
ASTRA 6

User's Guide

Version 6
(M1000 Rev. I)



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A variety of U.S. and foreign patents have been issued and/or are pending on various aspects of the apparatus and methodology implemented by this instrumentation.

Contents

Chapter 1: About ASTRA 6.....	1-1
What is ASTRA?	1-2
Using This Manual	1-3
User Modes	1-3
Operating Tiers	1-3
User Account Levels	1-4
How This Manual is Organized	1-5
Manual Conventions	1-5
Glossary	1-6
Batch Mode vs. Online Mode	1-9
Getting More Help	1-10
Contacting Technical Support	1-10
Where to Go from Here	1-10
Chapter 2: Installing and Setting Up ASTRA	2-1
System Requirements	2-2
Installing the ASTRA Software	2-2
Upgrading to a New Version of ASTRA	2-3
Migrating the System Database	2-4
Activating Optional ASTRA Features	2-6
Setting Up User Accounts	2-7
Setting Up Groups	2-7
Creating Users	2-8
Assigning Users to Groups	2-8
Note for Networked Accounts	2-8
Setting a Validation Domain for User Accounts and Groups	2-9
Running ASTRA	2-11
Accessing and Viewing Hardware	2-12
Viewing the Instruments Pane	2-12
Adding an Instrument or Computer to the Instrument List	2-13
Removing an Instrument or Computer from the Instrument List	2-15
Chapter 3: Getting Started.....	3-1
Starting ASTRA	3-2
ASTRA Tutorials on the Support Center	3-4

Performing a Simple Light Scattering Experiment	3-5
Checking the Instrument Connection	3-5
Creating an Experiment	3-5
Modifying the Configuration	3-7
Modifying Procedure Settings	3-9
Running the Experiment	3-10
Viewing Reports	3-12
Summary	3-12
More About the ASTRA Environment	3-13
User Modes	3-13
User Account Levels	3-13
Customizing the Environment	3-14
Command Reference	3-16
Printing	3-16
Getting Help	3-16
Exiting from ASTRA	3-17
Chapter 4: ASTRA Administration.....	4-1
21 CFR Part 11 Support Overview	4-2
Background and Reasons for Compliance	4-2
21 CFR Part 11 Support in ASTRA 6	4-3
Connecting to a Database	4-3
Viewing the Current Experiment Database	4-5
Creating a Microsoft Access Experiment Database	4-5
Managing User Accounts	4-6
Using the System and Experiment Logs	4-8
Viewing the System Log	4-8
Viewing an Experiment Log	4-9
Viewing a Sequence Log	4-9
Working with Logs	4-9
Deleting Experiments	4-10
Performing Database Maintenance	4-10
Chapter 5: Interfaces to Instruments.....	5-1
Instrument Server Interface Overview	5-2
Installing the ISI on Other Computers	5-4
Using the Diagnostic Manager	5-5
Viewing Graphical Data with the Diagnostic Manager	5-5
Viewing Numeric Data with the Diagnostic Manager	5-6
Sending Commands with the Diagnostic Manager	5-7
Viewing and Setting Properties with the Diagnostic Manager	5-9
Viewing the Log with the Diagnostic Manager	5-10
WCS Client Application	5-11
Chapter 6: Creating & Running Experiments	6-1
About Experiments	6-2
Creating New Experiments	6-4
Creating Default Experiments	6-4
Creating Experiments from Methods	6-5
Creating Experiments from Scratch	6-6

Opening an Experiment from a File	6-7
Opening an Experiment from the Database	6-8
Importing an Experiment from a File	6-9
Importing ASTRA 4 Files	6-9
Running an Experiment	6-11
Validating an Experiment	6-11
Starting a Data Collection Run	6-12
Signing Off on an Experiment	6-13
Stopping an Experiment	6-13
Re-Running an Experiment for Data Processing	6-14
If a Crash Occurs... ..	6-14
Viewing Experiment Properties	6-14
Closing an Experiment	6-15
Saving an Experiment to a File	6-15
Saving an Experiment to the Database	6-16
Exporting an Experiment	6-17
Creating a Method	6-18
Setting a Default Method	6-19
Deleting a Method	6-20
Deleting an Experiment	6-20
Copying Data	6-21
Applying a Method	6-22
Applying a Method to Multiple Experiments	6-22
Adding Elements to an Experiment	6-24
Adding to the Configuration	6-24
Adding Procedures	6-26
Setting the Procedure Order	6-27
Adding Data Set Definitions	6-28
Adding Reports and Graphs	6-28
Chapter 7: Configuring Experiments.....	7-1
About Configurations and Profiles	7-2
Configuration Example	7-4
Using Configurations	7-5
Editing a Configuration	7-5
Adding Instruments and Connections	7-5
Removing Instruments and Connections	7-7
Replacing an Experiment Configuration or Item	7-7
Exporting a Profile	7-9
Setting a Default Configuration	7-10
Using the Configuration Wizard	7-11
Experiment Configuration	7-14
Light Scattering Instrument Profiles	7-16
DAWN HELEOS and DAWN HELEOS 8 Profiles	7-16
DAWN EOS, DAWN DSP, DAWN DSP-F, and DAWN 8 Profiles	7-17
miniDAWN and miniDAWN TREOS Profiles	7-19
WyattQELS Profiles	7-21

Refractive Index Instrument Profiles	7-22
Optilab rEX and Optilab T-rEX Profiles	7-22
Optilab DSP Profiles	7-23
Optilab 903 Profiles	7-23
Generic RI Instrument Profiles	7-24
Viscometry Instrument Profiles	7-26
ViscoStar Profiles	7-26
Generic Viscometer Profiles	7-27
UV Absorption Instrument Profiles	7-29
Generic UV Detector Profiles	7-29
Injector Profiles	7-31
Sample Profiles	7-32
Sample Profiles	7-32
Molecular Standard Profiles	7-33
Pump Profiles	7-33
Solvent Profiles	7-34
Column Profiles	7-36
Connection Profiles	7-38
Fluid Connection Profiles	7-38
AUX Connection Profiles	7-39
Autoinject Connection Profiles	7-40
Chapter 8: Editing Procedures.....	8-1
About Procedures	8-2
About Data Processing in ASTRA	8-3
Working With Procedures	8-4
Editing Procedure Settings	8-4
Working with Procedure Graphs	8-5
When to Modify Procedures	8-7
Advanced Procedure Editing	8-8
Adding Procedures	8-8
Deleting Procedures	8-9
Changing Procedure Order	8-9
Validating a Procedures After Modification	8-10
Collection Procedures	8-11
Basic Collection	8-11
Script Collection	8-13
Configuration Procedures	8-15
LS Calibration	8-15
Alignment (Interdetector Delay)	8-18
Band Broadening	8-20
Normalization	8-23
RI Calibration	8-29
Absolute RI Calibration	8-35
Calibrate Column	8-39
Column Plate Count	8-43

Transformation Procedures	8-45
Despiking	8-45
Smoothing	8-46
Baselines	8-48
Blank Baseline Subtraction	8-51
Peaks	8-54
Broaden	8-60
Convert to Physical Units	8-60
Convert to Concentration	8-61
Convert Specific to Intrinsic Viscosity	8-61
Results Fitting	8-62
Analysis Procedures	8-64
Molar Mass and Radius from LS Data	8-64
A2, Molar Mass, and Radius from LS Data	8-69
Online A2	8-71
Molar Mass from VS	8-75
Number from LS Data	8-75
Radius from LS Data	8-77
Distributions and Moments	8-78
Dn/dc from RI	8-79
Dn/dc from Peak	8-80
RI Calibration from Peak	8-81
UV Extinction from Peak	8-81
UV Extinction from RI	8-81
Rh from QELS	8-82
Rh from VS Data	8-85
Branching	8-85
Parametric Plot	8-88
Peak Areas	8-88
Mark-Houwink-Sakurada	8-88
Distribution Analysis	8-90
Copolymer Analysis	8-91
Protein Conjugate Analysis	8-93
Regularization	8-95
Cumulants	8-99
Molar Mass from Column Calibration	8-101
Administration Procedures	8-105
Sign Off	8-105
Chapter 9: Using Sequences.....	9-1
About Sequences	9-2
Creating New Sequences	9-3
Creating Default Sequences	9-3
Creating Blank Sequences	9-3
Creating a Sequence from a Sequence Template	9-4
Setting a Default Sequence	9-4
Opening a Sequence	9-5
Importing an ASTRA Sequence	9-5
Importing an Empower Sequence	9-6

Editing a Sequence	9-7
Configuration Tab	9-7
Samples Tab	9-8
Collection Tab	9-10
Running Sequences	9-11
Validating a Sequence	9-11
Running a Sequence	9-11
Stopping a Sequence	9-11
Viewing a Sequence Log	9-12
Saving Sequences	9-12
Saving Sequences as Templates	9-13
Exporting Sequences	9-13
Deleting a Sequence	9-13
Chapter 10: Working With Reports	10-1
About Reports	10-2
Operator Names in Reports	10-2
Viewing a Report	10-3
Abbreviations Used in Reports	10-4
Setting Report Options	10-5
Using the Report Designer	10-6
Printing a Report	10-7
Exporting a Report	10-7
Adding a Report	10-8
Applying Report Formats From a Method	10-8
Chapter 11: Working With Graphs and Tables.....	11-1
About ASTRA Graphs and Tables	11-2
Using the EASI Graph	11-3
Using the EASI Table	11-6
Using Custom Plots and Data Set Definitions	11-8
Data Collection and Storage	11-8
Creating Data Set Definitions	11-9
Creating Custom Plots	11-11
Creating Surface Plots	11-13
Viewing and Modifying Graphs	11-15
Viewing Data Values	11-15
Customizing Line Colors, Widths, and Fonts	11-15
Zooming In and Out Graphs	11-16
Scrolling Within Graphs	11-16
Axis Settings	11-16
Copying Graphs to the Clipboard	11-17
Printing Graphs	11-17
Exporting Graphs	11-19
Exporting Pictures	11-19
Exporting Data	11-20
Chapter 12: Working with Profiles.....	12-1
About Profiles	12-2

Creating Profiles	12-3
Creating a New Profile	12-3
Saving as a Profile	12-4
Modifying Profiles	12-5
Opening a Profile	12-5
Editing a Profile	12-6
Saving a Profile	12-6
Duplicating a Profile with Save As	12-7
Closing a Profile	12-7
Deleting a Profile	12-7
Using Profiles	12-8
Adding an Item to a Configuration	12-8
Replacing a Single Configuration Item	12-8
Replacing an Entire Configuration	12-9
Appendix A: Menu Quick Reference	A-1
Modes and User Levels	A-2
File Menu	A-2
Edit Menu	A-4
View Menu	A-4
Experiment Menu	A-5
Processing Menu	A-6
Sequence Menu	A-7
System Menu	A-7
Window Menu	A-8
Help Menu	A-9
Appendix B: System Methods.....	B-1
Overview	B-2
General	B-3
Concentration Determination (AM)	B-3
Peak Areas (AM)	B-3
Column Plate Count (CM)	B-3
Light Scattering	B-4
Baseline Subtraction	B-4
Calibration (CM)	B-4
Utilities (CM)	B-4
Diagnostics	B-5
With QELS	B-6
Batch (Debye plot) (CM)	B-7
Batch (Zimm plot) (CM)	B-7
Branching (AM)	B-7
Online (CM)	B-8
Online (Zimm Plot) (CM)	B-8
Online A2 (CM)	B-8
Protein Conjugate (AM)	B-9
Particles (AM)	B-9
Number Density (AM)	B-10

RI Measurement	B-11
100% Mass Recovery Methods	B-11
Optilab rEX Specific	B-11
Diagnostics	B-12
Batch (Determine dn/dc) (CM)	B-12
Calibrate Conventional Column (CM)	B-13
Conventional Column Data Acquisition (CM)	B-13
Copolymer Analysis (AM)	B-13
RI Calibration (CM)	B-13
RI Peak Areas (AM)	B-13
UV Measurement	B-14
UV Extinction from RI Peak (AM)	B-14
Viscometry	B-14
Diagnostics	B-14
With Light Scattering	B-15
Utilities	B-15
Branching from Column Calibration (AM)	B-16
Branching from VS Data (AM)	B-16
Calibrate Universal Column (CM)	B-16
Copolymer Analysis (AM)	B-16
Mass from VS Data (AM)	B-16
Online (CM)	B-16
Universal Column Data Acquisition (CM)	B-16
Appendix C: Data Collection with Scripts	C-1
Introduction	C-2
Collection	C-3
Interacting with Instruments	C-3
Common Instrument Commands	C-4
Static Light Scattering Instrument Commands	C-5
Dynamic Light Scattering, Refractometer, and Viscometer Commands	C-5
Examples	C-5
Appendix D: Light Scattering Theory	D-1
Introduction	D-2
Basic Quantities	D-3
Measured Quantities and Calibration	D-4
Normalization	D-8
Implementation	D-9
Concentration Calculation Methods	D-11
Known dn/dc and Known AUX Calibration Constant	D-11
Known AUX Calibration Constant and 100% Mass Recovery	D-12
Determination of Molar Mass and Sizes	D-13
Conventional Method	D-14
Zimm (Reciprocal) Method	D-15
Berry (Square Root) Method	D-16
Assuming a Molecular Structure	D-16
Other Structures Method	D-17
Molar Mass and RMS Radius Moments	D-18

Uncertainties in Calculated Quantities	D-21
Out of Range Values	D-22
Differential Distribution Calculations	D-22
Branching Calculations	D-23
Branching Ratio: Radius Method	D-23
Branching Ratio: Mass Method	D-23
Branching Ratio: Viscosity Method	D-24
Branching Per Molecule	D-25
Long Chain Branching	D-26
Appendix E: Particles	E-1
Determination of Sizes	E-2
Conventional Mean Square Radius Determination	E-2
Zimm and Berry Mean Square Radius Determinations	E-3
Mean Square Radius Determination from an Assumed Random Coil	E-3
Size from a Known Structure	E-3
Homogeneous Spheres Using Exact Lorenz-Mie Theory	E-4
Radius Moments	E-5
RMS Radius	E-5
Geometric and Hydrodynamic Radius	E-6
Theory	E-8
Appendix F: QELS Theory	F-1
Cumulants	F-2
Theory	F-2
Application of the Method of Cumulants	F-4
Interpretation of Cumulant Results	F-5
Regularization	F-7
Theory	F-7
Implementation of Regularization in ASTRA	F-9
Interpreting Regularization Results	F-9
Appendix G: Viscosity Theory	G-1
Calculating Intrinsic Viscosity	G-2
Huggins	G-2
Kraemer	G-3
Solomon-Gatesman	G-3
Intrinsic Viscosity and Molecular Parameters	G-4
Flory-Fox Relation	G-5

1 About ASTRA 6

This chapter provides an overview of the ASTRA 6 software and this manual. It also tells you how to contact Wyatt Technology for support.

CONTENTS	PAGE
What is ASTRA?	1-2
Using This Manual	1-3
Glossary	1-6
Getting More Help	1-10
Where to Go from Here.....	1-10

What is ASTRA?

The ASTRA software collects and processes data from dilute macromolecular solutions. It uses this data to calculate the molar mass, radius moments, and other results. In addition, it controls the data acquisition performed by various instruments.

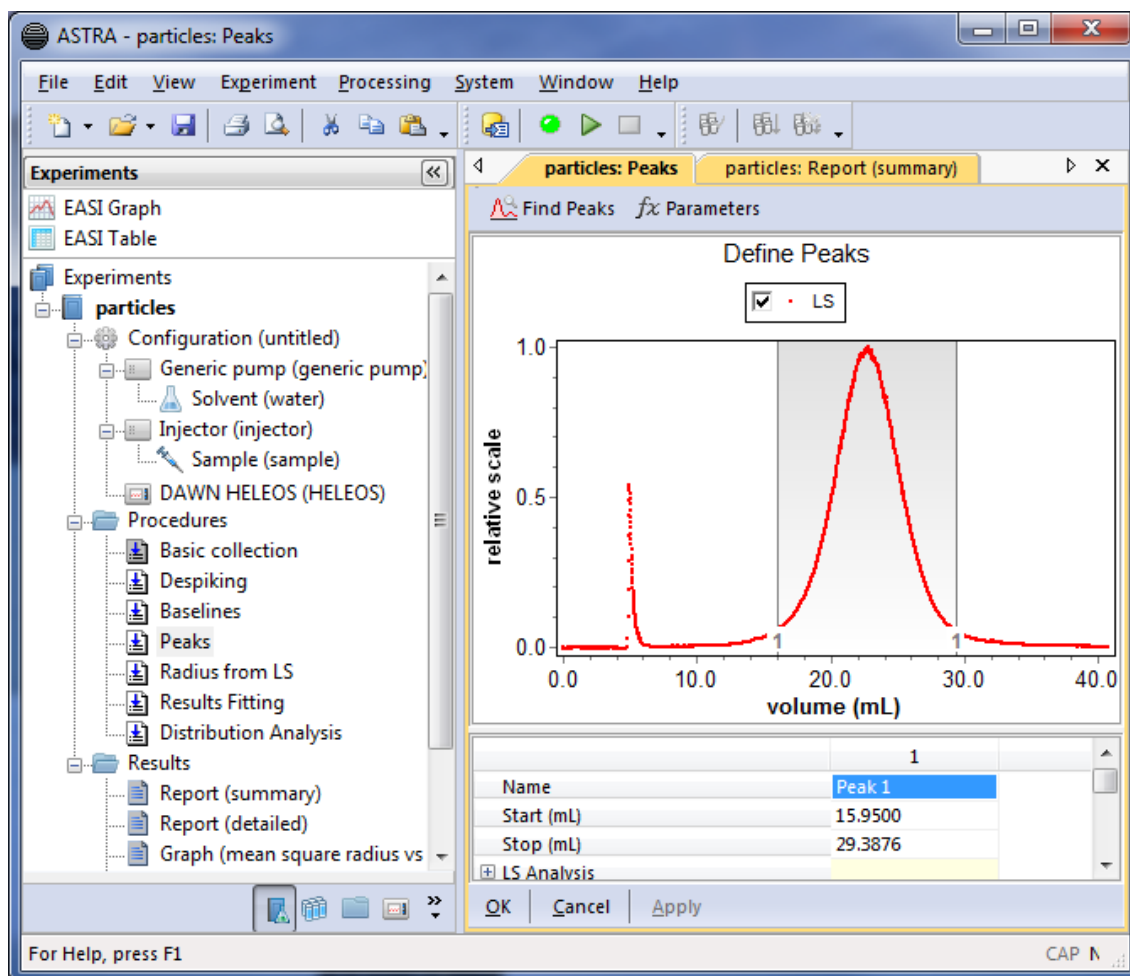


Figure 1-1: ASTRA Environment

Notice the icons at the bottom of the left pane. These provide access to the Experiments, Sequences, Profiles, and Instruments navigation panes.



Using This Manual

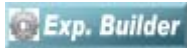
This manual describes how to install and configure the ASTRA software for collecting and processing data. It is meant to be used in conjunction with the hardware manual that came with your Wyatt instrument (for example, a DAWN or miniDAWN instrument).

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

User Modes

You can use ASTRA in “Run” mode or “Experiment Builder” mode.

Run mode makes it easier to learn to use ASTRA. It may be the mode you prefer even after you are an experienced user. In Run mode, you create experiments using the configuration and procedure methods provided with ASTRA. You can modify configuration and procedure properties, but cannot add or delete instruments or procedures.



Experiment Builder mode allows you to modify the configuration and procedures in a method. The icon to the left identifies portions of this manual that apply only if you turn on Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

In addition, Experiment Builder mode allows you to open multiple procedure windows at once. However, you should be careful with this feature, since changing and applying properties in one window does not generally result in changes to other open procedure windows. To see such changes reflected in other procedure windows, you should close and reopen them.

Operating Tiers

ASTRA 6 can be purchased with any of the following operating tiers:

- **ASTRA 6 Basic:** Saves experiments to files. In this manual, the “Basic” icon applies to this mode.
- **ASTRA 6 with Research Database:** Saves experiments to an experiment database. Does *not* provide 21 CFR Part 11 compliance. In this manual, the “Database” icon applies to this mode.
- **ASTRA 6 with Security Pack:** Provides 21 CFR Part 11 compliance. This includes saving experiments to an experiment database, user accounts with access levels, and sign off procedures. In this manual, the “Database” and “Security” icons both apply to this mode.

This manual identifies information that is specific to these operating tiers using the following icons:



- Identifies information that applies only to the **ASTRA 6 Basic** operating tier.



- Identifies information that applies to both of the following operating tiers: **ASTRA 6 with Research Database** and **ASTRA 6 with Security Pack**.



- Identifies information that applies only to the **ASTRA 6 with Security Pack** operating tier.

User Account Levels



As part of the 21 CFR Part 11 compliance of ASTRA 6 with Security Pack, all users must log in with a unique user id and password. The administrator sets up accounts with one of the following user account levels:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.



Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the “Security” icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA 6 with Security Pack. There are no access restrictions when ASTRA 6 is used in other operating tiers.

How This Manual is Organized

The first three chapters of this manual provide an overview of ASTRA, explain how to install ASTRA and prepare it for use, and how to get started using ASTRA.

The remaining chapters in this manual correspond to items in the ASTRA environment as shown in Figure 1-2.

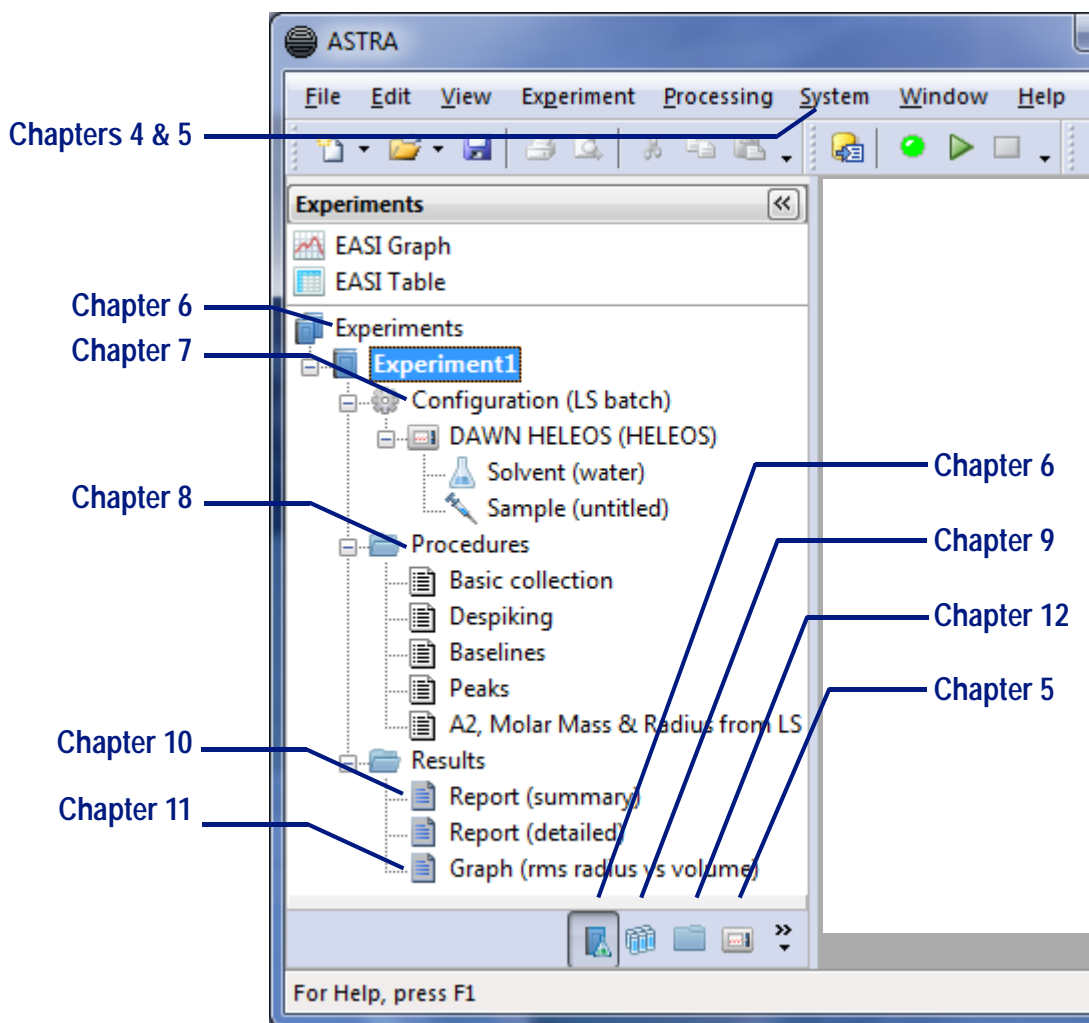


Figure 1-2: Workspace Items and the Chapters that Discuss Them

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.

- **Folder or Link Hierarchies.** This manual indicates a sequence of choices when navigating a tree, browsing for a folder, or following links in a web browser using a > sign. For example, an instruction to “Open the System > Solvents folder” indicates that you should go to the System folder and then open the Solvents folder.
- **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.
- **DAWN instrument.** Except where there are details for a particular instrument, when the name will be given, we will refer to the DAWN EOS and miniDAWN instruments simply as the *DAWN*.
- **Molar mass versus Molecular weight.** The IUPAC Definition Committee specifies the term *molar mass* for the sum of the atomic weights of all atoms in a mole of molecules. The term *molecular weight* has the same meaning. You will see *molar mass* used in this manual.
- **RMS Radius vs. Radius of Gyration.** The root mean square (RMS) radius is sometimes called the “radius of gyration” or R_g . The RMS radius is a measure of size weighted by the mass distribution about the center of mass. Radius of gyration is actually a misnomer, since it describes a kinematic measure of a molecule rotating about a particular axis in space. You will see *RMS radius* used in this manual.

Tip: See Appendix A, “Menu Quick Reference” for a complete list of keystroke alternatives to the mouse pointer for selecting menu options.

Glossary

The following terms are used in this manual:

- **A2, molar mass, and radius:** Results produced by a traditional Zimm plot analysis.
- **ASTRA:** The data collections, analysis, and lab control software for Wyatt Technology Corporation instruments.
- **batch mode:** Data collection performed in a stand-alone fashion—fluid injected into a cell, not being pumped through plumbing. For example, using the MicroCuvette with the DAWN. Batch measurements are typically on unfractionated samples. Batch mode is also called off-line.

- **configuration:** ASTRA's description of the physical apparatus used to collect data. It is an assembly of profile units describing sample, solvent, and instruments, as well as the connections (both fluid and signal) between them.
- **data set:** The grouping of data produced by a procedure. For example, a typical light scattering measurement might produce a set of data including molar mass and RMS radius.
- **DAWN:** Multi-angle light scattering detectors from Wyatt Technology Corporation. They are used to determine the molar mass, size, and second virial coefficient for macromolecules in solution. Versions include the DAWN HELEOS and miniDAWN.
- **DCOM (Distributed Component Object Model):** A Microsoft technology for communication between software (and instrument software) components across a network. Newer instrument connections use Microsoft's .NET Framework instead of DCOM.
- **dn/dc:** The change in a solution's refractive index with a change in the solute concentration. Measured in mL/g.
- **experiment database:** The database in which ASTRA 6 with Research Database and ASTRA 6 with Security Pack store experiment information and data.
- **fractionation:** The separation of a polydisperse solution of macromolecules by some physical property of the macromolecules. For example, size-exclusion chromatography (SEC) and field flow fractionation (FFF) separate macromolecules based upon size.
- **HPLC (High-Performance Liquid Chromatography):** A chromatography technique in which a pump, rather than gravity, provides pressure through a column.
- **intrinsic viscosity (IV):** A measure of the capability of a polymer in solution to enhance the viscosity of the solution. Derived using specific viscosity and concentration data.
- **ISI (Instrument Server Interface):** Connectivity software provided on newer Wyatt instruments or installable on computers connected to older Wyatt instruments.
- **light scattering:** A technique whereby the intensity of scattered light from a macromolecule in solution is measured at multiple angles to determine a molar mass, RMS radius, and second virial coefficient. Also called classical, Rayleigh, or static light scattering.
- **MALS (Multiangle Light Scattering):** A technique for finding the absolute molar mass and average size of particles in solution by measuring how they scatter light.
- **miniDAWN:** A DAWN instrument with three detectors. It is used primarily for characterizing small (less than 50 nm in RMS radius) macromolecules.

- **non-fractionated:** Samples that are typically polydisperse solutions containing a range of macromolecules with different weights, sizes, or conformations.
- **ODBC (Open Database Connectivity):** A software standard for database access.
- **online:** Data collection in which a fractionated solution flows through the instrument. For example, a pump pushes solvent through an injector, and the resulting solution is passed through a fractionation device and then characterized by instruments downstream. Online measurements are usually for fractionated samples, but can also be for non-fractionated samples. Note that a batch experiment can have a solution flowing through the instrument if the solution is not fractionated.
- **Optilab:** The Optilab instruments (T-rEX, rEX, DSP, and 903) are differential refractometers. These instruments can be used to determine the concentration of a macromolecule in solution, and to measure the dn/dc value necessary for determining molar mass in light scattering measurements.
- **physical units:** Units of measurement that have scientific meaning. For example, the DAWN instrument produces voltage signals that must be converted to the physical units of Rayleigh ratio before they can be analyzed to determine mass and radius.
- **procedure:** ASTRA's representation of a process in the collection and analysis of the data. A procedure can be either for collection, data transformation, data analysis, display, instrument configuration, or administrative purposes.
- **profile:** A reusable description of a physical entity in an ASTRA experiment. For example, an instrument, solvent, or sample. These are saved in the system database and can be imported into experiments or sequences. For example, common instrument configurations might be saved as profiles so they can later be easily used in different experiments.
- **QELS (Quasi-Elastic Light Scattering):** This technique is also known as dynamic light scattering or photon correlation spectroscopy. The WyattQELS instrument measures rapid fluctuations in scattered light intensity to determine the translational diffusion coefficient and hydrodynamic radius for macromolecules in solution.
- **Rh or R_h (hydrodynamic radius):** The radius of a hard sphere that diffuses at the same rate as the molecule. This is also sometimes called the Stokes radius or the spherical equivalent radius. The hydrodynamic radius is generally different from the RMS radius, depending on the shape of the molecule. The ratio of R_g to R_h increases as the object becomes less compact. A solid sphere has an R_g/R_h ratio of 0.77, while a linear coil polymer has an R_g/R_h ratio of about 1.5. A hollow sphere has a ratio of 1.0.

- **RI:** Refractive index. Used to describe differential refractometer instruments or data from the Optilab rEX, DSP, or 903.
- **SEC (Size-Exclusion Chromatography):** A chromatography technique in which molecules in solution are separated by size using a column. When organic solvents are used, SEC is also called Gel Permeation Chromatography (GPC).
- **system database:** The database in which ASTRA stores methods and profiles. This is separate from the experiment database.
- **Title 21 CFR Part 11:** United States Food and Drug Administration (FDA) guidelines on electronic records and electronic signatures. Part 11, as it is commonly called, is supported by ASTRA 6 with Security Pack.
- **ViscoStar:** On-line differential viscometer that measures the intrinsic viscosity and Mark Houwink-Sakurada (MHS) parameters of polymers.
- **user mode:** You can choose to use ASTRA in “Run” mode or “Experiment Builder” mode. Run mode is easier to use. Certain options are not available in Run mode.
- **WCS (Wyatt Communication Server):** Server software run by the Wyatt Instrument Server Interface (ISI).
- **workspace:** The portion of the ASTRA interface that shows the Experiments, Sequences, Profiles, and Instruments navigation panes and their contents.

Batch Mode vs. Online Mode

The distinction between batch mode and online mode is an important one for all types of instruments.

- **Batch Mode:** In a batch mode experiment, the measurement instrument stands alone, and is not hooked up to a pump. Samples are introduced into the instrument via vials or by injecting slugs of sample that completely fill the sample cell. The concentration of the sample is known, since the researcher has prepared it. Also, the solvent for the sample now needs to be associated with the instrument, since it no longer comes from the pump. Batch mode is also called “non-fractionated”.
- **Online Mode:** In an online mode experiment, a measuring instrument such as the Optilab rEX or DAWN HELEOS is connected to a pump, injector, and fractionation module, such as a column or a field-flow fractionator (FFF). The solvent flow is controlled by the pump, and the sample is added by the injector. The solution continually flows through the system. The concentration needs to be measured via an RI or UV instrument. Flow mode is also called “flow” or “fractionated”.

Light scattering, ultra-violet, RI, and viscometry instruments can all be run in either batch or flow mode.

Getting More Help

If you have a question about ASTRA, first look in this manual or consult the online help. You can also find late-breaking updates and technical information about your version of ASTRA in the readme file.

Also, be sure to register for and use your Wyatt Technology Support Center account. Go to www.wyatt.com to log in. You'll find FAQs, tutorials, software downloads, newsletters, and ways to order supplies.

If you still cannot find an answer, please contact Wyatt Technology Technical Support.

Contacting Technical Support

Please be prepared to provide the following information when you contact technical support. If you e-mail or fax your question in to us, include *all* of the following information.

- Wyatt Technology instrument serial numbers (located on the “System Tab” on the instrument’s front panel or the label on the back panel).
- ASTRA software version number. You can view the software version number by selecting **About** from the **Help** menu. The version of ASTRA used to collect the data is included in all reports.
- The type of computer hardware you are using.
- Microsoft Windows version number.
- Exact wording of any messages that appear on your computer screen.
- What you were doing when the problem occurred.
- How you tried to solve the problem.

Website	http://www.wyatt.com
E-Mail Support	astra.support@wyatt.com
FAX Support	(805) 681-0123
Telephone Support	(805) 681-9009
Corporate Headquarters	Wyatt Technology Corporation 6300 Hollister Ave. Santa Barbara, CA 93117 USA

International Support: Outside the USA, you may use one of the contact methods listed here, or you may contact the Wyatt Technology Distributor in the country where you bought your product.

Where to Go from Here

Continue to Chapter 2, “Installing and Setting Up ASTRA”.

Read your hardware manual(s) before attempting to collect data using the software. They contain important safety and operational information.

2

Installing and Setting Up ASTRA

This chapter provides instructions for installing ASTRA on your computer and instructions for preparing it for use.

The ASTRA administrator in your organization should follow the steps in all sections of this chapter to make ASTRA ready for use as described in the remaining chapters.

CONTENTS	PAGE
System Requirements.....	2-2
Installing the ASTRA Software.....	2-2
Activating Optional ASTRA Features.....	2-6
Setting Up User Accounts.....	2-7
Running ASTRA.....	2-11
Accessing and Viewing Hardware.....	2-12

System Requirements

In order to use the ASTRA software, you must have the following:

- Microsoft Windows® XP Professional, Microsoft Windows Vista, or Microsoft Windows 7
- An Intel Core 2 Duo processor or equivalent (or better)
- A graphics card compatible with Direct X 9/Open GL 1.5 (or better)
- At least 150 MB free hard disk space
- At least 512 MB of RAM (1 GB recommended)
- Microsoft Access or Microsoft SQL Server 2005 or higher (for ASTRA 6 with Research Database or ASTRA 6 with Security Pack only)
- A CD-ROM drive
- Available communication ports as required for data collection. (See the manuals for your instruments for details.)
- A Windows-supported printer and/or plotter
- A Windows-supported mouse

Additionally, adequate experiment storage space is required. Each experiment run with ASTRA will use on average of about 0.5 MB of storage.

Installing the ASTRA Software

This section provides basic instructions for installing ASTRA. You must use the ASTRA installation program to install ASTRA, rather than simply copying the files to your hard disk.

To install ASTRA, do the following:

1. Log in to Windows using an account with Administrator or Power User privileges.
2. Place the ASTRA disk in your CD drive. On most systems, the ASTRA setup procedure will start automatically. (If you downloaded an update, double-click on the downloaded ASTRA_6xxx_Setup.exe file.)

If the setup procedure does not start automatically, use Windows Explorer or the Run dialog to run setup.exe in the CD's ASTRA folder.

3. Answer the prompts in the setup procedure.

Choose to perform a full installation so that all the components of ASTRA will be installed. (See "Installing the ISI on Other Computers" on page 5-4 for other types of installations.)

After you install ASTRA, the Windows **Start** menu contains a folder called Wyatt Technology.

Upgrading to a New Version of ASTRA

You can check for newer versions of ASTRA by choosing **Help→Check for Updates** from the ASTRA menus. (You can also use this command to control how often ASTRA checks for updates automatically.)

You can use installations of both ASTRA 6 and ASTRA V on the same computer. Experiment templates/methods, logging, and data acquisition are not shared between the two revisions, and will therefore not affect one another.

Installing a new version *does not* update the system database, which is where experiment methods and profiles are stored. This is because you likely want to keep custom experiment methods and profiles. See “Migrating the System Database” on page 2-4 to update your system database so you have all the latest experiment methods and system profiles provided with ASTRA.

It is possible to have both ASTRA V and ASTRA 6 installed on the same computer and to use both simultaneously. The system and experiment databases are not shared between the two versions.

Note:	If you have an older version of ASTRA 6, you may be prompted to uninstall the old version of ASTRA before installing the new version. When you uninstall, any files you have created or modified (such as experiment files, your experiment database, and the system database) are not deleted.
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To uninstall an old version of ASTRA 6, follow these steps:

1. Choose **Start→Control Panel** from the Windows Start menu.
2. Double-click the **Add or Remove Programs** icon.
3. Scroll down in the Add or Remove Programs list to Wyatt Technology ASTRA.
4. Click the **Remove** button.
5. Install the new version of ASTRA as described in the previous section.

You can choose to install the new version of ASTRA in the same location as the previous version. This allows you to easily continue using the same experiment database and other files.

Migrating the System Database

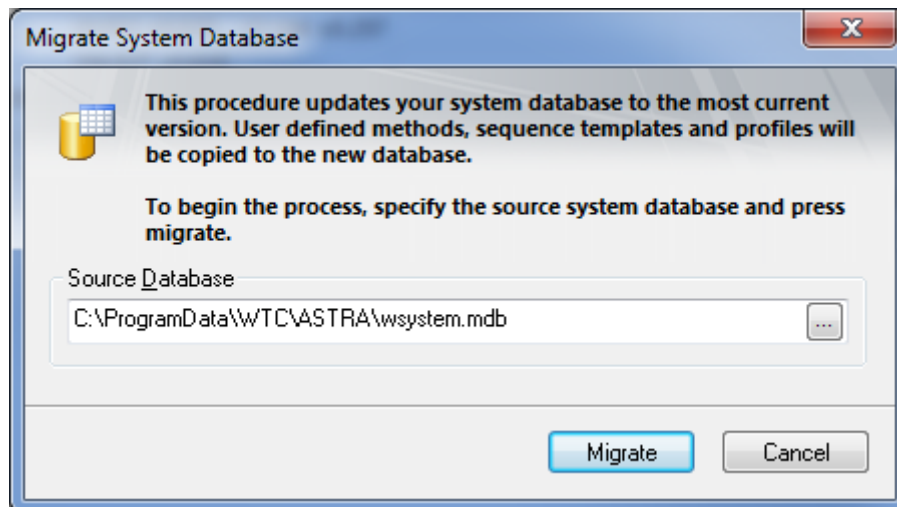


You must use an account with ASTRA Administrator access to follow the steps in this section.

The ASTRA system database stores experiment methods, sequence templates, profiles, solvents, and molecular standards. It includes both items provided with ASTRA and items you customize and save. All versions of ASTRA use a system database.

The installation does not overwrite an existing system database. To update your system database so you have all the latest experiment methods and system profiles, follow these steps:

1. Choose **System→Database Administration→Import System Database**.
2. You see a message that says the procedure will update to the most recent version of the system database and copy any methods, sequence templates, and profiles you have saved in your old system database to the new system database.
3. Click the “...” button and browse to the location of your old system database. For example, if you are upgrading from ASTRA V to ASTRA 6, your old system database is likely to be in a directory similar to C:\Program Files\WTC\ASTRA 5.3\Database and is likely to be named ASTRA_System2.mdb.



ASTRA 6 stores its system database in the Windows ProgramData directory, which is likely to be C:\ProgramData\WTC\ASTRA. The ASTRA 6 system database filename is wsystem.mdb.

4. Click **Migrate** to continue. Any custom experiment methods, sequences, and profiles in your old system database are copied into the new version of the system database.

5. You may see a warning that system log entries (such as login attempts and database connections) are not copied to the new database. To perform the migration, click **Yes**.

Hint:

You can use database migration to purge system databases that contain a large number of system log records. Note that the migration *does* copy 21 CFR log information related to methods and profiles it migrates.

6. As your database is migrated, you see progress information. Messages identify any methods or profiles that are not updated because you have customized them.

You can migrate an existing system database created with ASTRA v5.3 or higher.

Whenever you migrate the system database, a backup is created in a “Backup” subfolder with a filename of wsystem.bak (or wsystem_#.bak, where # is a sequence number, if you do multiple migrations). You can click **Show backup** to open a Windows Explorer view of the Database folder of the ASTRA installation, which contains the system database and backup files.

You can rollback a migration by deleting the new wsystem.mdb file and renaming the backup file to wsystem.mdb.

Activating Optional ASTRA Features

To activate optional ASTRA features—such as QELS—you use the Feature Activation dialog. In this dialog, you enter a license key provided to you by Wyatt Technology Corporation based on your licensing agreement.

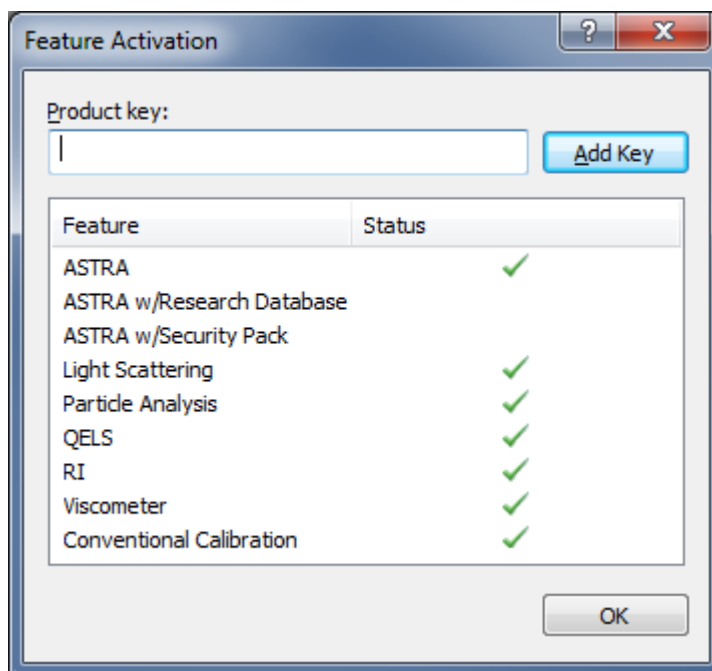


Figure 2-1: Feature Activation Dialog

To activate a feature, follow these steps:

1. Double-click the **ASTRA 6** icon on your desktop.
2. Choose **System→Feature Activation** to open the dialog above.
3. Type or paste your activation key into the **Product key** field.
4. Click **Add Key**. You will see checkmarks next to the features that are enabled.
5. Click **OK** when you are finished.
6. If your key enables **ASTRA w/Security Pack**, go to “Setting Up User Accounts” on page 2-7 for instructions on creating user accounts.

You can open and process experiments that use features for which you do not have a key, however, any procedures and results that are not licensed cannot be viewed.

When ASTRA is installed, the entire program and all modules are placed on the computer. The license key you receive when you purchase ASTRA unlocks a set of modules. If you want to unlock another module, contact Wyatt Technology Corporation.

Setting Up User Accounts



As part of the 21 CFR Part 11 compliance of this software, ASTRA 6 with Security Pack requires that all users log in with a unique user id and password.

User accounts in ASTRA 6 with Security Pack are managed as Microsoft Windows user accounts. You create the following four Windows groups, and then assign each user that should be able to access ASTRA to one of the following groups:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.

In operating tiers other than ASTRA 6 with Security Pack, users are not prompted to log in with a user name and password.



Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the “Security” icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA 6 with Security Pack. There are no access restrictions in other operating tiers.

Setting Up Groups



To create the groups you will use with ASTRA, follow these steps:

1. Log in using a Windows account that has administrator privileges.
2. Right-click on **My Computer** in the Windows Start menu, and select **Manage**. This opens the Computer Management window.
3. In the tree on the left, expand the Local Users and Groups item (which is within the System Tools list).
4. Right-click **Groups** under Local Users and Groups. Select **New Group**.

5. Create the following groups in the New Group dialog, and click **Create** after each one. Use the exact capitalization and spacing shown here.

Group name	Description
ASTRA Administrator	Administers ASTRA accounts and database
ASTRA Researcher	Creates and modifies experiments and profiles.
ASTRA Technician	Runs experiments and saves data.
ASTRA Guest	Read-only access to experiments and results.

6. Click **Close** after you have created all four groups.

If you want to use different group names than the ones in the previous table, choose **System**→**Security** and more to the **Groups** tab. See “Setting a Validation Domain for User Accounts and Groups” on page 2-9 for details.

Creating Users



Security

You can use existing Windows user accounts or create special accounts for ASTRA access. To create a new user account, follow these steps:

1. In the Computer Management window, right-click on **Users** under Local Users and Groups, and select **New User**.
2. In the New User dialog, type a User name, Full name, Description, and Password as desired.
3. Click **Create**.

Assigning Users to Groups



Security

To assign user accounts to an ASTRA group, follow these steps:

1. In the Computer Management window, right-click on one of the ASTRA groups you added and select **Properties**.
2. Click **Add**.
3. In the “Enter the object names to select” field, type a user name you want to add to this group.
4. Click **OK** in the Select Users dialog.
5. Click **Add** again if you want to add other users to this group.
6. Click **OK** in the Properties dialog when you have finished adding users to a group.

Note for Networked Accounts



Security

You can use a similar procedure to set ASTRA privileges for networked accounts. Log in to the server that you will use for account validation. Perform steps similar to those described in the previous sections on that server.

Setting up accounts locally or using networked accounts determines which domain name users need to type when logging in to ASTRA.

Add groups to either the corporate domain or the local machine, depending on where the user logs in. By default, ASTRA expects to find the Group and User information in the same place. So, for example, you cannot create a local group and add a domain user to this group.

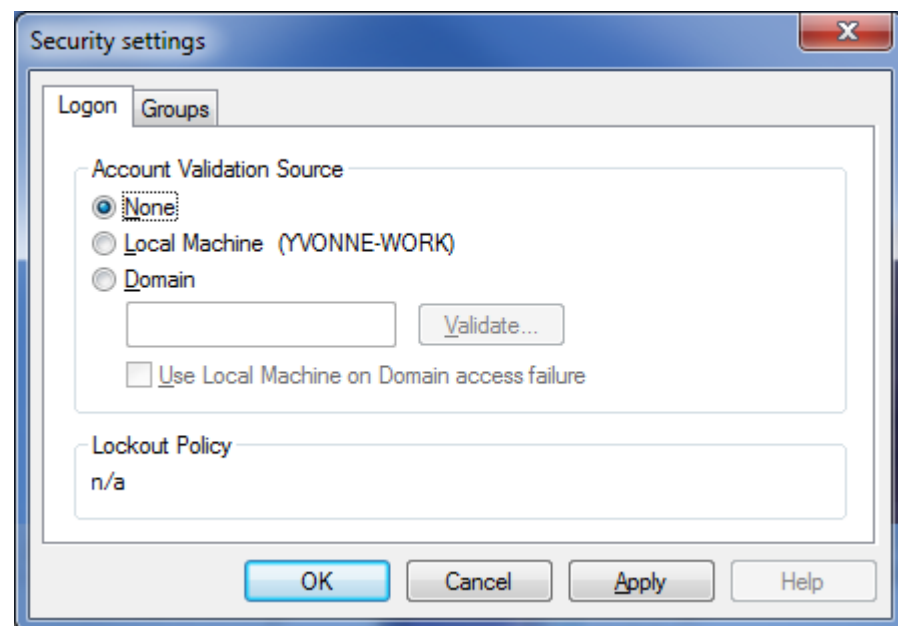
Setting a Validation Domain for User Accounts and Groups



You can specify the PC or domain to use for user and group authentication. This allows you to prevent security problems where a user with a lower security group on the corporate domain could create a local group called “ASTRA Administrator” and use that local group to log into ASTRA.

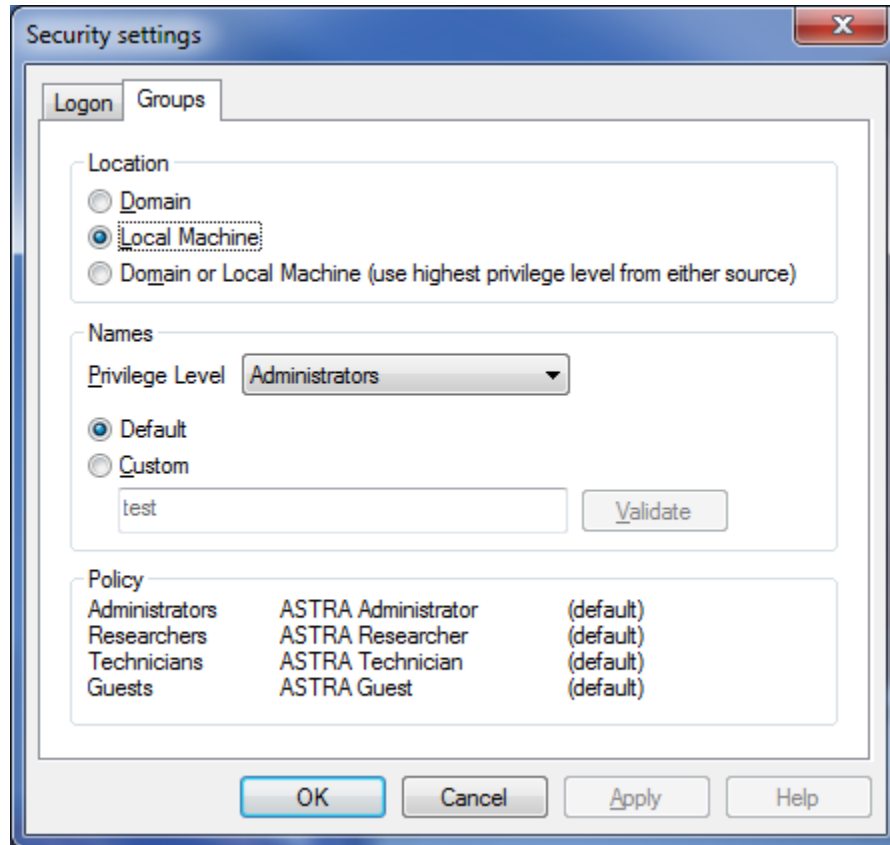
Follow these steps:

1. Choose **System**→**Security** to open the following dialog.



2. If the **Account Validation Source** is set to “None” (the default), accounts are validated from any source that has valid ASTRA groups. If you both the local machine and the enterprise domain have ASTRA groups, users get the highest privilege level set for them on either machine.
3. Alternately, you can choose to validate user accounts against those set up on the local machine or on a specified domain. If you choose “Domain”, type the name of the domain you want to use to authenticate users. Then, click **Validate** to confirm that the domain name you typed is accessible on the network.
4. If you want users to be able to use ASTRA if the domain is not available, check the **Use Local Machine on Domain access failure** box.
5. Click **Apply**.

6. Move to the **Groups** tab.



7. Select the location you want to use to check which groups the user belongs to. This can be the domain specified in the **Logon** tab, the user's local machine, or both. If you choose both locations, the user gets the highest privilege level on either machine.
8. If you want to modify the group names that ASTRA looks for, select a **Privilege Level** and the **Custom** option. Type the group name you want to use for that level. Then click **Apply**. This is useful if you already have users assigned to groups that correspond to the ASTRA groups. The list in the Policy section shows the currently specified group names for each privilege level.

Running ASTRA

To run ASTRA, do one of the following:

- Double-click the **ASTRA 6** icon on your desktop.
- Choose **Programs→Wyatt Technology→ASTRA 6** from the Windows Start menu.

It may take a minute or so for ASTRA to open. Avoid closing the initial startup window while waiting.



If you are using ASTRA 6 with Security Pack, you will be prompted to log in. Use a User Name / Password combination set up as described in “Setting Up User Accounts” on page 2-7.

If ASTRA privilege groups were set up on your local computer, type the name of your local computer for the domain. Otherwise, if the ASTRA privilege groups were added for your networked account, type the domain of your networked account.



By default, ASTRA 6 with Security Pack and ASTRA 6 with Research Database store experiments in a Microsoft Access database called ASTRA Experiment. A user with ASTRA Administrator privileges can change to another database by following the steps in “Connecting to a Database” on page 4-3. If you plan to change the database, it is best to do so before you start using ASTRA for experiments.

Accessing and Viewing Hardware

ASTRA's instrument list provides the following capabilities:

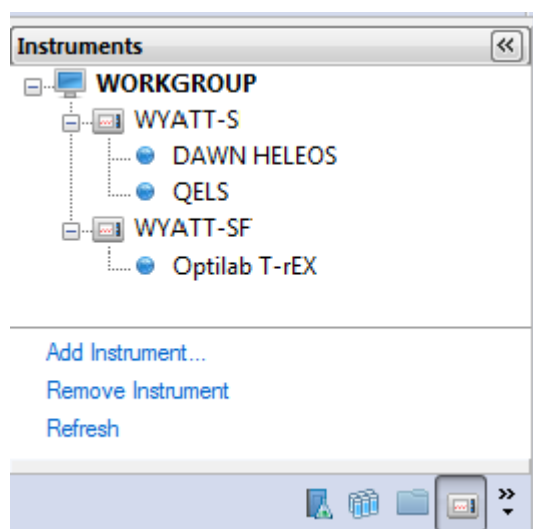
- Allows you to add and delete computers or instruments that run an Instrument Server Interface (ISI) to the set available to ASTRA on your computer.
- Shows instruments connected to your network and allows you to launch the Diagnostic Manager for each instrument.

This section gives step-by-step instructions for actions you need to perform to make instruments visible to ASTRA. For more details about the ISI and the Diagnostic Manager, see Chapter 5, "Interfaces to Instruments".

When you create an experiment from a method, ASTRA uses your instrument list to match up your physical instruments with the instruments in the method's configuration.

Viewing the Instruments Pane

To see a list of the instruments currently available to you, move to the Instruments navigation pane.



If an instrument becomes available or goes off-line, the Instruments navigation pane is automatically updated to show the current state of the instruments. ASTRA 6 checks the instrument status in the background, so keeping this list updated does not affect the performance of other things you are doing. The experiment log for an experiment also shows if instruments went offline during data collection.

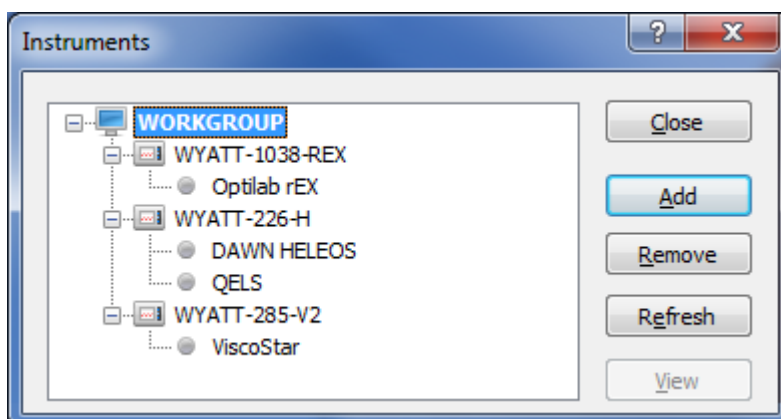
If you point to an instrument with your mouse, any active alarm conditions for that instrument are shown in the hover text.

You can allow ASTRA to send alarm messages from Wyatt instruments (with Ethernet connections) to your Windows desktop by toggling on ASTRA's **System→Preferences→Show Desktop Alerts** menu option. Messages will be shown for a few second near the Windows taskbar. The messages fade automatically if you do not click on them.

You can double-click an instrument in the Instruments navigation pane to open the Diagnostic Manager described in “Using the Diagnostic Manager” on page 5-5.

The list shows the instruments and computers with an ISI installed that your copy of ASTRA knows about. You see instruments that support a direct data connection to ASTRA or instruments that are connected (via USB) to computers you have added to the list.

Another way to view the list of instruments available to your copy of ASTRA is to choose **System→Instruments** from the ASTRA menus. You will see the Instruments dialog.



In the Instruments dialog, you need to click **Refresh** if you want to update the list. The **View** button in the Instruments dialog opens the Diagnostic Manager described in “Using the Diagnostic Manager” on page 5-5.

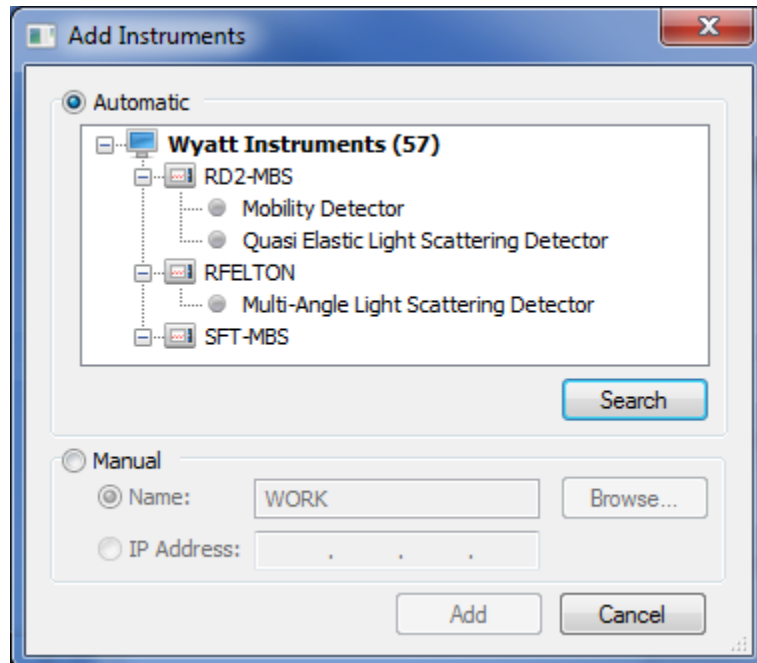
Adding an Instrument or Computer to the Instrument List

If you are collecting from a DAWN or WyattQELS instrument connected to your local computer, the first computer you should add to the instrument list is your own local computer. You should also add other computers that have or will have instruments connected to them.

To add an instrument to the instrument list, follow these steps:

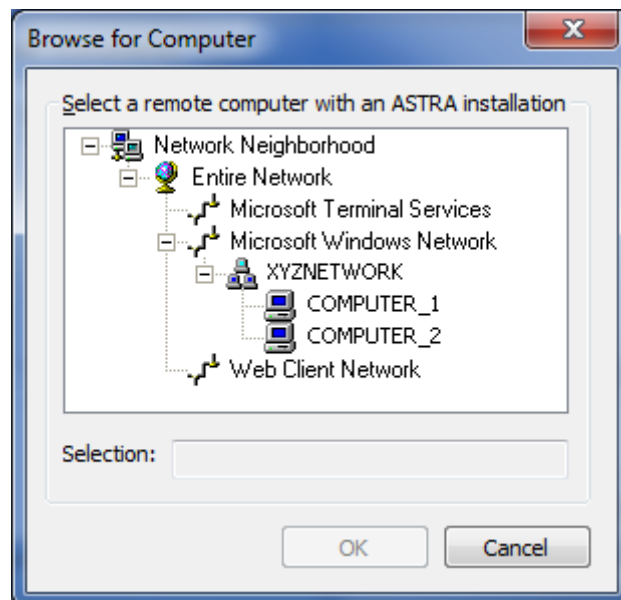
1. In the Instruments navigation pane, click **Add Instrument**. Or, in the Instruments dialog, click **Add** to open the Add Instruments dialog.

2. Click **Search** to search for available instruments.



3. If the instrument you want to add was found, select that instrument and click **Add**.
4. If your instrument was not found, you can add a computer or instrument by selecting the **Manual** option. Then either type the network name of the computer or instrument, or click the **Browse** button, or select the **IP Address** option and type the numeric IP address. Then click **Add**.

If you click the **Browse** button, expand the network listing so that you can see the instruments and computers on your network.



Instruments and computers that you add to the Instrument dialog are still connected to ASTRA in subsequent ASTRA sessions unless you delete them. Any supported instruments connected to those computers will be available within ASTRA.

See Chapter 5, “Interfaces to Instruments” for more about using the ISI, Instruments, and Diagnostic Manager dialogs.

Once instruments are visible in the Instruments dialog, ASTRA is ready to use for collecting data. Please note that ASTRA can still be used for the analysis of already collected data files without any connection to an instrument.

Removing an Instrument or Computer from the Instrument List

To remove an instrument or computer from the Instrument list (shown in the Instruments pane or opened with **System→Instruments**), select the name of that resource in the instrument list and click **Remove**.

3

Getting Started

This chapter shows you how to create and run a simple experiment. It assumes that ASTRA has been set up as described in Chapter 2, “Installing and Setting Up ASTRA”.

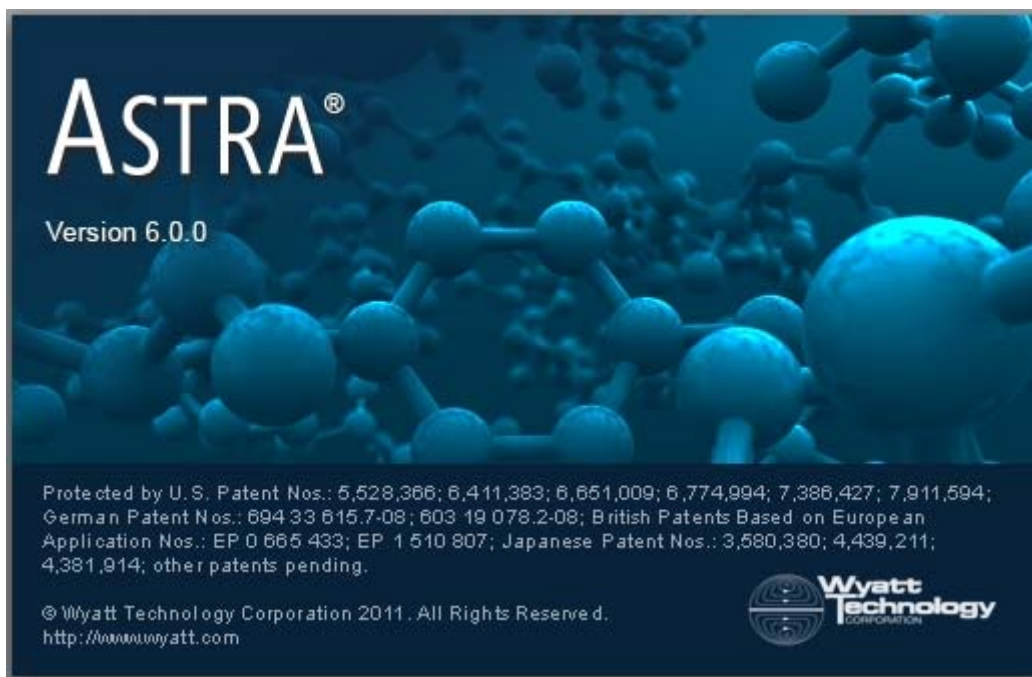
CONTENTS	PAGE
Starting ASTRA	3-2
ASTRA Tutorials on the Support Center	3-4
Performing a Simple Light Scattering Experiment	3-5
More About the ASTRA Environment	3-13

Starting ASTRA

To run ASTRA, do one of the following:

- Double-click the **ASTRA 6** icon on your desktop.
- Choose **Programs→Wyatt Technology→ASTRA 6** from the Windows Start menu.

It may take a minute or so for ASTRA to open.



If you are using ASTRA 6 with Security Pack, you will be prompted to log in. Use a User Name / Password combination given to you by the ASTRA administrator. This may be the same as your Windows user name and password.

If ASTRA privilege groups were set up on your local computer, type the name of your local computer for the domain. Otherwise, if the ASTRA privilege groups were added for your networked account, type the domain of your networked account.



The account you use determines the types of actions you can perform within ASTRA. The user levels are as follows:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.



Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the “Security” icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA 6 with Security Pack. There are no access restrictions in other operating tiers.

ASTRA Tutorials on the Support Center

Several tutorials are provided for ASTRA on the Wyatt Technology Support Center. Log in and click the “Tutorials” item and then the “ASTRA Tutorials” item.

If you don’t already have a support center account, please sign up for one.

Welcome to the Wyatt Technology Support Center

Be sure to let us know when you publish light scattering results from your Wyatt hardware! Email us for a FREE t-shirt, mug, or laser pointer!



Currently, the support center provides the following tutorials. We strongly encourage you to use these tutorials to learn how to use ASTRA.

- **ASTRA 6 Presentation:** This PowerPoint presentation provides an introduction to ASTRA and the terminology it uses.
- **ASTRA 6 Templates Exercise:** This tutorial shows you how to connect to instruments, create experiments from a saved method, adjust experiment configurations, save experiment methods and profiles, and run experiments.
- **ASTRA 6 SEC-LS Characterization:** This tutorial uses sample experiments that have already been run to show how to use data analysis procedures. You’ll need to download and unzip the data files provided. The steps of the tutorial show how to set baselines, normalize the detectors, set peaks, set delay volumes (alignment), set band broadening parameters, assessing whether the flow cell was clean, and viewing molar mass results.
- **ASTRA 6 Skill Building Exercise:** This tutorial contains a number of brief exercises that ask you to answer questions about the results of various experiments that have already been run. It also contains questions that help you learn to assess the quality of data and troubleshoot detector problems using data. It uses the same experiment and data files as the previous tutorial.

Click the “ASTRA’s Newest Features” link in the left column for links to videos about recently-added ASTRA features.

Additional information about various ASTRA features is provided at <http://www.wyatt.com/solutions/software/ASTRA.cfm>.

Performing a Simple Light Scattering Experiment

In this section, you will use ASTRA to perform a simple batch light scattering experiment, such as calibrating your light scattering instrument or measuring a Zimm plot. For this experiment, you need to have one of the following instruments connected to either your local computer or one that can be accessed over the network:

- DAWN HELEOS
- DAWN EOS
- miniDAWN
- miniDAWN TREOS

For this introductory experiment, it is best to set the instrument up for a simple batch collection. You do not need to connect pumps or other instruments at this time. If you have already set up a flow experiment, you may choose the appropriate “online” method for your setup.

Note: If you don’t have a DAWN or miniDAWN light-scattering instrument, you can still follow the steps in this example by selecting an experiment method appropriate to your instrument and setting properties that correspond to the ones described here.



You must use an account with ASTRA Researcher or ASTRA Administrator access to follow the steps in this section.

Checking the Instrument Connection

Your ASTRA administrator has probably already set up connections to computers with instruments you will access with ASTRA. You can confirm this in the Instruments navigation pane (choose **View→Instruments**). This pane lists instruments connected to computers that can currently be accessed by ASTRA.

If the instrument you want to use for this experiment is not listed, follow the steps in “Accessing and Viewing Hardware” on page 2-12.

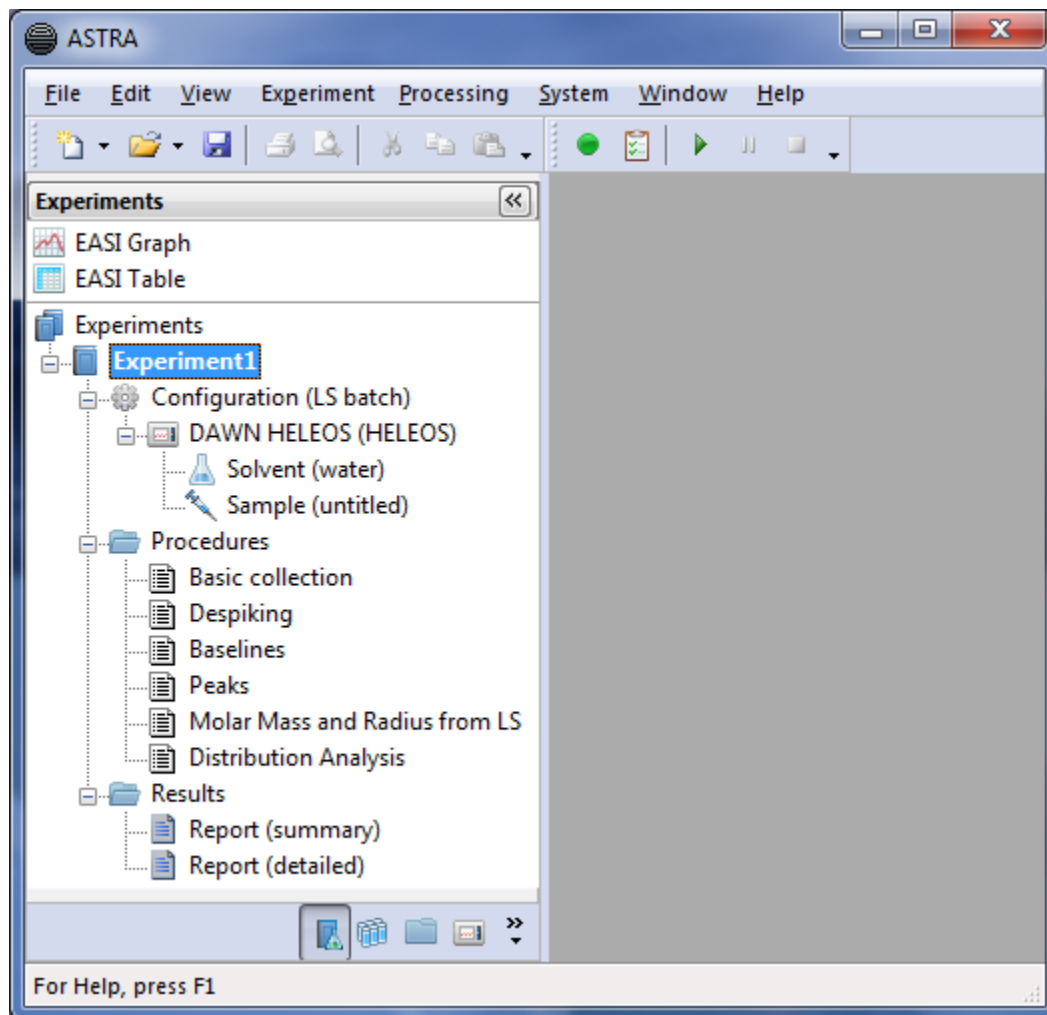
Creating an Experiment

ASTRA provides a large number of experiment methods. Most users will be able to find a method that defines a configuration identical or close to their own experimental setup.

To create an experiment, follow these steps:

1. Choose **File→New→Experiment From Method**. (Ctrl+Alt+T)
Appendix B, “System Methods” lists methods provided with ASTRA.
2. In the “New from Existing” dialog, open the “System” folder, then the “Methods” folder.
3. Open the “Light Scattering” folder.
4. Select a “batch” method and click **Create**.

A new experiment called Experiment1 is created based on the method you selected. The Experiments navigation pane in ASTRA shows the parts of the experiment.



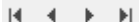
You can expand or collapse nodes in an experiment as desired by clicking on them. Each experiment contains the following categories of items:

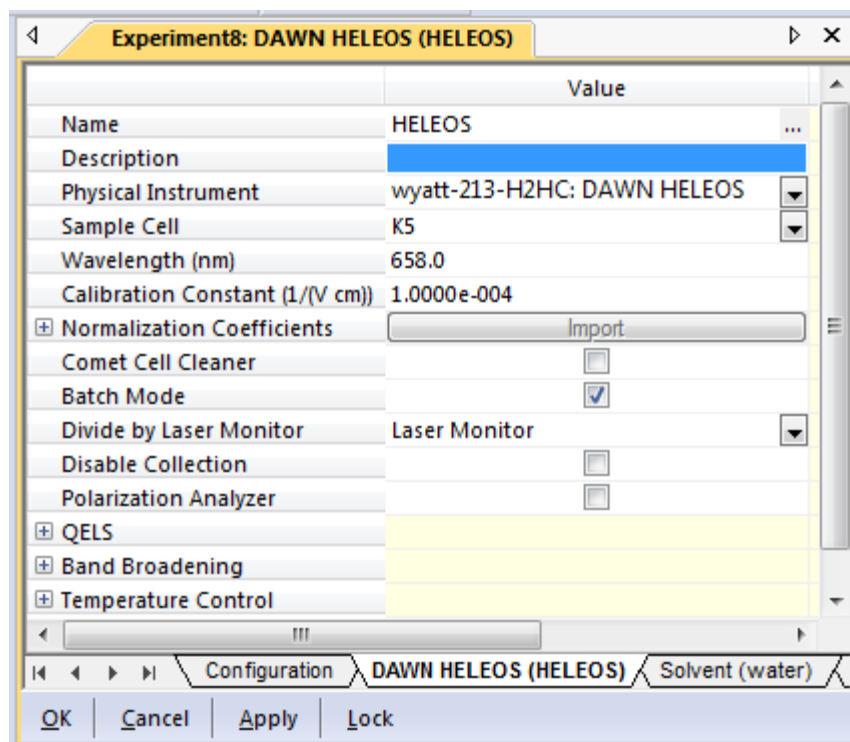
- **Configuration:** Hardware devices and connections used in the experiment. For online (fractionated) experiments, this may include a pump, injector, solvent, sample, DAWN, Optilab, and data connections. For details on all types of items that may be configured and their properties, see Chapter 7, “Configuring Experiments”.
- **Procedures:** Actions to be performed in order when the experiment is run. There are configuration, collection, transformation, analysis, and administrative procedures. For details on all types of procedures, see Chapter 8, “Editing Procedures”.
- **Results:** Reports and graphs to be produced after the experiment has been run. For details, see Chapter 10, “Working With Reports”.

Modifying the Configuration

The experiment method sets most of the properties to values you are likely to use. There are just a few properties you typically need to set.

For the example LS Batch experiment, follow these steps:

1. Choose **Experiment→Configuration→Edit**. This opens the properties page for the configuration. (You can also open this page by double-clicking on any part of the Configuration tree in the Experiments navigation pane.)
2. Notice that this page has a tab along the bottom for each item in the configuration.
3. Near the bottom of the page, select the tab for the DAWN or miniDAWN instrument. You can use the  arrows to the left of the tabs to scroll to the right to find a tab that you can't see.



	Value
Name	HELEOS
Description	
Physical Instrument	wyatt-213-H2HC: DAWN HELEOS
Sample Cell	K5
Wavelength (nm)	658.0
Calibration Constant (1/(V cm))	1.0000e-004
Normalization Coefficients	Import
Comet Cell Cleaner	<input type="checkbox"/>
Batch Mode	<input checked="" type="checkbox"/>
Divide by Laser Monitor	Laser Monitor
Disable Collection	<input type="checkbox"/>
Polarization Analyzer	<input type="checkbox"/>
QELS	
Band Broadening	
Temperature Control	

Configuration | **DAWN HELEOS (HELEOS)** | Solvent (water)

OK | Cancel | Apply | Lock

4. In the Physical Instrument row, make sure the correct instrument is selected. (When you create an experiment from a method, ASTRA automatically sets the Physical Instrument value to match instruments of the appropriate type in your instrument list.)

5. Select the **Solvent** tab from the tabs at the bottom of the page.

	Value
Name	water
Description	System solvent
Refractive Index (@ 658.0 nm)	1.3309
Viscosity (cP @ 25.0 °C)	0.8945
Rayleigh Ratio (@ 658.0 nm)	7.6603e-007
Refractive Index Model	Polynomial
Rayleigh Ratio Model	Corrected lambda^4
Viscosity Model	Linear
Thermal Expansion Model	Polynomial

Configuration | DAWN HELEOS (HELEOS) | **Solvent (water)** | Sample (untitled)

6. In the Name row, click the “...” button on the far right.
7. In the Copy from Existing dialog, open the System > Solvents folder and select the solvent you are using. For example, you may be using toluene. Then click **Copy**. The properties for the solvent you select automatically replace those of the default solvent.
8. Click **Apply** at the bottom of the page. (**Apply** saves changes without closing the page; **OK** saves changes and closes the page.)
9. Select the **Sample** tab from the tabs at the bottom of the page.

	Value
Name	untitled
Description	
dn/dc (mL/g)	0.000
A2 (mol mL/g ²)	0.0000e+000
UV Extinction Coefficient (L/(g cm))	0.0000
Concentration (mg/mL)	0.0000e+000
Mark-Houwink-Sakurada K (mL/g)	0.0000e+000
Mark-Houwink-Sakurada a	0.0000e+000

Configuration | DAWN HELEOS (HELEOS) | Solvent (toluene) | **Sample (untitled)**

10. Type a name for your sample and specify the dn/dc and concentration values. (For batch experiments with multiple peaks, you can override these settings in the “Define Peaks” procedure after collecting data.)
11. Click **OK** at the bottom of the properties page.

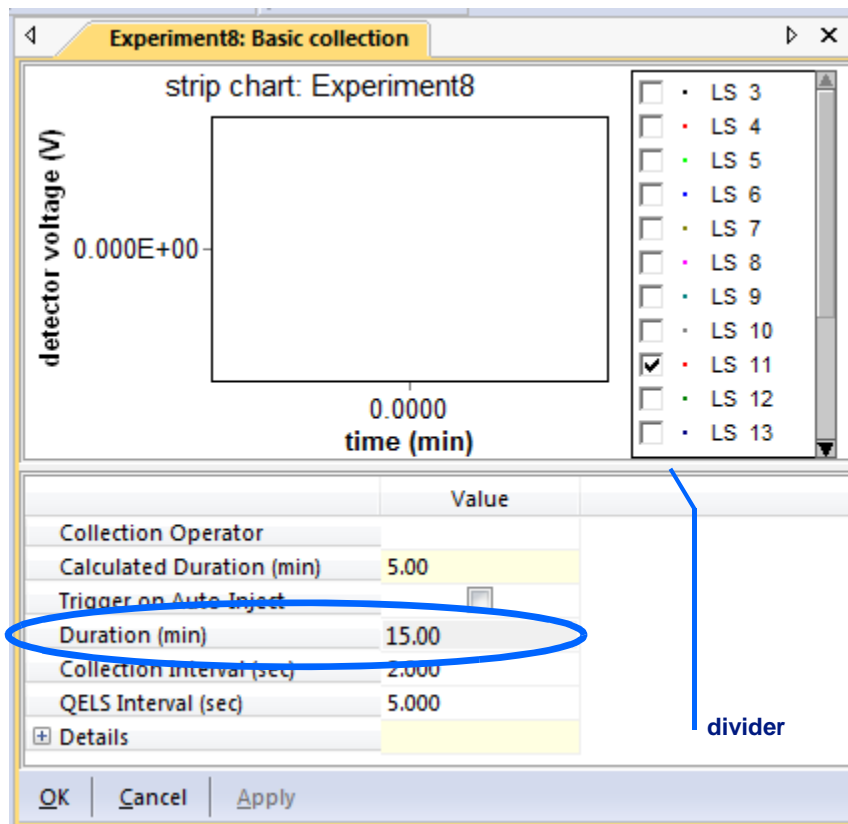
Just by setting a few properties you have created an experiment that can be run. The methods provided with ASTRA make it as simple as possible to get to the point where you can run an experiment.

You can set other properties for the experiment if you like. Configurations are described in detail in Chapter 7, “Configuring Experiments”.

Modifying Procedure Settings

At this point, you could run the default experiment. However, to show you more about using ASTRA, we'll set the duration of the data collection. Follow these steps:


1. In the Procedures node, double-click the Basic collection procedure. This opens the Basic collection page.
2. If necessary, resize or scroll the page to see the Duration property. You can also drag the divider between the graph and the property list to resize the graph.



3. Type a new duration for the collection. For example, since you are simply learning to use ASTRA, you might collect data for only one minute. When performing your own experiments, you will collect data for longer durations.
4. Click **Apply**.

Running the Experiment

Experiment procedures prompt you for any information they need in order to run successfully. To run the experiment, follow these steps:

1. Click the  **Run** icon in the ASTRA toolbar (Ctrl+Shift+R).

Note:

If you are using ASTRA without access to a light-scattering instrument, you can open an experiment with pre-collected data by choosing **File→Open→Experiment** (Ctrl+O) and opening the “Sample Data” folder, then “Practice Experiments”, then “batch processing example.afe6”. Skip to step 4.

2. Watch the data as it appears in the Basic collection graph. You can enable and disable detector displays in real-time.

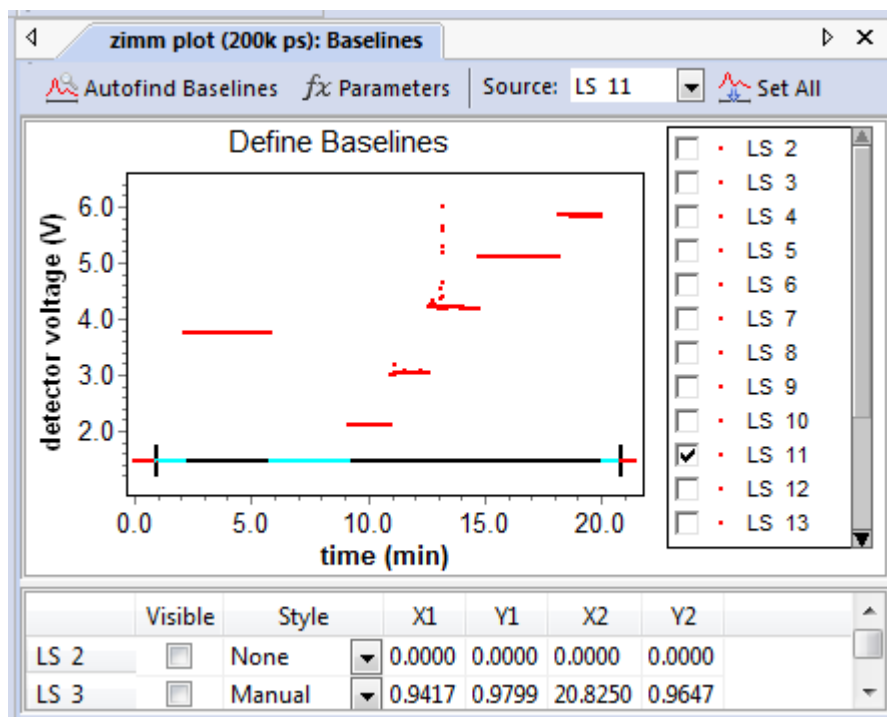
Note:


While you are collecting data, you can work on other experiments. You cannot modify an experiment that is running.

3. Inject samples and/or start pumps as needed to run the experiment.

After data is collected, you see a message that says a baseline needs to be set. Set a baseline by following these steps:

- a. Click **OK** to open the page for setting baselines.

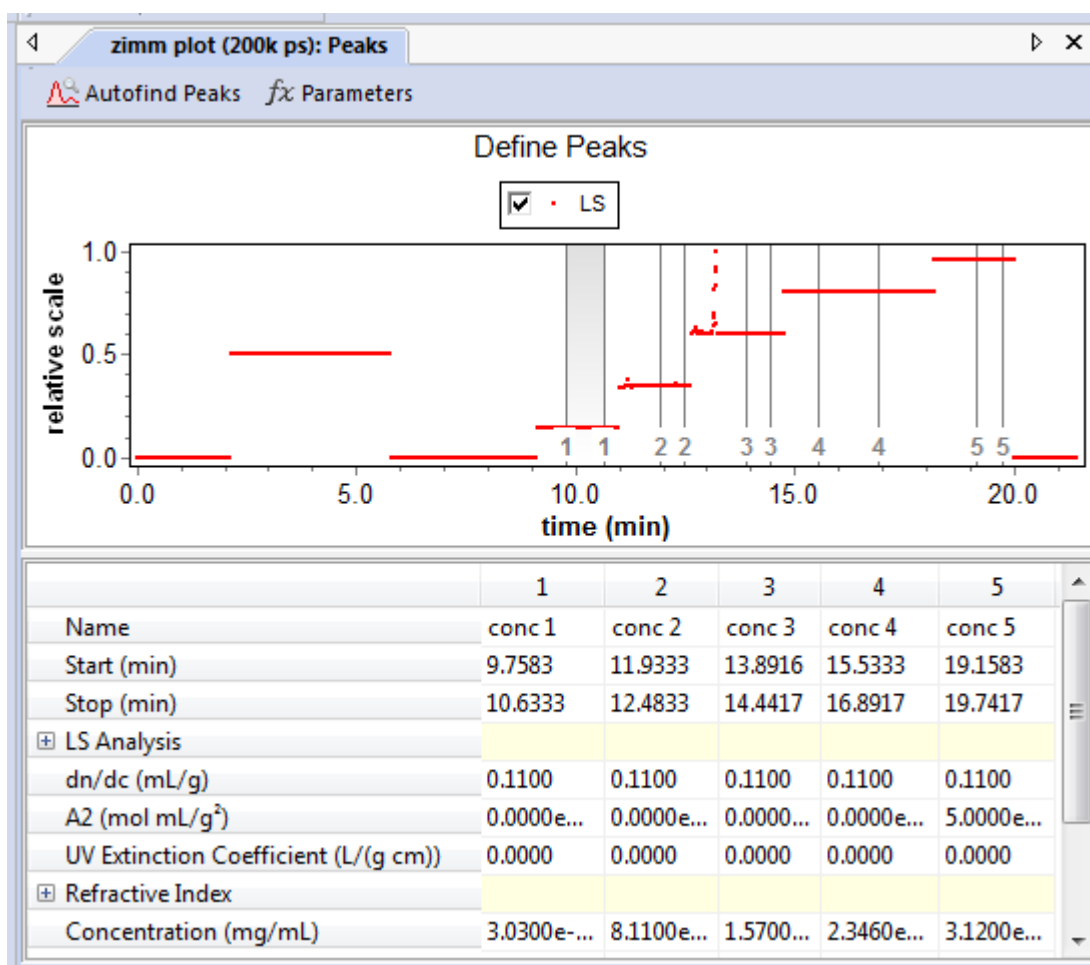


- b. The easiest way to set baselines is to click the  **Autofind Baselines** button at the top of the page. This automatically finds the optimal baselines for all detectors. You can tune the optimization parameters for baseline detection as described in “Baselines” on page 8-48.


- c. Alternately, you can select a detector signal to view in the right column and use your mouse to click on the baseline of the graph at one location and drag to another location on the baseline.


By default, baseline ends snap to the voltage level for a particular time. If you hold down the Shift key, you can then drag the end of a baseline to any location.

- d. Click **OK** to continue running the experiment.
4. You next see a message that says peaks need to be specified. Set peaks by following these steps:
- a. Click **OK** to open the page for setting peaks.







- b. Use your mouse to click on one end of a peak range. Then drag to the other end of that peak range. Add additional peak ranges as needed for your batch experiment.

Note: You can use the  **Autofind Peaks** button only if you are running an online (fractionated) experiment. See “Peaks” on page 8-54 for more about peak detection.

- c. A number is shown for each peak. This number corresponds to the column for that peak below the graph. The selected peak is shaded. You can modify dn/dc, concentration, and other known values for samples below the graph.
 - d. If you want to zoom in on the graph, hold down the Ctrl key and use your mouse drag an outline around the area you want to see. To zoom back out, hold down the Ctrl key and click your right mouse button.
 - e. You can delete peak range selections by highlighting a peak selection and pressing the Delete key.
 - f. Click **OK** to continue running the experiment.
5. The experiment runs to completion, and all the  procedure icons in the experiment show that they have been run.

A procedure's state is always indicated by its icon, as follows.

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid location.

For more about running experiments, see Chapter 6, “Creating & Running Experiments”.

Viewing Reports

To view a report, simply double-click on it in the experiment tree. You can scroll down to read the results of the data analysis.

For more about setting up and viewing results, see Chapter 10, “Working With Reports”.

Summary

In a few minutes, you've created and run an experiment. You have modified the properties of a configurations and set properties such as baselines for a procedure. These are the main types of tasks you will perform when setting up and running your own experiments.

As you become a more advanced user, you may want to learn to perform tasks that are available in Experiment Builder mode. For details, see “User Modes” on page 3-13.

More About the ASTRA Environment

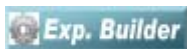
This section explains some general tasks you may perform within ASTRA that were not covered in the sample experiment in the previous section—such as customizing the ASTRA environment and getting help.

For an overview of ASTRA features, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the link to Features.

User Modes

You can use ASTRA in “Run” mode or “Experiment Builder” mode.

Run mode makes it easier to learn to use ASTRA and may be the mode you prefer even after you are an experienced user. In Run mode, you create experiments using the configuration and procedure methods provided with ASTRA. You can modify properties of the configuration and procedures, but cannot add or delete instruments or procedures.



Experiment Builder mode allows you to modify the configuration and procedures in a method. The icon to the left identifies portions of this manual that apply only if you turn on Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. This mode allows you to open multiple procedure windows at once. However, you should be careful with this feature, since changing and applying properties in one window does not generally result in changes to other open procedure windows. To see such changes reflected in other procedure windows, you should close and reopen them.

User Account Levels



As part of the 21 CFR Part 11 compliance of ASTRA 6 with Security Pack, all users must log in with a unique user id and password. The administrator sets up accounts with one of the following user account levels:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.



Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the “Security” icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA 6 with Security Pack. There are no access restrictions in other operating tiers.

Customizing the Environment

You can customize the way the ASTRA window looks to suit the way you use it. For example, you might want to hide items to allow you to make a graph display as large as possible on a small computer monitor. ASTRA remembers such settings the next time you run ASTRA.

Controlling Navigation Panes

Notice the icons at the bottom of the left pane. These provide access to the Experiments, Sequences, Profiles, and Instruments navigation panes.



You can also select a navigation pane using the menu commands:

- **View→Experiments** selects the Experiments navigation pane.
- **View→Sequences** selects the Sequences navigation pane.
- **View→Profiles** selects the Profiles navigation pane.
- **View→Instruments** selects the Instruments navigation pane.

You can display buttons instead of icons for one or more navigation panes by clicking the >> arrows in the lower toolbar and selecting **Show More Buttons**. The buttons show the number of currently open items of that type.



To further control the navigation panes, click the >> arrows in the lower toolbar and select **Navigation Pane Options**. This opens a dialog that lets you hide or change the order of the navigation panes.


Controlling Toolbars

- **View→Toolbars→Standard Toolbar** hides the standard toolbar. This bar contains icon buttons for creating, opening, and saving experiments, using the clipboard, printing, and getting help.
- **View→Toolbars→Processing Toolbar** hides the experiment/sequence toolbar. This bar contains icon buttons for creating, running, and stopping experiments and sequences.
- **View→Toolbars→Graph Toolbar** hides the toolbar that contains icon buttons for zooming and scrolling within graphs.
- **View→Toolbars→Customize**. The Toolbars tab in the Customize dialog lets you add text labels to the toolbar icons. The Keyboard tab lets you assign key sequences to commands you use often (see below).
- **View→Status Bar** hides the bar at the bottom of the window. This bar shows messages about the experiment status and the access level the current user has.

Controlling Window Display

- **View→Visual Manager** opens the Application Look dialog. You can use this dialog to change a number of Microsoft Windows-related aspects of the ASTRA display.
- **View→Full Screen** allows you to maximize the size of the ASTRA window and hide the left pane and toolbars. This makes the property pages and graphs as large as possible.
- **Window→Tab Groups** toggles between display modes for property pages, reports, and graphs. When Tab Groups is on, pages are shown at the full size of the document area and you use tabs to move between them. When Tab Groups is off, each page has a separate sub-window.
- You can arrange the open windows by using the **Window→Cascade**, **Window→Tile Horizontal**, **Window→Tile Vertical**, and **Window→Arrange Icons** menu commands.
- You can move to a different window by using the **Window→Next**, **Window→Previous**, and **Window→Windows** menu commands.
- You can close windows by using the **Window→Close** and **Window→Close All** menu commands.

Controlling Menus

By default, menus in ASTRA show all the commands. You can shorten the menus to show only the common commands (and let you click the  icon to extend a menu temporarily).

If you would prefer to always see the short menus, choose **View→Toolbars→Customize** and go to the Options tab. Check the “Menus show recently use commands first” box.

Controlling Key Sequence Assignments

You may want to add keystrokes for various menu commands you use frequently. To add a command, follow these steps:

1. Choose **View→Toolbars→Customize** and go to the Keyboard tab.
2. Select a menu in the Category pull-down list.
3. Select a command in the Commands list.
4. Click in the “Press New Shortcut Key” field.
5. Use your keyboard to press a key combination. The key names are shown in the field, and any command to which they are already assigned is shown below.
6. To assign the key combination to the selected command, click **Assign**.

Command Reference

See Appendix A, “Menu Quick Reference” lists of all menu commands, tool bar buttons, and key sequences provided in ASTRA.

Printing

You can print configuration and procedure windows, reports, and graphs from ASTRA. To print, choose **File→Print** (Ctrl+P) and use the Print dialog as in other Windows applications. For reports, you can choose **File→Print Preview** to examine the page breaks before printing.

You can choose **File→Page Setup** to choose a paper size and source, page orientation, and margin widths. You can choose **File→Print Setup** to choose a printer and set properties for your printer.

Getting Help

The online help for the ASTRA software contains all the information in this manual. Property pages are linked to context-sensitive help, so you can quickly learn about individual properties.

Note:	The online help system does not contain hardware-related information. Please refer to the User’s Guide for your specific instrument for more detailed information.
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To access the online help system, do one of the following:

- Choose **Help→Contents** to open the help system at the first topic.
- Choose **Help→Search** to open the full-text search for the help system.
- Choose **Help→Index** to open the index for the help system.
- Press F1 in any page to open help about that page.

Exiting from ASTRA

When you have finished working with ASTRA, exit it just as you would any other Windows application. If a file is open, ASTRA closes it. If any changes to an open file haven't been saved, you are prompted to save the changes or cancel the closing of ASTRA.

To close ASTRA, do one of the following:

- Choose **File→Exit**.
- Press ALT+F4.
- Press Alt, F, X.
- Click the X button in the upper-right corner of the ASTRA window.

4

ASTRA Administration

This chapter tells how to administer ASTRA 6 experiment databases. Such databases are used in ASTRA 6 with Research Database and in ASTRA 6 with Security Pack.

CONTENTS	PAGE
21 CFR Part 11 Support Overview.....	4-2
Connecting to a Database.....	4-3
Managing User Accounts	4-6
Using the System and Experiment Logs	4-8
Deleting Experiments	4-10
Performing Database Maintenance.....	4-10

21 CFR Part 11 Support Overview



21 CFR Part 11 contains regulations by the U.S. Food and Drug Administration (FDA) concerning electronic records and electronic signatures. FDA-regulated companies in pharmaceutical, biotechnology, and other industries are under increased scrutiny to comply with 21 CFR Part 11.

Background and Reasons for Compliance

Title 21 of the Code of Federal Regulations includes regulations for food and drugs regulated by the Food and Drug Administration. Part 11 of this title establishes the criteria under which electronic records and signatures can be considered equivalent to paper records and handwritten signatures in processes regulated by the FDA.

FDA-regulated industries must document that proper processes have been followed to insure that products are consistent. Signed documents about various points in the manufacturing processes must be reviewed, securely stored and available for review by the FDA. Reviewing these records was time consuming and required manual searches. 21 CFR Part 11 makes record handling more accurate and efficient for all parties because all of the records stored are digital.

The benefits of becoming 21 CFR Part 11 compliant include the following:

- **Compliance:** This may be a requirement for conducting business. Compliance provides better preparation for FDA inspections.
- **Improved Efficiency:** Electronic records can be searched quickly.
- **Faster Time to Market:** Time delays in approval cycles can be reduced because records can be transferred electronically.
- **Better Quality and Consistency:** Products may be improved and are manufactured in a consistent manner.
- **Improved Research Data:** Compliant electronic records provide better data integration and allow trending information to be better examined.
- **Reduced Cost:** Storage space for hardcopy records is more costly than electronic storage.
- **Reduced Risk:** Compliant electronic records are less vulnerable to signature fraud and misfiling.

For details from the FDA about 21CFR Part 11, see http://www.fda.gov/ora/compliance_ref/part11.

Making use of the 21 CFR Part 11 support in ASTRA 6 with Security Pack makes your experimental data collection, analysis, and storage compliant with the FDA ruling.

21 CFR Part 11 Support in ASTRA 6



Security

ASTRA 21 CFR Part 11 compliance features related to user accounts and logging activities are available only in ASTRA 6 with Security Pack. The icon to the left identifies information that is specific to ASTRA 6 with Security Pack.

If you are using these features, you must have an ASTRA administrator to manage 21 CFR Part 11 compliance. That manager will perform the following actions, which are described in this chapter:

- “Connecting to a Database” on page 4-3
- “Managing User Accounts” on page 4-6
- “Using the System and Experiment Logs” on page 4-8
- “Deleting Experiments” on page 4-10



Database

ASTRA features related to the use of an experiment database to store experiments and sequences are available in both ASTRA 6 with Security Pack and ASTRA 6 with Research Database. The icon to the left identifies information that applies to both of these software versions.

For more about 21 CFR Part 11 compliance in ASTRA 6 including a detailed white paper, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the link to Compliance.

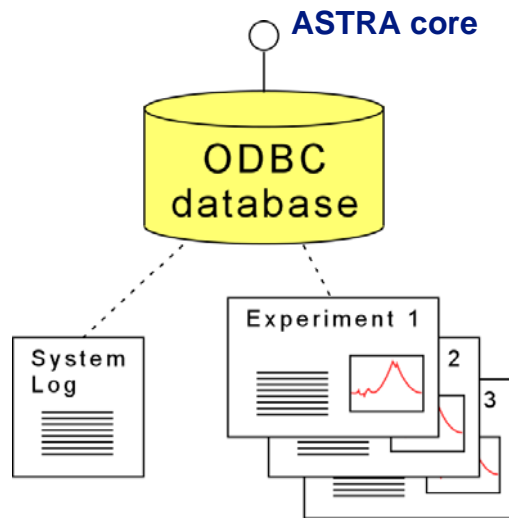
Connecting to a Database

ASTRA 6 uses two databases:

- **System database:** This database stores experiment methods, sequence templates, profiles, solvents, and molecular standards. A system database is used by *all* ASTRA 6 operating tiers. Each installation of ASTRA 6 uses its own, local system database. You cannot specify a different database to use as the system database. See “Migrating the System Database” on page 2-4 for information about updating the system database.
- **Experiment database:** This database stores information about experiments, database actions, warnings, and more. This database logs list all activities performed with ASTRA that must be logged for 21 CFR Part 11 compliance. An experiment database is used *only* in ASTRA 6 with Security Pack and ASTRA 6 with Research Database. You can specify which experiment database to use.

The rest of this section talks about ASTRA’s experiment database, which is sometimes called simply the “database”.

ASTRA uses Open DataBase Connectivity (ODBC) to connect to databases. ASTRA has been tested with Microsoft Access and Microsoft SQL Server, version 7 or higher databases.



Database

By default, the experiment database is a Microsoft Access database called ASTRA Experiment.

If you have Microsoft SQL Server, Wyatt recommends that you use this for your experiment database. Microsoft Access is a light-weight database that cannot handle the large amounts of data generated by ASTRA; the database operations will become slow as the amount of stored data increases.

Ideally, the experiment database will be a networked database that is backed up regularly by the IT department. Note that adequate database storage is required. If the database is networked, the database server must be accessible to the PC running ASTRA via the network.

If you have no networked Microsoft SQL Server installation available, you can install SQL Server locally. There is a free version of SQL Server called SQL Server 2005 Express Edition that is available from Microsoft.

For more information, see the “ReadMe Files” directory of your ASTRA installation, which contains information about database and network issues. In addition, you can go to the Wyatt Support Center website (www.wyatt.com/support) and follow the links to Software Support > ASTRA Support > Troubleshooting Topics for more about dealing with database issues.

Each experiment run with ASTRA uses an average of about 0.5 MB of storage, so database size should be based on estimated number of experiments to be saved. Also, a database user account and password must be created for the database. The user account must have privileges to create, delete, and modify tables in the database.

Viewing the Current Experiment Database

To see the current experiment database path, follow these steps:

1. Log in to ASTRA using an account with ASTRA Administrator access.
2. Choose **System→Database Administration→Connections**. See the Data tab for the experiment database and the System tab for the system database.

ASTRA 6 with Security Pack and ASTRA 6 with Research Database must be connected to an experiment database. Only one database can be used at a time. However, it is possible to connect to different databases for different types of experiments.

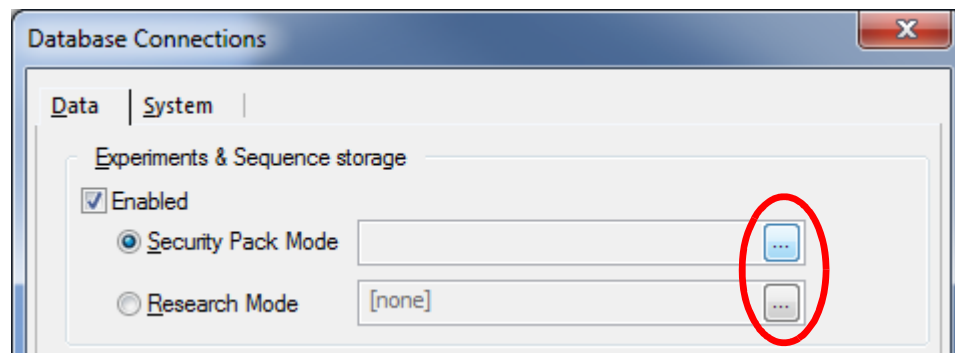
Creating a Microsoft Access Experiment Database

SQL Server is strongly recommended over Microsoft Access for the experiment database format. However, you can explore the experiment database features of ASTRA using Microsoft Access.

Warning: Once you are ready to use ASTRA for actual experiments, it is strongly recommended that you move to a SQL Server experiment database. If you continue to use Microsoft Access, you will likely encounter problems due to limitations on database size, performance degradation, and issues with multi-user database access.

To create a new Microsoft Access experiment database and connect to it with ASTRA, follow these steps:

1. Log in to ASTRA using an account with ASTRA Administrator access.
2. Choose **System→Database Administration→Connections**. This opens the Database Connections dialog.
3. In the Data tab, click the “...” button next to either **Security Pack Mode** or **Research Mode**, depending on your license.



4. Connect to an existing ODBC data source or create a new one using this dialog. Remember that only Microsoft Access and SQL Server databases are supported. For example, you might follow these steps:
 - a. Move to the Machine Data Source tab in the Select Data Source dialog and click **New**. (You may see an ODBC System DSN Warning; you can click **OK** and ignore this message.)
 - b. Select **System Data Source** and click **Next**. (Alternately, you can select **User Data Source** if you only want this account to access this database.)
 - c. Select **Microsoft Access Driver (*.mdb)** and click **Next**. (Alternately, you could select SQL Server.)
 - d. Click **Finish**.
 - e. Type a **Data Source Name**. For example, `astra_exp_db`.
 - f. Type a **Description**. For example, ASTRA Experiment Database.
 - g. Click **Create**.
 - h. In the New Database dialog, type a database name. For example, `astradb.mdb`. Also, browse for a location to store your database. For example, `c:\Program Files\WTC\ASTRA 6\Database`.
 - i. Click **OK** four times (in the New Database dialog, the success message, the ODBC Microsoft Access Setup dialog, and the Select Data Source dialog).
5. In the Database Connections dialog, click **Apply**.

Managing User Accounts



Security

User accounts in ASTRA 6 with Security Pack are managed as Microsoft Windows user accounts. You assign each user account that can access ASTRA to one of the following four groups:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.

With operating tiers other than ASTRA 6 with Security Pack, users are not prompted to log in with a user name and password.

Chapter 2, “Installing and Setting Up ASTRA” contains a section on “Setting Up User Accounts” on page 2-7 since that portion of ASTRA administration needed to be done during initial setup. That section provides steps for setting up the groups used by ASTRA, creating user accounts, and assigning users to groups. You can modify and delete user accounts using the same Windows tools.

The status bar at the bottom of the ASTRA window shows the name of the user account that is currently logged in. It also shows the ASTRA group to which that user is assigned.

A user should not be assigned to more than one ASTRA group.

Using the System and Experiment Logs

Database

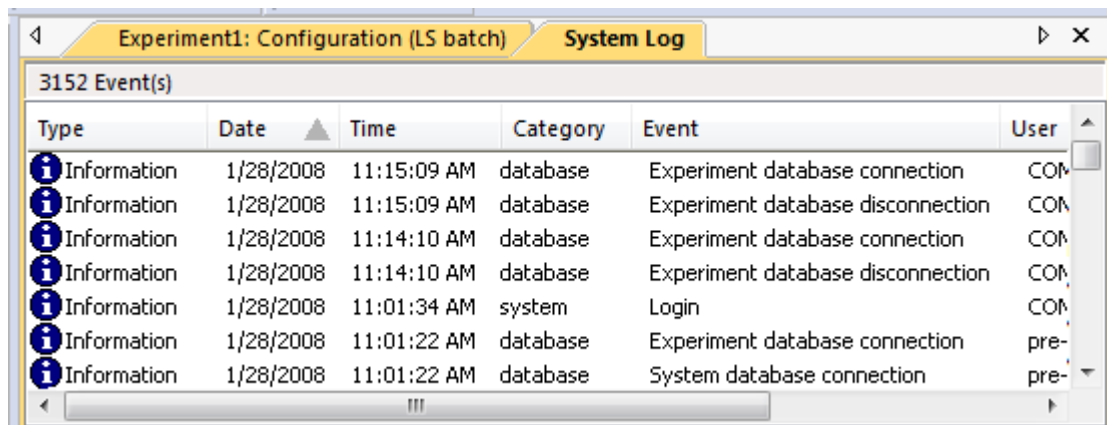
ASTRA 6 with Security Pack and ASTRA 6 with Research Database log information about actions performed that relate to experiments. There are two types of logs used by ASTRA:

- **System log:** Shows global actions that include: logging in, logging out, creating, importing, exporting, opening, saving, and deleting an experiment, and signing off on an experiment.
- **Experiment logs:** Shows actions that relate to a particular experiment. These include actions involving procedures, data set definitions, reports, methods, and more. This log shows when the procedures that make up an experiment were run.

As ASTRA Administrator, you should communicate to researchers about the Sign Off procedures they should add to experiments they create. These are likely required by your 21 CFR Part 11 policies and procedures. Sign offs can store information about the user responsible for the experiment, the user who approved the experiment, or the user who reviewed the experiment.

Viewing the System Log

To view the system log, choose **System→Log→Open** or **View→Logs→System→Open**.



The screenshot shows a window titled "Experiment1: Configuration (LS batch)" with a tab labeled "System Log". Below the tab, it says "3152 Event(s)". The main area is a table with the following columns: Type, Date, Time, Category, Event, and User. The table contains several rows of log entries, each starting with an information icon (i).

Type	Date	Time	Category	Event	User
Information	1/28/2008	11:15:09 AM	database	Experiment database connection	COM
Information	1/28/2008	11:15:09 AM	database	Experiment database disconnection	COM
Information	1/28/2008	11:14:10 AM	database	Experiment database connection	COM
Information	1/28/2008	11:14:10 AM	database	Experiment database disconnection	COM
Information	1/28/2008	11:01:34 AM	system	Login	COM
Information	1/28/2008	11:01:22 AM	database	Experiment database connection	pre-
Information	1/28/2008	11:01:22 AM	database	System database connection	pre-

If you make changes that affect the system database while the log is displayed, you can view the latest log entries by choosing **System→Log→Refresh** or **View→Logs→System→Refresh**.

You can save the system log to a CSV or text file by choosing **System→Log→Save As** or **View→Logs→System→Save As**.

Viewing an Experiment Log

Database

To view the entire experiment log, choose **System→Database Administration→Log→Open** or **View→Logs→Database→Open**.

You can save the entire experiment log to a CSV or text file by choosing **System→Database Administration→Log→Save As** or **View→Logs→Database→Save As**.

If you make changes to experiments while the experiment log is displayed, you can view the latest log entries by choosing **System→Database Administration→Log→Refresh** or **View→Logs→Database→Refresh**.

To view the log for the current experiment, select the experiment and choose **Experiment→Log→Open** or **View→Logs→Experiment→Open** from the menu bar. For information about experiments, see Chapter 6, “Creating & Running Experiments”.

You can save an individual experiment log to a CSV or text file by choosing **System→Database Administration→Log→Save As**.

Viewing a Sequence Log

Database

To view a log for a sequence, select the sequence and choose **Sequence→Log→Open** or **View→Logs→Sequence→Open** from the menu bar. For more about sequences, see Chapter 9, “Using Sequences”.

You can save an individual sequence log to a CSV or text file by choosing **Sequence→Log→Save As**.

Working with Logs

Database

In any log, the most recent action is shown at the top by default. You can click the column headings to sort the log in other ways, such as by category, event, or user.

You can double-click an entry to view a dialog with more detailed information about that log entry. The buttons in this dialog act as follows:

- **Up Arrow:** Go to the previous entry in the list. Unless you changed the sorting, this is the next entry in time.
- **Down Arrow:** Go to the next entry in the list. Unless you changed the sorting, this is the previous entry in time.
- **Copy Icon:** Copy the text of the event to the clipboard. You can then past the text into another application, such as a word processor.

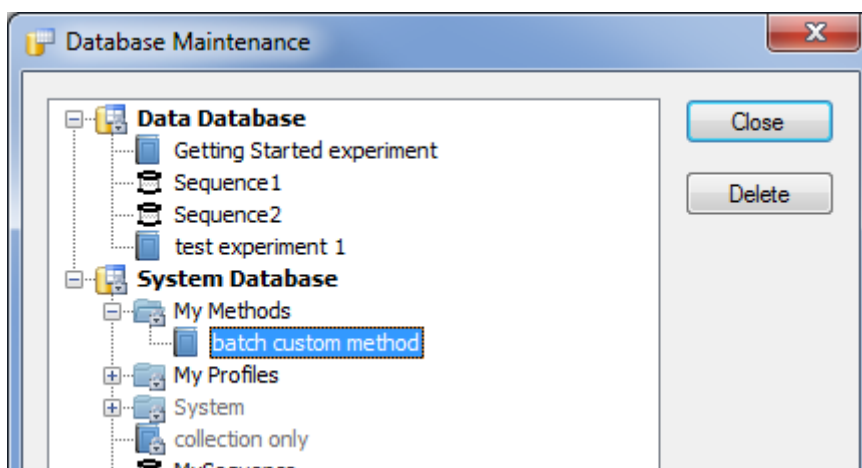
Deleting Experiments



Only an ASTRA Administrator is permitted to delete experiments from the experiment database. You should only delete an experiment if it is permitted by your 21 CFR Part 11 policies and procedures.

To delete an experiment, follow these steps:

1. Choose **System→Database Administration→Maintenance**. This opens the Database Maintenance dialog. Experiments you have saved are in the “Data Database” folder. (This dialog also lists profiles, methods, sequences, and other items stored in the experiment and system databases.)



2. Highlight the experiment you wish to delete in the list.
3. Click **Delete**.
4. Click **Close** when you have finished deleting experiments.

This command can also be used to delete sequences, profiles and experiment methods. For more information, see “Deleting a Sequence” on page 9-13, “Deleting a Profile” on page 12-7, and “Deleting a Method” on page 6-20.

Performing Database Maintenance

If you are having problems with your experiment database, you can choose **System→Database Administration→Automatic Maintenance** to attempt to correct the problem.

We do not recommend that you use this command unless you are having problems with the experiment database.

5

Interfaces to Instruments

This chapter explains the Instrument Server Interface (ISI), the Diagnostic Manager, and the Wyatt Communication Server (WCS) Client. These can all be accessed through ASTRA. The ISI allows you to connect to, administrate, and acquire data from instruments connected locally or over your network.

CONTENTS	PAGE
Instrument Server Interface Overview.....	5-2
Installing the ISI on Other Computers	5-4
Using the Diagnostic Manager	5-5
WCS Client Application	5-11

Instrument Server Interface Overview

ASTRA can collect data from various instrument types. More importantly, it can combine this data with analysis procedures. Connections to instruments can be made either locally or through the network.

For instructions for connecting to instruments, see “Accessing and Viewing Hardware” on page 2-12.

The Instrument Server Interface (ISI) is installed as part of the ASTRA installation process. The ISI allows you to access instruments connected locally or across your internal Local Area Network (LAN). That means the instruments you use with ASTRA need not be directly connected to the computer you are using.

Next-generation instruments from Wyatt Technology Corporation, such as the following, can be controlled directly over the network or a local USB connection. They have an integrated ISI that can be accessed directly through the network. You do not need to install an ISI on your local computer to access these instruments.

- DAWN HELEOS
- miniDAWN TREOS
- Optilab rEX/T-rEX
- ViscoStar
- Wyatt Internal QELS

The following instruments can also be accessed locally or through the network, in much the same way that a printer can be shared on a LAN. For these instruments, you must install the ISI (or ASTRA, which includes the ISI) on the computer to which the instrument is connected. See “Installing the ISI on Other Computers” on page 5-4.

- DAWN EOS, DSP, and DSP-F
- miniDAWN
- WyattQELS (QELS Slab)

Other instruments—including the following—may be connected through the AUX input of another instrument. As a result, the instruments will be shown as part of an experiment configuration.

- Optilab DSP
- Optilab 903
- Generic RI Instrument
- Generic UV Detector
- Generic Viscometer

In Figure 5-1 you see a summary of these types of connections:

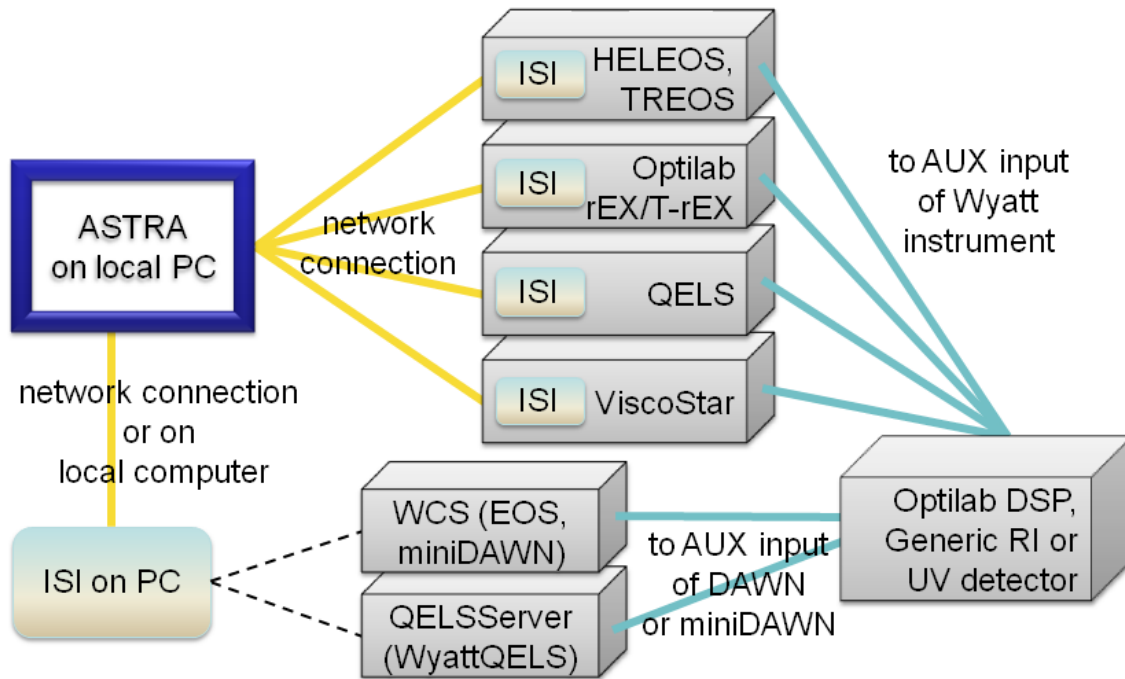


Figure 5-1: Connections Between ASTRA, the ISI, and Instruments

In Figure 5-2 you see the ASTRA 6 with Research Database and ASTRA 6 with Security Pack architecture in more detail. The circles represent interfaces presented by the ASTRA core, the ISI, and ODBC for use by other parts of the architecture.

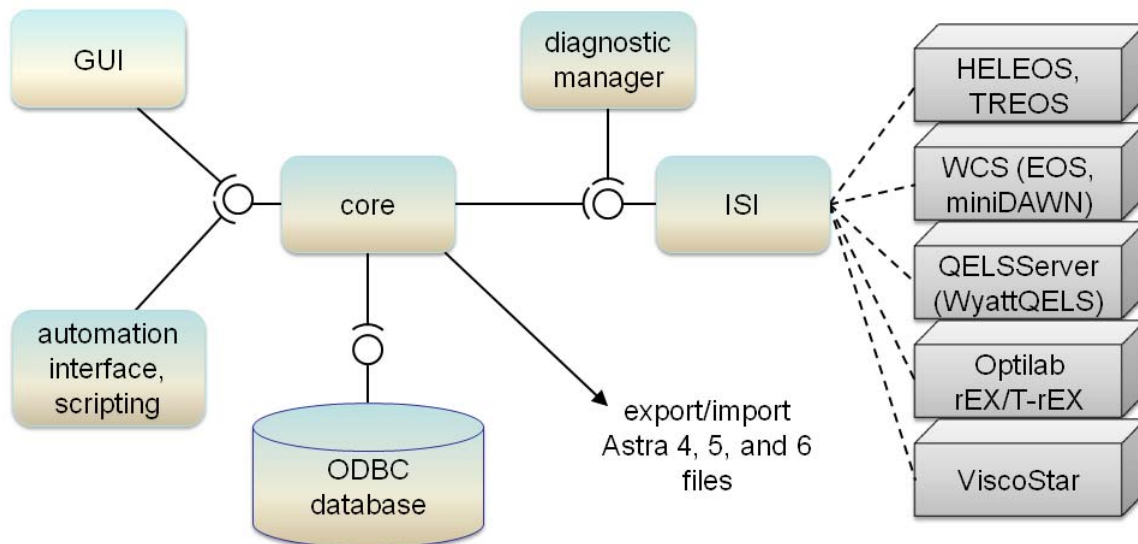


Figure 5-2: ASTRA System Architecture

The core of ASTRA is used by the ASTRA user environment. Console and scripting applications also access the ASTRA core. The ISI presents an interface that is used by both the ASTRA core and the Diagnostic Manager. The ISI, in turn, connects to various instruments.

If you use ASTRA 6 Basic, the ODBC database shown in Figure 5-2 would be replaced by file access to separate experiment files.

Installing the ISI on Other Computers

When you install the ASTRA software on a computer, the ISI software is automatically installed along with ASTRA.

The ASTRA installation CD allows you to install only the diagnostic manager and the appropriate instrument controllers. If you choose to do this, the ISI is installed without the graphical interface and other components of the ASTRA 6 software.

Your network administrator may want more technical information about the ISI. The ISI connects to HELEOS, TREOS, T-rEX, and QELS instruments via network sockets. Connections to ViscoStar and rEX instruments is done via DCOM.

If you want to connect to ViscoStar and/or original rEX instruments, the ISI must run a DCOM server on each machine where the ISI is installed. To successfully connect to an ISI, the computer should allow DCOM connections. This is the standard configuration for Windows XP Professional prior to Service Pack 2. Windows XP Professional SP2 and newer operating systems require manual configuration of the DCOM settings to allow external data connections. For more on DCOM and firewall issues, use your Windows Start menu to select All Programs > Wyatt Technology > ASTRA > Configuration Documents and select from the documents listed. You can also access these documents by going to the Wyatt Support Center website (www.wyatt.com/support) and following the links to Software Support > ASTRA Support > Troubleshooting Topics.

The “ReadMe Files” directory of your ASTRA installation contains additional information about database and network issues.

The ISI runs a server called the Wyatt Communications Server. In the Processes tab of your Windows Task Manager, this process is called WCS.exe. If ASTRA quits unexpectedly, you may need to end the WCS process through the Task Manager.

Using the Diagnostic Manager

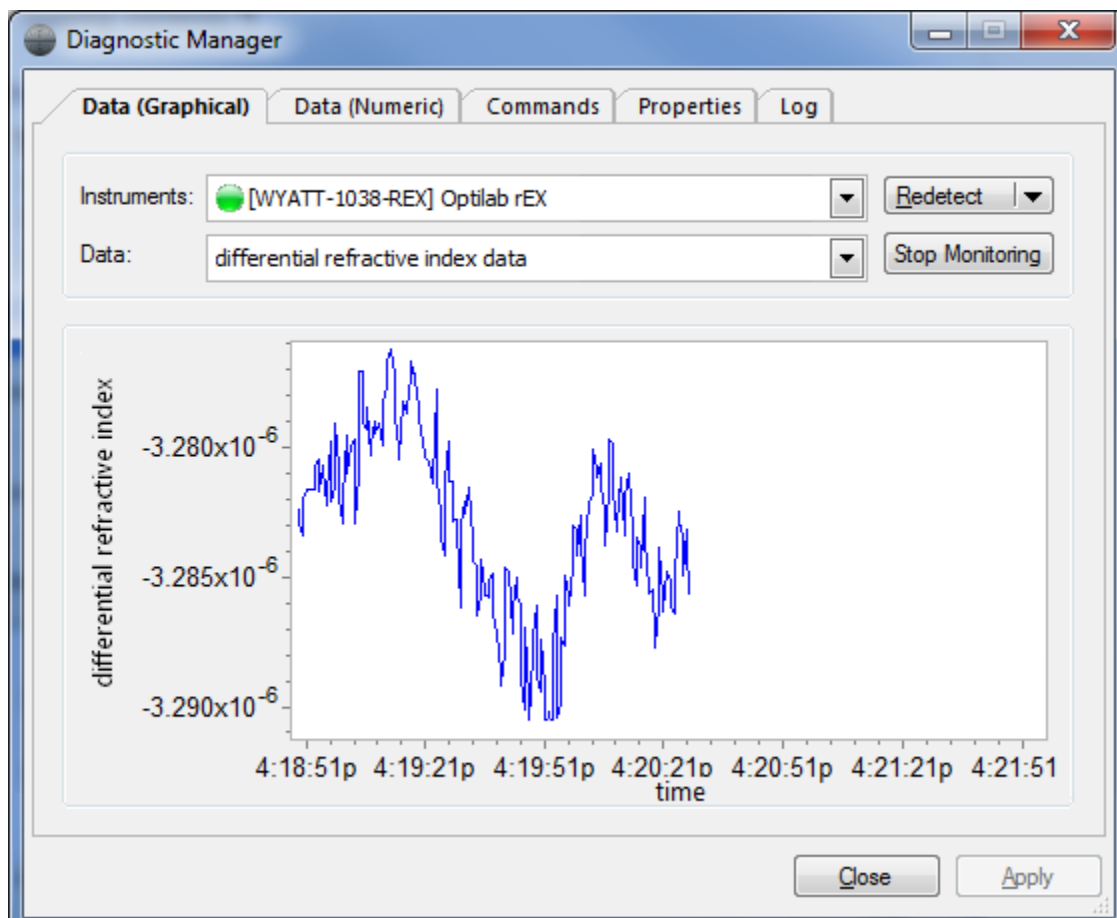
If you choose **System→Instruments**, select an instrument and click **View**, you see the Diagnostic Manager for that instrument. You can also start the Diagnostic Manager from the Windows Start menu by choosing **Programs→Wyatt Technologies→ASTRA→Diagnostic Manager**.

You can use the Diagnostic Manager utility to monitor and control instruments. For example, you can start a collection in your lab, then monitor the progress in your office over the network. Alarms and other state information are reported directly to the Diagnostic Manager, and you can use the Diagnostic Manager to send commands and configuration information to any instrument available within ASTRA.

This manager is not intended for viewing and interpreting data. Instead, it can be used to determine if your instruments are connected and functioning correctly.

Viewing Graphical Data with the Diagnostic Manager

The **Data (Graphical)** tab of the Diagnostic Manager allows you to view a real-time graph of data received from an instrument. The type of data collected is different for each type of instrument. The following figure shows data collected by an Optilab rEX instrument.



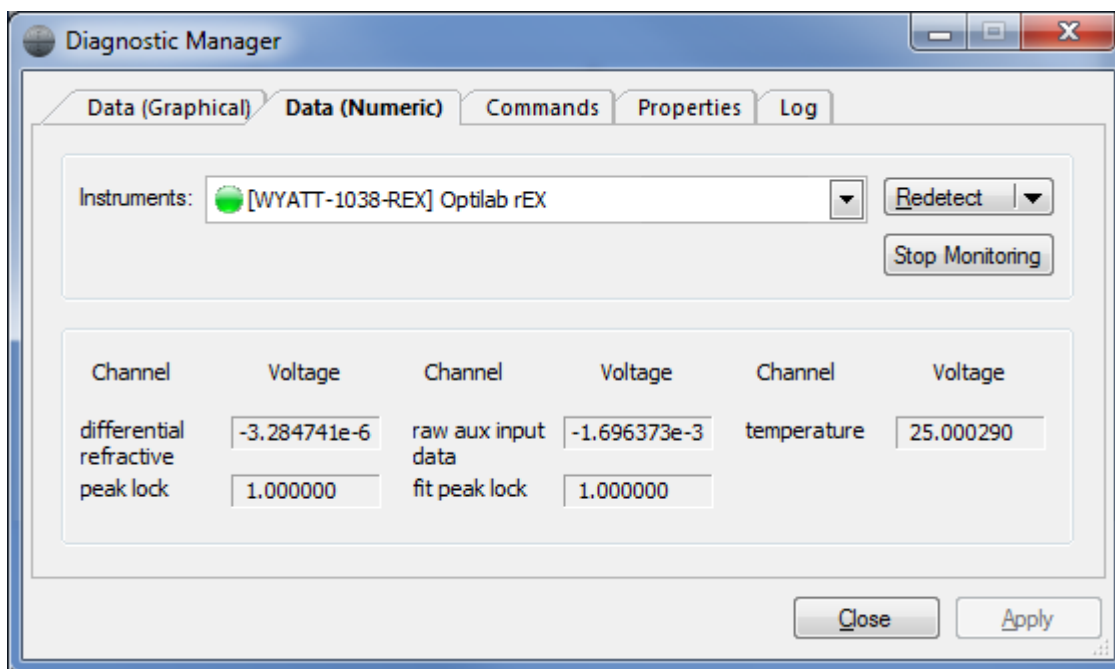
To monitor data, follow these steps:

1. Select an instrument to monitor from the Instrument drop-down list.
If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Redetect** to update the drop-down list.
2. Select the type of data to monitor. The choices differ depending on the type of instrument. For more about viewing "live data" for light-scattering instruments, see "Viewing and Setting Properties with the Diagnostic Manager" on page 5-9.
3. Click **Start Monitoring**.
4. To stop the graph, click **Stop Monitoring**.

You can modify the appearance of the graph just as you would in ASTRA. For details, see "Working with Procedure Graphs" on page 8-5.

Viewing Numeric Data with the Diagnostic Manager

The **Data (Numeric)** tab of the Diagnostic Manager allows you to view real-time numeric data received from an instrument. The type of data collected is different for each type of instrument. The following figure shows data collected by an Optilab rEX instrument.

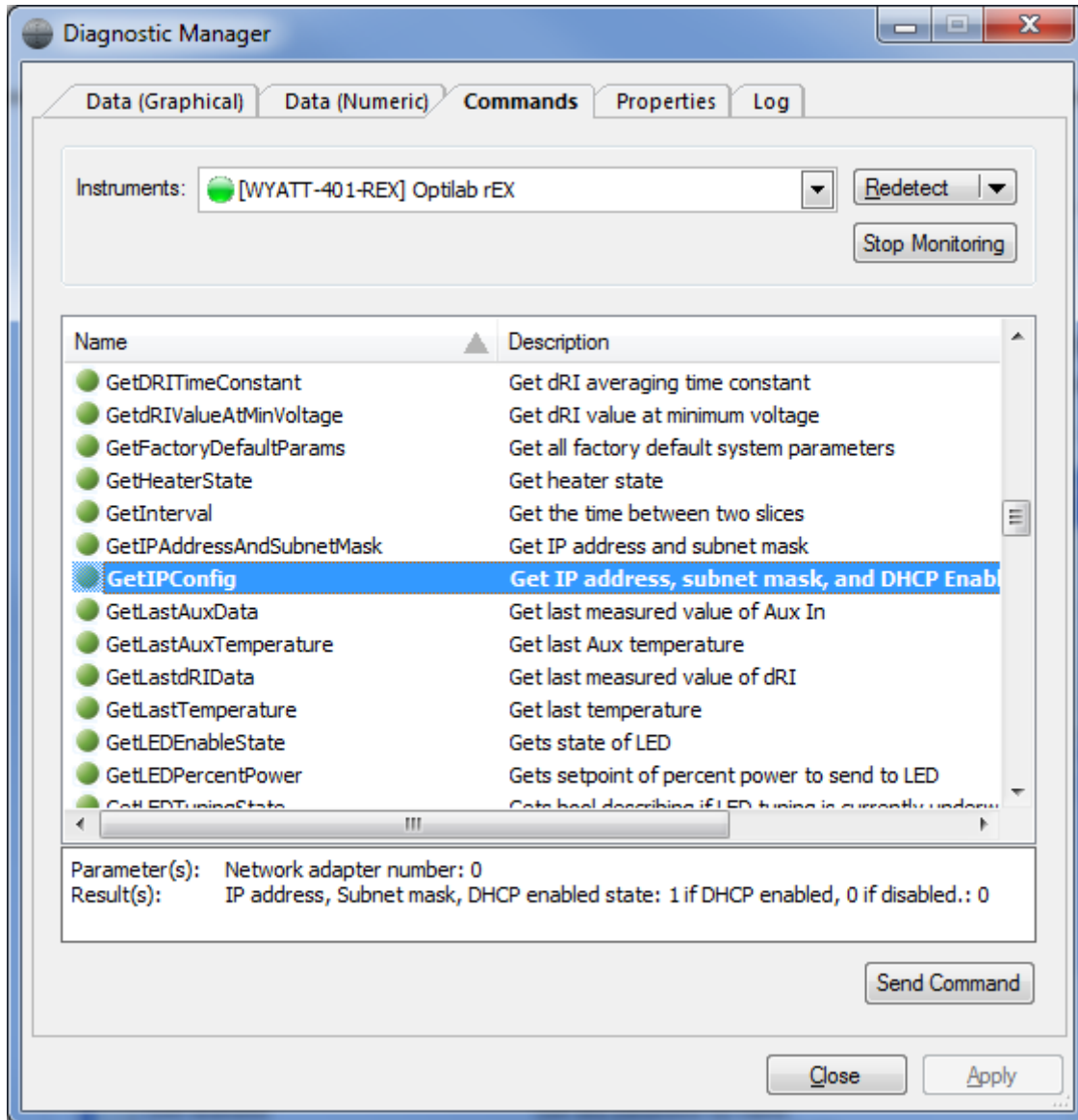


To monitor data, follow these steps:

1. Select an instrument to monitor from the Instrument drop-down list.
If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Redetect** to update the drop-down list.
2. Click **Start Monitoring**.
3. To stop the data updates, click **Stop Monitoring**.

Sending Commands with the Diagnostic Manager

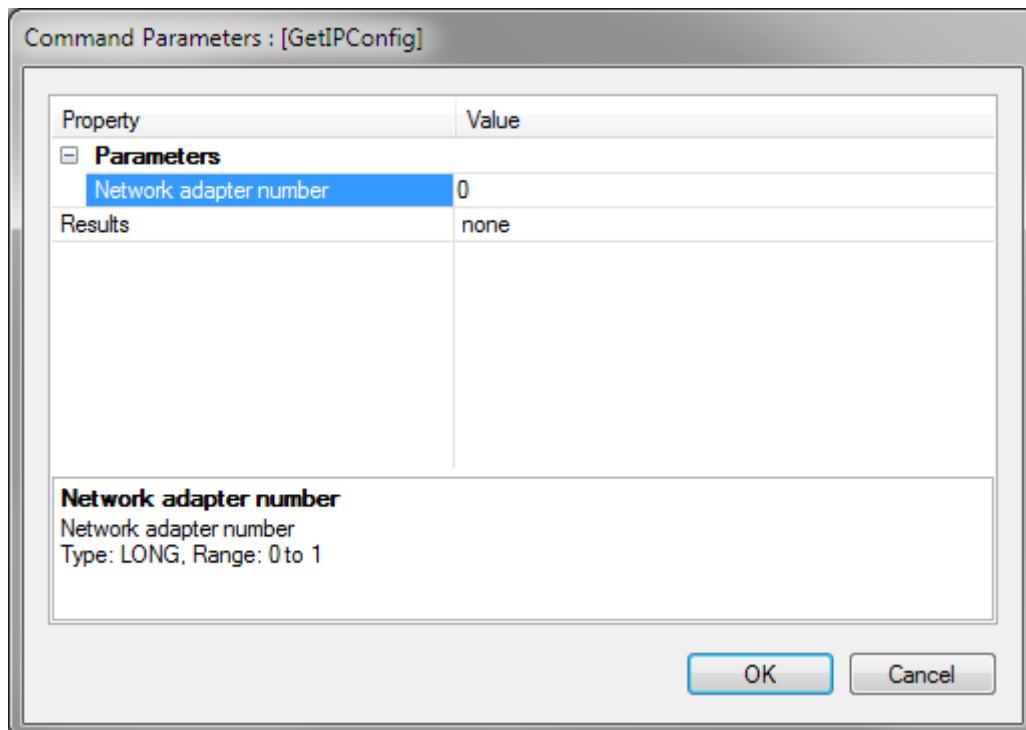
The **Commands** tab of the Diagnostic Manager allows you to send commands to instruments. The list of commands you can send is different for each type of instrument. The following figure shows the commands available for the Optilab rEX instrument.



To send a command, follow these steps:

1. Select an instrument from the drop-down Instruments list.
If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Redetect** to update the drop-down list.
2. Select the command you want to send to the instrument and click the **Send Command** button.

3. If the command you selected requires any parameters, you see the Command Results dialog. Type the values you want to use for the parameters listed. Then click **OK**. For example, the GetIPConfig command requires a network adapter number and the default value is 0.



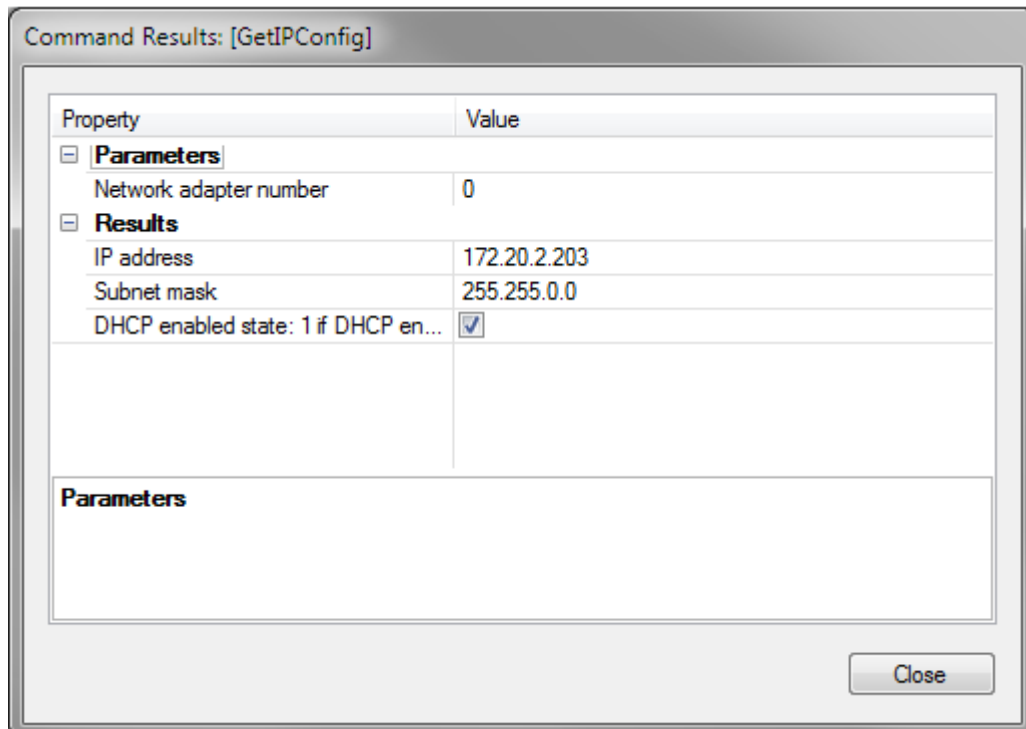
Command Parameters : [GetIPConfig]

Property	Value
Parameters	
Network adapter number	0
Results	none

Network adapter number
 Network adapter number
 Type: LONG, Range: 0 to 1

OK Cancel

4. The results of the command, if any, are shown in the Command Results dialog.



Command Results: [GetIPConfig]

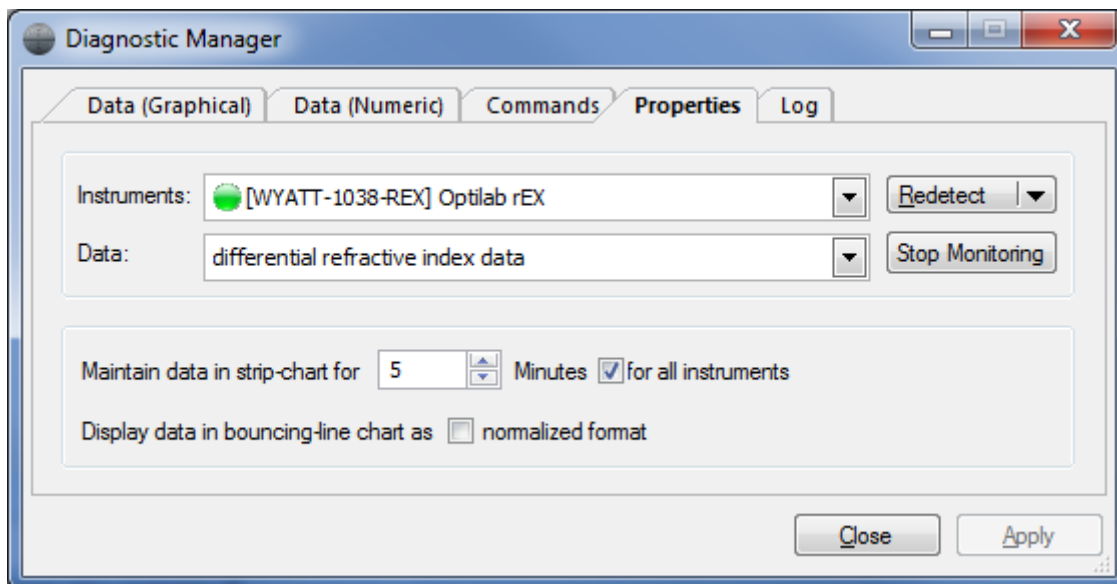
Property	Value
Parameters	
Network adapter number	0
Results	
IP address	172.20.2.203
Subnet mask	255.255.0.0
DHCP enabled state: 1 if DHCP en...	<input checked="" type="checkbox"/>

Parameters

Close

Viewing and Setting Properties with the Diagnostic Manager

The **Properties** tab of the Diagnostic Manager allows you to view and set instrument properties. The list of properties you can set is different for each type of instrument. The following figure shows properties available for the Optilab rEX instrument.



Properties are stored in the Windows registry of the computer to which the instrument is connected. The ISI running on that computer gets the information from the registry.

To set one or more properties, follow these steps:

1. Select an instrument from the drop-down Instruments list.

If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Redetect** to update the drop-down list.

2. In the "Maintain data in strip-chart" area, set fields as follows:
 - **Minutes:** Select the number of minutes for which you want data to be displayed in the graphical data page for each strip chart graph.
 - **For all instruments:** Put a checkmark in this box if you want the number of minutes to apply to all instruments accessible through the Diagnostic Manager. This setting affects only your view of the data; this setting does not affect users on other computers.
 - **Display data in bouncing-line chart as normalized format:** This checkmark is used for Wyatt light-scattering instruments only. If you put a checkmark in this box, the normalization coefficients shown here are applied in the Data - graphical tab (see page 5-5) when "live data" is selected as the data type.
 "Live data" displays the detector voltages as a function of detector number. Applying the normalization constants should make the line essentially flat when looking at the scattering from a solvent.

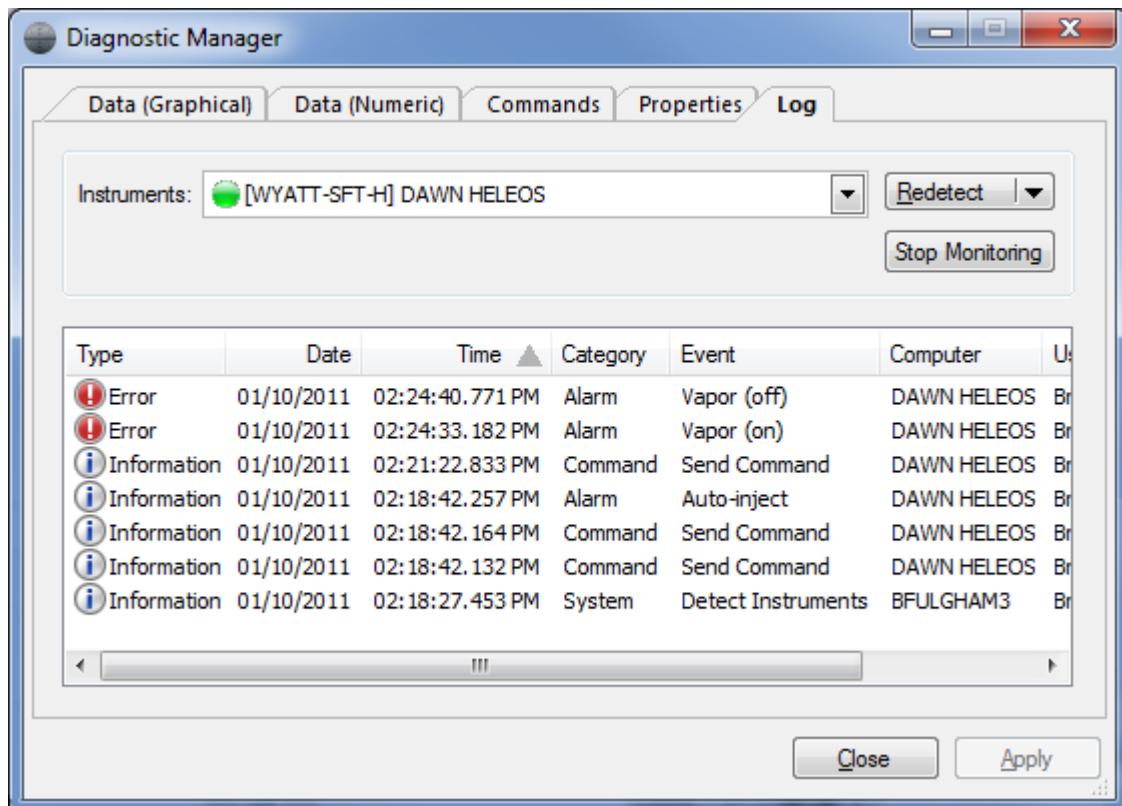
You can use this feature as a diagnostic tool when trying to set the orientation of a scintillation vial on the DAWN. Rotate the vial, view the live data, and try to put the vial in a position such that the live data line is flat.

3. For the remaining fields, change values for any properties you want to modify. The properties shown are different for each instrument type. Refer to the hardware documentation for details.
4. Click **Apply**.

Viewing the Log with the Diagnostic Manager

The **Log** tab of the Diagnostic Manager shows commands you have sent to the instrument via the Diagnostic Manager and responses provided by the instrument. It also shows any alarm conditions that have occurred.

Only commands and errors from the current Diagnostic Manager session are shown. The following figure shows some commands sent to a HELEOS instrument and some errors conditions reported by the instrument.



You can drag the borders between column headings to resize the columns.

You can copy data from this log for pasting into other applications.

You can allow ASTRA to sent alarm messages from Wyatt instruments to your Windows desktop by toggling on ASTRA's **System→Preferences→Show Desktop Alerts** menu option. Messages will be shown for a few second near the Windows taskbar. The messages fade automatically if you do not click on them.

WCS Client Application

For additional diagnostics, you may choose to run the WCS Client, which reports on the activities of the Wyatt Communications Server. This server manages communication between ASTRA and instruments.

You can start the WCS Client from the Windows Start menu by choosing **Programs→Wyatt Technologies→WCS→WCS Client**.

Typically, you would use this in cooperation with Wyatt Technical Support.

6

Creating & Running Experiments

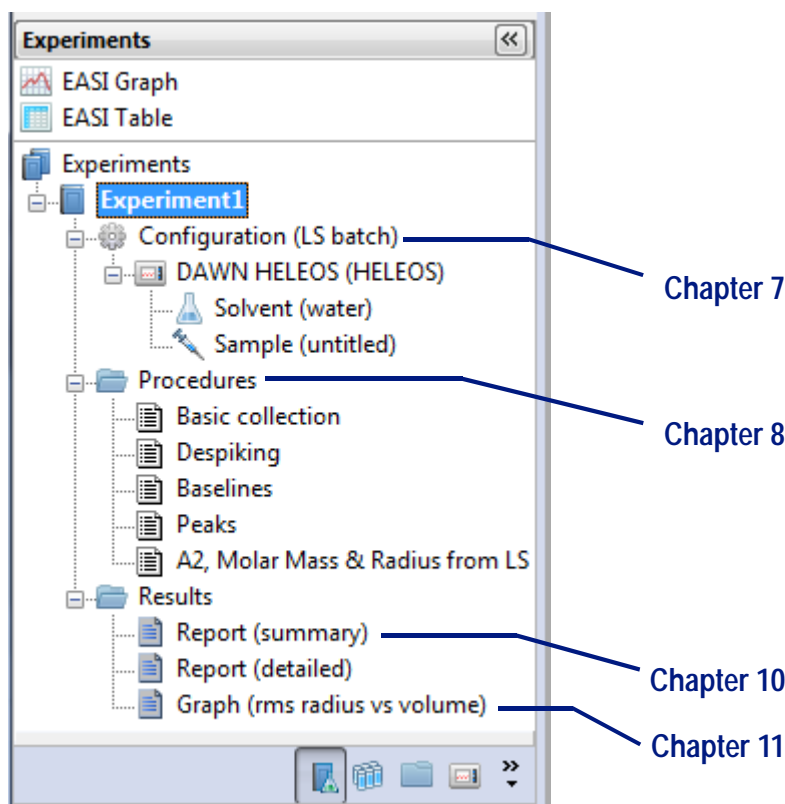
This chapter tells how to work with ASTRA experiments. The details of items contained in an experiment are covered in other chapters. This chapter describes actions you perform with the entire experiment, such as creating a new one, saving it, running it, or exporting it.

CONTENTS	PAGE
About Experiments.....	6-2
Creating New Experiments	6-4
Opening an Experiment from a File	6-7
Opening an Experiment from the Database.....	6-8
Importing an Experiment from a File	6-9
Running an Experiment	6-11
Viewing Experiment Properties	6-14
Closing an Experiment	6-15
Saving an Experiment to a File	6-15
Saving an Experiment to the Database.....	6-16
Exporting an Experiment.....	6-17
Creating a Method	6-18
Deleting an Experiment.....	6-20
Copying Data	6-21
Applying a Method	6-22
Adding Elements to an Experiment.....	6-24

About Experiments

The ASTRA user environment is centered around a structure we call an “experiment,” which contains all the information needed to run an experiment and produce results. After you run an experiment, the experiment structure contains the results.

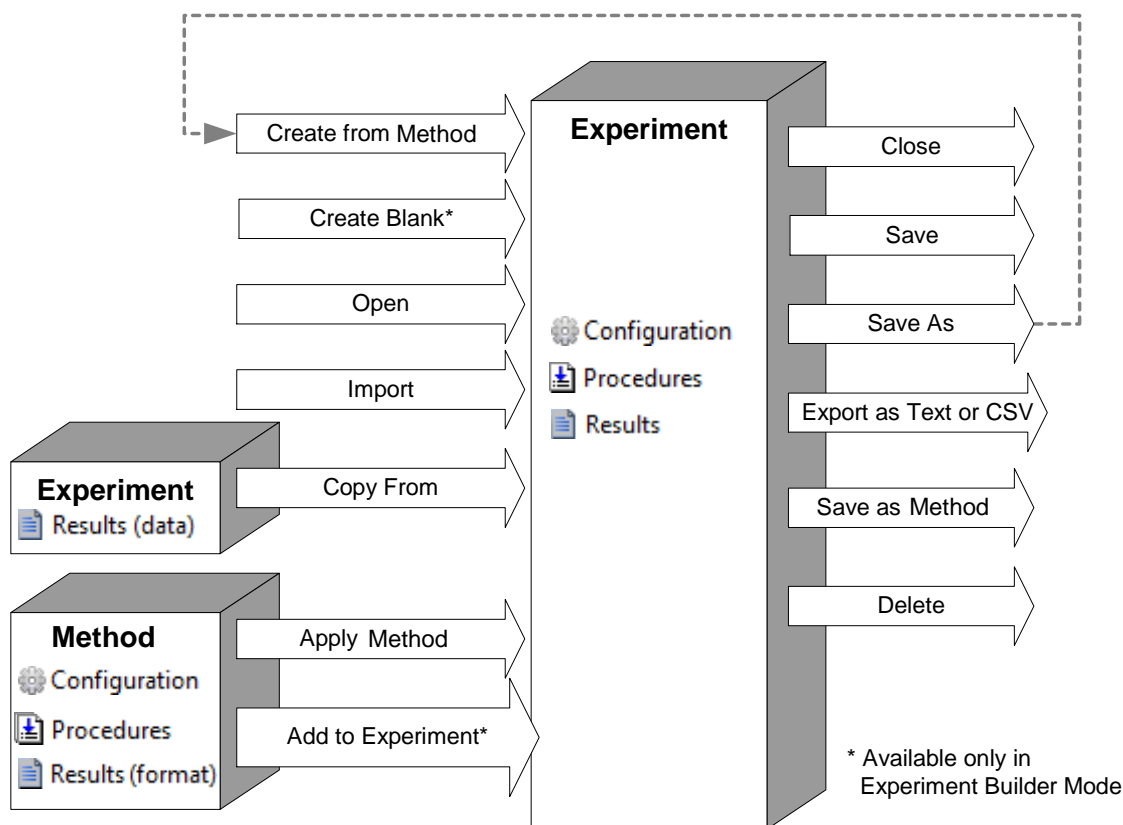
The Experiments navigation pane in ASTRA shows the parts of the experiment.



You can expand or collapse the folders in an experiment as desired. Each experiment contains the following categories of items:

- **Configuration:** The hardware devices and connections used in the experiment. For online (fractionated) experiments, this may include a pump, injector, solvent, sample, DAWN, Optilab, and data connections. For details on all types of items that may be configured and their properties, see Chapter 7, “Configuring Experiments”.
- **Procedures:** The actions to be performed in order when the experiment is run. There are configuration, collection, transformation, analysis, and administrative procedures. For details on all types of procedures, see Chapter 8, “Editing Procedures”.
- **Results:** The reports and graphs to be produced after the experiment procedure has been run. For details, see Chapter 10, “Working With Reports” and Chapter 11, “Working With Graphs and Tables”.

The actions you can perform on an experiment are shown in the following diagram. The arrows that point to the main experiment show ways to open, create, or bring information into an experiment. The arrows that point away from the main experiment show ways to close, save, or export information from an experiment.



The sections in this chapter listed below correspond to the actions shown in the previous diagram. Some commands behave differently depending on whether you are using ASTRA 6 Basic or a version that uses an experiment database (ASTRA 6 with Research Database or ASTRA 6 with Security Pack).

Table 6-1: Actions to Perform on Experiments

Action	Description	See
Create From Default	Make a new experiment based on a method that was saved as the “default method”.	page 6-4
Create From Method	Make a new experiment based on a configuration, procedure, and results method.	page 6-5
Create Blank	Make a new experiment with no default configuration, procedure, or results.	page 6-6
Open	Basic: Open an experiment from a file. This may include experiments saved with previous versions of Wyatt software. Database: Open an experiment from the experiment database.	page 6-8 and page 6-7
Import	Database: Open an experiment saved in a file. This may include experiments saved with previous versions of Wyatt software.	page 6-9

Table 6-1: Actions to Perform on Experiments


Action	Description	See
Export	Save an experiment to a file, or save experiment data to a tab-delimited or comma-separated values file.	page 6-17
Close	Close the current experiment.	page 6-15
Save	Basic: Save an experiment to a file. Database: Save an experiment to the experiment database.	page 6-16 and page 6-15
Save As	Basic: Save an experiment to a file with a different name. Database: Save experiment to the database with different name.	page 6-16 and page 6-15
Export as Text or CSV	Create a text or comma-separated file containing experiment data.	page 6-17
Save As Method	Save the configuration, procedure, and results formats so that they can be used as the basis for future experiments.	page 6-18
Delete	Delete the experiment from the experiment database.	page 6-20
Run	Run the experiment procedure.	page 6-11
Run Indefinitely	Run the experiment procedure ignoring the Duration.	page 6-11
Copy From	Copy results data from one experiment to another.	page 6-21
Apply Method	Create a copy of the experiment. Then apply the analysis procedures and result presentation from the selected method to the copy of the experiment.	page 6-22
Add to Experiment	Add items to the configuration, procedure, or reports. (Experiment Builder mode only.)	page 6-24
Editing Configuration	Add, remove, or change items in the experiment configuration.	page 7-5

Creating New Experiments

The recommended way to create an experiment is from a system method provided with ASTRA. For more customization, you may want to save experiments as methods and use those. Experiment Builders may choose to create experiments from a blank method; however, using a provided method saves time.

Creating Default Experiments

If you have specified a “default” experiment method (see page 6-19), you can choose **File→New→Experiment from Default** to quickly create a new experiment from this method.

You can quickly create and start running an experiment using the default method by choosing **Processing→Run Default** (Ctrl+J) or clicking the  toolbar icon. The experiment is created and begins data collection automatically. See page 6-19 for information on creating default methods.

Creating Experiments from Methods

Methods can be used to create new experiments, or to re-analyze data in a different way. ASTRA comes with over three dozen system methods that allow you to start using ASTRA at its full potential immediately.

Experiment methods set properties to values you are likely to use. There are just a few properties you typically need to modify.




You must have at least Technician access to create an experiment from a method. You must have at least Researcher access to modify an experiment that was created from a method.

To create an experiment from a method, follow these steps:

1. Choose **File→New→Experiment From Method**.

Shortcuts: Press Ctrl+Alt+T.

Click the down-arrow next to the  icon.

2. In the New from Existing dialog, open the folder that contains the experiment method you want to use. You can choose from the following folders:

- **System > Methods folder:** These methods are provided with ASTRA for your use. A set of experiment methods is provided for each Wyatt instrument. These methods typically provide a starting point for most experiment types you might perform.
- **Other folders:** You may have saved your own methods in a folder you created, such as **My Methods**. See “Creating a Method” on page 6-18.

If you read about an experiment method you want to use, but don't see it in the New from Existing dialog, see “Migrating the System Database” on page 2-4 to update your system database so you have all the latest experiment methods and system profiles.

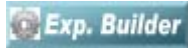
3. Select a method and click **Create**.

A new experiment is created based on the method you selected.

Methods you use to create experiments are stored in the ASTRA system database. They are not stored in separate files in the installation tree.

When you create an experiment from a method, the Physical Instrument value configured for instruments in the method are automatically set to match instruments of the appropriate type that are in your instrument list. See “Viewing the Instruments Pane” on page 2-12.

Creating Experiments from Scratch



You can create and modify blank experiments only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.




You must have at least Researcher access to create an experiment from scratch.

You can modify methods and save experiments as methods, so it is unlikely that you will want to work starting from an empty experiment. However, if you want to create an empty experiment, follow this step:

- Choose **File→New→Blank Experiment**.

Shortcuts: Press Ctrl+N.

Click the down-arrow next to the  icon.

Right-click “Experiments” in the workspace and choose **New→Blank**.

Opening an Experiment from a File




You can open and work with any experiment you have saved with ASTRA 4, ASTRA V, or ASTRA 6. If you are using ASTRA 6 Basic, experiments are stored in separate files with an extension of *.afe6.

To open an experiment, follow these steps:

1. Choose **File→Open→Experiment**.

Shortcuts: Press Ctrl+O.

Click the down-arrow next to the  icon.

Right-click “Experiments” in the workspace and choose **Open**.

Drag-and-drop an experiment file from Windows Explorer or the desktop to the ASTRA window.

Open a recently used experiment from the **File→Recent Files** list.

2. In the Open dialog, navigate to the folder that contains the experiment you want to import.
3. Select a file and click **Open**.

You can open any of the following types of files:

File Extension	Description
*.afe6	Experiment file saved or exported by ASTRA 6.
*.vaf	Experiment file saved or exported by ASTRA V.
*.vrf	ASTRA V or ASTRA 6 crash recovery file. (See page 6-14.)
*.adf	File saved by ASTRA version 4.70 or higher for a DAWN EOS, DAWN DSP, or DAWN DSP-F. See “Importing ASTRA 4 Files” on page 6-9 for information about fixing any problems with these files.
*.mdf	File saved by ASTRA 4 for a miniDAWN.
*.nwf	File saved by DNDC 5.
*.rwf	File saved by RICAL 5.

Imported experiments have a complete set of configuration items, procedures, and results needed to view the experiment.

Opening an Experiment from the Database

Database You can open and work with any experiment you have saved.


If you are using ASTRA 6 with Research Database or ASTRA 6 with Security Pack, experiments are stored in the experiment database, and you open experiments from that database. To open experiments stored in separate files (such as exported experiments or experiments saved with ASTRA 4), see “Importing an Experiment from a File” on page 6-9.

Security There are no access level restrictions on opening an experiment.

To open an experiment, follow these steps:

1. Choose **File→Open→Experiment**.

Shortcuts: Press Ctrl+O.


Click the down-arrow next to the  icon.

Right-click “Experiments” in the workspace and choose **Open**.

Open a recently used experiment from the **File→Recent Files** list.

2. In the Open dialog, select the experiment you want to import.

Unless you have created a folder in the database, all the experiments are in the top-level “/” folder.

As in standard file selection dialogs, you can click the  icon to change the way the list of experiments in the database is viewed. In the detail view, the last data and time the experiment was modified is shown.

3. Select an experiment and click **Open**.

Tips: You can open multiple experiments by holding down Shift key (for a range) or the Ctrl key (for individual experiments) while selecting experiments.

Importing an Experiment from a File



This item is disabled in ASTRA 6 Basic since it is identical to **File→Open→Experiment**.



You can import experiments stored in files. This includes experiments saved with ASTRA 4, ASTRA V, and ASTRA 6 Basic. It also includes experiments exported by ASTRA V and ASTRA 6.



You must have at least Researcher access to import an experiment.

To import an experiment, follow these steps:

1. Choose **File→Import→Experiment**.

Shortcuts: Press Ctrl+I.

Right-click “Experiments” in the workspace and choose **Import**.
Drag-and-drop an experiment file from Windows Explorer or the desktop to the ASTRA window.

2. In the Import Experiment dialog, navigate to the folder that contains the experiment you want to import.
3. Select a file and click **Open**.

You can open any of the following types of files:

File Extension	Description
*.afe6	Experiment file saved or exported by ASTRA 6.
*.vaf	Experiment file saved or exported by ASTRA V.
*.afs6	ASTRA 6 sequence file.
*.vsf	ASTRA V sequence file.
*.vrf	ASTRA V or ASTRA 6 crash recovery file. (See page 6-14.)
*.adf	File saved by ASTRA version 4.70 or higher for a DAWN EOS, DAWN DSP, or DAWN DSP-F. See “Importing ASTRA 4 Files” on page 6-9 for information about fixing problems with these files.
*.mdf	File saved by ASTRA 4 for a miniDAWN.
*.nwf	File saved by DNDC 5.
*.rwf	File saved by RICAL 5.

Imported experiments have a complete set of the configuration items, procedures, data set definitions, and results needed to view the experiment.

Importing ASTRA 4 Files

ASTRA 6 can automatically import most ASTRA 4 experiments. However, some issues may occur when importing certain files. For this reason, an Import Wizard allows you to attempt to fix such problems when importing the file.

Typical issues that may cause problems are that some ASTRA 4 files store smoothed data, but not the original data. Also, some files may not contain information about how AUX channels were used to receive RI or UV data for concentration calculations. Click the **Details** button for a description of the results fitting changes needed by your experiment.

When you open or import an ASTRA 4 file (*.adf or *.mdf), you see this dialog. The default is an automatic import; most other options are grayed out.

To import ASTRA 4 files:

1. If your ASTRA 4 file had a standard configuration (such as, batch, particles, or RI detector alone), click **OK** in the Import Wizard to try an automatic import.
2. If you had a UV detector instead of an RI detector, or in addition to an RI detector, choose the **Specify AUX Detectors** item. Then specify how your RI and/or UV detectors were connected. Note that only one detector should have the **Concentration Detector** box checked. For an RI detector, select the model. For a UV detector, specify the cell length and response value. Then click **OK**.
3. Double-click on a procedure with a graph (such as Peaks) or a results graph. If the graph makes sense, the experiment was imported correctly and you need not repair the file. If the graph looks like a scatter plot (when it should not), close the experiment without saving, and continue to the next steps.
4. Reimport the ASTRA 4 file. Check the box in the **Repair** area to remove smoothed or despiked data. Click **OK**.
5. Again, check to see if the resulting graphs look valid. If they do, the file was imported correctly.
6. If you continue to encounter difficulties importing a file created with ASTRA 4, DNDC, or RICAL, please contact Wyatt Technology Corporation for assistance.


Running an Experiment

Once you have set up an experiment in ASTRA (and the corresponding instruments, connections, solvents, and samples are ready), you can run the experiment.

Security You must have at least Technician access to run experiments.

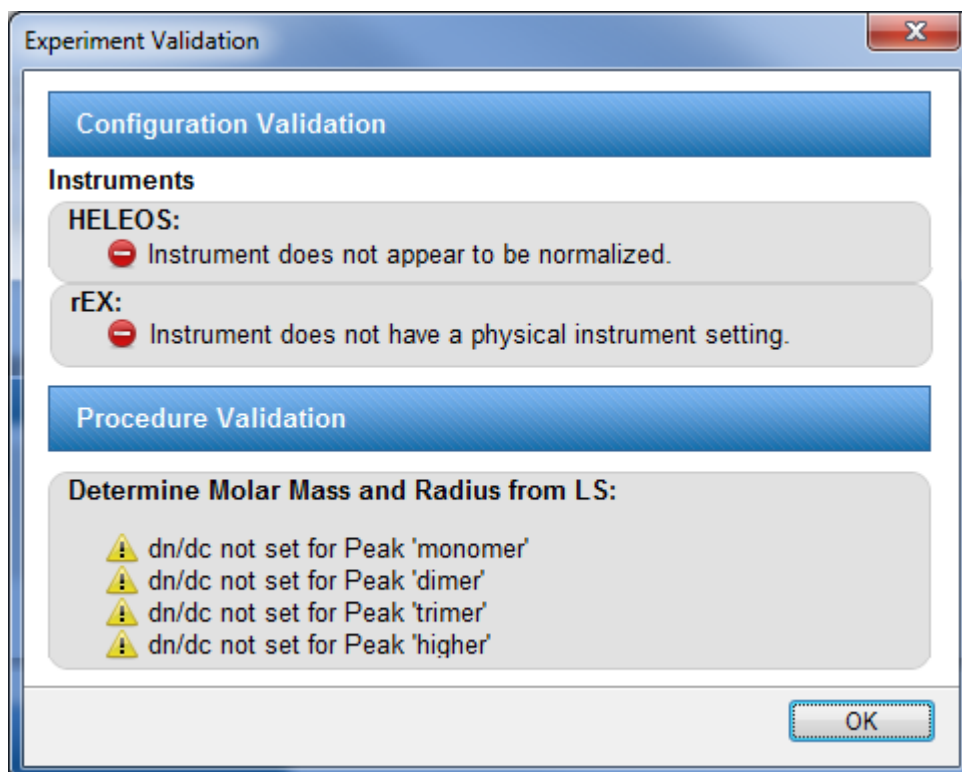
Validating an Experiment

You can validate an experiment's procedure list and configuration by choosing **Processing→Validate**.

Shortcuts: Press Ctrl+Shift+V.
Click the Validate icon  in the experiment toolbar.





Validation checks the procedure order for conflicts. If the experiment collects data, validation also checks that the necessary instruments are connected and available. It checks to make sure light scattering instruments are normalized. It checks to make sure the experiment configuration contains a solvent and a sample. It checks for values that may be required depending on the instruments you are using and the procedures to be run. For example, these values may include dn/dc, UV extinction, and solvent viscosity. In addition, validation checks the collection script if you write a custom script.

The results of a validation are shown in an Experiment Validation dialog:



If any procedure in the list has a red X on its icon, it is in an invalid location in the experiment or the configuration is missing instruments that produce data needed by the procedure. Modify the procedure list as described in “Setting the Procedure Order” on page 6-27 or revise the experiment configuration to include the appropriate instruments.

A procedure’s state is always indicated by its icon, as follows. (Collection procedures have a special two-page icon for all states.)

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid location or does not have the necessary data to run.

Starting a Data Collection Run

To start the experiment run, follow these steps:

1. Begin by turning on, warming up, and stabilizing your experimental apparatus. When everything is ready to go, continue with the following steps in ASTRA.
2. Choose **Processing→Run**.


Shortcuts: Press Ctrl+Shift+R.
Click the Run icon  in the experiment toolbar.

You can alternately choose **Processing→Run Indefinitely** to run the experiment until you stop it. This command ignores the Duration property for the collection. (The experiment will also stop collecting data if your disk or database runs out of storage space.)

3. For an experiment with a basic collection procedure (as opposed to a custom script), you will be prompted to click **OK** and then inject the sample.
4. The spinning hourglass icon on the experiment node in the workspace shows that the experiment is running. Alternately, the drop-down in the experiment status toolbar shows the current state of the experiment. You see data as it is collected and can use the checkboxes to turn on or off data traces from various sources. Light scattering data is red; RI data is blue; UV data is green, QELS data is magenta, and viscosity data is black. Markers are shown for any autoinjection signals.
5. During the experiment, you are prompted for any information a procedure needs in order to run. For example, for light-scattering experiments, you will probably need to set baselines and peaks after the data is collected.



6. If you are using ASTRA 6 with Security Pack, you will likely be prompted to sign off on an experiment after it runs.

You can quickly create and start running an experiment using the default method by choosing **Processing→Run Default** or clicking the  toolbar icon. The experiment is created and begins data collection automatically. See page 6-19 for information on creating default methods.

Signing Off on an Experiment



Security If you see a message that says some parameters are not set for the Sign off procedure, follow these steps:

1. Click **OK**. You will see the Sign off procedure page.

2. In the Sign off page, choose a sign off category from the list. The categories are as follows:


Category	Description
Unsigned	This is the initial state for a sign off. You must select another category.
Responsibility	Selecting this category indicates responsibility for performing the experiment according to procedures.
Approval	Selecting this category indicates approval of the experiment.
Review	Selecting this category indicates review of the experiment.

3. In the Comments field, type any additional information required by your standard operating procedures.
4. In the User id, Password, and Domain fields, type the values for your valid ASTRA account, as described in the section on “Starting ASTRA” on page 3-2. Be sure to use uppercase and lowercase correctly in your password. Note that it is not necessary for the person signing off on the experiment to be the same person who logged in initially to begin the ASTRA session.
5. Click **OK**.

Stopping an Experiment

To stop a running experiment, choose **Processing→Stop**.


Shortcuts: Press Ctrl+Shift+S.

Click the Stop icon  in the experiment toolbar.

Stopping an experiment with ASTRA stops only the selected experiment from executing. This includes the collection and analysis of data. It does not affect the execution of other experiments in ASTRA, nor does it affect any activity going on outside of ASTRA's control.

See your hardware documentation for information about alarms, emergency stops, and setting up safety interlocks. Alarms may be monitored via the Diagnostic Manager. See “Viewing the Log with the Diagnostic Manager” on page 5-10 for details.

Re-Running an Experiment for Data Processing

If you modify one or more procedures in an experiment, you can re-run the experiment using the **Run** command. This time, instead of collecting data, only the procedures marked with the  not-run icon are performed.

If a Crash Occurs...

While data is being collected, ASTRA stores data in a “crash recovery file” in your public documents folder. This is usually C:\Users\Public\Documents\ASTRA 6 Recovered Files. The filename contains the current date and time and have a file extension of .vrf. For example, 20080324_221518237Experiment.vrf.

When the data collection is complete and data is successfully saved, this file is automatically deleted.

When saving over the network, a problem may occur, for example, if the network connection fails or if you do not have permission to save to a particular location. When this happens, the save fails and the crash recovery file is retained.

Each time ASTRA starts, it checks for crash recovery files in the public documents location. If a .vrf file is present, ASTRA asks if you want to see the recovered data file.

Viewing Experiment Properties

You can find details about how the current experiment is stored by choosing **File→Properties**. The General tab of the Properties dialog shows information like the location, version of ASTRA used, and creation and access dates for the experiment. The Summary tab lets you enter information about the experiment, such as an author, manager, company, keywords, and description.

Note:	Property viewing works only with ASTRA 6 files. Older (ASTRA 4/V) files do not contain the necessary information.
--------------	---

For information about how the experiment was performed, see “Experiment Configuration” on page 7-14.

Closing an Experiment

You can work with multiple experiments open in ASTRA.

To close an experiment without exiting from ASTRA, follow these steps:

1. In the experiment tree, select an item in the experiment you want to close.
2. Choose **File→Close**.

Shortcuts: Right-click the experiment name in the tree and choose **Close**.
Choose **File→Close All** to close all experiments at once (unless data collection is in progress).

3. If you have made unsaved changes, you are asked whether you want to save them.

Saving an Experiment to a File




It is a good idea to save experiments frequently.

If you are using ASTRA 6 Basic, experiments are stored in files with an extension of *.afe6.

To save an experiment, follow these steps:

1. Choose **File→Save**.

Shortcuts: Press Ctrl+S.
Click the  icon.
Right-click the experiment name in the tree and choose **Save**.

2. If this is the first time you have saved this experiment, you see the Save As dialog. Otherwise, you are finished saving the file.
3. In the Save As dialog, navigate to the folder you want to contain the file.
4. In the File Name field, type a file name for the experiment. The following characters may not be used in ASTRA file names:

colon	:
question mark	?
quote	"
asterisk	*
forward slash	/
backslash	\
less than	<
greater than	>
pipe	

5. The Save As Type field shows that the file will be saved with an extension of *.afe6. You can choose an older version of ASTRA if you like. To save to an ASTRA 4, tab-delimited text, or comma-separated values format, see “Exporting an Experiment” on page 6-17.
6. Click **Save**.

To save an experiment with a different name or location, choose **File→Save As** and follow steps 3 through 6 above.

Shortcuts: Right-click the experiment name in the tree and choose **Save As**.

Saving an Experiment to the Database


Database If you use ASTRA 6 with Research Database or ASTRA 6 with Security Pack, experiments are saved in the ASTRA database. To save experiments in separate files, see “Exporting an Experiment” on page 6-17.


It is a good idea to save experiments frequently.

Security You must have at least Technician access to save an experiment.

To save an experiment, follow these steps:

1. Choose **File→Save**.

Shortcuts: Press Ctrl+S.
Click the  icon.
Right-click the experiment name in the tree and choose **Save**.

2. If this is the first time you have saved this experiment, you see the Save As dialog. Otherwise, you are finished saving the file.
3. Type a name for the experiment.
4. If you want to store this experiment in a subfolder, click the  New Folder icon and type a name for the folder. Then open the folder.
5. Make sure the Of Type field shows “Experiments”. For information about saving methods, see “Creating a Method” on page 6-18.
6. Click **Save**.

To save an experiment with a different name or location, choose **File→Save As** and follow steps 3 through 6 above.

Shortcuts: Right-click the experiment name in the tree and choose **Save As**.

Exporting an Experiment

You can export experiment data to separate files. These files can be imported by ASTRA, or you can save experiments in formats that can be imported by spreadsheets.



If you are using ASTRA 6 Basic, this command allows you to export experiments as ASTRA 4, tab-delimited, and comma-delimited files. To save an experiment to a different *.afe6 file in the current ASTRA format, use the Save As command instead.



You must have at least Researcher access to export an experiment.

To export an experiment, follow these steps:

1. Choose **File→Export**.

Shortcuts: Right-click the experiment name in the tree and choose **Export**.

2. In the Export Experiment dialog, navigate to the folder you want to contain the exported file. (Do not export the experiment to a folder that is a read-only folder, such as Sample Data or Analyzed Experiments.)
3. In the File Name field, type a file name for the experiment.
4. In the Save As Type field, select a type. The formats you can export are as follows:

Type	Description
*.afe6	ASTRA 6 file that can be imported by ASTRA 6 on this or another computer. (Use Save As instead for ASTRA 6 Basic.)
*.vaf	ASTRA V file. You can choose a version of ASTRA for compatibility with earlier versions.
*.adf	An ASTRA 4 format for use with earlier versions of ASTRA.
*.txt	Tab-delimited text file for exporting a data set defined by the selected data set definition. This format is easily imported into most spreadsheets.
*.csv	Interpolated comma-delimited text file for exporting a data set defined by the selected data set definition. This format is easily imported into most spreadsheets. Data traces are interpolated so that all measurements are on the same time scale, allowing for easy display in software such as Excel.

5. If you choose a .txt or .csv format, you can also choose a data set definition to export with the experiment. See “Creating Data Set Definitions” on page 11-9 for information about data set definitions.
6. Click **Save**.

If you do not find the organization of the tab-delimited or comma-delimited output useful, try the output described in “Exporting Data” on page 11-20.

Creating a Method

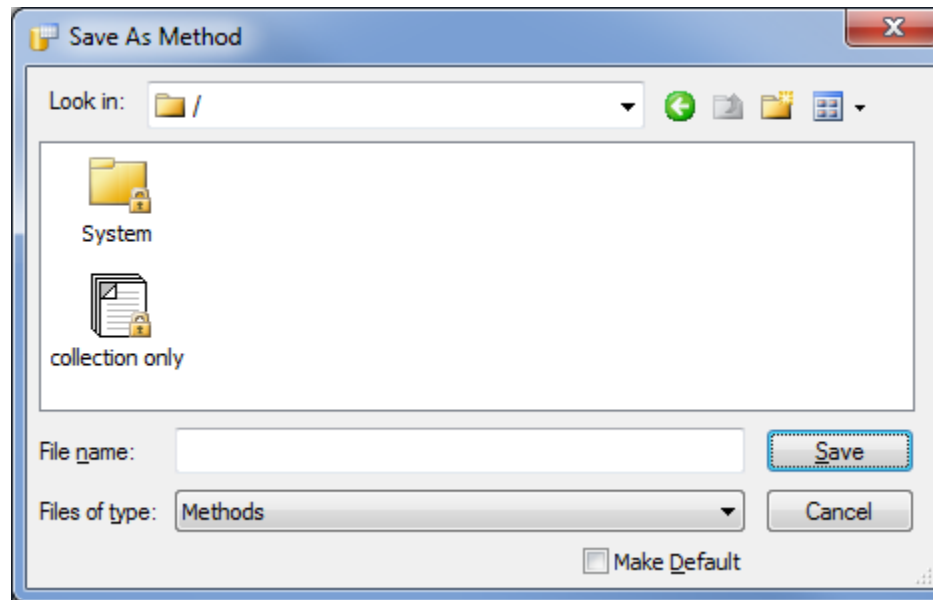
After you modify an experiment, you may want to save it as a method for other experiments. The saved method includes the configuration, procedure, and result formats.




You must have at least Researcher access to save methods.

To save a method, follow these steps:

1. Choose **File→Save As Method**.



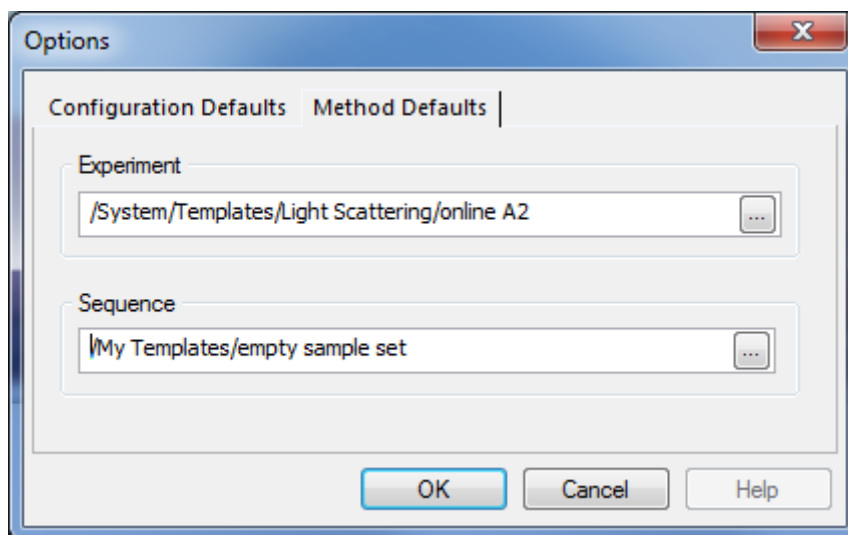
2. Navigate to the location in the system database where you want to save your method.
You can create a subfolder by clicking the  New Folder icon and typing a folder name. Then open the new folder. For example, you might want to create a new folder called **My Methods** and store your methods in that folder.
3. Check the **Make Default** box if you want this method to be the default for use with the **File→New→Experiment from Default** command.
4. Click **Save**.

Note: Methods are saved in the system database. This database is separate from the experiment database.

Setting a Default Method

If you have not yet set a default method, you are asked if you want to set one when you start ASTRA. If you click **Yes**, the Configuration Wizard opens and you can use it to define your default method. See “Using the Configuration Wizard” on page 7-11 for details.

You can also specify the default experiment method by choosing **System→Preferences→Options**. Select the **Method Defaults** tab.



The **Experiment** field shows the path to the current default method, if one is selected.

Next to the **Experiment** field, click the “...” button. In the Select Template dialog, browse for the existing method that you want to use as the default for new experiments.

When you choose **File→New→Experiment from Default**, the default method will be used to create a new experiment. The default method is also used in blank sequences as the default method for all samples in the set.

You can also set the default method when saving an experiment method by checking the **Make Default** box.

See “Setting a Default Sequence” on page 9-4 for information about setting a default sequence and “Setting a Default Configuration” on page 7-10 for information about setting configuration defaults.

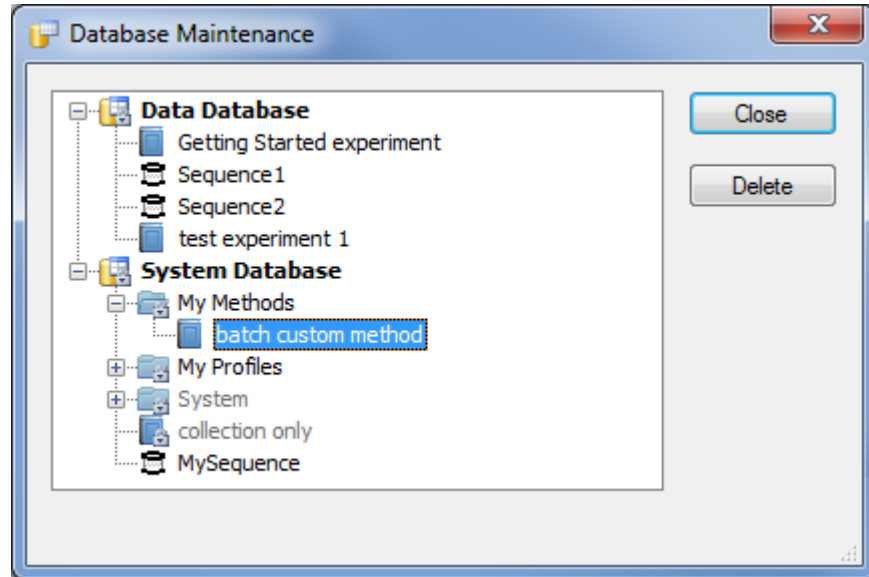
Deleting a Method



You must have at least Researcher access to delete methods.

To delete an experiment method, follow these steps:

1. Choose **System→Database Administration→Maintenance**. This opens the Database Maintenance dialog.



2. Find and highlight the method you want to delete. Methods are generally in the Data Database or the “My Methods” folder (if you have created one). You cannot delete methods that were saved as “read-only” methods. This includes the methods provided with ASTRA.
3. Click **Delete**.
4. Click **Close** when you have finished deleting methods.

Deleting an Experiment



If you are using ASTRA 6 with Security Pack, experiments can only be deleted by an ASTRA administrator. See “Deleting Experiments” on page 4-10 for details.



If you are using ASTRA 6 Basic, any user may delete an experiment by deleting the *.afe6 file that contains the experiment.

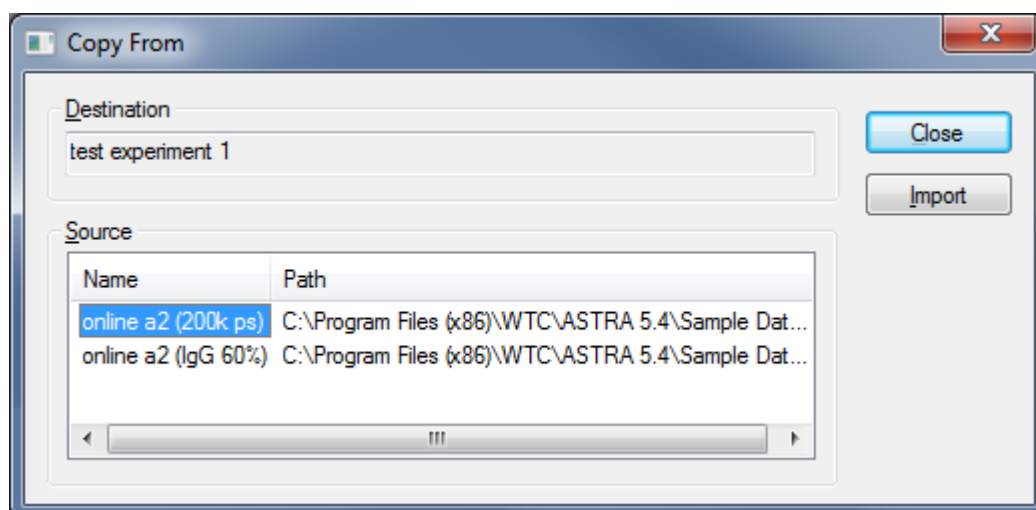
Copying Data

You can copy data generated for one experiment into another experiment. You might do this to create combined plots of results from several experiments, or to use the data in a procedure such as branching.

Note that the procedure described below is rarely needed. ASTRA provides buttons for importing data in various procedure pages for which is common to want to combine data. For example, see the **Import Linear** button described in “Branching” on page 8-85.

To copy data into an experiment, follow these steps:

1. Open both the source and destination experiments. The source experiment contains the data you want to copy. You will copy the data to the destination.
2. Activate the destination experiment by clicking on any part of the experiment in the workspace.
3. Choose **Experiment→Copy From**. The Copy From dialog appears.



4. Select the source experiment you want to use, and click **Import**. A Data Set Definition dialog for the source experiment is shown.
5. Select the data you want to copy into the destination experiment. See “Creating Data Set Definitions” on page 11-9 for information on using this dialog.
6. Click **OK** in the Data Set Definition dialog.
7. Repeat steps 4 through 6 for any additional data you want to copy.
8. When finished, click **Close** in the Copy From dialog.

After you have copied data, you can access it using a standard data set definition in the source experiment. The data set definition allows you to display the data in graphs. In addition, the data is available for procedures such as branching.

Applying a Method

You can apply the procedures and result formats from a method to an experiment you have already run to collect data. This allows you to perform multiple sets of procedures on the same set of raw data. (To apply a method to multiple experiments, see “Applying a Method to Multiple Experiments” on page 6-22.)

For example, after using the “LS batch (Debye plot)” method when collecting data, you might want to apply the “LS batch (Zimm plot)” method to the same data so that you can view the results differently.

Applying a method creates a separate experiment, so you do not lose any of the information in the original experiment. The new experiment has a name that reflects both the original experiment and the method.

If you apply a method that contains procedures intended for use with online data to an experiment that contains batch data (or vice versa), messages identify the procedures that are not compatible with the data.

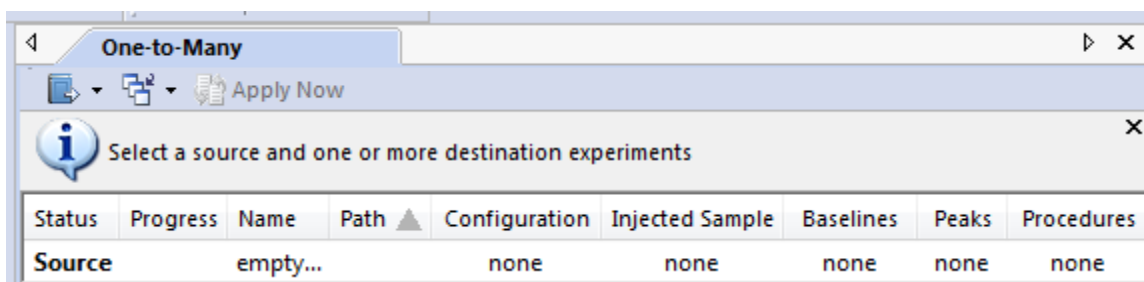
To apply a method, follow these steps:

1. Open the experiment that contains the raw data you want to use.
2. Choose **Experiment→Apply Method**. The New From Existing dialog appears. This is the same dialog you use to create an experiment from a method before data collection.
3. Choose a method to apply to the data. The procedure and result formats in the method will be used. Typically, you would choose a method from the “System > Methods” or “My Methods” folder.
4. Click **Create**. A new experiment is created and is run automatically. The name of the new experiment combines the names of the method used to create the experiment and the original experiment.
5. After the applied procedure runs, you can view the new results.

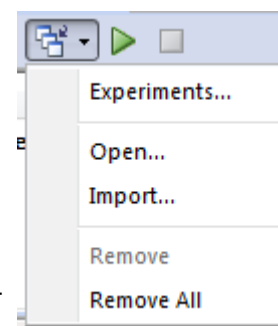
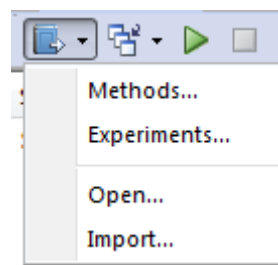
Applying a Method to Multiple Experiments

If you want to apply the same experiment method (or some aspect of an experiment or method) to multiple experiments, follow these steps:


1. Open all the experiments to which you want to apply a new experiment method.
2. Choose **File→One-to-Many**.



3. Click the **Source** icon and then select a method or experiment as the source of the method you want to apply to other experiments. If you choose **Methods**, you can browse the system database for a saved method. If you choose **Experiments**, you can select from the currently open experiments. You can also choose **Open** to open an experiment from the experiment database or **Import** to open an experiment stored in a file.
4. Click the **Add File(s)** icon and select an experiment to which you want to apply the source method. Choose **Experiments** to select one of the currently open experiments. You can also choose **Open** to open an experiment from the experiment database or **Import** to open an experiment stored in a file.
5. If you want to remove experiments from the destination list, click the **Add File(s)** icon and select **Remove** or **Remove All**.
6. Check the boxes for the items you want to apply from the source experiment or method. The choices are: the configuration, the injected sample, the baseline settings, the peak selections, and the procedures. By default, the configuration and procedures are applied, but the sample, baselines, and peaks are not.

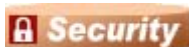


Status	Progress	Name	Path	Configuration	Injected Sample	Baselines
Source		vs + ri online (bsa).vaf	C:\Prog...	<input checked="" type="checkbox"/> Viscostar ...	<input type="checkbox"/> untitled2	<input type="checkbox"/> none
		online a2 (200k ps).vaf	C:\Prog...			
		online a2 (IgG 60%).vaf	C:\Prog...			

7. Click the Run icon  in the One-to-Many Processing window. The progress column will show which experiment is being processed.
8. A new experiment is created for each old experiment and is run automatically. The names of the new experiments combine the names of the method used to create the experiment and the original experiment.

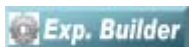
Adding Elements to an Experiment

This chapter provides simple examples for adding elements to the folders in an experiment tree. Other chapters are referenced to provide details about all the various configurations, procedures, data sets, and results that can be added and how to work with these things after adding them to an experiment.



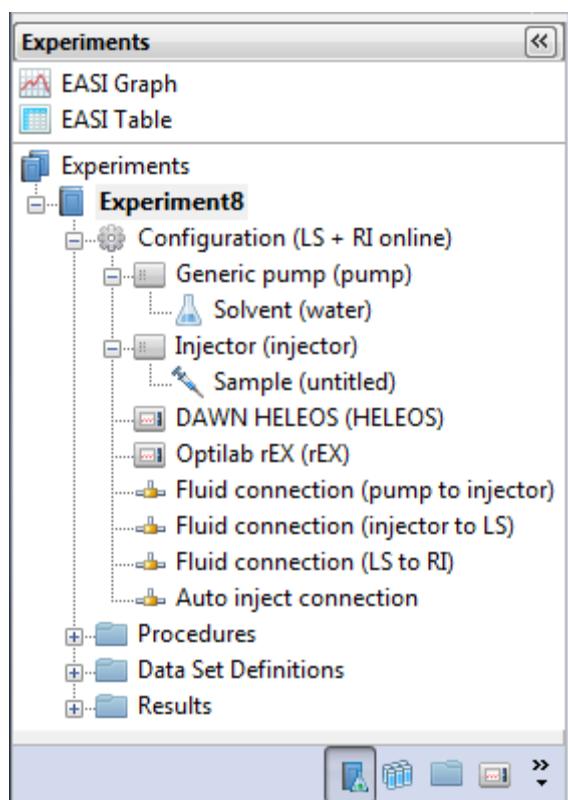
You must have at least Researcher access to add elements to experiments.

Adding to the Configuration



You can add items to the configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

The Configuration tree in the Experiments navigation pane shows the hardware configured to be used in the experiment. The methods provided with ASTRA include most instruments and connections you use in a typical experiment. Chapter 7, “Configuring Experiments” contains details about the properties that can be set for each instrument type.

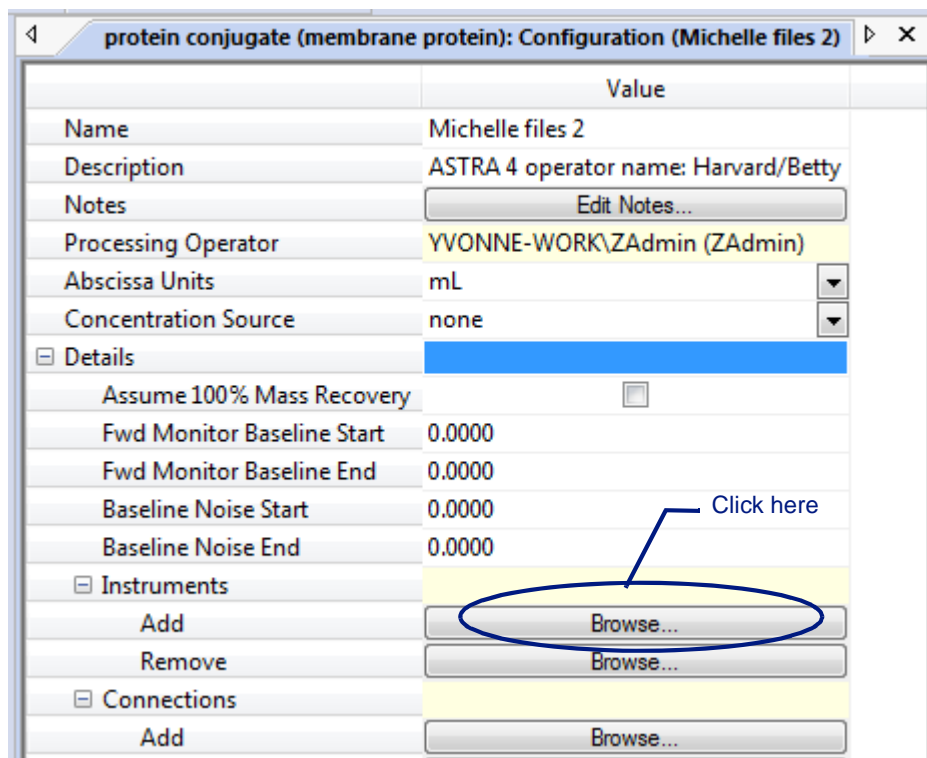


To add instruments and connections, you can specify them in the Configuration tab of the Experiment Configuration properties page. To add an instrument, follow these steps:

1. Choose **Experiment→Configuration→Edit**. This opens the properties page for the experiment configuration, which has a tab for each item in the configuration tree.

Shortcuts: Double-click any part of the configuration tree in the Experiments navigation pane.

2. In the Configuration tab, click the **Browse** button in the row to add instruments.



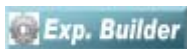
3. In the Add Instrument dialog, find a profile for the instrument you want to add to the experiment. For example, you might navigate to the /Profile/Instrument/UV Instrument folder to select a profile of a UV instrument.

Note: If you want to have custom instrument profiles available in addition to the profiles provided with ASTRA, see “Saving as a Profile” on page 12-4.

4. Select the instrument profile you want to add and click **Open**. The instrument is added to your experiment configuration and you can edit its properties by double-clicking it in the configuration tree.
5. To add a connection, follow the same steps but click the **Browse** button in the row to add connections.

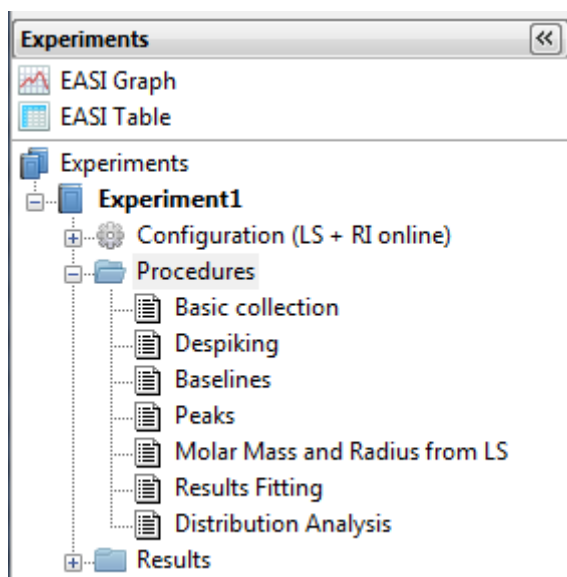
Importing an experiment configuration (with **Experiment→Configuration→Replace**) replaces the entire experiment configuration (all instruments and connections) with a different experiment configuration. Adding an instrument or connection adds only that item without replacing or removing other items.

Adding Procedures



You can add procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

The Procedures folder in the experiment tree shows actions ASTRA performs in order when you run the experiment. For details on all types of procedures, see Chapter 8, “Editing Procedures”.

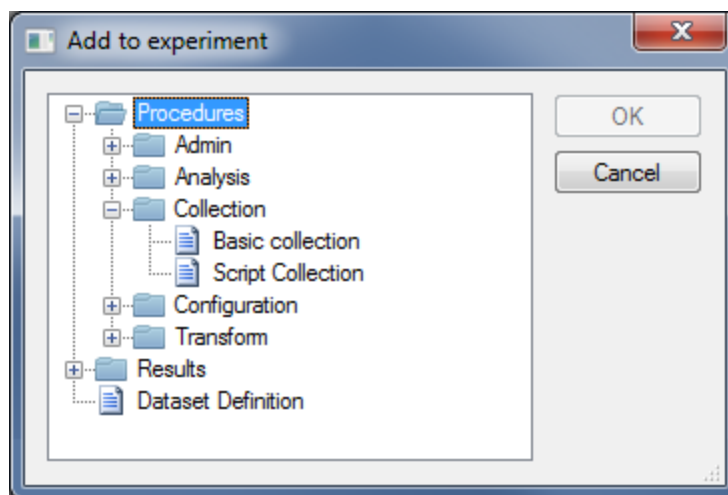


To add a procedure to an experiment, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results folders of the experiment.

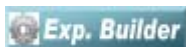
Shortcuts: Press Ctrl+Shift+P.
Right-click on experiment name in the experiment tree and choose **Add to Experiment**.

2. Open a folder under Procedures and select a procedure you want to add. See Chapter 8, “Editing Procedures” for detail on all procedures.



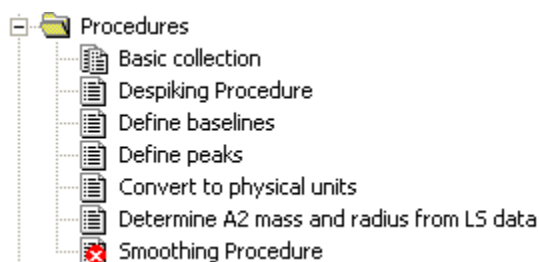
3. Click **OK**.

Setting the Procedure Order



You can change the order in which procedures are performed if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

If a procedure is in an invalid location in the experiment, it will have a red X on its icon.



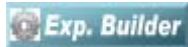
To correct the problem, drag the procedure to a location in the list where its icon and the icons that follow it have no red X.

Chapter 8, “Editing Procedures” has details on the correct location in the list for each procedure, and the data required for each procedure to be run.

Note that the standard experiment methods already contain the necessary procedures for collecting and analyzing the data in the correct order.

Adding Data Set Definitions

Data set definitions are a definition of data that ASTRA uses to create graphs. For example, a data set definition is needed to specify the contents of a custom plot. For details about creating and using data set definitions, see Chapter 11, “Working With Graphs and Tables”.



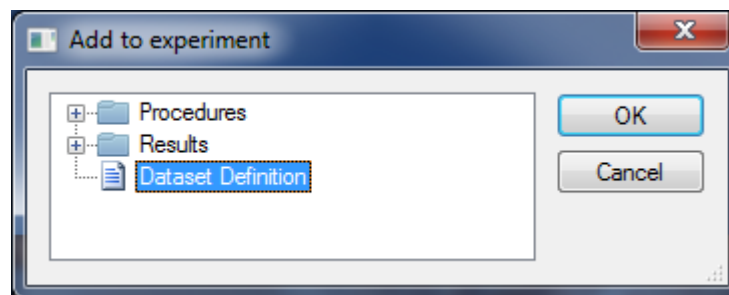
You can create data set definitions only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. You can see the Data Set Definitions folder in the workspace only if you opened the experiment while in Experiment Builder Mode.

To add a data set definition to an experiment, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog.

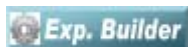
Shortcuts: Press Ctrl+Shift+P.

2. Select Data Set Definition and click **OK**. The data set definition is added to the Data Set Definitions folder of the experiment tree.



3. See Chapter 11, “Working With Graphs and Tables” for details about specifying the data in the set.

Adding Reports and Graphs



You can add reports only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

In Run mode, you can add graphs by choosing **Experiment→Graph→Add Custom Plot** or **Experiment→Graph→Add Parametric Plot**.

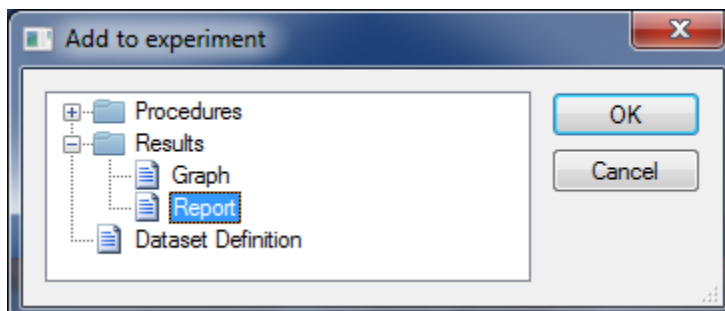
The results of an experiment are available through the reports and graphs you add to an experiment. For details about customizing reports and graphs, see Chapter 10, “Working With Reports”.

To add a report or graph to an experiment, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog.

Shortcuts: Press Ctrl+Shift+P.

2. Open the Results folder and select Graph or Report.



3. Click **OK**. The graph or report is added to the Results folder of the experiment tree.
4. See Chapter 10, "Working With Reports" for details about reports and Chapter 11, "Working With Graphs and Tables" for details about graphs.

7

Configuring Experiments

This chapter explains how to configure your experiments in ASTRA to reflect the instruments, connections, solvents, and samples you use to collect and process data. This is accomplished using ASTRA 6 configurations and profiles.

CONTENTS	PAGE
About Configurations and Profiles.....	7-2
Using Configurations.....	7-5
Using the Configuration Wizard	7-11
Experiment Configuration.....	7-14
Light Scattering Instrument Profiles	7-16
Refractive Index Instrument Profiles	7-22
Viscometry Instrument Profiles	7-26
UV Absorption Instrument Profiles.....	7-29
Injector Profiles	7-31
Sample Profiles.....	7-32
Pump Profiles.....	7-33
Solvent Profiles	7-34
Column Profiles.....	7-36
Connection Profiles	7-38

About Configurations and Profiles

The configuration of an experiment reflects not only the physical apparatus used to collect the data, but also elements such as the solvent and sample. In describing the configuration, ASTRA 6 breaks up the different parts of the experiment into logical units called *profiles*.

The types of profiles that are available are shown in Figure 7-1. Each instrument has a profile that contains parameters specific to that instrument. In addition, connections between instruments are represented by profiles. Finally, elements used in the apparatus, such as the solvent and sample, are represented by profiles as well.

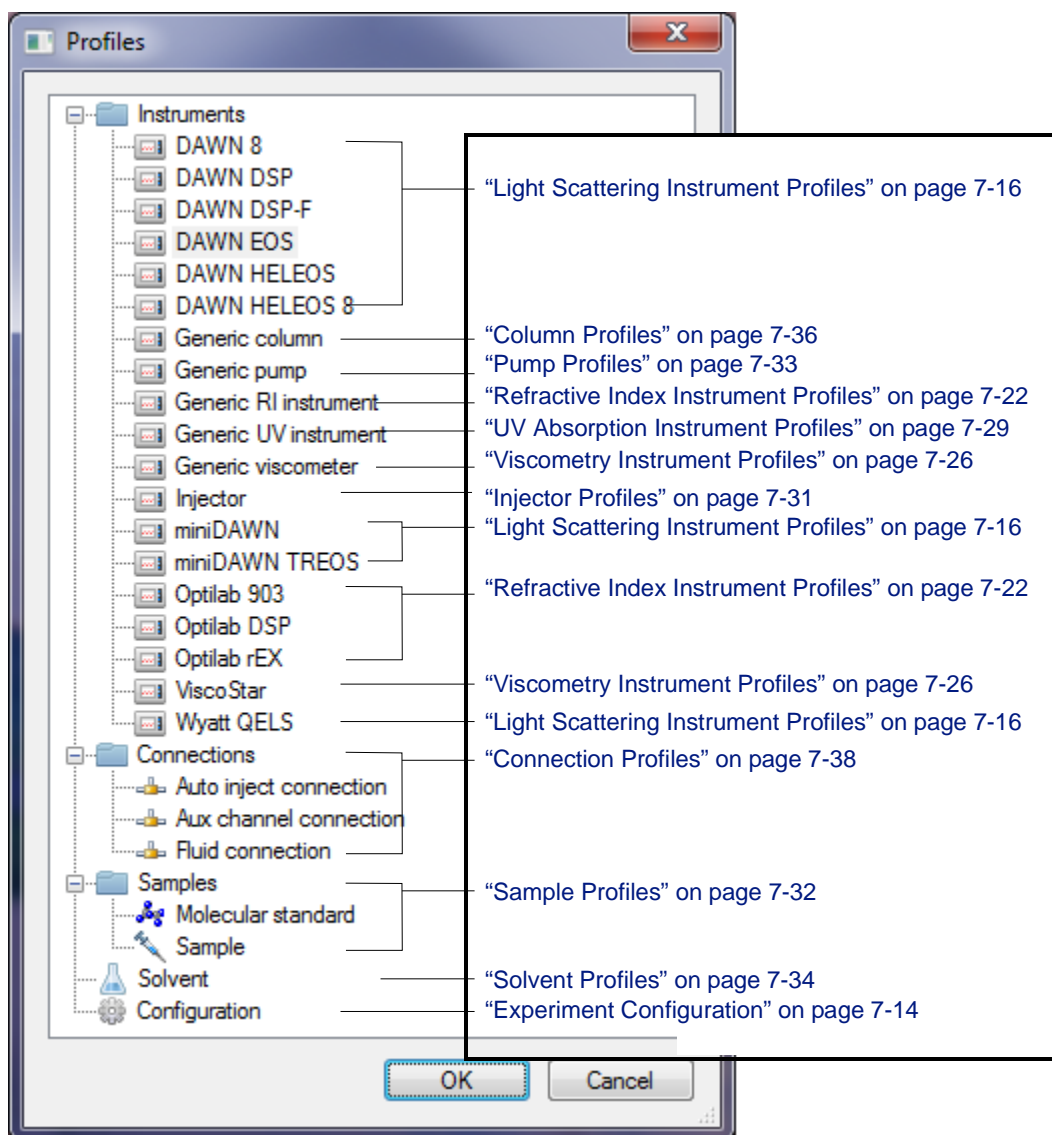
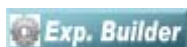


Figure 7-1: Profile types available in ASTRA 6

In an experiment, the set of profiles describe how an experiment is set up. This is called the *configuration*. You can also create profiles that are stored outside of experiments and can be copied into experiments as needed; these are called *profiles*.

This chapter focuses on using profiles in configurations. The experiment methods provided with ASTRA contain commonly used configurations. However, as you gain more experience using ASTRA, you may want to use profiles in conjunction with your experiments. See Chapter 12, “Working with Profiles” when you are ready to learn more about using profiles.

In Run mode, you use the configuration items provided in the methods. While you can modify the properties of items, you cannot add items to or remove items from a configuration.



You can add items to a configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties page, you need to close and reopen it after enabling Experiment Builder mode.



You must have at least Researcher access to work with configurations and profiles. If you are a Technician or Guest, you have read-only access to profiles.

This chapter describes the types of profiles contained in configurations and how their properties can be modified. Each profile type has a property list similar to that shown in Figure 7-2.

	Value
Name	injector to LS
Description	
Source Device	Injector
Destination Device	DAWN EOS
Volume (mL)	0.5000
<input type="checkbox"/> Temperature Control	
Enable	<input checked="" type="checkbox"/>
Temperature (°C)	25.000

Fluid connection (injector to LS) | Fluid connection (LS to RI) | Auto inject

OK | Cancel | Apply | Lock

Figure 7-2: Typical property dialog (fluid connection example)

Configuration Example

The Configuration tree in the Experiments navigation pane shows both the hardware configured to be used in the experiment, as well as additional elements such as the solvent and sample. The experiment methods provided with ASTRA 6 include most common configurations. A configuration for an online light-scattering experiment is set up as shown in Figure 7-3. The same experiment is shown in the schematic of Figure 7-4. Comparing these two figures highlights the logical structure of the configuration and its constituent profile elements in the workspace.

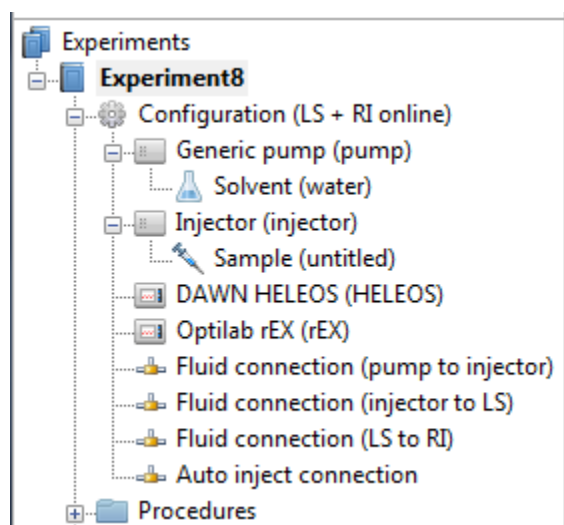


Figure 7-3: Configuration for an LS Online Experiment

The hardware setup for the configuration in Figure 7-3 would be organized similar to Figure 7-4:

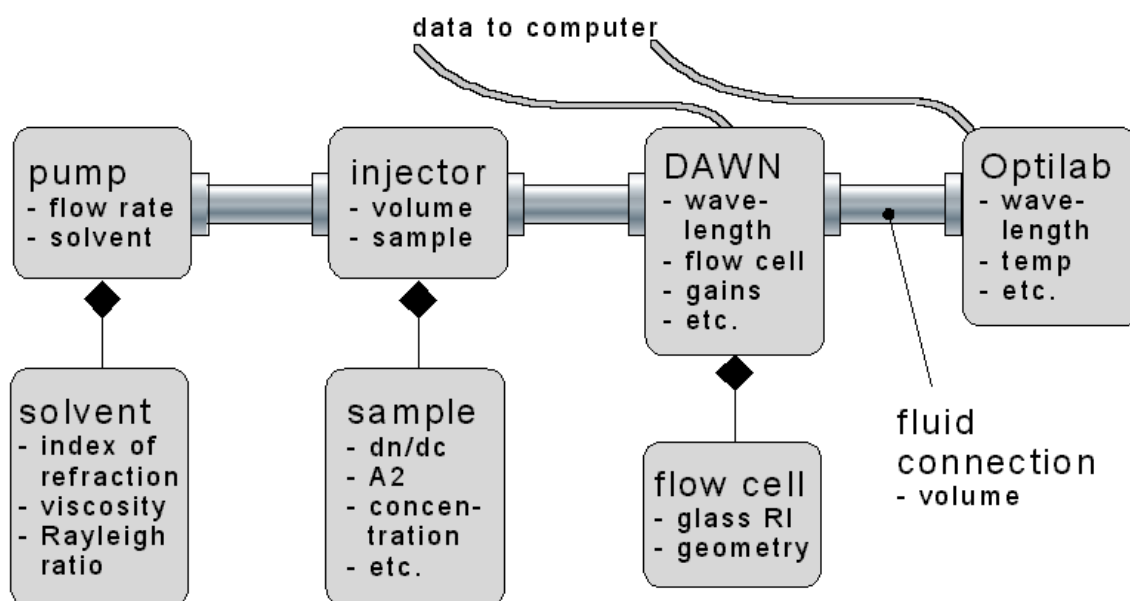


Figure 7-4: Hardware Connections for LS Online Experiment Setup

Using Configurations

Each experiment has a Configuration section that contains descriptions of all of the physical components used in the experiment.

The actions you can perform on a configuration are simple. The complexity comes from the large number of physical components that can be used and the large number of properties some of these components have.



You must have at least Researcher access to work with configurations and profiles. If you are a Technician or Guest, you have read-only access to profiles.


Editing a Configuration

To set properties of a configuration component, follow these steps:

1. Double-click on a component in the Configuration node of the Experiments navigation pane. This opens the properties page and selects the tab for that component. See Figure 7-2 for an example.

Shortcuts: Choose **Experiment→Configuration→Edit**.

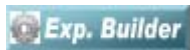
2. Set properties by typing, selecting from a list, or checking a box.
You can expand or hide lists of related properties if there is a + or - sign next to a property.
3. Alternately, you may click the browse button (“...”) to the right of the Name property and locate a profile to use to replace the existing property values for this item.
4. You can move to other tabs to view or set properties for other items.

Shortcuts: Double-click on an item in the Configuration tree to move to its tab. Move to a tab using the  tab arrows.

5. Click **Apply** or **OK** to make the changes.

The remaining sections of this chapter contain details about the properties you can set in the various tabs.

Adding Instruments and Connections



You can add items to a configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties page, close and reopen it after enabling Experiment Builder mode.

Note: If you want to have custom instrument profiles available in addition to the profiles provided with ASTRA, see “Saving as a Profile” on page 12-4.

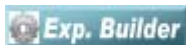
To add instruments and connections, you can specify them in the Experiment configuration tab of the Configuration properties page. To add an instrument, follow these steps:

1. Choose **Experiment→Configuration→Edit**. This opens the properties page for the configuration, which has a tab for each item in the configuration tree. Alternately, double-click the Configuration node in the Experiments navigation pane.
2. In the Experiment configuration tab, click the **Browse** button in the row to add instruments.

	Value
Name	Michelle files 2
Description	ASTRA 4 operator name: Harvard/Betty
Notes	Edit Notes...
Processing Operator	YVONNE-WORK\ZAdmin (ZAdmin)
Abcissa Units	mL
Concentration Source	none
<input type="checkbox"/> Details	
Assume 100% Mass Recovery	<input type="checkbox"/>
Fwd Monitor Baseline Start	0.0000
Fwd Monitor Baseline End	0.0000
Baseline Noise Start	0.0000
Baseline Noise End	0.0000
<input type="checkbox"/> Instruments	
Add	Browse...
Remove	Browse...
<input type="checkbox"/> Connections	
Add	Browse...

3. In the Add Instrument dialog, find the instrument you want to add to the experiment. This must be an instrument profile you have saved as described in “Creating Profiles” on page 12-3. For example, you might navigate to a “My Profiles” folder if you have created one to select a custom profile.
4. Select the instrument profile you want to add and click **Open**. The instrument is added to your configuration and you can edit its properties by double-clicking it in the configuration tree.
5. To add a connection, follow the same steps but click the **Browse** button in the row to add connections.

Removing Instruments and Connections



You can remove items from a configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties page, close and reopen it after enabling Experiment Builder mode.

1. Choose **Experiment→Configuration→Edit**. This opens the properties page for the configuration, which has a tab for each item in the configuration tree.
2. In the Experiment configuration tab, click the **Browse** button in the row to remove instruments or the row to remove connections.
3. In the Remove Instrument Profile or Remove Connection Profile dialog, check the box next to the item you want to remove and click **OK**.

Replacing an Experiment Configuration or Item

It is possible to replace an *entire configuration* with an experiment configuration stored as a profile. A number of experiment configurations are provided with ASTRA. You can also save your own experiment configurations as described in “Creating Profiles” on page 12-3. For example, you may have a standard experiment configuration you want to use in many different experiments.

You can also replace a *single instrument or other item* with a saved profile.

See Chapter 12, “Working with Profiles” for more about profiles.

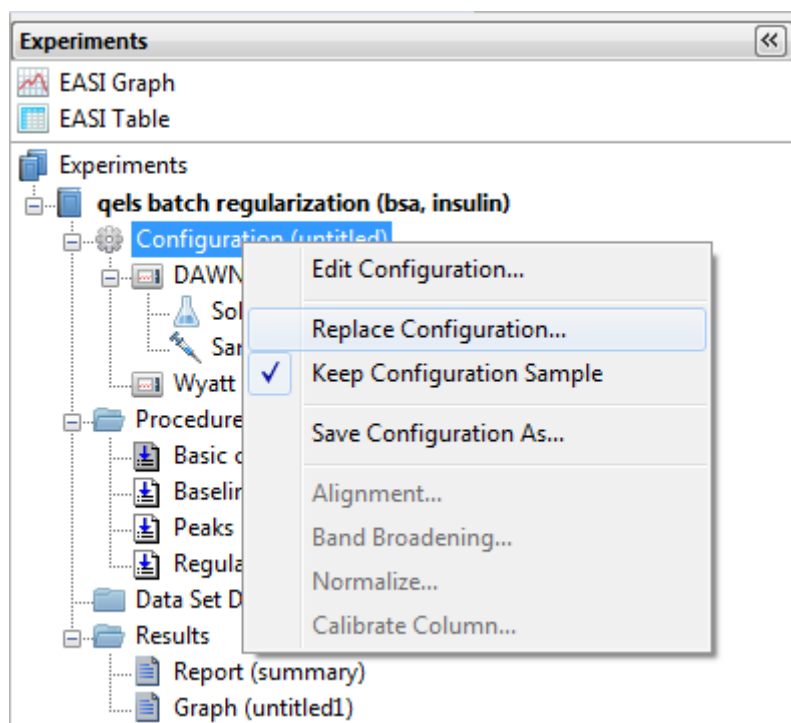
Replacing configurations is common when using sequences. For example, a typical workflow for replacing complete configurations in a sequence is as follows:

- First, run a sequence containing one normalization standard and a number of samples.
- Then, normalize and configure the experiment based on the standard run.
- Finally, replace the experiment configuration in all the sample runs in the sequence with the configuration from the “standard” run.

To import a *complete configuration*, follow these steps:

1. If you want to import an example configuration, but keep the sample(s) the same as when you ran the experiment, right-click on the Configuration node of the experiment and make sure the checkmark next to the **Keep Configuration Sample** is toggled on.

2. Choose **Experiment→Configuration→Replace**. (Or, right-click on the “Configuration” node in the experiment, and choose **Replace Configuration**.) You see the Open dialog.



3. Browse the system database for a configuration to import. In addition to any experiment configurations you have saved, ASTRA provides a number of configurations in the “System > Configurations” folder. These are organized by the experiment type and instruments involved.
4. When you find a profile, select it and click **Open**. The experiment configuration you selected replaces the existing one.

To replace an *individual item* in the configuration with another item of the same type, follow these steps:

1. Right-click on a node in the configuration and choose the **Replace** command for that item from the pop-up menu.
2. Browse the database for an item to import. You can only select from items of the corresponding type. That is, you can replace a sample with a sample, a solvent with a solvent, and so on for connections and instruments. If you are replacing an instrument, you can select any type of instrument.

Note: If you want to have custom profiles available in addition to the profiles provided with ASTRA, see “Saving as a Profile” on page 12-4.

3. When you find the profile you want to use, select it and click **Open**. The item you selected replaces the existing item.

If you later edit properties of items you imported, there is no effect on the profile from which it was imported. Likewise, modifying a profile does not affect experiments that imported that profile.

Exporting a Profile

One way to create a profile is to export items from an experiment. To do this, follow these steps:

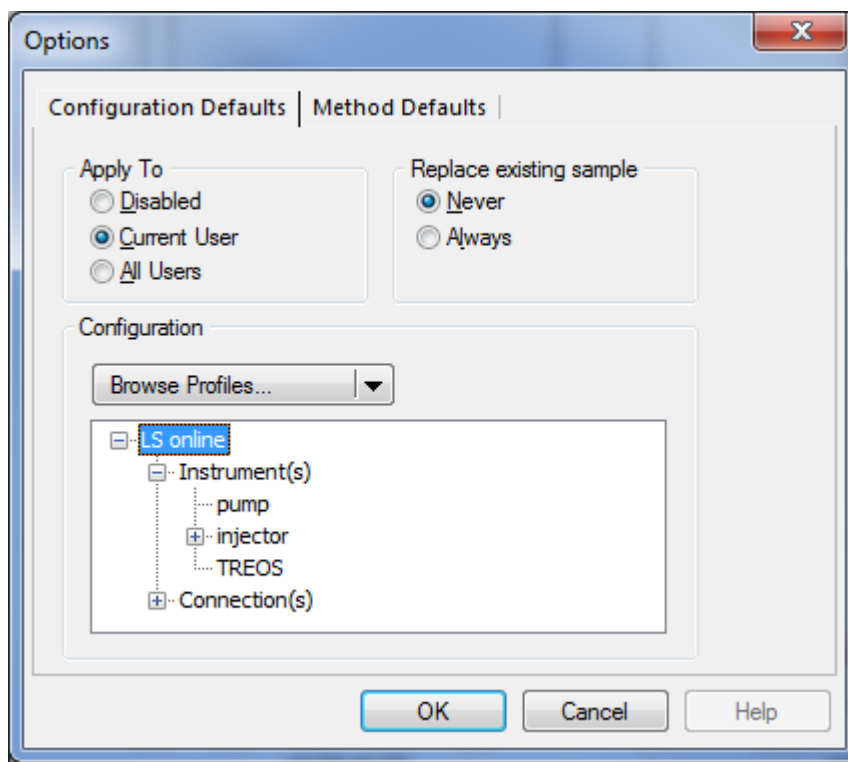
1. If you have more than one experiment open, make sure the one you want to export from is selected in the Experiments navigation pane.
2. Select the item in the configuration you want to export. (Any items nested at a lower level will be exported along with the item you select. For example, exporting an injector creates a profile that contains the injector and its sample. If you export the configuration item, the entire experiment configuration is saved as a profile.)
3. Choose **Experiment→Configuration→Save As**. Or right-click on an item and choose the **Save As** item from its right-click menu.
4. In the Save As dialog, choose the folder where you want to save the profile. Then type a name for the profile you are creating, and click **OK**.

See Chapter 12, “Working with Profiles” for more about using a profile you export.

Setting a Default Configuration

When you choose **File→New→Experiment from Default**, the default configuration (if you have specified one) is used in the new experiment. The configuration in the default configuration (if specified) overrides the configuration in the default method.

You can set the default configuration for new experiments by choosing **System→Preferences→Options**. Go to the **Configuration Defaults** tab.



To select a configuration, click **Browse Profiles**. Locate the profile you want to use in the system database. It may be one provided with ASTRA or one you have saved as describe in “Creating Profiles” on page 12-3.

In the **Apply To** area, choose whether you want the default configuration to be used by all users on this computer or only the currently logged in user. If you choose Disabled, the configuration in the default method is used as the default configuration when you create a new experiment.

In the **Replace existing sample** area, you can choose whether the sample in the profile you select as the default should take the place of the sample in the default method when you create a new experiment. You can control this for an individual experiment by right-clicking on the Configuration node of the experiment and making sure the checkmark next to the **Keep Configuration Sample** is toggled on or off, as needed.

See “Setting a Default Method” on page 6-19 for information about setting method defaults and “Setting a Default Sequence” on page 9-4 for information about setting a default sequence.

Using the Configuration Wizard



You must have at least Researcher access to use the Configuration Wizard.

You can use the Configuration Wizard to quickly specify the instruments and settings you want to use for data collection.

Follow these steps to use the Configuration Wizard:

1. To start the Configuration Wizard, choose **System→Configuration Wizard**.
2. On the first page of the wizard, select the **Solvent** you are using. If your solvent isn't on the list, select the solvent that is closest to your solvent, and you can adjust the settings later.

Select Solvent - Step: 1 of 3

Solvent

- methanol
- methylene chloride
- NMP
- PBS, Aqueous
- tcb (135 C)
- thf
- toluene
- water**

Select a solvent that most closely matches the mobile phase you will be using for analysis. You can always change this later.

Experiment Mode

☒ **Flow (Online)**
Select this option if you are using a pump to flow sample through a series of instruments.
0.00 HPLC Pump Flow Rate (mL/min)

☐ **Batch**
Select this option for unfractionated measurements, such as with cuvettes or direct injections from a syringe into a flow cell.

☐ **Calibration**
Select this option to generate a calibration method for your light scattering instrument.

< Back Next > Cancel

3. Choose whether to run in **Flow mode** (also called “online” or “fractionated”) or **Batch mode**. You can also use this wizard to create a light scattering instrument calibration method. Flow mode uses a pump, and batch mode typically uses cuvettes and/or injectors. If you are using Flow mode, you should also specify the flow rate in mL/min.

4. Click **Next**.
5. Check boxes next to categories of instruments you want to use. Then, select the instruments you want to use and any available options.

Select Instruments - Step: 2 of 4

Select the instruments you would like to use for analysis. Any Wyatt Technology Instruments found on the network are presented in the drop-downs in each category.

If one or more instruments send data via the AUX channel, be sure to select the appropriate target instrument (e.g., your HELEOS) and the AUX channel.

☒ Column

☒ UV Detector

Generic UV Instrument DAWN HELEOS @ WYATT-3 On Aux Channel 1

☒ Light Scattering

DAWN HELEOS @ WYATT-310-HHC ☐ QELS

☒ Refractometer

Generic RI DAWN HELEOS @ WYATT-3 On Aux Channel 2

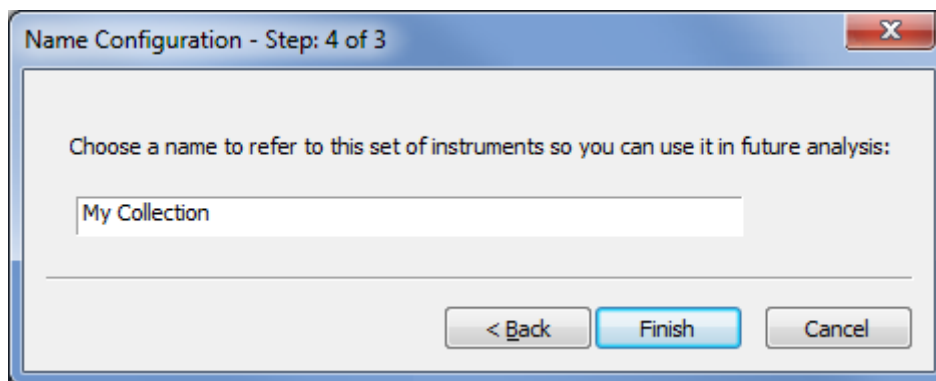
☐ Viscometer

ViscoStar @ WYATT-142-V

< Back Next > Cancel

6. For non-Wyatt instruments, such as a generic UV detector, use the second column to select the Wyatt instrument that receives this instrument's signal via an AUX input. Use the third column to specify which of the AUX inputs on the receiving instrument gets the signal from this non-Wyatt instrument. For example, if you connected the analog output of a UV instrument to AUX input one on a HELEOS, you would check the box for a UV Detector, select the HELEOS in column two, and select AUX 1 in column three.
7. Once you have selected all your instruments, click **Next**.
8. If this is an online experiment, you see Page 3 of the wizard. Otherwise, skip to Step 9.
 - a. On Page 3 of the wizard, use arrows to the right of the instrument list to match the fluid flow through the instruments.
 - b. Also on Page 3, if an instrument provides an auto-injection signal, select that instrument and click **Injection Signal**.
 - c. Click **Next**.

9. On the next page of the wizard, type a name to describe this set of instruments.



10. Click **Finish**.

Both a configuration (containing only instrument profiles) and a method (containing instrument profiles and procedures) are created when you click **Finish**. Both have the name that you specified, but are stored in different places in the system database:

- The instrument configuration profile is stored in /User/Configurations. See Chapter 12, “Working with Profiles”. You can set this configuration as the default configuration as described in “Setting a Default Configuration” on page 7-10.
- The method is stored in /User/Methods. You can set this method as the default method for new experiments as described in “Setting a Default Method” on page 6-19.

Experiment Configuration

An *experiment configuration* groups together all the profile components used in a particular experiment. For information about creating a new experiment and the associated configuration, see “Creating New Experiments” on page 6-4.

	Value
Name	Michelle files 2
Description	ASTRA 4 operator name: Harvard/Betty
Notes	Edit Notes...
Processing Operator	YVONNE-WORK\ZAdmin (ZAdmin)
Abcissa Units	mL
Concentration Source	none
Details	
Assume 100% Mass Recovery	<input type="checkbox"/>
Fwd Monitor Baseline Start	0.0000
Fwd Monitor Baseline End	0.0000
Baseline Noise Start	0.0000
Baseline Noise End	0.0000
Instruments	
Add	Browse...
Remove	Browse...
Connections	
Add	Browse...
Remove	Browse...

Navigation: Configuration / Generic pump (generic pump) / Solvent (water)

Buttons: [OK](#) [Cancel](#) [Apply](#)

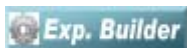
You can set the following properties for an experiment configuration:

Table 7-1: Experiment Configuration Properties

Field	Description
Name	Name of the experiment configuration. Make this name brief enough to be easily selected from your list of experiment configurations.
Description	Description of the experiment configuration, which typically contains more information than the Name.
Edit Notes	You can type longer notes to be stored with the experiment in the dialog that opens when you click the Edit Notes button.
Processing Operator	The current user. This changes each time you load the experiment. It is the source for the Processing Operator field in reports. In ASTRA 6 Basic, you can edit this field to show the name you want listed in a report. See “Operator Names in Reports” on page 10-2 for details.

Table 7-1: Experiment Configuration Properties

Field	Description
Abscissa Units	The x axis units for display. The default units are milliliters for an online (flow) experiment and minutes for a batch experiment. Also available are milliseconds, seconds, and hours. This setting affects the units for a number of fields in the experiment procedures.
Concentration Source	Select the source of concentration data you want to use in this experiment. The list shows concentration sources, such as RI and UV, currently in your experiment configuration (if any).
Details	
>Assume 100% Mass Recovery	<p>RI detector as concentration source: By default, the concentration at each data slice is determined based on the dn/dc and the calibration constant for the RI detector. Alternately, you can enable the 100% Mass Recovery option to determine the concentration by estimating the dn/dc based on the injected mass. In either case, the RI instrument's calibration constant must be known. See "Concentration Calculation Methods" on page D-11.</p> <p>UV detector as concentration source: By default, the concentration at each data slice is determined based on the UV extinction coefficient. Alternately, you can enable the 100% Mass Recovery option in an experiment configuration to determine the concentration by estimating the UV extinction coefficient based on the injected mass. In either case, the UV instrument's cell length and UV response factor must be known.</p>
>Fwd Monitor Baseline Start / End	If you selected "Forward Monitor" in the Divide by Laser Monitor field of the configuration for your light scattering instrument (see page 7-16), you should specify the start and end points for a region that corresponds to pure solvent. This pure solvent region acts as a baseline for the forward laser monitor signal. If you do not specify a region, the average of the forward laser monitor signals for the first 10% of the collected data is assumed to be the default "pure solvent" range for calculating the average forward monitor signal.
>Baseline Noise Region Start / End	Specify the start and end points for a region to be used for the baseline noise computation. If you do not specify a region, the first and last 10% of the run are used to assess baseline noise. These fields allow you to override the default if there are artifacts in these regions.
Add Instruments	Click Browse to select an instrument profile to add to the experiment. This property and the ones that follow are available only in Experiment Builder Mode.
Remove Instruments	Click Browse to select an instrument profile to remove from the experiment.
Add Connections	Click Browse to select a connection profile to add to the experiment.
Remove Connections	Click Browse to select a connection profile to remove from the experiment.



Buttons to add and remove instruments and connections are visible only in Experiment Builder mode, which you enable by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties page, close and reopen it after enabling Experiment Builder mode.

Light Scattering Instrument Profiles

An *instrument* is any hardware device used in an experiment. Light scattering instruments measure the molar mass, RMS radius, and second virial coefficient of a sample via Rayleigh scattering.

DAWN HELEOS and DAWN HELEOS 8 Profiles

The DAWN HELEOS is the default light scattering instrument in most light scattering experiment methods. You can set the following properties for a DAWN HELEOS or DAWN HELEOS 8 instrument:

Table 7-2: DAWN HELEOS Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” on the far right, and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose “Browse...” to open the Instruments dialog. (See “Accessing and Viewing Hardware” on page 2-12.)
Sample Cell	Select the type of sample cell used during data collection. The options are: Fused Silica, Scintillation vial, MicroCuvette, K5, NK5, F2, and Magic glass.
Wavelength	The wavelength of the laser that produces scattered light from the sample cell and its contents. (nm)
Calibration Constant	Type the Instrument Specific Calibration Constant (ISCC) value ($1/(V \text{ cm})$). Light scattering instruments use the ISCC in the computation of the Configuration Specific Calibration Constant (CSCC). See “LS Calibration” on page 8-15 for a way to determine this value. The factory calibration constant of your instrument can be found in the instrument's Certificate of Performance.
Normalization Coefficients > 1-18 (1-8 for HELEOS 8)	Type the normalization coefficients for the detectors or use the normalization procedure (see page 8-23) to set these values. Detector 11 always has a normalization coefficient of 1. (This is Detector 5 on a HELEOS 8.) Normalization is the process by which each detector signal is related to the 90° detector signal and the Instrument Specific Calibration Constant. Click the Import button to import normalization coefficients from an open experiment.
Comet Cell Cleaner	Check this box if a COMET cell cleaner is to be used with the DAWN instrument. Please see the COMET hardware manual for more information about the COMET cell cleaner.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a sample and solvent pair with the instrument profile, rather than with pumps and injectors (respectively). For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.

Table 7-2: DAWN HELEOS Properties

Field	Description
Divide by Laser Monitor	<p>Select the laser monitor option you want to use. The options are “Laser Monitor”, “Forward Monitor”, and “none”.</p> <ul style="list-style-type: none"> -- “Laser Monitor”: The light scattering signals are divided by the laser monitor, which corrects for fluctuations in the laser intensity due to power fluctuations. This is the default setting. -- “Forward Monitor”: The light scattering signals are divided by the forward laser monitor, which corrects for both laser intensity fluctuations and absorbance by the sample. -- “none”: No correction is performed for laser intensity fluctuations or sample absorbance. Use this option only if signal levels are so low that digital noise from the laser monitor signal can contaminate data. <p>No additional wiring is required for the DAWN HELEOS, DAWN HELEOS 8, or miniDAWN TREOS to collect forward laser monitor data. For other types of instruments, please refer to the User's Guide for your instrument for details on how to collect forward monitor data.</p>
Disable Collection	Check this box to disable data collection for this instrument. For example, if the light scattering instrument has the QELS option, it is possible to disable the DAWN collection and collect QELS data alone.
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the DAWN hardware manual for details.
QELS > Option	Check this box if the DAWN instrument has a detector replaced with a QELS fiber.
QELS > Replaced Detector	If QELS is enabled, type the number of the detector replaced for the QELS fiber.
Band Broadening > Enabled	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.
Temperature Control > Enable	When data collection begins, ASTRA gets the temperature control setting from the instrument.
Temperature Control > Temperature	When data collection begins, ASTRA gets the configured temperature from the instrument and stores it here.

DAWN EOS, DAWN DSP, DAWN DSP-F, and DAWN 8 Profiles

The properties that may be defined for a DAWN EOS, DAWN DSP, DAWN DSP-F, and DAWN 8 are identical.

You can set the following properties for a DAWN instrument:

Table 7-3: DAWN Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” on the far right, and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.

Table 7-3: DAWN Instrument Properties

Field	Description
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose "Browse..." to open the Instruments dialog. (See "Accessing and Viewing Hardware" on page 2-12.)
Sample Cell	Type of sample cell used during data collection. The options are: Fused Silica, Scintillation vial, MicroCuvette, K5, NK5, F2, and Magic glass.
Wavelength	The wavelength of the laser that produces scattered light from the sample cell and its contents. (nm)
Calibration Constant	Type the Instrument Specific Calibration Constant (ISCC) value ($1/(V \text{ cm})$). Light scattering instruments use the ISCC in the computation of the Configuration Specific Calibration Constant (CSCC). See "LS Calibration" on page 8-15 for a way to determine this value. The factory calibration constant of your instrument can be found in the instrument's Certificate of Performance.
Normalization Coefficients > 1-18 (1-8 for DAWN 8)	Type the normalization coefficients for the detectors or use the normalization procedure (see page 8-23) to set these values. Detector 11 always has a normalization coefficient of 1. (This is Detector 5 on a DAWN 8.) Normalization is the process by which each detector signal is related to the 90° detector signal and the Instrument Specific Calibration Constant. Click the Import button to import normalization coefficients from an open experiment.
Comet Cell Cleaner	Check this box if a COMET cell cleaner is to be used with the DAWN instrument. Please see the COMET hardware manual for more information about the COMET cell cleaner.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-9.
Divide by Laser Monitor	Select the laser monitor option you want to use. The options are "Laser Monitor", "Forward Monitor", and "none". <ul style="list-style-type: none"> -- "Laser Monitor": The light scattering signals are divided by the laser monitor, which corrects for fluctuations in the laser intensity due to power fluctuations. This is the default setting. -- "Forward Monitor": The light scattering signals are divided by the forward laser monitor, which corrects for both laser intensity fluctuations and absorbance by the sample. -- "none": No correction is performed for laser intensity fluctuations or sample absorbance. Use this option only if signal levels are so low that digital noise from the laser monitor signal can contaminate data. To use the Forward Monitor, you must route the instrument's forward laser monitor signal through one of the AUX inputs on the instrument.
Fwd Monitor Aux Channel	If you selected "Forward Monitor" in the field above, specify which AUX input on the instrument receives the forward laser monitor signal.
Disable Collection	Check this box to disable data collection for this instrument. For example, if the light scattering instrument has the QELS option, it is possible to disable the DAWN collection and collect QELS data alone.
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the DAWN hardware manual for details. (Not available for the DAWN 8.)
QELS > Option	Check this box if the DAWN instrument has a detector replaced with a QELS fiber.
QELS > Replaced Detector	If QELS is enabled, type the number of the detector replaced for the QELS fiber.

Table 7-3: DAWN Instrument Properties

Field	Description
Band Broadening > Enabled	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See "Band Broadening" on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See "Band Broadening" on page 8-20 for an explanation of the mixing term. The units are in microliters.
Temperature Control > Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled).
Temperature Control > Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.
Temp Controlled Line > Enable	Check this box if the plumbing line in the instrument is set to maintain a specified temperature (heated or cooled).
Temp Controlled Line > Temperature	If the line is temperature controlled, specify the temperature to which it is set. Use °C.
Detector Amp Gains > 1-18 (1-8 for DAWN 8)	Select the detectors gains to use for each laser detector. The options are 1, 21, and 101.
Auxiliary Channel Gains > 1-2	Select the auxiliary channel gains for channels 1 and 2. The options are 1, 10, 100, and 1000.

miniDAWN and miniDAWN TREOS Profiles

You can set the following properties for a miniDAWN or miniDAWN TREOS instrument:

Table 7-4: miniDAWN and miniDAWN TREOS Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click "..." and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose "Browse..." to open the Instruments dialog. (See "Accessing and Viewing Hardware" on page 2-12.)
Sample Cell	Type of sample cell used during data collection. The options for the miniDAWN are: K5, F2, and MicroCuvette. The options for the miniDAWN TREOS are: Fused Silica, Scintillation vial, MicroCuvette, K5, NK5, F2, and Magic glass.
Wavelength	The wavelength of the laser that produces scattered light from the sample cell and its contents. (nm)
Calibration Constant	Type the Instrument Specific Calibration Constant (ISCC) value (1/(V cm)). Light scattering instruments use the ISCC in the computation of the Configuration Specific Calibration Constant (CSCC). See "LS Calibration" on page 8-15 for a way to determine this value. The factory calibration constant of your instrument can be found in the instrument's Certificate of Performance.

Table 7-4: miniDAWN and miniDAWN TREOS Instrument Properties

Field	Description
Normalization Coefficients (1-3)	Type the normalization coefficients for the detectors or use the normalization procedure to set these values. Detector 2 always has a normalization coefficient of 1. Normalization is the process by which each detector signal is related to the 90° detector signal and the Instrument Specific Calibration Constant. Click the Import button to import normalization coefficients from an open experiment.
Comet Cell Cleaner	Check this box if a COMET cell cleaner is to be used with the miniDAWN instrument. Please see the COMET hardware manual for more information about the COMET cell cleaner.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.
Divide by Laser Monitor	Select the laser monitor option you want to use. The options are “Laser Monitor”, “Forward Monitor”, and “none”. -- “Laser Monitor”: The light scattering signals are divided by the laser monitor, which corrects for fluctuations in the laser intensity due to power fluctuations. -- “Forward Monitor”: The light scattering signals are divided by the forward laser monitor, which corrects for both laser intensity fluctuations and absorbance by the sample. -- “none”: No correction is performed for laser intensity fluctuations or sample absorbance. Use this option only if signal levels are so low that digital noise from the laser monitor signal can contaminate data. To use the Forward Monitor with the miniDAWN (but not the miniDAWN TREOS), you must route the instrument’s forward laser monitor signal through one of the AUX inputs on the instrument. No additional wiring is required for the miniDAWN TREOS to collect forward laser monitor data.
Fwd Monitor Aux Channel	If you selected “Forward Monitor” in the field above, specify which AUX input on the instrument receives the forward laser monitor signal. (Used for the miniDAWN only.)
Disable Collection	Check this box to disable data collection for this instrument. For example, if the light scattering instrument has the QELS option, it is possible to disable the collection and collect QELS data alone.
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the hardware manual for details. (Not available for the miniDAWN TREOS.)
Temperature Control > Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled). (Available for the miniDAWN only.)
Temperature Control > Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C. (Available for the miniDAWN only.)
Band Broadening > Enabled	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.
Auxiliary Channel Gains > 1-2	Select the auxiliary channel gains for channels 1 and 2. The options are 1, 10, 100, and 1000. (Available for the miniDAWN only.)

WyattQELS Profiles

A WyattQELS device is a Quasi-Elastic Light Scattering device. You can set the following properties for a WyattQELS instrument:

Table 7-5: WyattQELS Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose “Browse...” to open the Instruments dialog. (See “Accessing and Viewing Hardware” on page 2-12.)
Model	The instrument model. Options are: Wyatt QELS, Flex 99, and Flex 99 ADN.
Use QELS Temperature Probe	Check this box if the instrument is set to use a temperature probe signal during collection. Uncheck this box if you have a temperature-controlled DAWN or miniDAWN. If this box is unchecked, the temperature is taken from the temperature set for the DAWN or miniDAWN instrument.
Use QELS Dithering	WYATT instruments use a patented laser stabilization algorithm that dithers the laser intensity by a small amount. This algorithm ensures that the static scattering results are unaffected by laser mode hops. While dithering keeps the average intensity extremely stable, it can create a small artifact in the QELS baseline. The time scale of this artifact is widely separated from that of the diffusing molecule so it does not affect the accuracy of the measured Rh results. When performing QELS-only measurements or when the laser intensity is less than 50%, laser dithering is automatically disabled. In most cases, you should leave this option enabled.

Refractive Index Instrument Profiles

A *refractive index instrument* measures the differential refractive index (dRI) of a solution in order to calculate the concentration of the sample. In order to calculate the concentration from the differential refractive index, it is necessary to know the dn/dc value for the sample.

Optilab rEX and Optilab T-rEX Profiles

You can set the following properties for a Optilab rEX and Optilab T-rEX instruments:

Table 7-6: Optilab rEX Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Click “...” and select from the Instruments dialog. (See “Accessing and Viewing Hardware” on page 2-12.)
Wavelength	The wavelength (nm) of the light used in the instrument. When data collection begins, ASTRA gets this information from the instrument.
Temperature	When data collection begins, ASTRA gets the configured temperature from the instrument and stores it here.
Calibration Constant	When data collection begins, ASTRA gets the configured calibration constant from the instrument and stores it here.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.
Band Broadening > Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.

For Optilab rEX and T-rEX users, there are several utility methods in the **System > Methods > RI Measurement > Optilab rEX Specific** folder. These experiment methods include “Purge On”, “Purge Off”, and “Zero dRI”. We recommend that you purge the Optilab rEX when not running samples; the “Purge On” method is a convenient way to automate this as part of a sequence. In addition, Optilab rEX methods for absolute RI calibration and RI calibration from a peak are included in this folder.

The purge valves on Optilab rEX instruments are automatically closed at the start of data collection. The exception to this is when absolute RI analysis is conducted, where the Optilab rEX purge valve must be left open.

Optilab DSP Profiles

You do not select a Physical Instrument for the Optilab DSP because ASTRA 6 does not support a direct data connection to this instrument. When using the Optilab DSP, it is necessary to add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the Optilab DSP signal. See “AUX Connection Profiles” on page 7-39 for details.

You can set the following properties for a Optilab DSP instrument:

Table 7-7: Optilab DSP Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Cell	Type of sample cell used during data collection. The options are: P2, P2L, P10, P100, P10L, P20, P12, and ENGRCELL.
Temperature	If this instrument is temperature controlled, specify the temperature to which it is set.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.
Band Broadening > Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.

Optilab 903 Profiles

You do not select a Physical Instrument for the Optilab 903 because ASTRA 6 does not support a direct data connection to this instrument. When using the Optilab 903, it is necessary to add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the Optilab 903 signal. See “AUX Connection Profiles” on page 7-39 for details.

You can set the following properties for a Optilab 903 instrument:

Table 7-8: Optilab 903 Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Cell	Type of sample cell used during data collection. The options are: P2, P2L, P10, P100, P10L, P20, P12, and ENGRCELL.
Scale	The scale corresponds to the scale selected on the Optilab 903 instrument. Possible values are 2, 5, 10, 20, 50, and 100.
Offset	The offset voltage is determined during the Optilab 903 setup procedure. Please see the Optilab 903 hardware manual for instructions on determining the offset.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.
Band Broadening > Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.

Generic RI Instrument Profiles

You can create a Generic RI Instrument profile for any third-party refractive index instrument for which data is collected through the AUX input of another instrument.

You do not select a Physical Instrument for a Generic RI Instrument profile because ASTRA 6 does not support a direct data connection to such instruments. Instead, add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the signal. See “AUX Connection Profiles” on page 7-39 for details.

You can set the following properties for a generic RI instrument:

Table 7-9: Generic RI Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Temperature Control > Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled).

Table 7-9: Generic RI Instrument Properties

Field	Description
Temperature Control > Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-9.
Band Broadening > Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See "Band Broadening" on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See "Band Broadening" on page 8-20 for an explanation of the mixing term. The units are in microliters.

Viscometry Instrument Profiles

A *viscometer* measures the specific viscometry of a solution. See Appendix G, “Viscosity Theory” for a review of the theory of viscosity-related calculations.

Wyatt’s ViscoStar measures specific viscosity. When combined with concentration data from an RI or UV concentration detector, specific viscosity can be used to calculate intrinsic viscosity. Intrinsic viscosity, in turn, combined with data from light scattering measurements, can be used to derive the hydrodynamic radius (r_h) and molecular shape information.

ViscoStar Profiles

You can set the following properties for a ViscoStar instrument:

Table 7-10: ViscoStar Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Click “...” and select from the Instruments dialog. (See “Accessing and Viewing Hardware” on page 2-12.)
Dilution Factor	If you are using a UV detector plumbed before the ViscoStar in the flow sequence, use the default dilution factor of 1.00. If you are using an RI detector (or any other instrument) plumbed after the ViscoStar, see “Measuring the Dilution Factor” on page 7-27 to determine the value to enter here.
Temperature	When data collection begins, ASTRA gets the configured temperature from the instrument and stores it here.
Capillary Volume	The internal capillary volume of the ViscoStar instrument. This value is used to correct for certain types of mixing effects. This does not include the volume of the adjustable reservoir. You can compute the internal capillary volume using the provided experiment method.
Specific Viscosity Mode	If you are not using a concentration detector with the ViscoStar, check the Specific Viscosity Mode box. Intrinsic viscosity can only be calculated using both specific viscosity and concentration data.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.
Band Broadening > Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.

The purge valves on ViscoStar instruments are automatically closed at the start of data collection.

Measuring the Dilution Factor

If an RI detector (or other instrument) is plumbed after the ViscoStar in the flow sequence, the sample exiting the ViscoStar is diluted by approximately a factor of 2. Therefore the RI detector does not measure the same concentrations that flowed through the LS and ViscoStar instruments. To correct for this, you should measure the dilution factor experimentally.

To measure the dilution factor, use a sample that is known to elute 100%. The detailed report shows the resulting Dilution Factor, which you can enter in the ViscoStar or Generic Viscometer profile.

You should check the dilution factor occasionally, since it will change over time as samples that coat the tubing slowly build up.

To learn more, see the “Measuring the System Dilution Factor” section in the ViscoStar User’s Guide.

Generic Viscometer Profiles

You can create a Generic Viscometer profile for any high-temperature third-party viscometer for which data is collected through the AUX input of another instrument.

To create a generic viscometer profile, choose **File→New→Profile** and select Generic Viscometer and click OK. Name your viscometer profile. Then, you can double-click the viscometer profile in the Profiles tab to set its properties.

You can set the following properties for a generic viscometer:

Table 7-11: Generic Viscometer Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Dilution Factor	If you are using a UV detector plumbed before the ViscoStar in the flow sequence, use the default dilution factor of 1.00. If you are using an RI detector (or any other instrument) plumbed after the ViscoStar, see “Measuring the Dilution Factor” on page 7-27 to determine the value to enter here.
AUX input mode	Specify the data provided by the AUX input from the viscometer. The options are “differential and inlet pressure” (default), “specific viscosity”, and “differential pressure alone”. The Waters viscometer can be set to provide either of the first two types of data; other viscometers may provide differential data pressure alone.
Fixed IP (psi)	If you chose “differential pressure alone”, specify the fixed inlet pressure for the viscometer in psi.

Table 7-11: Generic Viscometer Profile Fields

Field	Description
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.
Band Broadening > Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.

UV Absorption Instrument Profiles

A *UV absorption instrument* measures the absorbance of a sample in the ultra-violet region of the spectrum. The absorbance can be converted to a concentration if the cell length of the UV absorption instrument is known and if the UV extinction coefficient for the sample is known.

Generic UV Detector Profiles

You can create a Generic UV Instrument profile for any third-party UV instrument for which data is collected through the AUX input of another instrument.

You do not select a Physical Instrument for a Generic UV Instrument profile because ASTRA 6 does not support a direct data connection to such instruments. Instead, add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the signal. See “AUX Connection Profiles” on page 7-39 for details.

You can set the following properties for a generic UV instrument:

Table 7-12: Generic UV Instrument Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Cell Length	The length of the sample cell in cm.
Channels (1-4)	Many UV detectors measure UV on several wavelengths at the same time. For such detectors, you can enable multiple channels and specify the wavelength and UV response for each channel.
Channels > Enable	Check this box to enable a channel.
Channels > Wavelength	Specify the UV wavelength (in nm) for this channel. See the hardware manual for your UV detector.
Channels > UV Response	The conversion factor from absorbance units (AU) to volts for the UV aux output for this channel. See the hardware manual for your UV detector.
Active Channel	If you have enabled multiple UV channels, select the channel for which you want to perform processing and UV extinction factor calculations. If you want to see data from additional UV channels in your ASTRA experiment, you must make additional AUX connections between the UV detector and a Wyatt instrument (one connection per channel). For example, to use all four channels, you would need four AUX connections between the UV instrument and the Wyatt instrument. Then, use the AUX profile (page 7-39) to identify which AUX channel is associated with which UV channel. Once the connections are made, UV traces in ASTRA graphs are listed as “UV 1”, “UV 2”, etc. Some graphs (such as “Peaks” and “Alignment”) only show the active UV trace.
Temperature Control > Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled).

Table 7-12: Generic UV Instrument Profile Fields

Field	Description
Temperature Control > Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-9.
Band Broadening > Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-20). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See “Band Broadening” on page 8-20 for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See “Band Broadening” on page 8-20 for an explanation of the mixing term. The units are in microliters.

Injector Profiles

An *injector* consists of an injection loop that injects the sample into the flowing solvent or mobile phase stream from the pump. ASTRA supports both manual and autoinjectors, each of which may provide an auto-inject signal from which data collection can be triggered.

You can set the following properties for an injector:

Table 7-13: Injector Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the injector, which typically contains more information than the Name.
Injected Volume (μL)	The volume of the solution injected in microliters. This is the same as the sample loop volume.
Auto-Inject Signal	Check this box if the injector provides an auto-inject signal.

An injector configuration always has a sample configuration associated with it in a profile. See “Sample Profiles” on page 7-32.

If the injector provides an auto-inject signal, you should also have an auto-injector connection in your configuration as described in “Autoinject Connection Profiles” on page 7-40.

Sample Profiles

A *sample* is the substance being tested. It is dissolved in the solvent, forming a solution. The solution is placed in or flows through a sample cell. A sample may be injected or may be a molecular standard used as a reference standard.

A *sample profile* stores information about samples to be used in experiments. Sample profiles are used by injector profiles and by instruments configured to run in batch (that is, standalone) mode.

Sample Profiles

A *sample* profile describes a sample for which you are determining properties.

You can set the following properties for a sample:

Table 7-14: Sample Properties

Field	Description
Name	Name of the sample. If you have already created a profile for this sample, click "... " and select a profile to use.
Description	Description of the sample, which typically contains more information than the Name.
dn/dc	dn/dc value associated with the sample in mL/g. The dn/dc value is used when the sample concentration is to be determined using a refractive index instrument. The value entered for the profile is used as a default value when peaks are set for the data.
A2	Second viral coefficient value associated with the sample in mol mL/g ² . The value set here is used as a default value for peaks set in the experiment.
UV Extinction Coefficient	The extinction coefficient in mL/(mg cm). The extinction coefficient is used when the concentration of the sample is to be determined using a UV absorption instrument. The value entered here is used as a default value when peaks are set for the data.
Concentration	The concentration of the sample in mg/mL.
Mark-Houwink-Sakurada K	The known Mark-Houwink-Sakurada K parameter of the sample. This parameter is used in the Mass from VS and Branching Analysis procedures. It is also used if you choose the Universal without Viscometer Data calibration technique. In this case, the equation used is: $[\eta] = K M^a$
Mark-Houwink-Sakurada a	The known Mark-Houwink-Sakurada a parameter of the sample. This parameter is used in the Mass from VS and Branching Analysis procedures. It is also used if you choose the Universal without Viscometer Data calibration technique.

An injector configuration always has a sample configuration associated with it in a profile. See "Injector Profiles" on page 7-31.

An autoinjector configuration always has a configuration for a sample associated with it in a profile. The properties tab for samples has a table with a row of the properties in Table 7-14 for each sample well. See “Autoinject Connection Profiles” on page 7-40.

Molecular Standard Profiles

A *molecular standard* profile describes a commonly used sample—such as BSA monomer—that has well-known properties. Such profiles are used as reference standards for processes such as normalization with a light scattering instrument. This profile allows you to quickly specify common standards during analysis runs, rather than manually entering values such as dn/dc and concentration in the peak definition.

Molecular standard profiles are associated with a peak in the data. The values set for the molecular standard profile will be used in the peak.

You can set the following properties for a molecular standard:

Table 7-15: Molecular Standard Properties

Field	Description
Name	Name of the standard. If you have already created a profile for this sample, click “...” and select a profile to use.
Description	Description of the standard, which typically contains more information than the Name.
Reference Wavelength	The wavelength at which the dn/dc or UV extinction value is accurate. (nm)
dn/dc	dn/dc value associated with the sample. (mL/g)
A2	Second virial coefficient value associated with the sample. (mol mL/g ²)
Molar Mass	Molar mass value associated with the sample. (g/mol)
Intrinsic Viscosity	A measure of the capability of a polymer in solution to enhance the viscosity of the solution. Derived using specific viscosity and concentration data.
Radius > Type	Type of radius specified. May be RMS, Hydrodynamic, or Geometric.
Radius > Value	Radius value associated with the sample. (nm)
UV Extinction Coefficient	The extinction coefficient in mL/(mg cm). The extinction coefficient is used when the concentration of the sample is to be determined using a UV absorption instrument.

For information about creating your own molecular standard profiles, see “Creating Profiles” on page 12-3.

Pump Profiles

Pumps move the mobile phase or solvent through the experimental apparatus. Therefore, a pump has a solvent profile associated with it. When setting up a pump profile, you select an available solvent profile to associate with the pump.

ASTRA supports profiles for generic third-party pumps. You can set the following properties for a generic pump:

Table 7-16: Generic Pump Profile Fields

Field	Description
Name	Name of the pump. If you have already created a profile for this instrument, click “...” and select a profile to use.
Description	Description of the pump, which typically contains more information than the Name.
Flow Rate	The rate at which the pump runs in mL/min.

A pump configuration always has a solvent configuration associated with it in a profile. See “Solvent Profiles” on page 7-34.

Solvent Profiles

A *solvent* is a substance in which another substance is dissolved, forming a solution. The solution is placed in or flows through a sample cell.

A *solvent profile* stores information about solvents used in experiments, such as toluene. Profiles for common solvents (water, toluene, THF, etc.) are supplied with ASTRA. You cannot change the values in existing solvent profiles. You can build and modify custom profiles for any other solvents you use.

You can set the following properties for a custom solvent:

Table 7-17: Solvent Properties

Field	Description
Name	Name of solvent profile. Typically, this is the name of the chemical. You can click “...” to choose from a list of common solvents in the System Solvents folder or solvent configurations you have saved as profiles.
Description	Description of the solvent, which may show more information than the Name.
Refractive Index	Displays the computed refractive index of the solvent at the wavelength used in the experiment. This property is shown only if this profile is part of an experiment configuration. When editing a solvent profile that is not part of an experiment configuration, this value is not displayed.
Viscosity	Displays the computed viscosity of the solvent at the temperature used in the experiment. This property is shown only if this profile is part of an experiment configuration.
Rayleigh Ratio	Displays the computed Rayleigh ratio of the solvent at the wavelength used in the experiment. This property is shown only if this profile is part of an experiment configuration.
Refractive Index Model	The model used to specify the refractive index. May be Fixed or Polynomial . Set the model type before setting the parameters required for that model. <ul style="list-style-type: none"> • If Fixed, specify the Reference Refractive Index. • If Polynomial, specify the Refractive Index Model Parameters. These are used to compute the Refractive Index.

Table 7-17: Solvent Properties

Field	Description
Refractive Index Model > Reference Refractive Index	Specify the refractive index of the solvent at the wavelength and temperature you will use. This property is required for both Fixed and Polynomial models.
Refractive Index Model > Parameters 2-5	<p>If the Refractive Index model is Polynomial, set Parameters 2-5 using the following polynomial model, which is used to compute the Refractive Index.</p> $n(L) = P_1 + \frac{P_2}{L^2} + \frac{P_3}{L^4} + \frac{P_4}{L^6} - P_5 T$ <ul style="list-style-type: none"> • P_1 is the reference refractive index. • P_2 through P_5 are solvent-specific constants. • T is the temperature • L is the wavelength in micrometers (μm) of the laser in the light scattering instrument. This is taken from the light scattering instrument profile as part of the configuration, so it does not need to be specified here.
Refractive Index Model > Reference Temp.	If the Refractive Index model is Polynomial , set the Reference temperature (T in the previous equation) ($^{\circ}\text{C}$) for which this fit is valid.
Rayleigh Ratio Model	The model used to specify the Rayleigh ratio of the solvent. May be Fixed or Corrected Lambda⁴ . Set the model type before setting the parameters required for that model.
>Reference Rayleigh Ratio	<p>If the Rayleigh Ratio Model is Fixed, specify the Rayleigh ratio of the solvent in $1 / (\text{cm})$ at the wavelength and temperature you will use.</p> <p>If the Rayleigh Ratio Model is Corrected Lambda⁴, specify the Rayleigh ratio of the solvent at the reference wavelength in $1 / (\text{cm})$.</p>
>Reference Wavelength	<p>If the Rayleigh Ratio Model is Corrected Lambda⁴, set the reference wavelength in μm.</p> <p>R_{θ} is the calculated solvent Rayleigh ratio using this formula:</p> $R(\theta) = P_1 \times \left(\frac{P_2}{L} \right)^4 \times \left(\frac{n_L}{n_{L_0}} \right)^2 \times \left(\frac{(n_L - 1)}{(n_{L_0} - 1)} \right)^2$ <ul style="list-style-type: none"> • P_1 is the Reference Rayleigh Ratio of the solvent • P_2 is the Reference Wavelength in μm • L is the wavelength in μm of the laser in the light scattering instrument. This is taken from the light scattering instrument profile as part of the configuration, so it does not need to be specified here. • n_L is the refractive index of the solvent at the wavelength of the laser in the light scattering instrument. This is calculated using the formula specified for the Polynomial Refractive Index Model. • n_{L_0} is the refractive index of the solvent at P_2 (the reference wavelength). This is calculated using the formula specified for the Polynomial Refractive Index Model.
Viscosity Model	The model used to specify the viscosity of the solvent. May be Fixed , Linear , or Exponential . Set the model type before setting the parameters required for that model.
>Reference Viscosity	<p>If the Viscosity Model is Fixed, specify the viscosity of the solvent in cP (centipoise) at the temperature you will use.</p> <p>If the Viscosity Model is Linear or Exponential, specify the viscosity of the solvent in cP (centipoise) at the reference temperature.</p>

Table 7-17: Solvent Properties

Field	Description
>Model Parameter 2	<p>If the Viscosity Model is Linear, set this parameters using the following model, where $n(T)$ is the viscosity as a function of temperature.</p> $n(T) = P_1 + P_2(T - P_3)$ <ul style="list-style-type: none"> P_1 is the viscosity in P at the reference temperature. P_2 is linear temperature dependence of the viscosity (cP/°C). P_3 is the reference temperature for the model in °C. T is the temperature as determined by the appropriate device's temperature probe in °C. <p>If the Viscosity Model is Exponential, set this parameters using the following model, where $n(T)$ is the viscosity as a function of temperature.</p> $n(T) = P_1 \exp(-P_2(T - P_3))$ <ul style="list-style-type: none"> P_1 is the viscosity in P at the reference temperature P_2 is the exponential temperature dependence of the viscosity in 1 / °C. P_3 is the reference temperature for the model in °C. T is the temperature as determined by the appropriate device's temperature probe in °C.
>Reference Temp.	If the Refractive Index model is Linear or Exponential , set the Reference temperature (P_3 in the previous equations) (°C) for which the reference viscosity is valid.
Thermal Expansion Model	The model to use for the thermal expansion of the solvent. May be Fixed or Polynomial . Set the model type before setting the parameters required for that model.
>Ref. Thermal Exp.	If the Thermal Expansion model is Fixed , specify the a known value for the thermal expansion of the solvent as the percent per °C of change.
>Reference Temp.	If the Thermal Expansion model is Fixed , set the Reference temperature (°C) for which the specified Reference Thermal Expansion is valid.
>Parameters 1-4	<p>If the Thermal Expansion model is Polynomial, set the parameters according to the following equation, where T is the temperature in °C and the parameter values are based on a regression analysis of experimental data:</p> $n(T) = P_0 + P_1T + P_2T^2 + P_3T^3$

Column Profiles

Columns are used in size-exclusion chromatography (SEC) to fractionate a mixture of polymer sizes.

In ASTRA, both conventional and universal column calibration can be performed. In conventional calibration, the analyzed polymer is the same as the polymer used for calibration. In universal calibration, the polymers may be different. Universal calibration requires either a viscometer (and concentration detector) or known values for the Mark-Houwink-Sakurada K and a coefficients. The dn/dc value is required for universal calibration (as it is necessary for intrinsic viscosity calculations), but not for conventional calibration.

ASTRA lets you store a profile for a generic column that contains coefficients obtained from column calibration experiments (see page 8-39). You can set the following properties for a generic column:

Table 7-18: Generic Column Profile Fields

Field	Description
Name	Name of the column. If you have already created a profile for this instrument, click "... " and select a profile to use.
Description	Description of the column, which typically contains more information than the Name.
Plate Count	The column manufacturer provides the initial plate count as documentation, but this value changes over time. You may enter the current plate count here as documentation when you perform an experiment. The plate count is sometimes called "Efficiency". It quantifies the separating efficiency of the column in terms of the "number of theoretical plates (N)". The specific calculation varies by column manufacturer, but generally measures how well the column is packed and its kinetic performance. In general, higher plate counts indicate more efficient columns. More efficient columns yield narrower peaks than less efficient ones.
Asymmetry Factor	The column manufacturer provides the initial asymmetry factor as documentation, but this value changes over time. You may enter the current asymmetry factor here as documentation when you perform an experiment. The asymmetry factor describes the shape of peaks generated by the column. The distance between the elution volume at the peak apex (V_a) and the front of the chromatogram at 10% of the peak apex (V_f) is divided by the distance between V_a and the backside of the chromatogram at 10% of the peak apex (V_b). A value greater than one indicates a "tailing" peak, in which the bulk of material elutes after the apex. Likewise, a value less than one indicates a "leading" peak in which the bulk of the material elutes prior to the apex.
Resolution	The column manufacturer provides the initial resolution as documentation, but this value changes over time. You may enter the current resolution here as documentation when you perform an experiment. The resolution quantifies the ability of a column to separate different species. This is typically measured for a column by injecting two different species into the column, and then measuring the distance between the peaks and the peak widths. The relation is $R_s = 2(V_2 - V_1)/(W_2 + W_1)$, where V is the elution volume for each species and W is the width of each peak at the baseline.
Calibration Technique	The type of column calibration performed. The options are: none, Conventional, Universal with Viscometer Data, and Universal without Viscometer Data.
Flow Marker	The elution volume of the flow marker, which is used when combining peak data from multiple experiments. If zero, the flow marker correction is not used.
Mark-Houwink-Sakurada K	The known Mark-Houwink-Sakurada K parameter of the polymer used for calibration. This parameter is used in the Mass from VS and Branching Analysis procedures. It is also used if you choose the Universal without Viscometer Data calibration technique. In this case, the equation used is: $[\eta] = K M^a$
Mark-Houwink-Sakurada a	The known Mark-Houwink-Sakurada a parameter of the polymer used for calibration. This parameter is used in the Mass from VS and Branching Analysis procedures. It is also used if you choose the Universal without Viscometer Data calibration technique.

Table 7-18: Generic Column Profile Fields

Field	Description
Conventional Calibration Function	Expanding this row shows the A_i coefficients of the following linear regression equation, where M is the polymer molar mass and V is the elution volume: $\log(M) = A_0 + A_1V + A_2V^2 + A_3V^3 + \dots$ Note that a column profile can contain results both for a conventional and universal calibration.
Universal Calibration Function	Expanding this row shows the A_i coefficients of the following linear regression equation, where M is the polymer molar mass, η is the intrinsic viscosity, and V is the elution volume: $\log(M[\eta]) = A_0 + A_1V + A_2V^2 + A_3V^3 + \dots$ Note that a column profile can contain results both for a conventional and universal calibration.

Connection Profiles

A *connection* is an interface between two instruments. There are three types of connections:

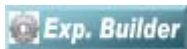
- A *fluid connection* represents a piece of physical tubing that routes the solution from one instrument to the next.
- An *AUX connection* represents a physical wire from the AUX output of one instrument to the AUX input of another instrument.
- An *auto-inject connection* represents a physical connection between the auto-inject output of an injector and the auto-inject input of an instrument.

A *connection profile* stores information about a connection between two specific instruments. Connection profiles are referenced by configurations. Connections must be specified in experiments that use more than one type of instrument. (The WyattQELS instrument is an exception, since it is associated with a DAWN or miniDAWN instrument.)

See Figure 7-3 in “Configuration Example” on page 7-4 for a diagram that shows the typical connections in an online light scattering experiment.

Fluid Connection Profiles

A *fluid connection profile* describes a plumbing (tubing) connection through which solvent or a solution flows between instruments.



Fluid connections are hidden in Run mode. To see them you must enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

You can set the following properties for a fluid connection:

Table 7-19: Fluid Connection Properties

Field	Description
Name	Name of the connection. If you have already created a profile for this connection, click “...” and select a profile to use.
Description	Description of the connection, which typically contains more information than the Name.
Source Device	Select the type of source instrument. The drop-down list shows the instrument profile types that are available for a connection.
Destination Device	Select the type of destination instrument. The drop-down list shows the instrument profile types that are available for a connection.
Volume (mL)	The fluid volume displacement that is a result of the plumbing (tubing) between instruments. This can be set manually or determined via the alignment procedure. Typically, the volume only needs to be set for fluid connections between instruments that collect data. (mL)
Temperature Control > Enable	Check this box when using insulated temperature controlled fluid lines (heated or cooled).
Temperature Control > Temperature	If the fluid line is temperature controlled, specify the temperature to which it is set. Use °C.

AUX Connection Profiles

An *AUX connection profile* describes a connection from the analog output of the source instrument to the analog input of the destination instrument.

If there is no AUX connection and the instrument is capable of collecting data and communicating data to the PC, the instrument-to-PC connection is implied and does not require a profile.

You can set the following properties for an AUX connection:

Table 7-20: AUX Connection Properties

Field	Description
Name	Name of the connection. If you have already created a profile for this connection, click “...” and select a profile to use.
Description	Description of the connection, which typically contains more information than the Name.
Source Device	Select the type of instrument that sends analog data over this connection. The drop-down list shows the instrument profile types that are available for a connection.
Destination Device	Select the type of instrument that receives analog data over this connection. The drop-down list shows the instrument profile types that are available for a connection.
AUX Channel	The input AUX channel number on the destination instrument. If you are using a UV instrument with multiple channels, see “Generic UV Detector Profiles” on page 7-29 for details about AUX connections to UV detectors.
Calibration Constant	Constant value by which the AUX signal should be scaled. This constant can be set manually or determined through one of the calibration procedures. The default value is 1.0. See “RI Calibration” on page 8-29.

Autoinject Connection Profiles

An *Autoinject connection profile* describes a physical connection between the auto-inject output of an injector and the auto-inject input of an instrument.

You can set the following properties for an Autoinjector connection:

Table 7-21: Autoinject Connection Properties

Field	Description
Name	Name of the connection. If you have already created a profile for this connection, click “...” and select a profile to use.
Description	Description of the connection, which typically contains more information than the Name.
Source Device	Select the type of instrument that sends the auto-inject signal over this connection. The drop-down list shows the instrument profile types that are available for a connection.
Destination Device	Select the type of instrument that receives the auto-inject signal over this connection. The drop-down list shows the instrument profile types that are available for a connection.

When a collection is running, the collection graph shows markers for auto-injection signals.

8

Editing Procedures

This chapter explains how to set up your experiment in ASTRA 6 to collect and analyze data. This is done using ASTRA 6 procedures.

CONTENTS	PAGE
About Procedures	8-2
Working With Procedures	8-4
Advanced Procedure Editing	8-8
Collection Procedures	8-11
Configuration Procedures	8-15
Transformation Procedures.....	8-45
Analysis Procedures	8-64
Administration Procedures	8-105

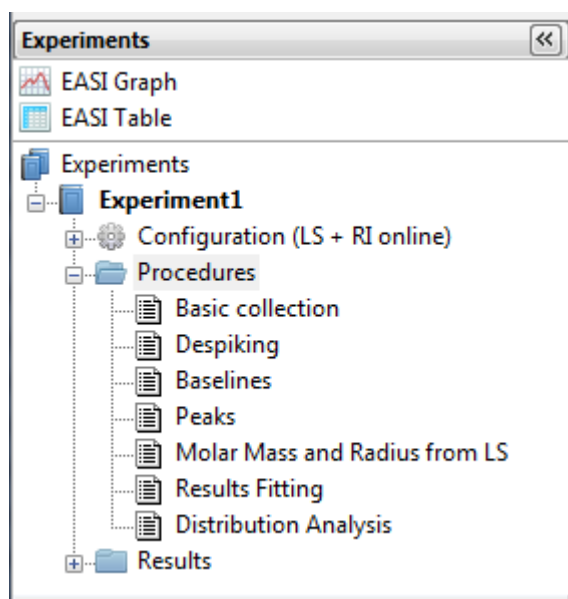
About Procedures

Data collection and analysis in an experiment are broken down into a logical set of units called *procedures* in ASTRA 6. The procedures are performed in order when the experiment is run. There are configuration, collection, transformation, analysis, and administrative procedures.



You must have at least Researcher access to add procedures, and at least Technician access to modify existing procedures. If you are a Guest, you have read-only access to procedures.

The Procedures node in the experiment tree shows actions ASTRA performs in the order shown when you run the experiment.

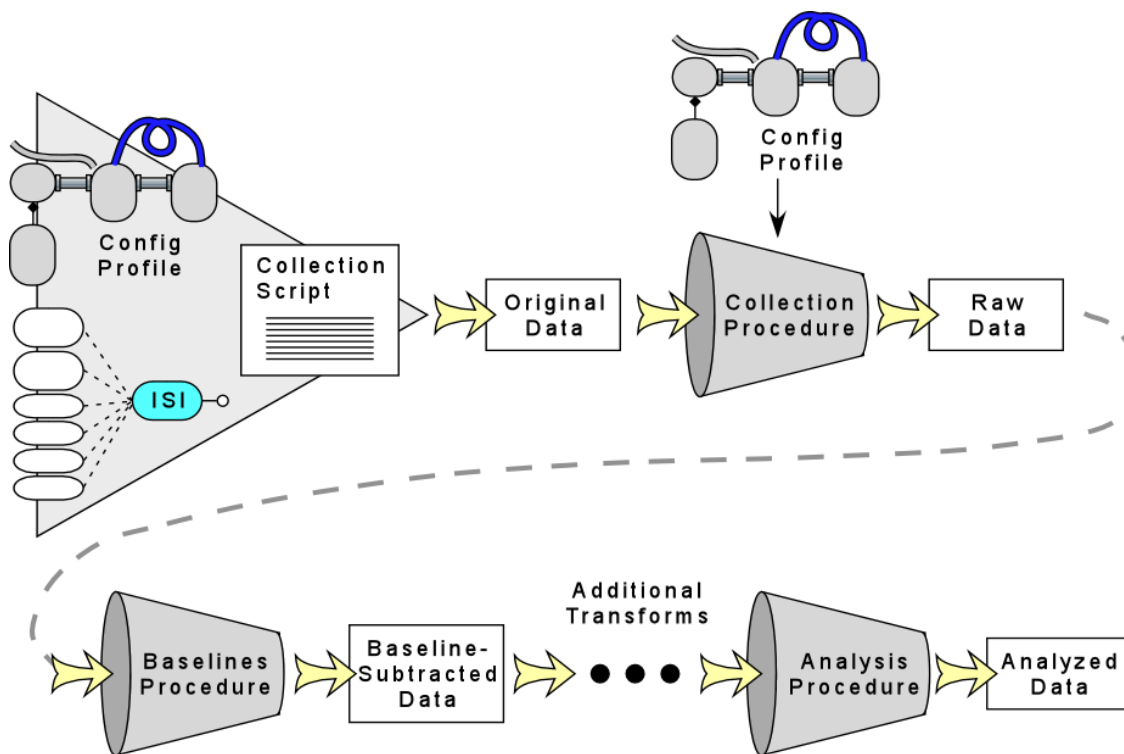


A procedure's status is indicated by its icon, as follows. (Collection procedures have a special two-page icon for all states.)

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid location or does not have the necessary data to run.

About Data Processing in ASTRA

When ASTRA runs a procedure, the data is modified in the order specified by the set of procedures in the experiment. The following figure shows a typical procedure order.



Initially, a collection procedure is responsible for gathering data through the ISI from instruments specified in the configuration. The *original data* created by the collection procedure is then forever kept with the experiment in an unmodified state. The same procedure then performs preprocessing on this original data based on the configuration to create what is called the *raw data*. For example, the original data contains the AUX channel traces from an instrument, but the raw data uses the experiment configuration to route that data to the appropriate instrument specified by the AUX connection.

After a transformation procedure runs—such as setting baselines—the data used by subsequent procedures has the transform applied. A number of transformations can be applied in series to the data.

After an analysis procedure runs, the experiment also contains *analyzed data* that can be displayed in reports.

Working With Procedures

Most users will not need to add, remove, or change the order of procedure items. The methods provided with ASTRA 6 contain procedures for most common experiments.



You must have at least Researcher access to add procedures, and at least Technician access to modify existing procedures. If you are a Guest, you have read-only access to procedures.

Editing Procedure Settings

To set properties of a procedure, follow these steps:

1. Double-click on a procedure in the experiment. This opens the properties page for that procedure.


The properties page shows different types of information depending on the type of procedure. Some procedures have a graph and properties; some have only properties; some have a message that says the procedure has no user configurable parameters.

2. Set properties by typing, selecting from a list, checking a box, or clicking a “...” browse button. You can paste numeric values into a property cell, including values from in scientific notation from ASTRA reports.

Rows shaded in yellow are read-only. You cannot change the value.

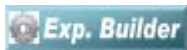
You can expand or hide lists of related properties if there is a + or - sign next to a property name.

3. Click **Apply** or **OK** to make the changes. (**Apply** saves changes without closing the page; **OK** saves changes and closes the page.)

In Run mode, you cannot open a page for a procedure unless that procedure has already been run (has the  run icon) or the procedure is the first one in the list that needs to be run.

In Run mode, you can open only one procedure at a time. When you open a procedure page, any other procedure page you have opened closes automatically. This prevents you from relying on information that may no longer be true due to changes in the settings for other procedures.

Certain procedures are hidden in Run mode if you do not need to interact with them. All procedures are shown in Experiment Builder mode.

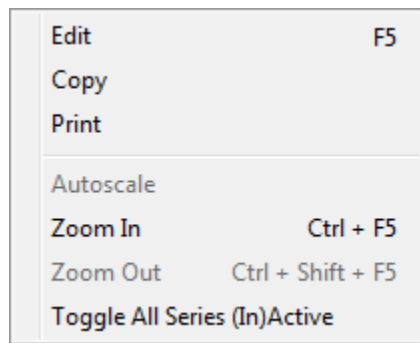


You can open any set of procedure pages if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. Remember that changes to the settings for one procedure affect other procedures. The data shown for later procedures may be incorrect if you have changed earlier procedure settings.

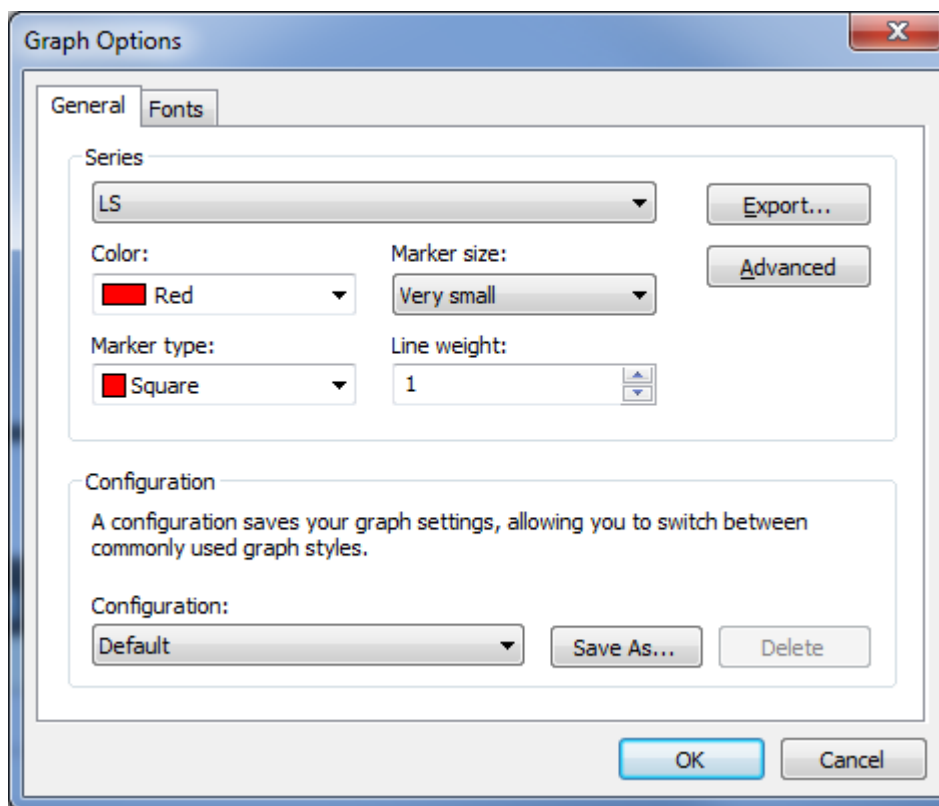
Working with Procedure Graphs

A number of procedure pages contain graphs. You can manipulate these graphs in the following ways:

- **View Data Values:** Point to a line on a graph and hold down the Shift key or press the Caps Lock key. The X and Y coordinates and the type of data are shown as you move your mouse. You can copy these coordinates to your clipboard by pressing Caps Lock and then using Ctrl+C. Press Caps Lock again to turn off the data value display so that you can paste the values into another application.
- **Copy to Clipboard:** Right-click on a graph and choose **Copy** from the pop-up menu. This places the graph in your “clipboard” and you can paste the graph into another program.
- **Print the Graph:** Right-click on a graph and choose **Print**. This displays the Print Preview dialog, which you can use to change the orientation, margins, and printer. See “Printing Graphs” on page 11-17 for details.
- **Zoom In:** Hold down the Ctrl key and your left mouse button. Drag a rectangle around the data you want to view larger. Or, press Ctrl+F5 to zoom in. Press F5 to open the Scale Graph dialog.
- **Zoom Out:** Hold down the Ctrl key and click your right mouse button. Each click undoes one zoom in action. Alternately, you can press Ctrl+Shift+F5 to zoom out one level. If you have zoomed in, you can right-click on a graph and choose **Autoscale** to automatically scale the graph to show all data.
- **Select Detectors or Data:** If there is a list of detectors or data sources above or to the right of the graph, you can use checkmarks to indicate the data you want to view. Some graphs can display multiple data sets in different colors (for example, collection). Others display only one data set at a time (for example, defining baselines). You can right-click and choose **Toggle All Series (In)Active** to check or uncheck all the boxes for data sources.



- **Edit:** Double-click on a graph (or right-click and choose **Edit**) to open the Graph Options dialog. This dialog lets you modify a variety of aspects of a graph's styles.



You see the effects of your changes as you make them without closing this dialog. Click **OK** to save your changes for use until you close the page containing the graph.

Graph customizations—such as line weight, color, marker style, and title changes—are not saved when you close pages that contain graphs. You can save graph settings as a “configuration” that you can then apply to a currently viewed graph. To save a configuration, click the **Save As** button and specify the name of an XML file to contain your current graph settings. To load a configuration, select one you have saved from the **Configuration** list.

The **General** tab of the Graph Options dialog has the following fields:

Table 8-1: Edit Graph Fields

Field	Description
Series	Choose the data series for which you want to change the color or marker type.
Color	Select the line or marker color you want to use in the graph. Changing this property changes the line color for all chromatogram traces.
Marker Size	Choose the marker size you want to use. The options are: very small, small, medium, and large. Use Marker Size for lines made up of individual data points; use Line Point Size for fitted curves.
Marker Type	Select the marker type you want to use in the graph. The default is square.

Table 8-1: Edit Graph Fields

Field	Description
Line Weight	Set the width of the line when the line is a fitted curve. Use Marker Size if the line is made up of individual data points.
Configuration	If you have saved graph configurations, you can select a different one from the drop-down list.

If you click the **Export** button, you can choose to save the graph in one of the following formats:

- data saved as Microsoft Excel file (.xls)
- data saved as comma-delimited text file (.csv)
- data saved as tab-delimited text file (.txt)
- data saved as tagged XML file (.xml)
- image saved as JPEG file (.jpg)



If you click the **Advanced** button, you have much more control over the graph display is provided than is described in this manual. For help on settings in the Advanced dialog, move to a field and press F1.


The **Fonts** tab of the Graph Options dialog lets you select the font, font size, font color, and font style for various text that appears in graphs.

For more about modifying graph displays, see “Viewing and Modifying Graphs” on page 11-15.

When to Modify Procedures

Some procedures prompt you to perform some action, such as marking baselines, when the experiment runs. Other procedures have default values for all their properties.

For procedures other than data collection, you can easily modify the properties after the initial experiment run. Procedures not affected by your changes still show the  run icon. Procedures that need to be re-run show the  not run icon.

Then, you can re-run the experiment using the **Run** command. This time, instead of collecting data, only the procedures marked with the  not run icon are performed.

Advanced Procedure Editing

The following subsections discuss advanced ways of managing procedures in an experiment.

Most users will not need to add, remove, or change the order of procedure items. The methods provided with ASTRA 6 contain procedures for most common experiments. You may contact Wyatt Technical Support if you are having difficulty creating an experiment method for your setup.



If you want to add, remove, or reorder procedures as described in this section, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.



You must have at least Researcher access to add, delete, or reorder procedures.

Adding Procedures

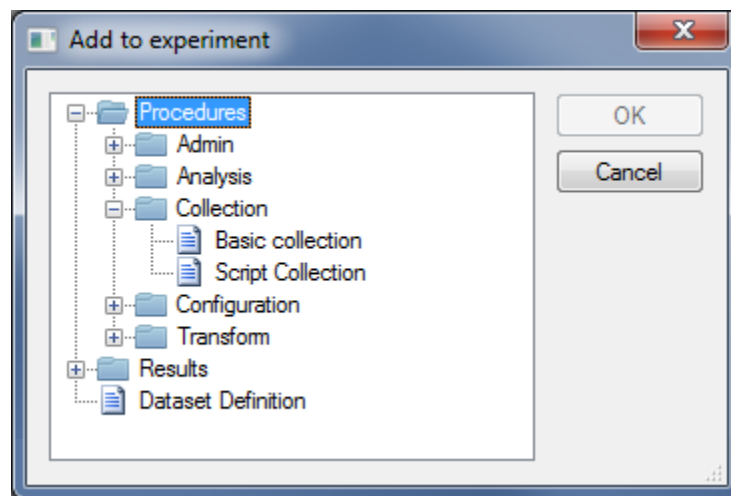


You can add procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

To add a procedure to an experiment, follow these steps:

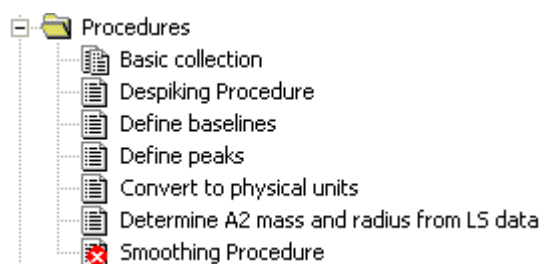
1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results nodes of the experiment.

Shortcuts: Press Ctrl+Shift+P.



2. Open a folder under Procedures and select a procedure to add.
3. Click **OK**. The procedure is added to the end of the experiment.

4. The procedure is likely to have a red X on its icon when it is placed at the end of the experiment. The red X means the procedure is in an invalid location in the list (or it requires data from an instrument that is not in the configuration).

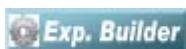


A procedure's state is always indicated by its icon, as follows. (Collection procedures have a special two-page icon for all states.)

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid location or does not have the necessary data to run.

5. Drag the procedure to a position in the list where its icon changes to show it is in a valid location.

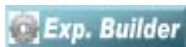
Deleting Procedures



You can delete procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

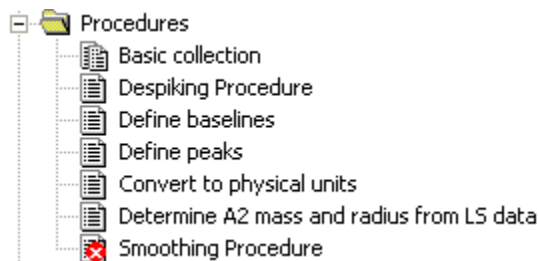
To delete a procedure from an experiment, select the procedure and press the **Delete** key.

Changing Procedure Order



You can reorder procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

If a procedure is in an invalid location in the list of experiment procedures, it will have a red X on its icon.



To correct the problem, drag the procedure to a location in the list where its icon and the icons that follow it have no red X. See the section about the specific procedure in this chapter for information about its valid positions in an experiment.

Validating a Procedures After Modification

To check the procedure and configuration, choose **Processing→Validate**.

Shortcuts: Press Ctrl+Shift+V.

In addition to checking for a correct procedure order, validation also tests to make sure instruments in the configuration are connected and available when you are getting ready to collect data. It checks for values that may be required depending on the instruments you are using and the procedures to be run. For example, these values may include dn/dc, UV extinction coefficients, and solvent viscosity. It also validates collection scripts for experiment builders. If you use the basic collection procedure, the collection script is built automatically, and validation never finds any problems with the script.

See “Validating an Experiment” on page 6-11 for more about validation results.

If any procedure in the list has a red X on its icon, it is in an invalid location in the experiment or it requires data from an instrument that is not in the configuration. Modify the order as described in “Changing Procedure Order” on page 8-9 or revise the experiment configuration to include the appropriate instruments.

Collection Procedures

Your experiment will typically contain a collection procedure as the first procedure in the experiment. The following types of collection procedures are available:

- “Basic Collection” on page 8-11
- “Script Collection” on page 8-13

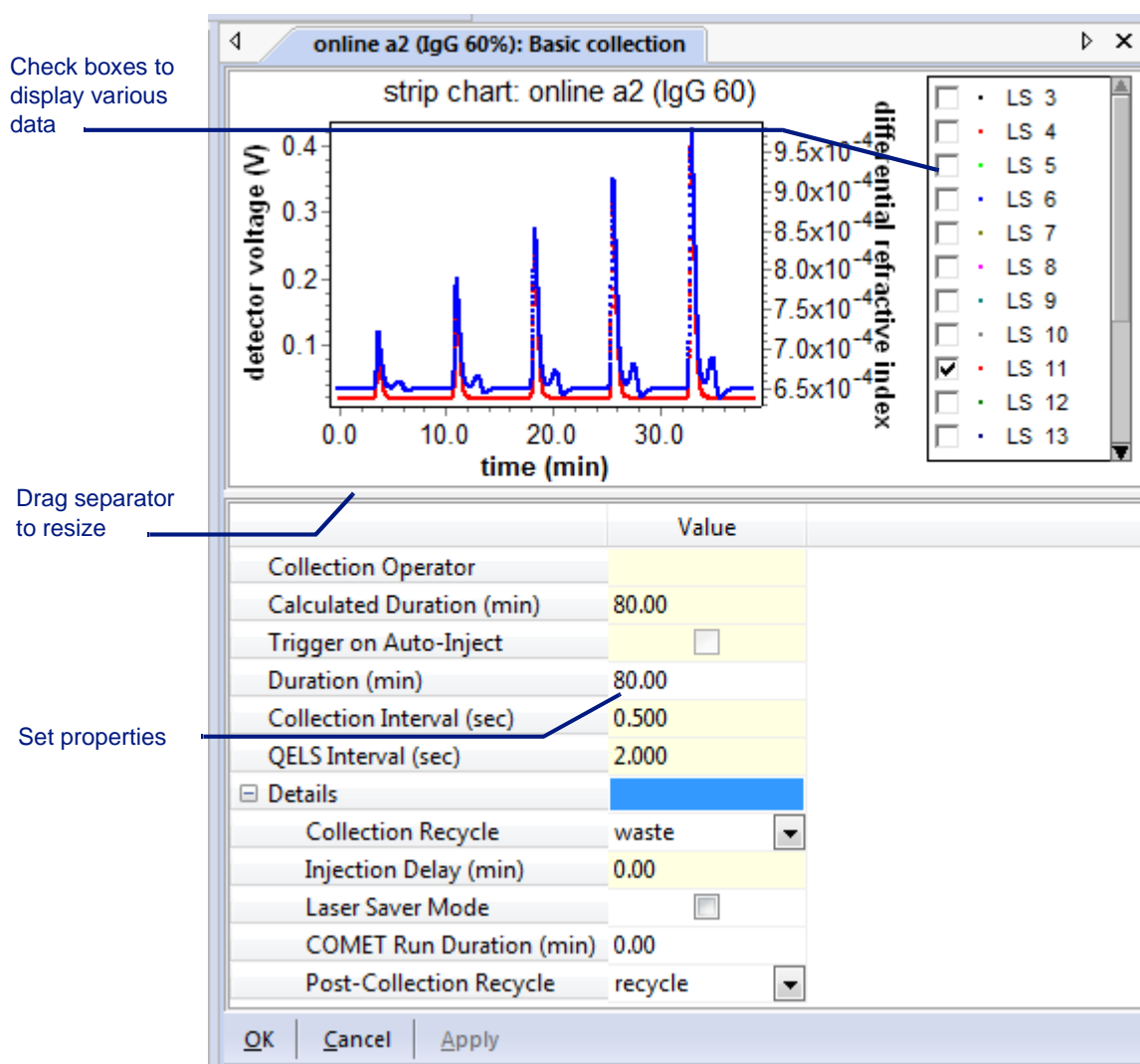
Basic Collection

The Basic collection procedure collects data from the instruments specified in the Experiment Configuration. You can set properties for this procedure before running the experiment. When you run the experiment, this procedure runs automatically, without prompting for parameters. You can use this page to view data as it is being collected.

If multiple instruments are collecting data, you can choose to view any set of signals in the checkboxes to the right of the graph. You may need to scroll to see some checkboxes. Signals are typically displayed in flow order. UV is generally at the top of this list, followed by light scattering (LS) and other signals. The other signals may include the forward laser monitor (FM) and refractive index. Light scattering data is shown in red; RI data is blue; UV data is green, QELS data is magenta, and viscosity data is black.

The x-axis of the graph uses units specified by the Abscissa Units property of the experiment configuration (see page 7-14). By default, the units are milliliters for an online experiment and minutes for a batch experiment.

The y-axis of the graph uses raw data units. For light-scattering experiments, this is in volts. The data is converted to other units by a later transformation procedure.



The properties you can set are as follows:

Table 8-2: Basic Collection Properties

Field	Description
Collection Operator	The user at the time data collection is started. See "Operator Names in Reports" on page 10-2 for details.
Calculated Duration	Shows the total duration of the collection based on the Duration, Injection Delay, and COMET Run Duration.
Trigger on Auto-Inject	Check this box if an auto-inject signal will signal the start of data collection.
Duration	The time or fluid volume for which data is to be collected. The units are determined by the Abscissa Units property of the experiment configuration.
Collection Interval (sec)	How often the light-scattering or other instrument should collect data. The default is every 0.5 seconds. This interval may be set to a multiple of 0.125 seconds. This interval is used to set the collection interval for Wyatt LS instruments, ViscoStars and/or the Optilab rEX.
QELS Interval (sec)	How often the QELS instrument (if there is one) should collect data. The default is every 2 seconds. This interval may be set to a multiple of 1 second. The maximum interval is 1 hour.

Table 8-2: Basic Collection Properties

Field	Description
Details	
>Collection Recycle	Controls the Recycle valve position during the collection. This may be set to "waste" or "recycle". The default is "waste"
>Injection Delay	The delay in time or fluid volume between injection and the start of data collection. The units are determined by the Abscissa Units property of the experiment configuration. The default is zero.
>Laser Saver Mode	Turn the light-scattering instrument's laser off after collection is finished. If you will not collect more data for at least an hour or so after finishing this collection, it is best to turn off the laser. However, you want to be sure not to cycle the laser frequently, since this will shorten the life-span of the laser. If you are using this collection as part of a sequence, do not check the Laser Saver Mode box. Instead, use one of the System > Methods > Light Scattering > Utilities > turn laser off methods as the last experiment method in the sequence.
>COMET Run Duration (min)	After data collection, run the COMET cell cleaner for a specified duration. You should also check the Comet Cell Cleaner box in the configuration page for the light-scattering instrument. See the COMET hardware manual for more information about the COMET cell cleaner.
>Post-Collection Recycle	Controls how the Recycle valve is set at the end of the collection. This may be set to "waste" or "recycle". The default is "recycle."

You can change the Duration while an experiment is running. However, you cannot change the collection interval or auto-inject signals after collection has started.

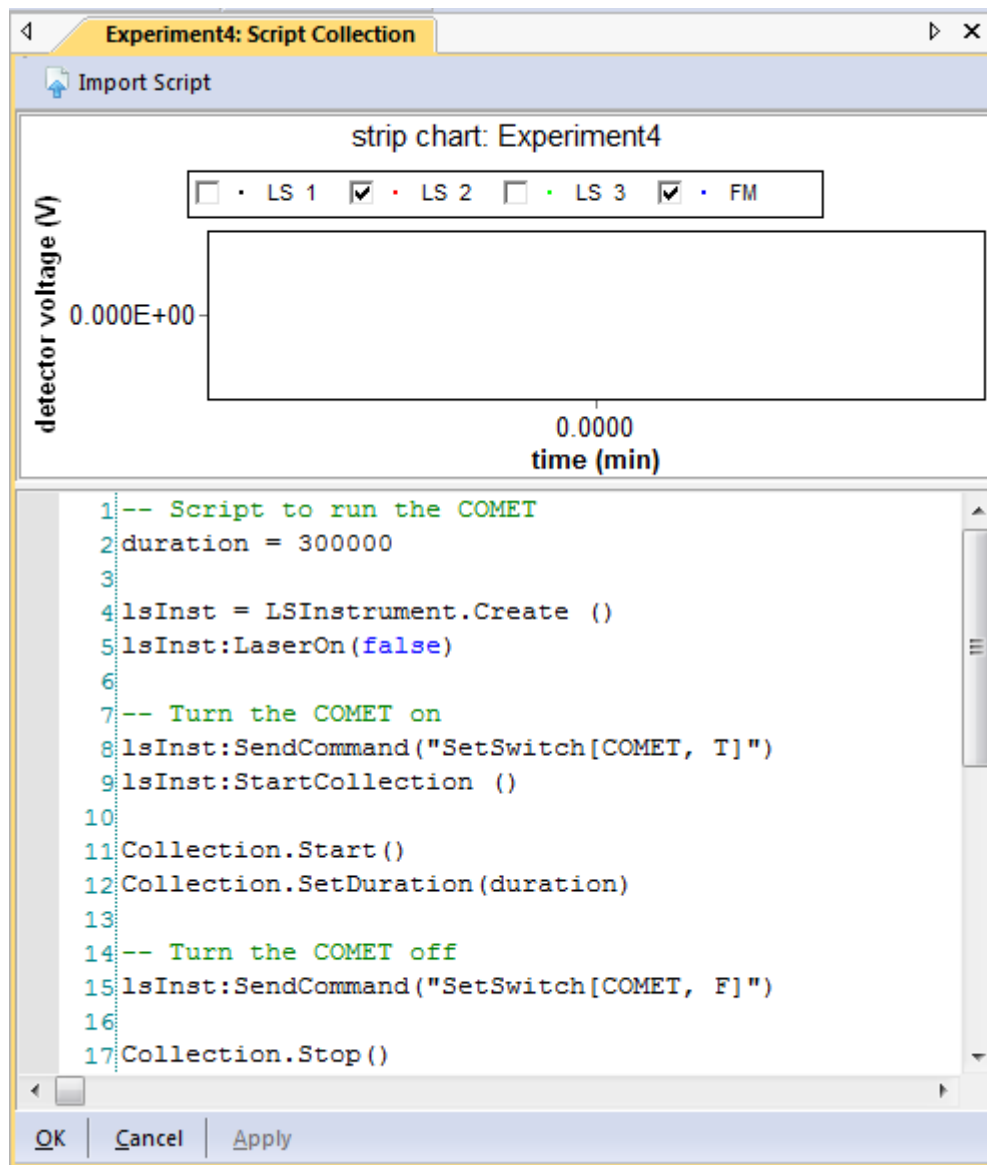
The "Basic Collection" procedure automatically closes all purge valves, switches recycle valves to waste (by default), and deactivates the COMET feature prior to data collection. The exception to the closing of purge valves is when absolute RI analysis is conducted, the Optilab rEX purge valve is left open as required.

Script Collection

You can customize your data collection by using a script collection. To learn more about writing collection scripts, and the scripting language, see Appendix C, "Data Collection with Scripts". Several simple collection scripts are included with ASTRA 6, and are used, for example, in the experiment methods for light scattering calibration and in utility methods for turning off the laser and for using the COMET cell cleaner.

The graph in the Script collection procedure page behaves the same as the graph in the Basic collection procedure page.

To select a script for the procedure, click the “...” button for the File Name property. In the Open dialog, select the script you want to use and click **OK**. Collection scripts typically have a file extension of *.col. You can type a script directly in the Script property row, but using a separate text editor is recommended.



Configuration Procedures

You may need to calibrate your instruments or measure various aspects of their behavior. These procedures may be used in separate calibration experiments, or integrated into other experiments.

The following configuration procedure types are available:

- “LS Calibration” on page 8-15
- “Alignment (Interdetector Delay)” on page 8-18
- “Band Broadening” on page 8-20
- “Normalization” on page 8-23
- “RI Calibration” on page 8-29
- “Absolute RI Calibration” on page 8-35
- “Calibrate Column” on page 8-39
- “Column Plate Count” on page 8-43

LS Calibration

A DAWN instrument needs to be calibrated to enable ASTRA to convert its signals to Rayleigh ratios. You must determine its calibration constant before using ASTRA to calculate absolute molar masses.

Calibration should be performed in batch mode; that is, before connecting the DAWN to a fractionation system.

This section describes the behavior of the separate procedure that performs calibration. In practice, this procedure is not used in isolation. Instead, you create an experiment using the calibration method for your instrument. That experiment contains a number of procedure items that run in order to turn the laser on and off, set peaks, and more.

When to Calibrate

Wyatt Technology calibrates each DAWN during manufacture and includes the calibration constant on the Certificate of Performance shipped with the instrument. However, you should calibrate the DAWN in your own lab and compare the value you obtain with the value on the QC report to verify that no internal damage occurred during shipment.

A DAWN should be recalibrated for any change that may affect the value of the scattering signal at the 90° detector. Calibrate if you:

- Disassemble the flow cell.
- Change the 90° detector photo diode.
- Change the laser gain.
- Have not calibrated in the last 6 months to 1 year (older DAWN EOS and DSP models only). The passage of time may affect the signal.

- Change the jumper setting on the amplifier PCB for the 90° detector. If you do this, make sure you change the corresponding detector gain in the DAWN profile.
- Realign the laser beam. (older DAWN DSP models only)

See “Measured Quantities and Calibration” on page D-4 for a discussion of calibration theory.

How to Calibrate

To perform a calibration experiment, follow these steps:

1. Set up your equipment for a batch (non-flow) experiment.
2. Create a new calibration experiment by choosing **File→New→Experiment From Method**.
3. In the New from Existing dialog, open the “System > Methods” folder, then “Light Scattering”, then “Calibration”, and then the experiment for your light-scattering instrument.
4. Click **Create**.
5. Double-click the light-scattering instrument in the configuration.
6. In the properties page, select the appropriate physical instrument, sample cell, and wavelength.
7. Select the Solvent tab or double-click the Solvent in the configuration.
8. The default solvent is toluene. If you would like to use a different solvent, click the “...” button for the Name property and open the System Solvents folder. Then select your solvent from the list of solvent profiles and click **Copy**.

You should calibrate the instrument using a pure solvent with a well characterized Rayleigh ratio. We recommend calibrating with HPLC-grade toluene for the following reasons:

- It has a high and accurately determined Rayleigh ratio.
 - It is generally a dust-free solvent.
 - Its refractive index is very similar to that of the flow cell windows.
9. Inject the solvent into the flow cell using a syringe pump. The solvent must be pure and free of particulates; we recommend that you use a 0.02 µm syringe filter attached to the syringe.
 10. Wait until solvent is flowing through the cell and the front panel display for the 90° detector (detector 11 on most DAWNs or detector 5 on a DAWN 8) is stable. The variation in the signal should be 5 µV or less.
 11. Choose **Processing→Run** to begin running the calibration experiment. The calibration constant is calculated and written to the DAWN instrument configuration property page and the final calibration report.
 12. Use the calibration constant in other experiment configurations in either of the following ways:

- Type the calibration constant in the property page for the DAWN instrument in other experiments.
- Export the calibrated DAWN profile as a profiles, then import the profile whenever you create a new experiment. See Chapter 12, “Working with Profiles” for details.

The accuracy of this constant may be improved by repeating the measurement a few times and averaging the results.

DAWN DSP and DAWN DSP-F: ASTRA prompts the user to turn the laser on and off at the appropriate times.

Setting Calibration Properties

The values for this procedure are set in the calibration method. Typically, you will not need to modify them. You can double-click on the LS calibration procedure to open its property page:

	Value
Baseline Peak Index	0
Calibration Peak Index	0
Calibration Constant (1/(V cm))	0.0000e+000

This procedure has the following properties:

Table 8-3: LS Calibration Properties

Field	Description
Baseline Peak Index	Number of the peak marker in the Peaks page that marks data collected with the laser off. The calibration method contains a pre-set baseline peak.
Calibration Peak Index	Number of the peak marker in the Peaks page that marks data collected with the laser on. The calibration method contains a pre-set calibration peak.
Calibration Constant	Shows the calibration constant that was computed.

The **Upload to Instrument** button above the list of properties updates the instrument with the computed calibration constant. (HELEOS, HELEOS 8, and TREOS instruments only.) When you begin collecting data in an experiment, ASTRA warns you if the calibration constant in the experiment configuration is different from the calibration constant stored on the instrument.

Alignment (Interdetector Delay)

If an online experiment has already been run, you can open the Alignment procedure view by choosing **Experiment→Configuration→Alignment**, or by right-clicking on the Configuration node in the experiment and selecting **Alignment** from the pop-up menu.

ASTRA uses the volume delay between different instruments to correlate their measurements. Determine the delay volumes by collecting data on a monodisperse sample, then aligning the resulting peak for each instrument collecting data.

Once delay volumes between instruments have been determined, ASTRA subtracts them from each instrument to correct for the time it takes fluid to propagate between instruments.

When to Determine the Delay

This procedure only needs to be performed once when you connect the instruments or change the tubing between the instruments. The volume delay will remain the same until you change the length of tubing between the instruments or change the instrument order.

How to Determine the Delay

To determine the delay volumes, follow these steps:

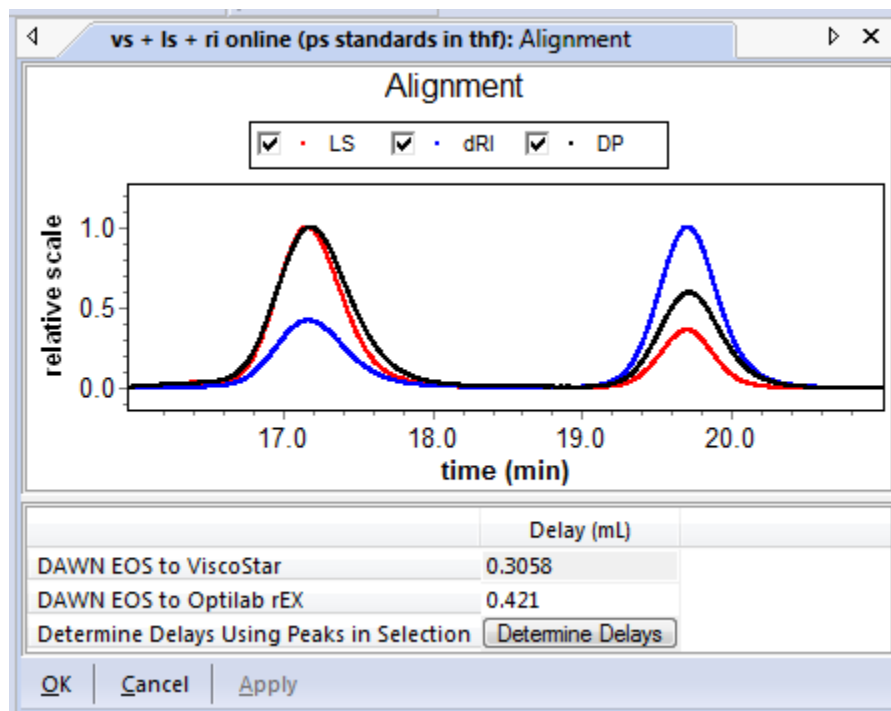
1. Prepare a monodisperse sample for data collection.

A monodisperse sample is necessary for determining the volume delay between the DAWN and other instruments. It should have a polydispersity of less than 1.05. A suitable sample for alignment may be a narrow polystyrene, polyethylene oxide or a non-aggregated protein, such as bovine serum albumin (BSA).

Broad standards will *not* have peaks overlaid even when the system is properly aligned. This is perfectly normal and due to the different characteristics of molar mass detectors and concentration detectors.

2. Create an experiment that includes the Alignment procedure. For best results, set the collection interval to 0.5 or 1.0 second. Intervals below this range are too noisy to provide good resolution.

- The page for this procedure looks as follows.



The relative heights of the peak for the traces are auto scaled to match each other.

- Use your mouse to select a range that contains the peaks you want to use to align the detector delays.
- Click the **Determine Delays** button. The delays between instruments are automatically calculated. This procedure stores the interdetector delays in the properties of the fluid connections in the experiment configuration.
- Alternately, you can type delay values directly in the property fields. The graph shows the peaks corrected for the delay values currently entered.

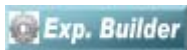
This procedure has the following properties:

Table 8-4: Alignment Properties

Field	Description
Instrument relation	A separate row is shown for each fluid connection between the light scattering instrument and other instruments.
Delay	The time or fluid volume between the two instruments. The units are determined by the Abscissa Units property of the experiment configuration.
Determine Delays	Click this button to automatically calculate the delays between instruments.

If you adjust the Alignment so that peaks are perfectly aligned and then go to the Band Broadening procedure (page 8-20), you may see a slight offset. This is because the analysis used in the Alignment procedure is slightly different from that in the Band Broadening procedure. In addition, the broadening of non-symmetric peaks causes the peak apexes to

shift slightly. In most cases, the Alignment procedure makes the initial adjustment and the Band Broadening procedure makes improvements. However, in rare cases, broadening causes enough of a peak shift that an error message occurs. If this happens, adjust the alignment manually to get the broadening procedure to function properly.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

Band Broadening

A chromatographic peak broadens as it passes through a series of in-line detectors. Consider a narrow sample “peak” moving through a series of instruments. Each instrument’s tubing and flow cell acts as a small mixing volume, causing the initially sharp peak to broaden with a slight exponential tail. Thus when ASTRA combines the light scattering and concentration data, it is likely that there will be mismatches in the peak traces, most noticeably at the edges of the peak.

A second effect also contributes to inter-detector mismatches. Each instrument contributes its own a broadening effect, regardless of its position in the flow path. As a peak enters an instrument’s measuring volume or cell, the instrument may or may not immediately register the sample’s presence, depending on the flow cell geometry and measurement type. For example, molecules must fully reach the illuminating beam in an optical instrument in order to be measured, yielding relatively sharp peaks. In contrast, in a viscometer the pressure signal begins increasing the instant a sample enters one end of the measuring capillary.

By fitting the change in the peak shape as the sample passes between pairs of detectors, one can determine the magnitude of these two effects. Since these effects are essentially independent of the composition of the sample, we can use the fit parameters determined from fitting a narrow standard to all subsequent data runs, whether they are narrow or not.

If left uncorrected, broadening can result in incorrect results for analysis methods that compare the signal of two different instruments. Using ASTRA’s Band Broadening procedure, you can correct for both the exponential mixing effect and the relatively Gaussian-shaped instrumental effect. For additional information, please refer to US Pat. No. 7386427.

For an example experiment that determines band broadening, choose **File→Open→Experiment** (or **File→Import→Experiment** if you use ASTRA 6 with Research Database). Open the “band broadening example (BSA).afe6 experiment in the Sample Data > Analyzed Experiments folder.

When to Determine Band Broadening

This procedure only needs to be performed once when you connect the instruments or change the tubing between the instruments. The band broadening remains the same until you change the length of tubing between the instruments or change the instrument order.

For more about how and why you need to correct for band broadening, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Band Broadening correction.

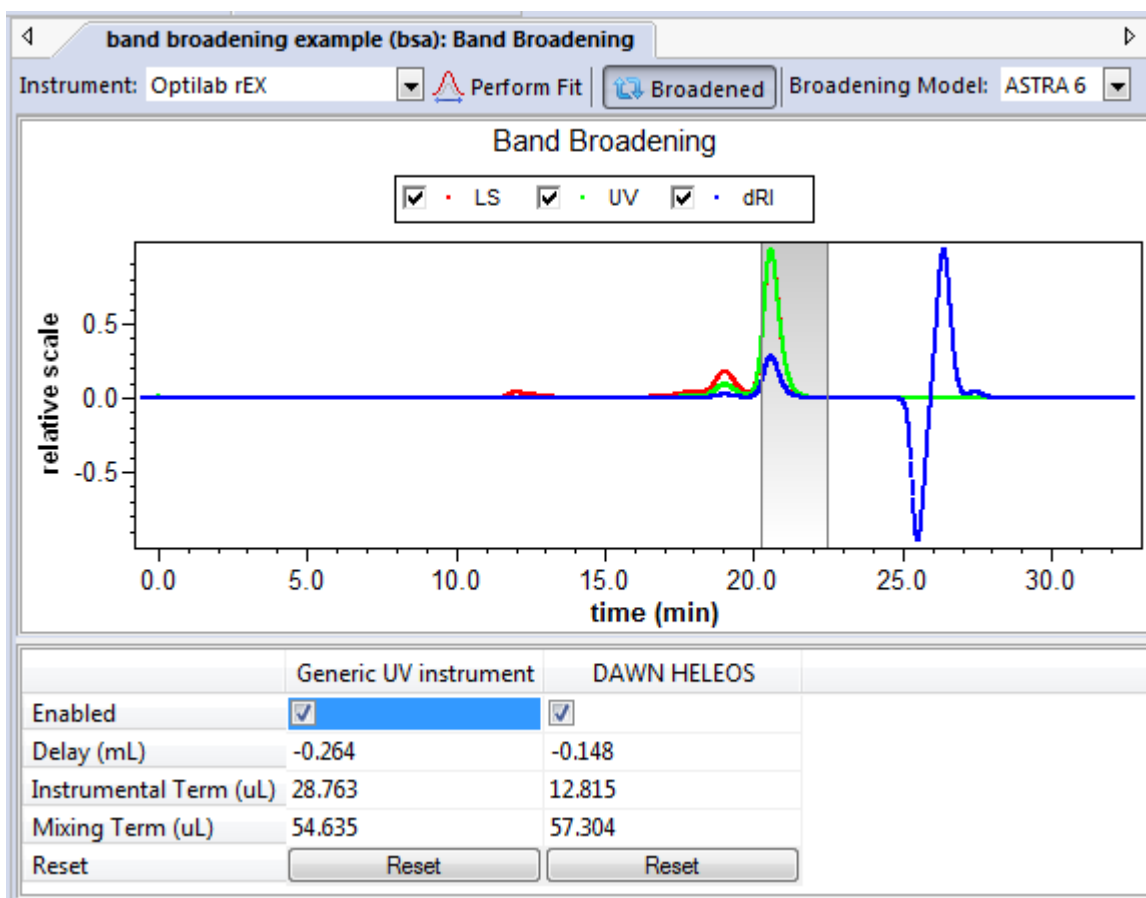
How to Determine Band Broadening

To use this procedure, collect data from a narrow, monodisperse sample for use in determining the band broadening parameters.

To calculate appropriate band broadening terms, follow these steps:

1. Open the Band Broadening procedure view. If an experiment has already been run, you can open this view by choosing **Experiment**→**Configuration**→**Band Broadening**, or by right-clicking on the Configuration node in the experiment and selecting **Band Broadening** from the pop-up menu.

The procedure view looks similar to the following:



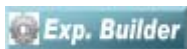
2. At the top of the view, select the **Instrument** that has the broadest signal. This is the reference instrument, against which others will be compared. ASTRA automatically selects the furthest downstream instrument, as it typically has the greatest broadening. However, if there is a viscometer in the flow path, choose the viscometer as the reference instrument. The inherent instrumental broadening in the viscometer is usually greater than the broadening resulting from flow between instruments.
3. Drag a peak range on the graph to create a range for determining the band broadening parameters. It is important that you select a mono-disperse peak. Be careful to select a region of the peak that is free from contamination by other eluting species. Typically, the range marker should be set from a position about halfway up the leading edge of the peak to a point just past the peak where all detector signals have returned to the baseline.
4. Click the **Perform Fit** button in at the top of the view to calculate the terms and update the graph.
5. You can click the **Broadened** button to toggle broadening on and off to compare the fit.
6. You can select the ASTRA 6 or ASTRA V **Broadening Model**. The band broadening logic is slightly different between the two versions of ASTRA. Band broadening corrections performed with ASTRA V are identical when opened with ASTRA 6. However, if you perform a broadening correction in ASTRA 6, and save the experiment as an ASTRA V data file, the results will be slightly different, because ASTRA V interprets band broadening terms differently. (The default model is the ASTRA 6 model. You can change the default using the **System→Preferences→Default to ASTRA V Band Broadening** command.)
7. Examine the fit between the two traces and the values for the Instrumental Term and the Mixing Term. The expected values vary depending on whether there is a viscometer in the instrument series and whether despiking or smoothing has been performed.
 - **No Viscometer and No Despiking or Smoothing:** The value of the instrumental term should be small (1 μ l).
 - **No Viscometer and Despiking or Smoothing Performed:** The value of the instrumental term will generally be larger than 1 μ l but smaller than the mixing term.
 - **Viscometer Used:** The instrumental term will generally be comparable to or larger than the mixing term. Despiking and smoothing should not make much difference if there is a viscometer.
8. If the resulting instrumental term is significantly larger than expected and the match between the peaks is not good, you should repeat the fit. To do this, click **Reset**, then enter seed values for the instrumental and mixing terms.

- **No Viscometer Used:** Use seed values of 1 μL for the instrumental term and 50 μL for the mixing term.
 - **Viscometer Used:** Use seed values of 20 μL for the instrumental term and 20 μL for the mixing term.
9. Click **Perform Fit** again.
 10. If repeated attempts to obtain a good fit fail, either verify that the baselines are set correctly or choose a different range for the fit.
 11. When the fit between the two traces looks good, click **OK** or **Apply** to re-run the experiment with the band broadening correction.

This procedure has the following properties:

Table 8-5: Band Broadening Properties

Field	Description
Instrument	The instrument with the largest degree of broadening in the instrument series. This is typically the last instrument in the series, unless a viscometer is present, in which case the viscometer should be chosen as the reference instrument.
Perform Fit	Click Perform Fit when you are ready to use the peak marker to calculate the band broadening terms. The graph shows the corrected data.
Traces	The instrument trace(s) to broaden. These columns are set automatically when you choose a reference instrument.
Enabled	Check this box to enable band broadening for specific instruments.
Delay (mL)	The interdetector volume (or time) between the reference instrument and the instrument to broaden. (The units are determined by the Abscissa Units field in Experiment Configuration.) You can set the initial seed value here or through the alignment procedure. The band broadening procedure recalculates the interdetector volume when determining the band broadening parameters. The resulting interdetector volume is generally different from that obtained from the alignment procedure.
Instrumental Term	A term that defines the degree of broadening due to instrumental effects—that is, not due to mixing—in the band broadening calculation.
Mixing Term	A term that defines the degree of broadening due to mixing in the band broadening calculation.
Reset	Click Reset to change the instrumental and mixing terms back to 1 μL and 50 μL (respectively) and update the graph.



This procedure is hidden in the Experiment tree in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure in the tree, choose **System→Preferences→Experiment Builder Mode**.

Normalization

If an experiment has already been run, you can open the Normalization procedure view by choosing **Experiment→Configuration→Normalize**.

Normalization is the process by which the various detectors' signals are related to the 90° detector signal and the instrument calibration constant. By definition, the 90° detector always has a normalization coefficient of 1.

(This is detector 5 on a DAWN 8 and detector 11 on all other DAWNs.) Good normalization is an important component in achieving good results from a DAWN.

When to Normalize

The first time you use ASTRA after installing your DAWN or miniDAWN, you need to normalize the detectors. Thereafter you will need to normalize only under certain conditions:

- Whenever you collect data from a sample whose solvent is different from that used for the previous normalization.
- For aqueous solvents, whenever the solvent offsets you just collected are better than those used for computing normalization coefficients earlier. (Better means lower voltages.)
- Whenever you reinstall the DAWN flow cell.
- Whenever you alter the laser alignment. (Laser alignment is necessary only for DAWN DSP and DSP-F instruments.)

See “Normalization” on page D-8 for details on how normalization coefficients are used in calculations.

Selecting a Normalization Standard

The normalization standard you use should have an RMS radius smaller than 10 nm and a low polydispersity. It should be highly concentrated and use the same solvent you plan to use for your experiments. The issues related to these requirements are discussed in the following list:

- **Isotropic Scattering:** The important concept to understand about normalization is that a very small molecule scatters light *isotropically*, meaning with equal intensity in all directions. So if we inject a very small molecule into the DAWN flow cell, we might expect to measure equal voltages at all detectors. This is not the case for several reasons:
 - Different detectors are collimated differently to improve performance and thus do not “see” equal lengths of the flow cell bore.
 - Refractive index effects come into play and change the light intensity and scattering angles.
 - Individual detectors vary somewhat in sensitivity.

We overcome these conditions by injecting a very small molecule and computing factors to force the light intensity to be equal for all detectors. As long as we inject a molecule whose size is too small to be measured accurately by the DAWN (smaller than 10 nm), the exact size does not matter.

A good isotropic standard is a sample molecule with a diameter of less than about 1/20th of the wavelength of the incident light, which is the case for random coil molecules with a molar mass below 50,000, and also for most proteins. Perform the normalization at the flow rate and in the solvent you intend to use to run samples.

- **Low Polydispersity:** It is inappropriate to normalize with a broad distribution (polydispersity greater than 1.2) or unknown sample. Always use a relatively narrow distribution (polydispersity less than 1.1), low molar mass sample for normalization.

It is usually appropriate to use the same sample for normalization that you used to determine the delay volumes between the DAWN and other instruments. If there are absolutely no narrow standards available in the solvent you are using, you may be able to normalize properly by setting the peak region to include only the central part. Nevertheless, use a narrow standard if one is available.

- **Same Solvent as Samples:** Due to the changes in scattering angle with solvent refractive index, the normalization needs to be performed in the same solvent as the samples you want to analyze. For chromatography, we recommend one of the following:
 - A 30,000 g/mol narrow polystyrene in toluene or THF, having an RMS radius of about 5 nm.
 - A 20,000–30,000 g/mol polysaccharide such as pullulan or dextran in water, or a PEO, also having an RMS radius of about 5 nm.
 - A monomer protein such as BSA in water, having an RMS radius of 3 nm.
- **High Concentration:** The standard you inject for normalization is at a higher concentration than normal. This is to improve the signal-to-noise ratio of the measurement. Aim for a ratio of at least 100:1 for the normalization peak.
- **Batch Mode Issues:** For batch measurements, you do not have the advantage of molar mass separation as you do in chromatography. Any aggregates in your sample will not be separated and may cause normalization errors. Therefore, we recommend higher concentrations of non-aggregating lower molar mass standards for normalization in batch mode. A 10–15 mg/mL solution of 4000 g/mol polystyrene in toluene or THF, or 5000 g/mol dextran, pullulan or PEO in water, works well. All of these have RMS radii of about 2 nm.

Running a Normalization Experiment

The sample and solvent you use for normalization are important. Follow these steps to normalize:

1. Choose a normalization standard as described earlier in this section.
2. Create a new experiment from the method appropriate for your type of experiment.
3. Run the experiment and set baselines and peaks as described for those procedures. Use a narrow peak for normalization.

For online experiments, set a peak symmetrically over a monomer peak (exclude any multimer peaks). For batch mode, set a peak region over the plateau corresponding to your normalization standard.

4. Perform the normalization by choosing **Experiment→Configuration→Normalize**, or by right-clicking on the Configuration node in the Experiment and selecting **Normalize**. You see the property view for the procedure.

The screenshot shows a dialog box titled "band broadening example (bsa): Normalization". It contains a table for coefficients and a "Details" section.

	Value	
Peak Name	Peak 1	
Radius (nm)	5.00	
Action	<input type="button" value="Normalize"/>	<input type="button" value="Import"/>
<input checked="" type="checkbox"/> Coefficients for	Old	New
Detector 1	282.720	282.720
Detector 2	2.727	2.727
Detector 3	1.233	1.233
...		
Detector 18	1.198	1.198
<input checked="" type="checkbox"/> Details		
Normalization Type	standard	
Model	Debye	
Radius Type	rms	
Percentage to Keep	n/a	

At the bottom of the dialog are buttons: , , and .

5. In the property view, specify the peak and radius for the sample. If you are using a Lorenz-Mie or sphere model for the sample, specify that the radius is a geometric radius.
6. If this is a batch experiment, specify the fraction of data to keep for the normalization.
7. Click the **Normalization** button.
8. The newly calculated normalization coefficients are displayed next to the previous coefficients. If you wish to use the new normalization coefficients, click **OK** or **Apply**. Otherwise, click **Cancel**.
9. You can view the calculated normalization coefficients in the Experiment Configuration page for the DAWN or miniDAWN.

Importing Normalization Coefficients

Instead of performing a normalization, you can import normalization coefficients from another experiment by clicking the **Import** button. The source experiment must contain normalization coefficients and must be open before you click the **Import** button.

Setting Normalization Properties

You can set properties for this procedure before or during the experiment. Double-click on the Normalization procedure to open its property page:



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

This procedure has the following properties:

Table 8-6: Normalization Properties

Field	Description
Peak Number	Type the number of the peak that corresponds to your normalization standard.
Radius	The radius of the normalization standard in nm.
Action	Click the Normalization button in this row when you are ready.
Coefficients for Detectors	The Old column shows previous normalization coefficients for each detector. The New column shows the computed normalization coefficients.
Normalization Type	The type of normalization to use. Options are standard and area. <ul style="list-style-type: none"> • “Standard” normalization uses the Rayleigh Ratio peak apex as the basis for normalizing. In effect, it divides the peak apex for each detector angle by the peak apex value for the 90-degree detector. • “Area” integrates Rayleigh Ratios over the entire peak. The Rayleigh Ratio peak areas for each detector angle are divided by the area for the 90-degree detector to yield the normalization coefficient. This method provides better performance than “standard” normalization.
Model	Displays the fitting model being used.
Radius Type	The type of radius specified. Options are RMS, geometric, and hydrodynamic. If you are using a Lorenz-Mie or sphere model for the sample, specify that the radius is a geometric radius.
Percentage to Keep	If this is a batch experiment, specifies the fraction of data to keep for performing the normalization. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.

Checking the Normalization Coefficients

After normalizing the detectors, you need to make sure that the coefficients you obtained are accurate. You can use the analysis plot (“About Analysis Plots” on page 8-66) to do this.

To use the analysis plot, inject a sample with a radius around 20 nm; a linear polymer with a molar mass about 200,000 g/mol is suitable. Set baselines and mark the peak, then use the Molar Mass and Radius from LS Data procedure (page 8-64) to display the analysis plot using the Debye model and a Fit Degree of 1. It is a good idea to step through several data slices at the top of the peak (use the left and right cursor keys) to get a

feeling for the random noise in the data. If one detector is consistently off the fitted line (above or below) its normalization coefficient needs to be redetermined.

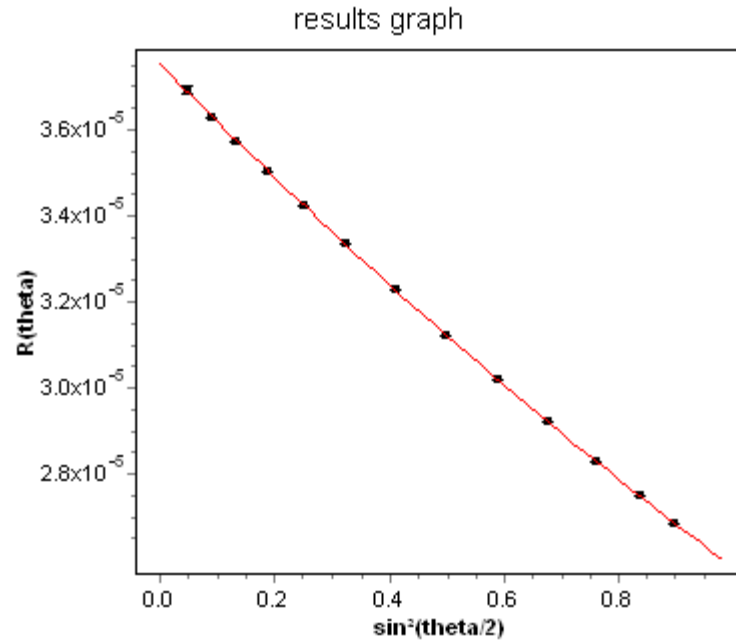


Figure 8-1: Analysis plot showing accurate normalization coefficients for all angles

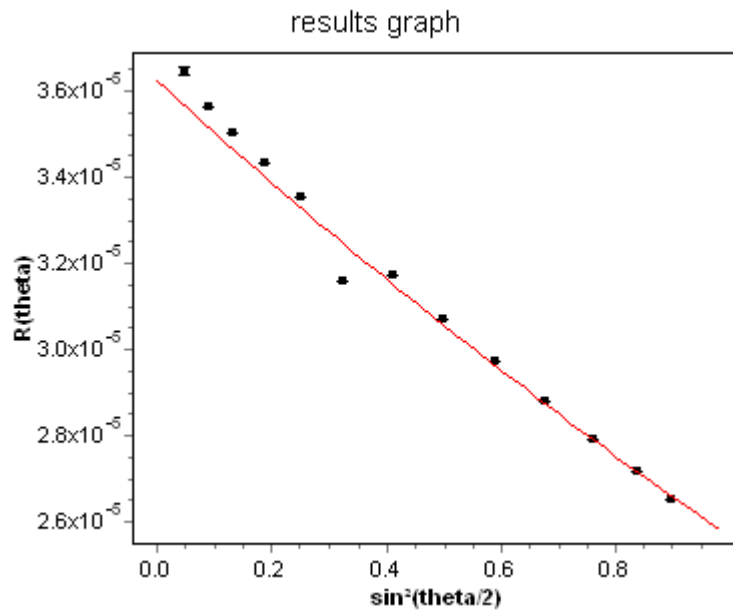


Figure 8-2: Analysis plot showing incorrect normalization coefficient for detector 9

RI Calibration

Most materials, when dissolved in a solvent, change the refractive index of the solution by an amount proportional to their concentration. This proportionality factor is called dn/dc , a factor that is usually independent of molar mass especially for molar masses greater than roughly 10,000 g/mol. The dRI detector's output is proportional to the change in refractive index, which in turn is equal to the product of the concentration and dn/dc .

For “analog” dRI instruments that send signals to the AUX input of another instrument, the proportionality factor relating detector output voltage to n is called the calibration constant. This constant is inversely proportional to dRI detector sensitivity. It is the number required by ASTRA to convert the voltage output of the dRI detector into changes in refractive index units when reading the dRI instrument signal through the AUX input of another instrument.

The Wyatt Optilab rEX is intended to communicate digitally with ASTRA in refractive index units. As such the Optilab rEX dRI calibration constant is an internal constant stored on-board the Optilab rEX itself.

When to Calibrate

dRI instruments manufactured by Wyatt Technology, including the Optilab rEX and the Optilab DSP, come pre-calibrated.

Other dRI instrument manufacturers might or might not supply an approximate dRI calibration constant. ASTRA needs an accurate dRI calibration constant since any error in the dRI calibration constant is directly proportional to the error in molar mass. The calibration constant can vary 10-15% from the manufacturer's approximate value, so calibration of any third-party dRI instrument is strongly recommend prior to use.

In all cases dRI measurement performance should be checked regularly (annually) against a standard with a known dn/dc value. Changing solvents does not affect the dRI calibration constant. If the calculated molar mass for a known sample changes over time, consider calibrating the dRI as part of your troubleshooting effort.

Note:	For use with certain chromatography systems and the ASTRA software, dRI calibration may be performed by injecting a suitable standard sample and verifying that the injected and calculated molar masses are equal. This option is often much more efficient than the off-line approach described below, particularly with organic mobile phases. Contact Wyatt Technical Support and review the section “RI Calibration from Peak” on page 8-81 for more information.
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Off-line dRI calibration may be performed using any substance with a well-characterized dn/dc value. In all cases the recommended standard is *anhydrous* sodium chloride dissolved in pure de-ionized water. This solution has a dn/dc of 0.172 mL/g at a wavelength of 690 nm (0.181 mL/g at 488 nm, 0.174 mL/g at 633 nm or 658 nm, and 0.170 mL/g at 900 nm).

Appropriate concentrations should be prepared using pure distilled water and *clean* glassware. The lowest and highest recommended concentrations vary by instrument.

- For the Optilab rEX: ~0.1 mg/mL and ~5mg/mL.
- For all other dRI instruments: ~0.1mg/mL to 1.2mg/mL.

Use six or more concentrations prepared within 1% or better accuracy to increase the precision of the final determination. Wyatt Technology provides validated, pre-mixed NaCl solutions in the ideal concentration range for calibration of any dRI instrument (Wyatt Technology part number P8400, NaCl Solutions Kit.) NaCl concentrations are as follows: 0.0 (blank), 0.1, 0.5, 1.0, 1.2, 2.0, 3.0, 4.0, and 5.0 mg/mL in nanopure water.

dRI instruments can differentiate between solvent that has been saturated with ambient gases and those that have been degassed. It is possible to detect concentration differences in the 1 ppm range. Consequently, standard samples and solvent blanks must be prepared from the same solvent stock. After preparing the standard solutions, fill two or three extra containers with water from the same solvent flask to use for “blank” analyses.

Samples should be kept well-sealed to avoid evaporation. Some solvents (such as water) keep fairly well for a number of months, but concentration changes may occur over time due to evaporation or contamination.

NaCl solutions may be infused into the dRI instrument directly via syringe pump or with an HPLC pump and an injector as described below.

HPLC Pump with Injector

This arrangement is shown in Figure 8-3. Use 0.01 in. ID tubing between the injector and the Optilab rEX, and 0.02 in. ID tubing elsewhere. A flow rate of approximately 0.5 mL/minute is ideal. Use a large sample loop, 0.5–1.0 mL to ensure that each injection produces a clear plateau and not a peak as it passes through the Optilab rEX.

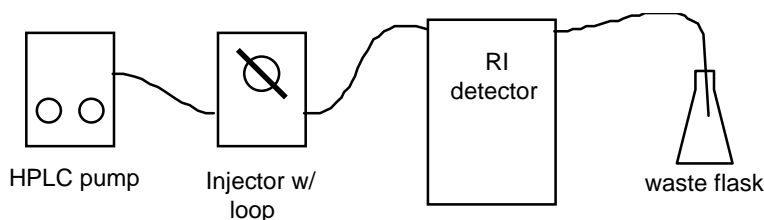


Figure 8-3: Setting up an HPLC pump and injector for calibration

The objective is to inject *known* concentrations into the detector. If a peak with a rounded top and no clear plateau is obtained, the concentration at the top of the peak will be unknown; flat-topped plateaus indicate that the cell has been fully flushed with sample solution.

A syringe pump fitted with a large syringe may be used instead of the HPLC pump.

Syringe Pump Infusion

Another option is to connect the Optilab rEX as shown in Figure 8-4, using a syringe pump. Use 0.02 in. ID tubing for both connections. A recommended flow rate is 0.2-0.5 mL/minute. The syringe must be rinsed and dried thoroughly or replaced between samples to avoid contamination of one concentration by the next sample. When the syringe is disconnected to change samples, the pressure change and injected air may cause an unstable baseline for several minutes.

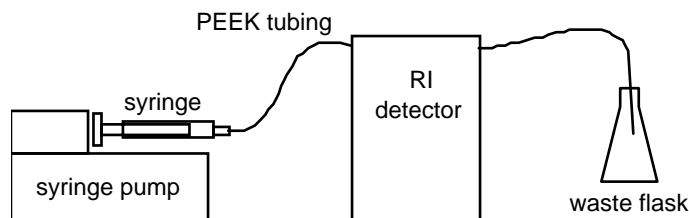


Figure 8-4: Setting up a syringe pump for calibration

Collecting Data

The dRI instrument's sample and reference cells should be flushed with high purity water (first solvent to be analyzed) for several minutes before making measurements. Confirm that the temperature is stable, and activate the instrument's purge feature while flowing pure water for several minutes. Deactivate the purge, flow with water for several more minutes, then zero the instrument.

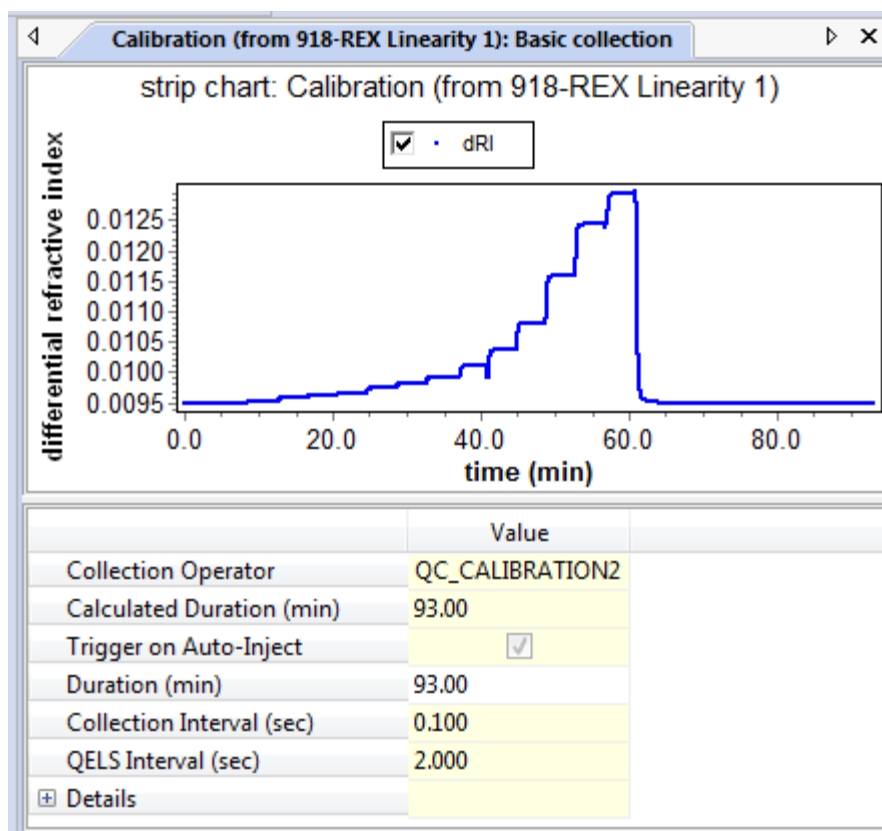
Collect data using ASTRA 6 software as follows:

1. Start the ASTRA software and select **File→New→Experiment from Method**. In the dialog box that opens, select the **System > Methods** folder, then the **RI Measurement** folder, and finally open the **RI calibration** method.
2. In the experiment, expand the **Configuration** node and verify that the proper experimental parameters have been entered. Update the configuration if necessary.
3. Expand the **Procedures** node of the method. Click on the **Basic collection** procedure and enter appropriate values for each of the fields. A typical duration for the dRI calibration experiment is 20 minutes or more. Close the dialog by clicking the **OK** button, and run the experiment.

4. Introduce pure solvent (blank) into the reference cell, making sure the solvent flows through the system at a constant rate.

dRI detectors do not react favorably to sudden changes in flow as the detector drift would overwhelm the signal. Thus, it is important to maintain a nearly constant flow through the instrument while measuring the solutions.

5. While ASTRA collects data, begin to introduce the series of prepared standards into the sample cell of the detector. Each standard solution is introduced one at a time, *beginning with the lowest concentration*.
6. Wait for the signal to stabilize (allow signal to reach a plateau), which may take several minutes, while the previous solution is completely rinsed out of the cell. After all the standards have been injected, re-inject a pure solvent sample (blank) to re-establish the baseline.
7. Once a good baseline signal is acquired, stop the ASTRA data collection.

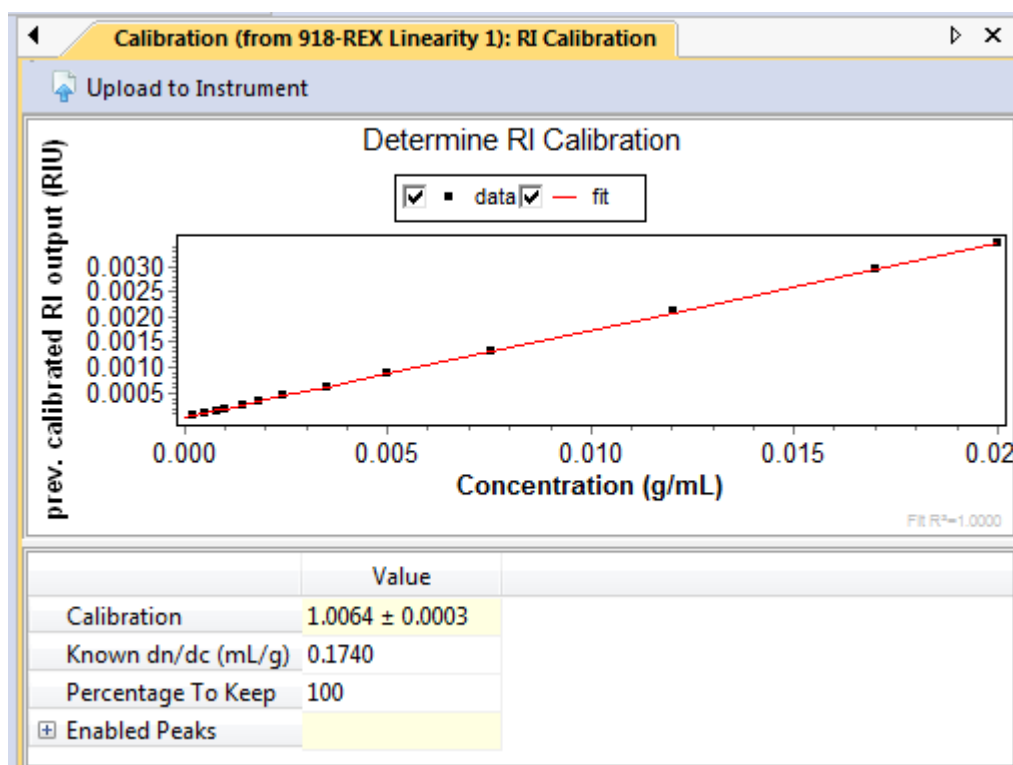


Data Processing

After the data collection has ended, follow these steps:

1. ASTRA may produce a message box informing you that baselines need to be set. Click **OK**, and the software will expand the Procedures section of the experiment and open the **Baselines** window. Otherwise click the **Run** button to proceed.

2. In the **Baselines** view, define the baseline using the solvent blanks that were injected before and after the standard solutions. Click and drag from one solvent plateau to the other, such that a baseline is drawn under the sample solution plateaus. Click **OK** to close the baselines window.
3. In the **Peaks** view, define a peak for each of the flat plateau regions associated with each injected standard. Click and drag over the maximum flat region for each sample injection. Do not set peak regions for the “blank” injections.
4. As peak regions are defined, a table beneath the Define peaks graph shows a new column associated with each respective peak. In these columns, under the Refractive index node, enter the known concentration for each standard in the row titled Concentration (mg/mL). Once you have assigned all the peaks their respective concentrations, click on the OK button at the bottom to close the pane.
5. Double-click the **RI Calibration** procedure to open its property page:



This procedure has the following properties:

Table 8-7: RI Calibration Properties

Field	Description
Calibration	The resulting calibration constant.
Known dn/dc	The known dn/dc value for the calibration standard used.

Table 8-7: RI Calibration Properties

Field	Description
Percentage to Keep	The percent of the marked peak data to use for calibration. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Enabled Peaks	This list shows the peaks used in the fit to determine the calibration constant. Checking or unchecking a peak adds or removes it from the fit to determine the calibration constant.

6. Enter the known dn/dc into the row titled Known dn/dc (mL/g) and click **Apply**. The value in the **Calibration** row should update once this known value is entered.
7. You can click the **Upload to Instrument** button above the list of properties if you want to update the instrument with the computed calibration constant. (rEX and T-rEX instruments only.)
8. Expand the **Results** node of the experiment and double-click the **Report (summary)** line. The report displays the new Calibration constant.

Optilab rEX dRI Calibration Results

For the Optilab rEX, the reported “Calibration Constant” is actually a correction factor. A value of 1.0000 would indicate that the Optilab rEX’s internal dRI calibration constant has remained unchanged. If the value is 1.0120, the Optilab rEX dRI calibration constant has increased by a factor of 1.2%.

1. Multiply the old dRI calibration constant by the correction factor to determine the new dRI calibration constant. The old dRI calibration constant is viewable in the **Constants** window on the Optilab rEX front panel.

The calculation is:

$$(\text{old dRI calibration constant}) \times (\text{ASTRA correction factor}) = \text{new dRI calibration constant}$$

For example:

$$3.3828\text{e-}3 \times 1.012 = 3.4234\text{e-}3$$

Note: You must calculate the new dRI calibration constant manually, using the old dRI calibration constant and the correction factor provided by ASTRA.

2. Tab to the **System** screen on the Optilab rEX front panel. Open the **Constants** window, and enter the new dRI calibration constant. Tab to the **Apply** button, and press Enter.

Optilab DSP or Third-Party Instrument dRI Calibration Results

Enter the reported dRI calibration constant for “analog” instruments in the “calibration constant” field of the AUX connection profile for the RI detector. This value is not set in the AUX connection profile automatically.

Absolute RI Calibration

You can calibrate the Optilab rEX aRI measurement as described in this section. This procedure is also summarized in the *Optilab rEX User Guide* section titled “Instrument Calibration for aRI.”

We recommend that you verify and/or calibrate the absolute Refractive Index (aRI) measurement before operating the instrument in aRI mode for the first time, and it is recommended that you check these values for accuracy on a periodic basis.

General Information

Optilab rEX aRI measurement performance should be checked against one or more standards. For a complete aRI calibration, at least 3 pure solvents with known aRI values (which are specific for the operating wavelength of the Optilab rEX) should be used. See Table 8-8 for recommendations. It is imperative that each solvent infused into the Optilab rEX must be miscible with the solvent that it replaces.

The Optilab rEX aRI is factory-calibrated using the following four solvents in the following order:

1. High Purity Water (NANOpure water with a final 0.2 micron filter)
2. Methanol (HPLC-Grade Fisher A452-4)
3. Tetrahydrofuran (GPC-Grade w/BHT Burdick & Jackson Cat. 341-4)
4. Toluene HPLC Grade (HPLC-Grade Burdick & Jackson Cat. 347-4)

Table 8-8: Known aRI Values for Various Solvents at Various Wavelengths

Solvent	785 nm	685 nm	658 nm	633 nm	532 nm	514 nm	488 nm
Water	1.3281	1.3303	1.3309	1.3316	1.3347	1.3354	1.3364
Methanol	1.3228	1.3242	1.3247	1.3253	1.3282	1.3289	1.3300
Tetrahydrofuran	1.3996	1.4015	1.4022	1.4029	1.4069	1.4079	1.4094
Toluene	1.4843	1.4882	1.4896	1.4910	1.4995	1.5017	1.5053

Note: It is imperative that each solvent be miscible with the solvent it replaces, whenever the Optilab rEX is flushed with a new solvent.

Collecting Data for Optilab rEX aRI Calibration

1. The Optilab rEX should be purged (flow liquid with the Purge on) with high-purity water for several minutes before beginning the aRI calibration procedure. Confirm that the temperature is stable at 25°C, and operate the Optilab rEX with the Purge ON for all data collection (the Purge indicator will be yellow on the **Main** tab).
2. On the Optilab rEX front panel, navigate to the **System** tab, and open the **Constants** dialog. Record the values listed for dRI calibration constant, dRI offset, aRI calibration constant, and aRI offset.

3. Collect data using the ASTRA software. As of the time of publication of this user's guide, the ASTRA collection procedure is as follows (refer to the current *ASTRA 6 User's Guide* for more information):
 - a. Start the ASTRA software and select **File→New→Experiment from Method**.
 - b. In the dialog that opens, select the **System > Methods** folder, then the **RI Measurement** folder, then the **Optilab rEX Specific** folder, and finally the **absolute RI calibration** method.
 - c. Expand the **Configuration** node and verify that the proper experimental parameters have been entered.
 - d. Expand the **Procedures** node of the method. Click on the **Basic collection** procedure and enter appropriate values for each of the fields. Close the pane by clicking the **OK** button.
 - e. Run the experiment.

Introduce Solvents

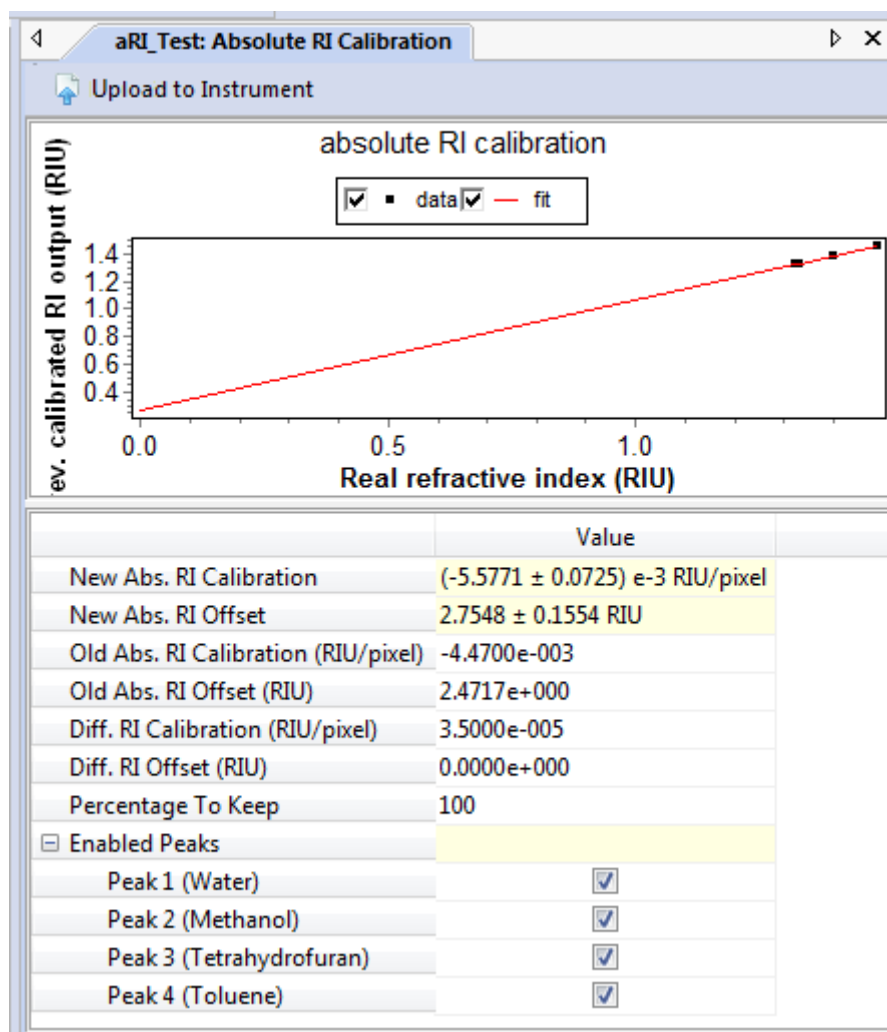
1. Flow the pure solvents directly into the Optilab rEX at a constant flow rate, using a syringe pump or an HPLC pump set in the flow rate range of 0.5mL/min to 1.0mL/min.
2. After flowing roughly 4-5 mL of solvent, toggle the purge valve to **PURGE OFF** for 15 seconds, and then toggle it back to **PURGE ON** for 15 seconds. Repeat this cycle for 2-3 minutes. Cycling the purge valve in this manner creates just enough agitation in the flow path to displace air bubbles and thoroughly remove the previous solvent.
3. On the Optilab rEX **System** tab, go to the **LED** button and press Enter. Adjust the **Percent max power** setting so that the **Light intensity** is close to, but not above, 7.8 Volts. Close the LED Intensity dialog and return to either the **Main** or **aRI** tab.
4. Complete the solvent introduction process by leaving the purge valve in the **PURGE ON** state for roughly one minute, and then turn the syringe pump off. After three minutes of stopped flow the Optilab rEX is stable. During this time ASTRA will record the stable, flat no-flow region. Note the time of this no-flow period, which will be referenced during data processing.
5. Repeat this solvent introduction procedure (beginning with step 1 of this list) for each remaining solvent.

ASTRA Calculations

Follow these steps in ASTRA:

1. After data collection, open the **Procedures** section of the experiment, and click **Define peaks**. On the graph, identify the flat no-flow regions associated with each solvent. Define a peak for each solvent by selecting a plateau of approximately 30 seconds located near the end of the flat region.

2. Beneath the graph a table is shown with a column for each defined solvent. Expand the **Refractive index** node. A row will now be visible titled **Real**. In each solvent's column, enter the known aRI value. Make sure that it is the correct aRI value for the Optilab rEX's operating wavelength. Table 8-8 lists aRI values for several solvents at various wavelengths. Click the **OK** button to close the window.
3. Click on the **Absolute RI Calibration** procedure to open its settings pane. Enter the **New Abs. RI calibration** and **Offset**, **Old Abs. RI Calibration** and **Offset**. These are the numbers recorded from the Optilab rEX front panel in Step 2 on page 8-35. Click **OK** to close the window.



4. You can click the **Upload to Instrument** button above the list of properties if you want to update the instrument with the computed absolute RI calibration constant. (rEX and T-rEX instruments only.)
5. Click the Run experiment icon on the icon toolbar at the top of the window. The method completes its data processing.

6. Expand the **Results** node and double-click the **Report** line. The report will display the new aRI calibration constant and new aRI offset. Save the ASTRA file. You may also want to print the report.
7. If you did not use the **Upload to Instrument** button on the Absolute RI Calibration page, go to the **System** tab on the Optilab rEX, tab to the **Constants** button and press Enter. Enter the new values into the Optilab rEX in the Constants dialog. Tab to the **Apply** button and press Enter. Tab to the **Close** button and press Enter. The Optilab rEX aRI is now recalibrated.
8. Measure and record the aRI value for pure ethanol, which should be near 1.35 after the correct constants are in place and after purging the rEX with ethanol as described in the section “Introduce Solvents” on page 8-36.

Setting Absolute RI Calibration Properties

This procedure has the following properties:

Table 8-9: Absolute RI Calibration Properties

Field	Description
New Abs. RI Calibration	The resulting absolute RI calibration constant. These are measured in refractive index units (RIU) per pixel on the photodiode.
New Abs. RI Offset	The resulting absolute RI offset in RIU.
Old Abs. RI Calibration	The previously used absolute RI calibration constant from the Optilab rEX.
Old Abs. RI Offset	The previously used absolute RI offset from the Optilab rEX.
Diff. RI Calibration	The differential RI calibration constant from which the absolute value was determined (from the Optilab rEX).
Diff. RI Offset	The differential RI offset from which the absolute value was determined (from the Optilab rEX).
Percentage to Keep	The percent of the marked peak data to use for calibration. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Enabled Peaks	This list shows the peaks used in the fit to determine the calibration constant. Checking or unchecking a peak adds or removes it from the fit to determine the calibration constant.

The graph shows a fit to the absolute RI values using the previous absolute RI calibration data and the measured refractive index data.

The **Upload to Instrument** button updates the instrument with the computed calibration constant. (rEX and T-rEX instruments only.)

For an example experiment that finds the absolute RI calibration, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the “absolute RI calibration.afe6” experiment in the Sample Data > Analyzed Experiments folder.

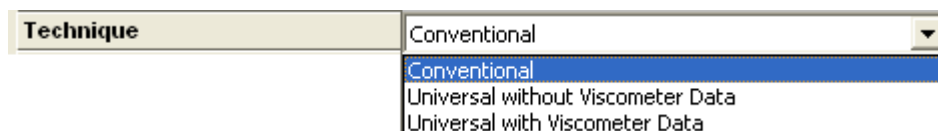
You can place this procedure with other analysis procedures and after all the transformation procedures. A procedure list can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine the dn/dc or RI calibration in a procedure, only the first one is valid.

Calibrate Column

ASTRA provides this procedure for developing a column profile. Such profiles can be used to compare the absolute molar masses derived from light-scattering results to the relative molar masses derived from conventional size-exclusion chromatography. Such comparisons can illustrate possible errors generated by relative molar mass measurements and may be useful for characterization of branching.

This procedure determines the calibration constants stored in the generic column profile (see page 7-36).

Two main types of column calibration are available. “Conventional calibration” and “universal calibration.” For conventional calibration, the polymer type of the analyzed polymer must be the same as the polymer used for calibration. For universal calibration, the polymer type of the analyzed polymer may be different.



The calculation for universal calibration requires either intrinsic viscosity data or known Mark-Houwink-Sakurada K and a coefficients for the polymers used for calibration and the polymer to be analyzed. The dn/dc value is required for universal calibration (as it is necessary for intrinsic viscosity calculations), but not for conventional calibration.

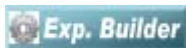
You can see example experiments that perform column calibration by choosing **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database) and opening the conventional calibration or universal calibration .afe6 file in Sample Data > Analyzed Experiments. For an experiment method choose **File→New→Experiment From Method**, and open the “universal calibration” method in the System > Methods > Viscometry folder.

For more, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Conventional and Universal Column Calibration.

When to Calibrate a Column

This procedure only needs to be performed when a new column is used, or if you think the column has aged to a point that its behavior needs to be reassessed.

Analysis of a sample using universal calibration takes place in two logical phases. First, the column profile must be determined by measuring the behavior of a set of known molecular standards when passed through the column. Once this "determine column calibration" phase is complete, the unknown sample can be analyzed in a separate experiment.



In an experiment, this procedure must come after the Baselines and Peaks procedures. If multiple detectors are used, this procedure must also come after the Alignment and Band Broadening procedures.

Running a Column Calibration

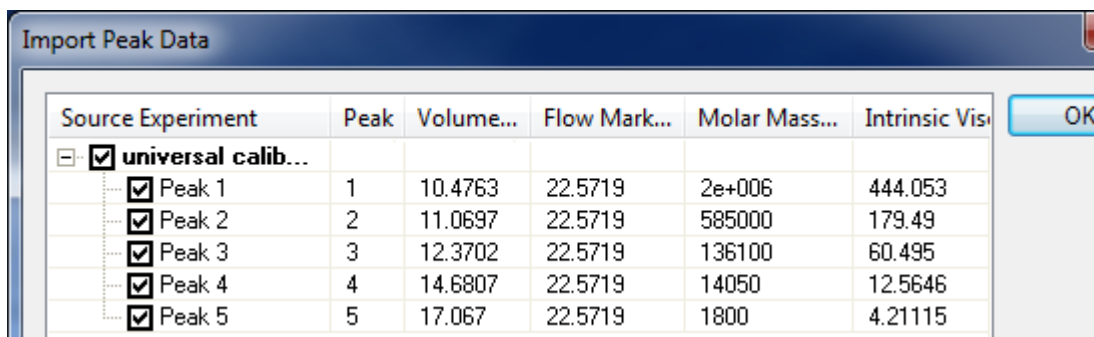
To calibrate a column, follow these steps:

1. Set up the equipment for an online experiment with your SEC column.
2. Choose **File→New→Experiment From Method** to open a new experiment. For conventional calibration, the method is in the System > Methods > RI Measurement folder. For universal calibration, the method is in the System > Methods > Viscometry folder.
3. Run the experiment using a set of known molecular standards.

If you are performing a universal calibration without viscosity data, use a standard that is available in several known molar masses and for which the Mark-Houwink-Sakurada K and a coefficients are known (for example, polystyrene).

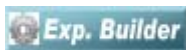
4. For each experiment, define peaks:
 - Enter molar masses or select predefined molecular standards for each peak in the Peak view.
 - For universal calibration, supply intrinsic viscosity information in the Extended Parameters if viscometry data is not present in the experiment.
5. Choose **Experiment→Configuration→Calibrate Column**. Examine the column calibration data fit. For the "Universal Calibration with Viscosity Data" technique, an additional column shows the intrinsic viscosity of the molecular standard.
 - You can specify a flow marker (peak) to use in the calibration. Select between positive and negative peaks to search for the peak maximum or minimum. If you select the same flow marker for each experiment, it is used when combining data to yield a more accurate curve.
 - You can view individual peak entries by pressing the "+" sign next to the Peak label.
6. Save the completed experiment.
7. Repeat the previous steps for any additional mixtures of known molecular standards as many times as necessary to cover the full column range. Use the flow marker you selected in each mixture.
8. Open all column calibration experiments you saved for this column.

9. In the Calibrate Column page, click the **Import Peak Data** button. You will see a dialog that lists other open experiments that contain column calibration data. Check the boxes next to any peaks you want to import. The grid shows the peak number, elution volume, flow marker, molar mass, and intrinsic viscosity for each peak. Then click **OK**.



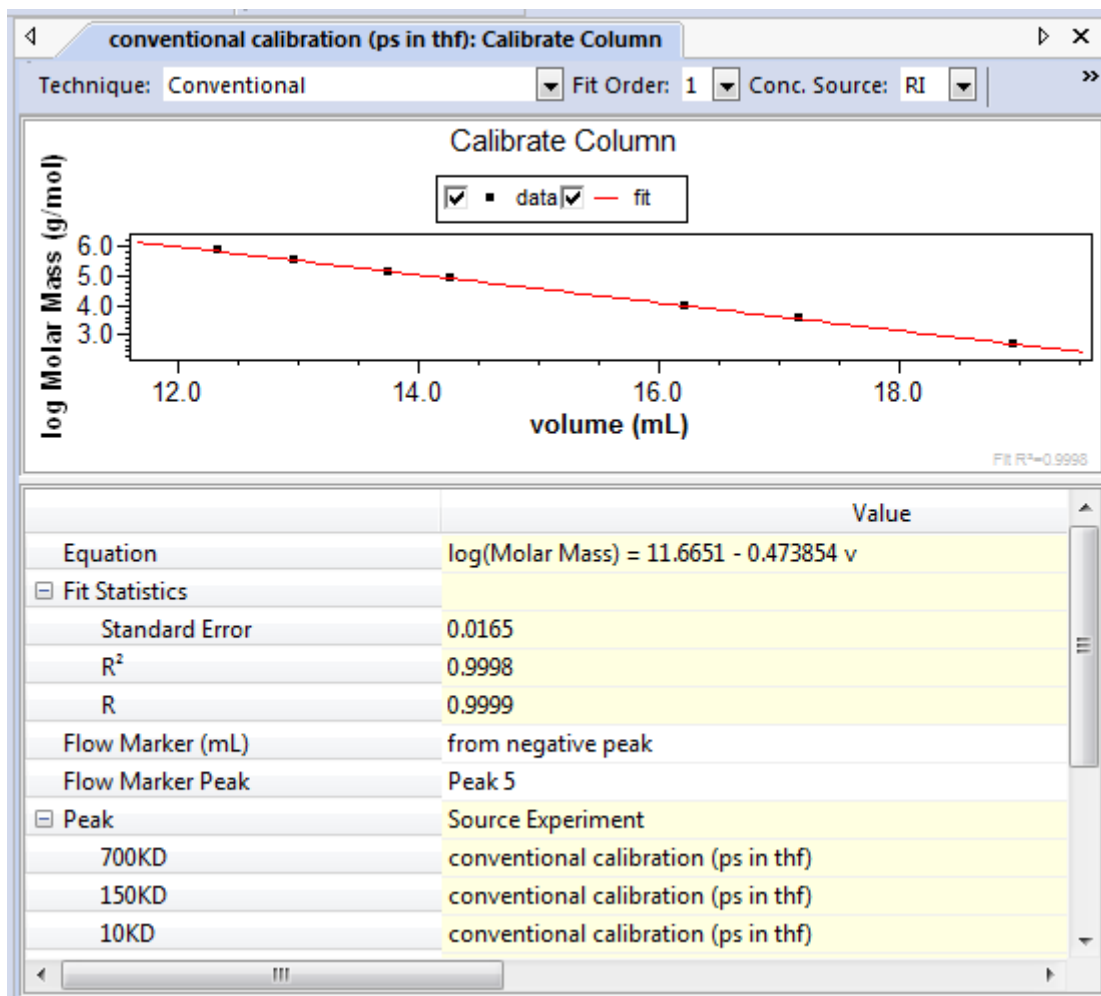
10. Confirm that the peaks have been added to the list and adjust the curve fit order. Then, click **Apply** to store the imported peaks.
11. Click **Export** in the Calibrate Column page to open the Save Calibrated Column Profile dialog. (You can click **Export** only after you have clicked **Apply**.)
12. Select a folder for your column profile, and type a name for the column. Click **Save** to store the profile. In future experiments, you can import this profile in the column profile (see page 7-36).

Setting Column Calibration Properties



This procedure is hidden in the Experiment tree in Run mode. However, you can still open it in Run mode by choosing **Experiment**→**Configuration**→**Calibrate Column**.

You can set properties for this procedure after you run the experiment collection. Double-click on the Calibrate column procedure to open its property page:



The graph shows a plot of the data and the linear regression. The red fit line provides visual feedback as to the quality of the fit.

This procedure has the following properties:

Table 8-10: Column Calibration Properties

Field	Description
Technique	Select the type of column calibration to perform above the graph. The options are Conventional, Universal with Viscometer Data, and Universal without Viscometer Data. For conventional calibration, the analyzed polymer must be the same as the polymer used for calibration. For universal calibration, the polymers may be different. Universal calibration requires either viscometer data or known values for the Mark-Houwink-Sakurada K and a coefficients.
Fit Order	Choose the linear regression to use above the graph, up to 7th order. If the fit order exceeds the number of degrees of freedom, the graph and equation portions of the display indicate that no fit is possible.

Table 8-10: Column Calibration Properties

Field	Description
Concentration Source	If multiple concentration instruments are present, select the one to use in determining the column calibration above the graph. You can use this field to switch between multiple concentration sources when deciding which peak to use. The setting here does not affect the setting in the "Experiment Configuration" on page 7-14.
Equation	Shows the resulting equation from the linear regression. The coefficients are the ones that will be stored in the Column profile.
Fit Statistics	
> Standard Error	Also known as the residual standard deviation, this shows the standard deviation of the observed data from the fit values. If the fit degree equals the number of degrees of freedom, this is zero (the fit is the same as the observed values).
> R^2	Shows the adjusted R squared value from the fit. This quantity can be used to gauge the quality of a linear fit. The closer this is to one, the better the fit. If the fit degree equals the number of degrees of freedom, this is set to zero to indicate that statistical interpretation of the results is not possible.
> R	Shows the square root of the adjusted R squared value. The closer this is to one, the better the fit. If the fit degree equals the number of degrees of freedom, this is set to zero to indicate that statistical interpretation of the results is not possible.
Flow Marker (mL)	Use the drop-down menu to select whether or not a flow marker is to be set, and how. Drop-down options are as follows: <ul style="list-style-type: none"> - None - no flow marker is to be used. The flow marker value is set to zero. - Enter value - enter the flow marker value in mL in the cell to the right of the drop-down. - From positive peak - the flow marker will be determined from the apex of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down. - From negative peak - the flow marker will be determined from the lowest point of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down.
Flow Marker Peak	Select the peak for the flow marker if you used one.
Mark-Houwink-Sakurada K	The known Mark-Houwink-Sakurada K parameter of the polymer used for calibration. This is only used if the Universal with or without Viscometer Data calibration technique is selected.
Mark-Houwink-Sakurada a	The known Mark-Houwink-Sakurada a parameter of the polymer used for calibration. This is only used if the Universal with or without Viscometer Data calibration technique is selected.
Peak	Expanding this row shows the peaks in the experiment. The enabled checkbox determines which peaks to include or exclude from the fit. The molar mass used for each peak comes from the Peaks page. For experiments with viscosity data, you can also enter Intrinsic Viscosity for each peak here.

Column Plate Count

The plate count is sometimes called "Efficiency". It quantifies the separating efficiency of the column, which is generally how well the column is packed and its kinetic performance. More efficient columns yield narrower peaks than less efficient ones.

This procedure lets you calculate the number of theoretical "plates" in a peak. The plate model supposes that a chromatographic column contains a large number of separate layers, called theoretical plates. Separate equili-

brations of the sample between the stationary and mobile phase occur in these imaginary plates. The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.

These imaginary plates can measure column efficiency in two ways:

- The number of theoretical plates in a column is N . A large number of plates is better.
- The plate height is called the Height Equivalent to a Theoretical Plate (HETP). A small HETP value is better.

If the length of a column is L , the HETP is computed as: $HETP = L / N$

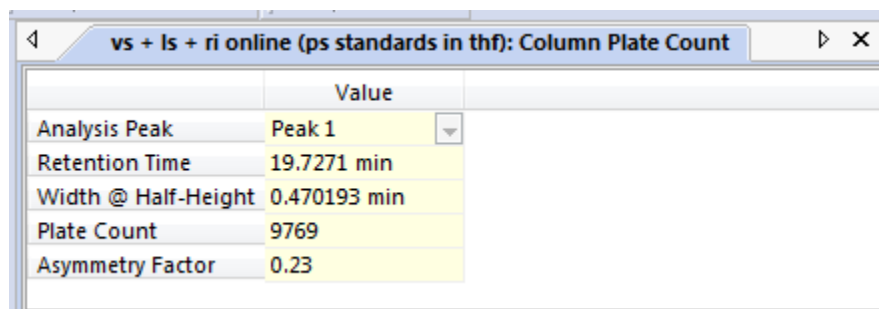
The column manufacturer provides the initial plate count as documentation, but this value changes over time. The current number of theoretical plates in a column can be found by examining a chromatographic peak after elution:

$$N = \frac{5.55t^2}{w^2}$$

where w is the peak width at half the column height, and t is the retention time—the elution time of the apex of the peak.

See <http://teaching.shu.ac.uk/hwb/chemistry/tutorials/chrom/chrom1.htm> for more about column plates.

Double-click on the Column Plate Count procedure to open its property page:



This procedure has the following properties:

Table 8-11: Column Plate Count Properties

Field	Description
Analysis Peak	The peak on which the analysis was performed.
Retention Time	The measured elution time of the apex of the peak. This is t in the previous equation.
Width @ Half-Height	The measured peak width at the midpoint below the peak apex. For example, if the apex of the peak measures 2 volts, the width of the peak is measured at the 1 volt level. This is w in the previous equation.
Plate Count	The calculated plate count for the column.
Asymmetry Factor	A factor that corrects for the asymmetry of the peak.

Transformation Procedures

The transformation procedures allow you to mark portions of the collected data or to convert the collected data in some way.

The following transformation procedure types are available:

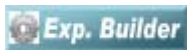
- “Despiking” on page 8-45
- “Smoothing” on page 8-46
- “Baselines” on page 8-48
- “Blank Baseline Subtraction” on page 8-51
- “Peaks” on page 8-54
- “Broaden” on page 8-60
- “Convert to Physical Units” on page 8-60
- “Convert to Concentration” on page 8-61
- “Convert Specific to Intrinsic Viscosity” on page 8-61
- “Results Fitting” on page 8-62

Despiking

The Despiking procedure removes spurious noise spikes from the collected data. Such spikes are likely caused by dust in the solvent.

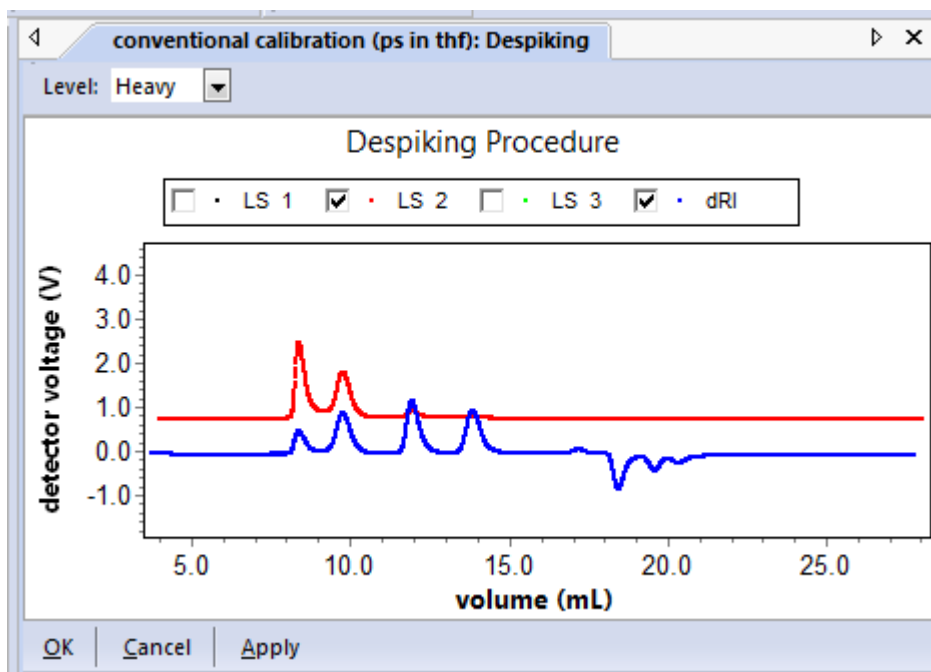
You can set the property for this procedure before running the experiment, or you can modify it after running the experiment and re-run the experiment to see the effects of changing the setting.

If your light scattering data is noisy, you may want to run one of the experiment methods provided with ASTRA for diagnostic purposes. For an experiment method choose **File→New→Experiment From Method** to open a method in the System > Methods > Light Scattering > Diagnostics folder. The “LS noise” method characterizes baseline detector noise.



This procedure may be placed at any point in the experiment before the analysis procedures that determine the final results. This procedure runs automatically without prompting for a value.

Double-click on the procedure to open its property page:



If data has already been collected for this experiment, the graph shows the data with the currently selected despiking level applied.

The property you can set at the top of the view is as follows:

Table 8-12: Despiking Properties

Field	Description
Level	Choose the degree of despiking. The options are None, Normal, and Heavy. The default is Normal.

Smoothing

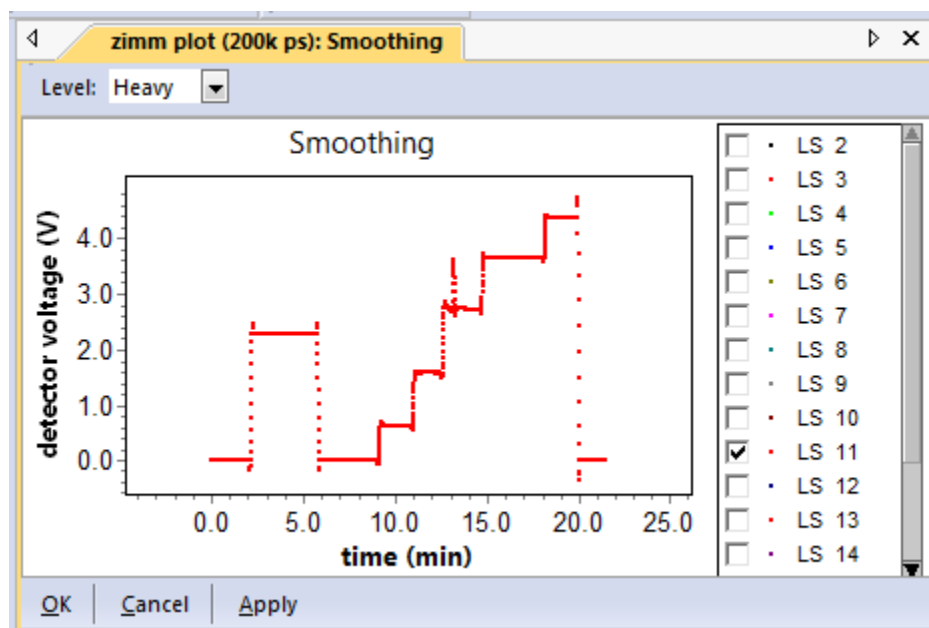
The Smoothing procedure smooths noisy data. Smoothing can be useful in certain circumstances, but in general it is better to remove the source of the noise, such as particulates in the mobile phase and pump pulsations, rather than to smooth the data.

ASTRA smooths data using the Savitsky-Golay technique of least-squares smoothing. Use this procedure with care—the height of very sharp peaks may be reduced somewhat by the smoothing process.

This procedure may be placed at any point in the experiment before the analysis procedures that determine the final results. This procedure runs automatically without prompting for a value.

You can set the property for this procedure before running the experiment, or you can modify it after running the experiment and re-run the experiment to see the effects of changing the setting.

Double-click on the procedure to open its property page:



If data has already been collected for this experiment, the graph shows the data with the currently selected smoothing level applied.

After you change the smoothing level, you should check the baselines and peaks to make sure no changes are needed because of the smoothing.

Smoothing always acts on the raw data. You cannot increase smoothing by re-smoothing.

The property you can set at the top of the view is as follows:

Table 8-13: Smoothing Properties

Field	Description
Level	Choose the degree of smoothing. The options are None, Normal, or Heavy. The default is None.

Note: Smoothing improves the appearance of the displayed data, but not the precision. To increase the precision of the calculated MM and radius you need to increase the signal to noise of the system by reducing the baseline noise and/or increasing the signal from the polymer.

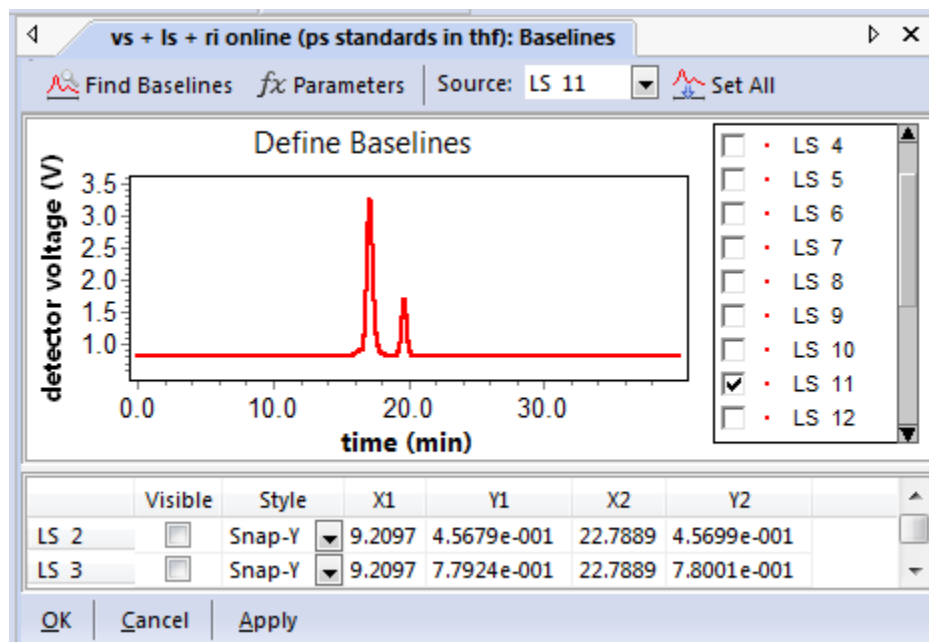
Baselines

Setting a baseline enables ASTRA to subtract the base signal from the collected data. For light-scattering experiments, the baseline level includes the photodiode dark offset and the solvent scattering.

This procedure is normally placed after any despiking or smoothing you want to perform and before conversion or analysis procedures.

When this procedure runs, you see a message that says a baseline needs to be set. Set a baseline by following these steps:

1. Click **OK** to open the page for setting baselines.

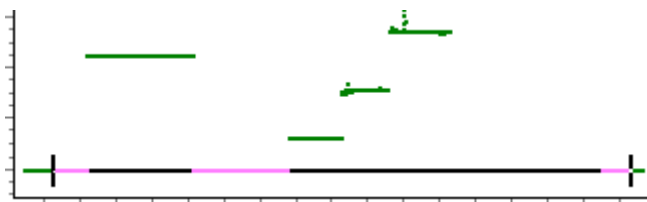


2. Click the **Autofind Baselines** button above the graph. Clicking this button automatically detects and applies the baseline for each of the source signals.
3. If you want finer control over how baselines are detected by the **Find Baselines** button, click the **Parameters** button above the graph. You can set the number of iterations to use when detecting baselines and the sensitivity to baseline noise for light scattering, RI, UV, and viscometry signals. The parameters you set are saved for use with future experiment runs. See “Parameters for Finding Baselines” on page 8-50.

If you do not want to use the automatic baseline detection, you can instead manually set baselines as follows:

1. In the list to the right of the graph, select the detector for which you set the baseline. (For example, detector 11.) To check the quality of your data, select different detectors from the detector list and examine the baselines. Normally, you should use the default trace when setting baselines. Note that if you select a different trace, it is important to set the Source field to match that trace before clicking **Set All**.

2. Click on the graph to add a baseline to the collected data. (You can press **Delete** to remove a baseline.)
3. Use your mouse to drag the baseline ends to appropriate locations.



Set the baseline ends far enough from the peak (where the baseline is flat) so they do not interfere with the signal.

Note: By default, baseline ends snap to the voltage level for a particular time. If you hold down the Shift key, you can drag the end of a baseline to any location. This may be useful if the collection was interrupted before the signal returned to the original baseline.

4. Select the signal for which you set a manual baseline in the **Source** field above the graph.
5. Click **Set All** to automatically set baseline ends at the same collection times for all other detector signals.
6. Check the automatic baseline settings by examining the baseline for each light scattering detector in turn. If necessary, you can modify the baseline for an individual detector.
7. If you are using multiple detector types (RI, UV, or viscometer), you should check their baselines independently. Other detectors are affected quite differently by chromatographic details such as injection peak, pump fluctuations, and baseline stability.
8. Click **OK** to continue running the experiment.

You can see the details of the baselines selected for each detector below the graph.

You can clear all baseline settings by deleting the baseline for the source detector and then clicking the **Set All** button.

When you position a baseline, the properties set for each detector are as follows:

Table 8-14: Baseline Properties


Field	Description
Visible	This box is checked if the data from this detector is shown in the graph.
Style	"None" indicated no baseline is set. "Snap-Y" indicates the Y value of the endpoint is calculated based on the Y value of the surrounding data points. "Manual" indicates that the X and Y endpoints are manually specified and are not taken from the Y value of the endpoint data.
X1	Shows the x-axis coordinate of the left end of the baseline for this detector.
Y1	Shows the y-axis coordinate of the left end of the baseline for this detector.

Table 8-14: Baseline Properties

Field	Description
X2	Shows the x-axis coordinate of the right end of the baseline for this detector.
Y2	Shows the y-axis coordinate of the right end of the baseline for this detector.

If your light scattering baseline drifts, you may want to run an experiment method provided with ASTRA for diagnostic purposes. Choose **File→New→Experiment From Method** to open a method in the System > Methods > Light Scattering > Diagnostics folder. The “LS noise” method reports baseline detector noise and drift.

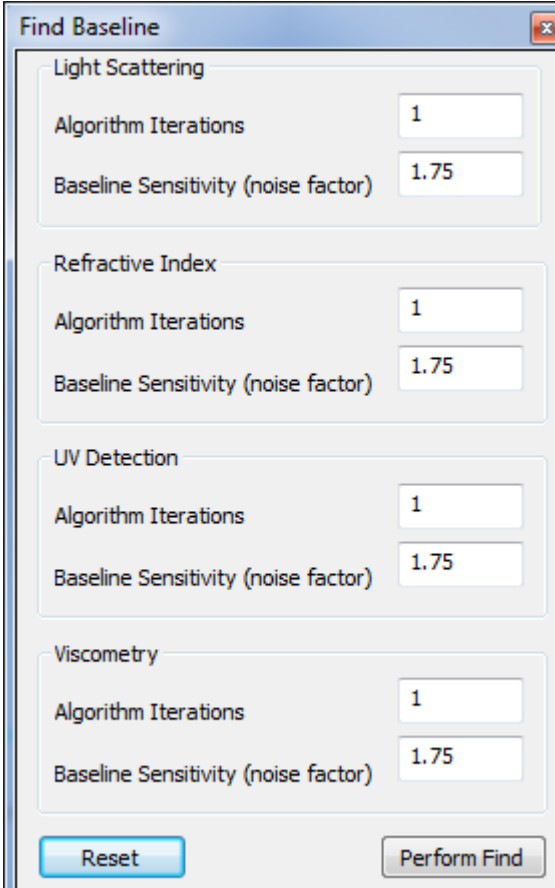
Parameters for Finding Baselines

If you want finer control over how baselines are detected, click the  **Parameters**

button above the Baselines graph. You can set the sensitivity and number of iterations for light scattering, RI, UV, and viscometry data. The parameters you set here are saved for use with future experiment runs.

The parameters are as follows:

- Baseline Sensitivity:** Adjusting this parameter allows you to tune how ASTRA differentiates between outliers or peak regions and actual baselines. The value is the maximum distance from the mean y-axis value that a point can have and still be considered part of the “baseline”. The units of this parameter are in standard deviations of the baseline noise.
- Algorithm Iterations:** Performing the baseline finding algorithm multiple times on a data set can potentially produce a more precise baseline. This parameter allows you to specify the number of times that the algorithm is run on a particular data set.



The **Find Baseline** dialog box contains four sections, each with two input fields: **Algorithm Iterations** and **Baseline Sensitivity (noise factor)**. The values are set to 1 and 1.75 respectively for all sections. At the bottom are **Reset** and **Perform Find** buttons.

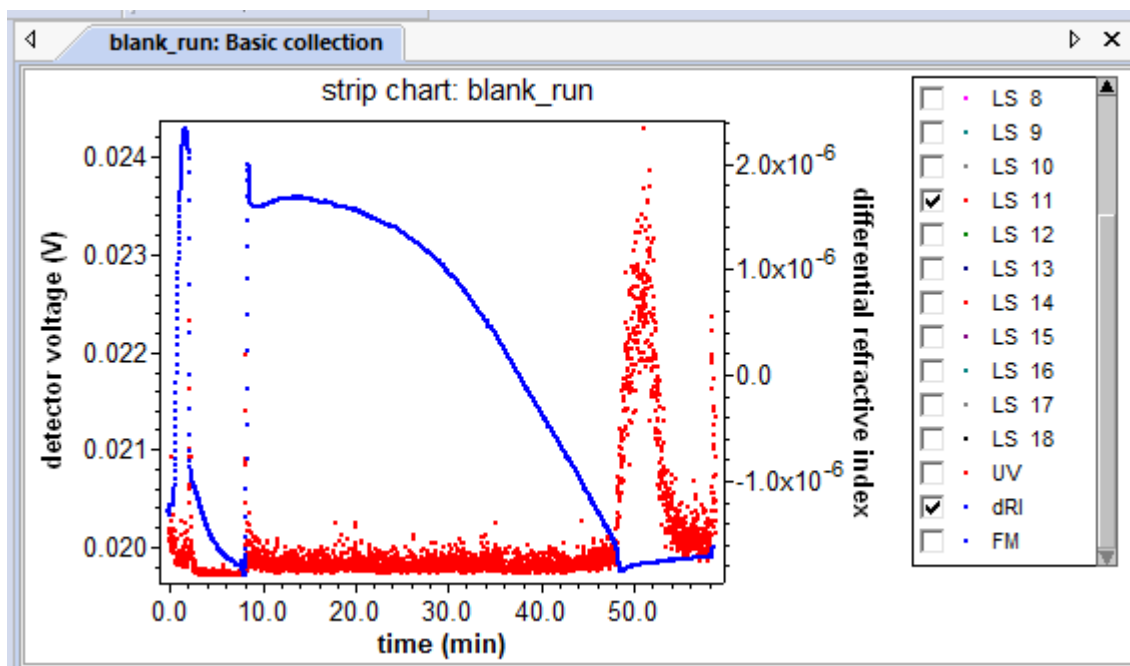
Method	Algorithm Iterations	Baseline Sensitivity (noise factor)
Light Scattering	1	1.75
Refractive Index	1	1.75
UV Detection	1	1.75
Viscometry	1	1.75

Blank Baseline Subtraction

While standard baseline subtraction is useful for combining instrument data during chromatography runs, there are many cases where predetermined changes in flow rate, temperature, and other effects can cause instrument baselines to drift such that the standard linear baseline subtraction feature cannot correct for the problem.

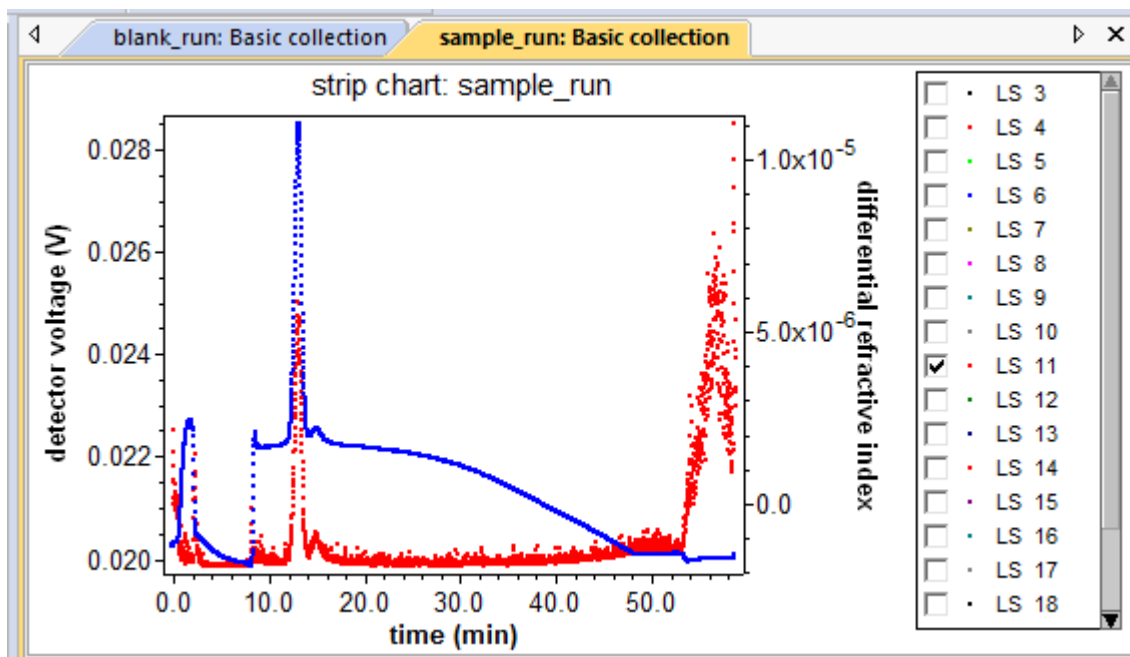
When the baseline changes are caused by a repeatable set of conditions, such as Eclipse instrument flow adjustments, a set of “blank” data can be collected that will model these effects. By subtracting this “blank” run, we can generate well-behaved result data.

1. Collect sample “blank” data for the experiment run by running an injection without any sample. ASTRA collects data about changes in baseline conditions caused by the collection environment. This collection should have the same characteristics—duration, solvent, instruments, etc.—as the sample runs, and should be subject to the same conditions—temperature control, use of Eclipse cross-flow levels, etc. For example, this blank run shows a fluctuating dRI baseline (blue):

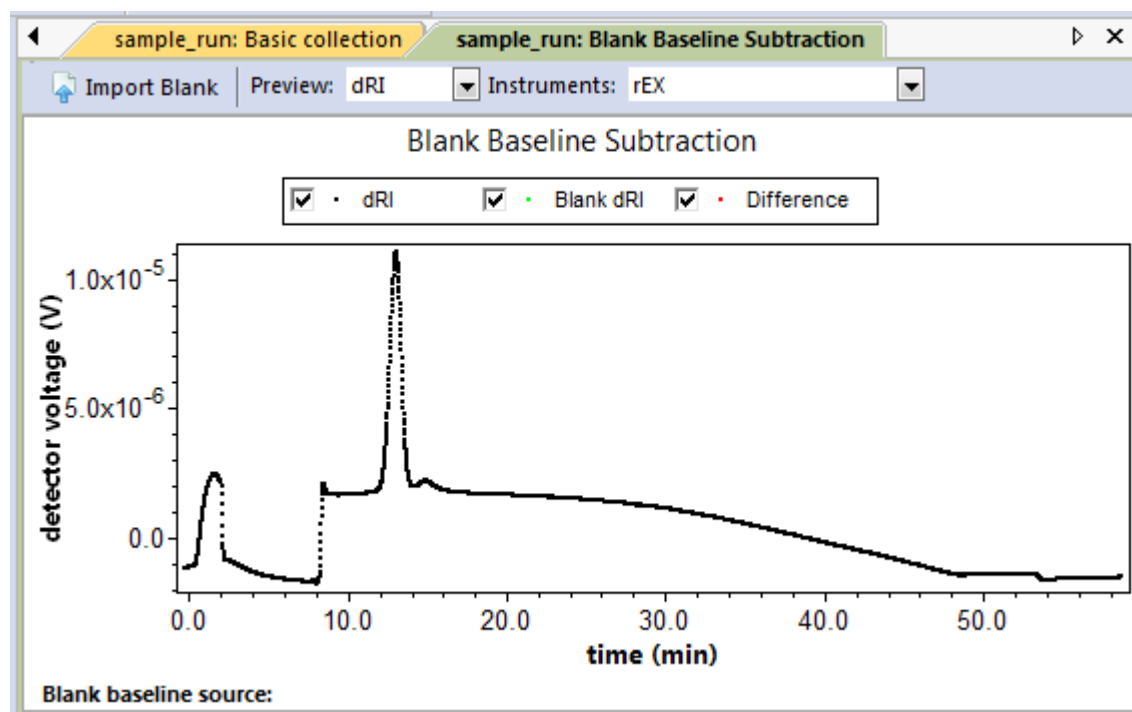


2. Choose **File→New→Experiment from Method**, and use the “online” experiment in the System > Methods > Light Scattering > Baseline Subtraction folder to create a new experiment.

- Run a data collection for the samples.

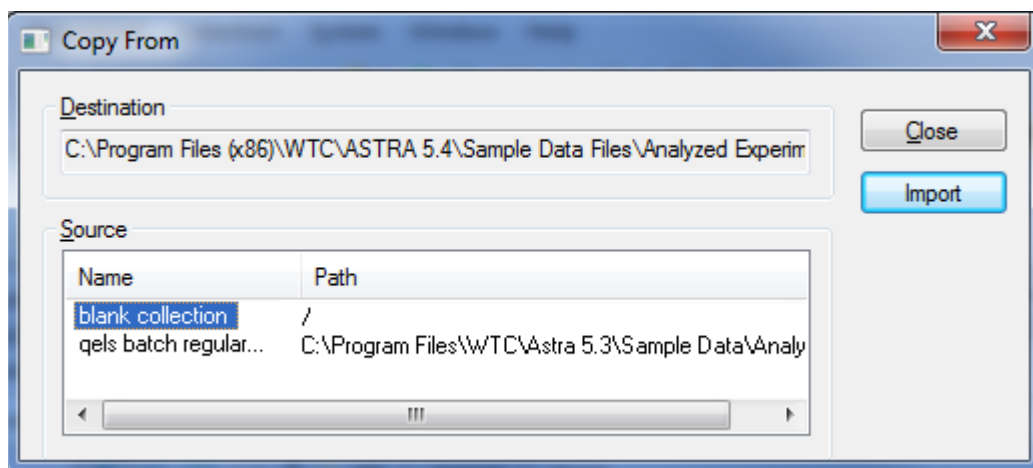


- Keep both the “blank” and “sample” experiments open in ASTRA.
- In the “sample” experiment, open the "Blank Baseline Subtraction" procedure.

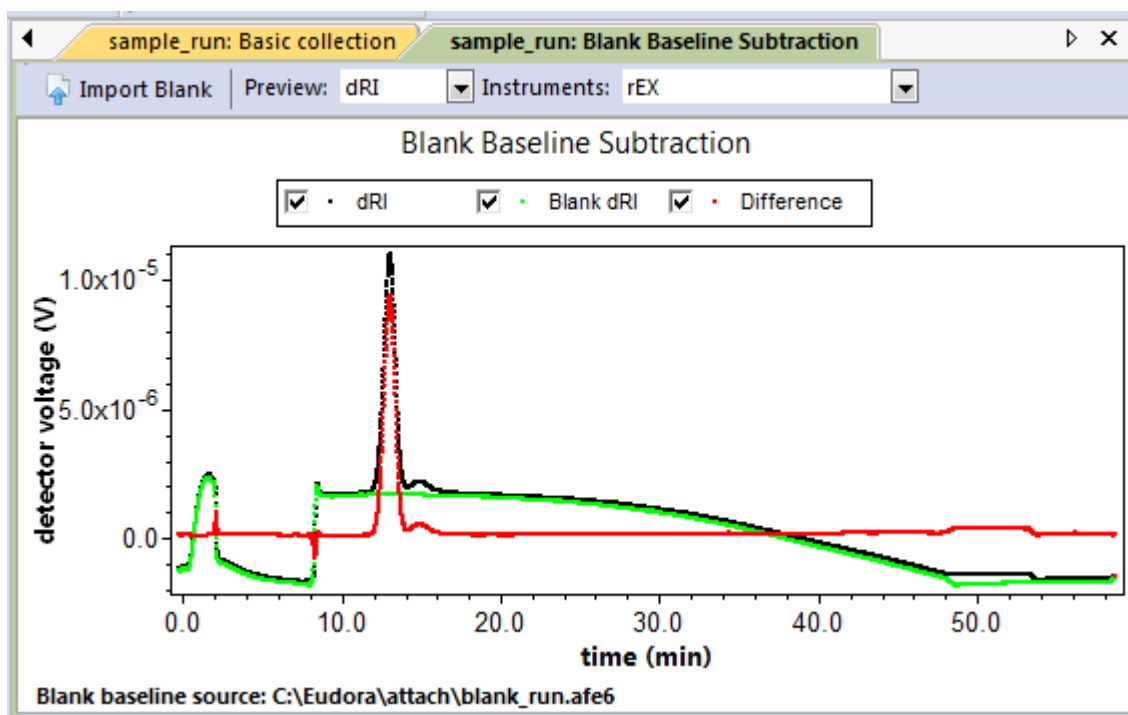


- Click the **Import Blank** button above the graph to open the Copy From dialog.

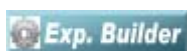
7. In the Source list, select the “blank” experiment and click **Import**. A compatible data set from the experiment you select is imported.



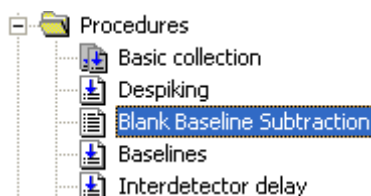
8. You can view the results of the subtraction in the Blank Baseline Subtraction procedure view. Use the **Preview** drop-down to see how the blank subtraction affects a particular signal.



9. In the **Instruments** drop-down list, choose one or more instruments that should have their baseline subtracted.



If you use Experiment Builder mode, you can add the Blank Baseline Subtraction procedure to experiments created using other methods by choosing the **Experiment→Add To Experiment** menu command. Position this added procedure right after the Despiking procedure.

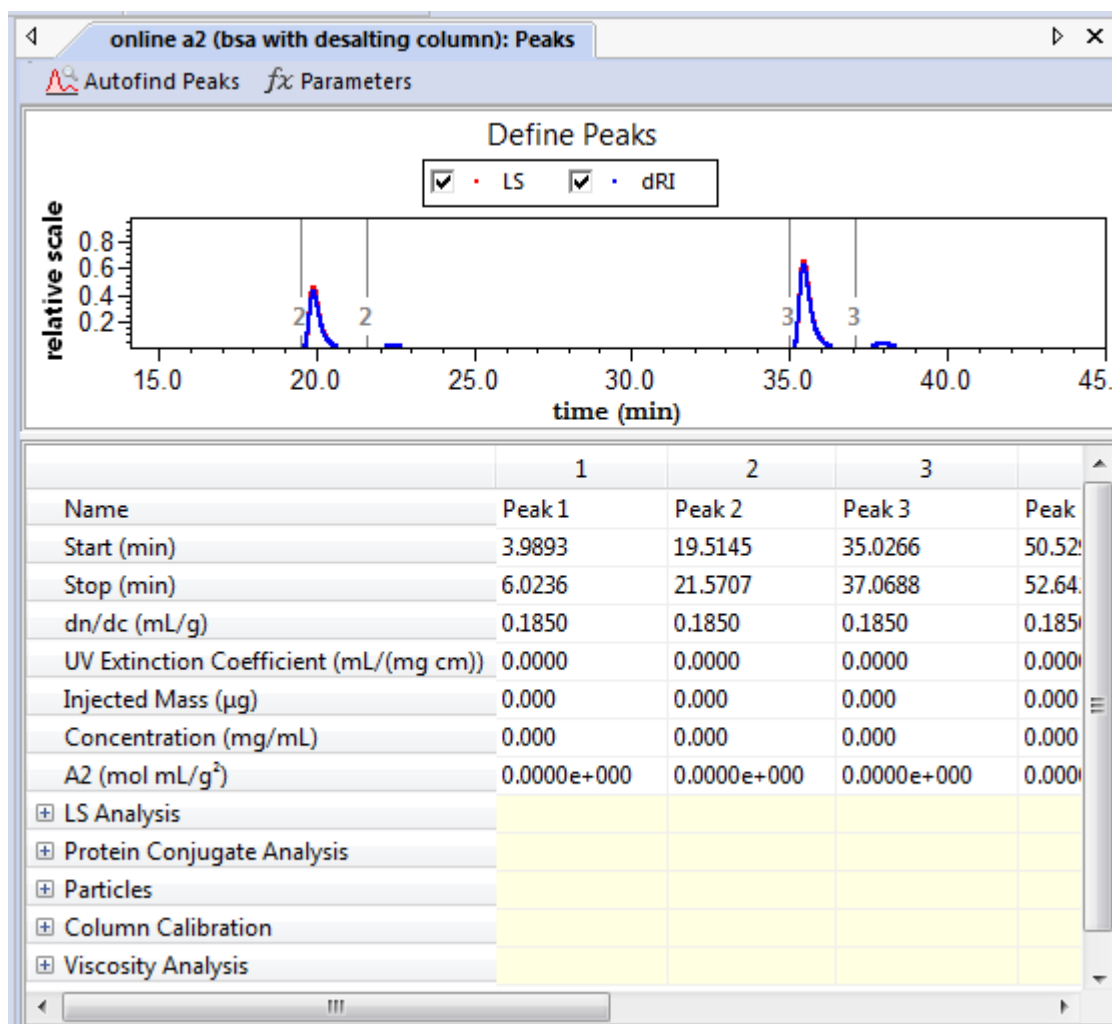



Peaks

After collecting data on your sample and setting baselines, you need to define the peak regions. This is done by marking the beginning and end point of every peak you want to include in processing.

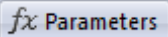
When this procedure runs, you see a message that says peaks need to be specified. Set peaks by following these steps:

1. Click **OK** to open the page for setting peaks.



- Click the  **Autofind Peaks** button above the graph. Clicking this button automatically detects and applies peaks to the data.

Note: **Autofind Peaks** can only be used with online (that is, fractionated) experiments. It does not work for batch experiments.

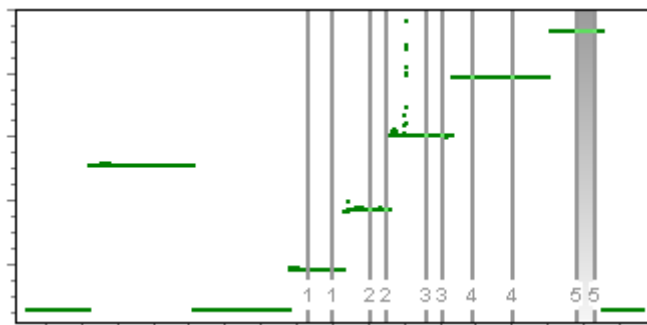
- If you want finer control over how peaks are detected, click the  **Parameters** button above the graph. You can set the peak sensitivity and peak approach. You can also specify a region of interest using X-axis units. The parameters you set here are saved for use with future experiment runs. See “Parameters for Finding Peaks” on page 8-59.

If you are running a batch experiment or do not want to use the automatic peak detection, you can manually set peaks as follows:

- Check detector boxes to choose which sets of data to view. The colors for data shown use the following default colors: light scattering data is red, refractive index data is green, UV data is blue, viscosity data is gray, and QELS data is magenta. Multiple detector angles use colors assigned by the graphing system.
- Click on the graph to add a peak range to the collected data.
- Use your mouse to drag the ends of the range to appropriate locations for the leftmost peak you want to analyze.

Note: For online experiments, we recommend setting peak endpoints so that the signal-to-noise ratio for both the light scattering and concentration detectors is greater than or equal to 2. This may necessitate excluding an aggregate peak (for which there may be a strong light scattering signal but no RI signal) or a low molar mass tail of a broad distribution sample (for which the light scattering signal will be small even though the RI signal is strong).

- Continue adding peak ranges for the rest of the collected data.
A number is shown for each peak that corresponds to the column for that peak below the graph. Peaks are numbered in the order you create them, not necessarily from left to right. The selected peak is shaded.



Note: If you want to zoom in on the graph, hold down the Ctrl key and use your mouse drag an outline around the area you want to see. To zoom back out, hold down the Ctrl key and click your right mouse button.

- If you selected “Forward Monitor” in the Divide by Laser Monitor field or the configuration for your light scattering instrument (see page 7-16), you should create a “peak” for pure solvent and specify the number of this peak in the “Experiment Configuration” on page 7-14. This pure solvent peak acts as a baseline for the forward laser monitor signal. If you do not specify such a peak, the average of the first ten percent of the forward laser monitor signal range is used as a baseline.

After setting peaks either automatically or manually, enter the relevant information for each peak as needed in the property list. Depending on the type of analysis to be performed, different properties need to be specified. See Table 8-15 to determine which properties you need to specify (the procedure lists for some properties may not be complete). The list contains the following fields for each peak:

Table 8-15: Peak Properties

Field	Description
Name	A name you can give to the peak for use in reports.
Start	The x-axis starting point for the peak. If you set peaks using the graph, the Start and Stop values are set automatically. Alternately, you can type values in these fields. The units are determined by the Abscissa Units property of the experiment configuration.
Stop	The x-axis ending point for the peak. The units are determined by the Abscissa Units property of the experiment configuration.
dn/dc	The dn/dc value for this peak. Set this parameter if you are performing any of the following analysis procedures: Molar Mass and Radius from LS Data RI Calibration UV Extinction from RI Protein Conjugate Analysis If you are performing a Protein Conjugate Analysis, this parameter corresponds to the dn/dc value for the protein.
UV Extinction Coefficient	The UV extinction coefficient for this peak in mL/(mg cm). Set this parameter if you are using a UV detector for concentration and are performing any of the following analysis procedures: Molar Mass and Radius from LS Data Protein Conjugate Analysis If you are performing a Protein Conjugate Analysis, this parameter corresponds to the extinction value for the protein.
Injected Mass (μg)	The mass of the sample injected in micrograms. If you do not enter a value in this field and you have provided all the necessary parameters (Concentration and sample volume), ASTRA computes the Injected Mass. Alternately, you can specify the value here. Procedures that use this injected mass value account for viscometer dilution factor effects if the concentration detector is downstream from a viscometer. Set this parameter if you are performing any of the following analysis procedures: Dn/dc from Peak UV Extinction from Peak

Table 8-15: Peak Properties

Field	Description
Concentration	The concentration of the sample for this peak. Set this parameter if you are performing any of the following analysis procedures: A2, Molar Mass, and Radius from LS Data Dn/dc from RI Molar Mass and Radius from LS Data (batch mode with no concentration detector)
A2	The second virial coefficient for this peak. Set this parameter if you are performing any of the following analysis procedures: Molar Mass and Radius from LS Data Protein Conjugate Analysis Caution: If you enter too large a second virial coefficient the molar mass may become negative or artificially large, depending on the method used. See Appendix D, "Light Scattering Theory".
LS Analysis	
>Model	Set this parameter when you perform any of the following analysis procedures: Radius from LS Data Molar Mass and Radius from LS Data Number from LS Data Protein Conjugate Analysis The available models are: Zimm (the default), Debye, Berry, random coil, sphere, Lorenz-Mie, coated sphere, and rod. See "Choosing a Fit Model" on page 8-58 for details.
>Fit Degree	If you selected Zimm, Debye, or Berry as the fit model, you must specify the fit degree here. The fit degree default is 1. The range is 0 to 5 for the DAWN, and 0 or 1 for the miniDAWN. See "Choosing a Fit Model" on page 8-58 for details.
Protein Conjugate Analysis	
>Modifier dn/dc	If you are using the Protein Conjugate Analysis procedure, specify the dn/dc value in mL/g for the modifier protein.
>Modifier UV Extinction Coefficient	If you are using the Protein Conjugate Analysis procedure, specify the extinction coefficient in mL/(mg cm) for the modifier protein.
Particles	
>Sphere Real RI	If you are using the Lorenz-Mie LS model, specify the real Refractive Index (RI) value of the entire sphere. If you are using the coated sphere LS model, specify the real RI of the core of the sphere. Set this parameter if you are performing any of the following analysis procedures: Molar Mass and Radius from LS Data Radius from LS Data (using Lorenz-Mie, sphere, or coated sphere) Number from LS Data
>Sphere Imaginary RI	If you are using the Lorenz-Mie LS model, specify the real RI value of the entire sphere. If you are using the coated sphere LS model, specify the real RI of the core of the sphere. This RI value should be corrected for absorption. Set this parameter for the same analysis procedures listed for the Sphere Real RI.
>Shell Thickness	If you are using the coated sphere LS model, specify the shell (coating) thickness in nm.
>Shell Real RI	If you are using the coated sphere LS model, specify the real RI of the shell.
>Shell Imaginary RI	If you are using the coated sphere LS model, specify an RI value for the shell. This RI value should be corrected for absorption.

Table 8-15: Peak Properties

Field	Description
>Rod Radius	For Rod LS model calculations, ASTRA assumes that the thickness of a rod-shaped particle is insignificant (0.0 nm) compared to its length. If the thickness is significant, enter its thickness or approximate thickness in nm.
Column Calibration	
>Molar Mass (g/mol)	If you are calibrating a column (or using molecular standards with known molar masses), enter the known molar mass of the molecular standard for this peak.
>Intrinsic Viscosity (mL/g)	If you are performing a universal column calibration (with viscosity data), enter the known intrinsic viscosity of the molecular standard for this peak.
>Mark-Houwink-Sakurada K	If you are performing viscosity-based calculations, enter the "K" fit parameter for the Mark-Houwink-Sakurada analysis for this peak in mL/g.
>Mark-Houwink-Sakurada a	If you are performing viscosity-based calculations, enter the "a" fit parameter for the Mark-Houwink-Sakurada analysis for this peak.
Viscosity Analysis	
>Model	Specify the model to use for intrinsic viscosity calculations for this peak. The model may be Huggins, Kraemer, or Solomon-Gatesman. The default is Huggins. ASTRA has historically used the Huggins relation with a "Huggins Constant" of zero. For more about viscosity analysis, see Appendix G, "Viscosity Theory" and go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis > Intrinsic Viscosity Models.
>Huggins Constant (k')	If you select the Huggins model, you can specify a Huggins constant here to be used in the calculation. The default is zero.
>Kraemer Constant (k'')	If you select the Kraemer model, you can specify a Kraemer constant here to be used in the calculation. The default is zero.

6. Click **OK** to continue running the experiment.

Choosing a Fit Model


The light-scattering fit models are as follows:

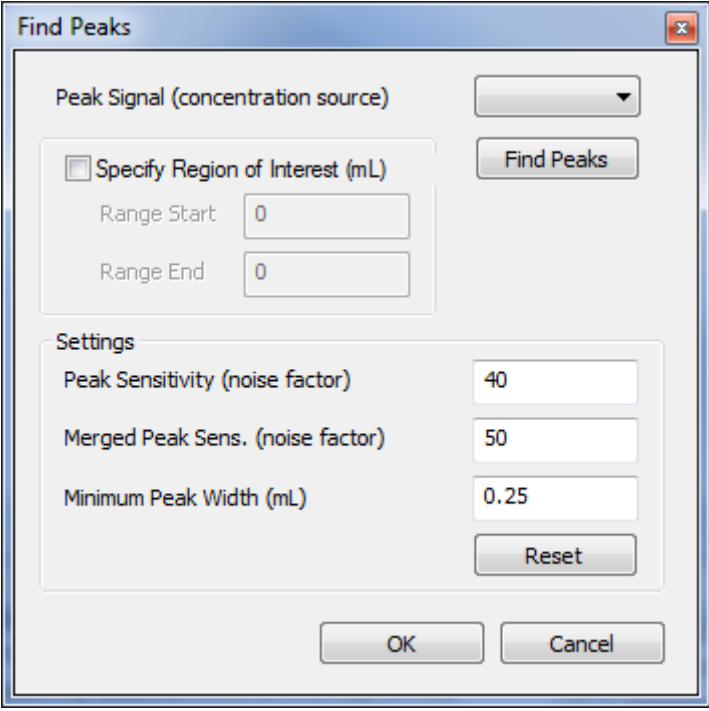
- **Zimm model:** uses the K^*c/R_θ formalism. The Zimm model should be used for molecules that have RMS radii smaller than 50 nm and that do not conform to another standard model such as random coil or sphere. The Zimm model has the advantage over the Debye model in that it often requires a lower fit degree for the same size molecule. For large (>50 nm) molecules, the Zimm model often produces a negative molar mass and should not be used.
- **Debye model:** Uses the R_θ/K^*c formalism. It gives better results over a wider range of molar mass, including the very large (greater than $\sim 10^6$ Daltons or ~ 100 nm RMS radius). But you may need to delete high angle detectors to improve the fit of the extrapolation since the curvature can be very large.
- **Berry model:** Uses the $\sqrt{K^*c/R_\theta}$ formalism. It can be useful, in combination with deleting high angle data, when analyzing molecules with RMS radii greater than 50 nm.
- **Random coil model:** Uses the formula for a theoretical random coil molecule rather than a polynomial to fit the angular light scattering data.

- **Sphere model:** Uses the analytical formula for a sphere rather than a polynomial to fit the angular light scattering data. Use this model only with known spherical samples, such as lattices. Note that if the spheres are aggregated, this model may not fit since the aggregated particles may be of any shape.
- **Rod model:** Uses the analytical formula for a rod rather than a polynomial to fit the angular light scattering data. It is necessary to specify the rod radius when using this model.
- **Coated sphere model:** Uses the analytical formula for a coated sphere rather than a polynomial to fit the angular light scattering data. It is necessary to specify the coating (shell) thickness and core and coating refractive indices when using this model.
- **Lorenz-Mie model:** Uses the Lorenz-Mie analysis for a sphere rather than a polynomial to fit the angular light scattering data. It is necessary to specify index of refraction when using this model. If you are using the Lorenz-Mie theory (as this model does), the particle need not satisfy the criteria for Rayleigh-Debye Gans scattering. As a result, this is the most general method for analyzing spheres of any size.

See the “Determination of Molar Mass and Sizes” on page D-13 for a discussion of the fit models.

Parameters for Finding Peaks

If you want finer control over how peaks are detected, click the  **Parameters** button above the Define Peaks graph. You can set the peak sensitivity and peak approach. You can also specify a region of interest using X-axis units. The parameters you set here are saved for use with future experiment runs.



The **Find Peaks** dialog box contains the following controls:

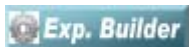
- Peak Signal (concentration source):** A dropdown menu.
- Specify Region of Interest (mL):** A checkbox.
- Range Start:** A text input field with the value 0.
- Range End:** A text input field with the value 0.
- Find Peaks:** A button.
- Settings:** A section containing:
 - Peak Sensitivity (noise factor):** A text input field with the value 40.
 - Merged Peak Sens. (noise factor):** A text input field with the value 50.
 - Minimum Peak Width (mL):** A text input field with the value 0.25.
 - Reset:** A button.
- OK:** A button.
- Cancel:** A button.

The parameters are as follows:

- **Peak Signal:** Choose the signal you want to use for identifying peaks.
- **Specify Region of Interest:** By default, the peak finding routine looks at all the data for peaks. You can set the starting and ending point in the data for finding peaks. Enter start and end points in units of time or volume, depending on the x-axis units in the graph.
- **Peak Sensitivity:** An average baseline is determined by plotting a histogram of the baseline points. Given enough baseline points, a standard deviation of the baseline noise can be determined. The Peak Sensitivity parameter is the number of standard deviations above or below the baseline at which data is considered a “peak” rather than baseline noise. In short, this parameter determines the minimum height of a peak.
- **Merged Peak Sensitivity:** This parameter is similar to Peak Sensitivity, but for Merged Peak Sensitivity, the derivative (slope) of the data and the baseline noise is used to determine cutoffs. The derivative of the data must go above a certain threshold and then below the negative of that threshold in order to be considered a peak. That threshold is measured in units of standard deviations of the derivative of the baseline noise.
- **Minimum Peak Width:** This parameter is the minimum width allowable for a region to be considered a peak. This is measured in either time or volume, depending on the x-axis units in the graph.

Click **Find Peaks** to use your current settings to find peaks. Click **OK** to save your settings without autofinding peaks. Click **Reset** to restore the default settings. Click **Cancel** to close the dialog without saving the settings.

Broaden



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. It applies the terms calculated by the “Band Broadening” procedure. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

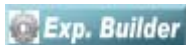
For an example experiment that corrects for band broadening, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the “band broadening example (BSA).afe6 experiment in the Sample Data > Analyzed Experiments folder.

Convert to Physical Units

This procedure is only visible in Experiment Builder mode. It converts instrument signals to physical units, if necessary. For example, light scattering values in volts are converted to Rayleigh ratios.

You may place this procedure in a location after the collection procedure and before the analysis procedures. Any procedures that follow this one will display detector data in physical units rather than voltages.

There are no properties to set for this procedure. It runs without prompting for any values.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

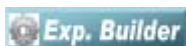
Convert to Concentration

This procedure converts the refractive indexes measured by an RI instrument or UV absorbance data to concentrations. It is only visible in Experiment Builder mode. The Experiment Configuration (see page 7-14) contains a Concentration Source field that allows you to choose between RI and UV data if both are available.

You may place this procedure in a location after the collection procedure and before the analysis procedures.

If the dn/dc value is specified for a peak region, any procedures that follow this one display RI data as concentrations for each peak region. If the UV extinction coefficient is specified for the peak region, any procedures that follow this one will display UV data as concentrations for each peak.

There are no properties to set for this procedure. It runs without prompting for any values.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

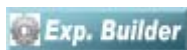
Convert Specific to Intrinsic Viscosity

This procedure converts the specific viscosity measured by a viscometer such as the ViscoStar to intrinsic viscosity using concentration data. See the *ViscoStar User's Guide* for details about the calculation that is performed.

You may place this procedure after the peaks are defined and before the analysis procedures. You must also place the “Convert to Physical Units” procedure before this procedure.

If both RI and UV concentration data were collected for this experiment, use the procedure “Convert to Concentration” on page 8-61 to specify which set of data to use for concentration calculations.

There are no properties to set for this procedure. It runs without prompting for any values. The procedure “Peaks” on page 8-54 lets you select a model to use for intrinsic viscosity calculations and any constants required by the selected model. The model may be Huggins, Kraemer, or Solomon-Gatesman.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

For more about viscometry data collection and analysis, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Viscometry.

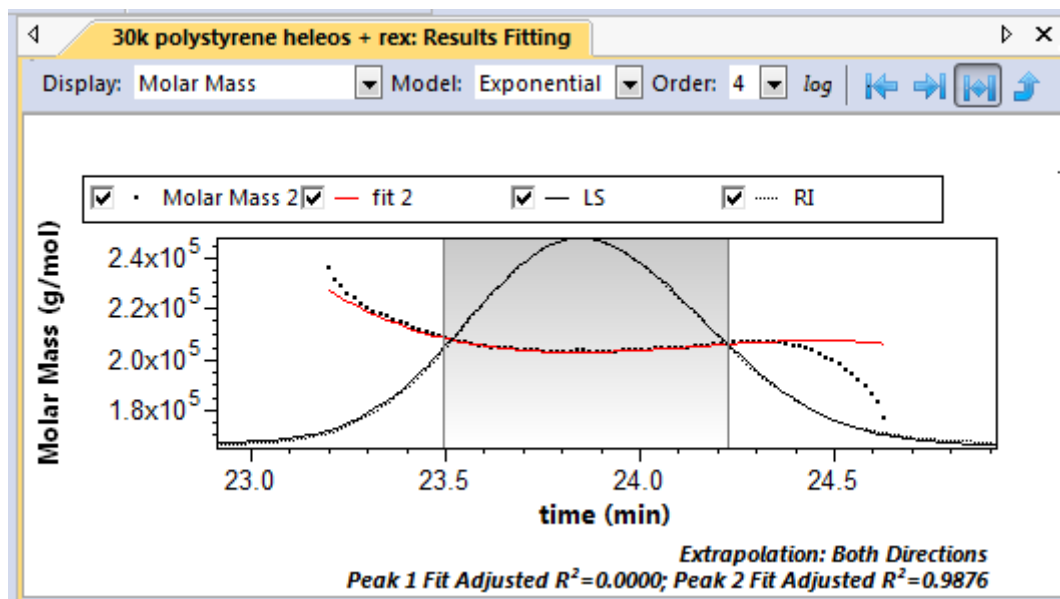
Results Fitting

You can use a curve fitting model to fit molar mass, radius, viscosity, branching ratio, and protein fraction results.

You can set the properties for this procedure before running the experiment, or you can modify them after running the experiment and re-run the experiment to see the effects of changing the settings.

If you set a model and fit order for a data type, the fit line is shown in graphs of that data, including EASI graphs.

To set fit properties, open the page for the procedure.



The fields above the graph are as follows:

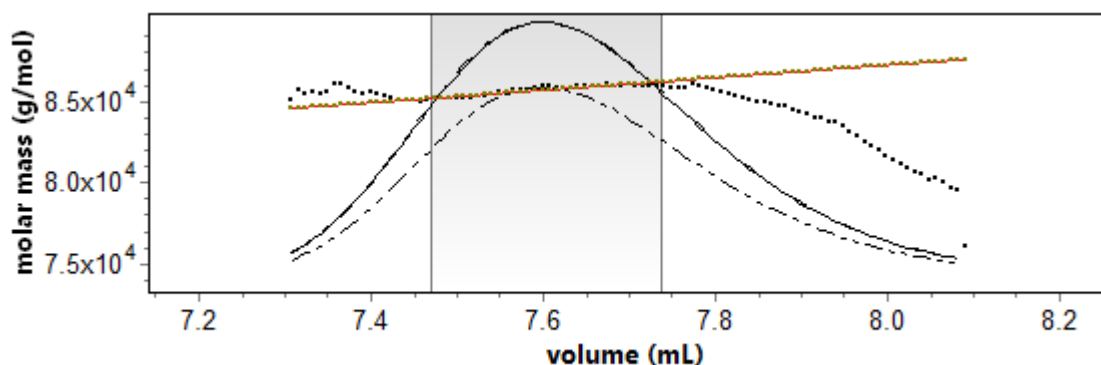
Table 8-16: Results Fitting Properties





Field	Description
Display	Choose the data to display and fit. The options depend on the data available in your experiment. For example, molar mass, mean square radius, hydrodynamic radius, geometric radius, number density, intrinsic viscosity, branching ratio, protein fraction, protein molar mass, and modifier molar mass.
Model	The model to use to fit the selected data. Options are None (the default) and Exponential.
Order	The fit degree for the selected data. The default is 1. The allowed range is 0 to 6. This field is disabled if the fit type is "None," which means no fit will be performed.
log	Click this button to change the Y-axis to a logarithmic scale.

Fit the molar mass or radius data if you wish to obtain more accurate peak moments and distribution results for molar mass and radius ranges that have significant scatter due to lower signal to noise ratios.

After choosing a fit, look at the graph to visually determine whether the fit is acceptable or not. If the fit is not good, ASTRA reports results that are meaningless. You should try both none and exponential fitting before deciding which one to use.

You can use your mouse to select a range over which the fit should be trained. Data outside the range is ignored for creating the fit trace.



You can extrapolate data backward, forward, or in both directions of the fit range you select. This allows you to use actual measurements for most of the analysis, and switch to a fit for regions with heavy scatter. You can click the    **Extrapolate Forward**, **Backward**, or **Both** icon to extrapolate and the  **Revert** icon to stop extrapolating. Extrapolation is available only for experiments with a single defined peak.

The extrapolation setting and fit adjusted R^2 value are shown on the graph.

Analysis Procedures

The analysis procedures calculate various results using the data.

The following analysis procedure types are available:

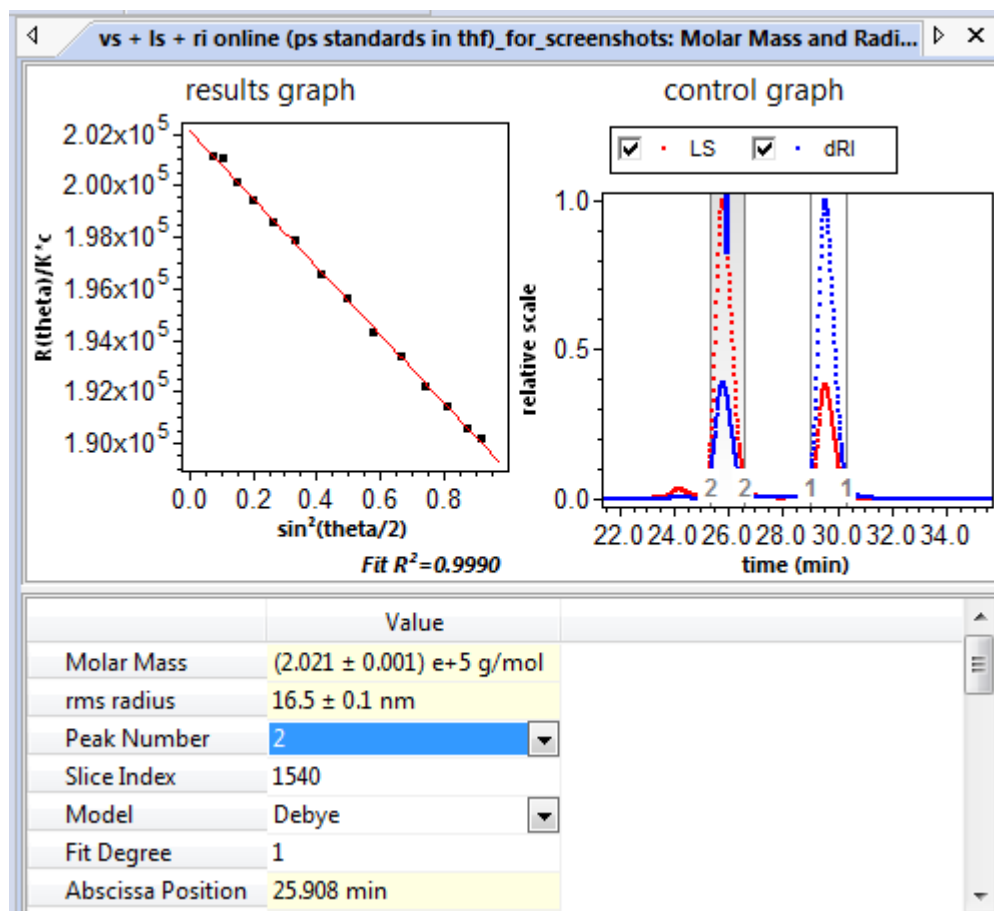
- “A2, Molar Mass, and Radius from LS Data” on page 8-69
- “Branching” on page 8-85
- “Copolymer Analysis” on page 8-91
- “Cumulants” on page 8-99
- “Distribution Analysis” on page 8-90
- “Distributions and Moments” on page 8-78
- “Dn/dc from Peak” on page 8-80
- “Dn/dc from RI” on page 8-79
- “Mark-Houwink-Sakurada” on page 8-88
- “Molar Mass and Radius from LS Data” on page 8-64
- “Molar Mass from Column Calibration” on page 8-101
- “Molar Mass from VS” on page 8-75
- “Number from LS Data” on page 8-75
- “Online A2” on page 8-71
- “Parametric Plot” on page 8-88
- “Peak Areas” on page 8-88
- “Protein Conjugate Analysis” on page 8-93
- “Radius from LS Data” on page 8-77
- “Regularization” on page 8-95
- “Rh from QELS” on page 8-82
- “Rh from VS Data” on page 8-85
- “RI Calibration from Peak” on page 8-81
- “UV Extinction from Peak” on page 8-81
- “UV Extinction from RI” on page 8-81

Molar Mass and Radius from LS Data

This procedure calculates the molar mass and RMS radius of the sample. Both light scattering and concentration data are required. For an online experiment, either an RI or UV detector provides the concentration data. For a batch measurement, the concentration can be specified for the peak ranges.

For more about light scattering analysis of molar mass, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Molar mass via light scattering.

The procedure has the following page:



The left graph shows the results in a Debye graph. See “About Analysis Plots” on page 8-66.

The right graph shows the baseline and peaks for the selected detector.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the molar mass. If you place multiple methods that determine molar mass in a procedure, only the first one will be valid.

The properties for this procedure are as follows:

Table 8-17: Molar Mass and Radius from LS Properties

Field	Description
Molar Mass	Shows the calculated molar mass. This field is display only.
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Peak Number	Type the number of the peak for which molar mass and RMS radius should be calculated. You can create additional peaks in this procedure.
Slice Index	Displays the selected slice number. alternately, you can enter the slice number for which to view results.
Model	This field shows and lets you change the light-scattering model selected for this peak.

Table 8-17: Molar Mass and Radius from LS Properties

Field	Description
Fit Degree	This field shows the fit degree selected for this peak. This setting is valid only if the Zimm, Debye, or Berry model has been selected for the peak.
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Concentration	This field shows the concentration of this peak. This field is display only.
dn/dc	Shows and lets you change the dn/dc value for this peak.
Enabled Detectors > 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.

About Analysis Plots

The following procedure views show an analysis plot with a Debye fit:

- “Molar Mass and Radius from LS Data” on page 8-64
- “Number from LS Data” on page 8-75
- “Radius from LS Data” on page 8-77

Analysis plots let you view the light scattering data for each light scattering detector for each slice of the peak and see the weighted least-squares fit to the data. These plots are a good place to check the appropriateness of the polynomial used for fitting. It also allows you to check visually the normalization coefficients for the DAWN.

When viewing an analysis plot, there are two separate graphs. On the right is the control graph (chromatograms). On the control graph, use the mouse to select a peak and slice to use for the results graph on the left. The selected peak is highlighted, and a cursor shows the selected slice. You can use the arrow keys to scroll through the various slices.

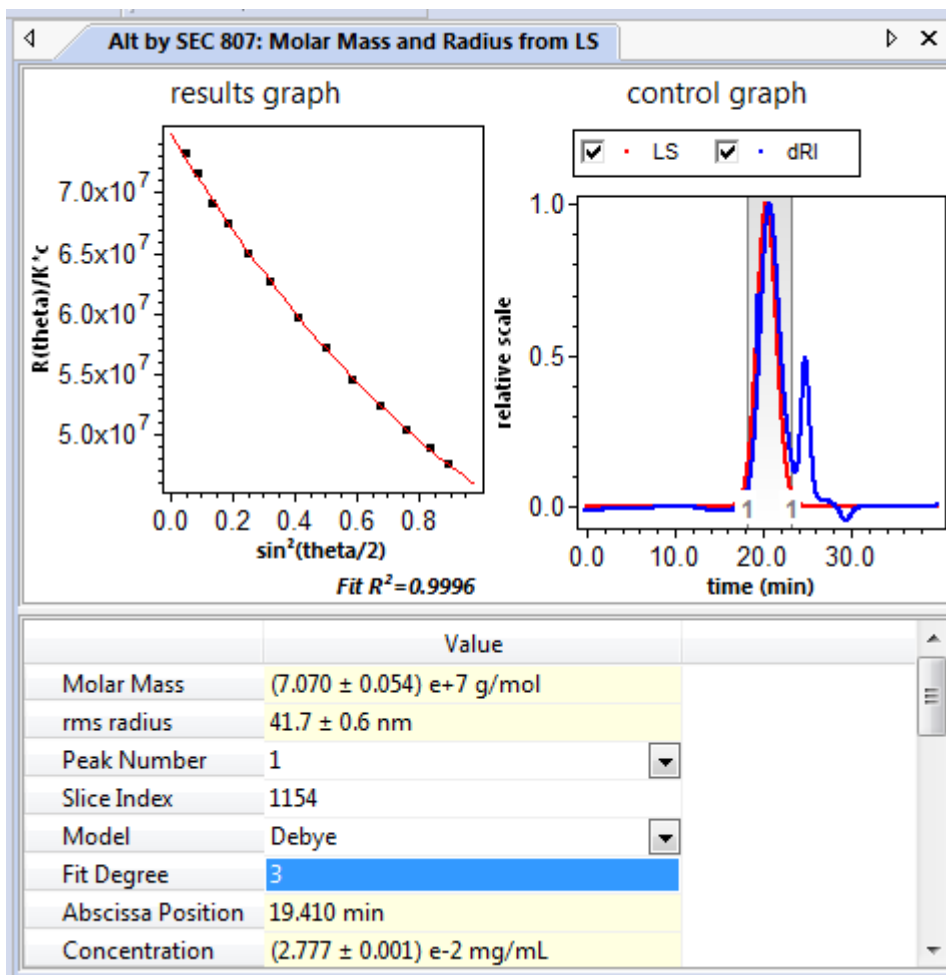


Figure 8-5: Analysis plot with fit degree of 3

Fitting the Light Scattering Data

Random coil, sphere, coated sphere, rod, and Lorenz-Mie models do not require a fit degree. Hence, the analysis plot can be used to assess the efficacy of the fit model and to flag noisy detectors or a poor normalization.

For the Debye, Zimm, and Berry models, the angular data is fit to a polynomial expansion. Hence it is necessary to specify a fit degree for these models. The fit degree can be set to a value from 0 to 5.

When using the Debye, Zimm, or Berry model and determining the fit degree, it is often sufficient to choose a fit degree that gives the smallest error.

Sometimes minimizing the error in the data is not a sufficient criterion. In fact, several polynomials may give very similar errors. This typically happens when you use the Debye model and have a lot of low angular curvature in the data. You must then visibly make sure that the low angle data is fit well, otherwise the fitted molar mass and radius will be incorrect. As an example, compare the result in Figure 8-5 with that of Figure 8-6. The only difference is the Fit Degree chosen for the calculation. In this example, using a Fit Degree of 1 is inappropriate due to the systematic deviations of the data with respect to the fit.

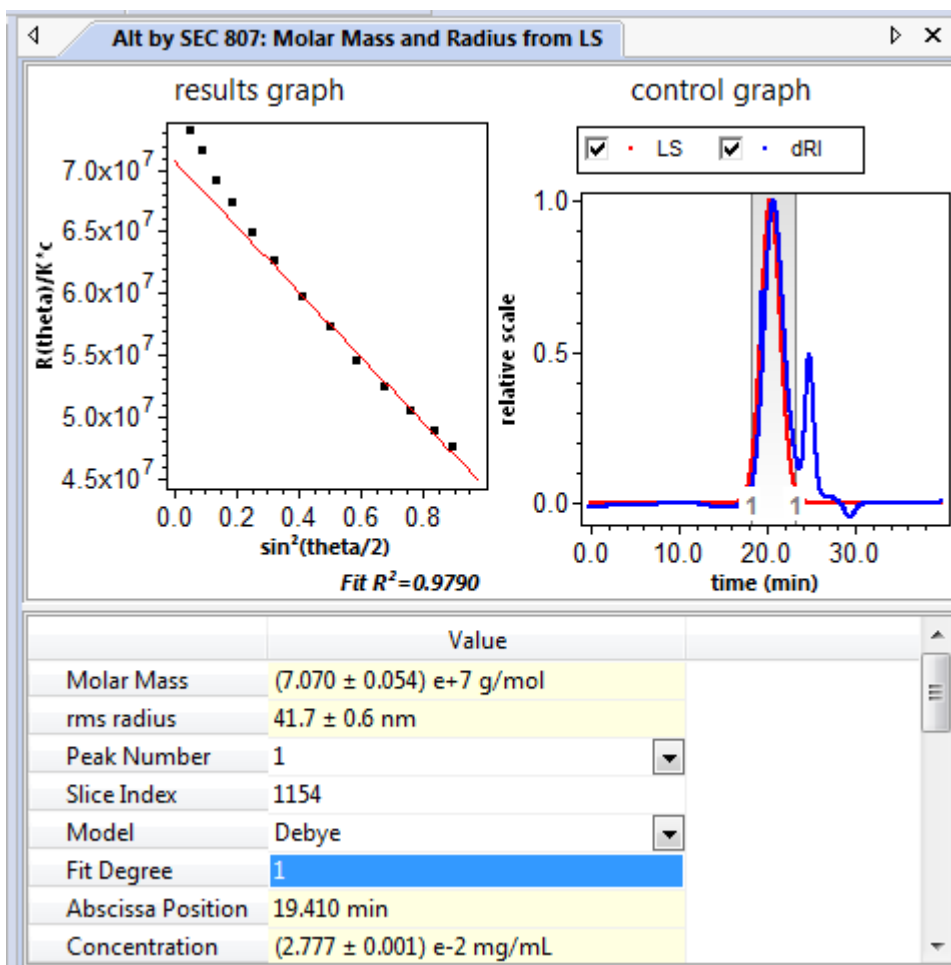


Figure 8-6: Analysis plot with fit degree of 1

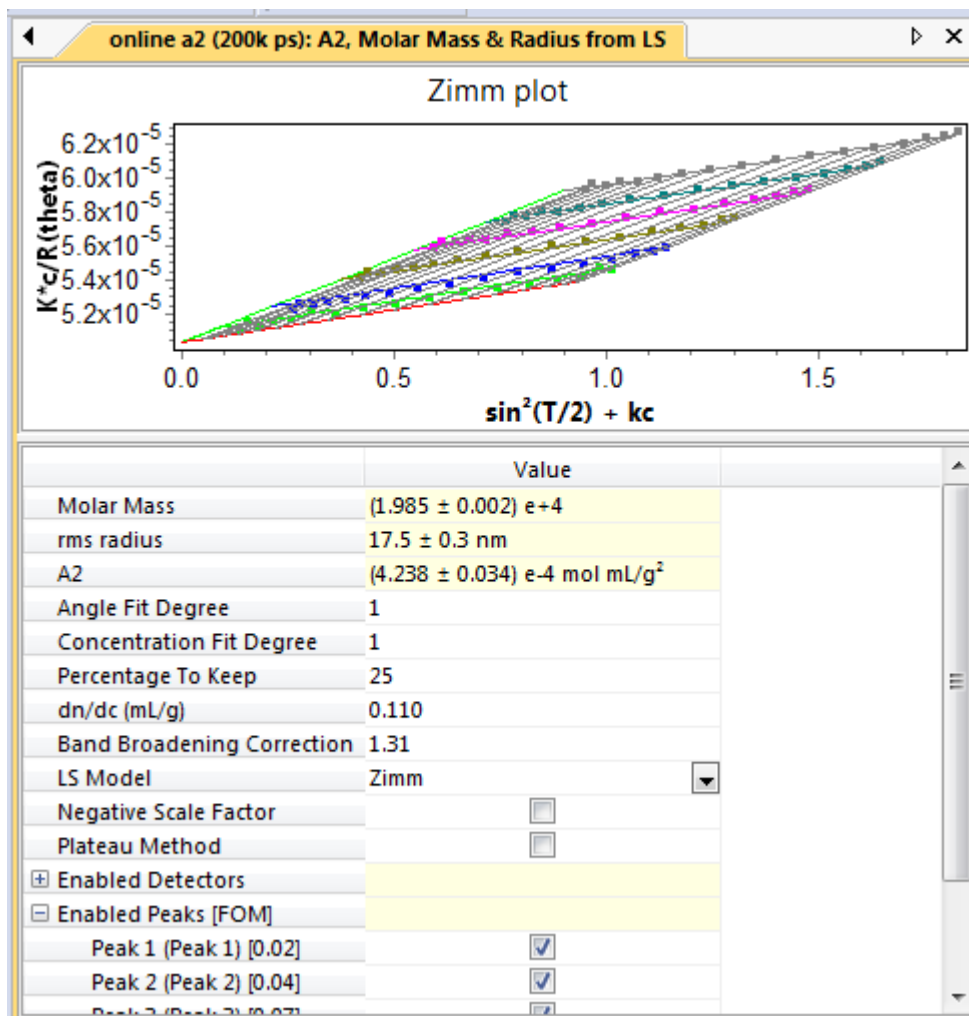
There are cases when no model accurately fits the angular curvature in the analysis plot. Typically, this is due not to a failure in the fit model, but to incomplete fractionation; the polydispersity in the sample cannot be reproduced by a model that assumes a monodisperse sample.

A2, Molar Mass, and Radius from LS Data

This procedure calculates the second virial coefficient (A_2), molar mass, and RMS radius of the sample based on light scattering data as a function of angle and concentration. For A_2 and A_3 calculation, see “Online A2” on page 8-71.

For more about determining the second virial coefficient, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Second virial coefficient.

The procedure has the following page:



By default, the graph shows the results in a Zimm plot style, although ASTRA 6 does not use a traditional Zimm plot analysis. Instead, a global fitting algorithm is used to present all concentration and angular data together. In the presentation, the grid represents the best fit results from the global fit. The quality of the fit can be assessed by seeing how the measured data points lie with respect to the best fit grid. The data points for each peak are shown in a different color.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the molar mass. If you place multiple methods that determine molar mass in a procedure, only the first one will be shown in the report.

Previously, A_2 measurements could only be done in batch (plateau) mode. ASTRA now supports A_2 measurement in online mode through use of the Plateau calculation method. Use the “online A_2 ” experiment method in the System > Methods > Light Scattering folder for online A_2 measurement. A completed “online A_2 ” example experiment is also available for importing.

The properties for this procedure are as follows:

Table 8-18: A_2 , Molar Mass, and Radius from LS Properties

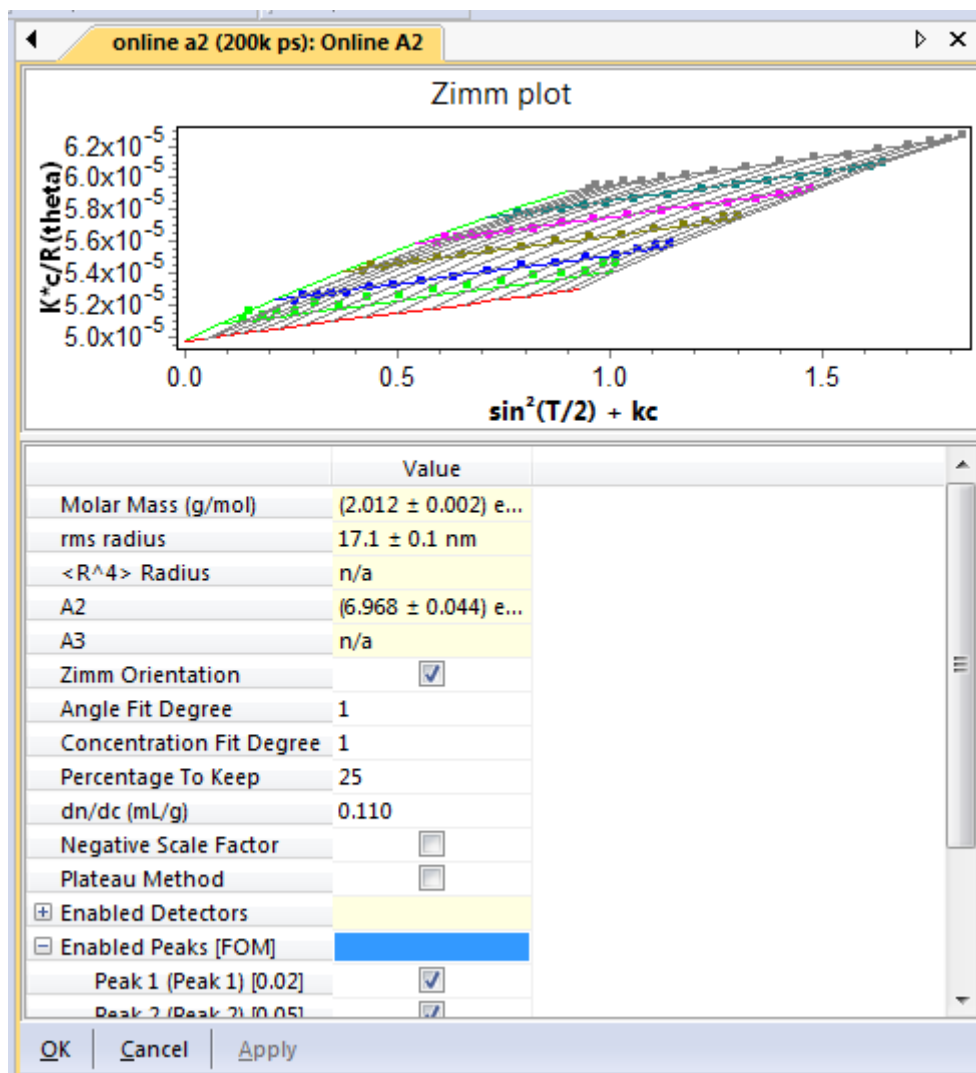
Field	Description
Molar Mass	Shows the calculated molar mass. This field is display only.
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
A_2 (mol mL/g ²)	Shows the calculated second virial coefficient. This field is display only.
Angle Fit Degree	The angular fit degree. May range from 0 to 5.
Concentration Fit Degree	The concentration fit degree. May range from 0 to 5.
Percentage to Keep	Type the percentage of each peak to use for calculating the A_2 molar mass and radius. The default is 25%. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
dn/dc (mL/g)	Specify the dn/dc value for the sample. If the dn/dc value is zero, the default is taken from the sample in the configuration.
Band Broadening Correction	If you are doing online Zimm plots, specify the band broadening correction factor. The corrupting effects of band broadening boil down to a single multiplicative correction factor. You can typically measure this by performing the Plateau method for a well-understood standard. Once this factor is measured, you can use this value on all subsequent analyses to correct for the effects of band broadening.
LS Model	Choose the calculation model to use. The units for the y-axis in the graph change as appropriate for the model you select. The options are Zimm (the default), Debye, Berry, Random Coil, and Sphere.
Negative Scale Factor	Put a checkmark in this box to use a negative scale factor for the Zimm plot.
Plateau Method	Put a checkmark in this box if this is a batch experiment (or an online experiment with plateaus rather than peaks).
Enabled Detectors > 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.
Enabled Peaks [A_2 FOM] > 1 to n	You can omit peaks from the plot by removing the checkmark next to the peak number. The [A_2 FOM] value shown for each peak is a “Figure of Merit,” which is a unitless value that reflects the ability to measure A_2 accurately. If the A_2 FOM for a peak is less than 1 or slightly greater than 1, then the peak will help measure A_2 accurately.

Online A2

This procedure calculates the second and third virial coefficients (A_2 and A_3 , respectively), molar mass, and RMS radius of the sample based on light scattering data as a function of angle and concentration.

For more about determining the second virial coefficient, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Second virial coefficient.

The procedure has the following page:



By default, the graph shows the results in a Zimm plot style, although ASTRA does not use a traditional Zimm plot analysis. Instead, a global fitting algorithm is used to present all concentration and angular data together. In the presentation, the grid represents the best fit results from the global fit. The quality of the fit can be assessed by seeing how the measured data points lie with respect to the best fit grid.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the molar mass. If you place multiple methods that determine molar mass in a procedure, only the first one will be valid.

Previously, A_2 measurements could only be done in batch (plateau) mode. ASTRA now supports A_2 measurement in online mode. Use the “online A_2 ” experiment method in the System > Methods > Light Scattering folder for online A_2 measurement. A completed “online A_2 ” experiment is also available for importing.

The properties for this procedure are as follows:

Table 8-19: Online A_2 Properties

Field	Description
Molar Mass	Shows the calculated molar mass. This field is display only.
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
R^4 Radius	Shows the calculated R^4 radius. This is a physical quantity derived from the Zimm series expansion. It is similar to the RMS or geometric radius, except that it is the "fourth-root-mean-fourth-power." This field is display only.
A_2 (mol mL/g ²)	Shows the calculated second virial coefficient. This field is display only.
A_3 (mol mL/g ³)	Shows the calculated third virial coefficient. This field is display only.
Zimm Orientation	Toggle the display between the Zimm and Debye plotting formalisms.
Angle Fit Degree	The angular fit degree. May range from 0 to 2.
Concentration Fit Degree	The concentration fit degree. May range from 0 to 2.
Percentage to Keep	When performing a batch analysis, type the percentage of each peak to use for calculating the A_2 molar mass and radius. The default is 25%. If the plateau is flat (not drifting) in the peak range, using the default value is recommended. For online analysis, this field is unused.
dn/dc (mL/g)	Specify the dn/dc value for the sample. If the dn/dc value is zero, the default is taken from the sample in the configuration.
Negative Scale Factor	Put a checkmark in this box to use a negative scale factor for the Zimm plot.
Plateau Method	Put a checkmark in this box if this is a batch experiment (or an online experiment with plateaus rather than peaks).
Enabled Detectors > 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.
Enabled Peaks [A_2 FOM] > 1 to n	You can omit peaks from the plot by removing the checkmark next to the peak number. The [A_2 FOM] value shown for each peak is a “Figure of Merit,” which is a unitless value that reflects the ability to measure A_2 accurately. If the A_2 FOM for a peak is less than 1 or slightly greater than 1, then the peak will help measure A_2 accurately.

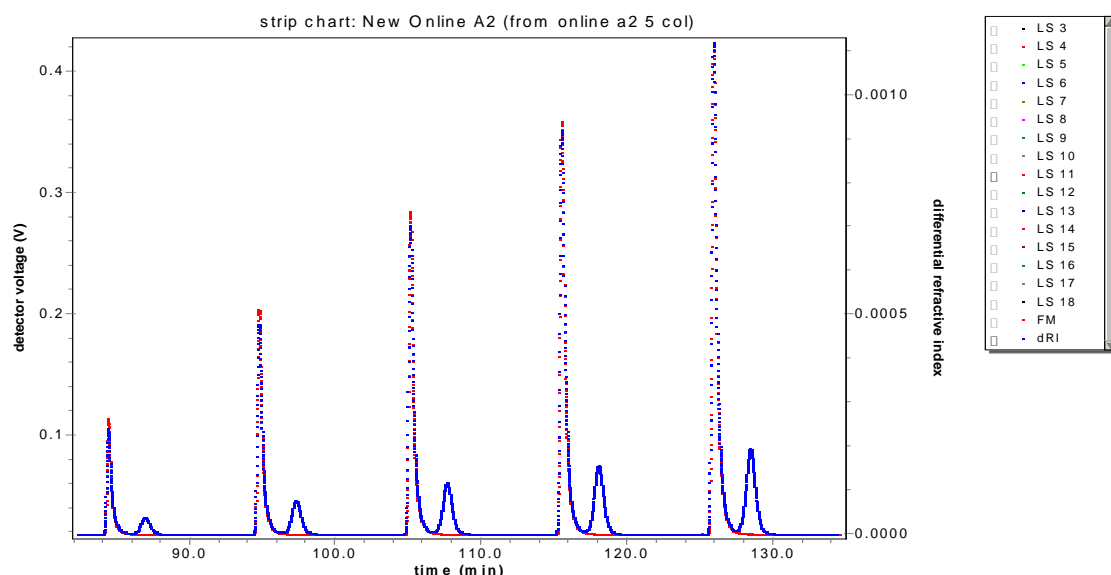
The Online A_2 model is based on a series expansion of the Zimm model in concentration. It is best suited for small molecules for which other models are not applicable. You cannot specify the light scattering fit model for this analysis.

Determining A₂ Using the Online A2 Procedure

The Online A2 procedure extends the analysis to include the third virial coefficient (A_3) and removes the need for a band broadening correction.

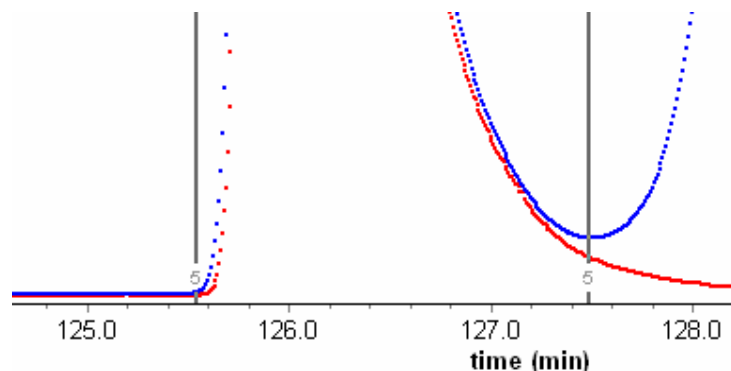
To analyze a sample for A_2 , follow these steps:

1. Create a new experiment using the System > Methods > Light Scattering folder > online A2 method.
2. Adjust the configuration to reflect your light scattering and concentration detectors (the default is a HELEOS and an Optilab rEX)
3. Run a data collection consisting of a series of injections of varying concentrations. The following example shows five samples. These injections should be collected during the same analysis run.



4. Set baselines as you would normally.
5. Select peak ranges for each of the peaks. It is important to make the peaks widths, and thus the analyzed volumes, as similar as possible.
 - Since the higher concentration sample peaks may be wider, you may wish to use this as the width to set all peaks.
 - Typically, you should start the leading edge of the peak right before the signal begins to rise from the baseline.
 - Likewise, you should choose the trailing edge of the peak to correspond to either the baseline or the inflection point if the signal rises towards a secondary peak.

For example, the following peak range was selected for Peak 5 starting just before the peak rises from the baseline and ending at the point where the concentration signal passes through zero slope to start the second peak:



Note that this corresponds to a peak width of $127.48 - 125.55 = 1.93$ min. You should keep the other peaks to as close to the same width as possible while still capturing the leading and trailing edges of the peak.

6. The “Online A2” procedure is now ready for processing. When you open the procedure, be sure the dn/dc value is set to the correct value for the sample being analyzed. If you have previously set a dn/dc value in the sample profile (in the experiment configuration) the number should already be filled in.
7. By adjusting the fit degree (from 0-2 for **Angle Fit Degree** and **Concentration Fit Degree**) you can change the types of results produced. The following table shows which results will be listed as “n/a” depending on your fit degree settings:

Table 8-20: Online A2 Results for Various Fit Degrees

Angle Fit Degree ($\text{Sin}^2(\theta/2)$)	Concentration Fit Degree	Results Excluded
0		R ⁴ Radius, RMS Radius
1		R ⁴ Radius
2		None
	0	A2, A3
	1	A3
	2	None

Molar Mass from VS

This procedure calculates the molar mass using viscosity data. It uses a Mark-Houwink-Sakurada analysis to calculate the intrinsic viscosity. See the “Operating Principles and Theory” appendix of the *ViscoStar User’s Guide* for details and Appendix G, “Viscosity Theory” in this manual.

You can use the Mark-Houwink-Sakurada procedure on page 8-88 to determine the appropriate Mark-Houwink-Sakurada parameter values for your polymer, solvent, and temperature combination. Then, you can use those values in this procedure to determine the molar mass.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the molar mass. If you place multiple methods that determine molar mass in a procedure, only the first one will be valid.

The procedure has the following page:

	Value
Mark-Houwink-Sakurada K (mL/g)	0.000e+000
Mark-Houwink-Sakurada a	0.000e+000

OK Cancel Apply

The properties for this procedure are as follows:

Table 8-21: Molar Mass from VS Data Properties

Field	Description
Mark-Houwink-Sakurada K	The “K” fit parameter for the Mark-Houwink-Sakurada analysis.
Mark-Houwink-Sakurada a	The “a” fit parameter for the Mark-Houwink-Sakurada analysis.

For more about viscometry data collection and analysis, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Viscometry.

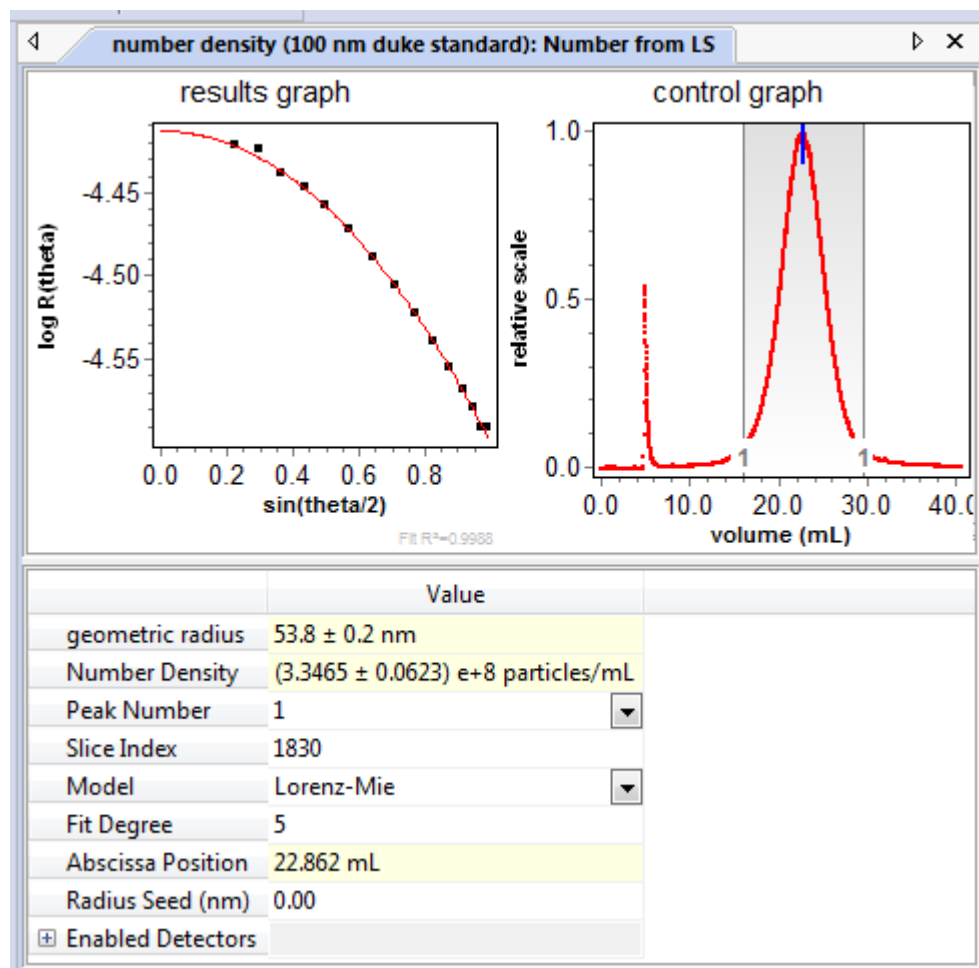
Number from LS Data

This procedure calculates the radius and the number of particles per mL (density) in the sample. It is necessary to specify the refractive index of the sample in the Peaks procedure to determine the number density. This procedure is normally used with online (fractionated) experiments.

For more about determining and using number density, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Particles and number density.

You can place this procedure with other analysis procedures, and after all the transformation procedures.

The procedure has the following page:



The graph display is a standard analysis plot. Click on the Control Graph to select a peak and a slice index to use for the Results Graph.

The properties for this procedure are as follows:

Table 8-22: Number from LS Data Properties

Field	Description
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Number Density	Shows the number density in particles per mL. This field is display only.
Peak Number	Displays the number of the peak for the results graph. You can create additional peaks in this procedure.
Slice Index	Displays the index for the slice displayed in the results graph. Alternately, you can type a slice index here.
Model	This field shows and lets you change the light-scattering model selected for this peak. Number density computations require a known shape model for the particle. If you choose the Debye, Berry, Zimm, or Random Coil model, you cannot use this procedure to compute the number density. If you choose the Rod model, you must first specify a Rod Radius in the Peaks dialog. See "Choosing a Fit Model" on page 8-58 for details.

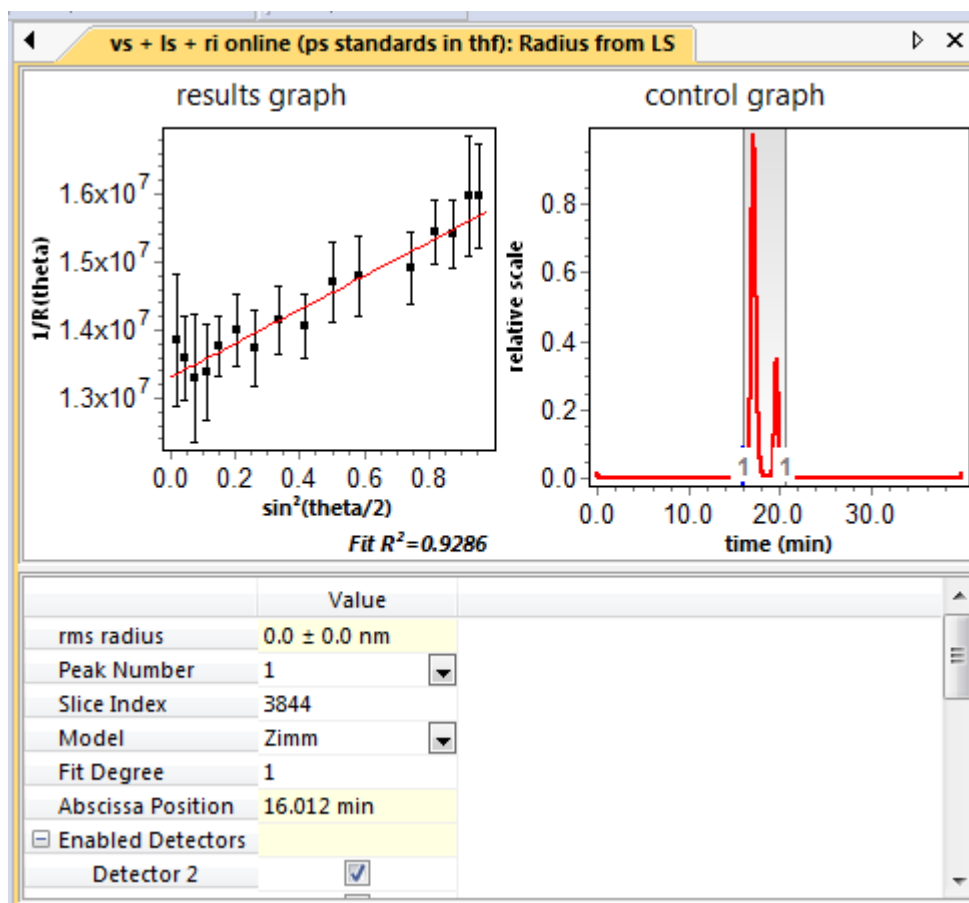
Table 8-22: Number from LS Data Properties

Field	Description
Fit Degree	This field shows and lets you change the fit degree selected for this peak. It is valid only if the Zimm, Debye, or Berry model is selected.
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Radius Seed	If you have a rough idea of the sample's radius, you can provide that value here. Providing a seed value helps the procedure look for a solution in the correct region.
Enabled Detectors > 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark..

Radius from LS Data

This procedure calculates the radius of the sample based on the light scattering signal alone. This procedure is normally used with online (fractionated) experiments.

The procedure has the following page:



You can place this procedure with other analysis procedures, and after all the transformation procedures.

The graph display is a standard analysis plot. The properties for this procedure are as follows:

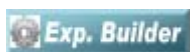
Table 8-23: Radius from LS Data Properties

Field	Description
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Peak Number	Select the number of the peak for the results graph. You can create additional peaks in this procedure.
Slice Index	Displays the index for the slice displayed in the results graph. Alternately, you can type a slice index here.
Model	This field shows and lets you change the light-scattering model selected for this peak.
Fit Degree	This field shows and lets you change the fit degree selected for this peak. It is valid only if the Zimm, Debye, or Berry model is selected.
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Enabled Detectors > 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.

Distributions and Moments

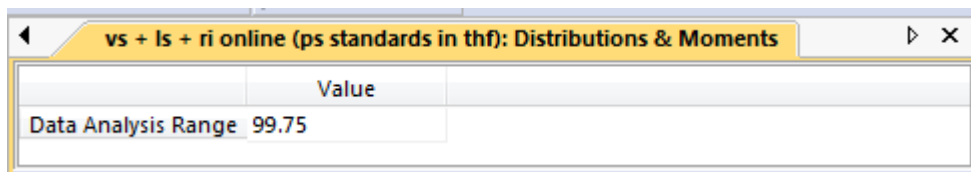
This procedure calculates the cumulative and differential molar mass and radius distributions, as well as the moments (number average, weight average, and Z-average) and averages for various quantities for each peak. This procedure is normally used with online (fractionated) experiments. It is used by the “Distribution Analysis” on page 8-90.

You can place this procedure with other analysis procedures, and after all the transformation procedures.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. To see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

The procedure has the following page:



The property for this procedure is as follows:

Table 8-24: Distributions and Moments Properties

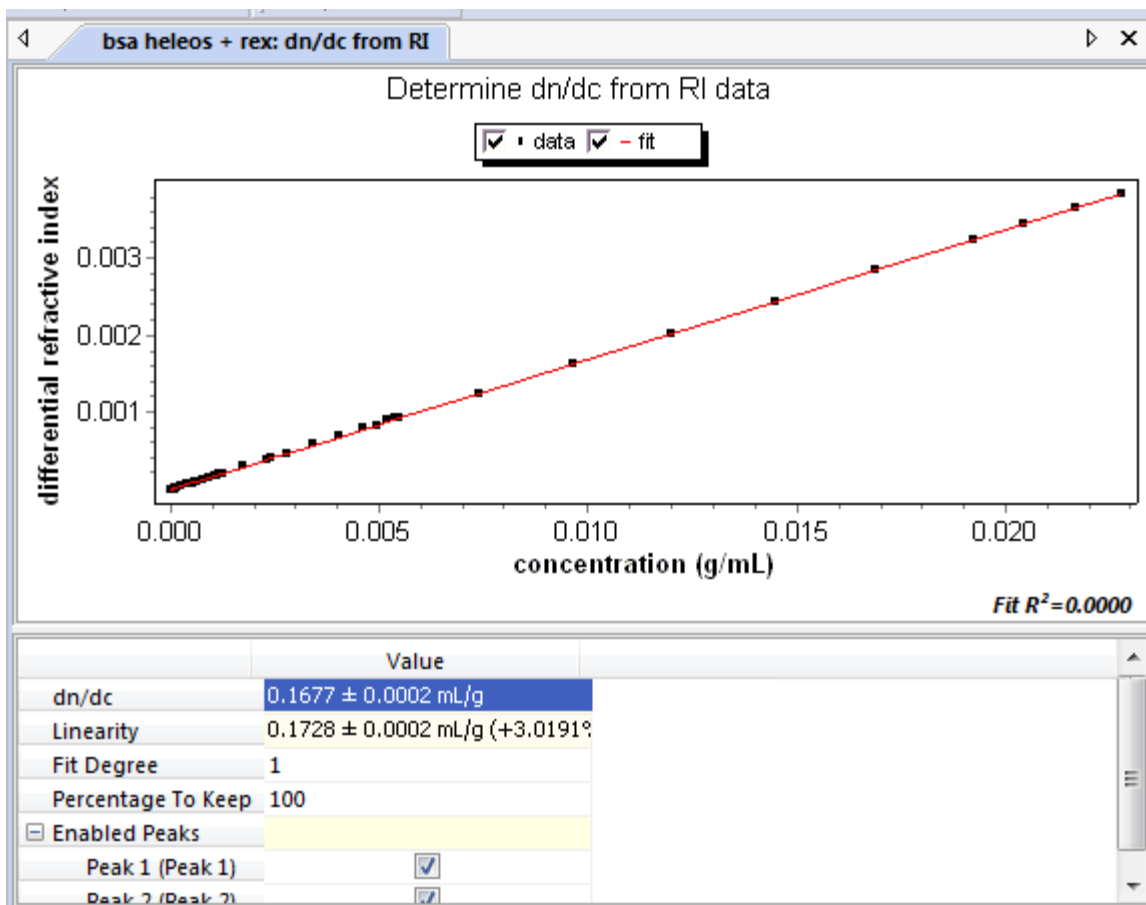
Field	Description
Data Analysis Range	Fine-tunes the percentage of data used in the distribution and moments calculation. For large amounts of data, the calculations can take a long time to perform; consequently, the range is set to 99.75 by default. If you wish, you can set it to 100% to use the full range of data in the analysis.

For more about distributions, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Distributions.

Dn/dc from RI

This procedure calculates the dn/dc of the sample and the linearity of the result using data from an RI detector.

The procedure has the following page:



The graph shows a fit to the differential refractive index and concentration data.

For an example experiment that determines dn/dc from RI data, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the "dndc measurement.afe6" experiment in the Sample Data > Analyzed Experiments folder.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine dn/dc or RI calibration in a procedure, only the first one will be valid.

The properties for this procedure are as follows:

Table 8-25: dn/dc from RI Properties

Field	Description
dn/dc	Shows the calculated dn/dc. This field is display only.
Linearity	Shows the linearity of the dn/dc result. This field is display only.
Fit Degree	Type a fit degree to use for fitting the data. The value may be either 1 or 2.
Percentage to Keep	Type the percentage of the peaks to use for calculations. By default, 100% is used. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Enabled Peaks > 1 to n	You can omit peaks you marked from the plot by removing the checkmark next to the peak number.

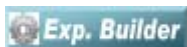
For more about determining dn/dc, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > dn/dc and UV extinction determination.

Dn/dc from Peak

This procedure calculates the dn/dc using the peak data from a batch mode run. You can use data from a batch mode RI run to calculate either the calibration constant (if dn/dc is known) or dn/dc (if the calibration constant is known). See the manual for your RI instrument for details.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine dn/dc or RI calibration in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

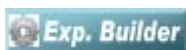
This procedure uses the injected mass value specified for the peak. The procedure adjusts for viscometer dilution factor effects if the concentration detector is downstream from a viscometer.

RI Calibration from Peak

This procedure calculates the refractive index calibration using the peak data from a batch mode run. You can use data from a batch mode RI run to calculate either the calibration constant (if dn/dc is known) or dn/dc (if the calibration constant is known). See the manual for your RI instrument for details.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine dn/dc or RI calibration in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.



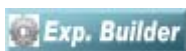
This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

UV Extinction from Peak

This procedure calculates the ultra-violet extinction using peak data.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the UV extinction coefficient. If you place multiple methods that determine the UV extinction coefficient in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

This procedure uses the injected mass value specified for the peak. The procedure adjusts for viscometer dilution factor effects if the concentration detector is downstream from a viscometer.

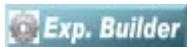
UV Extinction from RI

This procedure calculates the ultra-violet extinction using the RI data.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines the UV extinction coefficient. If you place multiple methods that determine the UV extinction coefficient in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.

For more about determining UV extinction, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > dn/dc and UV extinction determination.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

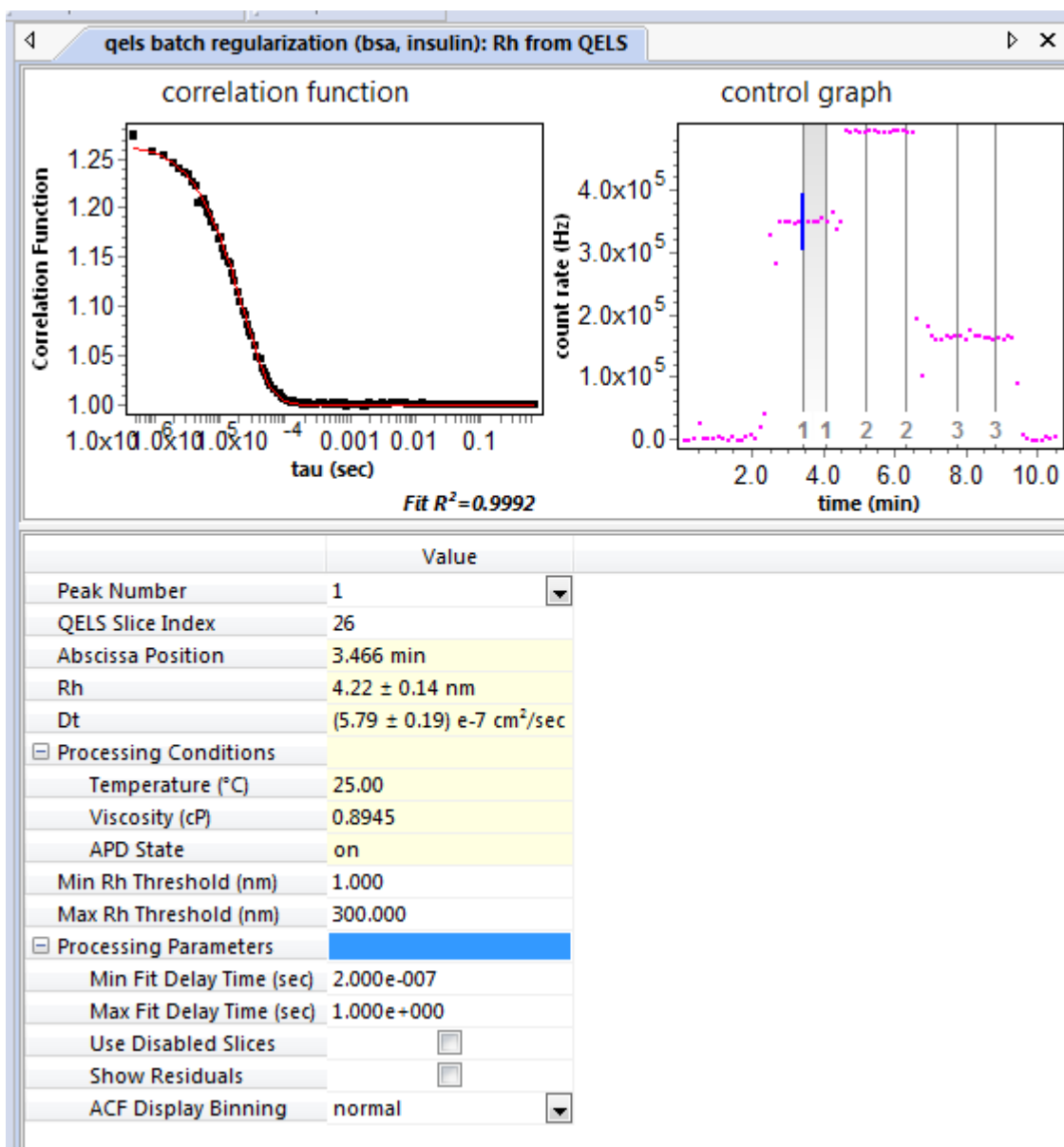
Rh from QELS

This procedure calculates the translational diffusion (Dt) and hydrodynamic radius (Rh) using QELS data. You can use this procedure if your experiment configuration uses the Wyatt QELS option in online mode. If you use QELS in batch mode, see “Regularization” on page 8-95.

For more about analyzing online QELS data, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Online QELS.

For an example experiment that determines Rh from QELS data, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the “band broadening example (BSA).afe6” experiment in the Sample Data > Analyzed Experiments folder. For an experiment method choose **File→New→Experiment From Method** to open a method in the System > Methods > Light Scattering > With QELS folder.

The procedure has the following page:



The graph is used to show the fit to the QELS correlation function. Use the graph on the right to select a peak and slice. The left graph shows the correlation function and fit results for the peak and slice you select. The quality of the fit can be determined from the left graph.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines Rh. If you place multiple methods that determine Rh in a procedure, only the first one will be valid.

Data points excluded from the calculations by the max and min properties are shown in red. QELS data collection and analysis tolerates gaps that may occur in QELS data collection due to instrument problems.

With QELS data, Rh can be determined for smaller particles (~10 nm) than with light scattering data. For example, if your experiment includes both the Number from LS and the Rh from QELS procedures, peaks for very small particles may have results for Rh but not the geometric radius.

The properties for this procedure are as follows:

Table 8-26: Rh from QELS Data Properties

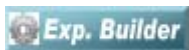
Field	Description
Peak Number	The peak number of the displayed correlation function. You can create additional peaks in this procedure.
QELS Slice Index	Displays the slice number for the correlation function. You may type a slice number here.
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Rh	Shows the calculated hydrodynamic radius (Rh) value. This field is display only.
Dt	Shows the calculated translational diffusion (Dt) value. This field is display only.
Processing Conditions	
>Temperature	Shows the temperature at which this slice was collected. This field is display only.
>Viscosity	Shows the viscosity at this slice index.
>APD State	Shows the state of the avalanche photo diode (APD) in the WyattQELS detector. If the QELS count rate exceeded the APD protection threshold, it is automatically turned off. This field displays the state of the APD for the correlation function. If the APD was off, the correlation function is probably not valid, and is by default excluded from the analysis. This field is display only.
Min Rh Threshold	Fitted Rh values with a lower radius than the value you type are not used in the analysis.
Max Rh Threshold	Fitted Rh values with a higher radius than the value you type are not used in the analysis.
Processing Parameters	
>Min Fit Delay Time	Data with a lower delay time than the value you type is not used in the fit to the correlation function.
>Max Fit Delay Time	Data with a higher delay time than the value you type is not used in the fit to the correlation function.
>Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photo diode (APD) is triggered or the delay time or Rh falls outside the specified ranges. So, any measurement where the APD detector protector was triggered is excluded from analysis unless you check the "Use Disabled Slices" check box. See "Rh from QELS" on page 8-82 for more about the avalanche photo diode (APD).
>Show Residuals	Check this box if you want to show residuals from the fit to the QELS correlation function.
>ACF Display Binning	Adjust the number of auto-correlation function bins to display. By default, data is shown as displayed by the correlator board. If you choose "heavy" a larger number of points is shown, which smooths the signal.

Rh from VS Data

This procedure calculates the hydrodynamic radius (Rh) using the viscosity data. You can use this procedure if your experiment configuration includes a viscometer.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure list can contain only one procedure that determines Rh. If you place multiple methods that determine Rh in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

For more about viscometry data collection and analysis, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Viscometry.

Branching

This procedure calculates the branching ratio between a linear (non-branched) and a branched polymer sample. This analysis is based on the fact that at a constant molar mass the molecular size decreases with increasing degree of branching.

Branching can be characterized by either the radius-based branching ratio (g), which is defined as the ratio of the mean square radius of a branched and linear molecule, or the intrinsic viscosity-based branching ratio (g'), which is defined as the ratio of the intrinsic viscosities of a branched and linear molecule. In both cases, the linear and branched molecules must have the same molar mass. The two types of branching ratios are related by a term called the “drainage parameter” (e).

Linear Models

Branching analysis requires the comparison of a branched sample to a linear reference of the same molar mass. The linear reference data can be obtained by performing a collection for a sample with the requisite molar mass, and then importing the data into the branching procedure.

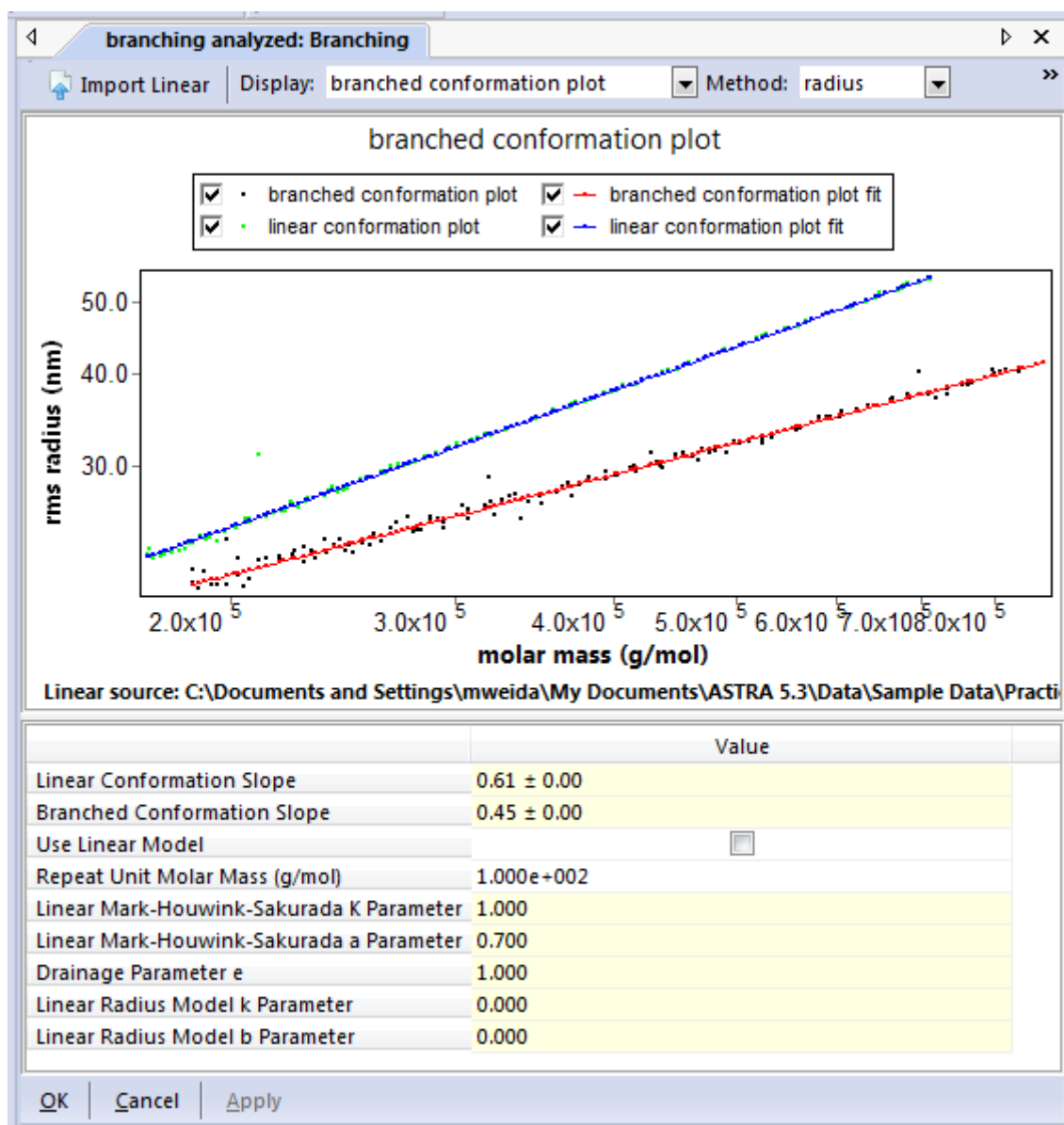
However, in many cases, it is sufficient to use an ideal linear model for comparison.

A linear model is used when the “Use Linear Model” check box is enabled. Depending on the type of analysis and instruments in the experiment, you can use a model based on radius data or intrinsic viscosity. The models may be based on measurements made in earlier experiments where a confirmation or Mark-Houwink-Sakurada plot were created, or from published values.

- **Radius Model:** The radius model is specified using a slope (k) and offset (b) for a conformation plot of RMS radius as a function of molar mass.
- **Viscosity Model:** The viscosity model is specified using the Mark-Houwink-Sakurada K and a parameters.

For an example experiment that determines branching, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the "branching analyzed.afe6" experiment in the Sample Data > Practice Experiments > Branching folder.

The procedure has the following page:



To import data from an experiment run using a linear reference sample of the same molar mass, follow these steps:

1. Run an experiment with a linear sample with the same range of molar masses as the branched sample.
2. Keep both experiments (for the linear and branched samples) open in ASTRA.
3. In the branched sample experiment, open the Branching procedure.
4. Click the **Import Linear** button above the graph and select the experiment containing data for the linear sample. Click **Import**.

The bar above the graph lets you make the following selections regarding what is graphed:

Field	Description
Display	Choose the type of graph to display. The choices are branched conformation, Mark-Houwink-Sakurada, branched volume-molar mass, branching ratio, branch units per molecule, and long chain branching.
Method	Select radius, molar mass, or viscosity as the method. If you select mass, you must also specify the Linear Mark-Houwink-Sakurada "a" and Drainage parameters. If the current experiment does not have a light scattering detector, the "radius" option is not available. Likewise, if a viscometer is not present, the "viscosity" option is not available.
Model	Select either trifunctional, tetrafunctional, star, or comb branching.
Slice	Select monodisperse or polydisperse. Note that the star and comb branching models require a monodisperse slice type.

The properties for this procedure are as follows:

Table 8-27: Branching Properties

Field	Description
Linear Conformation Slope	The slope of the linear conformation plot fit line. This is calculated from the graph, and is not settable.
Branched Conformation Slope	The slope of the branched conformation plot fit line. This is calculated from the graph, and is not settable.
Use Linear Model	Select this check box to use a linear model (rather than imported data) to perform the analysis.
Repeat Unit Molar Mass	Specify the repeat unit molar mass for long chain branching in g/mol.
Linear Mark-Houwink-Sakurada K Parameter	Specify this parameter if you chose the viscosity method and checked the "Use Linear Model" box.
Linear Mark-Houwink-Sakurada a Parameter	Specify this parameter if you chose the mass or viscosity method and checked the "Use Linear Model" box.
Drainage Parameter e	Specify this parameter if you chose the mass method.
Linear Radius Model k Parameter	Specify this parameter if you chose the radius or mass method and checked the "Use Linear Model" box.
Linear Radius Model b Parameter	Specify this parameter if you chose the radius or mass method and checked the "Use Linear Model" box.

For more about branching analysis, see "Branching Calculations" on page D-23 and go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Branching calculations.

Parametric Plot

The parametric plot procedure generates a new data set for two different types of x-y data that share the same x-axis. For example, you can use this procedure to create a plot of RMS radius vs. molar mass.

The properties for this procedure are as follows:

Table 8-28: Parametric Plot Properties

Field	Description
X Data	Choose the data that will be used for the x-axis of the parametric plot.
Y Data	Choose the first data set that will be used for the y-axis of the parametric plot.
Y Data2	Optionally choose a second data set to be used for the y-axis of the parametric plot.

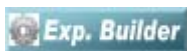
See “Creating Data Set Definitions” on page 11-9 for information about the dialog that appears when you click the “...” button for either the X Data or the Y Data property.

Peak Areas

This procedure calculates the area under the defined peaks. The values calculated by this procedure are peak area, % peak area, and retention time.

You can place this procedure after the Peaks procedure.

There are no properties to set for this procedure. It runs without prompting for any values.



This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

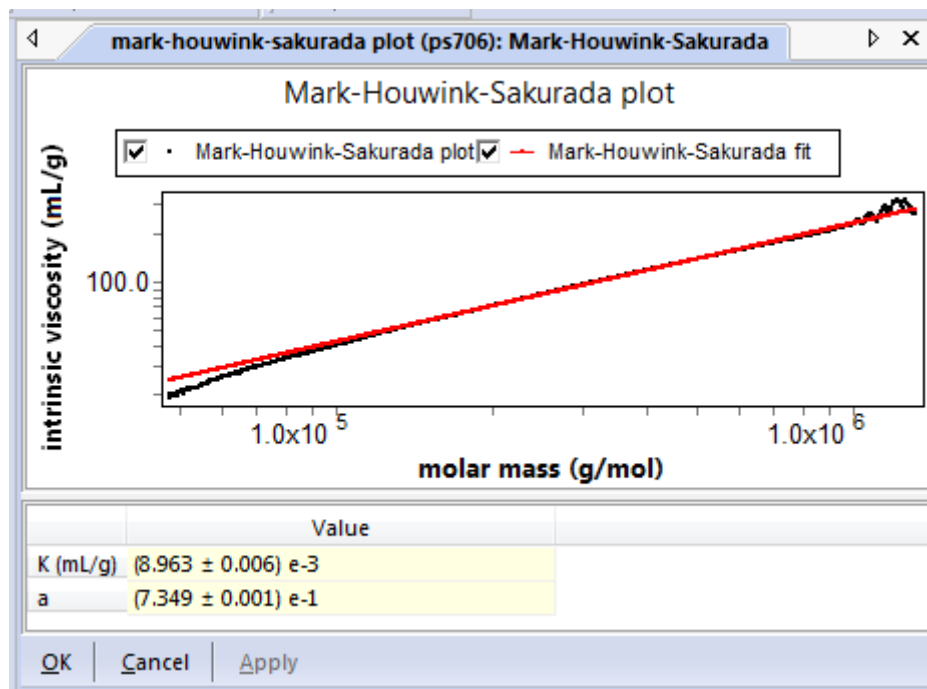
Mark-Houwink-Sakurada

This procedure calculates the Mark-Houwink-Sakurada K and a fit parameters using viscosity data.

For an example experiment that uses the Mark-Houwink-Sakurada procedure, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database) and open the “Mark-Houwink-Sakurada plot (PS706).afe6” experiment in the Sample Data > Analyzed Experiments folder. For an experiment method choose **File→New→Experiment From Method** to open the “online” method in the System > Methods > Viscometry > With Light Scattering folder.

You can place this procedure at the end of the list of procedures. This procedure can be used in the same experiment as “Distribution Analysis” and other analysis procedures.

The procedure has the following page:



The diagram shows the fit for the Mark-Houwink-Sakurada plot. The procedure selects K and a parameters to make the red line as close to straight as possible. The values of the K and a parameters vary depending on the polymer, solvent, and temperature.

The properties for this procedure are as follows:

Table 8-29: Mark-Houwink-Sakurada Properties

Field	Description
K	The resulting "K" fit parameter for the Mark-Houwink-Sakurada analysis.
a	The resulting "a" fit parameter for the Mark-Houwink-Sakurada analysis.

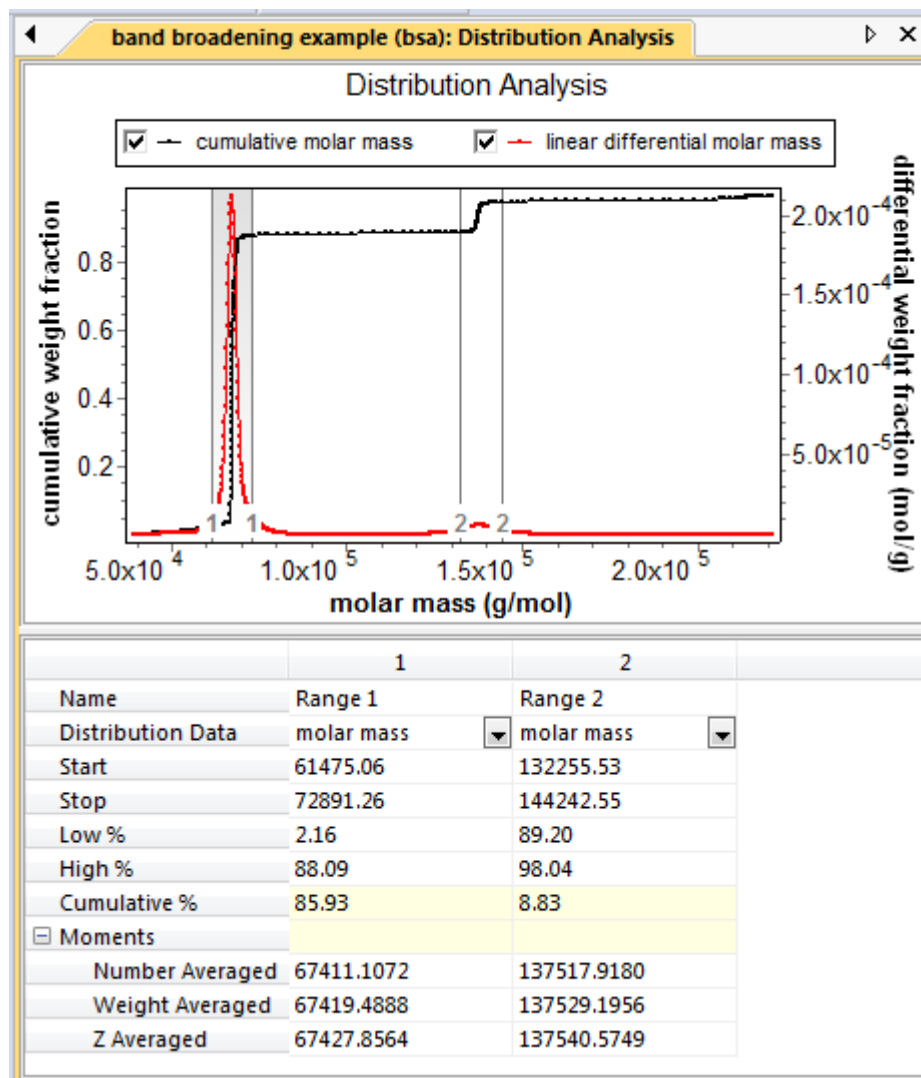
For more about viscometry data collection and analysis, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Viscometry.

Distribution Analysis

This procedure analyzes the distribution of the geometric radius or other selected data item within the sample for an online experiment.

You can place this procedure at the end of the list of procedures. Use your mouse to create ranges in the graph, for example where peaks occur. Data is shown in the table for each range you create.

The procedure has the following page:



The graph shows the linear and cumulative distribution of the geometric radius or other selected data.

For an example experiment that performs a distribution analysis, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the “band broadening example” experiment in the Sample Data > Analyzed Experiments folder.

The properties for this procedure are as follows:

Table 8-30: Distribution Analysis Properties

Field	Description
Name	Name of the range for this column. The default names are Range 1, Range 2, etc. You can change these as desired.
Distribution Data	The type of data to plot on the x-axis for distribution analysis. Options vary depending on the data in the experiment. Examples are molar mass, RMS radius, hydrodynamic radius, and translational diffusion.
Start	The starting point on the x-axis of the range.
Stop	The ending point on the x-axis of the range.
Low %	This field shows the cumulative number fraction at the start of the range you created with your mouse.
High %	This field shows the cumulative number fraction at the end of the range you created with your mouse. You can modify this value to change the location of the range end.
Cumulative %	This property shows the difference between the High % and Low %, which is the percent of the sample that falls within this range.
Moments	
>Number Averaged	This field reports the number-averaged value of the moment for the selected range.
>Weight Averaged	This field reports the weight-averaged value of the moment for the selected range.
>Z Averaged	This field reports the Z-averaged value of the moment for the selected range.

For more about distribution analysis, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Distributions.

Copolymer Analysis

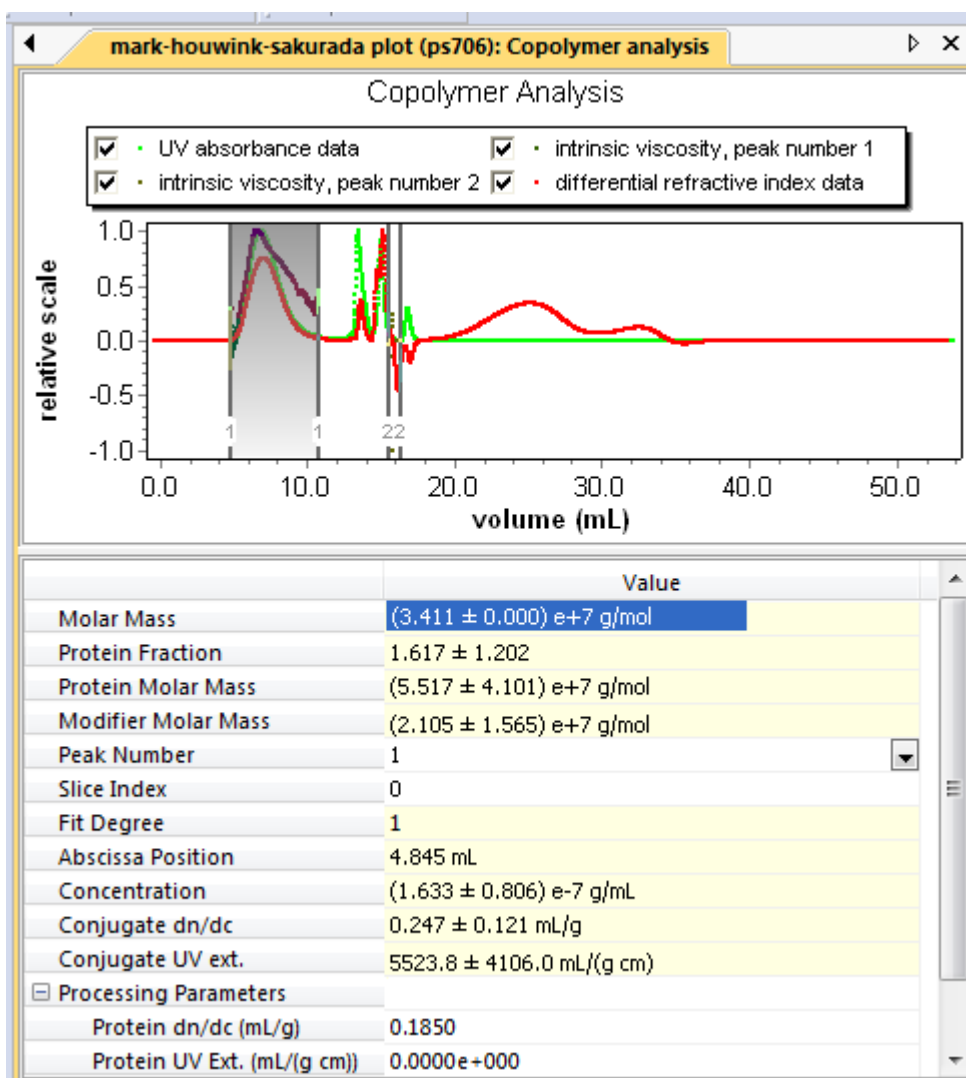
Like the “Protein Conjugate Analysis” on page 8-93, this analysis technique allows you to differentiate between two polymers with the same molecular size. This analysis procedure requires use of a viscometer in conjunction with a UV and RI detector.

The Copolymer Analysis page shows the total molar mass and protein fraction on a slice by slice basis. ASTRA calculates the size of the complex, molar mass of the complex, and molar masses of the constituents, and the uncertainties for these values.

This procedure should be placed in the list with the analysis procedures, but before the Results Fitting procedure if it occurs.

For an experiment method that performs a Copolymer Analysis, choose **File→New→Experiment from Method**, and open the “Copolymer Analysis” experiment in the System > Methods > Viscometry folder.

This procedure has the following page:



The properties for this procedure are as follows:

Table 8-31: Copolymer Analysis Properties

Field	Description
Molar Mass	Shows the total molar mass for the currently selected peak.
Protein Fraction	Shows the protein fraction for the currently selected peak.
Protein Molar Mass	Shows the molar mass of the protein indicated by the selected peak.
Modifier Molar Mass	Shows the molar mass of the protein modifier indicated by the selected peak.
Peak Number	Select the peak for which you want to view values. You can create additional peaks in this procedure.
Slice Index	Shows the current slice index, which is indicated by a vertical blue line in the graph.
Fit Degree	Shows the fit degree selected for this peak.
Abscissa Position	The value on the x-axis for the selected peak and slice.
Concentration	The concentration at the selected slice.
Conjugate dn/dc	The dn/dc at the selected slice.

Table 8-31: Copolymer Analysis Properties

Field	Description
Conjugate UV ext.	The UV extinction at the selected slice.
Processing Parameters	
>Protein dn/dc	Specify the dn/dc of the main protein in mL/g if it is known.
>Protein UV Ext.	Specify the UV extinction of the main protein in mL/(mg cm) if it is known.
>Modifier dn/dc	Specify the dn/dc of the modifier protein in mL/g if it is known.
>Modifier UV Ext.	Specify the UV extinction of the modifier protein in mL/(mg cm) if it is known.

Protein Conjugate Analysis

This procedure analyzes protein conjugates, which are an important class of copolymers. This analysis requires the use of a light scattering detector in conjunction with both a UV and RI detector.

The Protein Conjugate Analysis page allows you to see the total molar mass and protein fraction on a slice by slice basis. ASTRA calculates the size of the complex, molar mass of the complex, and molar masses of the constituents, and the uncertainties for these values.

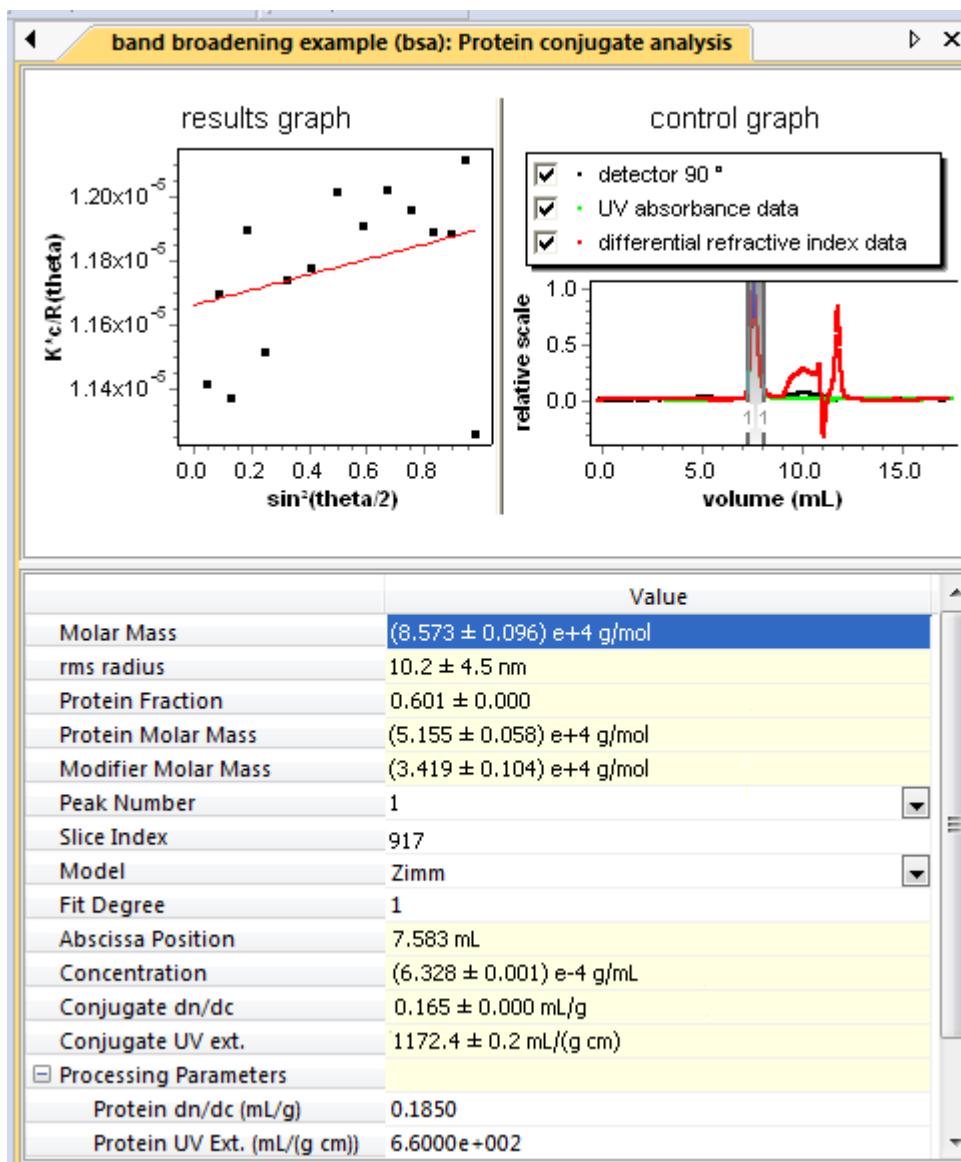
For an example experiment that performs a protein conjugate analysis, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the “protein conjugate” experiment in the Sample Data > Analyzed Experiments folder.

For an experiment method choose **File→New→Experiment From Method** to open the “protein conjugate” method in the System > Methods > Light Scattering folder or the “protein conjugate” method in the System > Methods > Light Scattering > With QELS folder.

For more about protein conjugate analysis, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Protein conjugates and copolymers.

This procedure should be placed in the list with the analysis procedures, but before the Result Fitting procedure if it occurs.

The procedure has the following page:



The left graph shows a Zimm plot of the results. The right graph shows collection data and peaks.

The properties for this procedure are as follows:

Table 8-32: Protein Conjugate Analysis Properties

Field	Description
Molar Mass	Shows the total molar mass for the currently selected peak.
Radius	Shows the calculated radius for the currently selected peak. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Protein Fraction	Shows the protein fraction for the currently selected peak.
Protein Molar Mass	The molar mass of the primary protein indicated by the selected peak.
Modifier Molar Mass	The molar mass of the protein modifier indicated by the selected peak.

Table 8-32: Protein Conjugate Analysis Properties

Field	Description
Peak Number	Select the peak for which you want to view values. You can create additional peaks in this procedure.
Slice Index	Shows the current slice index, which is indicated by a vertical blue line in the graph.
Model	The model selected for this peak.
Fit Degree	The fit degree selected for this peak.
Abscissa Position	The value on the x-axis for the selected peak and slice.
Concentration	The concentration at the selected slice.
Conjugate dn/dc	The dn/dc at the selected slice.
Conjugate UV ext.	The UV extinction at the selected slice.
Processing Parameters	
>Protein dn/dc	Specify the dn/dc of the main protein in mL/g if it is known.
>Protein UV Ext.	Specify the UV extinction of the main protein in mL/(mg cm) if it is known.
>Modifier dn/dc	Specify the dn/dc of the modifier protein in mL/g if it is known.
>Modifier UV Ext.	Specify the UV extinction of the modifier protein in mL/(mg cm) if it is known.
Enabled Detectors	The detectors to enable for this analysis.

Regularization

This procedure regularizes the results of a QELS batch experiment using the DYNALS regularization algorithm from ALANGO. See “Regularization” on page F-7.

The Regularization procedure supports the reporting the results that were previously available in the separate QELSBatch program. This procedure now reports the mean, peak, and standard deviation of the reported values (hydrodynamic radius and translational diffusion).

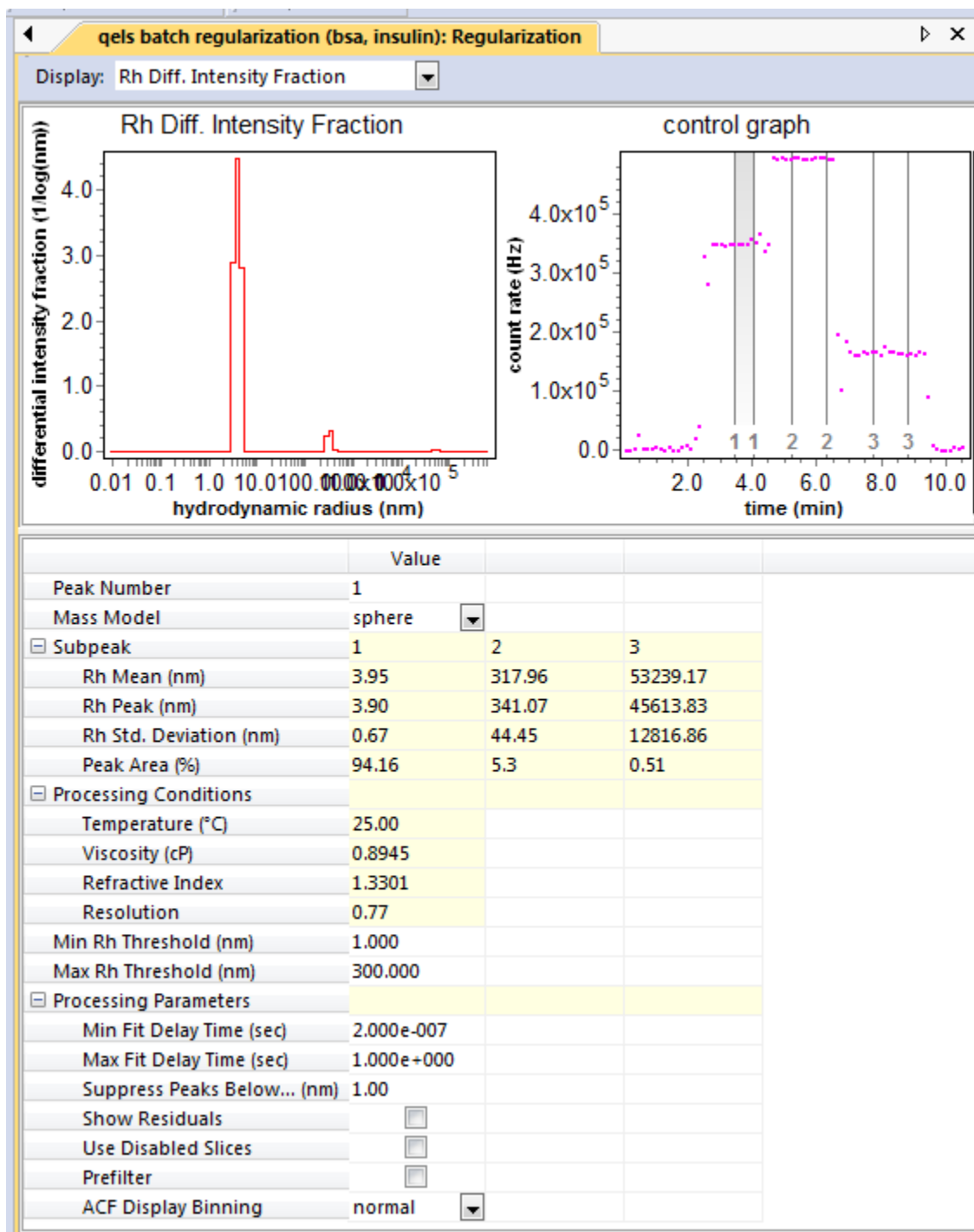
The regularization procedure provides a way to analyze a batch sample (a vial of some mixture of substances). Rather than setting up a chromatography system to separate the components using a column or membrane system, you can use the QELS data to identify the various Rh values of the mixture in the vial.

For an example experiment that performs regularization, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the “QELS batch regularization” experiment in the Sample Data > Analyzed Experiments folder. For an experiment method choose **File→New→Experiment From Method** to open the “regularization” method in the System > Methods > Light Scattering > With QELS folder.

For more about analyzing batch QELS data, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > QELS batch.

You can place this procedure at the end of a QELS batch experiment procedure.

This procedure has the following page:



The left graph shows various plots depending on the Display setting. The right graph shows the defined peaks for the batch experiment.

Above the graphs, choose the type of plot to display in the left graph. The options are Correlation Function, Rh Diff. Intensity Fraction, Rh Diff. Weight Fraction, Dt Diff. Intensity Fraction, Rh Cumulative Intensity, Rh Cumulative Weight Fraction, and Dt Cumulative Intensity Fraction.

The properties for this procedure are as follows:

Table 8-33: Regularization Properties

Field	Description
Peak Number	Click on a peak in the graph or type a number here to select a peak to regularize. You can create additional peaks in this procedure.
Mass Model	Select the mass model you want to use in the computation. Options are "sphere" and "random coil".
Subpeak	Summary of individual components identified by ASTRA in each analytical peak. You cannot modify these values.
>Rh Mean	The mean hydrodynamic radius of the subpeak.
>Rh Peak	The hydrodynamic radius at the peak of the subpeak.
>Rh Std. Deviation	The standard deviation of the peak hydrodynamic radius from the mean.
>Peak Area	The area under the subpeak as a percentage of the entire peak.
Processing Conditions	Conditions (per peak) during the regularization analysis. You cannot modify these values.
>Temperature	Shows the temperature at which the data was collected.
>Viscosity	Shows the viscosity of the solvent. This value comes from the solvent profile (page 7-34).
>Refractive Index	Refractive index of the sample for the given peak.
>Resolution	A value that represents the optimal smoothing of the distribution given the noise level of the correlation function. It varies from 0 (for very noisy data) to 1 (for data with very good signal-to-noise). In general, the lower the resolution value, the more uncertain the actual widths and structure of the final distribution.
Min Rh Threshold	Fitted Rh values with a lower radius than the value you type are not used in the analysis. The Min and Max Rh Thresholds are applied after all other analysis, to allow you to discard results that fall outside a desired range. For example, you could discard results greater than 300 nm). If the "Prefilter" box is not checked, the Min/Max Rh Thresholds are ignored.
Max Rh Threshold	Fitted Rh values with a higher radius than the value you type are not used in the analysis.
Processing Parameters	Various values that govern the processing of the data. Users do not frequently need to change these values.
>Min Fit Delay Time	Type the minimum number of seconds for the fit delay. The default is 0 seconds.
>Max Fit Delay Time	Type the maximum number of seconds for the fit delay. The default is 1 second.
>Suppress Peaks Below	Type a size in nanometers below which peaks should be omitted from the regularization.
>Show Residuals	Check this box if you want the correlation function graph to show residuals. The default is to omit residuals.
>Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photo diode (APD) is triggered or the delay time or Rh falls outside the specified ranges. So, any measurement where the APD detector protector was triggered is excluded from analysis unless you check the "Use Disabled Slices" check box. See "Rh from QELS" on page 8-82 for more about the avalanche photo diode (APD).

Table 8-33: Regularization Properties

Field	Description
>Prefilter	If the "Prefilter" box is checked, data points in the correlation function view that fall outside the minimum or maximum delay times (the "Min Fit Delay Time" and "Max Fit Delay Time" fields) are discarded from the fit. You can see the fit line stop short of these points, and the points themselves change color to red. The rest of the points are still included in the analysis. If this box is unchecked, data points outside the range are used in the fit.
>ACF Display Binning	Adjust the number of auto-correlation function bins to display. By default, data is shown as displayed by the correlator board. If you choose "heavy" a larger number of points is shown, which smooths the signal.

For an example of the results you can view with the regularization procedure, open the "QELS batch regularization (BSA, insulin)" sample file, and open its Regularization procedure. This procedure allows you to see the characteristics of three mixtures (the three peaks in the right graph).

- **Correlation function plot.** This graph gives an idea of how well the data fits the average correlation function over the peak. Peaks 1 and 2 match pretty well, while peak 3 shows some variation.
- **Rh diff intensity fraction plot.** This graph gives an idea of the number of each type of species by showing the intensity fraction as a function of hydrodynamic radius. For peak 1, there seems to be a lot of particles with a radius of 4 nm and a much smaller number with a 300 nm size. Peak 2 shows (again) a high number of ~4 nm particles and a smaller number of ~50 nm particles. The larger particles are on the right side of the graph.
- **Rh diff weight fraction plot.** This graph shows the distribution of Rh by weight fraction. In peaks 1 and 2 the larger particles don't even register on this view.
- **Dt diff intensity fraction plot.** This graph shows the intensity fraction as a function of translational diffusion. That is, the particles that move the most are toward the right-side of the graph, while the slower particles are toward the left side.
- **Rh cumulative intensity plot.** This graph gives an idea of the overall intensity produced by constituents below a specific size. For peak 1, most of the intensity seems to be delivered by particles in the range of 4 nm through 300 nm.
- **Rh cumulative weight fraction plot.** This graph shows the contribution to weight fraction for different sizes.
- **Dt cumulative intensity fraction plot.** This graph shows the cumulative intensity as a function of translational diffusion. Again, this graph shows that the bulk of the intensity is contributed by fast-moving (smaller) particles.

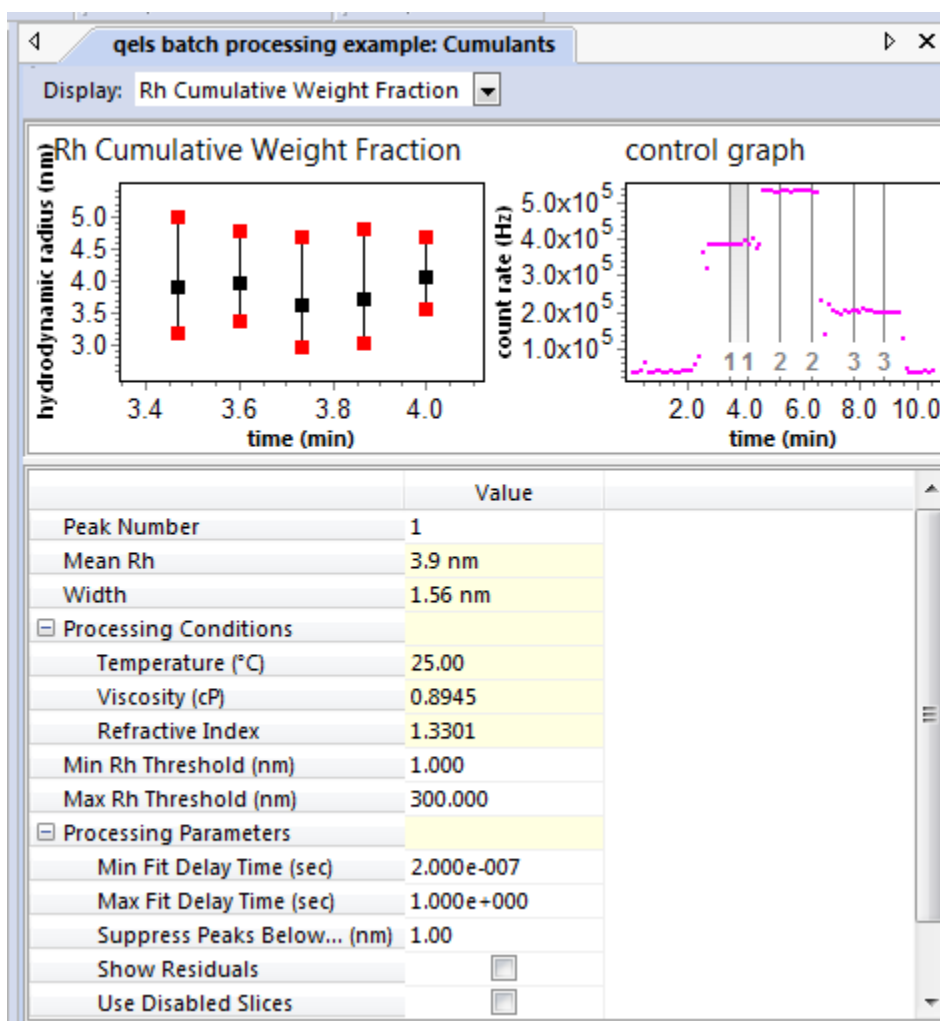
Cumulants

Cumulants analysis is a strategy for extracting information about the underlying size distribution of a polydisperse sample from the correlation function. The cumulants method involves fitting the correlation function not to a single decay time, but to a Gaussian distribution of decay times. This method retrieves the mean and variance for the distribution. “Cumulants” on page F-2 provides details on cumulants analysis.

Cumulants analysis is used with QELS data. Thus, an experiment configuration must include a light-scattering instrument and Wyatt QELS.

You can place this procedure with other analysis procedures and after all transformation procedures. A procedure list cannot contain both the Cumulants procedure and the procedure for “Regularization” on page 8-95.

This procedure has the following page:



The left graph shows various plots depending on the Display setting. The right graph shows the defined peaks for the batch experiment.

Above the graphs, choose the type of plot to display in the left graph. The options are Correlation Function and Rh Cumulative Weight Fraction.

The properties for this procedure are as follows:

Table 8-34: Cumulants Properties

Field	Description
Peak Number	Click on a peak in the graph or type a number here to select the peak for the cumulants calculation. You can create additional peaks in this procedure.
Mean Rh	Shows the mean hydrodynamic radius (Rh) for the peak. You can override the calculated value by typing a known value.
Width	Shows the width in nm.
Processing Conditions	Conditions (per peak) during the regularization analysis. You cannot modify these values.
>Temperature	Shows the temperature at which the data was collected.
>Viscosity	Shows the viscosity of the solvent. This value comes from the solvent profile (page 7-34).
>Refractive Index	Refractive index of the sample for the given peak.
Min Rh Threshold	Fitted Rh values with a lower radius than the value you type are not used in the analysis. The default is 1 nm. The Min and Max Rh Thresholds are applied after all other analysis, to allow you to discard results that fall outside a desired range. For example, you could discard results greater than 300 nm). If the "Prefilter" box is not checked, the Min/Max Rh Thresholds are ignored.
Max Rh Threshold	Fitted Rh values with a higher radius than the value you type are not used in the analysis. The default is 300 nm.
Processing Parameters	
>Min Fit Delay Time	Data with a lower delay time than the value you type is not used in the fit to the correlation function. The default is 0.
>Max Fit Delay Time	Data with a higher delay time than the value you type is not used in the fit to the correlation function. The default is 1.
>Suppress Peaks Below	Type a size in nanometers below which peaks should be omitted from the analysis.
>Show Residuals	Check this box if you want the correlation function graph to show residuals. The default is to omit residuals.
>Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photo diode (APD) is triggered or the delay time or Rh falls outside the specified ranges. So, any measurement where the APD detector protector was triggered is excluded from analysis unless you check the "Use Disabled Slices" check box. See "Rh from QELS" on page 8-82 for more about the avalanche photo diode (APD).
>Prefilter	If the "Prefilter" box is checked, data points in the correlation function view that fall outside the minimum or maximum delay times (the "Min Fit Delay Time" and "Max Fit Delay Time" fields) are discarded from the fit. You can see the fit line stop short of these points, and the points themselves change color to red. The rest of the points are still included in the analysis. If this box is unchecked, data points outside the range are used in the fit.
>ACF Display Binning	Adjust the number of auto-correlation function bins to display. By default, data is shown as displayed by the correlator board. If you choose "heavy" a larger number of points is shown, which smooths the signal.

In the cumulants plot, the average hydrodynamic radius and the distribution values at one standard deviation are presented. This creates an “error bar” appearance for the graph, but here the error bars indicate the width of the fitted distribution. They are asymmetric because the hydrodynamic radius is inversely proportional to the symmetric decay time distribution.

Molar Mass from Column Calibration

This procedure calculates the molar mass of a sample based on the elution volume of a peak through a column. You must have determined the response of the column to a series of known standards prior to using this procedure. See “Calibrate Column” on page 8-39 for details.

Two main types of column calibration are available. “Conventional calibration” and “universal calibration.” For conventional calibration, the polymer type of the analyzed polymer must be the same as the polymer type of the polymer used for calibration. For universal calibration, the polymers may be different.

For example experiments that perform this procedure, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Research Database), and open the “universal calibration” or “conventional calibration” experiment in the Sample Data > Analyzed Experiments folder. For an experiment method choose **File→New→Experiment From Method**, and open the “universal calibration” or “determine column calibration” method in the System > Methods > Viscometry folder.

For more, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Data Analysis > Conventional and Universal Column Calibration.

You can place this procedure with other analysis procedures, and after all transformation procedures. A procedure list can contain only one procedure that determines the molar mass from column calibration. If you place multiple methods that determine molar mass in an experiment, only the first one will be valid. Your experiment may or may not contain the procedure “Calibrate Column” on page 8-39. If your experiment does not contain the Calibrate Column procedure, you should import the results of a column calibration using “Column Profiles” on page 7-36.

This procedure has the following page:

	Value
Mark-Houwink-Sakurada K (mL/g)	1.832e-002
Mark-Houwink-Sakurada a	6.903e-001
Flow Marker (mL)	from positive peak ▼ 13.97
Flow Marker Peak	Peak 1 ▼

OK Cancel Apply

The properties for this procedure are as follows:

Table 8-35: Molar Mass from Column Calibration Properties

Field	Description
Mark-Houwink-Sakurada K	Type the known Mark-Houwink-Sakurada K parameter of the polymer used for calibration. This is only used if the Universal without Viscometer Data calibration technique was used.
Mark-Houwink-Sakurada a	Type the known Mark-Houwink-Sakurada a parameter of the polymer used for calibration. This is only used if the Universal without Viscometer Data calibration technique is selected.
Flow Marker	Use the drop-down menu to select whether or not a flow marker is set and how. Drop-down options are as follows: <ul style="list-style-type: none"> - None - no flow marker is to be used. The flow marker value is set to zero. - Enter value - enter the flow marker value in mL in the cell to the right of the drop-down. - From positive peak - the flow marker will be determined from the apex of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down. - From negative peak - the flow marker will be determined from the lowest point of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down.
Flow Marker Peak	Select the peak for the flow marker if you used one.

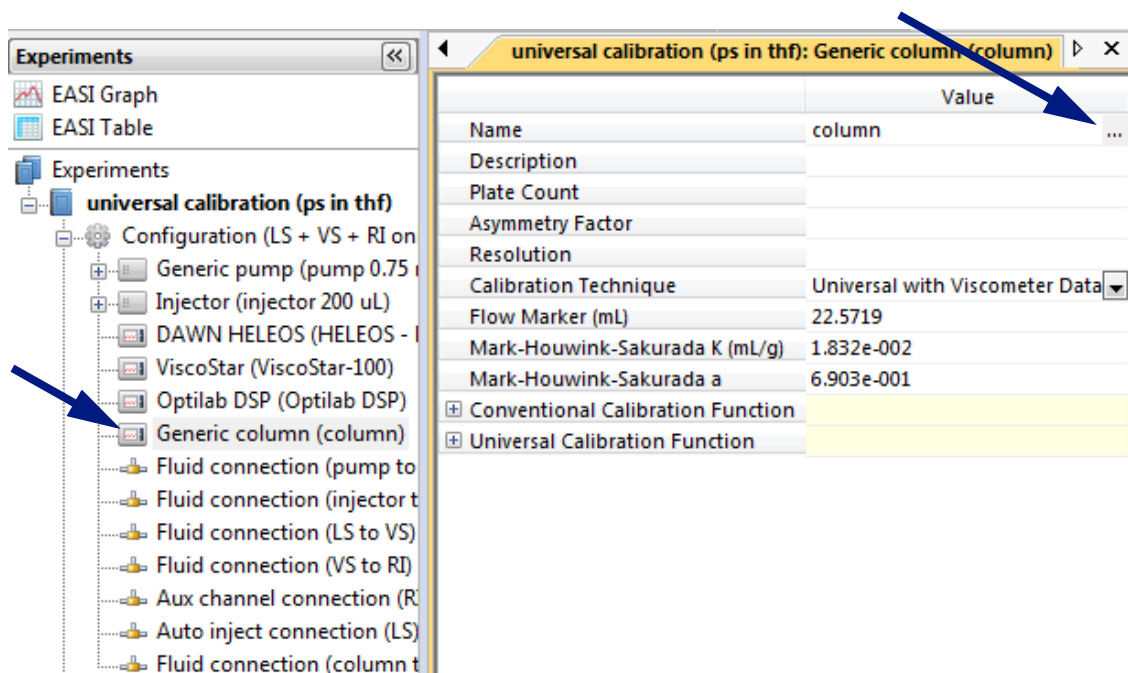
The error in the molar mass is estimated from the standard error of the calibration fit. If the fit degree is equal to the degrees of freedom, a default lower limit uncertainty of 0.001 is used in place of the standard error.

Using a Column Profile to Determine Molar Mass

To use a column calibration to measure the characteristics of an unknown sample, follow these steps:

1. Choose **File→New→Experiment From Method**, and open the “universal calibration” or “determine column calibration” method in the System > Methods > Viscometry folder.
2. Expand the configuration, and double-click on the “Generic Column” item.
3. In the Calibration Technique field, select the type of calibration you performed on this column.

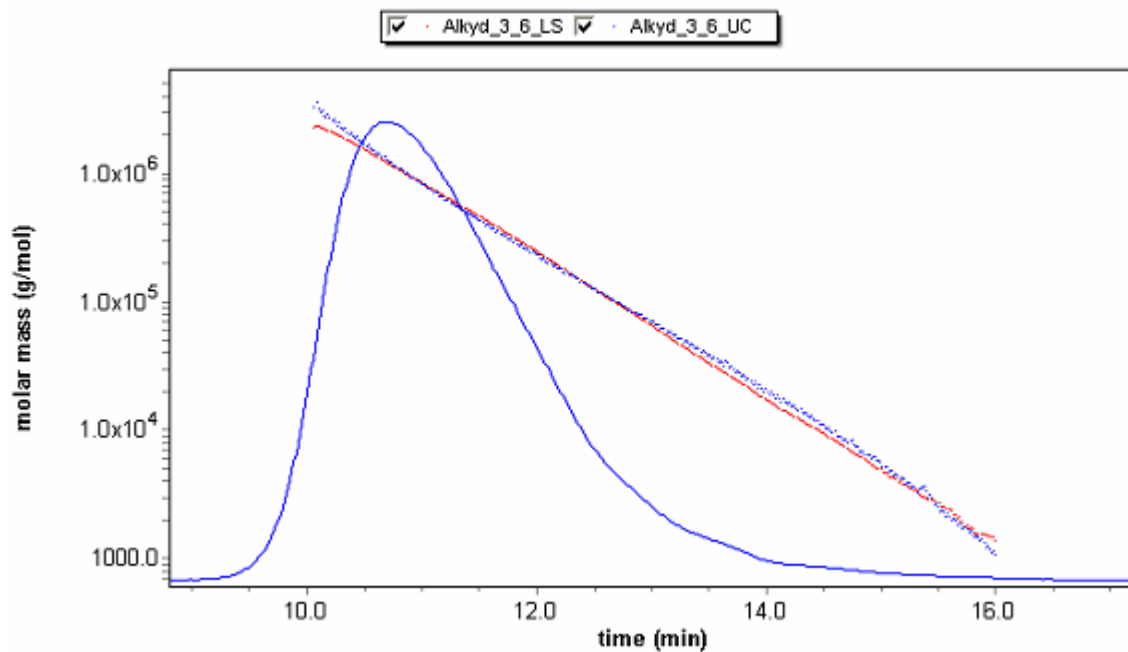
- Press the “...” button next to the Column name, and select a column calibration you performed earlier.



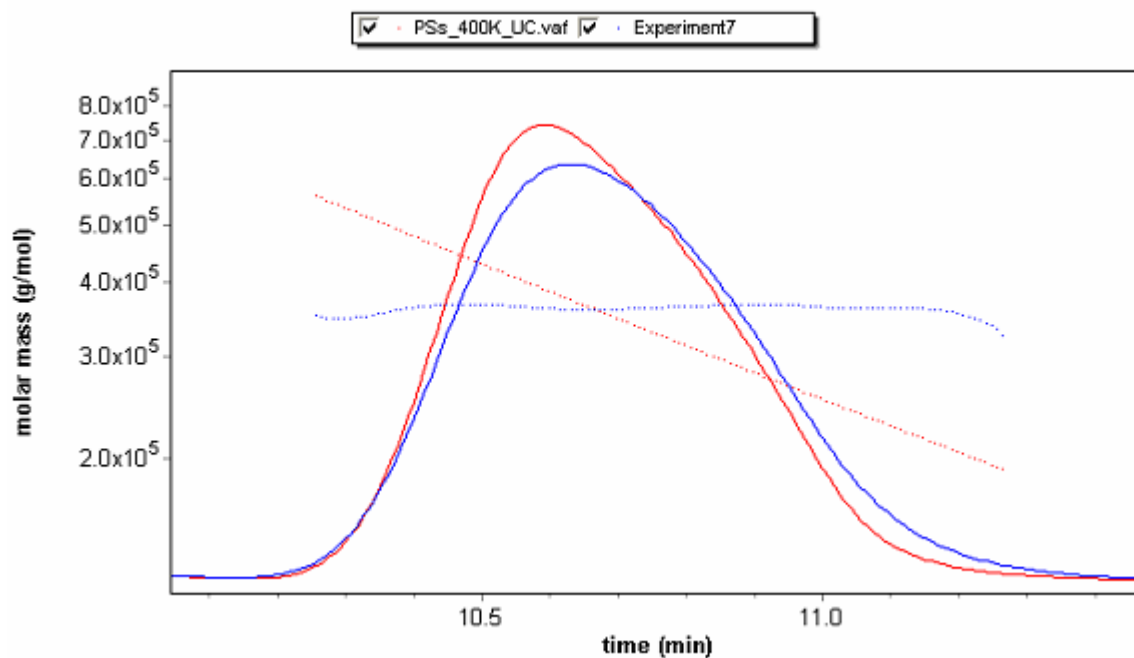
The column profile is updated with the coefficients from the known sample run.

- Select the particular instruments used at your location, configure the alignment, and make any other experiment configuration changes you normally make.
- Choose **Experiment**→**Configuration**→**Save As** to save this experiment as a method for later use.
- Now use this experiment to collect data on an unknown sample, or apply this method to already-collected data that was collected with the column and configuration used in the method. The results are calculated using the calibrated column values.
- In the “Molar mass from column calibration” procedure, choose a Flow Marker type and Flow Marker Peak (if any).

9. Finally, you can evaluate the molar mass of the unknown sample. In the following image, the mass characteristics are very close to the light scattering values:



As a counter-example, the following plot illustrates the false polydispersity displayed by universal calibration:



Administration Procedures

Administration procedures add an experiment log entry for the audit trail.

Sign Off



In order to comply with 21 CFR Part 11, your operating procedures may require that one or more electronic sign offs be associated with each experiment. A Sign off procedure may be located at any position in the procedure list specified by your policies and procedures.

The user who runs the experiment is prompted to set parameters for the Sign off procedure. The sign off page looks similar to the following:

Category	Value
Responsibility	
Comments	Run conditions changed to account for solvent change.
User Id	researcher_2
Password	*****
Domain	WYATT

OK Cancel Apply

The properties to enter are as follows:

Table 8-36: Sign Off Properties

Field	Description
Category	The type of sign off. Options are Responsibility, Approval, and Review.
Comments	Any comments about this experiment.
User Id	A valid ASTRA user ID. This need not be the same user ID used to log into this ASTRA session or this Microsoft Windows session.
Password	The password for the specified user ID. This is case-sensitive.
Domain	The domain for the specified user ID.

Electronic signatures can be executed for any data collected in ASTRA. The electronic signature is saved in the experiment log associated with the data, and shows up in the report associated with the experiment.

Multiple electronic signatures can be executed for an experiment. In the reported results, ASTRA flags any electronic signatures that were made before the last modification. Failed electronic signature attempts are recorded in the system log with an alarm status for immediate notification of system administrators.

9

Using Sequences

This chapter explains how to configure and run sequences in ASTRA 6.

CONTENTS	PAGE
About Sequences.....	9-2
Creating New Sequences	9-3
Opening a Sequence	9-5
Editing a Sequence	9-7
Running Sequences.....	9-11
Saving Sequences	9-12
Deleting a Sequence.....	9-13

About Sequences

ASTRA sequences provide an easy way to manage experiment sequences with multiple samples. A sequence can be configured with a default experiment method, such that all samples are collected in the same fashion. Or, a different experiment method can be specified for each sample, making it possible to collect different types of data for each sample. For example, you might do a QELS collection on two out of ten runs.

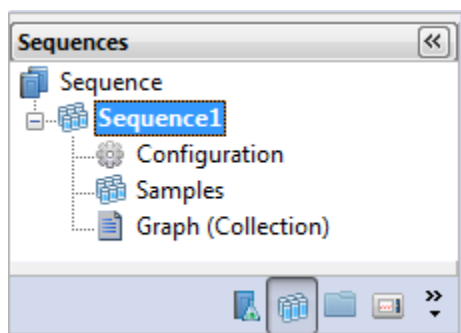
A sequence is used to collect data from a sequence of injections, usually from an autosampler. The sequence creates a new experiment from a pre-defined method for each injection, and then runs the experiment to collect and analyze the data. Therefore, a sequence can create many experiments.



Security

You must have at least Researcher access to create sequences. If you are a Technician, you can run sequences. If you are a Guest, you have read-only access to sequences.

ASTRA 6 has a Sequences navigation pane in the workspace that allows you to create, edit, and run sequences.



The procedures run for a particular sample are determined by referencing an experiment method for each sample. The samples may reference the same or different experiment methods.

Creating New Sequences

You can create sequences starting from a blank configuration or from a sequence template you have created. Blank sequences are easy to work with. If you often perform experiments with the same set of samples, using a sequence template can save time in setting up the sequence.



You must have at least Researcher access to create sequences.

Creating Default Sequences

If you have specified a default sequence template, you can choose **File→New→Sequence from Default** to quickly create a new sequence of this type.


If you have not yet set a default sequence template, you are asked if you want to specify it. If you click Yes, you see the Method Defaults tab of the Options dialog (see “Setting a Default Sequence” on page 9-4).

Creating Blank Sequences

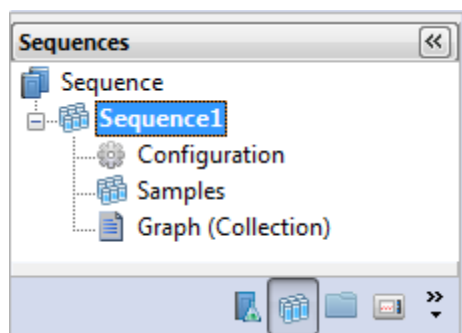
Create an empty sequence by following this step:

1. Choose **File→New→Blank Sequence**.

Shortcuts: Press Ctrl+Shift+N.

Click the down-arrow next to the  icon.

You see a sequence configuration in the Sequences navigation pane.




If you have defined a default experiment method (see page 6-18 and page 6-19), that method is the default for all samples in the new blank sequence.

Creating a Sequence from a Sequence Template

To create a sequence from a sequence template, follow these steps:

1. Choose **File→New→Sequence from Template**.

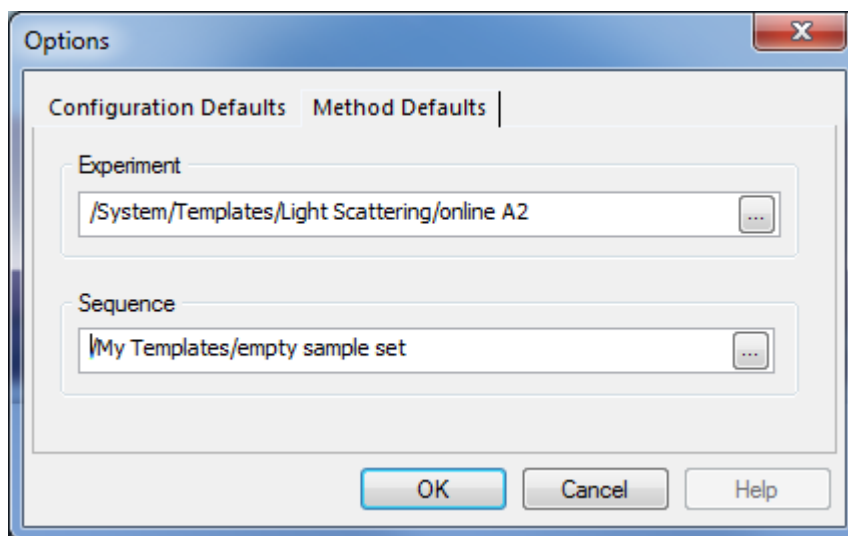
Shortcuts: Click the down-arrow next to the  icon.

2. In the New from Existing dialog, browse to and select the sequence template you want to use. Sequence templates are available only if you have saved them as described in “Saving Sequences as Templates” on page 9-13.
3. Click **Create**.

A new sequence is created based on the sequence template you selected.

Setting a Default Sequence

You can specify the default sequence by choosing **System→Preferences→Options**. Select the **Method Defaults** tab.



The **Sequence** field shows the path to the current default sequence definition, if one is selected.

Next to the **Sequence** field, click the “...” button. In the Select Template dialog, browse for the existing sequence that you want to use as the default sequence template for new experiments.

When you choose **File→New→Sequence from Default**, the default sequence will be used to create a new sequence.

See “Setting a Default Method” on page 6-19 for information about setting a default method and “Setting a Default Configuration” on page 7-10 for information about setting configuration defaults.


Opening a Sequence

You can open and work with any sequence you have saved. If you are using ASTRA 6 with Research Database or ASTRA 6 with Security Pack, experiments are stored in the experiment database, and you open experiments from that database. If you are using ASTRA 6 Basic, sequences are stored in separate files with an extension of *.afs6.

To open an experiment, follow these steps:

1. Choose **File→Open→Sequence**.

Shortcuts: Press Ctrl+Shift+O.

Click the down-arrow next to the  icon.

2. In the Open dialog, navigate to the folder that contains the sequence you want to open.
3. Select a file and click **Open**.

Importing an ASTRA Sequence



You must have at least Researcher access to import sequences.

You can import ASTRA sequences saved with ASTRA 6, ASTRA V, and ASTRA 4. ASTRA 6 and ASTRA V sequences have a file extension of *.vsf. ASTRA 4 sequences have a file extension of *.ss.



This item is disabled in ASTRA 6 Basic since it is identical to **File→Open→Sequence**.

To import an ASTRA sequence, follow these steps:

1. Choose **File→Import→Sequence**.

Shortcuts: Press Ctrl+Shift+I.

2. In the Import dialog, navigate to the folder that contains the sequence you want to import.
3. In the Files of type field, select the type of sequence file you want to import.
4. Select a file and click **Open**. The sequence is shown in your Sequences navigation pane with the filename you imported.
5. To save the sequence in your experiment database, choose **File→Save**.

Importing an Empower Sequence

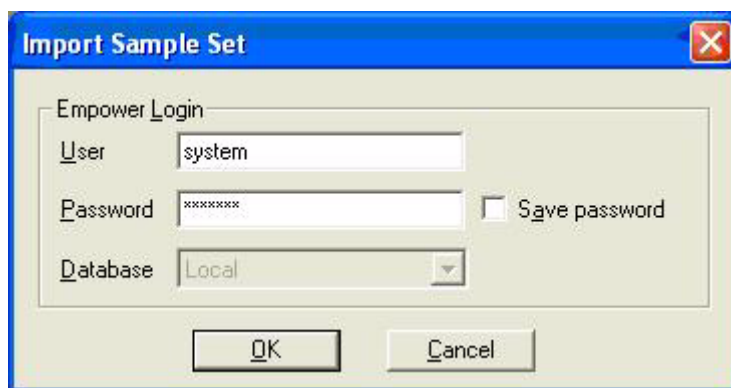


You must have at least Researcher access to import sequences.

ASTRA can connect directly to the database for the Waters Empower™ chromatography software to read in Empower sequences. So, you can set up your chromatography run in Empower, and then import the data into ASTRA for light scattering and related data analysis.

To import an Empower data set, follow these steps:

1. Create the sequence definition in Empower.
2. Choose **File→Import→Empower Sequence**.
3. Log in to the Empower database using your Empower user ID and password.



4. Find and select the sequence you want to import from the import dialog and click **Open**. The sequence is shown in your Sequences navigation pane with the name of the sequence you imported.

ASTRA converts the Empower sequence to a full-featured ASTRA sequence. Sample names and identifying information are all brought over from Empower.

5. To save the sequence in your experiment database, choose **File→Save**.

For more about using ASTRA with Empower, go to <http://www.wyatt.com/solutions/software/ASTRA.cfm> and follow the links to Connectivity > Interoperability with Waters Empower Software.

Editing a Sequence

To edit a sequence, you use the property page for the sequence.

To use this page, follow these steps:

1. Choose **Sequence→Edit**. This opens the properties page for the sequence. You will see the Sequence property page, which contains the “Configuration”, “Samples”, and “Collection” tabs.

Shortcuts: Double-click “Configuration”, “Samples”, or “Collection” in the sequence tree in the Sequences navigation pane.

2. You can set properties by typing, selecting from a list, or checking a box. Use the + or - next to a property to expand or hide lists of related properties.
3. You can move to other tabs in the page to view or set properties.
4. Click **Apply** or **OK** to make the changes.

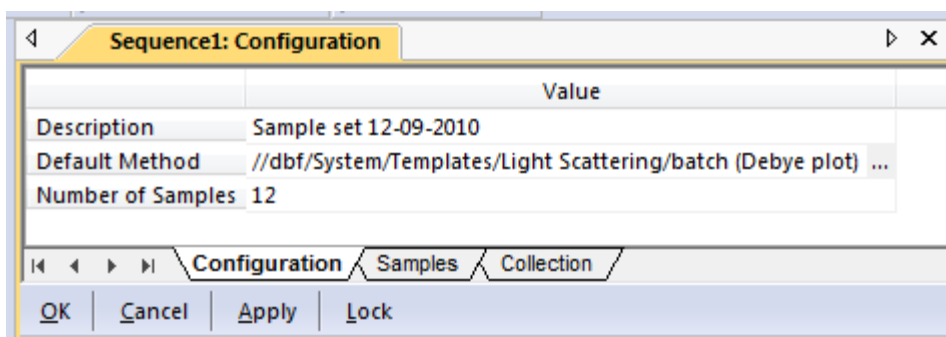
The remaining sections of this chapter contain details about the properties you can set in the various tabs.

The Sequence property page has tabs for the following items:

- **Configuration:** Sets global properties for the sequence configuration.
- **Samples:** Sets properties for samples in each well of the sample tray. Also specifies the experiment method to be used for each sample.
- **Collection:** Shows collection data as samples are being run.

Configuration Tab

The Configuration tab of the Sequence property page looks like this.



You can set the following properties for a sequence configuration:

Table 9-1: Experiment Configuration Properties

Field	Description
Description	Description of the sequence configuration.
Default Method	The experiment method to assign initially to all the samples. The method can be overridden on a sample-by-sample basis in the Samples tab. For information on choosing experiment methods, see “Creating Experiments from Methods” on page 6-5. For information on creating experiment methods, see “Creating a Method” on page 6-18.
Number of Samples	The number of samples in the sequence. This can be changed in the Samples view after the initial configuration.

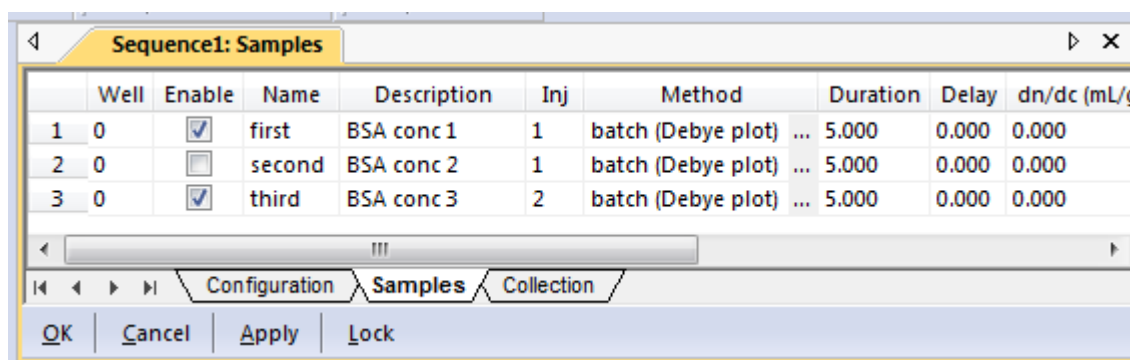
Replacing configurations (page 7-7) is common when using sequences. For example, a typical workflow for replacing complete configurations in a sequence is as follows:

1. First, run a sequence containing one normalization standard and a number of samples.
2. Then, normalize and configure the experiment based on the standard run.
3. Finally, replace the experiment configuration in all the sample runs in the sequence with the configuration from the “standard” run.

Note: If you want to import an example configuration, but keep the sample(s) the same as when you ran the experiment, right-click on the Configuration node of the experiment and make sure the checkmark next to the **Keep Configuration Sample** is toggled on.

Samples Tab

The Samples tab of the Sequence property page looks like this.



While a sequence is being run, you can change the values for a sample that has not yet been run. Once a particular sample has been run, its fields become noneditable.

If you right-click on a sample row, the pop-up menu allows you to add, delete, and reorder the samples before they are run. These operations are all available even while the sequence is being run. Note that you must

select the **Apply** button to apply any changes you make to the sequence while it is running.

You can set the following properties for a sample:

Table 9-2: Sample Properties

Field	Description
Seq #	The sequence number in the sequence. This field is non-editable.
Well	The number of the injection well in the sample tray. This is for informational purposes only. You need not use this field.
Enable	If this box is checked, the sample is enabled for the sequence run.
Name	Name of the file to be generated by the sequence. The sequence name is appended to this file name. If multiple injections are requested for a sample, the injection number is also appended to the name. If no name is specified, ASTRA generates a unique name for the generated file.
Description	Description of the sample, which typically contains more information than the Name.
Inj	The number of injections for the sample.
Method	The experiment method to use as the source for instrument configuration, procedure, and result formatting information for this sample. The default experiment method is set in the Configuration tab, but you can override it on a sample-by-sample basis here. If the experiment method itself is modified after you choose it here but before the sequence is run, the modified version of the experiment method is used. For information on choosing experiment methods, see "Creating Experiments from Methods" on page 6-5. For information on creating experiment methods, see "Creating a Method" on page 6-18.
Duration	The time or fluid volume for which data is to be collected. The units are determined by the Abscissa Units property of the experiment configuration.
Delay	The delay in time or fluid volume between injection and the start of data collection. The units are determined by the Abscissa Units property of the experiment configuration. The default is zero.
dn/dc	dn/dc value associated with the sample in mL/g. The dn/dc value is used when the sample concentration is to be determined using a refractive index instrument. The value entered for the profile is used as a default value when peaks are set for the data.
A2	Second viral coefficient value associated with the sample, measured in mol mL/g ² . The value set here is used as a default value for peaks set in the experiment.
UV Ext	The UV extinction coefficient in mL/(mg cm). The extinction coefficient is used when the concentration of the sample is to be determined using a UV absorption instrument. The value entered here is used as a default value when peaks are set for the data.
Conc	The concentration of the sample in mg/mL.
Vol	The injection volume of the sample in μ L.

In order to avoid cycling the laser frequently, which will shorten its lifespan, you should make sure the Laser Saver Mode box is not checked in the Basic Collection configuration of the methods you use for the samples.

Instead, you can add one more sample to the sequence and use one of the System > Methods > Light Scattering > Utilities > turn laser off methods as the last experiment method in the sequence.

Seq	Well	Enable	Name	Description	Inj	Template	d
1	0	<input checked="" type="checkbox"/>			1	/My Templates/collection only	...
2	0	<input checked="" type="checkbox"/>			1	/My Templates/collection only	...
3	0	<input checked="" type="checkbox"/>			1	/My Templates/collection only	...
4	0	<input checked="" type="checkbox"/>			1	/My Templates/collection only	...
5	0	<input checked="" type="checkbox"/>			1	/My Templates/collection only	...
6	0	<input checked="" type="checkbox"/>			1	/My Templates/collection only	...
7	0	<input checked="" type="checkbox"/>			1	/My Templates/collection only	...
8	0	<input checked="" type="checkbox"/>			1	/System Templates/Light Scattering/Utilities/turn laser off	...

Note: If you use any of the methods in System > Methods > Light Scattering > Utilities, you must specify a physical instrument for that method.

The order of precedence for values such as dn/dc, A2, UV extinction, concentration, and so on is as follows:

1. Peak values defined in the specified experiments are used if they exist.
2. Values from the Samples tab are used next.
3. The sample profile is used if no other values are set.

Collection Tab

The Collection tab of the Sequence property page allows you to view data as it is collected during a sequence run.


While a sequence is running, the collection data is displayed for the current sample. If an autoinject signal is expected to trigger the injection, this view displays a message indicating the state of the collection.

Running Sequences

Sequences act as sets of experiments that can be run as a group. Rather than running each separate experiment from the Experiments navigation pane, you run the collection from the Sequences navigation pane.

Validating a Sequence

You can validate an entire sequence by choosing **Processing→Validate**.

Shortcuts: Click the Validate icon  in the experiment toolbar.


Validation checks for conflicts in the procedure sequences for all samples in the sequence. If an experiment collects data, validation also checks that the necessary instruments are connected and available. In addition, validation checks any custom collection scripts.

Procedures are not shown in the Sequences navigation pane. You can view them by creating new experiments from methods as described in “Creating Experiments from Methods” on page 6-5. For information about modifying the procedure sequence, see “Setting the Procedure Order” on page 6-27.

Running a Sequence

To start the sequence run, follow these steps:

1. Begin by turning on, warming up, and stabilizing your experimental apparatus. When everything is ready to go, continue with the following steps in ASTRA.
2. Choose **Processing→Run**.

Shortcuts: Click the Run icon  in the experiment toolbar.


3. During a sequence run, the live data can be viewed in the Collection tab, and the state of the sequence execution can be monitored in the Samples tab.

After a sample has been run, its row in the Samples tab is shown with a blue background.

After the full sequence has been run, a message says “Sequence run complete.”

Stopping a Sequence

To stop a running sequence, choose **Processing→Stop**.

Shortcuts: Click the Stop icon  in the experiment toolbar.

A message says “Sequence run manually stopped.”

Stopping a sequence with ASTRA stops only the collection and analysis of data. It does not affect any activity going on outside of ASTRA’s control.

See your hardware documentation for information about alarms, emergency stops, and setting up safety interlocks. Alarms may be monitored via the Diagnostic Manager. See “Viewing the Log with the Diagnostic Manager” on page 5-10 for details.

Viewing a Sequence Log

Database

To view a log for a sequence, right-click any item in the Sequences navigation pane, and choose **Manage→Log→Open**. Alternately, you can choose **Sequence→Log→Open** or **View→Logs→Sequence→Open** from the menu bar. For more about sequences, see Chapter 9, “Using Sequences”.

Double-click on a line in the log to see more details.

You can save an individual sequence log to a CSV or text file by choosing **Sequence→Log→Save As**.

Saving Sequences

To save a Sequence, follow these steps:

1. Choose **File→Save**.

Shortcuts:	Press Ctrl+S.
	Click the  icon.

2. If this is the first time you have saved this sequence, you see the Save As dialog. Otherwise, you are finished saving the file.
3. In the Save As dialog, browse to the folder you want to contain the file.
4. In the File Name field, type a name for the sequence.

Basic

The Save As Type field shows that the file will be saved with an extension of *.vsf if you are using ASTRA 6 Basic.

Database

If you are using ASTRA 6 with Research Database or ASTRA 6 with Security Pack, the sequence is saved in the experiment database.

5. Click **Save**.

To save a sequence with a different name or location, choose **File→Save As** and follow steps 3 through 6 above.

Saving Sequences as Templates

A sequence can be saved as a sequence template right-clicking on the sequence in your Sequence navigation pane and selecting **Save As Template**. This allows you to save the sequence as a template in the system database.

Once you have configured a sequence, save it as a sequence template before running it. You can then create new sequences from the sequence template without building a new one from scratch each time. See “Creating a Sequence from a Sequence Template” on page 9-4 for more information.

Exporting Sequences

You can export a sequence from the experiment database to a file with an extension of *.afs6. This file is a binary file that can only be imported by ASTRA.

To export a sequence, follow these steps:

1. Select the sequence you want to export in your Sequence navigation pane.
2. Choose **File→Export**.
3. Select the directory where you want to save the file, and type a file-name for the sequence.
4. Click **Save** to create the file.

Deleting a Sequence

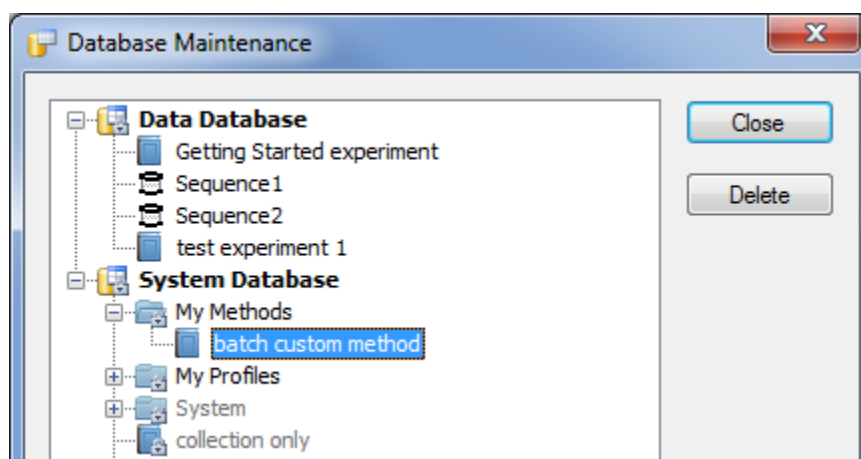


If you are using ASTRA 6 Basic, you may delete a sequence by deleting the *.vsf file that contains the sequence.



If you are using ASTRA 6 with Research Database or ASTRA 6 with Security Pack, delete a sequence by following these steps:

1. Choose **System→Database Administration→Maintenance**. This opens the Database Maintenance dialog.



2. Highlight the sequence you wish to delete in the list.
3. Click **Delete**.
4. Click **Close** when you have finished deleting sequences.



If you are using ASTRA 6 with Research Database or ASTRA 6 with Security Pack, you must have Administrator access to delete a sequence.

You can delete sequence templates by choosing **System→Database Administration→Maintenance** and using the dialog to delete sequence templates you no longer need.

10 Working With Reports

This chapter explains how to create, customize, and print reports with ASTRA.

CONTENTS	PAGE
About Reports	10-2
Viewing a Report.....	10-3
Abbreviations Used in Reports.....	10-4
Setting Report Options.....	10-5
Using the Report Designer.....	10-6
Printing a Report	10-7
Adding a Report	10-8
Applying Report Formats From a Method	10-8

About Reports

The methods provided with ASTRA 6 include reports that show the results computed for the experiment. Most methods include a summary report and a detailed report. Typically, these reports will need little or no modification. You can simply view and print the reports produced when you run an experiment.

To view a report, double-click on its name in the Results list of the experiment tree in the workspace.

Operator Names in Reports



If you are using ASTRA 6 Basic, the operator names shown in reports are assigned as follows:

- **Processing Operator:** This is determined at the time the report is generated. It is the “currently logged in user” obtained from Microsoft Windows.
- **Collection Operator:** This is determined at the time data collection is started. It is also the “currently logged in user” obtained from Microsoft Windows. This username is stored with the collected data, so that it is retained even if processing and report generation is performed by another user.

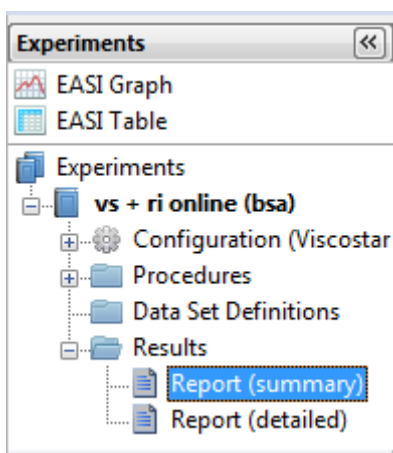


If you use ASTRA 6 with Research Database or ASTRA 6 with Security Pack, the operator names shown in reports are assigned as follows:

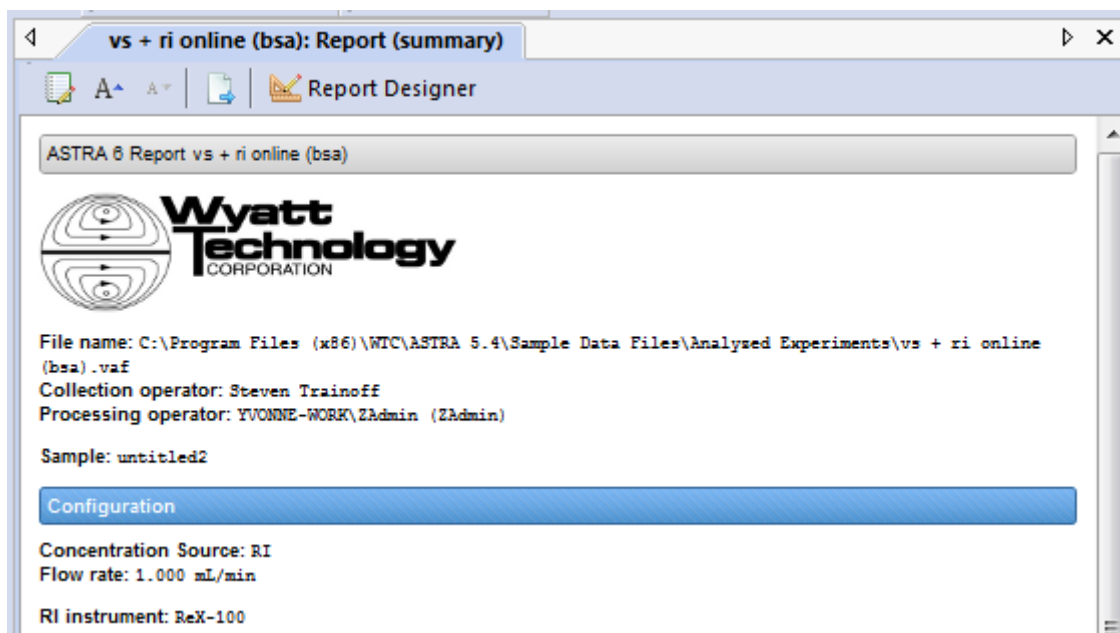
- **Processing Operator:** This is determined at the time the report is generated. It is the domain and username for the user currently logged into ASTRA. The Microsoft Windows “currently logged in user” is ignored.
- **Collection Operator:** This is determined at the time data collection is started. It is also the domain and username for the user currently logged into ASTRA. The Microsoft Windows “currently logged in user” is ignored. This username is stored with the collected data, so that it is retained even if processing and report generation is performed by another user.

Viewing a Report

To open a report, double-click on it in the Experiments navigation pane.



You will see the default report for your experiment.



You can use this view as follows:

- Click the  **Report Options** icon to open the Report Options dialog. See page 10-5 for details.
- Click one of the  **Font Size** icons to make the text larger or smaller.
- Click the  **Export** icon to save the report to an XML file. See page 10-7 for details.
- Click the  **Report Designer** icon to open the Report Designer pane. See page 10-6 for details.
- Print the report as described on page 10-7.

Abbreviations Used in Reports

The following are some of the abbreviations used in report results. See “Molar Mass and RMS Radius Moments” on page D-18 for details about how many of these values are calculated. In general, $M_n < M_v < M_w < M_z < M_{z+1}$. For monodisperse samples, the averages are identical.

- **M_n**: Number-average molar mass. M_n is sensitive to fractions with low molar masses. See Eq. (38).
- **M_p**: Molar mass at the apex of the concentration (RI or UV) peak. If the sample is polydisperse, the concentration peak apex does not necessarily coincide with the LS peak apex.
- **M_v**: Viscosity-average molar mass. This result is computed only if you set the Mark-Houwink-Sakurada parameters in the Peaks procedure or you compute Mark-Houwink-Sakurada terms using one of the online viscometry methods.
- **M_w**: Weight-average molar mass. This result is sensitive to fractions with high molar masses. See Eq. (39).
- **M_z**: z-average molar mass. This result is sensitive to fractions with high molar masses. See Eq. (40).
- **M_{z+1}**: Rarely used; z-average molar mass at one moment higher. This result is sensitive to fractions with high molar masses. The calculation performed is as follows:

$$M_{z+1} = \frac{\sum_i c_i M_i^3}{\sum_i c_i M_i^2}$$


- **M(avg)**: uncertainty-weighted average molar mass.
- **R_n**: Number-average mean square radius. See Eq. (45).
- **R_w**: Weight-average mean square radius. See Eq. (46).
- **R_z**: z-average mean square radius.
- **R(avg)**: Uncertainty-weighted average radius. See Eq. (47).
- **η_n**: Number-average intrinsic viscosity. See Appendix G.
- **η_w**: Weight-average intrinsic viscosity.
- **η_z**: z-average intrinsic viscosity.
- **η(avg)**: uncertainty-weighted average intrinsic viscosity.
- **R_h(n)**: Number-average hydrodynamic radius.
- **R_h(w)**: Weight-average hydrodynamic radius.
- **R_h(z)**: z-average hydrodynamic radius.
- **R_h(avg)**: uncertainty-weighted average hydrodynamic radius.

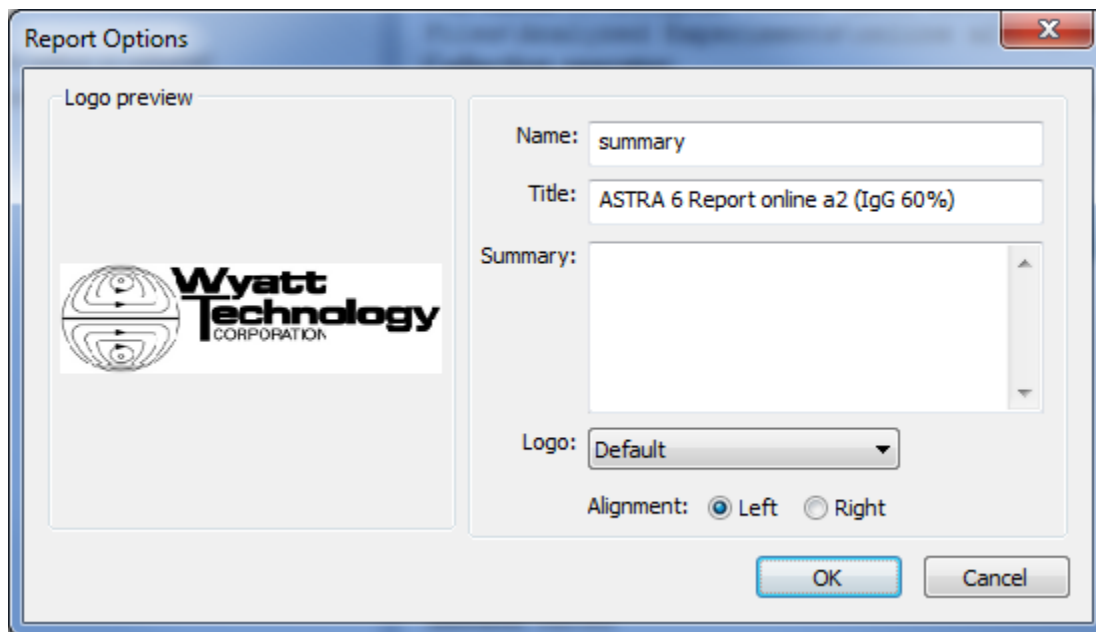
Setting Report Options


Security

You must have at least Researcher access to modify report formats.

You can use the Report Options dialog to change the report title, summary, and alignment. You can also change or remove the logo shown at the top of the report.

Click the  **Options** icon in a report window to open the Report Options dialog.




- The **Name** is shown in parenthesis in the experiment tree.
- The **Title** is shown at the top of the report in a gray bar. You can modify it as needed.
- The **Summary** is shown to the right of the logo. By default, there is no summary, but you can add any text you like here. If your summary text is long, it wraps below the logo.
- The **Logo** is shown just below the title. The default is the Wyatt Technology logo. To format the report with no logo, choose **None**. To use your own logo, choose **Browse**. Your logo can be in any of a number of image formats.
- The **Alignment** lets you change from left-justified to right-justified if desired.

Click **OK** or **Apply** to save the report.

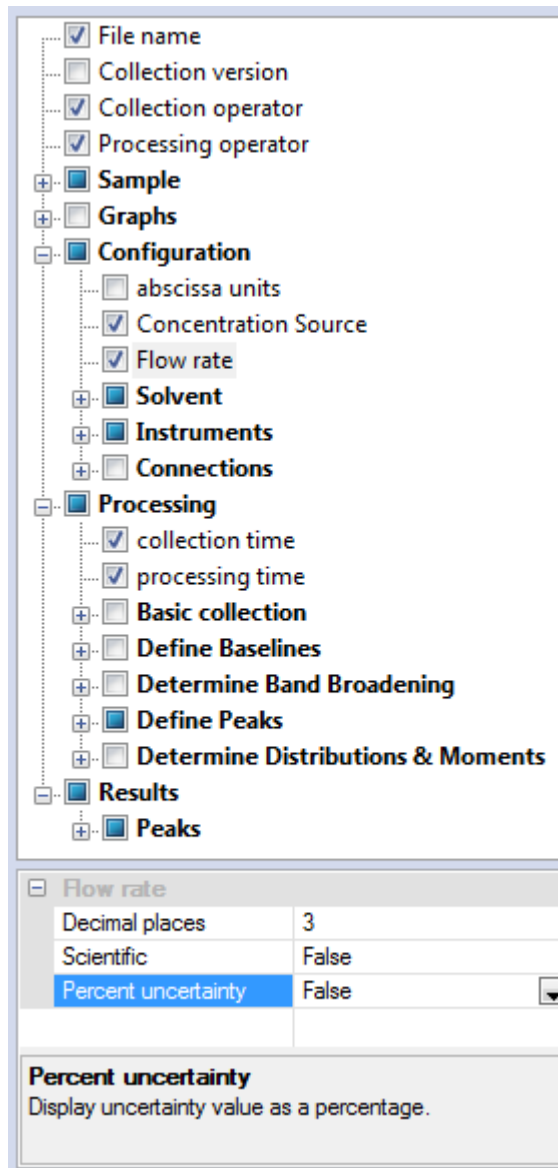
Using the Report Designer

Security You must have at least Researcher access to modify report formats.

You can add and remove report information using the Report Designer.

Click the  **Report Designer** icon to open the Report Designer pane, which opens to the right of the report.

- Adding Information:** Check or uncheck items to include or exclude them from the report. You can select all the items within a category by checking the box next to the parent item of that category.
- Formatting Data:** When you select a numeric data item, formatting settings for that item are shown below the list of report items. For example, you can change the number of places shown after the decimal point or whether a value is shown in scientific notation.
- Formatting Graphs:** You can zoom in on graphs in a report and set graph options as described in “Viewing and Modifying Graphs” on page 11-15.
- Select an item in the report view by clicking on it. You can remove the item from the report by clicking the “X” next to your selection.



The screenshot shows the Report Designer interface. On the left is a tree view of report items with expand/collapse icons. On the right is a settings table for the selected 'Flow rate' item.

Flow rate	
Decimal places	3
Scientific	False
Percent uncertainty	False
Percent uncertainty Display uncertainty value as a percentage.	

Click **OK** or **Apply** to save the report.

If you save an experiment as a method, the customizations you have made to the reports in that experiment are saved as part of the method. If you later create a new experiment using that saved method, the new experiment will have the report customizations.

Printing a Report

To print a report, do one of the following:

- Choose **File→Print**.
- Right-click in the report window and select **Print**.

Tip:	To copy a report to the Windows clipboard, highlight text, right-click, and choose Copy . Then, move to another application and paste.
-------------	---


To preview the report layout, do the following:

1. Choose **File→Print Preview**.
2. You can use the Print Preview window to change the print setup or to print the report.

You can choose **File→Page Setup** to choose a paper size and source, page orientation, and margin widths. You can choose **File→Print Setup** to choose a printer and set properties for your printer.

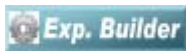
Exporting a Report

If you want to save a report as a word processor document, press Ctrl+A to select the entire report or use your mouse to select a portion of the report. Use Ctrl+C to copy from ASTRA and Ctrl+V to paste into a word processor document.

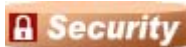
To save a report as an XML file, click the  **Export** icon. Choose the location of the file you want to create. The report is exported in XML format whether you create a .xml or .txt file.

To export experimental data only, see “Exporting Data” on page 11-20.

Adding a Report





You can add reports only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.



You must have at least Researcher access to add reports to experiments.

To create a new report, follow these steps:

1. Choose **Experiment→Report→Add Report**.
2. Double-click on the “Report (untitled1)” item that was added to the Results node in the experiment tree.
3. Click the  **Options** icon in a report window to open the Report Options dialog. Provide a report title as described in “Setting Report Options” on page 10-5.
4. Click the  **Report Designer** icon to open the Report Designer pane. Add fields to your report as described in “Using the Report Designer” on page 10-6.
5. Click **OK** or **Apply** to save the report.

Applying Report Formats From a Method


You can apply report and graph formats (along with procedures) from a method to an experiment you have already run to collect data. For example, after using the “LS batch (Zimm plot)” method when collecting data, you might want to apply the “LS batch (Debye plot)” method to the same data so that you can view the results differently.

Applying a method creates a separate experiment, so you do not lose any of the information in the original experiment.

To apply a method, follow these steps:

1. Open an experiment containing raw (source) data you want to use.
2. Choose **Experiment→Apply Method**. The New From Existing dialog appears. This is the same dialog you use to create an experiment from a method before data collection.
3. Choose a method to apply to the data. Typically, you would choose a method from the System > Methods or My Methods folder.

The procedures and result formats (reports and graphs) in the method are used in place of those in your source experiment. The source data and the source experiment configuration are not changed.

4. Click **Create**. A new experiment is created.
5. Select the new experiment and click the Run icon  in the toolbar.
6. After the applied procedure runs, you can view the new results.

11

Working With Graphs and Tables

This chapter describes how to create and use EASI graphs, data set definitions (DSDs), and DSD-based graphs.

CONTENTS	PAGE
About ASTRA Graphs and Tables.....	11-2
Using the EASI Graph.....	11-3
Using the EASI Table	11-6
Using Custom Plots and Data Set Definitions.....	11-8
Creating Surface Plots	11-13
Viewing and Modifying Graphs	11-15
Copying Graphs to the Clipboard.....	11-17
Printing Graphs	11-17
Exporting Graphs	11-19

About ASTRA Graphs and Tables

You can use the following ways to create graphs using ASTRA:

- **Procedure Graphs.** Many procedure pages contain graphs that you can print. See “Working with Procedure Graphs” on page 8-5 for information about these graphs.
- **EASI Graphs.** These graphs are flexible and easy to create. You make selections and see the resulting graph in the same page. EASI graphs are not saved with the experiment. See “Using the EASI Graph” on page 11-3 for details.
- **EASI Tables.** These tables let you view numeric data for multiple experiments. You can use controls to interactively display or hide portions of the data. EASI tables are not saved with the experiment. See “Using the EASI Table” on page 11-6 for details.
- **Custom Plots.** These graphs are more powerful (and more complicated to create). They use a data set definition to identify the data to plot. You can use custom plots to graph multiple types of data against any x-axis values you choose. See “Creating Data Set Definitions” on page 11-9 and “Creating Custom Plots” on page 11-11 for details.
- **Parametric Plots.** The parametric plot procedure generates a data set for two different types of x-y data that share the same x-axis. For example, you can use this procedure to create a plot of RMS radius vs. molar mass. See “Parametric Plot” on page 8-88.
- **Surface Plots.** You can create a 3D surface plot of detector data. See “Creating Surface Plots” on page 11-13.

For all types of graphs, see the following sections:

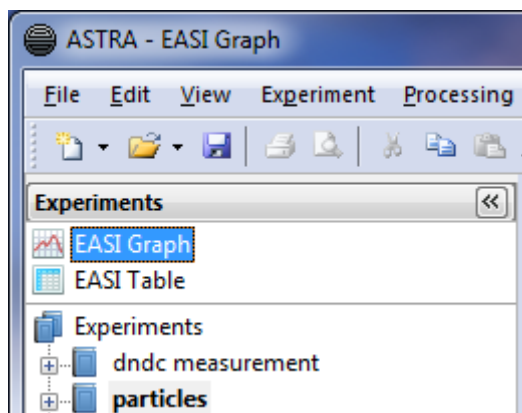
- “Viewing and Modifying Graphs” on page 11-15 for information about how to zoom in and out and change the look of the graph.
- “Printing Graphs” on page 11-17 for information about printing any type of graph.
- “Exporting Graphs” on page 11-19 for information about saving graphs to image files.

Using the EASI Graph

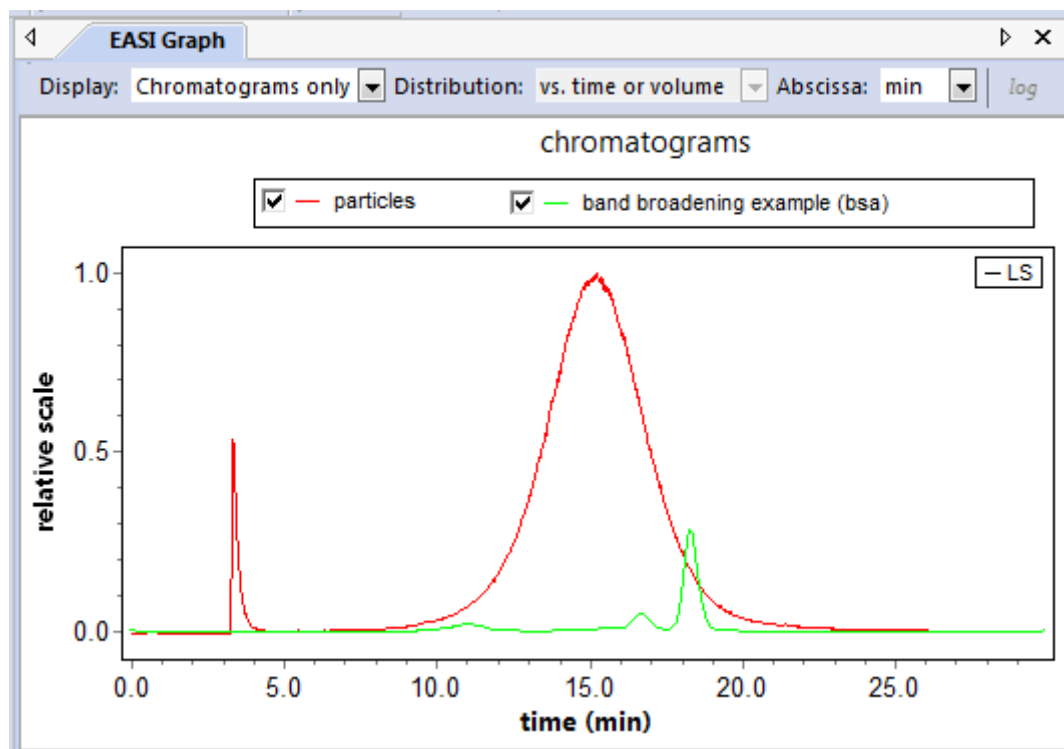
EASI graphs provide a way to quickly visualize results. You can use them to compare data from multiple experiments. You can view an EASI graph of chromatograms during data collection. Your settings are saved for the next time you view an EASI graph. The actual graph is not saved. You can only open one EASI graph.

To view the EASI Graph, do one of the following:

- Choose **View→EASI Graph**.
- Double-click the **EASI Graph** item at the top of the Experiments navigation pane.



The EASI Graph window opens. The first time you open the EASI graph, it displays chromatograms for all the experiments you have open.



You can print an EASI graph by right-clicking on the graph and choosing **Print** from the pop-up menu. You can also copy the graph to your clipboard and paste it into another application, such as a document.

You can use the following controls to modify the graph:

- **Display:** Choose the type of display you want. This will be the main data set plotted. (The solid line.) The options are: chromatograms, concentration, molar mass, RMS radius, Rh, RMS conformation plot, Rh conformation plot, RMS vs. Rh plot, Burchard-Stockmayer plot (R_g/R_h vs. time), intrinsic viscosity, Mark-Houwink-Sakurada plot, geometric radius, mean-square radius, translational diffusion, branching ratio (g and g'), branch units per molecule, long-chain branching, protein conjugate, and instrument voltages.
- **Distribution:** Choose the item you want to plot the display against. The options are: vs. time or volume, cumulative weight fraction, differential weight fraction, cumulative number fraction, and differential number fraction. If you choose “vs. time or volume”, that will be the x-axis of the plot. If you choose one of the fractional options, your choice will be the y-axis of the plot. (Some display options can only be plotted against time or volume.)
- **Abscissa:** Choose units to use on the x-axis for a Distribution of time or volume. The options are msec (milliseconds), sec (seconds), min (minutes), h (hours), or mL (milliliters).
- **Log:** Click the “log” button above the graph if you want a log scale used for the y-axis of the graph. The axis that log scaling applies to depends on the type of plot.
- **Visibility:** Check or uncheck the boxes in the graph legend to display or hide the trace(s) for that experiment.
- **Zooming:** Right-click on the EASI graph and use the Autoscale, Zoom In, and Zoom Out commands as you would with other graphs.
- **Graph Options:** To further customize the appearance of an EASI graph, right-click on the graph and choose **Edit** from the pop-up menu. This opens the Edit Graph dialog, which has the following fields:

Table 11-1: Edit Graph Fields

Field	Description
Marker Size	Choose the marker size you want to use. The options are: very small, small, medium, and large. Use Marker Size for lines made up of individual data points; use Line Weight for fitted curves.
Line Weight	Set the width of the line when the line is a fitted curve. Use Marker Size if the line is made up of individual data points.
1st Chromatogram	Choose a data set you want to plot along with the main display data. A thinner solid line is used for this data set. The options are: none, Rayleigh ratio, differential refractive index, UV absorbance, QELS count rate, and specific viscosity.
2nd Chromatogram	If you want to plot a third set of data, choose an item here and check the Show Chromatogram 2 property. A dashed line is used for this data set. The options are the same as for Chromatogram 1.

Table 11-1: Edit Graph Fields

Field	Description
3rd Chromatogram	If you want to plot a fourth set of data, choose an item here and check the Show Chromatogram 3 property. The options are the same as for Chromatogram 1.
Scaling	Choose whether to use normalized or relative scaling. "Normalized" means all data of a particular type (e.g., RI data) is scaled against the largest value of that type across all experiments. "Relative" means values in each experiment are scaled from 0 to 1, allowing all traces to have the same magnitude regardless of the actual values compared between experiments.
Experiment	Choose the experiment for which you want to modify the graphing style.
Color	Select the color you want to use for this experiment in the graph. Changing this property changes the color for all chromatogram traces.
Marker Type	Select the marker type you want to use in the graph. The default is square.
Marker Spacing	Choose how often you want markers to appear on the line. The options are every 1, 2, 3, 5, 10, 20, 30, or 50 data points.
X-axis Shift	Type a numeric value by which the x-axis values should be shifted. The number will be added to the existing times or volumes. You can use a negative value, for example, if 15 mL of solvent were run through the system initially, you might use an X Axis Shift of -15.

You can see the effects of your changes as you make them without closing this dialog.

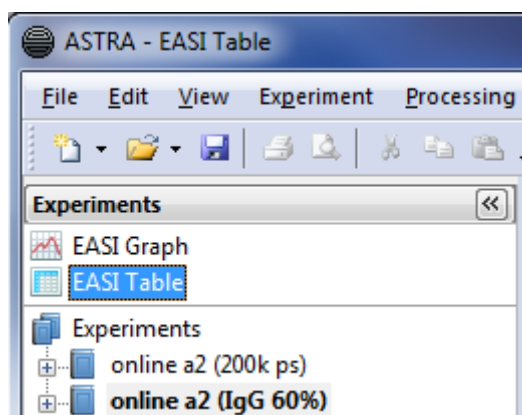
If you click **Advanced**, you have much more control over the graph display is provided than is described in this manual. For help on settings in the Advanced dialog, move to a field and press F1.

Using the EASI Table

You can use the EASI table to view numeric data for multiple experiments in a table. You can use controls to interactively display or hide portions of the data, and then export the data to a file that can be read by a spreadsheet or other application.

To view the EASI Graph, do one of the following:

- Choose **View→EASI Table**.
- Double-click the **EASI Table** item at the top of the Experiments navigation pane.



The EASI Table window opens. The first time you open the EASI table, it displays data for all the experiments you have open.

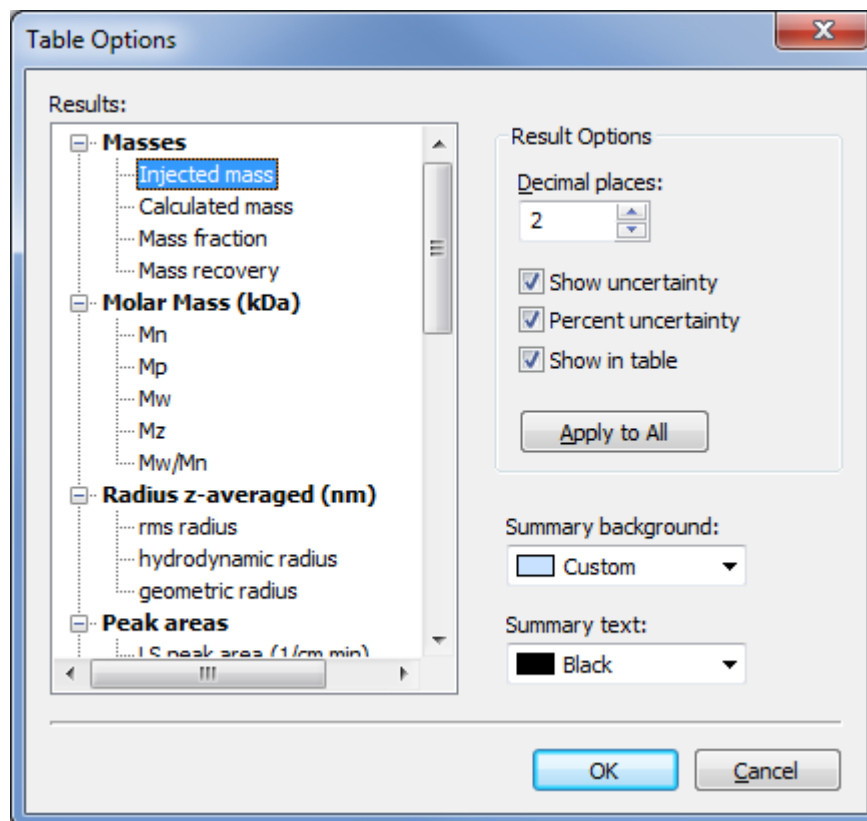
EASI Table						
Experiments: All Peaks: All Scalars: All						
	General			Peak 1		
	Mw (kDa)	Rz rms (nm)	Rz geo	Injected mass	Calculated mass	M _r
online a2 (200k ps)	198.5 (±0.1%)	17.5 (±1.5%)		0.00e000	2.03e01	3.0
online a2 (bsa with desalting)	68.6 (±0.1%)	3.1 (±15.9%)	0.0	0.00e000	8.51e02	6.6
online a2 (IgG with column)	143.7 (±0.1%)	0.0	0.0	0.00e000	3.34e02	3.6
Average	136.9	6.9	0.0	0.00e000	4.01e02	4.4
Standard deviation	86.6	10.3	0.0	0.00e000	5.12e02	2.1
% Standard deviation	63.3	149.5	n/a	n/a	1.28e02	47.
Minimum	68.6	0.0	0.0	0.00e000	2.03e01	3.0
Maximum	198.5	17.5	0.0	0.00e000	8.51e02	6.6

You can use the following controls to modify the graph:

- **Experiments:** Check the boxes for open experiments you want to include in the table.
- **Peaks:** Check the boxes for peaks you want to include in the table.
- **Scalars:** Check the boxes for fields you want to include in the table. A column is only shown in the table if that type of data is available in one or more open experiments.


For each column in the table the minimum, maximum, average, and standard deviation values are shown.

To format data, click the  **Numeric Formatting** icon above the table.



The Table Options dialog appears. To format data, follow these steps:

1. Select a type of data in the **Results** list. These items correspond to columns that can be displayed in the table.
2. In the **Results Options** area, modify how the column is displayed. You can adjust the number of places shown after the decimal point, toggle whether the uncertainty is shown in a column, toggle whether uncertainty is shown as a percent or as a value, and toggle whether that column is shown in the table.
3. Click **Apply to All** to apply the current settings in the Results Options area to all columns. Click **OK** to apply all changes you have made to individually selected columns.
4. You can use the **Summary background** and **Summary text** drop-down lists to select colors to use for the values in the Average, Standard deviation, Minimum, and Maximum rows.

To export data from the EASI table, click the  **Export** icon above the table. You can browse for a location, type a filename, and choose to export the data to a CSV file (for use in a spreadsheet), a text file, or a web page.

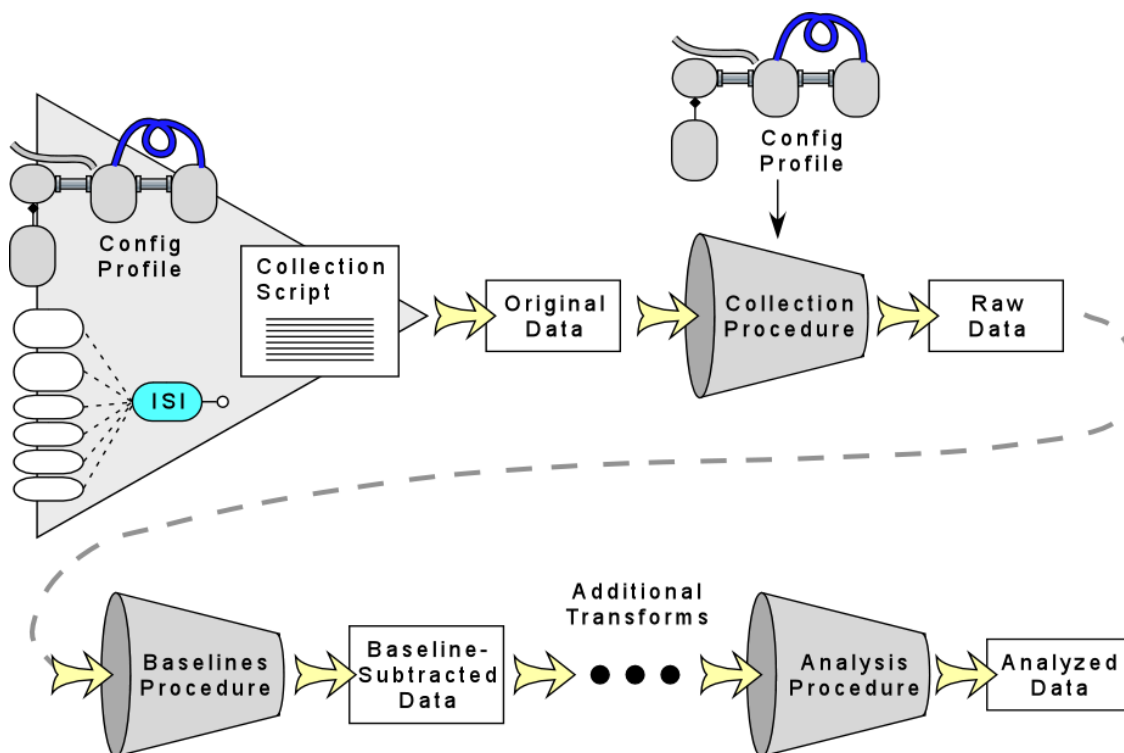
You can also use your mouse to select a block of cells in the table and press Ctrl+C. You can then paste the data into another application.

Using Custom Plots and Data Set Definitions

Data set definitions are a definition of data that ASTRA uses to create graphs. For example, a data set definition is needed to specify the contents of a custom plot. When you create and use data set definitions, you have access to more data sets stored within an experiment and have more control over which data is plotted.

Data Collection and Storage

ASTRA experiments store a set of data for each procedure in the experiment. Each set represents the data after the procedure ran. The following figure shows some of the typical data sets.

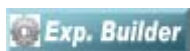


The data sets include the following:

- **Original data:** In addition to raw detector voltages, this data set typically includes the laser monitor signal and any auxiliary input data.
- **Raw data:** This is the data gathered by the data collection procedure. For a light-scattering experiment, this is the detector voltages. The raw data is kept with the experiment, so that data can be reprocessed if you modify the procedure and can be viewed in reports if desired.
- **Raw data after each transform:** This data has the results of despiking, smoothing, baselines, peaks, and other transformations. A separate data set for each transformation is stored in the experiment.
- **Data after conversion:** This data has the results of conversions, such as from detector voltages to Rayleigh ratios.

- **Analyzed data:** This data has been processed to arrive at results such as molar mass, RMS radius, or other values.

Creating Data Set Definitions



Data set definitions are visible in the Experiments navigation pane and can be created only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

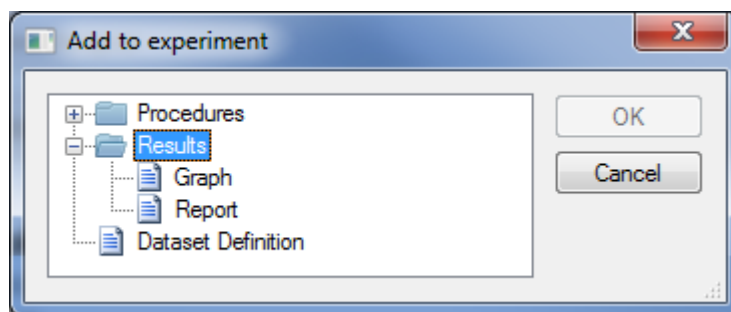
You can create a data set definition before or after you run an experiment.

To create a new data set definition, follow these steps:

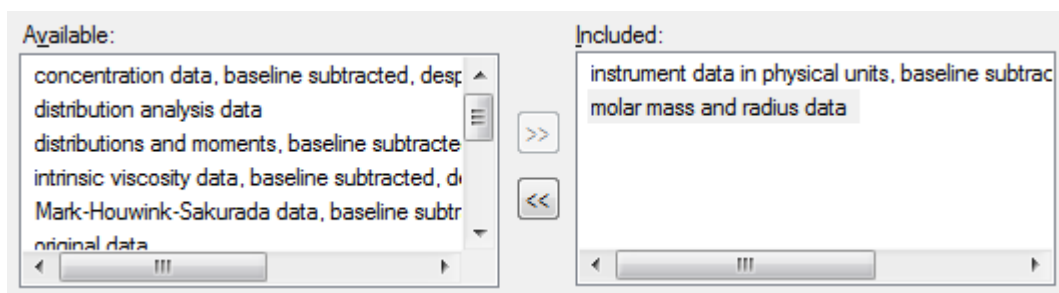
1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results nodes of the experiment.

Shortcuts: Press Ctrl+Shift+P.

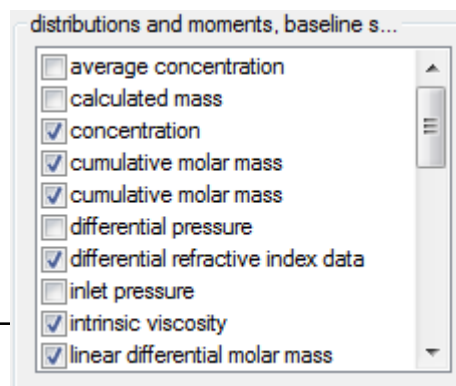
2. Close or scroll past the Procedures node, select Data Set Definition, and click OK.



3. Double-click the “untitled” item that is added to the Data Set Definitions folder in the experiment tree.
4. In the Data Set Definition dialog, type a name for this data set. Typically the name should describe what you want to graph. For example, “molar mass vs. volume”.
5. In the Available list, set one or more data set stages you want to graph data from. Each of these stages represents the data after an individual procedure runs. See “Data Collection and Storage” on page 11-8.
6. Click the >> button to move the selected items to the Included list.

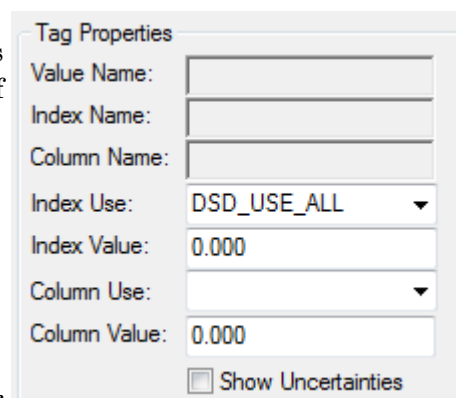


7. Select an item you have moved to the Included list. In the list of data in that stage, place checkmarks next to the data you want to graph. The first data set is graphed against the left y-axis. The second data set is graphed against the right y-axis.

**Note:**

Be aware that all the data items you select must be graphable against the same x-axis (index) values. For example, both molar mass and concentration can be graphed against volume (if that was the abscissa unit selected in the experiment configuration). However, the “fit of RMS radius vs. molar mass” can only be graphed against an x-axis of the molar mass.

8. When you select an item in the checkmark list, the Tag Properties fields change to describe aspects of that data. Some fields are modifiable for some items. Some fields vary depending on the item selected.
9. Modify values as needed for the data items you selected. All the changes you make for various items will be saved when you later click **OK**.



The properties in the list are as follows:

Table 11-2: Data Set Definition Properties

Field	Description
Value Name	The name of the selected item. (The y-axis values.)
Index Name	The index against which the item can be plotted. (The x-axis values.)
Column Name	Some data set items have column names that describe the type of data.
From Data	If you have selected a function tag, this box specifies whether the function is calculated from the x-axis data values. If not, you need to specify the index start and increment in the following fields.
Index Use	This property is not yet implemented.
Index Value	This property is not yet implemented.
Column Use	If you are using a matrix tag, you can specify how the columns are to be used. Select which column values to use. The options are: DSD_USE_ALL: Use all column values. DSD_CONTROL_VARIABLE: This option is not yet implemented. DSD_AT_VALUE: Use only one column with the value specified below.

Table 11-2: Data Set Definition Properties

Field	Description
Column Value	If you are using a matrix tag and are using only one column, this is the value of the column to use. For example, 90 degrees for the right-angle detector.
Index Start	If you are using a function tag and are not calculating the function from data, specify the starting index.
Index Space	If you are using a function tag and are not calculating the function from data, specify the range the x-axis should span.
Index Steps	If you are using a function tag and are not calculating the function from data, specify the total number of index points.
Show Uncertainties	Check this box if you want the graph to contain uncertainty error bars for this item.

10. Put a checkmark in the **Iterate Experiment Data Over Injection** box if there are multiple injections in the experiment that are to be displayed all at once.

11. If you checked the box to iterate, select the items to iterate.

12. Click **OK** to save your changes.

Using Data From Multiple Experiments

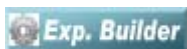
If you want to graph data from multiple experiments, you can copy data from one experiment to another. The copied data is shown in the “Available” list in the Data Set Definition dialog. See “Copying Data” on page 6-21 for details.

Other Uses for Data Set Definitions

When you export an experiment, you can choose to export the data matching a data set definition as a tab-delimited or comma-delimited text file. For details, see “Saving an Experiment to a File” on page 6-15 or “Exporting an Experiment” on page 6-17.

Creating Custom Plots

In Run mode, you can add custom graphs by choosing **Experiment→Graph→Add Custom Plot** from the menus. This opens the Data for Custom Plot dialog, and you can specify the data to display. Both the data set definition and results graph are created.

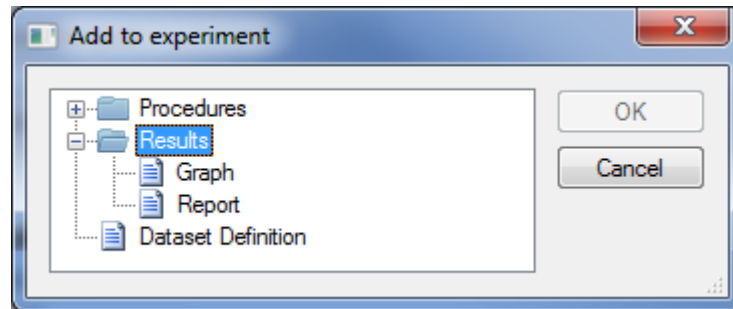


You can also add graphs as follows if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**:

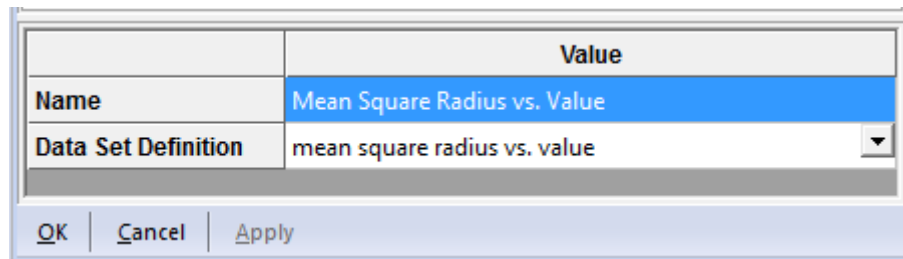
1. Create a data set definition for the data to be graphed as described in “Creating Data Set Definitions” on page 11-9.
2. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results folders of the experiment.

Shortcuts: Press Ctrl+Shift+P.

3. Select Graph (in the Results folder) and click OK.



4. Double-click the "Graph (untitled1)" item that was added to the Results folder in the experiment tree.
5. In the property list for the graph, type a Name to appear at the top of the graph.
6. For the Data Set Definition, select a data set definition you have already created.



7. Click **OK**.
8. Choose **Processing→Run** to run or re-run the experiment. After the procedure runs successfully, the graphs and reports are generated.

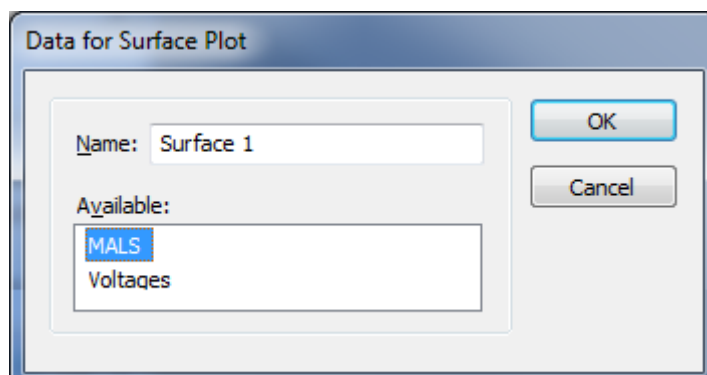
Creating Surface Plots

You can create a 3D surface plot of detector data.

Note: Surface plot generation is processor intensive. You may want to avoid displaying 3D surface plots during data collection.

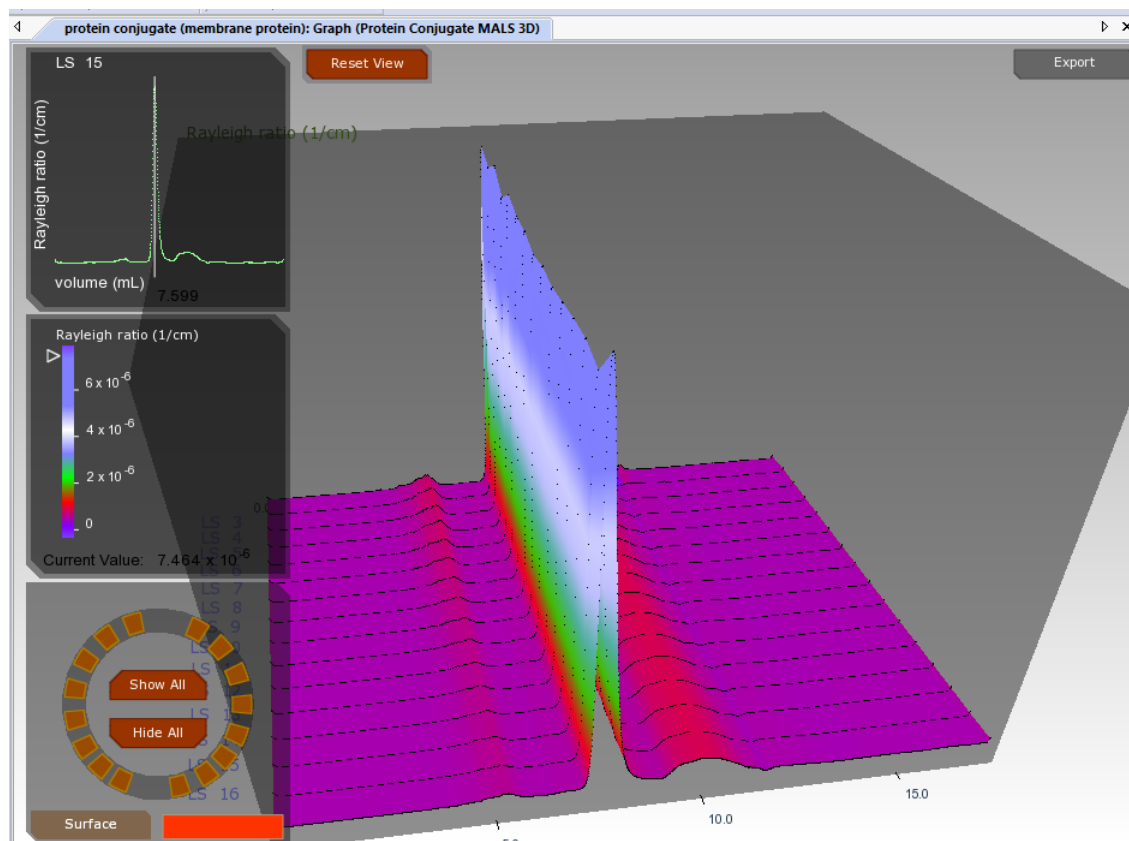
To create a 3D surface plot, follow these steps:

1. Choose **Experiment→Graph→Add Surface Plot** from the menus.
2. In the Data for Surface Plot, type a name for the plot and select the type of data you want to display. For example, light-scattering experiments allow you to select MALS or voltage data.



3. Click **OK**. Both a data set definition and the 3D graph are created.
4. Double-click on the new graph listed in the Results folder for this experiment in your workspace. It may take some time to load graphs of large amounts of data.

5. Within the surface plot window, you can do any of the following:



- See the Rayleigh ratio for the current surface location by moving your mouse around the graph. Two smaller graphs in the upper left show the Rayleigh ratio for that detector and at that point.
- Turn on and off detectors by toggling markers in the ring display in the lower right or clicking **Show All** or **Hide All**.
- Toggle between a wire frame graph and a colored surface by clicking the **Surface** button.
- Change the surface transparency by sliding the red bar next to the **Surface** button.
- Rotate or tilt the graph by holding down the left mouse button and dragging.
- Slide the graph around by holding down the right mouse button and dragging.
- Zoom in and out by holding down the Ctrl key and the left mouse button while moving your mouse up and down. You can also zoom in or out by right-clicking and choosing **Zoom In** or **Zoom Out**.
- Copy the currently displayed graph to the clipboard by pressing Ctrl+C or right-clicking and choosing **Copy**.
- Save a copy of the current plot to a file by clicking the **Export** button in the upper-right corner of the window.


Viewing and Modifying Graphs

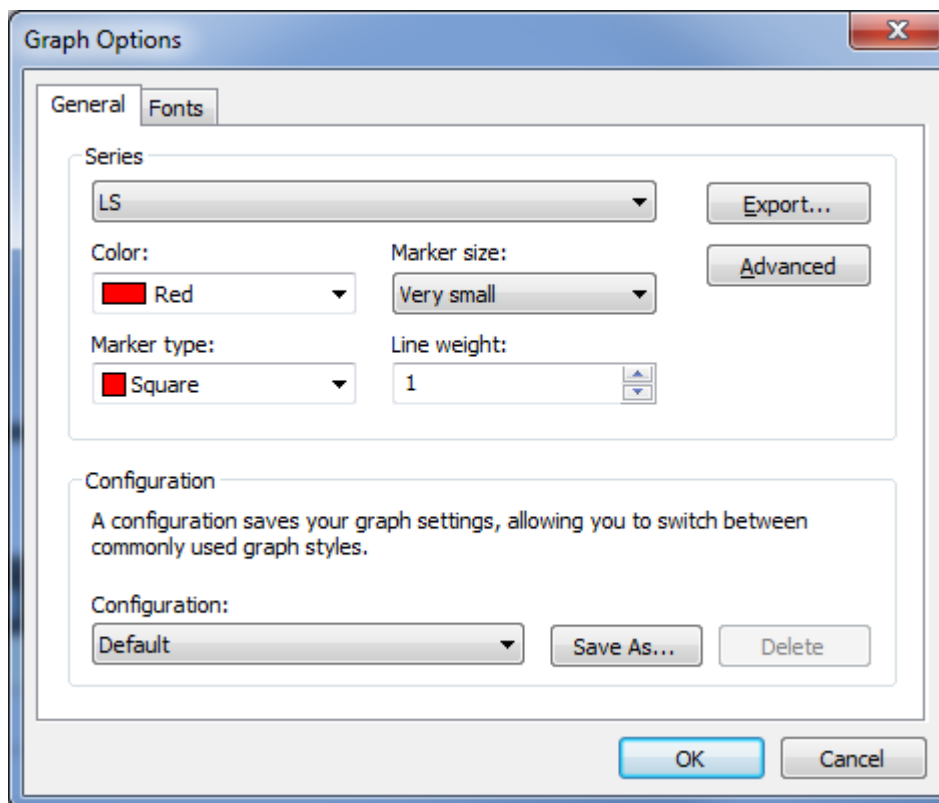
Graphs you generate can be manipulated in a number of ways.

Viewing Data Values

Point to a data trace on a graph and hold down the Shift key. The X and Y coordinates for the point and the type of data are shown. The values change as you move your mouse. If the Y axis has more than one scale, the left axis is shown as Y(L) and the right axis is shown as Y(R).

Customizing Line Colors, Widths, and Fonts

To change the line colors and widths in a graph, click the  Edit Chart icon, double-click on the graph, or right-click and choose **Edit**. You will see the Graph Options dialog.



To change the appearance of the plot of a data set, first choose a data **Series**.

In the **Color** field, select a defined color or “Custom” to choose other colors. The **Marker Size**, **Marker Type**, and **Line Weight** fields let you set the size and shape of the data points or width of the line. Some lines are made up of individual data points and some are drawn as a line. Use the fields that apply to your graph.

You see the effects of your changes as you make them without closing this dialog. Click **OK** to save your changes for use until you close the page containing the graph.

Graph customizations—such as line weight, color, marker style, and title changes—are saved for EASI graphs, custom plots, parametric plots, and surface plots. However, such graph customizations are not saved when you close procedure pages and reports that contain graphs.



You can save graph settings as a “configuration” that you can then apply to a currently viewed graph. To save a configuration, click the **Save As** button and specify the name of an XML file to contain your current graph settings. To load a configuration, select one you have saved from the **Configuration** list. For example, you might want to save graph settings that you use for research papers in one configuration file and graph settings you use for presentation slides in another configuration file.


If you click **Advanced**, you have much more control over the graph display is provided than is described in this manual. For help on settings in the Advanced dialog, move to a field and press F1.

The **Fonts** tab of the Graph Options dialog lets you select the font, font size, font color, and font style for various text that appears in graphs. Font settings are also saved as part of a graph configuration file.


See page 11-4 for details about the EASI Graph version of the Edit Graph dialog.

Zooming In and Out Graphs

- **Zooming in:** Click the  Zoom In icon or press Ctrl+F5 to zoom in one level. To zoom in on a specific area, hold down the Ctrl key and your left mouse button while dragging a rectangle around the data you want to zoom in on.
- **Zooming out:** Click the  Zoom Out icon or hold down the Ctrl key and click your right mouse button. Each click undoes one zoom in action. Alternately, you can press Ctrl+Shift+F5 to zoom out one level.

Click the  Autoscale icon or press Ctrl+right mouse button to restore the graph to its original zoom level.

Scrolling Within Graphs

If you have zoomed in on a graph, you can scroll around the graph by holding down the Spacebar and dragging the graph around with your mouse. Or, click the  Scroll Chart icon and drag the graph with your mouse.

Axis Settings

To control the values shown on each axis, follow these steps:

1. Double-click on a graph to open the Edit Graph dialog. Then click **Advanced**.

2. In the **Chart** tab, choose the **Axis** tab. (The editing dialog has multiple levels of tabs.)
3. In the **Axis** tab, you can select an axis and then change many aspects of how that axis is displayed. For example, click **Change** under Minimum or Maximum to change the range of values for that axis. Also, this tab has a checkbox to turn logarithmic scaling on and off.
4. If you drag the Edit Graph dialog to the side, you can see the effects of changes in your graph as you make them.
5. Click **Close** when you are finished changing the display.

Other tabs of interest in the advanced Edit Graph dialog are as follows:

- **Chart→Axis→Title:** Modify axis title display.
- **Chart→Axis→Labels:** Modify axis label formats and font.
- **Chart→Axis→Ticks:** Modify major ticks on selected axis.
- **Chart→Axis→Minor:** Modify minor ticks on selected axis.
- **Chart→Titles:** Modify graph title text, location, and format.
- **Chart→Legend:** Modify graph legend location and format.

For help on settings in the advanced Edit Graph dialog, move to a field and press F1.

Copying Graphs to the Clipboard

To copy a graph to the clipboard for pasting into another applications, press Ctrl+C while in the page that contains the graph. Alternately, right-click on the graph and choose **Edit** from the pop-up menu. Then, click the **Copy** button in the Edit Graph dialog.

By default, graphs that you copy use the current display size of the graph. You can set a specific size for all graphs you copy by choosing **Edit→Copy as Image Settings** and selecting the image size you want to be able to paste into other applications. For example, you can choose to copy graphs as 4"x6" images, no matter what size graphs are on your screen display.

Printing Graphs

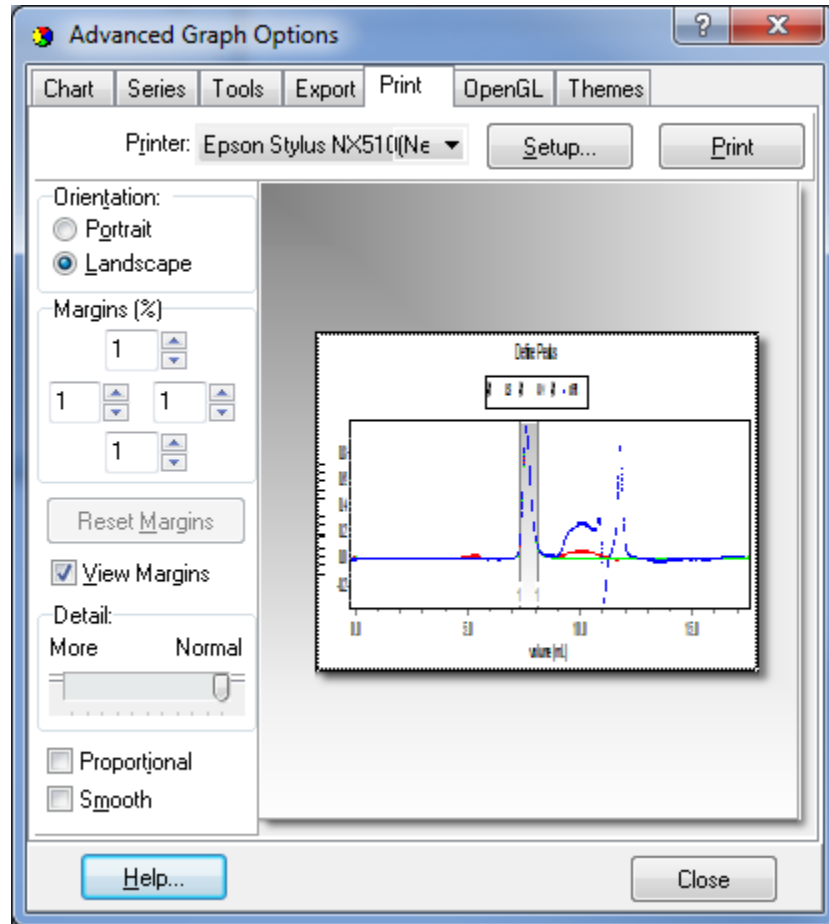
To print a graph, do either of the following:

- Right-click on the graph and choose **Print**.
- Choose **File→Print** from the menus.

You can choose **File→Page Setup** to choose a paper size and source, page orientation, and margin widths. You can choose **File→Print Setup** to choose a printer and set properties for your printer.

Alternatively, you can print a graph with more control over the output by following these steps:

1. Double-click on a graph to open the Edit Graph dialog. Then click **Advanced**.
2. Choose the **Print** tab from the top row of tabs.



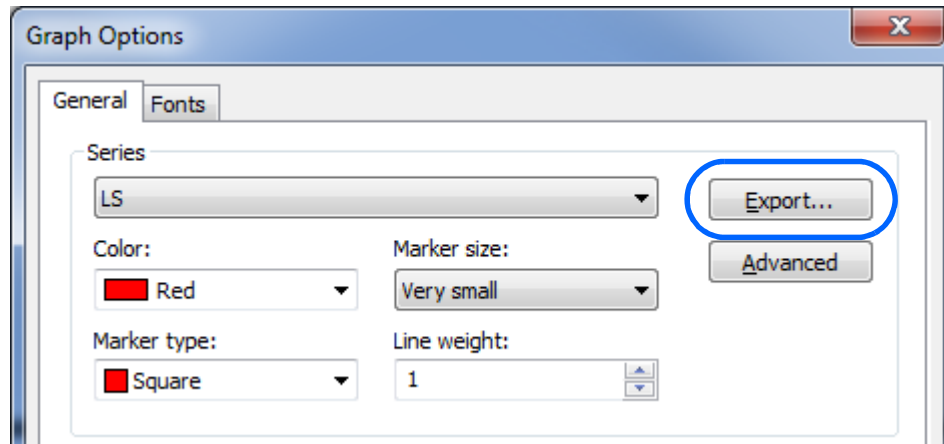
3. In the Printer list, choose the printer you want to send the graph to. Click **Setup** if you want to adjust printing properties.
4. Choose an orientation of Portrait or Landscape. The default is Landscape, which is appropriate for most graphs.
5. Adjust the **Margins** as needed. Note that margins are specified as a percentage of the page size.
6. Use the **Detail** slider to adjust the quality of the output and number of major tick marks on the axes.
7. Put a checkmark in the **Proportional** box if you want the graph to have a height and width proportional to the current graph display. After you check this box, you can drag the dotted lines on the preview to resize the graph on the page.
8. Put a checkmark in the **Smooth** box if you want data smoothing applied to the printout.
9. When you are ready to print, click **Print**.

Exporting Graphs

You can export graphs as pictures or data for use in other applications. You do this with the dialog you see when you double-click on a graph.

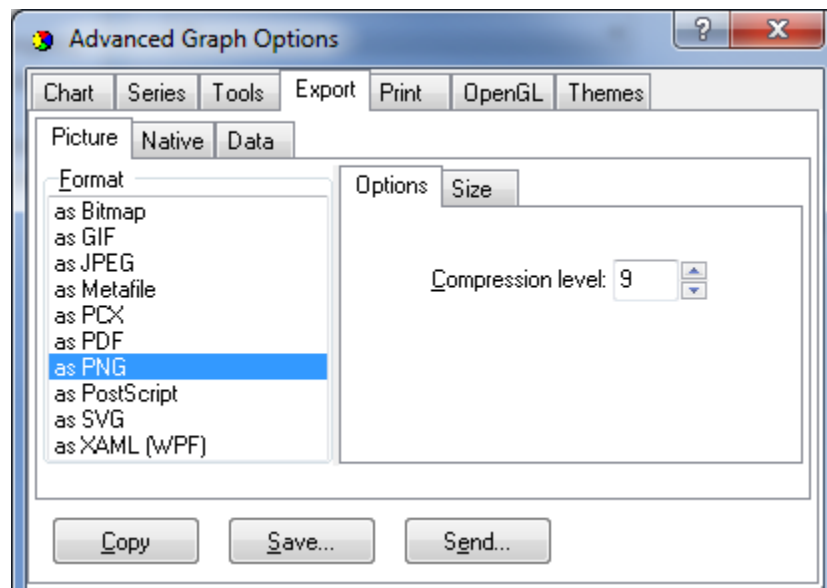
Exporting Pictures

To quickly export a graph image, double-click on a graph to open the Edit Graph dialog. Click the **Export** button and choose the JPEG output file type. See “Working with Procedure Graphs” on page 8-5 for details.



Alternatively, you can export a graph to a graphics file with more control over the output by following these steps:

1. Double-click on a graph. In the Edit Graph dialog, click **Advanced**.
2. Choose the **Export** tab from the top row of tabs.
3. Choose the **Picture** tab from the second row of tabs.



4. Select a file format to export. Metafile is a vector-based Windows Metafile (.wmf) used by applications such as Microsoft Word. SVG is also a vector-based format. Bitmap, GIF, JPEG, PNG, and PCX are all pixel-based image formats with different types of compression. PDF is the Adobe Acrobat format. PostScript is an output format used by many printers. Since encapsulated PostScript is created, some applications can import graphics in this format. XAML (WPF) is a Windows-supports derivative of XML.
5. The **Options** and **Size** tabs offer different settings depending on the format you select.
6. Once you have set the format and options, click one of these buttons:
 - **Copy:** Store the graphic on the clipboard in this format for pasting into another application.
 - **Save:** Send the graphic to a file of this type. You are prompted for the file name and location.
 - **Send:** Send the graphic to an application such as Microsoft Outlook. This is typically used to email the graphic.

Exporting Data

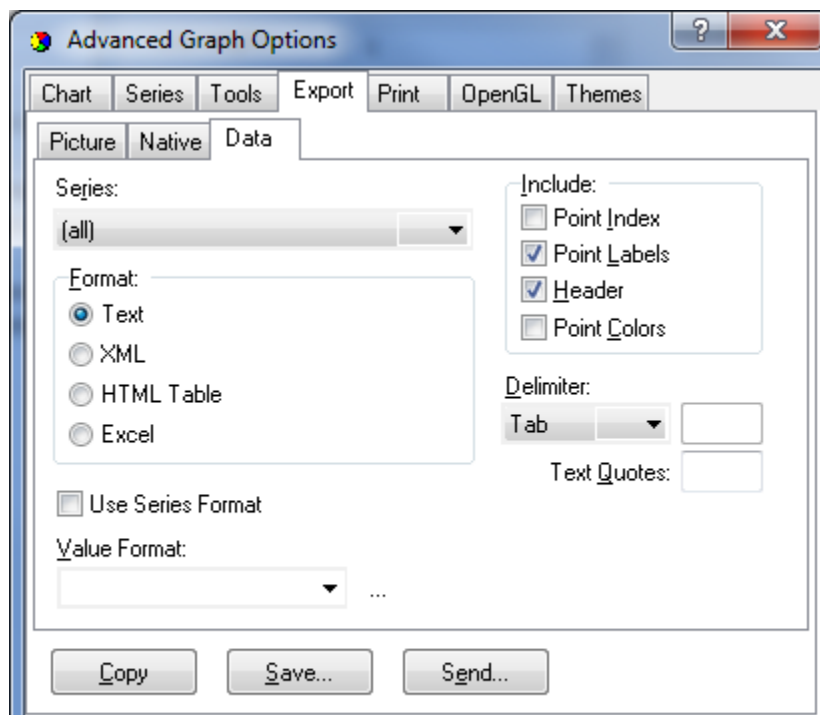
To quickly export graph data, double-click on a graph to open the Edit Graph dialog. Click the **Export** button and choose a data output file type. You can choose to save the graph in one of the following formats:

- Data saved as Microsoft Excel file (.xls)
- Data saved as comma-delimited text file (.csv)
- Data saved as tab-delimited text file (.txt)
- Data saved as tagged XML file (.xml)
- Image saved as JPEG file (.jpg)

Alternatively, you can export a graph to a data file with more control over the output by following these steps:

1. Double-click on a graph to open the Edit Graph dialog. Then click **Advanced**.
2. Choose the **Export** tab from the top row of tabs.

3. Choose the **Data** tab from the second row of tabs.



4. Select the output format you want: text, XML, HTML table, or Microsoft Excel. If you select Text, you can select a Delimiter to separate the fields. Delimiters are commonly used if you will be importing the data into a spreadsheet or database. You can also specify a quote character (usually blank, ', or ") to use around text in the output.
5. Select any other information you want to include in the data file, such as headers.
6. Once you have set the format and other options, click one of the following buttons:
 - **Copy:** Store the data on the clipboard in this format for pasting into another application.
 - **Save:** Send the data to a file of this type. You are prompted for the file name and location. The default file extension matches the format you selected in the Edit Graph window.
 - **Send:** Send the data to an application such as Microsoft Outlook. This is typically used to email the data.

If you do not find the organization of data output useful, try the output described in “Exporting an Experiment” on page 6-17.

12

Working with Profiles

This chapter explains how to create and use profiles in ASTRA 6.

CONTENTS	PAGE
About Profiles.....	12-2
Creating Profiles	12-3
Modifying Profiles.....	12-5
Using Profiles	12-8

About Profiles

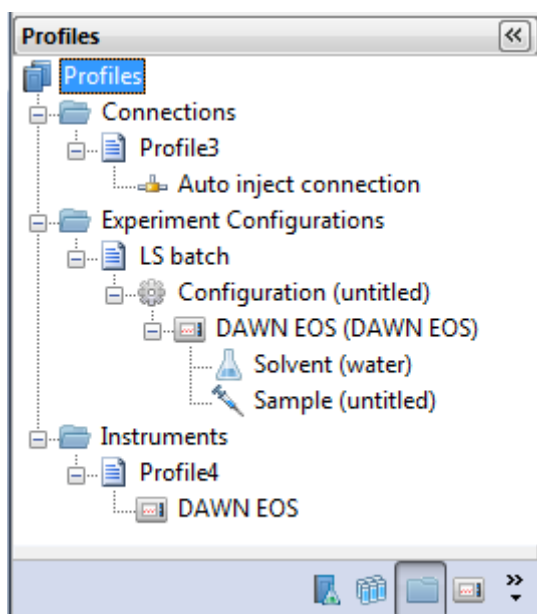
In an experiment, a set of instrument and connection profiles describes how an experiment is set up. This is called the *configuration*. You can also create *profiles* that are stored outside of experiments and can be copied into experiments as needed. Profiles provided with ASTRA are called *system profiles*.

The experiment methods provided with ASTRA contain commonly used configurations. However, if your instrument setup differs from the default methods, you may find that you need to modify new experiments in the same way each time you create one.

Profiles can save time by creating reusable blocks of information about your instruments and experiment setup. You can copy this information into new experiments. (Another way to save time is to save experiments as methods as described in “Creating a Method” on page 6-18.)

This chapter focuses on profiles. See Chapter 7, “Configuring Experiments” for information about configurations and for reference information about the properties of all the available profile items that make up configurations and profiles.

You can work with profiles in the Profiles navigation pane.



Creating Profiles


There are several ways to create profiles. These profiles are stored in the system database, rather than in separate files. Experiments use copies of profiles, but modifying the portion of an experiment configuration that came from a profile does not affect the profile itself.

Creating a New Profile

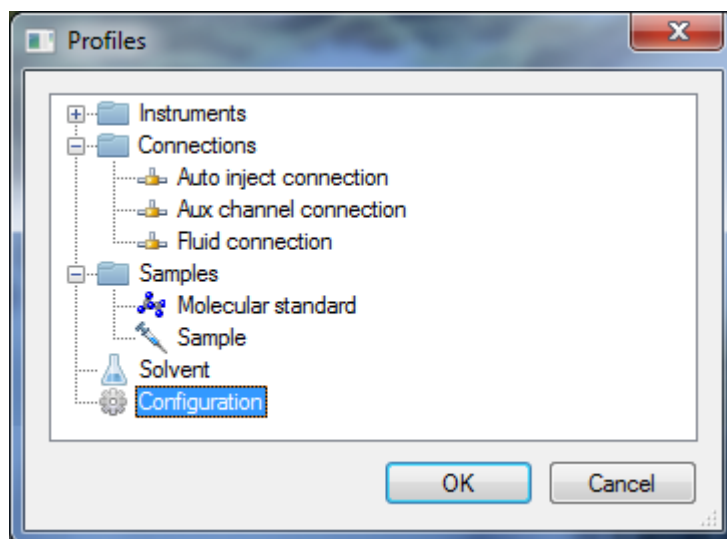
To create a profile with settings used in your experiments using the default profiles as a starting point, follow these steps:

1. Choose **File→New→Profile**. You will see the Profiles dialog.

Shortcuts: Press Ctrl+Alt+N.

Click the down-arrow next to the  icon.

Right-click on any profile item in the workspace, and choose **New**.



2. In the Profiles dialog, select the type of profile you want to create.
3. Click **OK**.
4. In the Save As dialog, type a name for the profile. You can create a folder (such as “My Profiles”) in the system database to store profiles.
5. Double-click the item you created in the Profiles navigation pane to open its property page.

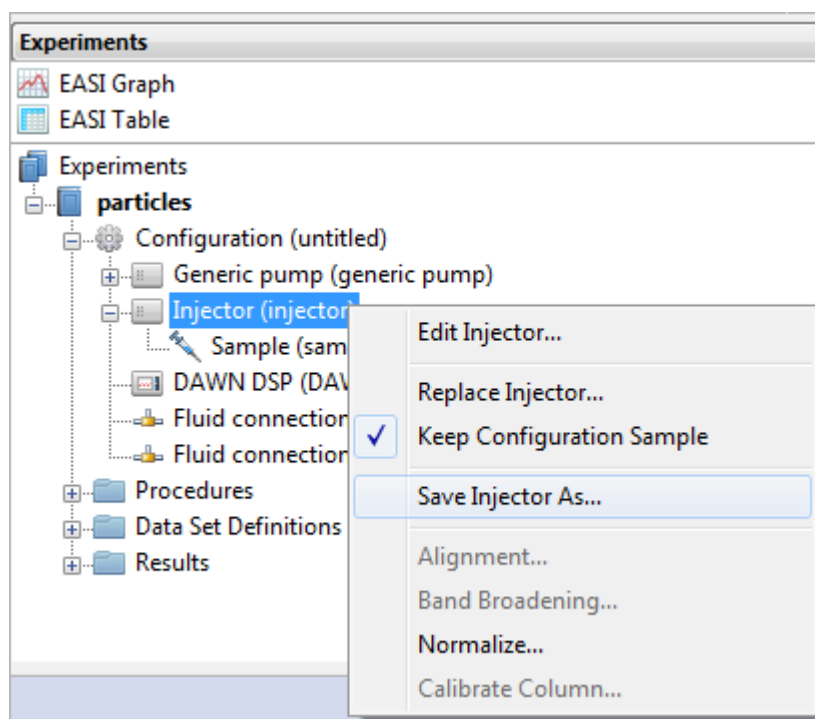
If you created a profile type that has multiple components, there will be a tab for each component. For example, a light scattering instrument in batch mode has a tab for a solvent and a sample. This information is stored with and imported with the instrument profile.

6. Edit the properties as needed. See Chapter 7, “Configuring Experiments” for details about the properties of all profile types.
7. Click **OK** or **Apply** to save your changes.

Saving as a Profile

You can create a profile by saving it from an experiment. To do this, follow these steps:

1. If you have more than one experiment open, make sure the one you want to export from is selected in the Experiments navigation pane.
2. Select the item in the configuration you want to export. (Any items nested at a lower level will be exported along with the item you select. For example, in the following figure, exporting the injector creates a profile that contains the injector and the sample. If you export the configuration item, the entire configuration is saved as a profile.)



3. Choose **Experiment**→**Configuration**→**Save As**. Or right-click on an item and choose the **Save As** item from its right-click menu.
4. In the Save As dialog, choose the folder where you want to save the profile. You might want to create a folder called “My Profiles” to contain your custom profiles. Then type a name for the profile you are creating, and click **OK**.

Modifying Profiles

The profiles are contained in the system database. To work with a profile you have already created or one of the system profiles provided with ASTRA 6, you first open the profile in the Profiles navigation pane. Then you can view, edit, or rename the profile.


Opening a Profile

If you just created a profile, it is open and listed in the Profiles navigation pane. If the profile is not listed, you must open it before you can modify it.

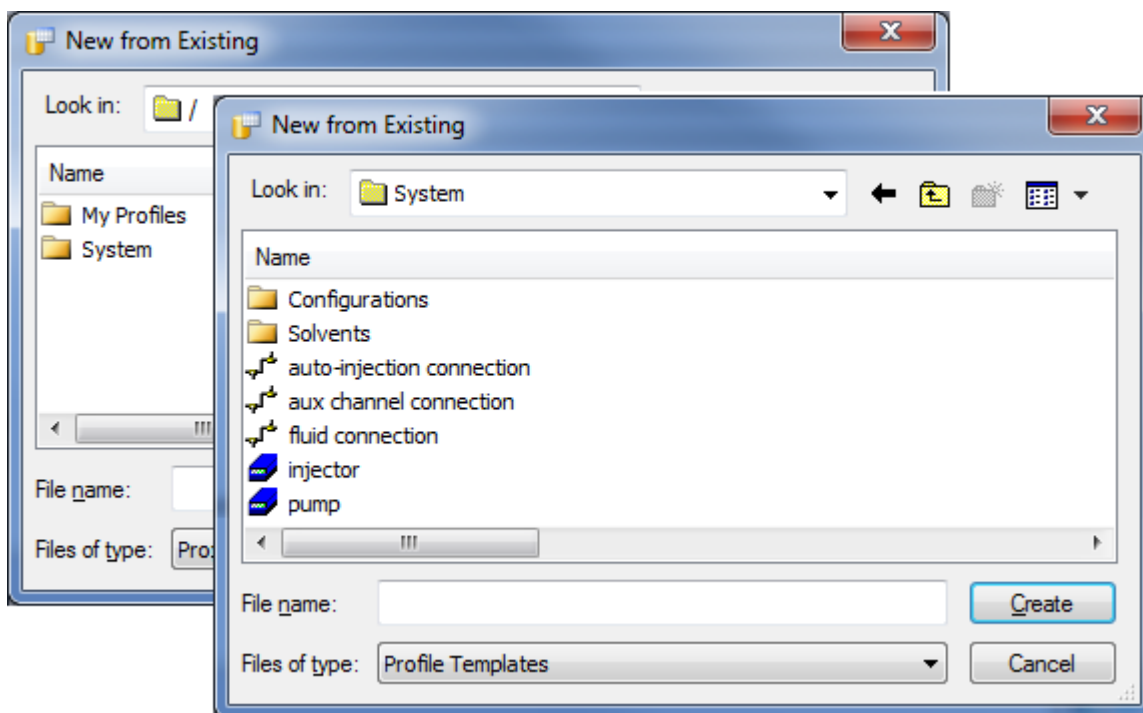
To open a profile, follow these steps:

1. Select **File→Open→Profile**.

Shortcuts: Press Ctrl+Alt+O.


Click the down-arrow next to the  icon.

2. In the Open dialog, locate the profile you want to open.



Profiles may be stored in any of the following folders:

- **My Profiles:** A handy place to save profiles you create.
 - **System > Configurations:** A set of complete configurations.
 - **System > Solvents:** A collection of solvent profiles.
3. You can select the type of profile you want to find in the Of Type list.

As in standard file selection dialogs, you can click the  icon to change the view of the list of experiments in the database. In the detail view, the last data and time the experiment was modified is shown.

4. Click **Open**. You see the profile listed in the Profiles navigation pane.

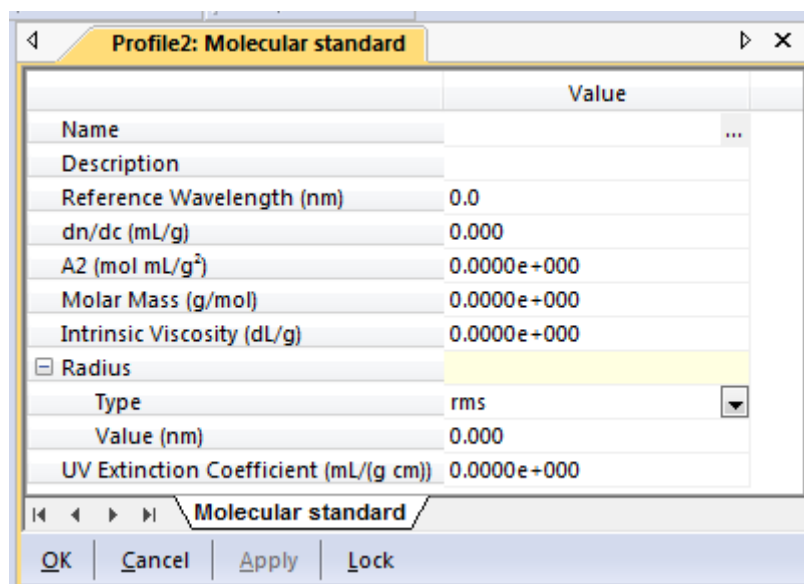
Editing a Profile

To modify a profile that you have opened, follow these steps:

1. Double-click the item you created in the Profiles navigation pane to open its property page.

If you created a profile type that has multiple components, there will be a tab for each component. For example, a light scattering instrument in batch mode has a tab for a solvent and a sample. This information is stored with and imported with the instrument profile.

2. Edit the properties as needed. See Chapter 7, “Configuring Experiments” for details about the properties of all profile types.



Name	Value
Name	...
Description	
Reference Wavelength (nm)	0.0
dn/dc (mL/g)	0.000
A2 (mol mL/g ²)	0.0000e+000
Molar Mass (g/mol)	0.0000e+000
Intrinsic Viscosity (dL/g)	0.0000e+000
<input checked="" type="checkbox"/> Radius	
Type	rms
Value (nm)	0.000
UV Extinction Coefficient (mL/(g cm))	0.0000e+000

3. Click **OK** or **Apply** to save your changes.

Information from a profile is copied when it is used in an experiment. After being copied, there is no link between the profile and the experiment. So, editing a profile has no effect on experiments to which the profile was previously copied. In addition, modifying portions of an experiment configuration that came from a profile has no effect on the original profile.

Saving a Profile

Changes to profiles are automatically saved to the system database when you click **OK** or **Apply** in their property page. If you attempt to close the page without saving, you are asked whether to save the changes.

Duplicating a Profile with Save As

To save a profile with another name, follow these steps

1. Select a profile in the Profiles navigation pane.
2. Choose **File→Save As**.

Shortcuts: Right-click on any profile in the workspace, and choose **Save As**.

3. In the Save As dialog, select the system database location where you want to save the new profile.
4. Type a name for the new profile.
5. Click **Save**.

You can use Save As and then delete the original profile to rename a profile.

Closing a Profile

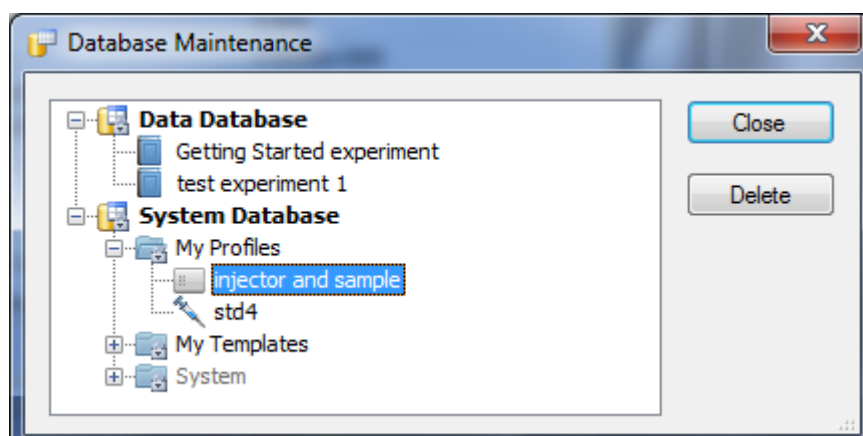
To close a profile, select the profile you want to close and choose **File→Close**. The profile is still available for use in the system database; it is simply not shown in the Profiles navigation pane.

Shortcuts: Right-click on any profile in the workspace, and choose **Close**.

Deleting a Profile

To delete an existing profile, follow these steps:

1. Select **System→Database Administration→Maintenance**.
2. In the Database Maintenance dialog, select one or more profiles to delete. Read-only profiles, such as those provided with ASTRA are shown in gray and cannot be deleted.



3. Click **Delete**.
4. You are asked if you are sure you want to delete the selected profile. Click **Yes** if you are sure.
5. Click **Close** when you are finished deleting profiles.

Using Profiles

The benefit of creating profiles is that you can use them to save time when configuring your experiments.

If you later edit properties of items you import or copy from a profile, there is no effect on the profile from which it was obtained.

For more about using profiles, see “Using Configurations” on page 7-5.

Adding an Item to a Configuration

You might want to add a component to an experiment. For example, you may have added a UV instrument to an experiment.

To add an item to an experiment configuration using a profile, follow these steps:

1. In the Experiments navigation pane, select the item that you want to replace. For example, this may be a solvent, instrument, sample, or even the entire experiment configuration.
2. Choose **Experiment→Configuration→Replace**. You see the Select Profile dialog.
3. In the Of Type field, select the type of item you are looking for: Connections, or Instruments.
4. Browse the system database for a profile to import.
5. When you find the profile, select it and click **Open**.

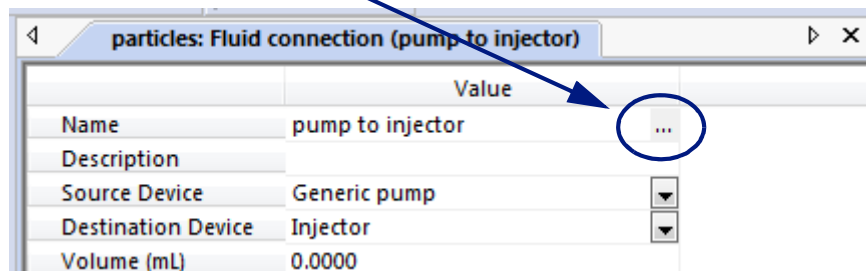
The item is added to your experiment. (If you want to import an entire configuration, see “Replacing an Entire Configuration” on page 12-9.)

Replacing a Single Configuration Item

You can copy all the properties set for a profile into the property page for an item in an experiment configuration. This has the effect of replacing the item in the experiment with a profile.

To copy from a profile to an experiment, follow these steps:

1. Double-click on an item in Configuration tree in the Experiments navigation pane to open its property page.
2. Click the browse button (“...”) to the right of the Name property.



3. In the Copy from Existing dialog, find the profile you want to copy from. The Of Type field is automatically set to match only the type of item you are editing.
4. Click **Copy**. The values of properties in the profile are copied to the experiment.

Replacing an Entire Configuration

You can replace the entire configuration with an experiment configuration that has been saved as a profile. For example, you might have a standard experiment configuration that you want to use in many different experiments. You can import that configuration into an existing experiment.

To import a completely new configuration, follow these steps:

1. If you have more than one experiment open, make sure the one you wish to modify is selected in the Experiments navigation pane.
2. Select the name of the experiment (that is, the top-level node of the experiment) in the Experiments navigation pane.
3. If you want to import an entire configuration, but keep the sample(s) the same as when you ran the experiment, right-click on the Configuration node of the experiment and make sure the checkmark next to the **Keep Configuration Sample** is toggled on.
4. Choose **Experiment→Configuration→Replace**. You see the Select Profile dialog.
5. In the Of Type field, select Experiment Configurations.
6. Browse the system database for a profile to import. A number of configurations are provided with ASTRA 6 in the Example Configurations folder.
7. When you find a profile, select it and click **Open**.
8. If you select the profile of an instrument or connection, the item is added to your experiment. If you select the profile of an entire configuration, that configuration replaces the existing one.

For more information about replacing a configuration or part of a configuration with a profile, see “Replacing an Experiment Configuration or Item” on page 7-7.

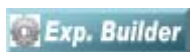
A Menu Quick Reference

This chapter contains a quick reference for ASTRA 6 menu commands and keyboard shortcuts.

CONTENTS	PAGE
Modes and User Levels	A-2
File Menu	A-2
Edit Menu	A-4
View Menu	A-4
Experiment Menu	A-5
Processing Menu	A-6
Sequence Menu	A-7
System Menu	A-7
Window Menu	A-8
Help Menu.....	A-9

Modes and User Levels

The following tables list the commands in the ASTRA 6 menus. The Description column includes a link to more information about the command. The Modes column identifies when the command is available as follows:



- **Builder:** This command is inactive unless you enable **System→Preferences→Experiment Builder Mode**, which is only available to researchers and administrators.



- **DB and Security:** This command exists only when using ASTRA 6 with Research Database or ASTRA 6 with Security Pack.



If a command is not limited to “DB and Security”, it is always available in ASTRA 6 Basic, even if user levels are listed. The user levels apply only to ASTRA 6 with Research Database and ASTRA 6 with Security Pack.

- **G:** Guest user level
- **T:** Technician user level
- **R:** Researcher user level
- **A:** Administrator user level

File Menu

The File menu contains the following commands:

Command	Keyboard Shortcut	Description	Modes
File→New			
→Experiment from Default		Create an experiment from the default method. See page 6-4.	T, R, A
→Experiment from Method	Ctrl+Alt+D	Create an experiment from a method. See page 6-5.	T, R, A
→Blank Experiment	Ctrl+Shift+V	Create an empty skeleton of an experiment. See page 6-6.	Builder: R, A
→Sequence from Default		Create a sequence from the default sequence template. See page 9-4.	T, R, A
→Sequence from Template		Create a sequence from a sequence template. See page 9-4.	T, R, A
→Blank Sequence	Ctrl+Shift+N	Create an empty skeleton of a sequence. See page 9-3.	R, A
→Profile	Ctrl+Alt+N	Create a profile. See page 12-3.	R, A
File→Open			
→Experiment	Ctrl+O	Open an existing experiment. See page 6-8 and page 6-7.	All

Command	Keyboard Shortcut	Description	Modes
→Sequence	Ctrl+Shift+O	Open an existing sequence. See page 9-5.	All
→Profile	Ctrl+Alt+O	Open an existing profile. See page 12-5.	All
File→Close		Close the active item. See page 6-15 and page 12-7.	All
File→Close All		Close all items in the current tab. See page 6-15.	All
File→Save	Ctrl+S	Save the selection. See page 6-16 and page 6-15.	T, R, A
File→Save As		Save the selection with a new name. See page 6-16 and page 6-15.	T, R, A
File→Save As Method		Save the experiment as a method. See page 6-18.	T, R, A
File→Import			
→Experiment		Import an experiment from a file. See page 6-9.	DB and Security: R, A
→Sequence		Import a sequence from an ASTRA 4 or ASTRA 5 file. See page 9-5.	DB and Security: R, A
→Empower Sequence		Import a sequence created with Waters Empower. See page 9-6.	R, A
File→Export		Save the selected experiment or sequence to a separate file. See page 6-17 and page 9-13.	R, A
File→One-to-Many		Apply experiment method to multiple experiments. See page 6-22.	T, R, A
File→Print	Ctrl+P	Print the currently active view. See page 3-16.	All
File→Print Preview		Preview the currently active report. See page 10-7.	All
File→Page Setup	Ctrl+Alt+P	Set up paper size, orientation, and margins. See page 3-16.	All
File→Print Setup		Set up page formatting for printing. See page 3-16.	All
File→Properties		View the properties of the current experiment. See page 6-14.	All
File→Recent Files		Open a recently used experiment. See page 6-8 and page 6-7.	All
File→Exit		Close all windows and exit from ASTRA 6. See page 3-17.	All

Edit Menu

The Edit menu contains the following commands:

Command	Keyboard Shortcut	Description	
Edit→Undo	Ctrl+Alt+Backspace	Undo the previous action. This command is currently disabled.	All
Edit→Cut	Ctrl+Shift+Delete	Cut the currently selected item and place it on the clipboard. This command is available only as appropriate.	All
Edit→Copy	Ctrl+C	Copy the currently selected item to the clipboard. This command is available only as appropriate.	All
Edit→Copy as Image Settings		Set the size of the image you want when you copy a graph. See page 11-17.	All
Edit→Paste	Ctrl+V	Paste the contents of the clipboard to the current location. This command is available only as appropriate.	All
Edit→Delete		Delete the currently selected item. This command is available only as appropriate.	All
Edit→Select All	Ctrl+A	Select everything in the active view. This command is available only as appropriate.	All

View Menu

The View menu contains the following commands:

Command	Keyboard Shortcut	Description	
View→EASI Graph		Open a quick, customizable graph that can compare data across experiments. See page 11-3.	All
View→EASI Table		Add a customizable table that lists data from multiple experiments. See page 11-6.	All
View→Experiments		Select the Experiments navigation pane. See page 3-14.	All
View→Sequences		Select the Sequences navigation pane. See page 3-14.	All
View→Profiles		Select the Profiles navigation pane. See page 3-14.	All
View→Instruments		Select the Instruments navigation pane. See page 3-14.	All
View→Toolbars			All
→Standard Toolbar		Hide and show toolbar with new, open, save, and print icons. See page 3-14.	All
→Processing Toolbar		Hide and show toolbar with begin, validate, run, pause, and stop icons. See page 3-14.	All

Command	Keyboard Shortcut	Description	
→Graph Toolbar		Hide and show toolbar with icons for use with graphs. See page 3-14.	All
→Customize		Open dialog to customize toolbars and keyboard shortcuts. See page 3-14.	All
View→Status Bar		Hide and show status bar at bottom of main window. See page 3-14.	All
View→Visual Manager		Open dialog to set Windows display options. See page 3-14.	All
View→Full Screen		Toggle between normal display and display that maximizes room for graph display. See page 3-14.	All
View→Logs			
→Experiment		Display experiment log for a single experiment. See page 4-9.	All
→Sequence		Display sequence log. See page 4-9 and page 9-12.	All
→System		Display and save system database log. See page 4-8.	All
→Database		Display and save full experiment log. See page 4-9.	All

Experiment Menu

The Experiment menu contains the following commands:

Command	Keyboard Shortcut	Description	
Experiment→Configuration			
→Edit		Open the property page for configuration of the currently selected experiment. See page 7-5.	All (view-only as Guest)
→Replace		Import a configuration item or entire configuration from a profile. See page 7-7 and page 12-4.	R, A
→Save As		Export a configuration item or an entire configuration to a profile. See page 7-9 and page 12-3.	R, A
→Alignment		Opens procedure page for determining the interdetector delay. See page 8-18.	R, A
→Band Broadening		Opens procedure page for correcting effects of fluid mixing between instruments. See page 8-20.	R, A
→Normalize		Opens procedure page for relating detector signals to the 90 degree detector signal and the instrument calibration constant. See page 8-23.	R, A
→Calibrate Column		Perform a column calibration for a SEC column. See page 8-39.	R, A

Command	Keyboard Shortcut	Description	
Experiment→Add To Experiment	Ctrl+Shift+P	Add a procedure, report, graph, or data set definition to an experiment. See page 8-8, page 10-8, and page 11-11.	Builder: R, A
Experiment→Copy From		Copy data from one experiment to the current experiment. See page 6-21.	R, A
Experiment→Apply Method		Apply procedures and results to the current experiment, creating a new experiment. See page 6-22.	T, R, A
Experiment→Report			
→Add Report		Add a report to the experiment. See page 10-8.	R, A
→Export		Export a report to a text file or a comma-separated values file. See page 10-7.	R, A
→Report Options		Set name, title, summary, and logo for the current report. See page 10-5.	--
Experiment→Graph			
→Add Custom Plot		Add a custom plot to the results. See page 11-11.	R, A
→Add Parametric Plot		Add a parametric plot to the results. See page 8-88.	R, A
→Add Surface Plot		Add a surface plot to the results. See page 11-13.	R, A
Experiment→Sign Off		Allow user to sign off experiment for 21 CFR Part 11 compliance. See page 6-13.	T, R, A
Experiment→Log→Open		Display experiment log. See page 4-9.	All
Experiment→Log→Save As		Save experiment log to a file. See page 4-9.	All

Processing Menu

The Processing menu contains the following commands:

Command	Keyboard Shortcut	Description	
Processing→Run Default	Ctrl+J	Create an experiment from the default method and start running it. See page 6-4.	T, R, A
Processing→Validate	Ctrl+Shift+V	Validate the experiment procedure and instrument availability. See page 6-11. Also validates sequences. See page 9-11.	T, R, A
Processing→Run	Ctrl+Shift+R	Start the experiment or sequence run. See page 6-11 and page 9-11.	T, R, A
Processing→Run Indefinitely		Run the experiment data collection until manually stopped. See page 6-12.	T, R, A
Processing→Pause		Temporarily stop data collection.	T, R, A
Processing→Stop	Ctrl+Shift+S	Halt the experiment or sequence. See page 6-13 and page 9-11.	T, R, A

Sequence Menu

The Sequence menu contains the following commands:

Command	Keyboard Shortcut	Description	
Sequence→Edit		Open the property page for configuration of the currently selected sequence. See page 9-7.	All (view-only as Guest)
Sequence→Log→Open		Display sequence log. See page 9-12.	All
Sequence→Log→Save As		Save sequence log to a file. See page 9-12.	All

System Menu

The System menu contains the following commands:

Command	Keyboard Shortcut	Description	
System→Instruments		View list of connected instruments. See page 2-12.	All
System→Configuration Wizard		Create a configuration using step-by-step dialogs. See page 7-11.	R, A
System→Database Administration			
→Connections		Connect to a different experiment database. See page 4-5.	DB and Security
→Maintenance		Delete experiments, sequences, profiles, or methods from database. See page 4-10, page 6-20, page 9-13, and page 12-7.	DB and Security: R, A
→Automatic Maintenance		Perform database maintenance. See page 4-10.	DB and Security: All
→Import System Database		Update system database with latest ASTRA methods and profiles. See page 2-4.	DB and Security: A
→Log→Open		View experiment database log. See page 4-9.	DB and Security: All
→Log→Refresh		Get latest entries for experiment database log. See page 4-9.	DB and Security: All
→Log→Save As		Save experiment database log to a file. See page 4-9.	DB and Security: All
System→Feature Activation		Activate features by providing activation key. See page 2-6.	DB and Security: A
System→Security		Specify a domain to use for user authentication. See page 2-9.	DB and Security: A

Command	Keyboard Shortcut	Description	
System→Preferences			
→Options		Set defaults for configurations and methods. See page 6-19 and page 7-10.	All
→Experiment Builder Mode		Set to Experiment Builder mode. See page 1-3.	DB and Security: R, A
→Show Desktop Alerts		Show instrument alarms in Windows taskbar. See page 2-12.	All
→Default to ASTRA V Band Broadening		Toggle the default between ASTRA V and ASTRA 6 Band Broadening calculations. See page 8-20.	All
→Reset Warnings		This will reset the state of any “Do not show me again” flags that the user sets when prompted with a warning.	All
System→Log→Open		View system database log. See page 4-8.	All
System→Log→Refresh		Get latest entries for system database log. See page 4-8.	All
System→Log→Save As		Save system database log to a file. See page 4-8.	All

Window Menu

The Window menu contains the following commands:

Command	Keyboard Shortcut	Description	
Window→Close	Ctrl+F4	Close current view. See page 3-15.	All
Window→Close All		Close all views. See page 3-15.	All
Window→Next	Ctrl+Tab	Move next view to front. See page 3-15.	All
Window→Previous	Ctrl+Shift+Tab	Move previous view to front. See page 3-15.	All
Window→Cascade		Arrange open views in cascading fashion. See page 3-15.	All
Window→Tile Horizontal		Arrange open views in column (wide views). See page 3-15.	All
Window→Tile Vertical		Arrange open views in row (tall views). See page 3-15.	All
Window→Arrange Icons		Line up minimized windows. See page 3-15.	All
Window→Tab Groups		Toggle between tabbed pages and separate windows in workspace. See page 3-15.	All
Window→Windows		Open a list of windows to select from. See page 3-15.	All

Help Menu

The Help menu contains the following commands:

Command	Keyboard Shortcut	Description	
Help→Contents	F1	Open help table of contents. See page 3-16.	All
Help→Search		Open search tab of online help.	All
Help→Index		Open index tab of online help.	All
Help→Wyatt Online		Open Wyatt Support Center website.	All
Help→Check for Updates		Look for newer versions of ASTRA available for download. See page 2-3.	All
Help→About ASTRA		Show version and copyright information about ASTRA 6.	All
Help→What's New		Display a window showing important changes in the current ASTRA version.	All

B System Methods

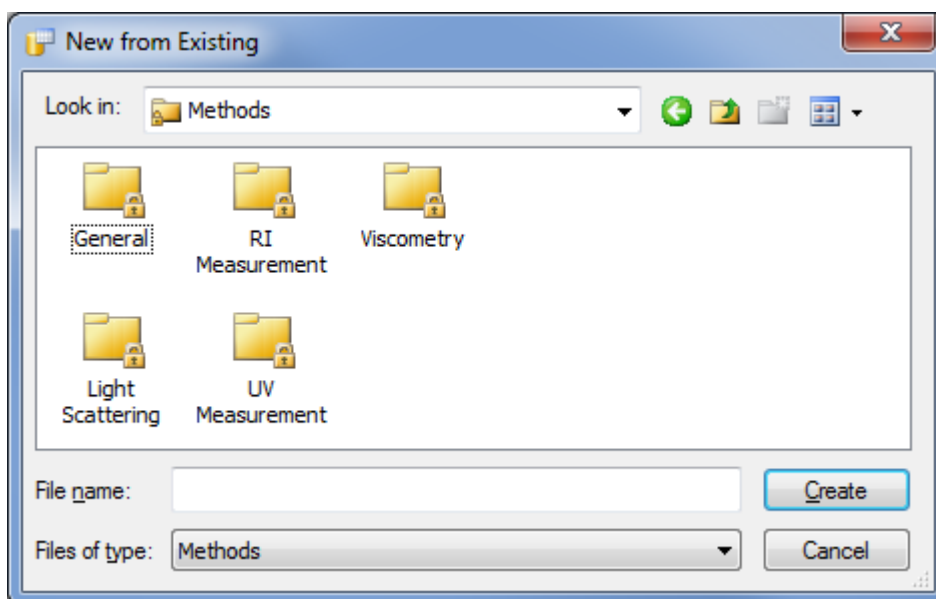
This appendix provides an overview of the ASTRA 6 System Methods

CONTENTS	PAGE
Overview	B-2
General	B-3
Light Scattering	B-4
RI Measurement	B-11
UV Measurement	B-14
Viscometry	B-14

Overview

This appendix provides an overview of the ASTRA 6 system methods. These methods are stored in five different folders: General, RI Measurement, Viscometry, Light Scattering, UV Measurement. This appendix is organized according to the folders that contain the methods.

To see the available methods, choose **File→New→Experiment from Method** and browse to the System > Methods folder.



Some methods are only usable with a feature activation keys.

Some methods need to be applied to an already existing experiment and some are used to run a new experiment. This information is provided along with the name of each method, using these abbreviations:

- **CM = Collection Method.** Methods used to collect new data.
- **AM = Analysis Method.** Methods that are generally applied to an already run experiment to analyze data in an additional way.

Hint:	If you read about an experiment method you want to use, but don't see it in the New from Existing dialog, see "Migrating the System Database" on page 2-4 to update your system database so you have all the latest experiment methods and system profiles.
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General

The following methods are provided in the General folder.

Concentration Determination (AM)

After you select the peaks and enter the dn/dc value for each peak, the average concentration and calculated mass is displayed in the report for each peak.

Results

Peak Results

	Peak 1	Peak 2
Concentration (mg/mL)		
Average concentration (mg/mL)	0.094 (±0.0%)	0.019 (±0.0%)
Masses (µg)		
Calculated mass (µg)	167.75	28.76

Peak Areas (AM)

This method includes the Peak Areas procedure (page 8-88) and reports data about the area under the selected peaks. The peak area units depend on the units you have chosen in the configuration for the abscissa unit.

Results

Peak Results

	Peak 1
Masses	
Injected mass (µg)	100.80
Calculated mass (µg)	106.67
Concentration (mg/mL)	
Average concentration	0.110 (±0.0%)
Refractive index moments (min)	
peak area	3.947×10^{-5}
Light scattering moments (1/cm min)	
peak area (detector 1)	1.465×10^{-6}
peak area (detector 2)	1.459×10^{-6}
peak area (detector 3)	1.456×10^{-6}

Column Plate Count (CM)

This method includes the Column Plate Count procedure (page 8-43) and reports the values it calculates in the results.

Light Scattering

The following methods and folders are provided in the Light Scattering folder.

Baseline Subtraction

The following method is provided in the Light Scattering > Baseline Subtraction folder.

Online (CM)

Use this method to run an online light scattering experiment to measure the baseline for later baseline subtraction.

Calibration (CM)

To calibrate a Wyatt Light scattering instrument, you need to create a new experiment (**File→New→Experiment from Method**) and choose the method corresponding to your light scattering detector.

The calibration is done with a batch injection of pure and filtered toluene.

Globally the method first collects data for 30 seconds with the laser on and then does a second measurement with the laser off (dark voltage). The intensity is measured at the 90° angle. ASTRA 6 analyzes the difference between both signals to convert the volt signal into meaningful units. The resulting calibration constant is shown in the report.

Results

Calibration constant: 1.8417×10^{-4} 1/(V cm)

Methods are available for the following instruments:

- Dawn 8
- Dawn DSP
- Dawn DSP-F
- Dawn EOS
- HELEOS
- HELEOS 8
- miniDAWN
- TREOS

Utilities (CM)

When you run a single experiment or sequence, the Comet methods allow you to program the Comet cell cleaner for a certain amount of time (shown in the name of the method). Note that in these methods the laser is automatically turned off.

- HELEOS or TREOS Comet 5min
- HELEOS or TREOS Comet 10min
- HELEOS or TREOS Comet 2hour

Laser off methods can only be used with a sequence. In the United States, the recommendation is not to turn the laser off if it will be off for less than 3 weeks.

Before using a “Laser off” method, you need to customize it. This means to perform the physical instrument connection and save the resulting experiment as a method before running a sequence.

- Turn EOS laser off
- Turn HELEOS or TREOS laser off
- Turn miniDawn laser off

The “Orbit on” method places the Orbit device in Recycle mode for the TREOS and HELEOS.

Diagnostics

The following methods are provided in the Light Scattering > Diagnostics folder.

Detector Overlay (AM)

This method allows you to visualize an overlay of the light scattering detectors. It is a very useful tool for trouble shooting:

- You can check the photodiodes’ normalization.
- You can check for laser misalignment. All the detector signals should look the same for a monodisperse sample.
- You can check the for a dirty cell. If the cell is dirty, the peaks are not the same shape for all detectors.

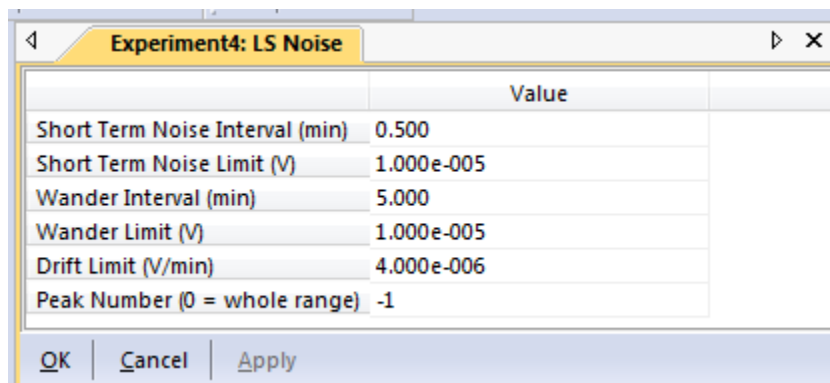
View 2 (Overlay Test) (CM)

This is similar to the Detector Overlay method.

Noise (AM)

- HELEOS Noise
- TREOS Noise
- EOS Noise
- miniDawn Noise

The noise methods analyze the noise level of the light scattering detectors. To do so, you must measure a stable baseline. Some criteria are already entered in the method in the “LS Noise” procedure.



	Value
Short Term Noise Interval (min)	0.500
Short Term Noise Limit (V)	1.000e-005
Wander Interval (min)	5.000
Wander Limit (V)	1.000e-005
Drift Limit (V/min)	4.000e-006
Peak Number (0 = whole range)	-1

OK Cancel Apply

The report indicates whether the measured noise levels are within specification.

With QELS

Batch (CM)

Use this method to do batch QELS measurements. Inject the sample directly into the light scattering detector.

The Rh from QELS graph is displayed in the “Rh from QELS” procedure.

Online (CM)

Use this method to do online QELS measurements. Run the sample through the chromatography system and detectors.

The Rh from QELS graph is displayed in the “Rh from QELS” procedure.

Regularization (AM)

Apply this method to perform a regularization regression, which permits the calculation of the size distribution of a sample.

For example, if you have a bad separation and several entities leave the column at the same time, regularization reveals this co-elution because there will be several peaks with the regularization.

Cumulants (AM)

Similarly to the Regularization analysis, applying this method calculates the size distribution of the sample. This method fits the correlation function data to a cumulant distribution.

Particles (AM)

In particles mode, it is possible to measure the size (radius) and number density of a sample using just a light scattering detector without any concentration detector. Note that you won't be able to measure the molar mass using this method.

In addition, this method computes R_h using QELS data.

Protein Conjugate (CM)

This method permits measurement of R_h from QELS data as well as RMS radius and molar mass using Protein Conjugate analysis.

Batch (Debye plot) (CM)

Use this method to do batch injection of a sample of known concentration (only one concentration).

The Debye plot is displayed together with the molar mass and radius.

Batch (Zimm plot) (CM)

Light scattering can be used to measure the second virial coefficient (A_2) of a macromolecule. ASTRA supports the analysis of rapid injections of small volumes of a sample. This proprietary online analysis can retrieve A_2 using a fraction of the sample needed for traditional measurements.

ASTRA uses a proprietary global fitting method to upgrade the Zimm plot to a more robust, modern analysis. The global fitting method removes all extrapolations from the Zimm plot, helps identify inconsistent data sets, and provides more precise and robust results for A_2 . As with all analysis procedures in ASTRA, the global fit view offers immediate visual confirmation of fit quality using a visual representation of the Zimm plot. You can change parameters in the grid view and view fit results immediately.

It is recommended that you inject six concentrations of the sample.

For this method you need to manually enter the concentration of each injected sample.

Branching (AM)

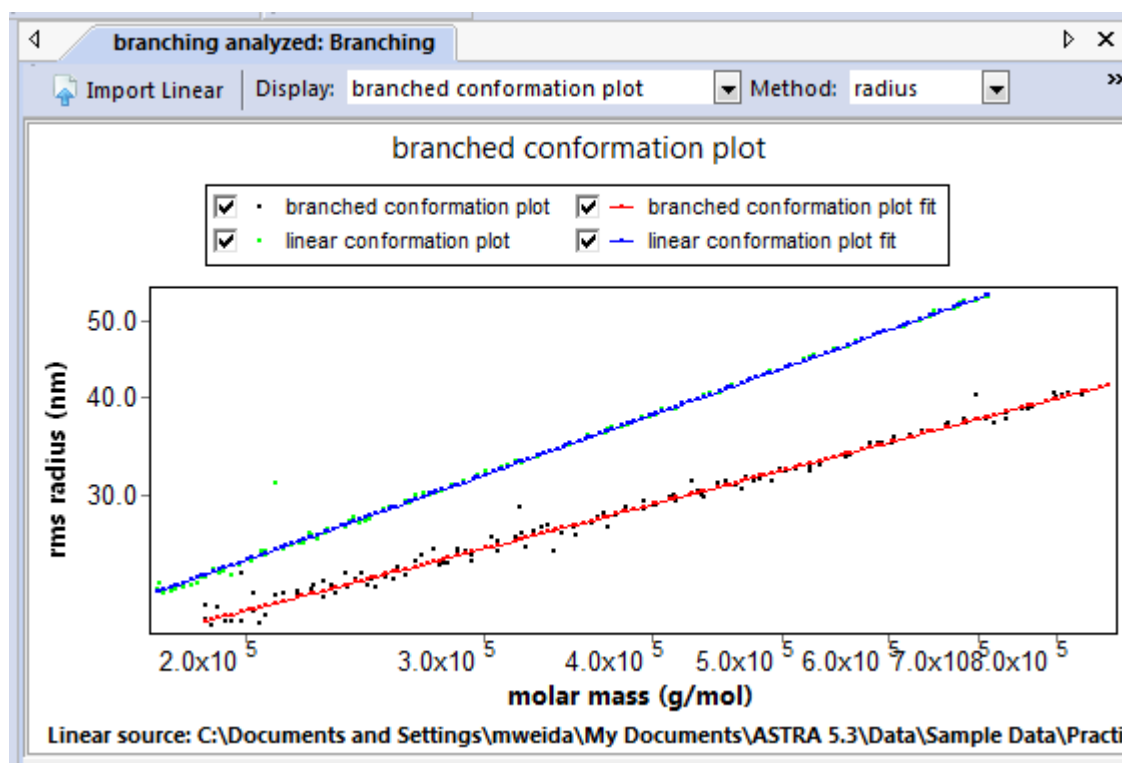
The branching characteristics of a polymer can only be determined if MALS (Multi-Angle Light Scattering) data of a linear example of the polymer exists.

Branching ratio, g
(Branching parameter)

$$g = \left(\frac{R_{br}^2}{R_{lin}^2} \right)_M$$

R = root mean square radius
(radius of gyration)

The “Branching” procedure shows the branched conformation plot and the slope of the linear and branched polymer.



The branching ratio is displayed in the report.

Online (CM)

Use this method to run basic online light scattering experiments.

With this method, the sample is injected online, using a concentration detector to measure the actual concentration (and mass) for each peak.

The RI or UV signal is used to determine the concentration and the light scattering for the intensity. With this method, the concentration doesn't need to be entered manually.

The results graph shows the mean square radius vs. volume.

Online (Zimm Plot) (CM)

This method is similar to the Online method except that the data is displayed in a Zimm plot using physical units.

Online A2 (CM)

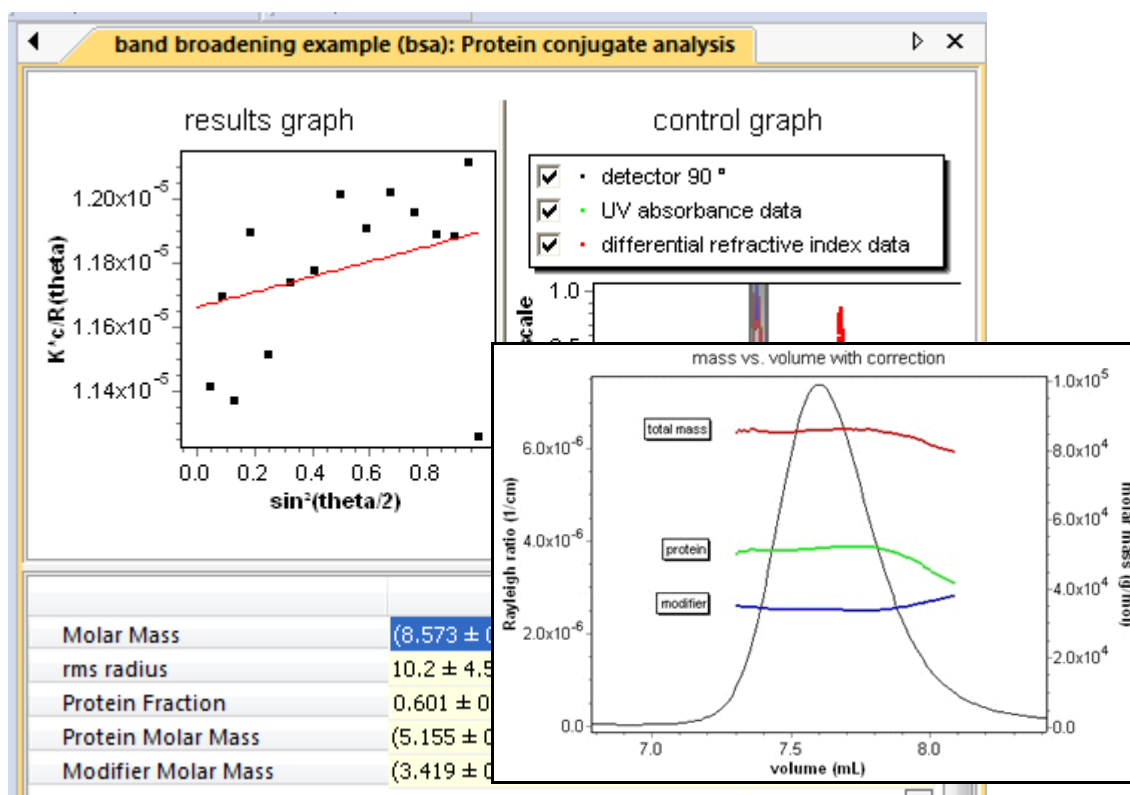
This method is similar to the Online method. In addition to mass and radius, the A2 value is computed and a Zimm plot is shown. This is similar to the Zimm batch analysis, except that it is performed in an online mode.

Protein Conjugate (AM)

This method allows you to determine the molar mass, size, and relative polymer fractions of a copolymer using light scattering. All that is required are two additional detectors that have differing sensitivities to the constituent polymers. Traditionally, light scattering has been used in conjunction with an RI and UV detector for this purpose.

An important class of copolymers are protein conjugates. For example, researchers often need to determine the fraction of protein in glycosylated and pegylated proteins, as well as membrane protein-detergent complexes. ASTRA has native support for protein conjugate and copolymer analysis using a light scattering detector in conjunction with a UV and RI detector.

You need to enter the dn/dc and UV extinction values for the protein and modifier. Total mass and protein fraction on a slice-by-slice basis are displayed. ASTRA calculates the size of the complex, mass of the complex, and masses of the constituents, displaying them with rigorous uncertainties. In addition, the concentration and calculated dn/dc values are displayed for that elution volume.



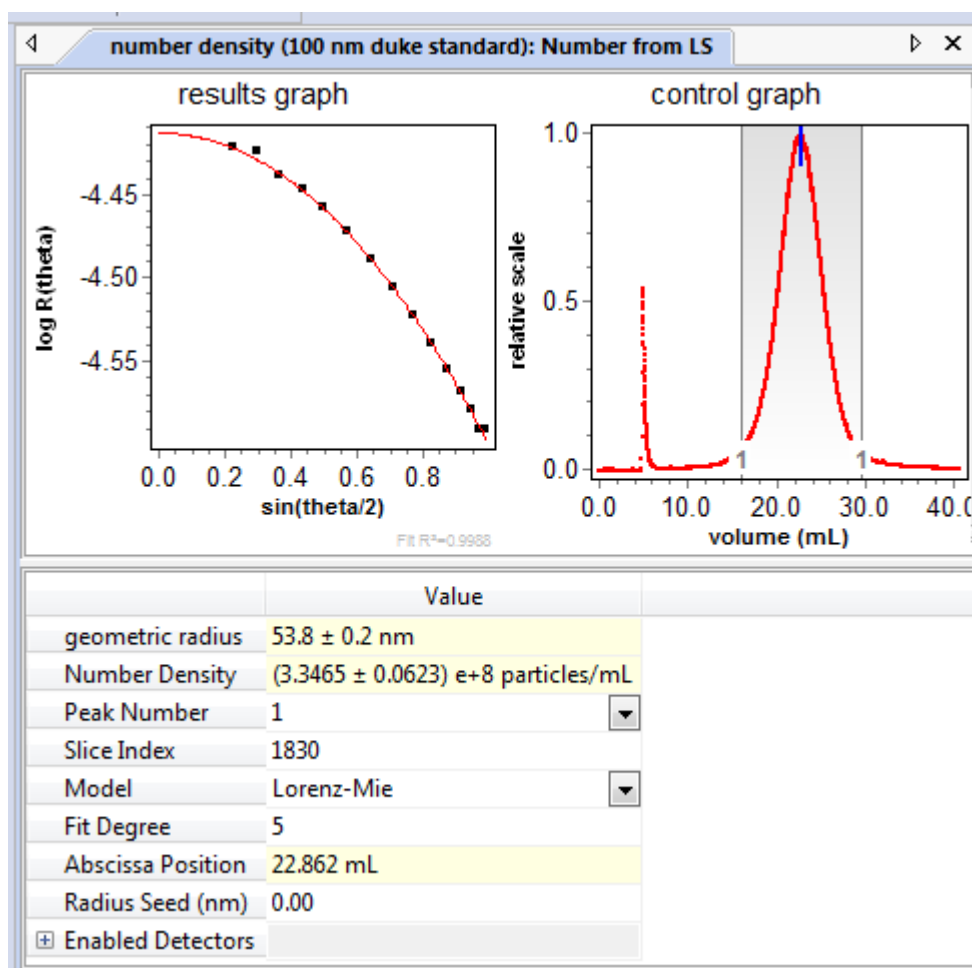
Particles (AM)

In particles mode, it is possible to measure the size (radius) and number density of a sample using just a light scattering detector without any concentration detector. Note that you won't be able to measure the molar mass using this method.

Number Density (AM)

This method provides a procedure to calculate number density, which means how many particles you have in your sample. Particle measurements are especially suited for use when a light scattering instrument is coupled to a fractionation technique such as Field Flow Fractionation (FFF) or Capillary Hydrodynamic Fractionation (CHDF), but concentration is not measured.

This procedure calculates the radius and the number of particles per mL (density) in the sample. You must specify the refractive index of the sample in the Define Peaks procedure to determine the number density. This method is normally used with online (fractionated) experiments.



Remarks:

- Model: This field shows (display only) the light-scattering model selected for this peak in the Define Peaks page.
- Fit Degree: This field shows (display only) the fit degree selected for the peak.

RI Measurement

The following methods and folders are provided in the RI Measurement folder.

100% Mass Recovery Methods

The following methods are provided in the RI Measurement > 100% Mass Recovery Methods folder.

dn/dc from Peak (AM)

Keep in mind that this method works on the hypothesis that all the mass injected is retrieved under the peak. This may not be a valid assumption for certain types of samples.

For each measurement slice of the peak, the refractive index is known due to the refractometer. You then need to enter the amount of injected sample in grams and the flow rate. The software can calculate the dn/dc value since it is proportional to these parameters.

RI Calibration from Peak (CM)

This method is used to calibrate any refractometer (Wyatt or generic) EXCEPT for the Optilab rEX, which uses a special method. Note that the main difference is in the experiment configuration. For this method there is a pump, injector, light scattering instrument, and refractometer.

To perform the calibration, you need to enter the precise injected mass and to know the dn/dc of the solvent. The calculation of the calibration constant is based on finding the same calculated mass.

Optilab rEX Specific

The following methods are provided in the RI Measurement > Optilab rEX Specific folder.

Absolute RI Calibration (CM)

The method is specifically for calibrating the Optilab rEX for absolute refractive index measurements. Absolute measurements means that there is no reference to a standard. So for these analyses, the purge valve must be ON.

Inject at least three different solvents with known (and different) refractive indexes (e.g., toluene, THF, and water).

RI Calibration from Peak (AM)

This method is only used to calibrate the Optilab rEX. For this method there is a pump, injector, and an Optilab rEX. No other instruments are necessary.

To do the calibration, you need to enter the precise injected mass and to know the dn/dc of the molecule in the specific solvent. The calculation is based on the assumption of 100% mass recovery, and the aim is to find the calibration constant needed to have the calculated mass equal to the injected mass.

Zero dRI (CM)

This method sets the dRI offset value to zero.

Orbit On (CM)

This method places the Orbit device in Recycle mode for the Optilab rEX.

Purge On (CM)

We recommend that you purge the Optilab rEX when not running samples; the “Purge On” method is a convenient way to automate this as part of a sequence.

Purge Off (CM)

You can use this method in combination with the “Purge On” method in a sequence to close the purge valves on an Optilab rEX instrument.

The purge valves on Optilab rEX instruments are automatically closed at the start of data collection. The exception to this is when absolute RI analysis is conducted, where the Optilab rEX purge valve must be left open.

Diagnostics

The following method is provided in the RI Measurement > Diagnostics folder.

Grimace (AM)

The Grimace method shows an overlay (to display it use an EASI graph). Molar masses are plotted.

The method allows you to determine if there is secondary band broadening. If there is secondary band broadening, for a monodisperse sample, the flat line will become a curve (that is, a “grimace” shape).

Batch (Determine dn/dc) (CM)

The determination of dn/dc for a specific sample in a solvent is done by manually injecting several concentrations of the sample. Additionally, the solvent used to dissolve the sample should also be injected before and after the samples.

When you have run the experiment, define the peaks and the concentration of each sample. These concentrations have to be very precise.

In the “ dn/dc from peak” procedure, the dn/dc value, errors, and graph are displayed.

Calibrate Conventional Column (CM)

Analysis of a sample using a universal or conventional column calibration takes place in two distinct phases. First, the response of a column to a set of standards with known molecular weights must be measured. Once this “determine column calibration” phase is complete, the unknown sample can be analyzed.

The “determine column profile” method allows you to set up the column profile for Conventional Calibration.

Conventional Column Data Acquisition (CM)

Use this method for configurations that contain only a refractometer.

Inject several monodisperse samples with well-known molar masses. Then do a calibration curve; this will allow you to compare the elution volume for your sample.

If two molecules have the same molar masses but different radii, there is an error in the calculation.

Copolymer Analysis (AM)

This method is for experiments without a light scattering instrument.

To use this method, a conventional column calibration profile is required. The molar mass is determined from the elution time and not from light scattering data.

For example, you might use this method to determine the quantity of monomer A and monomer B.

RI Calibration (CM)

Use this method only with batch measurements. The experiment configuration contains only an Optilab rEX.

Inject at least three concentration of sodium chloride, which has a well-known dn/dc . The slope allows ASTRA to determine the calibration constant.

RI Peak Areas (AM)

When this method is applied to an experiment, only the refractometer peak area for each selected peak is shown in the report.

RESULTS

	Peak 1	Peak 2	Peak 3
RI Instrument (RIU min)			
Peak Area	2.943e-5	3.170e-6	4.204e-7

UV Measurement

The following folder is provided in the UV Measurement folder.

UV Extinction from RI Peak (AM)

First select the peak of interest. You need to know and enter the dn/dc value of the molecule and the calibration constants of the UV and RI. The report shows the UV extinction coefficient in $mL/(mg\ cm)$ calculated from the refractometer signal.

If you know the dn/dc of the molecule of interest, it is better to determine the extinction coefficient using this method and not the 100% mass recovery method where the 100% mass recovery is hardly verified.

The advantage of using this method is that there is no effect based on the flow rate, the recovery, etc. That is, you don't have to make assumptions for these parameters.

Results	
Peak Results	
Peak 1	
General	
UV ext. coef.	0.662

Viscometry

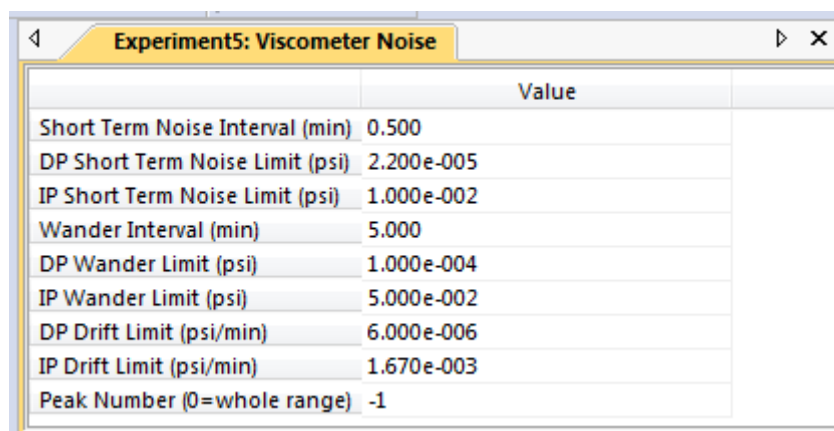
The following methods and folders are provided in the Viscometry folder.

Diagnostics

The following method is provided in the Viscometry > Diagnostics folder.

VS Noise (CM)

This method measures the noise of the viscometer and analyzes its level. To do so, a stable baseline needs to be measured. Some criteria are already provided in the method, they can be found in the “Viscometer noise” procedure. The report indicates whether the measured noise levels are within specification.



	Value
Short Term Noise Interval (min)	0.500
DP Short Term Noise Limit (psi)	2.200e-005
IP Short Term Noise Limit (psi)	1.000e-002
Wander Interval (min)	5.000
DP Wander Limit (psi)	1.000e-004
IP Wander Limit (psi)	5.000e-002
DP Drift Limit (psi/min)	6.000e-006
IP Drift Limit (psi/min)	1.670e-003
Peak Number (0=whole range)	-1

With Light Scattering

The following methods are provided in the Viscometry > With Light Scattering folder.

Branching (VS+LS) (AM)

This method lets you determine the branching ratio using either viscosity or light scattering data. It does not require conventional or universal calibration, as it measures the mass using the light scattering signal.

Online (CM)

This is the standard online method for use when you have a light scattering detector and a viscometer.

Copolymer Analysis (AM)

This method is for use with viscometer data. It is the equivalent of the Protein Conjugate method, but for viscometry.

Utilities

The following methods are provided in the Viscometry > Utilities folder.

Orbit On (CM)

This method places the Orbit device in Recycle mode for the ViscoStar.

Valve Conditioning (CM)

This method cycles the V1-V3 purge valves on the ViscoStar. You should flush with solvent while running this method. Cycling helps to dislodge any debris on the pressure transducer.

Branching from Column Calibration (AM)

This method is the equivalent of the branching method found in the Light Scattering folder. However, this one measures branching using viscometer data. It calculates molar mass based on the column calibration.

To use this method you must first do a column calibration and set a column profile.

Branching from VS Data (AM)

This method is the same as the Branching from Column Calibration method, however it calculates molar mass based on viscometer data.

To use this method you must first do a column calibration and set a column profile.

Calibrate Universal Column (CM)

Analysis of a sample using universal or conventional column calibration takes place in two distinct phases. First, the response of a column to a set of standards with known molecular weights must be measured. Once this “determine universal column profile” phase is complete, the unknown sample can be analyzed.

This method allows you to set up the column profile for Universal Calibration.

Copolymer Analysis (AM)

This method is for use with viscometer data. It is the equivalent of the Protein Conjugate method, but for viscometry.

Mass from VS Data (AM)

This method calculates the molar mass distribution all along the chromatogram based on the viscometer data.

Online (CM)

This is the standard online method for use when you have only a viscometer and no light scattering instrument.

Universal Column Data Acquisition (CM)

To use this method, you must have a saved column profile.

In the Generic Column of the experiment configuration, select the corresponding column profile by clicking the “...” button.

C Data Collection with Scripts

This appendix describes the scripting language you can use for script-based data collection.

CONTENTS	PAGE
Introduction	C-2
Collection	C-3
Interacting with Instruments	C-3
Examples	C-5

Introduction

ASTRA 6 embeds a powerful, general-purpose programming language called Lua in its data collection system. Scripts can be written using the normal syntax and features present in the core Lua language version 5.1.3. For details about Lua, see <http://www.lua.org>.

You use scripts with the procedure described in “Script Collection” on page 8-13. When you run a script, any syntax errors are reported in a message.

The following sections provide an overview of the features available for creating custom collection scripts.

Note:	Writing scripts is an advanced feature, almost never needed for typical data collection tasks. For users who want to construct novel collections involving significant interaction with the instrument settings, valve position, laser power levels, and so forth, it can provide a powerful means of interacting with your Wyatt instruments.
--------------	--

Scripts allow you to issue commands to instruments to prepare a collection, collect data for a specific period of time, and so forth. This provides a powerful mechanism for customizing data collection. Internally, scripts form the foundation of the Basic Collection procedure, which is simply a graphical interface to the features of the script collection interpreter.

Collection

The overall collection is controlled by issuing commands in the Collection namespace. These commands are used to interact with the user, and to control the collection status.

Commands are issued using the following syntax:

```
Collection.[command]
```

where [command] is one of the following commands:

Command	Effect
PromptUser ("text")	Pop up a message to the user that must be responded to (via the "OK" button) for the collection to proceed.
Start ()	Tell ASTRA to begin listening for data from instruments.
SetDuration ([milliseconds])	Wait for the specified number of milliseconds (while collecting data).
SetInjectToCollectDelay ([milliseconds])	Set the number of milliseconds ASTRA will wait after receiving an auto-injection signal before proceeding.
Stop ()	Tell ASTRA to stop listening for data.
WaitForMessage ([instrument], "[message]")	Hold the collection until the string [message] is received by [instrument]. The [instrument] is an instrument name, retrieved using one of the "Create" commands described in the next section. The only [message] currently available is ISI_INSTRUMENT_AUTOINJECT, which indicates that an autoinject signal has been received by the specified instrument.

Interacting with Instruments

To issue commands to an instrument, you must first obtain an instrument reference that links the specific physical instrument in the experiment configuration to the instructions in the script. This is done using one of the following commands:

Command	Effect
LSInstrument.Create ()	Get a reference to the experiment configuration's static light scattering instrument.
QELSLInstrument.Create ()	Get a reference to the experiment configuration's dynamic light scattering instrument.
RIInstrument.Create ()	Get a reference to the experiment configuration's refractive index instrument.
VIIInstrument.Create ()	Get a reference to the experiment configuration's viscometer.

Once you have obtained a reference to one or more instruments, you can issue commands to the instrument. Instruments respond to a general set of messages, as well as some instrument-specific commands.

Common Instrument Commands

All instruments understand the following messages. Commands are issued using the following syntax:

```
[instrument] : [command]
```

where [instrument] is the instrument reference obtained by the "Create" method defined in "Interacting with Instruments" on page C-3.

For example, the following commands turn the recycle valve of a light scattering instrument to the "waste" position:

```
lsInst = LSInstrument.Create ()
# Turn the recycle valve off
lsInst.SendCommand("SetSwitch[Recycle, F]")
```

The complete set of instrument commands is shown in the following table:

Command	Effect
GetInstrumentLabel ()	Get a string version of the instrument's name. Useful for generating messages to the user.
Enabled ()	Return true or false, indicating whether the "Disable Collection" flag in the instrument configuration is enabled.
SetCollectionInterval ([seconds])	Set the instrument data collection interval in to the specified number of seconds.
SendCommand ([command])	Tell the instrument to perform a particular command. The [command] is an instrument command string (described elsewhere).
StartCollection ()	Tell the instrument to begin transmitting data to ASTRA. This provides finer control over when instruments begin transmitting collection data.
Recycle()	Sets the instrument's recycle valve control output to its "Recycle" position. For example, if you have an Orbit accessory valve, this command places it in the correct position to recycle solvent.
Waste()	Sets the instrument's recycle valve control output to its "Waste" position. For example, if you have an Orbit accessory valve, this command places it in the correct position to send solvent to waste.

Static Light Scattering Instrument Commands

All static light scattering instruments understand the following messages.

Commands are issued using the following syntax:

```
[instrument] : [command]
```

where [instrument] is the instrument reference obtained by the "Create" method defined in "Interacting with Instruments" on page C-3.

Command	Effect
LaserOn ([state])	Set the laser status to on (true) or off (false). The [state] is either true or false.
RunCOMET ([state])	Sets the COMET to on (true) or off (false). The [state] is either true or false. This command has no effect on instruments without a COMET device.
DitherOn ([state])	Sets the laser dithering status to on (true) or off (false). The [state] is either true or false.

Dynamic Light Scattering, Refractometer, and Viscometer Commands

There are currently no Dynamic Light Scattering Instrument, Refractometer, or Viscometer-specific instrument commands.

Examples

The following examples show some useful collection scripts. You can learn about additional features of the Lua programming language by visiting the website at <http://www.lua.org>, or by reading the book *Programming in Lua*, second edition, by Roberto Ierusalimsky.

The following script collects data for three auto-injections:

```
lsInst = LSInstrument.Create ()
lsInst:LaserOn(true)
lsInst:SetCollectionInterval(0.5)
lsInst:StartCollection ()

for i=1,3 do
    Collection.WaitForMessage(lsInst,
        "ISI_INSTRUMENT_AUTOINJECT")
    if i == 1 then -- start collection on 1st iteration
        Collection.Start()
    end
    Collection.SetDuration(30000)
end

Collection.Stop()
```

The following script collects data for a light scattering instrument calibration. It prompts the user to press **Enter** to continue once the instrument is ready.

```
lsInst = LSInstrument.Create ()
lsInst:LaserOn(true)
lsInst:SetCollectionInterval(0.125)
lsInst:StartCollection ()
Collection.PromptUser("Press Enter to Start Calibration.")
Collection.Start()

-- Run 30 seconds with laser
Collection.SetDuration(30000)

-- Run 30 more seconds without laser ...
lsInst:LaserOn(false)
Collection.SetDuration(30000)
lsInst:LaserOn(true)
Collection.Stop()
```

The following script turns COMET features on and off as needed during the collection (see the **bold** portions in the example).

```
-- Script to run the COMET
duration = 7200000
lsInst = LSInstrument.Create ()
lsInst:LaserOn(false)

-- Turn the COMET on
lsInst:RunCOMET(true)
lsInst:StartCollection ()
Collection.Start()
Collection.SetDuration(duration)

-- Turn the COMET off
lsInst:RunCOMET(false)
Collection.Stop()
```

Sample experiments with collection scripts for running the COMET cell cleaner and turning lasers on and off are provided in the **System > Methods > Light Scattering > Utilities** folder of the system database.

For Optilab rEX users, there are several experiment methods in the **System > Methods > RI Measurement > Optilab rEX Specific** folder. These methods have scripts for “Purge On”, “Purge Off”, and “Zero dRI”.

You may contact Wyatt Technical Support if you have a specific need to create additional collection scripts.

D Light Scattering Theory

This appendix reviews the basic theory of light scattering and how the ASTRA software determines the molar masses and root mean square radii of a sample whose light scattering properties have been measured. The text covers basic quantities, the relation to measurements, calibration and normalization, and determination of molar masses, sizes and distributions.

CONTENTS	PAGE
Introduction	D-2
Basic Quantities	D-3
Measured Quantities and Calibration.....	D-4
Normalization	D-8
Concentration Calculation Methods	D-11
Determination of Molar Mass and Sizes	D-13
Molar Mass and RMS Radius Moments.....	D-18
Uncertainties in Calculated Quantities	D-21
Out of Range Values	D-22
Differential Distribution Calculations	D-22
Branching Calculations	D-23

Introduction

Perhaps the most important application of the ASTRA program is its ability to convert measurements of a fractionated sample, passing through appropriate instrumentation, into an accurate representation of the molar masses and sizes present in the sample.

Although size-exclusion chromatography (SEC) provides good separation of molecules based on their hydrodynamic radii, before the advent of light scattering techniques it had been necessary to calibrate SEC columns using standard samples of known molar mass in order to determine only the molar masses present in an unknown sample. Unfortunately, appropriate standards having the same composition and effective conformations as the unknown specimen have never really been available.

On the other hand, if the value of the differential refractive index increment (dn/dc) or the total mass of eluting solute is known, light scattering measurements can provide an absolute measurement of molar mass when used in series with a concentration-sensitive detector such as a refractive index (RI) detector.

Thus, light scattering measurements effectively provide a column “calibration curve” for every sample, obviating time-consuming, conformation-dependent calibration procedures. When techniques such as reverse phase chromatography are used, separation is not based on molecular hydrodynamic size, and calibration techniques based on known standards are useless.

Basic Quantities

The light scattered into a detector centered at angle θ per unit solid angle subtended by said detector is called the Rayleigh ratio or R_θ , and is defined as:

$$R_\theta = \frac{I_\theta r^2}{I_0 V} \quad (1)$$

where I_θ is the scattered intensity, I_0 is the intensity of the incident beam, V is the illuminated volume of the scattering medium from which the detector at θ collects light, and r is the distance between V and the detector.

Eq. (1) implies that the dimension of R_θ is (length)⁻¹.

Note: It is assumed in Eq. (1) and throughout this manual that the incident light is always vertically polarized; that is, its electric field is perpendicular to the plane in which the angular variation of the light scattered from the sample is measured. All DAWN instruments are supplied with such vertically polarized laser sources.

More generally, R_θ usually refers to the *excess* scattering from a solution above that scattered by the solvent alone as follows:

$$R_\theta = \frac{(I_\theta - I_{\theta, \text{solvent}}) r^2}{I_0 V} \quad (2)$$

where $I_{\theta, \text{solvent}}$ is the scattered intensity into angle θ from the solvent.

Thus scattering from a pure solvent is described by Eq. (1), while the scattering in excess above the solvent is described by Eq. (2).

Eq. (2) effectively describes the scattering after subtracting the “baseline” corresponding to the pure solvent.

As we shall see in the sections that follow, measurement of R_θ at a number of different angles combined with the corresponding molecular concentration provides the data by which the weight average molar mass and size of the solute molecules are determined.

Measured Quantities and Calibration

How do we measure R_θ ? If we try to use Eq. (1) or Eq. (2) directly, we run into trouble immediately. First, the quantities we can measure directly are detector voltages, not light intensities. Because the measured voltages are proportional to light intensities, we must calibrate our detectors. This calibration is not difficult and will be explained shortly.

A more serious problem is that due to the geometry of the sample cell in the DAWN or miniDAWN instruments in which each detector “sees” a slightly different scattering volume V and subtends a different solid angle with respect to the scattering volume. Refractive index differences among various solvents and sample cells exacerbate this problem. Calculating V for each detector is thus extremely complex and prone to inaccuracies.

The solution is to use the proportionality between R_θ and I_θ in Eq. (1) to derive a calibration factor which gives the correct value of R_θ for a known scatterer. Fortunately, some common solvents have been thoroughly studied, and their Rayleigh ratios are well known, allowing us to use an SEC solvent itself as the calibration standard. Using pure solvent as the scattering standard makes the calibration completely independent of any particular sample.

Let us first consider scattering at 90° . We integrate not only the detector sensitivity but also all the geometrical volume and solid angle factors into a single Configuration Specific Calibration Constant, called A_{CSCC} .¹ By using the proportionality between detector voltage and light intensity, Eq. (1) may be expressed as:

$$R_{90^\circ} = A_{CSCC} \left(\frac{V_{90^\circ} - V_{90^\circ \text{ dark}}}{V_{\text{laser}} - V_{\text{laser, dark}}} \right) \quad (3)$$

where V_{90° and $V_{90^\circ \text{ dark}}$ are the 90° detector signal voltage and its dark offset voltage, respectively. V_{laser} and $V_{\text{laser, dark}}$ are the laser monitor signal and its dark offset, respectively. Dark offsets are obtained with the laser turned off. Note that dividing by the laser monitor signal compensates for any changes in laser intensity due to power supply fluctuations, temperature drift, laser aging, etc.

As an example, suppose we calibrate with toluene. Pure filtered toluene has a Rayleigh ratio of $1.406 \times 10^{-5} \text{ cm}^{-1}$ at a wavelength of 632.8 nm.² Suppose that using our DAWN or miniDAWN sample cell we observe a 90° scattering signal of about 1 V. The laser monitor signal is factory-set to be near 5 V, and the dark offsets are much smaller than this, so Eq. (3)

1. The symbol A_{CSCC} is not displayed in the software.
2. W. Kaye and J.B. McDaniel, “Low-angle laser light scattering—Rayleigh factors and depolarization ratios,” *Applied Optics*, vol. 13, No. 8, 1974, pp. 1934–1937.

implies $A_{CSCC} \approx 7.0 \times 10^{-5} \text{ cm}^{-1}$. Of course, this is just an example, and the measured constant may be quite different depending on the instrument and conditions (laser wavelength, etc.).

Toluene provides a large scattering signal; in fact, toluene has the highest Rayleigh ratio of any of the common solvents, and is thus highly desirable for use as a calibrator. Many other solvents can, theoretically, be used for calibration of the DAWN or miniDAWN but we do not recommend them.

The astute reader will point out that since we know the Rayleigh ratio for toluene, and since the scattering from toluene is relatively large, we ought to be able to calibrate with toluene, measure our samples in water, and still obtain correct results. The complication lies in the geometrical factors which describe the volume of scattering molecules seen by the 90° detector as well as the solid angle it subtends. These factors depend on the refractive index of both the solvent and the glass of which the sample cell is made (see the DAWN or miniDAWN Hardware Manual).

Thus the “constant” A_{CSCC} is really dependent on the solvent type and cell type. That is why we call it a configuration specific calibration constant. To allow users to calibrate with one solvent and/or cell and make measurements with another, we must have an “instrument” constant that is truly independent of these changing factors and is instead only a function of the particular instrument and the sample cell geometry.

This instrument constant A_{inst} is related to A_{CSCC} as follows:

$$A_{CSCC} = A_{inst} (\text{Reflection correction})(\text{Geometry correction}) \quad (4)$$

First, the “Reflection correction” will be considered. The reflection correction represents the reflective losses at each interface in the sample cell. For example, the incident laser beam loses intensity at the air-glass interface of the sample cell, and the glass-solvent interface as well. Similarly, the scattered light that is to be detected also suffers from reflective losses at the solvent-glass and glass-air interfaces as it leaves the sample cell. If the solvent is changed, or a different cell is used, these reflective losses will change, and hence it is necessary to correct for them if A_{inst} is to be independent of solvent and cell glass.

The reflection correction can be considered in terms of the transmitted intensity between media 1 and 2 with indices of refraction n_1 and n_2 , respectively. The transmitted intensity from medium 1 into medium 2 is given by the Fresnel equations as:

$$T_{12} = \frac{4n_1n_2}{(n_1 + n_2)^2} \quad (5)$$

If g represents the flow cell glass, s represents the solvent, and a represents air, then the reflection correction can be written as:

$$\text{Reflection correction} = \frac{1}{T_{sg}^2 T_{ga}^{N^*}} \quad (6)$$

where N^* is the number of uncoated glass-air interfaces the incident and scattered light have to traverse. It is assumed that the reflective losses at a coated, i.e., antireflection coated, interface are negligible.

The “Geometry correction” for a sample cell is not as easily determined as the reflection correction. There are examples of analytical expressions derived for simple cell geometries^{1,2}, but there are no simple analytical expressions for more complicated geometries such as that of the K5 and F2 flow cells. (The symbols K5 and F2 refer to the Schott glass names.) In addition, these analytical expressions are valid for conditions in which the source of scattered light is either a point source or a completely illuminated volume, neither of which hold for the current scattered light source—a collimated laser beam. Therefore, the geometry correction has been calculated for each sample cell using computer ray-tracing simulations based upon the exact geometry of the sample cell, laser beam, and detection optics for the 90 degree detector in the DAWN and miniDAWN instruments.

For the K5 and F2 flow cells, the resulting geometry correction goes as $n_s n_g$, that is, the index of refraction of the solvent times the index of refraction of the glass, respectively. The complete expression taking into account the reflection and geometry corrections is therefore:

$$A_{\text{CSCC}} = A_{\text{inst}} \frac{n_s n_g}{T_{sg}^2 T_{ga}^{N^*}} \quad (7)$$

for the standard flow cell with an antireflection coated entrance window and uncoated exit surface, and the transmission terms are calculated using Eq. (5).

When using a scintillation vial, both the geometry and solvent-glass reflection corrections were folded into the ray tracing calculations, so only the factor for the reflection correction due to the two uncoated glass-air interfaces of the scintillation vial are in the final expression. The resulting complete formula for the scintillation vial is:

$$A_{\text{CSCC}} = A_{\text{inst}} \frac{n_s^{1.797}}{T_{ga}^2} \quad (8)$$

-
1. C.I. Carr, Jr. and B.H. Zimm, “Absolute Intensity of Light Scattering from Pure Liquids and Solutions”, *J. Chem. Phys.*, vol. 18, pp. 1616-1626 (1950).
 2. J.J. Hermans and S. Levinson, “Some Geometric Factors in Light-Scattering Apparatus”, *J. Opt. Soc. Am.*, vol. 41, pp. 460-464 (1951).

For the MicroCuvette, both the geometry and solvent-glass reflection corrections were folded into the ray tracing calculations. Both the entrance and exit windows for the MicroCuvette are anti-reflection coated, so there are no explicit reflection correction terms in the final equation. The resulting complete formula for the MicroCuvette is

$$A_{\text{CSCC}} = A_{\text{inst}} n_s^{1.983} \quad (9)$$

During the performance of a calibration, ASTRA calculates a configuration-specific constant from Eq. (3), but this number is never seen. It is immediately converted to the instrument constant A_{inst} via Eq. (7), Eq. (8), or Eq. (9) depending on the cell type. The A_{inst} value is reported as the “Calibration Constant” and is the value entered in the DAWN or miniDAWN profile.

If at some later time A_{inst} is changed in the instrument profile, ASTRA will recalculate A_{CSCC} . ASTRA also recalculates A_{CSCC} if the solvent or cell type is changed. This process may sound complicated, but it enables one to calibrate with one solvent and make measurements with another, while the software efficiently handles all the details.

Note:	Since changing the sample cell may require a realignment of the laser, we recommend you always recalibrate after changing the sample cell.
--------------	--

The calibration measurements should be made with *great care* as the accuracy of all other measurements depends upon them. As long as the system is left undisturbed it is not necessary to recalibrate, but we advise making occasional checks using a standard reference molecular species, as photodiode sensitivity may change with age. The calibration should be performed with HPLC-grade toluene filtered through the smallest available filter (0.02 μm) immediately before making the measurement using the ASTRA program. The cleanliness of the cell is vital for this purpose. Be sure to leave the DAWN or miniDAWN instrument and the laser switched on for one hour before making any measurements.

Normalization

At this point we have calibrated the 90° detector in an absolute sense: the calibration is totally independent of any sample we might wish to study. In other words, we can measure R_θ accurately for any solvent or sample, assuming it gives a large enough signal. Furthermore, the calibration can be traced directly to the scattering from pure, well-understood solvents.

So far we have ignored all angles except 90°. Each detector has its own geometrical factors and angular sensitivity to measured light intensity. Furthermore, these effects vary from solvent (and sample) to solvent. We would like to quantify this effect so that we can correct for it. If not, we will mistake solvent and geometric readhead effects for characteristics of our sample, resulting in poor data.

Therefore, we use a set of *normalization coefficients* N_θ to relate each detector to the 90° detector. These coefficients must be determined using the *same flow rate* (same pressure) and the *same solvent* that are used for the SEC measurement, since the refractive index of the solvent changes the scattering angles and the geometrical factors for each detector.

For purposes of normalization, we must employ a sample that is an isotropic scatterer (one which scatters equally in all directions), so that we can be sure that the variations measured are due to detector geometry and not some interaction of the sample with the light. Particles whose size is much smaller than the wavelength of the vertically polarized light incident upon them are often called Rayleigh particles and scatter such incident light isotropically. The normalization coefficient for the 90° detector is assigned a value of 1.0, while the other detectors are adjusted by varying amounts to yield identical values of R_θ at all angles.

The process of normalization is quite simple. We assume that the 90° detector has already been calibrated as described above. To normalize, we introduce an isotropic solute (*i.e.*, producing an R_θ is independent of θ) and compute a set of coefficients so that each detector gives the same R_θ as the 90° detector when its signal is multiplied by its correct normalization coefficient. Expressed algebraically we have:

$$R_\theta = N_\theta A_{CSCC} \left(\frac{V_\theta - V_{\theta, dark}}{V_{laser} - V_{laser, dark}} \right) \quad (10)$$

For Eq. (3) and Eq. (10) to agree when $\theta = 90^\circ$, N_{90} must be exactly unity.

Thus Eq. (10) gives us a way to calculate Rayleigh ratios at any detector angle. We recommend normalizing with a low molar mass sample whose constituents all have radii less than about 5 nm. (Molecules this small scatter nearly isotropically as discussed previously.) For organic solvents, small polystyrene samples are generally used with molar masses less than

about 30,000 g/mol. For aqueous solvents and buffers, dextran with a weight average molar mass of 10,000 g/mol or bovine serum albumin (BSA) with a molar mass of 66,400 g/mol may be used.

In practice we need not measure the various detector dark offsets $V_{90,dark}$ of Eq. (10). This is because the instrument is typically used to study samples in solution, not solvents by themselves. Thus we are interested in the *excess* Rayleigh ratio of the eluting sample, compared with the baseline of solvent alone. We therefore use an alternative form of Eq. (10):

$$R_{\theta} = N_{\theta} A_{CSCC} \left(\frac{V_{\theta} - V_{\theta,baseline}}{V_{laser} - V_{laser,dark}} \right) \quad (11)$$

where R_{θ} is the excess Rayleigh ratio, and $V_{\theta,baseline}$ is the detector voltage at a great distance from any solute peak. The quantity $V_{\theta,baseline}$ is the scattering signal from the solvent alone [cf. Eq. (2)] and the detector dark offset. Eq. (11) is used by ASTRA.

Implementation

ASTRA provides two normalization techniques: “Standard” normalization, which uses the normalization calculation used since ASTRA 4, and “Area” normalization, which uses a new method based on integration over the peak.

Standard Normalization

In practice, $V_{\theta} - V_{\theta,baseline}$ is not determined from a single data slice, but from the result of the following steps:

1. Select a sample peak to use for normalization.
2. Using the collected data points for the center half of the peak (that is, the half of the peak centered on the peak apex), fit them to a 6th order polynomial of the form.

$$y = a_0x + a_1x + a_2x^2 + a_3x^3 + a_4x^4 + a_5x^5 + a_6x^6 \quad (12)$$

Note that there must be at least seven points in the “center half” of the selected peak for the normalization calculation to run.

3. The apex of the fit curve, y in Eq. (12), provides $V_{\theta} - V_{\theta,baseline}$. The maximum y is found iteratively by using the x value for each slice used in the fit in Eq. (12), and selecting the largest resulting y . This method is used due to the relatively small number of points typically involved, and to preclude the chance of encountering another local maxima.
4. Repeat steps 2 and 3 first for the 90° degree detector, then for each light scattering detector for which a normalization coefficient is to be calculated.
5. Set any negative y values to 1.0.

6. Finally, divide the results of Eq. (12) for all detectors by the result of Eq. (12) for the 90° detector. This yields the desired normalization coefficients.

Area Normalization

Better normalization results were found when the results were calculated from the integration of the Rayleigh Ratio peak as follows, rather than just using the peak apex:

1. Select a sample peak to use for normalization.
2. Integrate the Rayleigh Ratios over the entire peak.
3. Repeat the integration for each light scattering detector.
4. Any negative results are set to 1.0.
5. Finally, set the normalization coefficients for all detectors equal to the result of the integration for the current detector divided by the result for the 90° detector. This forces the 90° detector to equal 1.0.

Concentration Calculation Methods

By default, the concentration at each data slice is determined from the dn/dc value previously determined and the calibration constant for the RI detector. Alternately, the 100% Mass Recovery option may be enabled in an experiment configuration to determine the concentration without knowledge of dn/dc .

Known dn/dc and Known AUX Calibration Constant

Sample concentration for each data slice is determined from the RI concentration detector assuming a constant dn/dc value across the sample peak.

This is the default method, and is the one we recommend. It requires known values for the “RI” calibration constant α and the differential refractive index increment dn/dc (in mL/g). It does not require that the total injected mass be known and is independent of an accurate flow rate. The quantity α can be determined by injecting a sample with known dn/dc into the refractometer at a few different concentrations, and the dn/dc value may be found in the literature or measured using an Optilab instrument.

For the i^{th} slice, the change in refractive index compared to pure solvent is given by:

$$\Delta n_i = \alpha(V_i - V_{i,\text{baseline}}) \quad (13)$$

where V_i and $V_{i,\text{baseline}}$ are the RI signal and baseline voltages, respectively. Dividing Δn_i by dn/dc gives the change in concentration of solute, compared to the baseline, for that slice:

$$\Delta c_i = \frac{\Delta n_i}{dn/dc} = \frac{\alpha(V_i - V_{i,\text{baseline}})}{dn/dc} \quad (14)$$

Since the baseline represents the signal from the pure solvent, $\Delta c_i = c_i$. Once the concentration is known, the mass w_i of solute in the slice is clearly:

$$w_i = c_i \Delta v_i \quad (15)$$

where Δv_i is the volume of the slice. Note that Δv_i is calculated from the elution time \times the flow rate, and therefore requires that an accurate flow rate is known. The calculated mass W for the peak is then

$$W = \sum_{\text{peak}} w_i \quad (16)$$

where the sum is over the slices in the peak. When using this method, ASTRA calculates the peak mass according to Eq. (16) where the sum is over the slices in the peak within the limits set in the Peaks graph. Com-

paring this value with the injected mass for the peak, given an accurate injection volume and assuming that no part of the sample remains on the column(s), the calculated eluted mass should agree with the injected mass.

Known AUX Calibration Constant and 100% Mass Recovery

If you enable the “Assume 100% Mass Recovery” option in an experiment configuration, ASTRA assumes that 100% of the injected mass elutes in the peak area selected. In addition, the following information must be known:

- The total eluted mass for each peak (assumed to be equal to the injection mass).
- The detector’s calibration constant, α .
- The flow rate with sufficient accuracy.

If you use this method, knowing the dn/dc is not required.

Using the known values, ASTRA calculates dn/dc by inverting Eq. (14) and using Eq. (17):

$$dn / dc = \frac{\alpha}{W_{injected}} \sum_{peak} \Delta v_i (V_i - V_{i,baseline}) \quad (17)$$

You must be sure that all the injected mass elutes in the peak area selected and that the flow rate is accurate.

Determination of Molar Mass and Sizes

We begin by reviewing briefly the relationship between the data collected by the DAWN system and the molecular parameters derived from them. We start with Eq. (18)¹:

$$\frac{R_\theta}{K^*c} = MP(\theta) - 2A_2cM^2P^2(\theta) \quad (18)$$

Where:

- $\lambda_0 = \lambda / n_0$, where n_0 is the solution's refractive index and λ_0 is the wavelength of the incident light in vacuum.
- $K^* = 4\pi^2 n_0^2 (dn/dc)^2 / (\lambda_0^4 N_A)$
- c is the mass concentration of the solute molecules in the solvent (mg/mL).
- M is the weight average molar mass (g/mol).
- A_2 is the second virial coefficient (mol ml / g²).
- K^* is an optical constant where N_A is Avogadro's number, equal to $6.022 \times 10^{23} \text{ mol}^{-1}$, and dn/dc is the differential refractive index increment of the solvent-solute solution with respect to a change in solute concentration, expressed in mL/g (this factor must be measured independently using a dRI detector).
- $P(\theta)$ is the theoretically-derived form factor, given by $P(\theta) = 1 - 2\mu^2 \langle r_g^2 \rangle / 3! + \dots$, where $\mu = (4\pi / \lambda) \sin(\theta / 2)$, and $\langle r_g^2 \rangle$ is the mean square radius. $P(\theta)$ is a function of the molecules' size, shape, and structure.
- R_θ is the excess Rayleigh ratio (cm⁻¹).

Eq. (18) is the basis of the calculations in ASTRA as well as of the Zimm plot technique, which is often implemented in a batch sample mode.

Eq. (18) assumes vertically polarized incident light and is valid to order c^2 . The task is now to determine, for each slice, the molar mass and mean square radius.

It is possible to solve Eq. (18) in a variety of ways, leading to a number of different methods. We shall consider the Conventional, Zimm, Berry, and Assumed Model methods.

1. B.H. Zimm, "The scattering of light and the radial distribution function of high polymer solutions," *J. Chem. Phys.*, vol. 16, pp. 1093-1099 (1948).

Conventional Method

First, construct a conventional plot. That is, plot $R_\theta / (K^*c)$ against $\sin^2(\theta/2)$. (This method is often referred to as the “Debye” method.) Next, fit a polynomial in $\sin^2(\theta/2)$ to the data for each of the m angles θ_i where measurements are collected for angles $i = 1, 2, \dots, m$. From these fits, obtain the intercept, $R_\theta / (K^*c)$, at $\theta = 0$ and the slope at zero angle, given by:

$$s = d[R_\theta / (K^*c)] / d[\sin^2(\theta/2)]_{\theta \rightarrow 0} \quad (19)$$

Note that as θ approaches zero, the form factor $P(\theta)$ approaches unity. Therefore, Eq. (18) becomes:

$$\frac{R_0}{K^*c} = M - 2A_2cM^2 \quad (20)$$

If $A_2 = 0$, then

$$M = \frac{R_0}{K^*c} \quad (21)$$

On the other hand, solving Eq. (20) for M yields:

$$M = \frac{2 \left(1 - \sqrt{1 - 8A_2c \left(\frac{R_0}{K^*c} \right)} \right)}{8A_2c} \quad (22)$$

Note that only one of the two solutions of Eq. (20) is physically reasonable. For very small values of A_2 , we may rewrite Eq. (22) as follows:

$$M = \frac{2 \left(\frac{R_0}{K^*c} \right)}{1 + \sqrt{1 - 8A_2c \left(\frac{R_0}{K^*c} \right)}} \quad (23)$$

Eq. (23) is not susceptible to round-off error. Note that Eq. (23) reduces to $R_0 / (K^*c)$ as $A_2 \rightarrow 0$.

To find the mean square radius $\langle r_g^2 \rangle$ for the slice, we note that at very small angles $P(\theta) = 1 - 2\mu^2 \langle r_g^2 \rangle / 3! + \dots$ and Eq. (18) may be written approximately as:

$$\begin{aligned} \frac{R_\theta}{K^*c} &= MP(\theta) - 2A_2cM^2P^2(\theta) \approx M \left[1 - 2\mu^2 \langle r_g^2 \rangle / 3! \right] - 2A_2cM^2 \left[1 - 2\mu^2 \langle r_g^2 \rangle / 3! \right]^2 \\ &\approx M \left[1 - 2\mu^2 \langle r_g^2 \rangle / 3! \right] - 2A_2cM^2 \left[1 - 4\mu^2 \langle r_g^2 \rangle / 3! \right] \end{aligned} \quad (24)$$

Eq. (19) may be written in terms of $\mu^2 = (4\pi/\lambda)^2 \sin^2(\theta/2)$. That is:

$$\begin{aligned} s &= d[R_\theta / (K^*c)] / d[\sin^2(\theta/2)] = \frac{16\pi^2}{\lambda^2} d[R_\theta / (K^*c)] / d[\mu^2] \\ &= -\frac{16\pi^2}{\lambda^2} \frac{M \langle r_g^2 \rangle}{3} \{1 - 4A_2cM\} \end{aligned} \quad (25)$$

Therefore:

$$\langle r_g^2 \rangle = \frac{-3s\lambda^2}{16\pi^2 M(1 - 4A_2Mc)} \quad (26)$$

Zimm (Reciprocal) Method

To perform calculations with the Zimm method, which begins with a plot of $(K^*c) / R_\theta$ against $\sin^2(\theta/2)$, we expand the reciprocal of Eq. (18) to first order in c :

$$\frac{K^*c}{R_\theta} = \frac{1}{MP(\theta)} + 2A_2c \quad (27)$$

By following the procedures described for the conventional plot, we obtain the following results:

$$M = \left(\frac{K^*c}{R_0} - 2A_2c \right)^{-1} \quad (28)$$

and

$$\langle r_g^2 \rangle = \frac{3sM\lambda^2}{16\pi^2} \quad (29)$$

where:

$$s = d[K^*c / R_\theta] / d[\sin^2(\theta/2)]_{\theta \rightarrow 0} \quad (30)$$

Berry (Square Root) Method

To perform calculations with the Berry method, which begins with a plot of $\sqrt{K^*c/R_\theta}$ against $\sin^2(\theta/2)$, we must expand the square root of the reciprocal of Eq. (18) to first order in c :

$$\sqrt{\frac{K^*c}{R_\theta}} = \frac{1}{\sqrt{MP(\theta)}} + A_2c\sqrt{MP(\theta)} \quad (31)$$

In this case the results are:

$$M = \frac{4}{\left(\sqrt{K^*c/R_\theta} + \sqrt{K^*c/R_\theta - 4A_2c}\right)^2} \quad (32)$$

and

$$\langle r_g^2 \rangle = \frac{3\lambda^2 s}{8\pi^2 \sqrt{M(1/M - A_2c)}} \quad (33)$$

where:

$$s \equiv \frac{d\left[\sqrt{K^*c/R_\theta}\right]}{d\left[\sin^2(\theta/2)\right]_{\theta \rightarrow 0}} \quad (34)$$

Assuming a Molecular Structure

For a variety of possible molecular structures, the interpretation of measurements may be simplified considerably if such structures are known in advance. Of course, if the scattering molecules are not of the assumed structure, significant errors can result. The key to their use, of course, requires the applicability of the Rayleigh Gans approximation (see “Theory” on page E-8). In that event, knowing the molecular structure in advance often results in an exact analytical expression for the form factor $P(\theta)$.

Consider first an assumed Random Coil structure. Returning again to Eq. (18), we replace the theoretical form factor $P(\theta)$ by its exact form first derived by Debye¹:

$$P(\theta) = \frac{2}{u^2} (e^{-u} - 1 + u) \quad (35)$$

where $u = (4\pi/\lambda^2) \langle r_g^2 \rangle \sin^2(\theta/2)$.

1. P. Debye, “Molecular-weight determination by light scattering,” *J. Phys. Coll. Chem.*, vol. 51, pp. 18-32 (1947).

Since $P(\theta)$ is a nonlinear function of the mean square radius $\langle r_g^2 \rangle$, we must use an iterative nonlinear least squares fit of the data to this model. Unlike the other methods, the Random Coil method assumes the polymers are approximately random coils. This can be an advantage for large random coil molecules because it allows the fit to proceed with fewer parameters than would otherwise be required in a simple polynomial fit, and the result can be lower estimated errors.

For the Peaks procedure (page 8-54), you can select which calculation type—Zimm, Berry, Debye, or random coil—you wish to employ. If you own a miniDAWN, the calculation type must be either Zimm or Random Coil.

The result of these calculations is that for each slice i we have the molar mass M_i and the mean square radius $\langle r_g^2 \rangle_i$.

Assuming good chromatographic separation, these quantities can be used together with the concentration c_i (measured with a concentration-sensitive detector) to find the molar mass and radius moments, as described next.

Other Structures Method

For certain other forms of molecular structures (for example, sphere, coated sphere, and rod), we fit the Zimm equation to R_θ / K^*c vs. $\sin(\theta/2)$. As in the Conventional method, we insert into Eq. (18) the theoretical form factor $P(\theta)$ for the desired model. Form factor models have been derived for spheres, coated spheres, and rods and are covered in the text by van de Hulst¹. Note that the sphere and coated sphere models yield geometric radii, while the rod model produces a length.

Spheres

$$P(\theta) = \frac{3}{u^3} (\sin u - u \cos u) \quad (36)$$

where $u = (4\pi r / \lambda) \sin(\theta/2)$.

Rods

$$P(\theta) = \int_0^{2u} \frac{\sin t}{t} dt - \frac{\sin^2 u}{u^2} \quad (37)$$

where $u = (2\pi / \lambda) L \sin(\theta/2)$, and L is the rod length, which is assumed to be much greater than its negligible radius.

1. H.C. van de Hulst, *Light Scattering by Small Particles*, Wiley, New York (1957)

Molar Mass and RMS Radius Moments

ASTRA calculates the following molar mass and RMS (root mean square) radius moments for each peak selected. Naturally, moments may be referenced to averages over the entire sample, which may include many peaks.

Number-average molar mass:

$$M_n = \frac{\sum_i n_i M_i}{\sum_i n_i} = \frac{\sum_i c_i}{\sum_i c_i / M_i} \quad (38)$$

Note that an ASTRA measurement usually requires an independent concentration determination. Since the relation between concentration (mg/mL) and number density (number/mL) is simply $nM = c$, the results of Eq. (38) follow immediately.

Weight-average molar mass:

$$M_w = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i} = \frac{\sum_i c_i M_i}{\sum_i c_i} \quad (39)$$

z-average molar mass:

$$M_z = \frac{\sum_i n_i M_i^3}{\sum_i n_i M_i^2} = \frac{\sum_i c_i M_i^2}{\sum_i c_i M_i} \quad (40)$$

The measurement of the mean square radius, $\langle r_g^2 \rangle$, by light scattering invariably requires measurement of the product of the molar mass times this quantity. The result depends also upon the concentration of the molecules. Thus measurement of the effective mean square radius is weighted by cM . Accordingly, we derive for each peak selected a scattered light weighting as:

$$\langle r_g^2 \rangle_{LS} = \frac{\sum_i c_i M_i \langle r_g^2 \rangle_i}{\sum_i c_i M_i} \quad (41)$$

This quantity is usually referred to as the z-average mean square radius, $\langle r_g^2 \rangle_z$, though this definition is quite strange. Specifically, it arises from the polymer chemistry nomenclature for a so-called ideal random coil structure whereby the molar mass is directly proportional to the mean square radius to the 0.5 power, that is:

$$M = a \langle r_g^2 \rangle^{0.5} \text{ or } \langle r_g^2 \rangle \propto M^2 \quad (42)$$

where a is a constant. Substituting this value of $\langle r_g^2 \rangle$ into Eq. (41) yields:

$$\langle r_g^2 \rangle_{LS} = \frac{\sum_i c_i M_i M_i^2}{a^2 \sum_i c_i M_i} = \frac{\sum_i c_i M_i^3}{a^2 \sum_i c_i M_i} \quad (43)$$

But this is identical (except for the constant a^2) to Eq. (40), the so-called z-average molar mass. This is the origin of the light scattering derived value of the mean square radius, that is:

$$\langle r_g^2 \rangle_z \equiv \langle r_g^2 \rangle_{LS} \quad (44)$$

Defining Eq. (41) as the z-average mean square radius suggests that there are number-average and weight-average possibilities, as well. These are reported by ASTRA, although their actual significance is unknown.

Number-average mean square radius:

$$\langle r_g^2 \rangle_n = \frac{\sum_i \frac{c_i}{M_i} \langle r_g^2 \rangle_i}{\sum_i \frac{c_i}{M_i}} \quad (45)$$

Weight-average mean square radius:

$$\langle r_g^2 \rangle_w = \frac{\sum_i c_i \langle r_g^2 \rangle_i}{\sum_i c_i} \quad (46)$$

The quantities c_i , M_i , and $\langle r_g^2 \rangle_i$ in these equations are respectively the mass concentration, molar mass (g/mol), and mean square radius of the i^{th} slice. The often referenced root-mean-square radii (rms) are simply the square roots of the associated mean square radii.

ASTRA also calculates two polydispersity values: $\rho = M_w / M_n$ and $\rho' = M_z / M_w$. Only the former is found in the literature.

All measurements processed by ASTRA are weighted by the standard deviations of the measured quantity. These standard deviations are then used to generate the expected standard deviations of all derived quantities. The uncertainty of the weight-average molar mass (M_{avg}) is then calculated as follows:

$$M_{w-avg} = \frac{\sum_i M_i \sigma_{M_i}^2}{\sum_i \sigma_{M_i}^2} \quad (47)$$

σ_{M_i} is the uncertainty in the value of M_i . The error in this calculation is defined as follows:

$$\sigma_{M_{w-avg}} = \frac{1}{\sum_i \sigma_{M_i}^2} \quad (48)$$

Uncertainties in Calculated Quantities

ASTRA calculates uncertainties for all reported quantities. By analyzing the baseline data at the beginning and end of the chromatogram, ASTRA determines the statistical fluctuation in each detector's output, including all photodiodes and the AUX signals.

Each detector is weighted based on the fluctuations (noise) seen in the first and last 10% of the data points, up to 100 data points. Whichever end is least noisy is used to calculate the weighting factor. (For batch mode calculations, data points within each plateau are used to calculate the detector weighting factors for each concentration.)

The error bars in the analysis plot do not represent this weighting factor directly. The analysis plot involves performing an n th order polynomial fit to $R_\theta / K^* c$ (for the Conventional Method), $K^* c / R_\theta$ (for the Zimm (Reciprocal) Method), $\sqrt{K^* c / R(\theta)}$ (for the Berry (Square Root) Method), or $P(\theta)$ (for the Other Structures Method). The error bar calculation therefore involves the weighting factor, the normalized R_θ value as well as a concentration uncertainty factor and the Chi-squared value returned from the fit. If the normalization is off for some detectors, then the Chi-squared value from the fit tends to increase, causing all error bars to grow. Hence, changes to the normalization coefficients will affect the error bars shown in the analysis plot, as well as the uncertainties in the overall peak results.

The different errors combine according to the usual rules for propagation of errors to yield a standard deviation (depending on calculation method) for each slice. These in turn allow calculation of uncertainties in the molar mass and size for each slice, and hence uncertainties in the calculated molar mass and size averages.

Remember, these uncertainties are statistical only, and do not include any of the many possible systematic errors that may be present. Examples are errors in dn/dc , the DAWN calibration constant, the AUX calibration constants, and the normalization coefficients.

Use the reported uncertainties as a measure of the statistical consistency of the data, never as an absolute limit on the error in your results.

Out of Range Values

Occasionally, electrical noise or a very low concentration or light scattering signal may cause the calculated molecular weight at a particular slice to be a negative number. For low molecular weights, often the mean square radius at a particular slice will be negative due to random noise in the analysis plot for that slice. Also, noise may cause both the calculated molecular weight and the mean square radius to have uncertainties larger than the values themselves. In these cases, special considerations are called for.

When calculating molecular weight averages, ASTRA first checks the calculated molecular weight values of all slices to be included in the calculations to find out if any of them are negative. ASTRA then removes slices that have negative values before calculating the averages.

When calculating mean square radius averages ASTRA includes values from all slices in the summation. If the sum of the mean square radii is positive, ASTRA will calculate the root mean square averages. If it is negative, the resulting root mean square averages will be set to zero.

In addition to the above, if any of the slices to be included in the averages have uncertainties larger than the values themselves, ASTRA will exclude them from the averages.

When plotting data in the Distribution Plots, ASTRA removes any slices that have negative values.

Differential Distribution Calculations

ASTRA 6 uses an adaptive binning technique for determining the differential distributions. It works both with the direct results, and with data that has been fit with results fitting.

Branching Calculations

ASTRA performs a number of sophisticated branching calculations. These are described below.

Branching Ratio: Radius Method

The branching ratio g_M is formally defined¹ as:

$$g_M = \left(\frac{\langle r^2 \rangle_{br}}{\langle r^2 \rangle_{lin}} \right)_M \quad (49)$$

where $\langle r^2 \rangle_{br}$ and $\langle r^2 \rangle_{lin}$ are the mean square radii of branched and linear (unbranched) polymer samples to be compared. Note that the ratio is taken at the same molar mass, *not* at the same volume. In general, for a given molar mass, the branched polymer will have a smaller radius, so g_M will lie between 0 and 1.

ASTRA calculates g_M this way: If no results fitting method has been selected, ASTRA uses the raw RMS Radius vs. MM data for both the linear and branched files. If a results fitting method has been selected, ASTRA uses the fitted data from MM vs. Volume and RMS Radius vs. Volume directly in the branching calculations. For a number of points (300 points per decade of molar mass), Eq. (49) is applied.

In order to obtain useful branching information, the two files (linear and branched) should overlap as much as possible in molar mass. The branching ratio g_M can only be calculated in this region of overlap, since only in this region can radii be found at the same molar mass.

To use this method, select the Radius method in the Branching properties view. See “Branching” on page 8-85.

Branching Ratio: Mass Method

If the molecular radii are too small to be calculated accurately, then we must use another method. Assuming the Flory-Fox equation is valid,² it can be shown that:

$$g_M = \left(\frac{M_{lin}}{M_{br}} \right)^{\frac{(a+1)}{e}}_V \quad (50)$$

1. B.H. Zimm and W.H. Stockmayer, “The dimensions of chain molecules containing branches and rings,” *J. Chem. Phys.*, vol. 17, pp. 1301-1314 (1949).
2. L.P. Yu and J.E. Rollings, “Low-angle light scattering-aqueous size exclusion chromatography of polysaccharides: Molecular weight distribution and polymer branching determination,” *J. Appl. Polym. Sci.*, vol. 33, pp. 1909–1921 (1987).

where M_{lin} and M_{br} are the molar masses of a linear and branched polymer, respectively, a is the Mark-Houwink-Sakurada parameter for the linear polymer, and e is the drainage parameter, ranging from 0.5 for a non-draining polymer to 1.0 for a free-draining polymer to 1.5 for a Flory-Fox polymer.¹ A value of 0.5–1.0 seems most used in the literature. The effect of the choice of e on the results can be seen in the figure below, which shows g_M for various values of e using a Mark-Houwink-Sakurada parameter a of 0.7, typical for a random coil. Notice that the ratio in Eq. (50) is taken at constant elution volume V .

If no results fitting method has been selected, ASTRA uses the raw MM vs. Volume data for both the linear and branched files. If a results fitting method has been selected, ASTRA uses the fitted data from MM vs. Volume in the branching calculations. For each slice of the branched file, ASTRA obtains the linear molar mass from the slice in the linear file having the elution volume closest to that of the branched slice.

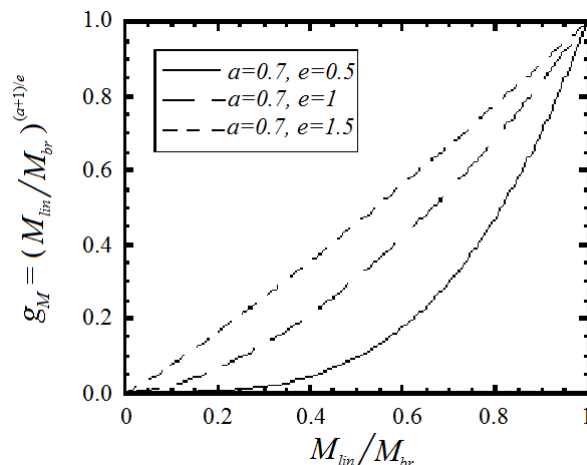


Figure D-1: Branching ratio g_M as a function of a and e for the Mass Method

For this method, the volumes should have a large region of overlap for an effective plot. To use this method, select the Mass method in the Branching properties view for each branched file. See “Branching” on page 8-85.

Branching Ratio: Viscosity Method

The branching ratio $g'M$ is formally defined as:

$$g'(M) = \frac{[\eta(M)]_{br}}{[\eta(M)]_{lin}} \quad (51)$$

where η_{br} and η_{lin} are the intrinsic viscosities of branched and linear (unbranched) polymer samples to be compared. Note that the ratio is taken at the same molar mass, *not* at the same volume. In general, for a given molar mass, the branched polymer will have a smaller radius, so $g'M$ will lie between 0 and 1.

1. B.H. Zimm and R.W. Kilb, “Dynamics of branched polymer molecules in dilute solution,” *J. Polym. Sci.*, vol. 37, pp. 19-42 (1959).

The Radius and Viscosity branching ratios are related by a term called the “drainage parameter” (e), as follows:

$$g^e = g'$$

ASTRA uses the raw intrinsic viscosity vs. MM data for both the linear and branched files. For a number of points (300 points per decade of molar mass), Eq. (51) is applied.

In order to obtain useful branching information, the two files (linear and branched) should overlap as much as possible in molar mass. The branching ratio $g'M$ can only be calculated in this region of overlap, since only in this region can intrinsic viscosity be found at the same molar mass.

To use this method, select the Viscosity method in the Branching properties view. See “Branching” on page 8-85.

Branching Per Molecule

The number of branches per molecule is related to the branching ratio, but some knowledge of the type of branching is necessary. You can choose either trifunctional (Y or T) or tetrafunctional (X) branching, and monodisperse or polydisperse slices. These formulas¹ relate g_M to B for randomly branched polymers:

Trifunctional

- Polydisperse:

$$g_M = \frac{6}{B_{3w}} \left\{ \frac{1}{2} \left(\frac{2 + B_{3w}}{B_{3w}} \right)^{1/2} \ln \left[\frac{(2 + B_{3w})^{1/2} + B_{3w}^{1/2}}{(2 + B_{3w})^{1/2} - B_{3w}^{1/2}} \right] - 1 \right\} \quad (52)$$

- Monodisperse:

$$g_M = \left[\left(1 + \frac{B_{3n}}{7} \right)^{1/2} + \frac{4B_{3n}}{9\pi} \right]^{-1/2} \quad (53)$$

Tetrafunctional

- Polydisperse:

$$g_M = \frac{\ln(1 + B_{4w})}{B_{4w}} \quad (54)$$

1. B.H. Zimm and W.H. Stockmayer, *ibid.*

- Monodisperse:

$$g_M = \left[\left(1 + \frac{B_{4n}}{6} \right)^{\frac{1}{2}} + \frac{4B_{4n}}{3\pi} \right]^{-\frac{1}{2}} \quad (55)$$

For each of these relations, the left hand side, g_M , is known already (see the previous sections). The appropriate equation is solved for B for each slice which produced a reasonable value of g_M . Note that if g_M falls outside the range 0 to 1, no value of B will be calculated for that slice.

The legend is labeled with the specified functionality: “3” for Trifunctional branching or “4” for Tetrafunctional branching; “n” for Monodisperse slices or “w” for Polydisperse. For each branched file to be plotted, select the branching functionality and whether the slices are monodisperse or polydisperse in the Branching property view.

The next figure, a plot of Eqs. (41)–(44), shows how the B 's are related to g_M for the various branching options. Note that these relations assume randomly branched polymers. Also note that different assumptions about functionality and dispersity yield quite different values of B for the same value of g_M . Thus some knowledge of the type of branching is necessary for a plot of branches per molecule to have any meaning.

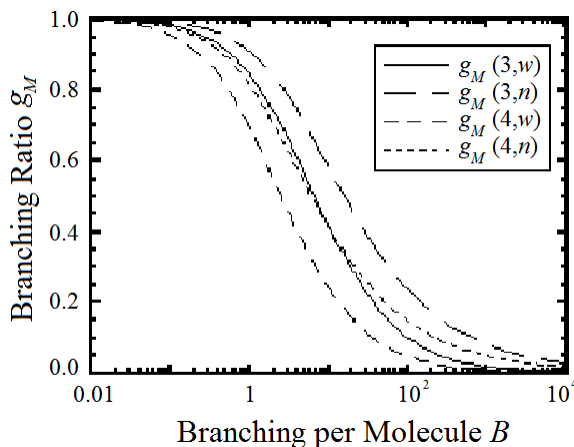


Figure D-2: Branching ratio g_M as a function of B for various branching options

Long Chain Branching

The long chain branching per 1000 repeat units is defined for each slice as

$$\lambda = 1000B \frac{R}{M} \quad (56)$$

where B is the branching per molecule for the slice (as calculated above), R is the repeat unit molar mass, and M is the branched molar mass for the slice. You must enter the repeat unit molar mass in the Unit MW box in the Branching property view for each branched file to be plotted.

Particles

Particles support is an add-on option for ASTRA. This option provides a procedure to calculate particle number densities (see “Number from LS Data” on page 8-75). Particles include latices, liposomes, and vesicles. Particle measurements are especially suited for use when a light scattering instrument is coupled to a fractionation technique such as Field Flow Fractionation (FFF) or Capillary Hydrodynamic Fractionation (CHDF), but concentration is not measured.

CONTENTS	PAGE
Determination of Sizes	E-2
Radius Moments	E-5
Theory	E-8

Determination of Sizes

As discussed in Appendix D, “Light Scattering Theory”, data collected by a DAWN system are used to derive molecular parameters. In the ASTRA Particles mode, however, concentration is not measured and dn/dc need not be known. It is assumed further that the particle concentrations are low enough that there are no interactions between them of consequence, and the 2nd virial coefficient is assumed to be zero. Most importantly, it is assumed that the particles present have been fractionated, so that each slice contains particles of identical size.

Only measurement of the variation of the scattered intensity with angle is made, that is, only the excess Rayleigh ratio need be measured. This angular variation is then matched to an assumed particle shape to obtain a corresponding size. If nothing is known of the particle shape then, for certain classes of particles, it may still be possible to obtain a measure of the particle’s mean square radius. Interpretation of these values in terms of explicit particle size characteristics again requires some assumed model.

The particles considered, except those analyzed by means of the Lorenz-Mie theory (discussed in “Homogeneous Spheres Using Exact Lorenz-Mie Theory” on page E-4) must satisfy the Rayleigh-Gans criteria:

$$\begin{aligned} |m - 1| &\ll 1, \text{ where } m = n / n_0 \text{ and} \\ ka|m - 1| &\ll 1, \text{ where } k = 2\pi / \lambda \end{aligned} \quad (1)$$

and a is a characteristic dimension of the particle.

Conventional Mean Square Radius Determination

First, construct a conventional plot excluding the optical constant and concentration terms, that is, create a plot of R_θ vs. $\sin^2(\theta/2)$. Second, fit a polynomial in $\sin^2(\theta/2)$ to the data, and thereby obtain the intercept at zero angle, R_0 , as well as the slope at zero angle, $s = d[R_\theta] / d[\sin^2(\theta/2)]_{\theta=0}$. Thus we have the following, which is in agreement with Appendix D.

$$\langle r_g^2 \rangle = \frac{-3s\lambda^2}{16\pi^2 R_0} \quad (2)$$

Note that this result is valid only in the Rayleigh-Gans limit wherein the excess Rayleigh ratio is assumed to be proportional to

$P(\theta) = 1 - 2\mu^2 \langle r_g^2 \rangle / 3! + \dots$ as given in “Determination of Molar Mass and Sizes” on page D-13.

Zimm and Berry Mean Square Radius Determinations

We again follow the Rayleigh-Gans approximation in Appendix D to obtain the following for the Zimm method:

$$\langle r_g^2 \rangle = \frac{3s\lambda^2 R_0}{16\pi^2} \quad (3)$$

and the following for the Berry method:

$$\langle r_g^2 \rangle = \frac{3s\lambda^2 \sqrt{R_0}}{8\pi^2} \quad (4)$$

Mean Square Radius Determination from an Assumed Random Coil

As discussed in “Determination of Molar Mass and Sizes” on page D-13, we insert the theoretical form factor $P(\theta)$ for random coils into Eq. (18).

$$P(\theta) = \frac{2}{u^2} (e^{-u} - 1 + u) \quad (5)$$

where $u = (4\pi / \lambda^2) \langle r_g^2 \rangle \sin^2(\theta / 2)$

Since $P(\theta)$ is a nonlinear function of its parameter, $\langle r_g^2 \rangle$, we use an iterative nonlinear least squares fit to the Zimm formalism. Unlike the other fit methods, the Random Coil method assumes the polymers are random coils. This can be an advantage for large random coil molecules, because it allows the fit to proceed with fewer parameters than would otherwise be required in a simple polynomial fit, and the result can be lower estimated errors. The only size derived, of course, is the mean square radius.

However, for a polymer comprised of N segments of length a , the mean square radius of such a polymer in a theta solvent is given by:

$$\langle r_g^2 \rangle = Na^2 / 6 \quad (6)$$

Size from a Known Structure

To derive a particle size based on a structure known *a priori*, we again plot R_θ vs. $\sin^2(\theta / 2)$ and replace the theoretical form factor $P(\theta)$ by the appropriate model assumed. Appropriate form factors have been derived

for spheres, coated spheres, and rods. They are covered in the text by van de Hulst¹. Note that the sphere and coated sphere models yield geometric radius, while the rod model produces a length.

Sphere

$$P(\theta) = \left[\frac{3}{u^3} (\sin u - u \cos u) \right]^2 \quad (7)$$

where $u = 2ka \sin(\theta/2)$.

Rod

$$P(\theta) = \int_0^{2u} \frac{\sin t}{t} dt - \frac{\sin^2 u}{u^2} \quad (8)$$

where $u = kL \sin(\theta/2)$, and L is the rod length, where L is assumed to be much greater than the rod radius.

Homogeneous Spheres Using Exact Lorenz-Mie Theory

If particles are known to be homogeneous spheres, their radii may be derived from the exact scattering theory developed by Ludvig Lorenz and referred to as the Lorenz-Mie theory. This theory represents an exact solution of Maxwell's electromagnetic theory. There are no restrictions on the particle's refractive index or size, so the ASTRA software may be used to determine the radius of homogeneous spherical particles including latex spheres and even gold and carbon particles whose refractive indices are complex.

From the Lorenz-Mie theory, the measured values of R_θ at the angular set measured are used to extract the radius producing the best fit to the theory in a least squares sense.

The extended Lorenz-Mie exact calculation for a coated sphere (single layer) is given in the text by Bohren and Huffman based on the paper of A. L. Aden and M. Kerker².

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1. H.C. van de Hulst, *Light Scattering by Small Particles*, Wiley, New York (1957)
 2. A. L. Aden and M. Kerker, "Scattering of electromagnetic waves from two concentric spheres," *J. Appl. Phys.*, vol. 22, pp. 1242-1246 (1951)

Radius Moments

ASTRA calculates the mean square radius moments for each peak as discussed in “Molar Mass and RMS Radius Moments” on page D-18.

Although only the LS average (also referred to as the z-average) is most commonly measured, the other two are shown for completeness. The specific type of radius (RMS radius, geometric radius, or hydrodynamic radius) depends on the type of analysis being performed. As in Appendix D, all summations are taken over one peak.

RMS Radius

LS Average

$$\langle r_g^2 \rangle_{LS} = \frac{\sum_i R_{0_i} \langle r_g^2 \rangle_i}{\sum_i R_{0_i}} \equiv \langle r_g^2 \rangle_z \quad (9)$$

The quantities R_0 , and $\langle r_g^2 \rangle_i$ in Eq. (9) and V_i in the equations that follow are respectively the Rayleigh Ratio, mean square radius, and volume of the i^{th} slice. The root mean square (rms) radii are simply the square roots of the appropriate mean square radii.

Number Average

$$\langle r_g^2 \rangle_n = \frac{\sum_i \frac{R_0 \langle r_g^2 \rangle_i}{V_i^2}}{\sum_i \frac{R_0}{V_i^2}} \quad (10)$$

Weight Average

$$\langle r_g^2 \rangle_w = \frac{\sum_i \frac{R_0 \langle r_g^2 \rangle_i}{V_i}}{\sum_i \frac{R_0}{V_i}} \quad (11)$$

Uncertainty Weighted Average

$$\langle r_g^2 \rangle_{avg} = \frac{\sum_i \langle r_g^2 \rangle_i \sigma_{\langle r_g^2 \rangle_i}^2}{\sum_i \sigma_{\langle r_g^2 \rangle_i}^2} \quad (12)$$

Where $\langle r^2 \rangle_i$ is as defined previously, and $\sigma_{\langle r_g^2 \rangle_i}^2$ is the uncertainty in the mean square radius measurement. The error in this calculation is defined as follows:

$$\sigma_{\langle r_g^2 \rangle_{avg}}^2 = \frac{1}{\sum_i \sigma_{\langle r_g^2 \rangle_i}^2} \quad (13)$$

Geometric and Hydrodynamic Radius

The quantities R_0 , r_i , and V_i in these equations are respectively the Rayleigh Ratio, radius (either geometric or hydrodynamic), and the volume of the i^{th} slice.

Number Average

$$R_n = \frac{\sum \frac{R_0 r_i}{V_i^2}}{\sum \frac{R_0}{V_i^2}} \quad (14)$$

Weight Average

$$R_w = \frac{\sum \frac{R_0 r_i}{V_i}}{\sum \frac{R_0}{V_i}} \quad (15)$$

Z-Average

$$R_z = \frac{\sum R_0 r_i^2}{\sum R_0} \quad (16)$$

Uncertainty Weighted Average

$$r_{avg} = \frac{\sum r_i \sigma_{r_i}^2}{\sum \sigma_{r_i}^2} \quad (17)$$

where r_i is as defined previously, and σ_{r_i} is the uncertainty in the radius measurement. The error in this calculation is defined as follows:

$$\sigma_{r_{avg}} = \frac{1}{\sum \sigma_{r_i}^2} \quad (18)$$

Theory

This section discusses how the distribution plots are calculated and why a model is needed.

The mean square radius is given by Eq. (19), where the distances r_i are measured from the particle's center of mass to the mass element m_i .

$$\langle r^2 \rangle = \frac{\sum_i r_i^2 m_i}{\sum_i m_i} = \frac{1}{M} \int r^2 dm \quad (19)$$

Eq. (19) refers to a single particle whereas the quantity actually measured from an ensemble of particles may be shown to be a so-called LS-average mean square radius.¹ Were the particles random coils in a theta solvent, then this would be the so-called z-average mean square radius. We assume that the particle size distribution within each slice of an eluting sample following separation is essentially monodisperse. Therefore the particles in slice i , each of mass M_i , are assumed to have the same mean square radius. We define the root mean square radius as the square root of the mean square radius or, simply, $r_g = \langle r^2 \rangle^{1/2}$.

The Rayleigh-Gans-Debye approximation (RGD):

$$\frac{K^* c}{R(\theta)} \approx \frac{1}{M_w P(\theta)} \quad (20)$$

can be re-written in the limit as $\theta \rightarrow 0$, $P(0) = 1$, and we have:

$$R(0) = K^* c_i M_i = K^* n_i M_i^2 \quad (21)$$

since the concentration of mass in the i^{th} slice is $c_i = n_i M_i$. If the elements of the particle whose molar mass is M_i are of uniform density and occupy a volume V_i , then the number of particles per mL in the i^{th} slice, n_i , is proportional to the extrapolated zero-angle Rayleigh ratio divided by the square of the particle's volume, i.e.:

$$n_i \propto R(0) / V_i^2 \quad (22)$$

Therefore we can write the *number fraction* of particles within slice i as n_i / D where:

$$D = \sum_j n_j \quad (23)$$

1. P.J. Wyatt, "New Insights into GPC Combined with MALS," *Waters Corporation GPC Symposium Proceedings* (San Diego, 1996).

is the summation taken over all slices in the selected region (or peak) of the eluting fractions. Note that although M_i is the *molar* mass of the particles, that value is proportional to the *mass* of the particles. Both are proportional to the *volume* of the particles if the volume is of uniform density.

Although the analysis of each slice results in a corresponding value of r_g , there may be other slices with similar sizes due to experimental fluctuations in the derived values. The expected monotonic variation of r_g with elution volume may be obtained by fitting the calculated values to a selected functional form using a least squares procedure. Alternatively, the slice data may be sorted into a set of size bins to obtain the differential number fraction after dividing each such fraction by the bin size. The fractions may also be distributed over the range of size bins included within the measured standard deviation associated with the particular contributing fraction.

In any event, the differential number fraction $n(r) dr$ of particles in the selected peak region between r and $r + dr$ now may be calculated explicitly without any advance knowledge of the mass concentration at each slice provided we know the particle structure and that the RGD approximation is valid. For example, if we know that the particles are homogeneous spheres, we may replace V_i^2 by r_g^6 . There are many other particle shapes where the relation between r_g and V_i^2 is known. The differential mass fractions may be generated in a similar manner without reference to a second detector.

What about particles whose shape is not known *a priori*? Although we may still calculate r_g as a function of elution volume (the r_g “calibration curve”), we cannot determine the differential number or mass fractions. Indeed, if we do not know the relation between the measured r_g and the particle's hydrodynamic radius, we cannot generate differential distributions. Were we to add a concentration detector following the LS detector, we could easily generate the differential mass fraction distributions of r_g .

A few other points must be discussed; most important among them is the applicability of the RGD approximation assumed in the preceding analysis. The simplest particles most frequently measured by particle sizing procedures are the polystyrene latex (PSL) spheres (emulsions) whose refractive index at wavelengths in the visible is about 1.59. Relative to water, whose refractive index is about 1.33, these spheres have a relative refractive index $m = 1.59/1.33 \approx 1.2$. Rigorous application of the RGD theory requires that $m - 1 \ll 1$, which is a slight stretch for these PSL spheres. Perhaps more importantly, the phase shift of a wave passing through the particle, $2\pi a[m-1]/\lambda_0$, where a is the sphere radius, also must be $\ll 1$. Even if we make the assumption that $0.2 \ll 1$, attempting to size larger submicron particles using this approximation will quickly lead us out of the range of RGD applicability!

The saving grace of this approach is twofold: first, the theory happens to work significantly better than one might expect, even when the RGD requirements are not strictly satisfied, and second, the pertinent values are calculated in the limit $\theta \rightarrow 0$ (as shown in Eq. (21)), a regime where the RGD requirements are much more easily satisfied. As the scattering angle becomes very small, the RGD approximation becomes more valid as was confirmed vividly by the analyses of Kerker et al.¹ The result is that values of R_θ may be generated directly from the measurements if the particle's structure is known, or from the more general expansion of the form factor $P(\theta)$.²

Furthermore, many of the calculated results for the analyses of distributions of PSL spheres may be checked with more exact LS theory to confirm the precision of the sizes measured using the RGD approximation. Applying the Lorenz-Mie theory confirms the results derived by the present treatment. In addition, average values measured by photon correlation spectroscopy (PCS) at individual slices also confirm the average values generated by the present implementation of RGD theory.

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1. M. Kerker, W.A. Farone, and E. Matijevic, "Applicability of Rayleigh-Gans Scattering to Spherical Particles," *J. Opt. Soc. Am.*, vol. 53, pp. 758-759 (1963).
 2. P.J. Wyatt, "Light scattering and the absolute characterization of macromolecules," *Analytica Chimica Acta*, vol. 272, pp. 1-40 (1993).

F QELS Theory

This appendix gives a quick overview of the theory behind cumulants and regularization, which are analysis techniques used with QELS data. This includes descriptions of the implementation in ASTRA and interpretation of results.

CONTENTS	PAGE
Cumulants	F-2
Regularization	F-7

Cumulants

The analysis of QELS data is straightforward for a monodisperse sample. For unfractionated, polydisperse samples, however, the analysis becomes much more complicated. The simplest approach to analyzing data from polydisperse samples is to assume that the sample is monodisperse, apply the analysis from ASTRA, and come up with some sort of mass-averaged result for the hydrodynamic radius. The measured correlation function for a polydisperse sample actually contains more information than this, and several strategies have been developed to extract more information about the underlying size distribution from the correlation function.

The next level of sophistication in QELS analysis for polydisperse, unfractionated samples is the method of cumulants. In a nutshell, the method of cumulants involves fitting the correlation function not to a single decay time, but to a Gaussian distribution of decay times. The method of cumulants retrieves the mean and variance for this distribution.

Theory

The result of a QELS measurement is a second order correlation function:

$$g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau) \rangle}{\langle I(t) \rangle^2} \quad (1)$$

where $I(t)$ is the intensity of the scattered light at time t , and the brackets indicate averaging over all t . The correlation function depends on the delay τ , that is, the amount that a duplicate intensity trace is shifted from the original before the averaging is performed. A typical correlation function for a monodisperse sample is shown in Figure F-1.

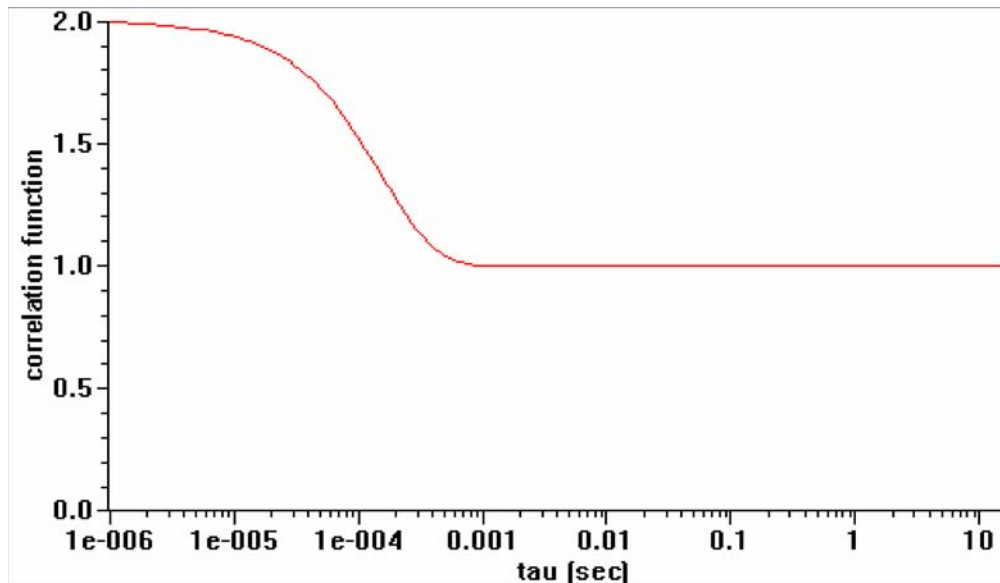


Figure F-1: Correlation function for a multi-tau correlator like that in WyattQELS

As described in various light scattering texts¹, the correlation function for a monodisperse sample can be analyzed via the equation:

$$g^{(2)}(\tau) = B + \beta \exp(-2\Gamma \tau) \quad (2)$$

where B is the baseline of the correlation function at infinite delay, β is the correlation function amplitude at zero delay, and Γ is the decay time. A nonlinear least squares fitting algorithm can be applied to Eq. (2) to retrieve the correlation function decay time Γ . This is exactly what is done in the ASTRA QELS analysis.

From this point, Γ can be converted to the diffusion constant D for the particle via the relation:

$$D = \frac{\Gamma}{q^2} \quad (3)$$

Here, q is the magnitude of the scattering vector, and is given by

$$q = \frac{4\pi n}{\lambda_0} \sin(\theta/2) \quad (4)$$

where n is the solvent index of refraction, λ_0 is the vacuum wavelength of the incident light, and θ is the scattering angle.

Finally, the diffusion constant can be interpreted as the hydrodynamic radius r_h for a diffusing sphere via the Stokes Einstein equation:

$$r_h = \frac{kT}{3\pi\eta D} \quad (5)$$

where k is Boltzmann's constant and η is the solvent viscosity.

The previous equations provide the tools for analyzing a correlation function from a monodisperse sample, but do not address the effects of polydispersity on the correlation function. One of the first attempts to analyze such data was the method of cumulants. First proposed by Koppel², the method of cumulants involves expanding Eq. (2) into the various moments of a distribution. In its simplest expression, this expansion turns Eq. (2) into the following:

$$g^{(2)}(\tau) = B + \beta \exp\left(-2\bar{\Gamma} \tau + \kappa_2 \tau^2 - \frac{\kappa_3}{3} \tau^3 \dots\right) \quad (6)$$

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1. B. Chu, *Laser Light Scattering: Basic Principles and Practice*, (Academic, Boston, 1991).
 2. D.E. Koppel, "Analysis of macromolecular polydispersity in intensity correlation spectroscopy: The method of cumulants," *J. Chem. Phys.* vol. 57, pp. 4814-4820 (1972).

Here, the decay time is now the average for the distribution, while the higher moments correspond to the variance, or width of the distribution (K_2), the skewness of the distribution (K_3) and so on.

In practice, it is usually only possible to determine the first two moments of the expansion in Eq. (6), that is, the average and variance. These are often referred to as the first and second cumulant. In this simplest form, the method of cumulants then boils down to fitting the correlation function to a Gaussian distribution of decay times; only the average and width of the distribution are obtained.

Application of the Method of Cumulants

In the ASTRA software, a variant of Eq. (6) is used to obtain the first and second cumulants in a nonlinear least squares fit of the correlation function. This variation was derived by Frisken¹, and is given by:

$$g^{(2)}(\tau) = B + \beta \exp(-2\bar{\Gamma}\tau) \left(1 + \frac{\mu_2}{2!} \tau^2 - \frac{\mu_3}{3!} \tau^3 \dots \right)^2 \quad (7)$$

Here, the moments μ_n correspond to the K_n terms in Eq. (6), and are the physical moments about the mean $\bar{\Gamma}$. Eq. (7) is inherently more stable than Eq. (6) when fitting at large delay times τ , thus leading to a more robust analysis of the correlation function than has traditionally been obtained from the method of cumulants.

The results obtained from the fit in the QELSBatch cumulant analysis are the first two moments, $\bar{\Gamma}$ and μ_2 in Eq. (7), as well as the baseline B and amplitude β . The baseline and amplitude values are used in the data filtering algorithm to reject QELS correlation functions after the initial cumulants analysis. However, the first two cumulants are the quantities of interest for assessing the polydispersity of the sample.

The first two moments define a Gaussian distribution in decay times, where the first cumulant gives the mean of the distribution, and the square root of the second cumulant gives the standard deviation. In terms of a distribution for sizes, the decay time distribution can be converted to

1. B.J. Frisken, "Revisiting the method of cumulants for the analysis of dynamic light-scattering data," *Applied Optics*, vol. 40, pp. 4087-4091 (2001).

hydrodynamic radius via equations 3 through 5. Since the radius is inversely proportional to the decay time, the distribution in radius is no longer a symmetric Gaussian. This can be seen in Figure F-2.

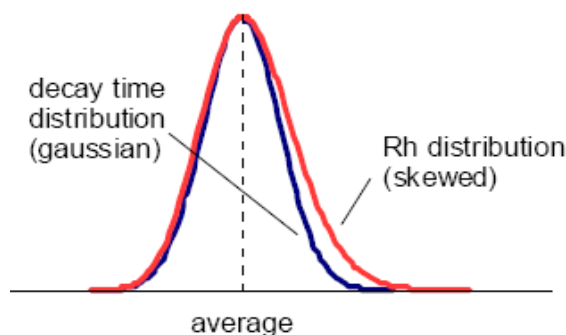


Figure F-2: Cumulants distributions in decay time and hydrodynamic radius

In the cumulants analysis results, the fitted first and second moments—that is the decay time distribution average and variance—are reported, as well as the uncertainties (one standard deviation) from the fit. The square root of the variance is used to determine the standard deviation in the decay time distribution. The average, the average plus the standard deviation, and the average minus the standard deviation are converted to hydrodynamic radius via Eq. 3 through 5, and are included in the results.

Interpretation of Cumulant Results

The cumulants method presents a challenge in displaying the results. ASTRA present the hydrodynamic radius results from the cumulants analysis, since size is more intuitive than decay time for most researchers. In the cumulant analysis graph, the average hydrodynamic radius and the distribution values at one standard deviation are presented. This creates an “error bar” appearance for the graph, but the error bars indicate the width of the fitted distribution. They are asymmetric because the hydrodynamic radius is inversely proportional to the symmetric decay time distribution, as shown in Figure F-2.

With cumulant results presented this way, it is important to remember that there is uncertainty in the first and second moments determined from the fit. This uncertainty translates into an uncertainty in the average hydrodynamic radius, but more importantly, an uncertainty in the effective width of the distribution implied by the cumulants analysis graph.

Therefore, an uncertainty in this width is estimated by calculating the spread in possible width values based on the fitted uncertainty in the second cumulant. The effective width implied by the cumulants is then compared to the spread of possible widths to derive a percentage uncertainty in the effective width. The average uncertainty in width is reported in the Width property, and should provide a good measure of how much to trust the widths that result from the analysis.

After all is said and done, the question remains how the cumulant analysis results relate to the actual polydispersity of the sample. Assuming that the size distribution in Figure F-2 reflects all samples is simply incorrect. Therefore, the cumulant results should be taken as a semi-quantitative estimate of the degree of polydispersity. It would probably be safe to assume that for two samples with the same average size, but different widths estimated from the cumulant analysis, that the sample with the greater width is more polydisperse. However, trying to define a rigorous polydispersity index from the cumulant analysis would probably lead to very inaccurate results when compared to a quantitative method such as fractionation followed by light scattering to determine the underlying distribution. Therefore, cumulant analysis results should only be used to assess the potential relative polydispersity of samples. Follow-up analysis, such as fractionation followed by light scattering, should be used to assess the reliability of the cumulant analysis results, particularly if they are to be used as the sole assay for polydispersity.

Regularization

Whereas the method of cumulants is one of the simplest approaches to analyzing QELS data from a polydisperse sample, the regularization analysis is one of the most sophisticated. There are many excellent references for the regularization method, and the theory is quite detailed.¹

Theory

As opposed to the method of cumulants, the regularization analysis makes far fewer assumptions about the underlying distribution of sizes that make up the polydisperse sample. A simple predecessor of the regularization method—the histogram method—demonstrates this nicely. In the histogram method, the distribution of decay times is not assumed to be Gaussian, as it is for the cumulant method with only the first two cumulants. Instead, the decay time distribution is divided into bins. Consider, for example, the model correlation function in Figure F-3 for a bimodal distribution consisting of widely separated sizes:

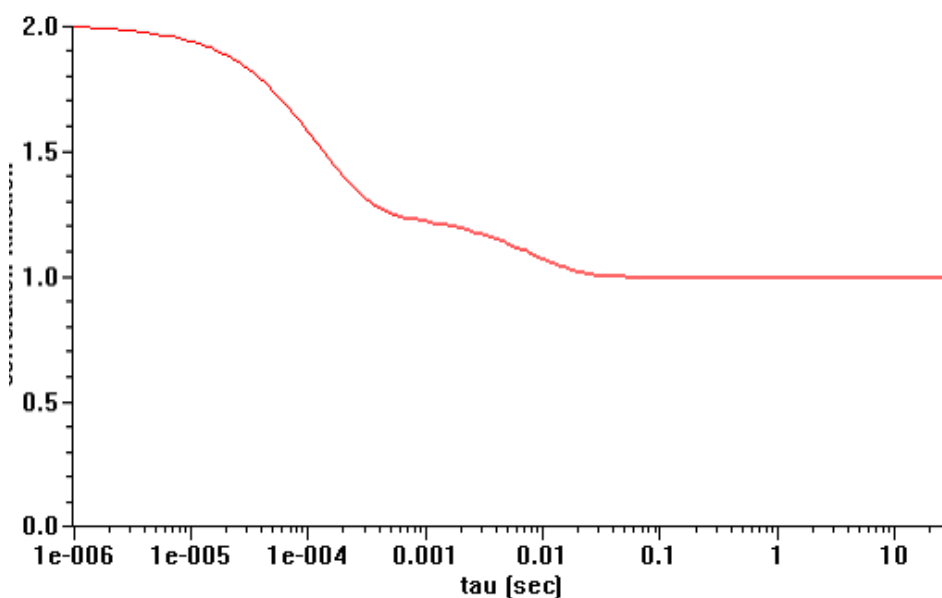


Figure F-3: Bimodal correlation function for mixture of 10 nm and 1 μ m particles.

Clearly, the correlation function in Figure F-3 would best be fit by a “sum” of two separate correlation functions, one with a short decay time, and one with a long decay time. In terms of the histogram method, the underlying distribution would appear as in Figure F-4, that is, only two bins would have any intensity. The correlation function is then modeled by “adding”

1. S.W. Provencher, “Inverse problems in polymer characterization: Direct analysis of polydispersity with photon correlation spectroscopy,” *Makromol. Chem.*, vol. 180, pp. 201-209 (1979).

the correlation functions for the two separate bins. (It is more complicated than this, since there is cross-correlation between the various components, but for the sake of pedagogy, the concept of adding is adequate.)

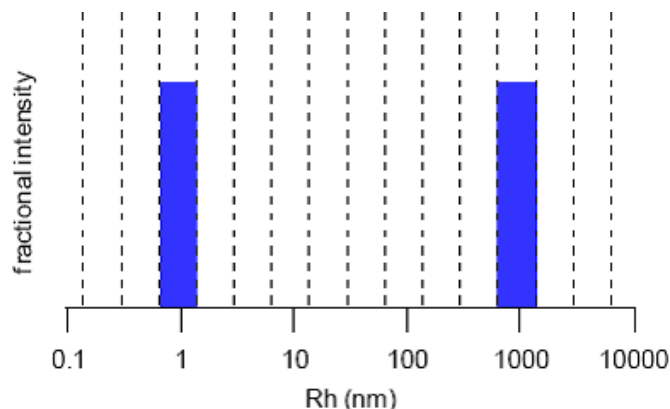


Figure B-2. Model histogram for bimodal size distribution.

Figure F-4: Model histogram for bimodal size distribution

More complicated correlation functions from more polydisperse samples could be modeled by the histogram method. Intensity would be shifted between bins until the right match was found. In so doing, the underlying distribution would be revealed, albeit in a somewhat jagged fashion from the bins. We can remedy this jaggedness by making our bin sizes smaller and smaller until we get the true distribution.

In reality, the histogram method breaks down long before enough bins can be added to accurately represent a distribution. The problem is that as more bins are added, the number of possible solutions explodes. There is not enough information in the correlation function to accurately distribute the intensity over hundreds of bins. In fact, for a standard correlator such as that in the WyattQELS instrument, the largest number of bins that can be handled is only about ten.

The regularization method makes it possible to have a finer mesh of bins. This is accomplished by constraining the types of distributions that can accurately reproduce the correlation function. The most common constraint, and the one employed in the DYNALS algorithm used in ASTRA, is that the distribution be smooth. This is accomplished by adding a regularization term that penalizes solutions that are not smooth. The magnitude of the regularization term determines how smooth the final result must be. The trick of every regularization algorithm is to determine the optimal amount of regularization such that the final solution captures as many features of the true distribution as possible, while balancing out the effects of noise in the correlation function. Noise can add spurious components to the calculated distribution, hence as the noise increases, the regularization term needs to increase to damp these spurious components.

Implementation of Regularization in ASTRA

The regularization algorithm in the ASTRA software is the DYNALS 2.0 algorithm supplied by Alango, Ltd.¹ The DYNALS algorithm sets the regularization level—referred to as the resolution—to the most appropriate value for the level of noise in the correlation function. The resolution value can range between 0 and 1, where 0 corresponds to the noisiest data, and 1 corresponds to the least noisy data. In ASTRA, the optimal value of the resolution is taken from the DYNALS algorithm and reported in the data window for the regularization analysis window.

The results of the regularization are an intensity distribution in hydrodynamic radius. However, in light scattering, the intensity distribution does not give an accurate representation of the number distribution. Therefore, intensity information can be converted to relative number by choosing a mass model for the particles, and applying a correction factor for the intensity. The mass models in ASTRA are sphere and random coil.

Interpreting Regularization Results

Regularization analysis results are more physical than results for the cumulants method. However, some care must be taken in interpreting these results. First, low size peaks (< 1 nm) often appear in the regularization results. These are sometimes attributed to solvent scattering, but are most likely due to avalanche photodiode afterpulsing picked up by the correlator. To exclude this from the correlation function, try setting a longer minimum delay time for the correlation function in ASTRA. Large size peaks are also common in the final distribution. These are usually real and correspond to dust.

Another issue of concern in interpreting regularization results is determining whether the resulting width of the distribution corresponds to an actual polydispersity. For example, applying the regularization analysis to a correlation function from a monodisperse sample often results in a distribution with some width. In general, the noisier the correlation function, the lower the optimal resolution of the regularization algorithm, and the broader the apparent width. Therefore, when interpreting distribution widths from regularization, always consider the resolution obtainable given the level of noise in the correlation function. Ideally, correlation functions for a monodisperse sample and the sample of interest can be obtained with comparable levels of noise, such that the regularization analysis resolution can be accurately assessed.

Finally, the smoothing nature of the regularization algorithm can mask features in the true distribution, even for correlation functions with very low noise. Therefore, if a very structured distribution in sizes is expected, regularization typically returns a much smoother distribution. In short, it

1. A.A. Goldin, "Software for particle size distribution analysis in photon correlation spectroscopy," website documentation at <http://www.softscientific.com/science/WhitePapers/dynals1/dynals100.htm>.

is prudent to compare regularization results with a quantitative method such as fractionation followed by light scattering to determine the true distribution. In general, regularization provides the most accurate analysis for samples that are broadly polydisperse over several orders of magnitude in size and that have intrinsically smooth distributions.

G Viscosity Theory

This appendix reviews the theory of viscosity-related calculations.

CONTENTS	PAGE
Calculating Intrinsic Viscosity	G-2
Intrinsic Viscosity and Molecular Parameters	G-4
Flory-Fox Relation.....	G-5

Calculating Intrinsic Viscosity

ASTRA 6 can process a wide variety of input viscosity sources, ranging from simple devices producing only a single pressure differential to more sophisticated devices that measure specific viscosity directly.

Once specific viscosity is measured, it is useful to compute the intrinsic viscosity. Intrinsic viscosity is defined as the limit of:

$$[\eta] = \lim_{c \rightarrow 0} \frac{\eta_{sp}}{c} \quad (1)$$

Of course, all real instruments measure the specific viscosity at finite concentrations. The concentration dependency of the specific viscosity is typically described using one of three formalisms: the Huggins equation, the Kraemer equation, and the Solomon-Gatesman equation. In all cases, the concentration of the sample must be derived from a detector, such as the Optilab rEX or a UV absorption detector.

Huggins

The Huggins equation is specified as:

$$\eta_{sp} = [\eta]c + k'[\eta]^2 c^2 + O(c^3) \quad (2)$$

The coefficient k' is the Huggins constant. For random coil polymers in good solvents, the Huggins constant typically has a value between 0.0 and 0.3. In size-exclusion chromatography, the concentration of the sample is usually so dilute that one can ignore the concentration terms of third power (or above) and use the approximation:

$$[\eta] = \eta_{sp} / c \quad (3)$$

Solving Eq. (2) for intrinsic viscosity yields:

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{\eta_{sp}^2 k'}{c} + O(\eta_{sp}^3) \quad (4)$$

Eq. (4) is simplified to the following for computation:

$$[\eta] \approx \frac{-1 + \sqrt{1 + 4\eta_{sp} k'}}{2k' c} \quad (5)$$

Kraemer

The Kraemer equation is:

$$\frac{\ln(\eta_{sp} + 1)}{c} \approx [\eta] + k''[\eta]^2 c \quad (6)$$

Solving Eq. (6) for intrinsic viscosity yields:

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{(1/2 + k'')\eta_{sp}^2}{c} + O(\eta_{sp}^3) \quad (7)$$

The expansion shows that for small values of specific viscosity, which is almost always the case for chromatography, the two formalisms are related:

$$k' = 1/2 + k'' \quad (8)$$

Eq. (7) is simplified to the following for computation:

$$[\eta] \approx \frac{-1 + \sqrt{1 + 4k''\ln(1 + \eta_{sp})}}{2k''c} \quad (9)$$

Solomon-Gatesman

The advantage of the Solomon-Gatesman equation is that it does not require empirical constants. However, for values of specific viscosity much less than one, it reduces to the Huggins Equation, with a value of $k' = 1/3$.

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{\eta_{sp}^2}{3c} + O(\eta_{sp}^3) \quad (10)$$

Eq. (10) is simplified to the following for computation:

$$[\eta] \approx \frac{\sqrt{2\eta_{sp} - 2\ln(\eta_{sp} + 1)}}{c} \quad (11)$$

Intrinsic Viscosity and Molecular Parameters

The simplest model of the intrinsic viscosity is due to Einstein and Simha¹. They considered the case of noninteracting rigid particles. They found that the viscosity can be related to the volume fraction of the fluid occupied by the particles. They found:

$$\eta = \eta_0(1 + \gamma\phi) \quad (12)$$

where ϕ is the volume fraction and $\gamma = 2.5$ for spheres and larger for non-spherical particles.

If the weight concentration of the molecule is c , then the number of molecules per unit volume is $N_A c/M$, where N_A is Avogadro's number and M is the molar mass as measured by light scattering. Therefore Eq. (12) can be written in terms of the measured intrinsic viscosity as:

$$[\eta] = \frac{\gamma N_A V_h}{M} \quad (13)$$

where V_h is the hydrodynamic volume of the molecules. Note that M/V_h is the molecular density, so in some sense, the intrinsic viscosity is measuring the molecular density.

The intrinsic viscosity often differs from the bulk density due to molecular shape, molecular density, and the effects of adsorbed or immobilized solvent on the surface of molecule. This so-called hydration layer moves with the molecule, so it affects measurement of the molecular density. In addition, when the molecule has an extended shape, penetration of non-immobilized solvent into the interior of the molecule similarly affects this measurement.

If we set $\gamma = 2.5$, this can be used to define the equivalent spherical volume of a nonspherical molecule. Similarly, it can be used to define the hydrodynamic volume r_h as:

$$r_h = \left[\frac{3V}{4\pi} \right]^{1/3} \quad (14)$$

When defined in this way, r_h is the radius of a sphere with the same intrinsic viscosity as the molecule under study.

-
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Flory-Fox Relation

While the Einstein-Simha relation can be used to define the hydrodynamic radius for solid molecules with adsorbed solvation layers, it not simply related to the molecular size of extended molecules such as random coil polymers. Several models have been developed to consider the effect the hydrodynamic drag on the intrinsic viscosity.

One of the most successful models comes from Flory and Fox who modeled the random coil as a series of “beads on a string” or a “jointed chain”. The string is flexible, but beads are rigid. Flory and Fox considered that hydrodynamic friction causes the solvent near the center of the molecule to move with the same velocity as the center of mass, but solvent near the edges is free to flow into and out of the molecule. This led them to a relationship between the intrinsic viscosity and the mean square radius of the polymer chain in a theta solvent. Their model is:

$$[\eta] = \Phi \langle r^2 \rangle^{3/2} / M \quad (15)$$

where $\langle r^2 \rangle$ is the mean squared end-to-end distance of the chain, and Φ_0 is a universal constant having the value 2.87×10^{23} . In practice, this constant varies somewhat from polymer to polymer with an experimental value closer to 2.5×10^{23} .

The Flory-Fox relationship is valid for polymers in theta solvents. Ptitsyn and Eizner considered the modification required to model other solvents. They found the following relationship:

$$[\eta] = \Phi(\varepsilon) \langle r^2 \rangle^{3/2} / M \quad (16)$$

$$\Phi(\varepsilon) = \Phi_0 (1 - 2.63\varepsilon + 2.86\varepsilon^2) \quad (17)$$

where Φ is now a function of the polymer-solvent interaction parameter ε , and Φ_0 is the Flory-Fox constant. When $\varepsilon = 0$, it reduces to the theta solvent result.

The ε parameter is experimentally measurable with a Mark-Houwink analysis. To perform a Mark-Houwink analysis, the data for a random coil polymer is fit to:

$$[\eta] = KM^a \quad (18)$$

where M is the molar mass. The K and a are fit parameters, which depend upon the polymer, solvent, and temperature. Traditionally, this data is also plotted as $\text{Log}[\eta]$ vs. $\text{Log}[M]$. If the data is fit well, this should be a straight line. The slope parameter a is related to ε by:

$$\varepsilon = (2a - 1)/3 \quad (19)$$

Index

Symbols

+ (plus sign), between key names 1-6

Numbers

100% Mass Recovery D-12

100% mass recovery D-12

21 CFR Part 11 compliance 1-9

benefits of 4-2

definition of 4-2

features enabled by 4-3

icons 1-4

installing module for 2-6

logging in for 2-11, 3-2

sign offs for 4-8, 6-13, 8-105

user accounts for 2-7, 3-3, 3-13

website for 4-2

3D surface plot 11-13

A

A2 calculation 8-71

A2 mass

definition of 1-6

A2 Mass and Radius from LS Data procedure 8-69

A2 radius 1-6

A3 calculation 8-71

Abscissa Units property, experiment configuration 7-15

access levels 2-7, 3-13

Access, Microsoft 4-5

activation keys for optional modules 2-6

Add Connections property, experiment configuration 7-15

Add Instruments property, experiment configuration 7-15

Add to Experiment command, Experiment menu

adding data set definitions 6-28, 11-9

adding graphs 6-29, 11-11

adding procedures 6-26, 8-8

adding reports 6-29, 10-8

.adf file extension 6-7, 6-9, 6-17

administration procedures 8-105

Administrator group 1-4, 2-7, 3-3, 3-13, 4-6

Administrator user level A-2

.afe6 file extension 6-7, 6-9, 6-15, 6-17

.afs6 file extension 6-9, 9-5, 9-13

alarms

desktop alerts 2-13

Diagnostic Manager 5-10

Instruments pane 2-13

alignment procedure 8-18

Alt+F4 keyboard shortcut 3-17

analysis plot

checking normalization coefficients using 8-27

definition of 8-66

error bars in D-21

procedures using 8-66

analysis procedures

data produced by 11-9

effect on data 8-3

list of 8-64

analyzed data 8-3

definition of 11-9

Apply Method command, Experiment menu 6-22, 10-8

Approval category for sign offs 6-13

area under peak, calculating 8-88

ASTRA

activating optional modules for 2-6

customizing environment 3-14

definition of 1-6

exiting 3-17

features of 1-2

installing 2-2

running 2-11, 3-2

system requirements for 2-2

upgrading version 2-3

ASTRA 4

exporting file 6-17

import wizard 6-9

importing sequence 9-5

ASTRA Administrator group 1-4, 2-7, 3-3, 3-13, 4-6

ASTRA Guest group 1-4, 2-7, 3-3, 3-13, 4-6

ASTRA Researcher group 1-4, 2-7, 3-3, 3-13, 4-6

ASTRA Technician group 1-4, 2-7, 3-3, 3-13, 4-6

auto-inject connections 7-38

- autoinjectors 7-31
 - profiles for 7-40
- AUX calibration constant D-11
- AUX connections
 - definition of 7-38
 - profiles for 7-39
- averages 10-4
- axis settings for graphs 11-16
- B**
- band broadening 8-20
- baseline drift 8-50
- Baseline Noise Region property 7-15
- Baselines procedure 3-10, 8-48
- baselines, setting 3-10, 8-48
- Basic collection page 3-9
- Basic Collection procedure 8-11
- Basic operating tier 1-4
- batch mode
 - definition of 1-6, 1-9
 - normalization and 8-25
 - performing experiment in 3-5
- Berry fit method 8-67, D-16
- Berry fit model 8-58
- bitmap files, exporting graphs to 11-20
- Blank Sequence, menu command 9-3
- Blank, Experiment, menu command 6-6
- branching calculations D-23
- branching per molecule D-25
- branching ratio -- mass method D-23
- branching ratio -- radius method D-23
- Browse for Computer dialog 2-14
- buttons, instruction conventions 1-6
- C**
- calculations (see analysis procedures; theory)
- calibration
 - calculating RI from peak data 8-81
- calibration constant
 - definition of D-4
 - LS 8-15
 - RI 8-29
- CFR Part 11 icons 1-4
- Chart tab, Editing dialog 11-17
- chromatography systems 9-6
- Close command, File menu 6-15
- Coated sphere fit model 8-59
- .col file extension 8-14
- collection procedures
 - list of 8-11
 - raw data produced by 11-8
- Collection tab
 - sequences 9-10
- column calibration 7-36
- columns
 - definition of 7-36
 - profiles for 7-36
- commands
 - log of, viewing 5-10
 - parameters for 5-8
 - sending to instruments 5-7
- Commands tab, Diagnostic Manager 5-7
- computers
 - adding to ISI list 2-13
 - removing from ISI list 2-15
 - viewing in instrument list 2-13
- Concentration Source 7-15
- concentrations
 - converting refractive indexes to 8-61
 - converting UV data to 8-61
- configuration
 - adding connections to 6-24
 - adding instruments to 6-24
 - default 7-10
 - definition of 1-7, 3-6, 6-2, 7-2
 - editing 3-7
 - example of 7-4
 - exporting to profiles 12-4
- Configuration folder, Experiments tab 6-2, 6-24
 - (see also configuration)
- configuration of graphs 8-6, 11-16
- configuration procedures
 - list of 8-15
 - (see also experiment configuration)
- Configuration tab
 - sequences 9-7
- Configuration Wizard 7-11
- connections
 - adding to configuration 6-24
 - adding to experiment configuration 7-6
 - definition of 7-38
 - profiles for 7-38
 - types of 7-38
- Connections, Database Administration, menu command 4-5
- contact information 1-10
- Contents command, Help menu 3-16
- conventional column calibration 7-36
- Conventional fit method D-14
- conventions used in this manual 1-5
- conversion procedures
 - Convert RI to Concentration procedure 8-61
 - Convert specific to intrinsic viscosity 8-61

- Convert to Physical Units procedure 8-60
- Convert UV to Concentration procedure 8-61
 - data produced by 11-8
- Convert RI to Concentration procedure 8-61
- Convert specific to intrinsic viscosity procedure 8-61
- Convert to Physical Units procedure 8-60
- Convert UV to Concentration procedure 8-61
- coordinates 11-15
- Copolymer analysis procedure 8-91
- Copy as Image Settings command, Edit menu 11-17
- Copy From command, Experiment menu 6-21
- Copy From dialog 6-21
- copy icon, in log dialog 4-9
- Correlation function plot 8-98
- crash recovery file 6-7, 6-9, 6-14
- .csv file extension 6-17
- Ctrl key
 - zoom 8-5
- Ctrl+Alt+N keyboard shortcut 12-3, 12-5
- Ctrl+Alt+T keyboard shortcut 3-5, 6-5
- Ctrl+I keyboard shortcut 6-9
- Ctrl+N keyboard shortcut 6-6
- Ctrl+O keyboard shortcut 6-7, 6-8
- Ctrl+P keyboard shortcut 3-16
- Ctrl+S keyboard shortcut 6-15, 6-16, 9-12
- Ctrl+Shift+N keyboard shortcut 9-3
- Ctrl+Shift+O keyboard shortcut 9-5
- Ctrl+Shift+P keyboard shortcut 6-26, 6-28, 6-29, 8-8, 11-9, 11-11
- Ctrl+Shift+R keyboard shortcut 3-10, 6-12
- Ctrl+Shift+S keyboard shortcut 6-13
- Ctrl+Shift+V keyboard shortcut 6-11
- cumulants analysis 8-99
- curve fitting model 8-62
- Customize command, View menu 3-15, 3-16
- D**
- data
 - after conversion 11-8
 - analyzed 11-9
 - copying to another experiment 6-21
 - exporting to file 11-21
 - monitoring while receiving from instruments 5-5, 5-6
 - original 11-8
 - precision of, increasing 8-47
 - processing of, while procedure runs 8-3
 - raw 11-8
 - raw after each transform 11-8
- Data (Graphical) tab, Diagnostic Manager 5-5
- Data (Numeric) tab, Diagnostic Manager 5-6
- data file, exporting graphs to 11-20
- data set definitions
 - adding to experiment 6-28
 - creating 11-9
 - definition of 11-8
 - exporting 11-11
 - (see also graphs)
- Data Set Definitions folder, Experiments tab 6-28
- data sets
 - definition of 1-7, 11-8
 - list of 11-8
- database
 - experiment 4-5
 - system 2-3
- Database Maintenance dialog 4-10, 6-20, 9-14, 12-7
- Database operating tier 1-4
- Database Properties dialog 4-5
- databases (see experiment database; system database)
- DAWN
 - calibrating 8-15
 - definition of 1-7
 - (see also miniDAWN)
- DAWN DSP
 - calibration and laser alignment 8-16
 - ISI installation for 5-2
 - profiles for 7-17
 - turning laser on/off and calibration 8-17
- DAWN DSP-F
 - ISI installation for 5-2
 - profiles for 7-17
- DAWN EOS
 - ISI installation for 5-2
 - profiles for 7-17
- DAWN HELEOS
 - profiles for 7-16
- DCOM (Distributed Component Object Model) 1-7, 5-4
- DCOM settings 5-4
- Debye fit method 8-67, D-14
- Debye fit model 8-58
- default configuration 7-10
- default method 6-18, 6-19

- default sequence 9-4
- defaults
 - experiment method 6-4
 - sequence template 9-3
- delay volumes, determining 8-18
- Delete command, File menu 12-7
- deleting
 - experiments 4-10
 - methods 6-20
 - profiles 12-7
 - sequences 9-14
- Description property, experiment configuration 7-14
- Description property, sequence configuration 9-8
- Despiking procedure 8-45
- detectors
 - interdetector delay, determining 8-18
 - normalizing 8-23
- Determining Rh from QELS Data procedure 8-82
- Diagnostic Manager
 - accessing 5-5
 - definition of 5-5
 - monitoring graphical data with 5-5
 - monitoring numeric data with 5-6
 - sending commands to instruments 5-7
 - viewing and setting properties with 5-9
 - viewing command log with 5-10
- diagnostics
 - baseline drift 8-50
 - noise 8-45
- differential distribution calculations D-22
- Distribution analysis procedure 8-90
- Distributions and Moments procedure 8-78
- dn/dc
 - calculation methods using D-11
 - definition of 1-7, 8-29
- dn/dc from peak procedure 8-80
- dn/dc from RI Data procedure 8-79
- DNDC, importing files from 6-7, 6-9
- domain
 - setting up users 2-8
 - specifying when logging in 2-11, 3-3
 - validation 2-9
- drift, diagnostics 8-50
- Dt (translational diffusion), calculating 8-82
- Dt cumulative intensity plot 8-98
- Dt diff intensity plot 8-98
- E**
- Edit menu, list of commands in A-4
- Edit, Experiment Configuration, menu
 - command 3-7, 6-25, 7-5, 7-6, 7-7
 - Edit, Sequence, menu command 9-7
- Editing dialog
 - Chart tab 11-17
 - Export tab 11-19, 11-20
 - opening 8-7, 11-5, 11-16
 - Print tab 11-18
 - tabs in 11-17
- e-mail support 1-10
- Empower
 - importing sequences 9-6
- Empower Sequence, Import, menu command 9-6
- Exit command, File menu 3-17
- Experiment Builder Mode
 - command list A-2
- Experiment Builder mode 1-3, 3-13
- experiment configuration
 - adding connections to 7-6
 - adding instruments to 7-6
 - column profiles 7-36
 - connection profiles 7-38
 - definition of 3-6, 6-2
 - editing 7-5
 - exporting to profiles 7-9
 - importing profiles into 7-7, 12-8, 12-9
 - injector profiles 7-31
 - light scattering instrument profiles 7-16
 - properties in 7-14
 - pump profiles 7-33
 - refractive index instrument profiles 7-22
 - sample profiles 7-32
 - solvent profiles 7-34
 - UV absorption instrument profiles 7-29
 - (see also configuration procedures)
- experiment database
 - changing 2-11, 4-5
 - database types supported by 4-4
 - default name for 2-11, 4-4
 - definition of 1-7, 4-3
 - files as alternative to 5-4
 - opening 6-8
 - saving experiments to 6-16
 - viewing current database 4-5
- experiment files
 - definition of 5-4
 - exporting experiments to 6-17
 - extensions for 6-16, 9-12
 - file extensions for 6-15, 6-17
 - importing 6-9
 - opening 6-7

- saving experiments to 6-15
 - types of 6-7, 6-9
- experiment logs
 - copying event text to clipboard 4-9
 - definition of 4-8
 - navigating 4-9
 - viewing 4-9
- Experiment menu
 - list of commands in A-5
 - (see also specific menu commands)
- Experiment, menu command 6-4
- Experiment, Open, menu command 6-7, 6-8
- experiments
 - baselines for 3-10, 8-48
 - closing 6-15
 - configuring (see configuration procedures; experiment configuration)
 - creating 3-5, 6-5, 6-6
 - data from (see data)
 - data set definitions for 6-28, 11-9
 - default configuration 7-10
 - default method 6-18, 6-19
 - definition of 6-2
 - deleting 4-10, 6-20
 - example of, running 3-5
 - exporting 6-17
 - importing 6-9
 - methods for 3-5, 6-5
 - opening 6-7, 6-8, 6-9, 9-5
 - opening multiple experiments 6-8
 - peaks for 3-11, 8-54
 - printing configuration for 3-16
 - procedures for
 - adding 6-26, 8-8
 - administration procedures 8-105
 - analysis procedures 8-64
 - collection procedures 8-11
 - configuration procedures 8-15
 - data processing during 8-3
 - data sets produced by 11-8
 - definition of 1-8, 8-2
 - deleting 8-9
 - editing 8-4
 - folder listing 3-6, 6-2, 8-2
 - graphs of 8-5
 - modifying 3-9, 8-7
 - sequencing 6-27, 8-9
 - status icons for 8-2, 8-9
 - transformation procedures 8-45
 - validating 6-12, 8-10
 - re-running 6-14, 8-7
 - results of (see graphs; reports)
 - running 3-10, 6-11, 6-12
 - saving 6-15, 6-16
 - saving as method 6-18
 - sign offs for 4-8, 6-13, 8-105
 - stopping 6-13
- Experiments tab
 - Configuration folder 3-6, 6-2, 6-24
 - Data Set Definitions folder 6-28
 - definition of 3-6, 6-2
 - Procedures folder 3-6, 6-2, 6-26, 8-2
 - Results folder 3-6, 6-2, 6-28, 11-11
 - viewing 3-14
- Export command, File menu 6-17, 9-13
- Export tab, Editing dialog 11-19, 11-20
- extensions (see file extensions)
- extinction
 - calculating from peak data 8-81
 - calculating from RI data 8-81
- F**
- F1 key 3-16
- F5 key 8-5
- FAX support 1-10
- Feature Activation command, System menu 2-6
- Feature Activation dialog 2-6
- file extensions
 - for experiment files 6-7, 6-15, 6-16, 9-12
 - for exporting experiments 6-17
 - for graphics files 11-20
 - for importing experiments 6-9
 - for scripts 8-14
 - for sequences 9-5
- File menu
 - (see also specific menu commands)
- File menu, list of commands in A-2
- firewall issues 5-4
- fit methods 8-67, D-14, D-15, D-16
- fit models 8-58
- flow mode (see online mode)
- fluid connections
 - definition of 7-38
 - profiles for 7-38
- fonts
 - graphs 11-16
 - reports 10-3
- Forward Monitor 7-15
 - baseline peak 7-15, 8-56
 - selecting signal 7-17, 7-18, 7-20
- fractionated mode (see online mode)
- fractionation 1-7
- From Method, Experiment, menu com-

mand 3-5, 6-5

From Template, Sequence, menu command
9-4

G

gel permeation chromatography (see SEC)

GIF files, exporting graphs to 11-20

GPC (gel permeation chromatography) 1-9,
D-2

Graph Toolbar command, View menu 3-15

graphics files, exporting graphs to 11-19

graphs

- adding to experiment 6-28, 11-11

- axis settings for 11-16

- coordinates 11-15

- copying 11-17

- of data from multiple experiments 11-
11

- exporting to data file 11-20

- exporting to graphics file 11-19

- modifying 8-7, 11-5, 11-16, 11-17

- printing 11-17

- of procedures 8-5

- saving configurations 8-6, 11-16

- saving format 11-16

- scrolling around 11-16

- viewing real-time 5-5

- zooming in and out of 11-16

- (see also data set definitions)

groups for user accounts

- assigning users to 2-8

- creating 2-7

- list of 2-7, 4-6

- number of groups a user can be as-
signed to 4-7

Guest group 1-4, 2-7, 3-3, 3-13, 4-6

Guest user level A-2

H

Help menu

- list of commands in A-9

- (see also specific menu commands)

help, using 3-16

Houwink, see also Mark-Houwink-Sakura-
da 8-88

HPLC (High-Performance Liquid Chroma-
tography) 1-7

HPLC pump with injector 8-30

hydrodynamic radius (Rh) 1-8

hydrodynamic radius (Rh), calculating 8-
82, 8-85

I

icons

Basic 1-4

Database 1-4

Security 1-4

icons in Procedures folder 8-2, 8-9

ID tubing 8-30, 8-31

Import command, Sequence menu 9-5, 9-6

Import Experiment command, File menu
6-9

Import, Experiment Configuration, menu
command 12-8, 12-9

Index command, Help menu 3-16

injector 8-30

injectors

- definition of 7-31

- profiles for 7-31

installation of ASTRA 2-2

Instrument list

- accessing Diagnostic Manager from 5-5

- definition of 2-12

- viewing 2-12

instruments

- adding to experiment configuration 6-
24, 7-6

- checking connections to 3-5

- connected through AUX input 5-2

- definition of 7-16

- ISI installation required for 5-2

- monitoring graph of data in real time 5-
5

- numeric data from 5-6

- properties of, viewing and setting 5-9

- sending commands to 5-7

- validating for experiment 6-11, 9-11

- viewing in Instruments dialog 2-12, 2-
13

- (see also specific instruments)

Instruments command, System menu 2-13,
3-5

Instruments dialog 2-13, 3-5

Instruments navigation pane 2-12

Instruments tab

- viewing 3-14, 3-15

Interdetector Delay procedure 8-18

intrinsic viscosity

- converting from specific 8-61

- definition of 1-7

ISI (Instrument Server Interface)

- definition of 1-7, 5-2

- installing on other computers 5-4

- integrated 5-2

- network requirements for 5-4

ISI list

- adding computers to 2-13

removing computers from 2-15
isotropic scattering, and normalization 8-24

J

JPEG files, exporting graphs to 11-20
jumper setting 8-16

K

Keep Configuration Sample command 7-7, 9-8, 12-9

keyboard shortcuts

- Alt+F4 3-17
- assigning to menu commands 3-16
- Ctrl 8-5
- Ctrl+Alt+N 12-3, 12-5
- Ctrl+Alt+T 3-5, 6-5
- Ctrl+I 6-9
- Ctrl+mouse button 3-12
- Ctrl+N 6-6
- Ctrl+O 6-7, 6-8
- Ctrl+P 3-16
- Ctrl+right mouse button 3-12
- Ctrl+S 6-15, 6-16, 9-12
- Ctrl+Shift+N 9-3
- Ctrl+Shift+O 9-5
- Ctrl+Shift+P 6-26, 6-28, 6-29, 8-8, 11-9, 11-11
- Ctrl+Shift+R 3-10, 6-12
- Ctrl+Shift+S 6-13
- Ctrl+Shift+V 6-11
- Shift key 3-11
- Shift+F5 8-5

keys for optional modules 2-6

keys, instruction conventions 1-6

L

laser

- turning off 8-13

Laser Monitor 7-17, 7-18, 7-20

level of user

- definition of 1-9

license keys for optional modules 2-6

light scattering instruments

- calibrating 8-15
- definition of 1-7
- performing experiment with 3-5
- profiles for 7-16
- (see also LS data)

light scattering theory

- Berry fit method D-16
- branching calculations D-23
- calculation methods D-11
- calibration D-4

Debye fit method D-14

differential distribution calculations D-22

molar mass and RMS radius moments D-18

molar mass and sizes D-13

normalization D-8

out of range values D-22

Random Coil fit method D-16

Rayleigh ratio

- equations for D-3

- measurement and calibration of D-4

summary of D-2

uncertainties in calculated quantities D-21

Zimm fit method D-15

linearity of result, calculating 8-79

Log command, Manage menu 4-9

Log command, Sequence menu 4-9, 9-12

Log command, System menu 4-8

Log tab, Diagnostic Manager 5-10

logging in 2-11, 3-2

logs (see experiment logs; system log)

long chain branching D-26

Lorenz-Mie fit model 8-59

LS Calibration procedure 8-15

LS data

- determining A2 mass and radius from 8-69, 8-71

- determining mass and radius from 8-64

- determining number of particles and RMS radius for 8-75

- determining RMS radius from 8-77

- fit methods 8-67

- fit models 8-58

- (see also light scattering entries)

M

Maintenance menu command 4-10, 6-20, 9-14, 12-7

Manage menu (see specific menu commands)

manual injectors 7-31

Mark-Houwink-Sakurada coefficients 7-36

- calculating 8-88

- column calibration 8-39

- intrinsic viscosity 8-75

Mark-Houwink-Sakurada procedure 8-88

mass

- calculating from viscosity 8-75

mass (see A2 mass; eluted mass; molar mass)

Mass from Column Calibration procedure

- 8-101
- Mass from VS data procedure 8-75
- mass recovery, 100% D-12
- .mdf file extension 6-7, 6-9
- menu commands
 - assigning keyboard shortcuts to 3-16
 - (see also specific menu commands)
- menus
 - instruction conventions 1-5
 - list of A-2
 - short vs. long 3-15
 - (see also specific menus)
- metafiles, export graphs to 11-20
- methods
 - creating 6-18
 - creating experiments from 3-5, 6-5
 - default 6-18, 6-19
 - deleting 6-20
 - for graphs 6-29
 - for reports 6-29
 - system 6-5
- Microsoft Access 4-5
- Microsoft Access database 4-4
- Microsoft Excel
 - exporting data to 11-21
- Mie fit model 8-59
- miniDAWN
 - definition of 1-7
 - ISI installation for 5-2
 - profiles for 7-19
- models, data fitting 8-58
- modules
 - activating 2-6
- molar mass
 - calculating 8-64
 - curve fitting model for 8-62
 - definition of 1-6
 - distribution D-22
 - in reports 10-4
 - light scattering theory and D-13, D-18
- Molar Mass and Radius from LS Data procedure 8-64
- molecular standards
 - profiles for 7-33
- molecular weight
 - compared to molar mass 1-6
 - (see also molar mass)
- moments 10-4
- Mp result 10-4
- Mv result 10-4

N

- Name property, experiment configuration 7-14

- networked accounts 2-8
- networked computers 2-13
- New Experiment, menu command 6-4
- New From Existing dialog 3-5, 6-22, 10-8
- New Sequence, menu command 9-3
- noise
 - diagnostics 8-45
 - removing spikes from collected data 8-45
 - smoothing data 8-46
- noise baseline 7-15
- non-fractionated samples 1-8
- normalization
 - coefficients 8-27
 - light scattering theory and D-8
 - standards 8-24
- Normalization procedure 8-23
- Number from LS Data procedure 8-75
- number of particles per mL, calculating 8-75
- Number of Samples property, sequence configuration 9-8
- numeric instrument data 5-6
- .nwf file extension 6-7, 6-9

O

- ODBC (Open Database Connectivity) 4-4
- off-line (see batch mode)
- One-to-Many command, File menu 6-22
- Online A2 procedure 8-71
- online help, using 3-16
- online mode 1-8, 1-9
- Open command, Sequence menu 9-5
- operating system requirements 2-2
- operating tiers 1-3
- Optilab 903
 - calibrating 8-29
 - connecting through AUX input 5-2
 - profiles for 7-23
- Optilab DSP
 - calibrating 8-29
 - connecting through AUX input 5-2
 - profiles for 7-23
- Optilab instruments 1-8
- Optilab rEX
 - ISI integrated 5-2
 - profiles for 7-22
- optional modules
 - activating 2-6
- order of precedence 9-10
- original data 8-3, 11-8

P

- Page Setup command, File menu 3-16, 10-

- 7, 11-17
- PCX files, exporting graphs to 11-20
- PDF files, exporting graphs to 11-20
- peak 9-10
- Peak areas procedure 8-88
- peaks
 - defining 3-11, 8-54
- Peaks procedure 3-11, 8-54
- phone support 1-10
- physical units 1-8
- plus sign (+), between key names 1-6
- PNG files, exporting graphs to 11-20
- polydispersity 8-104
 - and normalization 8-25
- PostScript files, exporting graphs to 11-20
- precedence 9-10
- Print command, File menu 3-16, 10-7
 - printing graphs 11-17
- Print Preview command, File menu 3-16, 10-7
- Print Setup command, File menu 3-16, 10-7, 11-17
- Print tab, Editing dialog 11-18
- printing
 - configuration 3-16
 - graphs 11-17
 - reports 3-16, 10-7
- procedures
 - adding to experiment 6-26, 8-8
 - administration procedures 8-105
 - analysis procedures 8-64
 - collection procedures 8-11
 - configuration procedures 8-15
 - data processing during 8-3
 - data sets produced by 11-8
 - definition of 1-8, 8-2
 - deleting 8-9
 - editing 8-4
 - folder listing 3-6, 6-2, 8-2
 - graphs of 8-5
 - modifying 3-9, 8-7
 - sequencing 6-27, 8-9
 - sign offs for 6-13
 - status icons for 8-2, 8-9
 - transformation procedures 8-45
 - types of 8-2
 - validating 6-12, 8-10
- Procedures folder, Experiments tab 6-2
 - adding procedures to 6-26
 - definition of 3-6, 8-2
 - icons in 8-2, 8-9
- Processing menu
 - list of commands in A-6
 - Run command 6-12, 9-11
 - Stop command 6-13, 9-11
 - Validate command 6-11, 9-11
- Processing Operator property, experiment configuration 7-14
- Processing Toolbar command, View menu 3-15
- Profile, New, menu command 12-3
- Profile, Open, menu command 12-5
- profiles 12-2
 - creating 12-3
 - creating by exporting from configuration 12-4
 - creating by exporting from experiment configuration 7-9
 - definition of 1-8
 - deleting 12-7
 - duplicating 12-7
 - editing 12-5
 - importing into experiment configuration 7-7, 12-8, 12-9
 - renaming 12-7
 - (see also configuration; profiles)
- Profiles tab
 - viewing 3-14
- properties of instruments 5-9
- Properties tab, Diagnostic Manager 5-9
- Properties, Database Administration, menu command 4-5
- Protein conjugate analysis procedure 8-93
- pump
 - HPLC pump with injector 8-30
 - syringe pump 8-31
- pumps
 - definition of 7-33
 - profiles for 7-33
- Q**
- QELS (Quasi-Elastic Light Scattering) 1-8
- QELS data
 - determining Dt from 8-82
 - determining Rh from 8-82
- Quasi-Elastic Light Scattering (see QELS)
- R**
- radius (see A2 radius; hydrodynamic radius; RMS radius)
- Radius from LS Data procedure 8-77
- radius of gyration vs. RMS radius 1-6
- Random Coil fit method D-16
- Random Coil fit model 8-58
- raw data 8-3, 11-8
- raw data after transform 11-8
- Rayleigh ratio

- converting voltages to 8-60
- equations for D-3
- measurement and calibration of D-4
- Reciprocal fit method D-15
- red X icon in Procedures folder 8-9
- refractive index (see RI entries)
- Regularization procedure 8-95
- Remove Connections property, experiment configuration 7-15
- Remove Instruments property, experiment configuration 7-15
- Replace existing sample field 7-10
- Replace, Experiment Configuration, menu command 7-8
- reports
 - abbreviations used in 10-4
 - adding to experiment 6-28, 10-8
 - copying to clipboard 10-7
 - exporting 10-7
 - preview 10-7
 - printing 3-16, 10-7
 - viewing 3-12
- repositioning graphs 11-16
- Research Database operating tier 1-4
- Research Database version 1-3
- Researcher group 1-4, 2-7, 3-3, 3-13, 4-6
- Researcher user level A-2
- Responsibility category for sign offs 6-13
- results fitting 8-62
- Results Fitting procedure 8-62
- Results folder, Add to experiment dialog 6-29, 11-12
- Results folder, Experiments tab
 - adding graphs to 6-28, 11-11
 - adding reports to 6-28
 - definition of 3-6, 6-2
- Review category for sign offs 6-13
- Rh (hydrodynamic radius) 1-8
- Rh (hydrodynamic radius), calculating 8-82, 8-85
- Rh cumulative intensity plot 8-98
- Rh cumulative weight plot 8-98
- Rh diff intensity plot 8-98
- Rh diff weight plot 8-98
- Rh from VS procedure 8-85
- RI (refractive index) instruments
 - calibration of 8-29
 - definition of 1-9, 7-22
 - generic, profiles for 7-24
 - profiles for 7-22
- RI calibration from peak procedure 8-81
- RI Calibration procedure 8-29
- RI data
 - converting to concentrations 8-61
 - definition of 1-9
 - determining dn/dc and linearity from 8-79
- RICAL, importing files from 6-7, 6-9
- RMS radius
 - calculating 8-64, 8-69, 8-71, 8-75, 8-77
 - curve fitting model for 8-62
 - light scattering theory and D-18
 - vs. radius of gyration 1-6
- Rod fit model 8-59
- Run button, ASTRA toolbar 3-10
- Run command, Processing menu 6-12, 9-11
- Run Default menu command 6-4
- Run Indefinitely command, Processing menu 6-12
- Run mode 1-3, 3-13
 - command list A-2
- .rwf file extension 6-7, 6-9
- S**
- Sakurada, see also Mark-Houwink-Sakurada 8-88
- samples
 - definition of 7-32
 - profiles for 7-32
- Samples tab
 - sequences 9-8
- Save As command, File menu 6-16, 6-18, 9-12, 12-7
- Save As dialog 6-15, 6-16, 9-12
- Save As, Experiment Configuration, menu command 7-9, 12-4
- Save command, File menu 6-15, 6-16, 9-12
- Script Collection procedure 8-13
- scrolling graphs 11-16
- Search command, Help menu 3-16
- SEC (size-exclusion chromatography) 1-9, D-2
- second virial coefficient 8-71
- security 2-9
- Security command, System menu 2-8, 2-9
- Security Pack operating tier 1-4
- Security Pack version 1-3
- sequence logs
 - viewing 4-9, 9-12
- Sequence menu
 - list of commands in A-7
 - (see also specific menu commands)
- Sequence menu (see specific menu commands)
- Sequence, Import, menu command 9-5
- Sequence, menu command 9-3
- sequences

- Collection tab 9-10
 - Configuration tab 9-7
 - creating 9-3
 - default 9-4
 - exporting 9-13
 - file type 9-5
 - importing 9-5
 - opening 9-5
 - running 9-11
 - Samples tab 9-8
 - stopping 9-11
 - Sequences tab
 - viewing 3-14
 - Show Desktop Alerts command, System menu 2-13
 - Sign off page 6-13
 - Sign Off procedure 8-105
 - sign offs 4-8, 6-13, 8-105
 - signal to noise ratio
 - increasing 8-47
 - peaks and 8-55
 - signal-to-noise ratio
 - and concentration 8-25
 - size-exclusion chromatography (see SEC)
 - Smoothing procedure 8-46
 - solvents
 - definition of 7-34
 - for normalization 8-25
 - profiles for 7-34
 - specific viscosity
 - converting to intrinsic 8-61
 - Sphere fit model 8-59
 - spikes, noise, removing from collected data 8-45
 - SQL Server database 4-4
 - Square root method D-16
 - .ss file extension 9-5
 - Standard Toolbar command, View menu 3-15
 - Status Bar command, View menu 3-15
 - status bar, hiding 3-15
 - Stop command, Processing menu 6-13, 9-11
 - support, technical 1-10
 - surface plot 11-13
 - syringe pump 8-31
 - system database 2-3
 - definition of 1-9, 4-3
 - deleting 2-3
 - saving methods to 6-18
 - system log
 - copying event text to clipboard 4-9
 - definition of 4-8
 - navigating 4-9
 - viewing 4-8
 - System menu
 - list of commands in A-7
 - (see also specific menu commands)
 - system methods 6-5
 - system profiles 12-2
 - system requirements 2-2
- ## T
- technical support 1-10
 - Technician group 1-4, 2-7, 3-3, 3-13, 4-6
 - Technician user level A-2
 - telephone support 1-10
 - templates
 - for graphs 11-12
 - templates, see methods 6-5
 - third virial coefficient 8-71
 - tiers, operating 1-3
 - toolbars
 - hiding 3-15
 - repositioning 3-14
 - transformation procedures
 - effect on data 8-3
 - list of 8-45
 - raw data produced by 11-8
 - translational diffusion (Dt), calculating 8-82
 - TREOS
 - profiles for 7-19
 - T-rEX, Optilab 7-22
 - tubing
 - between injector and Optilab rEX 8-30
 - for syringe pump infusion 8-31
 - .txt file extension 6-17
- ## U
- universal column calibration 7-36
 - Unsigned category for sign offs 6-13
 - updating 2-3
 - upgrading ASTRA 2-3
 - user accounts
 - assigning to groups 2-8
 - creating 2-8
 - displaying current user 4-7
 - list of groups for 2-7, 3-3, 3-13
 - managing 4-6
 - number of groups assigned to 4-7
 - setting up 2-7
 - user authentication 2-9
 - user level
 - definition of 1-9
 - UV absorption instruments
 - definition of 7-29

profiles for 7-29
UV extinction from peak procedure 8-81
UV extinction from RI procedure 8-81

zooming
graphs 11-16
procedure graphs 8-5

V

.vaf file extension 6-7, 6-9, 6-17
Validate command, Processing menu 6-11, 8-10, 9-11
View button, Instrument list 5-5
View menu
 (see also specific menu commands)
 list of commands in A-4
viscosity
 converting specific to intrinsic 8-61
 determining mass from 8-75
ViscoStar
 definition of 1-9
 ISI integrated 5-2
 profiles for 7-26
voltages, converting to Rayleigh ratios 8-60
.vrf file extension 6-7, 6-9, 6-14
VS data
 determining mass from 8-75
.vsf file extension 6-9, 9-5

W

warnings, resetting A-8
Waters Empower
 importing sequences 9-6
WCS Server 1-9, 5-4
WCS.exe process 5-4
websites
 21 CFR Part 11 compliance 4-2
 Wyatt Technology Corporation 1-10
Window menu
 list of commands in A-8
.wmf file extension 11-20
workspace
 customizing 3-14
 definition of 1-9
 hiding 3-15
Workspace command, View menu 3-15
Wyatt Technology Corporation
 contacting 1-10
WyattQELS
 ISI installation for 5-2
 profiles for 7-21

X

x axis units (see Abscissa Units property, experiment configuration)

Z

Zimm fit method 8-67, D-15
Zimm fit model 8-58