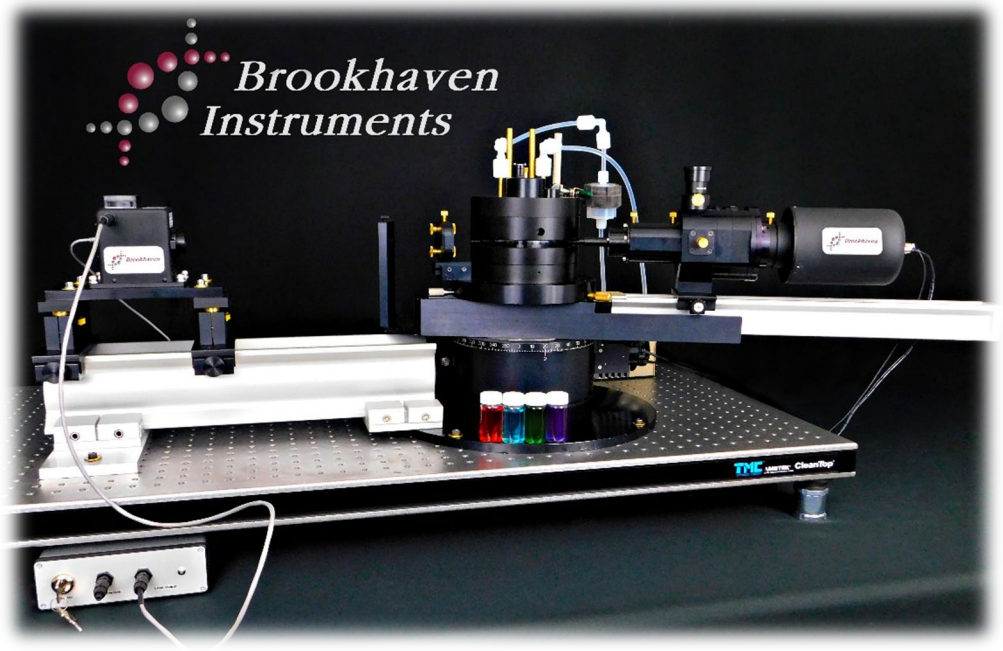
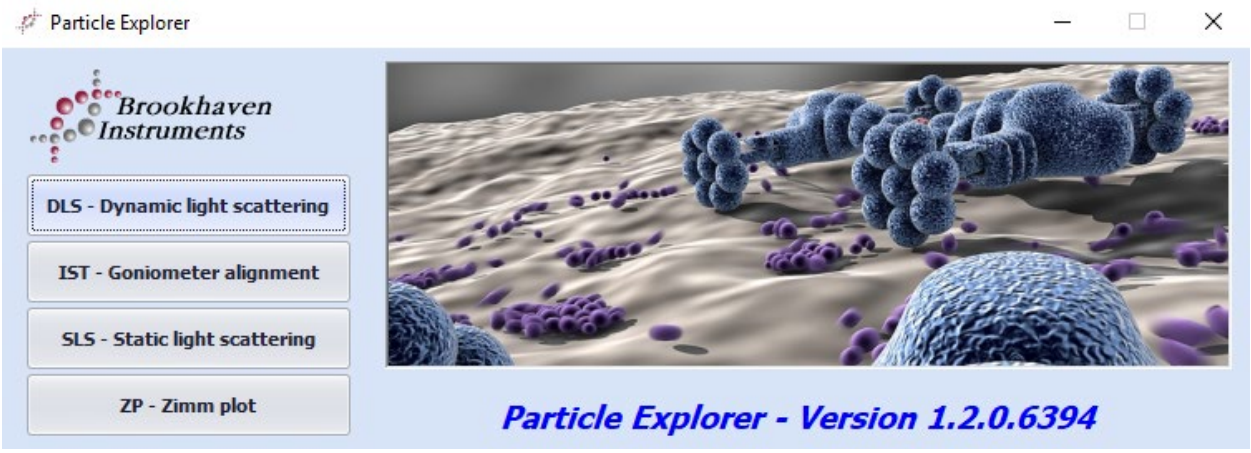
Particle Explorer
Software Manual





PLEASE READ

This is your instruction manual for the Brookhaven BI-200SM goniometer software, Particle Explorer. 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.

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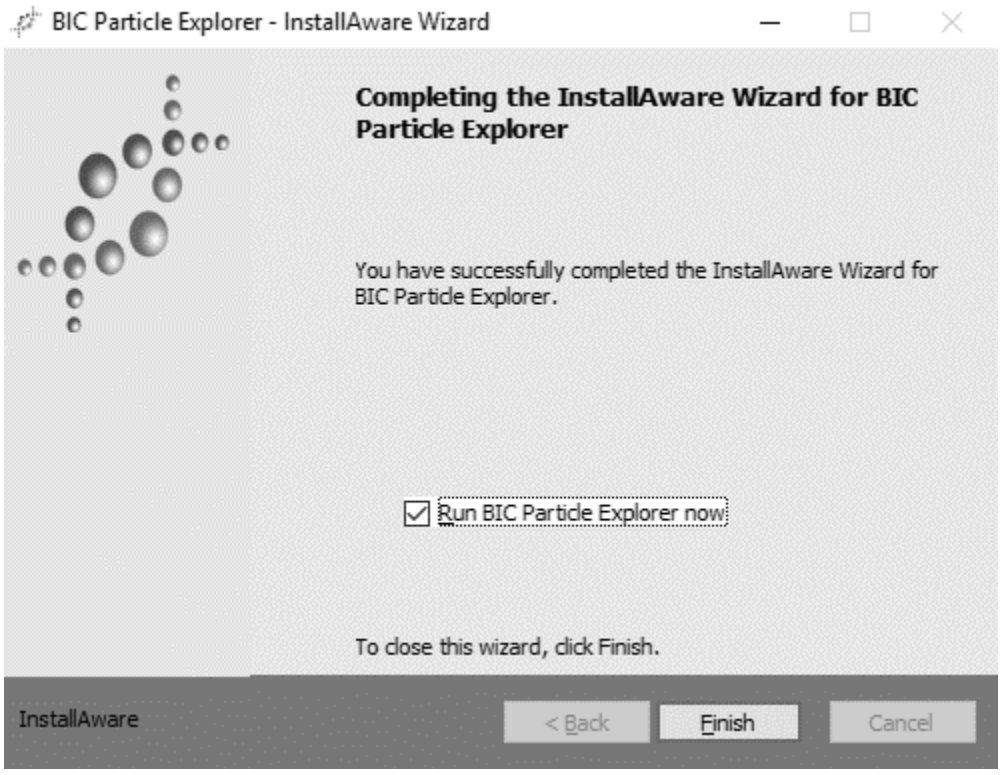
Installing the Instrument and Software

Installation of the BI-200SM goniometer is typically performed by a Brookhaven Instruments technician. Experienced users can refer to the **Goniometer Photo Alignment Manual** to perform their own installation and alignment. Contact Brookhaven Instruments for more information.

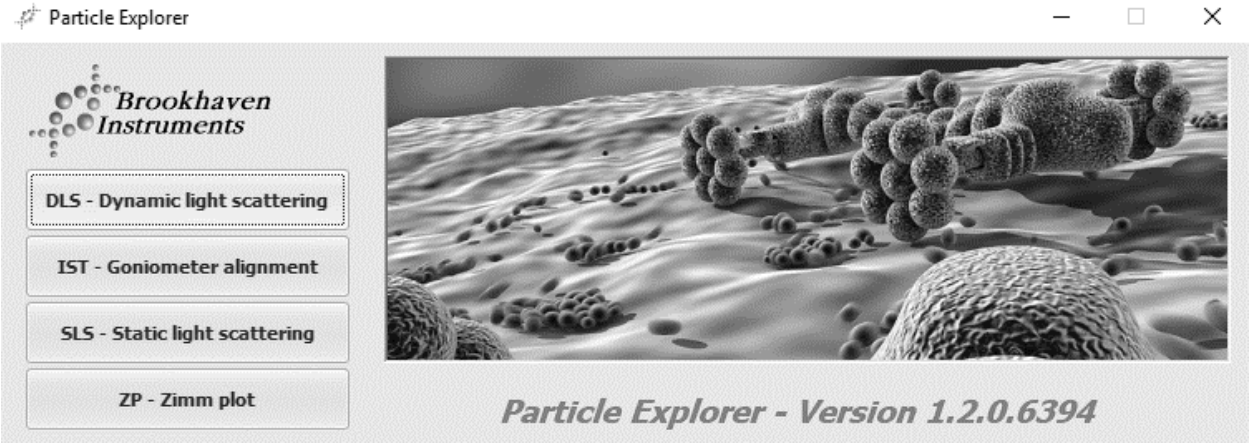
Once the BI-200SM goniometer has been installed, the software can be installed. **Be sure not to plug in any components of the instrument to the computer until all software is installed.**

1) Locate the USB drive included with the instrument. Plug into the computer designated for use with the BI-200SM. This computer should be operating with Windows 7 or better.
2) Open File Explorer to display the files on the USB drive.
3) Locate the file titled: "PE_x.x.x.xxxx_Setup"
4) Double click this Setup file.
5) Click "Yes" to allow the installation to begin.
6) When the installation wizard launches, follow the prompts to complete installation.
7) This window will appear when the software has been successfully installed:



8) Choose whether to run the software at this point. Click Finish.

9) If the software is set to run, this window will appear:



All measurement types available for the BI-200SM are displayed here.

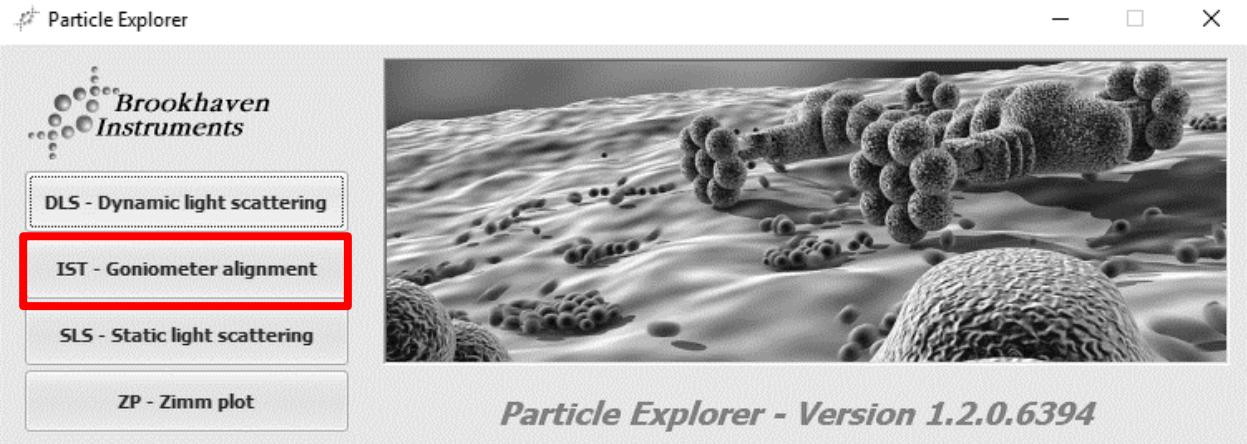
10) Prior to choosing a measurement type, plug in all components of the instrument.
11) As a test, click "DLS – Dynamic Light Scattering". A measurement window will open.
    If this is the first time an OBIS laser has been connected to this computer, the software will automate the process of installing the drivers.
    If no messages appear regarding instrument hardware, all instrument connections were recognized and measurements can be made. If any error messages appear, ensure all cables are connected to the instrument and computer properly and restart the DLS software.

Checking Instrument Alignment: IST

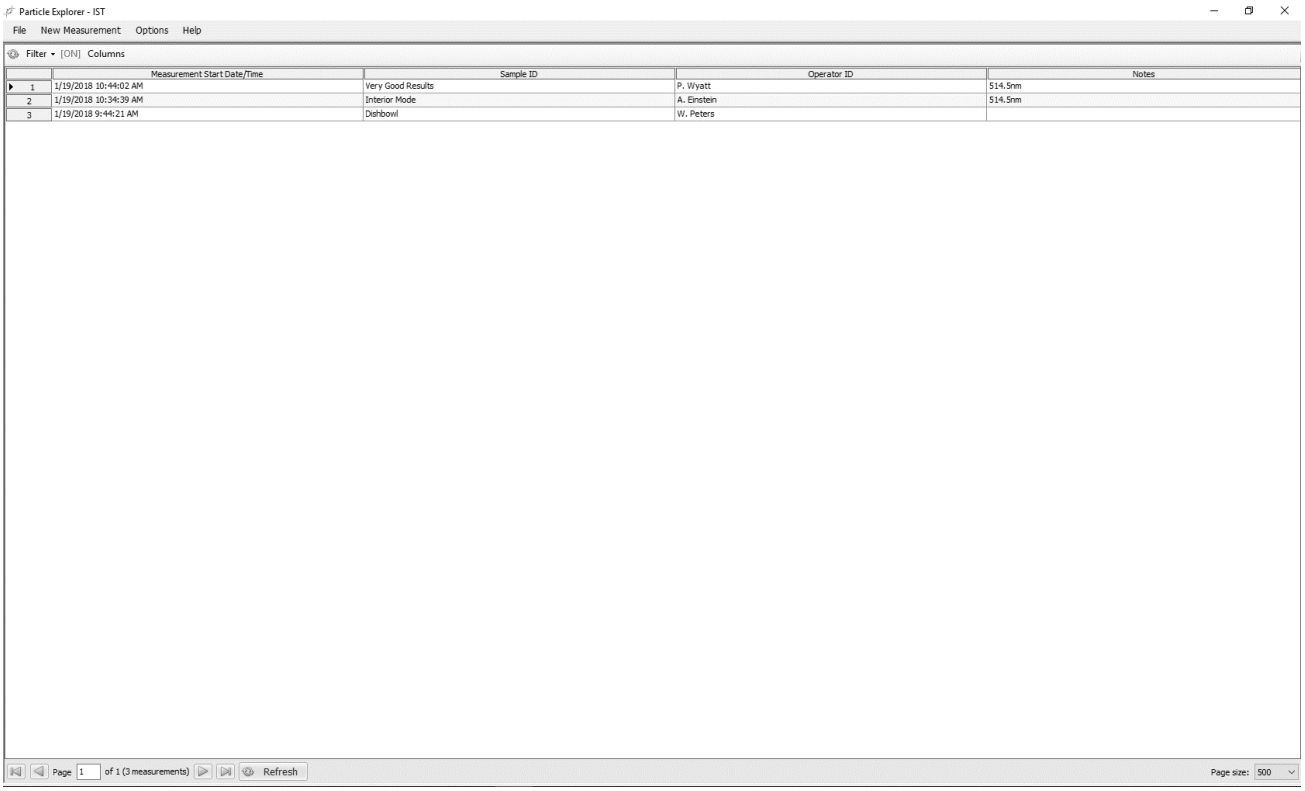
In order to make accurate and reliable light scattering measurements, the BI-200SM goniometer must be in proper alignment. This procedure is typically performed by a Brookhaven Instruments Technician or an experienced user. Contact Brookhaven Instruments for more information.

Once an alignment is completed, the IST Goniometer Alignment Software can be used to check how well the instrument is aligned. Follow these steps:

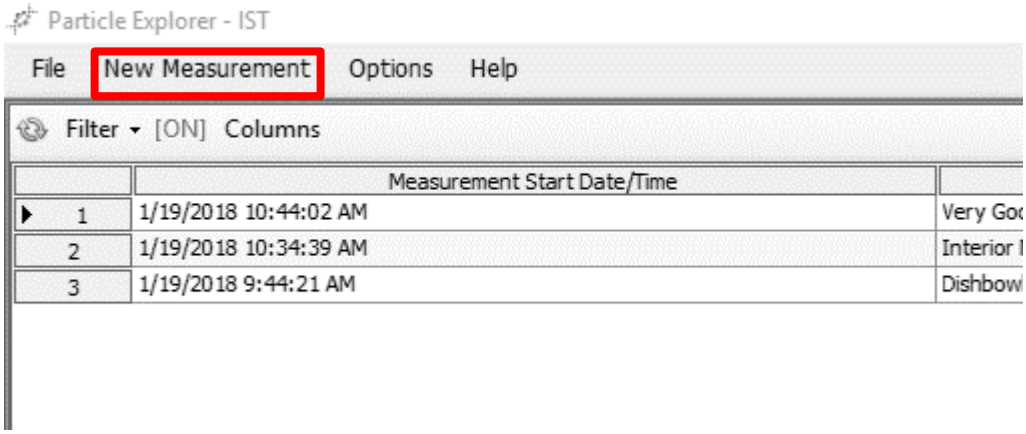
1) Click IST - Goniometer Alignment from the Particle Explorer splash screen.



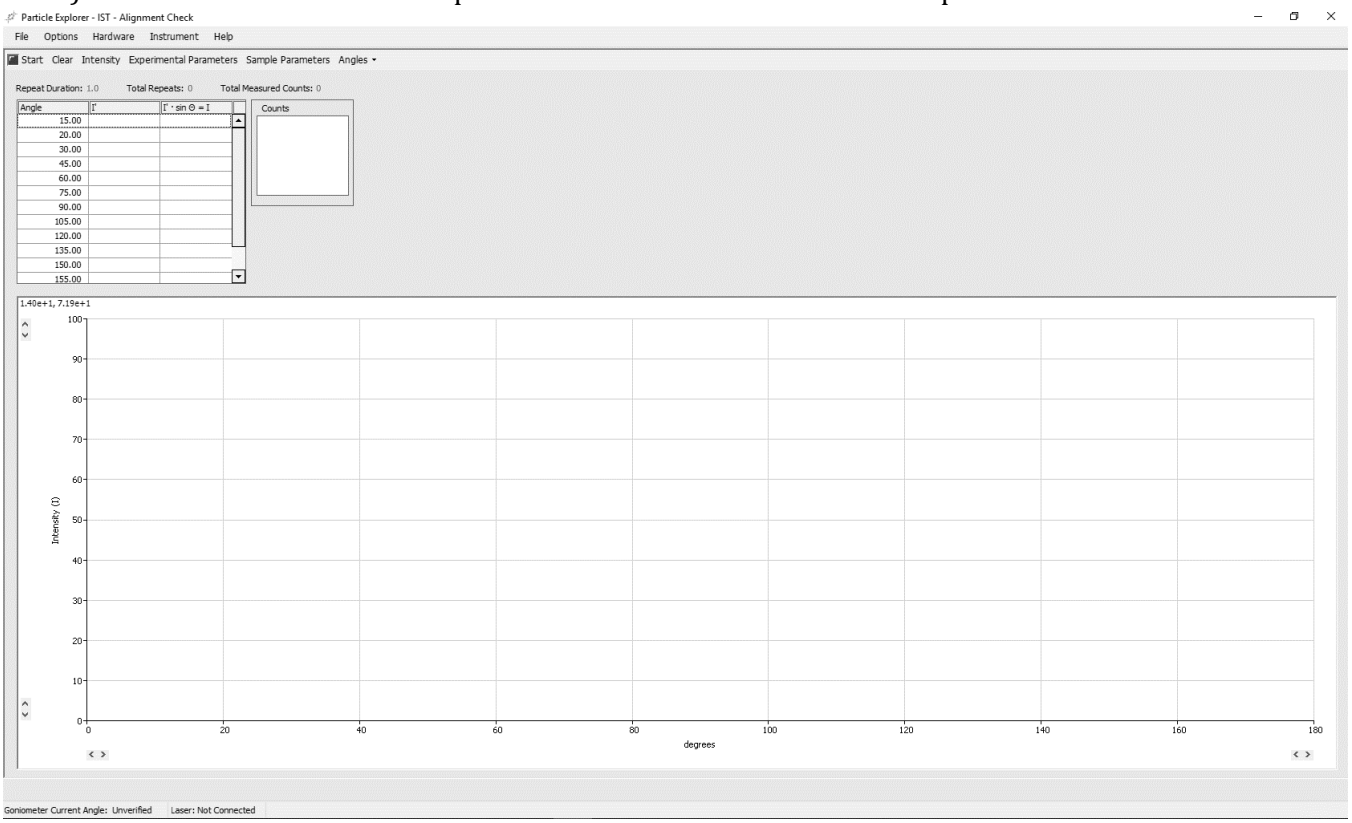
2) A new window with a database will open. This allows the review of previously recorded measurements.



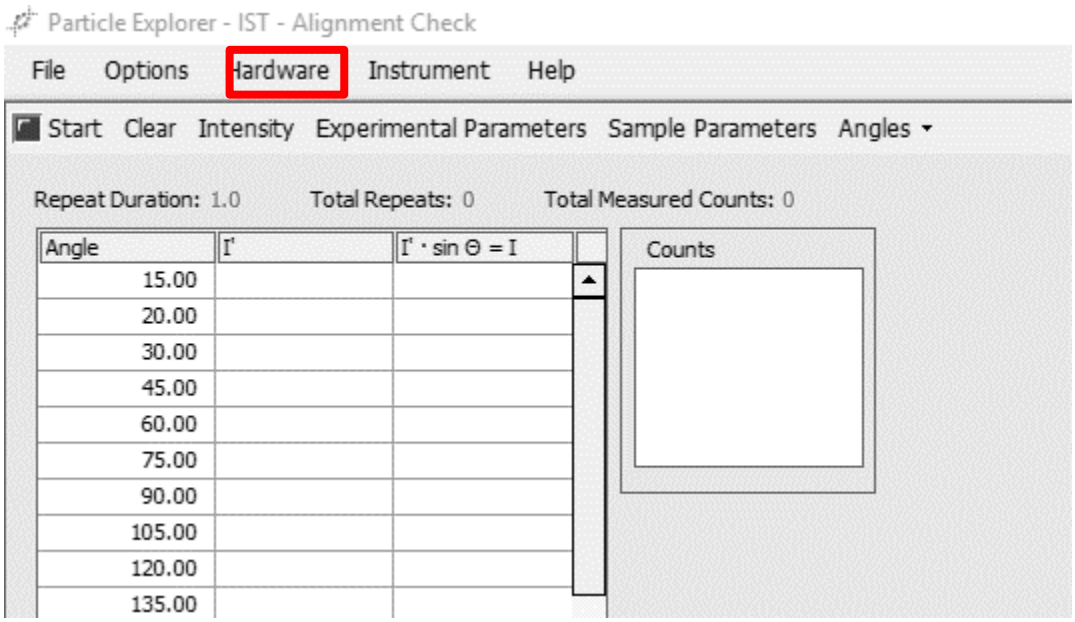
3) To setup a new alignment measurement, select "New Measurement" from the ribbon.



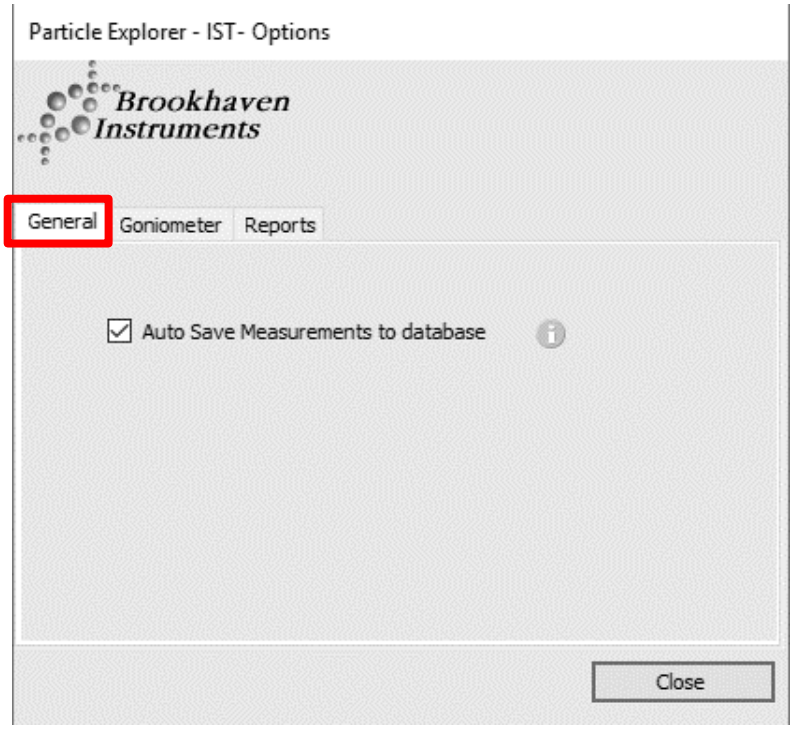
4) An additional window will open where a measurement can be set up and started:



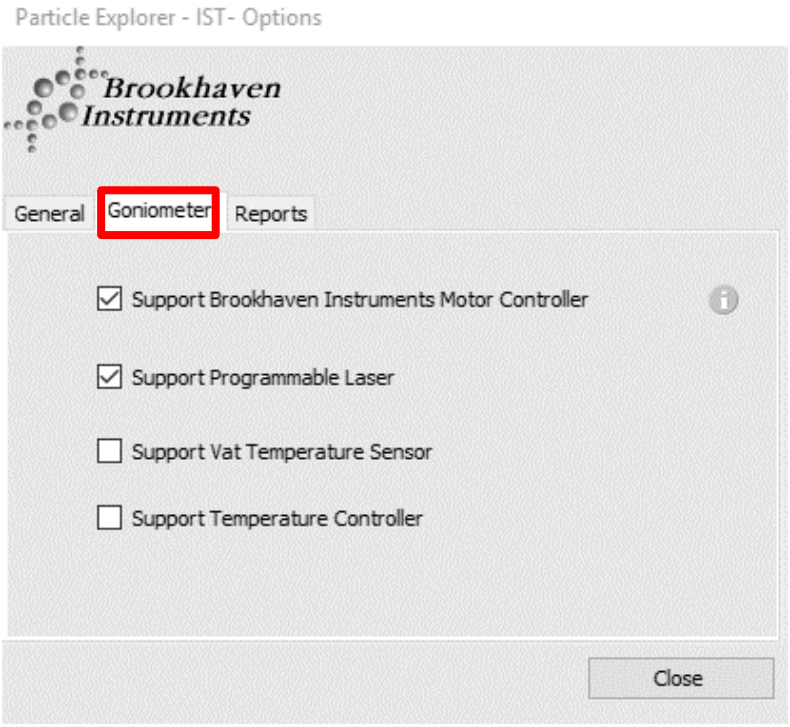
5) Click “Options” on the ribbon.



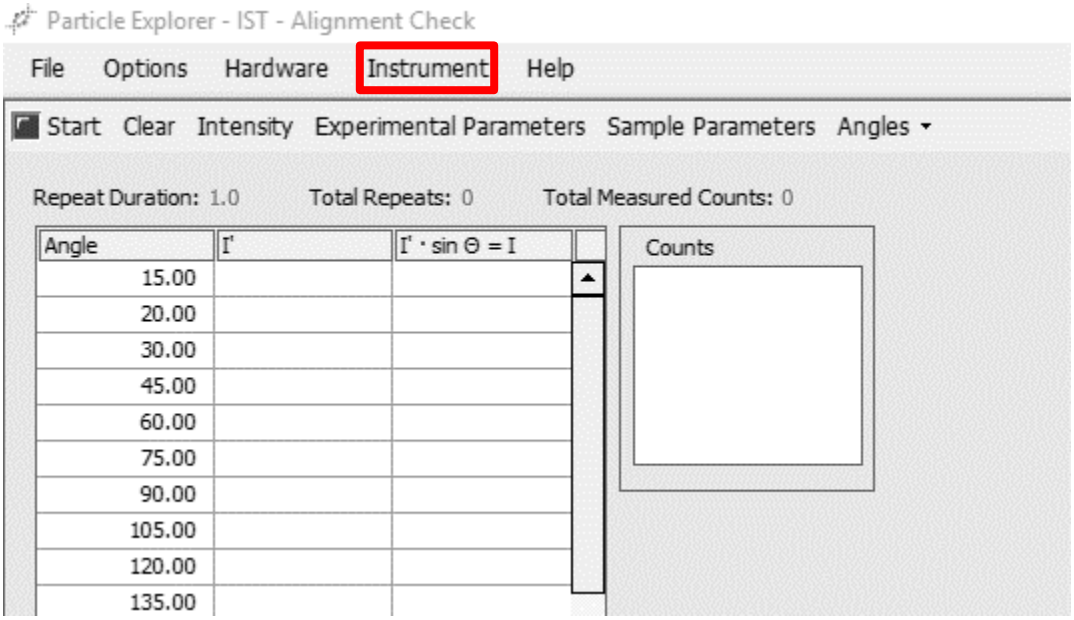
a) On the General tab, it is recommended to check the Auto Save option.
**There are information icons, , throughout the software. Click these for details about the setting or parameter. **



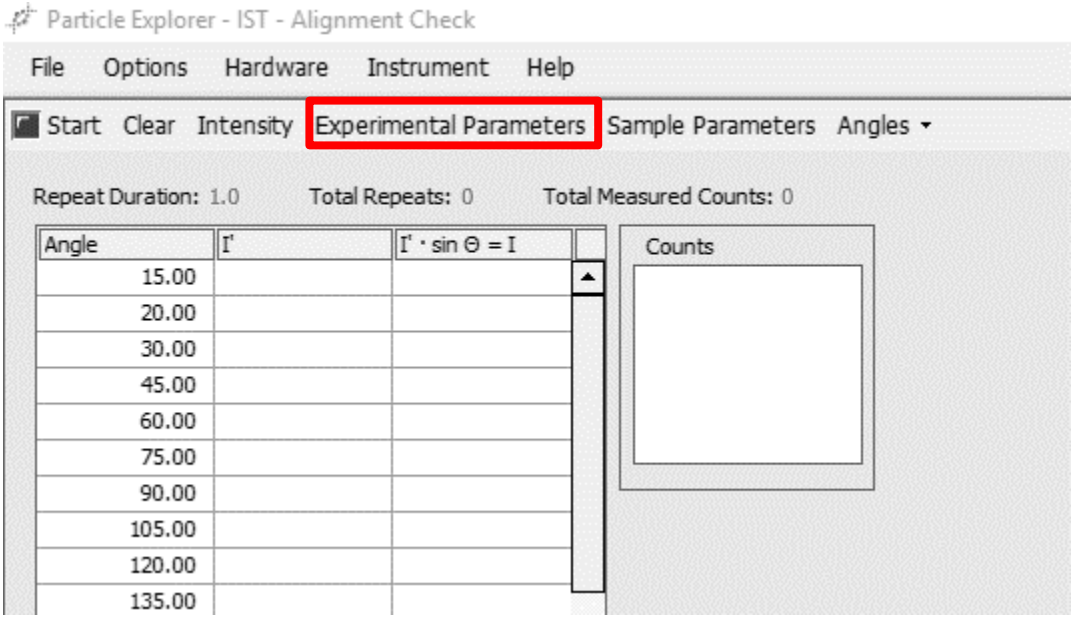
b) On the Goniometer tab, check what options the instrument is configured with. Close the window when all selections have been made.



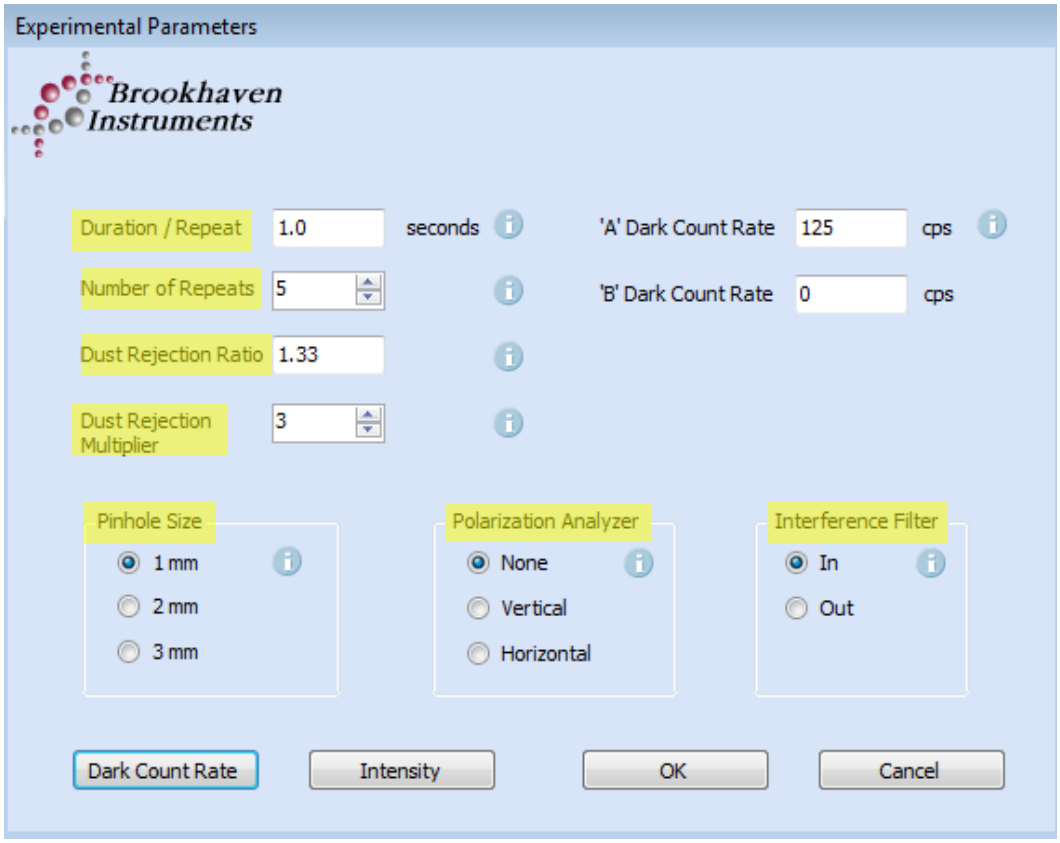
6) Click “Instrument” from the ribbon to connect a laser or temperature controller option. Follow the on-screen prompts. This is not a necessary step for the alignment check.



7) Click “Experimental Parameters”.

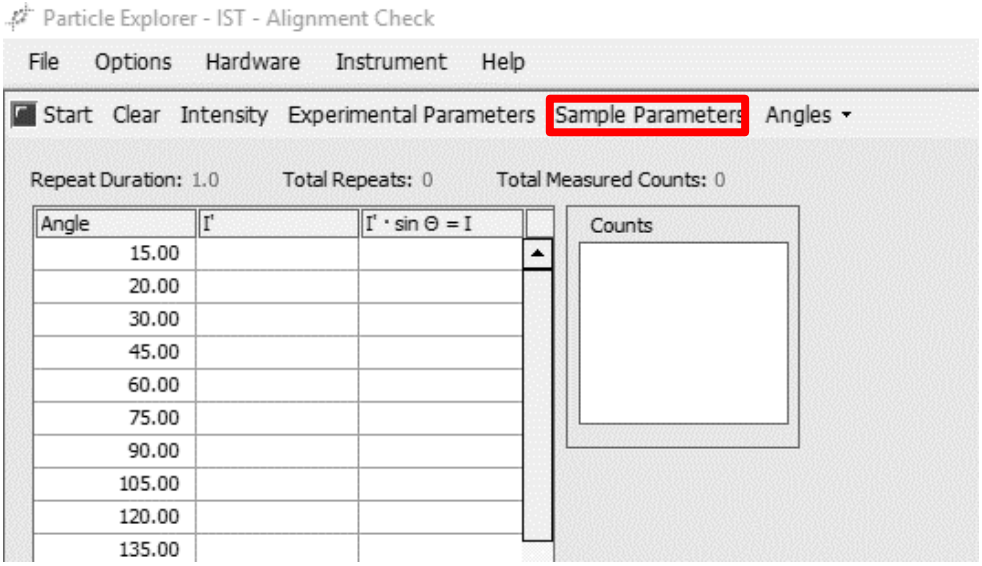


a) The default values shown in the highlighted sections below are recommended for an alignment check. Click the information icons for information about each parameter.

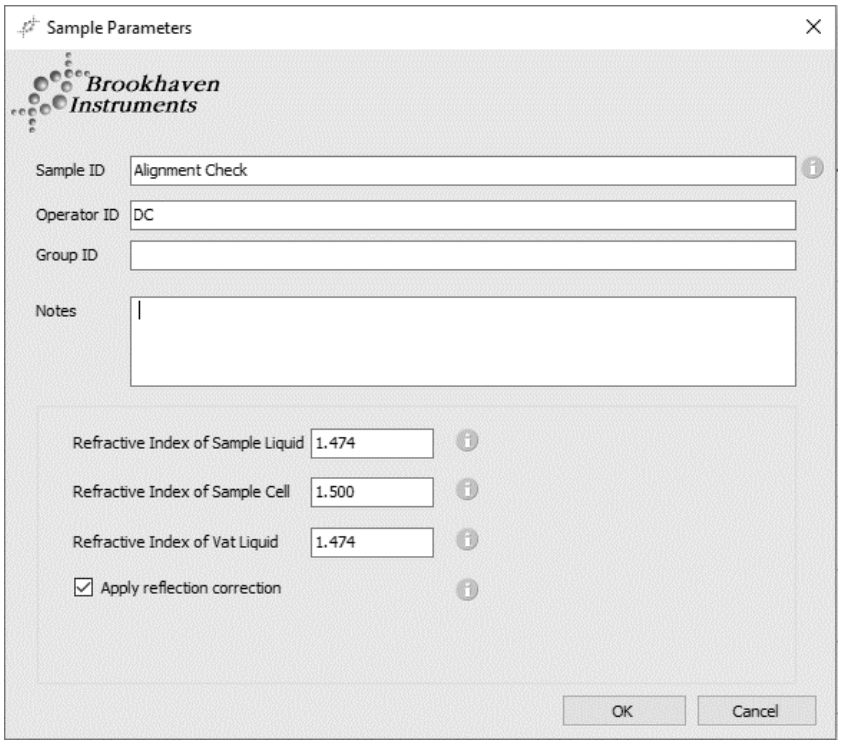


b) Prior to any SLS measurement, the Dark Count Rate must be measured. Turn the filter wheel on the detector optics to "C" for closed. Click "Dark Count Rate". The software will automatically measure the dark count rate, or the noise of the detector, so that it can subtract this value from the data collected during the measurement sequence. It is recommended to measure the Dark Count Rate a few times to make sure the value is repeatable. Once satisfied, click "OK" and the software will automatically fill in the field 'A' Dark Count Rate with the measured value.

8) Click “Sample Parameters”.

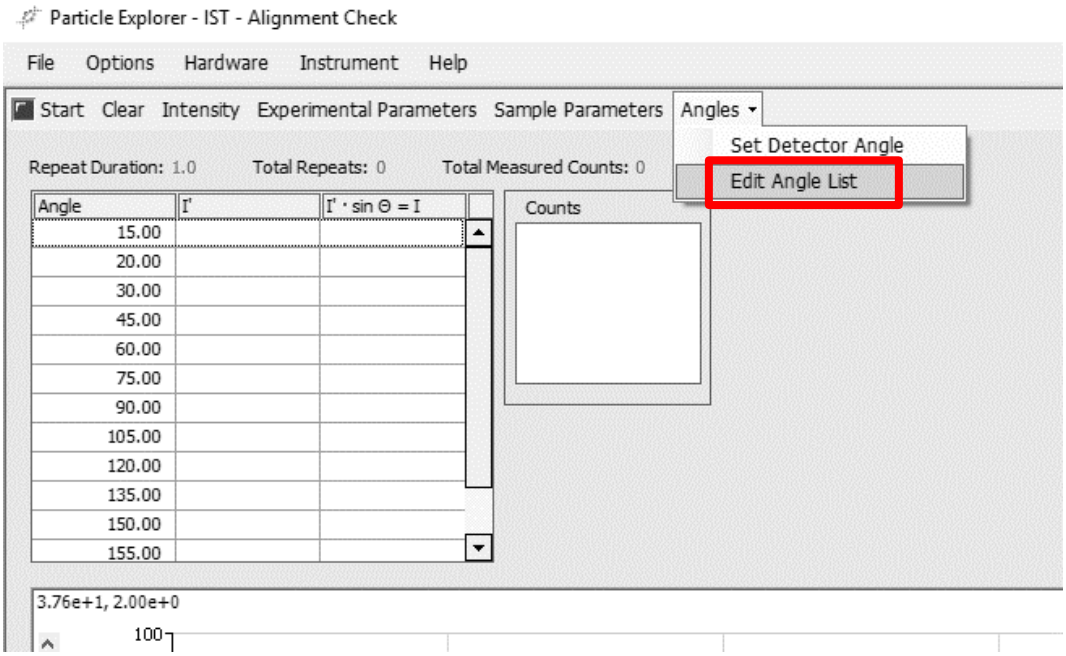


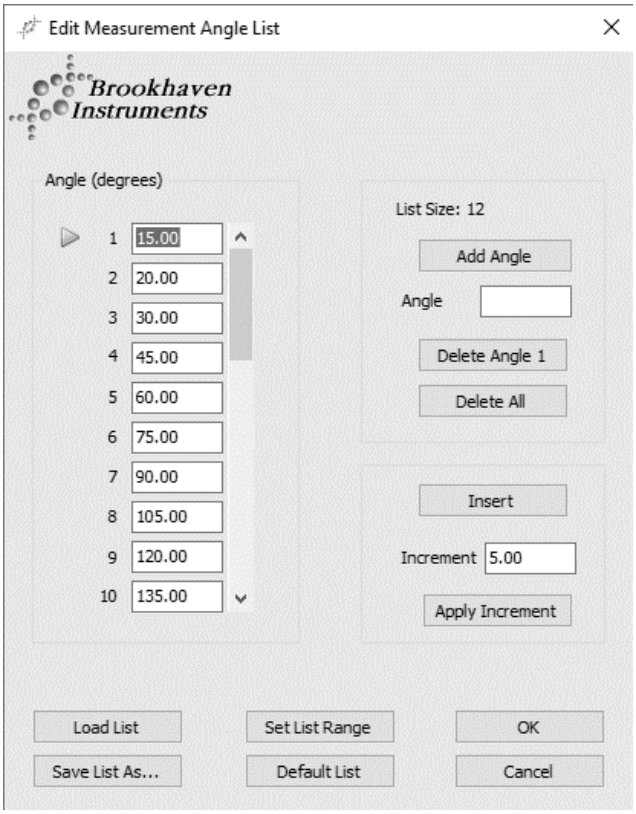
a) Sample ID, Operator ID, Group ID, and Notes are available fields for measurement identification purposes. Each field is not required but recommended so that reviewing past measurements is simple.



b) For an alignment check, no sample is required. In this case, the sample liquid and the vat liquid are both cis-trans decahydronaphthalene (decalin), which has a refractive index of 1.474. The glass vat has a refractive index of 1.500. Checking “Apply Reflection Correction” is recommended. These are the default values for an alignment check.

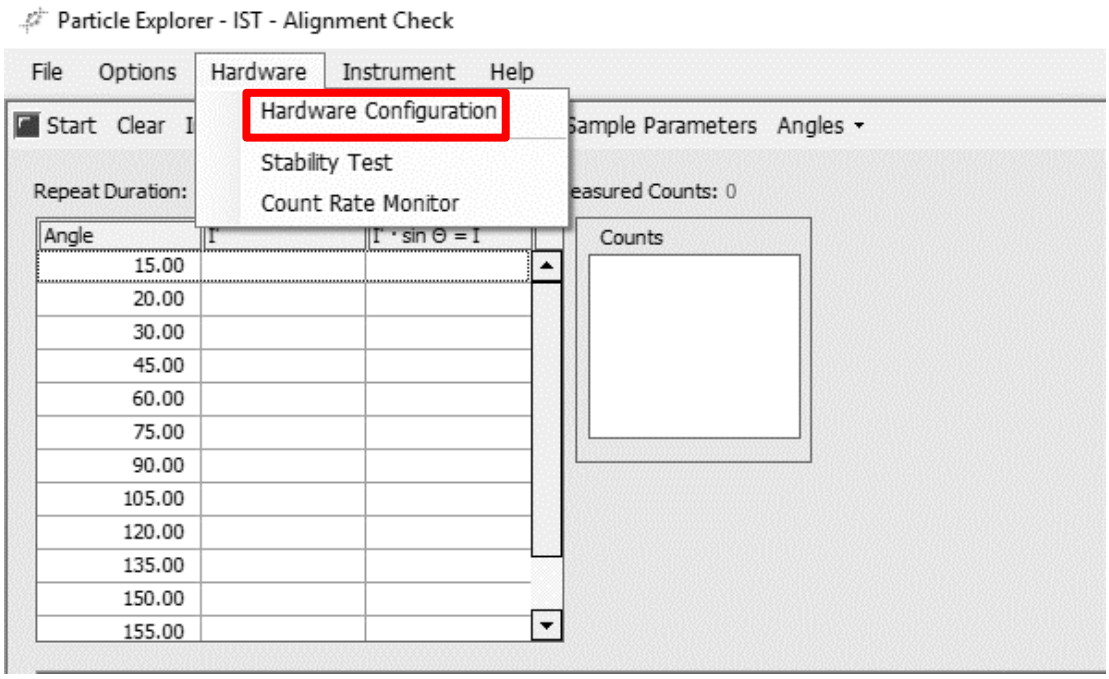
9) Click “Angles→ Edit Angle List”.

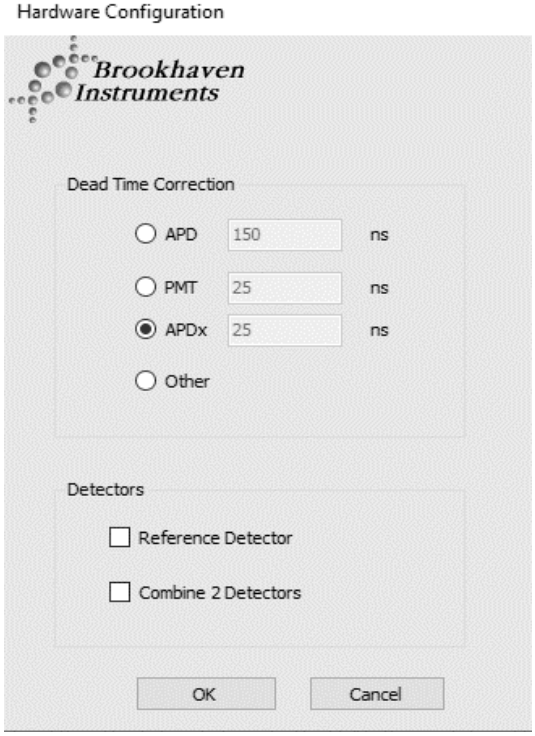




The default angle list is recommended for alignment checks. It thoroughly checks the full range of the instrument. Select Default List to import the recommended angle list. To check a different range of angles, less angles, or more angles, use the Add Angle, Delete Angle, and Set List Range buttons.

10) Click “Hardware→ Hardware Configuration”.

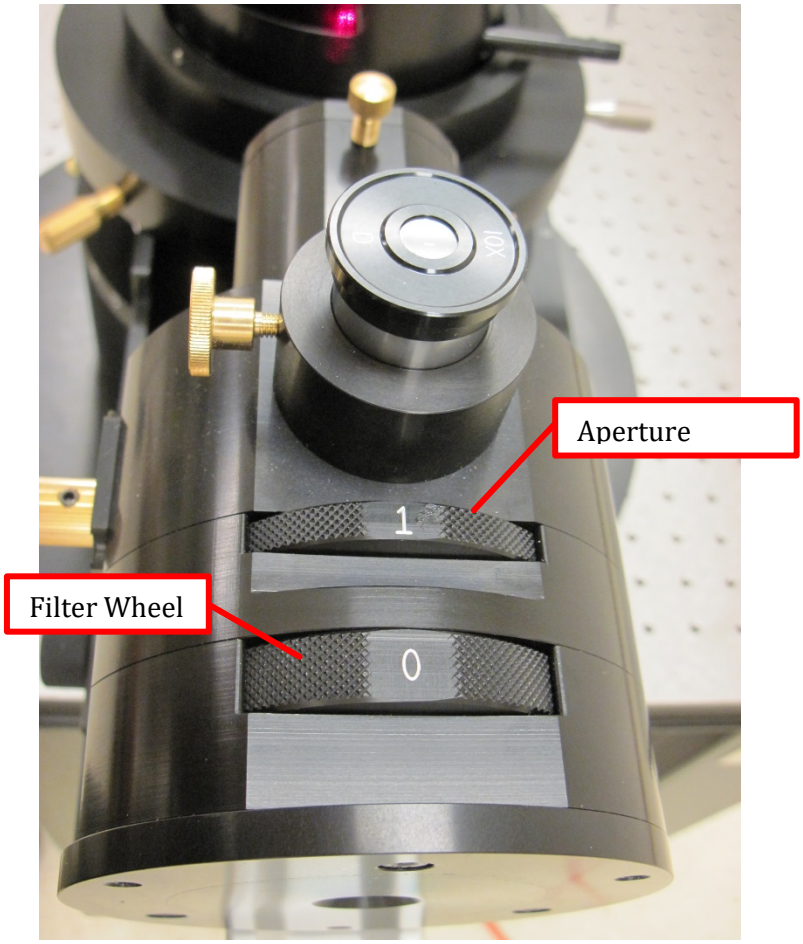




Choose the detector the instrument is equipped with. The dead time correction is automatically assigned. If the instrument has a detector or dead time not listed, select “Other” and fill in the dead time correction manually.

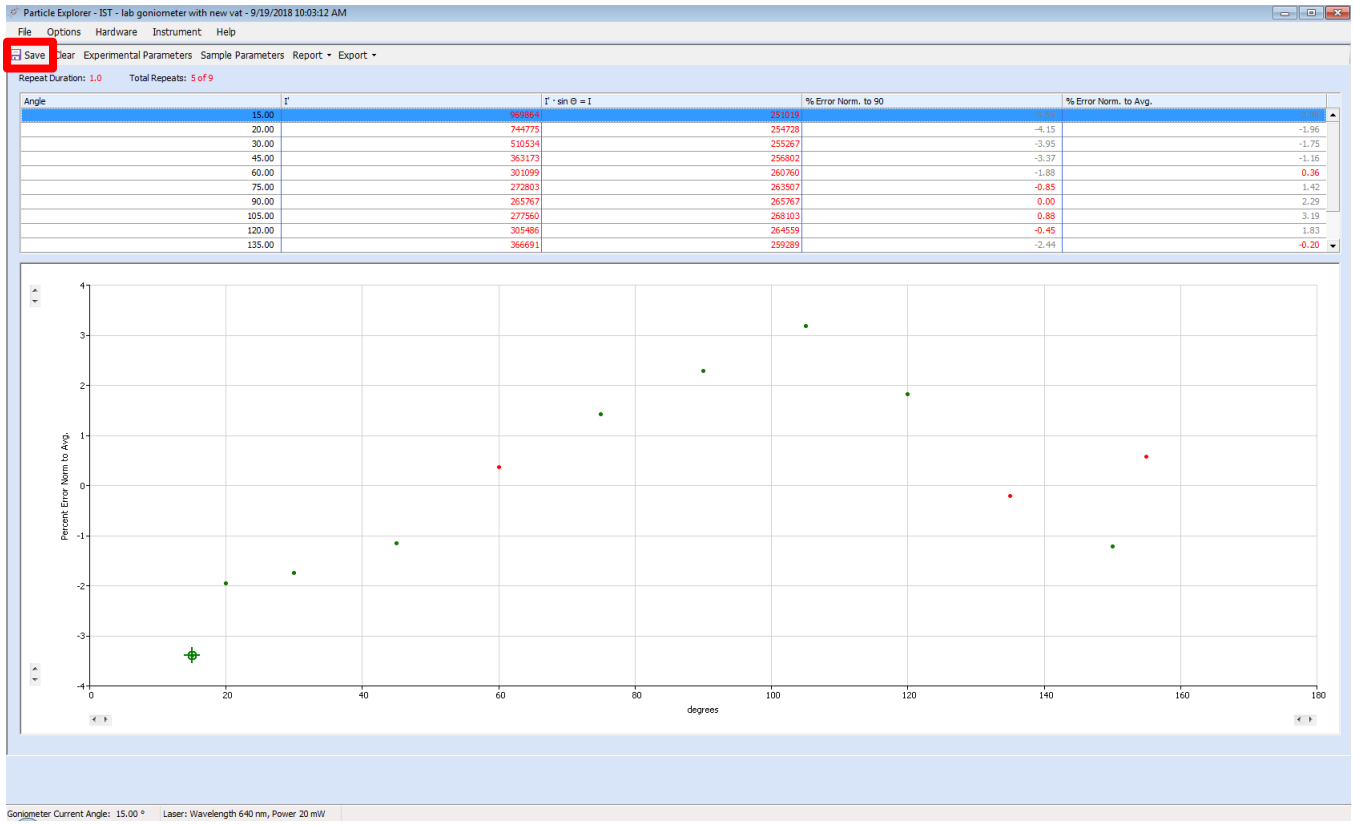
For specific applications, the instrument may be configured with a reference detector or two detectors. Check the appropriate box to perform an alignment check including these options.

11) Now, all of the alignment check parameters have been set. On the detector optics, open the filter wheel to the wavelength of the instrument's laser and the aperture wheel to the 1 mm pinhole. Click "Start" to begin the measurement.

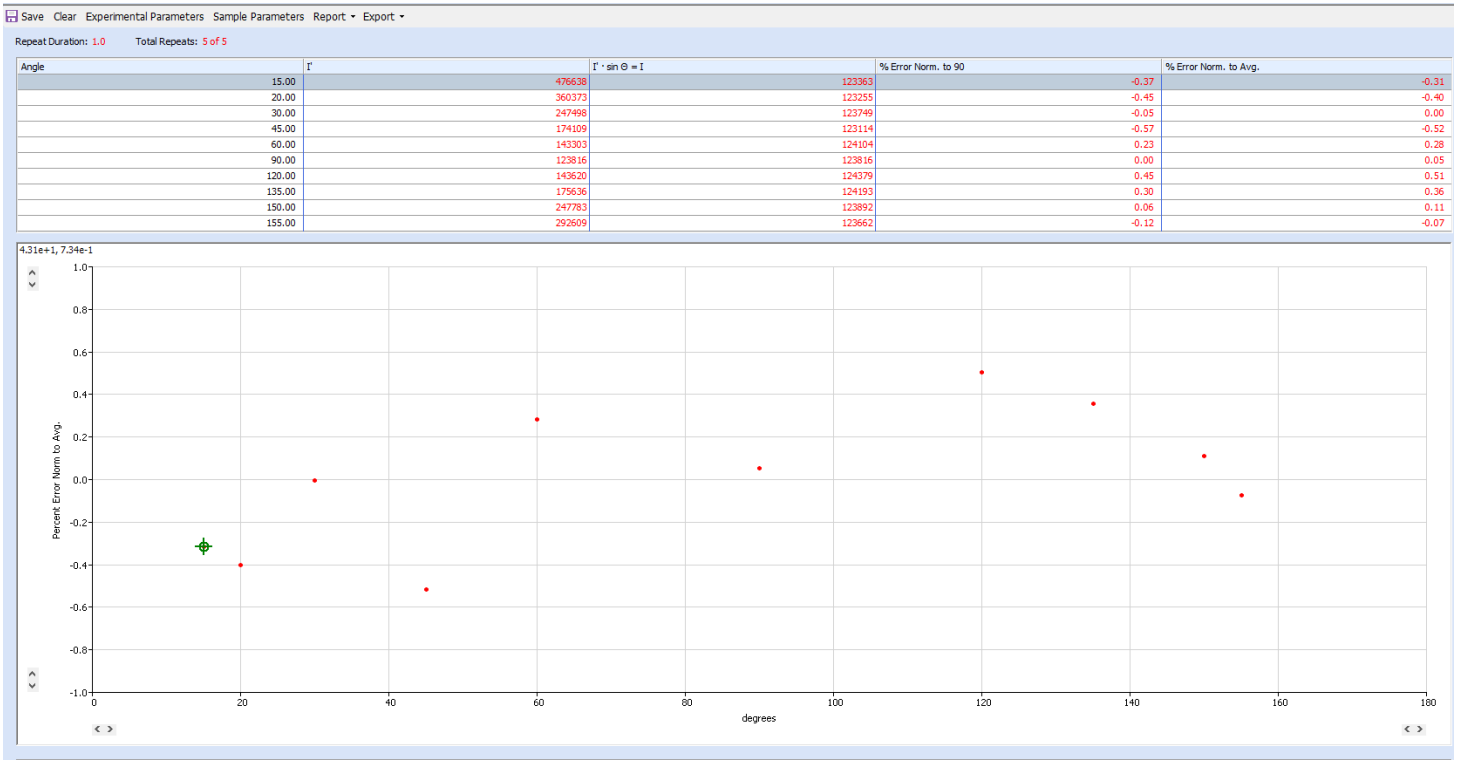


12) The software needs to know what angle the goniometer is at before it can begin the measurement. A window will appear asking for the current angle. Enter the angle and click OK to allow the measurement to proceed.

13) The rest of the measurement is automated. When complete, the measurement will be automatically saved to the database if selected in Step 5a. If not, click “Save” once the measurement is complete. Otherwise, the data will NOT be saved.

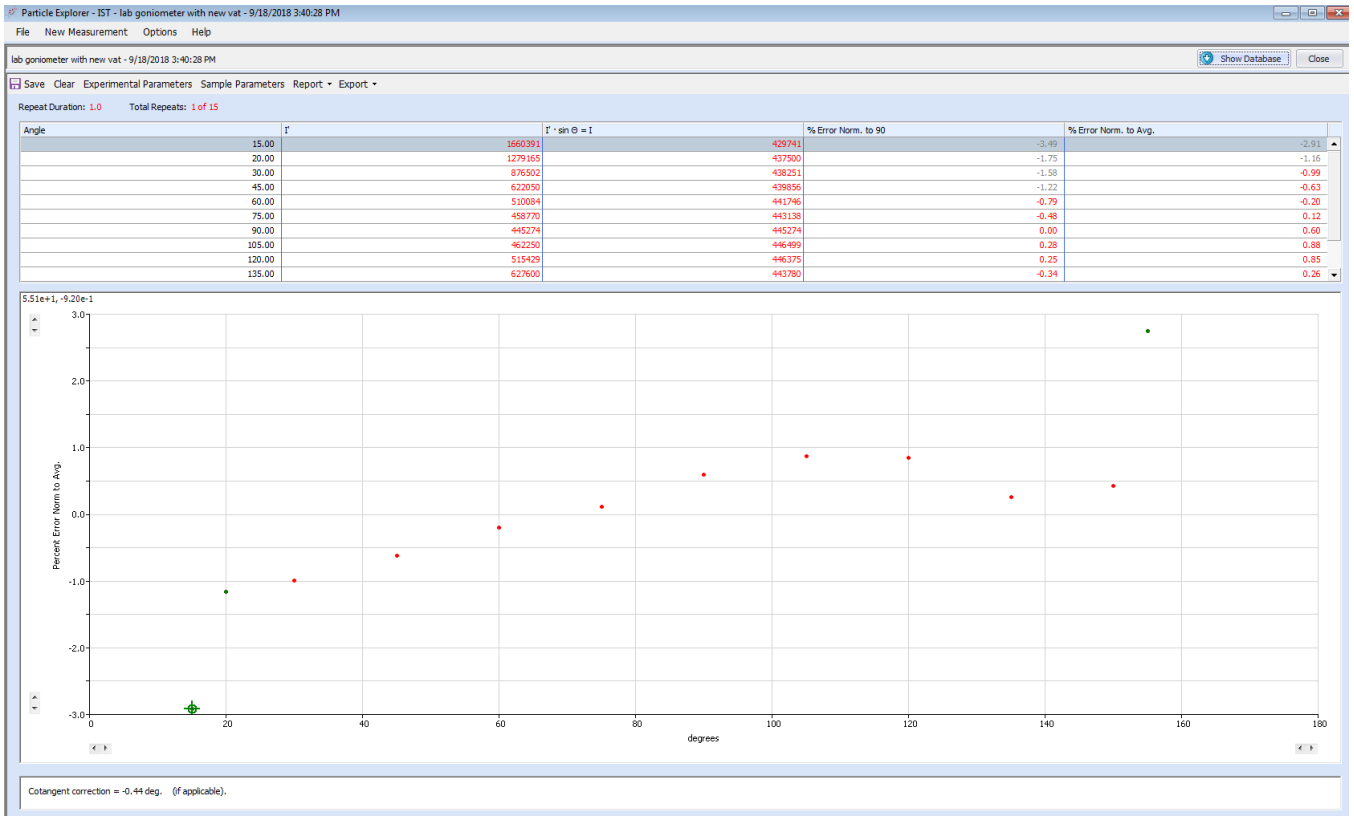


14) Below is an example of results from an instrument that is in good alignment. All angles are within 1% error when normalized to the average corrected scattering intensity. Make several measurements to ensure that the good alignment is repeatable.

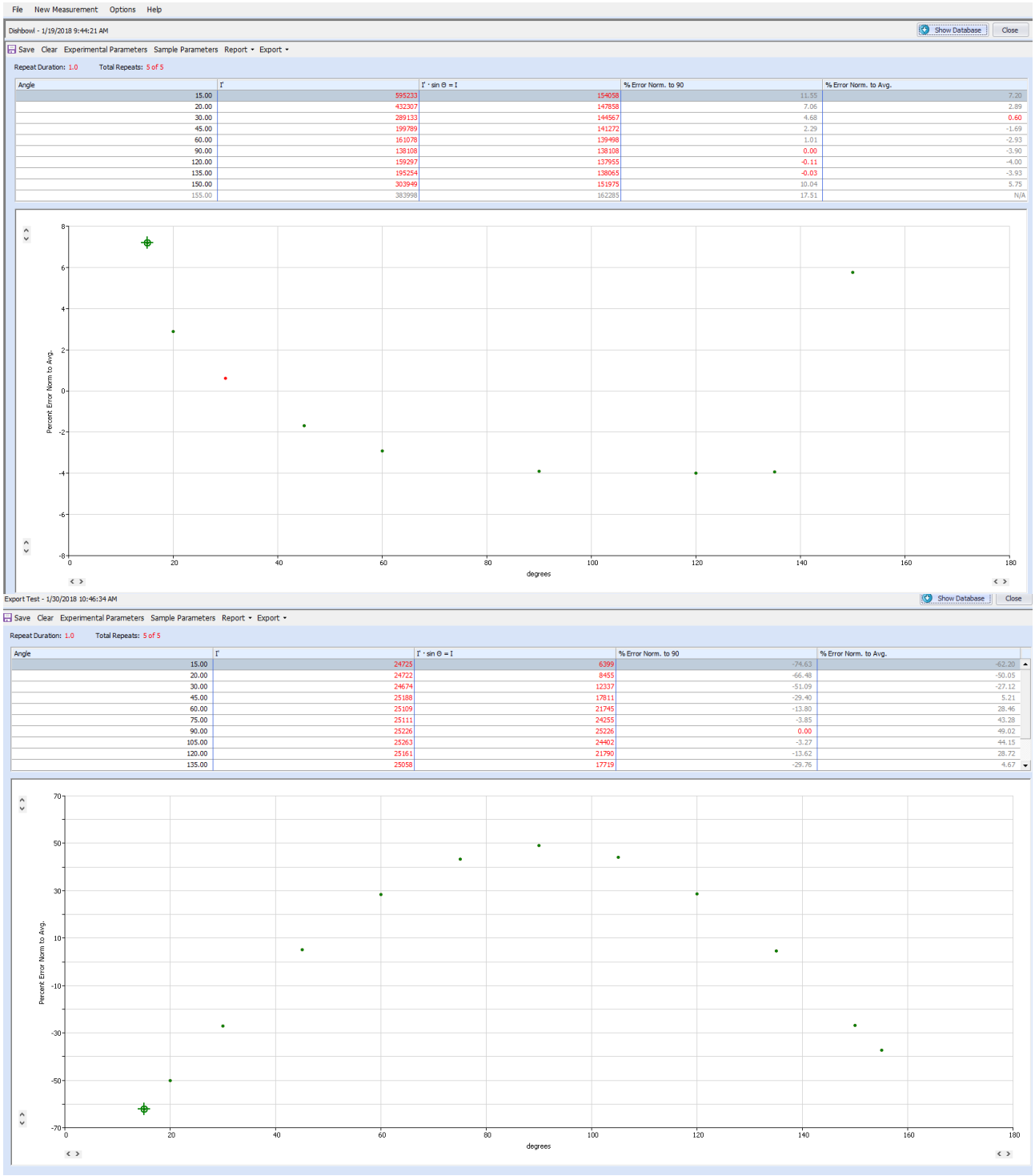


Angle	I	I · sin Θ = I	% Error Norm. to 90	% Error Norm. to Avg.
15.00	476638	123363	-0.37	-0.31
20.00	360373	123255	-0.45	-0.40
30.00	247498	123749	-0.05	0.00
45.00	174109	123114	-0.57	-0.52
60.00	143303	124104	0.23	0.28
90.00	123816	123816	0.00	0.05
120.00	143620	124379	0.45	0.51
135.00	175636	124193	0.30	0.36
150.00	247783	123892	0.06	0.11
155.00	292609	123662	-0.12	-0.07

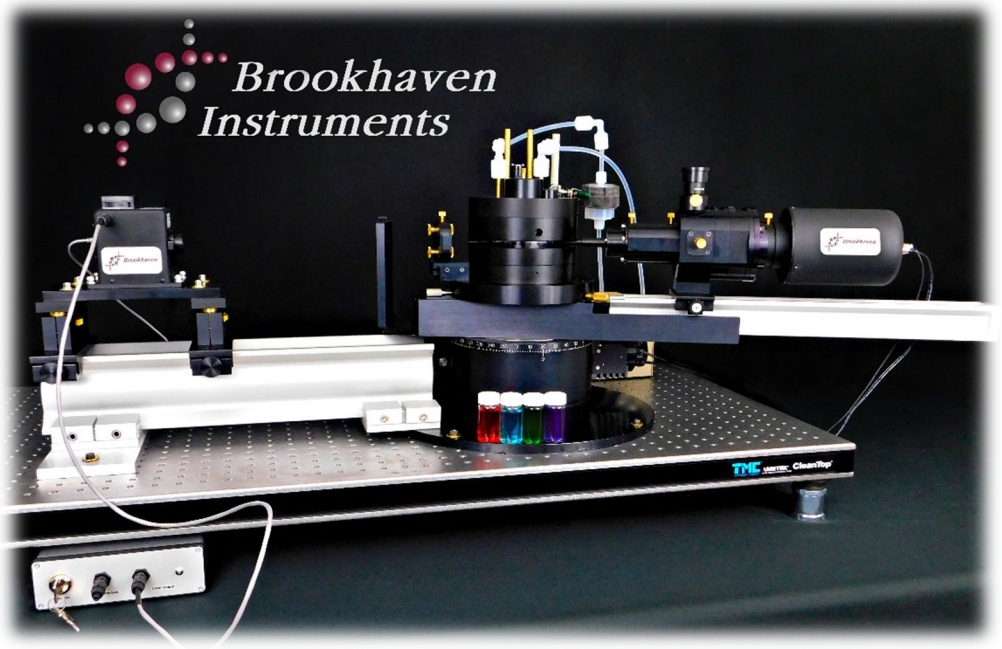
a) If the instrument is not in good alignment, the % error norm. to avg. will have values that are gray rather than red, meaning they are above 1%. There are a few situations that can cause this to happen.
    a. If the angle reflected on the goniometer is off by a small amount, the plotted data points will typically resemble a cotangent curve, as low. To correct for this, the software will suggest a cotangent correction. If this cotangent correction recommendation appears repeatedly after multiple alignment measurements, it is recommended to apply it. This will adjust the verniers on the goniometer to the new angle. For example, if the goniometer reads 155° and then a cotangent correction of -0.06° is applied, the goniometer will now read 154.94°. Once this correction is made, reset the verniers to read 155° again. See the photo alignment manual for vernier adjustment instructions. Start a new alignment measurement and check if this improved the alignment results. Occasionally, a second small cotangent correction may appear. If this is repeatable, apply it. Cotangent corrections will not usually be the solution for very poor alignment results.



b. If the steering lens needs a slight adjustment, the plotted data points typically form the shape of a parabola, facing either upward or downward, as shown below. In this case, the vertical on the steering lens can be adjusted. If it is facing upward, turn the vertical on the steering lens ¼ turn clockwise as a starting point and repeat the alignment to see if it improves. If it is facing downward, turn the vertical on the steering lens ¼ turn counterclockwise and repeat the alignment. See the photo alignment manual for more information. Contact Brookhaven Instruments with questions regarding the alignment.



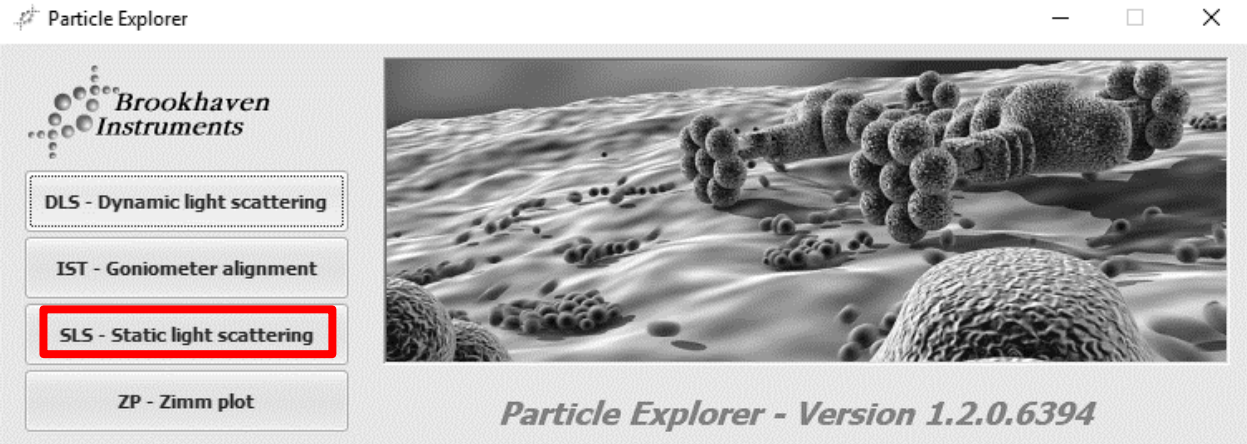
15) Once the instrument is in good alignment, reliable SLS and DLS measurements can be conducted. The alignment should be checked routinely. If the instrument is maintained well and no knobs are turned, the instrument can stay in good alignment for a long time.



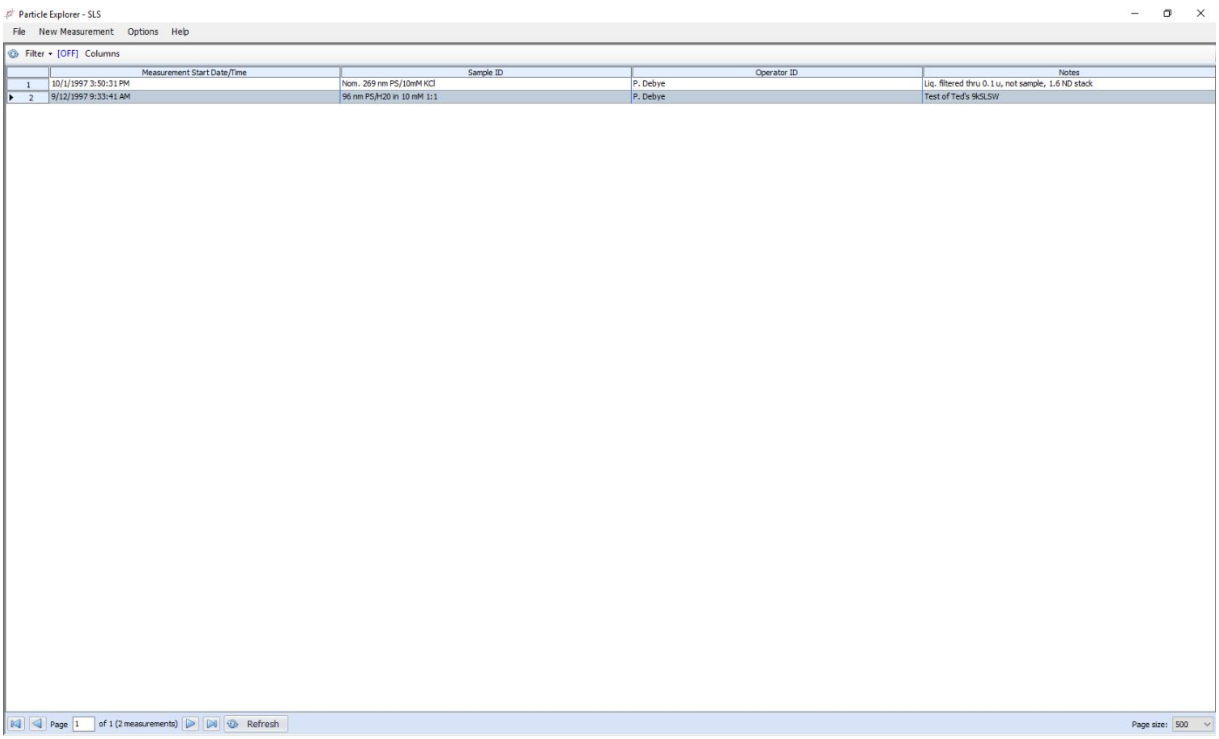
Making an SLS Measurement

The Static Light Scattering (SLS) software can be used to create different plots to determine properties of a suspension, such as radius of gyration (R_g). This measurement type requires one sample that will be measured at multiple angles. The measurement setup is very similar to that of the IST measurement. Follow these steps:

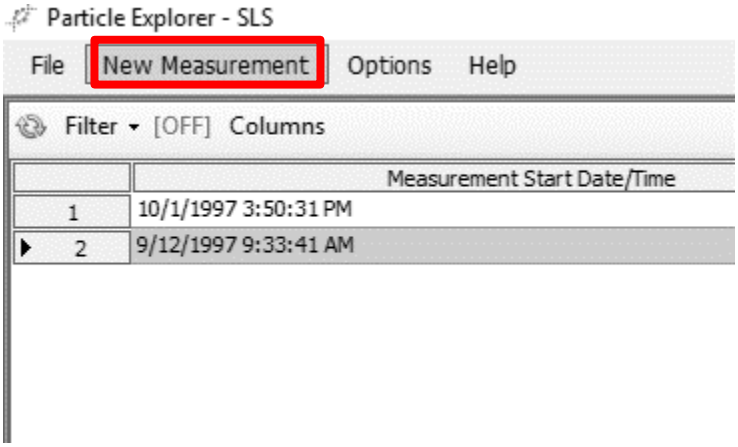
1) Choose SLS – Static Light Scattering from the splash screen.

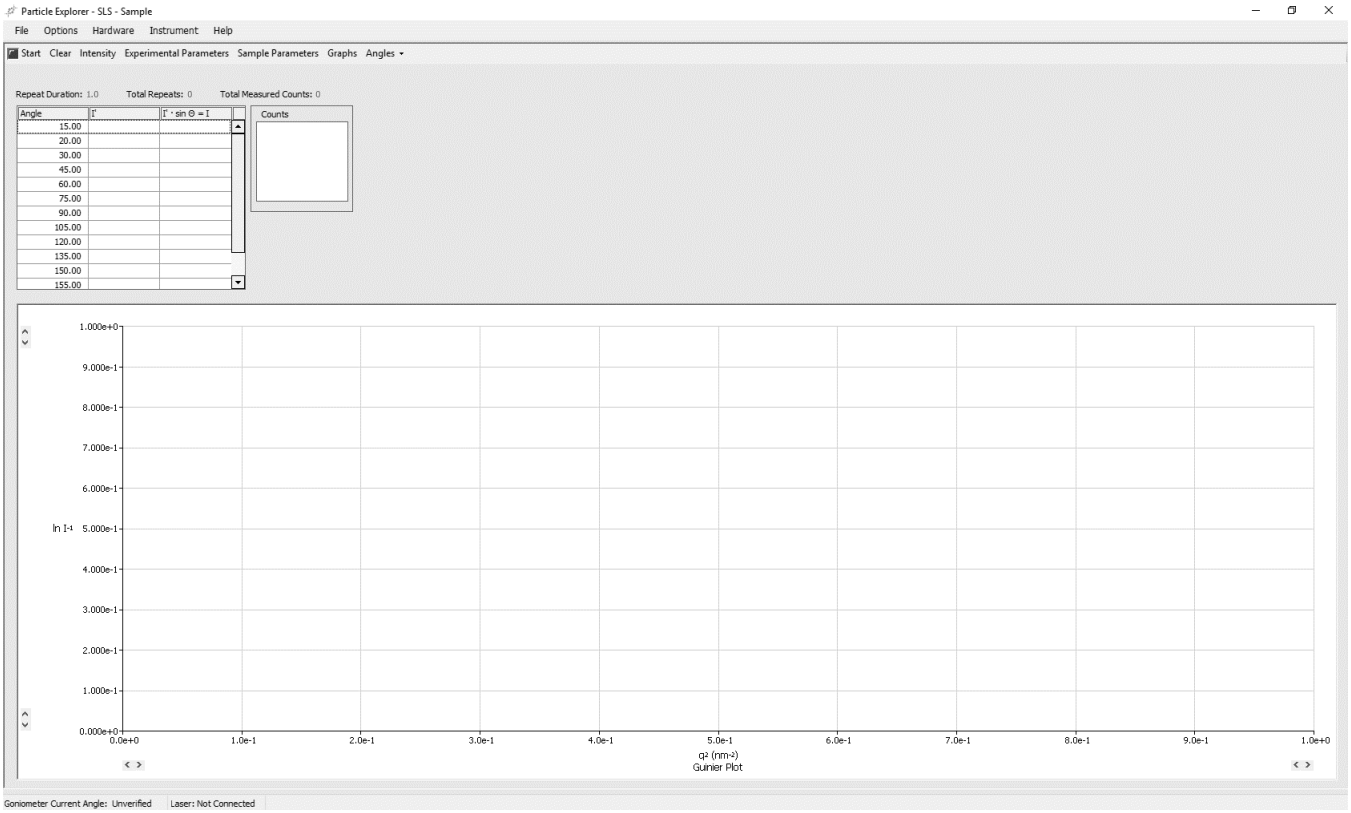


2) A new window with a database will open. This allows the review of previously recorded measurements.

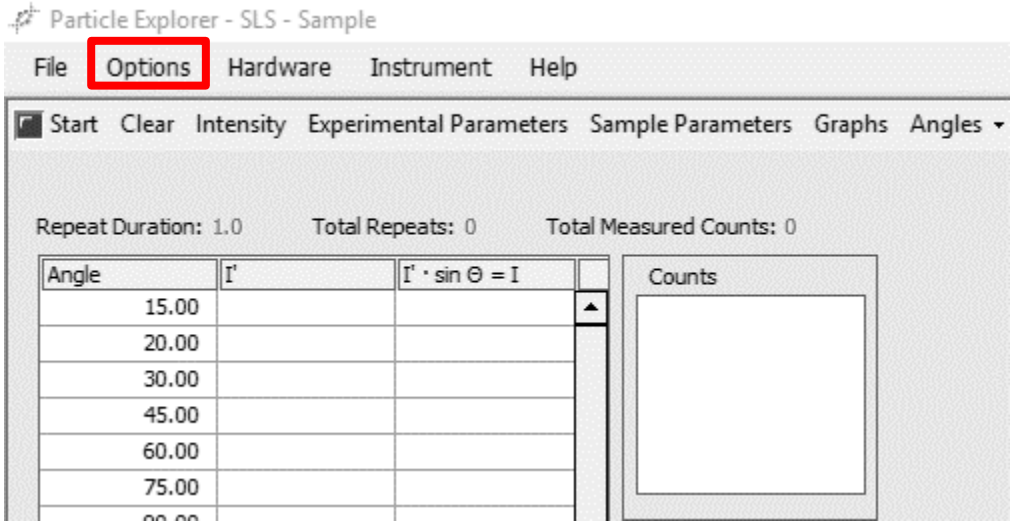


3) To setup a new SLS measurement, select “New Measurement” from the ribbon.

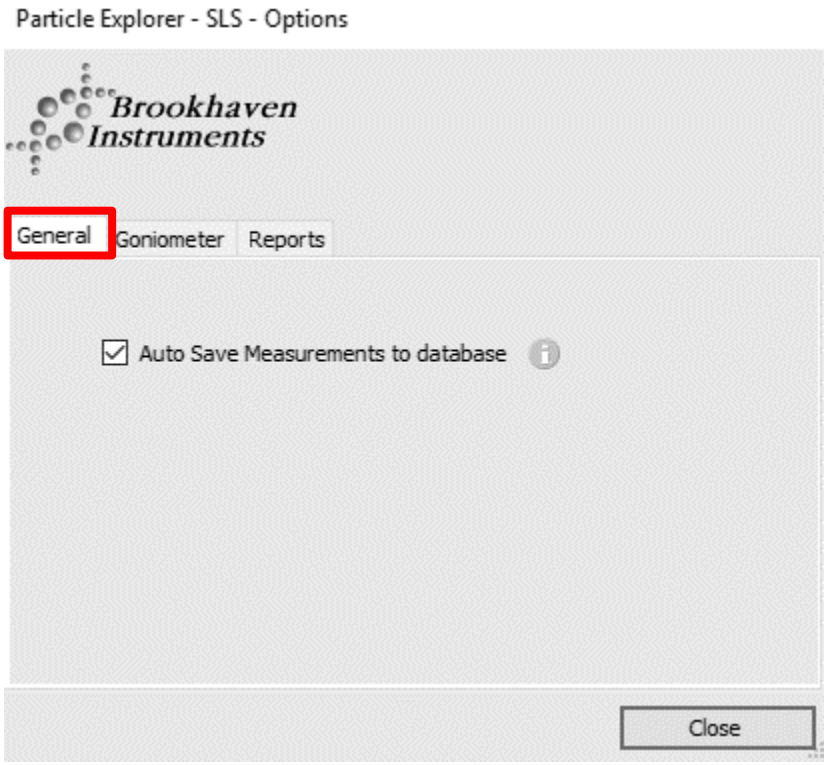




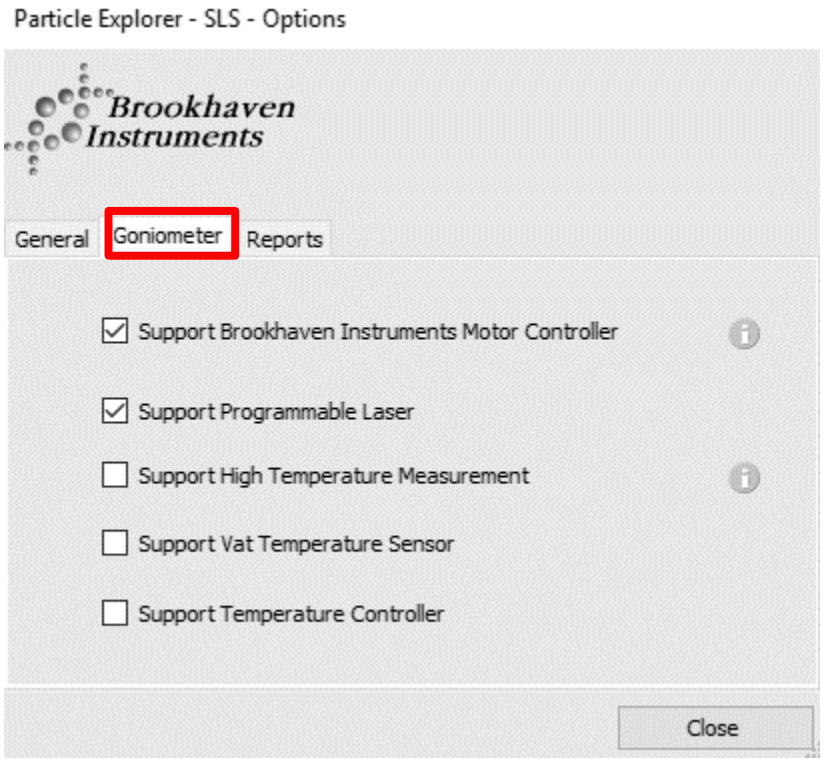
5) Click “Options” on the ribbon.



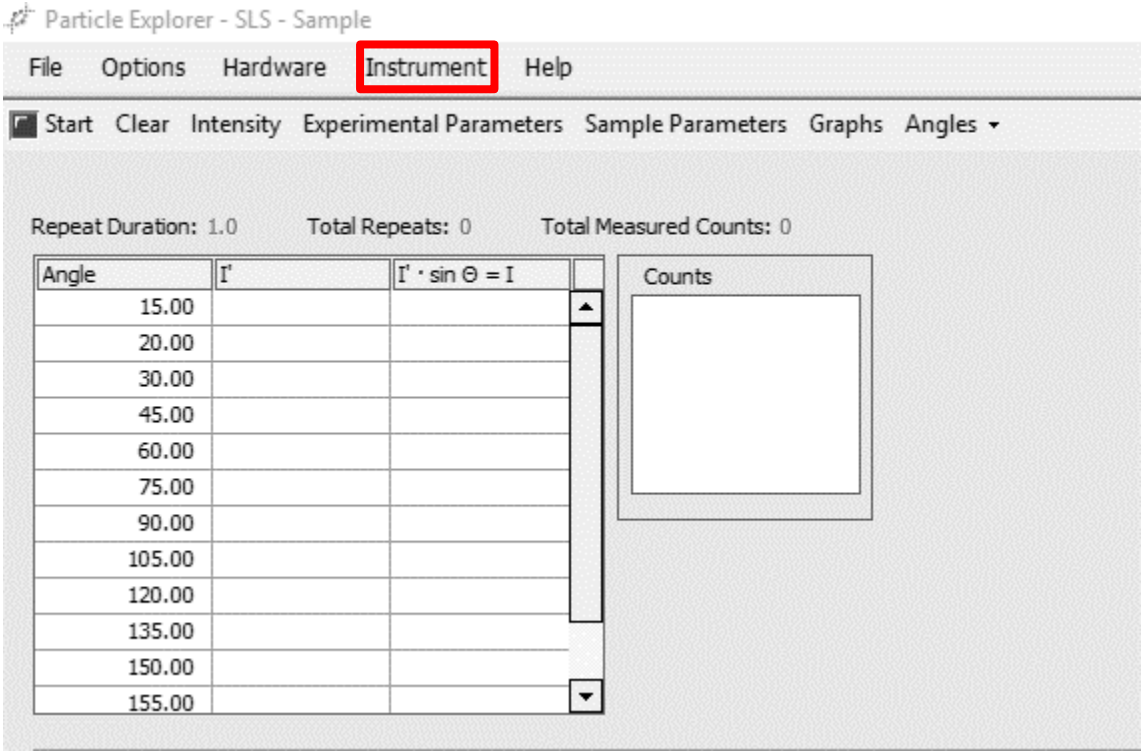
a) On the General tab, it is recommended to check the Auto Save option.



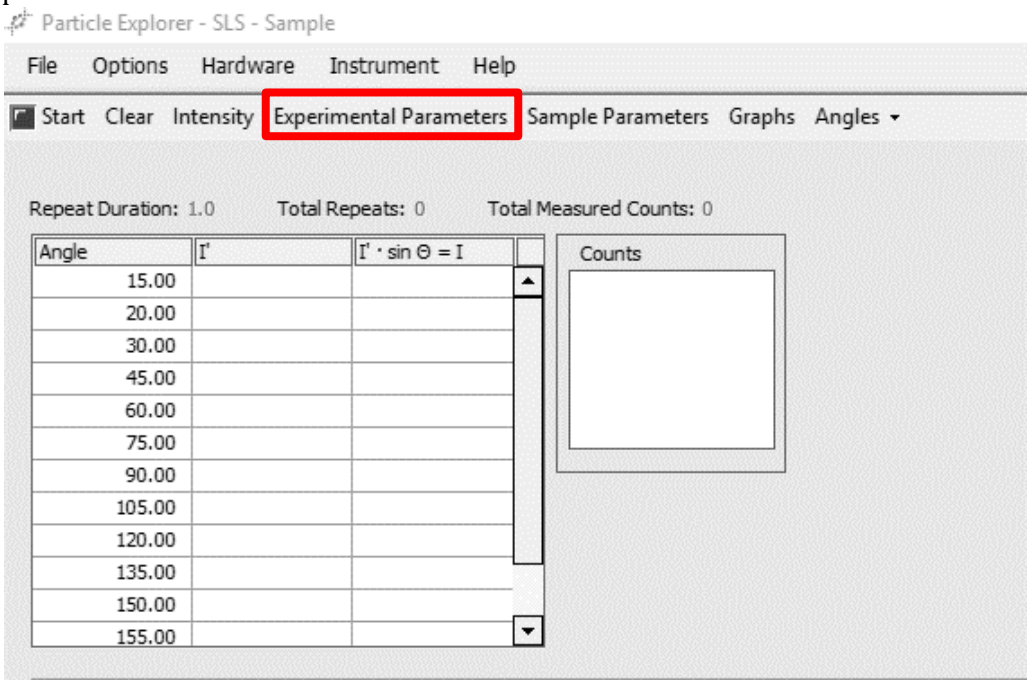
b) On the Goniometer tab, check off what options the instrument is configured with. Close the window when all selections have been made.



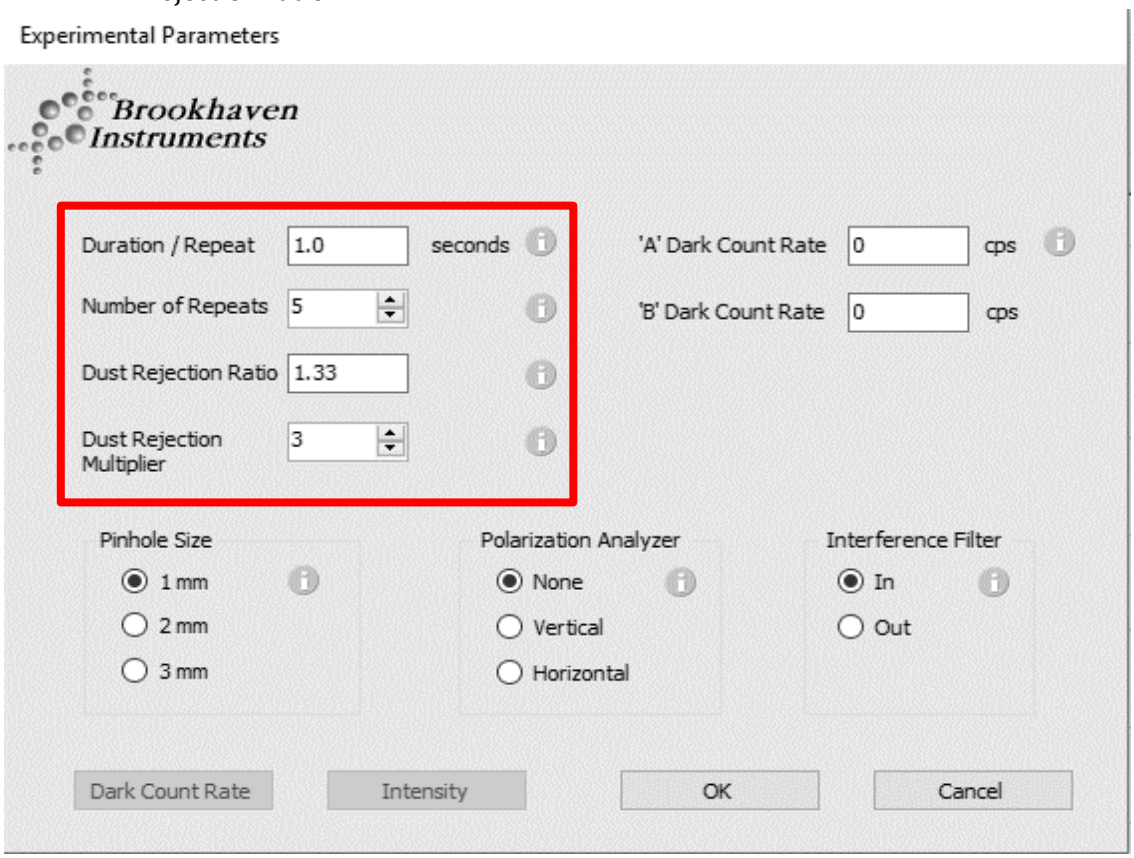
c) Click "Instrument" from the navigation bar to connect a laser or temperature controller option. Follow the on-screen prompts.



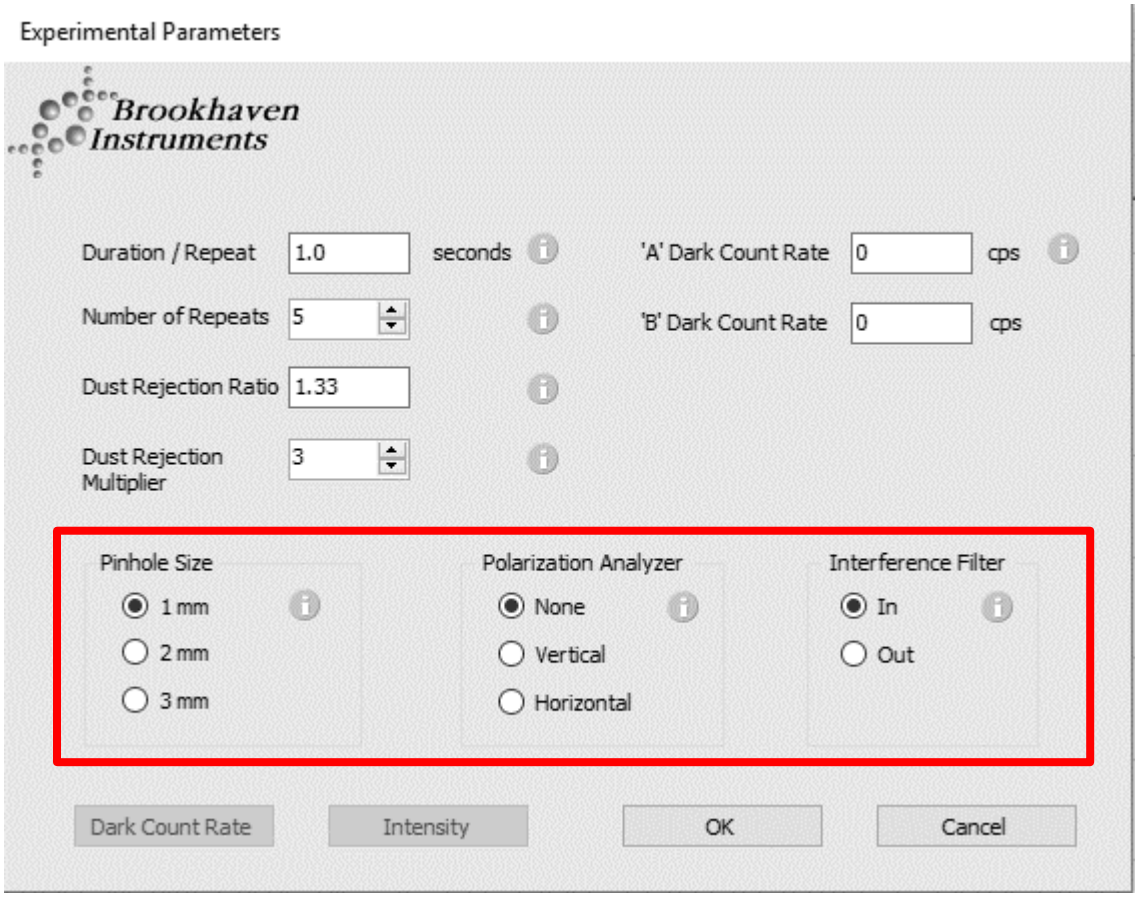
6) Click “Experimental Parameters”.



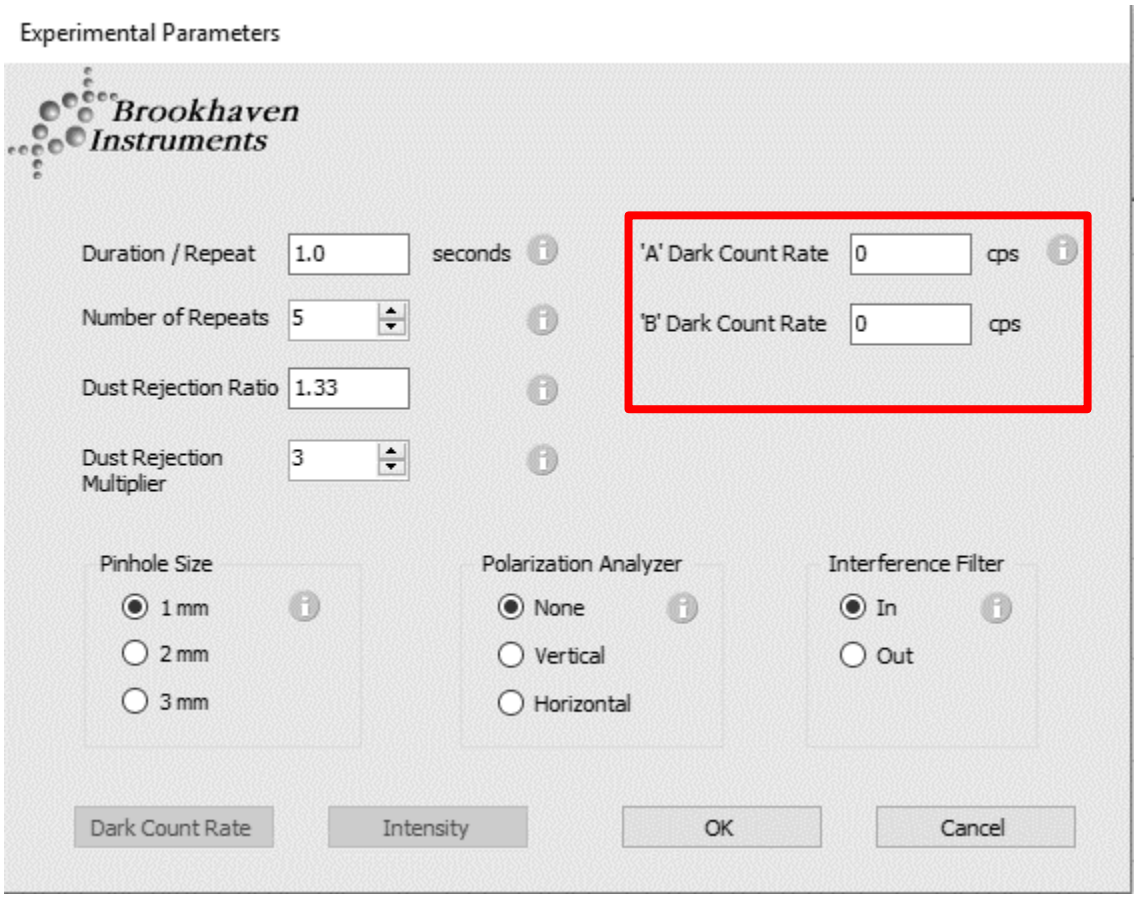
a) Use the following settings to adjust how long a measurement will be.
    a. Duration/Repeat: How frequently an intensity measurement will be taken.
    b. Number of Repeats: How many times an intensity measurement will be taken at the above frequency. For example, if the Duration/Repeat is 1 second and the Number of Repeats is 5, the software will collect five 1-second intensity measurements.
    c. Dust Rejection Ratio: Determines the average count rate and eliminates any count rates that are outside of this ratio. Brookhaven recommends a ratio of 1.33.
    d. Dust Rejection Multiplier: How many times the software will try to achieve "clean" data. For example, if the number of repeats selected is 5 and the dust rejection multiplier is 3, the software will try up to 5 x 3 = 15 times to have 5 measurements within the dust rejection ratio.



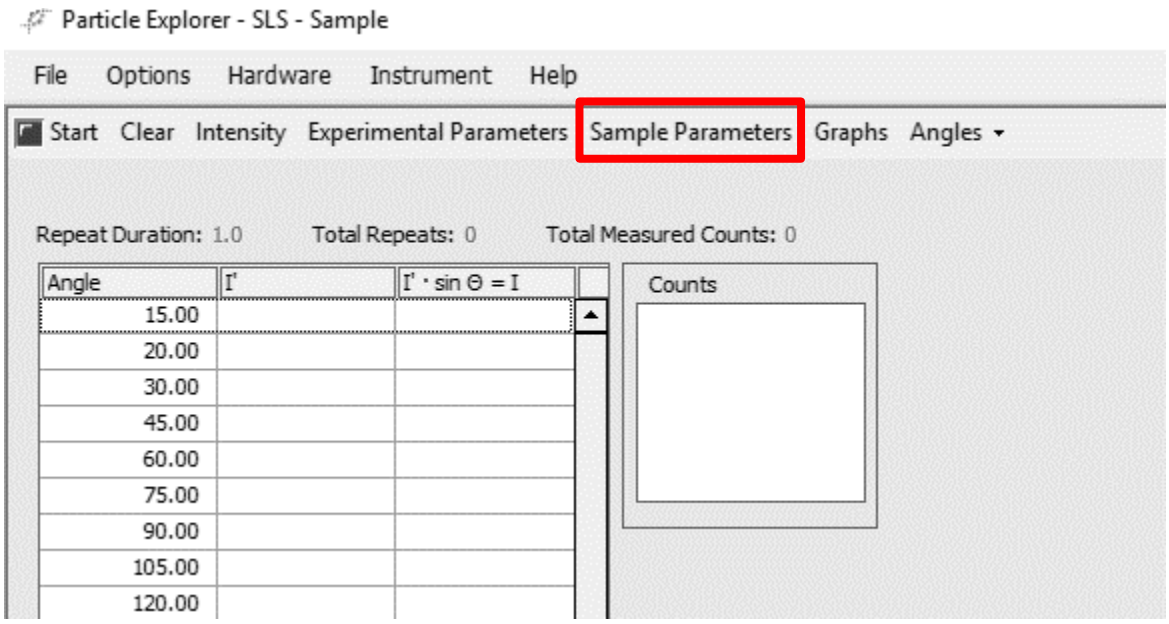
b) Use the following radio buttons for notetaking purposes.
   a. Pinhole Size: Choose the pinhole size that will be manually set on the detector optics.
   b. Polarization Analyzer: If the instrument is equipped with a Polarization Analyzer, select which direction it is going to be used in.
   c. Interference Filter: If a filter is being used to block excess light, choose "In". This is manually set on the detector optics by turning the wheel to the wavelength of the laser being used for the measurement. Choose "Out" if the wheel is set to "O" for Open.



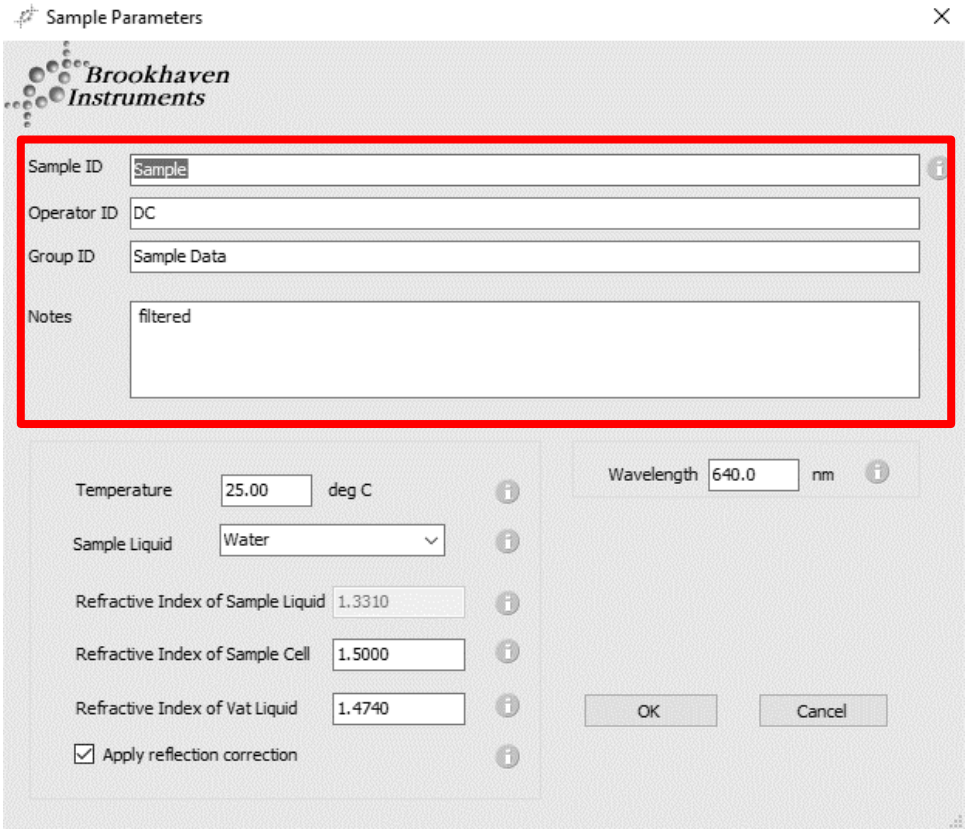
c) Prior to any SLS measurement, the Dark Count Rate must be measured. Turn the filter wheel on the detector optics to “C” for closed. Click “Dark Count Rate”. The software will automatically measure the dark count rate, or the noise of the detector, so that it can subtract this value from the data collected during the measurement sequence. It is recommended to measure the Dark Count Rate a few times to make sure the value is repeatable. Once satisfied, click “OK” and the software will automatically fill in the field ‘A’ Dark Count Rate with the measured number.



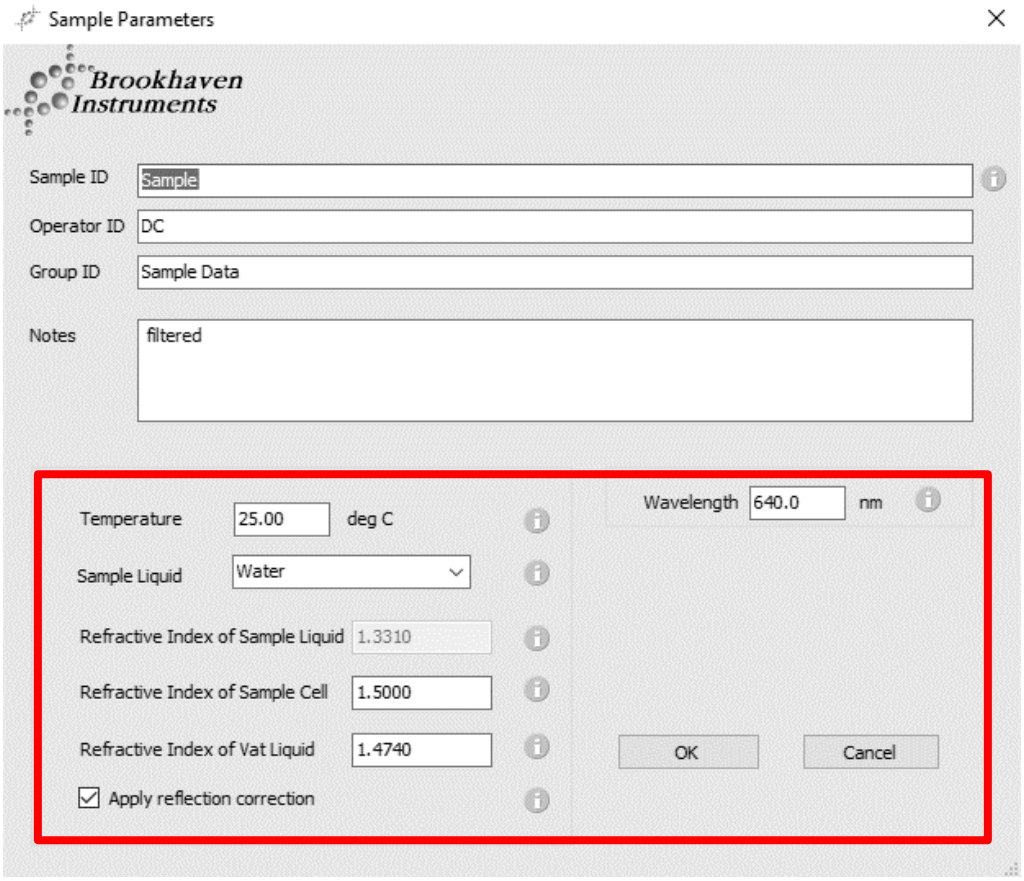
7) Click “Sample Parameters”.



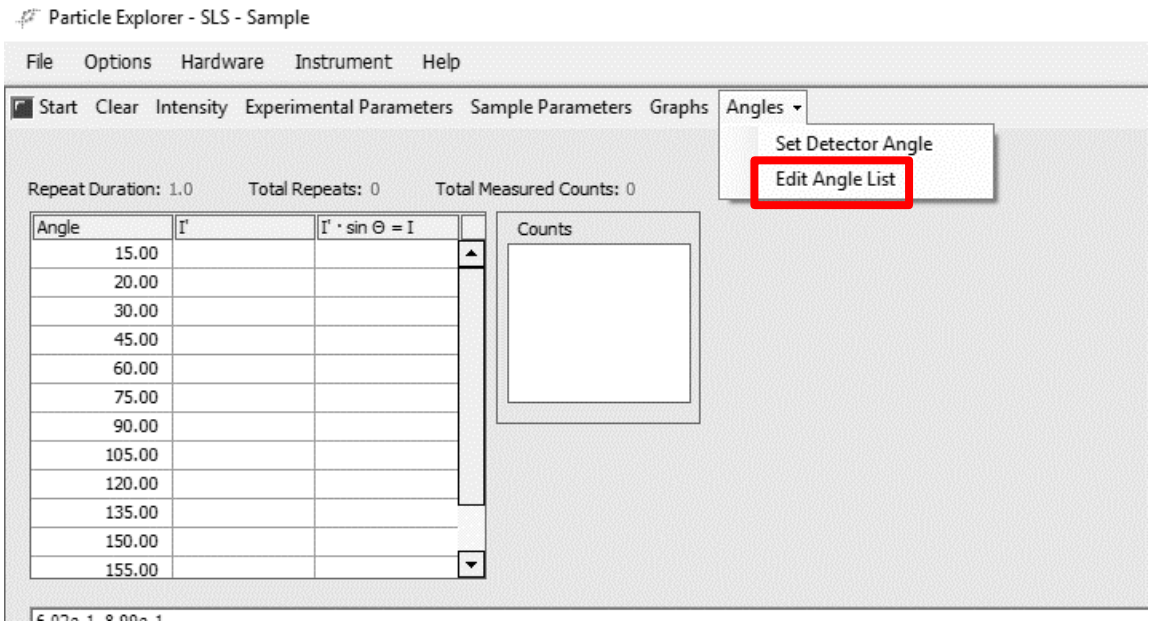
a) Sample ID, Operator ID, Group ID, and Notes are available fields for measurement identification purposes. Each field is not required but recommended so that reviewing past measurements is simple.

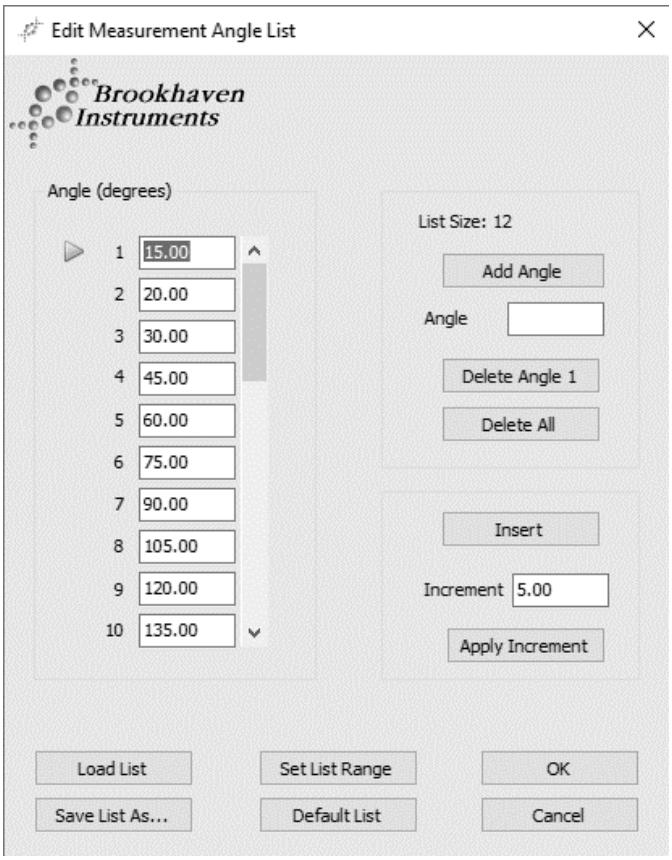


b) Fill in the following sample parameters to ensure that measurement calculations are performed properly.
    a. Input the temperature the measurement will take place at. If the instrument is equipped with a temperature sensor, this value will automatically be filled in once the measurement begins.
    b. Choose the liquid the sample is suspended in. If the liquid is not on the preloaded list, choose "Unspecified" to type the liquid name. Make sure to input the Refractive Index of Sample Liquid at the temperature the measurement will take place at.
    c. The Refractive Index of the Glass Sample Cells provided with the instrument is 1.5000. Only change this value if using different glass cells with different refractive index values.
    d. The recommended Refractive Index Matching Vat Liquid is cis-trans decahydronapthalene, or decalin. This has a refractive index of 1.4740. Only change this value if using a different refractive index matching vat fluid.
    e. Input the wavelength of the laser the instrument is equipped with.
    f. "Apply reflection correction" is recommended.



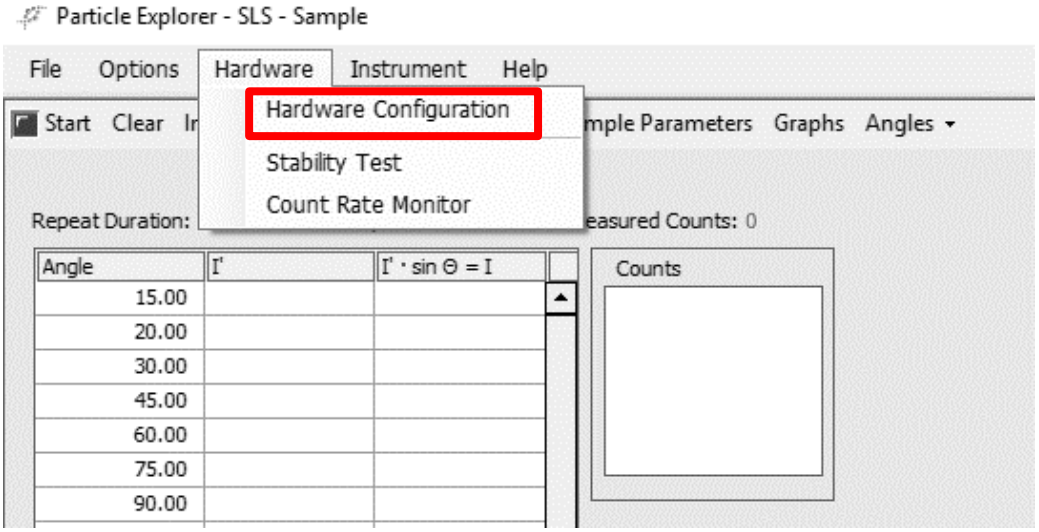
8) Click “Angles→ Edit Angle List”.



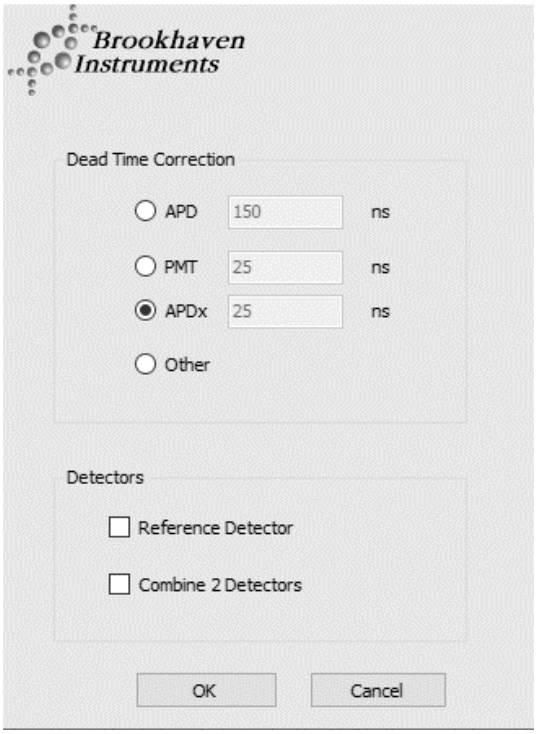


The default angle list spans nearly the full range of the instrument. To check a different range of angles, less angles, or more angles, use the Add Angle, Delete Angle, and Set List Range buttons.

9) Click “Hardware→ Hardware Configuration”.



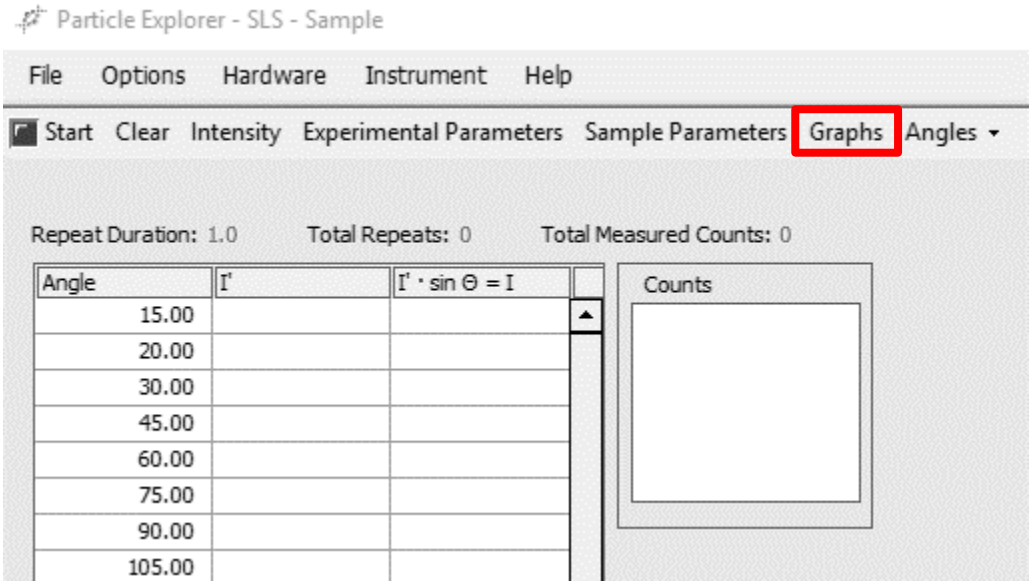
Hardware Configuration

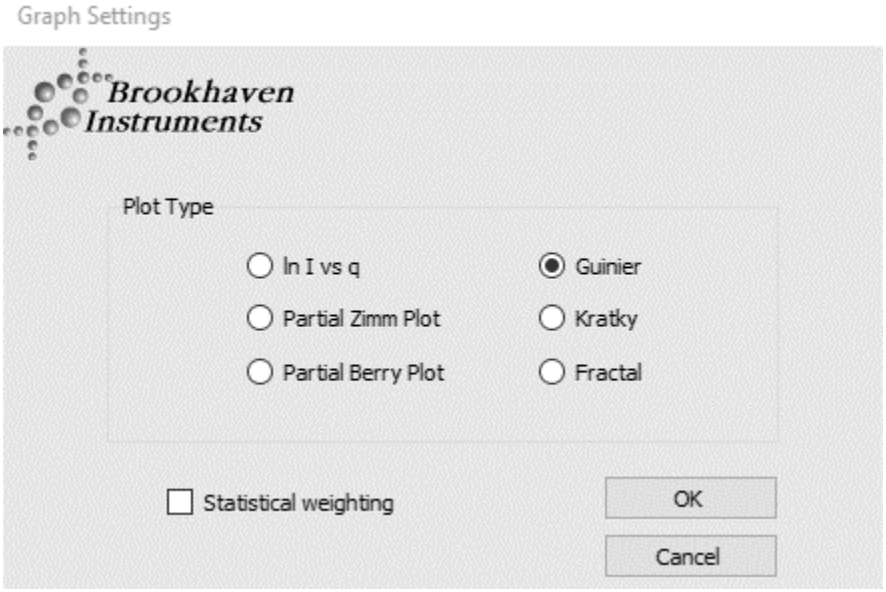


Choose the detector the instrument is equipped with. The dead time correction is automatically assigned. If the instrument has a detector or dead time not listed, select “Other” and fill in the dead time correction manually.

For specific applications, the instrument may be configured with a reference detector or two detectors. Check the appropriate box if using either configuration.

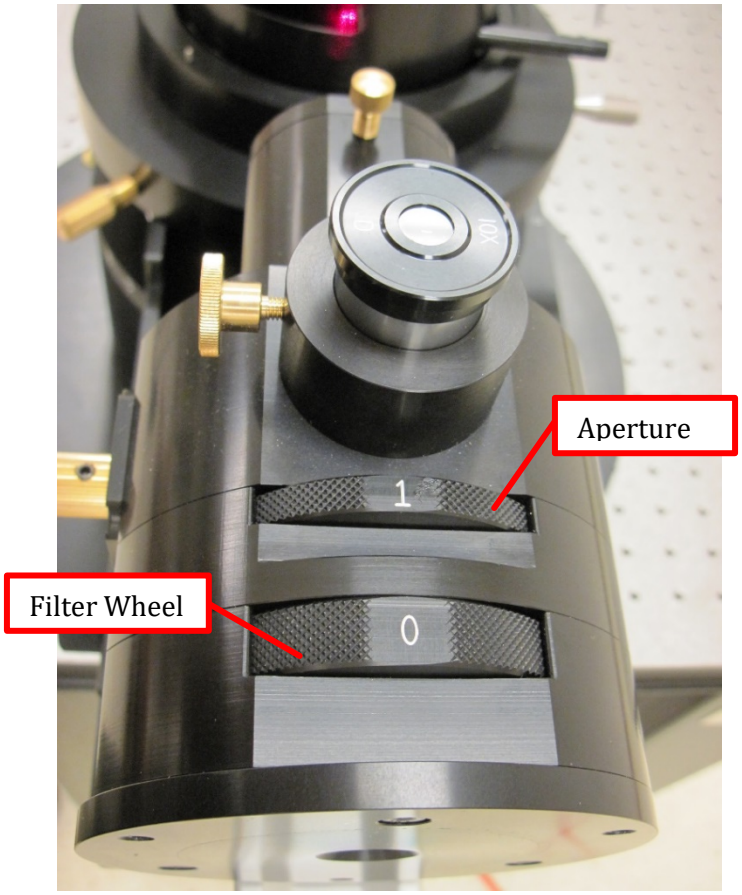
10) Click “Graphs”.



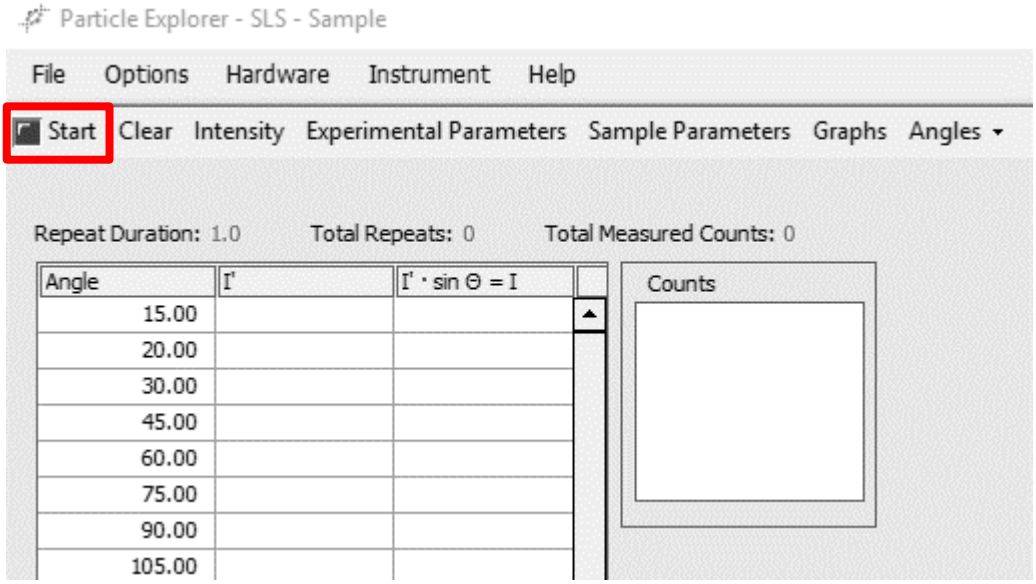


Choose which graph the software should plot during data collection. This can be changed before, during, or after the measurement.

11) Now, all of the SLS measurement parameters have been set. On the detector optics, set the filter wheel and pinhole wheel to the settings selected in Step 6b.



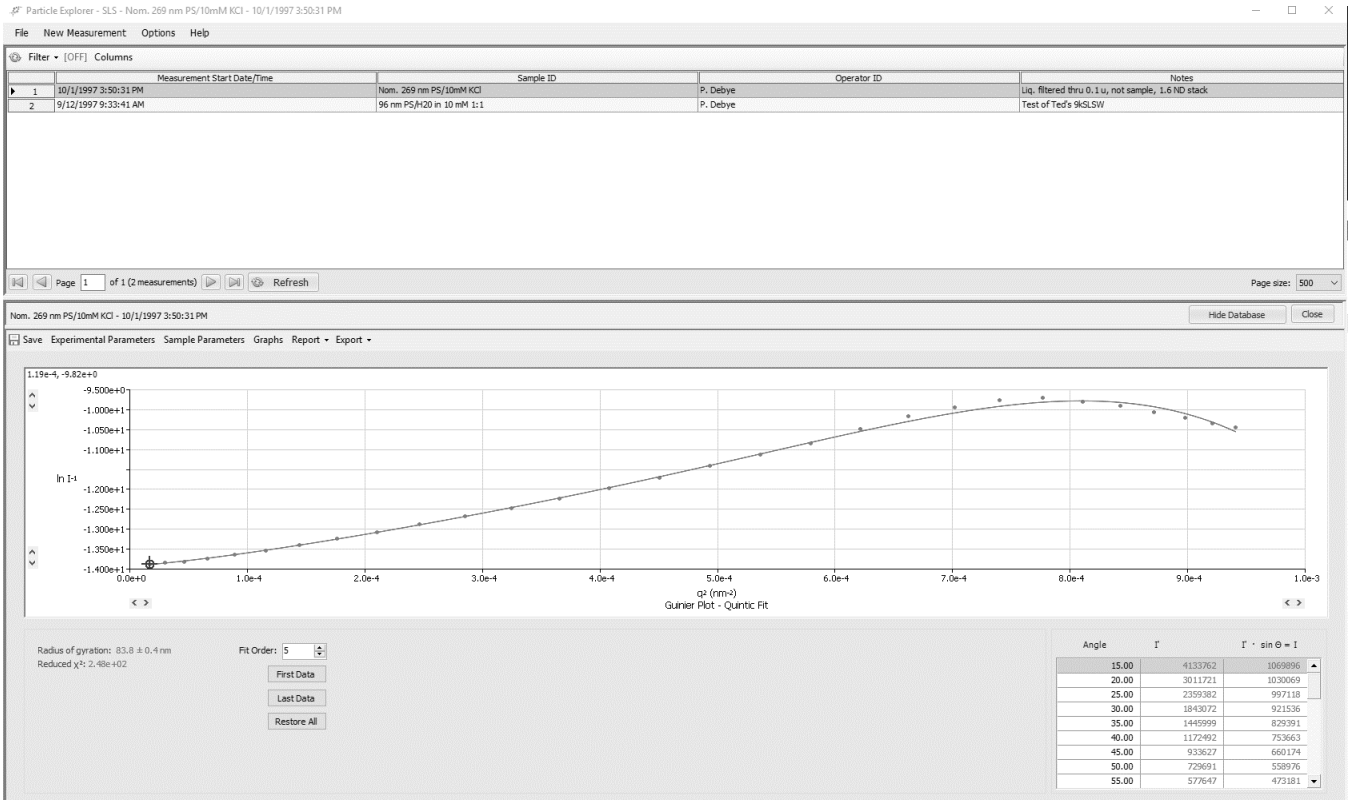
12) Click "Start" to begin the measurement.



The software needs to know what angle the goniometer is at before it can begin the measurement. A window will appear asking for the current angle. Enter the angle and click OK to allow the measurement to proceed.

13) The rest of the measurement is automated. When complete, the measurement will be automatically saved to the database if selected in Step 5a. If not, click “Save” once the measurement is complete. Otherwise, the data will NOT be saved.

14) Once the measurement is complete, the data can be reviewed. Double click the measurement from the database if the measurement is not already open.



a. Click “Sample Parameters” to modify any previously entered parameters. The data will then be reanalyzed with the new selections. For example, if the liquid was entered incorrectly initially, it can be corrected here and the data will be reanalyzed. Click “Save” to save the corrected measurement to the database. Right click on the incorrect measurement in the database to delete it.
b. Click “Graphs” to choose what graph analysis to display.
c. Click “Report” to preview a report printout of the data. Click “Print Report” to print the report to PDF or a printer.
d. Click “Export” to choose a format to export the graph and/or data as.
e. There are a few ways to remove outliers. The table on the bottom right-hand side lists angles with intensity values. Double-click to remove a value from the calculations. The value will turn gray, indicating it is no longer in the calculation.

Angle	I'	I' · sin Θ = I
15.00	4133762	1069896
20.00	3011721	1030069
25.00	2359382	997118
30.00	1843072	921536
35.00	1445999	829391
40.00	1172492	753663
45.00	933627	660174
50.00	729691	558976
55.00	577647	473181

Angle	I'	I' · sin Θ = I
15.00	4133762	1069896
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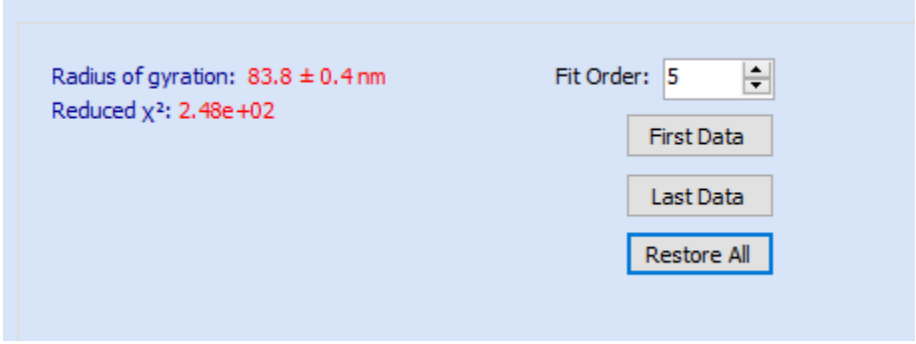
35.00	1445999	829391
40.00	1172492	753663
45.00	933627	660174
50.00	729691	558976
55.00	577647	473181

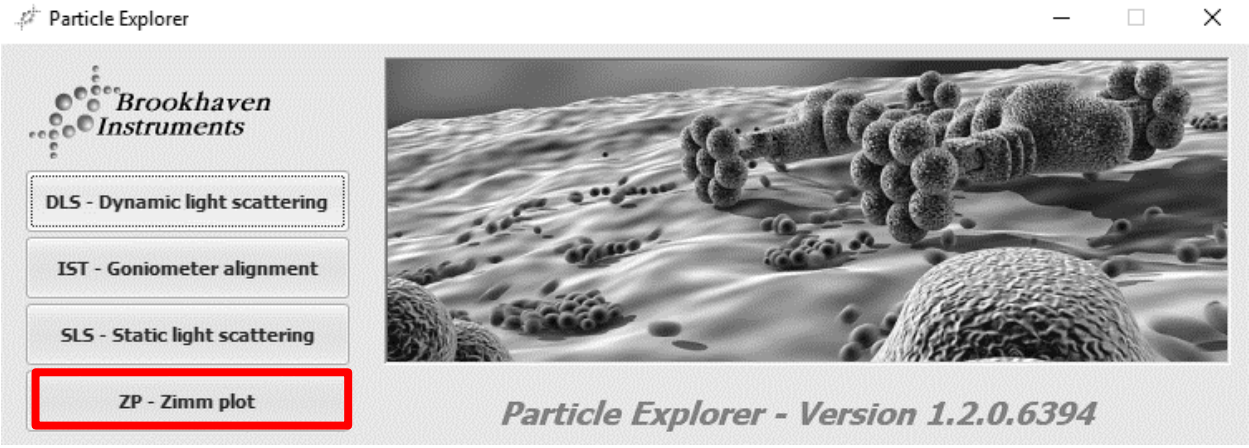
Click a data point on the graph. If the “First Data” button is clicked, the software will eliminate all data points to the left of the selected point. If “Last Data” is clicked, the software will eliminate all data points to the right of the selected point. Click “Restore All” to undo all changes.



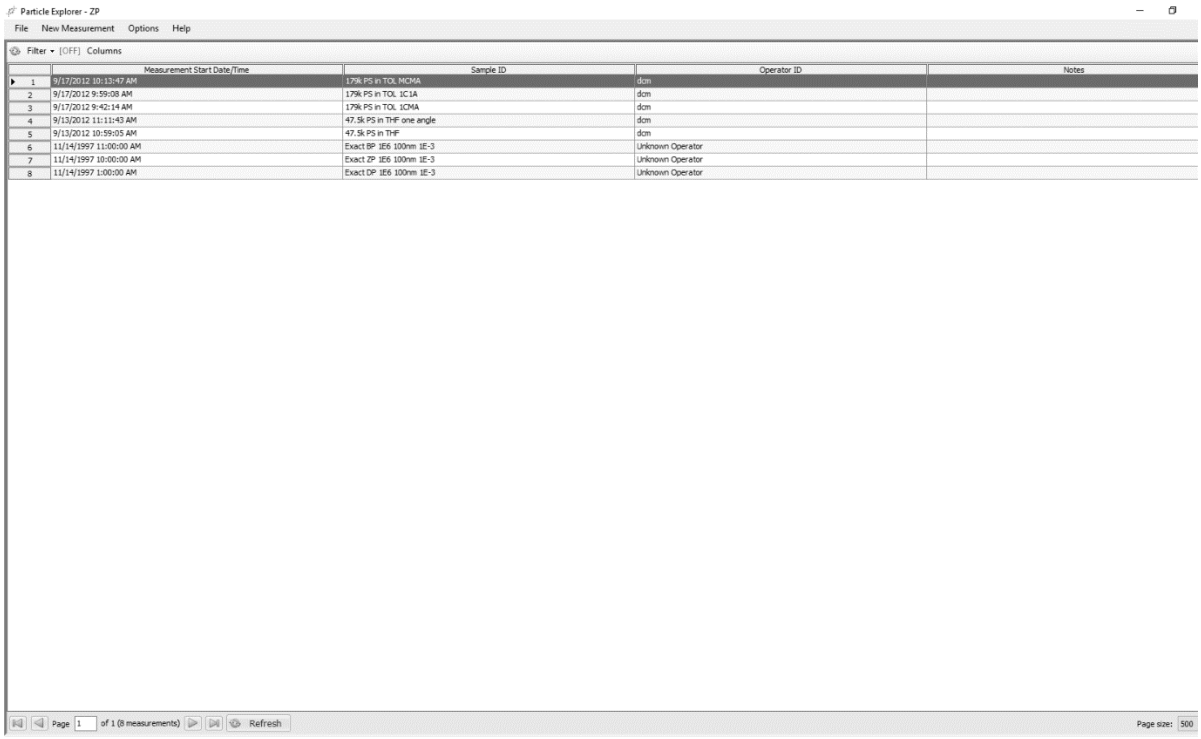
Making a Zimm Plot Measurement

The Zimm Plot measurement type can plot Zimm, Berry, and Debye plots to provide information on molecular weight (M_w), radius of gyration (R_g), and second virial coefficient (A_2). The Zimm plot requires at least 3 different concentrations of sample that will be measured at multiple angles. See Appendix A for sample preparation guidance.

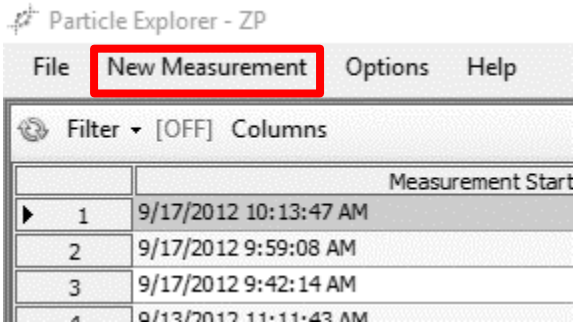
1) Click ZP – Zimm Plot button from the splash screen.



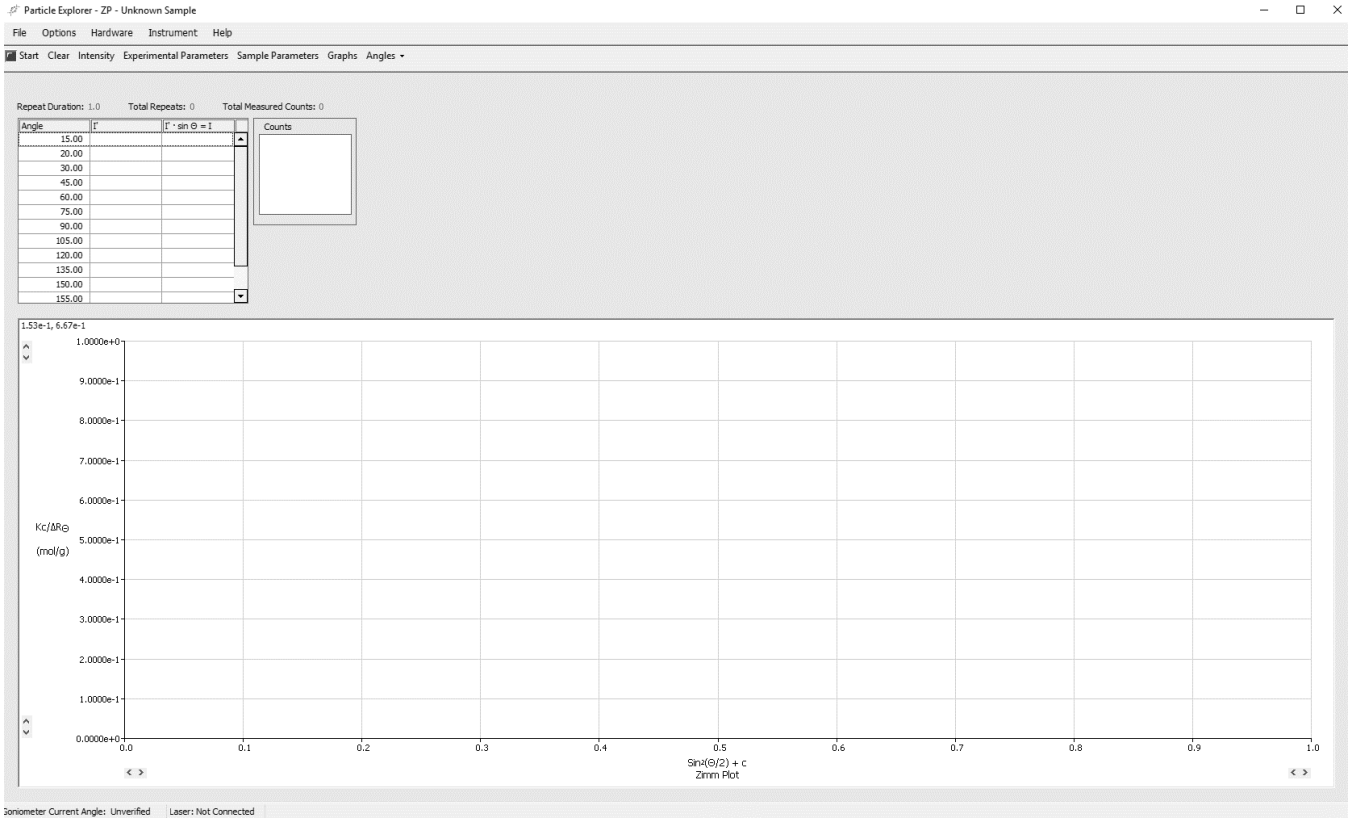
2) A new window with a database will open. This allows the review of previously recorded measurements.



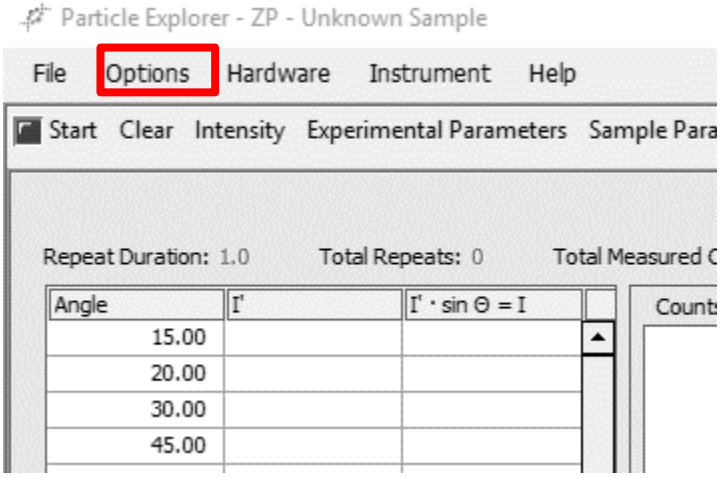
3) To setup a new ZP measurement, select "New Measurement" from the ribbon.



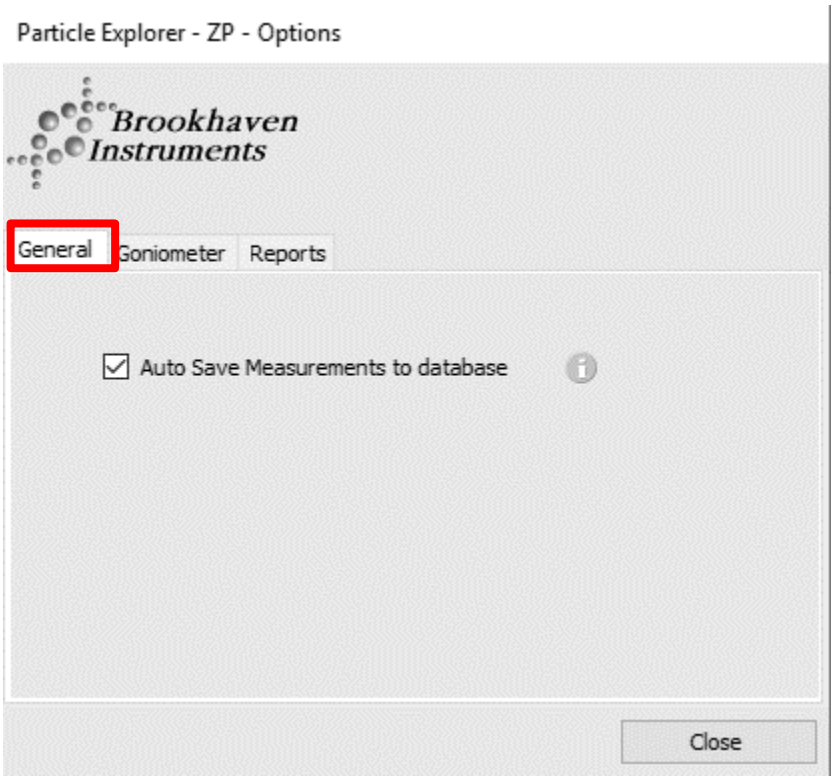
4) An additional window will open where a measurement can be set up and started.



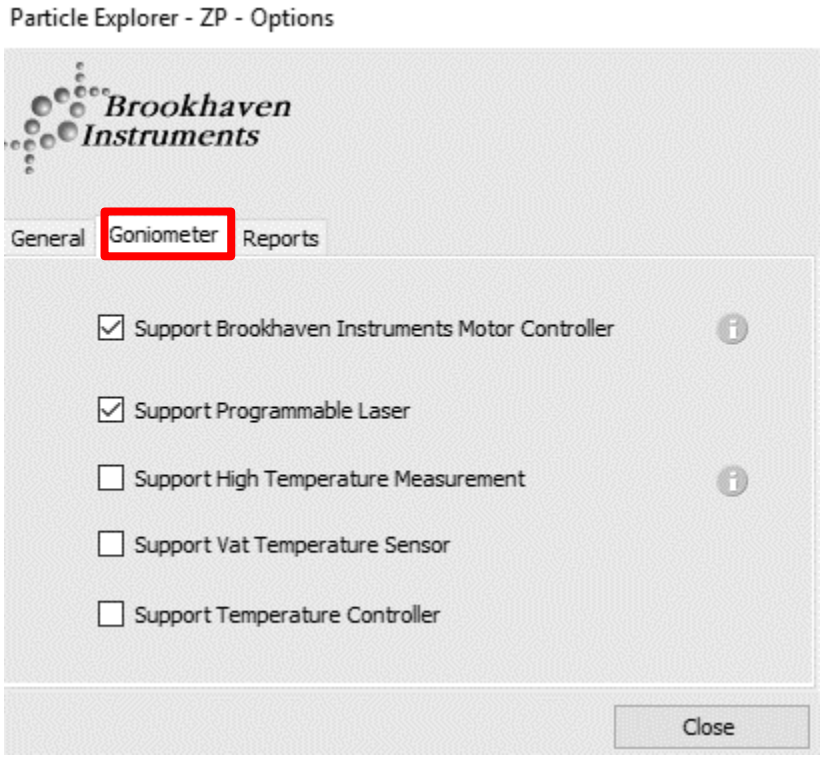
5) Click “Options” on the ribbon.



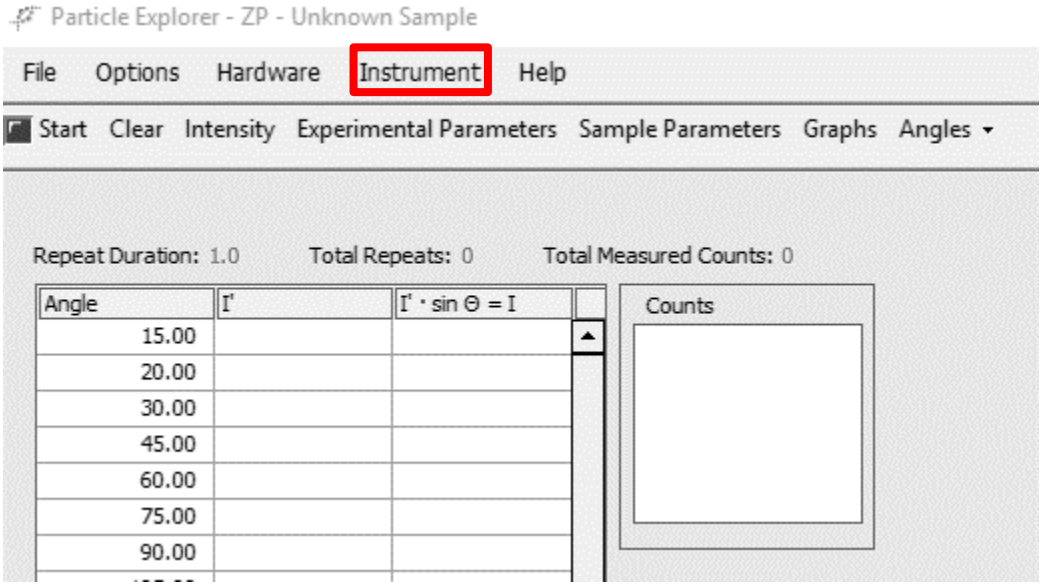
a) On the General tab, it is recommended to check off the Auto Save option.



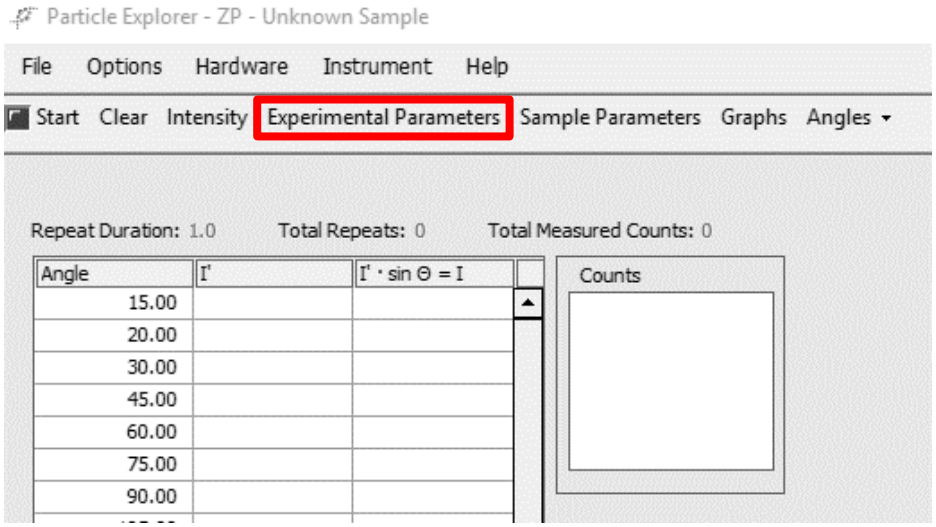
b) On the Goniometer tab, check off what options the instrument is configured with. Close the window when all selections have been made.



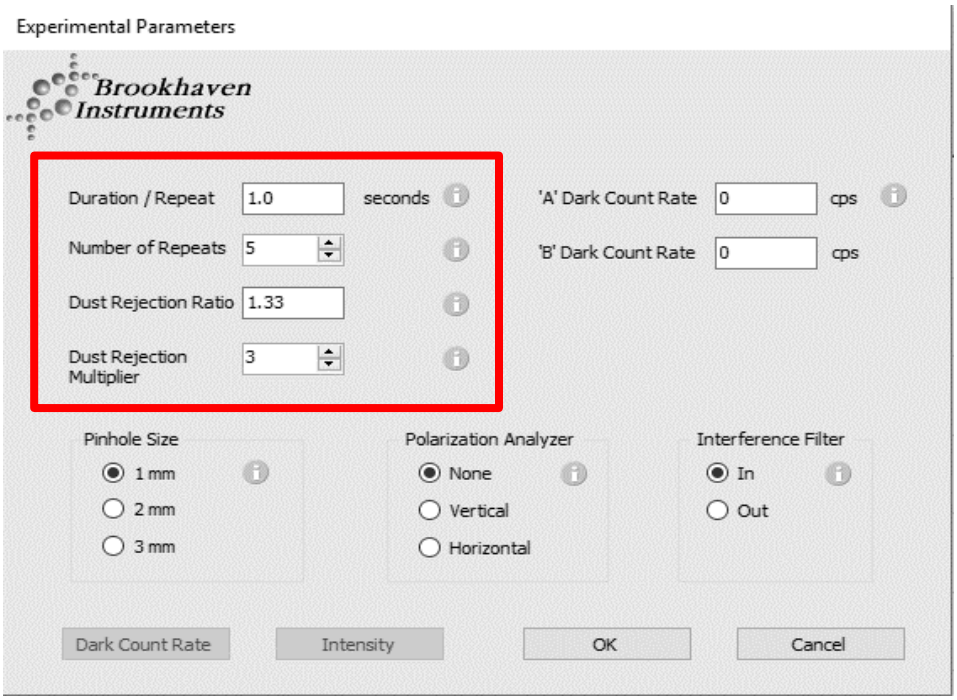
c) Click "Instrument" from the ribbon to connect a laser or temperature controller option. Follow the on-screen prompts.



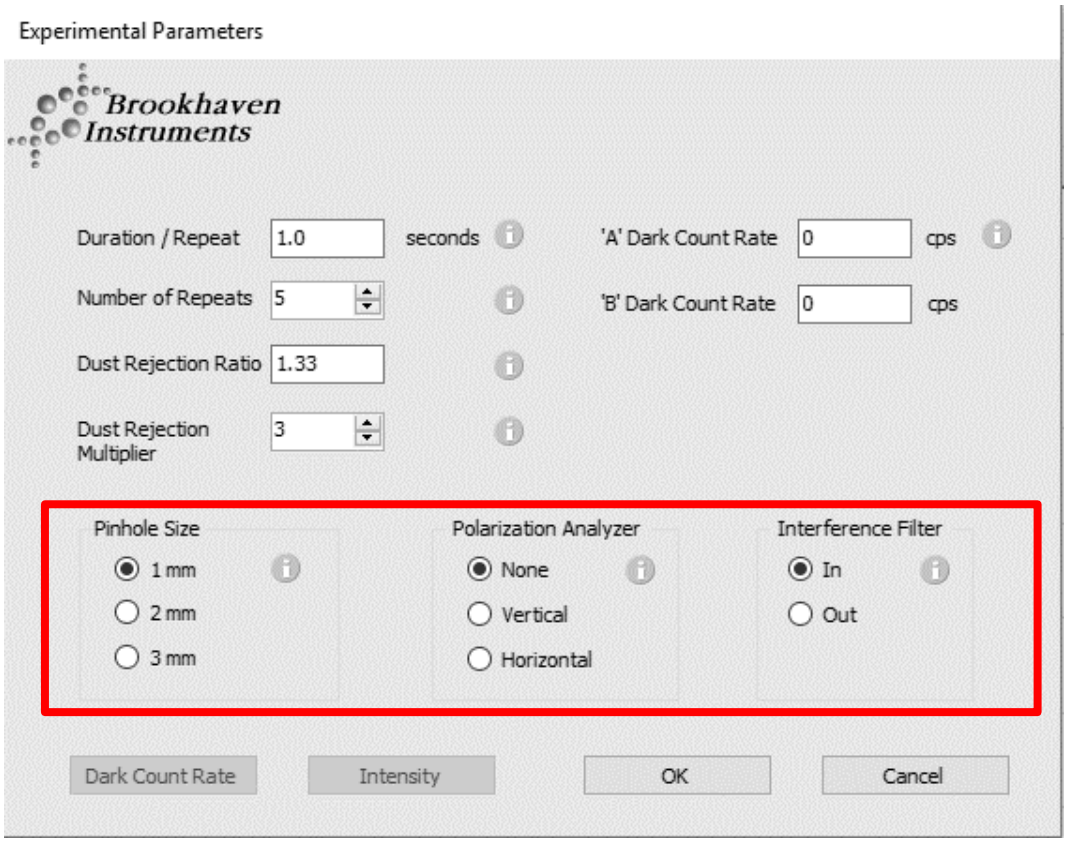
6) Click “Experimental Parameters”.



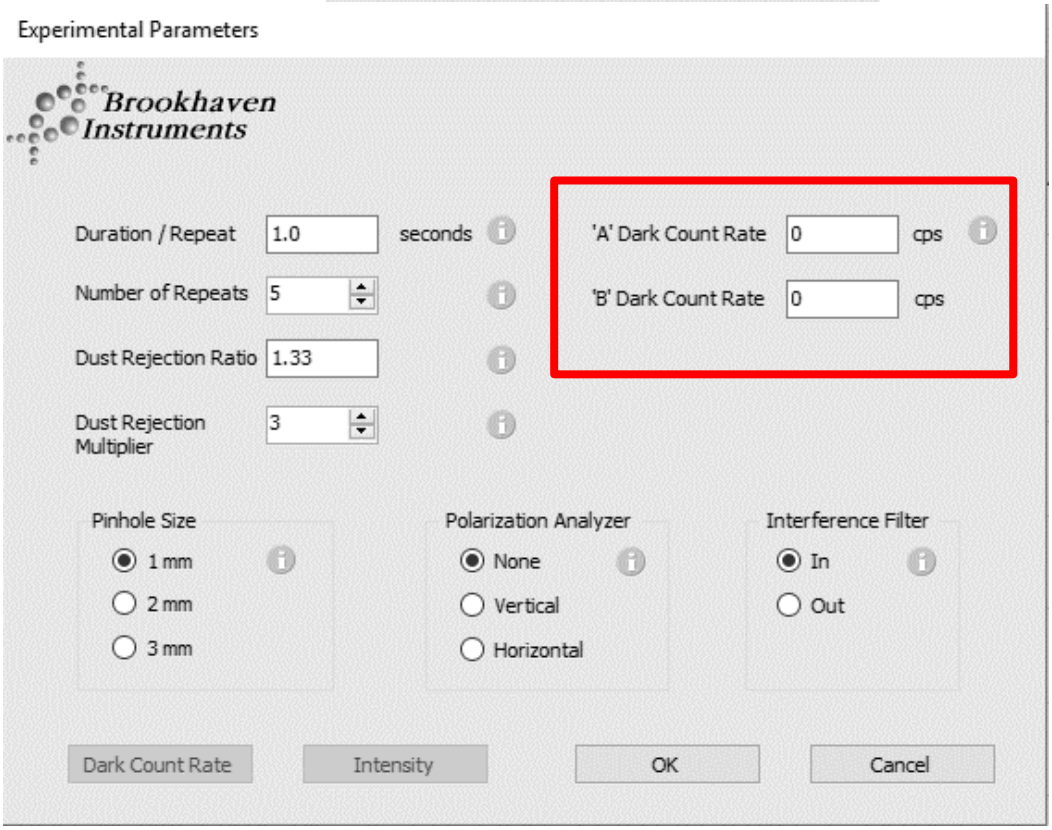
a) Use the following settings to adjust how long a measurement will be.
   a. Duration/Repeat: How frequently an intensity measurement will be taken.
   b. Number of Repeats: How many times an intensity measurement will be taken at the above frequency. For example, if the Duration/Repeat is 1 second and the Number of Repeats is 5, the software will collect five 1-second intensity measurements.
   c. Dust Rejection Ratio: Determines the average count rate and eliminates any count rates that are outside of this ratio. Brookhaven recommends a ratio of 1.33.
   d. Dust Rejection Multiplier: How many times the software will try to achieve “clean” data. For example, if the number of repeats selected is 5 and the dust rejection multiplier is 3, the software will try up to 5 x 3 = 15 times to have 5 measurements within the dust rejection ratio.



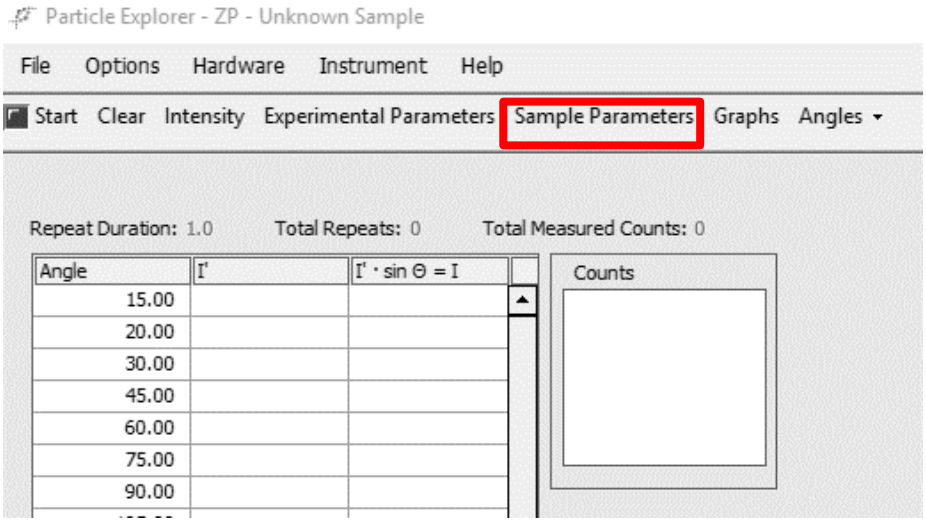
b) Use the following radio buttons for notetaking purposes.
   a. Pinhole Size: Choose the pinhole size that will be manually set on the detector optics.
   b. Polarization Analyzer: If the instrument is equipped with a Polarization Analyzer, select which direction it is going to be used in. This affects the calibration calculations, which in turn will affect the calculation of M_w.
   c. Interference Filter: If a filter is being used to block excess light, choose "In". This is manually set on the detector optics by turning the wheel to the wavelength of the laser being used for the measurement. Choose "Out" if the wheel is set to "O" for Open.



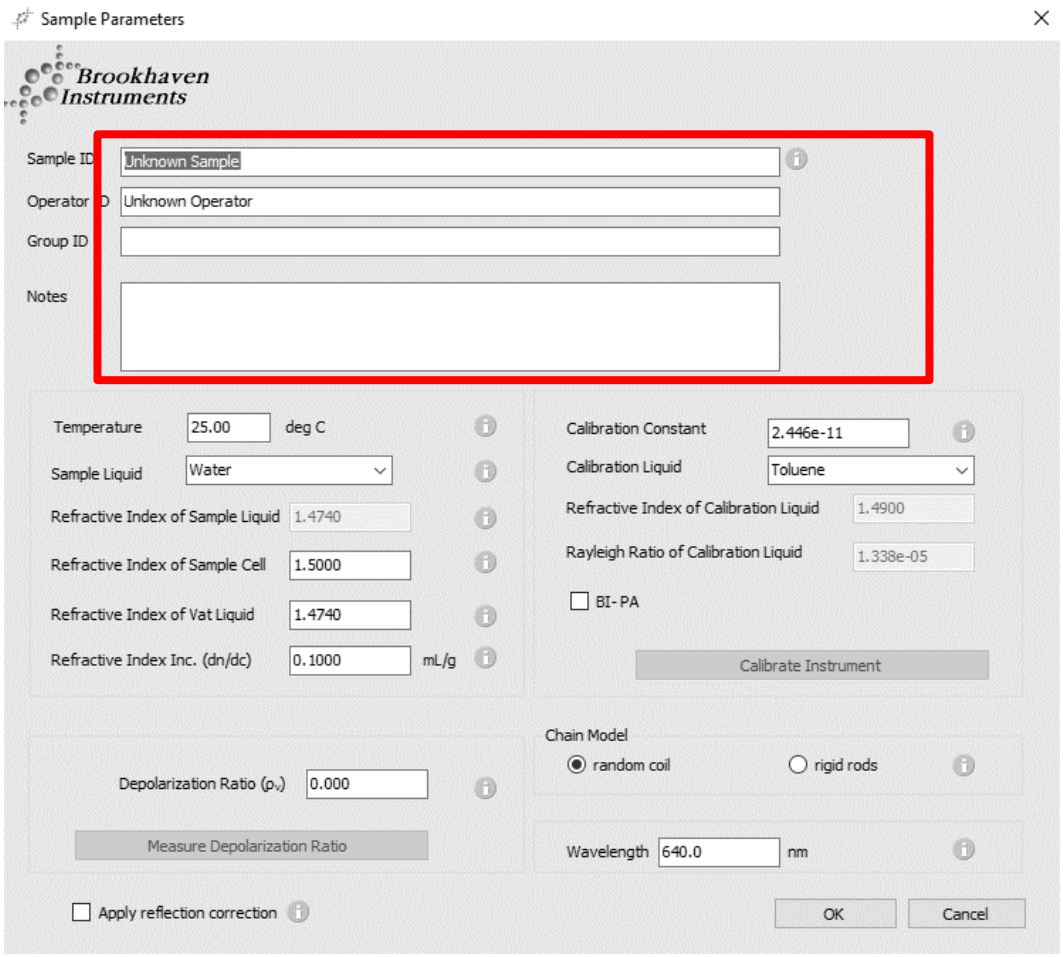
d) Prior to any SLS measurement, the Dark Count Rate must be measured. Turn the filter wheel on the detector optics to “C” for closed. Click “Dark Count Rate”. The software will automatically measure the dark count rate, or the noise of the detector, so that it can subtract this value from the data collected during the measurement sequence. It is recommended to measure the Dark Count Rate a few times to make sure the value is repeatable. Once satisfied, click “OK” and the software will automatically fill in the field ‘A’ Dark Count Rate with the measured number.



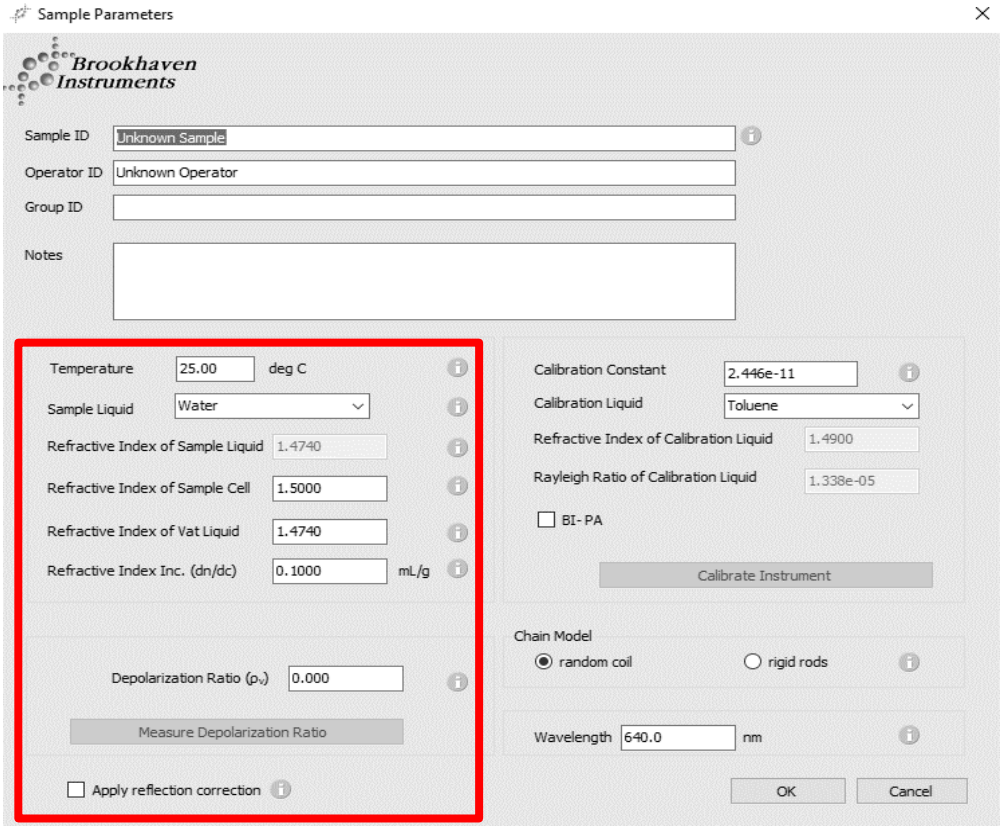
7) Click “Sample Parameters”.



a) Sample ID, Operator ID, Group ID, and Notes are available fields for measurement identification purposes. Each field is not required but recommended so that reviewing past measurements is simple.

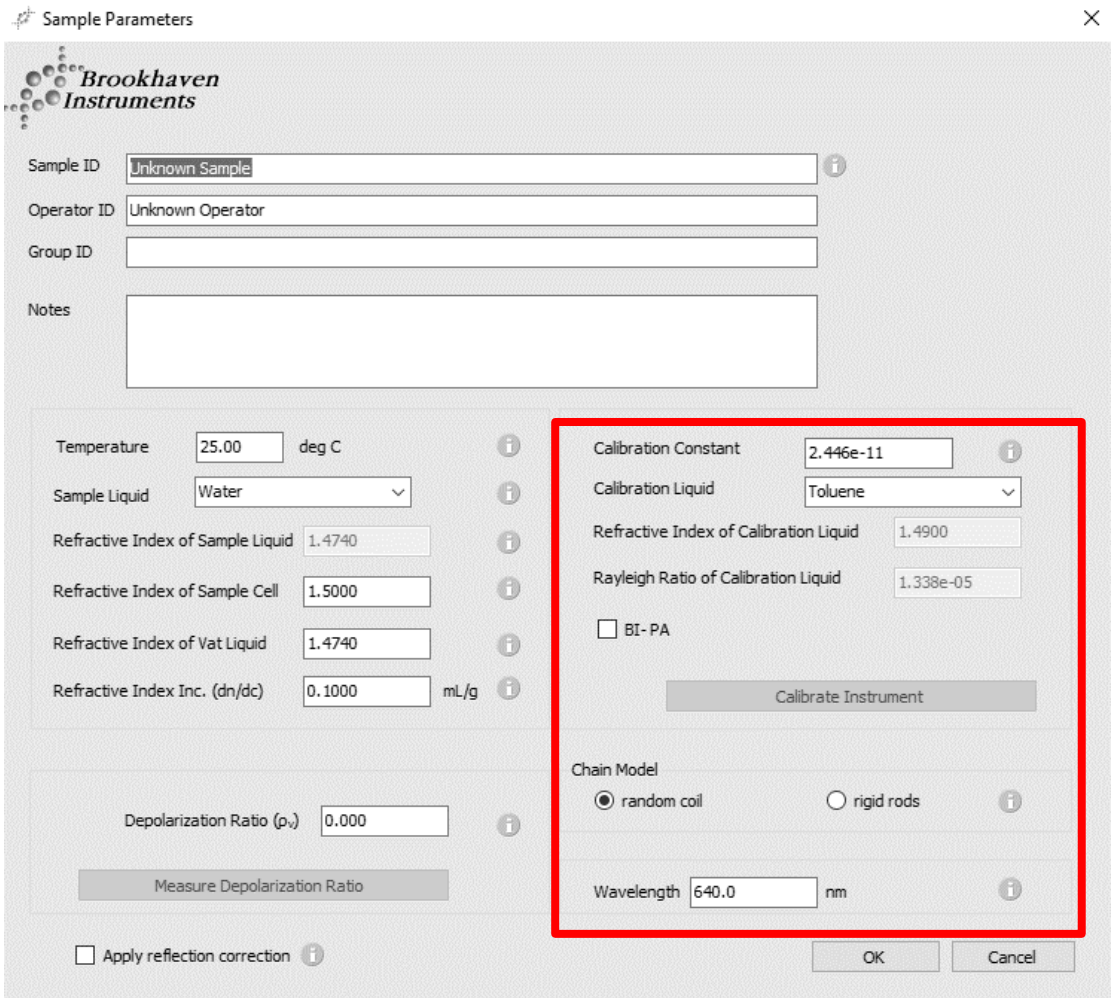


b) Fill in the following sample parameters to ensure that measurement calculations are performed properly.
    a. Input the temperature the measurement will take place at. If the instrument is equipped with a temperature sensor, this value will automatically be filled in once the measurement begins.
    b. Choose the liquid the sample is suspended in. If the liquid is not on the preloaded list, choose "Unspecified" to type the liquid name. Make sure to input the Refractive Index of Sample Liquid at the temperature the measurement will take place at.
    c. The Refractive Index of the Glass Sample Cells provided with the instrument is 1.5000. Only change this value if using different glass cells with different refractive index values.
    d. The recommended Refractive Index Matching Vat Liquid is cis-trans decahydronapthalene, or decalin. This has a refractive index of 1.4740. Only change this value if using a different refractive index matching vat fluid.
    e. The Refractive Index Inc. (dn/dc) is required for Zimm Plot measurements. Find a literature value at the temperature the measurement is taking place at and the wavelength of the laser being used. Or, measure the value with the BI-DNDC. See Appendix E for more information.
    f. Some samples need depolarization corrections. For most polymer samples, depolarization corrections are negligible. Input 0. For rod-like polymer molecules and short-chain molecules where end-groups may contribute significantly to depolarization, ρ_V may not be negligible and corrections are necessary. See Appendix F for more information.
    g. "Apply reflection correction" is recommended.

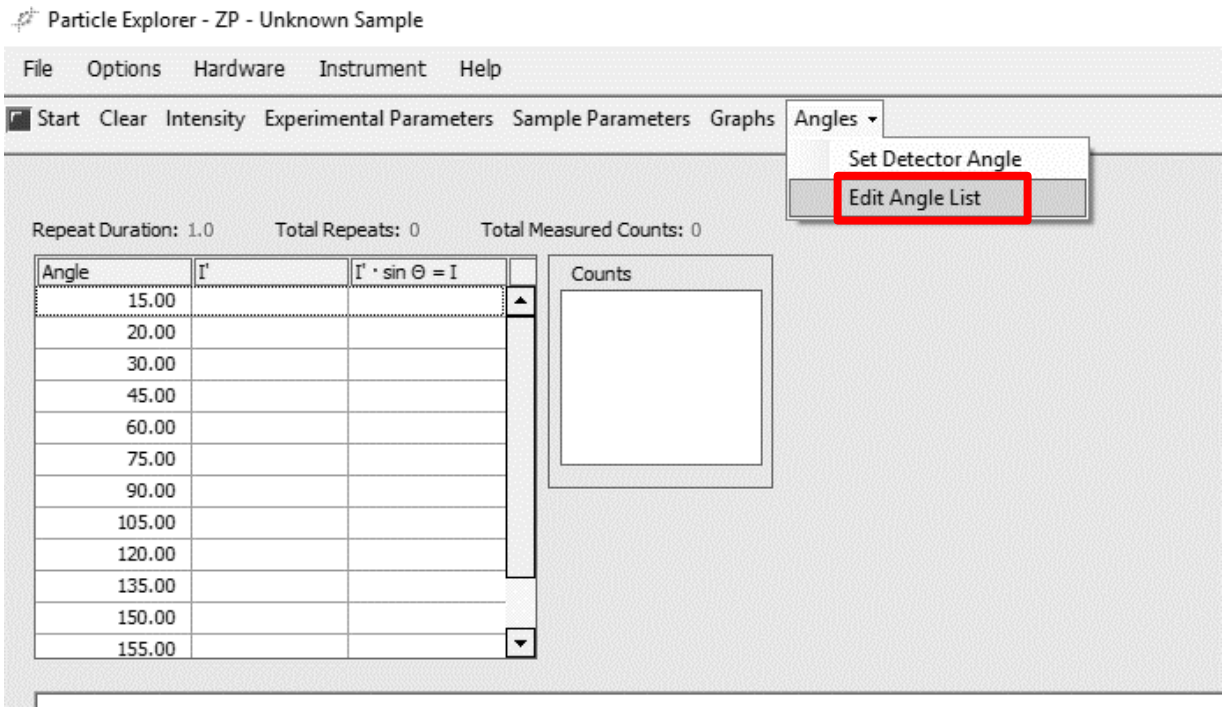


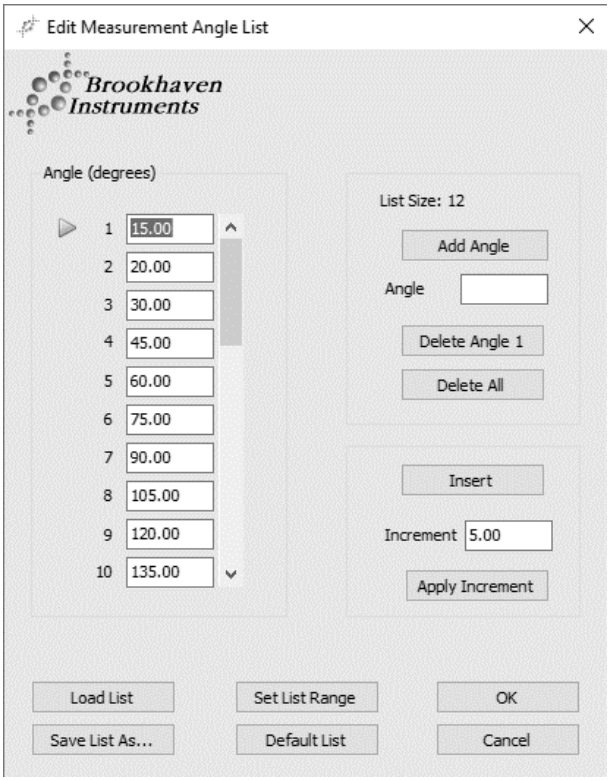
c)

a. For Zimm Plots, a calibration is required. This is done with a liquid with a known Rayleigh ratio, such as toluene. A calibration should be done at the start of each day, and MUST be done when the pinhole, interference filter, laser power, or laser wavelength have been changed. Choose the calibration liquid from the dropdown list. Then, click "Calibrate Instrument". Follow the on-screen prompts. It is recommended to repeat this step three times to make sure the calibration constant is repeatable.
b. Check BI-PA if a polarization analyzer is being used during the measurement.
c. Select the radio button that best describes the sample type. This will only affect calculations if a depolarization ratio other than 0 has been entered.
d. Enter the wavelength of the laser being used for the measurement.



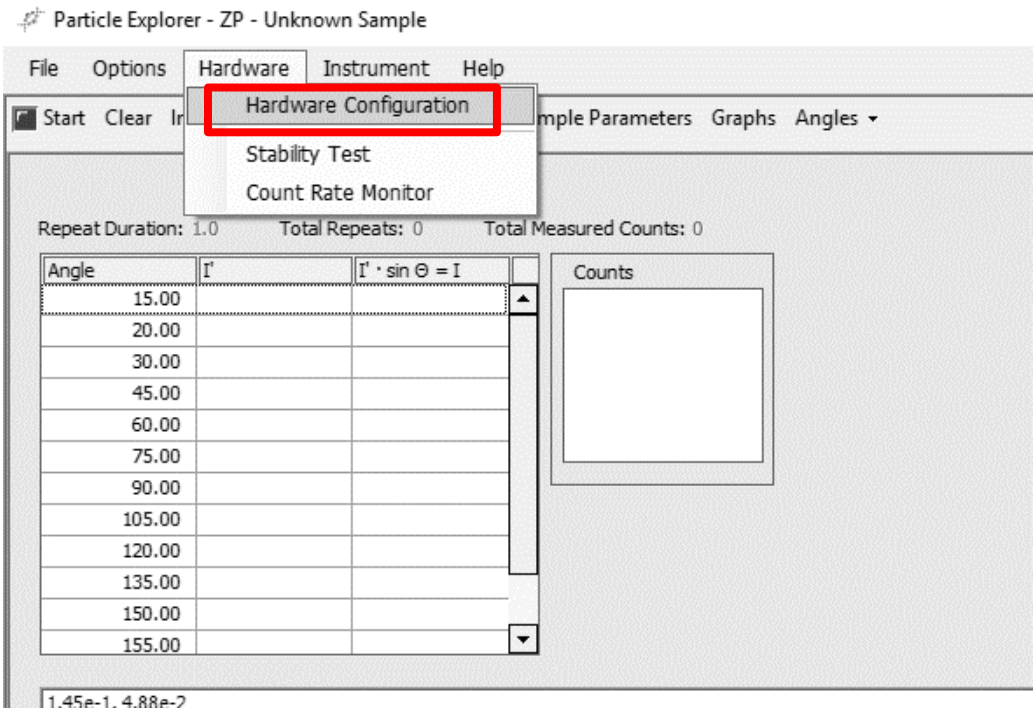
8) Click “Angles→ Edit Angle List”.

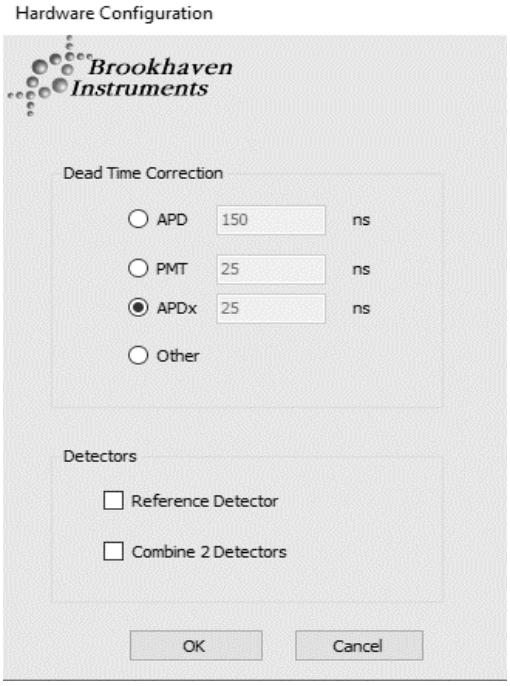




The default angle list spans nearly the full range of the instrument. To check a different range of angles, less angles, or more angles, use the Add Angle, Delete Angle, and Set List Range buttons.

9) Click “Hardware→ Hardware Configuration”.

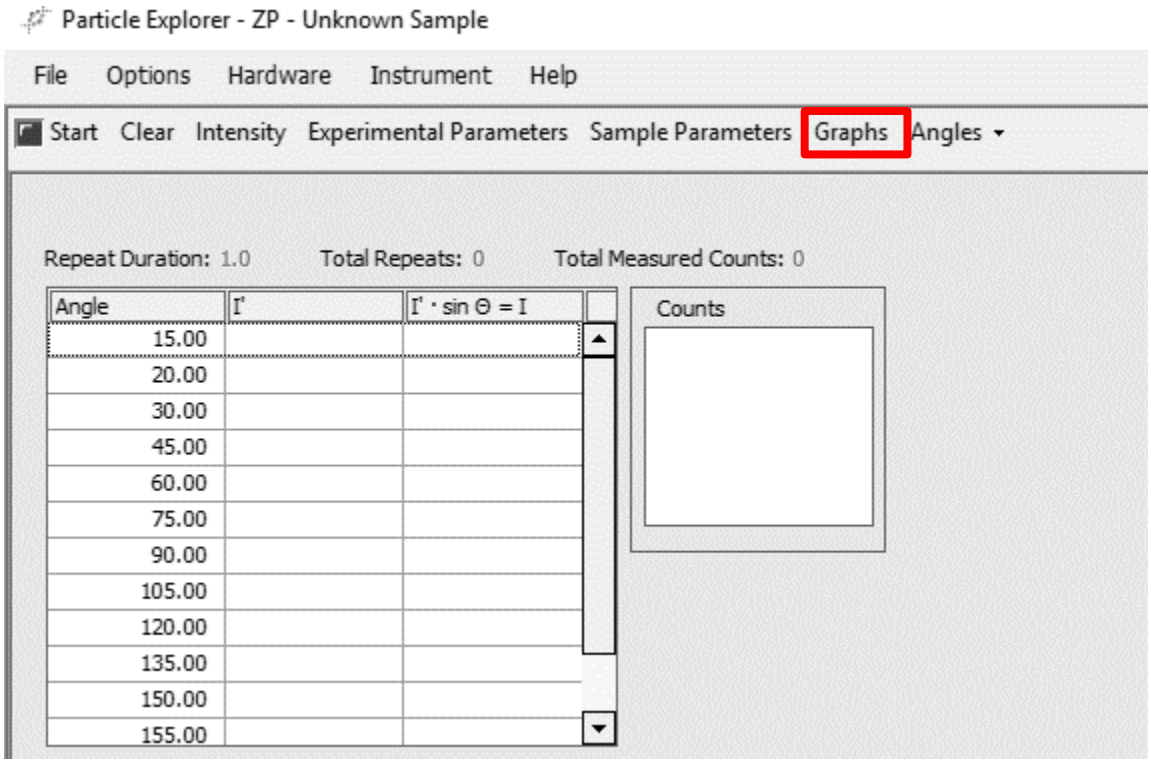


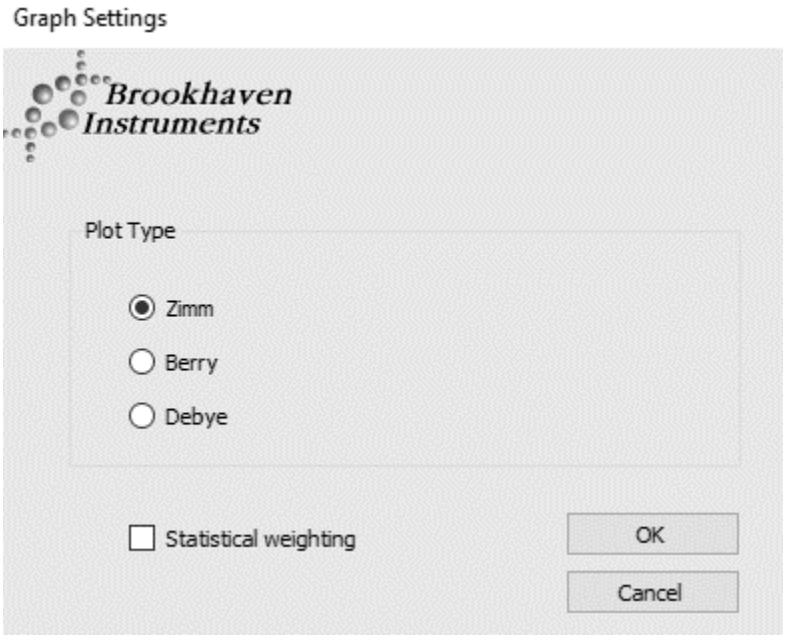


Choose the detector the instrument is equipped with. The dead time correction is automatically assigned. If the instrument has a detector or dead time not listed, select “Other” and fill in the dead time correction manually.

For specific applications, the instrument may be configured with a reference detector or two detectors. Check the appropriate box if using either configuration.

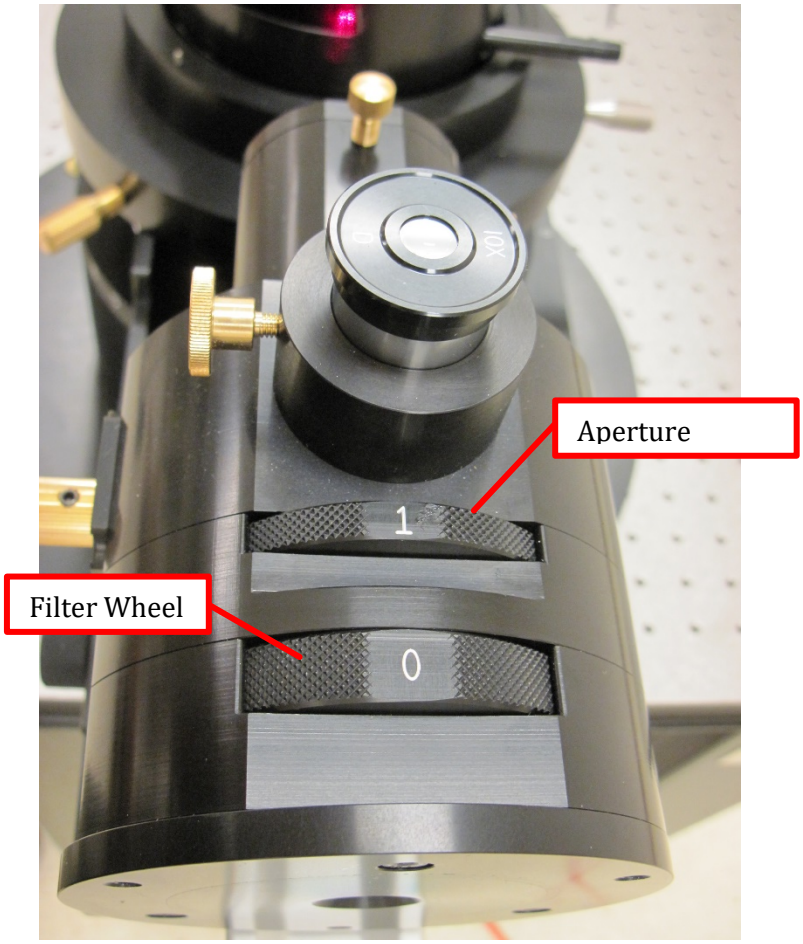
10) Click “Graphs”.



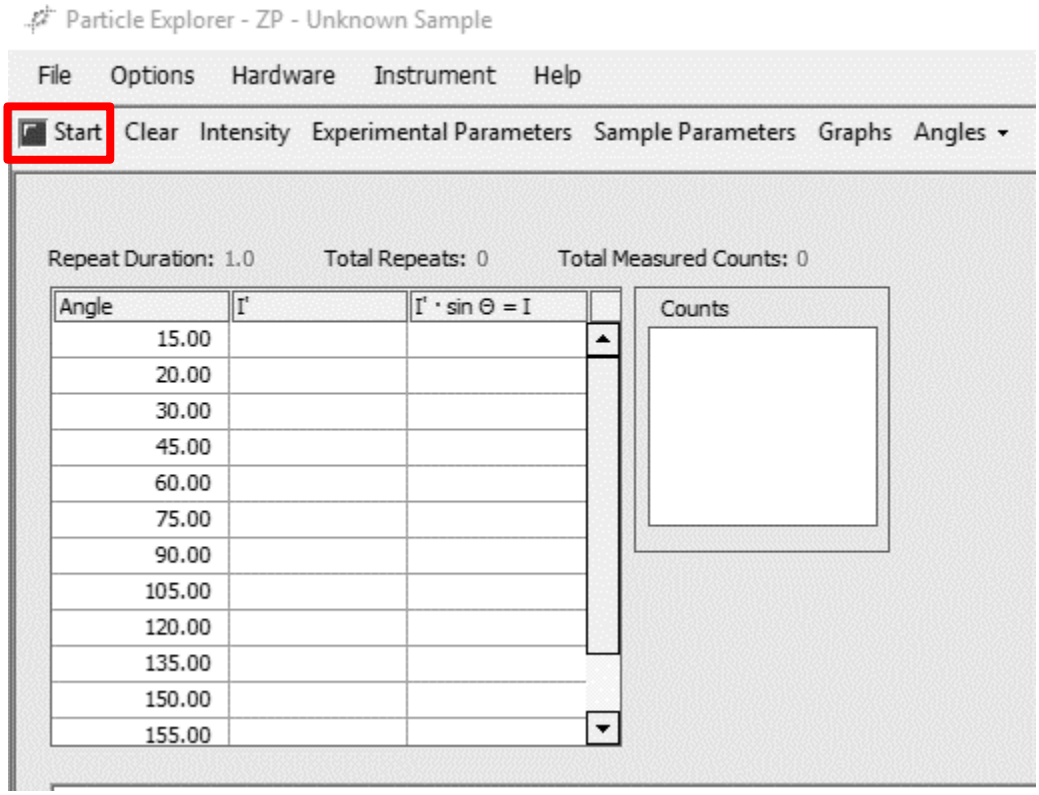


Choose which graph the software should plot during data collection. This can be changed before, during, or after the measurement.

11) Now, all of the ZP measurement parameters have been set. On the detector optics, set the filter wheel and pinhole wheel to the settings selected in Step 6b.



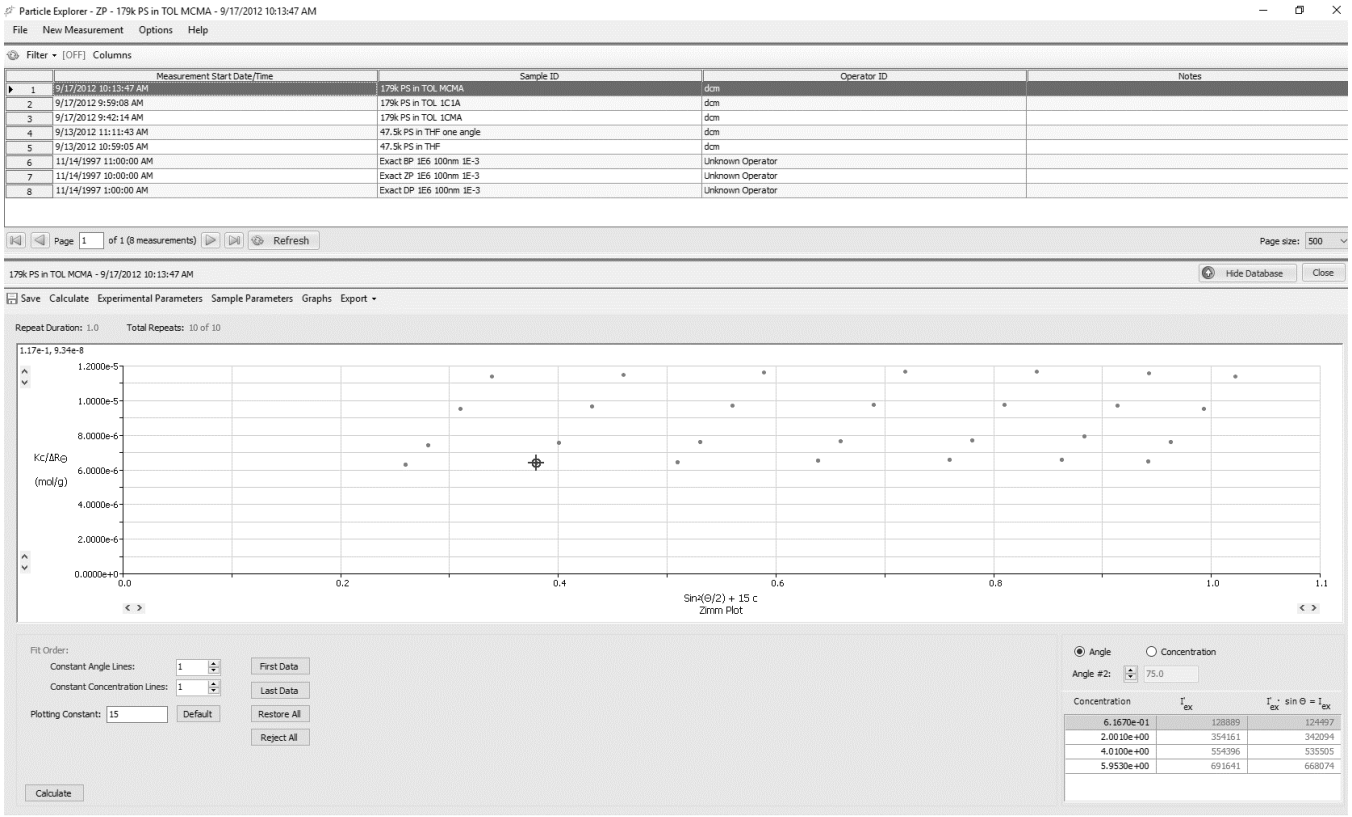
12) Click “Start” to begin the measurement.



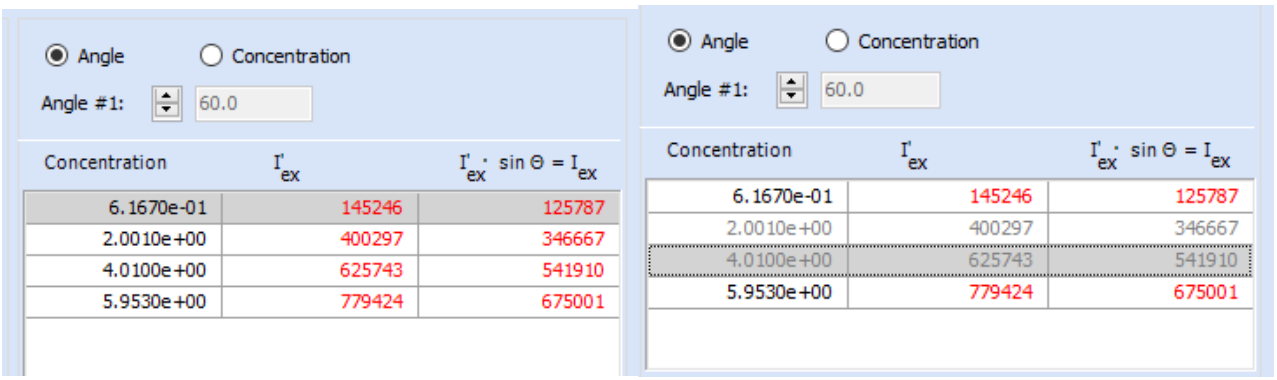
The software needs to know what angle the goniometer is at before it can begin the measurement. A window will appear asking for the current angle. Enter the angle and click OK to allow the measurement to proceed.

13) The rest of the measurement is automated. When complete, the measurement will be automatically saved to the database if selected in Step 5a. If not, click “Save” once the measurement is complete. Otherwise, the data will NOT be saved.

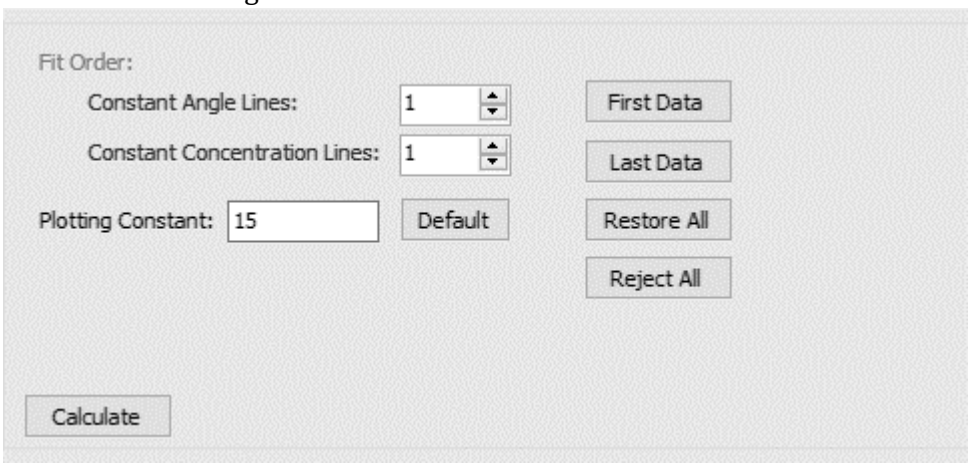
14) Once the measurement is complete, the data can be reviewed. Double click the measurement from the database if the measurement is not already open.



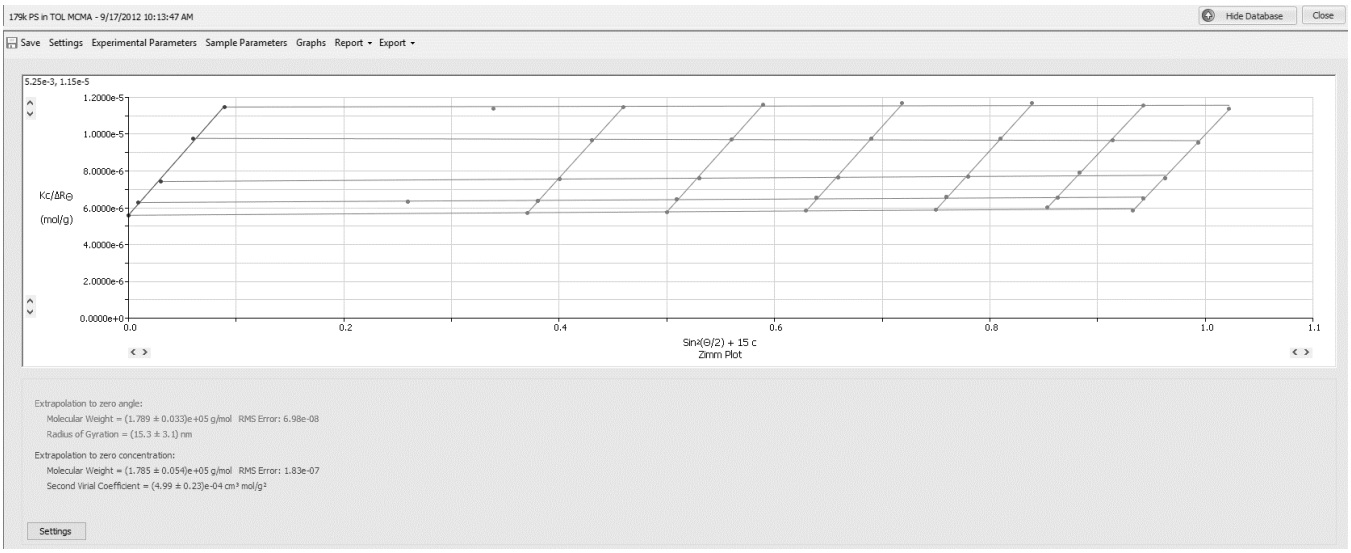
a. Click “Sample Parameters” to modify any previously entered parameters. The data will then be reanalyzed with the new selections. For example, if the liquid was entered incorrectly initially, it can be corrected here and the data will be reanalyzed. Click “Save” to save the corrected measurement to the database. Right click on the incorrect measurement in the database to delete it.
b. Click “Graphs” to choose what graph analysis to display.
c. Click “Report” to preview a report printout of the data. Click “Print Report” to print the report to PDF or a printer.
d. Click “Export” to choose a format to export the graph and/or data as.
e. There are a few ways to remove outliers. The table on the bottom right-hand side lists angles and concentrations with intensity values. Choose either angle or concentration and double-click to remove an entire concentration or angle from the calculations. The value will turn gray, indicating it is no longer in the calculation.



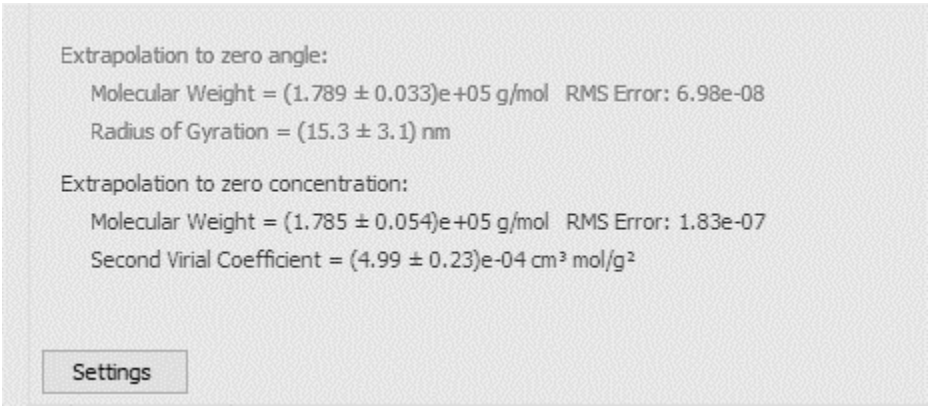
f. Click a data point on the graph. If the "First Data" button is clicked, the software will eliminate all data points to the left of the selected point. If "Last Data" is clicked, the software will eliminate all data points to the right of the selected point. Click "Restore All" to undo all changes.



g. Once any desired outliers are removed, click "Calculate" and the Zimm, Berry, or Debye plot will be created, displaying calculated values such as M_w.



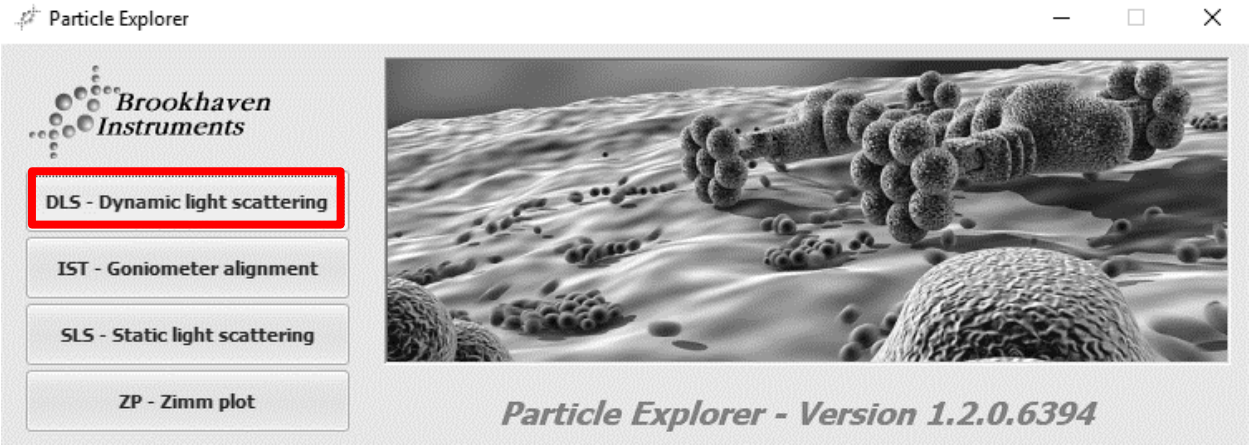
h. Toggle between “Settings” and “Calculate” to evaluate the data, outliers, and review other graph types.



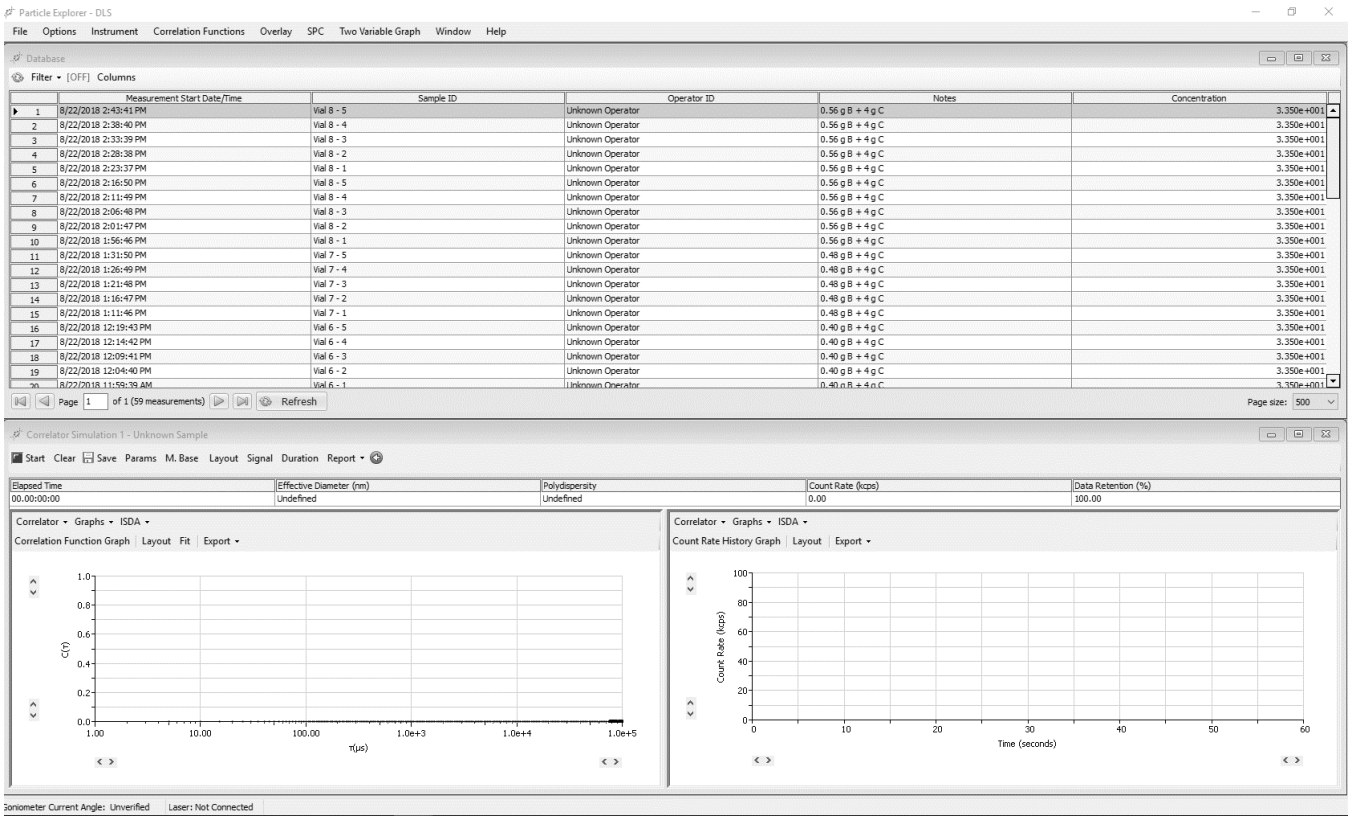
Making a DLS Measurement

Dynamic Light Scattering (DLS) measurements of suspensions provide information on the diffusion coefficient, effective diameter, and more. Follow the steps below to set up a DLS measurement using Particle Explorer software.

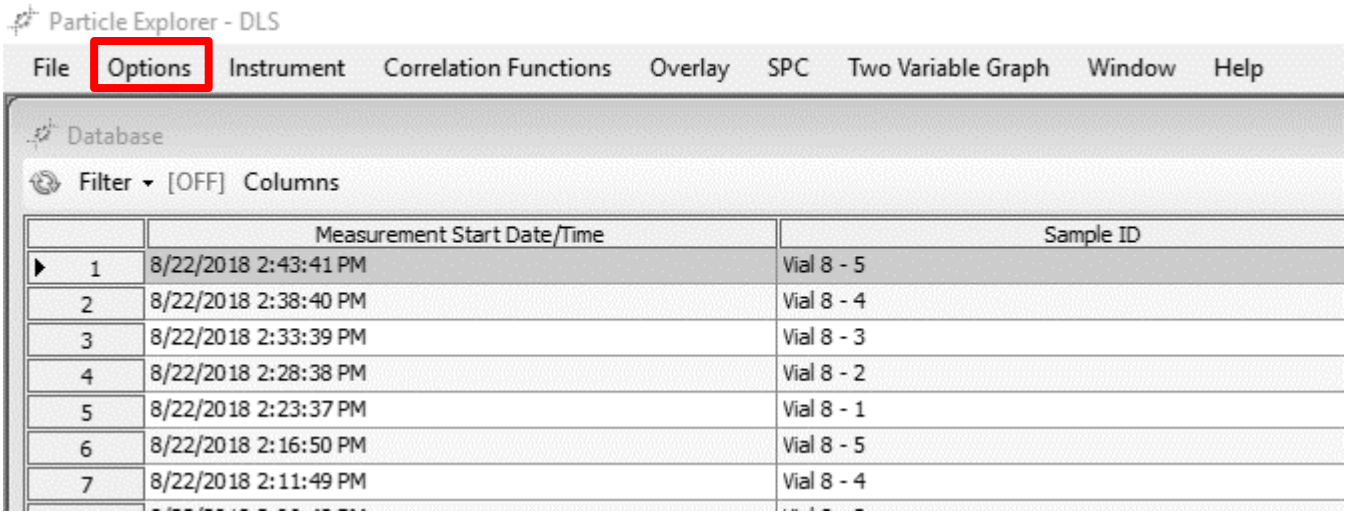
1) Choose DLS – Dynamic Light Scattering from the splash screen.



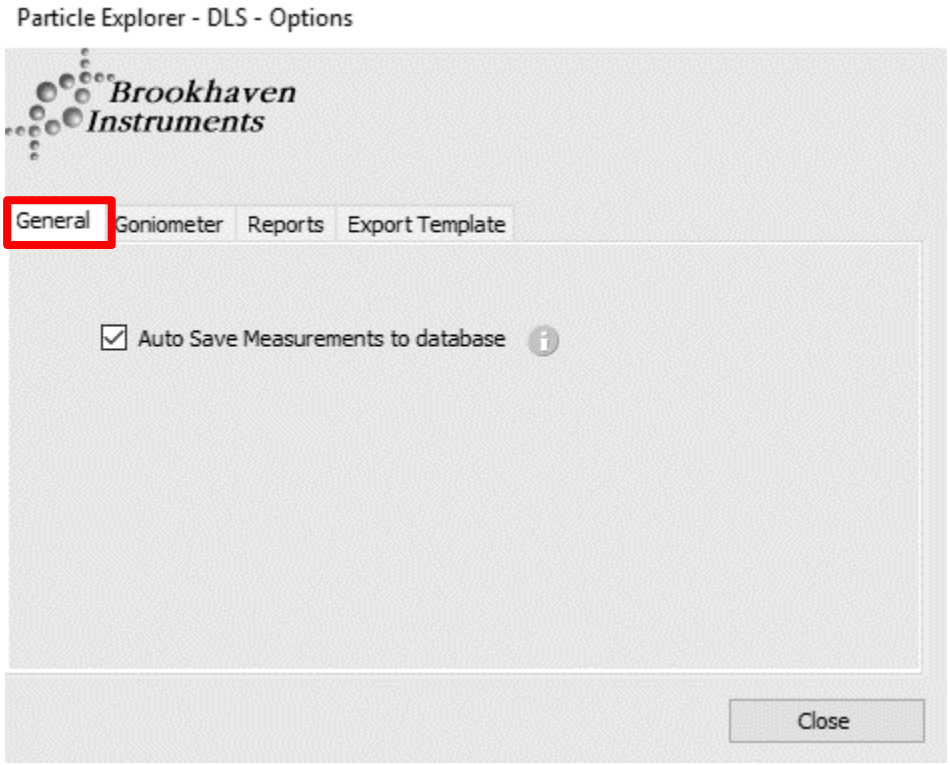
2) A new window will open showing the measurement database and two graphs ready to collect data.



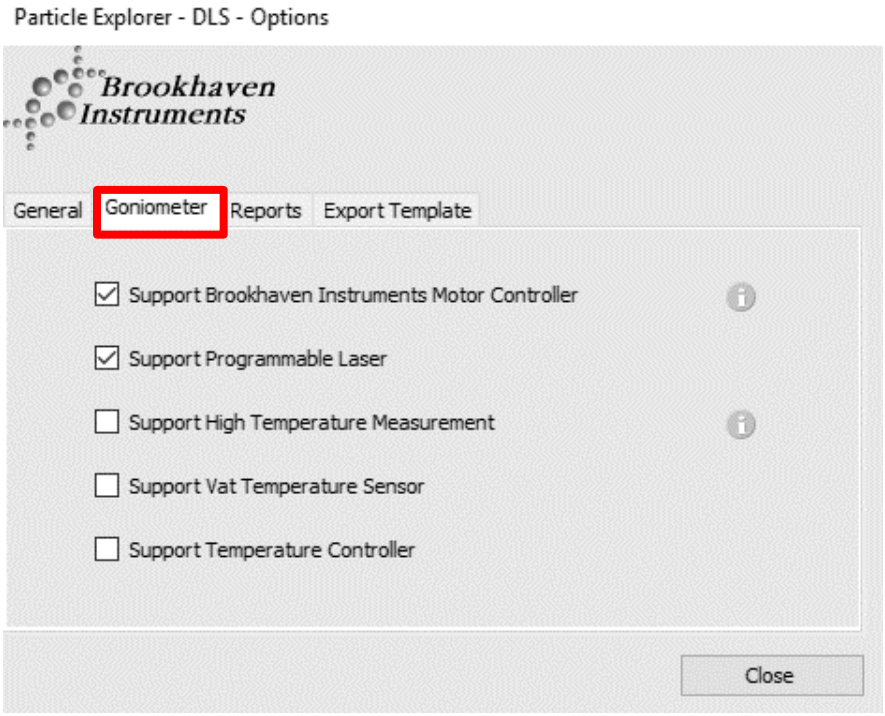
3) Click “Options” from the ribbon.



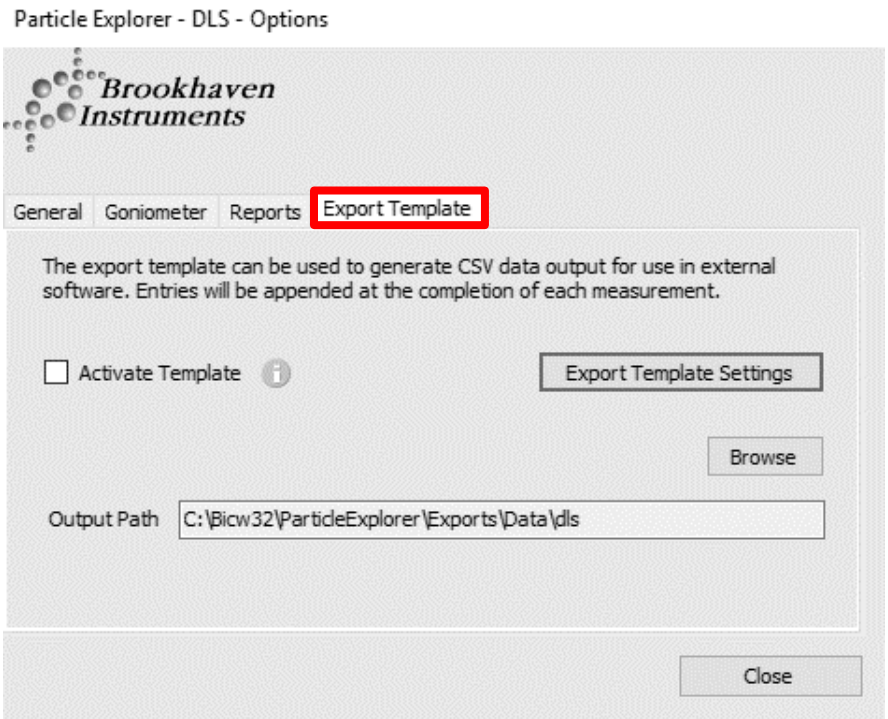
a) On the General tab, it is recommended to check off the Auto Save option.



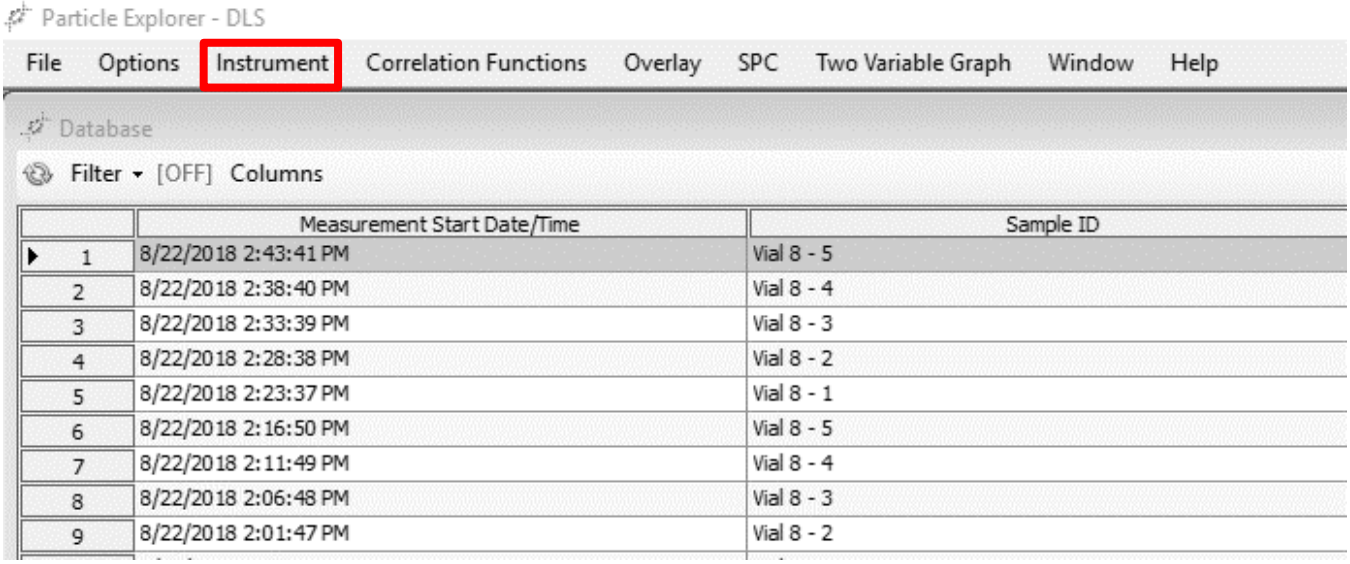
c) On the Goniometer tab, check off what options the instrument is configured with. Close the window when all selections have been made.



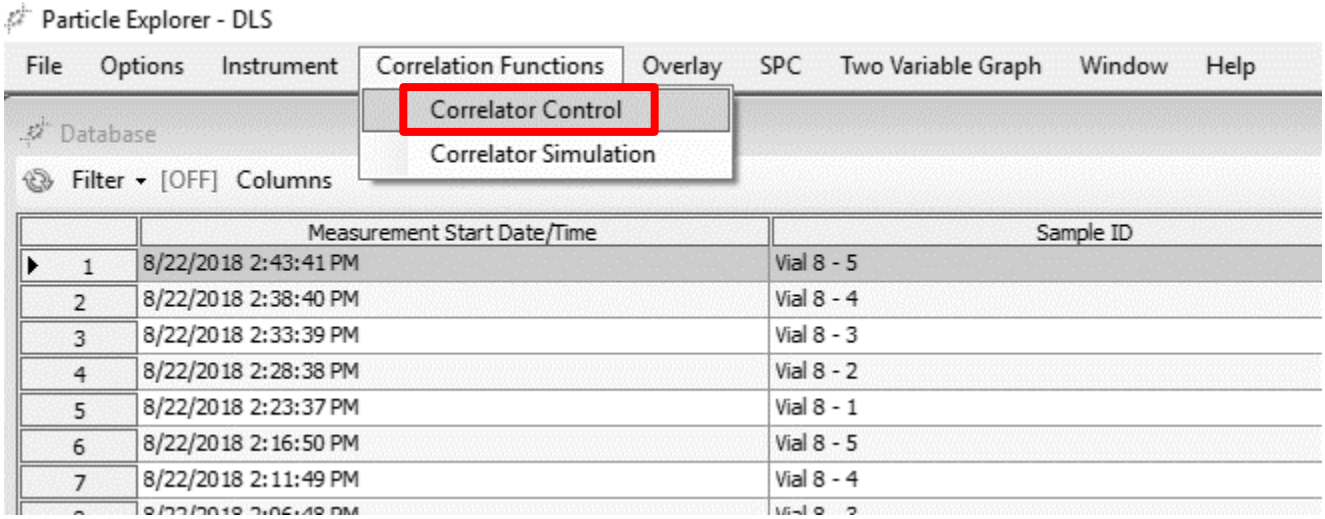
c) On the Export Template tab, there is an option to export the data collected during a measurement directly to an Excel file. Check "Activate Template" to enable this option. Click "Export Template Settings" to choose what data should be directly exported. Click "Browse" to choose an Output path where the software should save the exported data to.



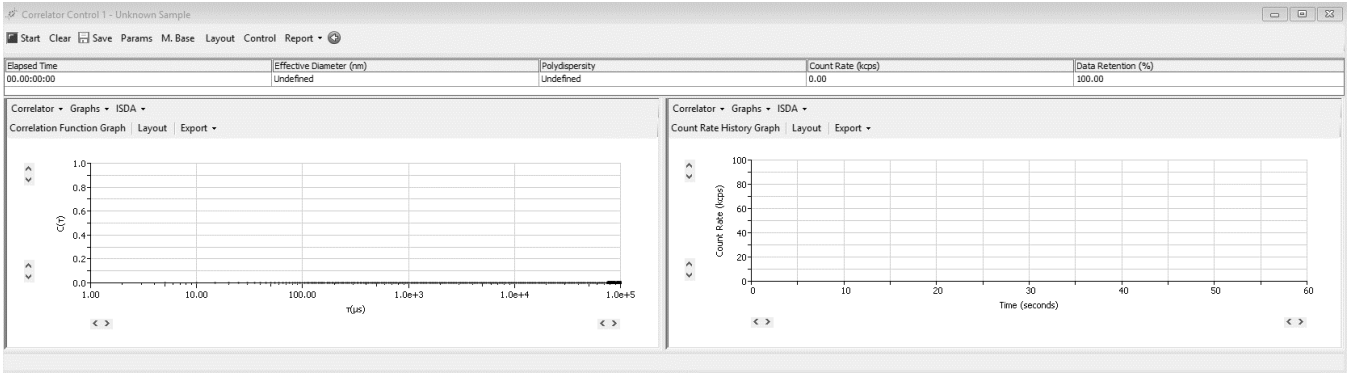
4) Click “Instrument” from the ribbon to connect a laser or temperature controller option. Follow the on-screen prompts.



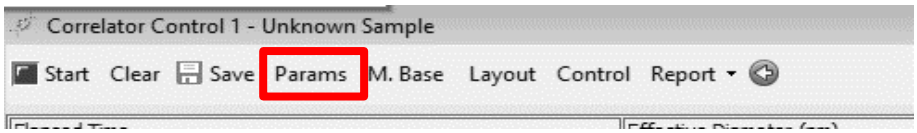
5) Under “Correlation Functions”, choose “Correlator Control” to open the measurement graphs if not already open.



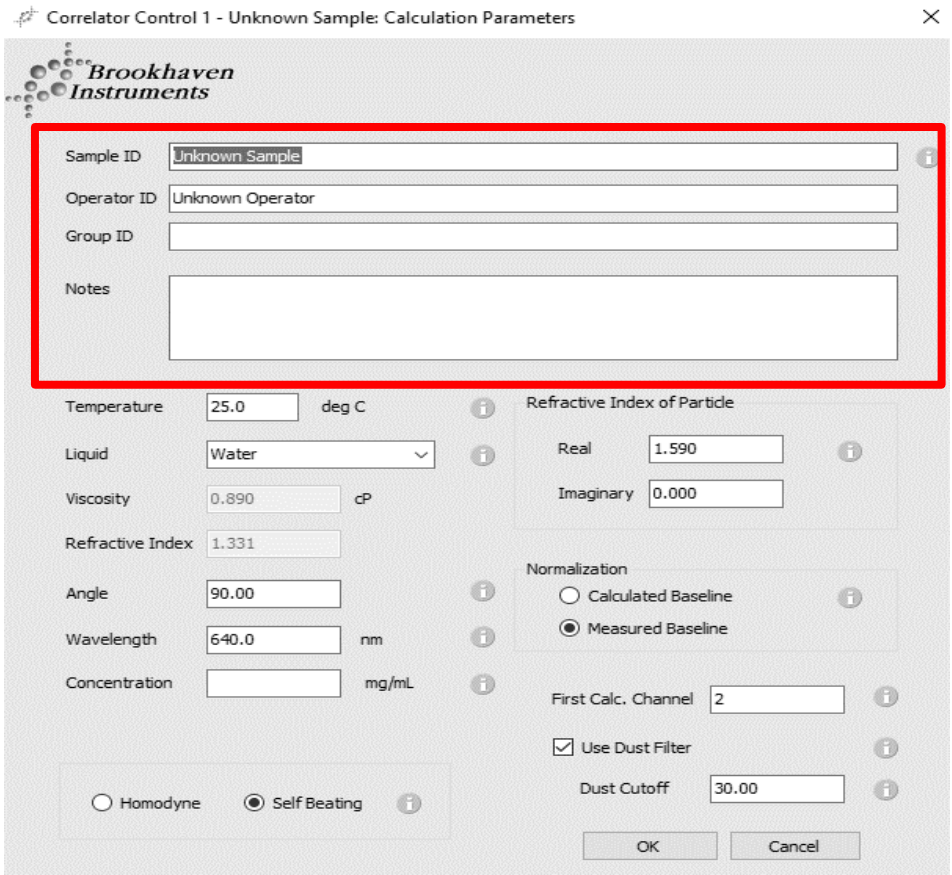
6) All parameters for a DLS measurement are in this window.



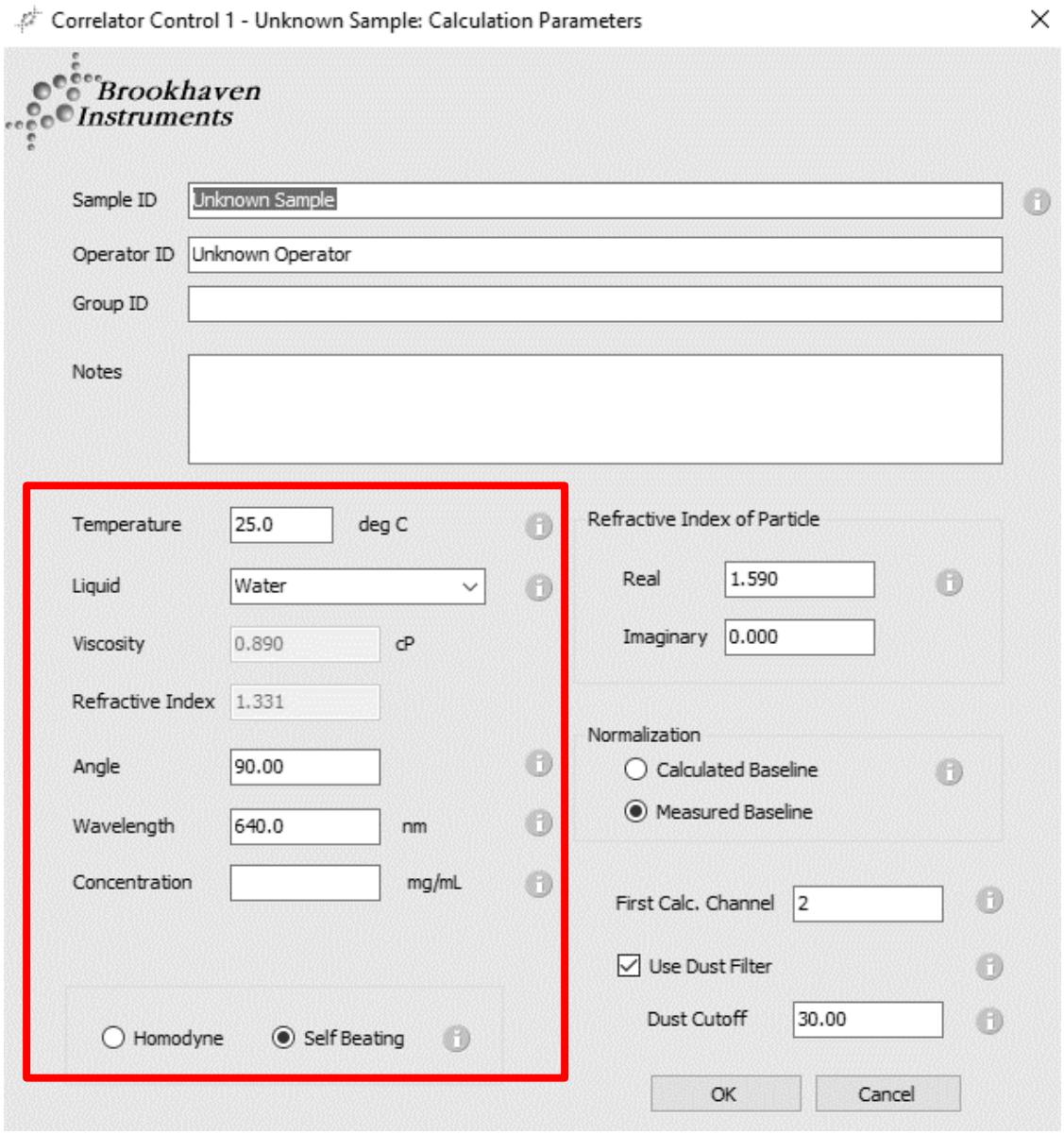
Select "Params" from the menu.



a. Sample ID, Operator ID, Group ID, and Notes are available fields for measurement identification purposes. Each field is not required but recommended so that reviewing past measurements is simple. It is helpful to record the laser power, pinhole size, and interference filter information in the Notes section.

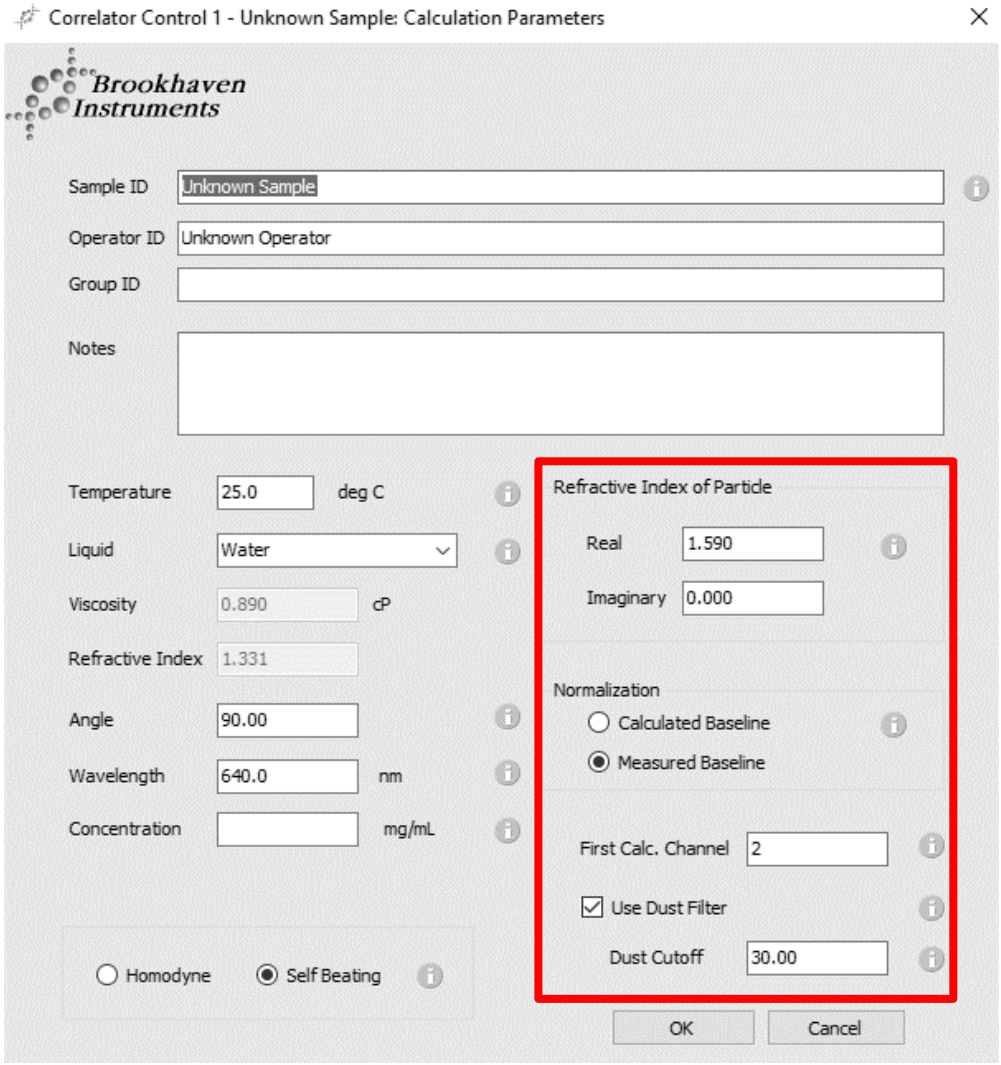


b. Fill in the following sample parameters to ensure that measurement calculations are performed properly.
    i. Input the temperature the measurement will take place at. If the instrument is equipped with a temperature sensor, this value will automatically be filled in once the measurement begins.
    ii. Choose the liquid the sample is suspended in. If the liquid is not on the preloaded list, choose "Unspecified" to type the liquid name. Make sure to input the Refractive Index and Viscosity of Sample Liquid at the temperature the measurement will take place at.
    iii. Input the angle the software should make the measurement at.
    iv. Input the wavelength of the laser being used for the measurement.
    v. Choose the "Self Beating" radio button for most DLS applications. See Appendix F for more information.

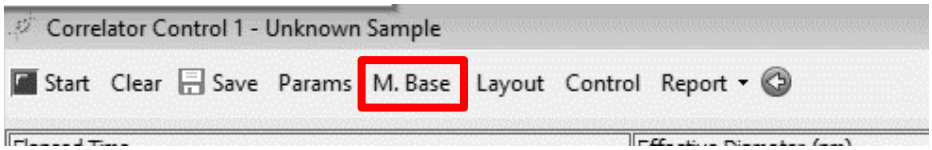


c.

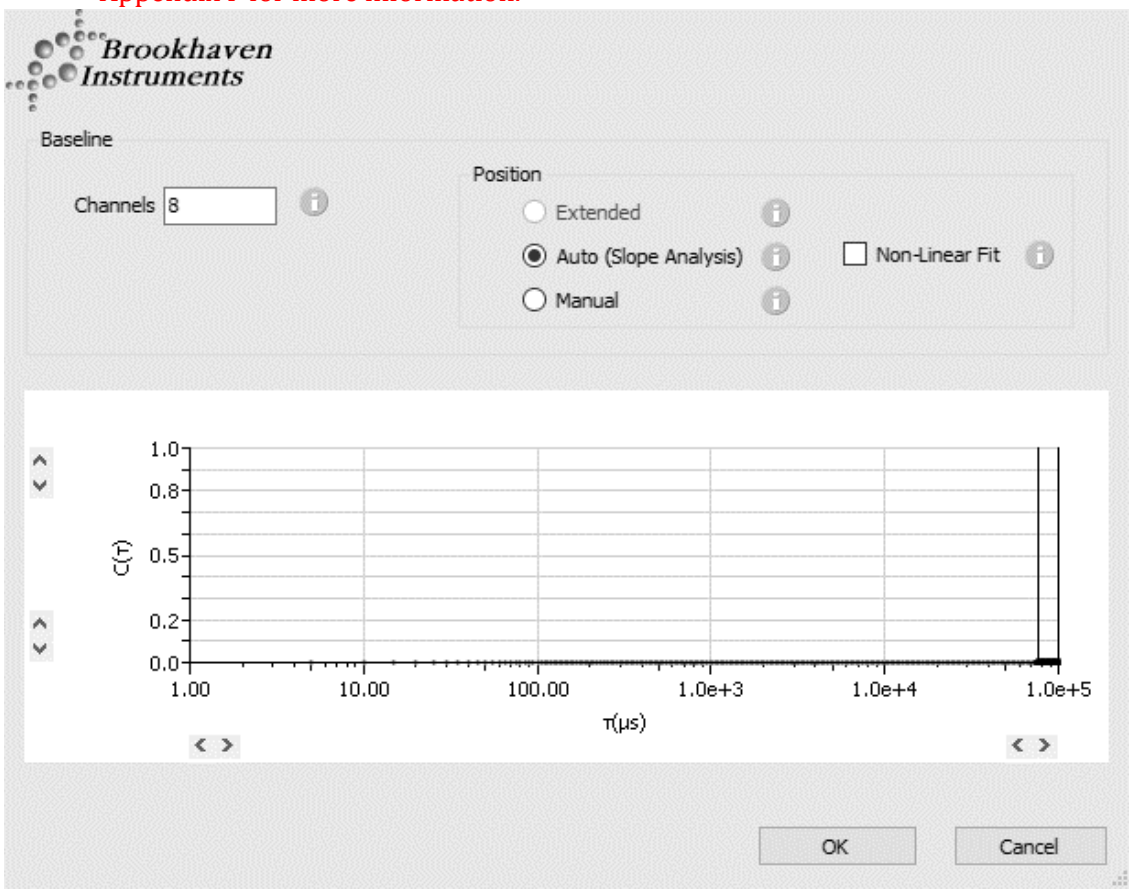
i. Input the real and imaginary refractive indices for the particle in the sample. These values allow for volume, surface area, and number transformations of the particle size distribution. They do not affect the effective diameter, polydispersity, or particle size distribution by intensity.
ii. Select the baseline to display during the measurement. Measured baseline is typically preferred to suppress the effect of residual dust. See Appendix F for more information.
iii. For First Calc. Channel, enter the number 1 for first delay times greater than 2 µs. Enter 2 or higher for first delay times less than 1 µs to avoid calculations that include after-pulsing effects associated with detectors.
iv. Check "Use Dust Filter" to implement the Dust Cutoff. This box toggles the display between results generated from the raw measured data and raw data that have been filtered during the collection of the measurement. Both sets of data will be preserved for all measurements regardless of the setting.
v. Fill in a value for the dust cutoff as described in the information icon text.



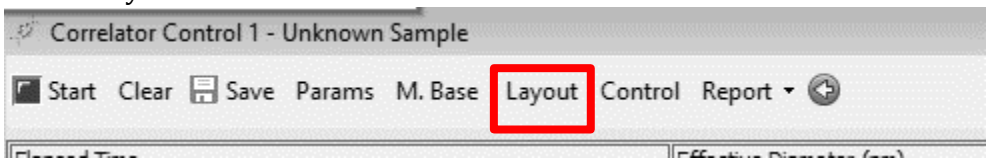
7) Select M. Base from the menu.



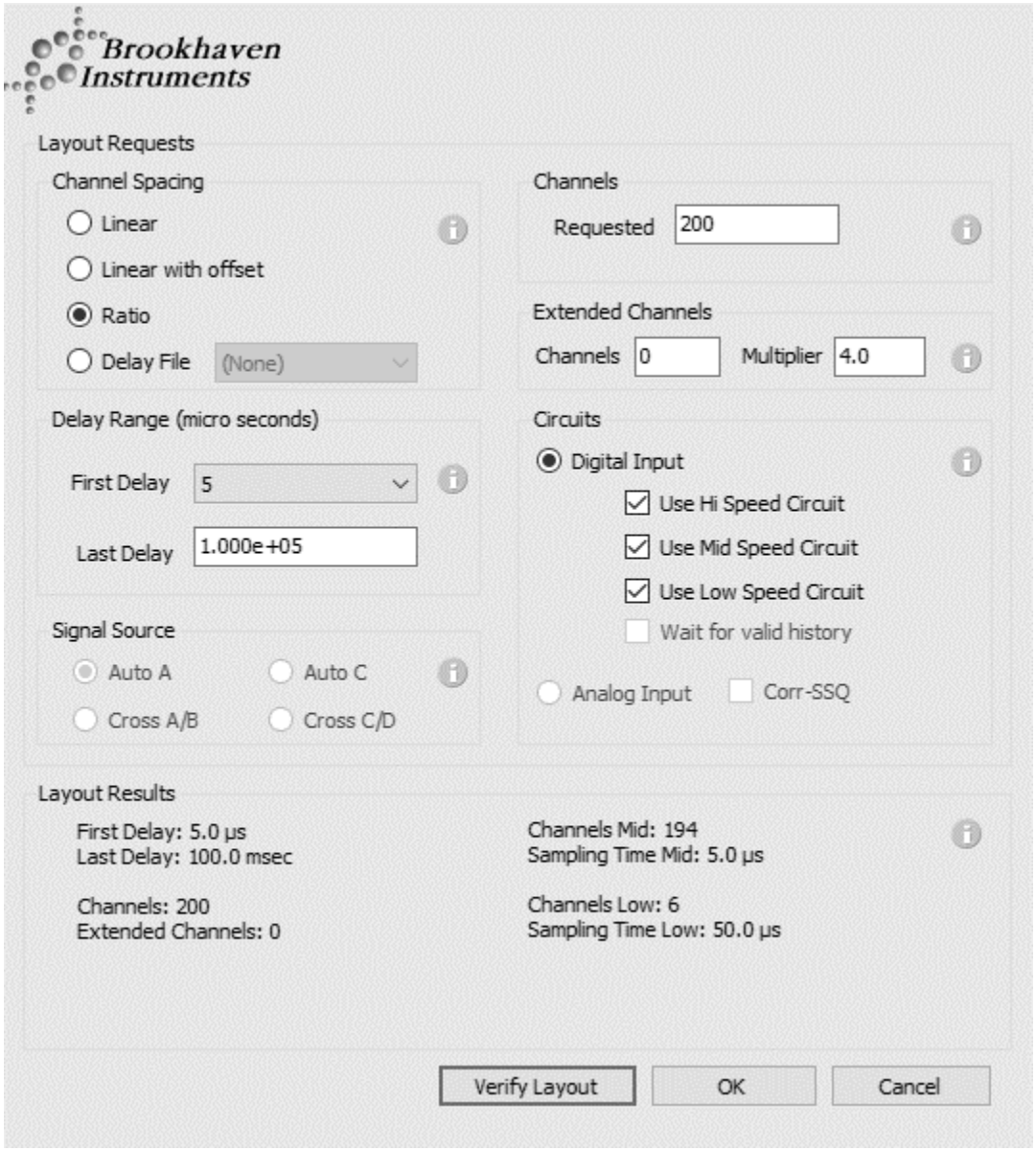
a. Choose how the software should assign the baseline. Auto (Slope Analysis) is recommended for most sample types. Manual will allow the cursors to be manually positioned. Non-Linear fit is not recommended for routine sample analysis. See Appendix F for more information.
b. The number of baseline channels can range from 2 to 32. The more channels selected, the better the autoslope calculation will be. Brookhaven recommends at least 4 channels. See Appendix F for more information.



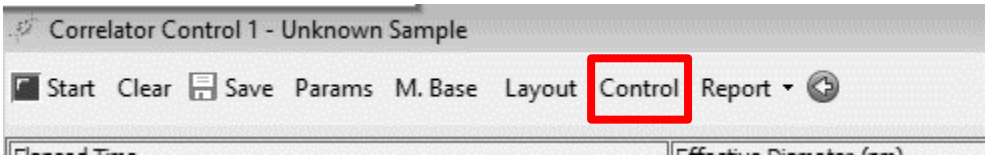
8) Select "Layout" from the menu.



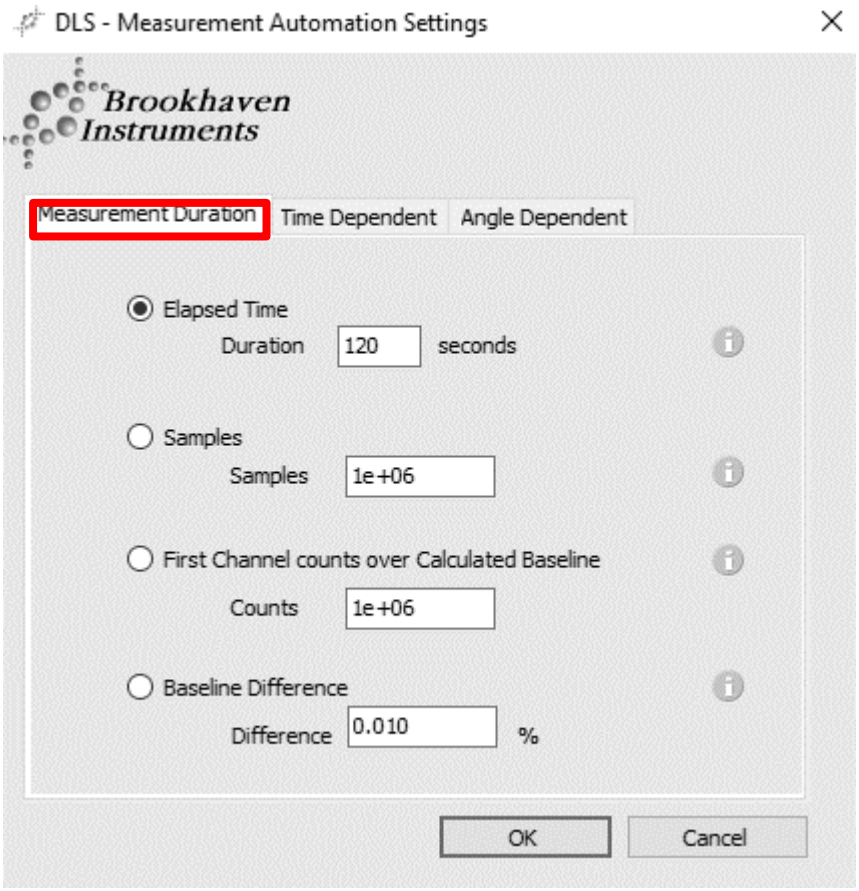
Keep the default layout as shown below for most samples. The first delay can be shortened for smaller particles and the last delay lengthened for larger particles. Ratio spacing is recommended. See Appendix F for more information on creating a correlator layout.



9) Click "Control" from the menu.

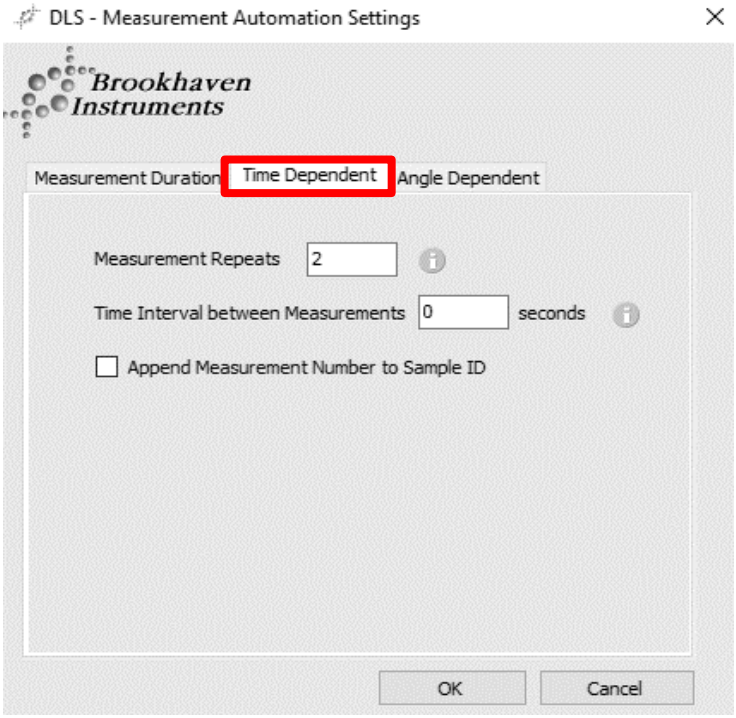


a. Under the “Measurement Duration” tab, select what method the software should use to determine the measurement duration. An elapsed time of at least 2 minutes is recommended.

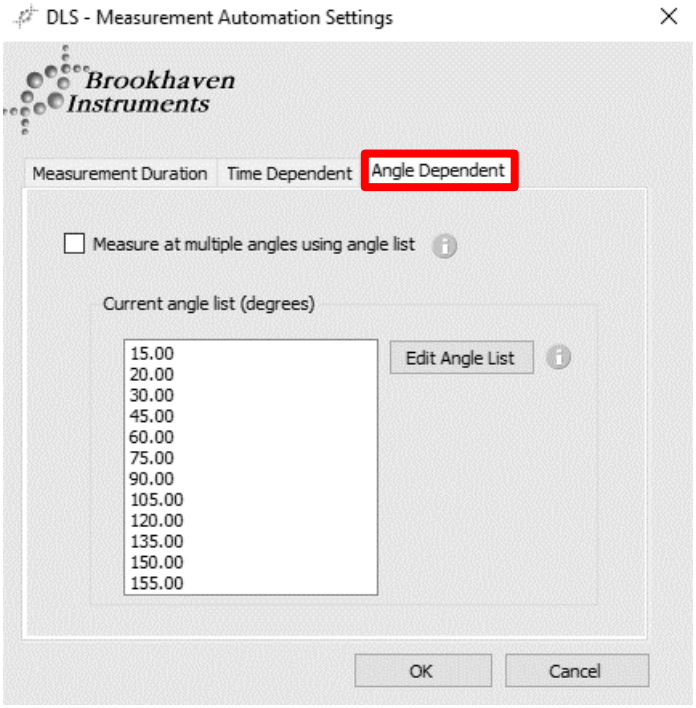


i. Samples: The measurement will end after the total number of samples entered is achieved. Total samples is the number of samples multiplied by the sampling time of the middle speed circuit (or the low speed if that is the only circuit enabled).
ii. First Channel: The measurement will end after the difference in the channel contents between the first delay channel and the infinite time delay (calculated baseline) reaches the entered value.
iii. Baseline Difference: The measurement will end after the entered percent difference between the measured baseline and the calculated baseline. This is essentially a signal to noise ratio limit.

b. Under the "Time Dependent" tab, automate a set of measurements. Input a number of measurement repeats the software will automatically collect. Input a time interval between measurements, if desired. Check "Append Measurement Number to Sample ID" so each measurement repeat is easy to find in the database.

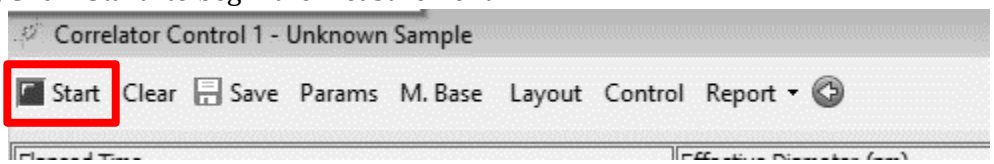


c. Under the "Angle Dependent" tab, check whether the measurement should occur at multiple angles. This will automate the process based on the angles selected. Click "Edit Angle List" to create or edit a list of angles.



10) Now, all of the DLS measurement parameters have been set. On the detector optics, set the filter wheel and pinhole wheel as desired. For DLS, it is recommended to start with the smallest possible pinhole size, 100 micron. If more signal is required for the sample, increase the pinhole size. Make sure the intensity does not exceed 1 Mcps at the angle(s) the measurement will take place at.
An interference filter of the wavelength of the laser is recommended.

11) Click "Start" to begin the measurement.



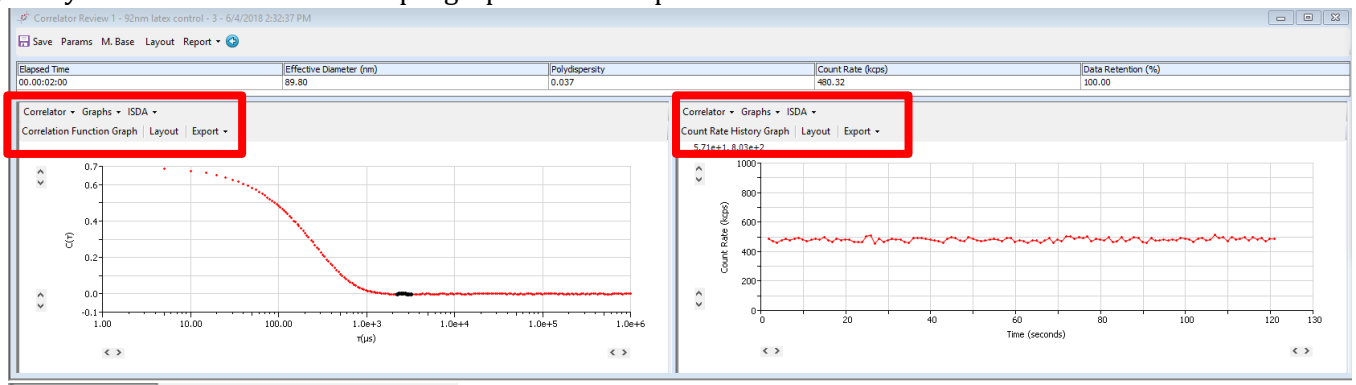
12) Once the measurement is complete, the data will be automatically saved in the database if the box was checked in Step 3a. If not, click "Save". Otherwise, the data will NOT be saved.

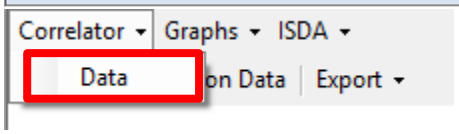
13) If the measurement is already open, data review is available. Otherwise, double-click on the measurement from the database to open it.

14) Click on "Params" or "M. Base" to modify any parameters for data reanalysis, such as correcting sample liquid.

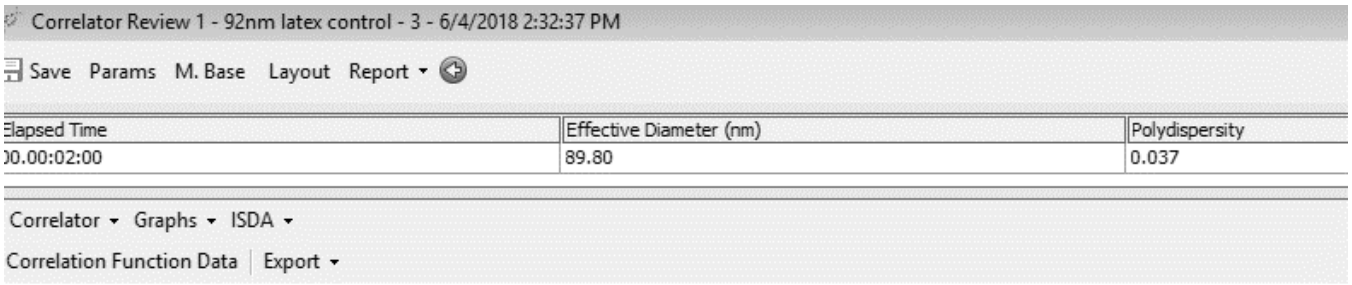
15) Data Review
    a. Click on "Report" preview or print a report of the results that are currently displayed.

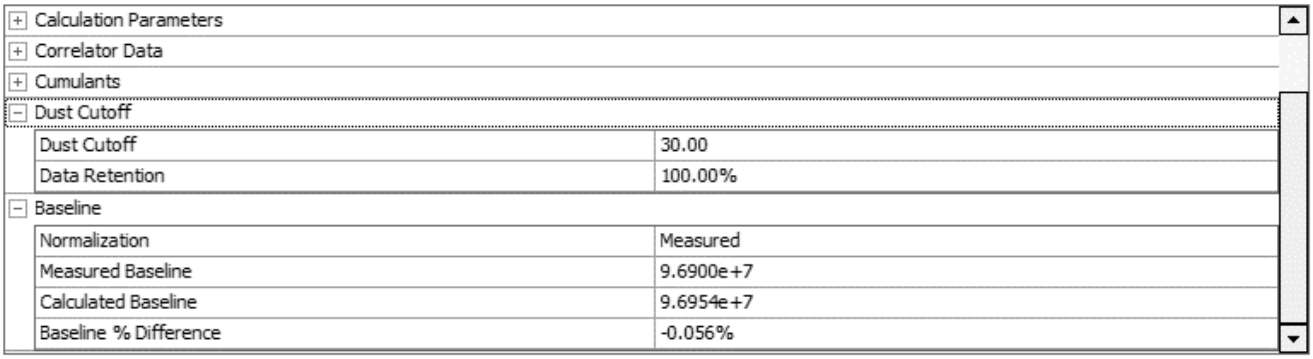
16) Analyze the data with the multiple graph and data options:



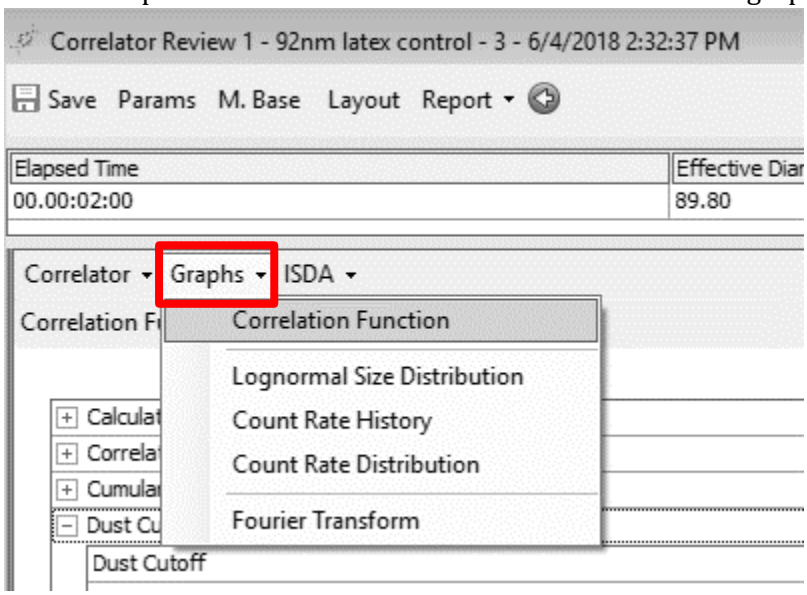


a. Click "Correlator→Data" to view different correlator data information, such as preset dust cutoff parameters and baseline calculation data. Click "Export" to choose a format to export this data to.





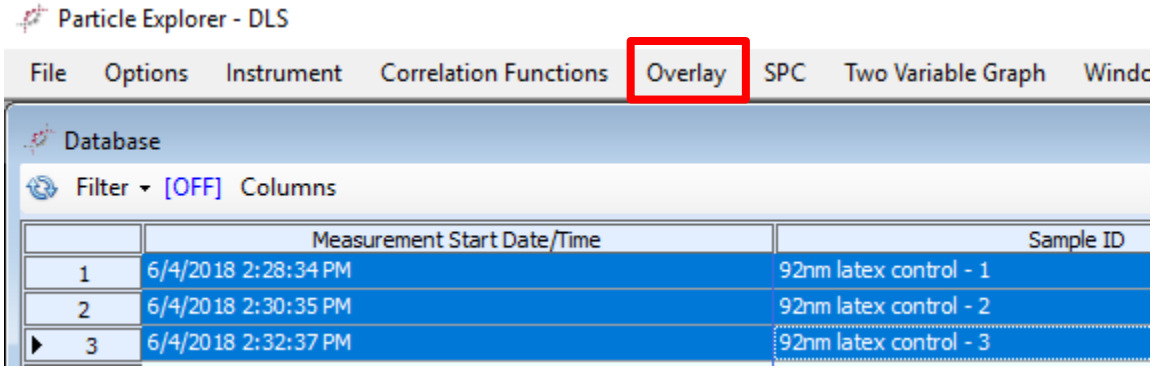
b. Click "Graphs" and choose from a list of different graph types for data review.



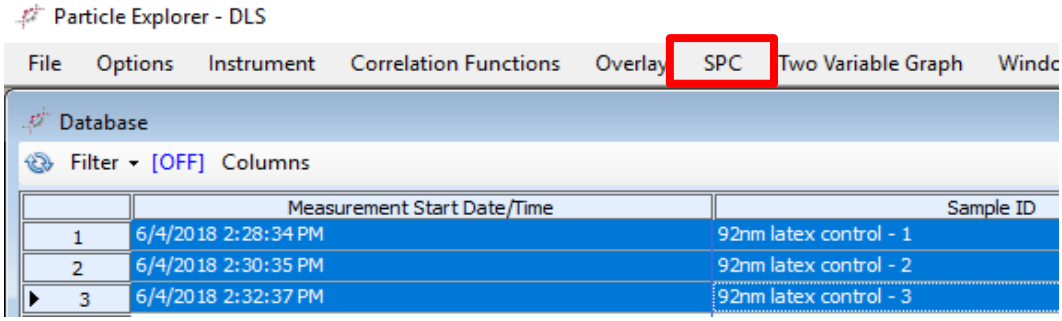
c. There are additional graph options under "ISDA" (Integrated Size Distribution Analysis). For accurate ISDA transformations by volume, surface area, or number, the refractive index of the particle MUST be entered under "Params". Click "Export" to export any graph or data to multiple formats.

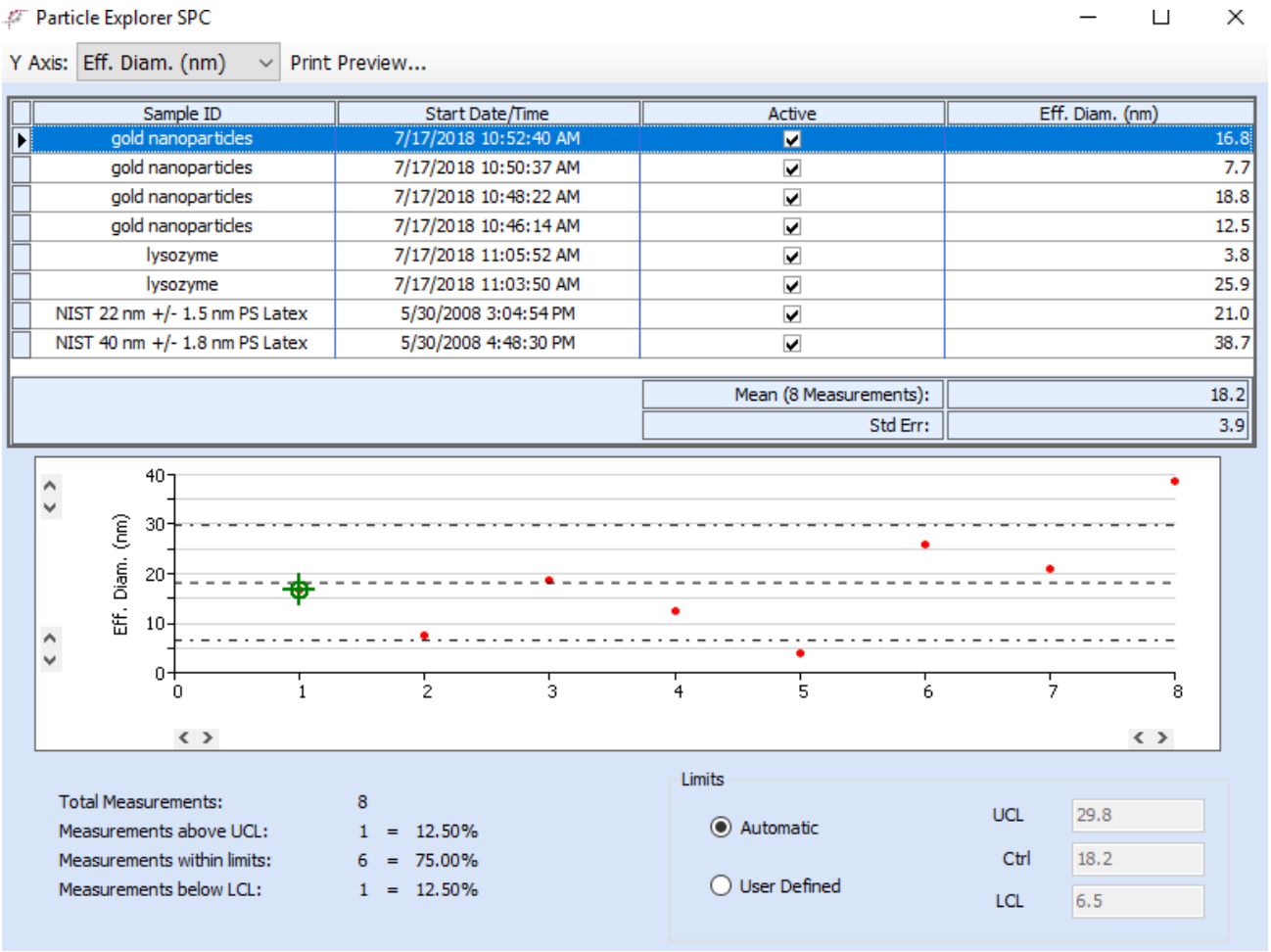
d. The data review window has two graph options to allow for side-by-side graph comparisons. Double-click the same measurement from the database to open additional graph types for review.

e. To overlay graphs, open all desired measurements from the database by double-clicking. Then, click "Overlay" from the top ribbon. This will display an overlay graph of the correlation functions and provide options for other graph overlays.



f. To perform Statistical Process Control (SPC), highlight measurements from the database by holding down Ctrl or Shift. Then, click "SPC" from the top ribbon. In this window, outliers of multiple measurements based on effective diameter or polydispersity can be determined either by automatically calculated upper and lower control limits or User Defined limits.





g. "The Two Variable Graph" is a built-in graphing tool that allows any two variables to be plotted. The two variables can be values that have been collected during the measurement or parameters that were entered prior to data collection (viscosity, etc.)

h. Use the "Window" tab to help organize the view of measurements. All options will fit all opened windows onto one screen for ease of review.

Appendix A: Sample Preparation

This appendix applies to all the software modules: IST, SLS, ZP and DLS.

The two biggest problems in light scattering are flare light, also called stray light, and "dust". If the instrument is properly aligned, cells are not scratched, and room light is block by use of the goniometers narrow band optical filter set to the wavelength of the laser, flare light is usually not a problem except, perhaps, at the highest and lowest angles. Use the goniometers eyepiece to check for flare light in the center of the cell.

Flare Light

Flare light can occur at the entrance and exit of the cell. The minimize this at the outer cell walls, an index matching liquid is used. At the inner walls, the amount of flare light depends on the refractive index of the sample liquid. For toluene and many other nonpolar liquids, their refractive index is close to that of the cell's, and flare light is a minimum. For water and other polar liquids, this is not true. Thus, if possible, use the largest diameter cell with such polar liquids to move the flare light further from the cell center. In any case, the angular range will be restricted by the distortion in the results caused by residual flare light.

Dust & Filtering

"Dust" is always a problem. Dust is a generic designation meaning particles much larger than those of interest and that cross the beam relatively infrequently. Dust can be removed by using clean cells, dilution vials, and transfer devices such as pipettes. Minimize contact between the liquid and surfaces such as pouring when possible instead of using a pipette or syringe. Filtering liquids helps to minimize dust. Be careful not to use a filter if the particles of interest will be affected. Thus, while filtering three times a 10 mM electrolyte such as KNO_3 will minimize dust in the liquid, and remove dust from the dilution vial walls, don't try it with 92 nm particle suspensions. They will block a 0.2 μ filter. Whereas, a 20 nm Anatop filter is good for globular protein solutions and other sub 10 nm particles, they can't be used with most other particles. Micelles can and should be filtered. While they break up going through the filter, they reassemble immediately afterwards, removing dust along the way. Liposomes (vesicles, exosomes), on the other hand, break up and remain so, or, worse, aggregate after filtration.

For nonpolar polymer solutions, a nylon or Teflon filter with a glass syringe works well. If any back pressure is felt, however, chances are the concentration is reduced by what is left on the filter.

The dust signature is easily monitored: spikes in the count rate history versus time. If there are relatively few, then the algorithms used in each of the software modules minimizes the effect of the residual dust on the end results. NOTE: It is better to remove dust through filtration and using clean surfaces rather than relying on algorithms to do so.

Light Scattering: A "Weak" Phenomena

Typically, light scattering is done with clear to ever so slightly turbid suspensions and solutions. Scattering is a weak phenomenon that is observed because the detectors are single photon counters. For example, a 40 mW, 640 nm wavelength laser has an output of 1.3×10^{19} photon/second. Whereas, strong scattering is a few million photons/second. While the laser power may be a factor of 10 lower, and further reduced by neutral density and optical filters, it remains huge compared to scattered light. And yet, in a dark room, the scattered light can be seen by eye going through a weakly scattering sample. This is because the human eye is a very

sensitive light detector too. These figures also explain why the tiniest amount of flare light (a tenth, of a tenth, of a tenth percent, for example) coming from the laser can still significantly affect the results.

Sample Concentration

For nonpolar polymer solutions, use typically five to seven concentrations ranging from 1 to 10 mg/mL for molecular weights from 10 kDa to 250 kDa; 0.5 to 5 mg/mL for molecular weights from 250 kDa and higher; and from proteins that may aggregate, use 0.3 to 3 mg/mL with higher laser power and a larger aperture to collect enough counts.

For polyelectrolytes, use the same concentration range as above, but add about 10 mg/mL of a salt like KNO_3. Without salt, the charges on the backbone of the polyelectrolyte cause the molecule to extend, resulting in a large radius of gyration (R_g) , and a, sometimes, difficult Berry Plot to interpret. The salt screens this effect. While the resulting R_g is smaller, the molecular weight is the same and much easier to find by linear extrapolation.

For colloids and nanoparticle and suspensions, the samples should look clear to ever so slightly turbid. If the concentration is too high, multiple scattering or particle-particle interactions occur and the end results are distorted. If in doubt, repeat at 20-30% less concentrated. If the results are the same, the first concentration was not too high. If the results change, dilute and check again.

For electrostatically stabilized suspensions, typically in water, where possible measure in about 10 mM of a 1:1 electrolyte such as KNO_3. This screens particle-particle interactions, resulting in free diffusion and better DLS particle sizing. Too much salt, however, can collapse the screening effect of charge around the particle (the Debye Double Layer) causing aggregation. Normally, this doesn't occur except at ionic strengths above about 150 mM.

As one example, often used to check DLS measurements, consider a stock suspension of 1%, 92 nm polystyrene in an aqueous solution of surfactants that prevent aggregation even when diluted. Since the density of PS is 1.05 g/cm^3, the 1% can be considered either 1% v/v or 1% wt/v (that is 10 mg/mL). A slightly turbid suspension can be made using 25 mL of 10 mM KNO_3 (three times filtered before adding the PS) and 1 drop of the 1% suspension. Now 1 drop is approximately 30 μL and there are about 30 drops/mL. So, the drop of concentrate is diluted by 1/(30 x 25) = 1/750, resulting in a concentration of 1.33×10^{-5} g/mL of latex for this slightly turbid suspension.

DLS optics requires a small scattering volume, and while it depends on the scattering angle and pinhole, a typical volume is on the order of 10 to 20 nL. Given the density and size of the latex, this amounts to 200×10^3 to 400×10^3 particles in the scattering volume. DLS theory requires that a minimum of 30 particles/scattering volume; yet, practically it is a minimum of 300 to 3,000. Anything smaller and the signal is too weak. But for this slightly turbid latex suspension, there are more than enough. If fact, it could have been diluted by much more. And yet, at the concentration made, results are good at this size with the added salt.

While DLS and light scattering in general are not sensitive to single particles, they are done in dilute suspensions and solutions.

Appendix B: Theory and Data Analysis for IST Alignment Software

The following sections apply to IST, SLS and ZP software modules.

Dark Count Rate

Click on the **Experimental Parameters** command button. Block the light to the PMT by turning the neutral density filter wheel to the closed, C, position on the BI-200SM. Enter 10 seconds in the **Duration/Repeat** field. Click on the **Dark Count Rate** command button. The dark count rate is the total photons counted divided by the duration in seconds. The units are counts/second, abbreviated cps. Click the **Close** command button and notice that the measured dark count rate is automatically transferred to the appropriate field. Since dark count rates are normally small, the fluctuations are large. Therefore, counting for ten seconds yields smaller fluctuations, and it is easier to estimate the true dark count rate. Return the duration to one second.

Photon Statistics

Select an appropriate Duration/Repeat. Select values in increments of 0.1 seconds up to a maximum of 10 seconds. One second is usually best. Photon counting follows Poisson statistics: the standard deviation of the distribution of repeated measurements equals the square root of the average. Here the measurements refer to the Total Measured Counts or, in the case of a reference detector, the Total Reference Counts. The intensity is the Total Counts divided by the duration (units of counts/second, cps, or kilocounts/second, kcps, or megacounts/second, Mcps). To repeat: total measured counts follow Poisson statistics; count rates--counts/second--do not.

As a result of the square root relationship, the relative error (std. dev. divided by the mean) is: 1% for 10,000 total counts; 0.32% for 100,000 total counts; and 0.1% for 1,000,000 total counts. Aim for 100,000 total counts. If this can be done in one second by increasing the incident laser power or selecting a larger pinhole (1, 2, or 3 mm for IST, SLS or ZP only, not DLS), do it. If not, increase the duration/repeat. This will result in an increase in the total experiment duration; it will also increase the probability that a measurement will be ruined by dust. With weak scattering and low laser power, it may be better to accept 50,000 or 20,000 or even 10,000 total counts rather than increase the duration/repeat. Experience will help you judge the best alternative.

Varying the Recorded Counts

The weakest scattering will come from a pure liquid without particles or polymers and at 90°. The strongest scattering will occur at the extreme angles. If you have not done so previously, move the goniometer to, and Set Detector Angle at, 90°. Click the Intensity command button in the main screen or from within the Experimental Parameters window. The Total Measured Counts are shown in the upper part of the main screen. Adjust the laser power, the pinhole, or the duration/repeat until a suitable total measured count is achieved as described in the previous paragraph. At all other angles, the Total Measured Counts will be higher and the corresponding relative error lower.

Dust Rejection

From within the Experimental Parameters window, select a value from 1 to 10 for the Number of Repeats. This value is used to discriminate against dust as explained below. This value may be adjusted up or down, depending on the cleanliness of the sample, the duration/repeat, and the intensity of scattered light. If the scattering liquid has been well filtered, the laser power is low (< 20 mW), and the pinhole is maximum (3 mm), select 3 to 5 repeats and 1 to 5 seconds duration to achieve 10,000 to 50,000 total measured counts. If

the laser power is high (> 50 mW), and the pinhole is set at 1 or 2 mm, select 5 to 10 repeats and 1 second duration to achieve 100,000 total measured counts.

With a well-filtered, organic liquid such as the vat liquid (for example, cis-trans decahydronaphthalene, also known as decalin) used as the scattering media for alignment purposes, enter 1.33 for the Dust Rejection Ratio. Values from 1 to 10 are allowed: small numbers are best for clean liquids; somewhat larger numbers (up to 2 or 3) may be used if dust is difficult to remove. NOTE: Using larger numbers may result in less than optimum alignment. But may be necessary if dust cannot be removed.

How the Count Rate is Corrected for Dust, Averaging, Dark Count, Dead Time, Reflection and Volume

At each angle, the following sequence will occur.

1. In the **Counts** box the total measured counts, raw, uncorrected counts, will appear and repeat measurements will follow up to the limit set by the Number of Repeats, 5 for example.

2. Dust discrimination is achieved using a software algorithm and is based on Poisson and Gaussian statistics as follows. The average and standard deviation of the set of N values are calculated as well as the square root of the average. The standard deviation is based on Gaussian statistics. Whereas, the square root of the average yields a standard deviation based on Poisson statistics. A dust event will increase the total measured counts during the duration. Assuming most measurements are not affected by dust, this increase only modestly affects the average and the Poisson standard deviation calculated from the average. But it increases significantly the Gaussian standard deviation.

3. The ratio of the Gaussian and Poisson standard deviations is formed. If this number is greater than or equal to the Dust Rejection Ratio, the largest of the total measured counts in the set of N is rejected and another one is collected. The calculation is repeated with this new set of values. The rejection criterion is applied again, and, if necessary, the highest value is again rejected. The procedure is repeated until either a set of N values is found that satisfy the criterion, or a total of 3N measurements have been made. If, after 3N measurements have been made, the remaining set of N values do not satisfy the criterion, the highest value is deleted, and the remaining N-1 are tested against the dust rejection criterion. This procedure is repeated until the criterion is satisfied or the last, representing the lowest, total measured count remains.

   This average is displayed in the upper right-hand corner of the main screen under the heading Total Measured Counts.

   [NOTE: *With clean liquids*, N values can normally be found to satisfy the criterion. At low angles, where dust scatters most, rejections will occur. If most of the measurements reach the limit of 3N measurements, consider aborting the run and cleaning the sample.

   This software criterion for rejecting residual dust works well when only a small amount of dust particles remains. It is no substitute for the mechanical removal of dust by filtration (most commonly) or, *under special circumstances, centrifugation.]*

4. The average is divided by the duration to form the average, uncorrected count rate (an intensity).

5. From the average, uncorrected intensity the dark count rate is subtracted. This is the measured, average intensity corrected for dark count rate.

6. The measured, average intensity corrected for dark count rate, I_m , is further corrected for the dead time, t_d , to produce the true count rate as follows: $I_t = I_m/(1-t_d.I_m)$. For the BI-9000AT correlator and photon counter, t_d is 25 ns.
   The true count rate is always larger than the measured, since a finite dead time means that no more than one pulse can be registered in a fixed interval. For dark count rates, and for low count rates in general, this correction is insignificant. At 10,000 cps the result is only 3 cps, an insignificant 0.03% correction. At 100,000 cps the result is 250 cps, a small 0.25% correction. At 1,000,000 cps the result is 25,640 cps, a significant 2.6% correction.

7. The true count rate is finally corrected for reflection where

$$I(\theta) = \left[I_t(\theta) - f \cdot I_t(180-\theta)\right]/\left(1-f^2\right)$$

   Here f is the combined reflection correction calculated from refractive index of sample cell, vat, and sample liquids using Fresnel's Law. It is generally a small correction. If the supplement of the scattering angle is not present in the list of angles measured, the nearest angle measured to the supplement is used. The error introduced by this is a second order correction to a small, first order reflection correction, and is, therefore, insignificant.

   This average, corrected, true count rate value is transferred to the column labeled I′ in the floating window in the middle of the main screen. It represents the intensity scattered by all the particles or molecules in the scattering volume as observed by the detector optics.

8. To form the intensity scattered per unit volume, one accounts for the different volumes observed at different scattering angles. Focusing of a TEM_{001} laser beam produces a hyperbola of revolution; however, with a long focal length lens, such as that used in the BI-200SM goniometer, the shape is cylindrical, at least over a millimeter or two around the center of rotation. Therefore, at any angle the length of the cylinder is larger by $1/\sin(\theta)$. Thus, to compare the intensity per unit volume at various angles, the average, corrected value is *multiplied* by $\sin(\theta)$. The last column in the floating window shows a list of $I'\sin(\theta)$ as a function of θ.

   The effect is the same as looking at a railroad track through a hollow cylinder. Starting with the axis of the cylinder perpendicular to the track, rotate around a fixed point on the track. The length of the track seen varies as $1/\sin(\theta)$. In fact, this effect has given its name to the procedure in Brookhaven's alignment software: $I\cdot\sin(\theta)$ or IST.

<u>IST Software</u>

Theory shows that for particles small compared to the wavelength of light, known as Rayleigh scattering, the scattered light is the same at all angles. Now small means 25 nm in diameter or less. The vat liquid molecules are much smaller than that and are therefore Rayleigh particles. This provides a method for testing whether the alignment is good. By plotting $I'\cdot\sin(\theta)$ vs θ, one can infer if the alignment is good or not. A good alignment has a flat, horizontal plot, with no deviation greater than $\pm$ 1%. The errors should be random. Common deviations are shown in the sample database for IST and include:

- Dish and inverted bowl shapes: Beam is not horizontal. Use vertical adjustment on focusing lens to correct,

- A single angle too high: Swab outside of vat with methanol if dirt or grease. If from a scratch on vat, program angular range to avoid that angle for a couple of degrees on either side, and

- S- and reversed S-shaped curves (measurement and true angle differ). A correction, called the cotangent correction after the S-shapes predicted, can be made. It is shown on the graph. Follow instructions for making the correction.

Appendix C: Theory and Data Analysis SLS Static Light Scattering Software

What is Probed with SLS and The Range (nm)?

Static light scattering yields information on structure: size and shape of molecules, colloids and nanoparticles; fractal dimensions of irregularly shaped particles; structure in liquids, especially complex fluids. However, there are usually limitations and readers are advised to consult the literature. The simple fits shown here, by themselves, may be misleading, and the user is forewarned.

In SLS the independent variable is q, the magnitude of the scattering wave vector. It is given by:

$$q = \frac{4\pi n_l}{\lambda_o} \sin(\theta / 2)$$

where n_l is the refractive index of the liquid, the continuous media, and λ_o is the wavelength of the light source in vacuo.

Typical laser wavelengths used in the visible include 488.0, 514.5, 532.0, 632.8, and diode laser values like 640 nm. Typical refractive indices vary from a low value of 1.33 (water and methanol) up to 1.6 (some halogenated solvents). The typical angular variation is from approximately 15^o to 155^o. The variation in q is from approximately 0.00345 nm^{-1} to 0.0258 nm^{-1} using a HeNe laser (632.8 nm) in water, a little less than a decade of variation. [Note: q is a function of wavelength and angle. Although one could vary the wavelength, and this has been done with a Krypton laser, it is the variation in angle that typically leads to the variation in q.]

When probing structure, the length scale of the probe is q^{-1}. Using the above example, the scale ranges from ~39 nm to ~290 nm. While one can probe systems whose length scale is smaller than ~39 nm using SLS, it becomes increasingly difficult to do so with any sensitivity. [The length scale is here defined as the longest dimension. For a sphere this is the diameter. The radius is half, about 20 nm for the above case. The Rg is smaller again, about 15 nm in this case.] Many interesting phenomena are below this range and other probes (small angle x-ray and neutron scattering) yield either complementary or better information at smaller sizes. Keep this in mind when interpreting data. [Note: Using DLS, dynamic light scattering, one can probe systems with sizes considerably smaller than 39 nm.]

Types of Plots Available with SLS

These include the following plots:

- $Ln(I_{ex})$ vs q
- Partial Zimm (angular variation only)

- Partial Berry (angular variation only)
- Guinier
- Kratky
- Fractal

Ln(I_{ex}) vs q

The first plot is the most general, In(I_{ex}) vs. q, where the expected variation in excess intensity is large, and a log plot is chosen as a convenience in scaling. The excess here means the scattering from the solvent has been subtracted. So, in all the measurements, the angular variation of the solvent is measured and that of the sample. With structure on the order of the wavelength, large variations in intensity are expected and a Mie scattering pattern, with minima and maxima, is expected. Fitting this type of data is beyond the scope of this software package, yet data can be collected and exported for use with user-supplied fitting routines.

NOTE: In this section on the interpretation of SLS plots, the excess intensity of scattered light, I_{ex}, has already been corrected for the change in the number of particles seen as a function of angle. That is, it has already been multiplied by sin(θ) to account for differences in scattering volume as a function of angle. This is also true for solvent scattering. Thus, in all equations and in all plots, I_{ex} is equal to $I' \cdot \sin(\theta)$, that is $I'_{ex} \cdot \sin(\theta)$. Where the prime indicates corrections for dust, averaging, dark counts and dead times have also been made.

In advanced texts on this subject it is the z-average particle scattering factor $P_z(q)$ that contains the variation of intensity with q after correction for sin(θ). $P_z(q)$ is also called the form factor or the intraparticle structure factor. It contains information on the structure or form of the particles. At higher concentrations, when interparticle interactions are important, there is another term called the static structure factor or the interparticle structure factor, S(q), which contains information on the distribution of the particles in the liquid. [There is also a dynamic structure factor, accessible using dynamic light scattering.] The intensity I(q) is, for randomly oriented, monodisperse particles, a product of Pz(q) and S(q). For the dilute systems considered here, S(q) is constant. Again, for convenience, we refer here just to intensities. It is up to the user to account for the more complex cases.

Partial Zimm Plot

The partial Zimm plot stems from the following approximate equation:

$$1 / I_{ex}(q) = C\left(1 + R_g^2 \cdot q^2 / 3\right)$$

Here R_g is determined from the slope and the intercept of a plot of $1/I_{ex}(q)$ vs. q^2. Zimm and partial Zimm plots are appropriate when the third virial coefficient is negligible. Virial coefficients describe the interaction between particles and are beyond the scope of this discussion. However, when the polymer concentration is too high or when the interactions are strong (polyelectrolytes at low salt concentration, for example), an approximation suggested by Berry (J.Chem.Phys., 44, 4550 (1966)) may be used.

Partial Berry Plot

The partial Berry plot follows from the following approximate equation:

$$\left(1 / I_{ex}(q)\right)^{1/2} = C\left(1 + R_g^2 \cdot q^2 / 6\right)$$

Here R_g is determined from the slope and the intercept of a plot of $(1/I_{ex}(q))^{1/2}$ vs. q^2. Note the 1/6 instead of 1/3. This arises naturally when taking the square root of the term in parenthesis in the full Zimm plot, assuming $(R_g \cdot q)^2/3$ is small compared to 1.

Guinier Plot

Guinier (see Guinier & Fournet, Small Angle Scattering of X-rays, Wiley Interscience, 1955) proposed the following simple variation for large and globular structures:

$$I_{ex}(q) = C \cdot \exp(-R_g^2 q^2 / 3)$$

Plotting $\ln(I_{ex})^{-1}$ vs. q2 yields a straight line when a Guinier plot is appropriate. From the slope R_g, the radius of gyration, can be determined. R_g is given by:

$$R_g^2 = \sum_i m_i r_i^2 \div \sum_i m_i$$

Here m_i is the i^{th} mass element of the scattering molecule or particle and r_i is the distance from the center of gravity to the i^{th} element. The interesting thing about this definition is that it is independent of the shape of the scattering particle, although to interpret the measured R_g further requires a model. For spherical particles $R_g^2 = (3/5)\cdot R^2$ where R is the radius of the sphere and for long, thin rods $R_g^2 = L^2/12$ where L is the length of the rod. Long means a length at least five times greater than rod diameter. For other shapes, consult specialized texts (Burchard , Static and Dynamic Light Scattering from Branched Polymers and Biopolymers, in Advances in Polymer Science, Vol. 48, Light Scattering From Polymers, table 3, pages 74-75, Springer-Verlag, New York, 1983).

Please note that R_g is also and more properly called the root-mean-square radius. Unfortunately, the majority of texts and publications use the term radius of gyration or root-mean radius of gyration, and it is difficult to break the habit. [Witness: molecular weight vs. relative molar mass.] In addition, for a polydisperse system, $R_g = \langle Rg^2 \rangle_z^{1/2}$, the z-average, root-mean-square radius. Z-averaging is a result of intensity weighting and has some interesting consequences which will not be explored further. Contrast this with the weight-averaging of the molecular weight also obtained from light scattering.

Kratky Plots

For branched polymers, information on the extent of branching may be obtained from the shape of the Kratky plot, where $q^2 I_{ex}^2(q)$ vs. q is plotted. Highly branched polymers display a distinct maximum and unbranched polymers do not. However, polydispersity also affects the shape of the plots, and the user should be careful in interpreting the shape of the plot without additional information. For more information consult Burchard, Macromolecules, 10, 919 (1977).

Fractal Plots

Some interesting colloidal particles result from the aggregation of smaller subunits. The structures obtained display a variety of irregular shapes, some more open than others, including branching. The openness of the aggregates is described by a non-integer number called the fractal dimension, d_f: the larger the fractal dimension, the more compact the structure and the lower the fractal number, the more open the structure.

The determination of the fractal dimension is appropriate when the size of the subunit, R_o (radius of an assumed spherical monomer), and the radius of gyration of the aggregate, R_g , satisfy the following criteria:

$$R_0 < q^{-1} < R_g$$

Fractal dimensions also indicate how the aggregate was formed: limiting, ideal cases are diffusion-limited, cluster-cluster aggregation (DLCCA or DLCA) and reaction-limited, cluster-cluster aggregation (RLCCA or RLCA). See Carpineti, Ferri and Giglio, Physical Review A, 42, 7347, (1990) for more information.

When a determination of the fractal dimension is appropriate, the following equation is applied:

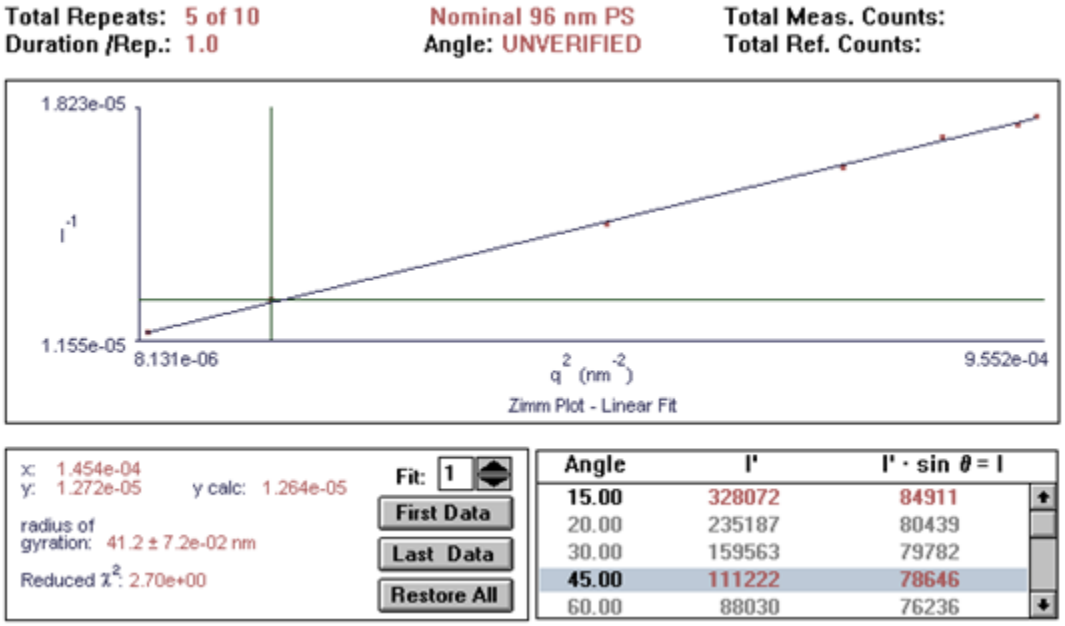
$$I_{ex}(q) = C \cdot q^{-d_f}$$

The fractal dimension is obtained as the slope of $\ln(I_{ex}(q))$ vs. $\ln(q)$. Ln-Ln plots are notorious for yielding seemingly straight lines. However, the reader is warned that such plots do not always yield straight lines over a sufficiently large ln(q) space for the resulting slope to be usefully interpreted as the fractal dimension.

General Fitting Considerations for All Plots

For those plots for which either R_g or d_f may be determined (Guinier, partial Zimm and Berry, Fractal), the software automatically fits a polynomial to all the points chosen for fitting. This may be all the data taken or a subset. Individual points may be eliminated by clicking on them in a displayed list or by selecting all the points above or below the current cursor position in the plot. An example is shown below for a partial Zimm plot of a nominal 98 nm (diameter) spherical latex.

The best fit of the low angle data yield 41.2 ± 0.1 nm; whereas, simple theory predicts 37 nm ($R_g = (3/5)^{1/2} \cdot R$), where R is 96/2 = 48 nm. The reduced $\chi 2$ is 2.7, indicating the fit is reasonable after deleting a few of the data points (greyed out entries in table) that lay obviously too far from the linear fit.



Perhaps the remaining difference is due to an error in the assumed particle diameter (TEM gives 96 nm, DLS gives 98 nm); perhaps, due to a relatively small number of doublets, it is due to the z-averaging; perhaps it is due to residual errors in the remaining data points; perhaps it is due to multiple scattering and the measurements should be done again at a lower particle concentration.

When fitting you can choose the order of the fit: 1 for a linear fit, 2 quadratic, 3 cubic, 4 quartic, and 5 quintic. While fits always improve, in an RMS sense, with more parameters, they do not necessarily yield better parameters in the physical sense. We strongly recommend using just the linear and quadratic fits. Why, you ask, did we include the others? Because someone out there in light scattering-land will ask for it. Use the higher order fits with great caution.

Each fit includes either R_g or d_f and +/- σ, where the error here is the standard deviation. In addition, either the root-mean-square error is given or the reduced $\chi 2$ (chi-squared). Both are measures of goodness-of-fit. The RMS is produced when you choose not to use the statistical weighting. This is accomplished by not clicking on the Statistical Weight box in Graph Plot Type window. The equivalent is too assume equal weighting in which case it is impossible to calculate the reduced $\chi 2$. Instead, the RMS error, a measure of the error of the overall fit, is used to determine the variances and covariances in the fitted parameters.

The advantage of using weighting is that reduced $\chi 2$ not only is a relative measure of goodness-of-fit (lower values mean better fits as with RMS values), but that a good fit should result in a reduced $\chi 2$ of 1 to 2. Smaller values are probably due to statistical fluctuations and not better fits, while significantly larger values indicate one of two possibilities. Either the equation chosen to fit the data is not appropriate, or the errors (systematic like flare or dust, and the random errors) are too large.
Whenever a data point is deleted (double click on the entry in the table in the row showing that angle) or undeleted (double click again on the row with the angle), a fit is changed (1, 2, ...5), or a change in the weighting is selected (Statistical Weighting or Equal Weighting), this forces a new least squares fit to the data with changes in the results.

Statistical Weighting

We use a least-squares fitting routine, where, when Statistical Weighting is selected, we calculate the weights as the reciprocal of the variance in each Y-axis value. This variance must be calculated for the actual quantity plotted ($1/I_{ex}$, $1/ I_{ex}^{1/2}$, or $Ln(I_{ex})$, considering that the variance for a counting experiment is equal to the square root of the total counts. In the table below the Y-axis variable, the X-axis variable, and the propagated statistical weight is shown for each case.

Weighted, Linear Least Squares Fitting			
Type	Y-Axis Variable	X-axis variable	Statistical Weights
Guinier	$Ln(I_{ex})$	q^2	$T\cdot(I_s - I_l)^2/(I_s + I_l)$
Fractal	$Ln(I_{ex})$	$Ln(q)$	$T\cdot(I_s - I_l)^2/(I_s + I_l)$
Berry	$1/(I_{ex})^{1/2}$	q^2	$4\cdot T\cdot \sin\theta\cdot(I_s - I_l)^3/(I_s + I_l)$
Zimm	$1/I_{ex}$	q^2	$T\cdot \sin^2\theta\cdot((I_s - I_l)^4/(I_s + I_l)$

In the case of $I_{ex} = I_s - I_l$, where s stands for either solution (dissolved polymer) or suspension (colloid suspended) and subscript l stands for the liquid without particles or polymer, the propagated weight if just Iex were plotted is $T\cdot \sin^2\theta/(I_s + I_l)$. The different weightings arise from propagating the errors arising from the different functional fits [$Ln(I_{ex})$, $1/(I_{ex})^{1/2}$, and $1/I_{ex}$]. The duration for counting is T and it is 0.1, 0.2...9.8, 9.9, or 10.0 seconds, though most commonly 1 second has been selected.

The weighted, least-squares fitting routine, using a straightforward matrix calculation, returns the intercept, the slope, the variances in each, the covariance between the two parameters, and the reduced $\chi 2$, which is also designated $\chi 2$ /(N-p) in some texts on data reduction and error analysis. Here N is the number of fitted data points and p is the number of fitted parameters. For polynomials p = n + 1, where n is the order of the polynomial fit. When the reduced $\chi 2$ is close to 1, the fit is good if and only if the data used in the fit contain only statistically weighted random errors and the equation chosen for the fit is appropriate. Values much greater than 1 indicate a poor fit. Please consult standard statistical textbooks for more information on the use of chi-square.

When we report R_g , we also report the propagated and random error from the fit. In the case of partial Zimm and partial Berry plots, the covariance as well as the variances are used to calculate the error in R_g. However, do not place undo emphasis on statistical weighting. It is less important than obtaining good data and choosing the appropriate fit. There is no substitute for making repeat measurements on fresh samples and comparing results to determine if the statistical error from a single fit, using weights determined by the measured intensities and the propagated errors, is about equal to the error from multiple determinations. If it is, you can place a great deal of confidence in the answers. If it is not, you will have to judge the quality of the data in some other way.

Appendix D: Theory and Data Analysis ZP Zimm Plot Software

Review the sections on how excess intensity is determined and corrected in Appendix B.

The Basic Equation

In the limit of low concentration and scattering angle the data obtained from SLS measurements for a dilute solution homopolymer in a single solvent can be written as:

$$H \cdot c/\Delta R(\theta,c) = 1/[M_w P_z(q)] + 2A_2 c \quad . \qquad (1)$$

The optical constant H is given by:

$$H = 4\pi^2 n^2_o (dn/dc)^2/(N_o \lambda_o^4) \quad ,$$

where

n_o	is the refractive index of the solvent. Values from 1.30 to 1.60 are typical.
dn/dc	is the specific refractive index increment of the solution. Values from 0.05 to 0.30 cm^3/g are typical. See Appendix E.
c	is the polymer concentration. Values from 0.01 to 10 mg/cm^3 are typical. The lower concentration range is more common when measuring high molecular weight polymers, above 1M Daltons for example, the upper concentration range is more common when measuring low molecular weight polymers, below 100K Daltons, for example.
N_o	is Avogadro's Number, 6.022×10^{23} mol^{-1}.
λ_o	is the wavelength in vacuum of the laser. For a HeNe laser it is 632.8nm, and for an Argon-ion laser it is either 514.5nm (green) or 488.0nm (blue). For frequency doubled, solid state laser it is 532 nm, and for diode lasers in the red it varies from 640 to 680 nm. The classical Hg lines are 436nm and 546nm.
θ	is the scattering angle in degrees.

ΔR	is the excess Rayleigh Factor (also called Rayleigh Ratio) in units of cm^{-1} calculated at different angles and concentrations. Excess means it is the difference between the value for the solution and the value for the solvent. R is proportional to the corrected, scattered light intensity, the proportionality constant is determined by calibration against a substance (toluene, benzene) with a known Rayleigh Ratio.
M_w	is the weight-average molecular weight (molar mass). Values in the range from a few thousand to a few million g/mol (Daltons) are measureable, more commonly 10^4 to 10^6.
$P_z(q)$	is the z-average particle scattering function or particle structure function. (In more concentrated and strongly interacting systems a solution structure function is also important.)
q	$= (4\pi n_o/\lambda_o)\sin(\theta/2)$, the magnitude of the scattering wave vector.
A_2	is the second virial coefficient, values in the range 0 to 10^{-4} $cm^3 \cdot mol/g^2$ are typical. For polydisperse systems, A_2 is an average quantity; however, the average is not a simply defined one like the weight- or z-average.

The factor 4 in the optical constant H is a result of using incident light that is linearly polarized perpendicular to the horizontal scattering plane. This is the most common configuration and is typically referred to as vertical polarization. For unpolarized incident light the factor is 2.

The particle scattering function can be expanded in terms of q^2, and the first three terms are

$$P_z(q) = 1 - (R^2_g \cdot q^2)/3 + O(q^4) \quad , \quad (2)$$

where R_g is shorthand for $\langle R^2_g \rangle^{0.5}_z$, the z-average root-mean-square radius of gyration, a measure of the molecular size. R_g is almost universally called the radius of gyration, even though that is a misnomer. It is a root-mean-square value. In fact, even for a monodisperse polymer system, where the z-averaging equals all other average values, R_g is still an average value. A random coil polymer takes up large numbers of configurations and R_g even for a single molecule is thus an average value.

The coefficient of q^2 is independent of the particle shape. Coefficients of higher order terms do depend on shape. Therefore, the radius of gyration (more properly, the z-average, RMS radius of gyration) is a primary parameter obtained from light scattering: primary in the sense that no model for the shape is assumed.

The Zimm Plot

Combining Equations 1 and 2, and expanding $1/P_z(q)$ to first order in q^2 with a Taylor series, gives the following:

$$H \cdot c/\Delta R(\theta,c) = [1/M_w] \cdot [1 + (R^2_g \cdot q^2)/3] + 2A_2 c \quad . \quad (3)$$

This equation is the basis for the famous Zimm Plot. Scattered light intensities are measured at several angles for each of several solution concentrations and for the pure solvent at each angle. Subtracting the solvent scattering from the solution value yields the excess intensity scattered by the polymer. A calibration constant is used to calculate excess Rayleigh Factors from measured intensities.

A calibration constant is obtained by measuring the intensity of light scattered from a standard. It is assumed that $R_c = k_c \cdot I_c$, where k_c is the calibration constant and R_c is the known Rayleigh Ratio. R_c is known for toluene and benzene at the laser wavelengths. These values are given in the BI-200SM manual. Rayleigh Ratios scale inversely with λ^4. Such a calibration is only good if the flare light at the calibration angle, typically 90°, is insignificant.

If the left-hand side of Equation 3 is plotted against $\sin^2(\theta/2) + k_p \cdot c$, where k_p is an arbitrary plotting constant chosen purely for convenience in viewing the results, a grid-like plot results. Two sets of, typically, parallel lines make up the grid. One set consists of angular measurements at each concentration, and one set consists of concentration measurements at each angle. Extrapolating the angular measurements to zero angle for each concentration yields a straight line in c. The slope of this line yields A_2. Extrapolating the concentration measurements to zero concentration for each angle yields a straight line in $\sin^2(\theta/2)$. The slope divided by the intercept of this line yields Rg.

The intercept of both extrapolated lines––the double extrapolation to zero angle and zero concentration––yields M_w the weight-average molecular weight of the polymer in solution. It is this parameter of a polymer distribution that is most often of interest. And light scattering offers an absolute method for its measurement.

The value of dn/dc, several other parameters in Equation 1, and a calibration constant must be known in advance in order to calculate M_w and A_2 from a Zimm Plot. This is also true for the Debye and Berry Plots described below. Interestingly, however, R_g can be calculated without knowing either dn/dc and without a calibration. Both parameters cancel from the calculation of R_g.

The Debye Plot

Rayleigh scattering particles are defined such that their size is small compared to the wavelength of light. In this case the angular term in Equation 3 is negligible, and it can approximated as:

$$H \cdot c/\Delta R(\theta,c) = 1/M_w + 2A_2c \quad . \quad (4)$$

This equation is the basis for the Debye Plot. At one angle intensities are measured as a function of concentration. The results are plotted according to Equation 4. A_2 is obtained from the slope of this plot; the intercept yields $1/M_w$. Typically, the angle used is 90°, but this is not necessary. This angle is convenient because flare light is often a minimum and because residual dust or microgel contributions are usually insignificant at such a high angle.

Whenever the molecular weight is small, roughly 100K Daltons and lower, consider collecting data for a Debye Plot. The measurement time is shorter; the interpretation of possible downward curvature at low angles is avoided. Downward curvature may be due to dust, microgels, colloids, or flare.

The ZP program allows one to make Debye Plots even from data collected at several angles. Click **Graphs** and choose the Debye plot. Then select the angle at which you want to plot the data. The converse is not possible: If you collect data at only one angle, you cannot make either Zimm or Berry Plots.

The Berry Plot

The term linear in c in Equation 1 is the first in a polynomial expansion in c. These terms come from the expansion of the osmotic pressure in c. At higher concentrations or for strongly interacting particles (polyelectrolytes not screened by free salt ions) they may be important. They are also important, for different reasons, for branched polymer systems. Whatever the reason, the Zimm Plot shows upward curvature, and it is no longer a simple set of straight lines.

The curved data may be fit to higher order terms in c and $\sin^2(\theta/2)$. Brookhaven software provides for fits up to 5th order in either or both variables. With few data points it is always possible to fit the measured data quite well with a high-order polynomial. Nevertheless, the parameters calculated with high-order fits are often not meaningful. Whenever possible use a linear fit or try to linearize the data. The Berry Plot was developed for this purpose. It is applicable to polyelectrolytes if the salt concentration has not sufficiently screened interactions.

The right-hand side of Equation 3 can be written as:

$$[1/M_w]\cdot[1+(R^2_g\cdot q^2)/3]\cdot[1+2A_2M_wc+3A_3M_wc^2+ ...] \quad . \qquad (5)$$

For hard spheres $A_3M_w = (5/8)\cdot(A_2M_w)^2$, while for flexible coils in a theta solvent the term is zero. In general, it can be written as $g(A_2M_w)^2$. When the 3rd order term is small but not negligible, an approximation for g might be 1/3, the approximate average between 0 and 5/8 (.625). With g = 1/3,

$$[1 + 2A_2M_wc + (A_3M_w)^2c^2 + ...] \cong [1 + A_2M_wc]^2 \quad ,$$

and Equation 5 becomes,

$$H\cdot c/\Delta R(\theta,c) \cong [1/M_w]\cdot[1 + (R^2_g\cdot q^2)/3]\cdot[1 + A_2M_wc]^2 \quad . \qquad (6)$$

This equation can be made to yield 2 sets of lines by taking the square root. The result, linear in q^2 and c, is

$$H\cdot c/\Delta R(\theta,c) = [1/M_w]^{0.5}\cdot[1+(R^2_g\cdot q^2)/6]\cdot[1+A_2M_wc] \quad . \qquad (7)$$

This equation is the basis of the Berry Plot. The square root of $H\cdot c/\Delta R$ is plotted against $\sin^2(\theta/2) + k_pc$.

In the polymer and light scattering literature, one often finds an alternate designation for the second virial coefficient, Γ, when referring to viscosity measurements. The value of Γ typically varies from about 10 to 1,000 cm^3/g, and it is a measure of molecular volume. The second virial coefficient A_2 is related to Γ as follows:

$$A_2 = \Gamma/M_w \qquad (8)$$

Corrections for Depolarization

For optically isotropic molecules, the polarization of the scattered light is parallel to the polarization of the incident light. The most common configuration with a laser source is incident light that is linearly polarized perpendicular to the scattering plane. This is commonly called vertical polarization. For many, large, flexible-chain polymers depolarization effects are negligible, and, as a result, no polarization corrections are necessary. M_w, R_g, and A_2 are correct as calculated from the Equations 3, 4, and 7. [Many solvent molecules are anisotropic and depolarize the scattered light significantly; however, these effects are negated upon

subtracting the solvent scattering from that of the solution.]

For optically anisotropic molecules, the polarization of the scattered light has components both parallel and perpendicular to the incident polarization. For short, flexible-chain polymers and chains in or near the rigid rod limit, depolarization effects are not negligible, and, as a result, polarization corrections are necessary. Under these circumstances M_w, R_g, and A_2, as used in the equations above, should be considered *apparent*, not *true* values.

To make the corrections, two limiting cases of the worm-like chain model must be distinguished: flexible chain and rigid rod. In addition, each of these two cases can be measured in either of two common experimental setups: measurements with linearly polarized incident light perpendicular to the scattering plane and no analyzer R_{uv}; or, measurements with an analyzer set to pass only scattered light with polarization parallel to the incident polarization R_{vv}.

For R_{uv} measurements on flexible chain molecules:

$$M_{w,app} = M_w \cdot [1 + (7/5)\delta^2] = M_w \cdot [(3 + 3\rho_v)/(3 - 4\rho_v)] \ , \qquad (9)$$

$$A_{2,app} = A_2 \div [1 + (7/5)\delta^2] = A_2 \div [(3 + 3\rho_v)/(3 - 4\rho_v)] \ , \qquad (10)$$

$$R_{g,app} = R_g \div [1 + (7/5)\delta^2]^{0.5} = R_g \div [(3 + 3\rho_v)/(3 - 4\rho_v)]^{0.5} \ . \qquad (11)$$

For R_{uv} measurements on rigid rod molecules:

$$R_{g,app} = R_g \cdot \{[1 - (4/5)\delta + (47/35)\delta^2]/[1 + (7/5)\delta^2]\}^{0.5} \ . \qquad (12)$$

For R_{vv} measurements on flexible chain molecules:

$$M_{w,app} = M_w \cdot [1 + (4/5)\delta^2] = M_w \cdot [3/(3 - 4\rho_v)] \ , \qquad (13)$$

$$A_{2,app} = A_2 \div [1 + (4/5)\delta^2] = A_2 \div [3/(3 - 4\rho_v)] \ , \qquad (14)$$

$$R_{g,app} = R_g \div [1 + (4/5)\delta^2]^{0.5} = R_g \div [3/(3 - 4\rho_v)]^{0.5} \ . \qquad (15)$$

For R_{vv} measurements on rigid rod molecules:

$$R_{g,app} = R_g \cdot \{[1 - (4/5)\delta + (4/7)\delta^2]/[1 + (4/5)\delta^2]\}^{0.5} \ . \qquad (16)$$

In these equations δ is the molecular anisotropy and ρ_v is the depolarization ratio. The depolarization ratio is an experimentally measured parameter. It is operationally defined as:

$$\rho_v = R_{hv}(90°) \div R_{vv}(90°) \ , \qquad (17)$$

where $R_{hv}(90°)$ is the excess scattered light intensity measured at $\theta = 90°$ with polarization perpendicular to the incident polarization, and $R_{vv}(90°)$ is the excess scattered light intensity measured at $\theta = 90°$ with polarization parallel to the incident polarization. The excess is defined by the difference between the value measured with the solution and the value measured with the solvent. Since many common solvents can

depolarize the light significantly, it is extremely important to use the excess values.

The molecular anisotropy can be written in terms of the experimentally determined depolarization ratio as:

$$\delta^2 = 5\rho_v \div (3 - 4\rho_v) \qquad (18)$$

The ZP software includes a routine for measuring ρ_v provided the optional BI-PA polarization analyzer is available. Alternatively, literature values of ρ_v can be input, and its effect on recalculated values M_w, R_g, and A_2 determined.

For most polymer samples the depolarization corrections are negligible. This is so because the normally random orientation of dipole moments from the anisotropic monomeric units in a polymer cancel as the molecular weight increases. In fact, it is often stated that if the measured depolarization ratio is not negligible, the measurement is probably wrong. The older literature values of depolarization ratios are suspect. Be careful in applying these corrections. If in doubt, do not apply them. This is accomplished by setting ρ_v in the program, to zero, the default value. With ρ_v equal to zero, there should be no effect on the calculation of M_w, R_g, or A_2 even if the flexible chain or rigid rod choices are selected.

For rod-like polymer molecules, for short-chain molecules where end-groups may contribute significantly to depolarization, ρ_v may not be negligible, and corrections are necessary.

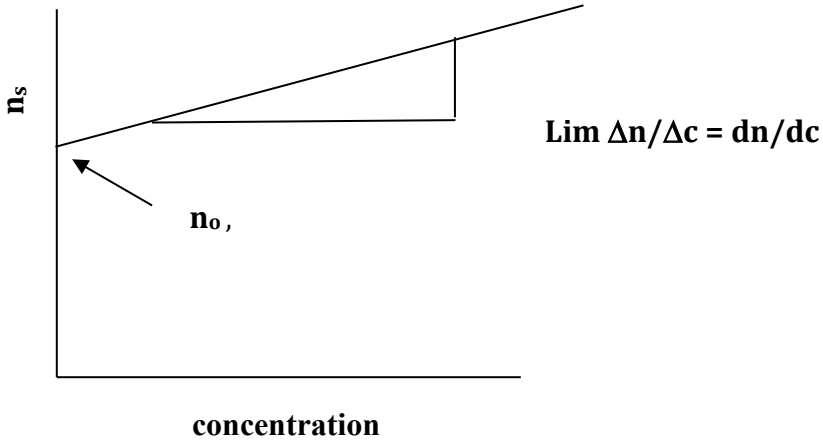
WARNING

Real samples and real measurements almost never fit exactly the simple theory just presented above. As the angle is decreased, the intensity is often anomalously large due to dust, scratched cells, microgels, other supramolecular entities, and flare light at the cell-solution interface. These effects cause a downward curvature in the Zimm and Berry Plots. The user is advised to consider whether this downward curvature is real. If real, it will lead to lower intercepts and greater slopes, resulting in anomalously large M_w and R_g values. Such curvature is easily fit to higher order polynomials that are available in the ZP program. Though the fit will certainly improve as the order of the polynomial is increased, the user must use judgment in accepting these results. Are they repeatable results? If not, then dust, microgels, and other supramolecular entities (colloids, perhaps) are most likely the problem. Look at the number of repeats necessary to accept a data point. If 1 of 30 instead of 10 of 10 measurements are required, dust or its equivalent is most likely the cause. Clean up the sample before proceeding. If the results are repeatable, do they make sense? If not, perhaps flare light is the problem; perhaps the calibration was not done with a clean solvent; perhaps the polymer concentrations are not correct; perhaps the value of dn/dc is wrong.

Appendix E: Differential Refractive Index Increment, dn/dc, Required for Calculation of Molecular Weight in Zimm Plot

Definition of dn/dc

The differential refractive index increment, sometimes referred to as the specific refractive index increment, or SRII, is the slope of n_s vs. concentration at zero concentration. Here n_s is the refractive index of a polymer solution. The SRII is a limiting slope at zero concentration often referred to as just dn/dc. However, in most polymer/solvent samples n_s vs. concentration is a straight line and the limiting slope is the same as the slope at finite concentrations, even up to 10's of mg/mL.

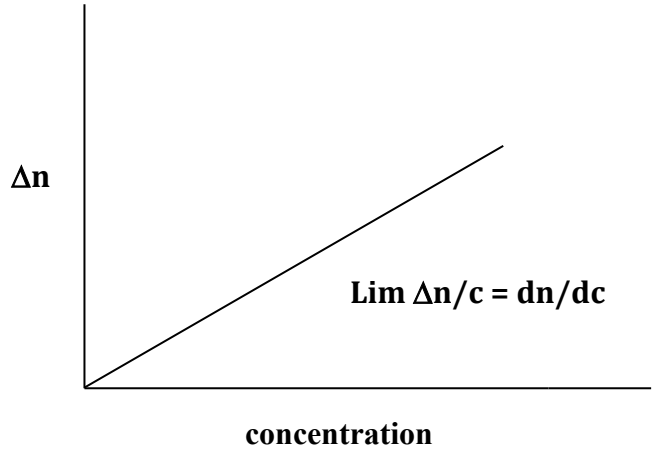


These concepts are illustrated above and serve to define conceptually dn/dc. In practice, the variation in n_s with temperature is larger than its variation with concentration, and the above plot is nearly impossible to obtain with precision unless extreme measures are taken for temperature stability. To avoid this problem, one should use a differential refractometer such as the BI-DNDC.

Differential Refractometer

In a differential refractometer, the solution and solvent are maintained at the same temperature in the same cell separated by a transparent partition. It is not important to know the exact temperature to within a few degrees; it is only important that the temperature difference between the two sides of the cell is small, ≤ 0.01 °C. This is not a particularly stringent condition. It can be accomplished in a well-insulated, small cell by waiting for thermal equilibrium or by controlling the cell temperature to within ± 0.01 °C. The BI-DNDC differential refractometer does maintain the solvent and solution sides of the cell within ± 0.01 °C of each other from about 5 °C above ambient to about 80 °C.

The signal in a differential refractometer is proportional to the displacement of a beam of light as it traverses the solution and solvents sides of the cell. To improve resolution, the beam is reflected, back through the cell to double the displacement. The displacement is proportional to the difference in refractive index of the solution and solvent-sides of the cell. $\Delta n \equiv n_s - n_o$. The basic plot is illustrated below.



The value of dn/dc normally varies from about 0.05 to 0.25 mL/g. Occasionally negative values occur when the refractive index of the solvent is greater than that of the polymer molecule. Since dn/dc is squared in the equations used to determine M_w, a negative dn/dc is physically acceptable.

Establishing Error Limits

When using the ZP module in the PE software, the relative error in calculating M_w is twice the relative error in dn/dc. For example, if you want to know M_w to 5% using static light scattering, a reasonable error limit on M_w, then the error contributed by dn/dc must be less than 2.5%.

Literature Values of dn/dc

Literature values for several common polymers in a variety of solvents are tabulated in:

1. Huglin, M.B., "Specific Refractive Index Increments", Chapter 6 in Light Scattering from Polymer Solutions, M.B. Huglin editor, Academic Press, London & New York, 1972.

2. Huglin, M.B., "Specific Refractive Index Increments of Polymers in Dilute Solution", pp. IV-267 to IV-308 in Polymer Handbook, 2nd ed., J. Brandrup, and E.H. Immergut, editors, Wiley-Interscience, NY, 1975. Essentially a repeat of values tabulated in reference 1.

3. Timasheff, S.N., p. 372-382 in the Handbook of Biochemistry and Molecular Biology, 3rd edition, Vol. II, edited by G.R. Fasman, 1976. Values for proteins in water.

4. Refractive Increment Data-Book for Polymer and Biomolecular Scientists, compiled by A. Theisen, C. Johann, M.P. Deacon, and S.E. Harding, Nottingham University Press, 2000. Many entries are more recent than the classic compilations above.

The classic values (before the widespread use of lasers, say before 1970) are given primarily at 436 nm and 546 nm, the mercury lines. Hg-arc sources were the light sources used in differential refractometers and light scattering instruments before lasers and LEDs became widely available. As shown below (**Wave Length Dependence of dn/dc)**, fitting to $A + B/\lambda^2$ using values known at two wavelengths is reasonable for calculating at laser wavelengths of 488 nm and 514.5 nm (Argon-ion laser lines) or 532 nm (newer,

frequency-doubled, solid state lasers). However, using the fit to predict dn/dc at 632.8 nm (HeNe laser line) or 640 nm ("red", diode lasers) is less acceptable, but may be necessary.

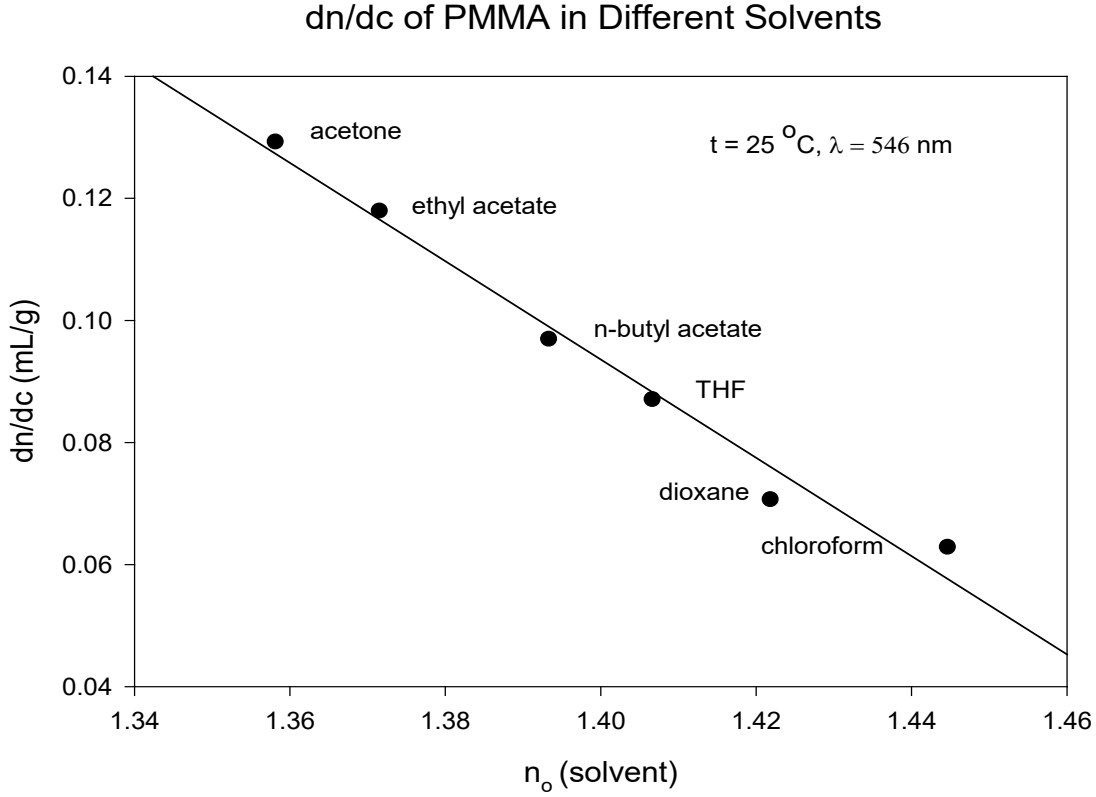
Without a direct measurement, literature values may be all that you have to work with. It is important, however, to understand that dn/dc varies with the solvent's refractive index, impurities in the solvent and polymer, molecular weight of polymer, temperature, and wavelength. Each of these variables is discussed below.

Effect of Solvent's Refractive Index

If a specific polymer can be dissolved in several different solvents, it is best to choose the one with the greatest difference in refractive index. In this case, dn/dc will be a maximum (scattering intensity varies as square of dn/dc), and errors in determining dn/dc will become less important. While the shape of the molecule (affecting R_g) and its interaction with the solvent (affecting A_2) may change from solvent-to-solvent, M_w will not.

The variation of dn/dc with solvent refractive index is roughly linear. Here is an example.

The data was from Huglin's book, reference 1 above. Note the approximate factor of two in the spread of



dn/dc. This leads to a factor of four in the scattered intensity. So, using acetone instead of chloroform will yield greater excess intensity, something easier to measure with greater confidence. Note that the near linearity for this polymer in several solvents suggests that the partial molar volume at infinite dilute is the same in all the solvents.

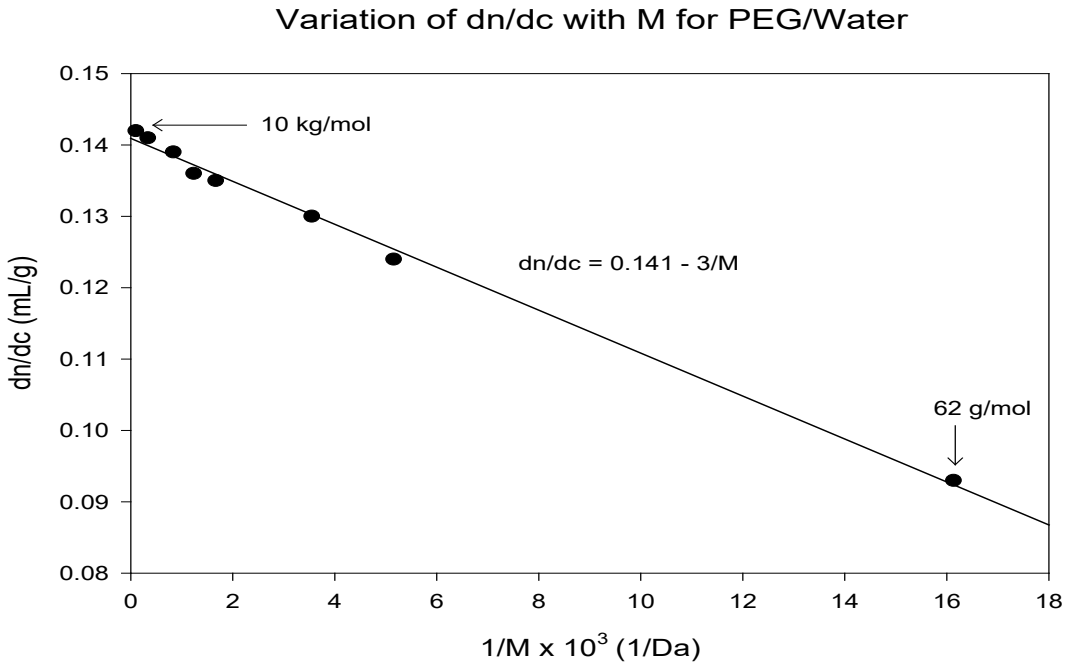
Effect of Impurities in Solvent and Polymer

Literature values from several authors for apparently the same polymer/solvent system often differ by several percent, even at the same temperature and wavelength. While this can be due to sample preparation, calibration, and measurement errors, it is sometimes due to impurities in the polymer or solvent. Solvent impurities will cancel when making the dn/dc measurements since they appear in both the pure solvent and the solvent used to prepare the solutions, but then your light scattering measurements have to be made with exactly the same polymer/solvent impurities as that used by the author of the literature article. As this is highly unlikely, it is better to measure dn/dc using your polymer/solvent.

Another manifestation of this same problem may occur if the solutions and solvent have not been kept under the same conditions for both the dn/dc and light scattering measurements. Ideally, the exact same solutions should be used for both measurements, and the solvent used to prepare the solutions should be kept under the same conditions (sealed, no additional moisture or other impurities allowed in contact). Otherwise, the subtraction involved in determining the excess scattered intensity and that involved in determining Δn may be different.

Molecular Weight Dependence of dn/dc

Generally, dn/dc increases with molecular weight and reaches a plateau when the end groups are sufficiently far apart. Below approximately 1,000 g/mol, dn/dc varies considerably. It increases by a few percent up to ~10,000 to ~ 20,000 g/mol, depending on the solvent, polymer shape and end-group contributions to the refractive index. Above this range it is, typically, constant. An early example from the literature involved polyethylene glycol in water. Here are the results:



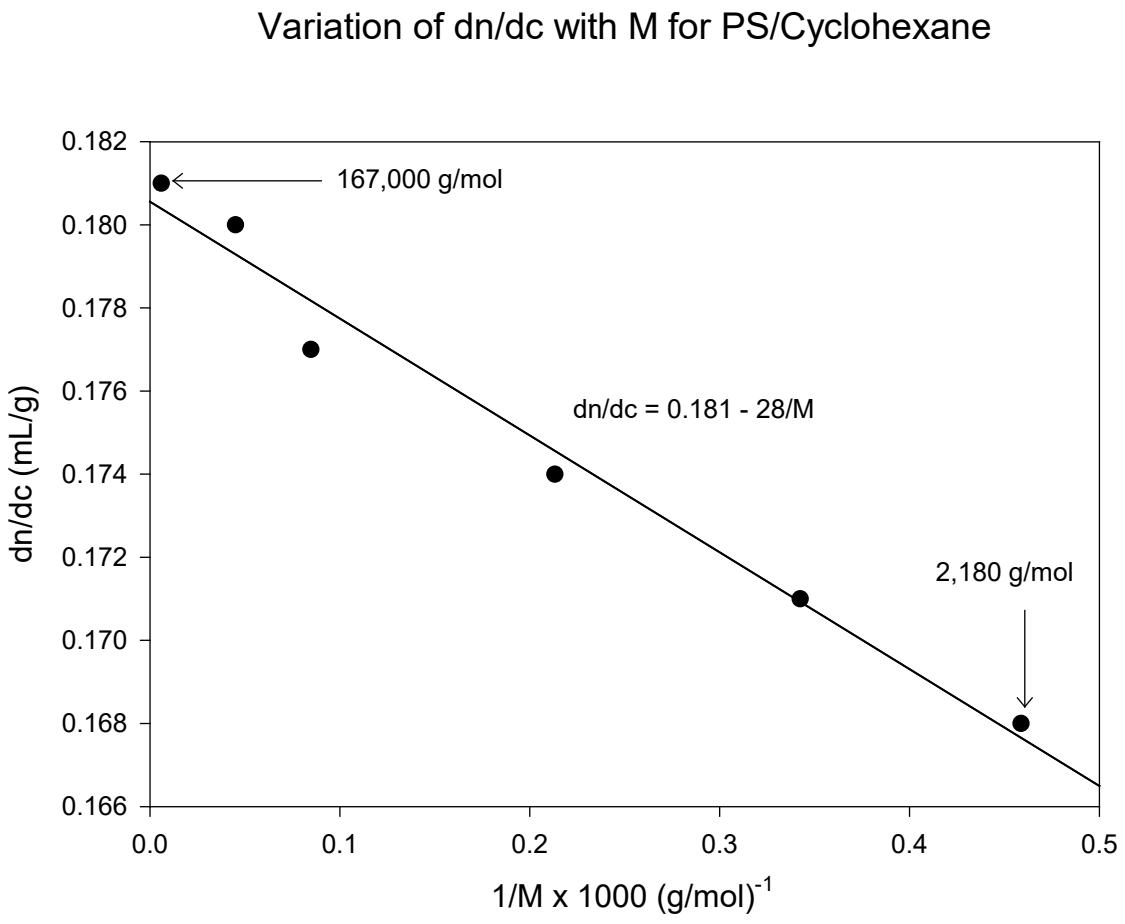
Generally, data like this fits reasonably well to $dn/dc = \alpha - \beta/M$, so when M is large enough, dn/dc does not change. Given the fit above, the change in dn/dc is less than 2% when M > 1,064 g/mol. This is an unusual case and may be because both solvent and polymer have hydroxyl groups.

Polystyrene in cyclohexane is a more common case as shown in the next graph. Here the rate of change with M is 9 times as large and the change in dn/dc is less than 2% when M > 7,750 g/mol. Both sets of data are

tabulated in Chapter 6 of Huglin's 1972 book and in the original literature, the references for which are found in Huglin's book.

It is particularly important to realize that each set of data plotted here was measured by the same authors (Kratohvil, J.P. 1968 for PS/cyclohexane and Rempp 1957 for PEG/H_2O). Therefore, it is safe to assume that the samples and corresponding solvents in each run were prepared under similar conditions for the different molecular weights.

If one simply compared a list of dn/dc values for the same solvent/polymer from different authors, all at the same wavelength and temperature, one would be surprised to find variations of several percent. While it is tempting to think that this variation can be explained by differences in molecular weight, more often the reason is that the impurity levels in the polymers and solvents are different from author-to-author. Therefore, if you want to deduce the molecular weight dependence, one must rely on data from the same author.



Temperature Dependence of dn/dc

The refractive index of liquids varies with temperature. For common organic solvents, the range is from -3 x 10^{-4} K^{-1} to -5 x 10^{-4} K^{-1}. Water's value, an exception, is -1.1 x 10^{-4} K^{-1}. Experimental temperature coefficients for dn/dc are usually linear from room temperature up to 100 °C and higher. The coefficients can be zero,

negative, or positive, but the variation in the absolute value is typically in the range of 1 to 5 x 10^{-4} $mL \cdot g^{-1} \cdot K^{-1}$. Thus, a few degrees difference in the absolute temperature between the measurement of dn/dc and the light scattering experiment is not significant.

Wavelength Dependence of dn/dc

Experimental observations show that dn/dc decreases with increasing wavelength, with variations of 1 to 3% over the range of 436 nm to 546 nm. These two wavelengths, from a Hg-arc source, represent the two most common ones used in SLS measurements prior to the use of lasers. Since they span the 488 nm and 514.5 nm lines of an Argon-ion laser, and the 532 nm line of a frequency-doubled, solid state laser, one could interpolate (not linear interpolation, see equation below) values of dn/dc from those measured at the classical values. More uncertainty would accrue to values extrapolated to 632.8 nm, the wavelength of a HeNe laser, or to values extrapolated to ~640 nm, a common wavelength for a "red" diode laser. (Again, the extrapolation is not linear. See below.)

Except for absorption peaks in the spectrum of either the solvent or the polymer, the variation in wavelength is given by:

$$\mathbf{dn/dc = A + B / \lambda^2}$$

This equation is also applicable to the variation in refractive index of solvent and polymer (again, assuming no absorption in the wavelength range of interest) and is known as the Cauchy equation. If dn/dc is known at two wavelengths, this equation can be used to calculate it at a third. However, it is much better if three or more measured values fit well to a straight line.

Some investigators ignore the wavelength dependence, and this is usually a mistake. Although there are some polymer/solvent pairs that exhibit little dispersion, there are others that exhibit quite a bit. For example, poly(vinyl acetate)/water has an 8.8% variation in dn/dc from 436 nm to 632.8 nm (see Page 40 of reference 4 listed above). In batch mode, such an error would result in nearly an 18% error in M_w; whereas, PVC/dioxane shows no change in dn/dc from 436 nm to 586 nm (see page 40 of reference 4 listed above). In addition, many researchers working with proteins and protein-like structures, assume a constant dn/dc of approximately 0.18, ignoring wavelength and temperature corrections.

Since the errors in dn/dc arising from differences in samples, even samples supposedly of the same chemical composition and with the same solvent, can be larger than the variations with temperature and wavelength, in order to find suitable A & B coefficients for the equation above, one must choose examples from the same author. The hope is that at least the polymer/solvents were the same. Here is a selection obtained from references 1 and 4 above.

Polymer/solvent	**A**	**B**
Polystyrene/toluene	+ 0.1015	+ 0.00200
Polystyrene/DMF	+ 0.1450	+ 0.00500
Polystyrene/MEK	+ 0.1963	+ 0.00675
Poly(methylmethacrylate)/Dioxane	+ 0.0486	+ 0.00031

Sucrose/water	+ 0.1392	+ 0.00115
Myosin/water	+ 0.1847	+ 0.00121
BSA/0.1M NaCl	+ 0.1791	+ 0.00378
Poly(acrylamide)/acetic acid	+ 0.1857	+ 0.00253
Poly(dimethyl siloxane)/toluene	- 0.0767	- 0.00504

The average value of B for all eight of the positive values is + 0.0028; for the non-aqueous samples the average is a bit higher at + 0.0035; and for the aqueous-based samples the average value of B is a bit lower at + 0.0022. The table is obviously not exhaustive, but the values do cover a range of polymer/solvents. B is calculated with λ in microns.

Here are examples of using the table to estimate small, first-order corrections.

Suppose you measure dn/dc = 0.1500 mL/g for a random-coil polymer in an organic solvent using the 620 nm source in a BI-DNDC. You want to estimate dn/dc at 640 nm, the wavelength of the laser in your BI-200SM. Assuming B = + 0.0035, calculate $A = 0.1500 - 0.0035/0.620^2$. The result is A = 0.1409. Now calculate dn/dc at 640 nm. The result is $dn/dc = 0.1409 + 0.0035/0.640^2 = 0.1494$ mL/g. The difference in dn/dc at 620 nm and 640 nm is 0.4%. The error in a calculated value of M_w using 0.1500 mL/g instead of 0.1494 mL/g is 0.8%, nearly insignificant compared to other errors. This case demonstrates why using the dn/dc values measured close to the laser wavelength is sufficient for many purposes.

Suppose you find a literature value of 0.1500 mL/g measured at 436 nm and you want to estimate the value at 640 nm. Aside from the fact that the literature value may correspond to a polymer/solvent system with different impurities or molecular weights than yours, this large wavelength difference cannot be ignored. In this case $A = 0.1500 - 0.0035/0.436^2 = 0.1316$, and the estimated value at 640 nm is $dn/dc = 0.1316 + 0.0035/0.640^2 = 0.1401$ mL/g. Now the difference between the two dn/dc values is 6.6%. This could lead to a 13% error in M_w in the batch mode, something that may not be acceptable.

Included in the table are two values that demonstrate atypical situations. First, note the negative value of A and B for poly(dimethyl siloxane) in toluene. This means that dn/dc is also negative. Negative values of dn/dc arise when the refractive index of the solvent is larger than that of the polymer. Since, typically, this is not the case, most dn/dc values are positive. Second, notice the rather small value of A and B for PMMA in dioxane. This means that dn/dc is also small, and it arises because the refractive indices of polymer and solvent are similar. It also demonstrates the fact that there exist polymer/solvent combinations with near-zero dn/dc values. In these cases, the errors in light scattering measurements will be large.

Appendix F: Theory and Data Analysis DLS Dynamic Light Scattering Software

Introduction

Particles move. In a gas, they move extremely fast and their mean-free path is much longer than their size. In a liquid, they also move fast, but their mean-free path is much shorter than their size. This has consequences for their time-dependent motion and the time dependence of scattered light. It leads, in a liquid, to diffusion coefficients from which particle or molecular size is calculated.

Because the incident light has an insignificant effect on the particle's movement, this type of scattering is almost purely elastic: it is quasi-elastic. Initially, the power spectral density function of the intensity of

scattered light, $P_I(\omega)$, was measured using a spectrum analyzer, but later the autocorrelation function of the intensity, $C_I(\tau)$,which is the Fourier transform of $P_I(\omega)$, was measured directly using a digital autocorrelator. This preserved the digital nature from photons exiting the laser to scattered photons from the particles to photons generating digital signals at square-law detectors (PMT's & APD's) that were correlated. This is many times more sensitive than the analog measurements of $P_I(\omega)$. As a result, for much of the 1970's, the technique was called photon correlation spectroscopy. [$P_I(\omega)$ re-enters the picture when discussing electrophoretic light scattering, ELS, and zeta potential.]

But by the early 1980's, the name dynamic light scattering, DLS, was catching hold. It makes a nice contrast with SLS and ELS, electrophoretic light scattering, or PALS, phase analysis light scattering, the later two used in zeta potential determination. DLS is a name that reflects the dynamics of the particle as it diffuses. And that dynamics is embodied in the translational diffusion coefficient, D_T. [For rod-like particles there is also rotational diffusion. For flexible coil polymers, there is also internal motion. These are special cases, but here we describe rigid, globular particle motion.]

General References

Much of the information in this can be seen in greater detail in one or more of the following references:

A. **Laser Light Scattering**, authors Charles S. Johnson Jr. and Don A. Gabriel, Dover Publications, Inc., New York. This monograph, originally published in 1981 by the CRC Press, was reissued in 1994 with corrections and added material. It is an excellent review of the earlier work including the frequency measurements before fast, digital correlators were available. Much of the emphasis is on biochemical applications, especially the early work from 1964 to 1980. A section on electrophoretic light scattering is also interesting.

B. Chapter 4, Data Analysis in Dynamic Light Scattering, Petr Štěpánek, in **Dynamic Light Scattering: The Method and Some Applications**, author Wyn Brown, Oxford Science Publications, Clarendon Press, Oxford, UK, 1993. This chapter is an excellent review with references for all the popular data analysis methods up to circa 1991. Other chapters in this book cover scattering from polymer solutions, both dilute and semi-dilute, scattering from gels and other complex fluids. It is an excellent, advanced text with many good references on these subjects.

C. **Dynamic Light Scattering by Macromolecules**, author Kenneth S. Schmitz, Academic Press, 1990. Despite its title, this book covers static light scattering, scattering from colloids and complex fluids as well as emphasizing the title material.

D. **Laser Light Scattering: Basic Principles and Practice, Second Edition**, author Benjamin Chu, Academic Press, 1991. An excellent, overall presentation it includes many of the earlier experimental designs, the overlap of x-ray, neutron, and light scattering. There is material devoted to using single mode fiber optics in SLS and DLS.

E. **Let There Be Light: Characterizing Physical Properties of Colloids, Nanoparticles, Polymers & Proteins Using Light Scattering**, author Bruce B. Weiner, available on Amazon Books, 2019. Chapters on DLS, SLS, and ZP. This book bridges the gap between introductions and advanced literature.

Brief Introduction to Intensity Power Spectral Density, $P_I(\omega)$, and Autocorrelation, $C_I(\tau)$, Functions

Time dependence of the scattered light intensity (subscript "I") can be determined in two ways: passing the current generated in a photomultiplier tube from light impinging on its photocathode to obtain the powder spectral density function of intensity, $P_I(\omega)$; or accumulating the autocorrelation function, $C_I(\tau)$, obtained from photons impinging on the PMT or on an avalanche photodiode (APD). Both techniques yield the same information. In fact, $P_I(\omega)$ and $C_I(\tau)$ are Fourier transform pairs related by the Wiener-Khinchine Theorem:

$$P_I(\omega) = \frac{1}{\pi} Re \int_0^\infty C_I(\tau)\, e^{-i\omega\tau} d\tau \qquad \text{(F-1)}$$

$$C_I(\tau) = \frac{1}{\pi} Re \int_0^\infty P_I(\omega)\, e^{i\omega\tau} d\tau \qquad \text{(F-2)}$$

Likewise, the power spectral density function of the scattered electric field, $P_E(\omega)$, and the autocorrelation function of the scattered electric field, $C_E(\tau)$, are also Fourier transform pairs.

The scattered electric field autocorrelation function $C_E(\tau)$ is also called the 1st-order autocorrelation function; and the scattered intensity autocorrelation function $C_I(\tau)$ is also called the 2nd-order autocorrelation function. It is the 1st-order autocorrelation function that is related to particle or molecular properties, but it is the 2nd-order autocorrelation function, or its transform pair, $P_I(\omega)$ that are measured.

There is a simple relationship (though difficult to derive[1]) between $g^{(1)}$, the normalized 1st-order (field) correlation function and $g^{(2)}$, the normalized 2nd-order (intensity) correlation function, namely the Siegert relationship:

$$g^{(2)}(\tau) = 1 + \left|g^{(1)}(\tau)\right|^2 \qquad \text{(F-3)}$$

The relationship is only true if the scattered field is a Gaussian random variable, and this is true only if there are enough particles contributing to the total scattered light. In statistics, according to the Central Limit Theorem, even if individual values follow a different probability distribution, if there are something like 30 or more, the sum follows Gaussian statistics. The sum is a Gaussian random variable. Now the scattered electric field is a sum over scattering from individual particles and many (30 is about the minimum theoretically) contribute to the sum. Thus, DLS is never a single particle counter.

What Is a Correlation Function?

Correlation is a measure of the similarity between two quantities. In the time domain it is useful, among other things, for establishing correlation between random signals and for establishing periodicity in the presence of noise. The correlation between two analog signals A(t) and B(t) is defined as:

$$C(\tau) = \lim_{T\to\infty} \left(\frac{1}{T}\right) \int_{t_0}^{t_0+T} A(t) \cdot B(t-\tau) dt \qquad \text{(F-4)}$$

T is the integration time; t_o is the initial time; A(t) and B(t) are the amplitudes of the time-varying signals; and $C(\tau)$ is called the cross-correlation function. A correlation function is a plot of $C(\tau)$ versus τ, where τ is called the delay time (also called the shift time or the lag time). If these signals arise from scattered light,

[1] Mandel, L, "Fluctuations in Light Beams", *Progress in Optics,* Vol. 2, Wolf, E., Ed., Wiley-Interscience, New York, 1963, p. 181.

then $C(\tau) = C_I(\tau)$. Here, and in the next few equations, when no subscript is given, reference is made to the general idea arising from any type of signal, not just scattered light intensity.

If A(t) and B(t) are the same function, that is B(t-τ) is simply a delayed version of A(t), then C(τ) is called the autocorrelation function, abbreviated ACF. The Fourier transforms of the autocorrelation and cross-correlation functions are the power spectral density and the cross power spectral density functions, respectively. As such, they contain, in principle, information in the frequency domain equivalent to the information the correlation functions contain in the time domain. See Eqs. (F-1) and (F-2).

For stationary signals (signals independent of the initial time) and for ergodic systems (systems in which time averages are equivalent to ensemble averages), the correlation function can be written as

$$C(\tau) = \langle A(0) \cdot B(t-\tau) \rangle = \lim_{T\to\infty} \frac{1}{T} \int_0^T A(t) \cdot B(t-\tau)\, dt \qquad \text{(F-5)}$$

where <A(0)·B(t-τ)> is the ensemble average.

The cross- or autocorrelation functions may be obtained with analog processing by multiplying the signal A(t) with a delayed version of signal B(t-τ) or itself, A(t-τ), and averaging this product over T seconds using, for example, an RC or operational amplifier integrator. However, analog integration can lead to distortion in the measured function. Because of the advances in digital electronics (1960's and onward), and since some important time-varying signals are discrete by nature (photons, for example), it is useful to approximate the integral by a finite sum of N products obtained by sampling the signal in discrete intervals. This discrete interval or sampling time (occasionally called the bin time) is designated as Δt.

In linearly-spaced correlators, the delay time is an integer multiple of the sampling time. The last delay time equals the sampling time multiplied by the number of hardware correlator channels. Because of the proportionality between the delay and the sampling times, these terms were often, and erroneously, used interchangeably. Sometimes the term sample time was used, even though such use is confusing and incorrect.

In modern correlators, while linear-spacing is still possible, the delay time and the sampling time are de-coupled, allowing for a much greater range of delay times with fewer channels.

When approximating the ACF by a sum of N products, also called the number of samples, the ACF is written as:

$$C(\tau_j) = \lim_{N\to\infty} \frac{1}{N} \sum_{i=1}^{N} n_i \cdot n_{i-j} \qquad j = 1, 2, 3 \ldots M \qquad \text{(F-6)}$$

where τ_j is the j^{th} delay time, n_i is the number of pulses during the sampling time Δt centered at some time t, n_{i-j} is the number of pulses during Δt centered at t-τ, and M is the number of correlator channels. Of course, when counting photons (pulses), the average over time, <n>/T, is in fact the average intensity of scattered light, <I>.

How is an Autocorrelation Function Accumulated?

See Figure F-1. It depicts a linear spacing of delay times τ using equal sampling (bin) times Δt. The delay time is an integer multiple of the sampling time.

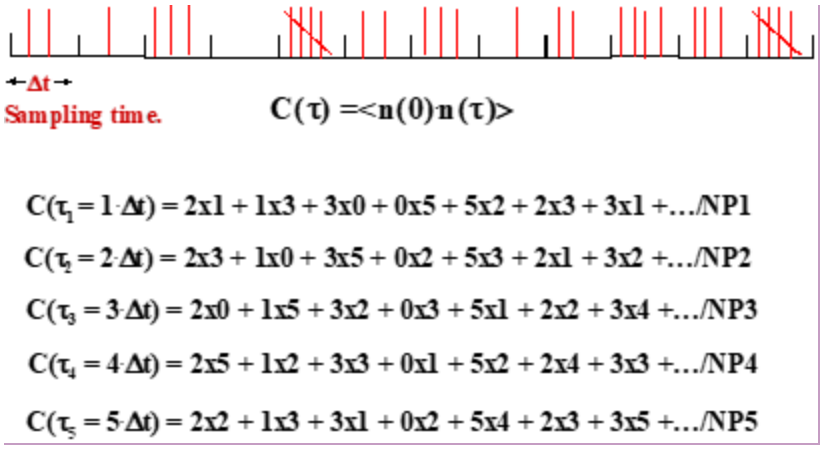


Figure F-1: Measurement (accumulation) of an autocorrelation function.

The signal is indicated by a fluctuation in the number of photo pulses registered in each bin: two in the first, one in the second, three in the third, zero in the fourth, five in the fifth, two in the sixth and so on. The ACF is the average of the product, <n(0)·n(τ)>, which is the sum over the product of the number of pulses at zero and a delay time later, divided by the number of products, NPX, where X is the channel number.

The first channel shows all products one delay time (1·Δt) apart divided by NP1. The second channel shows all products two delay times (2·Δt) apart divided by NP2. The third channel shows all products three delay times (3·Δt) apart divided by NP3, and so on.

Assuming the particles are rigid, globular, and all about the same size, it will be shown in the next section that the autocorrelation function (ACF) is a single exponential decay riding upon a substantial baseline. With modern correlators, however, and theory that shows linear spacing is unnecessary, most ACF's looks like the following (Figure F-2).

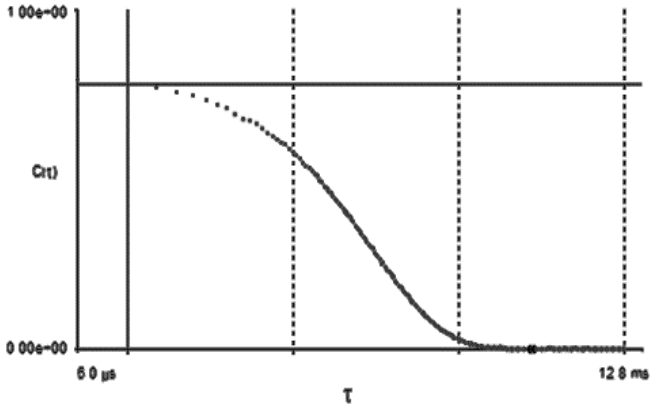


Figure F-2: Single exponential ACF with normalization and nonlinear channel spacing on log scale.

Single Decay Process Arising from Diffusion

The full development[2] is more advanced than the presentation here allows. However, a few steps are worthy of presentation. The normalized, first-order autocorrelation function is related

$$g^{(1)}(\boldsymbol{q},\tau) = exp(-D_o q^2 \tau) \qquad \text{(F-7)}$$

Typically, it is the translational diffusion coefficient D_o at infinite dilution that is desired, since it can be related to particle size. It is common to set $\Gamma = D_o q^2$, where Γ is called the linewidth. This reference to a spectral linewidth becomes more obvious when the electric field power spectral density function is calculated using the electric field Fourier transform analogous to Eq. (F-1).

The result is the positive half of a Lorentzian function (frequencies < zero have no physical meaning). The half-width at half-maximum represents the width, $\Delta\omega_{1/2} = \Gamma$. Therefore, Γ is called the linewidth.

From Eqs. (F-3) and (F-7), we find

$$g^{(2)}(\tau) = 1 + exp(-2D_o q^2 \tau) = 1 + exp(-2\Gamma\tau) \qquad \text{(F-8)}$$

Thus, the normalized, 2nd-order (intensity) ACF decays twice as fast as the 1st-order (electric field) ACF.

So far, the baseline used for normalization of the measured ACF has been neglected as has coherence properties. When included Eq. (F-8) becomes:

$$G^{(2)}(\tau) = B\left(1 + f^2 exp(-2D_o q^2 \tau)\right) = B\left(1 + f^2 exp(-2\Gamma\tau)\right) \qquad \text{(F-9)}$$

Where B and f are discussed in more detail in the section below entitled "Introduction to Data Analysis".

Introduction to Data Analysis

The most common starting point in the analysis of the measured function, the second-order or intensity autocorrelation function (ACF) is the full Siegert relationship:

$$G^{(2)}(\tau) = B \cdot \left(1 + f^2 \left|g^{(1)}(\tau)\right|^2\right) \qquad \text{(F-10)}$$

Here $G^{(2)}(\tau)$ is the un-normalized measured function, often designated as $C(\tau)$, where τ is the delay time. The baseline B is proportional to the square of the intensity. The amplitude f is related to the temporal and spatial coherence properties of the laser beam and the detector optics. It is roughly equal to the inverse of the number of detected coherence areas.[3] f is less than or equal to one and determines the intercept-to-baseline ratio. It is obtained during the fitting procedure and is here squared for convenience as it is always determined along with the normalized, first-order or electric field autocorrelation function $g^{(1)}(\tau)$. [Normalized correlation functions are traditionally shown as small g's and un-normalized ones as capital G's.

[2] Reference E, pages 46-47, of General References in this appendix.

[3] Define A_{det} as the area of the detector (PMT or APD) determined by pinholes, lenses, or single mode fiber optics. Define A_{coh} as the coherence area that can be estimated by λ^2/Ω where Ω is the solid angle subtended by the source at the detector. Then $N \approx A_{det}/A_{coh}$ and $f \approx A_{coh}/A_{det}$. Small pinholes decrease A_{det}, increase f as does focusing the beam so that A_{coh} increases. This maximizes the intercept-to-baselines ratio of the measured autocorrelation function. It is easier to obtain an accurate fit when f is maximized.

Thus $f \cdot g^{(1)}(\tau)$ = SqRt{$G^{(2)}(\tau)/(B-1)$}.] Physical parameters of interest are related to $g^{(1)}(\tau)$; whereas, the measured quantity is $G^{(2)}(\tau)$.

The Siegert relationship is rarely violated except when there are very few particles in the scattering volume and when the system is non-ergodic. The validity of the relationship rests on the assumption that the scattered electric field is a Gaussian random variable, and this is ensured when there are at least 30 particles at the intersection of the illuminated and detected volumes. [Note: Since the scattering volume is so small, 30 particles per scattering volume is typically $10^N/cm^3$, where N is 7, 8, 9 or 10. In other words, this is not a single particle counting technique!]

A system is ergodic when the ensemble average of a property (theoretically predicted) equals the time average of the property (measured). Except for gels and colloidal glasses, or in the case of very strongly interacting systems, or particles trapped in a network, ergodicity is assumed for the cases of interest here. Consult the references for information on these non-ergodic systems and how, in some cases, static light scattering is combined with dynamic light scattering measurements to gain more information.

Monodisperse, Rigid, Globular Particles: Spheres

The references will show that, in this case, a single exponential describes the correlation function:

$$\left|g^{(1)}(\tau)\right| = \exp(-\Gamma \cdot \tau) \qquad \text{(F-11)}$$

Where, Γ is called the decay or relaxation rate and $\tau_r = 1/\Gamma$ is called the decay or relaxation time. For dilute systems where the decay is entirely due to translational, diffusive motion of the center of mass of the particle, it can be shown that:

$$\Gamma = D_o \cdot q^2 \qquad \text{(F-12)}$$

Where, D_o is the translational diffusion coefficient and q is the magnitude of the scattering wave vector. This magnitude, discussed in Chapter II on SLS, and developed in Appendix SLS3, is given by:

$$q = \frac{4 \cdot \pi \cdot n_o}{\lambda_o} \cdot \sin(\theta/2) \qquad \text{(F-13)}$$

Where, n_o is the refractive index of the liquid, λ_o is the wavelength of the laser in a vacuum, and θ is the scattering angle. A length scale is defined by q^{-1} against which distances between particles is compared. When the distance is large compared to q^{-1}, then self-diffusion is measured. When the distance is small, then mutual diffusion is measured. Mutual and self-diffusion vary differently with concentration (particle-particle separation) depending on the attractive and repulsive forces involved. However, for very dilute samples, both asymptotically approach the same limit, $D_T \rightarrow D_o$, where D_o is given by the Stokes-Einstein equation:

$$D_o = \frac{k_B \cdot T}{3 \cdot \pi \cdot \eta \cdot d_H} \qquad \text{(F-14)}$$

Here k_B is Boltzmann's Constant, 1.3807 x 10^{-16} ergs/deg, T is the absolute temperature in Kelvin, η is the bulk viscosity of the liquid in which the particle moves, and d_H is the hydrodynamic diameter of the assumed sphere.

Hydrodynamic diameters include the particle "dry" diameter plus whatever is permanently attached to, and moves with, the surface. In the case of hard mechanical spheres with relatively short-chain surfactants added to impart stability (e.g. 100 nm PS and sodium dodecyl sulfate, approximately 2 nm), the difference between the "dry" and hydrodynamic diameters is usually not significant. However, when the particle is much smaller, and/or the attached species are much larger, as may be the case with long-chain polymer additives, then there may be a significant difference between the "dry" and hydrodynamic diameters. Interesting cases involve polyelectrolyte additives which exhibit isoelectric points. Such additives may change size significantly as a function of pH.

Model Fits[4]

These include cumulants, Williams-Watts, and the double exponential.

Cumulants

The best-known method for analyzing polydispersity is the method of cumulants.[5] For an assumed continuous, normalized distribution of decay rates G(Γ):

$$\left|g^{(1)}(\tau)\right| = \int_0^\infty G_I(\Gamma)\exp(-\Gamma \cdot \tau)d\Gamma \quad \text{, with} \qquad \text{(F-15)}$$

$$\int_0^\infty G_I(\Gamma)d\Gamma = 1 \qquad \text{(F-16)}$$

Do not confuse $G_I(\Gamma)$ with either $G^{(1)}(\tau)$ or $G^{(2)}(\tau)$. The first is the intensity-weighted distribution function of Γ's from which the distribution of diffusion coefficients, size or molecular weights is ultimately determined. The second and third represent the unnormalized electric field and intensity autocorrelation functions, respectively.

[4] Reference E, Chapter III of General References in this appendix, has more information on fitting,
[5] First proposed by D.E. Koppel, *J. Chem. Phys.*, **57**, 4814, 1972.

For a discrete distribution of particles or molecules, the distribution is written as,

$$G_I(\Gamma) = \frac{\sum_i I_i \cdot \delta(\Gamma - \Gamma_i)}{\sum_i I_i} = \frac{\sum_i N_i m_i^2 P_i \cdot \delta(\Gamma - \Gamma_i)}{\sum_i N_i m_i^2 P_i} = \frac{\sum_i c_i M_i P_i \cdot \delta(\Gamma - \Gamma_i)}{\sum_i c_i M_i P_i} \quad \text{(F-}$$

, where

I_i is the intensity scattered by, m_i is the mass of, and N_i is the number per scattering volume of the i^{th} particle with decay rate Γ_i. Also, if the particles are molecules, readers will recognize that c_i is the mass concentration of a polymer molecule with molecular weight M_i. The term $\delta(\Gamma - \Gamma_i)$ is the delta function and is used mathematically to select discrete values when summed in Eq. (F-17).

The factor P_i accounts for angular scattering effects for particles larger than about $\lambda/20$, the usual approximation for a non-Rayleigh particle. P_i is calculated from Mie scattering theory and requires the particles' refractive index. For Rayleigh particles and at sufficiently low angles $P_i = 1$, and experienced readers will realize that the formalism above will lead to a z-average diffusion coefficient from which an inverse, z-average particle diameter is calculated. More generally the averaging is by intensity and the resulting particle size is an inverse, intensity-weighted diameter. (It is sometimes claimed, incorrectly, that DLS measurements *always* yields a z-average result. This is only true when $P_i = 1$.)

Expanding the exponential in Eq. (F-15) about an average value, and integrating term-by-term one obtains:

$$\left|g^{(1)}(\tau)\right| = \exp\left(-\overline{\Gamma}\tau\right) \bullet \left[1 + \frac{\mu_2}{2!} \cdot \tau^2 - \frac{\mu_3}{3!} \cdot \tau^3 + \frac{\mu_4}{4!} \cdot \tau^4 + \ldots\right] \quad \text{(F-18)}$$

Here the first moment about the origin, the "average", is defined as

$$\overline{\Gamma} = \int_0^\infty \Gamma \cdot G_I(\Gamma) d\Gamma \quad \text{(F-19)}$$

The n^{th} moment about this average value is defined as

$$\mu_n = \int_0^\infty \left(\Gamma - \overline{\Gamma}\right)^n G_I(\Gamma)\, d\Gamma \quad \text{(F-20)}$$

Taking the natural logarithm of Eq. (F-18) and expanding in a Taylor series the term in brackets, under the assumption that the sum of the various terms involving powers of τ is small, one gets the following simple polynomial (after adding back the amplitude f):

$$\ln\left[f\left|g^{(1)}(\tau)\right|\right] = \ln(f) - \overline{\Gamma}\cdot\tau + \left(\mu_2/2!\right)\cdot\tau^2 - \left(\mu_3/3!\right)\cdot\tau^3 + \left((\mu_4 - 3\mu_2^2)/4!\right)\cdot\tau^4 + \ldots \quad \text{(F-21)}$$

The coefficient of τ^n is called a cumulant, though the first three are equal or proportional to simple moments.

The first cumulant, $\overline{\Gamma}$, is the most important term that is recovered. In polymer solutions, it can be shown that:[6]

$$\overline{\Gamma} = D_T \cdot R_g^{-2} \cdot \left(q \cdot R_g\right)^n \qquad \text{, where} \qquad \text{(F-22)}$$

Depending, on the magnitude of $q \cdot R_g$, n is either 2 or 3. R_g is the radius of gyration, a measure of polymer size. It is proportional to $R_H = d_H/2$, the hydrodynamic radius, but not equal to it. When n = 2, simple center-of-mass diffusive motion occurs, and $\overline{\Gamma} = \overline{D}_T \cdot q^2$. This occurs in dilute solution when $q \cdot R_g < 1$. When $q \cdot R_g > 1$, n = 3; the motion observed in DLS is not diffusive; the internal polymer modes are probed; and $\overline{\Gamma}$ varies as q^3. Furthermore, $\overline{\Gamma}$ is then no longer simply dependent on the particle size or molecular weight.

For slightly concentrated systems the measured and infinitely dilute diffusion coefficients are expressed as:

$$\overline{D}_T = \overline{D}_o \cdot \left(1 + k \cdot c + \ldots\right)$$

This equation can also be applied to suspensions of particles as well. However, in more (F-
suspensions of particles or solutions of polymers, the q^2 modes observed are not proportional to self-diffusion but to mutual (also called cooperative) diffusion. Self- and mutual diffusion vary differently with concentration, but this is a topic beyond the scope of this presentation. Suffice it to say that it is wrong to interpret every ACF in terms of particle size derived from a self-diffusion coefficient.

Assuming the ACF can be interpreted in terms of self-diffusion, then for particle sizing the intensity-weighted, self-diffusion coefficient is an average. After invoking the Stokes'-Einstein equation, Eq. (III-23), the following applies:

$$\textit{Effective Diameter} \equiv \left(\overline{\frac{1}{d_H}}\right)^{-1} = \frac{\sum_i N_i d_i^6 P_i}{\sum_i N_i d_i^5 P_i} \qquad \text{(F-}$$

The **Effective Diameter**, abbreviated Eff. Dia., is neither the mass nor volume nor number-weighted diameter. Nor is it the intensity-weighted diameter. Nor is it, as it is sometimes erroneously called, the z-average diameter. The intensity weighting is on the diffusion coefficient. Since that varies inversely with particle size, Eq. (F-24) results. (Note: The d^6 term arises from the m^2 term in Eq. (F-17) but is no surprise after reading Appendix C on SLS.)

The Eff. Dia. is normally larger than most other common, average diameters since the averaging is over the 6th and 5th powers. In certain cases, were P_i is a strong function of the angle, and a minimum in the Mie

[6] Doi and Edwards, The Theory of Polymer Dynamics, Clarendon Press, Oxford, U.K., 1986, page 107

scattering pattern obtains, it is possible to observe a reversal such that the Eff. Dia. is smaller than the volume average diameter. But generally, it is the other way around. This explains why dust and other, unwanted large particles, while few, can distort the results.

The second moment μ_2 is the intensity-weighted variance of the diffusion coefficient distribution when samples are dilute and interactions negligible. The relative variance, $\mu_2/\overline{\Gamma}^2$, also called the polydispersity index (PDI) or Poly (short for polydispersity), is a measure of the width of the intensity-weighted, decay-rate distribution, $G_I(\Gamma)$. [In early literature references this relative value is called the reduced second moment.] Therefore, when the distribution is one of particles, Poly is a measure of the size distribution width. It is the intensity-weighted relative variance of the diffusion coefficient, but nonetheless a measure of width. When the Poly equals zero, the sample is monodispersed, and as Poly increases so does the width of the distribution. It applies to monomodal as well as multimodal distributions since moments do not indicate distribution shape, only magnitudes. Sometimes that is enough since a sample such as a pure monoclonal antibody, a latex used as a reference material, or any other sample that is supposed to be narrowly distributed must have a low Poly, typically ≤ 0.025.

The validity of the cumulant expansion rests on the assumption that the higher order terms do not contribute significantly. As a generalization, it is found that the cumulant analysis works best when Poly $\leq$ 0.3, approximately.

A relative third moment, defined as the Skew = $\mu_3/(\mu_2)^{3/2}$, is a dimensionless number that can, if the skew is repeatable, indicate if the distribution is skewed to the left (negative skew) or to the right (positive skew) of the modal value.

A relative fourth moment, defined as the Kurtosis = $\mu_4/(\mu_2)^2$, is a dimensionless number that, for unimodal distributions only, if repeatable, indicates the relative peaked-ness of the distribution compared to a Gaussian distribution (Kurtosis = 3). The 4th moment obtained using the cumulant method is rarely of value and is included here for historic purposes only.

Using the Cumulant Fit: Interpretation

See Table F-1 for an example of a sample correlation function successively fit to 1st, 2nd, 3rd, and 4th order.

	Gamma (s^{-1})	Eff. Diam. (nm)	Poly	Skew	Kurtosis	RMS Error	Amplitude
Linear:	1.477e+03	116.1				9.3404e-04	0.748
Quadratic:	1.500e+03	114.3	0.039			1.5591e-05	0.750
Cubic:	1.500e+03	114.3	0.041	0.32		3.5361e-06	0.750
Quartic:	1.500e+03	114.3	0.040	0.06	1.48	4.7423e-05	0.750

Table F-1: Successive cumulant fits to the same ACF.

A standard polynomial least squares fitting procedure is used with weighting since the fit is to the logarithm of $g^{(1)}$ rather than the directly measured value $G^{(2)}$.

The linear fit just includes the linear term in τ. From the fitted Γ (capital "Gamma"), the diffusion coefficient can be calculated from Eqs. (F-12) and (F-13). The Eff. Dia. is calculated from Eq. (F-14). To determine **Poly** requires at least a second order fit; to determine the **Skew** requires at least a third order fit; and to determine the **Kurtosis** requires at least a fourth order fit.

The RMS error is the root-mean-square error. It is the square root of the sum of squares of the differences between the fitted ACF and the measured. Before taking the square root, the sum is divided by the number

of data points minus the number of fitted parameters. Smaller RMS errors means a better fit in the case of cumulants. But it should be a significantly smaller value as is the case from linear to quadratic and perhaps to cubic, but not for quartic. Thus, only the Eff. Dia., Poly and perhaps Skew are determined as well as f^2 = 0.75. This is not a monodispersed sample, but it is not overly broad either.

The amplitude is f^2 as in Eq. (F-10). It represents the amplitude above the baseline chosen for the fit. The amplitude is a number between 0 and 1. Here the value of 0.75 means the detected and coherence areas were reasonably well matched. The pinhole used was small enough.

This suggests that no more than three distribution parameters (ignoring f) can be usefully determined. For example, if another algorithm (CONTIN, NNLS, Exp. Sampling, for example) yielded a bimodal (two peak positions plus the ratio of amount in each peak), this is consistent with three parameters. A trimodal is not consistent (three peak positions and ratio of two independent areas, for a total of five distribution parameters). A broad unimodal distribution is also consistent with three distribution parameters.

Higher order fits are not always worthwhile and may lead to large RMS errors if the data cannot support the forced fit. Look for trends in the fitted parameters vs. fit order that are repeatable and consistent with any prior knowledge of the scattering system.

Do not expect the cumulant method to work well when the distribution is overly broad. This applies to broad particle size and polymer distributions, and it also applies to scattering from complex fluids and polymer solutions where more than diffusion is occurring.

Lognormal Approximation from Cumulants

While the method of cumulants is the oldest and still a quite useful technique for analyzing ACFs, it cannot yield the shape of the decay rate distribution; therefore, it cannot shed light on the shape of the size distribution. It can only yield, at best, moments of the distribution. A simple approximation, however, allows one to convert the first two moments into an intensity-weighted size distribution which can then be plotted. For particle size distributions, a common unimodal choice is the lognormal curve, which is a Gaussian or Normal curve in log space.

The n^{th} moment of an x-weighted, lognormal distribution of particle diameter is given by:

$$\text{where } \overline{d_x^n} = d_{gx}^n \cdot \exp\left(\frac{n^2}{2} \cdot \ln^2 \sigma_g\right) \qquad \text{(F-25)}$$

x signifies the weighting (intensity, volume, surface area, number), d_{gx} is the geometric mean diameter with x weighting (also equal to the median value on a log scale), and σ_g is the geometric standard deviation (also known as the harmonic standard deviation). A unique feature of a lognormal distribution is that its width, characterized by the parameter σ_g, is independent of the weighting.

The entire lognormal curve by intensity is determined with both d_{gI} and σ_g specified. Set the Eff. Dia. = d_{gI}; then calculate σ_g using the simplifying assumption that P_i = 1. The calculation is outlined as follows.

The Poly, Q, can be written as,

$$Q = \left(\overline{D_T^2}\right)_I / \left(\overline{D_T}\right)_I^2 - 1 \quad , \qquad \text{(F-26)}$$

where the subscript "I" means intensity weighting. Using Eq. (F-25) and since the intensity "I" is proportional to $N \cdot d^6 \cdot P$, where P is the Mie scattering factor which reflects any angular dependence, one can relate Q to the intensity-weighted moments and thus to the Lognormal distribution. Further progress requires values for P. Though strictly true only for Rayleigh particles or at low angles, the simplifying assumption that P = 1, allows one to derive the following simple formula relating the measured polydispersity index Q and the geometric standard deviation, σ_g:

$$\sigma_g = \exp\left\{\left[\ln(1+Q)\right]^{1/2}\right\} \qquad \text{(F-}$$

Please note that the Lognormal fit from cumulants cannot possibly describe any multimodal distribution, nor one that is skewed on a log scale, nor was it intended as anything more than a way to visualize graphically the relative width as contained in Poly. With the two parameters that define the Lognormal equation, any number of other values can be calculated. Yet, there is no more information than that contained in the two values: Eff. Dia. and Poly. The Lognormal distribution, and its assumptions stated above, may have value as a quality control tool only, nothing more.

Williams-Watts (W-W)

This function, also known as a stretched exponential, and related to the Tung-Weibull distribution in statistics, is useful when the ACF is a broad, non-exponential function such as those found when measuring complex fluids like viscous liquids and gels.[7] The Williams-Watts function is given by,

$$\left|g^{(1)}(\tau)\right| = \exp\left[-\left(\frac{\tau}{\tau_r}\right)^{\beta}\right] \qquad \text{(F-28)}$$

With $0 < \beta \leq 1$ describing the width of the distribution of relaxation times τ, and τ_r is a characteristic relaxation time. When $\beta = 1$, the distribution is exponential, but as β approaches zero the decay in the ACF covers many decades.

An average decay rate can be calculated by integrating Eq. (III-37) multiplied by τ. The result is:

$$\bar{\tau} = \frac{\tau_r}{\beta} \cdot \Gamma\left(1/\beta\right) \qquad \text{(F-29)}$$

[7] For more information consult Section 2, Photon Correlation of Bulk Polymers, page 127, G.D. Patterson, **Light Scattering from Polymers,** Springer-Verlag, 1983.

Here $\Gamma(1/\beta)$ represents the gamma function tabulated in statistical textbooks, not the linewidth associated with the decay of an ACF.

The Williams-Watts function is interesting in that it is used to fit the $g^{(1)}$ function directly rather than a size distribution.

Double-Exponential (D.E.)

Like the W-W and cumulant fits, the D.E. fit assumes a specific form for the first-order ACF, namely:

,where $$\left|g^{(1)}(\tau)\right| = G_I(\Gamma_1)\cdot\exp(-\Gamma_1\tau) + G_I(\Gamma_2)\cdot\exp(-\Gamma_2\tau) \quad \text{(F-30)}$$

$G_I(\Gamma_i)$ represents the relative intensity contributed by the i^{th} particle with decay rate Γ_i. This too is a non-linear least squares problem for which the Marquardt-Levenberg algorithm is used.

Note that this is a forced fit: do not be surprised when a bimodal answer is obtained. One can fit reasonably well some overly broad but unimodal distributions using a double exponential. Nothing can be said about the actual distribution shape when force-fit to a double exponential. The global mean and global variance, as determined by both resulting peaks, are useful for characterizing moments of, but not for determination of, the true distribution shape. One could not say, for example, because the RMS error is small (meaning a good fit) that the distribution is, therefore, a double exponential.

Fundamental Limit on Distribution Information from DLS

In simple linear least squares fitting, such as with a single exponential (monodisperse), with a polynomial in τ (cumulants), with the William-Watts, where the first two are linearized by fitting the log, and the third by fitting the log of the log, the fit proceeds by minimizing the sum S of the squares of the residuals (the basis for least squares fitting):

$$S = \sum_i w_i \cdot \left(g^{(1)}(\tau_i) - \sum_j G_I(\Gamma_j)\cdot exp(-\Gamma_j\cdot\tau_i)\right)^2, \text{ where} \quad \text{(F-31)}$$

the summation over i represents the sum over the correlation channels at τ_i, and the summation over j represents that over the assumed number of decay rates Γ_j. This is a weighted sum, with w_i the weighting factor. It is related to the inverse of the variance at each channel.

In simple linear or linearized cases, there is a single, well-defined minimum in S versus the fit parameters. But for non-linear functions such as the D.E., this may not be the case. There may be local minima with only a single absolute minimum. Thus, if the initial estimates are too far off, the iteration gets trapped in a local minimum with the wrong results.

This was but a prelude when attempts were made to deconvolute the more general equation relating the measured $g^{(1)}(\tau)$ to $G_I(\Gamma)$. To be sure, it is $G^{(2)}(\tau)$ [that is $C(\tau)$] that is measured, but if the baseline is well-determined, $g^{(1)}(\tau)$ = SqRt{$G^{(2)}(\tau)/(B-1)$}, aside from the optical constant f (so-called because of its relationship to the ratio of the detected to the coherence area). The more general problem is then to solve for $G_I(\Gamma)$. From it, the distribution weighted by intensity for Γ is determined. And once determined the intensity-weighted distributions for τ_r, D_T, d_H, and even M (in the case of where the Mark-Houwink-Sakurada equation holds, though using GPC/SEC is a better technique for finding the molecular weight distribution), follow.

Thus, the general problem is reduced to a deconvolution of,

$$\left|g^{(1)}(\tau)\right| = \int_0^\infty G_I(\Gamma)\exp(-\Gamma\cdot\tau)d\Gamma \qquad \text{(F-32)}$$

with

$$\int_0^\infty G_I(\Gamma)d\Gamma = 1 \qquad \text{(F-33)}$$

Those familiar with transforms will recognize the first equation as a Laplace transform with the exponential term as the kernel. Calculating $G_I(\Gamma)$ from the measured $g^{(1)}(\tau)$ is the equivalent of Laplace transform inversion.

Tables of inverse Laplace transform exist for many, many different kernels. But not for an exponential. Thus, it is called an ill-conditioned or ill-posed Laplace transform. There are other ill-conditioned Laplace transforms but this one, with an exponential as the kernel, is a prototype for the difficulties in dealing with them. [Note: Sums of exponentials also occur in circular dichroism measurements and spectra from mixtures of dye molecules with forbidden singlet-triplet transitions.] What are the consequences of this ill-conditioning and how does it limit size (and other) distribution information?

First there is no analytic solution, no simple functional form for the distribution. Thus, only numerical solutions are possible. If this were the only problem, living with a table of $G_I(\Gamma)$ vs. Γ would be quite acceptable. However, there is more to the limitation from inverting this ill-conditioned Laplace transform: minor differences in the noise along the correlation function, from one measurement to the next, can give rise to different results. Where one measurement might yield a unimodal set of values when plotted, the next might yield a bimodal or multi-modal. A slight difference in the measured baseline B used to calculate $g^{(1)}(\tau)$ can have the same results. And, finally, finding the minimum of S, the sum of the squares of the residuals, is no longer a guarantee of finding the right solution. Several solutions, all with approximately the same S, may exist but with different distributions.

Worse, it was also found that there was limited information to be found in such deconvolutions. For example, for a bimodal, each mode with a width, there are at least five parameters (degrees of freedom) necessary to describe it: two peak positions, two widths (a standard deviation or half-width at half height, both are widths), and the ratio of the area under each curve describing the relative scattered intensity for each peak. In DLS, rarely are more than three degrees of freedom found with any repeatability.

This—the ill-conditioning of the general problem of deconvolution—is the fundamental limit in DLS. It prevents DLS from being a high-resolution particle sizing technique. At best, DLS is a medium resolution technique.

Various Methods for Deconvolution of the Autocorrelation Function

A variety of numerical techniques for the deconvolution have been proposed:

- NNLS (Non-Negatively Constrained Least Squares)
- CONTIN (one type of Regularization)
- Maximum Entropy Method
- Singular Value Decomposition

- Exponential Sampling
- Histogram Method
- REPES

None are absolute as might be surmised from the limit placed on the solution by minor differences in noise from one measurement to the next. Given the limited resolution, all are usually employed with constant ratio (also called exponential) spacing on the Γ axis. And all can rarely find correct results when independent Γ values are closer than about 2:1. To be sure, finer steps can be filled in by shifting the original set of Γ values, but these are not independent of the original set. Thus, the resolution is only apparently improved. Unless the measured correlation function is smooth (minimizing noise), the various techniques do not converge, and often result in peaks that are broader than the true distribution results. In general, none of these techniques are superior in all cases to the rest. See Chapter 4 in reference C listed near the beginning of this chapter for more detail.

Non-Negatively Constrained Least Squares

One technique, NNLS[8], has something in common with cumulants, William-Watts, and an assumed single exponential: linearizing the functional form (either by taking the log or the log of the log) or fitting a polynomial, none of which are ill-conditioned as far as standard least squares is concerned. This is done by starting with the discrete form for the general solution by replacing Eqs. (F-32) and (F-33) with the following:

$$g^{(1)}(\tau) = \sum_{j=1}^{j=N} G_I(\Gamma_j) \cdot \exp(-\Gamma_j \tau) \qquad \text{(F-34)}$$

With the usual normalization condition that the sum over the relative intensities is unity:

$$\sum_{j=1}^{j=N} G_I(\Gamma_j) = 1 \qquad \text{(F-35)}$$

Next, a numerical set of Γ_j is chosen. Usually this is done in diameter space by selecting a minimum and maximum diameter, d_{min} and d_{max}. This might be done from prior knowledge of the sample, or by calculating a d_{min} by fitting the first few channels to a single exponential. (Remember: Γ_{max} corresponds to a d_{min} and is related to the fastest part of the correlation function decay at the smallest τ values.) Somewhat more arbitrarily a d_{max}, which corresponds to a Γ_{min}, is selected usually by multiplying d_{min} by 50 or 100. After the first results are obtained, if only zeros for the $G_I(\Gamma_j)$ are found at the beginning and end of the distribution, a second pass with a larger d_{min} and smaller d_{max} can be tried. However, it is fruitless to make the spread in these initial conditions too small to get a higher resolution result. Because, what usually happens is the results do not converge or converge to a non-physical result. Again, the limit is set by the ill-conditioned nature of the deconvolution problem, and the noise along the measured function.

Though possible with smooth correlation functions and a perfectly accurate baseline, rarely are reliable results closer than about 2:1 in size.

Given a max and a min value for Γ, the next decision is how many values (N) should be calculated. Again, somewhat arbitrarily, a number from 10 to 50 is selected. If the number is small, a second iteration starting with a separate set of max and min Γ values is selected, amounting to a shift from the first set. This does not

[8] Grabowski, E.F. and Morrison, I.D., in *Measurement of suspended particle by quasi-elastic light scattering*, B. E. Dahneke editor, Wiley-Interscience, New York (1983).

amount to obtaining a higher resolution result, but it does fill in results between widely spaced Γresults in the first set.

Finally, the spacing between a set of Γ_{min} and Γ_{max} is required. Early in the use of NNLS, linear spacing was used. But it was later found that this was overly optimistic. Once again, the limited resolution was better served by keeping a constant ratio between successive Γ values. This ratio can be calculated given Γ_{min}, Γ_{max}, and N. The stage is now set for the calculation. (Ratio spacing of results is also the reason that ratio spacing of correlator delay time values τ is available in advanced designs.)

This time, however, the parameters to be fit, the set of $G_I(\Gamma_j)$ are linearly related to $g^{(1)}(\tau)$ since the term $\exp(-\Gamma_j\tau)$ is just a number at each delay time τ and each one of the N values of Γ_j. Thus, the ill-conditioning has been lifted by this linearization trick.

Still, without using the non-negativity condition—the NN in NNLS—perfectly good fits can be obtained with some of the $G_I(\Gamma_j)$ being negative. But since these represent relative intensity values, which are inherently positive, adding the NN condition constrains the solution to a physically realistic set of positive $G_I(\Gamma_j)$. NNLS is useful if the distribution is known to have multiple peaks.

CONTIN (One form of Regularization)

As mentioned, one problem of ill-conditioning is that artifacts in the form of pseudo peaks can result, though they are usually not repeatable. One way around that is to add another term to the sum of the square of the residuals to smooth results and penalize spurious peaks.[9] See Eq.(F-36).

$$S = \sum_i w_i \cdot \left(g^{(1)}(\tau_i) - \sum_j G_I\left(\Gamma_j\right) \cdot exp\left(-\Gamma_j \cdot \tau_i\right)\right)^2 + \alpha \cdot f^{reg} \qquad \text{(F-36)}$$

The factor α is called the regularization parameter. It varies from 0 to 1. When it is zero, the result is the same as in Eq. (F-31). When it is 1, the smoothing term is as important as the minimization of the sum of the squares of the residuals. Over smoothing flattens peaks and makes it harder to separate them. Thus, compared to NNLS, CONTIN is best used on broad unimodal distributions (polymers, for example) or widely separated peaks such as 3:1 and higher.

Different forms for the regularization term f^{reg} are possible. The most common is the square of the sum of the 2nd derivative of $G(\Gamma_j)$. A program called CONTIN uses this method but allows for other choices instead of the 2nd derivative.[10] For each α, a different distribution is obtained. It is up to the user to determine which is appropriate. The default value for α is 0.5.

Summary Comments on Size Distributions using DLS

Before extracting a size distribution, make sure the measured correlation function is smooth and the baseline is flat. If not, getting more information than an average size and a polydispersity index is problematic. Distribution results will not be repeatable. Still, getting the first two moments to be repeatable is often enough to characterize the distribution.

Peaks closer than 2:1 apart are suspicious, and a few repeats should be attempted. A trimodal is almost always wrong, with one or more of the peaks being artifacts.

[9] Tikhonov, A.N, *Soc. Math*, **4**, 1035 (1963)
[10] Provencher, S.W., *Computer Phys. Comm.*, **27**, 213, (1982)

Problems with the baseline are apt to yield large diameter artifacts in the results. Recalculate with a different baseline to see if the large diameter peak appears again. Problems with high frequency noise along the correlation function are apt to yield small diameter artifacts in the results. Increase measurement duration, or laser power to see if they persist.

If two or more algorithms yield consistent results, then results are more believable. With multi-modals, if one peak's diameter is large enough that it will show an angular dependence, then make another measurement at a higher angle. The peaks should appear again, but the relative intensity (area under the curve in the differential distribution) should decrease at the higher angle for the larger particle.

Finally, compare the distribution with any other information you have. Is it consistent with other types of measurements including SLS results? Latex particles may aggregate but they do not usually fall apart. The appearance of a small-diameter peak is therefore probably an artifact.