DelsaMax Analysis Software Instructions For Use

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Manufactured for
Beckman Coulter, Inc.
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Brea, CA 92821 U.S.A.

BECKMAN COULTER

DelsaMax Analysis Software

Instructions for Use

B23931AA

Find us on the World Wide Web at:
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About DelsaMax Analysis Software

This chapter provides a brief overview of DelsaMax Analysis Software software and this manual. It also tells you how to contact Beckman Coulter for support.

What is DelsaMax Analysis Software?

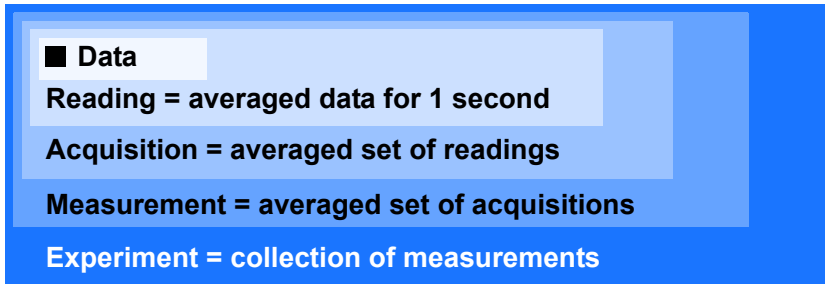
DelsaMax Analysis Software is proprietary light scattering instrument control software for nanoparticle research. It is used with the DelsaMax CORE and DelsaMax PRO instruments.

DelsaMax Analysis Software is focused on streamlining the process of data collection, analysis, and interpretation of the physical characteristics of solutions of particles. When used with the the DelsaMax CORE and DelsaMax PRO, DelsaMax Analysis Software provides a platform for analyzing individual samples over wide ranges of temperatures and laser intensities.

Special Terms

The following are special terms used in DelsaMax Analysis Software.

- **Dynamic Light Scattering (DLS):** The technique used by many DelsaMax instruments to determine the size distribution profile of particles by scattering laser light off the particles in suspension.
- **Data** - Instantaneous light scattering intensity values collected by an instrument. These data values are not stored by the DelsaMax Analysis Software software.
- **Reading** - Instantaneous light scattering intensity data collected and averaged over a one second interval.
- **Acquisition (Acq)** - A collection of readings and one correlation function collected over a user-specified period of time. For PALS data collected by the DelsaMax PRO, there is no acquisition level of data accumulation.
- **Measurement** - A collection of acquisitions (typically 5 to 10). The correlation functions are averaged and used to create the final intensity auto-correlation curve.
- **Experiment** - A set of measurements stored in a single experiment file.



The DeslsaMax instruments determine size distributions of particles in solution. Size distributions are defined by several terms:

- **Bin:** A discrete numerical particle size component of the histogram or size distribution that is defined by an x-axis value in nanometers (size), and an x-axis value in relative amount of light scattered by the bin to the other bins. The number of bins, the value or particle size represented by the bin, and the relative amount of scattered light are determined by numerical algorithms.
- **Mean Value:** The weighted average of the various size particles (bins or bars) in the distinct or resolvable population. The various sizes are weighted by their probability of being detected.
- **Modality:** Refers to the number of "peaks" in the size distribution. A size distribution with one peak is called Monomodal. A size distribution with more than one peak is called Multimodal. (Bimodal and Trimodal are common terms for size distributions with 2 or 3 peaks.)
- **Molar Mass:** The mass of a mole of the sample. It is shown in units of g/mol. Historically, the term "molecular weight" was sometimes used with the same meaning, but this has been deprecated in favor of "molar mass."
- **Peak:** A peak in a size distribution represents a distinct and resolvable species or population of analytes or particles. A peak is comprised of several size particles, represented by bins or bars, and is defined by a mean (average) value and polydispersity.
- **Polydispersity:** The standard deviation of the histogram that refers to the width of the peak. Sometimes referred to as the percent polydispersity (polydispersity divided by the mean value), it is a measure of the heterogeneity or homogeneity of the species comprising the population.
- **Size:** Refers to the radius or diameter of the particle modeled as a sphere that moves or diffuses in the solution (in contrast to the molar mass of the particle). Usually expressed as the mean value of the peak of the size distribution.
- **Size Distribution:** The manner in which the sizes of the particles are dispersed, spread, allocated among one or more peaks; presented in a graphical form known as a histogram.

The DelsaMax PRO determines electrophoretic mobility in solution. The following terms are use when describing data collected by the DelsaMax PRO:

- **Electrophoresis:** the migration of particles under the influence of an electric field.

- **Electrophoretic Mobility:** The ratio of a particle's velocity during electrophoresis to the applied electric field. This is a widely accepted proxy for molecular charge and interfacial potential, also known as the zeta potential.
- **Conductivity:** The ability of a solution to conduct electricity. Electrolytic conductivity is determined by measuring the resistance of the solution between two electrodes. It is commonly measured in units of siemens per meter (S/m).
- **Detector:** One of the photodetectors used to measure scattered light after it is recombined with the reference beam.
- **Dielectric Constant:** The relative permittivity under direct current of a substance.
- **Phase Analysis Light Scattering (PALS):** A light-scattering analysis technique in which a laser beam is split into a sample beam and a reference beam. The sample beam is scattered by moving particles, is collimated, and is recombined with the reference beam onto an array of photodetectors, each one of which makes an independent measurement of the mobility.
- **Quasi-Elastic Light Scattering (QELS):** Instruments with the optional QELS feature measure time-dependent fluctuations in scattered light using a fast photon counter. Since the fluctuations are directly related to the rate of diffusion of the molecule through the solvent, the fluctuations can be analyzed to determine a hydrodynamic radius for the sample.
- **Zeta Potential:** A measure of electrokinetic potential in a colloidal system. It is usually indicated by the Greek letter zeta. The zeta potential can be calculated using the electrophoretic mobility. Zeta potential is commonly expressed in units of millivolts.

Using this Manual

This manual describes how to use DelsaMax Analysis Software software for collecting and processing data. It is meant to be used in conjunction with the hardware manual that came with your instrument (for example, the *DelsaMax CORE Instrument for Use*). Setup and installation is covered in the hardware manual that came with your instrument.

This manual assumes a basic knowledge of Microsoft Windows features and mouse operations.

How This Manual is Organized

This manual is organized as follows:

Chapter 1, "About DelsaMax Analysis Software": provides a brief overview of DelsaMax Analysis Software software and this manual, and information on how to contact Beckman Coulter, Inc.

Chapter 2, "Getting Started": describes how to get started using DelsaMax Analysis Software.

Chapter 3, "Defining Instruments": describes how to define hardware.

Chapter 4, "Setting Parameters": provides information about the Parameters node including selecting solvents.

Chapter 5, "Automating Experiments": describes how to schedule events to occur during the course of an automated experiment. It also provides sample scripts of commonly scheduled events.

Chapter 6, "Recording Data": describes how to monitor data with the Instrument Control Panel and how to record data.

Chapter 7, "Displaying Data": describes how to manage and display large amounts of data captured by the instrument using the various data management and analysis tools available in DelsaMax Analysis Software.

Chapter 8, "Interpreting Data": helps you interpret the data obtained from the instrument by providing an overview of size distributions, correlation functions, and molar mass estimates.

Appendix A, "Analysis Methods": helps you understand the analysis methods employed by DelsaMax Analysis Software to generate size and size distribution information from autocorrelation function data, provides an overview of the mathematics and algorithms underlying the analysis, and describes when these methods are used by DelsaMax Analysis Software.

Appendix B, "Quick Reference": provides a list of menu bar commands.

Index: provides lookup assistance.

Manual Conventions

To make it easier to use this manual, we have used the following conventions to distinguish different kinds of information

- **Menu commands.** This manual indicates menu commands to use as follows: **File**—**Open**. This example indicates that you should open the **File** menu and select the **Open** command. You will see this style wherever menu commands are described.
- **Buttons.** In the text you will see instructions to "click" on-screen buttons and to "press" keys on the keyboard.
- **Key combinations.** A plus sign (+) between key names means to press and hold down the first key while you press the second key. For example, "Press ALT+ESC" means to press and hold down the ALT key and press the ESC key, then release both keys.
- **DelsaMax PRO:** Some parameters apply only to the QELS-enabled version of this instrument. Other parameters apply to all DelsaMax PRO instruments.

Contacting Beckman Coulter, Inc.

If you have a question about DelsaMax Analysis Software, first look in this manual or consult the online help that comes with DelsaMax Analysis Software for Windows. If you cannot find an answer, please contact your local Beckman Coulter representative.

Where to Go from Here

- Install DelsaMax Analysis Software software and set up your instrument(s). See the installation chapter in the hardware manual provided with your instrument(s).

- Continue to Chapter 2, "Getting Started" in this manual to get started using DelsaMax Analysis Software.
- Be sure to read your hardware manual before attempting to collect data using the software. It contains important safety and operational information.
- See the DelsaMax Analysis Software online help by choosing **Help ▸▸ Help Topics** from the menu bar.

2
Getting Started

This chapter shows you how to start DelsaMax Analysis Software and describes its various windows. It assumes that your instrument(s) have been set up as described in the installation chapter of the hardware manual provided with your instrument.

Installing DelsaMax Analysis Software

DelsaMax Analysis Software *must* be installed before connecting instruments to your PC.

System Requirements

As of the date of publication of this manual (January 31, 2013), the minimum system resources DelsaMax Analysis Software requires are listed below.

- DelsaMax Analysis Software requires either a 32-bit or 64-bit edition of Windows Vista (including the Business, Enterprise, and Ultimate versions), or Windows XP Professional 32-bit edition, or Windows 7 Professional 32-bit edition.
- Internet Explorer version 5.5 or higher
- Pentium IV or better processor
- 2 GHz or better processor speed
- 512 MB of RAM or better (1GB recommended)
- At least 75 MB of available hard-disk space
- CD-ROM Drive (optional for installation)

DelsaMax CORE and DelsaMax PRO must be connected to the PC via an Ethernet connection.

User Accounts with Restricted Privileges

If DelsaMax Analysis Software is to be run from a user account with restricted privileges, it is necessary to install DelsaMax Analysis Software under the account to be used. If DelsaMax Analysis Software is installed globally, you must have Windows Power User privileges to run DelsaMax Analysis Software.

Installing the Software

Install the software as follows:

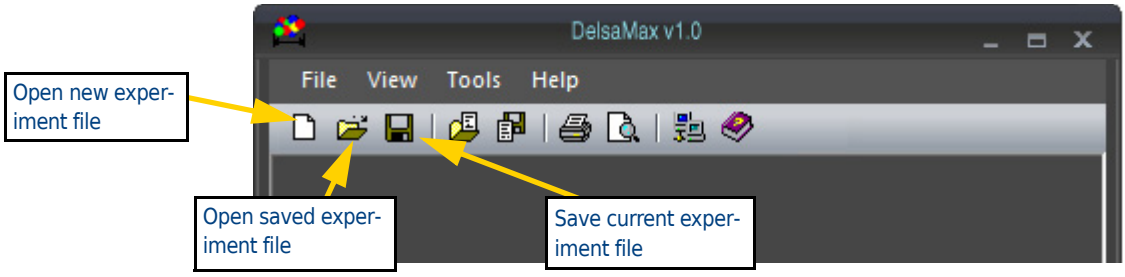
1. Restart your computer to ensure that no other programs are running, and that any previously installed DelsaMax Analysis Software components are not running.

2. Insert the DelsaMax Analysis Software CD in your CD drive. On most systems, the DelsaMax Analysis Software setup procedure will start automatically.
3. If the setup procedure does not start automatically, use Windows Explorer or the Run dialog to run the setup.exe file in the DelsaMax Analysis Software folder on the CD.
4. Answer the prompts in the setup procedure.
5. To verify installation of DelsaMax Analysis Software, open the Windows **Start** menu and look for **All Programs** ▸▸ **Beckman Coulter** ▸▸ **DelsaMax v1.0**

Starting DelsaMax Analysis Software

To start DelsaMax Analysis Software, choose
All Programs ▸▸ **Beckman Coulter** ▸▸ **DelsaMax v1.0** ▸▸ **DelsaMax v1.0**
from the Windows **Start** menu.

The main toolbar in DelsaMax Analysis Software holds a collection of shortcut buttons for performing various common tasks.



DelsaMax Analysis Software Windows

The main window in DelsaMax Analysis Software allows you to open multiple child windows from within the main window. You can move, rearrange, minimize or maximize the child windows. There are several window types:

- **Experiment Windows** are used to set up, run, and record/save data for experiments, and to view parameters and results of past experiments. Data recording (saving to memory) occurs from within an Experiment Window. See "Nodes in the Experiment Tree" on page 2-5 and "The Experiment Window Tool Bar" on page 2-6.
- The **Instrument Control Panel** is used to verify communications with the instrument and other external devices, to set basic parameters such as laser power, and to monitor data input. For more information, see "Monitoring Data with the Instrument Control Panel" on page 6-1.

Opening Experiment Files

If this is the first time you are running DelsaMax Analysis Software, please connect your instrument and power it on before creating a new experiment. This will enable DelsaMax Analysis Software to auto-detect your instrument settings.

If you forget to connect your instrument before creating a new experiment, you will see a message that says "No Hardware Defined." If this happens, exit DelsaMax Analysis Software, connect your instrument, then start DelsaMax Analysis Software again. Your instrument will now be auto-detected and available when creating a new experiment. Or, see "Adding Instruments to the List" on page 3-3 for information about defining instruments.

To open a new experiment, do one of the following:

- Select **File** ▸▸ **New** from the main menu bar.
- Click the new experiment icon  on the main toolbar.
- Press Ctrl+N.

To open an existing experiment file, do one of the following:

- Select **File** ▸▸ **Open** from the main menu bar.
- Click the open experiment icon  on the main toolbar.
- Press Ctrl+N.

Experiments you have opened recently are listed in the **File** menu.

Saving and Closing Experiment Files

To save the current experiment file, do one of the following:

- Select **File** ▸▸ **Save** from the main menu bar.
- Click the save icon  on the main toolbar.
- Press Ctrl+S.

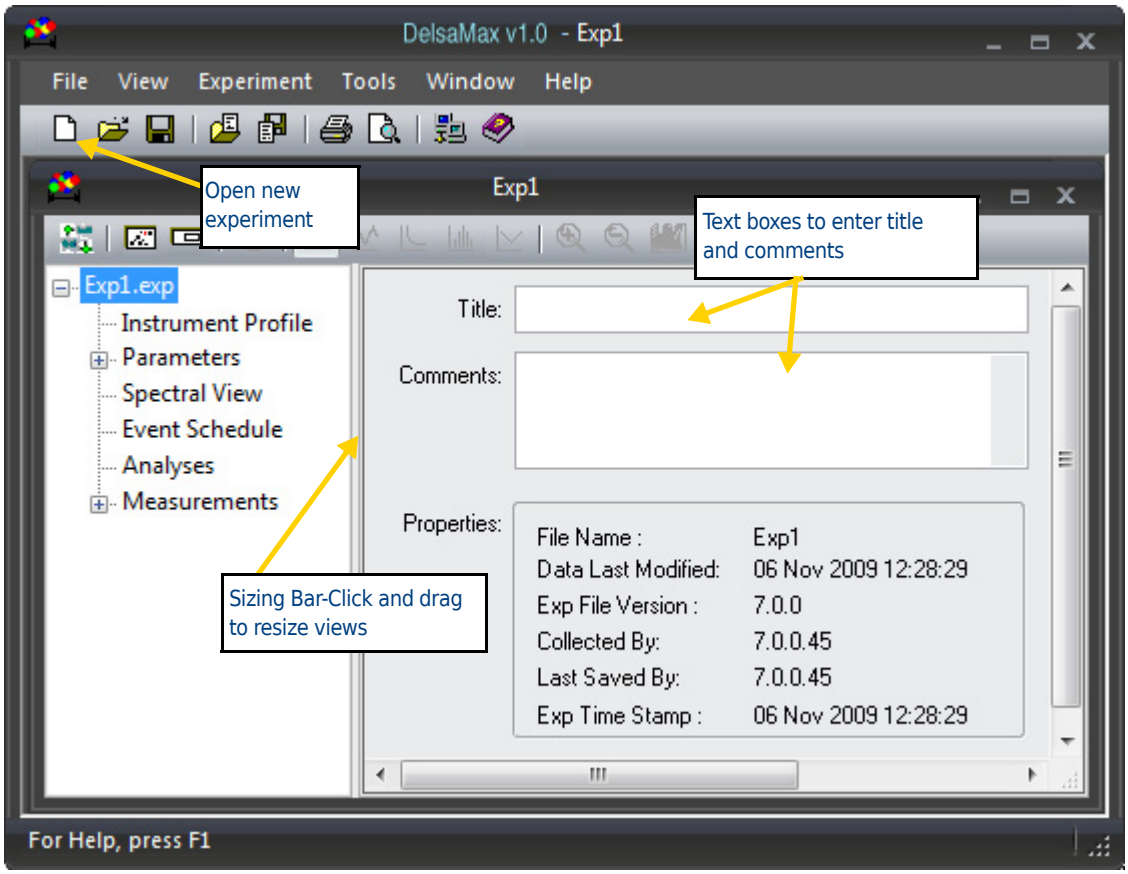
You can save the current experiment to a different file or location by selecting **File** ▸▸ **Save As** from the main menu bar.

You can save the current experiment in the DelsaMax Analysis Software format by selecting **File** ▸▸ **Save As** from the main menu bar.

You can close the current experiment file by choosing **File** ▸▸ **Close**. If you have not saved your changes, you will be asked if you want to save them. You are also prompted to save changes if you choose **File** ▸▸ **Exit** to exit from DelsaMax Analysis Software.

About the Experiment Window

An experiment window is opened within the main window. The experiment window is used to set up, run, and record/save data for new experiments, and to view parameters and results of past experiments.



The adjustable sizing bar in the experiment window separates the window into two areas—the experiment tree and the display.

- **Experiment tree** - The left side contains a list of categories within which the experimental information and data are grouped.
- **Display** - The right side displays the specific information, parameters, and/or data associated with the particular node selected in the experiment tree. Selecting a node in the experiment tree changes what you see in the display view.

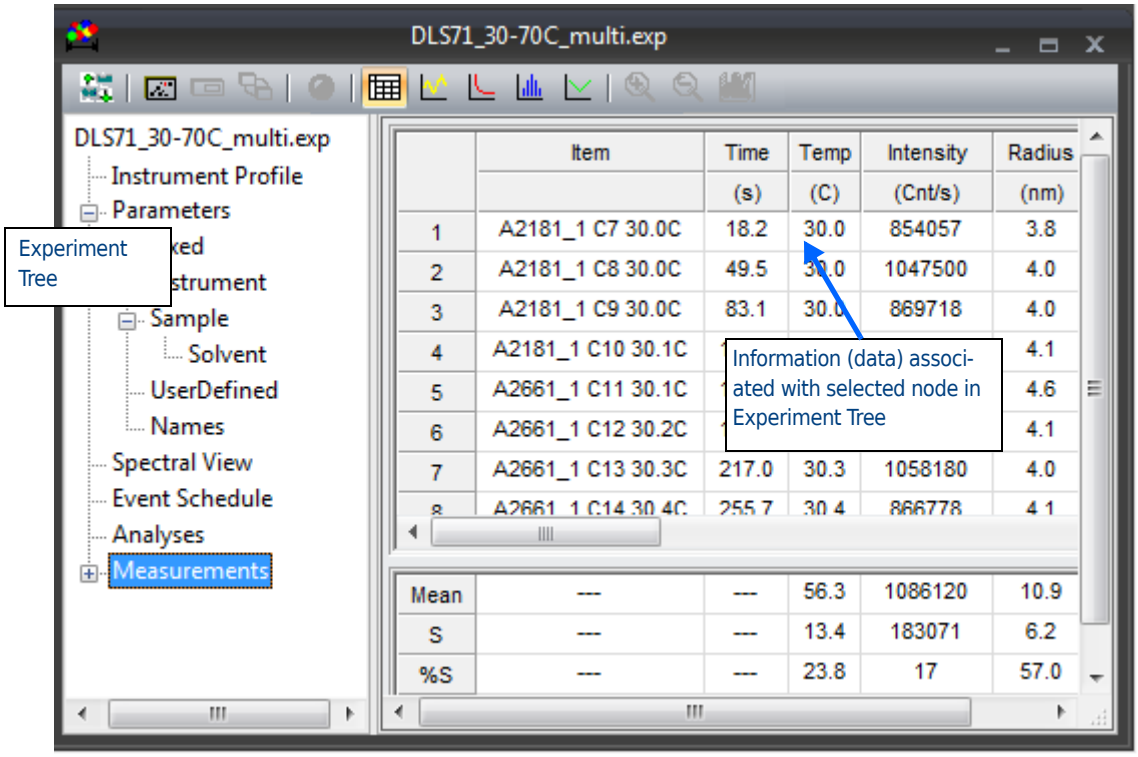
Note: If you have not yet set a "next" sample definition, as is the case the first time you use DelsaMax Analysis Software, you will see a message about the default sample definition that was created for this new experiment.

When the top node of the experiment tree is selected, you see information about the experiment file: including the filename, when the file was last modified, when the data was collected, and the versions of DelsaMax Analysis Software used to perform various actions.

You can open multiple windows for the same experiment. For example, you might want to do this so that you can view the Datalog Grid and Datalog Graph at the same time. To open another window for the current experiment, choose **Window** ▸▸ **New Window** from the main menu bar. The **Window** menu also provides the following commands for organizing multiple windows: **Cascade**, **Tile Horizontally**, **Tile Vertically**, and **Arrange Icons**.

Nodes in the Experiment Tree

The experiment tree in DelsaMax Analysis Software is used to select groups or categories of information for viewing in the display side of the experiment window. The main nodes in the experiment tree are: Instrument Profile, Parameters, Event Schedule (optional), Analyses, and Measurements. Some nodes are not available for certain types of instruments.



Instrument Profile Node

The Instrument Profile node contains parameters and settings necessary to describe the instruments associated with the experiment. You add and remove instruments to this node using the list boxes in the properties table. To view parameters and settings for a specific instrument, select a component in the Instrument Profile node. See "Using the Instrument Profile Node" on page 3-2.

Parameters Node

The Parameters node contains all settings needed to describe experiment conditions, such as instrument settings and time limits, along with user-defined parameters. It also contains all parameters and settings for calculations, such as the analysis to perform and the solvent viscosity. You edit parameters and settings by selecting the appropriate sub-category in the parameters node. See "Setting Experimental Parameters" on page 4-1.

Event Schedule Node

The Event Schedule node contains a schedule of user-defined actions or events that are to occur (or did occur) during the course of an automated experiment. There are no sub-categories associated with the event schedule. See "Automating Experiments" on page 5-1.

Analyses Node

The Analyses node lists any parameter analyses you have added to the experiment. For example, these may include an analysis of radius vs. temperature measurements. You can examine the data in sub-groups by sample. See "Analysis Views" on page 7-33.

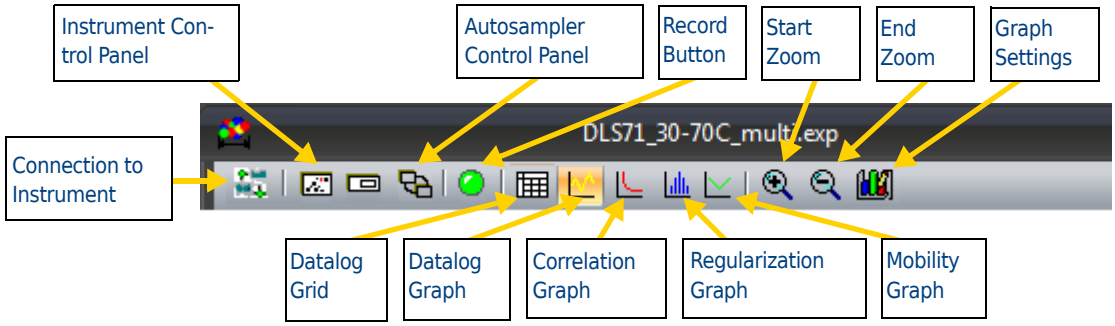
Measurements Node

The Measurements node contains all the measured and calculated data collected during the course of an experiment. For DelsaMax PRO instruments with a QELS unit, each measurement node has subnodes for DLS and PALS data. DelsaMax PRO instruments without QELS support show only PALS data. Other instruments show only DLS data. DLS data has subnodes for each acquisition. PALS data has subnodes for detectors and readings.

The display format for the information in the measurement node is dependent upon which view button is selected in the experiment window toolbar. See "Displaying Data" on page 7-1.

The Experiment Window Tool Bar

The icon buttons on the toolbar in the experiment window are used to select the display format of the data contained in the measurement node of the experiment tree, to start and stop data recording and automated experiments, and to open various worksheets and control panels. Brief descriptions of each button are given below.



Connect to Instrument - The Connect to Instrument button opens a connection to the selected instrument. DelsaMax Analysis Software must be connected to the instrument before you can collect data. See "Connecting to Instruments" on page 6-6.

Click the Connect to Instrument button once to begin collecting data from the instrument and storing it in the experiment file. Click again to disconnect if you wish to stop the flow of data into the experiment file.

Instrument Control Panel - The Instrument Control Panel displays the control panel for monitoring data and/or adjusting control parameters before recording data. See "Monitoring Data with the Instrument Control Panel" on page 6-1.

Autosampler Control Panel - Used with a DelsaMax PRO that is connected to an autosampler. See "Controlling the Autosampler" on page 6-8.

Record Button - The Record button is used to start and stop recording data into an experiment window, and to start and stop automated experiments. See "Recording Data" on page 6-1.

Datalog Grid View - The Datalog Grid View provides a table of the data and parameter values for the data selected within the Measurements node of the experiment tree. Other than direct data editing, the features and available functions in the Datalog Grid view are similar to those incorporated into standard spreadsheet type software packages. A statistical analysis of the data is also available in the grid view via the right-click menu. All data, including that contained in the parameters node, can be displayed in this view. See "Datalog Grid" on page 7-14.

Datalog Graph - The Datalog Graph displays a graph of the data and parameter values for the data selected within the Measurements node of the experiment tree. Format and display features are similar to those in standard graphing software packages, with the added benefit of having the displayed data linked to application-specific algorithms and worksheets. All data, including user-defined parameters, can be displayed in this view. See "Datalog Graph" on page 7-20.

Correlation Graph - The Correlation Graph displays the auto-correlation curve for the data selected within the Measurements node of the experiment tree. Overlay and complementary view options include: best fit curves, baselines, channel cutoffs, and residuals. See "Correlation Graph" on page 7-21.

Regularization Graph - The Regularization Graph displays the size distribution derived from a Regularization analysis of the auto-correlation curve for the data selected within the Measurements node of the experiment tree. Display options include: Radius, Diameter, Diffusion Coefficient, and Decay Time for the X axis, and %Intensity and %Mass for the Y axis. See "Regularization Graph" on page 7-25.

Mobility Graph - The Mobility Graph displays the electrophoretic mobility vs. time. See "Mobility Graph" on page 7-30.

Start Zoom - To zoom in on a graph, click this icon and drag the mouse over the area you want to enlarge. For more about resizing graphs, see "Scaling Graphs" on page 7-12.

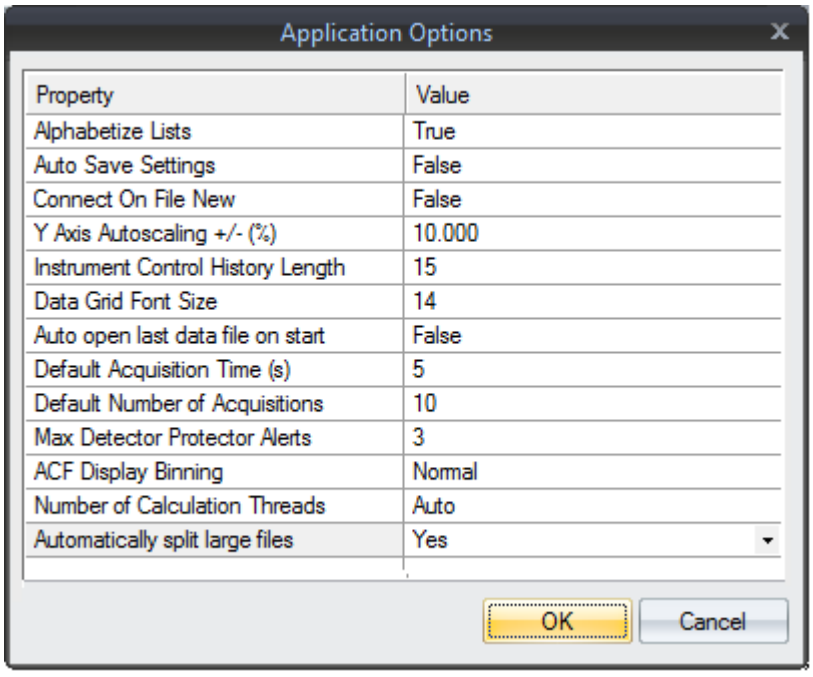
End Zoom - To zoom out on a graph, click this icon to return to autoscaling.

Graph Settings - Click this icon to open the Graph Setup dialog. For details about this dialog, see "Scaling Graphs" on page 7-12.

Setting Application Options

Use the Application Options window to view and edit control and display parameters that are applied throughout the DelsaMax Analysis Software application.

Select **Tools** ▸▸ **Options** from the main menu bar to open the Application Options dialog. Each property is described in the list that follows.



Alphabetize Lists: True or False setting indicating whether list boxes in the Table Settings window and the Datalog Graph are alphabetized.

Auto Save Settings: True or False setting indicating whether you want DelsaMax Analysis Software to automatically save the workspace settings as the defaults when you exit from the software.

Connect On File New: True or False setting indicating if DelsaMax Analysis Software will automatically connect to the instrument when a new file is opened.

Y Axis Autoscaling +/- (%): The percentage of 1) the maximum data value added to the maximum, and 2) the minimum data value subtracted from the minimum, to determine Y-axis scaling limits in the Trace, Correlation, and Regularization Graphs.

Instrument Control History Length: Number of instantaneous readings displayed during data monitoring with the Instrument Control Panel.

Data Grid Font Size: The font size for the Grid View.

Auto open last data file on start: Set to True to automatically open the last data file upon start up.

Default Acquisition Time (s): The default acquisition time used in the instrument parameters for a new experiment.

Default Number of Acquisitions: The default number of acquisitions in the instrument parameters for a new experiment.

Max Detector Protector Alerts: Sets the number of consecutive detector protector alerts that will be issued before the experiment is stopped.

ACF Display Binning: Choose None, Normal, or Heavy. Sets the amount of binning of adjacent X and Y values for display of the correlation graph. “None” results in a correlation graph display of strictly raw values. “Normal” results in moderate binning for some cases, while “Heavy” results in the greatest degree of binning. This parameter does not influence data analysis or data export, which always uses raw values.

Number of Calculation Threads: This parameter controls how many processing threads are used to perform calculations. If you use the default “Auto” setting, DelsaMax Analysis Software uses the maximum number of threads appropriate for the computer. You should change this setting only if you are running other processing-intensive programs at the same time as DelsaMax Analysis Software and notice the other programs running more slowly than needed.

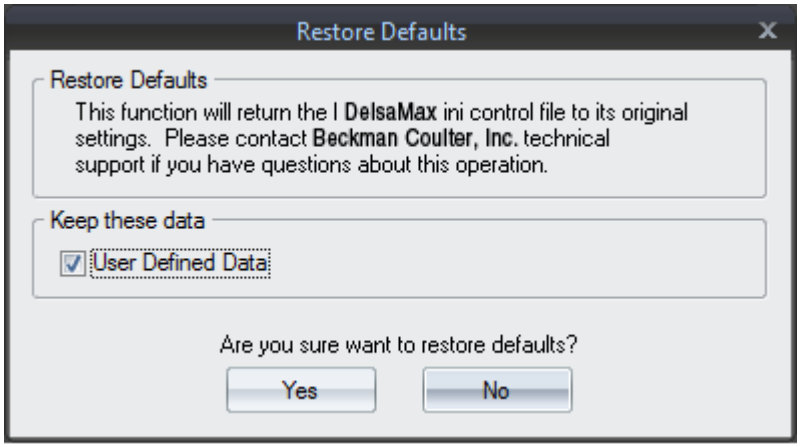
Automatically Split Large Files: If the experiment file becomes too large for the file format during data collection, the file is automatically split into separate files with sequential numbers added to the filename. For example, “experiment-1” and “experiment-02”. You can disable this behavior by changing the setting of this option. Other options are to stop the event schedule run when the file becomes too large or to ignore the problem and continue collecting data.

Diagnostic Tools

DelsaMax Analysis Software provides the following diagnostic tools.

Restoring Defaults

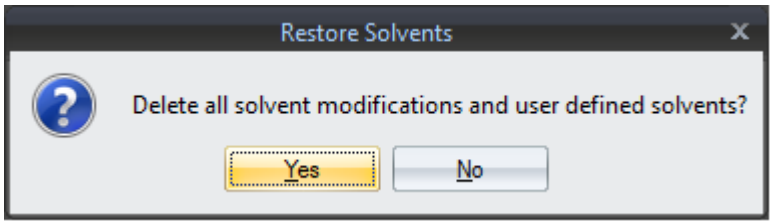
Select **Tools** ▸▸ **Diagnostics** ▸▸ **Restore Defaults.** You see this dialog:



If you click **Yes**, all defaults and instrument configurations that have been set on this computer will be deleted.

Restoring Solvents

Select **Tools** ▸▸ **Diagnostics** ▸▸ **Restore Solvents.** You see this message.



Clicking **Yes** deletes all user-defined solvents and solvent modifications.

Write EEPROM

Select **Tools** ▸▸ **Diagnostics** ▸▸ **Write EEPROM.** This functionality is reserved for Beckman Coulter use.

Running Calculations

Select **Tools** ▸▸ **Diagnostics** ▸▸ **Running Calculations** to see if any calculations have not finished running. This feature is used for diagnostic purposes by Beckman Coulter Technical Support.

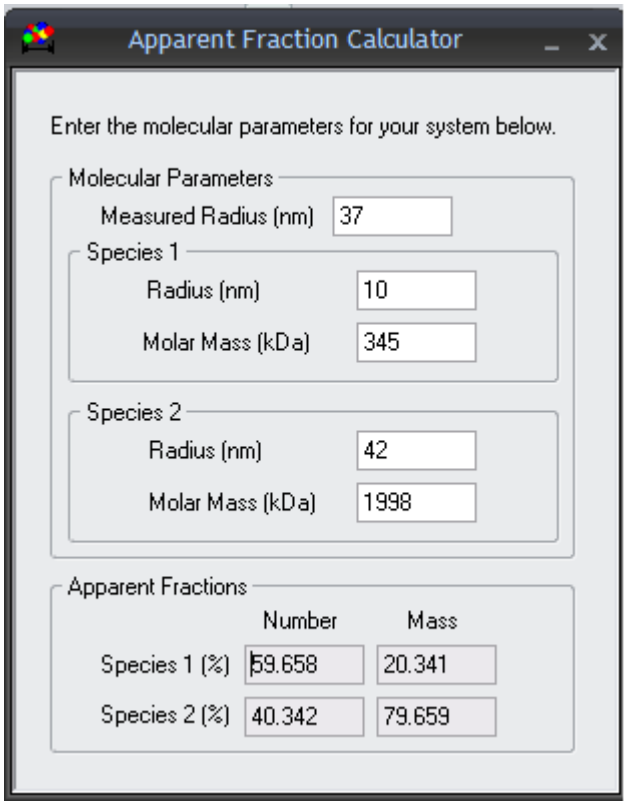
Calculators

DelsaMax Analysis Software provides several calculators you can use to compute values you may need.

Using the Apparent Fraction Calculator

The Apparent Fraction Calculator calculates the fractions of two species in a mixture by number and mass when given the overall measured radius (in nm) and the radius and molar mass (in kDa) for the two species.

1. Select **Tools** ▸▸ **Calculations** ▸▸ **Apparent Fraction** to calculate the fraction of two similar components mixed in the sample.



2. Type the measured hydrodynamic radius (in nm) for the overall mixture.
3. Type the hydrodynamic radius and molar mass (in kDa) for the two most common components of the mixture. The accuracy of the results is dependent on any other species being very uncommon in the mixture.
4. The measured hydrodynamic radius is interpreted as a combination of the scattering from species 1 and 2.

This calculator is helpful when a dimer/monomer (or trimerization or tetramerization) equilibrium is suspected. The need for this calculator arises when the regularization algorithm can not separate a distinct oligomeric peak. (This situation is, by itself, an indication that the worst case scenario is a hexamer/monomer mix.)

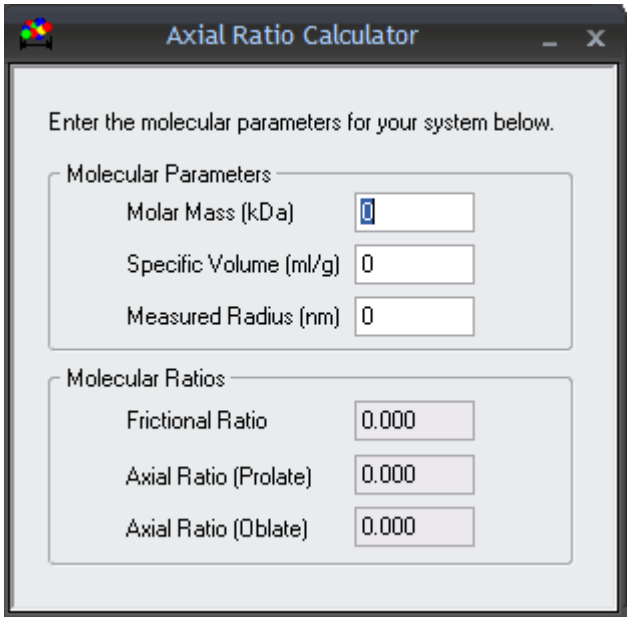
Note: Be aware that a hydrodynamic radius increase can also be the result of a shape change!

For more information, see Lunelli, L.; Bucci, E.; Baldini, G. "Electrostatic Interactions in Hemoglobin From Light Scattering Experiments", *Physical Review Letters* 1993, 70(4), 513-516.

Using the Axial Ratio Calculator

The Axial Ratio Calculator calculates an estimate of the shape of the molecule. The molar mass, specific volume (typically about 0.8 ml/g), and the measured hydrodynamic radius are used to generate a shape prediction. The two shape models are a prolate ellipsoid (an egg-shape) or an oblate ellipsoid (doughnut or saucer shape). The ratio of major to minor axis is reported.

1. Select **Tools** ▸▸ **Calculations** ▸▸ **Axial Ratio**.



2. Type the known or measured values for molar mass (in kDa), specific volume (in ml/g, the inverse of density), and the measured radius (in nm).
3. The calculator will compute the frictional ratio and the axial ratio for both prolate (elongated) and oblate (flattened) spheroids.

The Axial Ratio Calculator takes the inverse of the specific volume you enter to find the density of the protein. The density is a measure of "how much volume this protein occupies per mass," so the product of the molar mass and the specific volume is the calculated volume occupied by one ("solid") protein molecule. Using the standard equation for the volume of a sphere, an equivalent spherical radius of the molecule can be determined, under the assumption that the molecule is spherical.

The ratio of the measured hydrodynamic radius to the theoretical radius is the frictional ratio: the ratio of the true friction due to its hydrodynamic shape compared to its theoretical friction if it were a (solid) globular molecule. The frictional ratio is also called the Perrin factor. Tables of the Perrin factor for different shapes and axial ratios are published and lead to the result of this calculator.

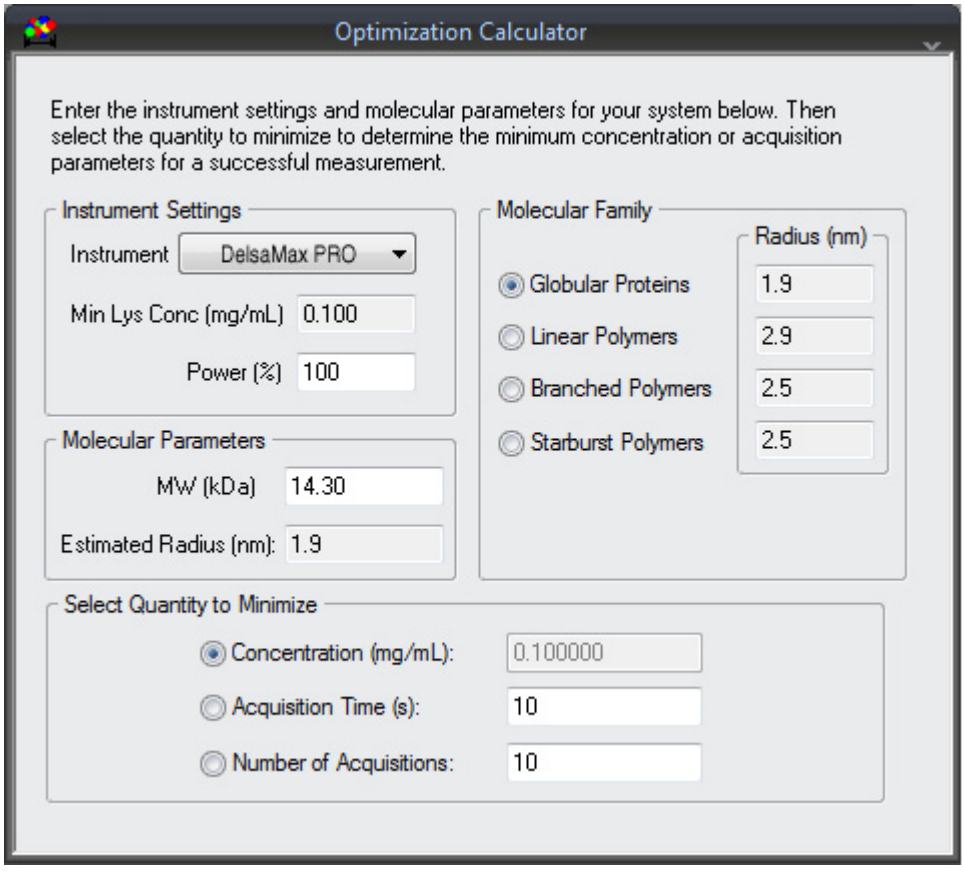
Note: Estimates from the Axial Ratio Calculator can be off due to hydration of the molecule. The frictional ratio reported by this calculator is really the product of a contribution due to the shape and a contribution due to the hydration of the molecule. The hydration contribution is not taken into account by this model.

For details about the model used in this calculator, see the chapter "Effects of shape on translational frictional properties" in *Biophysical Chemistry, Part II: Techniques for the study of biological structure and function* by Charles R. Cantor and Paul R. Schimmel, Freeman and Company publishers, New York 1980. Also of interest concerning shape determination is "Quasi-elastic light scattering and analytical ultracentrifugation are indispensable tools for the purification and characterization of recombinant proteins" by H.-J. Schönfeld, B. Pöschl and F. Müller in *Advances in Ultracentrifugation Analysis*, Biochemical Society Transactions, vol. 26, pp. 753-758, 1998.

Using the Optimization Calculator

The Optimization Calculator provides a convenient way to determine the concentration, acquisition time, and number of acquisitions that will be necessary to obtain a good correlation function.

1. Select **Tools** ▸▸ **Calculations** ▸▸ **Optimization**. The Optimization Calculator is displayed.



2. In the Instrument Settings area, select the type of instrument you are using. Type the laser Power (%) setting you will use—that is, the one you will set in the System tab on your instrument display.
3. Select the Molecular Parameters and Molecular Family to match your sample of interest.

4. In the portion of the dialog entitled **Select Quantity to Minimize**, select the item for which you want to find the minimum amount required to obtain good data. For example, in the previous figure, for the specified sample parameters, acquisition time, and number of acquisitions, the minimum concentration of sample needed for a good measurement is 0.1 mg/ml.

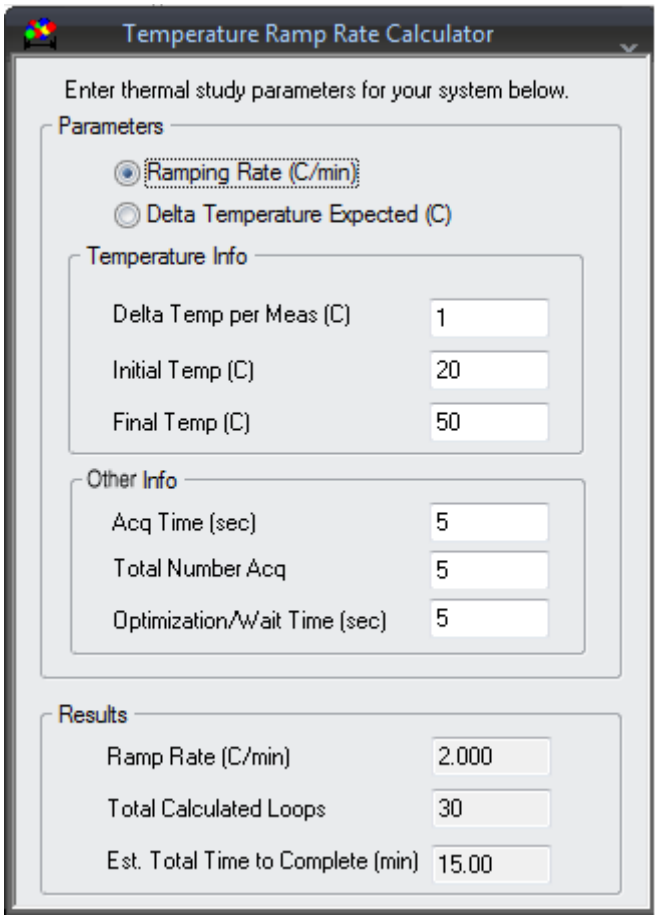
Using the Ramp Rate Calculator

In a "thermal ramping" experiment, data are collected while the system is continuously ramping or changing the temperature. The instrument does not equilibrate at each successive temperature "step." Instead, the instrument acquires data at the current temperature without "stopping" at the thermal step. The desired ramp rate is determined by the desired thermal resolution and acquisition time. Typical ramp rates range from 0.005 °C/min when measuring in 1 °C increments to 0.1 °C/min when measuring in 5 °C increments.

Temperature ramping is available only for the temperature-controlled DelsaMax CORE.

The Ramp Rate Calculator provides a convenient way to determine values for use in the Event Schedule, such as the temperature ramp rate, the change in temperature per measurement, the number of loops required, and the estimated total time for the run.

1. Select **Tools** ▸▸ **Calculations** ▸▸ **Ramp Rate** to open the calculator.



2. Choose whether you want to compute the ramp rate (in °C/min), the change in temperature (in °C) expected between measurements. The other fields in the calculator change slightly based on your choice of what to solve for.
3. Specify the temperature values for your experiment.

   Note: If you are using a DelsaMax CORE, set the **Total Number of Wells** to 1 when using this calculator.

The **Delta Temp per Meas** is the change in temperature between successive measurements. It is used, along with the difference between the **Final Temp** and **Initial Temp**, to calculate the **Total Calculated Loops**.

The **Ramp Rate** is calculated by dividing the difference between the **Final Temp** and **Initial Temp** by the **Est. Total Time to Complete**.

You can use the recommended ramp rate and other calculated values in your experiment.

Saving Parameters and Workspace Settings

If you routinely use a particular set of experimental parameters, you can save them for reuse as follows:

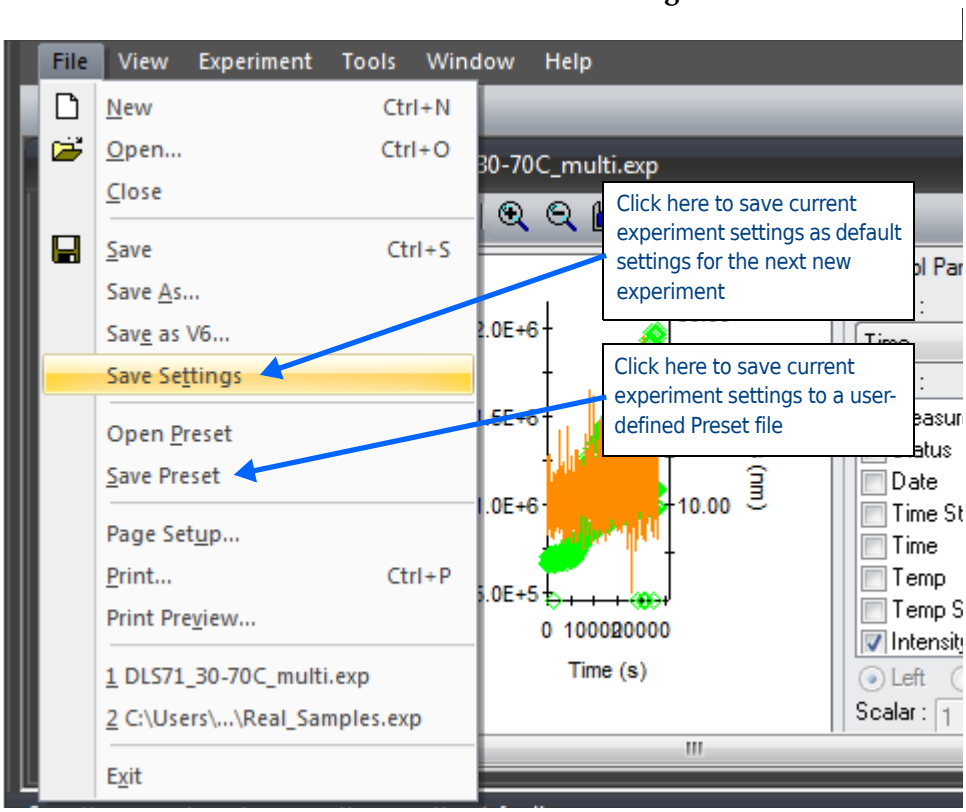
- Select **File** ▸▸ **Save Settings** from the menu bar. This saves the current settings as the defaults for any new experiments you create.
- Select **File** ▸▸ **Save Preset** (or use the  toolbar icon) from the menu bar. You are prompted to choose a file name and location for the Preset file. The file extension for these files is .pst. You can later reuse a Preset file by selecting **File** ▸▸ **Open Preset** (or use the  toolbar icon) from the menu bar.

A Preset file stores all of the following types of settings:

1. Instrument configuration
2. Application-wide options
3. Table and graph settings
4. Properties and values from the Parameter node and sub-nodes
5. Templates for samples, solvents, names, and user-defined values
6. Event Schedule commands

Some of these settings override values in the Application Options window.

When you choose **File** ▸▸ **Save Settings**, items 1 through 4 on the previous list are saved. Templates and Event Schedules are not saved with **File** ▸▸ **Save Settings**.



3

Defining Instruments

This chapter describes how to detect your default instrument configuration and define new instruments and configurations.

Some sections in this chapter are only applicable to specific instruments. The section title will contain the name of the specific instruments to which it applies.

Autodetecting Instruments

DelsaMax Analysis Software can autodetect your instrument configuration.

If you have not yet defined any instruments using **Tools** ▸▸ **Instruments**, and no USB instrument connection is found, DelsaMax Analysis Software automatically starts searching for an instrument on your network when you create a new experiment.

If you are using DelsaMax Analysis Software for the first time:

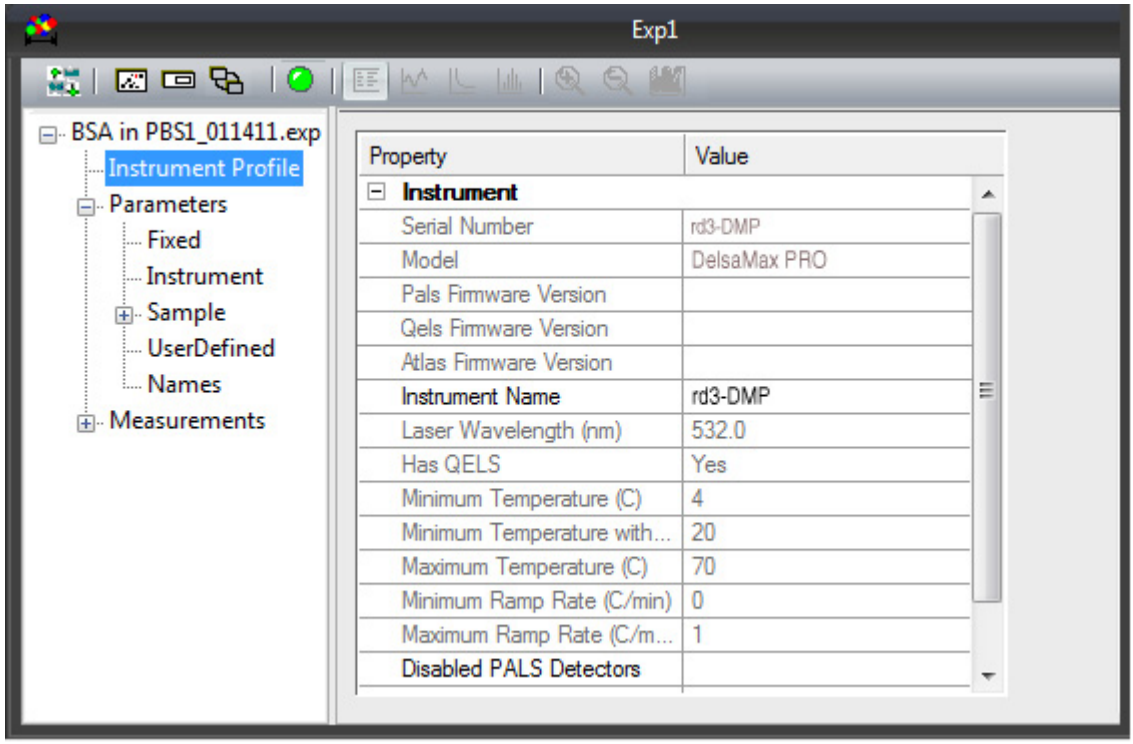
1. Connect your instrument and power it on.
2. Start DelsaMax Analysis Software and choose **File** ▸▸ **New**.
3. DelsaMax Analysis Software auto-detects the instrument configuration, which you can view either in the Instrument Profile node or by selecting **Tools—Instruments**.

Note: If the Add Instrument Profile dialog is displayed when you select **File** ▸▸ **New**, this indicates that your instrument is not connected and powered on. If this is the case, please select **Cancel** in the Add Instrument Profile dialog, then exit DelsaMax Analysis Software. Connect your instrument, verify that it is powered on, and confirm that the drivers have been installed correctly. (Please see the installation chapter in the hardware manual provided with your instrument.) Restart DelsaMax Analysis Software. Your instrument will now be auto-detected and available.

If you want to autodetect a new instrument that is different from an earlier instrument you used with DelsaMax Analysis Software, simply connect the new instrument, then click the **Detect** button in the Create Instrument Profiles dialog. DelsaMax Analysis Software first detects USB connected instruments, then any network-connected instruments.

Using the Instrument Profile Node

The Instrument Profile node for an experiment shows the instrument currently selected for use in the experiment.



The list of properties is different depending on the type of instrument you are using.

If you have not yet run the experiment, you can select a different instrument from the drop-down list of defined instrument serial numbers. If the experiment has already been run, you cannot change the instrument.

Some default instrument parameters can be changed on a per experiment basis.

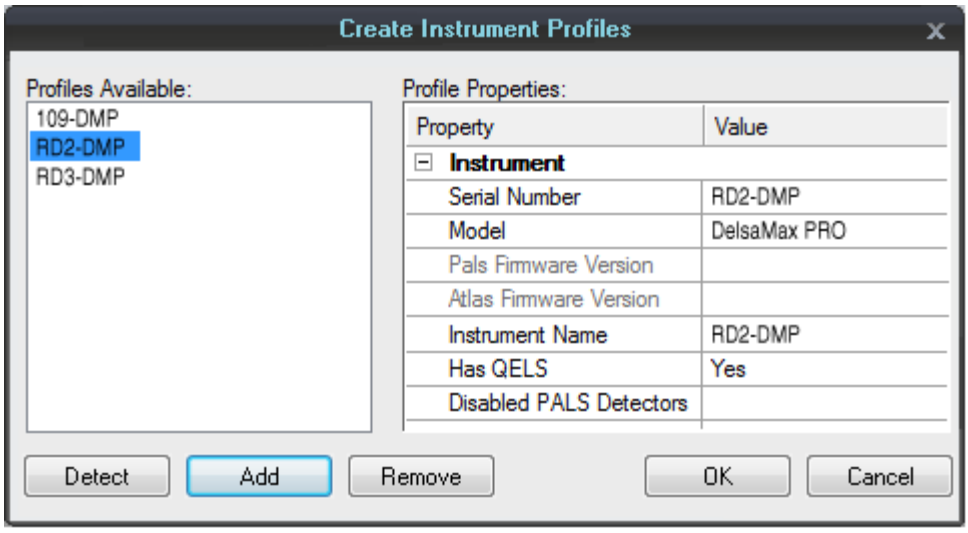
For DelsaMax PRO instruments, you can disable one or more PALS detectors in this node by clicking "..." and checking boxes next to detectors you want to disable for this experiment.

The minimum and maximum supported temperatures and ramp rates are shown in this node for temperature-controlled instruments.

If a DelsaMax PRO instrument is connected to an autosampler, select the autosampler using the **Serial Number** field below the Autosampler node. The autosampler may have been auto-detected or added manually.

Adding Instruments to the List

You can define and detect new instruments or edit existing components using the **Tools** ▸▸ **Instruments** window, which is available from the main menu bar.

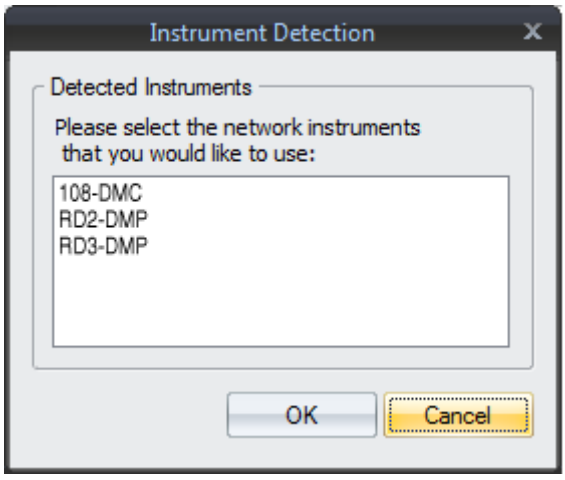


To find instruments connected to the network, click **Detect**. To add instruments manually, click **Add**.

Detecting Equipment

To look for instruments you can used with DelsaMax Analysis Software, follow these steps:

1. Choose **Tools** ▸▸ **Instruments** from the main DelsaMax Analysis Software menu.
2. Click the **Detect** button in the Create Instrument Profiles dialog. If an instrument is connected to a USB port, that instrument will be detected first, before network-connected instruments are detected. You see a list of the instrument serial numbers that were found.



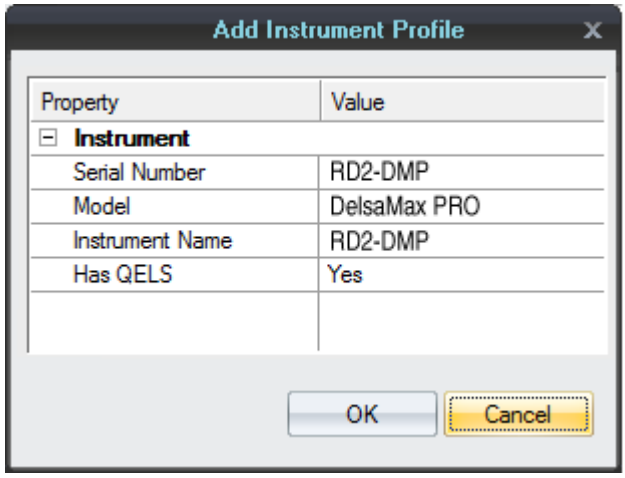
3. In the **Instrument Detection** dialog, choose your instrument and click **OK**.

4. Information about the Instrument is shown in the Create Instrument Profiles dialog. For host instruments, you can edit the instrument name. For instruments with optics blocks, you can select a different optics serial number if you have more than one.
5. Click **OK** in the **Create Instrument Profiles** dialog to save your selection.

Adding Instruments Manually

To specify information about an instrument, follow these steps:

1. Choose **Tools** ▸▸ **Instruments** from the main DelsaMax Analysis Software menu.
2. Click the **Add** button in the Create Instrument Profiles dialog. You see the Add Instrument Profile dialog.



Provide information about your instrument as follows. Different fields are shown for different host and optics models.

General Settings

- **Serial Number** - Type the serial number for your host unit. This number will be used to identify the instrument in DelsaMax Analysis Software.
- **Model** - Select the model of the host unit.
- **Pals Firmware Version** - Displays the detected version of any PALS firmware on the instrument.
- **Qels Firmware Version** - Displays the detected version of any QELS firmware on the instrument.
- **Instrument Name** - For networked instruments, type the instrument's network name. For USB-connected instruments, type nothing.

DelsaMax PRO Settings

- **Has QELS** - If your instrument has the QELS option, select Yes for this field.
- **ASSIST Firmware Version** - Displays the detected version of the firmware on any ASSIST instrument connected to the DelsaMax PRO.

Autosampler Controller Settings

- **Autosampler Firmware Version** - Displays the detected version of the firmware on any autosampler connected to the DelsaMax PRO.
- **Hardware ID** - Displays the detected hardware ID of any autosampler connected to the DelsaMax PRO.

To use an autosampler with the DelsaMax PRO, you must have installed the autosampler control service on the computer that runs the autosampler. This may be a different computer than the one running DelsaMax Analysis Software. See the documentation provided with the autosampler control service software for details.

DelsaMax instrument Settings

- **Laser Wavelength (nm)** - Type the laser wavelength in nanometers for the system.
- **Internal Laser** - Check this box if the laser is contained in the host unit (not the optics block).

Optics Settings

- **Optics Serial Number** - Type the serial number for the optics block.
- **Optics Model** - Select the model of the optics block. Ignore this field if you have a DelsaMax CORE.
- **Temperature Control** - Highlight this button if the optics block includes temperature control.
- **Scattering Angle** - Enter the scattering angle in degrees for the optics.

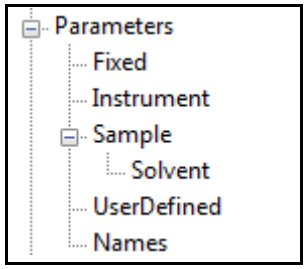
4

Setting Parameters

This chapter describes how to set experimental parameters, including selecting and defining solvents.

Setting Experimental Parameters

Experimental parameters are defined in the Parameters node, which can be expanded into several sub-nodes: **Fixed**, **Instrument**, **Sample**, **UserDefined**, and **Names**. The **Sample** node has a **Solvent** sub-node and may have a **Cuvette** sub-node.



Fixed Parameters

The **Fixed** sub-node contains parameters that are applied to all measurements within the experiment. You can change these parameters before or after data collection with no irreversible effects on data storage. The parameters are described in "Fixed Parameters Node" on page 4-3.

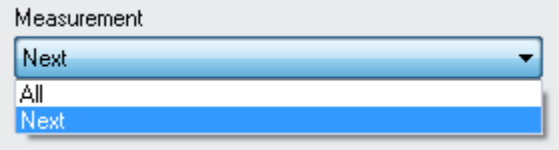
Variable Parameters

The **Instrument**, **Sample**, **Solvent**, **Cuvette**, **UserDefined** and **Names** sub-nodes contain variable parameters that can be different for each measurement.

- The **Instrument** parameters are described in "Instrument Parameters Node" on page 4-5.
- The **Sample** parameters are described in "Sample Parameters Node" on page 4-8. For details on setting samples, see "Managing Sample Definitions and Assignments" on page 4-11.
- The **Solvent** parameters are described in "Solvent Parameters Node" on page 4-10. For details on setting solvents, see "Managing Solvent Definitions and Assignments" on page 4-14.
- The **Cuvette** sub-node is available only if you are using a DelsaMax CORE. For details on working with cuvettes, see "Managing and Calibrating Cuvettes" on page 4-18.
- The **UserDefined** sub-node is described in "Creating User-Defined Parameters" on page 4-25.
- The **Names** sub-node is described in "Creating a Measurement Naming Template" on page 4-27.

Setting Parameter Values

1. Select the appropriate Parameters sub-node in the experiment tree.
2. Double-click in the value cell and type or select the new value. Then move to another cell (or use the Enter button on your keyboard).
3. For the **Instrument** and **UserDefined** sub-nodes, the **Measurement** list box at the bottom of the property table lets you vary parameters between measurements. Choose **Next** (the default) to apply your property changes only to future measurements taken. Choose **All** to apply your property changes to both previously collected measurements and to future measurements. If you have already collected data in this experiment, you can also choose to apply a parameter change to a specific measurement only.



Saving Parameters

If you routinely run experiments with the same parameter settings, use the **File** ▸▸ **Save Settings** command in the menu bar to save the current experiment settings (including parameter values) as the defaults for new experiments.

Parameter Descriptions

The following sections describe the parameters defined in the Parameters node of the DelsaMax Analysis Software software. User-defined parameters are described in "Creating User-Defined Parameters" on page 4-25.

Fixed Parameters Node

The **Parameters** ▸▸ **Fixed** node of the experiment tree contains the following parameters. Different parameters are listed for different types of instruments. These are called "fixed" parameters because your settings for these parameters are the same for all measurements in an experiment. You can change these parameters before or after data collection with no irreversible effects on data storage.

Property	Value
Real Time Data Filter	Yes
⊟ **Cutoff**	
Correlation function low cut-off (µs)	1.44
Correlation function high cut-off (µs)	6.29e+004
⊟ **Peak Radius Cutoffs**	
Peak Radius Low Cutoff(nm)	1.000
Peak Radius High Cutoff(nm)	5000.000
Analysis Type	Dynals
Measurement Time Limit Factor	5.000
Auto-attenuation Time Limit(s)	60.000
Calculate D10/D50/D90	No
Calculate Polydispersity	Yes
Event Schedule	Yes

General Parameters

Real Time Data Filter: Choose Yes or No for whether or not the data filter algorithms should be applied in real time (that is, while the data are being collected). See "Real Time Data Filtering" on page 7-45.

Correlation Function Low Cut-off: The lower fit limit of the time-delay (in microseconds for the x-axis range) of the autocorrelation function that is analyzed. Time delay values on the x-axis below the specified Correlation Function Low Cut-off, and the corresponding intensity autocorrelation coefficients on the y-axis, are ignored in the Cumulants and Regularization algorithm analysis of the autocorrelation function.

Correlation Function High Cut-off: The upper fit limit of the time-delay (in microseconds for the x-axis range) of the autocorrelation function that is analyzed. Time delay values on the x-axis above the specified Correlation Function High Cut-off, and the corresponding intensity autocorrelation coefficients on the y-axis, are ignored in the Cumulants and Regularization algorithm analysis of the autocorrelation function.

Peak Radius Low Cutoff (nm): Sets the lower limit for the peak values determined by the Regularization algorithm that are displayed in the regularization graph. Peaks below the Peak Radius Low Cutoff value will not be displayed, nor will they be included in the %I and %M calculations.

Peak Radius High Cutoff (nm): Sets the upper limit for the peak values determined by the Regularization algorithm that are displayed in the regularization graph. Peaks above the Peak Radius High Cutoff value will not be displayed, nor will they be included in the %I and %M calculations.

Measurement Time Limit Factor: This parameter determines the maximum time allotted to the instrument for completing a measurement. If the time to complete the measurement exceeds the maximum time allotted, the measurement is stopped and marked as "Incomplete" in the data file. The maximum time allotted is determined by multiplying the Measurement Time Limit Factor by the product of the specified values for "Acq Time" and "Num Acq".

Event Schedule: Set to Yes or No to specify whether or not to activate the Event Scheduler. Setting this to Yes also shows the Event Schedule node in the experiment tree. The default is Yes.

DelsaMax CORE-Only Parameters

Analysis Type: Specifies whether the Dynals™ analysis or the original Legacy analysis is applied to the autocorrelation for the calculation of Dt, Rh, and other parameters determined by the technique of Dynamic Light Scattering. This parameter is available for DelsaMax CORE instrument only.

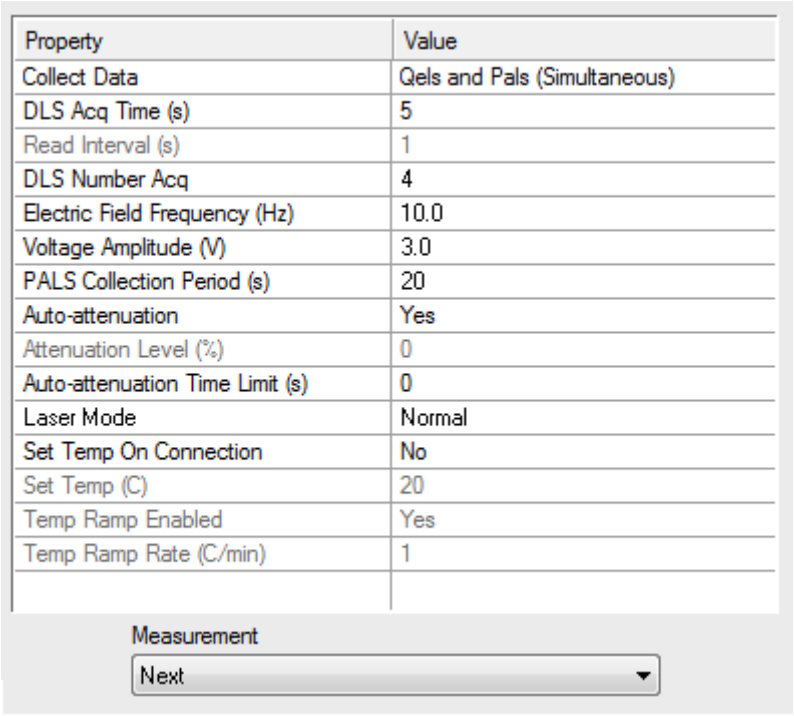
Auto-attenuation Time Limit(s): This is the number of seconds that DelsaMax Analysis Software waits before deciding that auto-attenuation has failed. This parameter is available for DelsaMax CORE instrument only.

Calculate D10/D50/D90: Set to Yes or No to specify whether or not to automatically calculate the radius/diameter values below which 10%, 50%, and 90% of the cumulative distribution is contained. The default is No. Note that setting this parameter to Yes causes calculations to take considerably longer since the number of bins for the calculations is increased. This parameter is available for DelsaMax CORE instrument only.

Calculate Polydispersity: Set to Yes or No to specify whether or not to automatically calculate the polydispersity for each measurement. The default is Yes. This parameter is available for DelsaMax CORE instrument only.

Instrument Parameters Node

The **Parameters** ▸▸ **Instrument** node of the tree contains parameters related to the type of instrument selected. The values of these parameters may be different for different measurements in an experiment. This example shows the parameters available for the DelsaMax PRO instrument with the QELS option.



After an experiment has been performed, you can change these parameters for the "Next" measurement, but not for measurements that have already been performed.

DelsaMax CORE and DelsaMax PRO with QELS Parameters

The following parameters are available for instruments that perform dynamic light-scattering (DLS or QELS) analysis:

DLS Acq Time (s): The amount of time in seconds to collect or acquire a single auto-correlation curve; this is also referred to as the "integration time". Larger acquisition times may result in better signal averaging, but also increase the likelihood of a "dust event" occurring during the course of the acquisition, which may adversely affect data analysis.

Read Interval (s): The time in seconds for one DLS or QELS reading. This value cannot be changed. The number of readings in an auto-correlation curve is equal to the DLS Acq Time divided by this value.

DLS Num Acq: The number of acquisitions to be collected for the measurement.

Auto-attenuation: The instruments are equipped with integral algorithms to automatically determine the Laser Power (%) and Attenuation Level (%) for each measurement in real time. You may enable or disable Auto-attenuation by setting this field to Yes or No.

Attenuation Level (%): The percentage of detected light attenuated by the digitally-controlled optical attenuator during a measurement. When "Auto-attenuation" is enabled, the Attenuation Level (%) is automatically determined by the instrument. Otherwise, you may manually select the Attenuation Level (%) through the software or front panel control. You cannot change the Attenuation Level (%) during data acquisition.

Auto-attenuation Time Limit (s): This value controls how long DelsaMax Analysis Software waits (in seconds) for the instrument to perform auto-attenuation before it is assumed that the auto-attenuation failed. A value of zero means that there is no time limit.

Temperature-Controlled Instrument Parameters

The following parameters are available for instruments that allow you to set the temperature:

Set Temp On Connection: Set to Yes or No to set whether DelsaMax Analysis Software sets the instrument temperature when you click the **Instrument Connect** button. See "Connecting to Instruments" on page 6-6.

Set Temp (C): The user-defined target temperature for temperature-controlled systems. The value entered in Set Temp (C) is applied when Set Temp on Connection is enabled, or when the operator manually enters a new value prior to manually acquiring data.

Temp Ramp Enabled: Set to Yes or No to enable or disable temperature ramp mode.

Temp Ramp Rate (C/min): The Temp Ramp Rate (C/min) determines the rate at which the temperature changes when Temp Ramp Enabled is set to Yes. See "Using the Ramp Rate Calculator" on page 2-14 to calculate an appropriate value.

DelsaMax PRO Parameters

Note: The Electric Field Frequency, Voltage Amplitude, and PALS Collection Period parameter settings should be considered as a whole. For example, while it is generally advisable to apply low to moderate voltages to conductive samples, sometimes you can gather better data by applying a slightly higher voltage with a shorter PALS collection period. The guidelines given here are not absolute rules; you should modify the settings as needed to fit your samples.

The following parameters are available for DelsaMax PRO instruments only:

Collect Data: Choose whether you want to acquire data for both QELS (dynamic light scattering) and PALS (mobility measurements) simultaneously, only QELS data, or only PALS data. Other instrument parameters are enabled or disabled depending on your choice. This parameter is shown only if your DelsaMax PRO supports QELS.

Electric Field Frequency (Hz): Choose the frequency for cycling the applied electric field. The choices are 10 Hz and 20 Hz. In theory, this frequency should not affect the measured mobility results. Nevertheless, the presence of counter-ions in the sample solution can complicate this matter, because they give rise to electrode polarization. The lower the electric field frequency, the more polarized the electrodes will be. Since the number of counter ions increases with the ionic strength of the solution, you may see a better quality PALS V-graph with 20 Hz for solutions with higher ionic strengths. For most applications, 10 Hz is a good choice.

Voltage Amplitude (V): Set this parameter to the desired voltage for the applied electric field. DelsaMax PRO can apply up to 10 volts for PALS measurements of conductive samples and up to 100 volts for non-conductive samples. A minimum voltage of 1-1.5 volts should be applied to

overcome the overpotential at the electrode-fluid interface. An applied voltage between 2.0 volts and 3.0 volts typically gives satisfactory results for most conductive aqueous solutions. Higher voltages can be applied for low-conductivity samples (< 1 mS/cm). The optimal value will be sample-dependent. Some samples are robust and can withstand high electric voltages and fields, while others should be treated more delicately. Organic and non-conductive samples are, in general, much more robust under electric fields because very little electrical current, if any, is present.

PALS Collection Period (s): Set this parameter to the amount of time to apply the electric field for the electrophoretic mobility measurement. The optimal value will be sample-dependent. Some samples are more vulnerable to applied electric fields and can degrade, denature, and/or aggregate in the presence of electrical current. While you can test a sample under different conditions to find a threshold below which the sample is not damaged, the general rule is that the higher the conductivity of the sample, the shorter the optimal collection period should be. The PALS Collection Period is unrelated to the DLS Acq Time; you can set either one to a longer value than the other.

Laser Mode: The DelsaMax PRO laser can be used either in Normal mode (45 mW) or Low mode (4.5 mW). Set the laser to Low mode here if you have a highly absorptive or strongly scattering sample. This mitigates sample heating due to the absorbed laser light and the accompanying thermal lensing effect. The instrument's sensitivity is reduced when you use the laser in Low mode, so use this mode only if your sample is so strongly absorbing that thermal lensing occurs, or if the sample is so strongly scattering that the QELS fiber attenuator cannot bring the count rate within range for the photodetectors.

DelsaMax PRO with Autosampler Parameters

Flow Rate (mL/min): Specify the rate at which solvent and sample should flow through the autosampler.

Injection Volume (µL): Specify the amount of sample that should be injected each time an injection is performed.

Run Time (min): Specify how long the pumps should run during an injection.

Adjust these parameters so that the sample is in the DelsaMax PRO cuvette when the Run Time ends. The values you will need to use depend on the fluid volume in your system between the autosampler and the DelsaMax PRO.

If you use the Event Scheduler, you can specify separate autosampler parameter values for sample and wash injections.

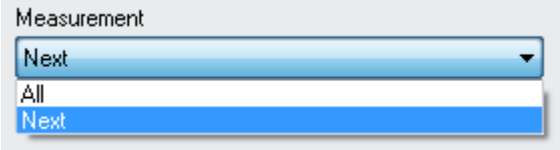
DelsaMax CORE Parameters

Laser Power (%): The percentage of full laser power used during a measurement. When "Auto-attenuation" is enabled, the Laser Power (%) is automatically determined by the instrument. Otherwise, the operator may manually select Laser Power (%) through the software or front panel control. The operator cannot change the laser power during the data acquisition process.

DLS Only: For the DelsaMax CORE only. Set to Yes or No to indicate whether the proprietary intensity stabilization algorithm is turned off, which will slightly improve dynamic light scattering data. The effect of disabling intensity stabilization (**DLS Only** = **Yes**) will be a slight improvement in the DLS baselines, but the static scattering results will no longer be reported.

Measurement List Box

The **Measurement** list box at the bottom of the property table lets you vary instrument parameters between measurements. Choose **Next** (the default) if you want to change the property values for future measurements.

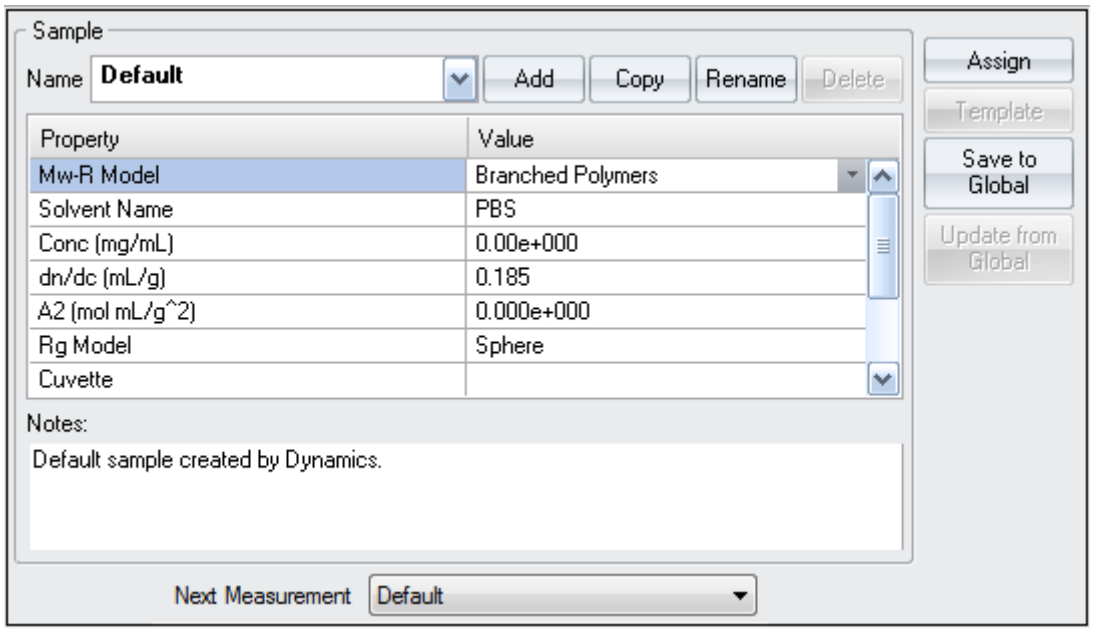


If you have already collected data in this experiment, you can use the **Measurement** list box to see what property values were used when collecting the data for a specific measurement. If different settings were used for different measurements, you see "Variable" for that property when you select **All** from the list box.

Sample Parameters Node

See "Managing Sample Definitions and Assignments" on page 4-11 for information about using sample definitions.

The **Parameters** ▸▸ **Sample** node of the tree contains the following fields, buttons, and parameters. These may be different for different measurements in an experiment:



General Parameters

Solvent: The name of the solvent used with the designated sample.

Conc (mg/mL): The concentration of the sample in units of mg/mL. This parameter is used for static mass calculation (along with dn/dc, A2, and Rg Model).

dn/dc (mL/g): The specific refractive index increment for the sample in units of mL/g. This parameter is used for static mass calculation (along with Conc, A2, and Rg Model).

Mw-R Model: The weight-averaged molar mass estimated from the measured hydrodynamic radius of the analyte. Available options are No Mw-R Model, Globular Proteins, Linear Polymers, Branched Polymers, and Starburst Polymers.

Notes: You can type information about the sample as further documentation.

DelsaMax CORE Parameters

A2 (mol mL/g^2): This is the second viral coefficient, which is used for static mass calculation (along with dn/dc, Conc, and Rg Model).

Rg Model: This parameter is used for static mass calculation (along with dn/dc, A2, and Conc). The options are Hollow Sphere, Sphere, Random Coil, and Regular Star (2-5 arms).

Cuvette: Select a defined cuvette to be used with this sample. See "Managing and Calibrating Cuvettes" on page 4-18 for information about defining cuvettes. For the DelsaMax PRO, the instrument provides information about the cuvette in use to DelsaMax Analysis Software internally.

DelsaMax PRO-Only Parameters

Rh (nm): Specify the known hydrodynamic radius of the sample. This value is ignored if the Ignore DLS Rh parameter is set to No (the default).

Ignore DLS Rh: For the DelsaMax PRO with QELS only. A DelsaMax PRO instrument with a QELS unit calculates the hydrodynamic radius of the sample and uses that value in the PALS calculations if this parameter is set to No (the default). If you set this parameter to Yes, the Rh parameter value is used instead of the value calculated from QELS data.

Zeta Potential Model: For the DelsaMax PRO only. Select the Smoluchowski, Huckel, or Henry model for determining the zeta potential. The default is Henry. If the size and ionicity of the sample are known, the Henry model is recommended. If these values are not known, the Smoluchowski model works best for large particles—those with a hydrodynamic radius that is over 50 nm or much larger than the Debye length. The Huckel model is best for small molecules—those with a hydrodynamic radius that is less than 5 nm or much less than the Debye length.

Ionic Strength (mM): For the DelsaMax PRO only. The ionic strength of a solution is a measure of the concentration of ions in the solution. If you are using the Henry model for determining the zeta potential, specify the known millimolar concentration of ions in the solution. The Debye length, which is the inverse of the Debye parameter (κ), is inversely proportional to the square root of the ionic strength.

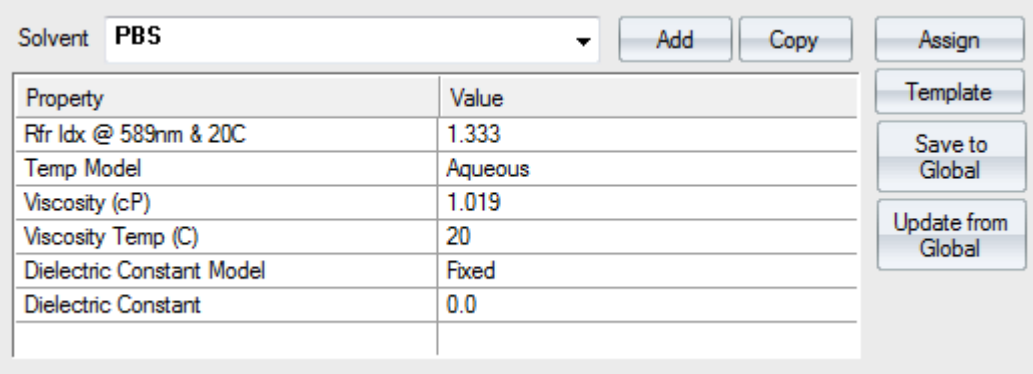
Measurement List Box

The **Next Measurement** list box (below the **Notes** field) lets you select which sample definition will be assigned to the next measurement for which you collect data.

Solvent Parameters Node

See "Managing Solvent Definitions and Assignments" on page 4-14 for information about using solvent definitions.

The **Parameters** ▸▸ **Sample** ▸▸ **Solvent** node of the tree contains the following fields, buttons, and parameters. These may be different for different measurements in an experiment:



Solvent The name of the solvent.

Rfr Idx @ 589nm & 20C: The refractive index of the solvent at 20 degrees Celsius using a 589 nm light source.

Temp Model: The temperature model used to estimate the solvent refractive index and viscosity at temperatures other than the temperature specified in the Viscosity Temp (C) field. The choices are Fixed and Aqueous. If the Temp Model is set to Aqueous, the Datalog Grid displays the temperature-corrected values for the viscosity and refractive index.

Viscosity (cp): The viscosity of the solvent at the temperature specified in the Viscosity Temp field located directly below this field in units of centipoise.

Viscosity Temp (C): The temperature that corresponds to the viscosity value specified in the Viscosity (cp) field located directly above this field.

Note: The **Rrf Idx** (refractive index), **Viscosity**, and **Temp Model** are predetermined and locked for all standard solvents defined in DelsaMax Analysis Software. You can edit these values if necessary, but such changes are not encouraged.

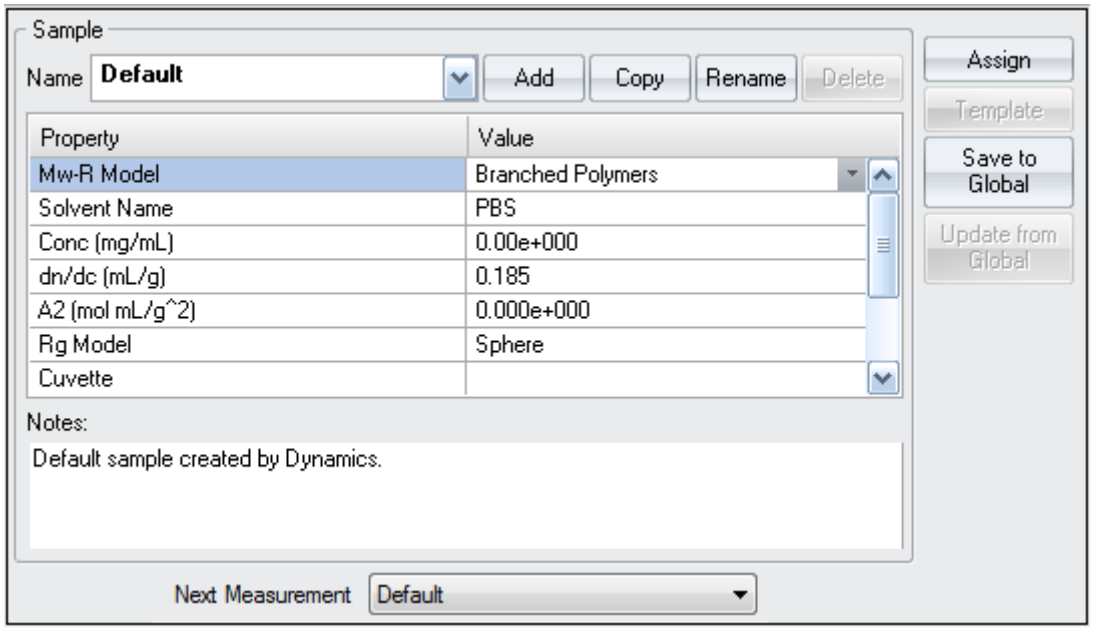
DelsaMax PRO-Only Parameters

Dielectric Constant Model: The choices are Fixed and Aqueous. If you choose Fixed, specify the value of the dielectric constant in the row that follows. If this model is set to Aqueous, the Datalog Grid displays the temperature-corrected values for the dielectric constant.

Dielectric Constant: If you are using the Fixed Dielectric Constant Model, specify the dielectric constant for the solvent at the temperature at which the measurement is taken.

Managing Sample Definitions and Assignments

The **Parameters** ▸▸ **Sample** node of the experiment tree lets you manage information about samples used. If you are using a DelsaMax CORE, the default screen looks like the following. Different parameters are available for other instruments.



You can use this node for the following tasks:

- "Defining Samples" on page 4-11
- "Assigning Samples to Measurements" on page 4-12
- "Using Global Sample Definitions" on page 4-13

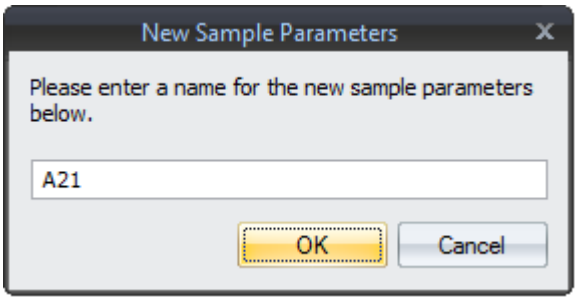
Defining Samples

Note: If you use multiple solvents (including multiple solvent concentrations) with the same solute, you must create a separate "sample" definition for each solute-solvent pair.

To assign a single sample (that is, a single solute-solvent pair) to all the measurements in an experiment before performing the experiment, simply specify the parameters for the sample using the default "Sample 1" sample. Make sure the **Next Measurement** field has "Sample 1" selected. (Or use whatever sample you want to automatically assign to measurements when you run the experiment.)

If your experiment will use multiple samples in different measurements, use the following steps to define all the samples:

1. For each different sample you will use, click the **Add** button. Type a name for the sample, and click **OK**.



2. Set parameters as needed for your sample. For all instruments, you need to select the Mw-R (weight-averaged molar mass estimated from hydrodynamic radius) model and the solvent that is always used with that solute. See "Managing Solvent Definitions and Assignments" on page 4-14 for information on adding solvent definitions. For the DelsaMax CORE, you set additional parameters.
3. You can further manage the list of samples in the Name drop-down list by using the **Copy** and **Rename** buttons.

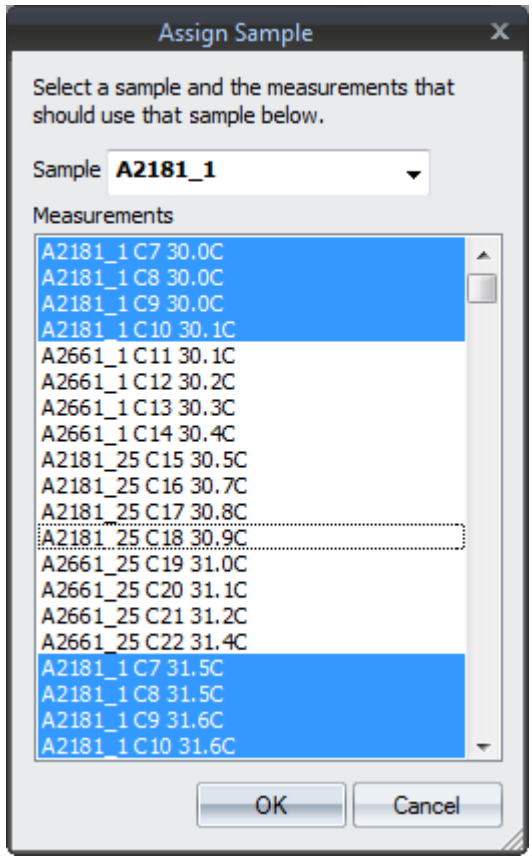
See "Using Global Sample Definitions" on page 4-13 for information on defining samples globally (not just for a single experiment).

Assigning Samples to Measurements

An experiment stores assignments of samples to each measurement. After you have run an experiment, you can create such assignments by following these steps:

1. In the **Sample** node of the experiment tree, click the **Assign** button.
2. From the drop-down list, select the sample you want to assign to measurements.
3. In the scrolling list, select all the measurements that used this sample. You can hold down the Ctrl key on your keyboard to select multiple items or the Shift key to select a range of items.
4. Click **OK** to save your assignments.

You can also assign samples to measurements in the Datalog Grid for the top-level Measurements node by using the pull-down menu in the "Sample" column. See "Datalog Grid" on page 7-14.



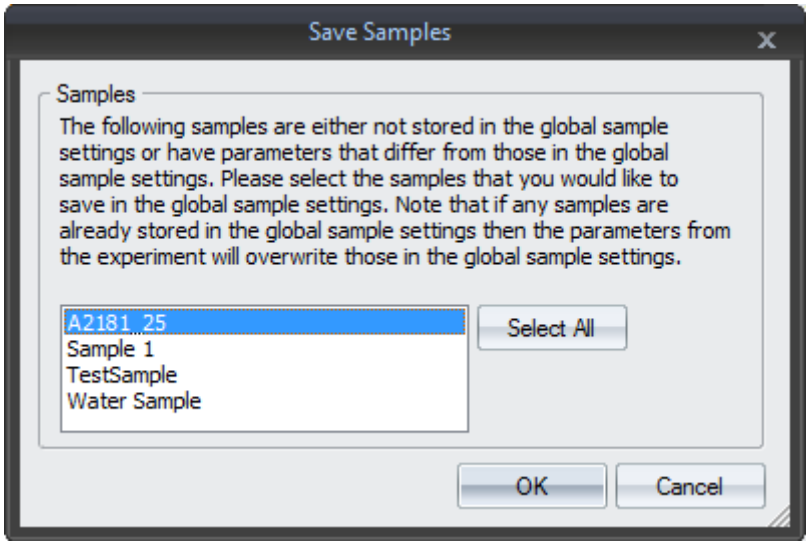
Using Global Sample Definitions

DelsaMax Analysis Software stores a list of global sample definitions that can be used by any experiment. Normally, when you create a sample definition, that definition is stored in the experiment only.

In the Sample node of an experiment, if the Name of the sample is shown in **bold** type, the sample is defined locally in the experiment. If the Name in shown in regular type, the sample is defined globally in DelsaMax Analysis Software.

If you want to be able to use your sample definitions in other experiments, follow these steps:

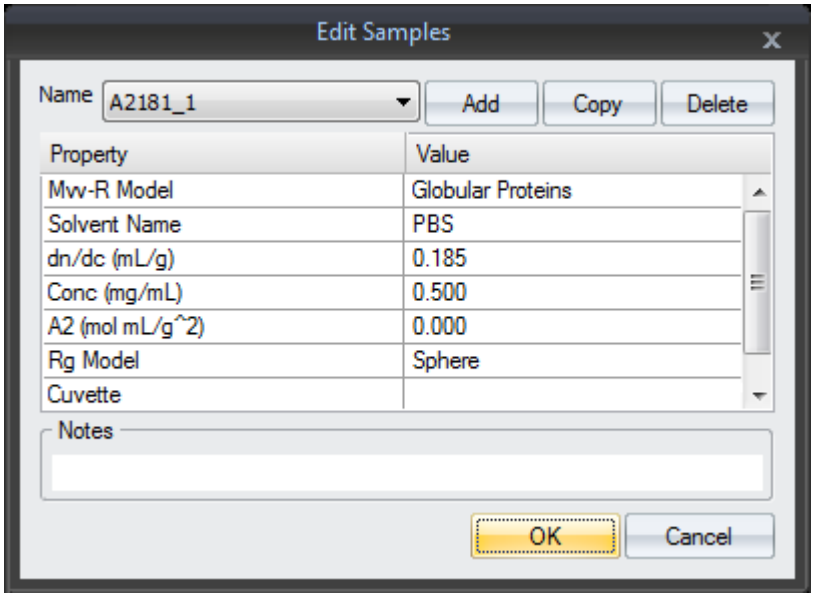
1. In an experiment that has the sample definitions you want to make global, go to the **Parameters ▸▸ Sample** node of the experiment tree.
2. Click the **Save to Global** button.
3. In the Save Samples dialog, select the sample definitions you want to make global. (Definitions that have already been saved globally are not listed here.) You can hold down the Ctrl key on your keyboard to select multiple items or the Shift key to select a range of items.



4. Click **OK**.

If an experiment contains a sample definition that does not match the global definition, you can import the global definition into the experiment by clicking the **Update from Global** button. You will be asked to select the sample definitions you want to import.

You can manage global Sample definitions by choosing **Tools** ▸▸ **Parameters** ▸▸ **Samples** from the menus. You see the Edit Samples dialog:

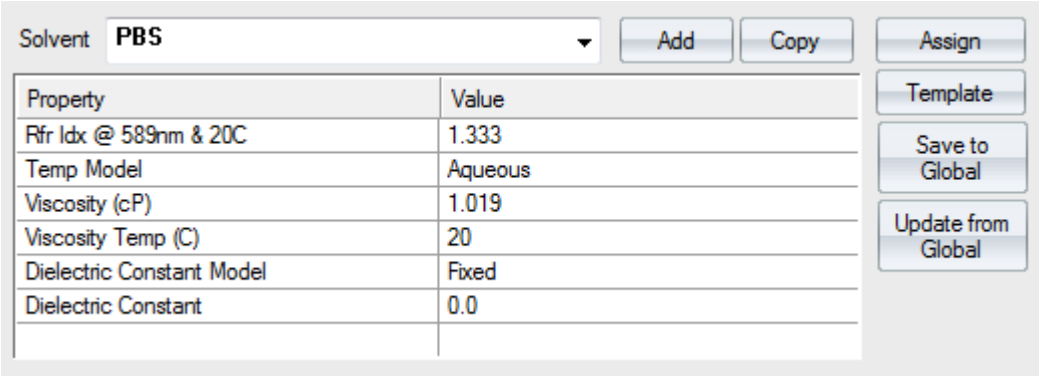


You can use this dialog to add, copy, and delete global sample definitions. You can change the parameters. (All sample parameters are visible here.) Additionally, you can add a text description of the sample in the Notes field.

Managing Solvent Definitions and Assignments

Many of the calculations and data transforms in DelsaMax Analysis Software require solvent-related information. DelsaMax Analysis Software is delivered with an integrated solvent database, containing roughly 100 predefined solvents.

The **Parameters** ▸▸ **Sample** ▸▸ **Solvent** node of the experiment tree lets you manage information about solvents used. The default screen looks like the following:



See "Solvent Parameters Node" on page 4-10 for descriptions of the parameters.

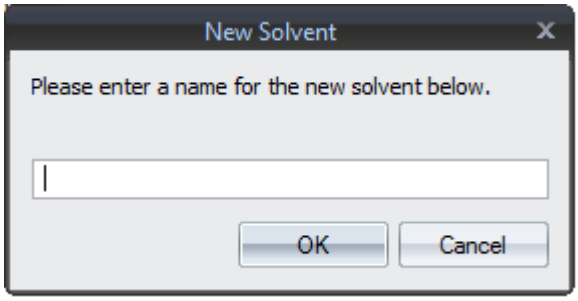
You can use this node for the following tasks:

- "Defining Solvents" on page 4-15
- "Assigning Solvents to Samples" on page 4-15
- "Using Global Solvent Definitions" on page 4-16

Defining Solvents

DelsaMax Analysis Software is delivered with a solvent database, containing roughly 100 predefined solvents. If you use a solvent or solvent concentration that is not in the database, you can create a definition as follows:

1. Open the **Parameters** ▸▸ **Sample** ▸▸ **Solvent** node of the experiment tree.
2. For each new solvent you will use, click the **Add** button. Type a name for the solvent, and click **OK**.



3. Set parameters as needed for your solvent. See "Solvent Parameters Node" on page 4-10 for descriptions of the parameters.
4. You can further manage the list of solvents by using the **Copy** button.

See "Using Global Solvent Definitions" on page 4-16 for information on defining solvents globally (not just for a single experiment).

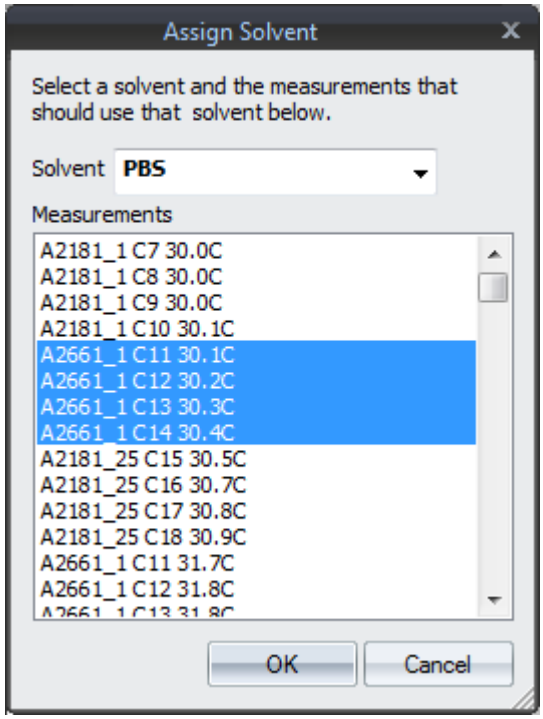
Assigning Solvents to Samples

An experiment stores assignments of solvents to each sample in the experiment. You can create such assignments for a single experiment in the **Parameters** ▸▸ **Sample** node of the experiment tree by selecting the solvent for a sample from the drop-down list.

You can assign solvents to samples globally (for all experiments) by choosing **Tools** ▸▸ **Parameters** ▸▸ **Samples** from the menus.

To assign solvents to samples in an experiment that has already been run, follow these steps:

1. In the **Solvent** sub-node of the experiment tree, click the **Assign** button.
2. From the drop-down list, select the solvent you want to assign.
3. In the scrolling list, select all the measurements that you want to use the selected solvent. You can hold down the Ctrl key on your keyboard to select multiple items or the Shift key to select a range of items.
4. Click **OK** to save your assignments.



You can also assign solvents to measurements in the Datalog Grid for the top-level Measurements node by using the pull-down menu in the "Solvent Name" column. See "Datalog Grid" on page 7-14.

Note: Although this dialog lets you choose solvents and measurements, the actual assignments made are from samples to measurements. If a sample is found that uses the selected solvent, then that sample is assigned to the selected measurements. If there is no sample that uses the selected solvent, then a new sample is created and that sample is assigned to the selected measurements.

Using Global Solvent Definitions

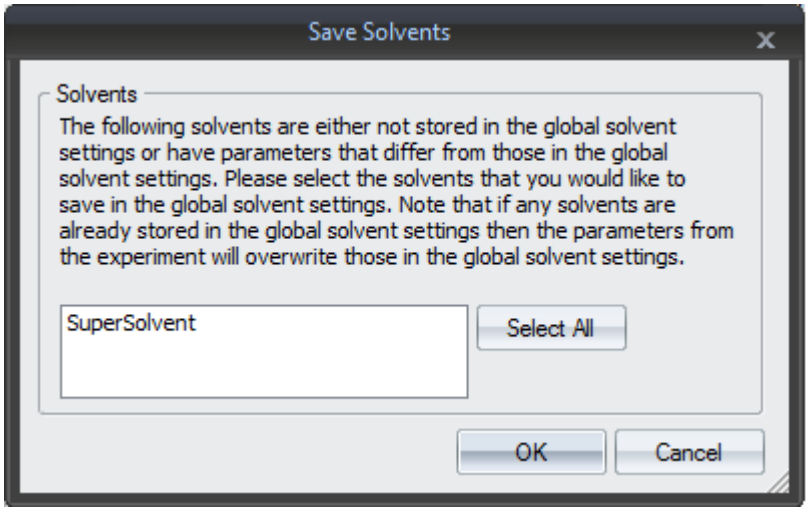
DelsaMax Analysis Software stores a list of global solvent definitions that can be used by any experiment. Normally, when you create a custom solvent definition, that definition is stored in the experiment only.

In the Solvent node of an experiment, if the Name of the solvent is shown in **bold** type, the solvent is defined locally in the experiment. If the Name in shown in regular type, the solvent is defined globally in DelsaMax Analysis Software.

If you want to be able to use your custom solvent definitions in other experiments, follow these steps:

1. In an experiment that has the solvent definitions you want to make global, go to the **Parameters** ▸▸ **Sample** ▸▸ **Solvent** node of the experiment tree.
2. Click the **Save to Global** button.

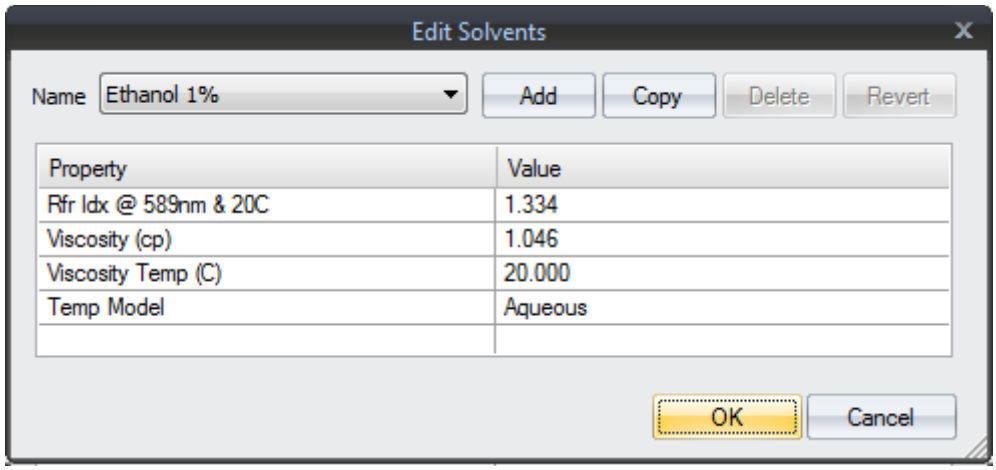
3. In the Save Solvents dialog, select the solvent definitions you want to make global. (Definitions that have already been saved globally are not listed here.) You can hold down the Ctrl key on your keyboard to select multiple items or the Shift key to select a range of items.



4. Click **OK**.

If an experiment contains a solvent definition that does not match the global definition, you can import the global definition into the experiment by clicking the **Update from Global** button. You will be asked to select the solvent definitions you want to import.

You can manage global Solvent definitions by choosing **Tools** ▸▸ **Parameters** ▸▸ **Solvents** from the menus. You see the Edit Solvents dialog:



You can use this dialog to add, copy, and delete global solvent definitions. You can change the parameters. Click **OK** to save your changes.

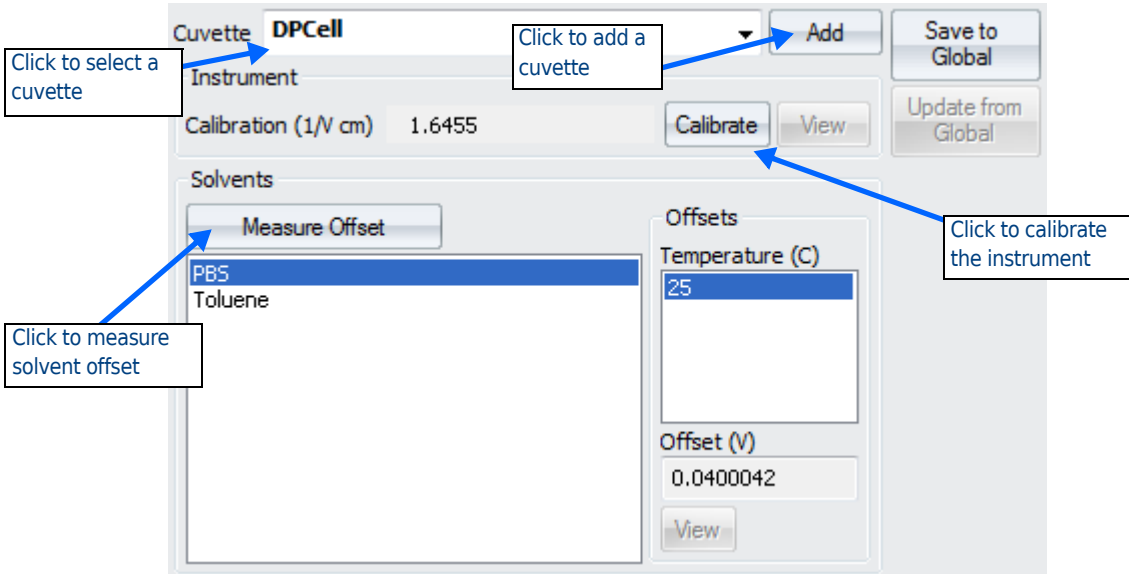
The **Revert** button changes the parameter values for the selected solvent back to the original values that were shipped with DelsaMax Analysis Software.

Managing and Calibrating Cuvettes

To perform static mass calculations when using the DelsaMax CORE instrument, DelsaMax Analysis Software needs more information about the instrument and the solvents being used. This information is managed using "cuvette" definitions, which are assigned to samples (just as solvents are assigned to samples). The cuvette definition can store detector baseline information related to calibrating the instrument and measuring temperature offsets.

Note: The DelsaMax PRO provides information about the cuvette in use to DelsaMax Analysis Software internally. You do not need to store cuvette information in DelsaMax Analysis Software. You can ignore any sections in this manual related to cuvettes.

The **Parameters** ▸▸ **Sample** ▸▸ **Cuvette** node of the experiment tree lets you manage information about cuvettes. The node looks similar to this:



The **Instrument** section displays the calibration constant and the **Calibrate** and **View** buttons. The **Calibrate** button starts an instrument calibration.

The **Solvents** section holds a list of calibrated solvents. When you select one of these solvents, the **Temperature** list shows temperatures that have been calibrated for the solvent. When you select a temperature, the **Offset** box shows the detector baseline value measured for this solvent at this temperature.

With a cuvette selected, you can perform an instrument calibration (toluene at 25 C), a solvent calibration, or **View** previous calibration data.

You can use this node for the following tasks:

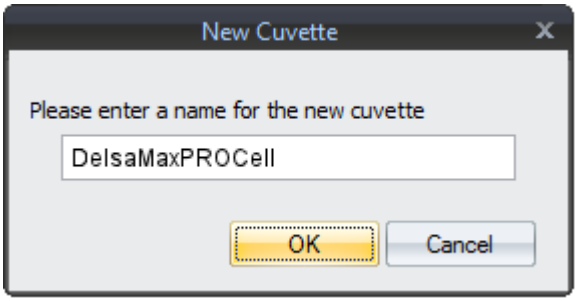
- "Defining Cuvettes" on page 4-19
- "Assigning Cuvettes to Samples" on page 4-19
- "Viewing Calibration Data" on page 4-19
- "Calibrating an Instrument" on page 4-19 and "Measuring Solvent Offsets" on page 4-22

- "Using Global Cuvette Definitions" on page 4-23

Defining Cuvettes

You can create a cuvette definition as follows:

1. Open the **Parameters** ▸▸ **Sample** ▸▸ **Cuvette** node of the experiment tree.
2. To create a cuvette definition, click the **Add** button. Type a name for the cuvette, and click **OK**.



See "Using Global Cuvette Definitions" on page 4-23 for information on defining cuvettes globally (not just for a single experiment).

Assigning Cuvettes to Samples

An experiment stores assignments of cuvettes to each sample in the experiment. You can create such assignments for a single experiment in the **Parameters** ▸▸ **Sample** node of the experiment tree by selecting the cuvette for a sample from the drop-down list.

You can assign cuvettes to samples globally (for all experiments) by choosing **Tools** ▸▸ **Parameters** ▸▸ **Samples** from the menus.

Viewing Calibration Data

You can click one of the **View** buttons in the Cuvette node or dialog to view stored calibration data for an instrument or a solvent.

Use the slider to control the **Despiking Filter**. Data points that have been removed by the filter are red on the graph while the points that the filter is keeping are blue.

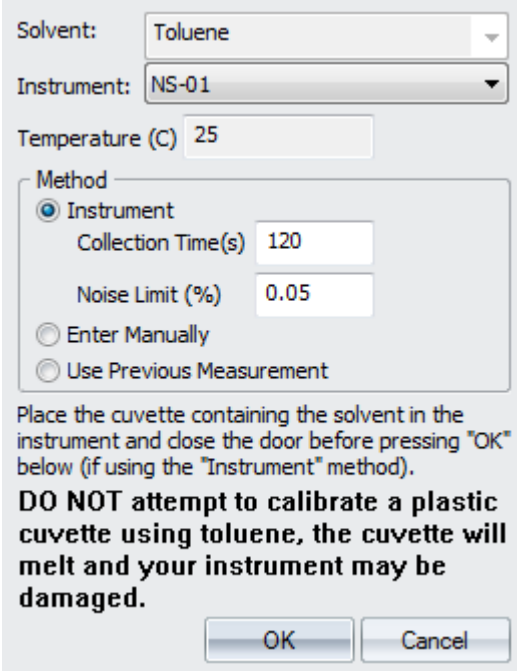
Click **OK** to save any changes. Click **Cancel** to return without saving changes.

Calibrating an Instrument

You can perform an instrument calibration (toluene at 25 °C) as follows:

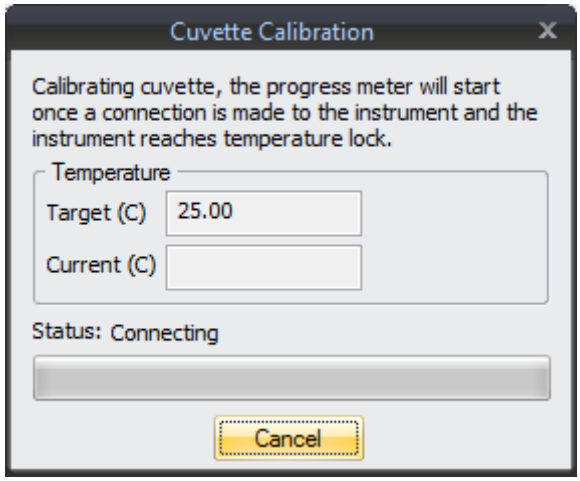
1. See the section on "Calibrating DelsaMax CORE" in the *DelsaMax CORE Instructions for Use* for sample preparation and hardware details about calibration.
2. Go to the Cuvette sub-node or choose **Tools** ▸▸ **Parameters** ▸▸ **Cuvettes** from the menus.

3. Click the **Calibrate** button to open the Cuvette Calibration dialog.
4. The **Solvent** is locked to toluene when doing an instrument calibration.
5. Select the **Instrument** to calibrate. If you are using the Cuvette sub-node, you can only calibrate the instrument selected for use in this experiment.
6. The **Temperature (C)** is locked to 25 °C when doing an instrument calibration.
7. Select the **Method** to use for the calibration.
    - **Instrument** does the calibration using the actual instrument. You can set a collection time (in seconds) and a noise limit (as a percentage of the data average that the standard deviation cannot exceed before DelsaMax Analysis Software issues a warning).

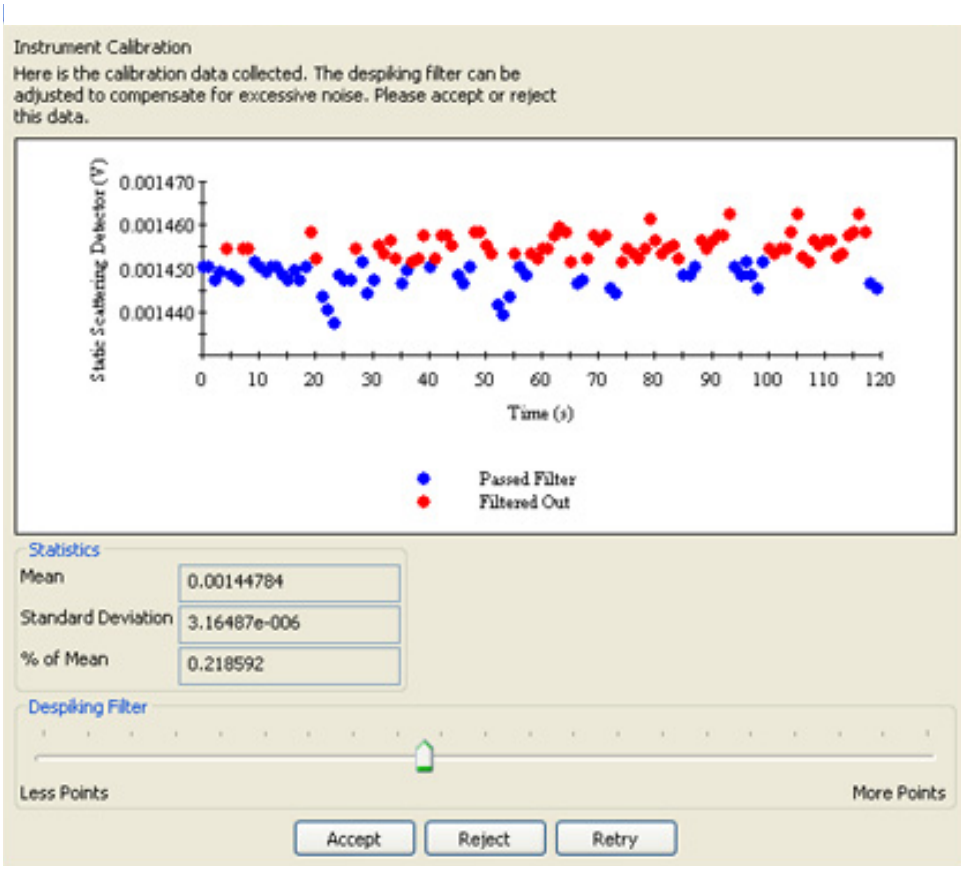


    - **Enter Manually** allows you to enter calibration constants by hand. See page 4-22.
    - **Use Previous Measurement** allows you to select a calibration that was used in the past. See page 4-22.

If you choose the **Instrument** method, a dialog is displayed so you can monitor the calibration progress. You can **Cancel** the calibration at this point if necessary.



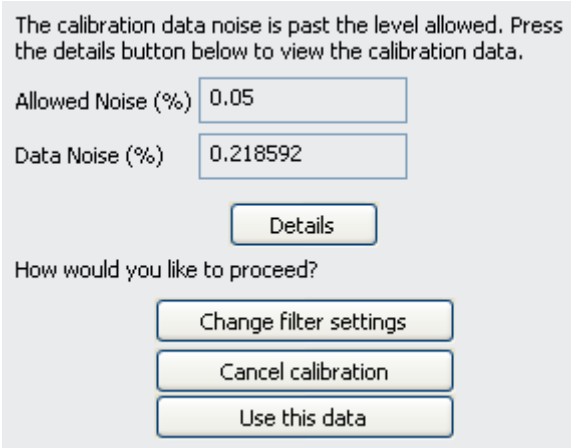
When the calibration finishes, the Cuvette Calibration Data dialog shows the collected data and statistics for the data.



Use the slider to control the **Despiking Filter**. Data points that have been removed by the filter are red on the graph while the points that the filter is keeping are blue.

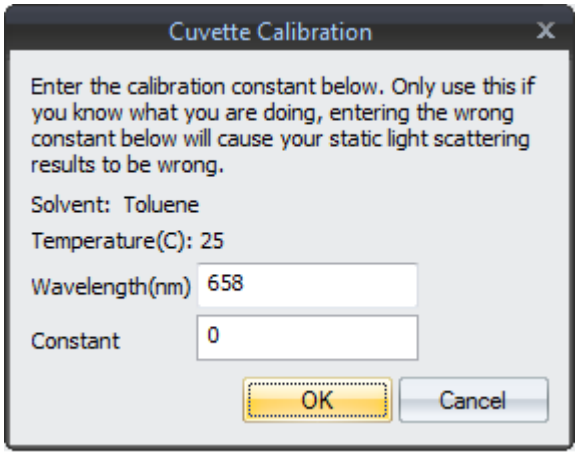
You can **Accept** or **Reject** the data. Click **Retry** to return to the **Cuvette Calibration** dialog with the same settings that were used for the last calibration.

If you accept calibration data with noise greater than the **Noise Limit (%)** you set, you see a warning message. Both the limit and the data noise are displayed. You can click **Change filter settings** to return to the Cuvette Calibration Data dialog and adjust the despiking filter; **Cancel calibration** to start over; or **Use this data** to accept the calibration data.



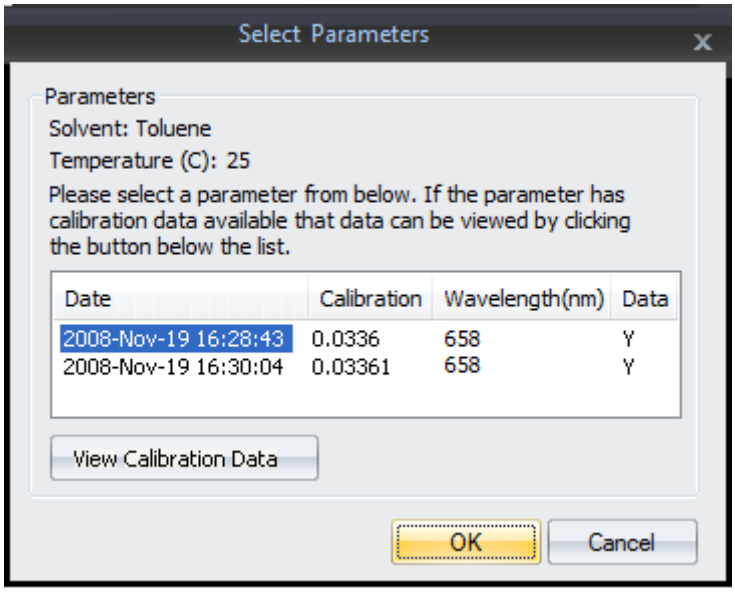
Manual Calibration

If you choose the **Enter Manually** method, you can type the laser wavelength and the calibration constant in the dialog shown.



Previous Measurement Calibration

If you choose the **Use Previous Measurement** method in the initial calibration dialog, then the **Select Parameters** dialog is displayed.



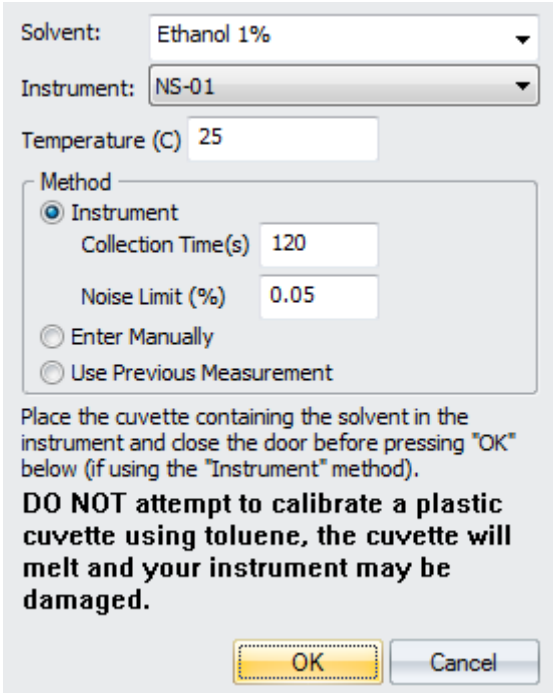
This dialog displays all the recorded calibrations for the selected cuvette/solvent/temperature combination. If calibrations have associated data, you can view the data by selecting the calibration in the list and clicking the **View Calibration Data** button.

Measuring Solvent Offsets

You can perform an solvent calibration as follows:

1. See the section on "Calibrating DelsaMax CORE" in the *DelsaMax CORE Instructions for Use* for sample preparation and hardware details about calibration.

2. Go to the Cuvette sub-node or choose **Tools**→**Parameters**→**Cuvettes** from the menus.
3. Click the **Measure Offset** button to open the Cuvette Calibration dialog.
4. Select the **Solvent** to calibrate.
5. Select the **Instrument** to use for the calibration.
6. Enter the **Temperature (C)** to do the calibration for.
7. Select the **Method** to use for the calibration.
   - **Instrument** does the calibration using the actual instrument. You can set a collection time (in seconds) and a noise limit (as a percentage of the data average that the standard deviation cannot exceed before DelsaMax Analysis Software issues a warning).
   - **Enter Manually** allows you to enter calibration constants by hand. See page 4-22.
   - **Use Previous Measurement** allows you to select a calibration that was used in the past. See page 4-22.



The calibration process continues as described in "Calibrating an Instrument" on page 4-19 but for the solvent you selected instead of toluene.

Note: When you perform temperature-dependent measurements, you must measure solvent offsets for temperatures covering the temperature range measured. For example, for a temperature scan between 4 °C and 95 °C, measure the solvent offset at 4 °C, 95 °C, and ideally at temperatures between, such as 25 °C and 50 °C. The software then calculates the solvent offset for all temperatures within the range. If solvent offsets have not been measured at relevant temperatures, the Mw-S column shows a "No Offset" message.

Using Global Cuvette Definitions

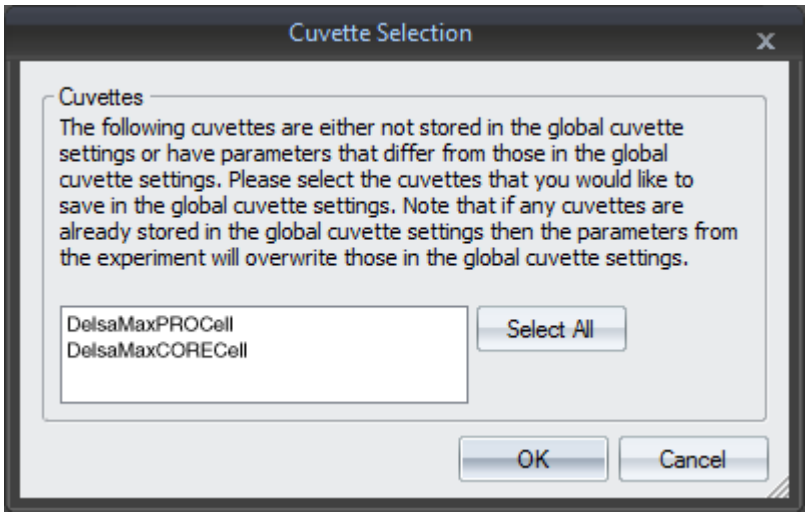
DelsaMax Analysis Software stores a list of global cuvette definitions that can be used by any experiment. Normally, when you create a cuvette definition, that definition is stored in the experiment only.

In the Cuvette node of an experiment, if the Name of the cuvette is shown in **bold** type, the cuvette is defined locally in the experiment. If the Name in shown in regular type, the cuvette is defined globally in DelsaMax Analysis Software.

If you want to be able to use your custom cuvette definitions in other experiments, follow these steps:

1. In an experiment that has the cuvette definitions you want to make global, go to the **Parameters** ▸▸ **Sample** ▸▸ **Cuvette** node of the experiment tree.
2. Click the **Save to Global** button.

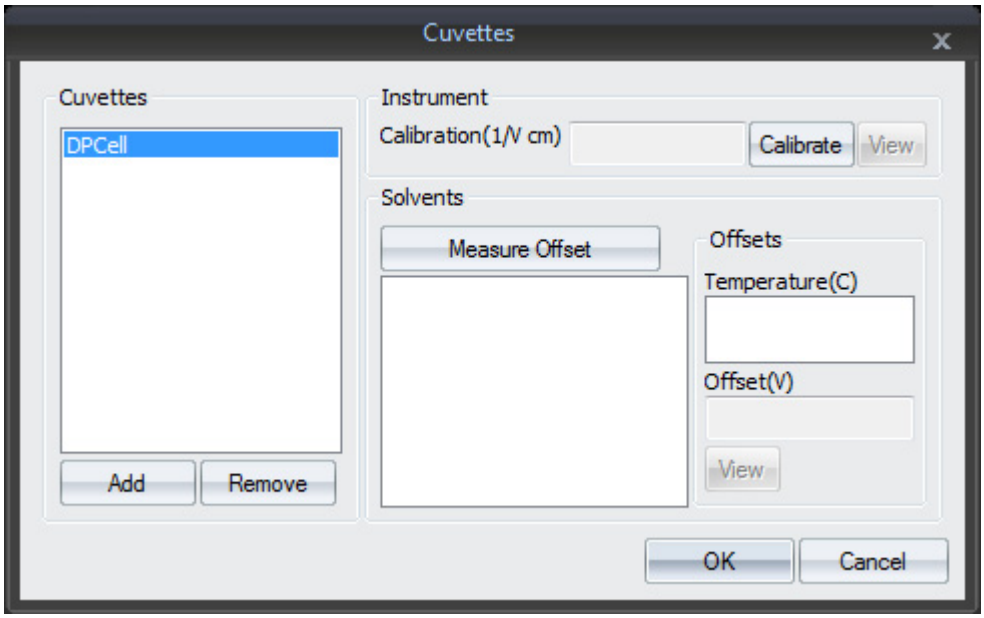
3. In the Cuvette Selection dialog, select the cuvette definitions you want to make global. (Definitions that have already been saved globally are not listed here.) You can hold down the Ctrl key on your keyboard to select multiple items or the Shift key to select a range of items.



4. Click **OK**.

If an experiment contains a cuvette definition that does not match the global definition, you can import the global definition into the experiment by clicking the **Update from Global** button. You will be asked to select the cuvette definitions you want to import.

You can manage global Cuvette definitions by choosing **Tools** ▸▸ **Parameters** ▸▸ **Cuvettes** from the menus. You see the Cuvettes dialog, which is very similar to the Cuvettes node:



You can use this dialog to add and delete global cuvette definitions. Click **OK** to save your changes.

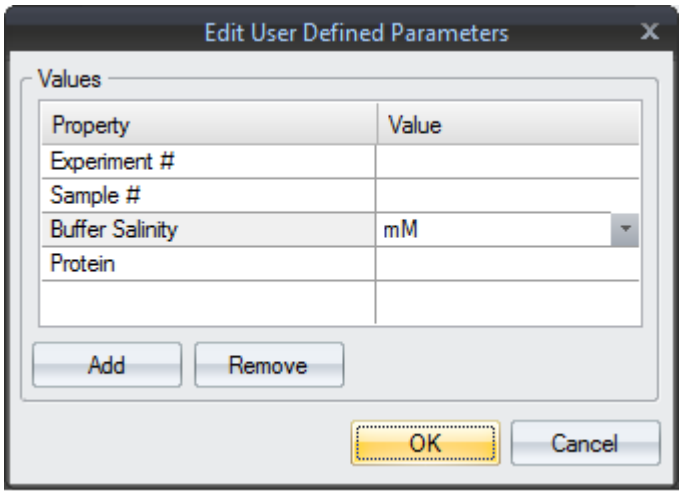
Creating User-Defined Parameters

The **UserDefined** parameters sub-node is used to store parameters and values that have special significance to an experiment or set of experiments. This feature can be used to create graphs with values that are not determined by DelsaMax Analysis Software. For example, a salinity vs. Rh graph would require a User-Defined salinity parameter.

Note: User-defined parameter values can be edited from the datalog grid. See page 4-26.

Adding User-Defined Parameters Globally

1. Select **Tools** ▸▸ **Parameters** ▸▸ **User Defined** from the main menu bar. You see the Edit User Defined Parameters dialog.



2. Click **Add** to create a user-defined parameter that will be available to all your experiments.
3. Type the new **Property** name.

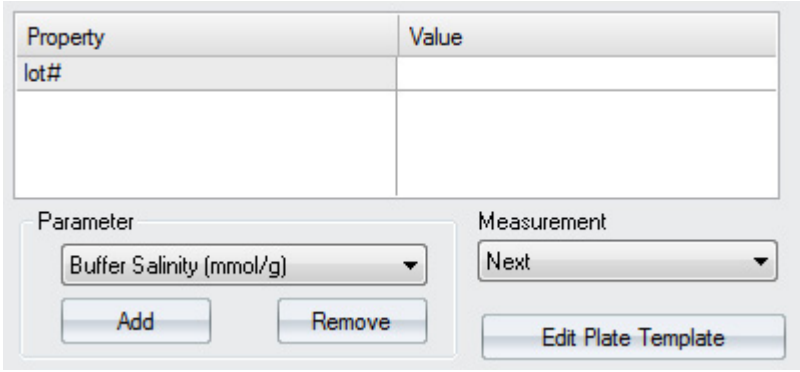
Note: Do not create user-defined parameters named “Sample”, “Solvent” or “Names”. These are reserved names.

4. Select units for the property’s **Value** from the pull-down menu. This list shows all DelsaMax Analysis Software supported units. Alternately, you can type your own units, although unit transforms in the Grid View will not be allowed. If the new parameter is unit-less, leave the Units field blank.
5. Click the **OK** button to save changes.

To delete a user-defined parameter from the global parameter list, select the row containing the parameter to be removed and click **Remove**.

Adding User-Defined Parameters to an Experiment

1. Highlight **UserDefined** in the Parameters node of the experiment tree. You see fields like the following:



2. Select a global parameter from the **Parameter** drop-down list.
3. Click the **Add** button to add it to the experiment's property table.
4. Type the **Value** for the selected parameter in the appropriate cell.
5. Select the **Measurement** to which this parameter and value should apply.

If you want to use different User-Defined parameters for different measurements, we recommend that you first use the **Measurement** field to set values for the "Next" measurement. Then collect data for the measurement.

If you forget to set User-Defined parameters before performing a measurement, you can set values for a specific measurement by selecting that measurement in the **Measurement** drop-down list and setting the values. The change is applied to the selected measurement when you click on something that forces a recalculation, such as a measurement node in the experiment tree.

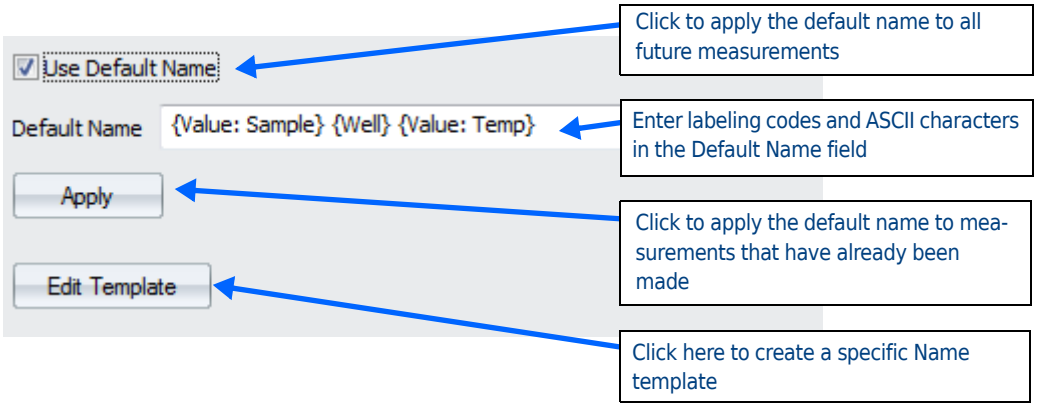
Note: DelsaMax Analysis Software doesn't use any of the parameters in the **UserDefined** sub-node for calculation purposes. If a parameter is needed for a calculation, the parameter is listed in one of the other **Parameter** sub-nodes.

Editing User-Defined Parameters from the Datalog Grid

1. Select the Measurements node in the experiment tree.
2. Right-click the datalog table and select **Table Settings**.
3. Add one or more User-Defined parameters to the table.
4. Edit any of the User-Defined values by double-clicking the appropriate boxes in the datalog grid.

Creating a Measurement Naming Template

DelsaMax Analysis Software allows you to easily automate the process of naming measurements to provide customized information about each measurement in the name.



1. In the **Parameters** node of the experiment tree, select **Names**.
2. If you want to label all measurements in the same manner, click the **Use Default Name** checkbox.
3. Fill in the **Default Name** field with any combination of the following labeling codes. These will name the measurements with meaningful values for each measurement. You may also place ASCII characters for formatting in the **Default Name** field. Click **Help** for an example.

{Solvent}	The measurement's solvent name.
{Value: <datalog table name>}	Any value from the datalog table. Appending units in parenthesis to the name causes the value to be converted to those units. When the value is put into the label it will have units appended to it.
{Number}	The measurement's index in the measurement list.
{OldName}	The measurement's old name. This is particularly useful if you decide to apply the default name to measurements that have already been taken.

4. Once you've created a default name you can simply take measurements and they will all be labeled as you specified. If measurements have already been taken and you would like to replace the names of these measurements with your default name, click the **Apply** button.

For example, suppose you enter the following in the **Default Name** field:

```
{Solvent}: {Value: Time} - {Value: Temp (F)}
```

A measurement taken using PBS as a solvent and taken at 28.4s at a temperature of 21.2 °C would be labeled as follows:

```
PBS: 28.4s - 70.2F.
```

Note: The Temp value is converted to Fahrenheit from the default units of Celsius used in the datalog table.

5 Automating Experiments

This chapter describes how to schedule events to occur during the course of an automated experiment. It also provides sample scripts of commonly scheduled events.

Scheduling Events

You can schedule events to occur during the course of an automated experiment using the Event Scheduler.

It may be easier to understand event scheduling by trying some of the event schedule templates that come with DelsaMax Analysis Software. See "Sample Scripts to Automate Experiments" on page 5-7.

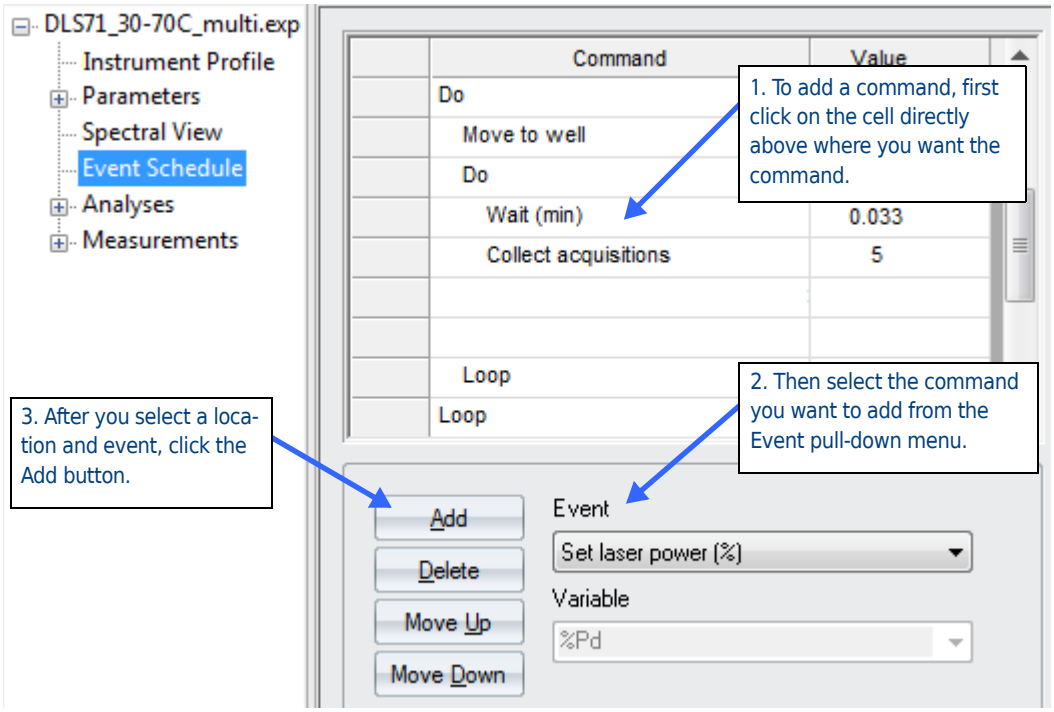
Note: You may set the acquisition time prior to starting the Event Schedule by entering a value in the **Parameters** ▸▸ **Instrument** node, or alternatively by including the "Set Acquisition Time" command in the Event Schedule.

Using the Event Scheduler

1. To open the Event Scheduler, select **Event Schedule** in the experiment tree.

   Note: If you don't see the Event Schedule node, right click in the experiment tree area and choose **Event Schedule** from the right-click menu or go to the **Parameters** ▸▸ **Fixed** node Fixed and set the **Event Schedule** parameter to Yes.

2. In the command list, click on the command directly above the position where you want to add a command.



3. Select a command from the **Event** drop-down list. See the "Event Schedule Commands" on page 5-3 for descriptions of the commands.
   - To add a command that requires a variable, select the variable from the **Variable** drop-down list and click **Add**.
   - To add a command that requires a value, click **Add** then enter the value in the **Value** column.
   - If the command requires neither a value nor a variable, click **Add**.
4. Double-click in the **Value** column to edit the values passed to the commands.
5. You can edit the event schedule by right-clicking in the command list. The right-click menu lets you **Cut**, **Copy**, **Paste**, and **Delete** in the command list.
6. Use the **Move Up** and **Move Down** buttons to change the sequence of events.

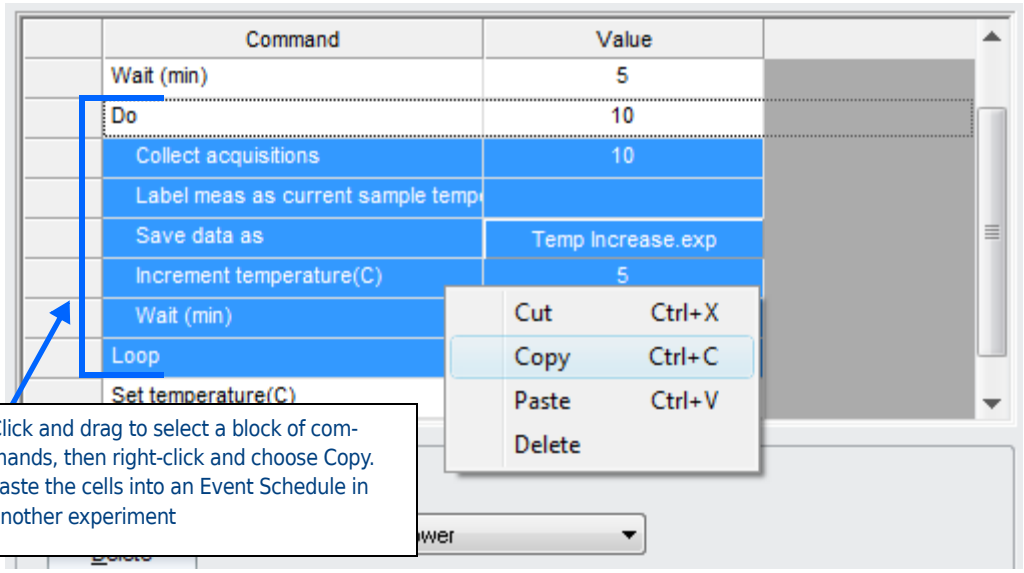
Cutting and Pasting Scripts Using DelsaMax Analysis Software Presets

You can also edit your Event Schedule by cutting and pasting from saved experiments and Presets.

A number of Presets are provided with DelsaMax Analysis Software in the `PreSets` subdirectory of the location where you installed DelsaMax Analysis Software. By default, this is the `C:\Program Files\BCI\DelsaMax v1.0\PreSets` directory.

1. Select **File** ▸▸ **Open Preset**.
2. Open the directory titled **DelsaMax v1.0\PreSets** and select a Preset file.

3. Select a range of commands in the Preset's Event Schedule to use in your experiment. Notice that the first command in the range you select has a white background, instead of blue. It is still part of the selection.
4. Right-click on a command and choose **Copy** in the right-click menu.



5. Move to your experiment and select the location in the Event Schedule where you want to place the commands.
6. Right-click on a command and choose **Paste** in the right-click menu.
7. Change values to suit your particular experiment.

Event Schedule Commands

This section describes the commands available in the Event Scheduler.

The event schedule commands in the following table are color coded to indicate which instruments can execute the commands:

- **Black** indicates the command is available for all instruments or only the instruments specified in the description.
- Orange indicates the command is only available for DelsaMax PRO or ASSIST instruments only. This includes DelsaMax PRO instruments connected to an autosampler.

Event Schedule Command	Description
Auto-attenuation disable	Disable auto-attenuation.
Auto-attenuation enable	Enable auto-attenuation.
Clear all data	Delete all data currently stored in the experiment file prior to collecting additional data, allowing the user to save independent data in separate experiment files.
Collect data	Collects the specified number of acquisitions for a measurement. See "Using the Optimization Calculator" on page 2-13 for help with determining the appropriate number of acquisitions.
Decrement temperature (C)	Decrease the current temperature by a user-defined amount specified in the value column.
Decrement temperature (C), don't wait	Set the target temperature to the current target temperature minus a user-defined value. The event scheduler continues to the next command without waiting for the instrument to reach the new temperature. This allows taking data while the instrument is changing temperature.
Depressurize	Depressurize the sample using the ASSIST accessory. (ASSIST only)
Do	Designate the beginning of a set of events or commands. The set of commands is performed or repeated a number of times as specified in the command. The "Do" command must be paired with the "Loop" command. The Do command is highlighted in **red** if there are not enough Loop commands.
Do until variable <	Designate the beginning of a set of repeated commands, which repeats until the specified Variable (e.g. Intensity) becomes less than the designated value. In other words, the customer instructs the DelsaMax CORE to "Repeat the following commands while the Variable is less than or equal to the value specified." The "Do until variable <" command must be paired with the "Loop" command.
Do until variable >	Designate the beginning of a set of repeated commands, which repeats until the specified Variable (e.g. Intensity) becomes greater than the designated value. The "Do until variable >" command must be paired with the "Loop" command.
Increment temperature (C)	Increase the current temperature by a user-defined amount specified in the value column.
Increment temperature (C), don't wait	Set the target temperature to the current target temperature plus a user-defined value. The event scheduler will continue to the next command without waiting for the instrument to reach the new target temperature. This allows the user to take data while the instrument is changing temperature.

Event Schedule Command	Description
Inject sample	Perform a sample injection with the current vial and the current sample parameters for the flow rate, injection volume, and run time. Use the “Move to vial” or “Move to next vial” command to set the vial number before each injection. If the injection parameters have not been specified in the Event Schedule, the parameters set in the experiment’s instrument parameters are used. Use either the “Inject sample” command (and its related commands) or the “Wait for auto-inject” command; do not use both of these commands in the same event schedule. (Autosampler with DelsaMax PRO only)
Inject sample from vial	Perform a sample injection as for the “Inject sample” command. However, you can specify the vial number as part of this command. (Autosampler with DelsaMax PRO only)
Inject wash	Perform a wash injection with the current vial and the current sample parameters for the flow rate, injection volume, and run time. Use the “Move to vial” or “Move to next vial” command to set the vial number before each injection. If the injection parameters have not been specified in the Event Schedule, the parameters set in the experiment’s instrument parameters are used. Note that the experiment’s instrument parameters do not store separate values for sample and wash injections. (Autosampler with DelsaMax PRO only)
Inject wash from vial	Perform a wash injection as for the “Inject wash” command. However, you can specify the vial number as part of this command. (Autosampler with DelsaMax PRO only)
Label meas as current sample temperature	Change the name of the current measurement from “Meas #” to the current temperature in degrees Celsius.
Label meas as temperature set point	Change the name of the current measurement from “Meas #” to the current temperature set point in degrees Celsius.
Label measurement	Change the name of the current measurement from “Meas #” to a name defined in the Value column.
Label measurement (formatted)	Change the name of the current measurement from “Meas #” to a name defined in the Value column. Measurement labeling codes can be placed in the Values column as well, refer to “Creating a Measurement Naming Template” on page 4-27.
Loop	Designate the end of a set of repeated commands, as defined by the “Do” designation (Do, Do Until). The Loop command is highlighted in **red** if there are not enough Do commands.
Move to next vial	Set vial number for the next injection to be the next vial in the sequence. (Autosampler with DelsaMax PRO only)
Move to vial	Set vial number for the next injection. (Autosampler with DelsaMax PRO only)
Pressurize	Pressurize the sample using the ASSIST accessory. (ASSIST only)
Save data	Save the collected data to the filename most recently specified by the “Set experiment name” command.
Save data as	Save the collected data into the designated path/filename.

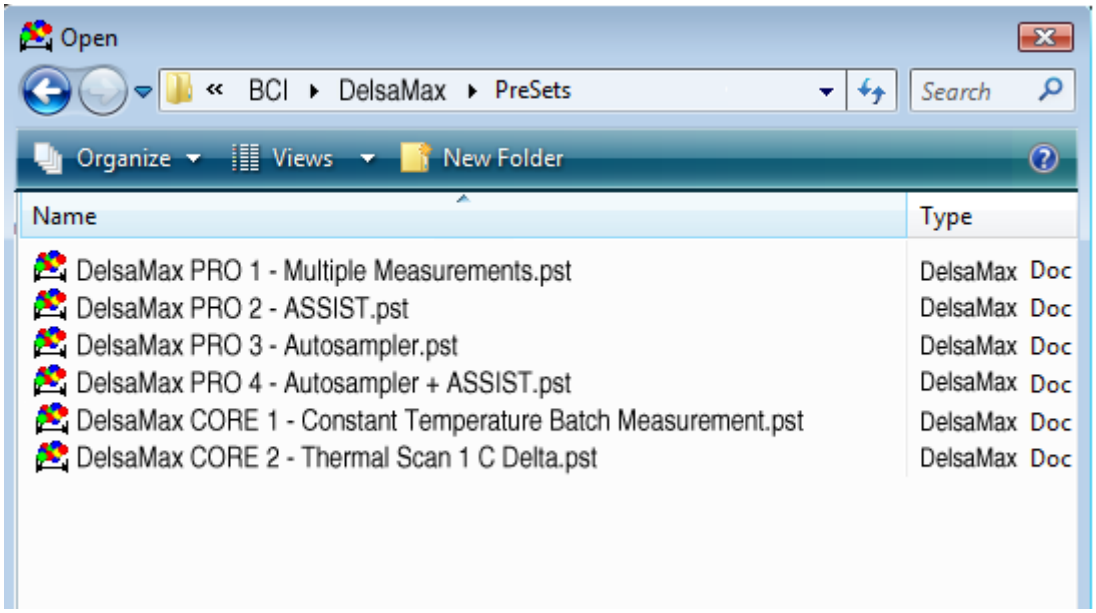
Event Schedule Command	Description
Send firmware command	Send a low-level command to an instrument. This command should not be needed by typical users.
Set attenuation (%)	Set the level of attenuation by the beam collector.
Set data type collected	Set whether to collect both QELS and PALs data, only QELS data, or only PALS data. Available only for DelsaMax PRO instruments with a QELS unit. The default is to collect both types of data. (DelsaMax PRO only)
Set DLS acquisition time (secs)	Change the default single acquisition collection time period. See "Using the Optimization Calculator" on page 2-13 for help with determining the appropriate acquisition time.
Set E field freq (Hz)	Set the electric field frequency for mobility measurements to the specified value in Hz. Use a value of 10 Hz or 20 Hz. (DelsaMax PRO only)
Set experiment name	Set the name of the file to which the experiment will be saved when the "Save data" command is run.
Set laser power (%)	Set the laser power to the specified, relative (percentage) power level, ranging from 0 to 100% of power.
Set number of DLS acquisitions	Set the number of dynamic light scattering acquisitions to be collected for the measurement.
Set PALS collection period (s)	Set the PALS collection period in seconds for mobility measurements. (DelsaMax PRO only)
Set sample flow rate (mL/min)	Set the flow rate for sample injections. (Autosampler with DelsaMax PRO only)
Set sample injection volume (µL)	Set the amount of sample injected with each sample injection. (Autosampler with DelsaMax PRO only)
Set sample pump run time (min)	Set the amount of time the pumps run during a sample injection. (Autosampler with DelsaMax PRO only)
Set temperature ramp rate (C/min).	Set the rate at which the instrument changes temperature to reach the target temperature. See "Using the Ramp Rate Calculator" on page 2-14 for help with determining the appropriate ramp rate. You cannot use this command until after the Temperature ramping enable command.
Set temperature (C)	Set the target temperature of the sample chamber to the specified value, proceeding only when the actual temperature is within close proximity to the target temperature (0.05 °C for two minutes for a DelsaMax CORE). If temperature ramping is disabled, adjusts the temperature set point immediately to the specified value, proceeding only when the actual temperature is within 0.1 °C of the target value. If temperature ramping is enabled, adjusts the temperature set point, along with the ramp rate, until the final set point is reached.
Set temperature (C), don't wait	Set the target temperature of the instrument to a user-specified value, and then proceeds immediately to the next command without waiting for the instrument to reach the target temperature. This allows the user to take measurements while the instrument is changing temperature.
Set voltage amplitude (V)	Set the voltage amplitude for the applied electric field for mobility measurements. (DelsaMax PRO only)
Set wash flow rate (mL/min)	Set the flow rate for wash injections. (Autosampler with DelsaMax PRO only)

Event Schedule Command	Description
Set wash injection volume (µL)	Set the amount of solvent injected with each wash injection. (Autosampler with DelsaMax PRO only)
Set wash pump run time (min)	Set the amount of time the pumps run during a wash injection. (Autosampler with DelsaMax PRO only)
Temperature ramping disable	Disable temperature ramping.
Temperature ramping enable	Enable temperature ramping. This command needs to be performed before the Set temperature ramp rate (C/min) command can be used.
Wait (min)	Instruct the instrument to Wait the specified time before proceeding to the next Event Schedule Command. This function allows the temperature to stabilize after a Set temperature (°C) command has been issued.
Wait for auto-injection	Instruct the instrument to wait until an auto-inject signal is received before proceeding to the next Event Schedule Command. Note that you can use either the "Inject sample" command (and its related commands) or the "Wait for auto-inject" command; do not use both of these commands in the same event schedule. (DelsaMax PRO only)

Sample Scripts to Automate Experiments

The following examples make use of common commands in the Event Scheduler. You can use these examples as templates to help you program your experiment.

These examples are provided as Presets with the DelsaMax Analysis Software package. These Presets can be found in the `PreSets` subdirectory of the location where you installed DelsaMax Analysis Software. You can use **File** ▸▸ **Open Preset** to open these examples:



Important: After opening a Preset, replace the default instrument in the Preset with your own Instrument Serial Number in the **Instrument Profile** node. Otherwise, you will not be able to connect to your instrument and run the Preset.

DelsaMax PRO 1: Multiple Measurements

Using this schedule, the DelsaMax PRO collects 5 PALS and DLS measurements. The preset for this schedule can be found in the Preset folder in the file "DelsaMax PRO 1 – Multiple Measurements.pst".

Table 5-1: Event Schedule for DelsaMax PRO Multiple Measurements

Command	Value	Description
Set PALS collection period (s)	20	Set PALS collection period to 20 seconds
Set voltage amplitude (V)	3	Set voltage amplitude to 3 V
Set E field freq (Hz)	10.0	Set the frequency of the electric field reversal to 10 Hz
Set DLS acquisition time (s)	2	Set the DLS acquisition time to 2 sec
Set number of DLS acquisitions	10	Set number of DLS acquisitions to 10
Wait (min)	0.5	Wait time before starting measurements (0.5 min)
Do	5	Number of measurements made (5); start of loop
Collect data		Perform DelsaMax PRO data collection
Label measurement (formatted)	[name]	Label measurements with user-specified name
Save data as	[file]	Save collected data into specified experiment file
Loop		End of loop

- The "Wait" command with a value of 0.5 minutes gives the sample time to equilibrate before the measurements.
- The Do-Loop has a repeat cycle of 5. It measures the sample five times to verify sample reproducibility.

Please see "Event Schedule Commands" on page 5-3 for details on each command in the Event Schedule.

DelsaMax PRO 2: ASSIST

Using this schedule, the DelsaMax PRO collects 3 PALS and DLS measurements while the sample is pressurized. The ASSIST accessory is required to pressurize the DelsaMax PRO flow cell. A Do-Loop command is used to repeat commands used multiple times within the same schedule.

The preset for this schedule can be found in the Preset folder in the file "DelsaMax PRO 2 – ASSIST.pst".

Table 5-2: Event Schedule Using ASSIST to Pressurize the DelsaMax PRO

Command	Value	Description
Set PALS collection period (s)	20	Set PALS collection period to 20 seconds
Set voltage amplitude (V)	3.0	Set voltage amplitude
Set number of DLS acquisitions	4	Set number of DLS acquisitions to 4
Set DLS acquisition time (s)	5	Set the DLS acquisition time to 5 sec
Pressurize		Sample is pressurized
Wait (min)	0.2	Wait time before starting measurements (0.2 min)
Do	3	Number of measurements made (3); start of loop
Collect data		Perform DelsaMax PRO data collection
Label measurement (formatted)	[name]	Label measurements with user-specified name
Loop		End of loop
Depressurize		Sample is depressurized

- The "Wait" command with a value of 0.2 min gives the flow cell time to pressurize before starting measurements.
- The Do-Loop commands with a repeat cycle of 3 measure the sample three times to verify sample reproducibility.

DelsaMax PRO 3: Autosampler

Using this schedule, the DelsaMax PRO uses the "Wait for auto-injection" command and careful timing to collect 3 PALS and DLS measurements for different samples delivered with an autosampler. First, an initial measurement of a wash solution is made. Subsequently, 4 samples and 4 buffer injections are measured. Each sample is measured three times.

The preset for this schedule can be found in the Preset folder in the file "DelsaMax PRO 3 – Autosampler.pst".

Table 5-3: Event Schedule for DelsaMax PRO with an Autosampler

Command	Value	Description
Set PALS collection period (s)	20	Set PALS collection period to 20 sec
Set number of DLS acquisitions	4	Set number of DLS acquisitions to 4
Set DLS acquisition time (s)	5	Set the DLS acquisition time to 5 sec
Wait for auto-injection		Wait to receive autoinject signal from the autosampler
Wait (min)	3.5	3.5 min wait time to flush the cell and stop the flow
Do	3	Number of measurements made (3); start of loop
Collect data		Perform DelsaMax PRO data collection
Label measurement	Initial Wash	Label measurements with user-specified name
Loop		End of loop
Do	4	Number of different samples injected (4); start of outer loop
Wait for auto-injection		Wait to receive autoinject signal from the autosampler
Wait (min)	1.5	1.5 min wait time to inject the sample into the cell and stop the flow
Do	3	Number of measurements made for each sample (3); start of inner loop
Collect data		Perform DelsaMax PRO data collection
Label measurement	Sample	Label measurements with user-specified name
Loop		End of inner loop
Wait for auto-injection		Wait to receive autoinject signal from the autosampler for next sample
Wait (min)	3.5	3.5 min wait time to flush the cell with buffer and stop the flow
Do	3	Number of measurements made (3); start of second inner loop
Collect data		Perform DelsaMax PRO data collection
Label measurement	Buffer Wash	Label measurements with user-specified name
Loop	[name]	End of second inner loop
Save data as	[file]	Save collected data into specified experiment file
Loop		End of loop

The autosampler method used with this preset should have the following flow rate changes for the wash injection.

Table 5-4: Wash Injection Flow Rates with Autosampler

Time (min)	Flow Rate (ml/min)
0.00	1.0
3.00	1.0
3.01	0.0
6.50	0.0

We recommend a 500 – 900 µL injection volume. This method will flush the wash injection through the DelsaMax PRO flow cell at 1.0 ml/min, stopping the flow after 3 minutes before data collection starts at 3.5 minutes. The stop time is 6.5 minutes.

The autosampler method used with this preset should have the following flow rate changes for the sample injection.

Table 5-5: Sample Injection Flow Rates with Autosampler

Time (min)	Flow Rate (ml/min)
0.00	0.5
1.00	0.5
1.01	0.0
6.50	0.0

We recommend a 500 – 900 µL injection volume. This method will flush the sample into the DelsaMax PRO flow cell at 0.5 ml/min, stopping the flow after 1 minute before data collection starts at 1.5 minutes. After data collection, the subsequent wash method is used to flush the sample out of the cell. The stop time of this method is 6.5 minutes.

Important: Contact Beckman Coulter if you need assistance synchronizing your HPLC method to your preset.

DelsaMax PRO 4: Autosampler + ASSIST

Using this schedule, the DelsaMax PRO uses the "Wait for auto-injection" command and careful timing to collect 3 PALS and DLS measurements for different samples delivered with an autosampler while the sample is pressurized using the ASSIST accessory. First, an initial measurement of a wash solution is made. Subsequently, 4 samples and 4 buffer injections are measured. Each sample is measured three times.

The preset for this schedule can be found in the Preset folder in the file "DelsaMax PRO 4 – Autosampler + ASSIST.pst".

Table 5-6: ASSISTEvent Schedule Using ASSIST with an Autosampler

Command	Value	Description
Depressurize		Depressurize before starting flow on the HPLC pump
Set PALS collection period (s)	20	Set PALS collection period to 20 sec
Set number of DLS acquisitions	4	Set number of DLS acquisitions to 4
Set DLS acquisition time (s)	5	Set the DLS acquisition time to 5 sec
Wait for auto-injection		Wait to receive auto-inject signal from the autosampler
Wait (min)	3.5	3.5 min wait time to flush the cell and stop the flow
Pressurize		Pressurize flow cell using the ASSIST accessory
Wait (min)	0.2	Wait time before starting measurements (0.2 min)
Do	3	Number of measurements made (3); start of loop
Collect data		Perform DelsaMax PRO data collection
Label measurement	Initial Wash	Label measurements with user-specified name
Loop		End of loop
Depressurize		Depressurize using ASSIST before starting pump flow
Do	4	Number of different samples injected (4); start of outer loop
Wait for auto-injection		Wait to receive auto-inject signal from the autosampler for next sample
Wait (min)	1.5	1.5 min wait time to inject the sample into the cell and stop the flow
Pressurize		Pressurize flow cell using the ASSIST accessory
Wait (min)	0.2	Wait time before starting measurements (0.2 min)
Do	3	Number of measurements made for each sample (3); start of inner loop
Collect data		Perform DelsaMax PRO data collection
Label measurement (formatted)	Sample	Label measurements with user-specified name
Loop		End of inner loop
Depressurize		Depressurize using ASSIST before starting pump flow
Wait for auto-injection		Wait to receive auto-inject signal from the autosampler for next sample

Table 5-6: ASSISTEvent Schedule Using ASSIST with an Autosampler

Command	Value	Description
Wait (min)	3.5	3.5 min wait time to flush the cell with buffer and stop the flow
Pressurize		Pressurize flow cell using the ASSIST accessory
Wait (min)	0.2	Wait time before starting measurements (0.2 min)
Do	3	Number of measurements made (3); start of inner loop
Collect data		Perform DelsaMax PRO data collection
Label measurement	Buffer Wash	Label measurements with user-specified name
Loop		End of inner loop
Depressurize		Depressurize flow cell before starting pump flow
Save data as	[file]	Save collected data into specified experiment file
Loop		End of outer loop

The autosampler method used with this preset should have the following flow rate changes for the wash injection.

Table 5-7: Wash Injection Flow Rates with Autosampler

Time (min)	Flow Rate (ml/min)
0.00	1.0
3.00	1.0
3.01	0.0
6.50	0.0

We recommend a 500 – 900 µL injection volume. This method will flush the wash injection through the DelsaMax PRO flow cell, stopping the flow after 3 minutes before the cell pressurization starts at 3.5 minutes. Data collection will commence. The stop time of this method is 6.5 minutes.

The autosampler method used with this preset should have the following flow rate changes for the sample injection.

Table 5-8: Sample Injection Flow Rates with Autosampler

Time (min)	Flow Rate (ml/min)
0.00	0.5
1.00	0.5
1.01	0.0
6.50	0.0

We recommend a 500 – 900 µL injection volume. This method will flush the sample into the DelsaMax PRO flow cell, stopping the flow after 1 minute before the cell pressurization starts at 1.5 minutes. Data collection will commence. After data collection and depressurization, the subsequent wash method is used to flush the sample out of the cell. Stop time is 6.5 minutes.

Important: Contact Beckman Coulter if you need assistance synchronizing your HPLC method to your preset.

DelsaMax PRO 5: Autosampler with Active Injection Control

The event schedules in "DelsaMax PRO 3: Autosampler" on page 5-10 and "DelsaMax PRO 4: Autosampler + ASSIST" on page 5-12 use the "Wait for auto-injection" command and careful timing to collect measurements for samples delivered with an autosampler. An alternate way to use an autosampler is to use commands to set injection parameters and the "Inject sample" and "Inject wash" commands to control the autosampler as described in "Controlling the Autosampler" on page 6-8.

The following event schedule begins by setting the injection parameters. It then performs injections from five sample vials and five wash vials.

Table 5-9: Event Schedule for DelsaMax PRO with Active Autosampler Control

Command	Value	Description
Set sample flow rate (mL/min)	0.6	Set the flow rate for sample injections
Set sample injection volume (µL)	60	Set the volume to inject for sample injections
Set sample pump run time (min)	0.6	Set the time to run the pump for sample injections
Set wash flow rate (mL/min)	0.4	Set the flow rate for wash injections
Set wash injection volume (µL)	40	Set the volume to inject for wash injections
Set wash pump run time (min)	0.4	Set the time to run the pump for wash injections
Move to vial	1	Move to the specified autosampler vial
Do	5	Number of sample and wash measurements made (5); start of loop
Inject sample		Inject from the current vial using the sample injection settings
Collect data		Perform DelsaMax PRO data collection
Label measurement (formatted)	Vial {value: Vial Number}	Label measurements with user-specified name
Save data as	[file]	Save collected data into specified experiment file
Move to next vial		Move to the next autosampler vial
Inject wash		Inject from the current vial using the wash injection settings
Move to next vial		Move to the next autosampler vial
Loop		End of loop

DelsaMax CORE 1: Constant Temperature Batch Measurement

Using this schedule, the DelsaMax CORE collects a batch of 3 measurements, with 10 acquisitions each, at 25 °C. A Do-Loop command is used to repeat commands used multiple times within the same schedule. At the end of the run, the laser is turned off. Auto-attenuation is active during the run to ensure optimal intensity count rates.

The Preset for this schedule can be found in the Preset folder in the file "DelsaMax CORE 1 - Constant Temperature Batch Measurement.pst".

Table 5-10: Event Schedule for Constant Temperature Batch Measurement

Command	Value	Description
Set laser power (%)	100	Set laser to maximum power level
Set temperature (C)	5	Initial temperature
Wait (min)	5	Temperature equilibration
Auto-attenuation enable		Activate laser auto-attenuation
Set acquisition time (secs)	5	Set single acquisition collection time period.
Set temperature (C)	25	Set initial temperature to 25 °C.
Wait (min)	5	Temperature equilibration
Do	3	Number of measurements made (3); start of loop
Collect acquisitions	10	Number of acquisitions
Label measurement (formatted)	[name]	Label measurements with user-specified name
Save data as	[file]	Save collected data into specified experiment file
Loop		End of loop
Set temperature (C), don't wait	25	Set temperature to 25 °C
Auto-attenuation disable		Deactivate laser auto-attenuation
Set laser power (%)	0	Set laser to minimum power level.

- The "Wait" command with a value of 5 minutes gives the sample time to equilibrate after inserting the cuvette into the compartment.
- The Do-Loop commands with a repeat cycle of 3 measure the sample three times to verify sample reproducibility.
- The "Set Temperature, don't wait" command is included in case you want to set the instrument to a different temperature upon completion of the data capture (that is, to return to ambient or cold storage temperature).

See "Event Schedule Commands" on page 5-3 for details on each command in the Event Schedule.

DelsaMax CORE 2: Thermal Scan, 1 °C Temperature Increments

Using this schedule, the DelsaMax CORE collects sample measurements over a range of temperatures in 1 °C increments. The initial temperature is set at 5 °C and increases to 70 °C at a rate of 2 °C/minute, resulting in 78 separate temperature measurements. The temperature is continuously ramping, so no time is spent equilibrating between acquisitions. Auto-attenuation is active during the run to ensure optimal intensity count rates. A Do-Loop command set is used to repeat commands used multiple times in the same schedule. At the end of the run, the temperature is set to 25 °C and the laser is turned off.

The Preset for this schedule can be found in the Preset folder in the file "DelsaMax CORE 2 - Thermal Scan 1 C Delta.pst".

Table 5-11: Event Schedule for Thermal Scan 1 °C Delta

Command	Value	Description
Auto-attenuation enable		Activate laser auto-attenuation
Set acquisition time (secs)	5	Set single acquisition collection time period.
Set temperature (C)	5	Set initial temperature to 5 °C.
Set temperature ramp rate (C/min)	2	Set temperature ramp rate to 2 °C/minute.
Set temperature (C), don't wait	70	Set final temperature to 70 °C.
Do	78	Number of measurements made (78); start of loop
Collect acquisitions	5	Number of acquisitions
Label measurement as current sample temperature		Automatically label measurements with current sample temperature
Save data as	[file]	Save collected data into specified experiment file
Loop		End of loop
Set temperature (C), don't wait	25	Set temperature to 25 °C
Auto-attenuation disable		Deactivate laser auto-attenuation
Set laser power (%)	0	Set laser to minimum power level.

- The "Set temperature ramp rate" value is critical to the experiment, producing the desired temperature increment associated with the total measurement time determined by the acquisition time and number of acquisitions. See "Using the Ramp Rate Calculator" on page 2-14 for how to calculate the appropriate value.
- The Do-Loop with a repeat cycle of 78 collects data over the desired temperature range. The value of 78 is 20% larger than the calculated number of loops required for a 1 °C increment (70 °C minus 5 °C is 65 loops) to provide a margin of safety in the event some measurements require longer optimization periods.
- The final "Set Temperature, don't wait" command is included in case you want to set the instrument to a different temperature upon completion of the data capture (that is, to return to ambient or cold storage temperature).

See "Event Schedule Commands" on page 5-3 for details on each command in the Event Schedule.

6
Recording Data

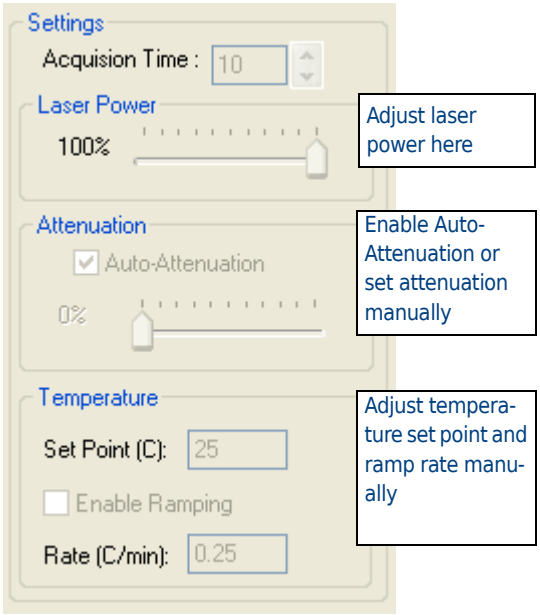
This chapter describes how to record data and how to monitor data with the Instrument Control Panels.

Monitoring Data with the Instrument Control Panel

Prior to starting an experiment, it is generally a good idea to check the quality of the data using the Instrument Control Panel.

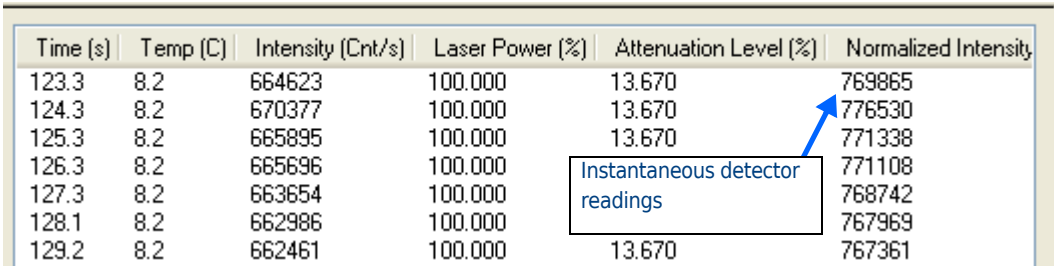
Use the toolbar icon in the toolbar or choose **View** ▸▸ **Instrument Control** in the menu bar to open the Instrument Control Panel.

Opening this panel automatically starts data monitoring.



In addition to the data monitoring, you can control parameters related to the instrument in the Instrument Control Panel. For example, for DelsaMax CORE instruments, you can set the acquisition time, laser power, attenuation, and temperatures.

For quick tests, you can adjust parameters in the Instrument Control Panel on the fly. If you are setting up an experiment that may be repeated, you should set the Laser Power and Acquisition Time values using the "Instrument Parameters Node" on page 4-5.

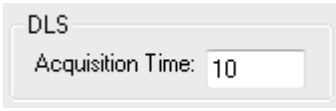


See the subsections that follow for details about categories of settings in the Instrument Control Panel. These categories depend on what instrument you are using.

- "Adjusting DLS Acquisition Time" on page 6-2
- "Adjusting Laser Power" on page 6-2
- "Adjusting Attenuation or Auto-Attenuation" on page 6-3
- "Adjusting Temperature Set Point and Ramp Rate" on page 6-3
- "Adjusting Electrophoresis Settings" on page 6-3
- "Adjusting Pressurization Settings" on page 6-4

Adjusting DLS Acquisition Time

The DLS acquisition time can be set in this screen. See "Using the Optimization Calculator" on page 2-13 for help in determining DLS acquisition times.



Adjusting Laser Power

We recommend using the default setting of 100% laser power for maximum sensitivity. This setting is suitable when working with dilute protein or other nanoparticle preparations. If you are working with a DelsaMax CORE, or DelsaMax PRO with a QELS unit, we recommend using the Auto-attenuation Function.

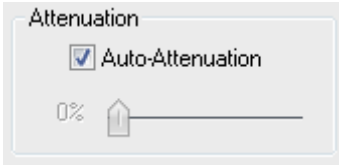
Otherwise, adjust laser power in the Instrument Control Panel so that the total intensity is below approximately 5 million but above approximately three times the count rate of the buffer solution (up to 100%). You may want to reduce the laser power prior to time or temperature experiments that may result in aggregation or increased particle size.

When working at high concentrations or with large particles, it may make sense to set the default laser power to 10-15% to avoid triggering the detector protector upon inserting the cuvette. If the detector protector triggers, it will automatically turn off the laser by disconnecting after three alerts are received. Alternately, select the "Stop" button to disconnect immediately upon receiving an alert.

See "DelsaMax PRO Parameters" on page 4-6 for the Laser Mode parameter that can be set for DelsaMax PRO instruments.

Note that the sensitivity scale is not necessarily linear.

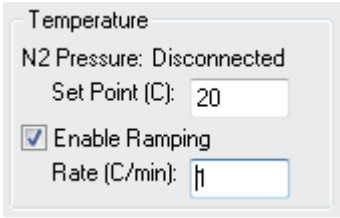
Adjusting Attenuation or Auto-Attenuation



The Attenuation functions reduce the signal that is received by the Single Photon Count Module, to lower the count rate. The Attenuation bar allows the user to manually attenuate the beam collector to a specific count rate.

The Auto-Attenuation button automatically reduces the signal so that the count rate will fall between an optimal, predetermined range. If the count rate drifts out of this range, the Auto Attenuator will activate, stop the current acquisition and zero it, move the signal back into range, and restart the acquisition. If the count rate is too high, the Auto-Attenuation will set to 100%, allowing no signal through, and will then back off to find the optimal count rate.

Adjusting Temperature Set Point and Ramp Rate



The Temperature area shows the pressure of the nitrogen gas, the current temperature set point, whether temperature ramping is enabled, and the current temperature ramp rate if any.

As long as a measurement is not in progress, the sample temperature set point and sample temperature set point ramp rate can be adjusted instantaneously. Temperature ramping can be enabled or disabled by clicking the "Enable Ramping" box. The temperature ramping option allows the user to change temperatures at a particular rate.

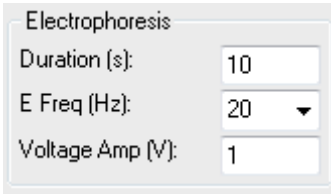
For the DelsaMax CORE, the maximum heating or cooling rate is 15 °C/min.

For the DelsaMax PRO, the maximum heating or cooling rate is 1 °C/min.

Be aware that the sample fluid temperature lags behind the cell temperature at high heating or cooling rates. Generally a ramp rate of 1 °C/min or less is recommended when measuring samples.

See "Using the Ramp Rate Calculator" on page 2-14 to calculate an appropriate value for the ramp rate.

Adjusting Electrophoresis Settings



You can change the duration, electric field frequency, and voltage amplitude use for measuring electrophoretic mobility with the DelsaMax PRO.

Duration: The duration can be an number of seconds. If the sample is subject to "cooking" by electric fields, the optimal value will be sample-dependent. Some samples are more vulnerable to applied electric fields and can degrade, denature, and/or aggregate in the presence of electrical current. While you can test a sample under different conditions to find a threshold below which the sample is not damaged, the general rule is that the higher the conductivity of the sample, the shorter the optimal collection period should be.

E Freq: The electric field frequency can be 10 Hz or 20 Hz. In theory, this frequency should not affect the measured mobility results. Nevertheless, the presence of counter-ions in the sample solution can complicate this matter, because they give rise to electrode polarization. The lower the

electric field frequency, the more polarized the electrodes will be. Since the number of counter ions increases with the ionic strength of the solution, you may see a better quality PALS V-graph with 20 Hz for solutions with higher ionic strengths. For most applications, 10 Hz is a good choice.

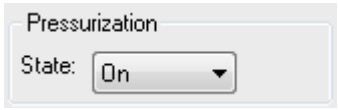
Voltage Amp: Set this parameter to the desired voltage for the applied electric field. DelsaMax PRO can apply up to 10 volts for PALS measurements of conductive samples and up to 100 volts for non-conductive samples. The optimal value will be sample-dependent. A minimum voltage of 1-1.5 volts should be applied to overcome the overpotential at the electrode-fluid interface. An applied voltage between 2.0 volts and 3.0 volts typically gives satisfactory results for most conductive aqueous solutions. Higher voltages can be applied for low-conductivity samples (< 1 mS/cm). Some samples are robust and can withstand high electric voltages and fields, while others should be treated more delicately. Organic and non-conductive samples are, in general, much more robust under electric fields because very little electrical current, if any, is present.

Note: The Duration, Electric Field Frequency, and Voltage Amplitude parameter settings should be considered as a whole. For example, while it is generally advisable to apply low to moderate voltages to conductive samples, sometimes you can gather better data by applying a slightly higher voltage with a shorter PALS collection period. The guidelines given here are not absolute rules; you should modify the settings as needed to fit your samples.

For each reading, the Instrument Control Panel shows the temperature, PALS amplitude (volts), forward laser monitor (volts), nitrogen gas pressure (in psi), the solution conductivity (in siemens/cm), and the electric field frequency (in Hz).

Adjusting Pressurization Settings

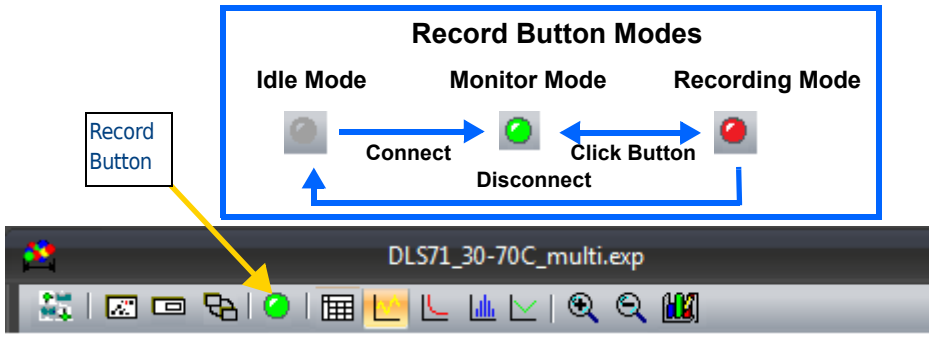
You can change the pressurization state if an ASSIST accessory is connected to a DelsaMax PRO or other instrument with a firmware upgrade to support the ASSIST.



You can toggle the Pressurization setting On when you are ready to take a measurement with the DelsaMax PRO and Off when you are finished taking the measurement.

Recording Data

Data recording in DelsaMax Analysis Software is controlled using the **Record** button on the experiment window toolbar. For batch mode experiments, the **Record** button exists in three states, representing the idle mode (gray button), monitor mode (green button), and recording mode (flashing red button) for the experiment window. For automated experiments, a yellow flashing button, representing the wait mode is also used.

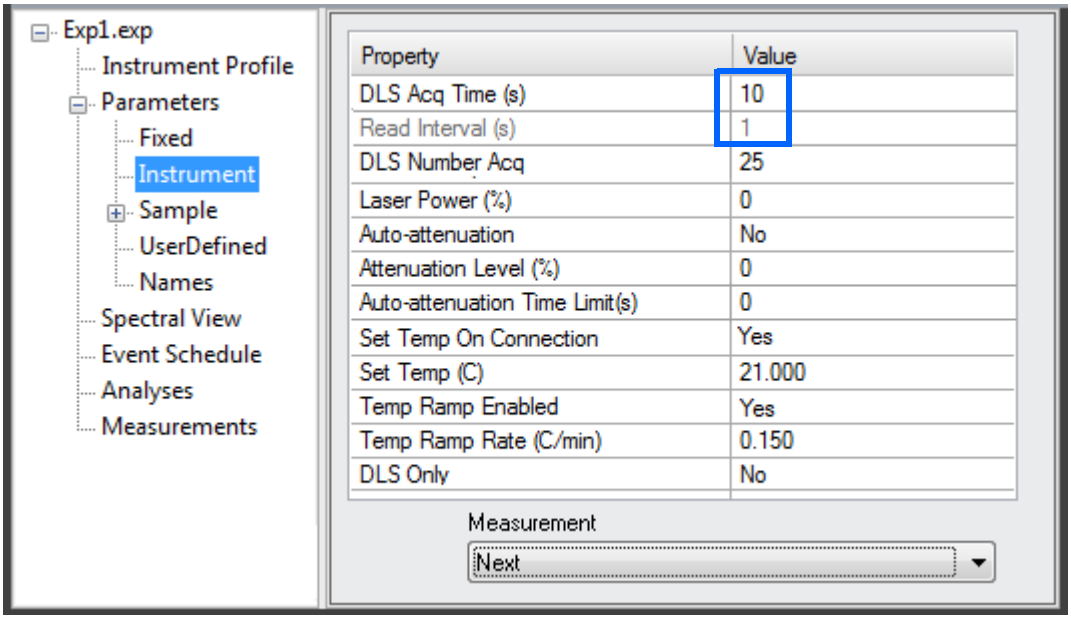


The basic steps for recording data are described in the subsections that follow. They are:

- "Setting the Run Length" on page 6-5
- "Connecting to Instruments" on page 6-6
- "Starting Data Recording" on page 6-6

Setting the Run Length

1. View the **Parameters** ▸▸ **Instrument** node in the experiment tree.
2. Set the acquisition time (**DLS Acq Time (s)**) to **5** seconds.
3. Set the number of acquisitions (**DLS Number Acq**) to **20**.

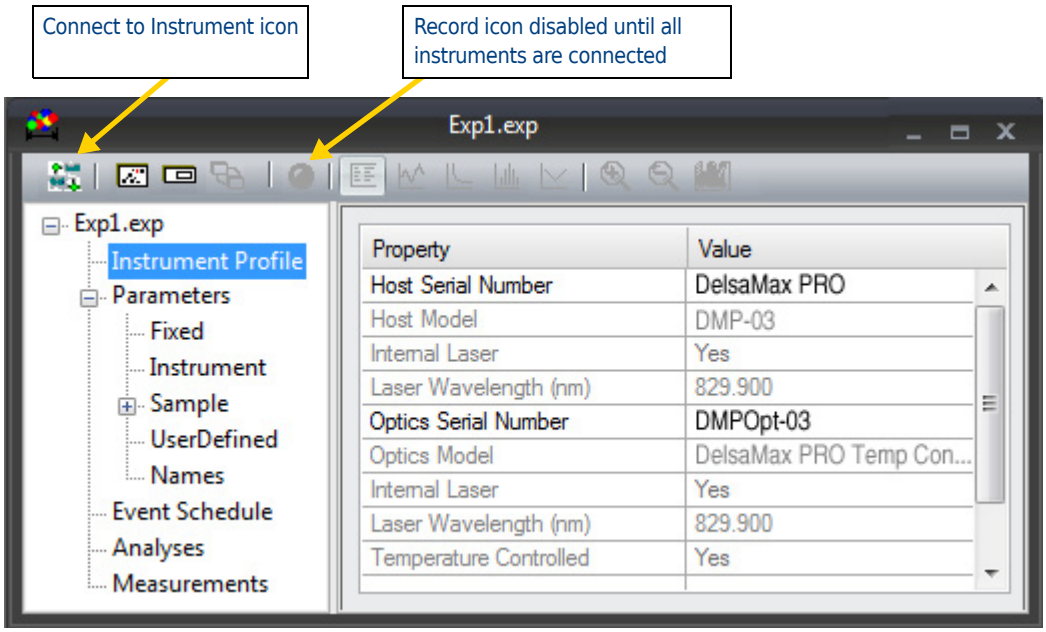


Connecting to Instruments

Connecting to an instrument places an experiment window in the monitor mode and changes the **Record** button color to green, indicating that the system is ready to begin recording. While the incoming data stream can be monitored from the Instrument Control Panel, none of the data is being saved to memory when the system is in the monitor mode.

1. Click the **Connect to Instrument** button to open communications between the software and the various instruments.
2. Once the instrument is connected, the **Record** button on the experiment window toolbar will turn green, indicating that the software is ready to begin recording data.

Note: Once the laser is enabled or turned on, a wait time of up to 30 seconds may be required before the laser is functional.



Starting Data Recording

1. To start recording data, click the green **Record** button.

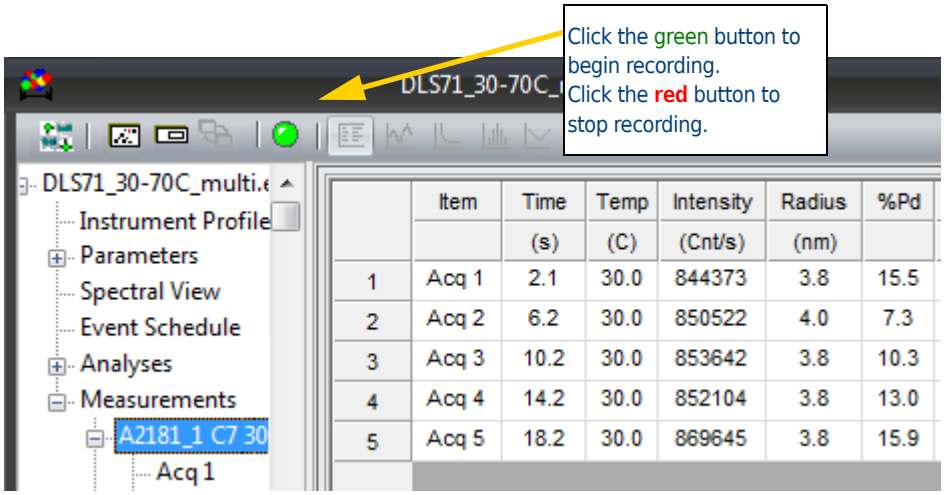
   The button face changes to a flashing red, indicating that DelsaMax Analysis Software is recording data. Incoming data are displayed in the Measurements grids of the experiment window.

   While the experiment window is in recording mode, incoming data are recorded, regardless of the view you are looking at. Incoming data can be displayed in any of the views available on the experiment window toolbar—Datalog Grid, Datalog Graph, Correlation, and Regularization.

   Note that DelsaMax Analysis Software does not permit Microsoft Windows to go into sleep mode while data is being collected.

2. To change the view, click a view icon in the toolbar. DelsaMax Analysis Software lets you view and even perform functions on data from another measurement while saving new data.

The button turns green again after the specified number of acquisitions and the specified acquisition time is met. This means the software is ready to begin recording data for the next measurement.



	Item	Time (s)	Temp (C)	Intensity (Cnt/s)	Radius (nm)	%Pd
1	Acq 1	2.1	30.0	844373	3.8	15.5
2	Acq 2	6.2	30.0	850522	4.0	7.3
3	Acq 3	10.2	30.0	853642	3.8	10.3
4	Acq 4	14.2	30.0	852104	3.8	13.0
5	Acq 5	18.2	30.0	869645	3.8	15.9

3. To stop recording data manually, click the flashing red **Record** button. The **Record** button face will then change to green, indicating that software is ready to begin recording data for the next measurement.

Notes & Tips

A new measurement category is auto-created in the Measurements node every time the experiment window is placed in the recording mode. New acquisitions cannot be added to an existing measurement.

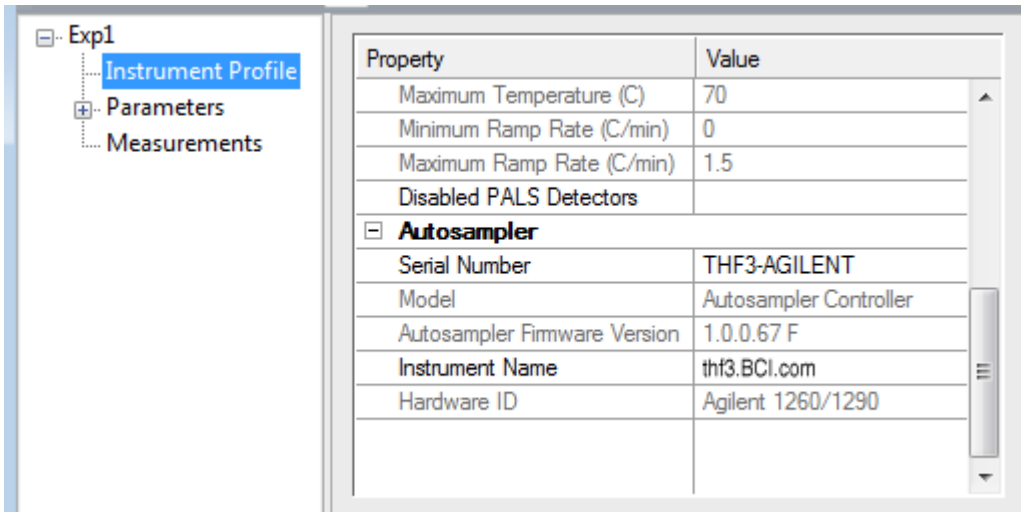
The acquisition time and laser power are fixed parameters for a given measurement, unless adjusted by auto attenuation. When the experiment window is recording, the Acquisition Time and Laser Power fields in the Instrument Control Panel are disabled. To adjust these parameters, you'll need to stop recording, make the adjustments, and then re-start recording.

While running an Event Schedule, the Record button flashes yellow unless data are recorded.

Controlling the Autosampler

To configure an experiment to use an autosampler with the DelsaMax PRO, follow these steps:

1. Install the autosampler control service on the computer that runs the autosampler. This may be a different computer than the one running DelsaMax Analysis Software. See the documentation provided with the autosampler control service software for details.
2. When you create an experiment that will use the autosampler, use the Instrument Profile node to select the autosampler in the **Serial Number** field below the Autosampler node (page 3-2). The autosampler may have been auto-detected or added manually (page 3-5).

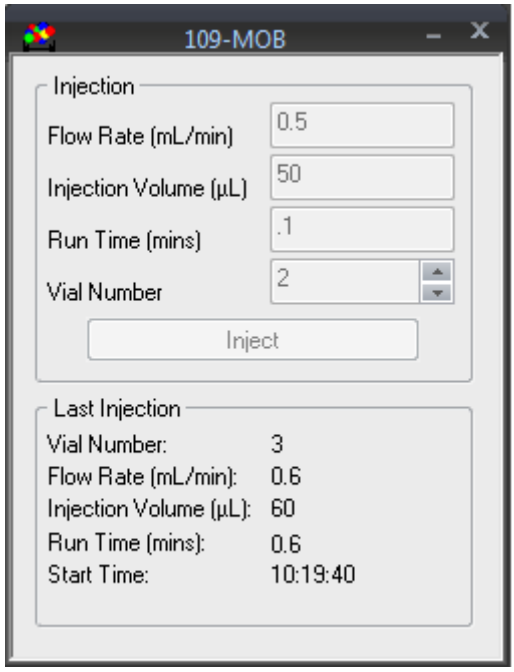


3. In the Instrument node for the experiment, adjust the **Flow Rate**, **Injection Volume**, and **Run Time** parameters so that the sample is in the DelsaMax PRO cuvette when the Run Time ends. The values you will need to use depend on the fluid volume in your system between the autosampler and the DelsaMax PRO.

Once the experiment is configured, one way to control the autosampler is by using the Event Scheduler to program a series of injection events. If you use the Event Scheduler, you can specify different autosampler parameter values for sample and wash injections. See "Event Schedule Commands" on page 5-3 for autosampler events.

Another way to control the autosampler is by using the Autosampler Control dialog to manually trigger injections. To open this dialog, choose **View** ▸▸ **Autosampler Control**. from the menus or click the  icon in the toolbar. Set parameters as follows:

- **Flow Rate (mL/min)**: Specify the rate at which solvent and sample should flow through the autosampler.
- **Injection Volume (µL)**: Specify the amount of sample that should be injected each time an injection is performed.
- **Run Time (min)**: Specify how long the pumps should run during an injection.
- **Vial Number:** Specify the vial number to use for the next injection.



Click **Inject** when you want to trigger the autosampler to perform an injection using the specified parameter values.

Once the injection is running, you can cancel it by pressing the **Stop Injection** button (the text on the **Inject** button changes). The dialog also shows the last vial injected, the injection settings used, and any errors from the last injection.

Typically, you will want to alternate between “sample” and “wash” injections. A common strategy is to set up the autosampler so that odd numbered vials contain detergent for washing the cuvette while even numbered vials contain sample. Wash injections are normally done with a longer run time than sample injections. You can use an Event Schedule to automate experiments that use different times and volumes for sample and wash injections (see page 5-3).

The vial number of the last injection is recorded in each experiment. You can see this number in the “vial number” column.

Deleting Data Measurements

You can delete unwanted or unnecessary measurements after they have been recorded.

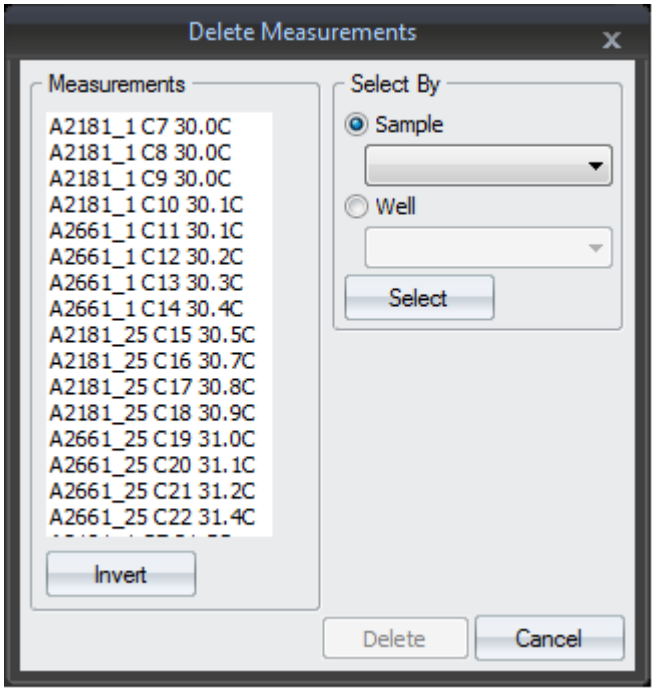
Entire measurements are deleted at once; you cannot delete individual acquisitions, though you can mark individual data points as outliers that should not be included in calculations (see page 7-41).

Note: We recommend that you make a backup copy of the experiment file before deleting any data.

To delete an individual measurement, follow these steps:

1. Click the + sign to expand the **Measurements** node and view the names of all the measurements that have been taken.
2. Right-click on a measurement you want to delete and select **Delete** from the right-click menu.

If you want to delete multiple measurements at once, select **Experiment** ▸▸ **Delete Measurements** from the main menu bar. In the **Delete Measurements** window, highlight the measurements you wish to delete and click the **OK** button.



Tip: If you change your mind, close the experiment file without saving and re-open it.

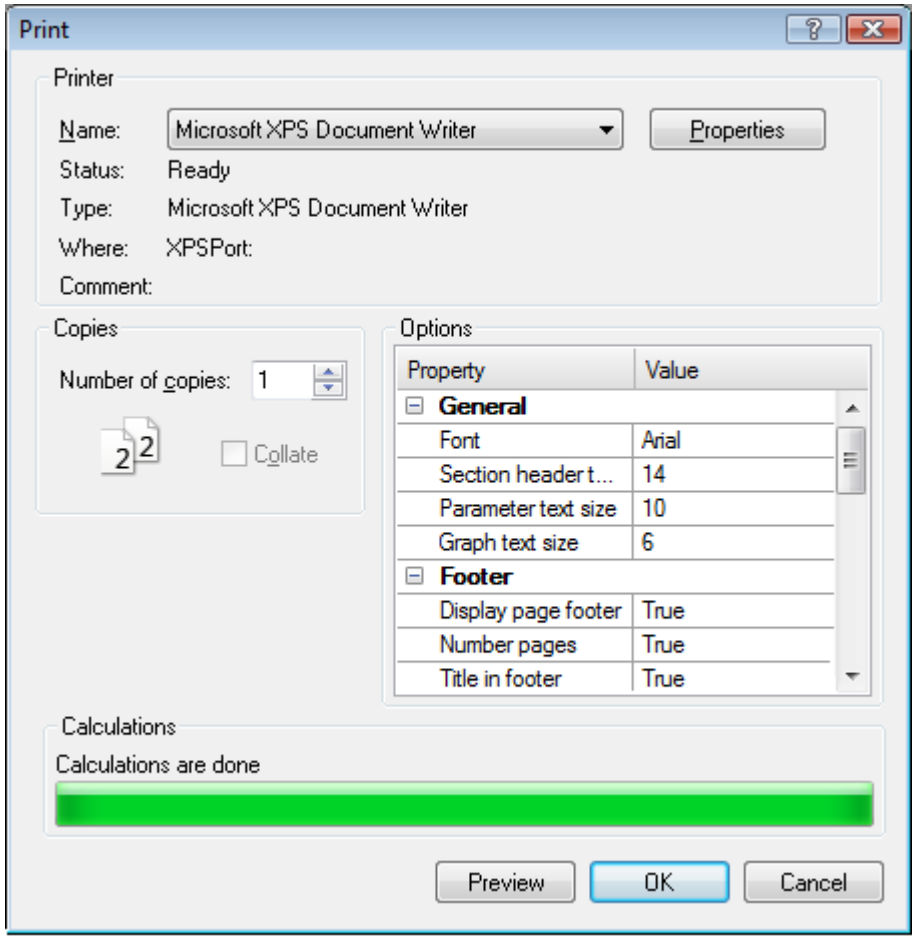
7
Displaying Data

This chapter describes how to manage and display large amounts of data captured by DelsaMax CORE or DelsaMax PRO using the various data management and analysis tools available in DelsaMax Analysis Software.

Printing Experiment Reports

DelsaMax Analysis Software provides the following commands for printing experiment information, data, and graphs:

- **File** ▸▸ **Page Setup** opens a standard Page Setup dialog that lets you set the paper size, source, orientation and margins.
- **File** ▸▸ **Print** (or Ctrl+P or the  toolbar icon) opens a Print dialog. In addition to standard options to select a printer, printer properties, and number of copies, DelsaMax Analysis Software lets you set a number of properties related to what the printout will contain. These differ depending on whether you are printing from a **Measurements** node or some other node. When you print from a **Measurements** node, the output contains data and graphs for the selected measurement or acquisition. When you print from a non-Measurements node, a general report about the experiment is produced. See page 7-2 for a list of properties you can set.
- **File** ▸▸ **Print Preview** (or the  toolbar icon) opens the currently selected print output based on the most recent settings in the Print dialog and the node you have selected in DelsaMax Analysis Software.



Report Printing Properties

When you print from a non-Measurements node of the experiment tree, a general report about the experiment is produced. By default, this report contains sections on Annotations (information about the experiment file), Instrument, Fixed, Event Schedule, Samples, Solvents, and Parametric Analysis. These report sections contain information that corresponds to that in the nodes of the experiment tree.

You can turn off creation of any report sections in the Print dialog. You can set the values of any of the following properties:

Property	Default
General	
Font	Arial
Section header text size	14
Parameter text size	10
Graph text size	6
Footer	
Display page footer	True

Property	Default
Number pages	True
Title in footer	True
Date in footer	True
Footer text size	6
Batch Printing	
Enable	False
Measurements	... (see page 7-5)
Collate by Measurement	True
Grid	
Minimum text size	6
Allow direction change	True
Annotations	True
Instrument	True
Fixed	True
Event Schedule	True
Samples	True
Solvents	True
Cuvettes	True
Instrument Parameters	True
Parametric Analysis	
Print	True
Print Graphs	False
Separate Replicate Graphs	False
Width	Full Page
Width (inches)	6
Fraction of page width	0.5
Height	Fraction of page
Height (inches)	6
Fraction of page height	0.3
Print grid	True
Show Replicates in grid	False

When you print from the **Measurements** node or any of its sub-nodes, the output contains data and graphs for the selected measurement or acquisition. In addition to the properties you can set for the general report, you can also set the following properties in the Print dialog for a Measurements report:

Property	Default
Datalog Table	
Print	True
Print data rows	True
Print statistics rows	True
Datalog Graph	
Print	True
Width	Full Page
Width (inches)	6
Fraction of page width	0.5
Height	Fraction of page
Height (inches)	6
Fraction of page height	0.3
Correlation Function	
Print	True
Width	Full Page
Width (inches)	6
Fraction of page width	0.5
Height	Fraction of page
Height (inches)	6
Fraction of page height	0.3
Print error graph	True
Error graph size	0.3
Mobility	
Print	False
Width	Full Page
Width (inches)	6
Fraction of page width	0.5
Height	Fraction of page
Height (inches)	6
Fraction of page height	0.3
Print error graph	True
Error graph size	0.3
Regularization Results	
Print	False
Print Graph	True

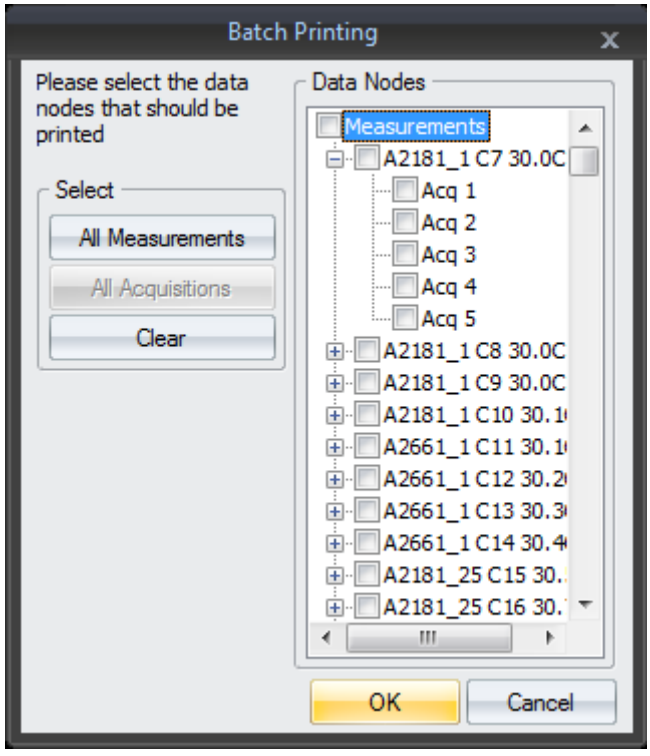
Property	Default
Width	Full Page
Width (inches)	6
Fraction of page width	0.5
Height	Fraction of page
Height (inches)	6
Fraction of page height	0.3
Print results grid	True

Batch Printing

One of the print properties is "Batch Printing". This option allows you to create a report that contains data and graphs for the Measurement sub-nodes that you select. This may include data and graphs for measurements and for individual acquisitions as desired.

To use batch printing, follow these steps:

1. Select **File** ▸▸ **Print** from the menu bar.
2. In the Options area, scroll down to find the "Batch Printing" category. Set the **Batch Printing** ▸▸ **Enable** property to True.
3. Select the **Batch Printing** ▸▸ **Nodes** property, and click the "..." button in the Value column. You see the Batch Printing dialog:



4. Check the boxes next to the nodes that you want to include separate data grids and graphs for in the report.
   - You can click the **All Measurements** button to select all the measurement nodes.

- If you select an individual measurement, you can click **All Acquisitions** to include separate data and graphs for all acquisitions in that measurement.
- Checking the box next to "**Measurements"** causes an overlay graph to be printed for the Datalog Graph, Correlation Graph, and Regularization Graphs if Print is set to True for those fields.

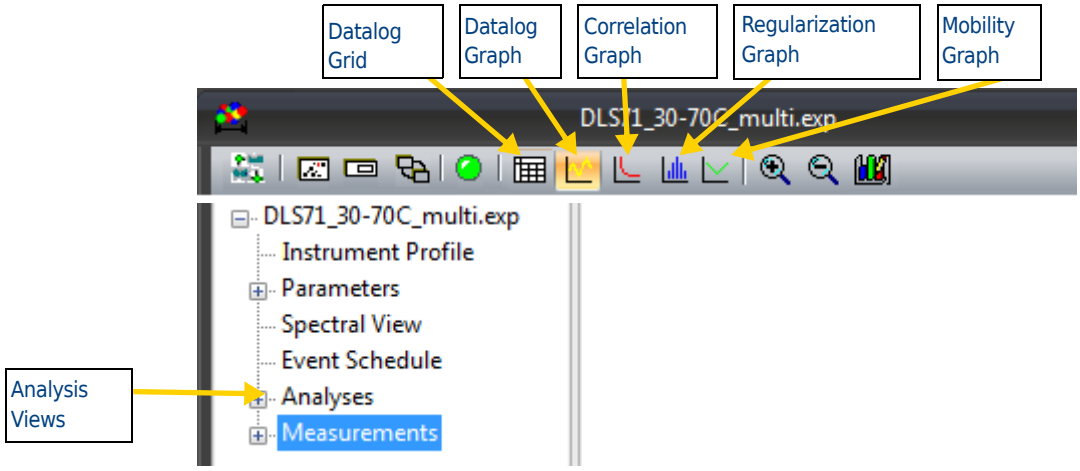
5. Click **OK**.
6. In the Print dialog, scroll down to set the **Print** property to **False** for any tables or graphs that you do not want to include in the report for all the nodes you selected.
7. When you are ready to print, click **OK**.

Note: Reports can become quite long if you select many measurements and acquisitions.

Displaying Data Views

While DelsaMax Analysis Software is in recording mode and after data has been collected, you can display data in the **Measurements** node and its subnodes using any of the views that can be accessed from the experiment window toolbar: **Datalog Grid**, **Datalog Graph**, **Correlation Graph**, **Regularization Graph** and **Mobility Graph** (for DelsaMax PRO). Additional views can be accessed from the experiment tree: **Temperature Dependence** and other parametric analysis views.

To display a view, click the appropriate view button on the toolbar as shown below.



The views available in DelsaMax Analysis Software are described in this chapter:

- Measurements node views
  - "Datalog Grid" on page 7-14
  - "Datalog Graph" on page 7-20
  - "Correlation Graph" on page 7-21
  - "Regularization Graph" on page 7-25
  - "Mobility Graph" on page 7-30

- Other node views
  - "Analysis Views" on page 7-33

Working with Grid Views

The following views contain grids of data cells (like a spreadsheet):

- Datalog Grid for Measurement node, measurements, and acquisitions (page 7-14)
- Results Summary table in the Regularization Graph (page 7-25)
- Statistics table in the Datalog Grid (page 7-14)
- Analysis view tables (page 7-33)

For example, this is part of a Datalog Grid for acquisitions in a measurement:

	Item	Time (s)	Temp (C)	Intensity (Cnt/s)	Radius (nm)	%Pd	Mw-R (kDa)	Amp	Baseline	SOS
1	Acq 1	2.100000	30.0	844373.000000	3.838810	15.5	78	0.154	1.000	0.291
2	Acq 2	6.200000	30.0	850522.000000	3.958390	7.3	84	0.153	1.000	0.240
3	Acq 3	10.200000	30.0	853642.000000	3.796470	10.3	76	0.152	1.000	0.388
4	Acq 4	14.200000	30.0	852104.000000	3.801710	13.0	77	0.150	1.000	0.178
5	Acq 5	18.200000	30.0	869645.000000	3.849870	15.9	79	0.152	1.000	0.220

You can work with the cells in grids in the following ways:

- Copying data for pasting as comma-separated values (CSVs) (page 7-7)
- Exporting a CSV file (page 7-8)
- Formatting table cells (page 7-8)
- Choosing table columns (page 7-9)
- Setting peak ranges (page 7-10)
- Marking data outliers (page 7-41)
- Filtering data (page 7-43)

Copying Data

You can copy selected data cells from any of the table views to the Windows clipboard. The data are in tab-separated format. You can then paste the data into other applications such as Microsoft Excel, Word, and/or PowerPoint.

To copy data, right-click in the view and select **Copy** from the right-click menu. If you use your mouse to select some table cells before selecting **Copy**, only the data from the cells you selected will be placed on the clipboard.

Exporting Data

You can export numerical data from grids to a CSV (comma-separated values) file for use with external analysis packages like Spotfire. Exporting from the Datalog Grid results in a CSV file that contains a row of data for each measurement that was taken. All the data for that measurement is provided in a row with commas between values. If a measurement has more than one regularization peak, the CSV file contains a separate row of values for each peak.

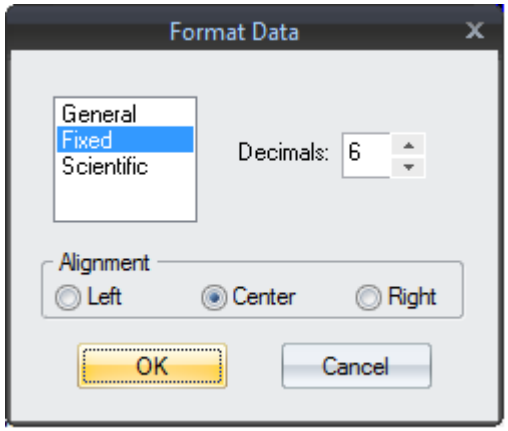
To export data, right-click the data grid and then select **Export** from the right-click menu.

- In a Datalog grid and the Results Summary table of the Regularization Graph, you can save the data to a CSV file.
- In the Statistics table of a Datalog grid, the exported file is saved in tab-separated format to a .dat file.
- You cannot export from the data grid in an analysis view.

Formatting Table Cells

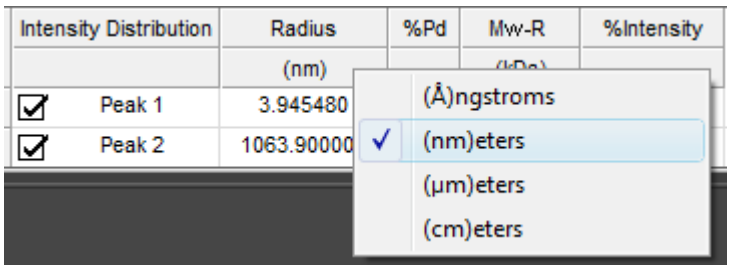
In a data grid you can choose a data format for numeric columns on a per-column basis by following these steps:

1. Right-click on a column heading and select **Format Data** from the right-click menu.
2. In the Format Data window, select **General**, **Fixed**, or **Scientific.**
3. Set **Decimals** to the number of digits to show after the decimal point.
4. Select the data **Alignment**.
5. Click **OK**.

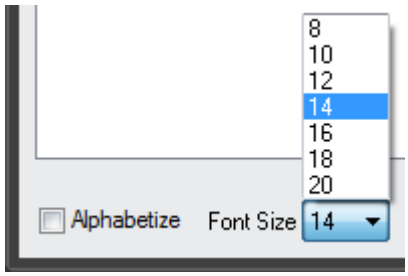


For columns that have a unit, the column heading also shows the unit currently used. To change the units, follow these steps:

1. Right-click on the unit in the column heading.
2. From the right-click menu, select the unit to which you want to convert the data.



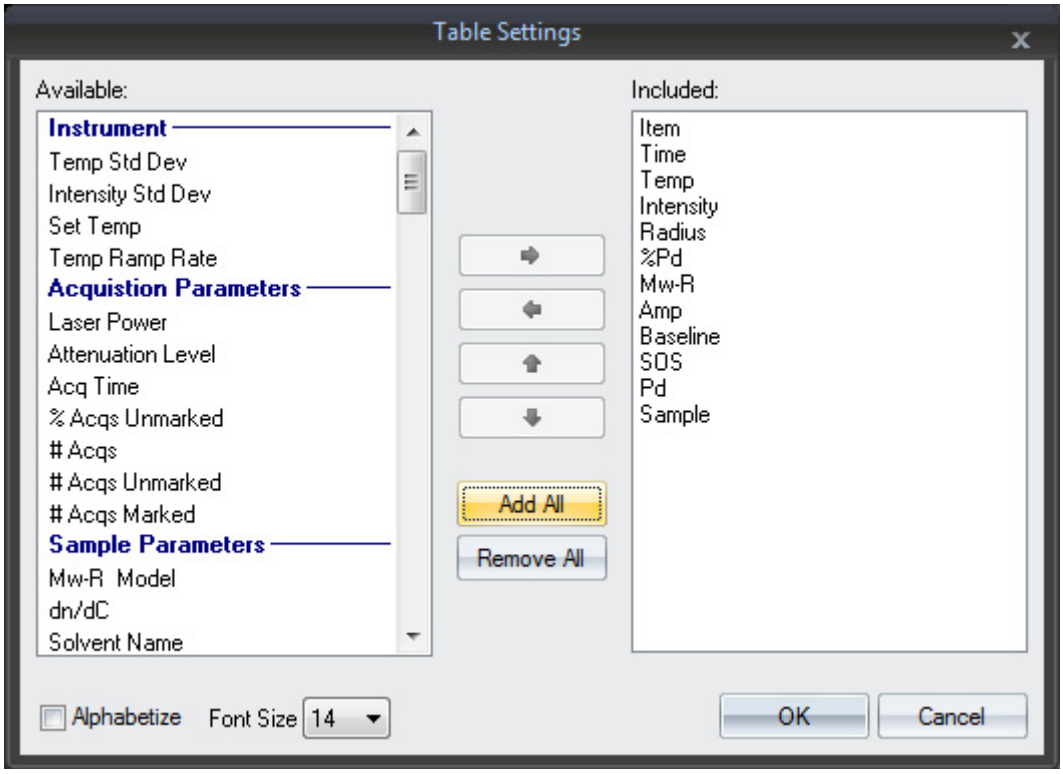
To change the font size of table cells, right-click on the data and choose **Table Settings** from the right-click menu. In the Table Settings dialog, choose a **Font Size** for the grid.



Selecting Columns in a Grid

You can choose which data columns to display in the Datalog grid and the Results Summary table of the Regularization Graph. Follow these steps:

1. Right-click on the data grid and select **Table Settings** from the right-click menu. The Table Settings dialog allows you to add, remove, and sort columns.



2. Select columns you want to add to the grid from the **Available** list and click the right-arrow button to move them to the **Included** list.
3. In the **Included** list, you can use the up and down arrows to modify the order of columns in the grid.
4. Put a check mark in the **Alphabetize** box to list the Available columns alphabetically. Otherwise they are sorted by category. See "Available Datalog Grid Columns" on page 7-15 for descriptions of the columns available in the Datalog Grid. See "Results Summary Table" on page 7-27 for descriptions of the columns available in the Regularization Graph.

You can remove a column from a data grid by right-clicking on the column heading and selecting **Remove Column** from the right-click menu.

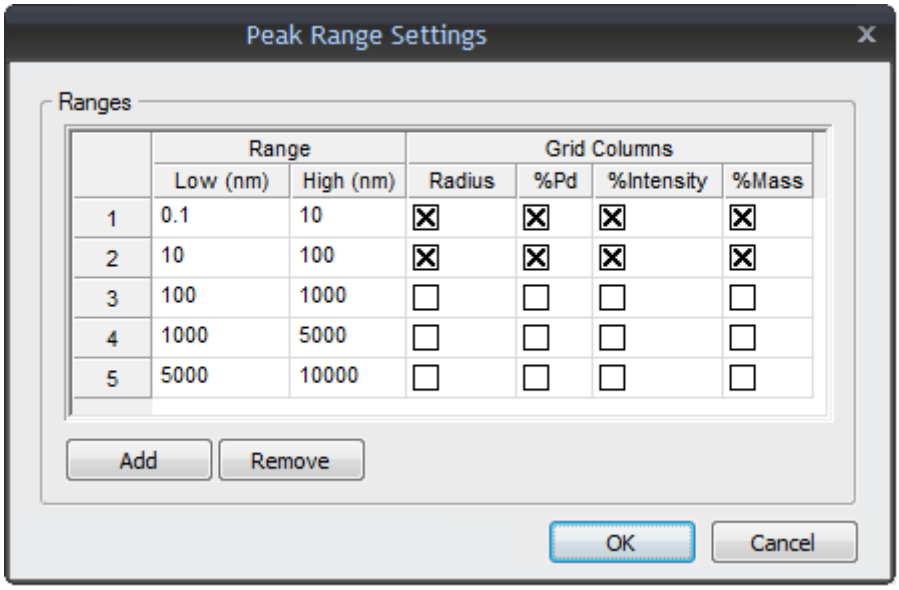
Setting Peak Ranges

You can use the Peak Range Settings dialog to specify the minimum and maximum radius values for the peak range. These settings are used in the Datalog Grid and the Results Summary of the Regularization Graph.

A "peak range" is a user-specified range of reported radii from regularization results. Any peak—or group of similar radii—within the range will be displayed in the Datalog Grid. If there is more than one peak in the range, the peak that corresponds to the lowest reported radii within the range will be displayed in a blue font to indicate that multiple peaks occur within the range.

To display the Peak Range Settings dialog:

1. Right-click in the Datalog Grid view (or the Results Summary of the Regularization Graph) and select **Peak Range Settings**.



2. Enter **Minimum** And **Maximum** size values in nm for each peak.
3. Use the checkboxes to select which quantities to calculate and display for a peak range in the Datalog Grid. The available options are:

   Radius: The average radius for the peak range.

   %Pd: The percent polydispersity for the range.

   %Intensity: The percentage of total scattering intensity in the range.

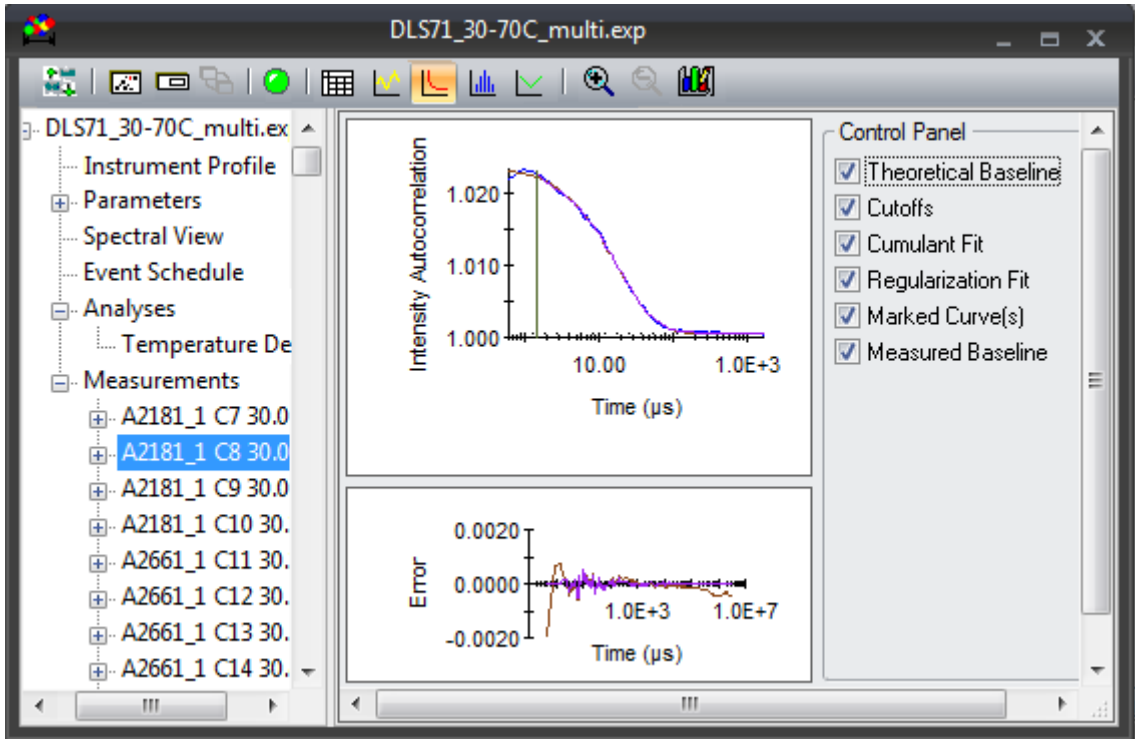
   %Mass: The percentage of the total mass in the range based on the Mw-R model selected in the Parameters Sample node.
4. To remove a peak range setting from the grid, deselect all the checkboxes for that peak range.
5. To specify more than 5 peaks, click **Add**.

Working with Graphs

The following views contain graphs:

- "Datalog Graph" on page 7-20
- "Correlation Graph" on page 7-21
- "Regularization Graph" on page 7-25
- "Mobility Graph" on page 7-30
- "Analysis Views" on page 7-33

For example, this is a Correlation Graph for a measurement:



You can work with the graphs in the following ways:

- Copying graphs for pasting as images (page 7-7)
- Exporting a CSV file (page 7-8)
- Scaling a graph (page 7-12)
- Changing the graph font (page 7-13)
- Filtering data (page 7-43)

Copying Graphs

You can copy a graph to the Windows clipboard for pasting into other applications such as Microsoft Word and/or PowerPoint.

To copy a graph, right-click on the graph and select **Copy as EMF** or **Copy as JPG** from the right-click menu. The EMF format stores the graph as an Enhanced Metafile, which is a Windows-based format that can store images as vectors. The JPG format stores the graph as a bitmap. In general, the EMF format will provide higher resolution graphics if you are pasting into an application that supports this format.

Exporting Graphs

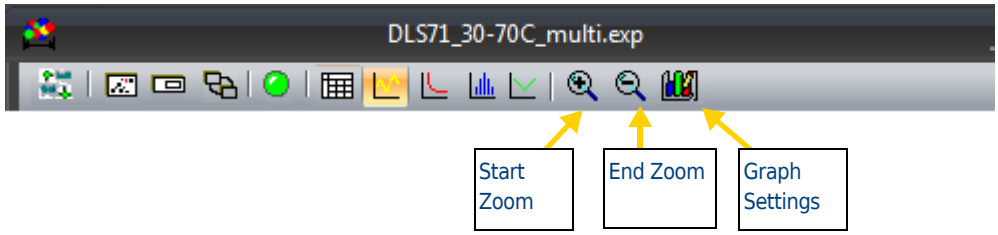
You can export the data used to create a graph to a CSV file for use with external analysis packages like Spotfire or Microsoft Excel.

To export data, right-click the graph and then select **Export** from the right-click menu. You are prompted to specify the file name and location.

The CSV file contains a comma-separated list of the y-axis values followed by the x-axis value. The default y-axis and x-axis values depend on the type of graph you are using, but the first row of the CSV file contains headings to identify each column.

Scaling Graphs

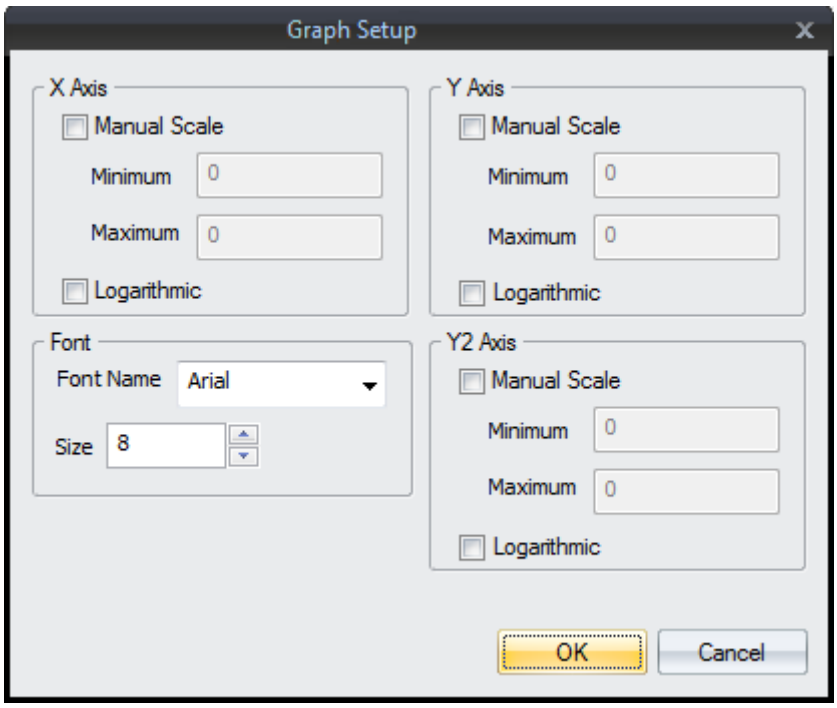
You can zoom in to expand a portion of a graph or zoom out to see the whole graph.



You can control the zoom level in any of the following ways:

- Click the **Start Zoom** icon in the toolbar and drag your mouse over the area of the graph that you want to expand.
- Click the **End Zoom** icon in the toolbar to return to the default zoom level.
- Right-click on a graph and select **Auto-scale** from the right-click menu to return to the default zoom level.
- Hold down the Ctrl key and select a region with the left mouse button to zoom in. To zoom out again, hold down the Ctrl key and click the right mouse button.
- Click the **Graph Settings** icon in the toolbar (or right-click and choose **Graph Settings**) to open the Graph Setup dialog.

The Graph Setup dialog gives you detailed control over the scaling of the X, Y, and Y2 axis for the graph. You can set the minimum and maximum values for the axis and can set the axis to logarithmic scaling.



Formatting Graphs

In addition to detailed scale control, the Graph Setup dialog lets you select the font and font size to use in the graph.

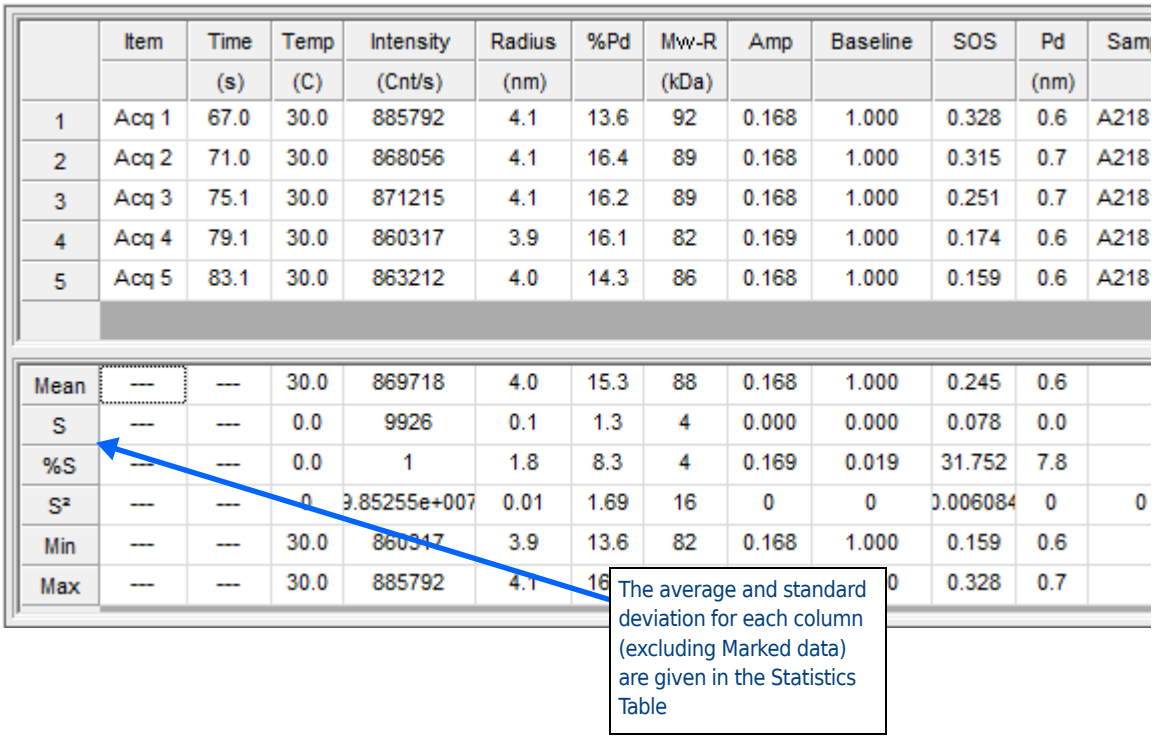
Additional graph formatting options are described in the sections for individual graph types.

Datalog Grid

The Datalog Grid displays measurement data in a configurable table format. The Datalog Grid View is designed to support database management tools available in Excel or other database or analysis programs.

To display the Datalog Grid:

1. Select a measurement or a sub-node in the **Measurements** node of the experiment tree.
2. Click the Datalog Grid icon in the experiment window toolbar or choose **View** ▸▸ **Datalog Grid** from the menu bar.
3. You should see two grids, the data table and the statistics table. If you only see one table, right-click and select **Statistics Table** to open the Statistics Table.



	Item	Time	Temp	Intensity	Radius	%Pd	Mw-R	Amp	Baseline	SOS	Pd	Sam
		(s)	(C)	(Cnt/s)	(nm)		(kDa)				(nm)	
1	Acq 1	67.0	30.0	885792	4.1	13.6	92	0.168	1.000	0.328	0.6	A218
2	Acq 2	71.0	30.0	868056	4.1	16.4	89	0.168	1.000	0.315	0.7	A218
3	Acq 3	75.1	30.0	871215	4.1	16.2	89	0.168	1.000	0.251	0.7	A218
4	Acq 4	79.1	30.0	860317	3.9	16.1	82	0.169	1.000	0.174	0.6	A218
5	Acq 5	83.1	30.0	863212	4.0	14.3	86	0.168	1.000	0.159	0.6	A218
Mean	---	---	30.0	869718	4.0	15.3	88	0.168	1.000	0.245	0.6	
S	---	---	0.0	9926	0.1	1.3	4	0.000	0.000	0.078	0.0	
%S	---	---	0.0	1	1.8	8.3	4	0.169	0.019	31.752	7.8	
S²	---	---	0	9.85255e+007	0.01	1.69	16	0	0	0.006084	0	0
Min	---	---	30.0	860317	3.9	13.6	82	0.168	1.000	0.159	0.6	
Max	---	---	30.0	885792	4.1	16				0.328	0.7	

The average and standard deviation for each column (excluding Marked data) are given in the Statistics Table

Within the Datalog Grid, you can use the techniques described in "Working with Grid Views" on page 7-7 to copy and export data, format cells, and select columns.

4. You can select the columns to display using the Table Settings dialog, see "Available Datalog Grid Columns" on page 7-15 for a list of columns available in the Datalog Grid.
5. You can change the units displayed in the grid by right-clicking on the unit heading and choosing the unit you want.
6. You can mark data to be ignored in calculations as described in "Marking Outlying Data Points" on page 7-41.
7. You can filter data as described in "Filtering Data" on page 7-43.

You can use the Datalog Grid for the top-level Measurements node to assign samples and solvents to measurements. Select the appropriate sample and/or solvent for each measurement using the pull-down menu in the "Sample" and "Solvent Name" columns. See "Selecting Columns in a Grid" on page 7-9 for how to display these columns in the Datalog Grid. If you change the solvent for a measurement, you are asked what sample to use with this solvent or you can create a new sample definition if the solvent is not already used in a sample definition.

Available Datalog Grid Columns

When you right-click on the Datalog Grid and select **Table Settings** from the right-click menu, the Table Settings dialog allows you to add, remove, and sort columns. See "Selecting Columns in a Grid" on page 7-9 for more about how to use this dialog.

You can check or uncheck the Alphabetize box to sort the list by name or by category.

The column names in the following alphabetized table are color coded to indicate which instruments support including that column in the grid:

- **Black**: All instruments or all instruments except DelsaMax PRO
- **Blue**: DelsaMax CORE instruments only
- **Orange**: DelsaMax PRO instruments only.

The columns available in the Datalog Grid are:

Column Name	Description
# Acqs Marked	Number of marked acquisitions (that is acquisitions excluded from calculations)
# Acqs Unmarked	Number of unmarked acquisitions
# Peaks	The total number of regularization graph peaks, including those excluded by setting the upper and lower limits for regularization graph peak display.
# Peaks in Range	The number of peaks within the exclusion range, which will be the number of peaks displayed in the regularization graph.
% Acqs Unmarked	The percentage of acquisitions that are unmarked in a measurement. This provides a quick view of the quality of the sample. Samples that are homogeneous and stable typically will have 100% or nearly 100% unmarked acquisitions.
% Pd	The polydispersity divided by the estimated hydrodynamic radius from the cumulants fit of the autocorrelation function multiplied by 100.
A2	For the DelsaMax CORE only. The second virial coefficient for the sample (in mol-mL/g^2). This is a thermodynamic term that is indicative of solvent-solute interactions. Positive A_2 indicates a high affinity for the solvent.
Acq Time	The integration time for each DLS correlation function in the measurement.
Amplitude	The amplitude of the correlation function at zero delay time.
Attenuation Level	The attenuation of the signal seen by the beam collector as a percentage.
Baseline	The measured value of the normalized intensity auto-correlation curve at the last channel used. Values of 1.000 indicate that the measured auto-correlation curve has returned to the baseline within the time encompassed by the defined number of channels. Deviations from the theoretical value of 1.000 indicate either a noisy baseline or a range of correlator channels that is too small.

Column Name	Description
Collection Period	DelsaMax PRO instruments only. The duration in seconds of the PALS collection period during which an electric field is applied to determine mobility. This is specified in the Instrument parameters.
Concentration	For the DelsaMax CORE only. The concentration of the sample. For informational purposes only.
Conductivity	DelsaMax PRO instruments only. The conductivity measured when the reading was taken. Any value below .05 mS/cm is shown as 0. You can display this in mS/cm or S/cm.
D10	D10 is the diameter below which 10% of the cumulative distribution is contained, where the distribution is determined by the regularization algorithm and y-axis value (%Intensity or %Mass). If the regularization analysis is intensity-weighted, D10 is the diameter below which 10% of the cumulative %Intensity is contained. If the regularization analysis is mass-weighted, D10 is the diameter below which 10% of the cumulative %Mass is contained. This column and the two that follow are available only if the Calculate D10/D50/D90 parameter is set to True in the Fixed parameters.
D50	Diameter below which 50% of the cumulative distribution is contained.
D90	Diameter below which 90% of the cumulative distribution is contained.
Date	Lists the date when a measurement was taken.
Debye Length	DelsaMax PRO instruments only. The distance over which significant charge separation can occur. In solution it is calculated using the ionic strength, dielectric constant, temperature, and various constants as shown in “Mobility Theory and Calculations” on page A-5. This is the inverse of the Kappa value.
Diameter	The diameter of the particle, determined by doubling the hydrodynamic radius estimate from the cumulants fit of the autocorrelation function.
Dielectric Constant	DelsaMax PRO instruments only. The value either entered as a fixed dielectric constant for the solvent or calculated using the aqueous model.
Dielectric Constant Model	DelsaMax PRO instruments only. The model selected in the Solvent parameters for determining the dielectric constant.
Diffusion Coefficient	The translational diffusion coefficient.
Disposable Cuvette	For the DelsaMax CORE only. Records that a measurement was taken using a disposable cuvette.
dn/dc	The refractive index increment for the sample.
Effective Charge	DelsaMax PRO instruments only. The effective charge of the particle. This is calculated using the viscosity, hydrodynamic radius, mobility, and the Kappa value as shown in “Mobility Theory and Calculations” on page A-5.
Electric Field Frequency	DelsaMax PRO instruments only. The frequency for reversing the electric field used to measure mobility. This is specified in the Instrument parameters.
Fit Error	DelsaMax PRO instruments only. The sum of squares error for the fit line shown in the mobility graph. The units are the same as for the mobility.
Forward Monitor	For the DelsaMax CORE only. The voltage reading of the detector directly across the sample from the laser. When compared with the Laser Monitor signal, it can provide a measurement of light absorbance by the sample.

Column Name	Description
Henry's Function	DelsaMax PRO instruments only. The value calculated for Henry's function for this reading. This value is used when calculating the effective charge of a particle. It is calculated using the light frequency, the inverse Debye length, and the hydrodynamic radius as shown in "Mobility Theory and Calculations" on page A-5.
Intensity	The measured intensity in counts/sec.
Intensity Std Dev	The standard deviation (counts/sec) in the measured intensity.
Ionic Strength	DelsaMax PRO instruments only. A measure of the concentration of ions in the solution. This is the value entered in the Sample parameters.
Item	This column lists the name of the measurement, acquisition, detector, or reading.
Kappa	DelsaMax PRO instruments only. The inverse of the Debye Length. This value is used when calculating Henry's function and the Effective Charge.
Lambda	Fit parameters from Cumulants analysis (1/sec). For details, please refer to Equations (5), (8), and (9) in Appendix A.
Laser Monitor	For the DelsaMax CORE only. The voltage reading of the detector reading the laser intensity prior to entering the sample cuvette.
Laser Power	The percentage of maximum laser power used for the measurement.
Mobility	DelsaMax PRO instruments only. The mobility is calculated by dividing the electrophoretic velocity by the strength of the applied electric field. By default the units shown are (m cm/s V).
Mw-R	The weight-averaged molar mass estimated based upon the particle conformation, size, and density.
Mw-R Model	The model used to estimate the molar mass from the hydrodynamic size of the analyte. This setting is defined for each sample in the Sample parameters node.
Mw-S	The molar mass derived from the static light scattering sensor in the DelsaMax CORE.
Number Acqs	Total number of acquisitions in the data shown
Normalized Intensity	The intensity after correcting for variations in laser power and attenuation. The calculation is as follows: I_norm = I_meas / (laser * transmission) where laser is the laser power and the transmission = 1 - attenuation. For example, for 40% laser power and 90% attenuation: I_norm = I_meas / (0.4 * (1-0.9)) = I_meas * 25
Normalized Intensity Std Dev	The standard deviation (counts/sec) in the normalized intensity.
Normalized Static Scattering Detector	For the DelsaMax CORE only, the static scattering detector voltage, corrected for variations in laser power.
PALS Amplitude	DelsaMax PRO instruments only. The average voltage measured by the detectors.
PALS Cell ID	DelsaMax PRO instruments only. This is used for diagnostic purposes only.
PALS FM Amplitude	DelsaMax PRO instruments only. The voltage reading of the forward monitor detector, which is directly across the sample from the laser. On the DelsaMax PRO, this value is used for diagnostic purposes.
PALS N2 Pressure	DelsaMax PRO instruments only. The nitrogen gas pressure measured at the time of the reading.
Pd	The polydispersity, or width of the distribution, in nm determined using a Cumulants analysis.

Column Name	Description
Pd Index	The polydispersity index based on a Cumulants analysis. This is comparable to the distribution width divided by the mean. If the value cannot be accurately determined, this column says “Multimodal”.
Peak # %Intensity	The light scattering signal intensity of the specified peak divided by the total signal intensity of the measurement multiplied by 100.
Peak # %Mass	The estimated total mass of the particles in solution corresponding to the user-specified peak divided by the estimated total mass of all particles in solution from the regularization data.
Peak # %Pd	The percent polydispersity within a user-defined peak.
Peak # Diameter	The regularization Diameter calculation for a user-defined peak range.
Peak # Diffusion Coefficient	The diffusion coefficient for a user-defined peak range.
Peak # Mw-R	The estimated molar mass for the peak based on the estimated hydrodynamic radius, particle density, and conformation model from the regularization fit.
Peak # Radius	The regularization Radius calculation for a user-defined peak range.
Polydispersity	The width of the distribution, determined using a cumulants analysis.
Pressurization State	Shows whether the sample was pressurized or not when the data was collected. (DelsaMax PRO with ASSIST accessory only)
Radius	The estimated hydrodynamic radius based on the cumulants fit of the autocorrelation function.
Ramp Rate	The speed at which temperature changes in time (°C/s).
Rfr Idx @ 589nm 20C	The solvent’s refractive index as specified in the Solvent parameters.
RG Model	The model specified for this sample for use in mass calculation. This setting is defined for each sample in the Sample parameters node.
RMS Error	The root-mean-square error in the cumulants fit of the correlation function.
Sample	The name of the sample for each measurement, acquisition, detector, or reading.
Sample Pressure	The pressure in the cell when the data was collected. If the ASSIST accessory was not connected when the experiment was run, this column is blank. (DelsaMax PRO with ASSIST accessory only)
Set Temp	The set temperature for the temperature-controlled MicroSampler.
Sigma	Fit parameters from Cumulants analysis (1/sec^2). For details, please refer to Equations (5), (8), and (9) in Appendix A.
Solv Rfr Idx	The solvent refractive index. For informational purposes only.
Solv Visc	The solvent viscosity in centipoise at the measurement temperature.
Solvent	The name of the solvent used for each measurement, acquisition, detector, or reading.
SOS	The sum-of-squares from the correlation function fit.
Span	Calculates the span of diameters. The calculation is (D90-D10)/D50. This column is available only if the Calculate D10/D50/D90 parameter is set to True in the Fixed parameters.
Static Scattering Detector	For the DelsaMax CORE only, the static scattering detector voltage, not corrected for variations in laser power.

Column Name	Description
Status	This column shows any error status if one applies to the calculations for the specific measurement, acquisition, detector, or reading.
Temp	The temperature of the measurement in Celsius.
Temp Std Dev	The standard deviation (C) in the measured temperature.
Temp Model	The temperature model used to estimate the solvent refractive index and viscosity at temperatures other than the temperature specified in the Viscosity Temp (C) field. This setting is defined for each solvent in the Solvent parameters node.
Time	The time at which the correlation function was measured from the start of the measurement.
Time Stamp	The time at which a measurement was taken.
Vial Number	The number of the vial injected by the autosampler. (Autosampler with DelsaMax PRO only)
Viscosity	DelsaMax PRO instruments only. This value is corrected for the temperature.
Voltage Amplitude	DelsaMax PRO instruments only. The applied voltage used to generate an electric field as specified in the Instrument parameters.
Zeta Potential	DelsaMax PRO instruments only. The calculated zeta potential value. See as shown in "Mobility Theory and Calculations" on page A-5 for the calculations used.
Zeta Potential Model	DelsaMax PRO instruments only. The model used to calculate the zeta potential as specified in the Sample parameters.

For the "Peak #" columns, up to 5 peaks are supported by default. (You can add more peaks in the Peak Range Settings dialog as shown on page 7-10.) The column headings for peak values contain "(I)" or "(M)" to indicate whether the intensity or mass distribution is selected in the Regularization Graph for this measurement or acquisition.

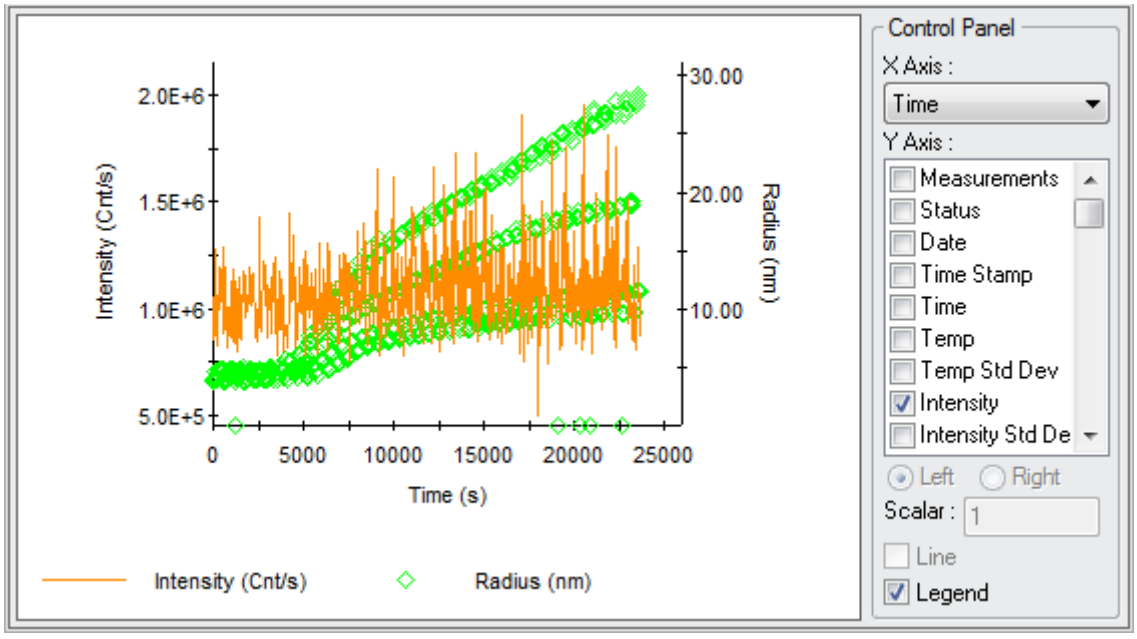
For DelsaMax PRO instruments with a QELS unit, the columns you select that apply to QELS are displayed in the Datalog Grids for DLS acquisitions. The columns you select that apply to electrophoretic mobility (shown in orange in the previous table) are displayed in the Datalog Grids for PALS readings.

Datalog Graph

The Datalog Graph presents and analyzes large quantities of experimental data in graphical form. You select the variables you want displayed on the X and Y axes. The Datalog Graph is easily exported to other Microsoft applications to create professional reports and presentations.

To display the Datalog Graph:

1. Select a measurement or a sub-node in the **Measurements** node of the experiment tree.
2. Click the Datalog Graph icon in the experiment window toolbar or choose **View** ▸▸ **Datalog Graph** from the menu bar.



Note: By default, Intensity and Radius are plotted vs. Time. If you are using the DelsaMax PRO to examine PALS data, you will need to select other data sets to plot on the y-axis.

To view information about a data point, press the Shift key and hover the mouse pointer over the point of interest.

- For information about saving, scaling, and formatting the graph, see "Working with Graphs" on page 7-11.
- For information about selecting particular data to graph, see "Filtering Data" on page 7-43.
- To mark points to be omitted from graphs and calculations, see "Marking Outlying Data Points" on page 7-41.

Using the Control Panel

1. If you do not see the Control Panel, right-click on the graph and select **Control Panel** from the right-click menu. The Control Panel in the Datalog Graph provides lists of variables for the X and Y axes as well as legend control.
2. In the Control Panel, select the data set you want to use for the **X axis**.

3. Put check marks in the boxes next to data sets you want to display on the **Y axis**. All of the columns described in "Available Datalog Grid Columns" on page 7-15 are available for the X and Y axes.

You can also do any of the following using the Control Panel:

- When you have an item selected in the **Y axis** list, you can select the **Left** or **Right** radio button to move that data set to the left or right Y axis.
- You can specify a value in the **Scalar** field to be used as a data multiplier. For example. value displayed = (true value) x (scalar). The graph legend shows any scalar values that have been applied.
- You can uncheck the **Line** checkbox to have data displayed as individual data points.
- You can uncheck the **Legend** checkbox to hide the graph legend. Note that if you click on an item in the graph legend, the Y axis list in the Control Panel scrolls to that item automatically.
- You can put a check mark in the **Alphabetize** box to list the Y axis options alphabetically.

Correlation Graph

The Correlation Graph displays the intensity auto-correlation curve, which is the raw dynamic light scattering data from which the hydrodynamics properties calculated within DelsaMax Analysis Software are derived.

Note: Correlation graphs do not apply to PALS data collected with the DelsaMax PRO.

While the SOS (sum of squares error for a Cumulants fit), amplitude, and baseline are fairly good parameters for judging the "goodness" of the auto-correlation curve, it is typically a good idea to also look at auto-correlation curves, to make sure that all are reasonable. For monomodal (single size) samples, the auto-correlation curve should be a smooth exponential, with an amplitude (intercept) ranging between ~1.1 and 2.0 and baseline of ~1.00.

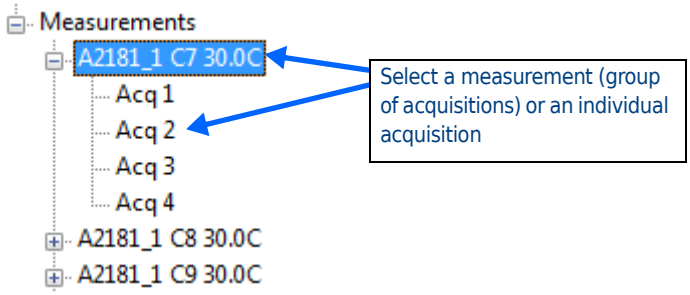
If you find a few "bad" auto-correlation curves in an experiment, they may be due to dust during the acquisition period.

You can view the correlation function of a single acquisition, a measurement, a set of measurements, or all measurements.

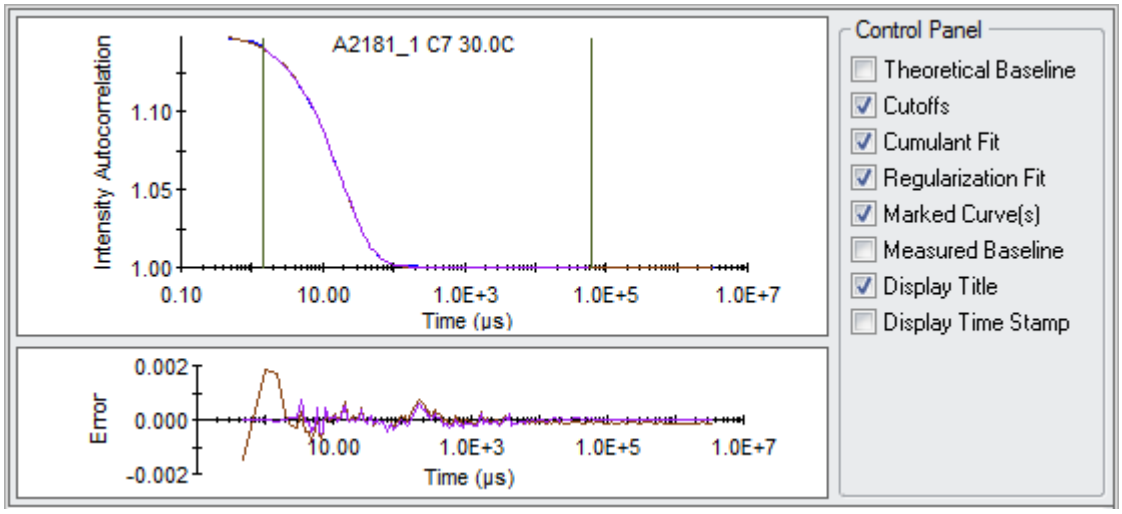
Displaying a Correlation Graph for a Single Measurement

To display the correlation graph for a single measurement or acquisition:

1. In the Measurements node of the experiment tree, select a measurement or individual acquisition.

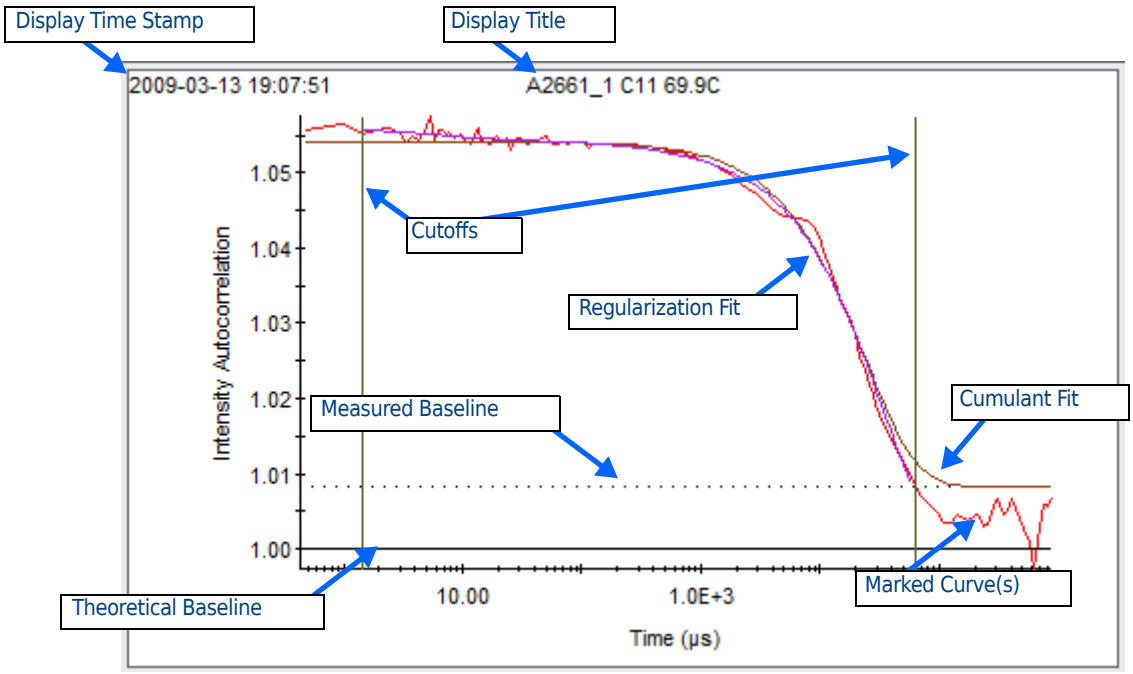


2. Click the Correlation Graph icon on the experiment window toolbar or choose **View→ Correlation Graph** from the menu bar to see the graph.

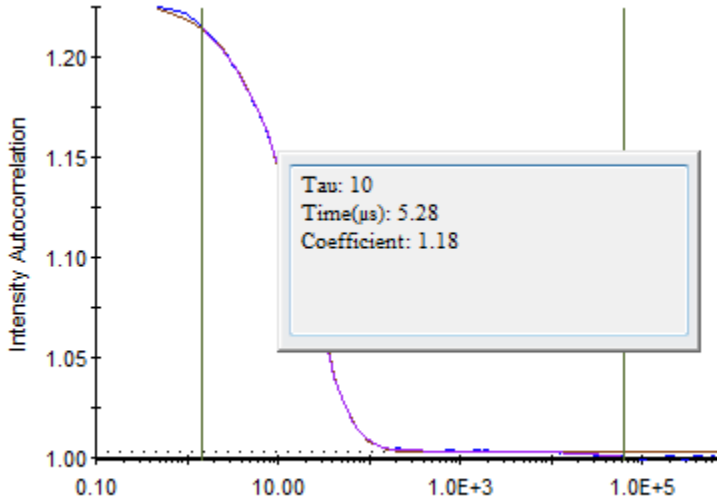


3. If you don't see the Control Panel to the right of the graph, right-click on the graph and select **Control Panel**.
4. If you don't see a graph of error vs. time below the graph, right-click and select **Residuals**. The Residuals graph shows the difference between the measured and fitted data at each point in time.

5. You can use the Control Panel to show or hide parts of the graph.



6. Experiment with the Cumulant Fit and Regularization Fit checkboxes to find the best fit for the data.
7. To view information about a data point, press the Shift key and hover the mouse pointer over the point of interest.



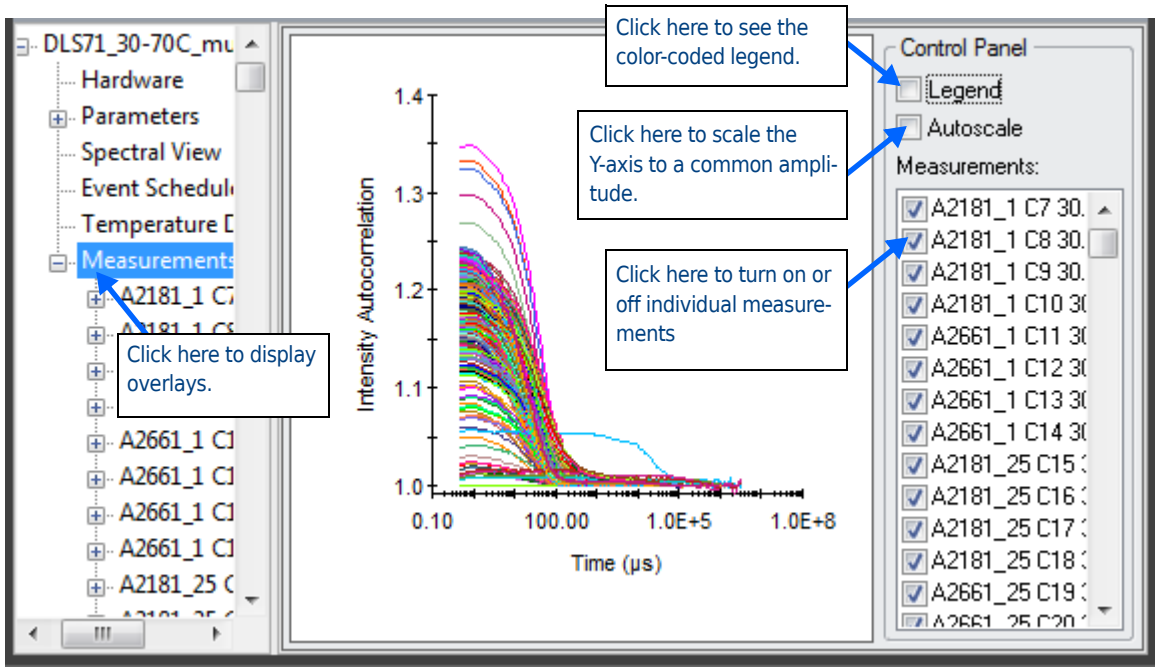
For information about saving, scaling, and formatting the graph, see "Working with Graphs" on page 7-11. For information about selecting particular data to graph, see "Filtering Data" on page 7-43. To mark points to be omitted from graphs and calculations, see "Marking Outlying Data Points" on page 7-41.

Hint: After selecting a measurement or acquisition in the experiment tree for a correlation graph, you can use the arrow keys on your keyboard to quickly scroll through a series of correlation graphs.

Displaying a Correlation Graph for Multiple Measurements

Viewing multiple measurements simultaneously as an overlay lets you quickly validate the quality of the correlation function prior to accepting the size distribution regularization results.

1. Highlight the **Measurements** node in the experiment tree.
2. Click the Correlation Graph icon on the experiment window toolbar. The overlays are color coded to match the Legend.



3. To select the measurements to display, check or uncheck boxes in the Measurements list in the Control Panel.
4. Move your cursor over any auto-correlation curve and hold the Shift key down to display the measurement that is associated with that curve.

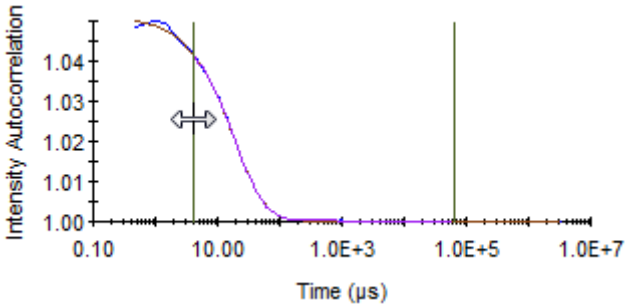
Adjusting the Cutoffs

The cutoffs are the time points on the X-axis of the Correlation Graph that tells DelsaMax Analysis Software where to begin and end the Cumulants and Regularization fit calculations. If you change the cutoffs for one measurement, the cutoffs are changed for all measurements.

You can change the cutoffs for the intensity auto-correlation curve from within the Correlation Graph as follows:

1. Put a check mark in the **Cutoffs** option box to display the cutoffs.

2. Center the mouse cursor over the cutoff to be changed. The mouse cursor will change to a double-headed arrow.



3. Click and drag the cutoff to the new value.

You can also specify values for the cutoffs in the **Parameters**—**Fixed** node of the experiment tree.

Regularization Graph

The Regularization Graph shows the calculated size distribution for the auto-correlation curve associated with the measurement or acquisition selected in the experiment tree.

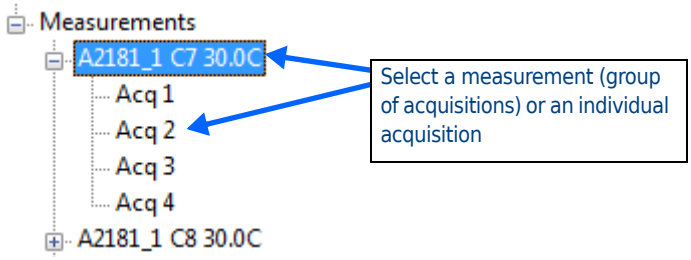
Note: Regularization graphs do not apply to PALS data collected by the DelsaMax PRO.

You can view the regularization functions of a single acquisition, a measurement, a set of measurements, or all measurements. You can display the results in graphical as well as tabular form.

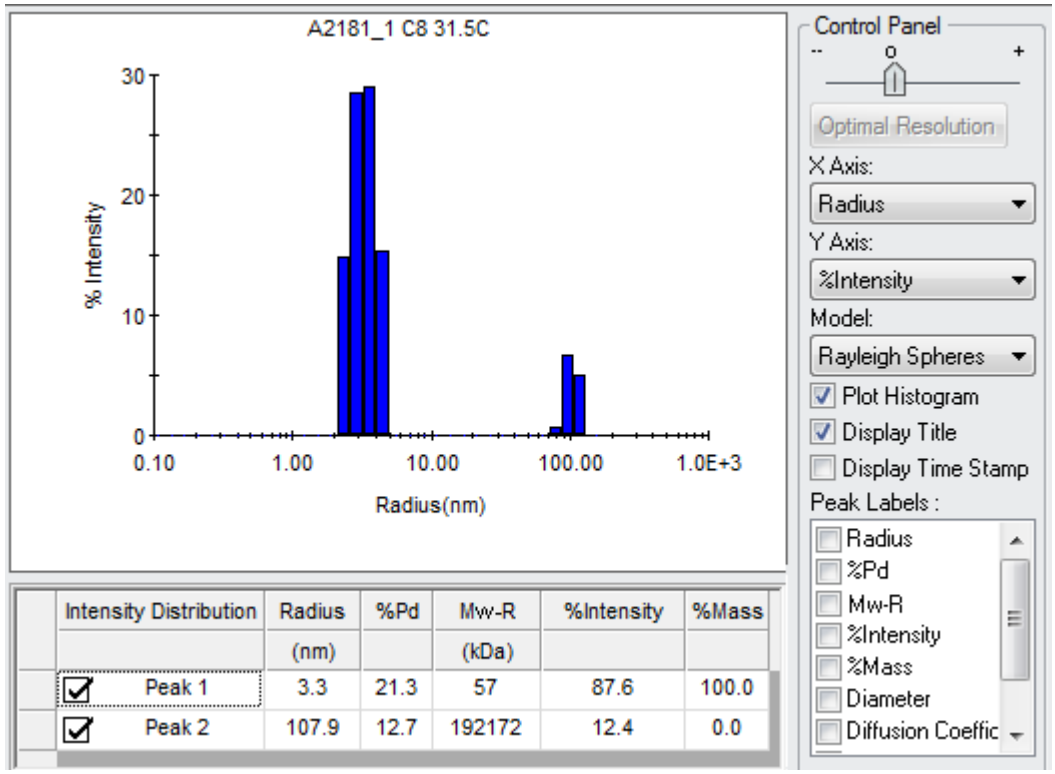
Displaying a Regularization Graph for a Single Measurement

To display the regularization graph for a single measurement or acquisition:

1. In the Measurements node of the experiment tree, select a measurement or individual acquisition.

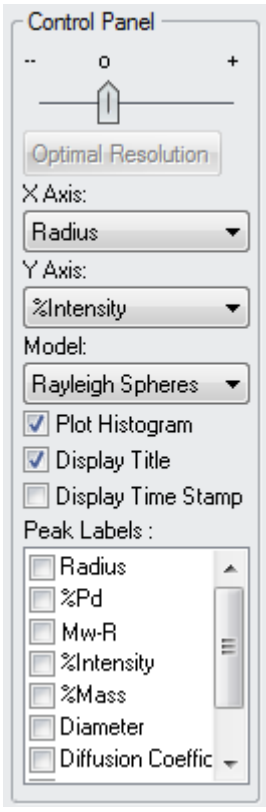


2. Click the Regularization Graph icon on the experiment window toolbar or choose **View** ▸▸ **Regularization Graph** from the menu bar to see the graph.



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
Peak 1	3.3	21.3	57	87.6	100.0
Peak 2	107.9	12.7	192172	12.4	0.0

3. If you don't see the Control Panel to the right of the graph, right-click on the graph and select **Control Panel**.

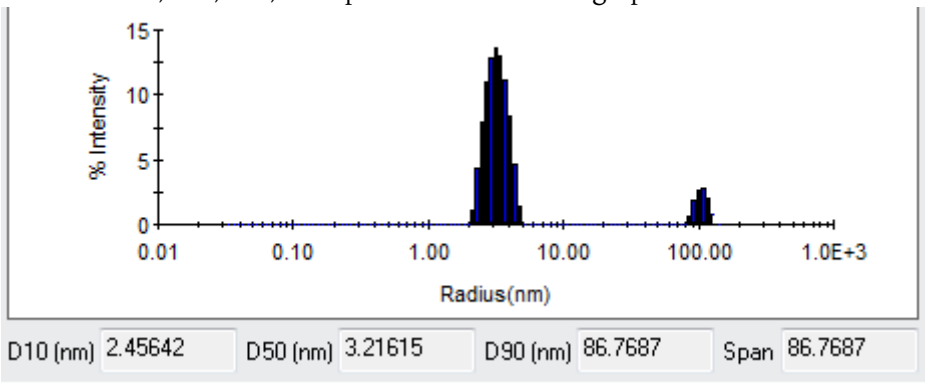


- To change the resolution, use the slider at the top of the Control Panel. See "Adjusting Resolution" on page 7-29 for more information.
- By default, the **X Axis** shows the radius. You can use the drop-down list to choose Decay Time, Diameter, or Diffusion Coefficient instead.
- For the **Y Axis** you can choose to display the distribution as a percentage of Intensity or Mass. The first column heading in the Results Summary table below the graph indicated whether intensity or mass is selected.
- Select a **Model** type from the Model drop-down list. The choices are Coils, Rayleigh Spheres, and Isotropic Spheres. The appropriate form factor for the model you select is applied when converting %Intensity to %Mass. (If you use %Intensity for the Y-axis, this value is independent of the model.)
- If **Plot Histogram** is checked, the graph is a bar graph. If this box is unchecked, the graph is a line graph.

- Check the **Display Title** box to show the name of the measurement above the graph. Check the **Display Time Stamp** box to show the date and time the measurement was made in the upper-left corner.
- Check boxes in the **Peak Labels** area to add text next to each peak with that information.

4. If you don't see a table below the graph, right-click and select **Results Summary**. See "Results Summary Table" on page 7-27 for more about using this table.

If you enabled the "Calculate D10/D50/D90" feature in the Fixed Parameters (see page 4-3), you also see the D10, D50, D90, and Span values below the graph.



Results Summary Table

To display the Results Summary table, right-click the Regularization Graph and select **Results Summary**. (There is no Results Summary table available when you are viewing the overlay graph for multiple measurements.)

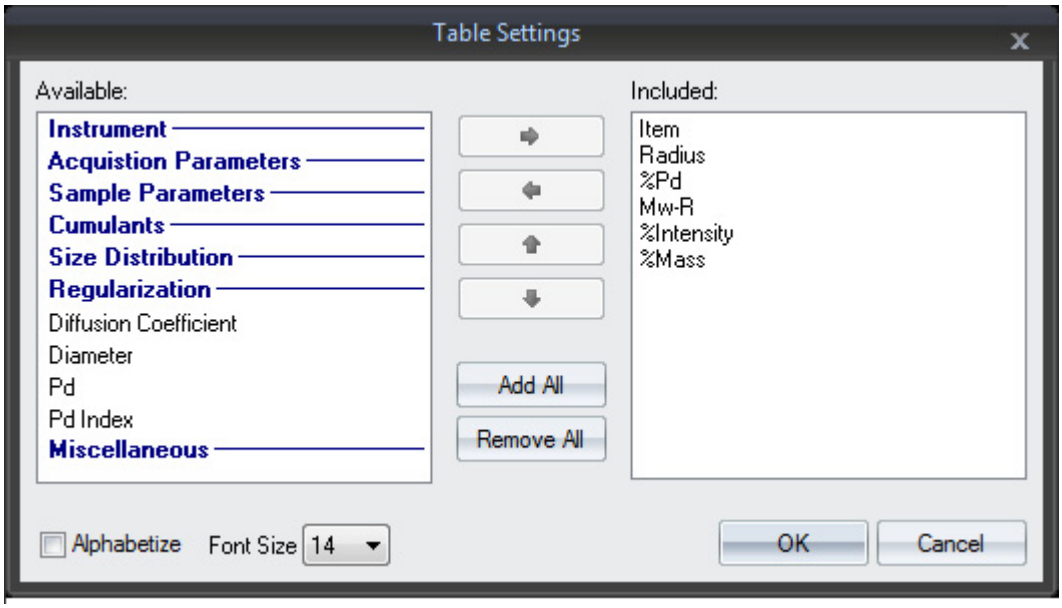
Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass	Diffusion Coefficient (cm²/s)	Diameter (nm)
☑ Peak 1	3.3	21.3	57	87.6	100.0	8.486e-007	6.7
☑ Peak 2	107.9	12.7	192172	12.4	0.0	2.633e-008	215.7

The **Results Summary** table gives a per peak breakdown of the size distribution. Right-click on the units heading to change units. You can customize this table to include any or all of the following columns:

Column Name	Description
% Intensity	The relative amount of light scattered by each population.
% Mass	The estimated relative amount of mass (concentration) of each peak or species.
% Pd	The polydispersity divided by the estimated hydrodynamic radius from the cumulants fit of the autocorrelation function multiplied by 100.
Diameter	The diameter of the particle in nm, determined by doubling the hydrodynamic radius estimate from the cumulants fit of the autocorrelation function.
Diffusion Coefficient	The translational diffusion coefficient for the peak.
Item	The column listing peak numbers. This column is labeled "Intensity Distribution" or "Mass Distribution" depending on the y-axis selection in the Control Panel.

Column Name	Description
Mw-R	The weight-averaged molar mass estimated based upon the particle conformation, size, and density.
Pd	The polydispersity, or width of the distribution, in nm determined using a Cumulants analysis.
Pd Index	The polydispersity index based on a Cumulants analysis. This is comparable to the distribution width divided by the mean.
Radius	The mean value of the radius.

To add columns to the table, right-click and select **Table Settings** from the right-click menu. The Table Settings window allows you to add, remove, and sort columns. See "Selecting Columns in a Grid" on page 7-9 for information on using the Table Settings dialog.



To remove a peak from the Regularization Graph and from consideration in the %Intensity and %Mass calculations, uncheck the box next to the peak number. The graph automatically scales to better display the remaining peaks.

This feature is particularly helpful for very low concentration samples, where noise in the auto-correlation curve at long delay times can lead to the erroneous appearance of small peaks at large sizes (>> 1 micron) well outside the range of dynamic light scattering instrumentation.

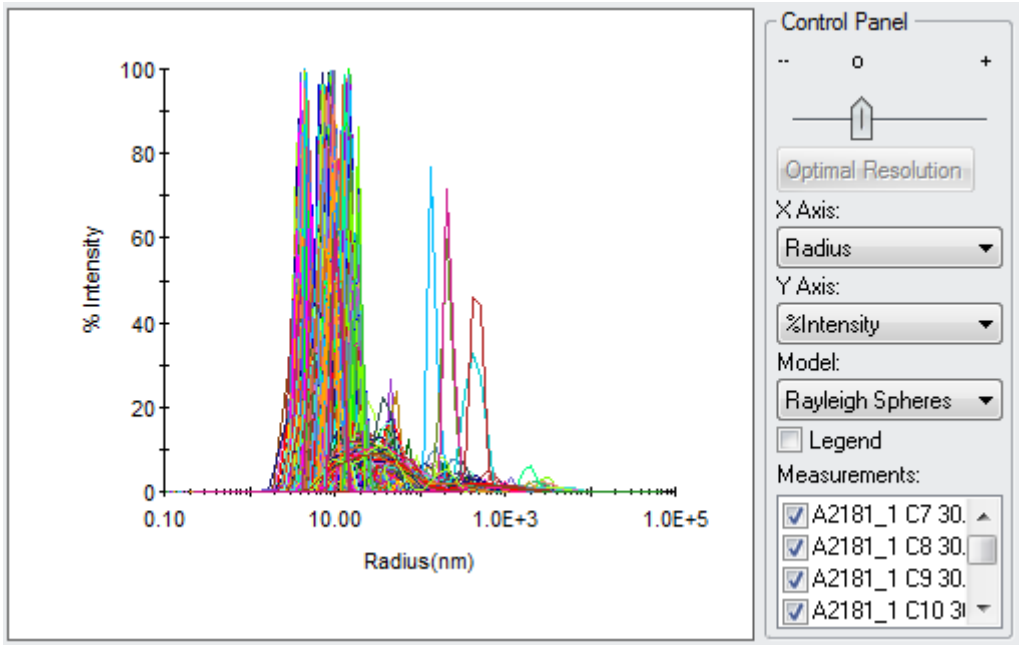
You can set peak ranges by right-clicking on a data cell in the Results Summary area and selecting **Peak Range Settings**. (See page 7-10 for details.)

Displaying a Regularization Graph for Multiple Measurements

You can view multiple measurements simultaneously as an overlay.

1. Highlight the **Measurements** node in the experiment tree.

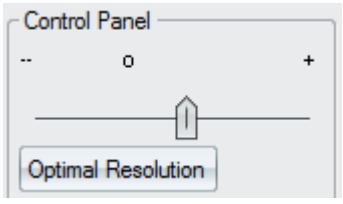
2. Click the Regularization Graph icon on the experiment window toolbar.



3. If you don't see the Control Panel to the right of the graph, right-click on the graph and select **Control Panel**. (There is no Results Summary table available when you are viewing the overlay graph for multiple measurements.)
4. You can use the resolution slider, **X Axis**, **Y Axis**, and **Model** lists as described in "Displaying a Regularization Graph for a Single Measurement" on page 7-25.
5. If you click the **Legend** checkbox, you see a list of the colors used for each measurement. The overlays are color coded to match the Legend.
6. To select the measurements you want to display, check or uncheck the appropriate boxes in the Measurements list in the Control Panel.

Adjusting Resolution

In Regularization Graphs, you can use the resolution slider to adjust the peak resolution.



Slide the pointer to the left for less resolution and to the right for more resolution. If you have moved the slider, you can click the **Optimal Resolution** button to return to the default setting.

The peak resolution limit in dynamic light scattering is 5x in size. Therefore, it is highly unlikely to resolve oligomers, such as dimers and trimers, from the monomer. Be aware that doubling the molar mass will result in a size increase much less than a factor of two.

The Resolution slider provides a small measure of peak resolution control in cases where previous information is available. The Regularization algorithm is a non-linear fitting routine that maximizes the "randomness" of the residual (the difference between the fitted and the measured auto-correlation curve).

The Optimal position on the Resolution slider is the resolution that achieves this maximum. If you know, however, that a peak in the histogram is comprised of multiple particle types, moving the Resolution slider to the right will relax the limits on the randomness of the residual and enhance the possibility of resolving the particles. If you are unsure, you should always use the Optimal Resolution position.

Mobility Graph

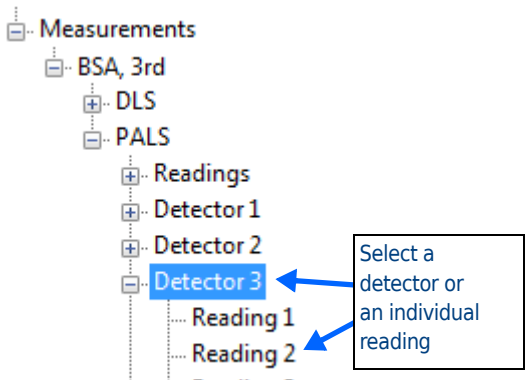
The Mobility Graph plots the electrophoresis per unit electric field (μm cm/V) vs. time.

You can select the **PALS** node to see all readings averaged for each detector and deselect various detectors. You can select the **Readings** node to see all detectors averaged for each reading and deselect various readings. Select individual detector and reading nodes to see the individual data points.

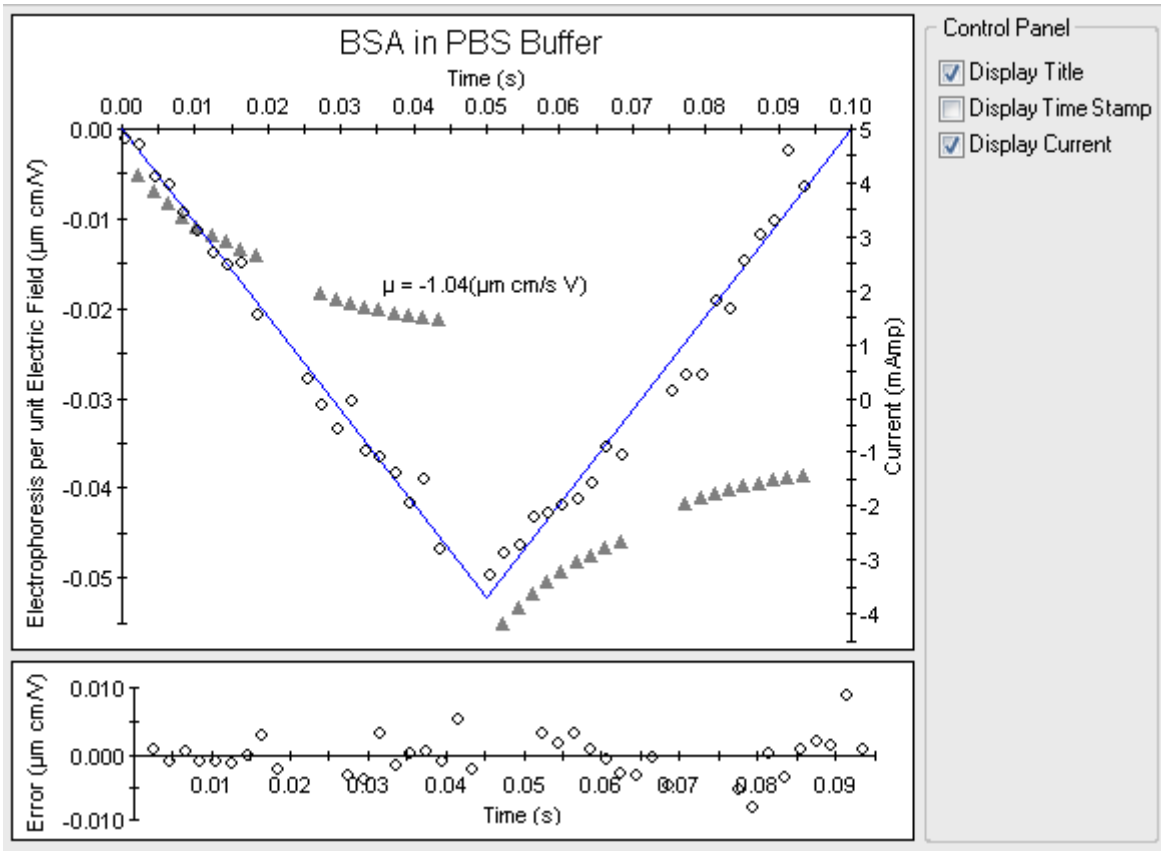
Displaying a Mobility Graph for a Single Detector or Reading

To display the mobility graph for a single detector or reading:

1. In the Measurements node of the experiment tree, expand the PALS node and select a detector or individual reading.



2. Click the Mobility Graph icon on the experiment window toolbar or choose **View—Mobility Graph** from the menu bar.

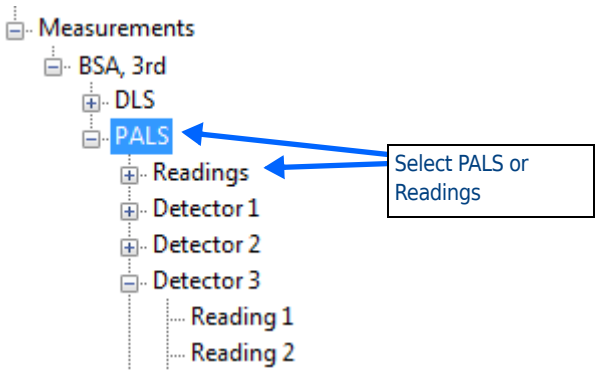


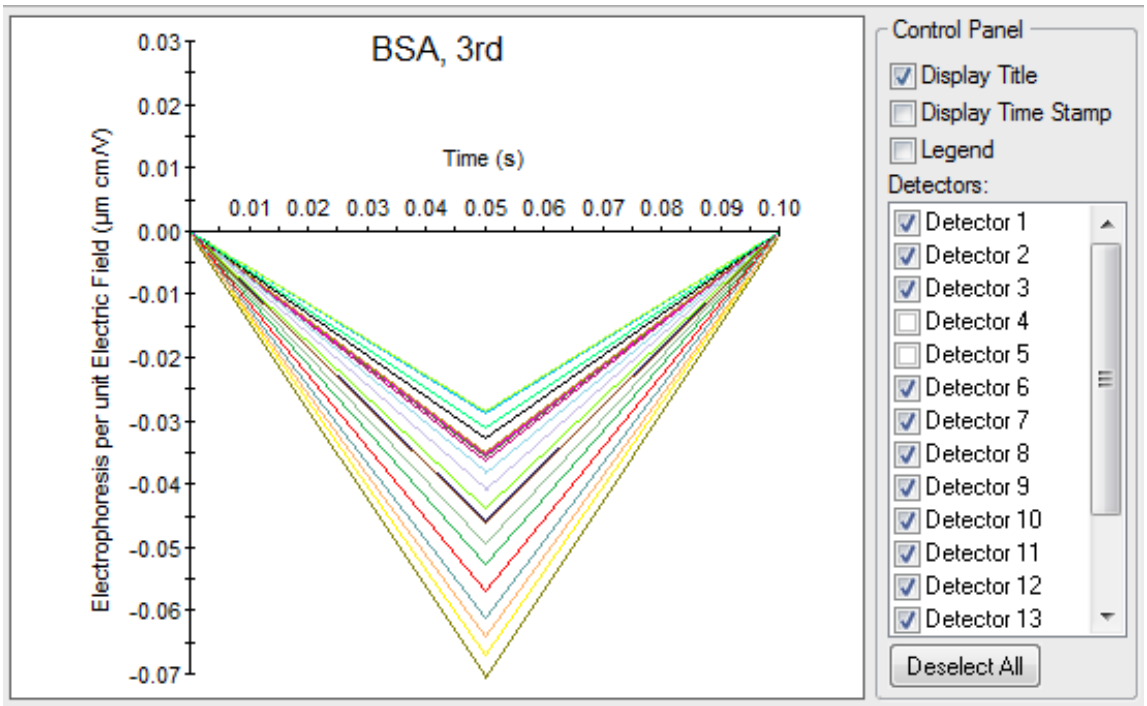
3. The blue line is the fit line through the mobility data values, which are shown as diamond outlines. Triangles show the current in amps.
4. If you don't see the Control Panel to the right of the graph, right-click on the graph and select **Control Panel**.
   - Check the **Display Title** box to show the measurement name.
   - Check the **Display Time Stamp** box to show or hide the date and time the measurement was made in the upper-left corner.
   - Check the **Display Current** box to show or hide the current in amps as the right Y-axis data set.
5. If you don't see the error graph, right-click and select **Residuals**.

Displaying a Mobility Graph for Multiple Measurements

You can view multiple measurements simultaneously as an overlay.

1. In the Measurements node of the experiment tree, expand the PALS node and select the **PALS** node or the **Readings** node.
2. Click the Mobility Graph icon on the experiment window toolbar or choose **View→Mobility Graph** from the menu bar.





3. If you don't see the Control Panel to the right of the graph, right-click on the graph and select **Control Panel**. (There is no Residuals graph when you are viewing the graph for multiple detectors and readings.)
4. Check the **Display Time Stamp** box to show or hide the date and time the measurement was made in the upper-left corner.
5. Check the **Legend** box to see a list of the colors used for each detector or reading.
6. To select the detectors or readings you want to display, check or uncheck the appropriate boxes in the Control Panel.

Spectral View

Spectral View is not available for the DelsaMax CORE and DelsaMax PRO instruments.

Analysis Views

Note: Analysis views, including Parametric Analysis, are not currently supported for DelsaMax PRO instruments. Parametric Analysis will be supported for the DelsaMax PRO in a future release.

DelsaMax Analysis Software provides "analysis views" that you can use to compare one set of data to another.

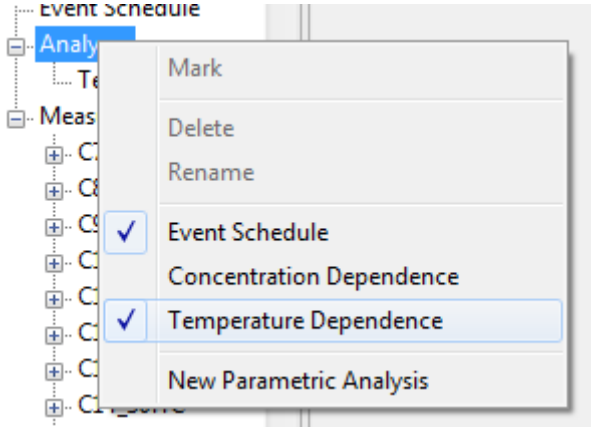
- **Temperature Dependence** compares temperature (for a ramp rate experiment) to hydrodynamic radius. See "About Temperature Dependence Analysis" on page 7-34.
- **Concentration Dependence** compares concentration to radius. See "About Concentration Dependence Analysis" on page 7-34.
- **New Parametric Analysis** lets you choose two parameters to plot in an x-y graph. See "About Custom Parametric Analysis" on page 7-34.

These are also called "parametric analyses". The parameter being analyzed may be temperature, concentration, or a number of other parameters.

Important: The parametric analyses require a minimum of four (4) data points to work. With fewer that four data points in the selected sample, it is not possible to fit the data. The fit improves as you provide more data points.

Adding an Analysis View

To add an analysis view to your experiment, right-click on the experiment tree and choose **Temperature Dependence**, **Concentration Dependence**, or **New Parametric Analysis** from the pop-up menu.



If you choose **Temperature Dependence** or **Concentration Dependence**, that analysis view is added to your experiment under the **Analyses** node of the experiment tree.

Removing an Analysis View

To remove an analysis from an experiment, choose **Experiment→Delete Parametric Analysis** from the menus. Select the analysis you want to delete and click **Delete** to remove it from the experiment.

About Temperature Dependence Analysis

Protein or other molecular systems unfold, or "melt" over a range of temperatures. The melting temperature, T_M, is defined as the temperature at which half of the molecules are unfolded. When a molecule unfolds, the measured hydrodynamic radius, r_h, increases, and this increase in r_h may be used to determine the melting temperature.

However, in addition to simply unfolding, many protein systems aggregate as they unfold. For a system that aggregates as it unfolds, r_h increases indefinitely as temperature increases, and dynamic light scattering or static light scattering measurements as a function of temperature generally are not sufficient to allow knowledge of T_M. However, such measurements may robustly estimate an onset temperature of unfolding/aggregation, T_{onset}.

For temperature ramps, begin the collection well below the expected onset temperature and extend the temperature ramp well above. A typical temperature ramp is 20 °C - 90 °C, with an acquisition every 1 °C, or possibly up to every 2 °C.

The Event Schedule node (see Chapter 5, "Automating Experiments") is ideal for creating collection routines with a temperature ramp or some other parameter variation.

About Concentration Dependence Analysis

If you add a **Concentration Dependence** analysis to your experiment, that analysis plots concentration on the x-axis and radius on the y-axis.

A typical use for a Concentration Dependence analysis would be to determine the Critical Micelle Concentration (CMC) of a surfactant. The CMC is the concentration of a surfactant above which micelles (an aggregate of surfactant molecules dispersed in a liquid) are spontaneously formed. Typically, as you add surfactants to a system, they initially partition into the interface between solvent and sample. As more surfactants are added, they eventually reach a point (the CMC) at which they begin to aggregate into micelles. Any additions above this point simply increase the number of micelles.

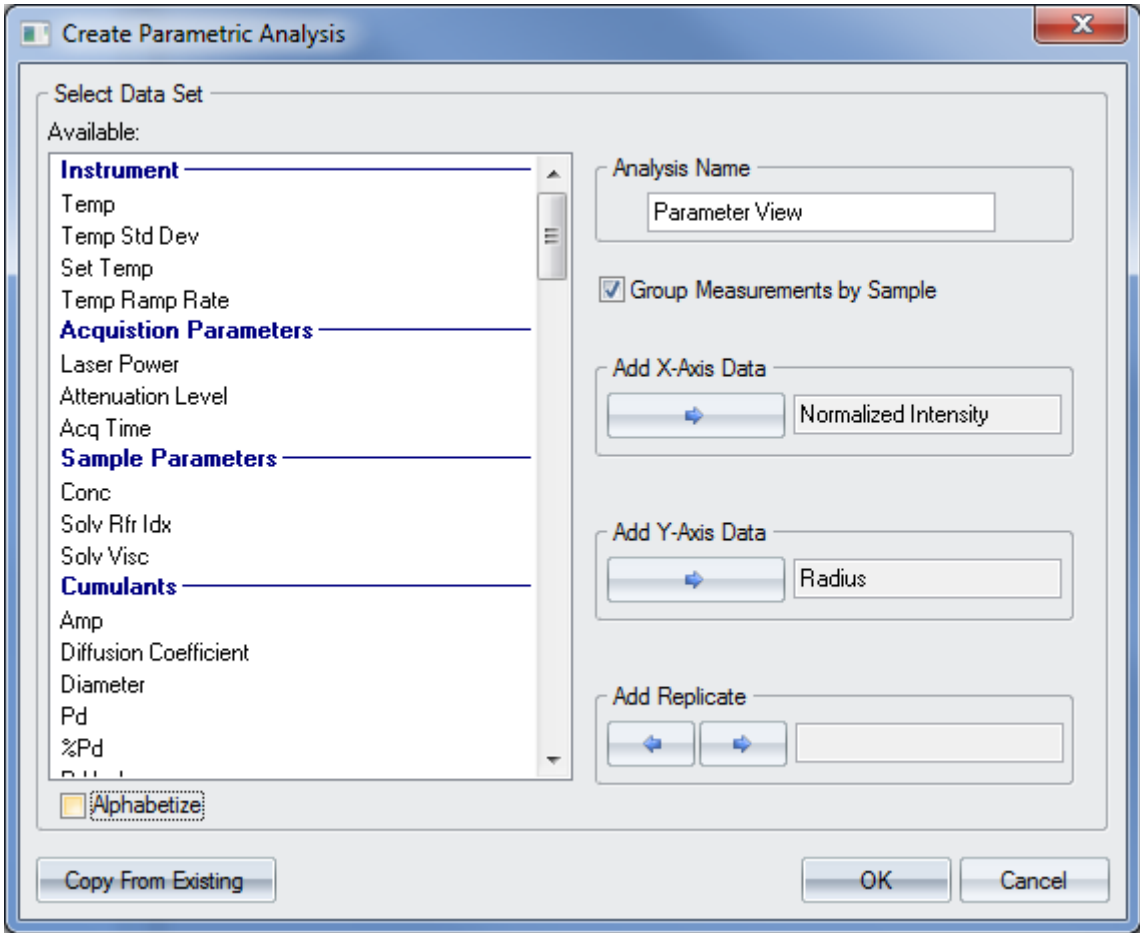
About Custom Parametric Analysis

Note: Parametric Analysis is not currently supported for DelsaMax PRO instruments. Parametric Analysis will be supported for the DelsaMax PRO in a future release.

A Parametric Analysis is a generalization of the temperature and concentration dependence analysis. This allows you to analyze the transition in any one parameter versus any other parameter. Parameters can include data sets stored by DelsaMax Analysis Software and any User Defined parameters such as pH or salt concentration.

Examples of experiments for which you might want to use a Parametric Analysis include pH dependent or salt concentration dependent monomer-dimer equilibrium or the change in molar mass (MW-S) vs. temperature.

If you choose **New Parametric Analysis**, you see the Create Parametric Analysis dialog. Use this dialog as follows:

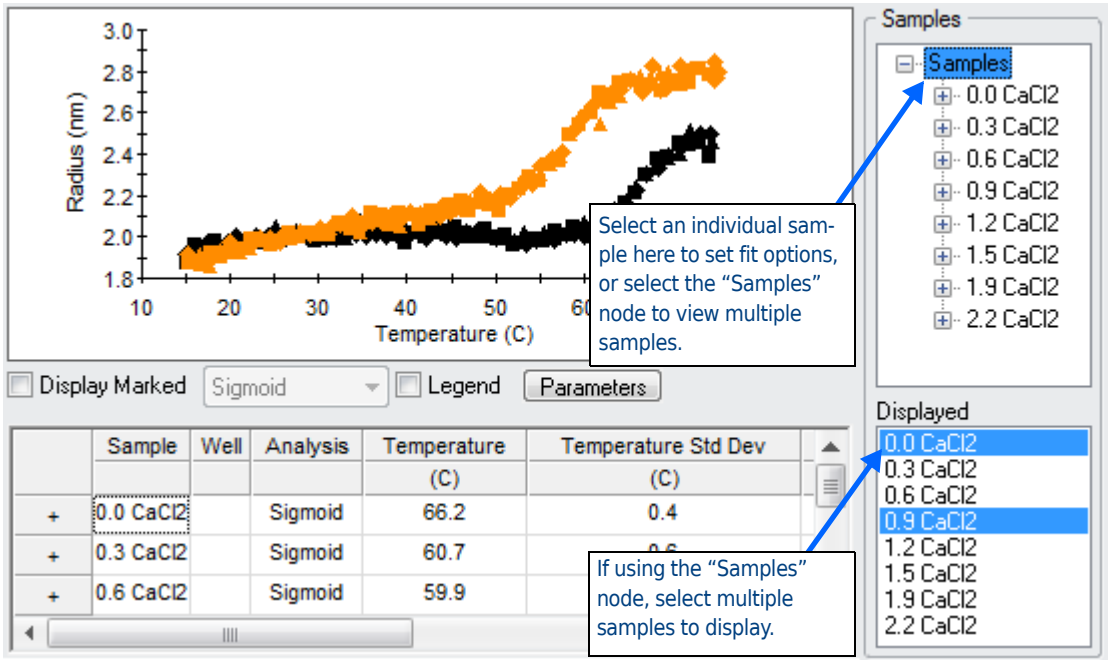


1. Type a name for your custom parametric analysis. This name will be shown in the experiment tree under the **Analyses** node.
2. In the list of available data sets, select the x-axis data you want to use. Then click the right arrow button next to **Add X-Axis Data**. Often, you will want to choose a value that varied in a controlled manner over the course of the experiment for the x-axis data.
3. In the list of available data sets, select the y-axis data you want to use. Then click the right arrow button next to **Add Y-Axis Data**. Often, you will want to use Radius, Diameter, or one of the other data sets in the Cumulants category for the y-axis data.
4. If your experiment repeats measurements with the same value for some variable, you can select that variable for averaging measurements in the **Add Replicate** area. Selecting a Replicate is optional.
5. You can click the **Copy From Existing** button to open a dialog that lets you select an existing analysis and copy the data set selections from that analysis to the new one. Then make changes as needed to define your modified parametric analysis.
6. Click **OK** to create the parametric analysis.

Using a Parametric Analysis

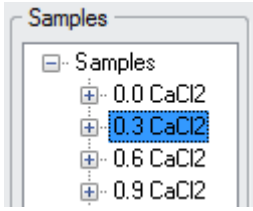
Follow these steps to use an analysis view:

1. Choose the item in the **Analysis** node for an analysis view you want to see. For example, if you select **Temperature Dependence**, you see a plot of radius vs. temperature similar to the following.

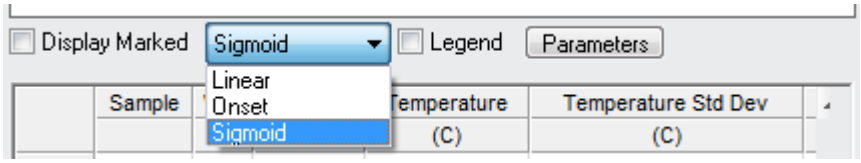


   You can save, scale, and format analysis view graphs as you would other types of graphs. For example, you can zoom in by holding the Ctrl key while dragging your mouse over the area of the graph you want to enlarge. For more information, see "Working with Graphs" on page 7-11.

2. If the experiment includes multiple collections or samples, select a sample from the **Samples** area to the right of the plot. (**Note:** You cannot change the fit settings if you have the main "Samples" node selected in the Samples tree.)

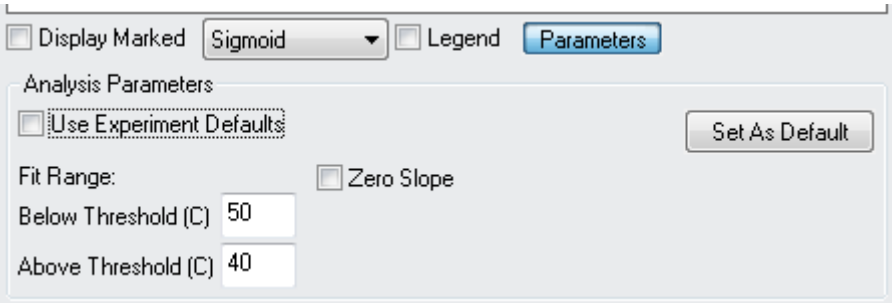


3. Select the type of fit you want to use from the drop-down list. The options are Linear, Onset, and Sigmoid. See "Fit Options" on page 7-39 for details about these fit types.



Important: The Linear and Onset fits identify an onset point, whereas the Sigmoid fit identifies a midpoint.

4. If you are using an **Onset** or **Sigmoid** fit, click the **Parameters** button to adjust the fit parameters. (The Parameters do not apply to a **Linear** fit. Instead, you can use your mouse to drag the ends of the two linear fit regions in the graph. See page 7-40.)



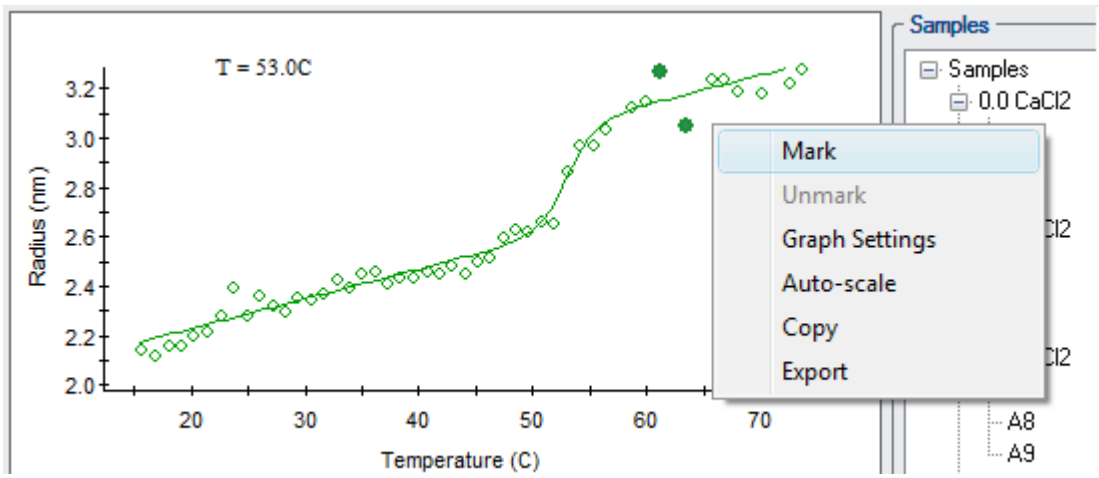
5. In the Analysis Parameters area, you can set the following values that apply to the curve fitting:
   - **Use Experiment Defaults.** This box is checked by default, which means that the default range shown will be used.
   - **Fit Range Thresholds.** Set the number of degrees above and below the threshold to include in the curve fitting range. For example, in a Temperature Dependence analysis, if the T_{onset} appears to be around 60 °C, you can set the range used for Onset or Sigmoid fitting to be approximately 30 °C to 80 °C by setting the **Below Threshold** to 30 and the **Above Threshold** to 20.
   - **Zero Slope.** If you check this box, an overall slope to the parametric dependence is not allowed. If this box is unchecked, the overall slope can be non-zero and is adjusted to find the best fit.
   - **Set As Default.** Click this button to apply the values you have set in the Analysis Parameters area for this sample to all samples that do not have their **Use Experiment Defaults** box unchecked.
6. In a Temperature Dependence analysis, the temperature displayed in the table below the graph and parameters is the sample's melting temperature (T_{onset} or T_m) as determined by the fit.

	Sample	Well	Analysis	Temperature (C)	Temperature Std Dev (C)	Radius (nm)	Radius Std Dev (nm)
+	0.0 CaCl2		Linear	44.2	24.5	1.7	0.6
+	0.3 CaCl2		Onset	51.3	1.8	2.0	0.0
+	0.6 CaCl2		Sigmoid	59.9	0.8	2.3	0.0
+	0.9 CaCl2		Sigmoid	58.0	0.5	2.5	0.0

Hint: You can resize the graph and table by positioning your mouse cursor just below the Parameters button (whether the Analysis Parameters area is visible or not). When you see the resize cursor, click and drag to make either the graph or table larger.

7. You can improve the quality of the fit by marking individual data points or groups of points as outliers. You can select individual data points in the graph for a single data collection by clicking on them. Select groups of points by using your mouse to drag a selection region

around them. After you have selected data points, right-click and choose **Mark** from the right-click menu. (Note that you can mark only individual measurements; you must click an item in the lowest measurement level in the "Samples" tree in the top right corner of the window to see individual measurements.)

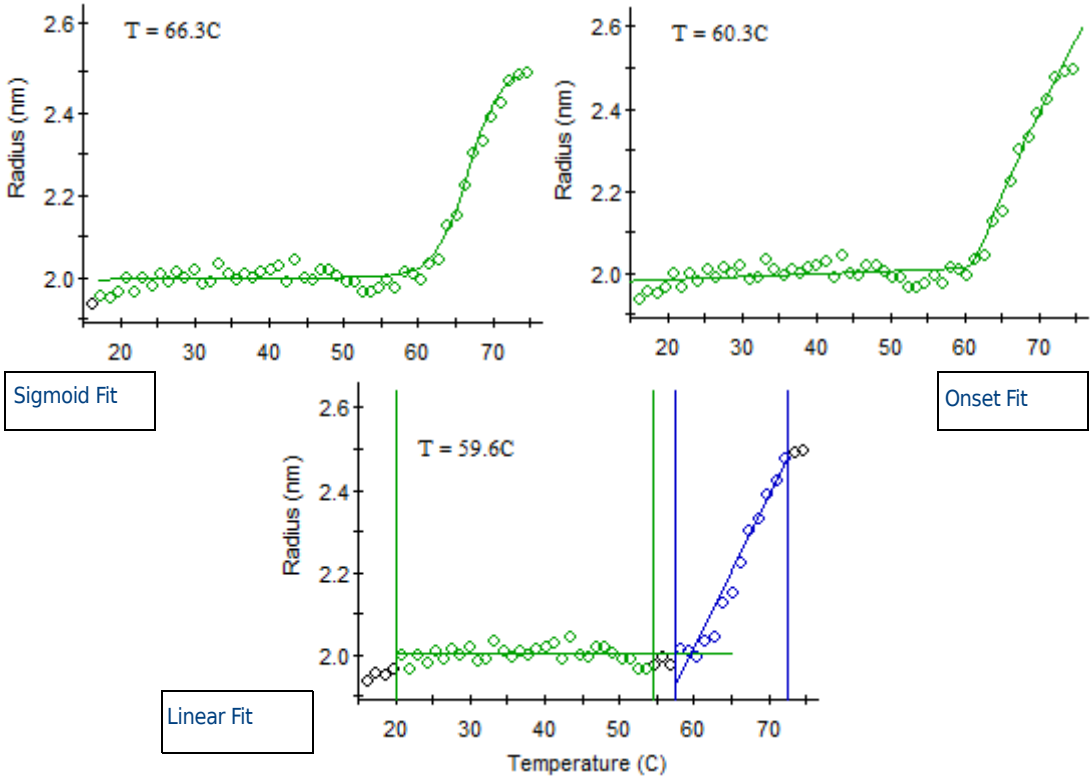


You can add marked data back to the plot by putting a check mark in the **Display Marked** box, which enables the points so you can select and unmark them. If you want to mark a sample result for exclusion, right-click on the sample result in the Samples tree and choose **Mark** from the right-click menu.

8. To view an overlay plot of multiple samples, choose the "Samples" selection at the top of the tree. Then select the samples you want to overlay in the **Displayed** list below the Sample tree. Use the Shift or Ctrl key to select multiple samples from the list.

Fit Options

DelsaMax Analysis Software provides several fitting methods to obtain quantities such as the melting temperature or the point of onset of aggregation. The three analysis methods provided to determine such parametric fits are: **Sigmoid**, **Onset**, and **Linear**.



Important: The Linear and Onset fits both identify an **onset** point, whereas the Sigmoid fit identifies a **midpoint**. If you analyze the same dataset with different methods, you will obtain quite different transition points!

For the Onset and Sigmoid functions, DelsaMax Analysis Software provides the option to not allow an overall slope to the parametric dependence, or having the slope as a free parameter in the fit.

Important: A fit requires a minimum of four (4) data points to work. With fewer that four data points in the selected sample, it is not possible to fit the data to lines or a curve. The fit improves as you provide more data points.

Sigmoid Fit

The Sigmoid function is appropriate for data that shows a change in r_h beginning at a certain temperature, concentration, or other characteristic parameter and a leveling off or only slight change in r_h above that threshold. Such data are characteristic of melting without aggregating, micelle formation, or having an aggregation time constant that is slow compared to the time over which the measurement occurs. Melting without aggregating often results in a relatively small increase in r_h, for example, a 25% change.

In a Temperature Dependence analysis, a fit to the Sigmoid function estimates a molecular melting temperature, T_M. The T_M found using this method is the midpoint temperature of the sigmoid curve, that is, the temperature at which r_h has risen halfway between the radius below the transition to that above the transition.

Onset Fit

The Onset function is appropriate for data that shows an increase in r_h at some characteristic temperature or other parameter and where r_h continues to increase significantly beyond that point without leveling off. Such data are characteristic of simultaneous melting and aggregation, for example.

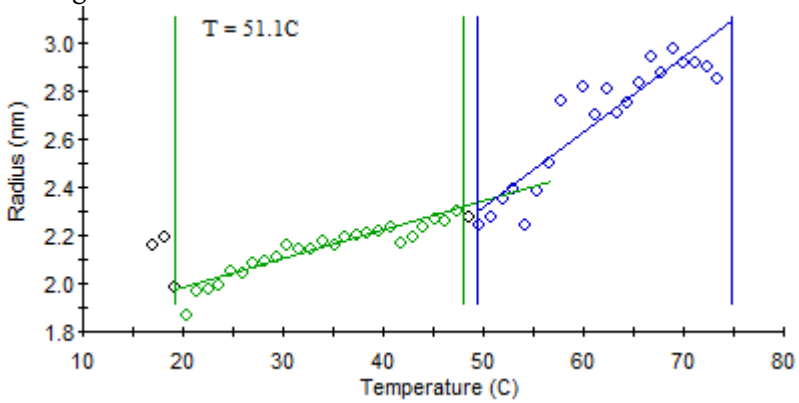
Fitting the data to the Onset functional form provides an estimate for the point of onset. Data through the transition are included when fitting, and the functional form works well in estimating the onset point of unfolding/aggregation both for data with linear regions above and below the onset point as well as data with continuous curvature above the transition.

Linear Fit

You can fit the data above and below the onset point linearly. The intersection of the two linear fits is reported as the onset value.

You can use your mouse to drag the ends of the two lines to adjust the regions used for the two linear fits. To do this, follow these steps:

1. Move to the analysis view graph for a single sample result (for which a Linear fit is used for the sample).
2. You should see four vertical lines that mark the edges of the linear fit regions. The green lines mark the ends of the left linear fit region, and the blue lines mark the ends of the right linear fit region.

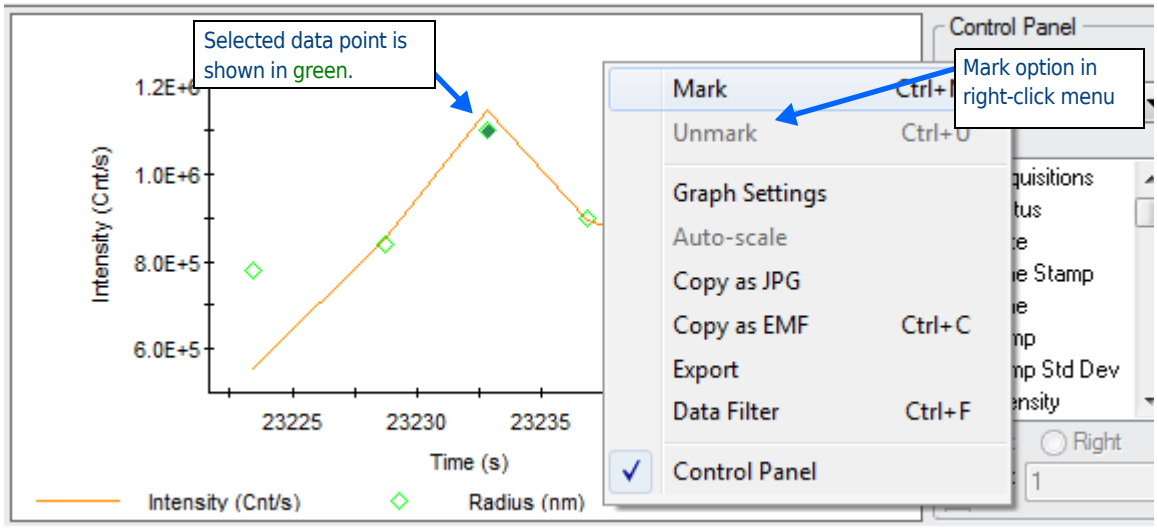


3. Drag the linear fit region edges as needed to best fit the data points. The Value reported at the top of the graph is the value at which the two lines intersect.

Marking Outlying Data Points

Occasionally, a dust particle may momentarily enter the laser beam path and cause a spike in the data.

In the screen shot of the data results shown in the following figure, both the Intensity and the Radius data indicate a spike during the third acquisition, shown at the 30 second mark on the X axis. The other acquisitions have similar values for both the Intensity and the Radius, suggesting that a "dust" particle may have momentarily entered the laser beam path during the third acquisition time period. If all ten acquisitions are considered for averaging purposes, the mean Intensity and Radius values for Measurement 1 would be skewed, due to the presence of a single outlier.



For low molar mass and low concentration samples, the standard approach of re-measuring the sample in the hopes of collecting a better data set, could be problematic, particularly for samples with time-dependent properties.

DelsaMax Analysis Software lets you mark selected outlying data points in the Datalog Grid, the Datalog Graph, analysis graphs, and within the Measurements node of the experiment tree, thereby removing outlying data from subsequent calculations and graphical display.

"Marking" and "filtering" (page 7-43) data are two ways of marking data that should not be included in graphs and result calculations. Marking is a manual process. In contrast, filtering excludes data automatically based on parameter values. Both manually marked and filtered data are shown in red in the Datalog Grid. Cells that are manually marked or unmarked also have a blue background to show that the data filter will not be applied to the cell.

To mark data manually:

1. Position your mouse cursor over a data point, and select the data point with a left click. Selected points in a graph are highlighted in dark green.

- In the Datalog Graph, you can select single data points by clicking the mouse or multiple points by dragging the mouse over an area of the graph (without holding down the Shift or Ctrl key). Notice that when you drag the mouse over an area of the graph, you are likely to be selecting both Intensity and Radius data points.
- In the Datalog Grid, you can select multiple data points by dragging your mouse over a set of data cells or by holding down the Ctrl key while selecting individual cells.
- On a Mobility Graph, you can mark the mobility results shown in that graph as bad. You cannot mark individual data points.

2. Right-click and select the **Mark** or **Unmark** option in the right-click menu.
3. After being marked, the outlying data points are removed from the Datalog Graph and ignored in subsequent calculations, such as the Regularization analysis at the Measurement level, CNF calculations, and baseline adjustments.

   In the Datalog Grid, marked cells are shown in **red**. Marking a data point in the Datalog Graph causes calculated values based on that data to also be shown in red in the Datalog Grid. In addition, manually marked or unmarked data cells have a **blue** background. This indicates that any data filtering will not be applied to the cell.

   In the Measurements node of the experiment tree, you can right-click to mark and unmark measurements and acquisitions. Note that marked data are not highlighted in the experiment tree, but you can see which data are marked in the Datalog Grid.

Tips on Marking Data

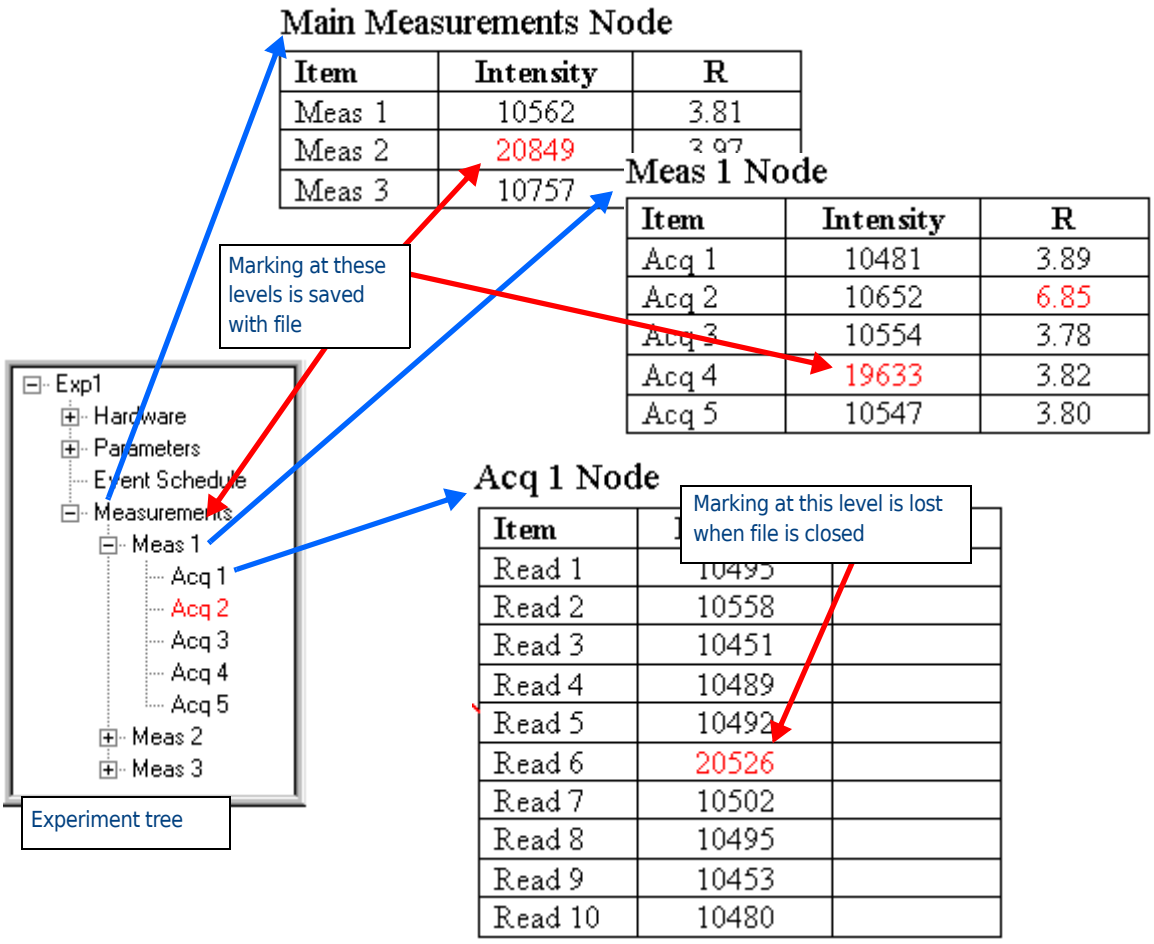
- You can choose **Unmark** to remove marking from a cell that was manually marked. You can also manually **Unmark** a cell that matches a data filter you have set; this causes the cell to be included in graphs and calculations despite matching the data filter.
- To remove the **Mark/Unmark** setting from selected cells, right-click and select **Enable Data Filter**. This resets the cells to allow any data filtering you have set to look at the values in the cells.
- You can mark outlying data points in real time (during data collection). However, because the figure is continuously updating during collection, real-time data marking is easier in the Datalog Grid.
- In DelsaMax Analysis Software, if any parameter associated with a auto-correlation curve is considered to be an outlier, then the auto-correlation curve itself is considered to be bad. If you mark a single parameter derived from a auto-correlation curve, all other parameters derived from that same auto-correlation curve will also be marked. Parameters associated with a auto-correlation curve are Radius, polydispersity (Pd), %polydispersity (%Pd), polydispersity index (Pd Index), amplitude, baseline, SOS, and molar mass estimated from the hydrodynamic radius (Mw-R).
- For information on automatically filtering data, see "Filtering Data" on page 7-43.

Saving Marked Data

One of the advanced features of DelsaMax Analysis Software is the ability to group an unlimited number of measurements (sets of acquisitions) into a single experiment file. A consequence of this versatility is that it is difficult to save all data marking permutations. While you can apply manual

or automatic data marking at any level of the experiment tree while the experiment file is open, once the file is saved, only marking at the main Measurements node level, measurement level, and acquisition level is saved. Marking for individual readings is not saved.

For example, consider the marking scheme shown here.



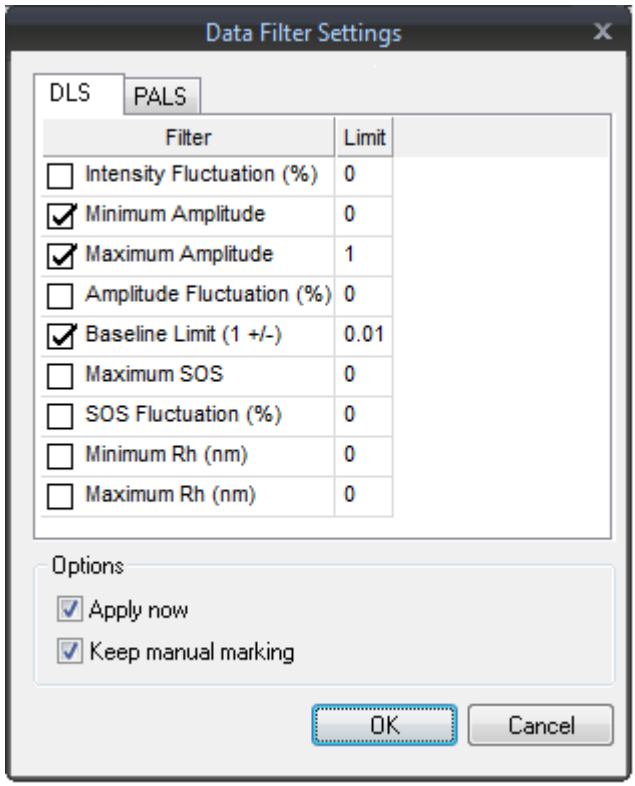
At the main Measurements node, the average (20849) Intensity for all the Acquisitions in Meas 1 has been marked as an outlier. In the Meas 1 node, the average (19633) Intensity for all readings in Acq 4 and the acquisition data for Acq 2 have been marked. In the Acq 1 node for Meas 1, the Intensity for Read 6 (20526) is also marked.

When the experiment file is saved, the marking associated with the Intensity for Meas 1, the Intensity for Meas 1, Acq 4, and the acquisition data for Meas 1, Acq 2 will be saved. The marking associated with Meas 1, Acq 1, Read 6 will be lost.

Filtering Data

The Data Filter is an automated routine for marking data outliers according to user-defined limits. For information on manually filtering data, see "Marking Outlying Data Points" on page 7-41.

1. Right-click in the Datalog Grid and select **Data Filter**. You see the Data Filter Settings dialog.



2. If you are using a DelsaMax PRO instrument with a QELS unit, you can choose the DLS or PALS tab to filter different types of data. For other instruments, only the DLS or PALS tab is available as needed. The filters in the DLS tab apply to dynamic light scattering data. The filters in the PALS tab apply to mobility data.
3. To apply a filtering limit, check the box to the left of a filter. Several filters are provided, and you can enable multiple filtering limits simultaneously.
4. Type the filtering limit next to boxes you have enabled. For example, if you type "10" in the **Intensity Fluctuation (%)** field, any Intensity readings that deviate from the mean by >10% are marked as outliers. Likewise, if you type 100 in the **Maximum SOS** field, the correlation data for any sum-of-squares values that are >100 are marked as outliers.
   - The other filters available for DLS data are: Minimum Amplitude, Maximum Amplitude, Amplitude Fluctuation (%), Baseline Limit, SOS Fluctuation (%), Minimum Rh, and Maximum Rh.
   - The filters available for PALS data are minimum and maximum values for the Amplitude (V), Forward Monitor Amplitude (V), Mobility (µm cm/s V), and Conductivity (mS/cm).
5. By default, the data filters you set are applied to all measurements when you click **OK**. If you want to apply the data filter only to the currently selected measurement, acquisition, detector, or reading, remove the check mark from the **Apply now** checkbox.

The filtered data will be highlighted in **red** in the Datalog Grid and **green** in all other views. Both manually marked and filtered data are shown in **red** in the Datalog Grid. Cells that are manually marked or unmarked also have a **blue** background to show that the data filter will not be applied to the cell.

6. If you have marked some data values manually to exclude them from calculations (or "unmarked" them to force them to be included), check the **Keep manual marking** option to keep the previous manual marking. If you uncheck this box, all manual marking will be discarded.
7. When you have defined the limits, click **OK** to initiate the automated data filtering routine.
8. To remove the manual **Mark/Unmark** setting from selected cells, right-click and select **Enable Data Filter**. This causes data filtering settings to be applied to the cell.

Real Time Data Filtering

You can select Data Filter options when experiment data are being recorded. However, we don't recommend using the Data Filter while you're collecting and recording data. For large data sets, the filter tends to slow the system down, especially if you're applying percent fluctuation limits.

An alternate approach is to set the **Real Time Data Filter** option in the **Fixed Parameters** to **True**. See "Parameter Descriptions" on page 4-2. This option enables a special form of the data filtering routine that is better suited for filtering data in real time.

When the Real Time Data Filter option is enabled, only the **Maximum** and **Minimum** limits that you've defined in the Data Filter Settings dialog are applied while the experiment window is in recording mode. Application of any percent fluctuation limits is deferred until data recording has stopped. At that point, all the limits enabled in the Data Filter Settings are applied to the data set.

You can still mark data manually while the experiment window is in the recording mode, even if the **Real Time Data Filter** option is set to True. However, if you wish to preserve your manual marking, turn on the **Keep Manual Marking** option in the Data Filter Settings dialog.

8
Interpreting Data

This chapter will help you interpret the data obtained from your instrument by providing an overview of size distributions, correlation functions, and molar mass estimates. Please review the "Special Terms" on page 1-1 of this book.

Interpreting a Measurement

DelsaMax Analysis Software defines a measurement as a collection of acquisitions for a particular sample. An acquisition is a period of time, typically 5 seconds, during which the light scattered by the sample is averaged and correlated. For low scattering samples (e.g. low concentration), we recommend a measurement time period of 50 seconds—10 acquisitions, 5 seconds each. For highly scattering samples of radius less than 50 nm, shorter acquisition times and fewer acquisitions may be sufficient—5 acquisitions, 1 second each. Larger radius samples require longer acquisition times, and lower concentration samples generally benefit from a longer total measurement time.

The result of a measurement contains N number of acquisitions, which are averaged and presented in a number of ways.

Dynamic light scattering autocorrelation data may be analyzed in several ways. Two principle methods of analyzing such data are cumulants and regularization analysis. The method of cumulants is a relatively simple and robust method whereby the data are fit to an assumed distribution of particle sizes, and the average radius and spread of radii (first and second moments of the distribution) are reported. The reported radius labeled as "Radius (nm)" and the polydispersity labeled as "%Pd" in the Table View are the results of a cumulants type analysis. As opposed to cumulants analysis, regularization analysis produces an estimate of the radii and relative abundance of all species present in solution without assuming an underlying distribution. Radius and polydispersity values labeled as Peak n, where n is 1, 2, 3, etc., in the Table View are the results of regularization analysis. Additional details regarding these analysis methods, including Legacy and Dynals methods, are given in Appendix A, "Analysis Methods".

The size distribution derived from a regularization analysis is shown in the Regularization Graph. Information on the distribution of the sizes of the analyte is applied to various processes, such as protein crystallization, protein-based drug development, drug delivery nanoparticle development, nanoparticle characterization, and many other areas of advanced materials characterization.

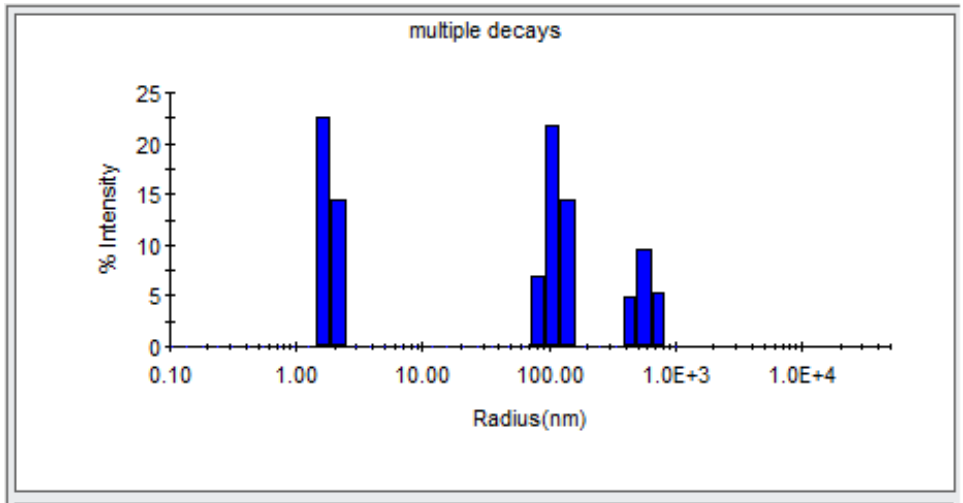
Size Distribution Results

You view the size distribution results in the Regularization Graph.

- Click the Regularization Graph button in the toolbar to display a Regularization Graph.

The Regularization Graph shows the calculated size distribution for the auto-correlation curve associated with the measurement or acquisition selected in the experiment tree, see "Regularization Graph" on page 7-25.

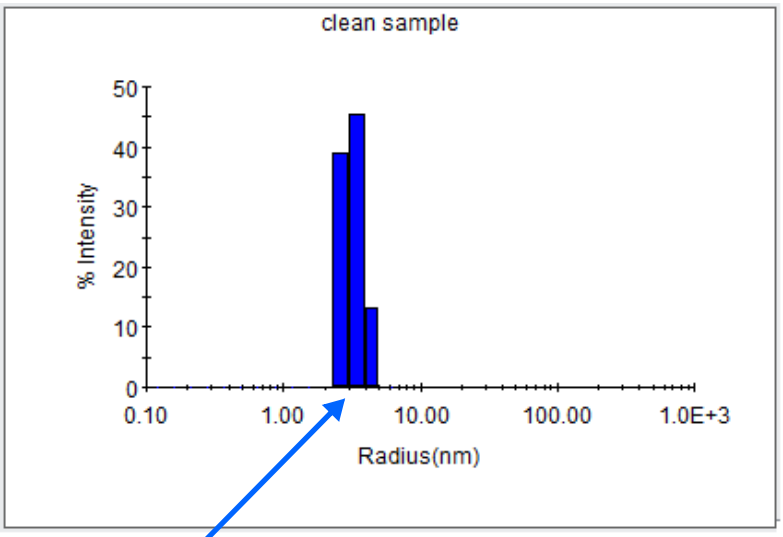
The Results Summary table located below the size distribution histogram describes the number of peaks and their mean value (Radius), % polydispersity (%Pd), molar mass estimated from the measured radius (Mw-R), relative amount of light scattered by each population (%Intensity), and estimated relative amount of mass (concentration) of each peak or species (%Mass).



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☑ Peak 1	1.9	14.0	15	37.2	99.9
☑ Peak 2	116.3	18.5	229231	43.0	0.0
☑ Peak 3	594.7	19.8	10434800	19.8	0.1

Monomodal Size Distribution

The following histogram has one peak, so it is called a monomodal size distribution. The peak is defined by the mean value and polydispersity. The width of the peak is the standard deviation of the weighted bin values, also known as the Polydispersity. The mean value of the peak is defined by a weighted average of the number of bins comprising the histogram, in this case three. The bins by themselves do not represent real, distinct, physical particles; however, their mean and standard deviation do.



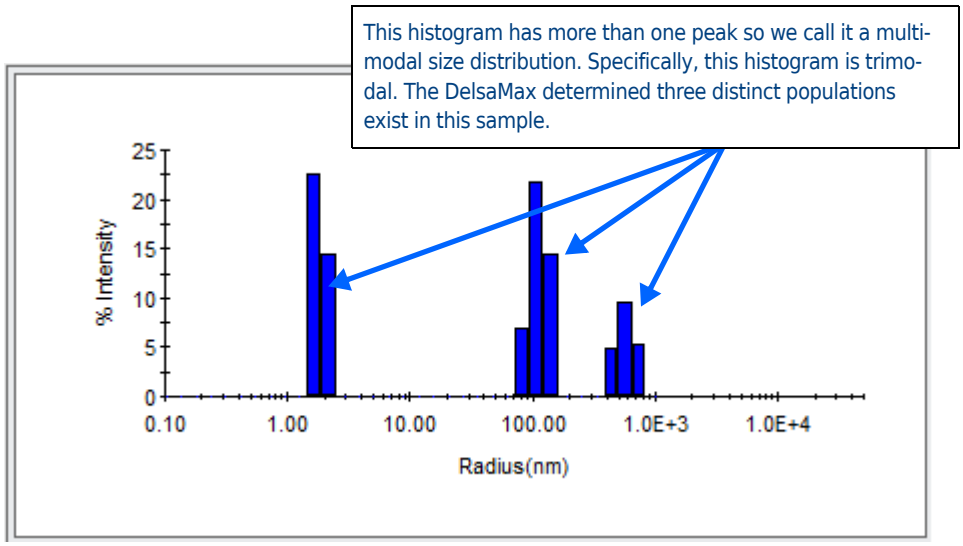
Y-axis
Relative amount of light scattered by each bin, %Intensity (% of Total Light Scattered). Represents the probability of existence of the species.

X-axis
Discrete particle sizes, in nanometers

The mean value of the peak is defined by a weighted average of the number of bins comprising the histogram, in this case three.

Multimodal Size Distribution

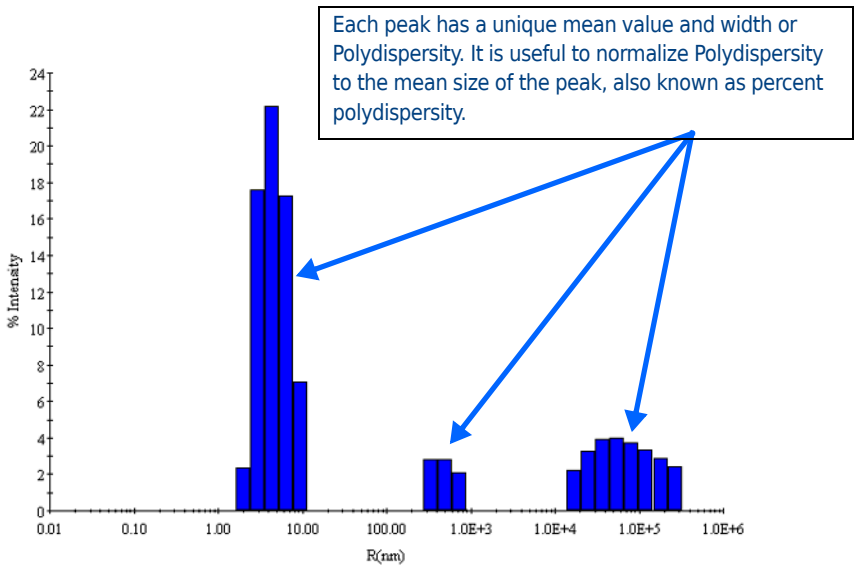
The following histogram has more than one peak, so we call it a multimodal size distribution. The presence of different and resolvable species in the sample cause modes in the size distribution. To be resolved as a separate peak, a species must have a size (radius) larger than another species by a factor of five or more, and be detectable (produce sufficient scattered light for detection by your instrument). When the sizes of the species are below this factor, a separate peak will not be resolved for each species.



By definition, a multimodal size distribution is heterogeneous—the sample contains distinct populations of particles that are not the same size. The DelsaMax instruments can resolve up to four or five modes in a size distribution. For each mode, DelsaMax estimates the relative amount of light scattered and the relative amount of mass based upon one of several possible particle scattering properties. Often, the relative amount of mass of a peak is quite small, for example, less than .1%, and is considered to be negligible.

Polydispersity

Polydispersity refers to the level of homogeneity of the sizes of the particles. When the level of homogeneity is high, the particles can be considered to be virtually identical in their size, or monodisperse. The level of homogeneity is considered high when the percent polydispersity is less than 15%. When the level of homogeneity is low (percent polydispersity greater than 30%), the particle population can be considered to contain significantly different sizes, or polydisperse.

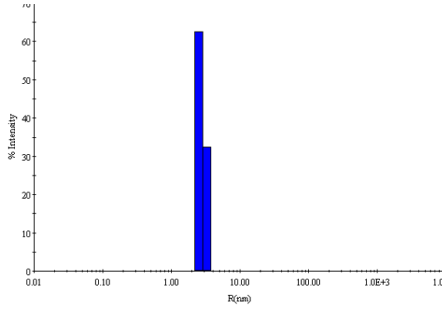


Polydispersity is caused by the presence of different species that cannot be resolved by the technique of dynamic light scattering (species with sizes less than a factor of two relative to other species exist in solution can not be resolved). A peak containing 100% monomer will have a smaller polydispersity than peak containing a mixture of monomer:octamer. The peaks shown here all have % Polydispersity greater than 30%.

Size Distribution Interpretations

Monomodal Monodisperse

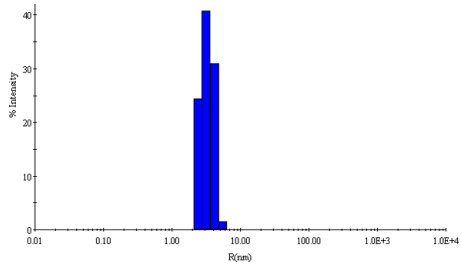
Figure 8-1:



BLGA, 4 mg/ml, PBS, T = 25 °C
Peaks: 1
Mean Radius: 2.8 nm
% Pd: 13.8%
Majority monomer

Monomodal Polydisperse

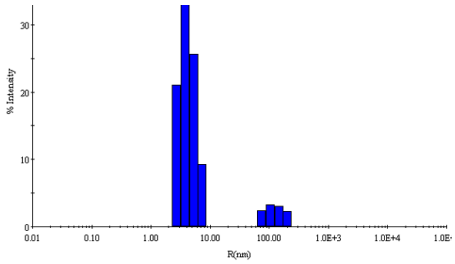
Figure 8-2:



BLGA, 4 mg/ml, PBS, T = 5 °C
Peaks: 1
Mean Radius: 3.4 nm
% Pd: 22.%
Increasing amounts of Dimer

Multimodal Polydisperse

Figure 8-3:

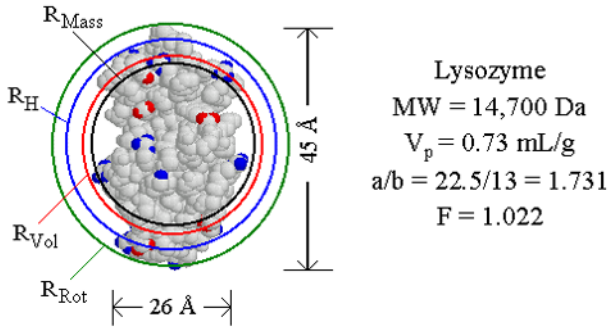


BSA, 2 mg/ml, PBS, T = 25 °C
Peaks: 2
Peak 1:
Mean Radius: 4.3 nm
% Pd: 32.1%
Monomer, Dimer, Trimer

Peak 2:
Mean Radius: 130.9
% Pd: 34.5%
Various non-specific aggregates

Hydrodynamic Radius: Physical Interpretation of Size

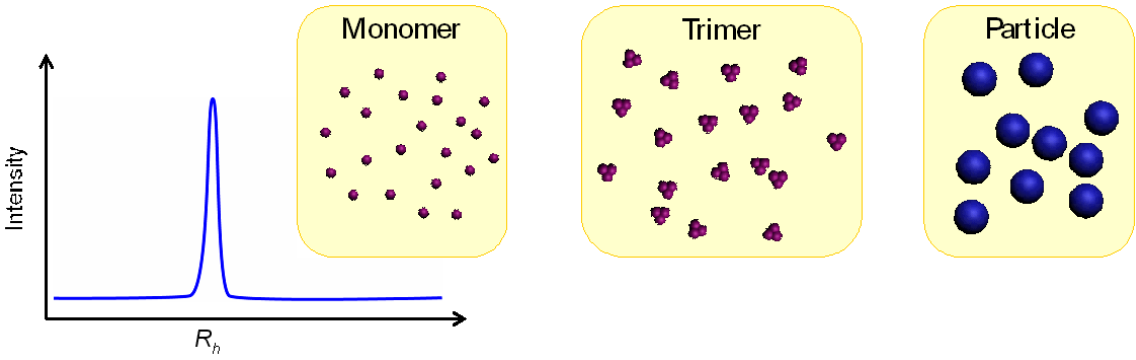
Dynamic Light Scattering measures the size distribution of the particles in the sample. The size, previously defined as the radius or diameter of the particle, is represented in this figure as Rh. Rh, or Hydrodynamic Radius, is the spherical equivalent radius of a hard sphere diffusing at the same rate as the particle of interest. The measured hydrodynamic radius includes any hydration or solvent layer that surrounds the particles.



Physical Interpretations of Size Distributions

Monomodal Monodisperse

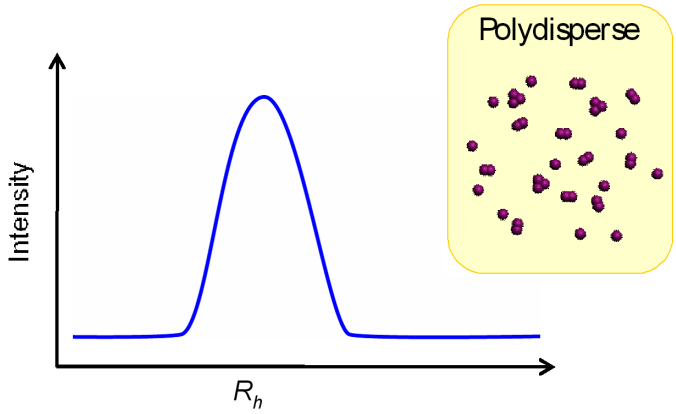
The sample contains one type of particle. The particles can be considered to be virtually identical in their size, or monodisperse. The following figure shows three examples: a protein monomer, a protein trimer, and a larger particle (such as a polystyrene nanosphere).



Monomodal Polydisperse

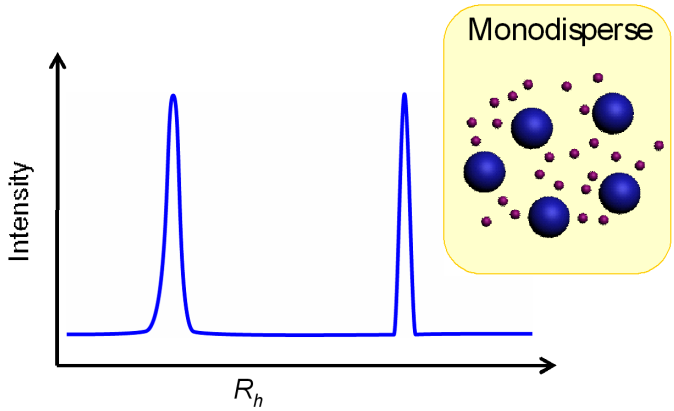
The sample contains three types of particles, monomers, dimers, and trimers. The radii of the dimer and trimer are less than five times the radius of the monomer, so only one peak is resolved and the distribution is monomodal. However, the population consists of two species and this

increase in size heterogeneity causes an increase in measured polydispersity compared to the samples containing pure monomer and pure trimer. Also, the mean radius of the peak will be larger than the radius of the pure monomer but smaller than the radius of the pure trimer.



Multimodal Monodisperse

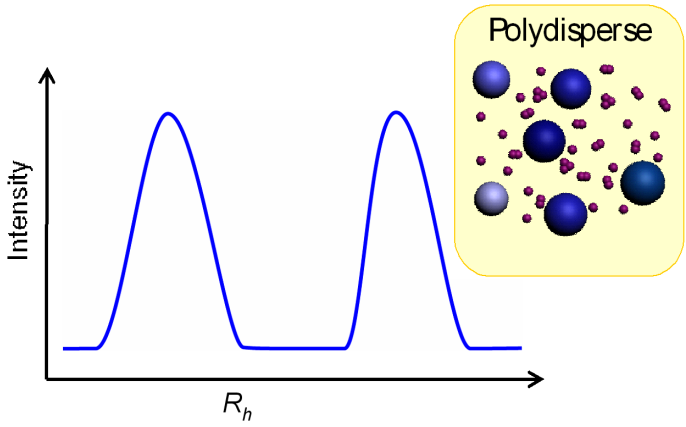
The sample contains two types of particles, the monomer and a large aggregate. This is a special case of a multimodal size distribution—a bimodal distribution. The large particle is more than five times the radius of the monomer and present in sufficient quantities to be measured, so two peaks are resolved by the DelsaMax instrument. Both species are homogeneous, so the measured polydispersity for each peak is low.



Multimodal Polydisperse

The sample contains four types of particles: monomers, dimers, trimers, and a larger aggregate. In this case the DelsaMax instrument resolves only two peaks. This is a special case of a multimodal size distribution—a bimodal size distribution—since two separate species are resolved. The

monomer, dimer, and trimer are not resolved from each other and form only one polydisperse peak. In this example, the second peak is formed by the larger particle, which is resolvable from the unresolved monomer and oligomer peak. The second peak is polydisperse.



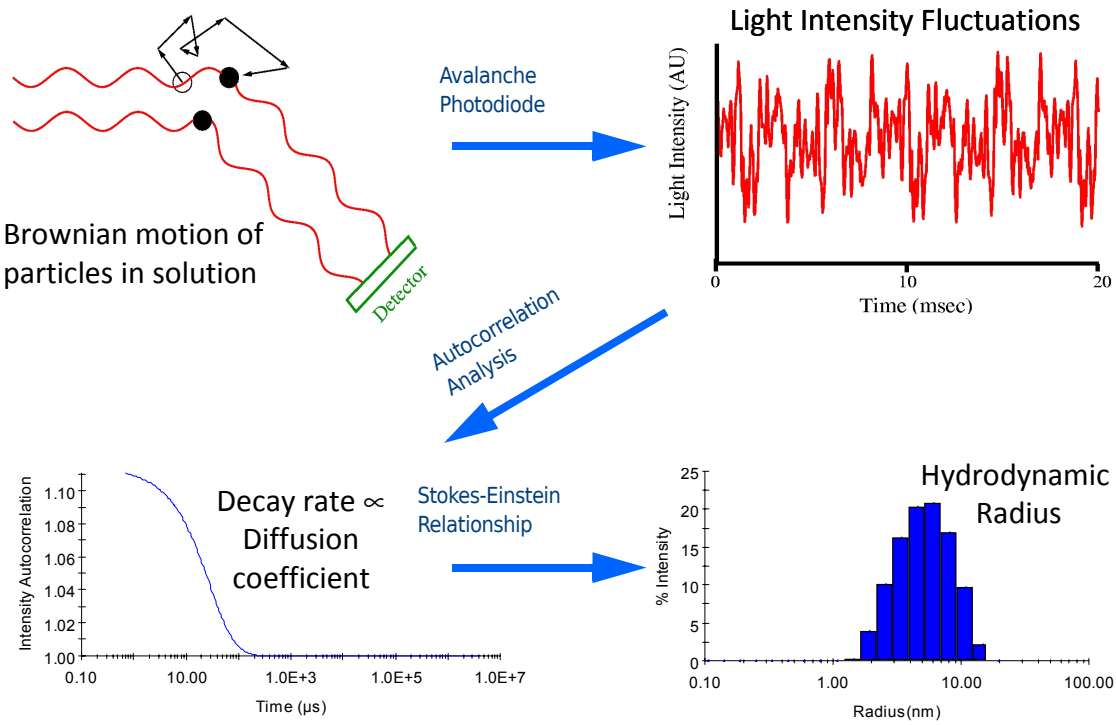
Good or Bad: Judging the Quality of Data

How do we determine if results are acceptable or unacceptable? The instrument software and DelsaMax Analysis Software provide data analyses that indicate if the data are in acceptable ranges. The analyses are based on simple numerical data filters or qualifiers. Yet these data filters do not always capture or allow for good and bad raw data.

In this section, we only briefly outline the principle of Dynamic Light Scattering (DLS). We will focus on how to interpret the raw data, which are the auto-correlation functions that are computed in the DelsaMax instrument and transmitted to the DelsaMax Analysis Software.

Dynamic light scattering measures the translational diffusion of molecules in solution due to Brownian motion. As the molecules diffuse, their relative positions change with time. This causes fluctuations in the intensity of the scattered light due to interference. Small molecules diffuse quickly and generate signals that fluctuate rapidly. Conversely, large molecules generate signals that fluctuate slowly. The diffusion coefficient from these fluctuations is determined by

autocorrelation analysis. If the molecule is assumed to be a uniform sphere, the Stokes-Einstein relationship enables the molecule's hydrodynamic radius to be determined from the diffusion coefficient.



Correlation Function

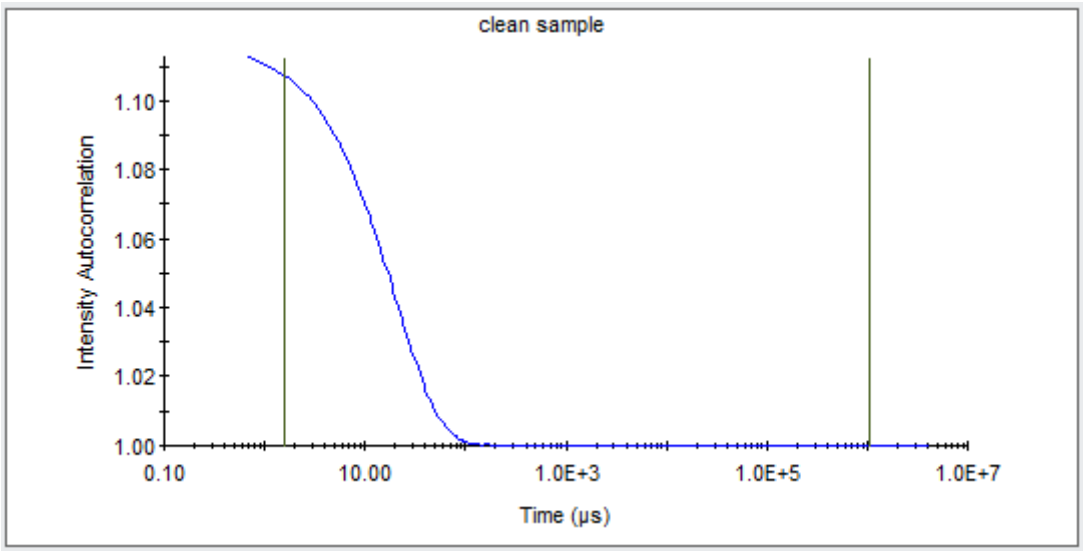
The DelsaMax instruments and the DelsaMax Analysis Software determine the size of particles in solution by exploiting the physical process of Brownian Motion: the particles are moving in solution as a function of time, and their rate of motion is related to their size. The rate of motion is measured by illuminating the particles with laser light and determining the rate at which light scattered by the particles changes with time.

The technique of auto-correlation determines the rate of these time intensity fluctuations, expressed as a correlation function (shown in the following figure). Correlation functions are computed by the correlator board in the DelsaMax instrument and transmitted to DelsaMax Analysis Software for subsequent calculations.

- Click the Correlation Graph button in the toolbar to display a Correlation Graph.

A correlation function is an exponential function comprised of correlation coefficients (y-axis) dependent upon the delay time (x-axis), the time-value separating the sets of data. The function can be mathematically described by one or more decays. The rate of decay is related to particle size. A faster decay indicates a smaller particle, a slower decay indicates a larger particle.

Correlation functions are determined during each acquisition comprising a measurement, as described earlier.



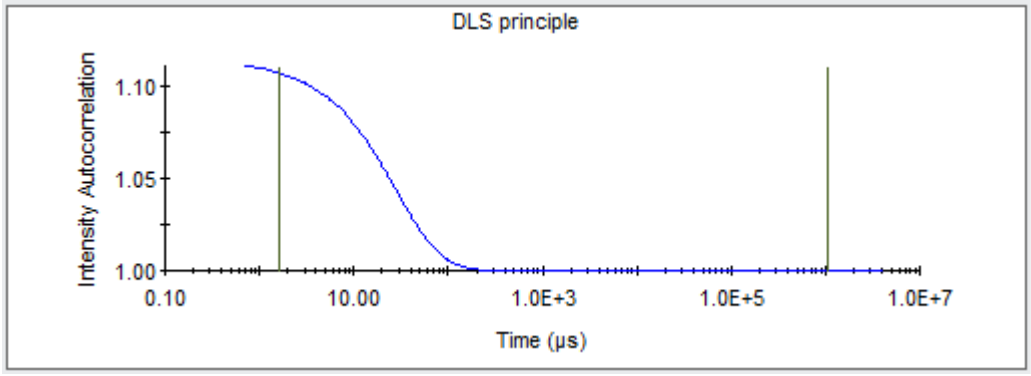
Numerical algorithms are applied to determine the rates of decay or size distributions of the exponential correlation functions. DelsaMax Analysis Software uses “regularization”, a method that finds the size distribution producing the smoothest distribution with the least amount of error (see Appendix A, “Analysis Methods” for details). The error is the difference between the measured correlation function and the fitted correlation function.

Sample vs. Solvent

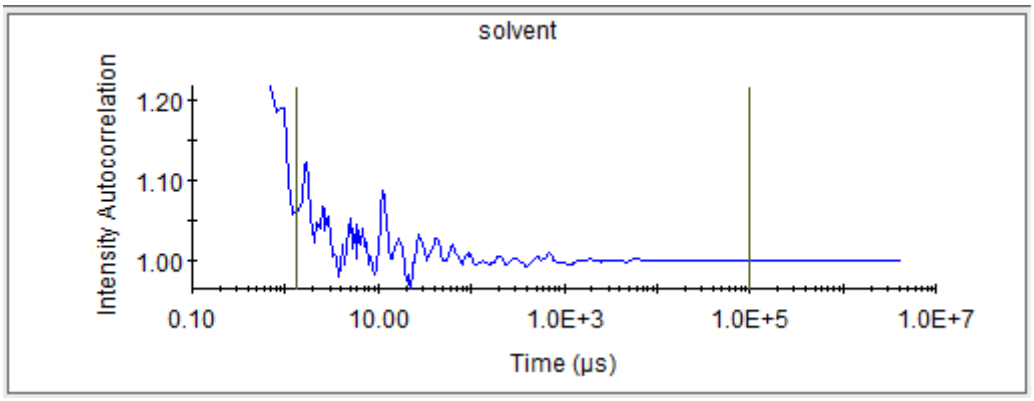
Not all samples can be measured, nor are all samples properly suited for measurement by DelsaMax instruments, and therefore not all samples produce valid correlation functions. Without a valid correlation function, it is not possible to determine a valid size distribution.

A valid correlation function is generally smooth and continuous, exponentially decaying from a maximum value of 2 to a value of 1.

The following figure shows a valid correlation function. Visually, we observe one decay in the function.

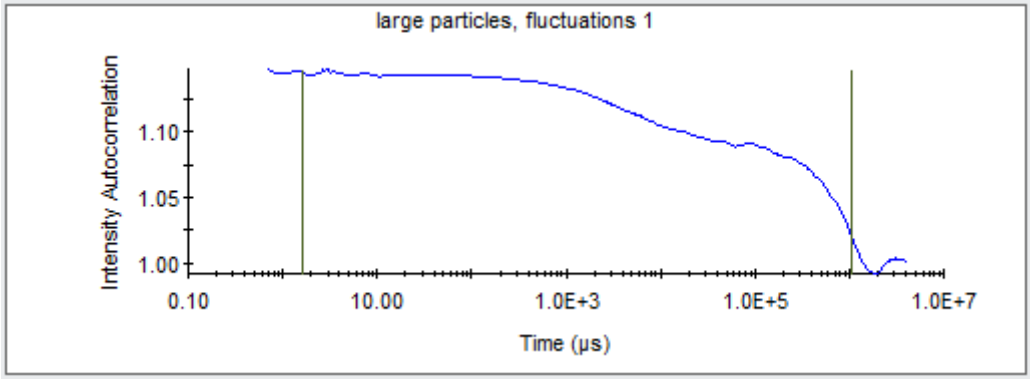


The function contains random values centered around 1, asymptotically reaching 1. Randomness represents the result from measuring pure solvent: solution containing zero analyte or analyte below the limits of detection. The size distribution analysis attempts to find a result for these functions. These must be marked and removed from the analysis. It is generally a good idea to measure the solvent to confirm its purity. If you unexpectedly see a function characteristic of a solvent, increase the laser power, measure the sample unfiltered (to avoid potential binding to the membrane) or uncentrifuged, and/or increase the concentration of the analyte.



Large Particles, Large Fluctuations

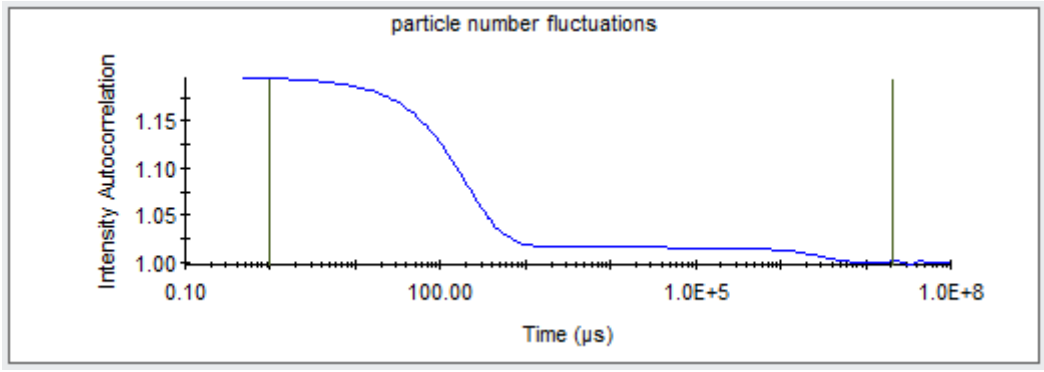
If during the measurement of a correlation function, the total intensity scattered by the population of particles fluctuates significantly, multiple decays with a fluctuating baseline might be observed as shown in the following figure.



These functions must be marked and removed from the size distribution analysis.

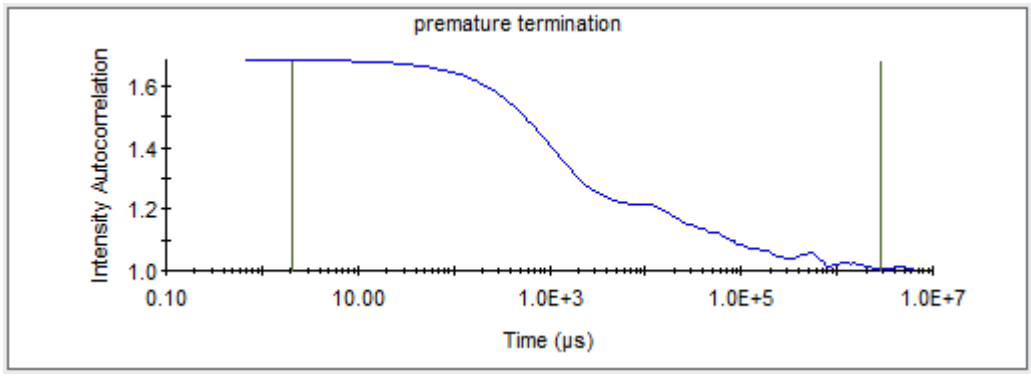
The situation can be remedied by removing bubbles, centrifuging or filtering the sample, or changing solvent conditions to remove large aggregates or particles.

The number of particles in the measurement volume of DLS instruments remains relatively constant at high sample concentrations. Particles diffuse in and out of the measured volume, but the change in particle number is negligible relative to the absolute number of particles. However, at very low particle concentrations, this changes. Diffusion causes the number of particles in the measurement volume to vary significantly through time. These “number fluctuations” affect the intensity of scattered light, and lead to an extra decay in the autocorrelation function. This appears as a “foot” at high delay time values as shown in the following figure.



In the following figure, the decay of this function has not been fully captured; it is prematurely terminated. This is caused by having an acquisition time too short relative to the long decay of the correlation function. Generally, a larger particle size requires a longer acquisition time. The size

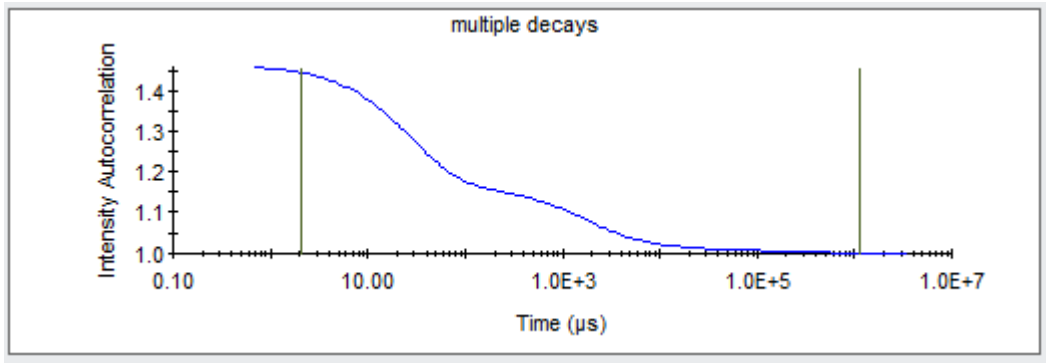
distribution analysis can be performed; however, there will be greater error in the results. The additional correlation coefficients can be captured by extending the acquisition time of the measurement.



Note: Increasing the number of acquisitions will not capture additional coefficients in the longer time delays.

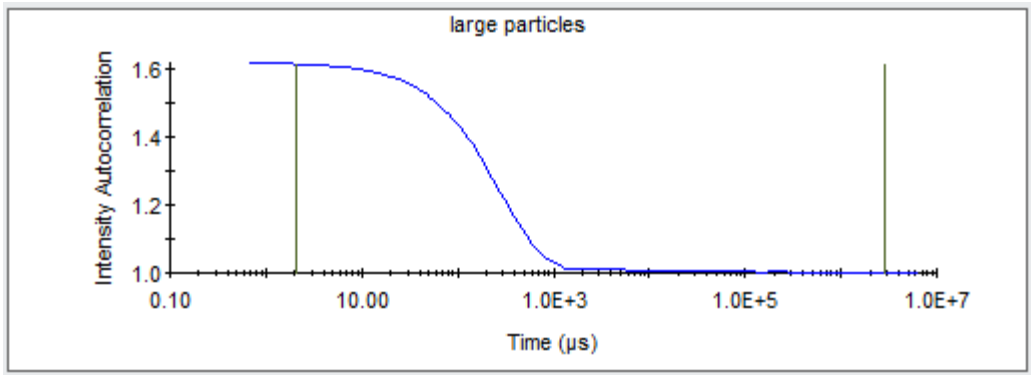
Large Particles, Multimodal Populations

The correlation function shown in the following figure contains at least two visually observable decays. One is faster, representing a smaller particle, and the other is slower, representing a larger particle. These functions are valid and can be analyzed.



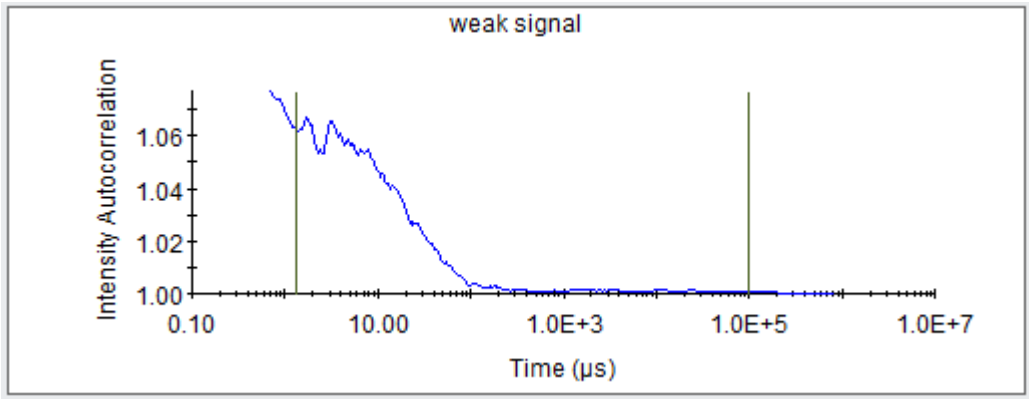
The correlation function associated with larger particles has a longer decay, as shown in the following figure. Note the y-value of the function has asymptotically reached a value of 1, yet the function has some variation at the larger time delays. The variation is referred to as ripple or

noise. The noise is due to insufficient numbers of correlation coefficients being collected and calculated. The noise can be reduced by collecting additional numbers of acquisitions. With less noise, the size distribution analysis will be of higher quality.



Weak Signal

In the following figure, ripple or a lack of smoothness of the function in the short time delay area indicates a weaker signal from the particles. These functions can be fitted; however, the polydispersity may be greater due to this noise.



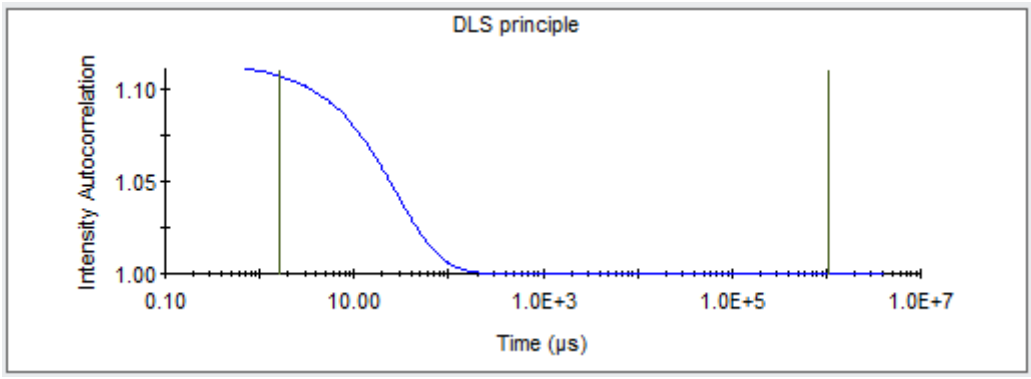
The remedy for this situation is to either extend the acquisition time, collect more acquisitions, increase laser power, and/or increase analyte concentration

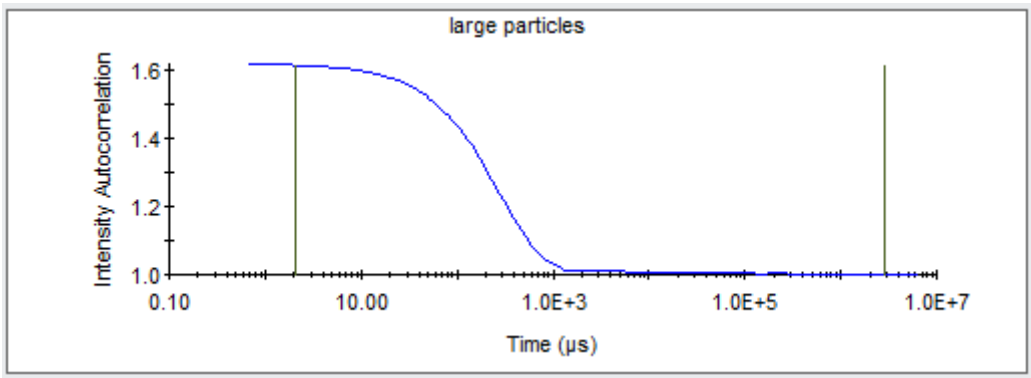
Evaluating Correlation Function

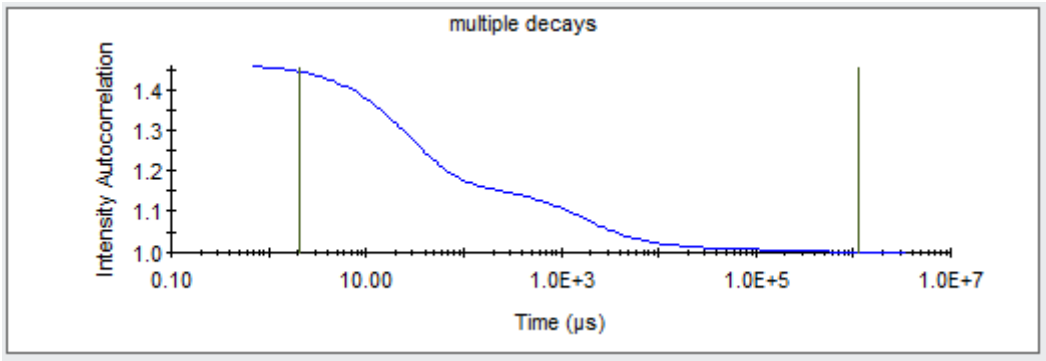
The following figures show examples of when you should continue with data interpretation, when you should try to improve the quality of the data before proceeding, or when you should stop.

Proceed Category

The following figures show examples of the correlation function in the “Proceed” category.If the correlation function is in the Proceed category, continue with the data interpretation.







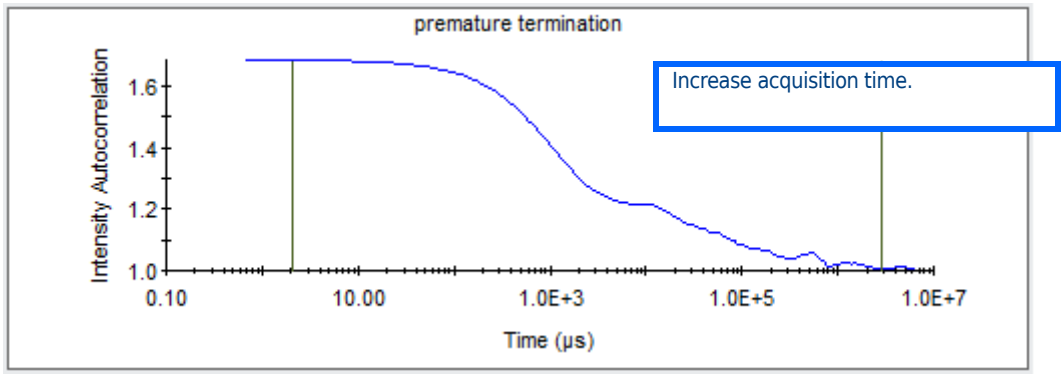
Caution Category

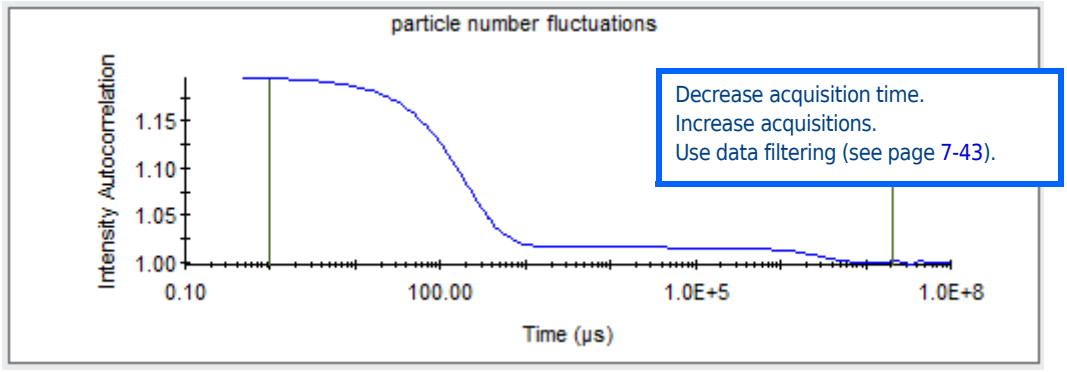
The following figures show examples of the correlation function in the caution category. Before proceeding, attempt to improve the quality of the data by following all or some of the recommended changes to the experiment.

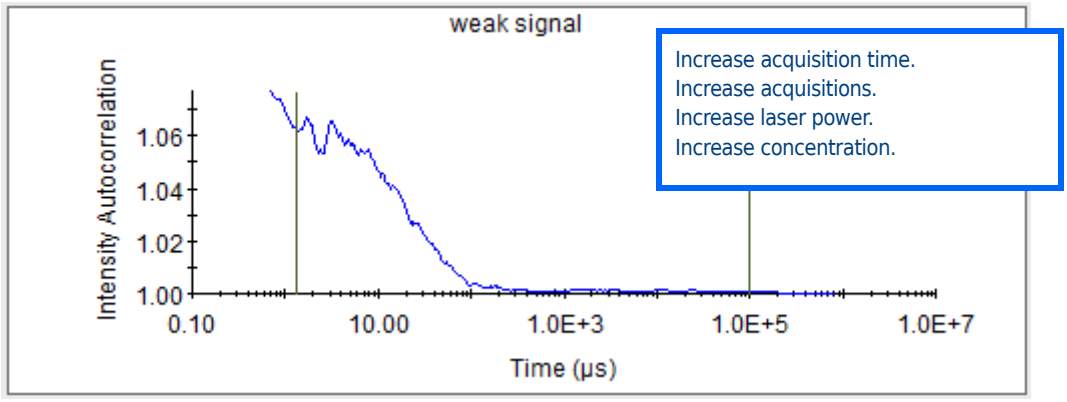
Leave the sample in the cuvette and follow these steps:

- Change the acquisition time: Increase the time for incomplete decays, or decrease the time if a number of fluctuations are present, and/or increase the number of acquisitions.
- Increase laser power (to maximum value of 100%).

If none of these steps lead to functions shown in the “Proceed” category, it may be necessary to increase the concentration of the analyte. Ultimately, you may accept the imperfect data from this category and continue.







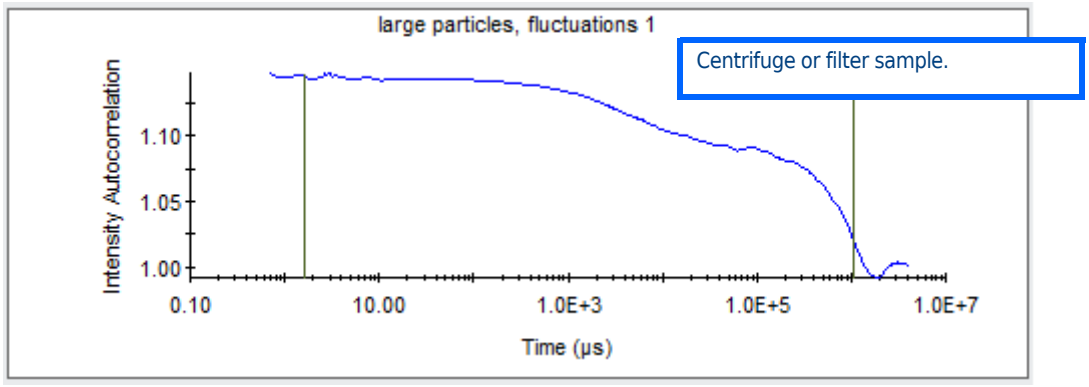
Stop Category

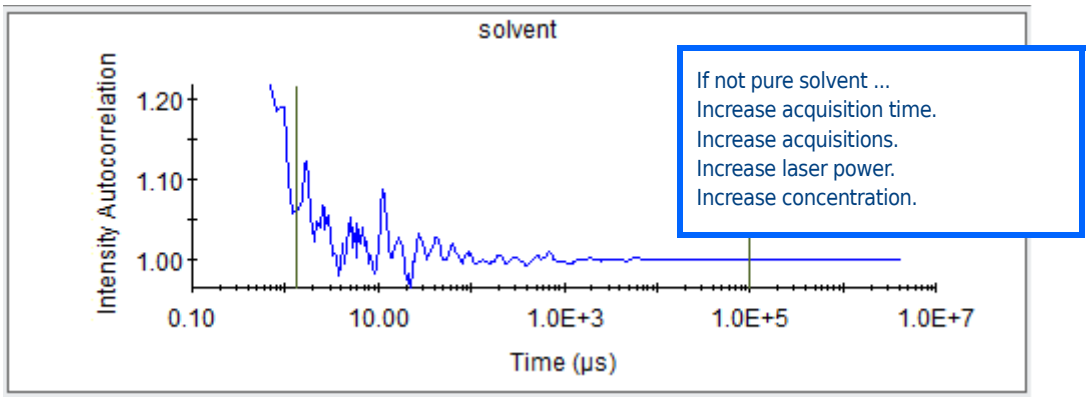
Do not proceed with data interpretation if the correlation function appears as shown in the following figures.

If the graph appears as in the first figure, the sample probably contains large particles, and should be centrifuged (6,000 x g for 10-30 min works well to remove micron size particles) or filtered using syringe filters (.1 micron).

If the graph appears as shown in the second figure, make sure the cuvette is inserted properly, the lid is closed, and that the sample is not pure solvent.

If all of these items check out OK, follow the recommendations under the “Caution” category. If these steps fail, contact your local Beckman Coulter representative.





Molar Mass Estimates

The molar mass of a biological molecule can be estimated from the measurement of the hydrodynamic radius. The estimate is based upon on an empirical curve of known proteins and measured hydrodynamic radius.

The error of the estimated molar mass from hydrodynamic radius ranges from several percent to over 100%. The wide range of error is due to the nature of the estimate. Not all proteins fall on the curve. The estimated value must be used with caution.

When applying the molar mass estimate, make sure the intensity weighted size distribution analysis is selected. The empirical curves are based upon the use of the intensity weighted calculation of the mean of the peak.

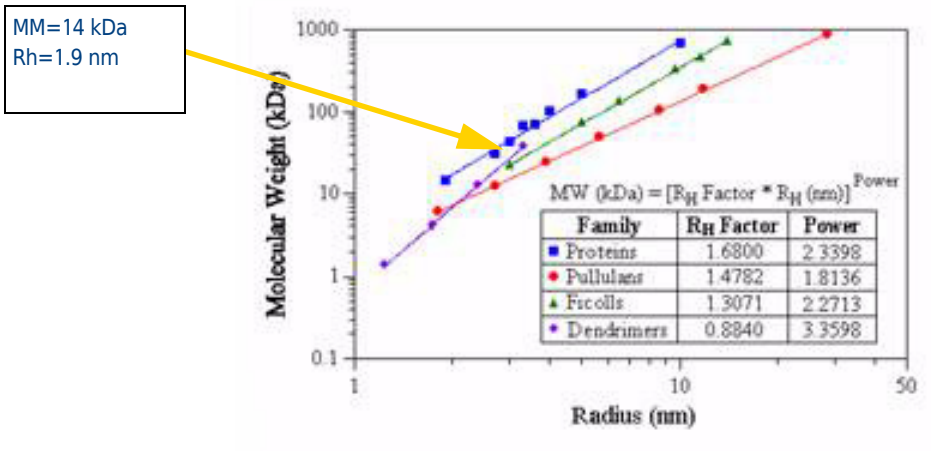
Also, if the peak is determined to be polydisperse by the DelsaMax size distribution analysis, then the mean radius is a weighted average of more than one species. The estimated molar mass will be a weighted estimate based upon the weighted average size.

The molar mass estimate can be qualified by examining the shape factor, the relationship between the measured hydrodynamic radius and the hard sphere radius calculated from the known molar mass and density of the protein.

Molar Mass Interpolated from Radius

Mw-R is the molar mass estimated based upon the measured hydrodynamic radius. Ideally, the size distribution is monodisperse; otherwise, the measured radius is a weighted average of more than one species, and the estimated molecular—even for a protein or other particles that falls on the empirical curve—will be in error.

Select the model that best fits the a priori knowledge of the sample. Or, match the model that best matches the known molar mass or oligomer to obtain an estimate on the shape or conformation of the sample.



A
Analysis Methods

This chapter helps you understand the analysis methods employed by DelsaMax Analysis Software to generate size and size distribution information from autocorrelation function data, provides an overview of the mathematics and algorithms underlying the analysis, and describes when these methods are used by DelsaMax Analysis Software.

Cumulants Analysis

Cumulants analysis of dynamic light scattering data was introduced by Koppel[1] as a means of determining general information regarding a distribution of exponential decay rates (e.g. mean and width of the distribution of decay rates), which may be expressed in terms of the distribution of sizes. For a complete cumulants expansion involving all (infinitely many) cumulants terms, the first, second, and third cumulants terms are equal to the first, second, and third moments (which are related to the center, width, and skew, respectively) of the intensity distribution of correlation rates. It is not feasible to use more than a few free parameters when fitting dynamic light scattering data, and so typically only the first two cumulants terms are used, resulting in the equation:

$$g^{(2)}(\tau) = 1 + \alpha e^{-2\kappa_1\tau + \kappa_2\tau^2} \quad (1)$$

where $g^{(2)}(\tau)$ is the intensity autocorrelation function measured by DelsaMax instrumentation, α is the amplitude of the autocorrelation function, and κ_1, κ_2 are the first and second cumulants. Limiting the cumulants expansion in this way to the first and second cumulants terms is equivalent to fitting data to a light intensity distribution, which is a Gaussian distribution of correlation rates (the correlation rate is proportional to 1/radius), including negative correlation rates, which are not physically possible. The presence of unphysical negative correlation rates causes Eq. (1) to diverge to infinity for large enough values of τ, which has various undesirable effects when analyzing data.

Eq. (1) may be modified to correspond to a physically possible distribution of sizes by assuming a Gaussian distribution of correlation rates truncated at zero, rather than extending to negative correlation rates. A truncated Gaussian distribution of correlation rates Γ is given by:

$$G(\Gamma) = \frac{1}{N} e^{-(\Gamma-\lambda_0)^2/2\sigma^2} \quad (2)$$

1. Koppel, D. E. (1972), *Journal of Chemical Physics*, vol. 57, no. 11, 4814-4820

where λ_0 is the decay rate corresponding to the center of the Gaussian distribution, σ is the width of the Gaussian distribution, and N is the normalization for the distribution computed as:

$$N = \int_0^\infty e^{-(\Gamma-\lambda_0)^2/2\sigma^2} d\Gamma = \sigma\sqrt{\frac{\pi}{2}} erfc\left(-\frac{\lambda_0}{\sigma\sqrt{2}}\right) \tag{3}$$

where $erfc(x)$ is the complimentary error function, defined as:

$$erfc(x) = 1 - \frac{2}{\sqrt{\pi}}\int_0^x e^{-t^2} dt \tag{4}$$

The autocorrelation function resulting from the truncated Gaussian distribution is given by:

$$g^{(2)}(\tau) = 1 + \alpha\left[\int_0^\infty e^{-\Gamma\tau} G(\Gamma) d\Gamma\right]^2 = 1 + \alpha e^{-2\lambda_0\tau+\sigma^2\tau^2}\left[\frac{erfc\left(\frac{\sigma\tau}{\sqrt{2}} - \frac{\lambda_0}{\sigma\sqrt{2}}\right)}{erfc\left(-\frac{\lambda_0}{\sigma\sqrt{2}}\right)}\right]^2 \tag{5}$$

Eq. (5) is well behaved and monotonically decreasing everywhere with increasing τ, as it must be since it is simply a combination of decaying exponential functions.

Traditional cumulants analysis attempts to determine the first and second cumulants terms, which are equal to the first and second moments of the intensity distribution, which are given as:

$$\mu_1 = \int_0^\infty \Gamma G(\Gamma) d\Gamma \tag{6}$$

and

$$\mu_2 = \int_0^\infty (\Gamma - \mu_1)^2 G(\Gamma) d\Gamma \tag{7}$$

For a Gaussian distribution truncated at zero, the center and width of the Gaussian does not directly yield the first and second moments of the distribution. Using Equations (2), (6), and (7), the first and second moments of the truncated Gaussian are calculated to be:

$$\mu_1 = \lambda_0 + \sqrt{\frac{2}{\pi}}\frac{\sigma e^{-\lambda_0^2/2\sigma^2}}{erfc\left(-\frac{\lambda_0}{\sigma\sqrt{2}}\right)} \tag{8}$$

and

$$\mu_2 = \lambda_0^2 - \mu_1^{\,2} + \sigma^2 \left(\frac{1 - \frac{2}{\pi}}{erfc\left(-\frac{\lambda_0}{\sigma\sqrt{2}} \right)} + \frac{2}{\sqrt{\pi}} \right) \quad (9)$$

The cumulants radius reported by DelsaMax Analysis Software with Dynals analysis selected is derived from μ_1, and the percent polydispersity is given by $\%Pd = 100\frac{\mu_2}{\mu_1^2}$.

The maximum possible polydispersity for a truncated Gaussian distribution is approximately 57%.

Regularization Analysis

Regularization analysis attempts to estimate the distribution of particle sizes, which gives rise to a particular autocorrelation function. Uniquely determining the distribution of particle sizes from autocorrelation data is possible only for theoretically perfect data, having zero noise, infinite extent in time, and infinite resolution in time. In the absence of such perfection, there are an infinite number of particle size distributions, quite disparate distributions in some cases, which all fit the data equally well. Some additional criteria, unsupported by the data, must be imposed to choose between these equivalently good solutions. The degree of "smoothness" of the particle distribution is the most commonly used criteria when choosing amongst equivalently good solutions, and it is the criteria used by the regularization algorithms in DelsaMax Analysis Software. For a more detailed discussion and references for this method see *Laser Light Scattering Basic Principles and Practice* by Chu, and S.W. Provencher[2].

2. Chu, B., *Laser Light Scattering Basic Principles and Practice*, Second Edition, Dover Publications, Mineola, 2007, "Inverse problems in polymer characterization: Direct analysis of polydispersity with photon correlation spectroscopy," Makromol. Chem. 180, 201-209 (1979)

Dynals vs. Legacy Analysis Methods

Note: Dynals is the name of a software application and analysis package distributed by Alango Ltd. DelsaMax Analysis Software 1.0 and higher incorporates the Dynals regularization analysis, but does not use the Dynals cumulants analysis.

DelsaMax Analysis Software versions 1.0 and higher with **Dynals** analysis selected in the Fixed Parameters node uses the following analysis methods:

- **Regularization:** The Dynals algorithm supplied by Alango, Ltd. is used. For additional information see A.A. Goldin, "Software for particle size distribution analysis in photon correlation spectroscopy"[3].
- **Cumulants Analysis:** With Calculate Polydispersity set to **False** in the Application Options window, the autocorrelation function data are fit to a simple exponential function. With Calculate Polydispersity set to **True** in the Application Options window, the autocorrelation function data are fit to Eq. (5).

DelsaMax Analysis Software versions prior to 6.10.0, and versions 6.10.0 and greater with **Legacy** analysis selected in the Fixed Parameters node use the following analysis methods:

- **Regularization:** Proprietary algorithm similar to both Dynals and CONTIN[4].
- **Cumulants Analysis:** With Calculate Polydispersity set to **False** in the Application Options window, the autocorrelation function data are fit to a simple exponential function. With Calculate Polydispersity set to **True** in the Application Options window, the autocorrelation function data are fit to a simple exponential, and a distribution of decay rates around that exponential are found.

3. A.A. Goldin, "Software for particle size distribution analysis in photon correlation spectroscopy," website documentation at:
www.softscientific.com/science/WhitePapers/dynals1/dynals100.htm
4. CONTIN is a regularization package that is publicly available at
http://s-provencher.com/index.shtml.

Mobility Theory and Calculations

The optical system of the DelsaMax Analysis Software implements the massively parallel phase analysis light scattering (MP-PALS) technique, a first-principle mobility-measuring method. This technique makes speedy measurements through massive parallelism of detection, and it extends the measurable molecular size below 2 nm. A much shortened measurement time—30 seconds or less—contributes to preservation of precious and fragile samples.

Electrophoresis and Electrophoretic Mobility

Electrophoresis is the migration of (macro-)ions under the influence of an electric field. For moderate field strengths (< 200 V/cm), the steady-state electrophoretic velocity[5] $\boldsymbol{v_e}$ attained by the migrating macro-ions is proportional to the applied electric field E, where μ is the electrophoretic mobility, or velocity per unit electric field.

$$\boldsymbol{v_e} = \mu \boldsymbol{E} \tag{10}$$

As shown in Figure A-1, a positively charged macromolecule in the solution is subject to an electric field $\boldsymbol{E}$.

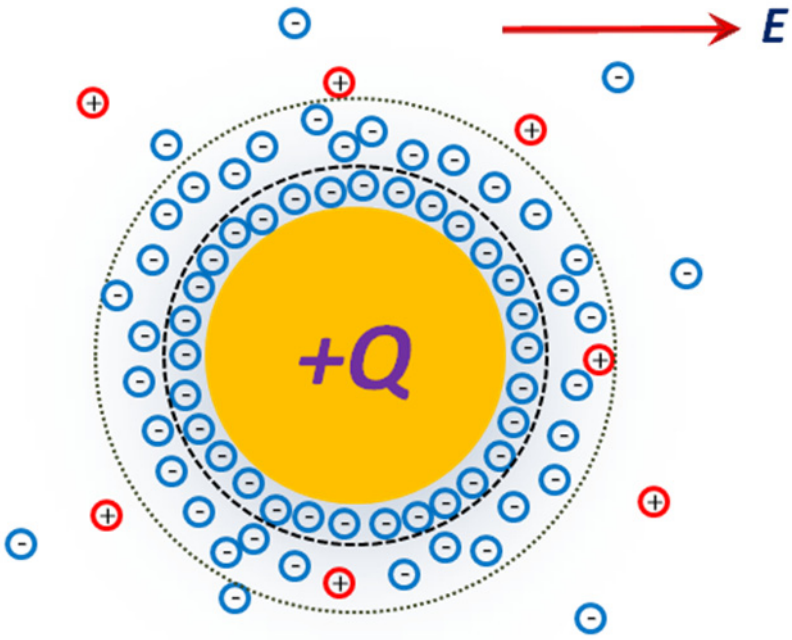


Figure A-1: A positively charged macromolecule surrounded by excess counter-ions in solution.

5. The time scale within which a steady-state electrophoretic velocity is attained is on the order of magnitude *m/f*, where *m* is the mass of the macro-ion and $f = 6\pi\eta R_h$ is the associated frictional coefficient; η stands for the solvent dynamic viscosity and R_h is the molecule's hydrodynamic radius. For BSA molecules in an aqueous solution, the quantity *m/f* is approximately 0.2 nsec.

In the steady state, the electric force QE will be exactly balanced by the frictional force $6\pi\eta R_h v_e$, where η is the solvent dynamic viscosity and R_h is the molecule's hydrodynamic radius. Eq. (11) shows the mobility to be proportional to the molecular charge and inversely proportional to the viscosity.

$$\mu = v_e / E = Q / 6\pi\eta R_h \tag{11}$$

However, this relationship is too simplistic for most cases, because the effects of the counter-ions have been ignored. The charged macromolecule is, in reality, surrounded by an excess of counter-ions, which inevitably screens out the electric field and reduces the electric field strength experienced by the macromolecule. As a result, the electrophoretic mobility is usually much lower than that predicted by the net molecular charge.

The value of electrophoretic mobility can be used to evaluate the amount of charge (Ze) carried by a macro-ion or its *zeta potential* ζ, which is defined as the electrostatic potential existing at the hydrodynamic plane of shear of a solvated macro-ion. Several relationships have been derived between μ, Ze, and ζ, but none of them is rigorous enough to be applicable under all circumstances. Various approximations and parameters such as Debye length, solution ionic strength, dielectric constant, viscosity, particle size, and macro-ion surface conduction all contribute to the complexity. Furthermore, non-idealities such as the *electrophoretic effect*[6] and *ion relaxation*[7] render an exact analytical expression between μ and ζ a formidable, if not impossible, feat.

While it is often not possible to compute the zeta potential rigorously, it is always possible to make first-principle physical measurements of the (electrophoretic) mobility, which conveys important information regarding the dispersed macromolecules.

6. Macro-ions are necessarily surrounded by low-molar-mass counter-ions and co-ions, usually derived from the electrolyte. An excess of counter-ions exists in the vicinity of each of the macro-ions. The electric field that drives the macro-ions also acts on these counter-ions in the opposite direction. The moving, solvated counter-ions drag the solvent along with them, and the solvent in turn acts on the macro-ions. The net effect is a secondary force that has a retardation effect on the movement of macro-ions. This is called the electrophoretic effect. (Adapted from *Physical Chemistry of Macromolecules* by C. Tanford.)
7. Ion relaxation refers to the perturbation from equilibrium of the distribution of co-ions and counter-ions around the macro-ions due to the same electric field that drives the movement of the macro-ions.

Introduction to Mobility Measurement with Light Scattering

Since the early 1970s, various techniques of laser light scattering have been developed for measuring electrophoresis. Light scattered from moving particles is Doppler-shifted and carries information about their movement. As shown in Figure A-2, the incident light is scattered by particles within the scattering volume into a new direction with a scattering angle θ_s.

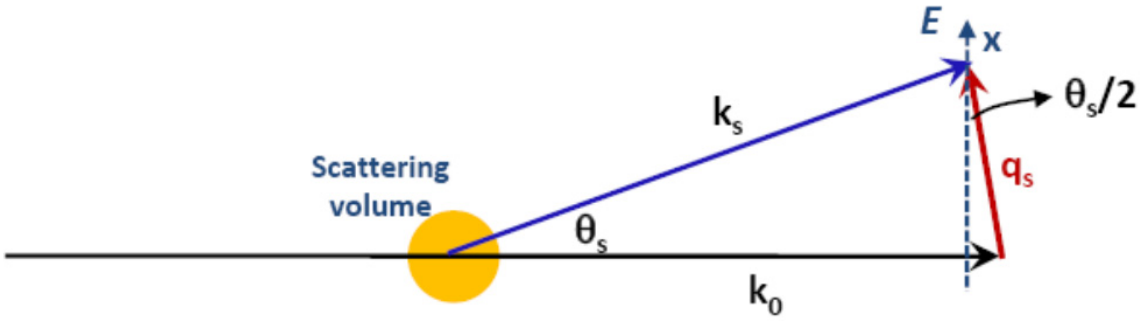


Figure A-2: Phase diagram for light scattering. The applied electric field **E** along the X-axis drives electrophoresis.

The scattering vector is defined as $\boldsymbol{q_s} = \boldsymbol{k_s} - \boldsymbol{k_0}$, the difference between the wave vectors of the scattered and incident light. Its direction is as shown in Figure A-1 and it has a magnitude $|q_s| = 4\pi n \sin(\theta_s/2)/\lambda$, where n is the refractive index of the solution and λ is the wavelength of the light in vacuum. The existence of an electric field $\boldsymbol{E}$ along the x-axis results in electrophoresis in the x direction with a velocity $\boldsymbol{v_e} = \mu \boldsymbol{E}$.

The optical phase ϕ_s of the scattered light is related to the positions of the scattering particles. Considering only electrophoretic movement, the optical phase difference between time 0 and Δt later is $\Delta\phi_s = \boldsymbol{q_s} \cdot \boldsymbol{v_e}\, \Delta t$ where $\boldsymbol{v_e}\, \Delta t$ is the displacement of the particles due to electrophoresis.

We obtain, after some straightforward algebraic operations, the Doppler shift:

$$\omega_s = d\phi_s/dt = \mu \cdot 2\pi n \cdot \sin(\theta_s) E / \lambda \qquad (12)$$

In general, the movement of particles can be collective (due to fluid flow, electrophoresis, etc.) and diffusional (due to Brownian motions). The diffusional component, being stochastic in nature, averages to zero over time, and the collective component can be revealed if enough measurement is available to average away the diffusional component. If one measures the average optical phase shift per unit time $d\bar{\phi}_s / dt$ due to the applied electric field, the electrophoretic mobility μ can be determined.

Figure A-3 shows how the optical phase associated with particle diffusion can be averaged away given enough measurement time.

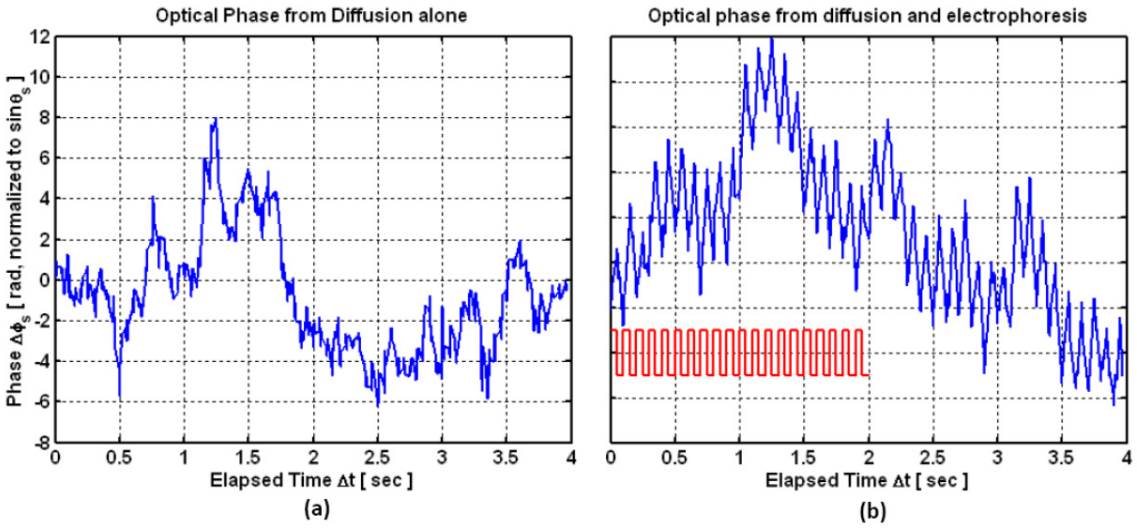


Figure A-3: Measured optical phase due to (a) particle diffusion alone and (b) electrophoresis and diffusion. For both (a) and (b), the sample is Titania (Anatase TiO_2 from Inframat® Advanced Materials™) dispersed in ethanol.

Figure A-3(a) shows the measured optical phase due to particle diffusion alone. The sample is titanium dioxide (TiO_2) dispersed in ethanol, $R_h \cong 100\text{nm}$. The phase has been measured and normalized to sin θ_s. As the figure shows, this diffusional component is indeed a random walk. The average displacement due to particle diffusion happens to average to zero at Δt = 4 sec. The smaller the particles' hydrodynamic radius, the more conspicuous the diffusion will be and the more time it will take to average this component away.

In Figure A-3(b), a 10 Hz, square-wave electric field with a magnitude of 18 V/cm is applied to drive electrophoresis. The scaled and offset electric field is plotted below the optical phase for visual reference. Here the optical phase consists of both an electrophoretic and a diffusional component. It is clear that the electrophoretic component reverses its direction every time the electric field switches polarity, while the diffusional component remains independent of the electric field and proceeds with its random walk. Since the diffusional component should average out in the order of 4 seconds, the electrophoretic mobility can be measured from Figure A-3(b) and is computed +0.46 μm•cm/V•sec.

In general, light scattering (LS) can be carried out in two different ways: light scattering in the *homodyne* mode mixes only light scattered from particles, while light scattering in the *heterodyne* mode mixes light scattered from particles along with some unscattered light, often referred to as the local oscillator. To appreciate the distinctions between these two methods, we consider the photocurrent $i(t)$ generated at the detector as shown in Eq. (13).

$$i(t) = \Re\left|\sqrt{P_{LO}}e^{i\omega t} + \sum_{j=1}^{N}\sqrt{P_0}\,e^{i\omega t}e^{i\varphi_j(t)}\right|^2 \qquad (13)$$

In Eq. (13), $\mathcal{R}$ is the detector responsivity, ω is the laser angular frequency, P_{LO} is the local oscillator power, P_0 is the power of the scattered light from each of the N particles in the scattering volume, and $\varphi_j(t)$ is the phase of the light scattered by the j^{th} particle.

Without loss of generality, we have assumed that all particles scatter equally efficiently; that is, they have the same *polarizability*. The phase $\varphi_j(t)$ is related to the position $\boldsymbol{x_j}(t)$ of particle j by $\varphi_j(t) = \boldsymbol{q_s}\cdot\boldsymbol{x_j}(t)$. Because both diffusional and collective movements contribute to $\boldsymbol{x_j}(t)$, we have the following relationship:

$$\varphi_j(t) = \boldsymbol{q_s}\cdot\boldsymbol{x_j}(t) = \boldsymbol{q_s}\cdot[\boldsymbol{x_{jD}}(t) + \boldsymbol{v_c}t] = \varphi_{jD}(t) + \varphi_c(t) \tag{14}$$

In Eq. (14) $\boldsymbol{x_{jD}}(t)$ and $\varphi_{jD}(t) = \boldsymbol{q_s}\cdot\boldsymbol{x_{jD}}(t)$ are the displacement and phase components associated with the Brownian motion of the j^{th} particle and $\varphi_c(t) = \boldsymbol{q_s}\cdot\boldsymbol{v_c}t$ is the phase component due to the collective velocity $\boldsymbol{v_c}$, which is common to all particles. Note that $\boldsymbol{x_{jD}}(0)$ stands for the initial position of the j^{th} particle. Expanding $i(t)$, we get Eq. (15).

$$\begin{aligned} i(t) = \mathcal{R}\Bigg\{ & P_{LO} + NP_0 + 2\sqrt{P_{LO}P_0}\sum_{j=1}^{N}\cos\left[\varphi_{in}(t) + \varphi_c(t)\right] \\ & + 2P_0\sum_{j=2}^{N}\sum_{k=1}^{j-1}\cos\left[\left(\varphi_{jD}(t) + \varphi_c(t)\right) - \left(\varphi_{kD}(t) + \varphi_c(t)\right)\right]\Bigg\} \end{aligned} \tag{15}$$

In the homodyne mode, $P_{LO} = 0$ and we end up with:

$$i(t) = \mathcal{R}\left\{NP_0 + 2P_0\sum_{j=2}^{N}\sum_{k=1}^{j-1}\cos\left[\varphi_{jD}(t) - \varphi_{kD}(t)\right]\right\} \tag{16}$$

Note that all of the collective phase components $\varphi_c(t)$'s cancel out in $i(t)$ for homodyne detection. Measurements obtained at all times t have the information-carrying terms $\varphi_{jD}(t) - \varphi_{kD}(t) = \boldsymbol{q_s}\cdot[\boldsymbol{x_{jD}}(t) + \boldsymbol{x_{kD}}(t)]$. It is now clear that LS in the homodyne mode is insensitive to collective particle movement since only diffusional information is available. This makes homodyne LS suitable for measuring hydrodynamic radii even when a flow is present, but it cannot measure electrophoretic mobilities.

On the other hand, signals obtained from heterodyne LS contain the terms $\cos[\varphi_{jD}(t) + \varphi_c(t)]$ and carry phase information of both collective and diffusional components. Therefore, in order to measure electrophoresis with light scattering, optical heterodyning is necessary[8]. Dynamic light scattering in the heterodyne mode had been used to measure the mobility of bovine serum albumin molecules (5% by weight, or about 50 mg/mL) in 4 mM NaCl solution[9].

Another method, *laser Doppler electrophoresis* (LDE), involves detecting light scattered from electric field-driven macro-ions moving within a stationary fringe pattern[10]. The fringe pattern is generated by the interference of two light beams derived from the same laser to ensure a good contrast ratio[11]. As macro-ions traverse the light and dark fringes, a temporally sinusoidal

8. B. J. Berne and R. Pecora, *Dynamic Light Scattering with Applications to Chemistry, Biology and Physics*, John Wiley & Sons Inc. 79 (1976).
9. R. Ware and W. H. Flygare, *J. Colloid Interface Sci.* **39**, 670 (1972).
10. E. E. Uzgiris, *Prog. Surf. Sci.* **10**, 53 (1981).

intensity signal is detected from the scattered light. Since the spacing between adjacent light and dark fringes is known from the angle of intersection between the interfering laser beams, the electrophoretic velocity can be measured and mobility can be obtained from the signal frequency.

LDE has important advantages over earlier techniques. It greatly reduces the volume of sample required for mobility measurement (to milliliters or less) because the fringe pattern can be generated within the space between electrodes that are merely 1–2 mm apart. Since the electric field is the voltage difference between the electrodes divided by their spacing, the reduced electrode spacing decreases the magnitude of the voltage that must be applied to generate observable electrophoresis. In addition, a large number of macro-ions contribute to the detected signal, which produces statistically sound measurements. To avoid electrode polarization, whereby macro-ions accumulate near the electrodes and shield the bulk from the applied field, an oscillating (AC) electric field is preferable to a DC field. The frequency of the field reversal should be made much lower than the Doppler frequency and much higher than the reciprocal of the time it takes for electro-osmosis to develop[12]. However, when it comes to measuring low-mobility species[13], LDE cannot satisfy both conditions without applying excessively high voltages and running into problems such as profuse electrolysis and fluid convection. Another limitation of LDE is that both positive and negative electric fields produce the same optical signal, so the sign of the mobility cannot be determined.

Massively-Parallel Phase Analysis Light Scattering (MP-PALS)

To address the limitations of LDE, *phase analysis light scattering* (PALS)[14] was developed in the late 1980's and early 1990's. PALS is derived from LDE; the major distinction between them is that in PALS a sweeping, instead of a stationary, fringe pattern is established in the scattering volume. Such fringe patterns can be generated by phase-modulating one or both of the interfering laser beams. A sweeping fringe pattern not only obviates the necessity of macro-ions having to traverse a few light and dark fringes during a single (electric) field period, but also enables the determination of the sign of mobility. Zero-mobility (and therefore stationary) particles give rise to a signal frequency equivalent to that of the sweeping fringes, and macro-ions migrating in the same/opposite direction of the sweeping fringes produce a signal frequency lower/higher than that of the sweeping fringes.

There is another incarnation of PALS: instead of interfering the two laser beams at the scattering volume, the second laser beam (or local oscillator) is mixed/heterodyned directly on the detectors with scattered light from the macro-ions and acts both as an optical amplifier and as a phase reference. The local oscillator is phase-modulated for the same reason explained above. In this configuration, PALS is really an interferometric method in which one arm is the modulated local

11. The direction of the driving electric field is almost always made perpendicular to the phase fronts of the fringes.
12. The characteristic time for development of electro-osmosis is d^2/ν, where d is the relevant linear dimension and ν is the kinematic viscosity of the solution. For aqueous solutions at 20° C, $\nu \approx 1.0\,\mathrm{mm^2/sec}$, and the time it takes for electro-osmosis to develop is about 1 sec for a 1 mm electrode gap.
13. Species where the absolute value of the mobility is smaller than 0.1–0.5 $\mu\mathrm{m \cdot cm/V \cdot sec}$.
14. J. F. Miller, K. Schätzel, B. Vincent, "The Determination of Very Small Electrophoretic Mobilities in Polar and Nonpolar Colloidal Dispersions Using Phase Analysis Light Scattering," *J. Colloid Interface Sci.* **143**, 532-554 (1991).

oscillator and the other is the scattered light. As the incident laser beam goes straight through the sample, the sample volume can be further reduced. The optical system of the DelsaMax Analysis Software builds upon this technique and takes it a step further.

The implementation of the PALS technique in the DelsaMax Analysis Software enables multiple simultaneous measurements with an array of detectors. As long as the active area of each detector element is greater than or equal to the coherence area of the scattered light[15], the signals collected from different detector elements are truly independent. Since each detector channel makes an independent measurement, N channels will reduce the measurement time by a factor of N. In the DelsaMax Analysis Software, N=31 (and can be increased in the future with available computation power). This parallelism is made possible by an innovative optical design, massively-parallel PALS (MP-PALS), which facilitates free space multiplexing of the interfering beams. Moreover, the detectors used in the DelsaMax Analysis Software are linear and robust over their excellent dynamic range (from nW all the way up to mW). This range ensures that the full advantage of the coherent amplification is fully utilized to achieve superior instrument sensitivity.

Parameters Derived from the Mobility

Several important molecular parameters can be derived from μ if appropriate models and/or theories are applied. Summarized below are the parameters the DelsaMax Analysis Software reports for mobility measurements:

Effective Charge

If the hydrodynamic radius R_h is known, the effective charge is computed through the relationship:

$$Ze = 6\pi\eta R_h \mu \frac{1+\kappa R_h}{f(\kappa R_h)} \qquad (17)$$

where Z is the valence of the macromolecules, e (~1.6 x 10^{-19} coulombs) is the elementary charge, η is the sample viscosity, κ is the Debye-Hückel parameter, and $f(\kappa R_h)$ is Henry's function. Both the Debye-Hückel parameter and Henry's function are explained in the section on "Henry's equation" on page A-12.

Zeta Potential

The DelsaMax Analysis Software provides the following three different formulas for calculating zeta potential ζ. Researchers should choose the most suitable model for their applications.

Smoluchowski's equation

In the limiting case where the molecular hydrodynamic radius R_h is much larger than the Debye length κ^{-1}, Smoluchowski's equation can be used:

15. When scattered light impinges on a screen, a diffraction/speckle pattern is produced which depends, among other things, on the extent of the scattering volume. That is, the intensity maxima and minima depend on the dimensions of the scattering volume. On any given point *P* on the screen, one could define an area near the point *P* such that the signals at all points within this area are partially coherent with that at *P*. This area would be called the coherence area. A useful estimate of the coherence area for light scattering is $A_{coh} = \lambda^2/\Omega$ where Ω is the solid angle subtended by the scattering volume at the detector(s). As Ω grows smaller, A_{coh} becomes larger. (Adapted from *Dynamic Light Scattering with Applications to Chemistry, Biology and Physics* by B. J. Berne and R. Pecora.)

$$\zeta = \frac{\eta\mu}{\varepsilon_0\varepsilon_r} \quad (18)$$

where ε_0 (~8.854 x 10^{-12} F/m) is the permittivity of free space, and ε_r (~80 for water at 20º C) is the solvent dielectric constant.

Hückel's equation

In the other limiting case where the molecular hydrodynamic radius R_h is much smaller than the Debye length κ^{-1},Hückel's equation can be used:

$$\zeta = \frac{3\eta\mu}{2\varepsilon_0\varepsilon_r} \quad (19)$$

Henry's equation

Henry's equation is the most general of the three formalisms. It includes both the Smoluchowski's and Hückel's equations as its limiting cases. Henry's equation is:

$$\zeta = \frac{3\eta\mu}{2\varepsilon_0\varepsilon_r f(\kappa R_h)} \quad (20)$$

To utilize Henry's equation, researchers need to know the *ionic strength I* of their sample solution. The ionic strength is defined as:

$$I = \frac{1}{2}\sum_{i=1}^{n} C_i z_i^2 \quad (21)$$

where C_i is the molar concentration of any mobile ion and z_i its valence. The summation extends over all mobile ion species present. (As examples, the ionic strengths of 10 mM NaCl, $CaCl_2$ and $MgSO_4$ are, respectively, 10 mM, 30 mM and 40 mM.)

In turn, the Debye-Hückel parameter κcan be determined from the ionic strength of the electrolyte, where N_A (~6.02 x 10^{23} mol^{-1}) is the Avogadro constant, k (~1.38 x10^{-23} J K^{-1}) is the Boltzmann constant, and T is the absolute temperature in Kelvin.

$$\kappa = \sqrt{\frac{2N_A e^2 I}{\varepsilon_0\varepsilon_r kT}} \quad (22)$$

The reciprocal of $\kappa$is defined as the *Debye length*, which corresponds to the double-layer thickness surrounding the molecules. The Debye length κ^{-1} of 10 mM NaCl is 3.05 nm, for example.

Figure A-4 shows a plot of Henry's function $f(\kappa R_h)$ for reference:

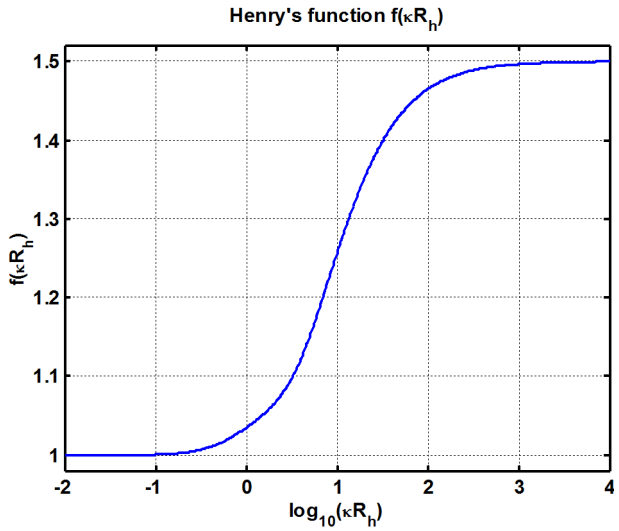


Figure A-4: Plot of Henry's function

As evident from the figure, when taken to one extreme, Henry's equation becomes Hückel's equation:

$$\lim_{\kappa R_h \to 0} f(\kappa R_h) = 1 \qquad (23)$$

When taken to the other extreme, Henry's equation reduces to Smoluchowski's equation:

$$\lim_{\kappa R_h \to \infty} f(\kappa R_h) = \frac{3}{2} \qquad (24)$$

Colloidal Stability

One of the most widely adopted uses for zeta potential is in the determination of colloidal stability. As the molecular charge increases, the probability of molecules aggregating and flocculating out of the solution decreases. Like charges repel each other. The widely accepted criterion for colloidal stability at room temperature is:

$$|\zeta| \geq \frac{kT}{e} \approx 25mV \qquad (25)$$

Using Smoluchowski's equation, a mobility of 1.00 μm•cm/V•sec corresponds to a zeta potential ζ of 12.7 mV at 25º C in aqueous solutions. Therefore most protein solutions, with $|\mu|$< 2.00 μm•cm/V•sec are considered borderline stable or unstable.

B

Quick Reference

This appendix provides a quick list of DelsaMax Analysis Software menu commands and keyboard shortcuts.

File Menu

The File menu contains the following commands:

Command	Keyboard Shortcut	Description
File ▸▸ New	Ctrl+N	Create an empty experiment. See page 2-3.
File ▸▸ Open	Ctrl+O	Open an existing experiment. See page 2-3.
File ▸▸ Close		Close the active experiment. See page 2-3.
File ▸▸ Save	Ctrl+S	Save the current experiment. See page 2-3.
File ▸▸ Save As		Save the experiment with a new name. See page 2-3.
File ▸▸ Save Settings		Save current settings as the default. See page 2-16.
File ▸▸ Open Preset		Load settings from a Preset file. See page 2-16.
File ▸▸ Save Preset		Save current settings to a Preset file. See page 2-16.
File ▸▸ Page Setup		Set up paper size, orientation, and margins. See page 7-1.
File ▸▸ Print	Ctrl+P	Print information about the current experiment. See page 7-1.
File ▸▸ Print Preview		Preview information about the current experiment. See page 7-1.
File ▸▸ Recent Files		Open a recently used experiment. See page 2-3.
File ▸▸ Exit		Exit from DelsaMax Analysis Software. See page 2-3.

View Menu

The View menu contains the following commands:

Command	Keyboard Shortcut	Description
View ▸▸ Toolbar		Display or hide the main toolbar. See page 2-2.
View ▸▸ Status Bar		Display or hide the status bar at the bottom of the window.
View ▸▸ Datalog Grid	Ctrl+Alt+D	Move to the Datalog Grid view. See page 7-14.
View ▸▸ Datalog Graph	Ctrl+Alt+G	Move to the Datalog Graph view. See page 7-20.
View ▸▸ Correlation Graph	Ctrl+Alt+C	Move to the Correlation Graph view. See page 7-21.
View ▸▸ Regularization Graph	Ctrl+Alt+R	Move to the Regularization Graph view. See page 7-25.
View ▸▸ Mobility Graph	Ctrl+Alt+M	Move to the Mobility Graph view. See page 7-30.
View ▸▸ Instrument Control	Ctrl+I	Open the Instrument Control Panel. See page 6-1.
View ▸▸ Autosampler Control	Ctrl+A	Open the Autosampler Control Panel. See page 6-8.

Experiment Menu

The Experiment menu contains the following commands:

Command	Keyboard Shortcut	Description
Experiment ▸▸ Delete Measurements		Delete multiple data measurements from the experiment. See page 6-10.
Experiment ▸▸ Delete Parametric Analysis		Select a parametric analysis to delete from the experiment. See page 7-34.

Tools Menu

The Tools menu contains the following commands:

Command	Keyboard Shortcut	Description
Tools ▸▸ Instruments		Define and detect instruments. See page 3-3.
Tools ▸▸ Parameters ▸▸ Samples		Edit sample definitions. See page 4-13.
Tools ▸▸ Parameters ▸▸ Solvents		Edit solvent definitions. See page 4-16.
Tools ▸▸ Parameters ▸▸ Cuvettes		Edit cuvette definitions. See page 4-23.
Tools ▸▸ Parameters ▸▸ User Defined		Edit user-defined parameters. See page 4-25.
Tools ▸▸ Calculations ▸▸ Apparent Fraction		Open the Apparent Fraction calculator. See page 2-11.
Tools ▸▸ Calculations ▸▸ Axial Ratio		Open the Axial Ratio calculator. See page 2-12.
Tools ▸▸ Calculations ▸▸ Optimization		Open the Optimization calculator. See page 2-13.
Tools ▸▸ Calculations ▸▸ Ramp Rate		Open the Ramp Rate calculator. See page 2-14.
Tools ▸▸ Diagnostics ▸▸ Restore Defaults		Reset defaults and configurations to the defaults. See page 2-10.
Tools ▸▸ Diagnostics ▸▸ Restore Solvents		Reset solvent definitions to the defaults. See page 2-10.
Tools ▸▸ Diagnostics ▸▸ Write EEPROM		Reserve for use when recommended by Beckman Coulter Technical Support. See page 2-10.
Tools ▸▸ Diagnostics ▸▸ Running Calculations		Show any calculations that are currently being run. See page 2-10.
Tools ▸▸ Options		Adjust settings that control the behavior of DelsaMax Analysis Software. See page 2-8.

Window Menu

The Window menu contains the following commands:

Command	Keyboard Shortcut	Description
Window ▸▸ New Window		Open the same experiment in a separate window. See page 2-4.
Window ▸▸ Cascade		Display or hide the status bar at the bottom of the window. See page 2-4.
Window ▸▸ Tile Horizontally		Arrange open views in cascading fashion. See page 2-4.
Window ▸▸ Tile Vertically		Arrange open views in column (wide views). See page 2-4.
Window ▸▸ Arrange Icons		Arrange open views in row (tall views). See page 2-4.
Window ▸▸ <select>		Move to a different window.

Help Menu

The Help menu contains the following commands:

Command	Keyboard Shortcut	Description
Help ▸▸ Help Topics	F1	Open help table of contents. See page 1-4.
Help ▸▸ About DelsaMax		Open version and copyright information about DelsaMax Analysis Software.

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