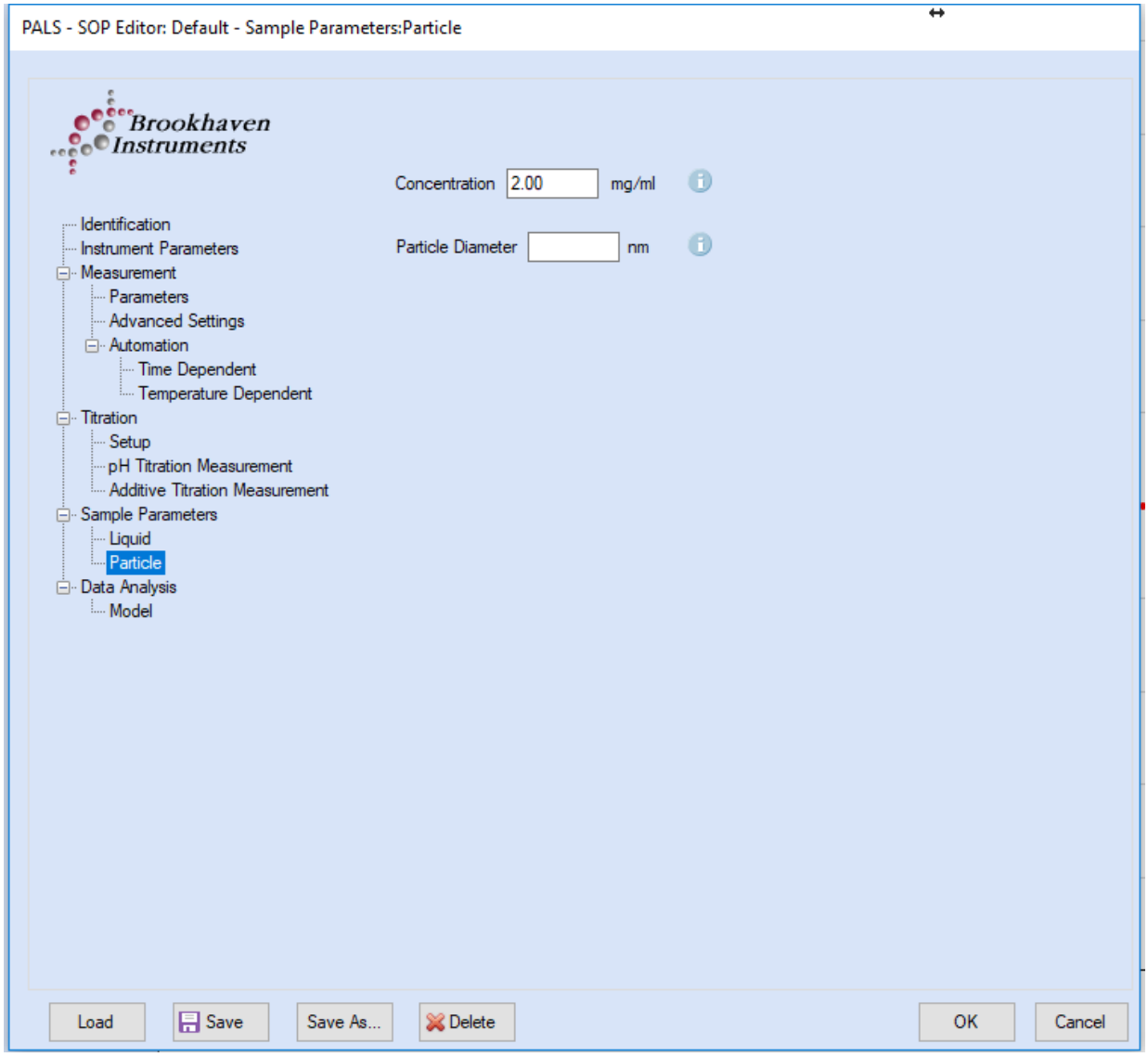
The final temperature that the instrument should return to at the end of the measurement sequence is also shown on this page.

PALS SOP Sample Parameters : Liquid Window



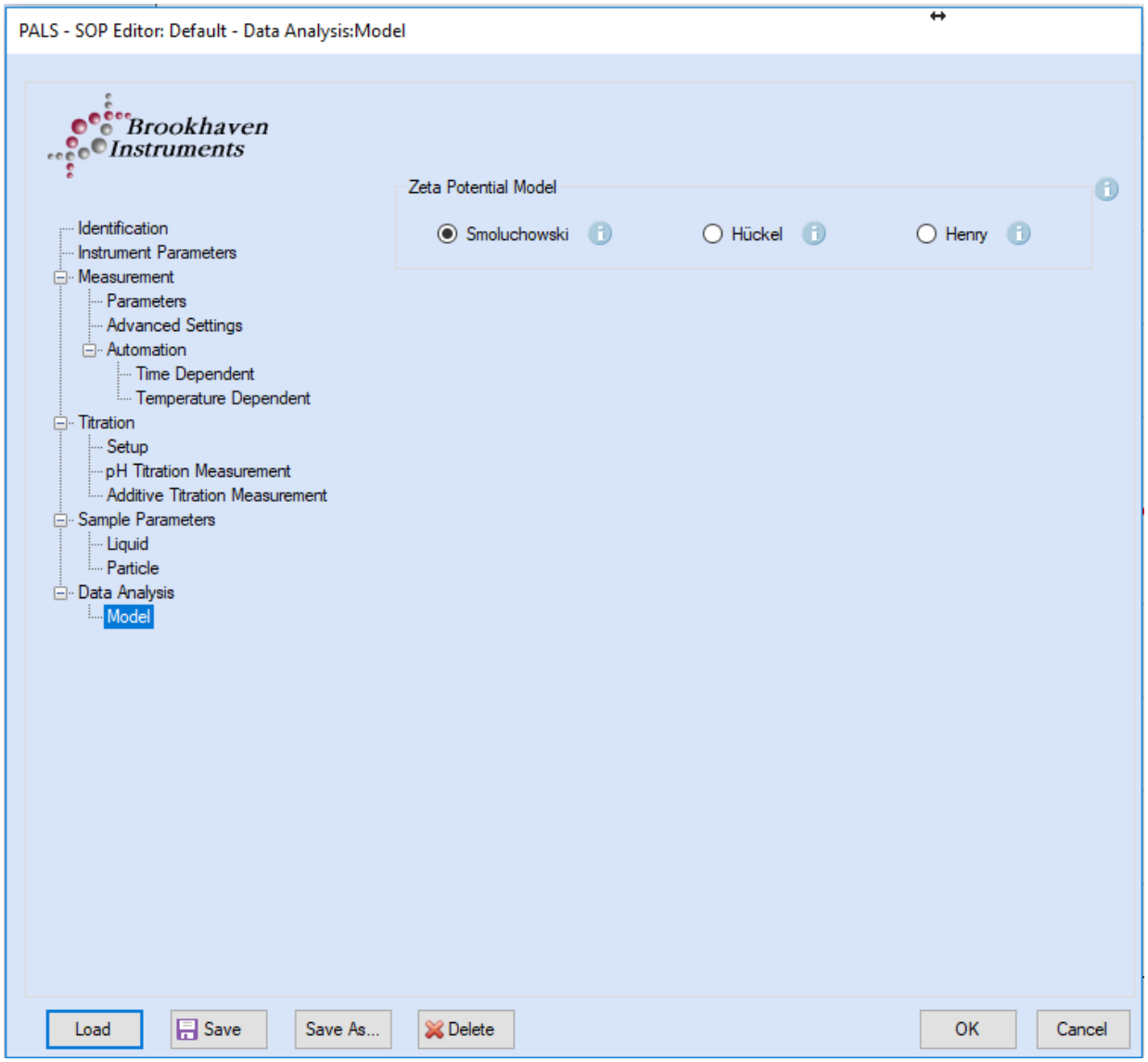
In this window the sample liquid properties are indicated.

PALS SOP Sample Parameters : Particle Window



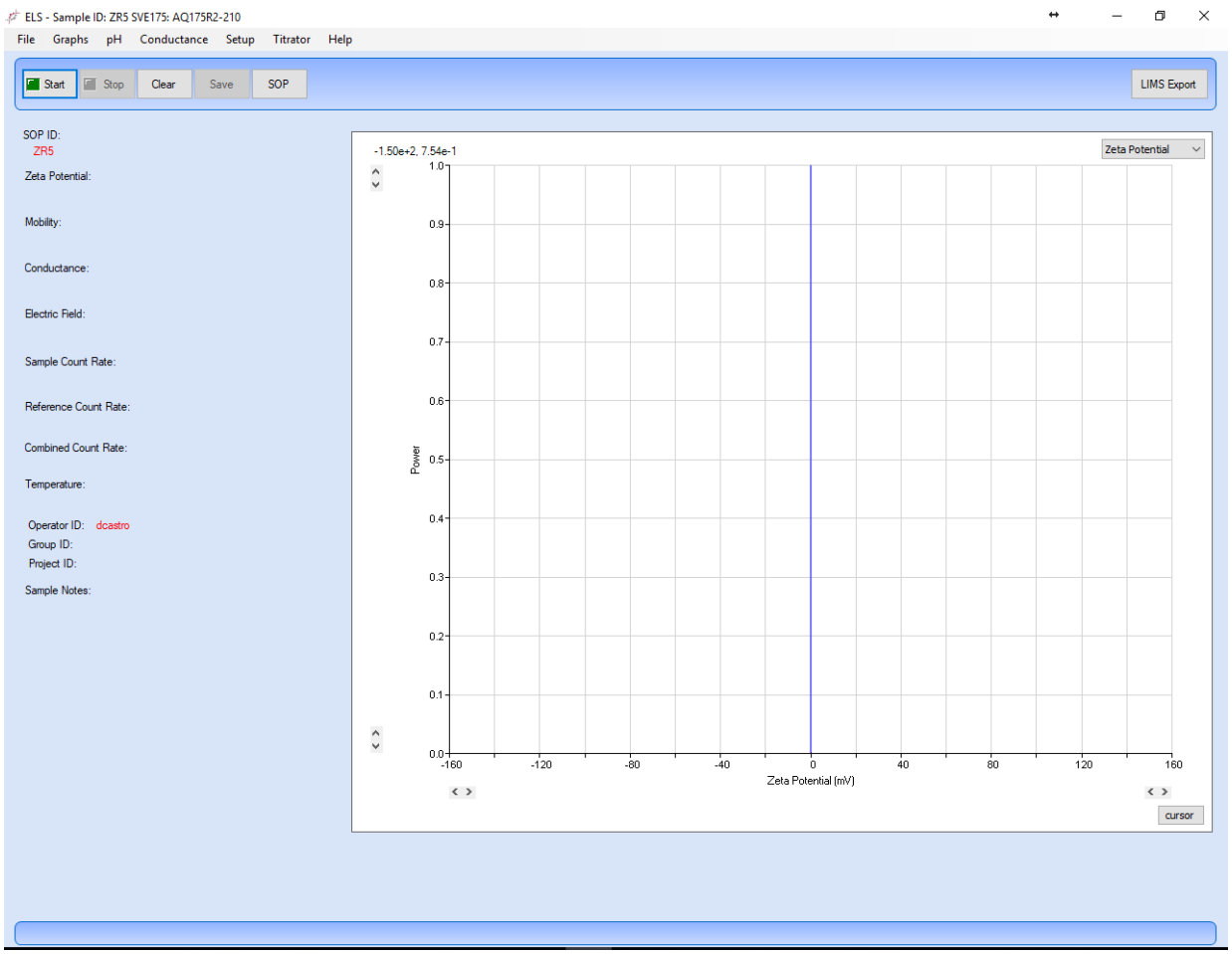
In this window, particle characteristics are defined. You may enter a particle size for your sample, but it is not required, and you may leave it blank if it is value is unknown.

PALS SOP Data Analysis : Model



In this window the zeta potential model is indicated.

ELS New Measurement Window

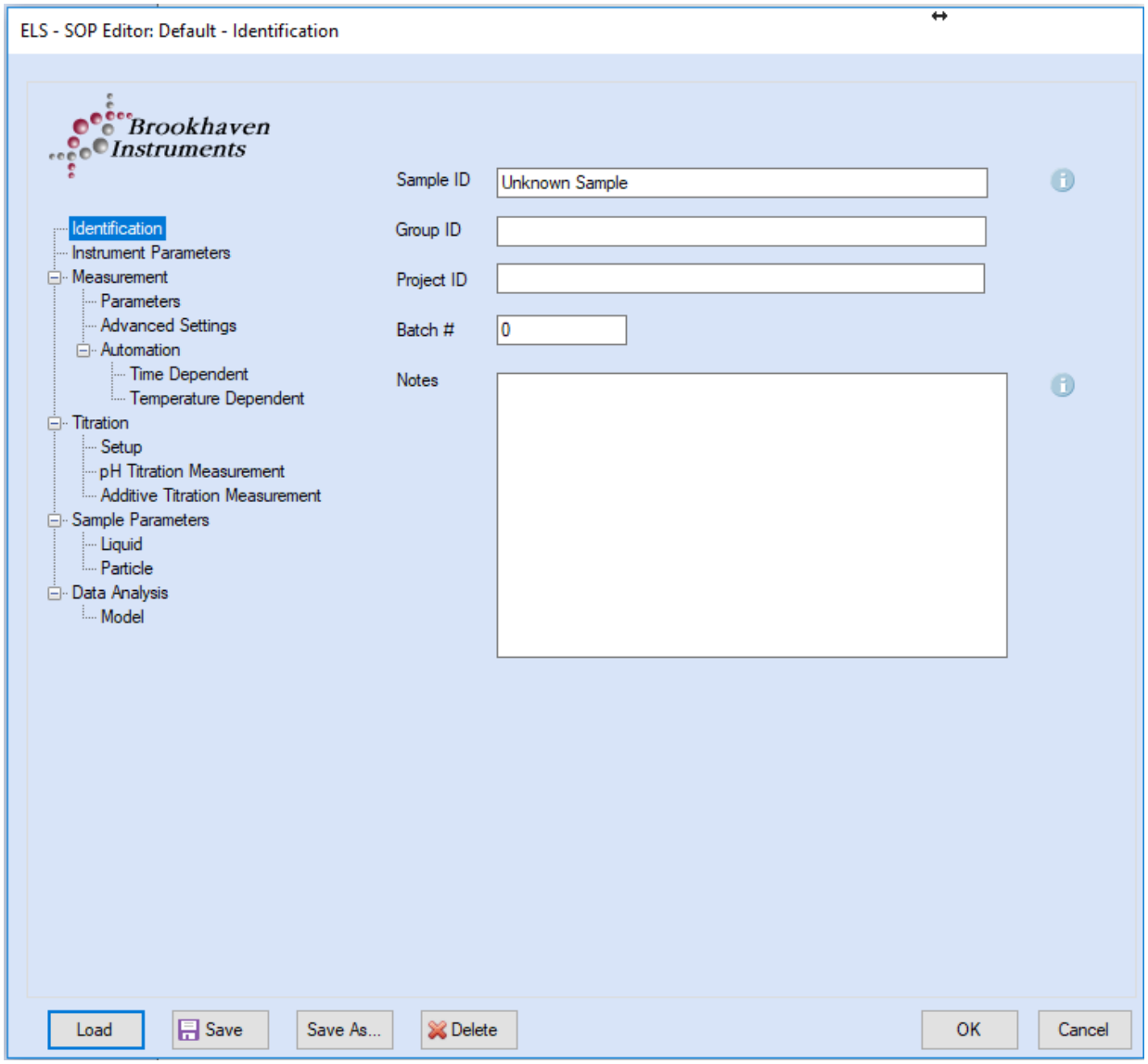


The ELS measurement window is used to collect new measurements.

The Start and Stop buttons are used to initiate and pause a measurement's progress. When Start is pressed, a sequence of one or more measurements will be collected as defined in the measurement SOP.

The Clear button can be used to clear the current measurement. Once a measurement sequence is completed, the final measurement from the sequence will be visible. It must be cleared before an operator can begin a new measurement sequence.

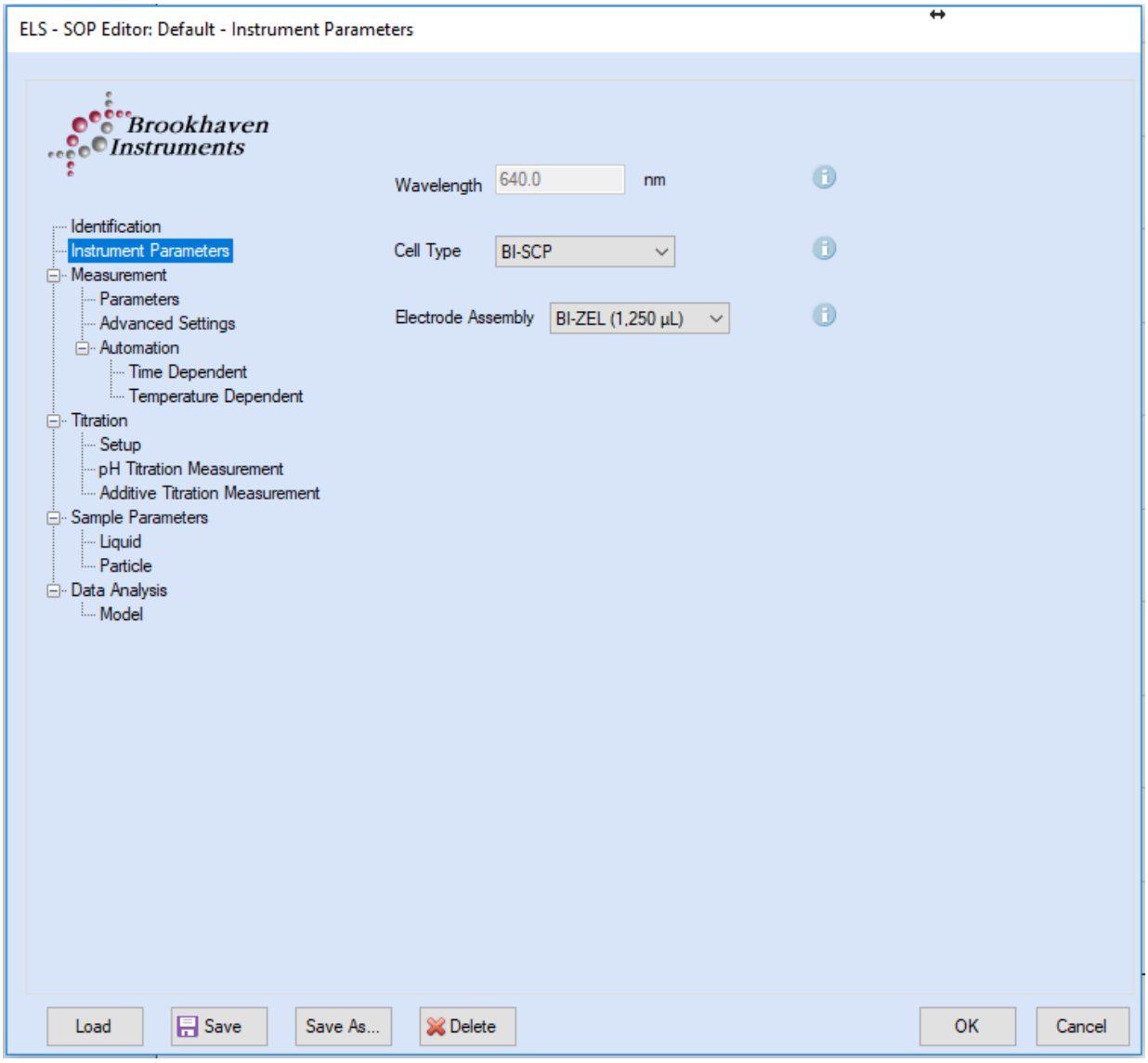
ELS SOP Identification Window



Measurement Identification parameters are indicated in the identification window.

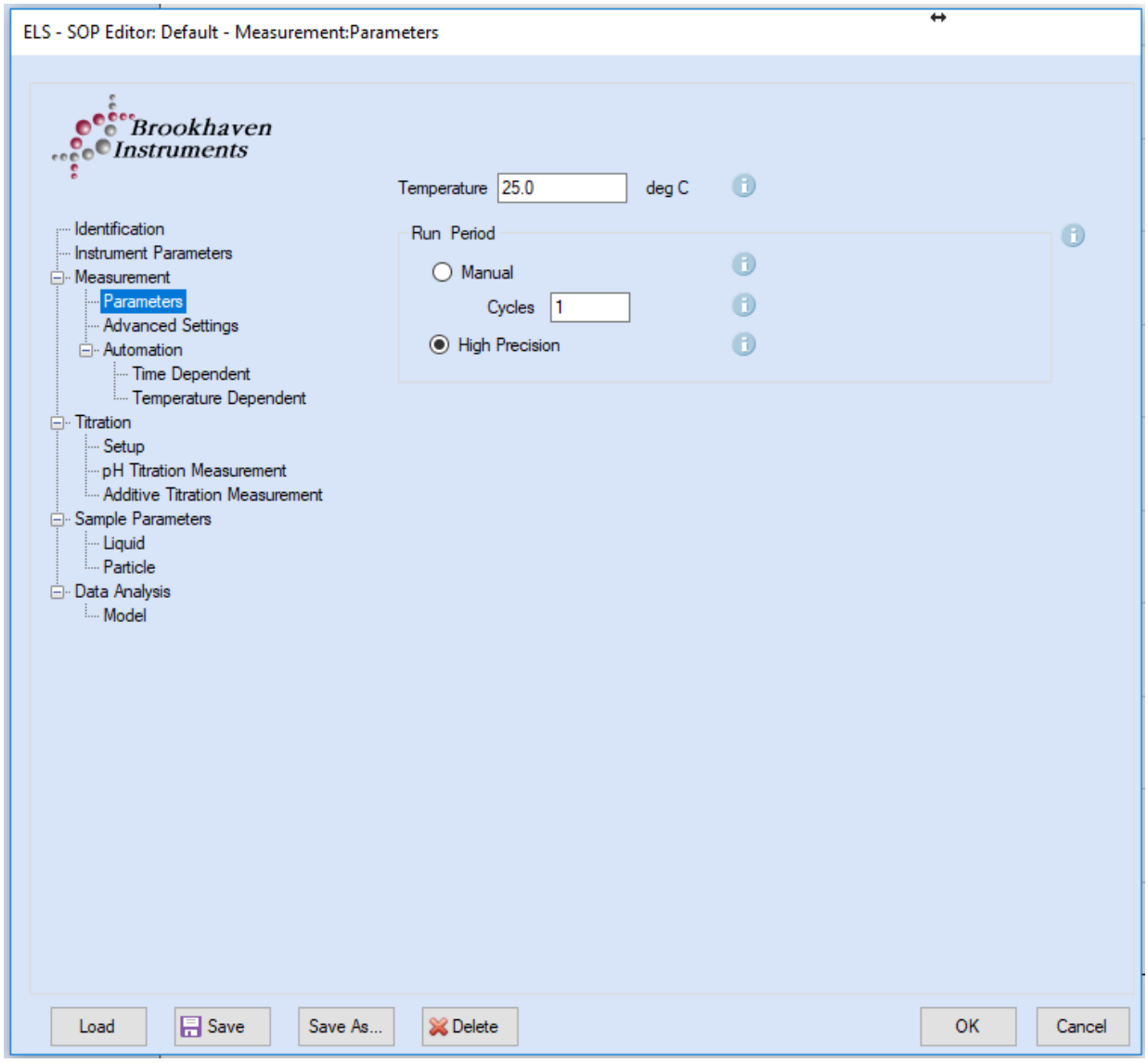
The SOP ID is the name of the SOP file that is currently in effect. You may load and save SOP files as needed before a measurement sequence is started. Once started, you may not change the SOP to a different file.

ELS SOP Instrument Parameters



Instrument parameters are indicated this window.

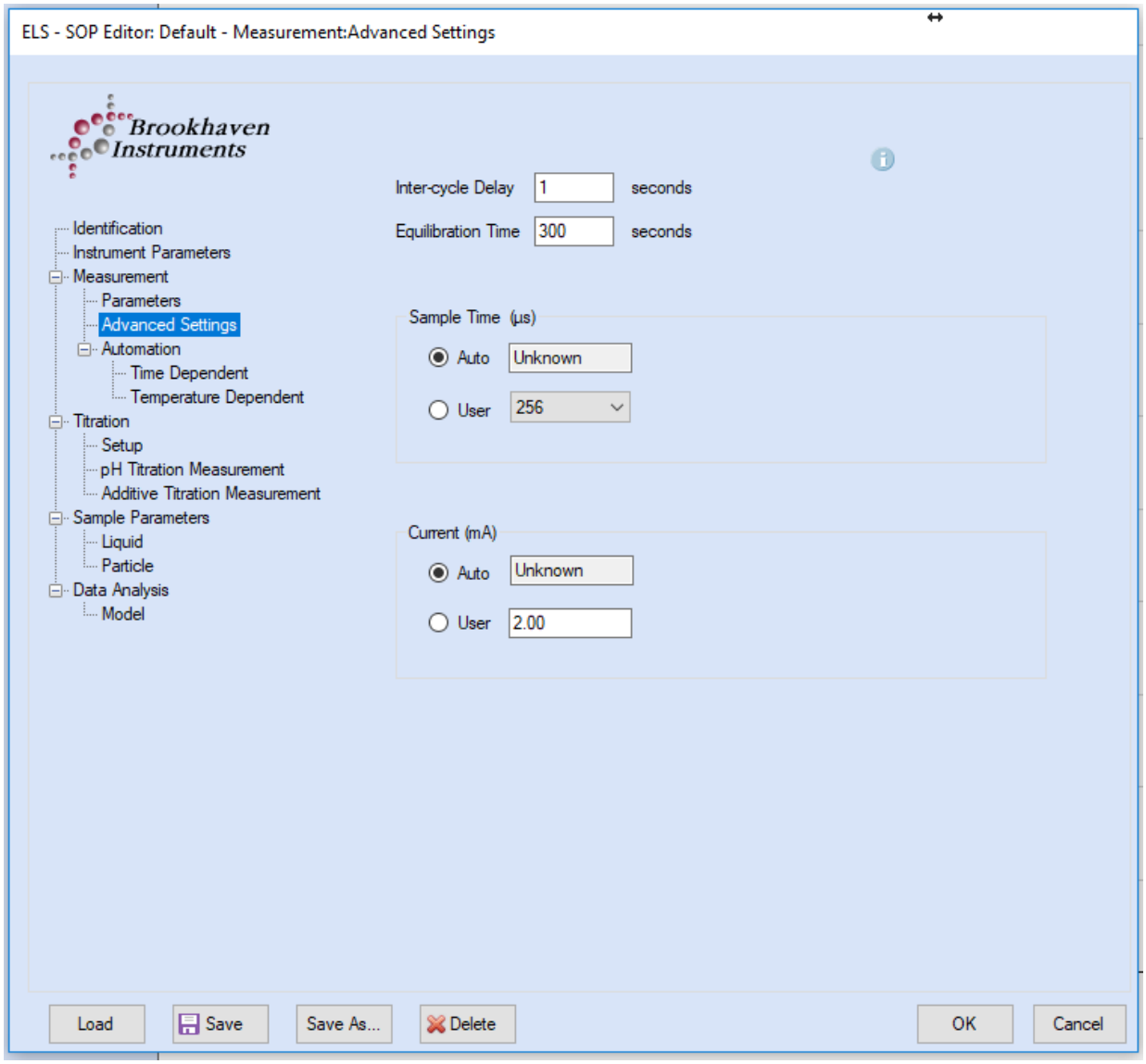
ELS SOP Measurement : Parameters Window



Use the Run Period settings to control the duration of the measurement.

Once a measurement sequence is started, many of the SOP parameters, such as measurement run period, cannot be changed and are used for the entire measurement sequence.

ELS SOP Measurement : Advanced Settings Window

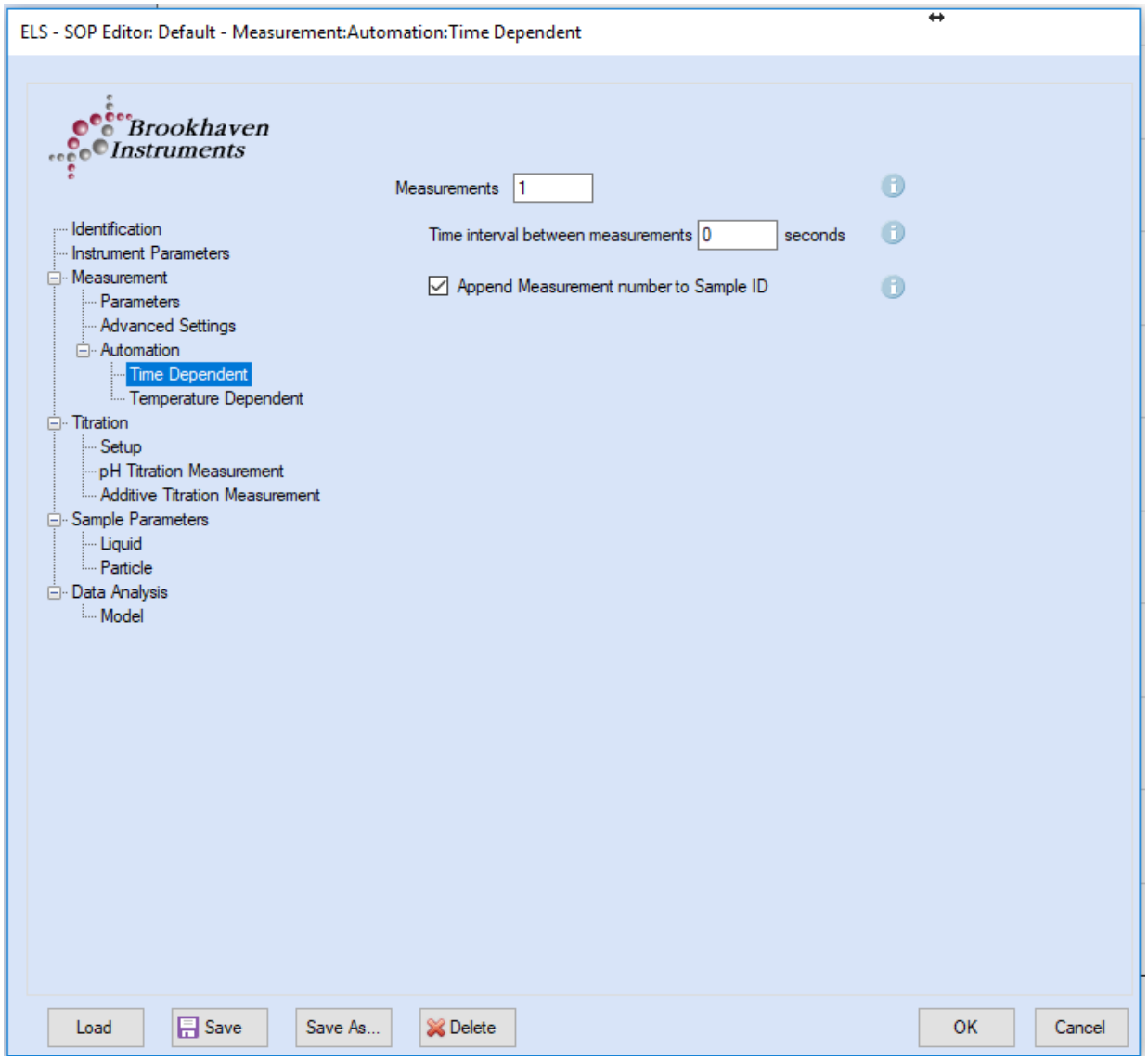


You may use the Advanced Settings Window to alter the measurement conditions.

The Equilibration Time may be used to invoke a delay after the temperature has been stabilized, before the actual measurement data acquisition begins.

The Inter-cycle Delay may be used to invoke a delay between the applications of the electric field during cycle acquisition.

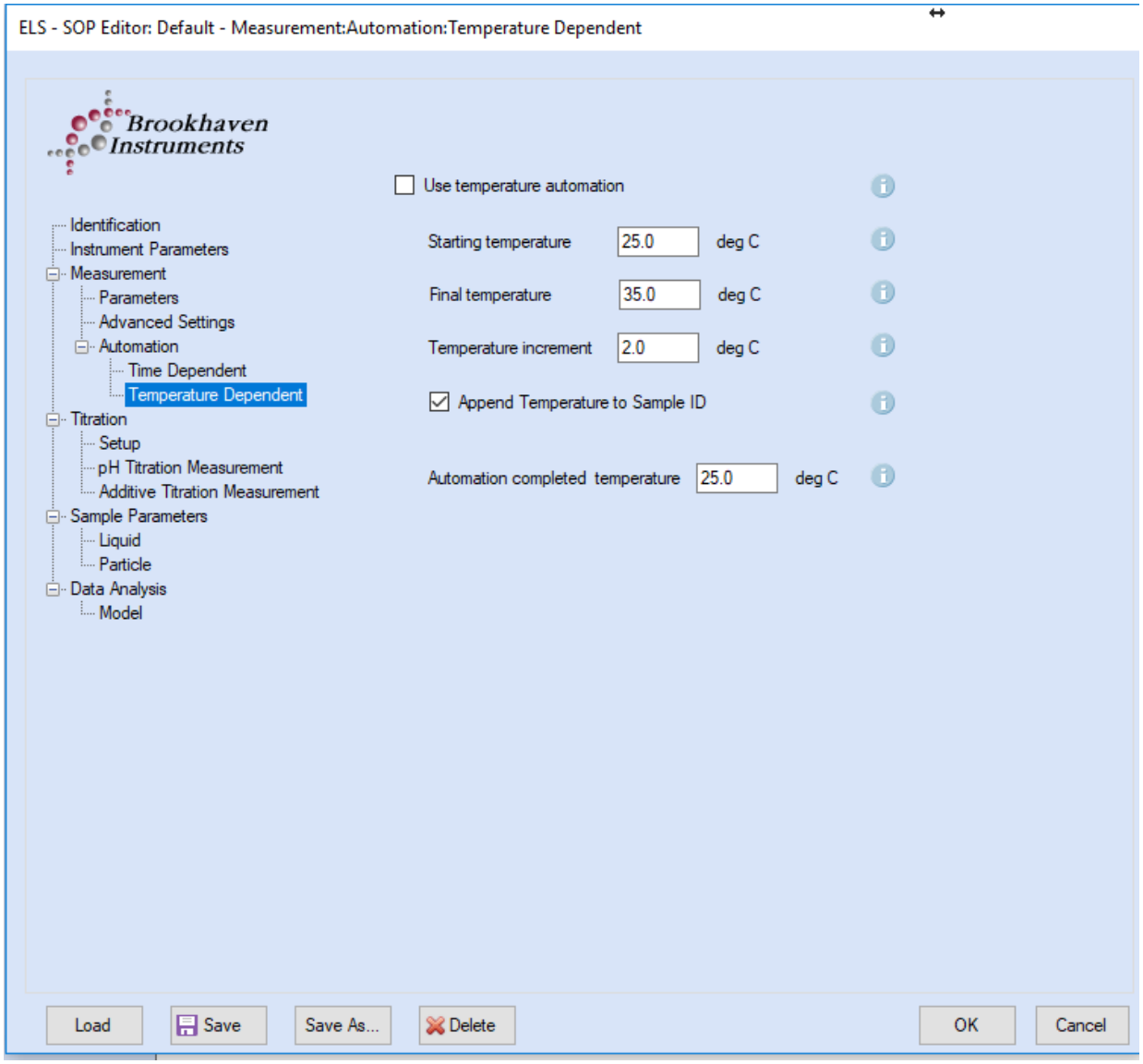
PALS SOP Measurement : Automation : Time Dependent Window



In this window, you may indicate the number of measurements to make using Total Measurements.

You may also indicate a time interval between measurements. The interval will control a delay between the start of a new measurement and the end of the prior measurement.

ELS SOP Measurement : Automation : Temperature Dependent Window

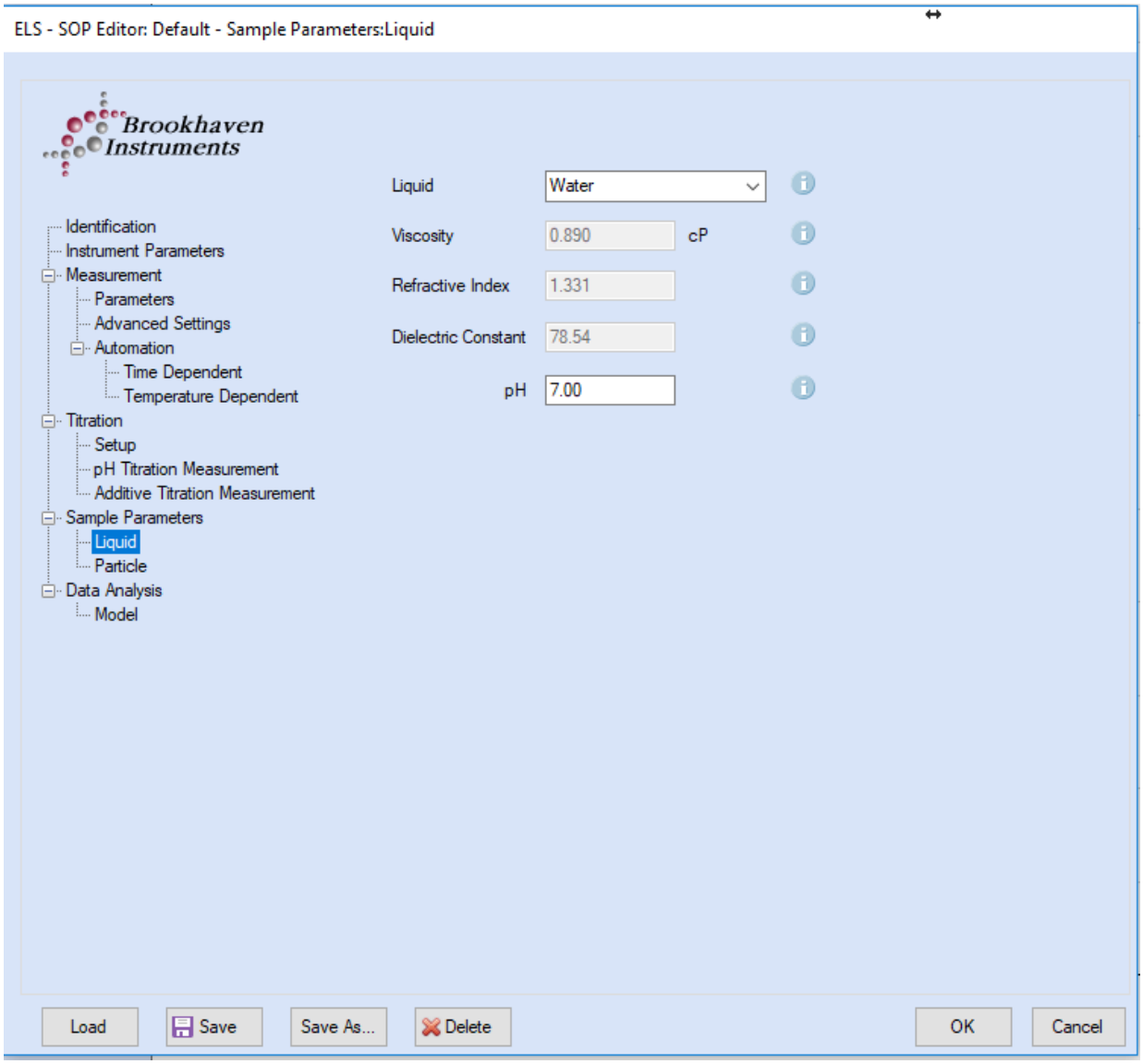


In this window, you may indicate a temperature automation sequence. You must indicate a start, and final temperature and a temperature increment. The Use temperature automation checkbox must also be checked in order to use the temperature automation settings in the measurement sequence.

Note that the number of measurements requested in the Measurement Automation : Time Dependent Window will be made for each temperature in the temperature automation sequence.

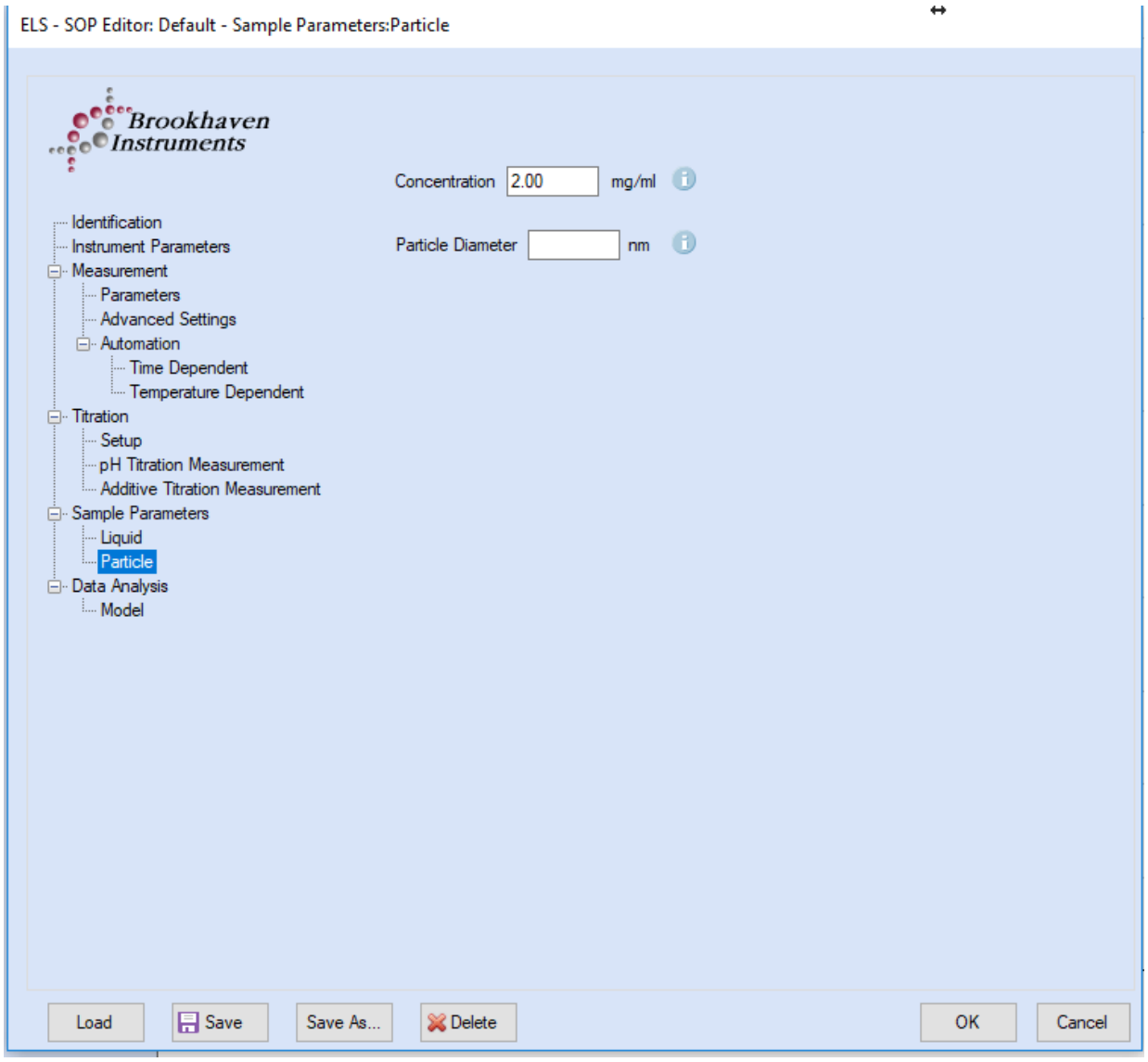
The final temperature that the instrument should return to at the end of the measurement sequence is also indicated on this page.

ELS SOP Sample Parameters : Liquid Window



In this window the sample liquid properties are indicated.

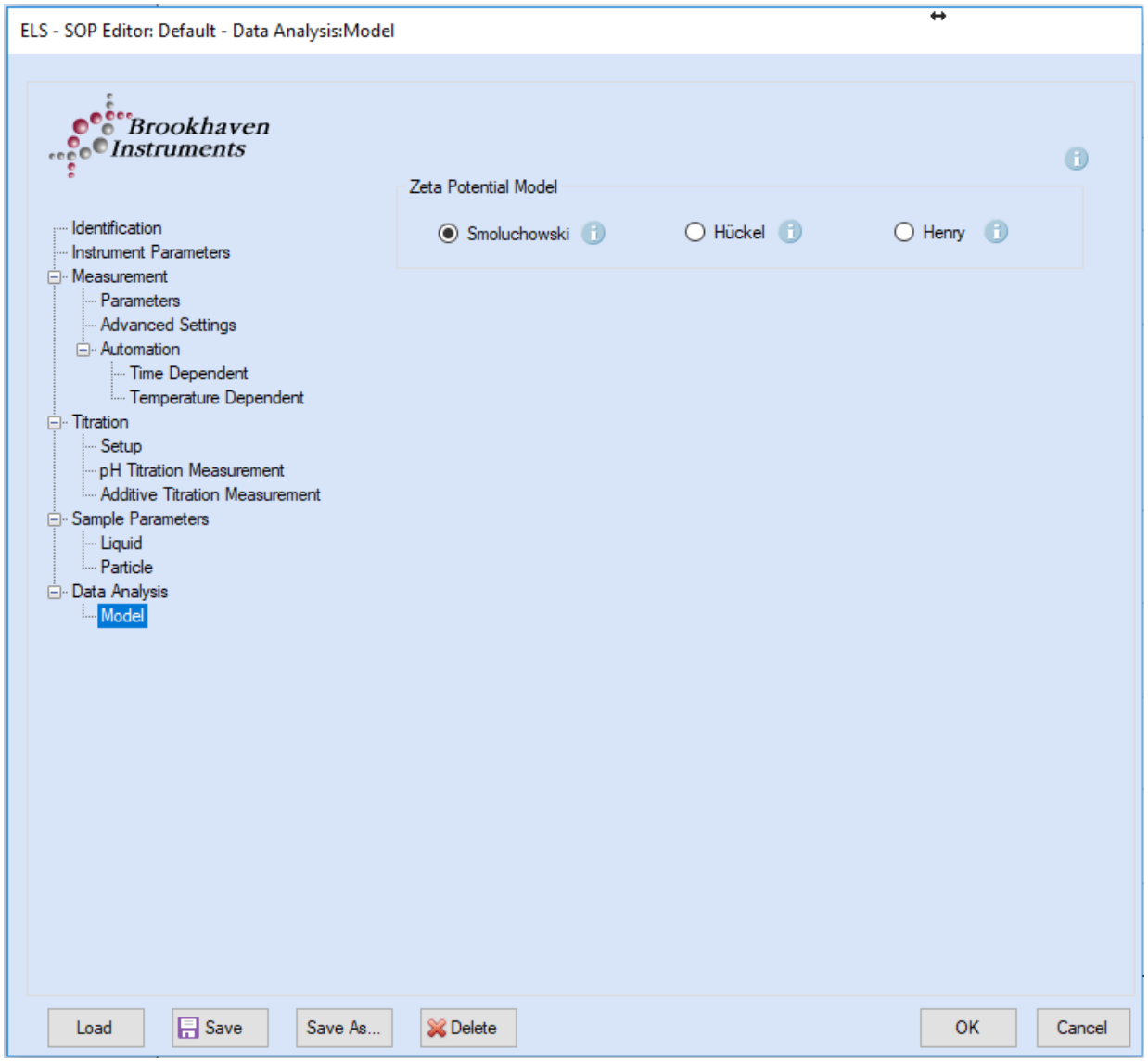
ELS SOP Sample Parameters : Particle Window



In this window, particle characteristics are defined.

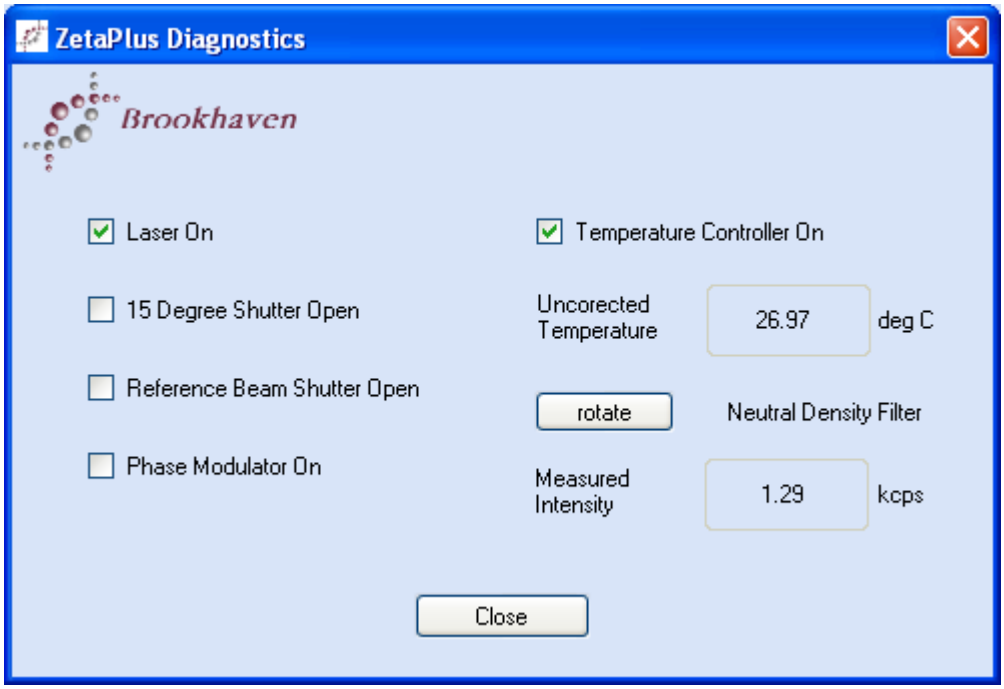
You may enter a particle size for your sample, but it is not required and you may leave it blank if it is value is unknown.

ELS SOP Data Analysis : Model



In this window the zeta potential model is indicated.

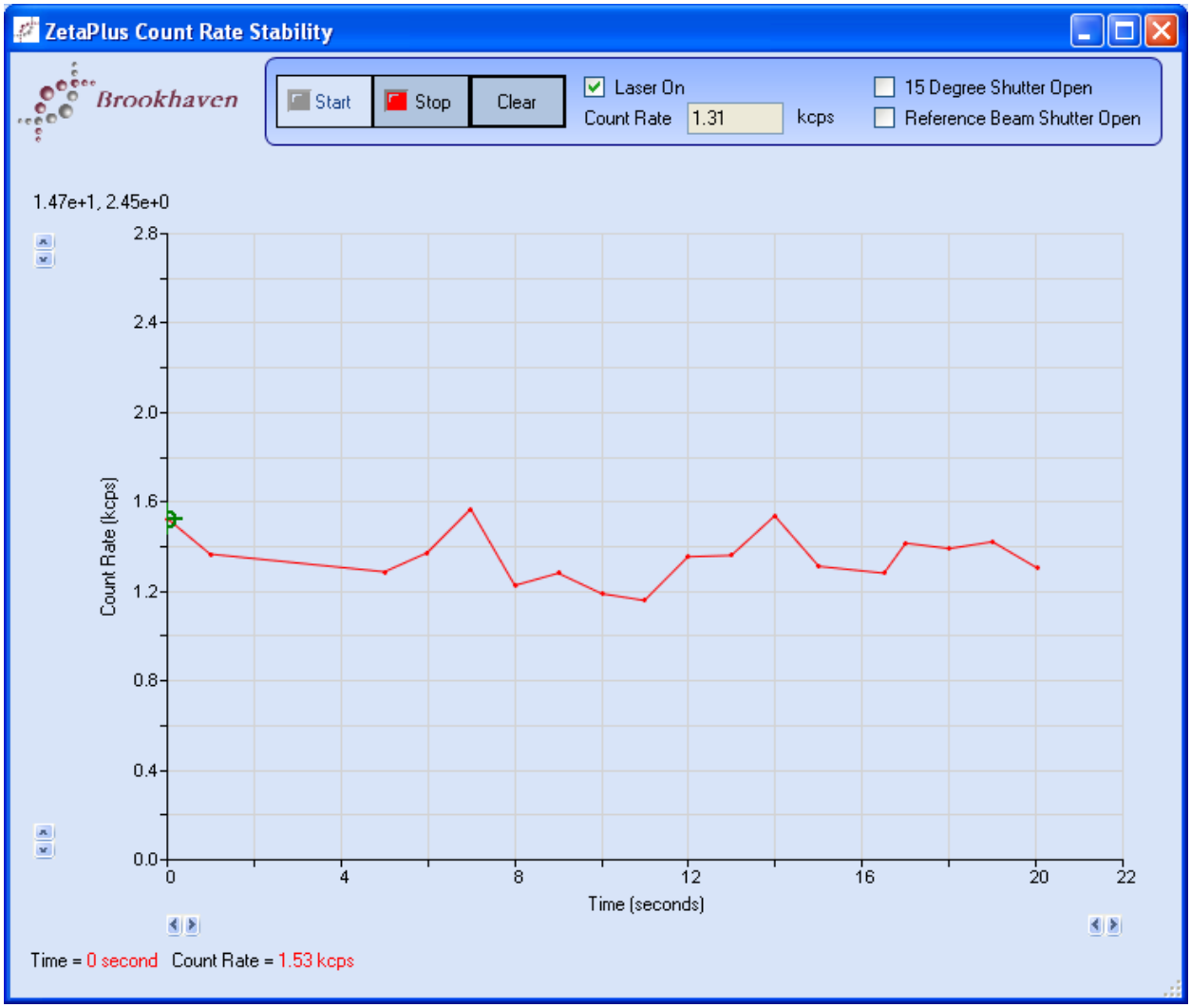
NanoBrook Setup : Diagnostics : Instrument Hardware Test



Each measurement application that uses a ZetaPlus, ZetaPALS or 90Plus has a diagnostic window that can be accessed from the application's main window by selecting Setup\Diagnostics\Instrument Hardware Test from the window's main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

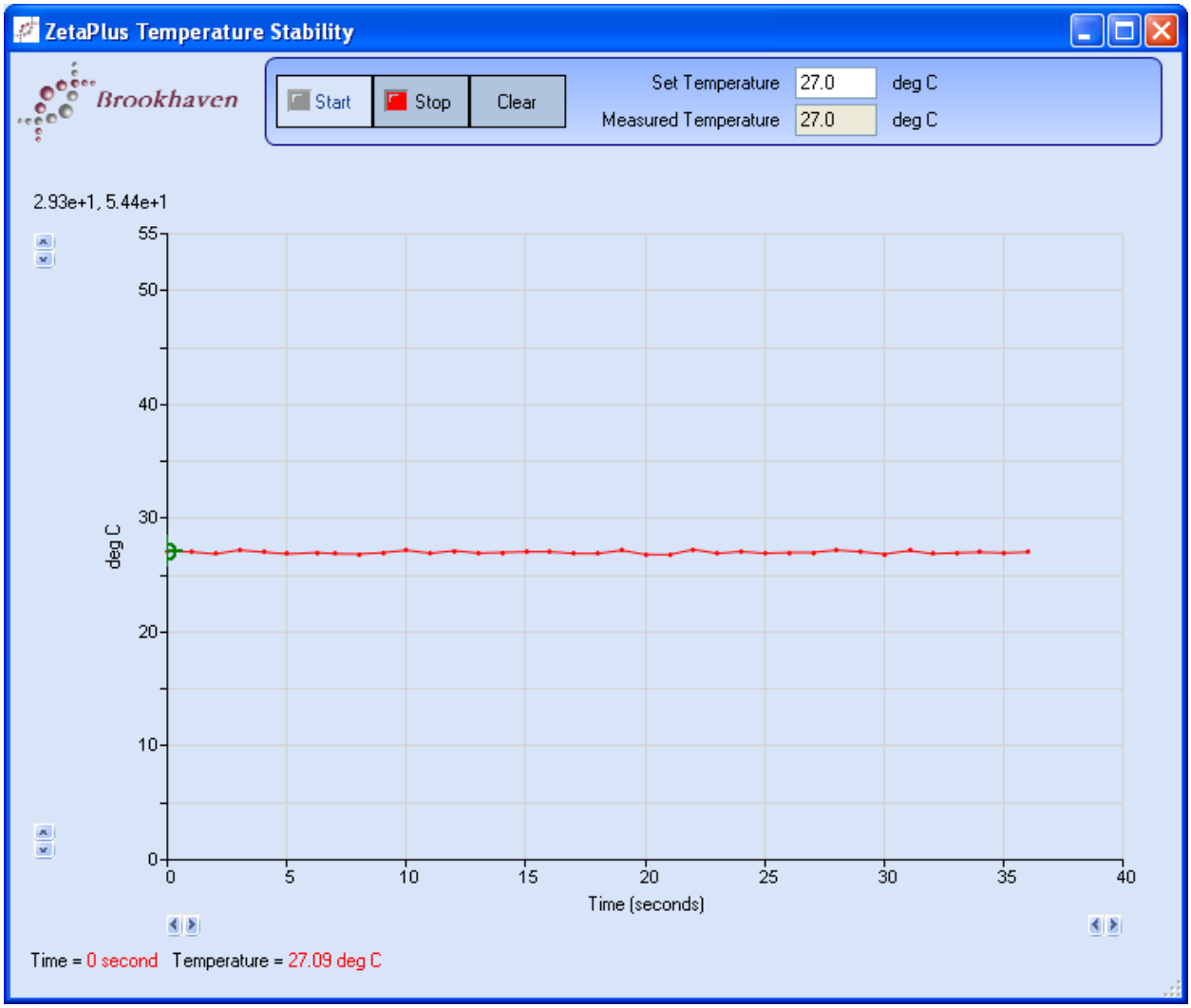
NanoBrook Setup : Diagnostics : Count Rate Stability



Each measurement application that uses a ZetaPlus, ZetaPALS or 90Plus has a count rate stability window that can be accessed from the application's main window by selecting Setup\Diagnostics\Count Rate Stability Graph from the window's main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

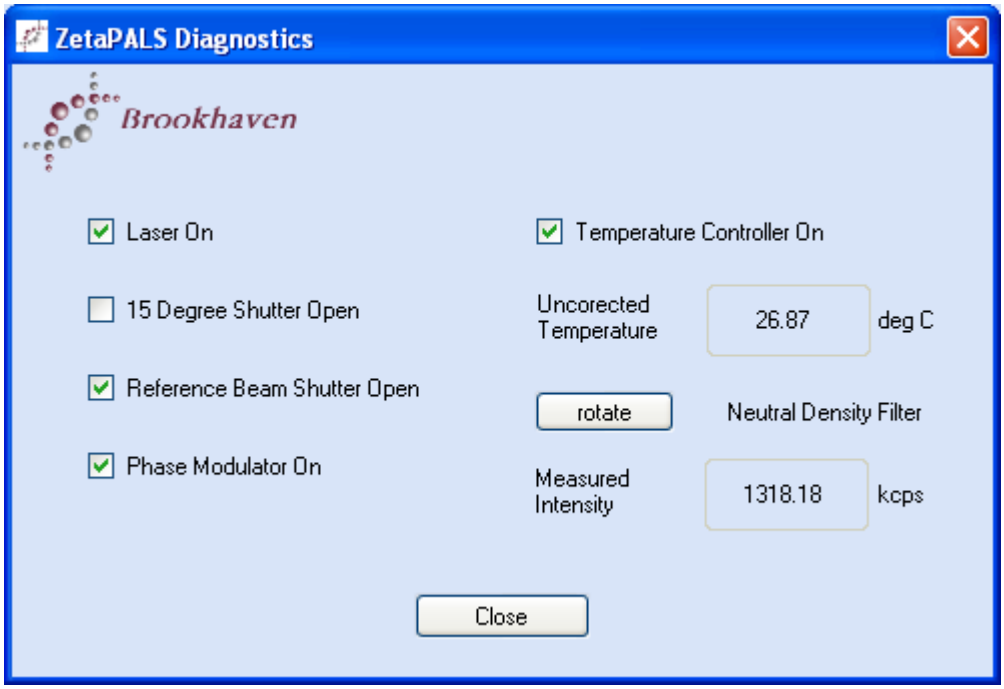
NanoBrook Setup : Diagnostics : Temperature Stability



Each measurement application that uses a ZetaPlus, ZetaPALS or 90Plus has a temperature stability window that can be accessed from the applications main window by selecting Setup\Diagnostics\Temperature Stability Graph from the window's main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

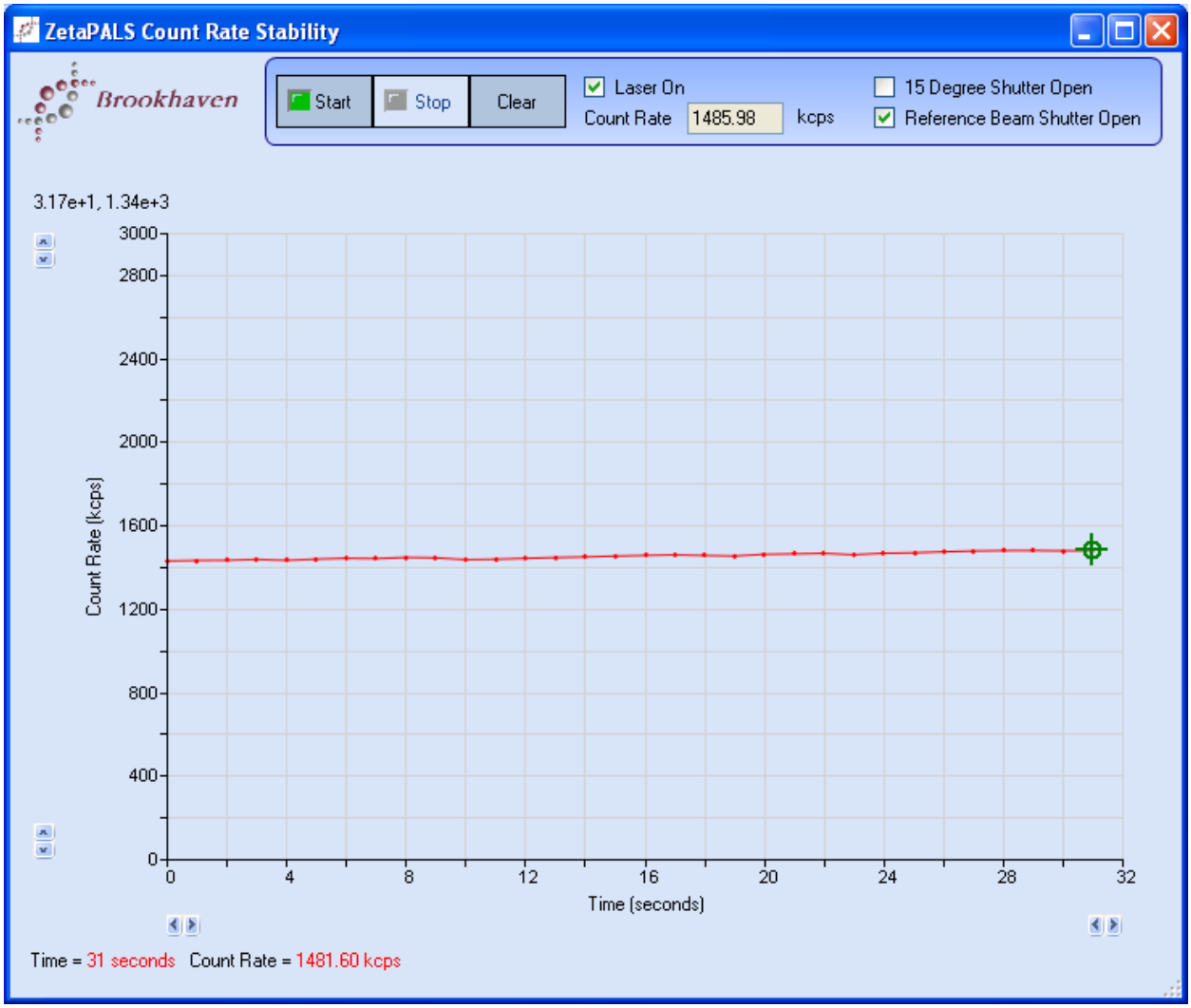
PALS Setup : Diagnostics : Instrument Hardware Test



The PALS software has a diagnostic window that can be accessed from the application's main window by selecting Setup\Diagnostics\Instrument Hardware Test from the window's main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

PALS Setup : Diagnostics : Count Rate Stability



The PALS software has a count rate stability window that can be accessed from the application's main window by selecting Setup\Diagnostics\Count Rate Stability Graph from the window's main menu.

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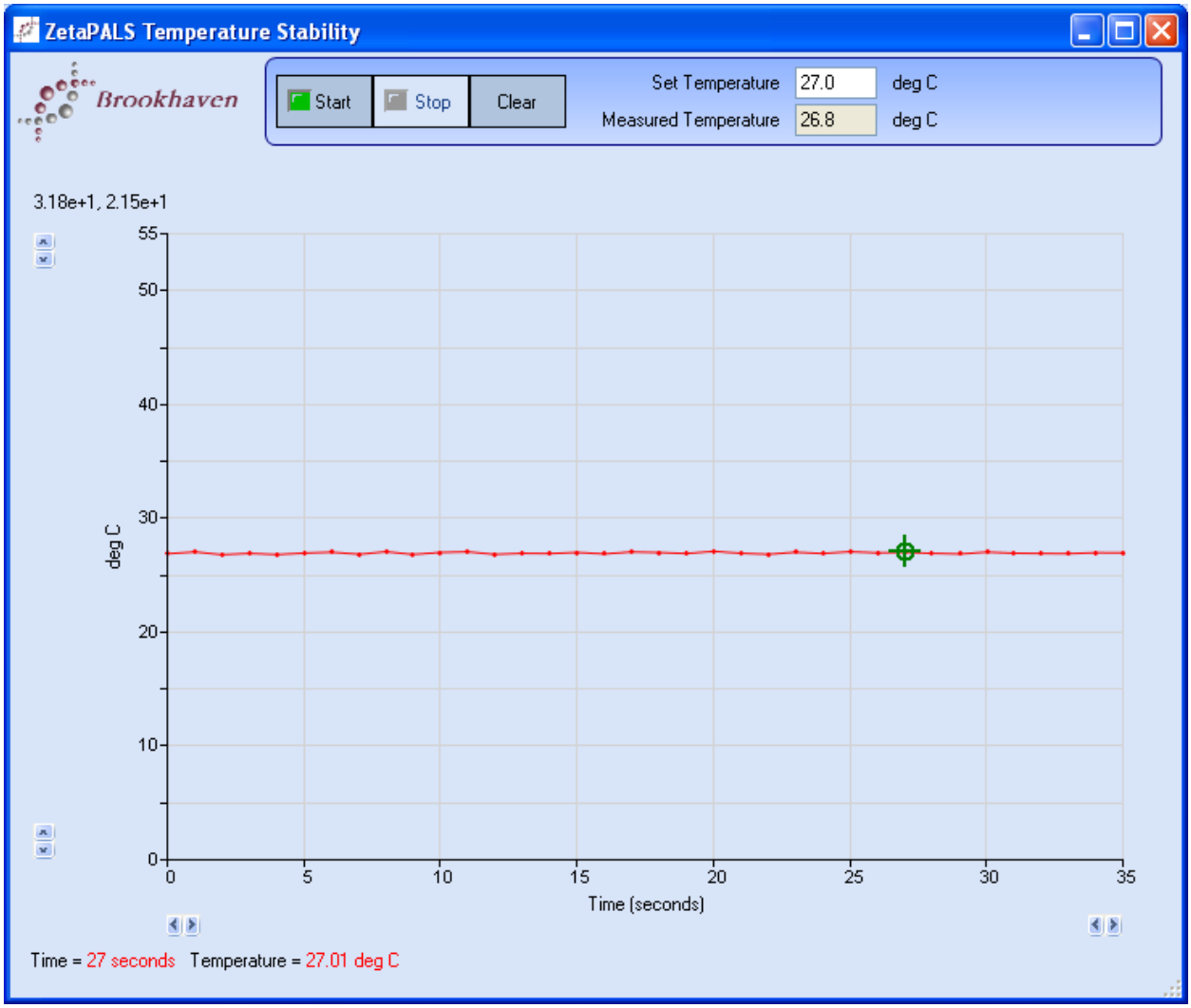
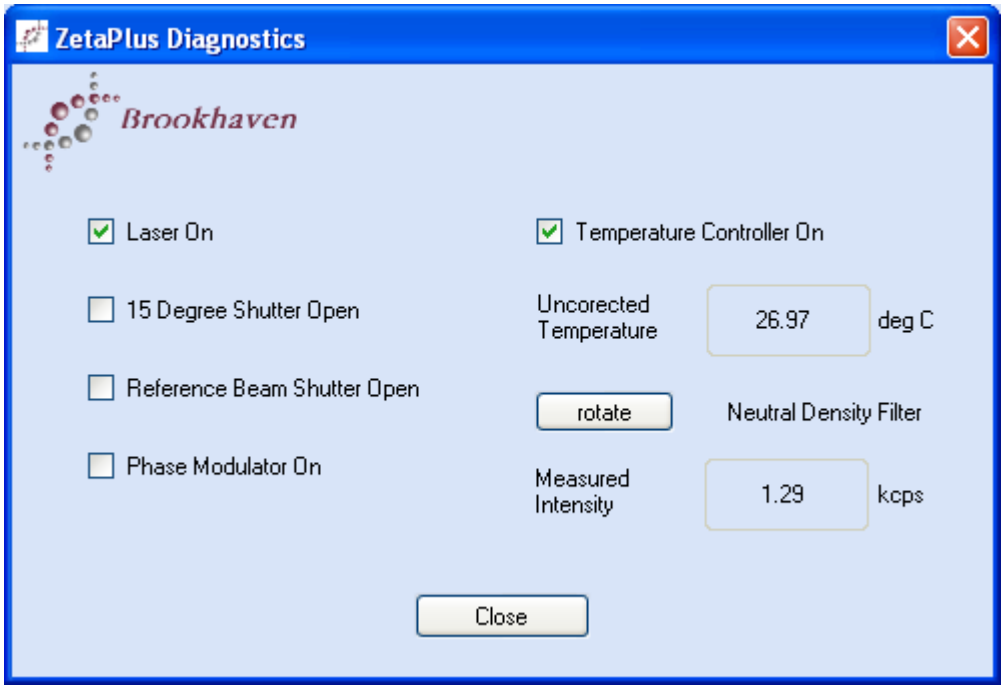
PALS Setup : Diagnostics : Temperature Stability



The PALS software has a temperature stability window that can be accessed from the application's main window by selecting Setup\Diagnostics\Temperature Stability Graph from the window's main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

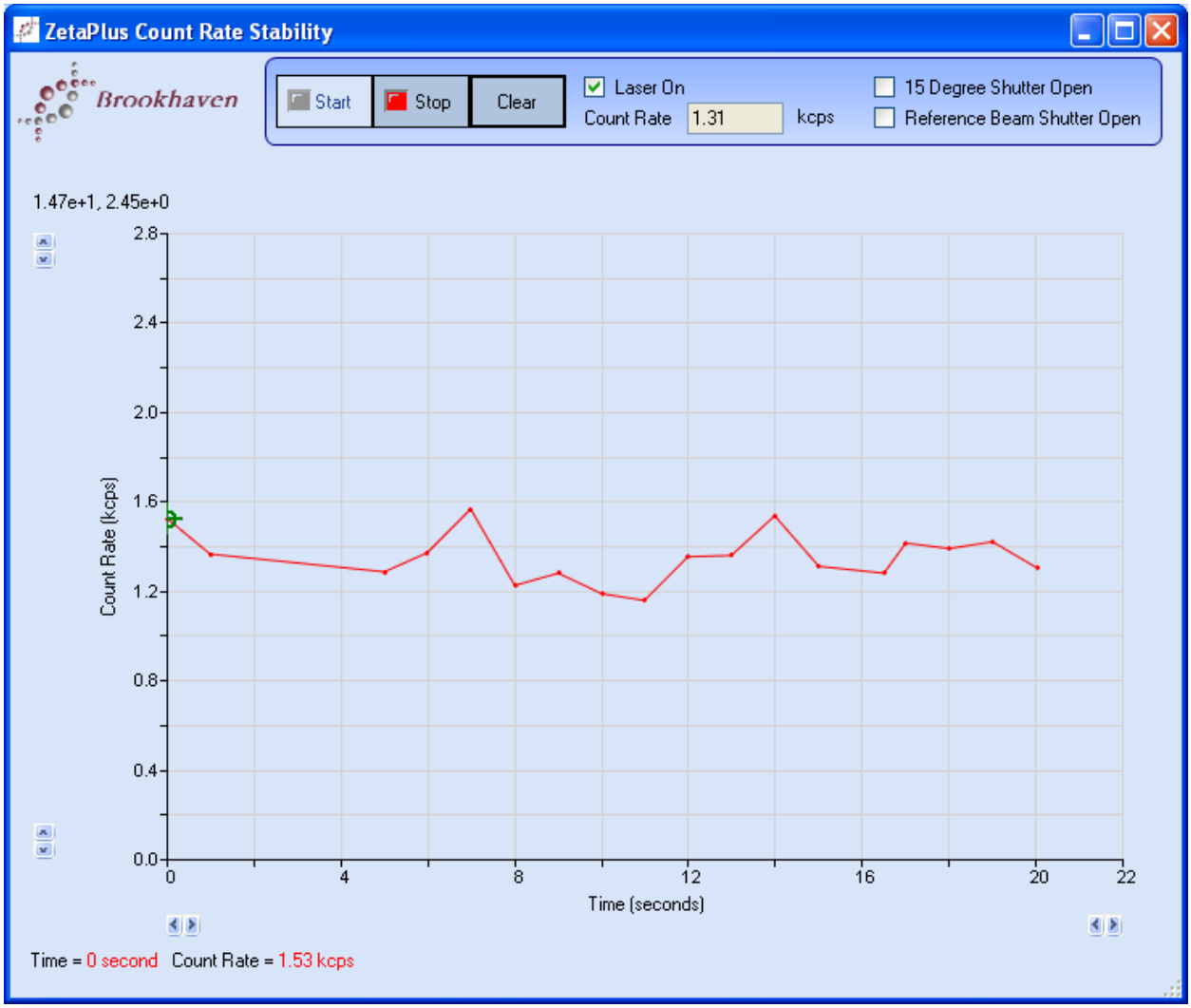
ELS Setup : Diagnostics : Instrument Hardware Test



The ELS software has a diagnostic window that can be accessed from the application's main window by selecting Setup\Diagnostics\Instrument Hardware Test from the window's main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

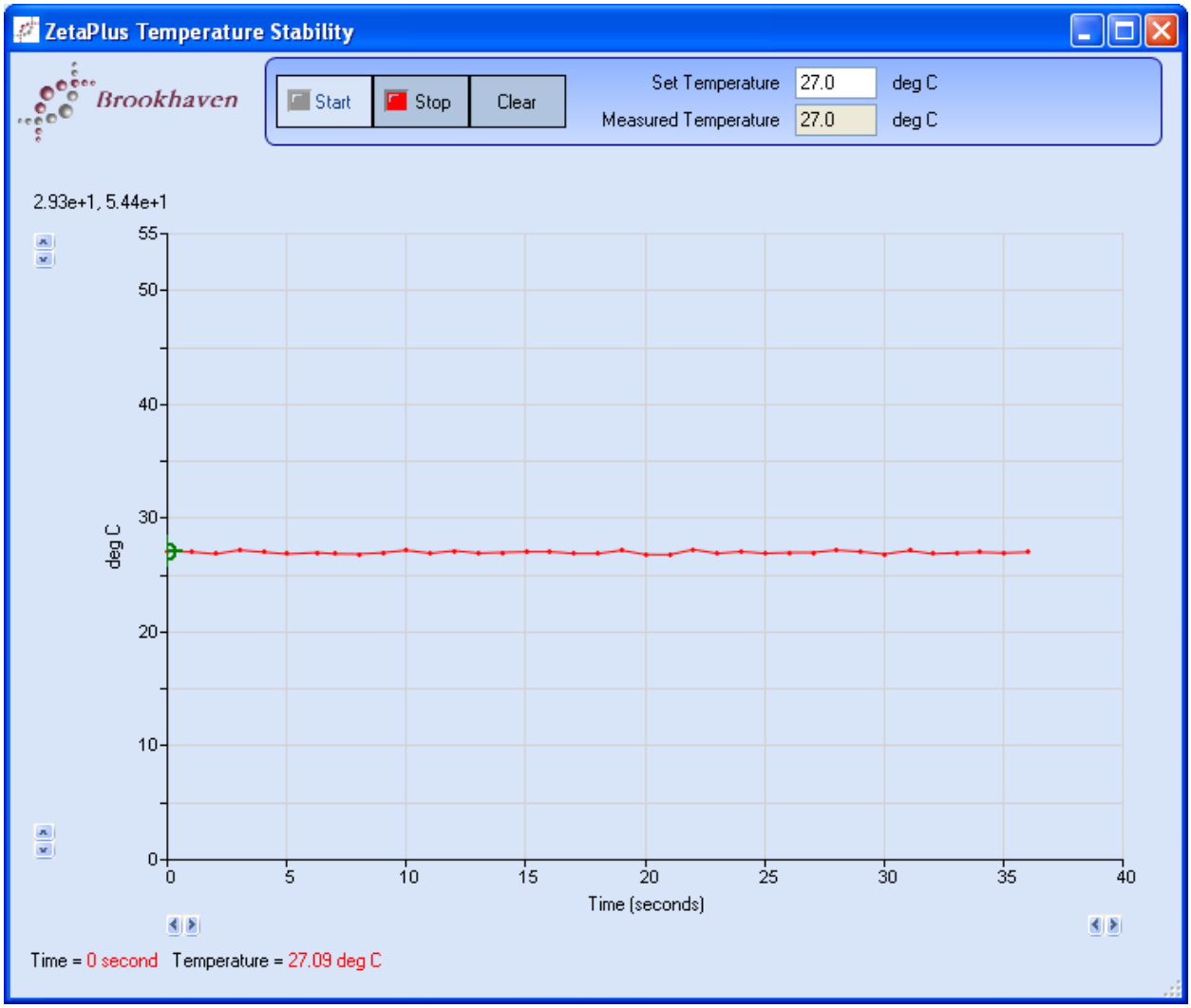
ELS Setup : Diagnostics : Count Rate Stability



The ELS software has a count rate stability window that can be accessed from the application's main window by selecting Setup\Diagnostics\Count Rate Stability Graph from the window's main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

ELS Setup : Diagnostics : Temperature Stability



The ELS software has a temperature stability window that can be accessed from the application's main window by selecting Setup\Diagnostics\Temperature Stability Graph from window's the main menu.

This window provides direct access to the instrument hardware for diagnostic purposes.

Zeta Potential Theory

The reason for performing a phase analysis measurement may be understood by examining the relationship shown as equation 1. This is the relationship between mobility and zeta potential as a function of the parameters of the suspending liquid: the dielectric constant e, and the viscosity h. The factor f(ka) is of the order unity. (For a discussion of f(ka) see the appendices to the *ZetaPlus* instruction manual).

$$\mu = \zeta \frac{\varepsilon}{\eta} f(ka) \qquad [1]$$

m is electrophoretic mobility. It is often expressed in practical units of (micron/second)/(Volt/cm), which for typical colloids in polar liquids like water, is typically in the range of 1 to 5. The correct SI formulation of meters^2/(Volt-second) gives these values scaled by a factor of 10^{-8}. z is zeta potential, usually expressed in millivolts (mV).

Table 1 Ratios of e/h For A Few Common Liquids

Liquid	e/h	Ratio To Water
Water	87.55	1
Methanol	61.11	0.7
Toluene	4.25	0.05
Ethylene Glycol	2.35	0.03
Oleic Acid	0.095	0.001

If we now consider a particle system in which the zeta potential is constant, despite the fact that very different liquids (see Table 1) are used to make the dispersion, we see that the mobility that will arise can be much smaller than the value when water is used. In general, dispersions in organic liquids may have mobilities lower by several factors of 10 than aqueous based systems. Low mobilities can also occur in dispersions of high salt content, in higher viscosity liquids, or

close to an isoelectric point. Conventional laser Doppler electrophoresis is limited to mobilities of the order of 0.5 x 10^{-8} $m^2/(V \cdot s)$ and cannot readily be used to measure such low mobilities due to limits of frequency resolution and background drifts in the sample due to thermal fluctuations. However, direct frequency analysis is not the only way of measuring the Doppler shift. If the Doppler signal is compared to a reference signal corresponding to stationary particles using phase quadrature, any small change in frequency eventually gives rise to a change in phase between the signals. This phase change Q(t) can be related to the Doppler shift and hence the electrophoretic mobility in the following way:

Measure a phase Q(t) in the scattered light field at scattering vector q which changes due the movement of particles with mobility m in an electric field E(t). Given that the scattered light has an amplitude of A,

$$\langle Q(t) - Q(0) \rangle = \langle A \rangle q \mu \int_0^t E(t')dt' \quad . \qquad [2]$$

For the case of a sinusoidal field the integral gives the following result,

$$\langle Q(t) - Q(0) \rangle = \langle A \rangle q \mu \{\cos(\phi) - \cos(\omega t + \phi)\} / \omega \, , \qquad [3]$$

where ω is the frequency of the applied electric field.

The ZetaPALS hardware incorporates a digital signal processor which can extract Q(t) directly from the Doppler signal. The other quantities are known or controlled so the mobility can be determined and an estimate of zeta potential by applying equation [1] in either the Smoluchowsky (f(ka)=1.5) or Hückel (f(ka)=1) limits.

One of the problems with microelectrophoresis is the presence of small thermal drifts in the sample. These manifest themselves as a collective velocity component added to the electrophoretic motion. Over short periods of time this component may be taken to be constant, but unknown.

If we add the effect of a constant velocity V_c we obtain equation [4].

$$\langle Q(t) - Q(0) \rangle = \langle A \rangle q \mu \{\cos(\phi) - \cos(\omega t + \phi)\} / \omega + V_c t \qquad [4]$$

The effect of the constant velocity is to add a slope to the phase signal. This can be removed experimentally by adjusting the reference frequency appropriately. This is known as '*Autotracking*' and improves the precision of the system. *'Autotracking'* is a useful feature that minimizes convection effects due to sample heating and other parasitic flows by tuning the phase analysis to remove any collective velocity. This shows up as a slope superimposed on the oscillating phase (AWPD) function. It should be left selected (checked) in normal circumstances.

Preparation and Measurement of the BIC Reference Material ZR5

BI-ZR5 is a zeta potential reference material for use with Brookhaven's ZetaPlus and ZetaPALS instruments. The sample is a colloidal pigment, blue in color. Since there are no universally accepted zeta potential standards, only reference materials are available to check the proper operation of zeta potential instruments. The BI-ZR5 is such a material. Brookhaven has made measurements on this sample over a long period of time, under a strict set of sample preparation conditions. As long as the user prepares the sample as described below, and as long as the materials is not used beyond the expiration date printed on the bottle, the value of any single zeta potential measurement using any ZetaPlus or ZetaPALS will be within the range whereas, the average of many runs should be -44 +/- 8 mV.

BI-ZR5 comes as a powder.

Preparing a sample and making a measurement from the concentrate.

A. Use only **de-ionized water** to prepare 100 mL of a 10 mM KCl solution. Since the molecular weight of KCl is 74.6 g/mol, add 74.6 mg to 100 mL of de-ionized water. This is the stock solution. Then add 90 mL of de-ionized water to 10 mL of the stock solution, resulting in a final dilution to 1 mM KCl. Or, directly make a 1 mM KCl solution by weight, taking care of measuring the salt accurately. **All measurements will be made with the 1 mM KCl.**

[DO NOT USE TAP WATER. The residual ions in tap water vary greatly from place-to-place. Furthermore, the residual metal ions found in tap water specifically absorb on the surface of the pigment, dramatically changing the zeta potential. For example, using tap water in our laboratory to make up the 1 mM KCl, reduces the zeta potential by 50%.]

B. Take a few flakes of the ZR5 powder and disperse it in 20 mL of the 1 mM KCl solution. Make sure to filter the 1 mM KCl solution through a syringe filter of 0.2 μm or less to ensure a clean sample prior to adding the ZR5 powder.

C. Clean a cell for use in the ZetaPlus or ZetaPALS. Add Approximately 1.5 mL of the diluted BI-ZR5, and slowly insert the electrodes into the cell. Take care that the electrodes are completely immersed in the sample, that no bubbles are trapped between or on the electrodes, and that any excess sample is wiped from the outside of the cell walls.

D. Attach the electrodes to the cable inside the ZetaPlus or ZetaPALS, and insert the cell into the cell holder.

E. With the temperature set at 25° C, wait several minutes for thermal equilibrium.

F. Initiate a measurement with the ZetaPlus or ZetaPALS of the ZR5. The conductance should be 320 μS (microsiemens) ± 10%, provided the electrodes are fully covered by the liquid in the cell. If it is not, something is either wrong with your instrument or the sample or your sample preparation. Check first for any entrapped air. Next, use the ZetaPlus or ZetaPALS to measure the conductance of the stock 1 mL KCl solution without particles. The reading should be 320 μS ± 10%.

G. For ELS, watch the reference frequency. It should settle down to 250 Hz, the peak shape being relatively sharp and clean, ignoring any noise below the 10% level. If, after several minutes, a sharp, clean peak is not centered at 250 Hz, replace the sample with a Static Scatterer that Brookhaven supplies at no charge. If the Static Scatterer shows a sharp, clean peak at 250 Hz, then the BI-ZR5 concentrate is no longer viable. Discard it, and prepare a fresh sample from the dry power as described below, or order a fresh sample from Brookhaven. If the Static Scatterer does not show a sharp, clean peak at 250 Hz, contact Brookhaven immediately for assistance.

H. Make ten single cycle ELS measurements, saving all of them. Make two more fresh samples, and repeat the measurements. If the instrument also has PALS capabilities, make 5 measurements of 30 cycles each to confirm that all technologies are within specification.

I. If your version number is less than 3.22, mark all measurements for use in the SPC table. If the average is -44 ± 8 mV, the results are acceptable. If you feel that any of the individual runs are outliers, either high or low values, reject them. We reject values that are higher than the average plus three times the standard error, or lower than the average minus three times the standard error. Average the remaining runs using the SPC feature.

If you have Version 3.22 or higher, mark the three average runs for use in the SPC tables. If the average is -44 ± 8 mV, the results are acceptable. If you feel that any of the individual runs are outliers, mark all for use in the SPC table. Examine these for any outliers, either high or low values. We reject values that are higher than the average plus three times the standard error, or

lower than the average minus three times the standard error. Average the remaining runs using the SPC feature.

Zeta Potential Electrode Conditioning

Electrode conditioning can help to greatly improve measurements of conductance and zeta potential. Contact Brookhaven Instruments before performing this procedure.

Electrode conditioning consists of running the electrodes in physiological saline (0.9 wt./vol.% NaCl). This procedure can be performed in both the ZetaPALS and the ZetaPlus. In the ZetaPALS, physiological saline is measured for 10 runs,
30 cycles each. In the ZetaPlus, physiological saline is measured for 10 runs, 5 cycles each. Below is our suggested protocol.

Procedure:

1. Using the electrode cleaning kit (BI-ELECCK) available from Brookhaven Instruments, remove any tarnish on electrodes.

2. Rinse the electrodes with deionized water.

3. Fill a plastic cuvette with approximately 1.5 mL of physiological saline (900 mg NaCl in 100 mL de-ionized water) and insert the electrode assembly into the cuvette.

4. With Particle Solutions, set up and make a measurement with 10 runs of 30 cycles. With the ZetaPlus, set up and make a measurement with 10 runs of 5 cycles each. If you have both modes of operation, always use the PALS mode. Afterwards, thoroughly rinse the electrodes with de-ionized water.

5. Fill a plastic cuvette with approximately 1.5 mL of physiological saline and insert the electrode assembly into the cuvette.

6. The measured conductance should be around 32 000 µS. If it is lower than 30 000 µS, repeat the procedure until the measured conductance is greater than 30 000µS.

NOTE: If the electrodes are used several times daily, repeat this procedure every day. If the electrodes are used infrequently, condition the electrodes before each important set of new measurements.

FAQ: Particle Sizing

1. What is the baseline index?
2. Why are baselines important?
3. How are baselines changed in the software?
4. Why are different baseline options available?
5. What is *Autoslope* (Slope Analysis) normalization?
6. What is *Last Channels* normalization?
7. Must a measurement be made again in order to use a different baseline?
8. What is the *Dust Filter*?
9. What is the exact algorithm for the *Dust Filter*?
10. What value is appropriate for the *Dust Filter*?
11. How do I know how much data is retained for a given *Dust Filter* setting?
12. What is the *Effective Diameter*?
13. What is the *Mean Diameter*?
14. What is the *Combined Diameter*?
15. Which one do I use, the *Mean Diameter* or the *Combined Diameter*?
16. What if there are outliers?
17. What is the upper limit for count rate?
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19. How do I send data to Brookhaven Instruments when I have a problem?
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21. Why is 10 mM KNO_3 used as a diluent for the latex reference material?
22. Why not just use distilled water as a diluent?
23. What is the Stokes-Einstein Equation?

1. What is the baseline index?

The baseline index (BI) is a measure of how much the autocorrelation function was distorted by a few large particles. Often referred to as "dust", they could be a few aggregates, too few for this technique to characterize properly. A high value (10) corresponds to no dust and a low value (0) corresponds to a noticeable amount of dust. During data analysis, the experimental autocorrelation function must be normalized. In theory, one should use the infinite time baseline B_∞ obtained from the square of the average counts per sampling time. However, in practice, in order to eliminate the effect of a few large particles (dust), a baseline obtained from the measured correlation function at some long delay time is a better choice for normalization. During measurement, this constant background term is measured using a variety of possible methods (see below) to obtain a measured baseline value B_m. For $(B_m/B_\infty-1) < 0.01$, that is a less than 1% difference in baselines, the index is defined by

$$\mathbf{BI = 10\,[\,1 - 100\,|(B_m/B_\infty - 1)|\,],} \qquad \mathbf{if\ |(B_m/B_\infty - 1)| < 0.01,\ otherwise,\ BI = 0.0}$$

If the measured baseline is 1% or more above the infinite time baseline, or the measured baseline is below the infinite time baseline, the baseline index is 0. If the baselines agree exactly (a good thing), the baseline index equals 10. At a 0.1% baseline difference, the baseline index is 9 (which is quite good). If the BI value is 8 or higher, it means that the two measures of the correlation function baseline are in good agreement. Even if the value is lower than 8, if you have reasonable results and repeatable results, then the software has taken care of the less than perfect sample. The low value for baseline index is to alert you about what is going on. If you make a new sample, consider removing the few large dust particles by, for example, filtration or centrifugation. In previous versions of the **90Plus** software the BI was referred to as Sample Quality. The two names refer to exactly the same value, only the name was changed.

2. Why are baselines important?

The first step of data analysis is to normalize the experimental autocorrelation function by dividing each data point by the baseline. These normalized data are then analyzed to obtain particle size. In the most common case, cumulant analysis, the logarithm of the normalized data minus one is fit to a second order power series in delay time. A good baseline index indicates that the data is of high quality, e.g., free of dust. Don't worry if the index is poor; the **90Plus** uses advanced algorithms to recover as much information as possible. If you have reasonable results and repeatable results, then the software has taken care of the less than perfect sample.

3. How are baselines changed in the software?

Click on the SOP, under Data Analysis select Normalization. Select the method desired. Normally the "Autoslope (Slope Analysis)" method is selected. This is described below.

4. Why are different baseline options available?

This is one way that you can recover from an imperfect sample. Varying the baselines changes the way large dust particles are treated in the analysis.

5. What is *Autoslope* (Slope Analysis) normalization?

In this case, the data are examined starting halfway down the autocorrelation function and a series of straight line fits are performed until the slope is less than a small, positive value. Effectively, this is where the decaying autocorrelation function is flat or nearly so. For most samples this works nicely and we recommend using the Autoslope baseline. Sometimes, there are correlation functions that are very noisy or have two distinct decays. In these cases, the Autoslope (Slope Analysis) routine may set the baseline prematurely. In such cases we recommend either "Last Channels" or "Calculated" as baseline choices.

6. What is *Last Channels* normalization?

This places the last eight baseline channels at the last eight delay times. If the baseline index (BI) improves and repeatability is acceptable then the results may be better. However, if there are large variations in Effective Diameter and Polydispersity then there may be some dust and you are better off with Autoslope.

7. Must a measurement be made again in order to use a different baseline?

No. Just recall the data (file/database) and change the normalization method to see the results.

8. What is the *Dust Filter*?

The dust filter is an algorithm that rejects data that are corrupted by scattering from dust. You can turn the "dust filter" on or off during a measurement and you can recall data and turn the dust filter on and off retroactively. However, during and after a measurement, a new filter value cannot be entered. If the dust cutoff value is too high, 100% of the light is accepted for correlation and you have not "clipped" the high or "dust" contributions. Note that for very clean samples, one expects that 100% of the data is retained, provided the dust cutoff is not set too low. If the dust filter value is too low, then the reported effective diameter will be too low.

9. What is the exact algorithm for the *Dust Filter*?

In the data collection the scattered intensity is measured over many short, consecutive time intervals. The data are divided into two sets. The first data set, "unfiltered" includes all of the collected data. The second data set, "filtered" is filled by using the following procedure. The moving mean, I_{mean}, and standard deviation, σ_m, of five sequential intensi- ty values is calculated. On the sixth measurement, the intensity, I_{new}, is compared with the average of the previous five using the dust filter cutoff value "C." If the following test is true, the data is accepted into the second "filtered" data set.

$$| I_{new} - I_{mean} | < C \sigma_m$$

If the test is false, then the data from this measurement is not added to the "filtered" data set. Thus, the "filtered" set is only the result of measurements that meet the inclusion criteria for the dust filter. The autocorrelation function is accumulated and kept for both sets. In this way, the user can turn the dust filter on or off during or after a measurement to observe the effects of data filtering on the results. However, the dust filter cutoff value cannot be changed once a measurement has begun.

10. What value is appropriate for the *Dust Filter*?

Start with a value of 30. The optimal value of the dust filter cutoff is actually related to the expectation of outliers in intensity, which is related to the size of particles being measured. For particle sizes on the order of 10 nm, use a value of about 15. For particle sizes on the order of several 100 nm, use a value of 30. For particle sizes on the order of 1 micron, use a cutoff value of 80. To truly optimize the dust cutoff value, lower the dust cutoff filter until about 98% of the data is retained.

11. How do I know how much data is retained for a given *Dust Filter* setting?

When the dust filter is on, the percent data retained is displayed to the right of the baseline index.

12. What is the *Effective Diameter*?

The effective diameter is calculated from the Stokes-Einstein equation (for spheres, see question 27) from the experimentally determined, translational diffusion coefficient. The translational diffusion coefficient is calculated from the accumulated autocorrelation function of the scattered light using the method of cumulants. Thus, the translational diffusion coefficient is intensity-weighted. The Stokes-Einstein equation shows that the hydrodynamic diameter is inversely proportional to the diffusion coefficient. Therefore, it is wrong to say the diameter determined this way is intensity-weighted. The weighting is actually the inverse of the intensity weighting of the reciprocal of the diameter. An average calculated in this way is sometimes called the harmonic mean.

13. What is the *Mean Diameter*?

Consider N runs, each yielding an Effective Diameter. The average of these Effective Di- ameters (sum divided by N) is called the Mean Diameter.

14. What if there are outliers?

If the Effective Diameter of a particular run differs from the Mean Diameter by more than twice the standard error, consider it an outlier. Delete the outlier by double clicking on the line of data corresponding to the run. The text on that line will turn gray. Deletion can be reversed by double clicking on the same line.

15. What is the upper limit for count rate?

The upper limit for count rate is 500 kcps (kilocounts per second) for the avalanche pho- todiode (APD) detector and 1 Mcps (1 million counts per second) for the photomultiplier tube (PMT) detector.

16. What is the lower limit for count rate?

The lower limit for count rate is 2 kcps, about five times the count rate for a typical liquid. A lower count rate means a measurement will take longer to complete. At low count rates, over a given time period, fewer photons are reaching the detector and there is less data with which to build the correlation function. Thus, at low count rates increase the du- ration to get more repeatable results.

17. How do I send data to Brookhaven Instruments when I have a problem?

See question 18, "How do I save the data so I can analyze it at my desk?" Then, e-mail the resulting file to Brookhaven Instruments.

18. How do I save the data so I can analyze it at my desk?

In the main database screen highlight the measurements you wish to save. Now, click on File -> Save Archive File. A standard save file dialog will appear. Choose a descriptive file name then save the file. The extension ".bak" will automatically be appended to the file name. This file contains many details about the measurement that are not available in a screen shot or printed report.

19. Why is 10 mM KNO_3 used as a diluent for the latex reference material?

See question 26, "Why not just use distilled water as a diluent?"

20. Why not just use distilled water as a diluent?

In order to calculate particle size from dynamic light scattering results, the Stokes-Einstein equation is used (see question 27). An underlying assumption in the calculation is that the particles are freely diffusing. For dilute electrostatically stabilized dispersions, long-range interactions distort free, translational diffusion. Adding salt screens the particle-particle interactions, making the assumption of free diffusion valid and therefore the use of the Stokes Einstein equation correct. 10 mM aqueous KNO_3 is often a good choice for a diluent. Most aqueous

dispersions, such as the anionic latex used to validate the performance of the 90Plus, are electrostatically stabilized.

21. What is the Stokes-Einstein Equation?

The Stokes-Einstein equation relates the diameter of a spherical particle to the translational diffusion coefficient.