

# ASTRA® 8 User's Guide

M1111 Rev. A



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## Notices

### ASTRA User's Guide

#### M1111 Rev. A

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A WARNING notice denotes a hazard and calls attention to an operating protocol that, if not correctly followed or adhered to, could result in personal injury or fatality. Please pay particular attention to WARNING notices and do not proceed until the indicated conditions are fully understood.

## **Using this Manual**

This user's guide describes the ASTRA® software.

The chapters and appendices in this manual are organized as outlined in the Contents that follow.

## **Manual Conventions**

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 is often used in the literature. The term molar mass will be used in this manual.

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## Preface

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### Wyatt Technology Corporation

Founded in 1982 by Dr. Phillip J. Wyatt, Wyatt Technology Corporation (WTC) formed around his patents, ideas, and inventions to commercialize the first light scattering instrumentation using lasers with initial support from the Small Business Innovation Research (SBIR) contracts. Since then we have defined and redefined the paradigm for laser light scattering hardware, software, training, and services to meet customer needs, including the development of related technologies such as dynamic light scattering, refractometry, viscometry, zeta potential, and field-flow fractionation. For additional information and the history of Wyatt Technology®, visit us online at <https://www.wyatt.com/>.

Today, Wyatt Technology is a family-owned and operated company that considers every customer to be part of the Wyatt Technology family. To this end, we take the successes of our customers personally and welcome opportunities to further develop our technology and strive for the utmost level of product quality and innovation.

Our DAWN® detectors are used in the world's most prestigious universities and some the most influential companies in the world, assisting in the research for over 16,000+ peer-reviewed articles. View our continuously growing body of citations with an online bibliography available online at <https://www.wyatt.com/library/bibliography.html>.

If you have a question about ASTRA, please refer to this manual or consult the ASTRA online help within the software. For additional assistance, please contact Wyatt Technology or take advantage of the online technical support options available to our customers in the following section.

## Wyatt Technical Support

Wyatt Technical Support offers a variety of support options.

Located online at <https://www.wyatt.com/Support>, our Support Center contains a wealth of useful resources on everything related to your Wyatt Technology instruments, software, and applications. This center is free for our customers and contains software updates and bug fixes, technical notes for connecting to and using your instruments, tutorials, webinars, certificates of analysis for Wyatt standards, and variety of additional reference materials. We are continuously adding resources to our Support Center.

Before contacting Wyatt Technical Support, try to resolve any issues or problems through the ASTRA online help system, this manual, or our Support Center online, where we provide both solutions and guidance through a library of detailed technical notes and tutorials. If you need additional assistance, please contact us online or by phone with the contact information provided below but please first gather *all* of the following information:

- Wyatt Technology instrument serial numbers. The serial number can be found on the front panel on the **Settings** tab under **Instrument** for NEON family instruments, on the **System** tab for other instruments, or for all instruments on the back panel.
- The computer hardware you are using.
- If the problem is software related, please have available your Microsoft Windows version, ASTRA version number, and software release version, and the exact wording or screenshots of messages.
- What you were doing when the problem occurred or how to reproduce the problem.
- How you have tried to resolve the problem before contacting us so that we may offer you the most pertinent and relevant advice moving forward.

## Wyatt Technology Technical Support Contact Information

**Electronic mail address:** support@wyatt.com

E-mailing our support team will generate a ticket number and log your request in our support system. Please be sure to whitelist [support@wyatt.com](mailto:support@wyatt.com). You are encouraged to attach a representative ASTRA data file for us to review and one of our scientific support team members will get back to you soon!

For customers outside the United States, please feel free to contact your local distributor for instrument support. You can find contact information for our global offices at [www.wyatt.com/Distributors](http://www.wyatt.com/Distributors).

European customers can reach Wyatt Technology Europe support at [support@wyatt.eu](mailto:support@wyatt.eu). Our Wyatt Technology UK office can be contacted directly at [WTUKsupport@wyatt.com](mailto:WTUKsupport@wyatt.com).

**Wyatt US & Canada Telephone Number:** +1 (805) 681-9009; Option #4

Based in Santa Barbara, California, our support team can be reached between the hours of 8:30 A.M. and 5:00 PM, Pacific Time (PT), Monday through Friday. A voicemail can be left at any time and our support team will return your call during business hours.

Wyatt Technical Support also offers remote support with an easy transition from a support phone call or e-mail to a screen sharing session via an internet connection for remote assistance with data acquisition and processing, instrument communication, and application support.

## Sales Support

For general inquiries, please contact [info@wyatt.com](mailto:info@wyatt.com) or call +1 (805) 681-9009. For information about purchasing additional instruments or accessories to aid your light scattering measurements, you can contact Wyatt Technology Corporation Sales or visit us online at <https://www.wyatt.com/products.html>. You can purchase parts, accessories, and columns at <https://store.wyatt.com/>.

**Sales Phone:** (805) 681-9009

**Sales email:** [sales@wyatt.com](mailto:sales@wyatt.com)

# 1

## About ASTRA 8

This chapter provides an overview of the ASTRA software.

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## What is ASTRA?

The ASTRA software controls data acquisition, collects data, and processes data from dilute macromolecular solutions. It uses this data to calculate the molar mass, radius moments, and other results.

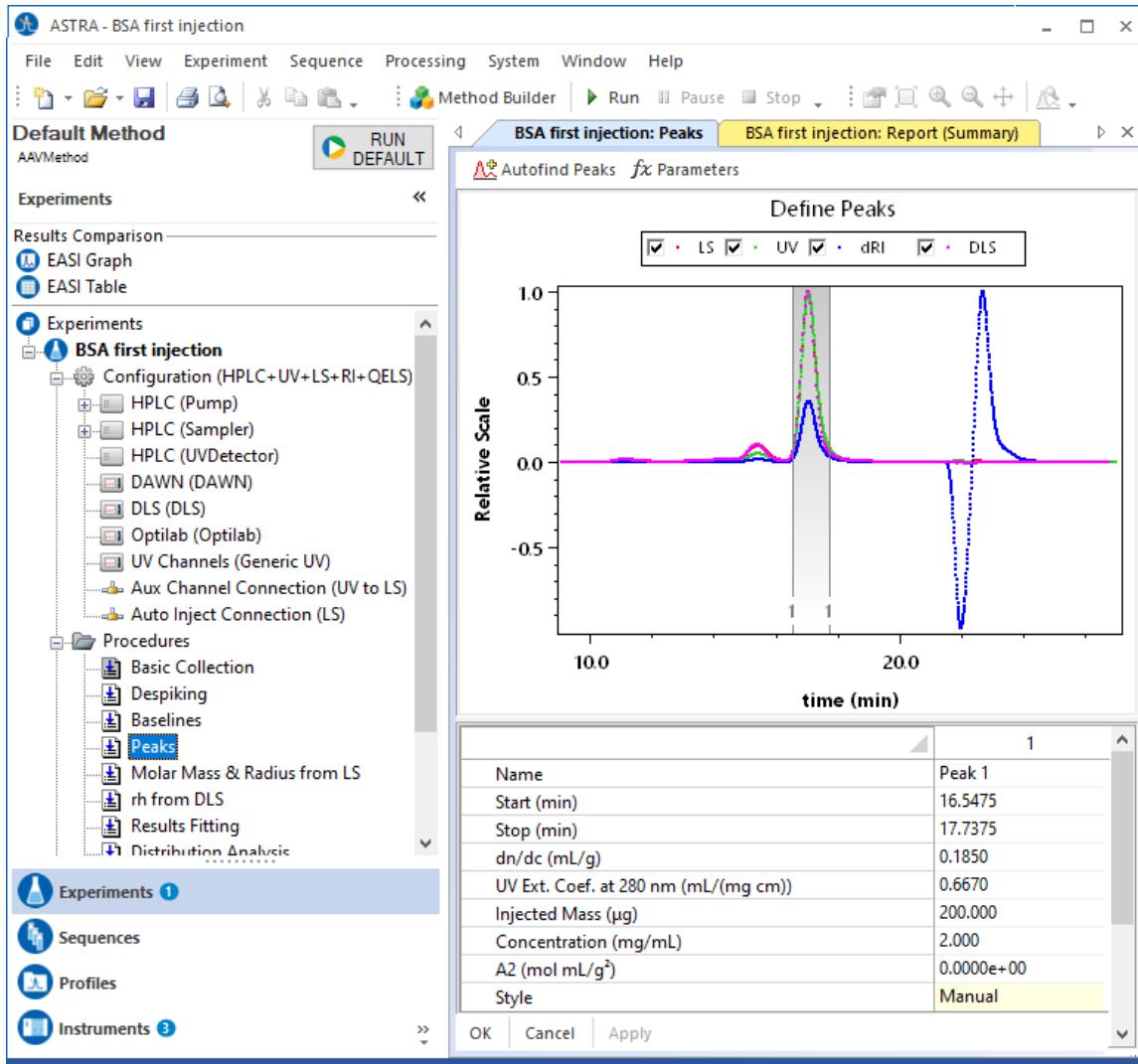


Figure 1-1: ASTRA Environment

## 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 for your Wyatt instrument.

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

### Left Margin Icons

You will see icons in this manual that indicate the information to the right of the icon applies only to specific operating modes.



- **Experiment Builder:** Indicates that a feature is available only if you have enabled Experiment Builder mode. See [User Modes on page 62](#).



- **ASTRA Basic:** Saves experiments to files. In this manual, the “Basic” icon applies to this mode.

Some features are not available unless enabled by entering an activation key (page 33).



- **ASTRA with Security Pack:** Provides 21 CFR Part 11 compliance. This includes experiments saved to a database, user accounts with access levels, and sign off procedures. As part of 21 CFR Part 11 compliance, all users of ASTRA with Security Pack must log in with a unique user id and password. See [Setting Up User Accounts on page 34](#) for details about the account levels.



- **HPLC:** This icon indicates that this feature is available only if Wyatt HPLC CONNECT™ software is installed.

This manual identifies information that is specific to these operating tiers using the icons shown above.

## 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.

### Chapters 5 & 6

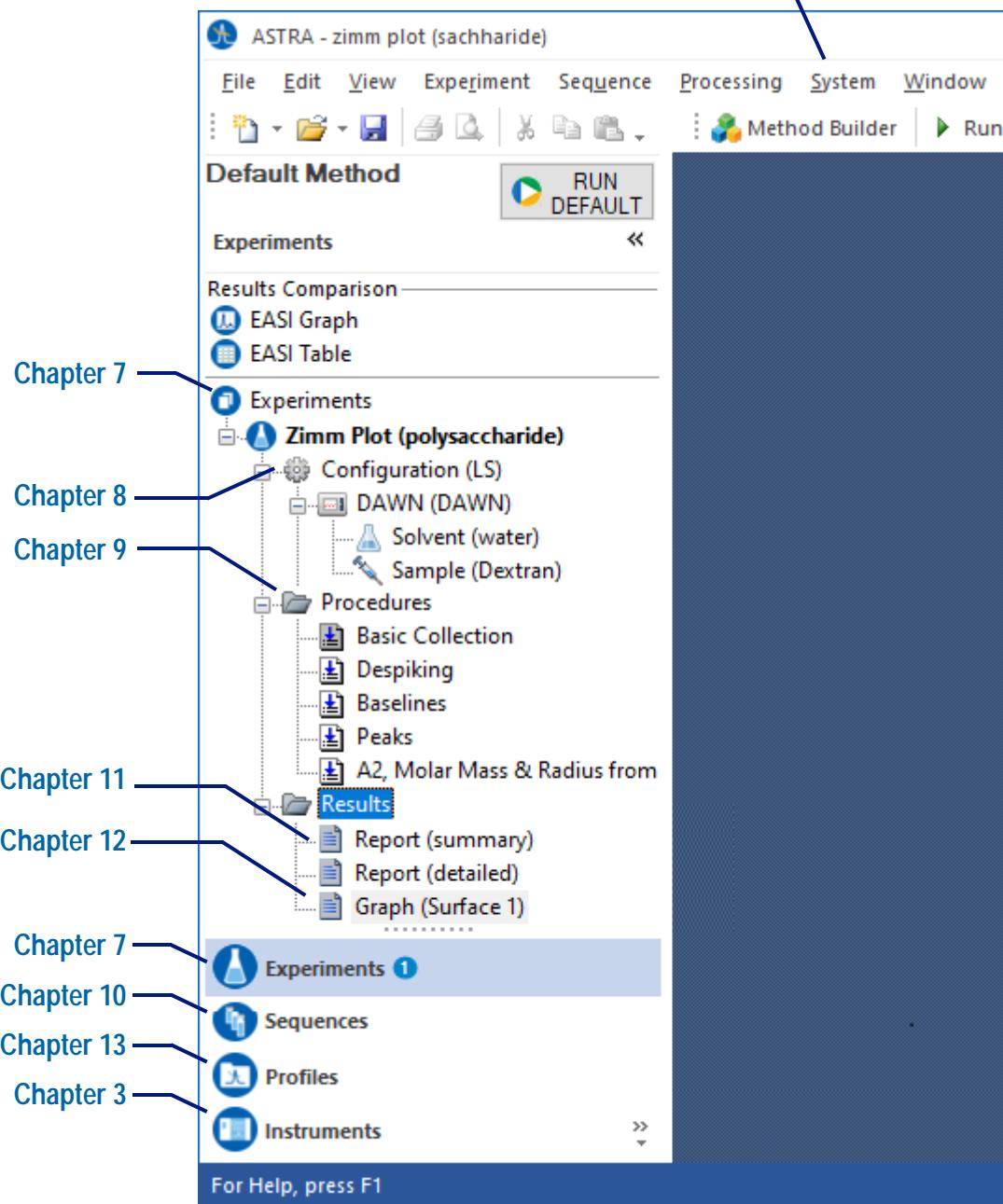


Figure 1-2: Workspace Items and the Chapters that Discuss Them

## 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.
- **MALS instrument.** Except where there are details for a particular instrument, when the name will be given, we will refer to the various DAWN models—DAWN, miniDAWN®, and microDAWN™—simply as the MALS instrument.
- **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.

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<b>Tip:</b>	See <a href="#">Appendix A, Menu Quick Reference</a> for a complete list of keystroke alternatives to the mouse pointer for selecting menu options.
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## 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 collection, analysis, and lab control software for Wyatt Technology Corporation instruments.
- **Batch mode:** Data collection in which an unfractionated sample (potentially polydisperse) is measured by injecting it into the cell by means of a syringe or using a cuvette. See [Batch Mode vs. Online Mode on page 25](#) for details.
- **Configuration:** ASTRA's description of the set of instruments used to collect data. It is an assembly of profile units describing the instruments as well as the sample and solvent used. The configuration also indicates the connections between these instruments both for the fluid pathway as well as any signals transmitted between instruments.
- **Data set:** The resulting data from a given series of procedures. For example, a typical light scattering measurement might produce a set of data including molar mass and rms radius.
- **DAWN:** Multi-angle light scattering (MALS) 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 and DAWN 8.
- **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 with Security Pack stores experiment information and results.
- **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, Eta, or  $[\eta]$ ):** A measure of the capability of a polymer in solution to enhance the viscosity of the solution. Derived using specific viscosity and concentration data.
- **Light scattering:** A technique for finding the absolute molar mass and average size of particles in solution by measuring how they scatter light.

- **MALS (Multiangle Light Scattering):** A technique whereby the intensity of scattered light from a macromolecule in solution is measured at multiple angles simultaneously to determine a molar mass, rms radius, and second virial coefficient. Also called classical, Rayleigh, or static light scattering.
- **microDAWN:** A MALS instrument with three detectors developed specifically for UHPLC applications.
- **miniDAWN:** A MALS instrument with three detectors. It is used primarily for characterizing small (less than 50 nm in rms radius) macromolecules.
- **ODBC (Open Database Connectivity):** A software standard for database access.
- **Online:** Data collection in which a pump is used to subject a sample to chromatographic techniques for separation (to yield a monodisperse sample) or to pass through several instruments in succession. See [Batch Mode vs. Online Mode on page 25](#) for details.
- **Optilab®:** The Optilab and microOptilab™ instruments 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 MALS 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:** An ASTRA process in the collection and analysis of the data. A procedure performs data collection, data transformation, data analysis, display, instrument configuration, or administrative purposes.
- **Profile:** A description of an entity in an ASTRA experiment. For example, a profile can be that of 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 (DLS) or photon correlation spectroscopy (PCS). The WyattQELS™ module measures rapid fluctuations in scattered light intensity to determine the translational diffusion coefficient and hydrodynamic radius for macromolecules in solution.

- **$r_h$  (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 obtained from differential refractometer instruments or from the Optilab instruments.
- **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 with Security Pack.
- **ViscoStar®:** On-line differential viscometer that measures the intrinsic viscosity and Mark Houwink-Sakurada (MHS) parameters of polymers.
- **UHPLC (Ultra-High Performance Liquid Chromatography):** An HPLC technique under high operating pressures to achieve high resolution LC separations in much shorter time frames.
- **Un-fractionated:** Samples that have not undergone any separation but are rather a mixture that is typically polydisperse containing a range of macromolecules with different weights, sizes, or conformations.
- **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.
- **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 fractionation module upstream. 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 “un-fractionated”.
- **Online Mode:** In an online mode experiment, a measuring instrument such as the Optilab or DAWN 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, UV, 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](http://www.wyatt.com) to log in. You'll find tutorials, software downloads, technical notes, newsletters, and more.

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

You can report problems from within ASTRA by choosing **Help→Report Software Issue** and filling out the form.

---

## 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.

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Installing the ASTRA Software .....	28
Activating ASTRA Features.....	33
Setting Up User Accounts.....	34
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## System Requirements

For the most current ASTRA system requirements please refer to our website: <https://www.wyatt.com/PCRequirements>.

The following lists provide the minimum and recommended specifications for a computer running ASTRA.

### **Minimum Computer Requirements for Wyatt Software**

ASTRA requires a PC with at least these minimal specifications:

- Intel® i5 processor or better
- Display with 1280-by-1024 resolution or higher
- Support for DirectX 9 graphics
- 4 GB of RAM;  
8 GB of RAM if additional software is to run simultaneously on the same PC, (for example, HPLC software or SQL Server® for ASTRA with Security Pack)
- 1 GB of available disk space for installation
- 1 Ethernet port
- Supported operating system mentioned below
- DVD drive for installation unless installing from a download
- For ASTRA with Security Pack: Microsoft® SQL Server or SQL Server Express 2012, 2014, 2016, 2017, or 2019

### **Recommended Computer Specifications for Wyatt Software**

For optimal performance, ASTRA should be installed on a PC with these extended specifications:

- Intel i7 Broadwell (5th generation) processor or better
- Display with 1920-by-1080 resolution or higher
- 16 GB or more of RAM
- 500 GB or larger Solid State Disk (SSD)
- For ASTRA with Security Pack: Microsoft SQL Server 2016, 2017, or 2019

### **Operating System**

ASTRA will run on the following operating systems:

- Recommended: Microsoft Windows® 10 64-bit Professional Edition
- Also compatible with all 32-bit and 64-bit versions of:
  - Microsoft Windows 10 Pro/Enterprise
  - Microsoft Windows 8 Pro/Enterprise

Note that ASTRA can be installed as either a 32-bit or 64-bit application. The 64-bit edition requires a 64-bit version of Windows. See the ASTRA ReadMe for additional information on these two editions.

ASTRA has been tested extensively with English versions of the Windows operating system. We do not guarantee absolute compatibility with all other language versions but in our experience, we have seen almost no issues.

---

## 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. Download ASTRA from the Support Center or place the ASTRA disk in your DVD drive. On most systems, the ASTRA setup procedure will start automatically. (If you downloaded an update, double-click on the `astra_8_#_#_#_setup.exe` file.)  
If the setup procedure does not start automatically, use Windows Explorer or the Run dialog to run `setup.exe` in the DVD'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.

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

---

## Upgrading to a New Version of ASTRA



If you are upgrading ASTRA with Security Pack, see [Upgrading ASTRA with Security Pack on page 80](#) instead of this section.

---

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.

If an ASTRA update or instrument firmware update is available, you will see instructions for downloading and installing the update.

You can have installations of ASTRA 8, ASTRA 7, ASTRA 6, and ASTRA V on the same computer and use them all simultaneously. Experiment templates and methods, logging, and data acquisition are not shared.

between the different revisions, and will therefore not affect one another. The system and experiment databases are not shared between the different versions of ASTRA.

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 [Importing a System Database on page 30](#) to update your system database so you have all the latest experiment methods and system profiles provided with ASTRA.

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

To deinstall an old version of ASTRA, 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.

## Importing a System Database

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

---

The ASTRA system database stores experiment methods, sequence templates, profiles, solvents, and molecular standards. It contains Wyatt system items for common tasks (such as, data collection, data analysis, and hardware control utilities). It is the storage location for items you customize and save. All versions of ASTRA use a system database.

The details of how to import/migrate an existing ASTRA system database depend on the location of the system database to be used with ASTRA:

- **During ASTRA 8 upgrade installations,** the installer does not overwrite your existing ASTRA system database. As a result, you have access to your existing custom items, but not to the latest Wyatt system items. To update your system database to include the latest Wyatt system items, use the **Import System Database** command and select your current system database file.

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**Note:** Wyatt Technology recommends that you base your custom methods on the latest Wyatt system methods or via the **System→Method Builder Wizard** command to take full advantage of the latest features and updates.

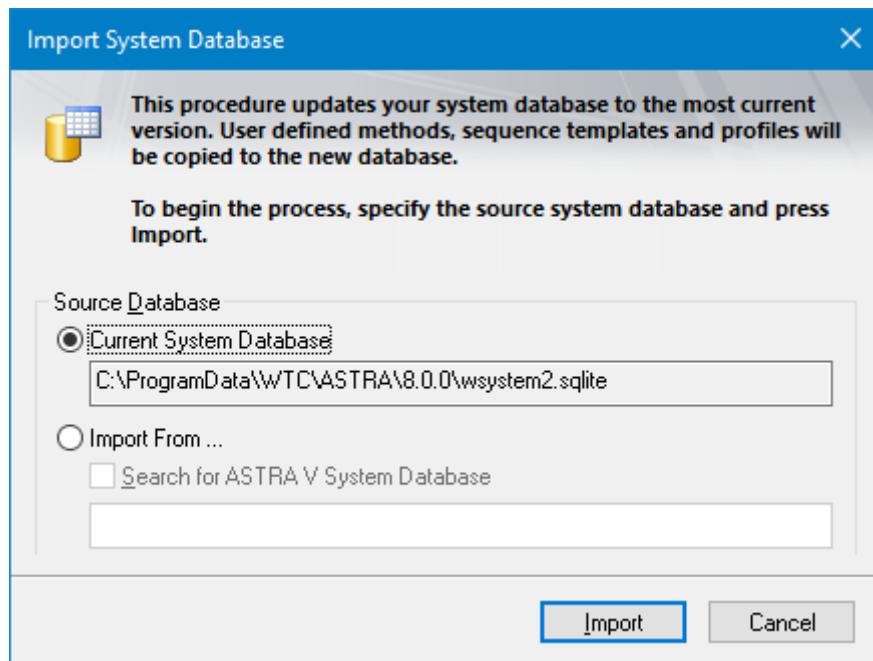
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- **When you launch ASTRA 8,** a search is performed for an existing ASTRA 8 system database. If it is found, the database is used for the ASTRA session.

If a system database file called “wsystem2.sqlite” does not exist in the ASTRA 8 system database location, the ASTRA 7 system database location is checked for a compatible ASTRA 7 system database that can be migrated to create the ASTRA 8 system database. If a suitable migration candidate is located, it is automatically merged with the latest Wyatt system items to create the ASTRA 8 system database. If a suitable candidate is not found, a clean copy of the default ASTRA 8 system database is created.

To manually update an existing ASTRA 8 system database with the latest Wyatt system items or to import an ASTRA system database from another computer or backup file, use the **Import System Database** command by following these steps:

1. Choose **System**→**Database Administration**→**Import System Database**.
2. To import a system database from another computer or backup file, select the **Import from ...** option. Click the “...” button and browse to select a system database file.



If you are upgrading from ASTRA 6 on Windows 7, the old system database is usually located in C:\ProgramData\WTC\ASTRA and has a filename similar to “wsystem.mdb” or “wsystem2.sqlite”.

If you are upgrading from ASTRA V, the old system database is usually located in a directory similar to C:\Program Files\WTC\ASTRA 5.3\Database (or Program Files (x86)) and has a filename similar to “ASTRA\_System2.mdb”.

You can import system databases created with ASTRA v5.3 or higher.

3. To update the existing ASTRA 8 system database with the latest Wyatt system items, select the Current System Database option in the Import System Database dialog.
4. Click **Import** 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**.

---

**Important:** Importing copies only log entries contained within individual experiment methods or sequence templates. It does not copy log entries for system-wide events. System-wide events include session information, system status messages, and system file operations (such as Open and Save).

---

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

Whenever you import the system database, a backup is created with a filename of wsystem2\_YYYY-MM-DD.bak (or wsystem2\_YYYY-MM-DD\_###.bak, where ### is a sequence number if you have multiple backups). You can click **Show Backup** to open a Windows Explorer view of the folder that contains the ASTRA system database and backup files.

Follow these steps to rollback an import:

1. Close ASTRA.
2. Rename or removing the existing wsystem2.sqlite file.
3. Rename the backup file to wsystem2.sqlite.
4. Launch ASTRA.

## Activating ASTRA Features

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.

To activate ASTRA features, 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.

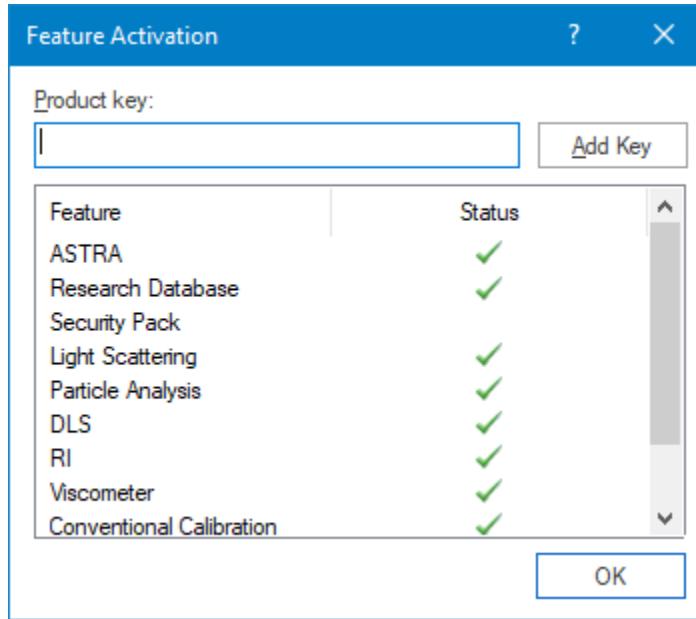


Figure 2-1: Feature Activation Dialog

To activate a feature, follow these steps:

1. Double-click the **ASTRA 8** 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 34](#) 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.

## Setting Up User Accounts



### Security

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

User accounts in ASTRA 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:

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

See [Managing User Accounts on page 75](#) for details about the actions that can be performed by each user level.

You can use different group names by following the instructions in [Customizing Windows Groups for ASTRA Privilege Levels on page 37](#).

In ASTRA Basic, users are not prompted to log in with a user name and password.



### Security

Where necessary, the user level required to perform an action is identified in this manual. The “Security” icon (shown here in the left margin) highlights such information. Security information is specific to ASTRA with Security Pack. There are no access restrictions if you are using ASTRA Basic.

## Setting Up Groups



### Security

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** or **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
AstraAdministrators	Administers ASTRA accounts and database
AstraResearchers	Creates and modifies experiments and profiles.
AstraTechnicians	Runs experiments and saves data.
AstraGuests	Read-only access to experiments and results.

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

You can use different group names by following the instructions in [Customizing Windows Groups for ASTRA Privilege Levels on page 37](#).

- 
- Note:** For backward compatibility with ASTRA V, the default group names for that version, which have a single space after the word “ASTRA” in the group name are still supported. These group names are “ASTRA Administrator”, “ASTRA Researcher”, “ASTRA Technician” and “ASTRA Guest”.
- 

## Creating Users



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



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



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 ASTRA security groups to either the corporate domain or the local machine. Note that ASTRA security groups that are set up on the corporate domain require domain user groups.

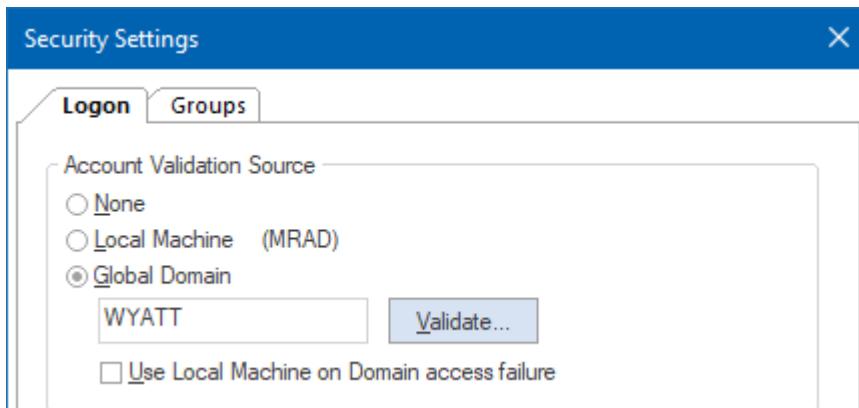
## Setting a Validation Domain for User Accounts and Groups



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 "AstraAdministrators" 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 both the local machine and the enterprise domain have ASTRA groups, users get the highest privilege level set for them on either machine.  
Leaving the account validation source set to "None" is not recommended, since it leaves the potential for a user to elevate their privileges by using a local login and security group.
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.

Using the “Local Machine” setting is recommended only if domain accounts are not used. If domain accounts are used, the Global Domain setting should be chosen.

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**.

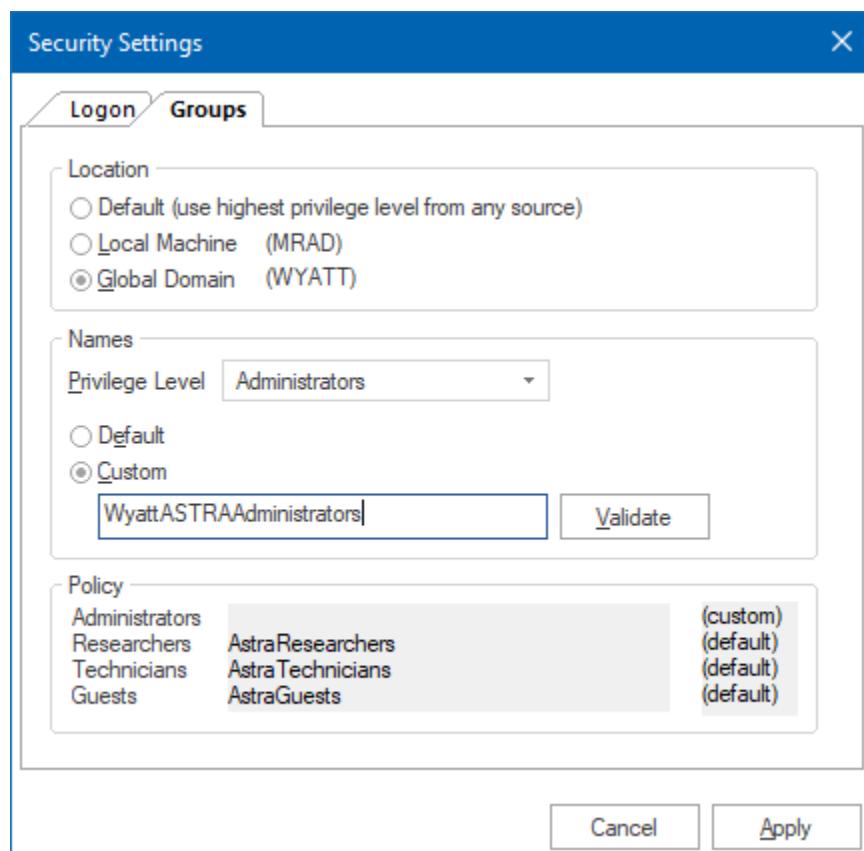
## Customizing Windows Groups for ASTRA Privilege Levels



You can specify different Microsoft Windows user groups to be used for the four privilege levels defined for ASTRA. This is useful if you already have users assigned to Windows groups that generally correspond to the ASTRA groups.

Follow these steps:

1. Start ASTRA and log in using a user account in the default “AstraAdministrators” group.
2. Choose **System→Security** to open the Security Settings dialog.
3. Move to the **Groups** tab.



4. 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.

We recommend choosing domain groups if you are using domain accounts. Local groups can be used if domain groups are not available. Choosing Default is not recommended since this potentially allows users to elevate their privileges.

5. If you want to modify a group name that ASTRA uses, select a **Privilege Level**.
6. Select the **Custom** option if you want to specify a new group name. (Select the **Default** option if you want to revert to the default group.)
7. Type the custom group name you want to use for that level. Group names are case-insensitive.
8. Click **Validate**. Validation checks to make sure the name does not contain invalid characters and fits within the required name length. Validation does not check to make sure the group exists. You can create the group as described in [Setting Up Groups on page 34](#) after specifying its name here.
9. Repeat these steps for each privilege level as needed.
10. Click **Apply**. 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:

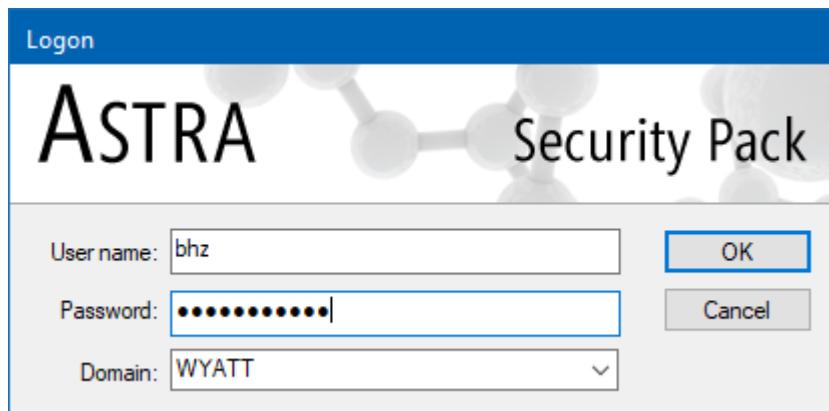
- Double-click the **ASTRA 8** icon on your desktop.
- Choose **Programs**→**Wyatt Technology**→**ASTRA 8** from the Windows Start menu.

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



If you are using ASTRA 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 34](#).

In the **Domain** field, if you are using a network user account to log in, choose the global domain name. If you are using a local user account to log in, choose the local computer name.



By default, ASTRA with Security Pack stores experiments in a Microsoft SQL Server database. A user with AstraAdministrators privileges can change to another database by following the steps in [Connecting to a Database on page 72](#). If you plan to change the database, it is best to do so before you start using ASTRA for experiments.

# 3

## Interfaces to Instruments

This chapter explains how instrument connections to ASTRA are made and how to use the Diagnostic Manager. These can be accessed through ASTRA. You can connect to, administer, and acquire data from instruments connected locally or over your network.

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Instrument Connection Overview .....	44
Using the Diagnostic Manager .....	45
About CheckPlus.....	54

## Accessing and Viewing Hardware

ASTRA's instrument list provides the following capabilities:

- Allows you to add, display, remove, or refresh the list of instruments available within ASTRA on your computer.
- Shows instruments connected to your network and allows you to launch the Diagnostic Manager for each instrument.

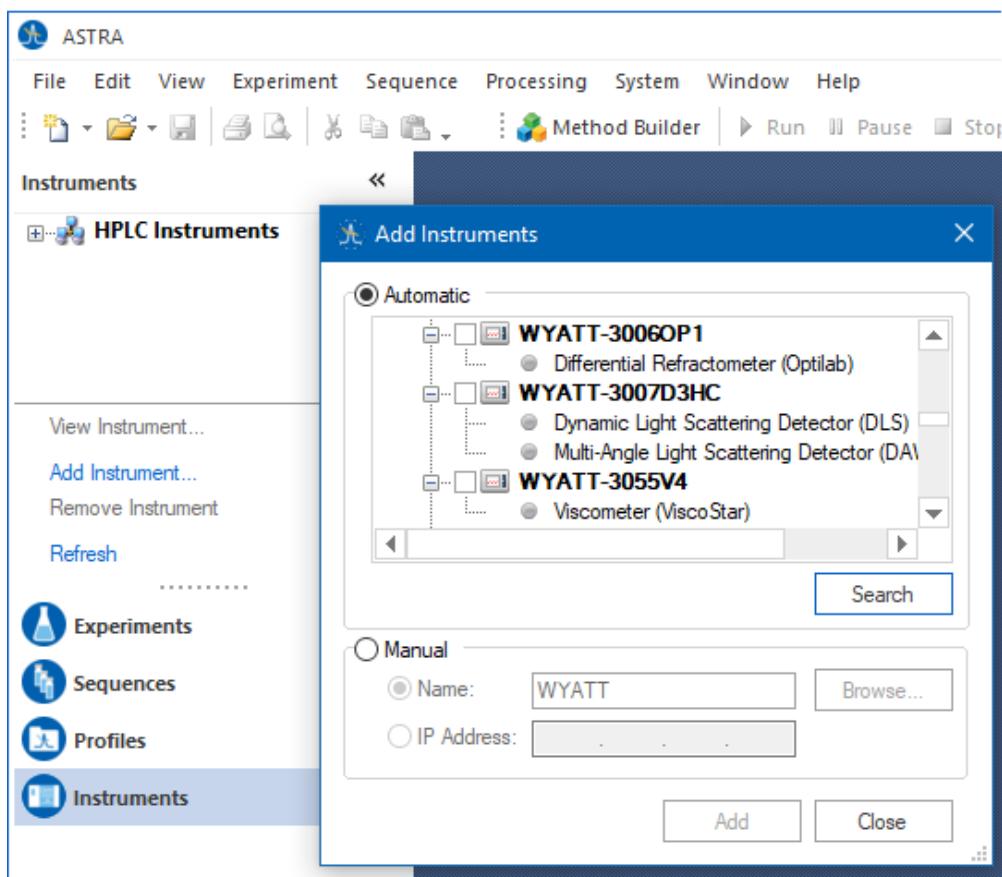
This section gives step-by-step instructions for actions you need to perform to make instruments visible to ASTRA. For more details about the Diagnostic Manager, see [Chapter 3, Interfaces to Instruments](#).

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.

### Adding an Instrument or Computer to the Instrument List

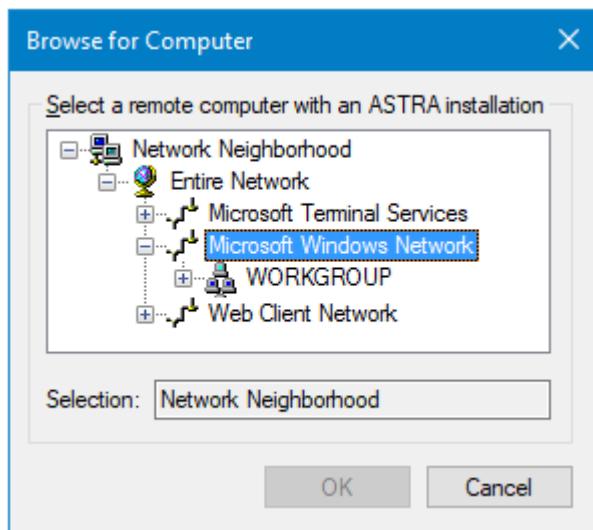
To add an instrument to the instrument list, follow these steps:

1. Select **Instruments** in the lower-left corner of the ASTRA window.
2. In the Instruments navigation pane, click **Add Instrument**. Or, in the **System > Instruments** dialog, click **Add**.
3. Click **Search** to search for available instruments.



4. If the instrument you want to add was found, select that instrument and click **Add**.
5. If your instrument was not found, you can add a computer or instrument by selecting the **Manual** option. You can 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 if allowed by your Windows settings.



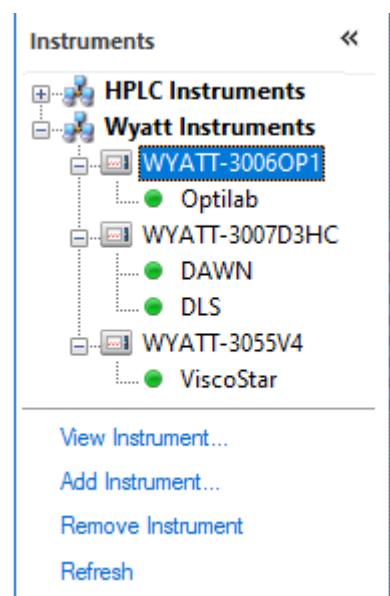
Instruments and computers that you add to the Instrument dialog remain connected to ASTRA in subsequent ASTRA sessions unless you delete them. Any supported instruments connected to those computers will be available within ASTRA.

See [Chapter 3, Interfaces to Instruments](#) for more about connecting to instruments and using the Diagnostic Manager dialogs.

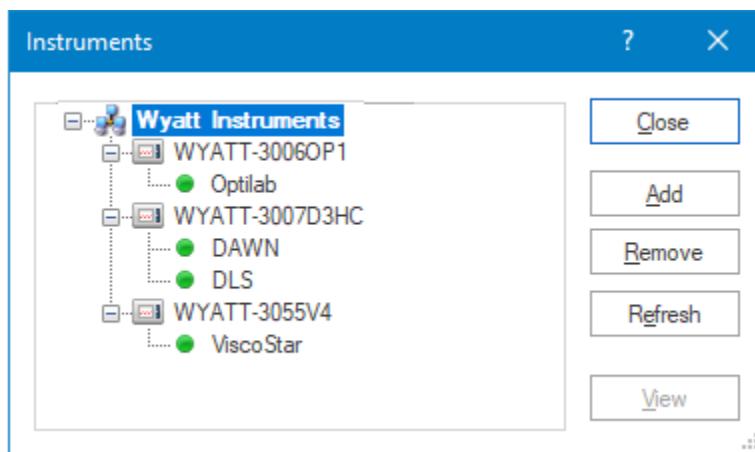
Once instruments are visible in the Instruments dialog, ASTRA is ready to use for collecting data. Please note that ASTRA can still be used for the analysis of already collected data files without any connection to an instrument.

## Viewing the Instruments Pane

To see a list of the instruments currently available to you, select **Instruments** in the lower-left corner of the ASTRA window to show the Instruments navigation pane.



Another way to view the list of instruments available to you 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 45](#).

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 8 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 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.



See [Configuring Instrument Profiles for Use with HPLC on page 177](#) for information about how to detect HPLC instruments.

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 seconds 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 45](#). You can also right-click on an instrument in the instrument list and click **View** to open the Diagnostic Manager.

## 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 Instrument**.

---

## Instrument Connection 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 41](#).

Instruments manufactured by Wyatt Technology Corporation can be controlled directly over the network.

Third-party instruments, such as generic RI instruments, generic UV detectors, and generic viscometers are connected through the AUX input of another instrument. As a result, these instruments will be shown as part of an experiment configuration.



See [Configuring Instrument Profiles for Use with HPLC on page 177](#) for information about how to detect third-party HPLC instruments after you have enabled Wyatt HPLC CONNECT as described in [Chapter 6, Setup for HPLC Functionality](#).

## Using the Diagnostic Manager

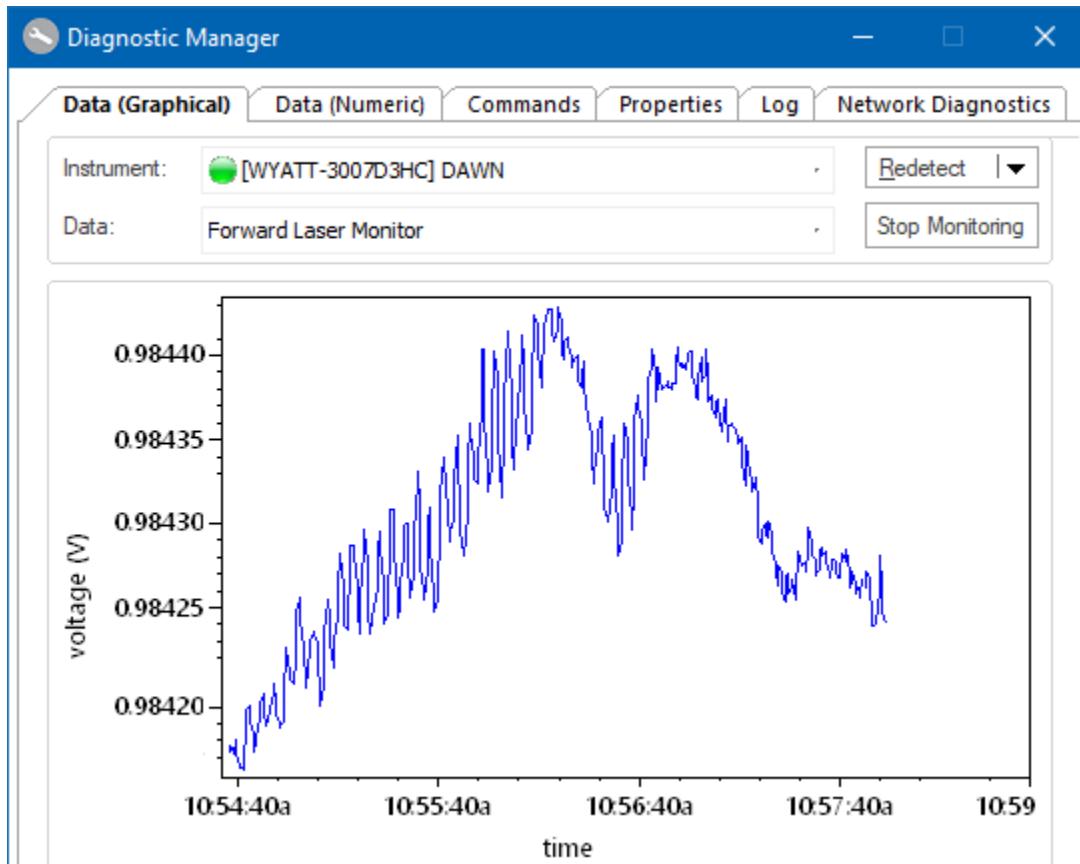
If you choose **System→Instruments**, select an instrument and click **View**, you see the Diagnostic Manager for that instrument. You can also start the Diagnostic Manager from the Windows Start menu by choosing **Programs→Wyatt Technologies→ASTRA→Diagnostic Manager**.

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 instrument.



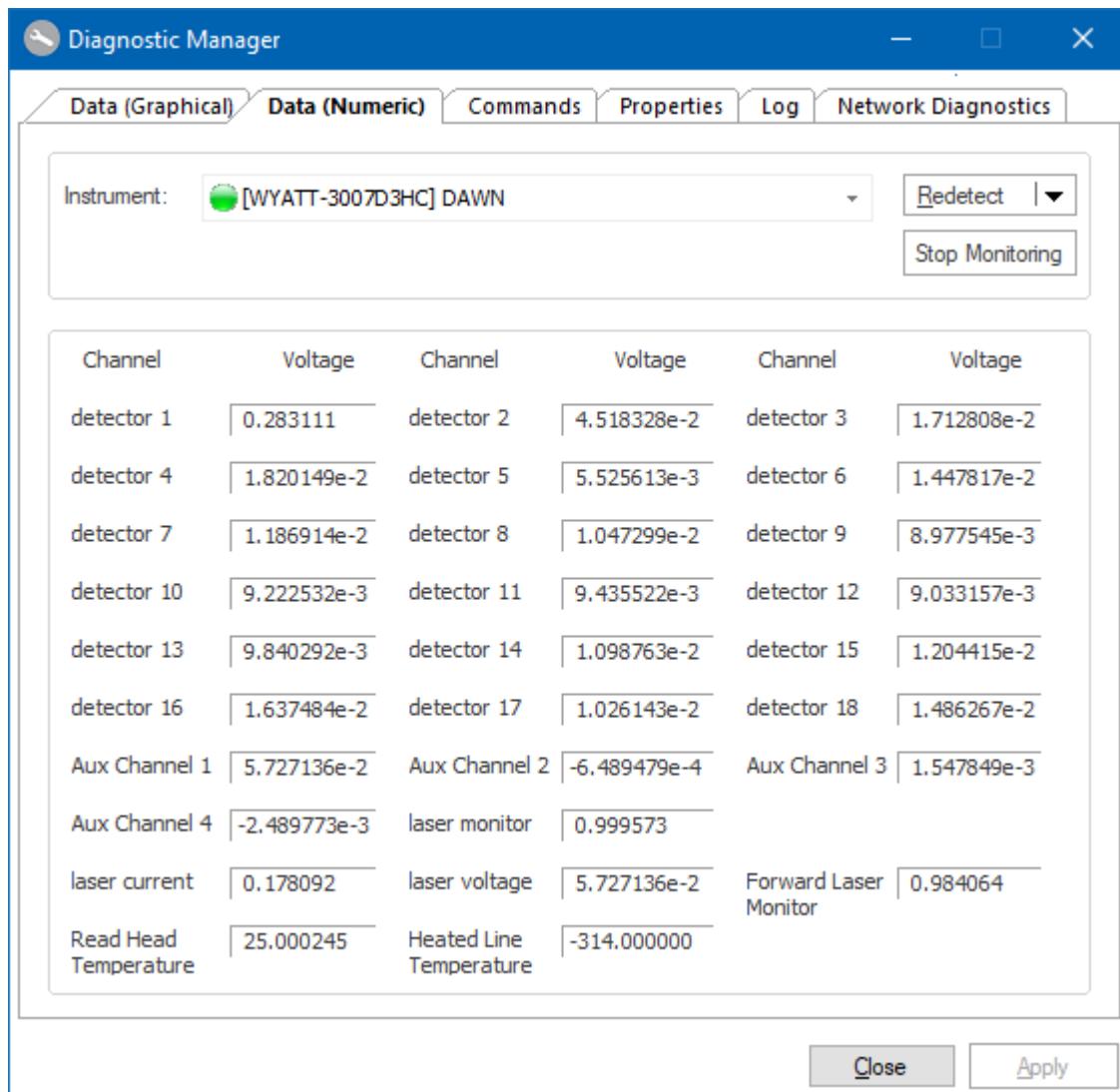
To monitor data, follow these steps:

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 50](#).  
If a MALS instrument is selected from the Instruments drop-down list, you can select “Calibration History” from the **Data** drop-down list.
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 192](#).

## 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 instrument.

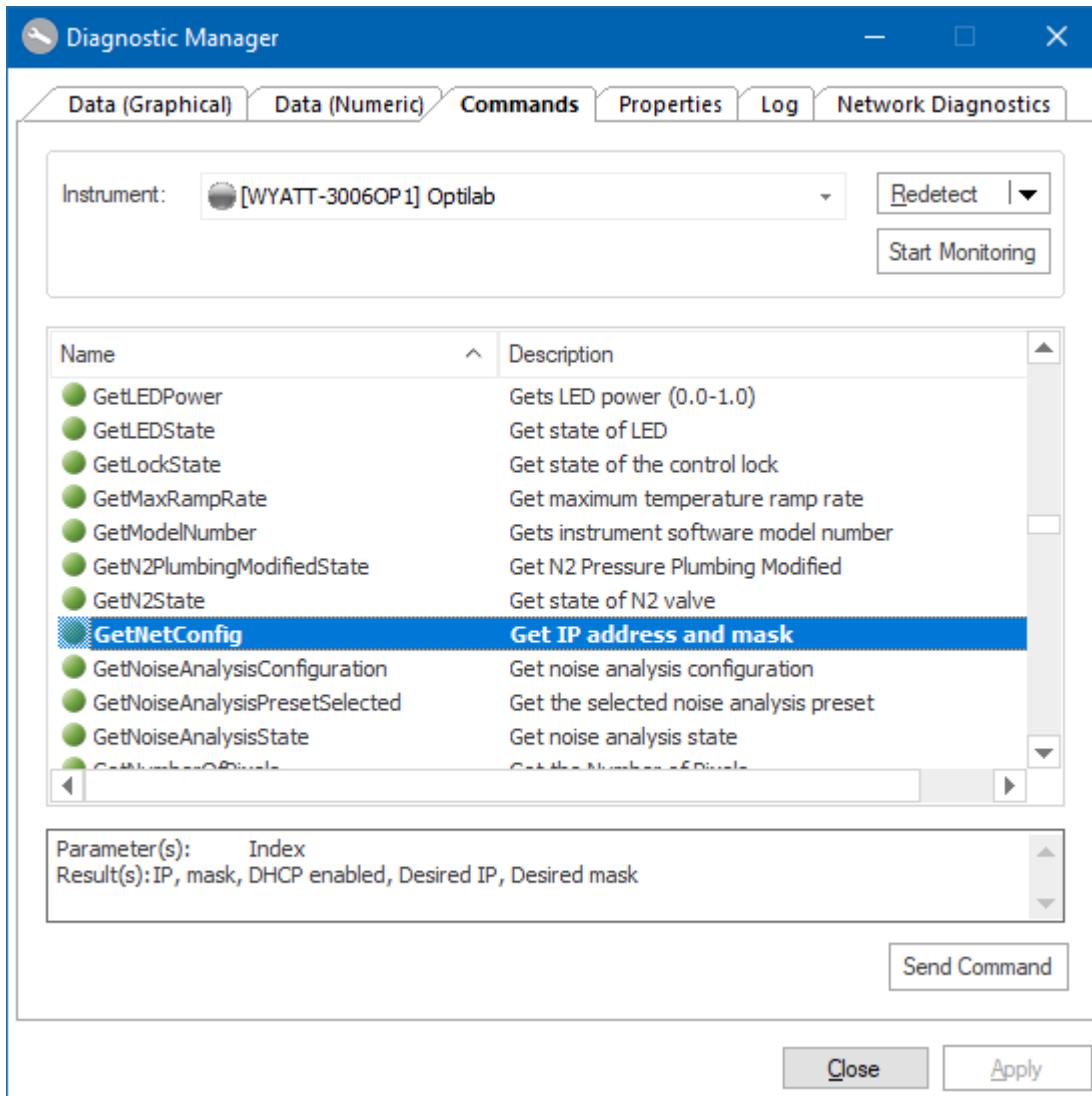


To monitor data, follow these steps:

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 instrument.



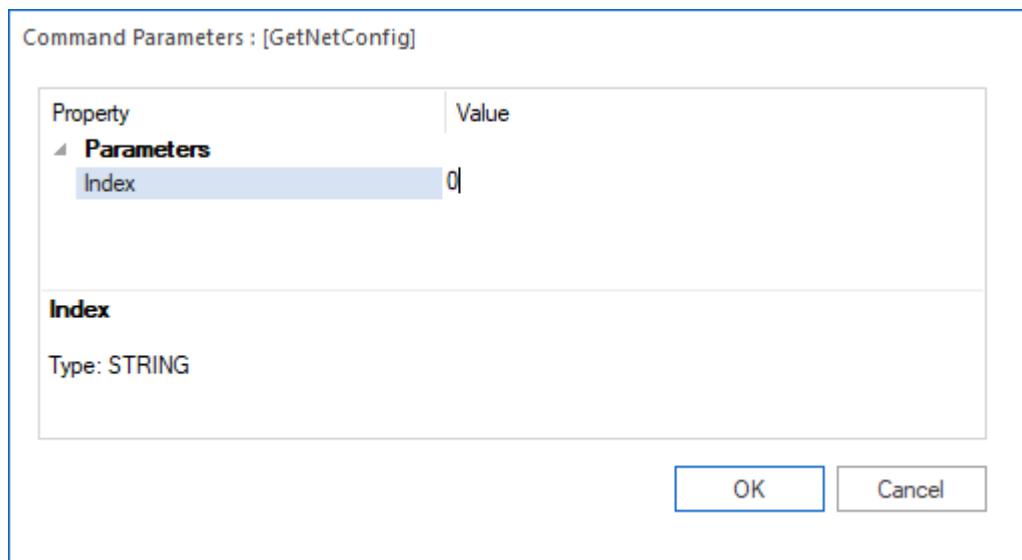
To send a command, follow these steps:

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

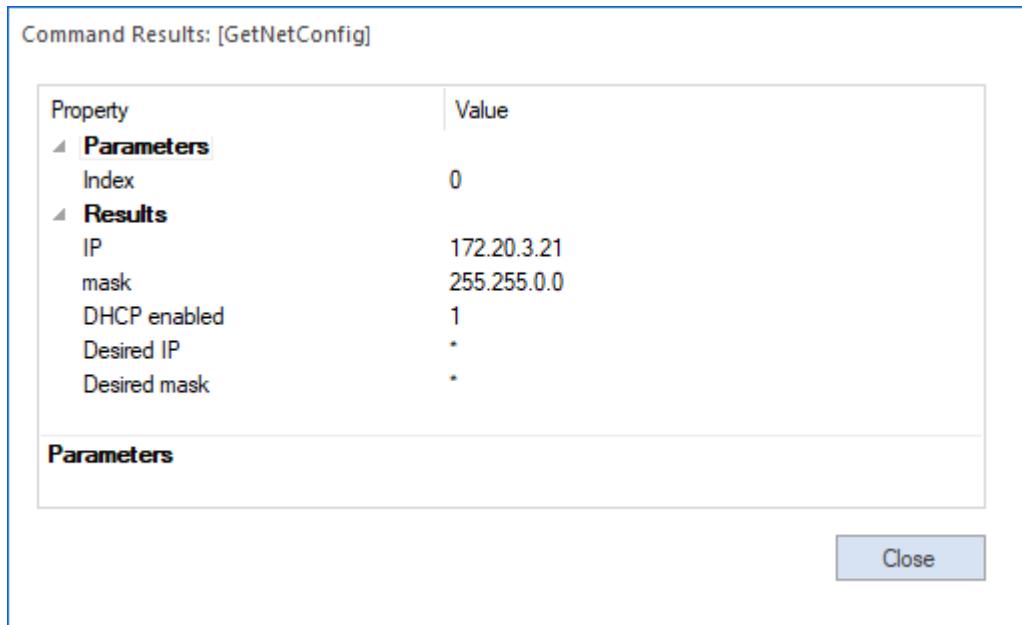
If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Redetect** to update the drop-down list.

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 Parameters dialog. Type the values you want to use for the parameters listed. Then click **OK**. For example, the GetNetConfig command requires an Index value, and 0 can be entered as the default.

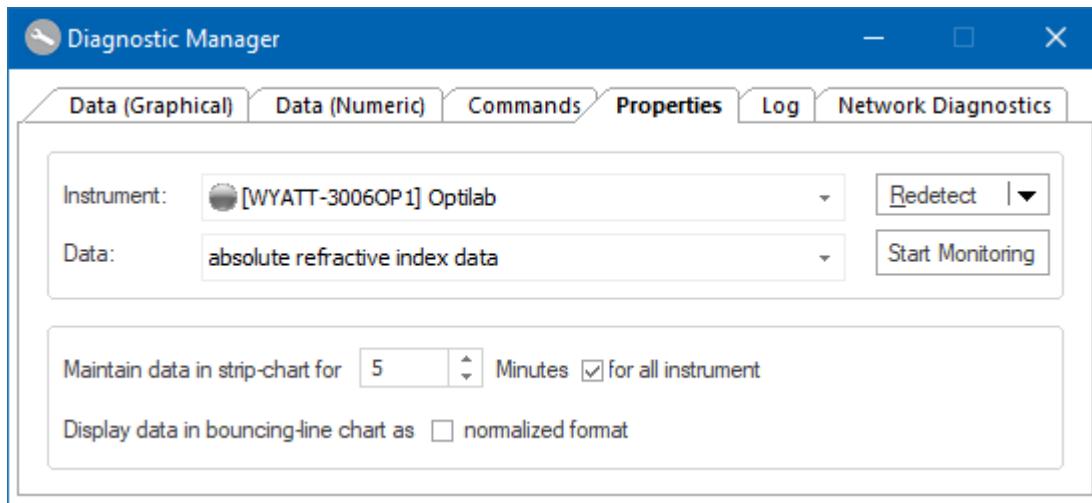


4. The results of the command, if any, are shown in the Command Results dialog.



## 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 instrument.



Properties are stored in the Windows registry of the computer to which the instrument is connected.

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 46) 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 ori-

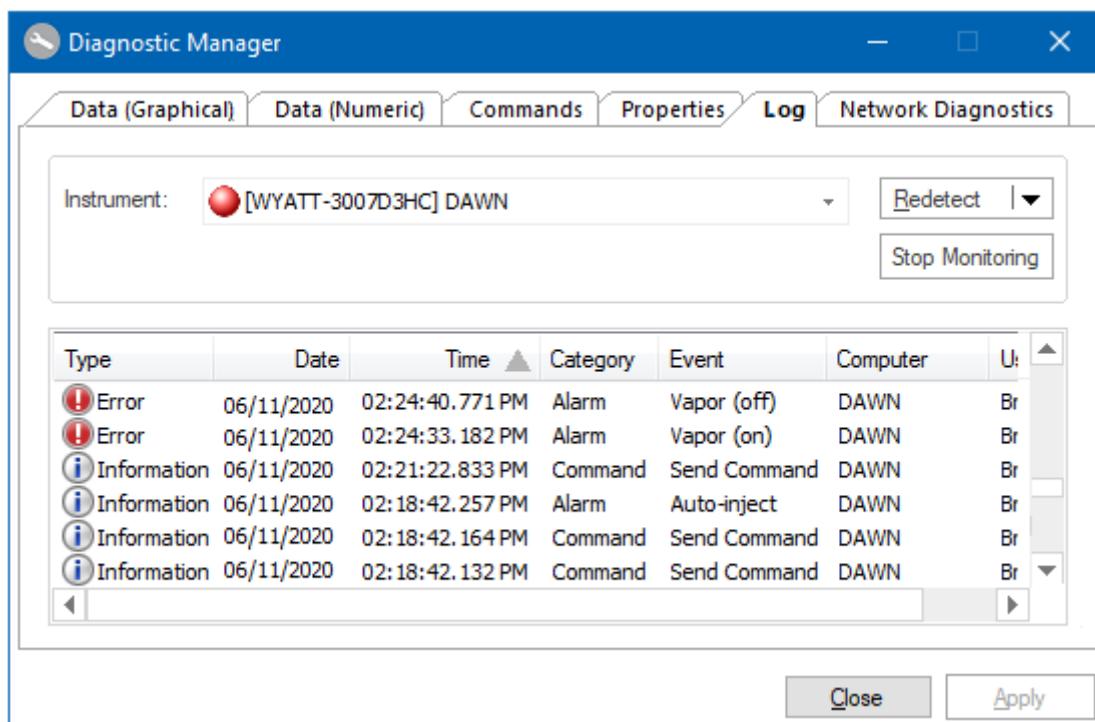
entation of a scintillation vial on the DAWN. Rotate the vial, view the live data, and try to put the vial in a position such that the live data line is flat.

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 DAWN instrument and some errors conditions reported by the instrument.



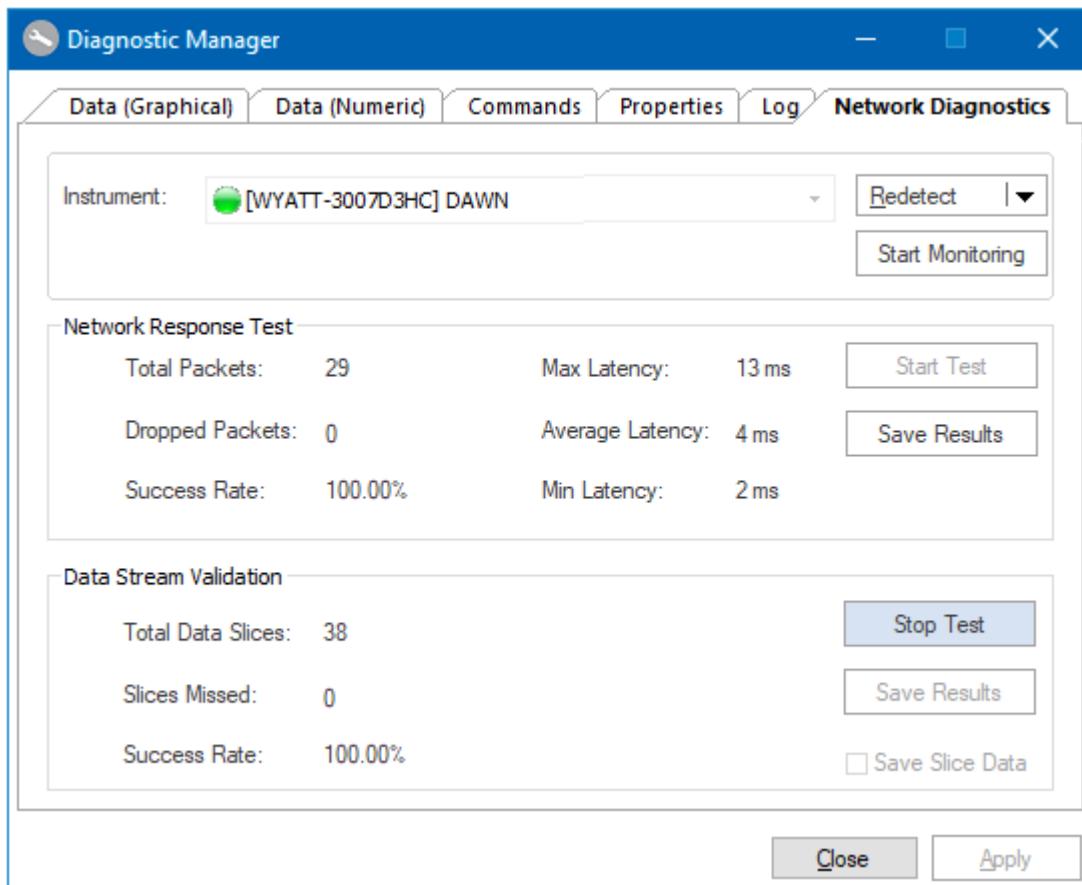
You can drag the borders between column headings to resize the columns.

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

You can allow ASTRA to send 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 seconds near the Windows taskbar. The messages fade automatically if you do not click on them.

## Network Diagnostics

The Diagnostic Manager provides built-in utilities to assess network reliability and performance. Two utilities are available on the **Network Diagnostics** tab: Network Response Test and Data Stream Validation.



### Network Response Test

This diagnostic verifies that the network can reliably communicate data between the workstation and the instrument. To conduct this test, follow these steps:

1. Click the **Start Test** button in the Network Response Test section.
2. Let the diagnostic test run. The test attempts to communicate with the instrument once per second to determine network reliability. We recommend you let the test run for 30 minutes or more to catch any transient network disruptions.
3. When you are done, click **Stop Test** to end the testing session.
4. If you want to save the statistics to a text file, click **Save Results**.

The key statistics to look for in the results are the number of dropped packets and the communication success rate. A solid network will have zero dropped packets and 100% success in communication.

## Data Stream Validation

This diagnostic verifies that all instrument data can reliably be received by ASTRA. To conduct this test, follow these steps:

1. If you want to save the raw instrument data during the collection, you can enable the **Save Slice Data** checkbox. Saving data is optional and can be left disabled.
2. Click the **Start Test** button in the Data Stream Validation section
3. Let the diagnostic test run. The test monitors the instrument data stream for any disruptions or lost data. We recommend you let the test run for 30 minutes or more to catch any transient network disruptions.
4. When you are done, click **Stop Test** to end the testing session.
5. If you want to save the statistics to a text file, click **Save Results**.

The key statistics to look for in the results are the number of missed slices and the communication success rate. A solid network will have no missed slices and 100% success in communication.

## Instrument Status Indicators

The following list shows the icons used by ASTRA to denote instrument status in the Diagnostic Manager.

Icon	Instrument Status	Details
	Unknown or Offline	Disconnected
	Unavailable	Unable to connect (connection failure)
	Online	Connected + zero or more information alarms
	Alarm Minor	Connected + one or more minor alarms
	Alarm Error	Connected + one or more error alarms
	Alarm Critical	Connected + one or more critical alarms

## About CheckPlus

CheckPlus is a standalone software application that helps you analyze and resolve Wyatt product issues at your site. CheckPlus operates in these three modes:

- Issue Reporting
- Issue Analysis
- Hardware Verification

Users can access the Issue Reporting function. The other functions are reserved for use by Wyatt representatives.

### Issue Reporting

You can use CheckPlus to report issues you encounter while using Wyatt instruments. CheckPlus automatically sends the report and diagnostic information to Wyatt Technology for analysis.



To use CheckPlus, follow these steps:

1. Launch CheckPlus from the desktop icon, the Microsoft Windows **Start** Menu, or by using the **System**→**CheckPlus** command in ASTRA.
2. Fill in the information requested in the Report Issue form.
  - a. Provide your contact information.
  - b. Specify the issue or symptom in detail.
  - c. Select the instrument for which an issue is suspected. (This is supported only for certain instrument models.)
  - d. Attach ASTRA or other files related to the issue and provide a description of the file. For example, attach an ASTRA file or screenshots.
3. Click **Next**.

CheckPlus packages the report and diagnostic information into a file. CheckPlus attempts to send the file directly to Wyatt Technology if an internet connection is available. If the file cannot be sent, a copy of the file is saved in  
`C:\Users\<user>\Documents\WTC\CheckPlus`. You can send the CheckPlus file to Wyatt Technology using another file sharing service, such as an FTP server.

# 4

## 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 .....	56
ASTRA Tutorials on the Support Center .....	57
Performing a SEC-MALS Experiment .....	58
More About the ASTRA Environment.....	62
Hardware Connections for Autoinject Signals.....	68

## Starting ASTRA

To run ASTRA, do one of the following:

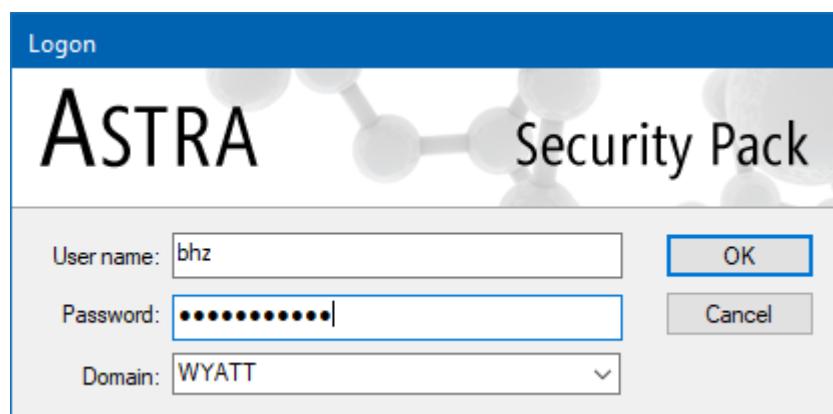
- Double-click the **ASTRA 8** icon on your desktop.
- Choose **Programs→Wyatt Technology→ASTRA 8** from the Windows Start menu.

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



If you are using ASTRA 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:

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

See [Managing User Accounts on page 75](#) for details about the actions that can be performed by each user level.



#### Security

Where necessary, the user level required to perform an action is identified in this manual. The “Security” icon (shown here on the left) highlights such information. Security information is specific to ASTRA with Security Pack. There are no access restrictions if you are using ASTRA Basic.

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## ASTRA Tutorials on the Support Center

Tutorials are provided for ASTRA on the Wyatt Technology Support Center. If you don’t have a Support Center account, please sign up for one.

The Support Center provides the latest self-guided tutorials and example files to assist you in getting the most out of your ASTRA software. We strongly encourage you to use these tutorials and other materials to learn how to use ASTRA.

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

# Performing a SEC-MALS Experiment



You must use an account with AstraResearchers or AstraAdministrators access to follow the steps in this section.

## Checking the Instrument Connection

Verify that your instrument connections to ASTRA are set up. 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 41](#).

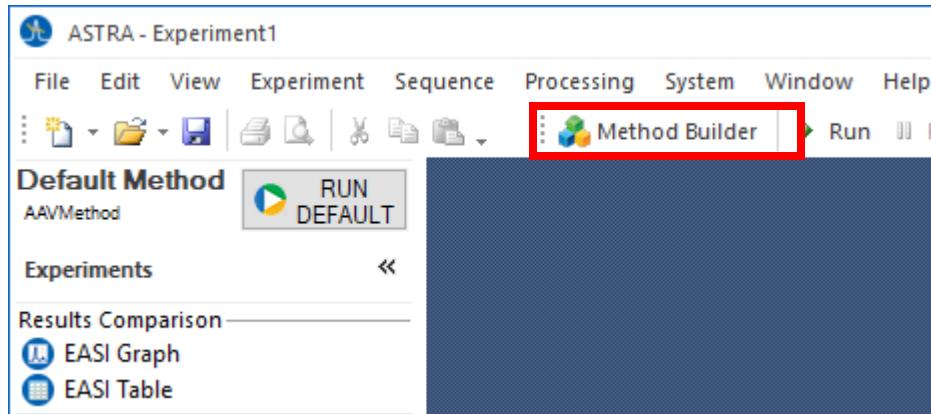
## Creating an Experiment Method

The fastest way to start an SEC-MALS experiment is to use the Method Builder Wizard. This multi-paged wizard guides you through the steps to create an experiment appropriate for your instruments and devices, analyzing the collected data, and reporting the results. The wizard can be used to set up experiments for flow separation, batch analysis, and instrument calibration.

After you create a method with the Method Builder Wizard, you can use it as the default method for future experiments and run it quickly.

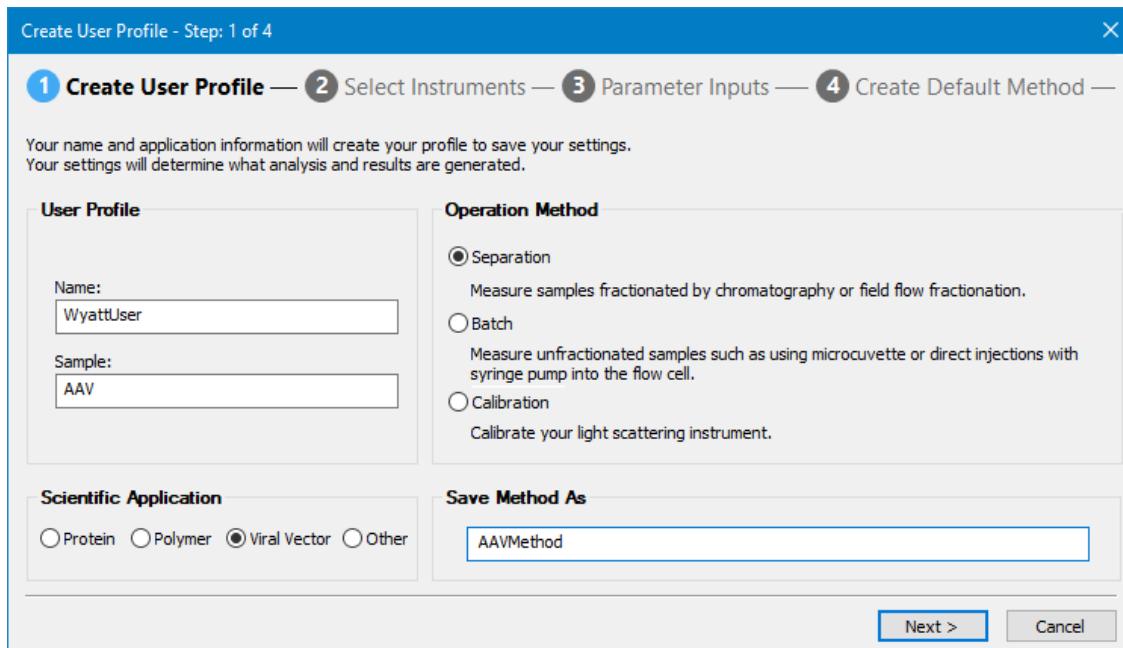
Detailed information about the Method Builder Wizard is available in [Using the Method Builder Wizard on page 129](#).

1. Launch the Method Builder Wizard by clicking the **Method Builder** button in the toolbar. (The Method Builder Wizard starts automatically the first time you launch ASTRA after the installation.)



## Method Builder: Step 1 of 4

Follow these steps in Step 1 of the Method Builder Wizard:



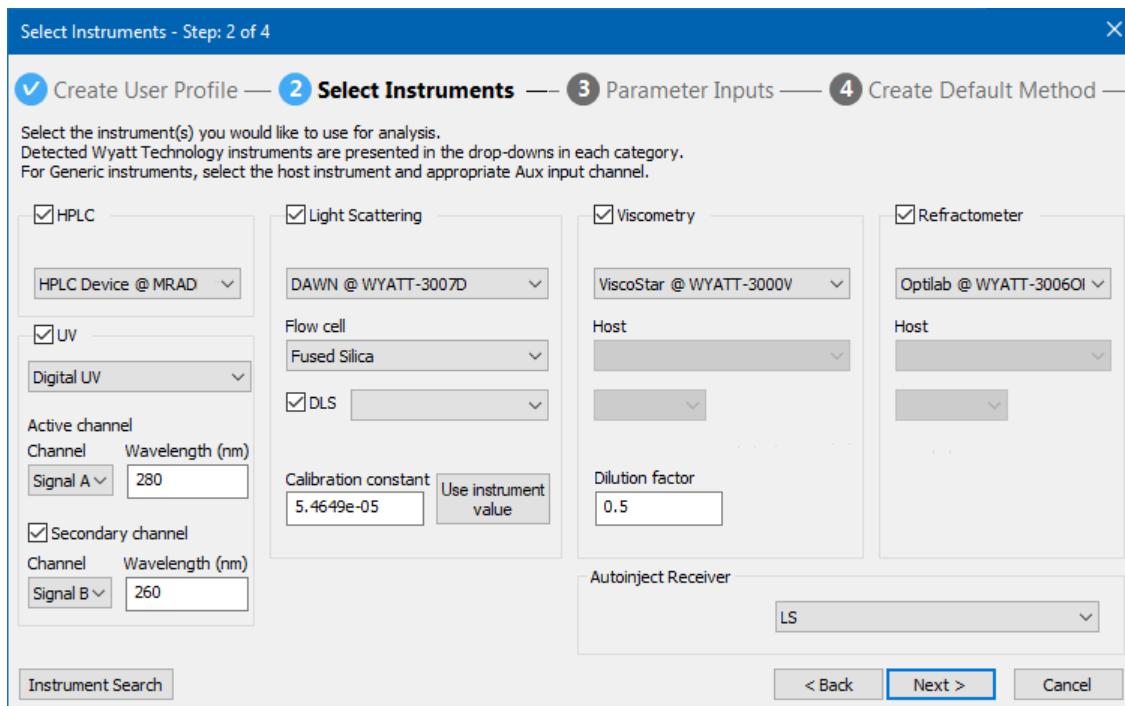
1. In the **User Profile** area, type a unique **Name** identifying yourself. Spaces are permitted. This acts as a username for storing methods in ASTRA's system database.
  2. In the **Sample** field, type the name of the sample being tested.
- The following example creates a “Jane Doe\MySample” folder in the ASTRA system database and store the generated method in that folder:

3. For **Operation Method**, select “Separation” for online experiments with SEC.
4. For **Scientific Application**, select the option (Protein, Polymer, Viral Vector, or Other) that most closely matches your sample. This choice affects the data analysis and results reported by the method. Viral Vector is grayed out if you do not have a license key for this option.
5. For **Save Method As**, type a name for the method.
6. Click **Next**.

For details, see [Method Builder Details: Step 1 of 4 on page 130](#).

## Method Builder: Step 2 of 4

Follow these steps in Step 2 of the Method Builder Wizard:



1. Select the instruments that are available and required for your experiment.
2. If you enable **HPLC**, choose the HPLC device.



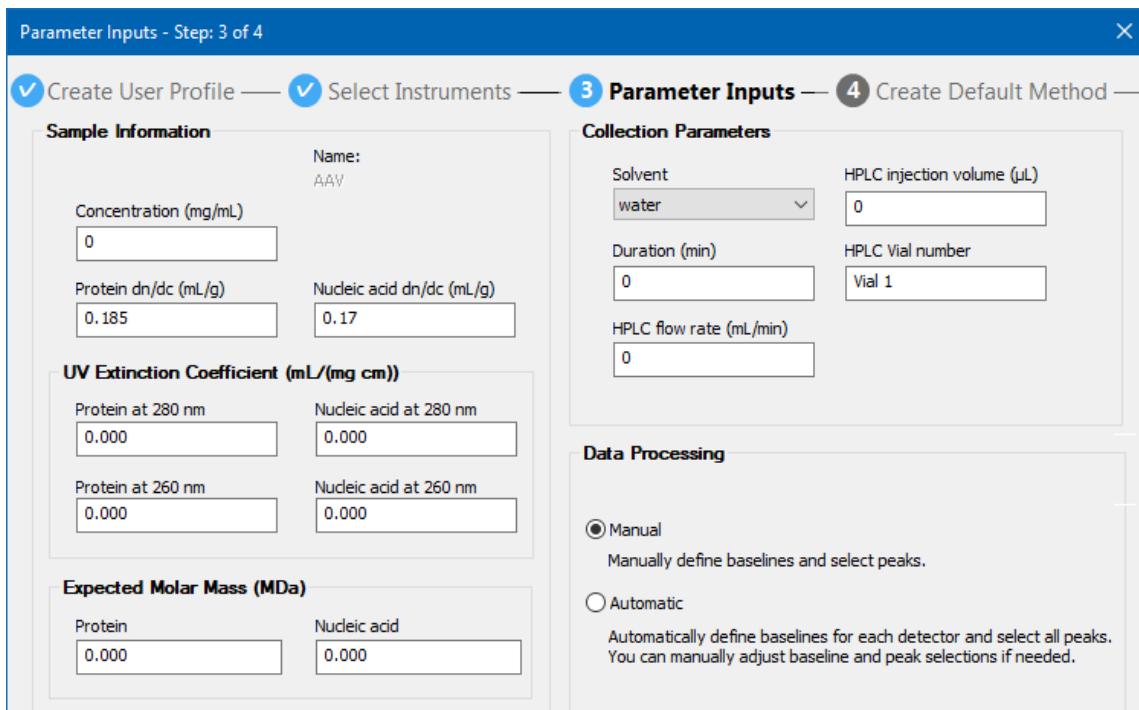
**Note:** When using HPLC CONNECT, ASTRA obtains the autoinject signal directly from the HPLC instrument. However, for optimal timing between instruments, we recommend connecting an autoinject cable between the HPLC Sampler and a Wyatt detector (usually the MALS detector) and using a daisy chain to send the signal to any other Wyatt instruments. Choose the instrument with the autoinject cable connection as the **Autoinject Receiver**.

3. In the **UV** area, if you have HPLC CONNECT you can choose to import a digital signal by choosing **Digital UV** in the drop-down list. Then, type wavelengths for the active and secondary channels.
4. In the **Light Scattering** area, type the **Calibration constant** as found on the Certificate of Performance or as determined from a calibration measurement.
5. In the **Autoinjector Receiver** field, select the instrument that should be used to trigger data collection to begin when a run is started.
6. Click **Next**.

For details, see [Method Builder Details: Step 2 of 4 on page 132](#).

## Method Builder: Step 3 of 4

The options shown for Step 3 depend on your selections in Steps 1 and 2. For example, selecting Viral Vector as the Scientific Application in Step 1 causes options to be shown for the dn/dc and UV extinction coefficients of the protein and nucleic acids.



You can click **Back** to change prior settings or **Next** to create the method.

For details, see [Method Builder Details: Step 3 of 4 on page 135](#).

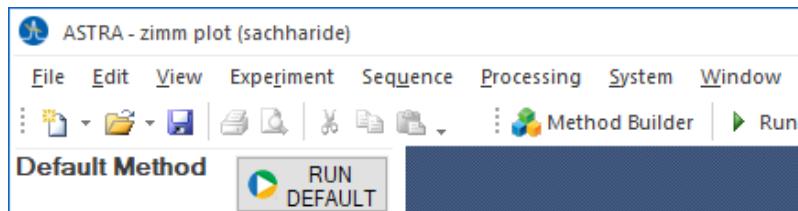
## Method Builder: Step 4 of 4

In this step, the wizard sets the method you created as the default method. The method can be manually run from the default method interface at the top of the ASTRA workspace. For details, see [Method Builder Details: Step 4 of 4 on page 137](#).

**Note:** Calibration methods will not be set as the default method.

Click **Run now** to create a new experiment using the newly created method and to immediately start a collection.

Click **Run later** to store the method for future use. The default method can be quickly run from the main ASTRA window.



## 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.

### User Modes

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

Run mode is the standard mode in ASTRA and is the recommended mode for running experiments. 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 within 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 also 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 with Security Pack, all users must log in with a unique user id and password. The administrator sets up accounts using the account levels described in [Managing User Accounts on page 75](#).



Where necessary, the user level required to perform an action is identified in this manual. The “Security” icon (shown here in the left margin) highlights such information. Security information is specific to ASTRA with Security Pack. There are no access restrictions if you are using ASTRA Basic.

### Switching Users



You can change which user is logged in by choosing **System→Switch Active User** from the menus.

Switching users can be done at any time, including when a collection is in progress. The new user must log in using their security credentials. After the new user is authenticated, all audit log statements will list the new user as the responsible party.

The new user must have a privilege level greater than or equal to the previous user.

## Interface Idle Lock

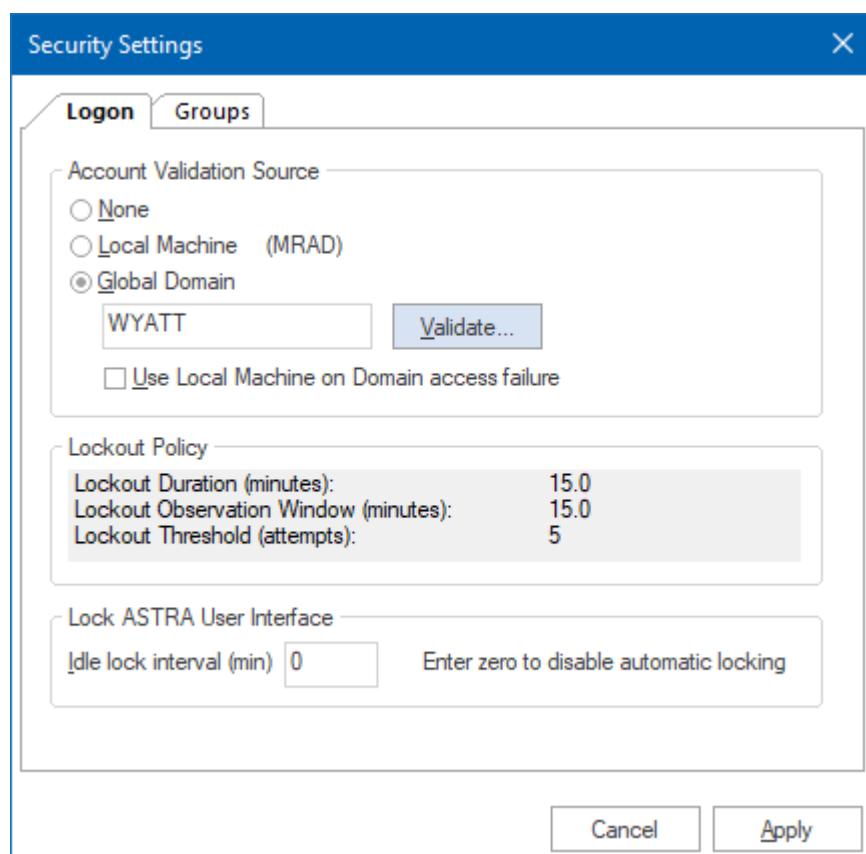


To prevent unauthorized use, ASTRA allows users to lock the user interface manually. In addition, ASTRA can be configured to automatically lock itself after being idle for some period of time. Collection continues if it is in progress while the user interface is locked.

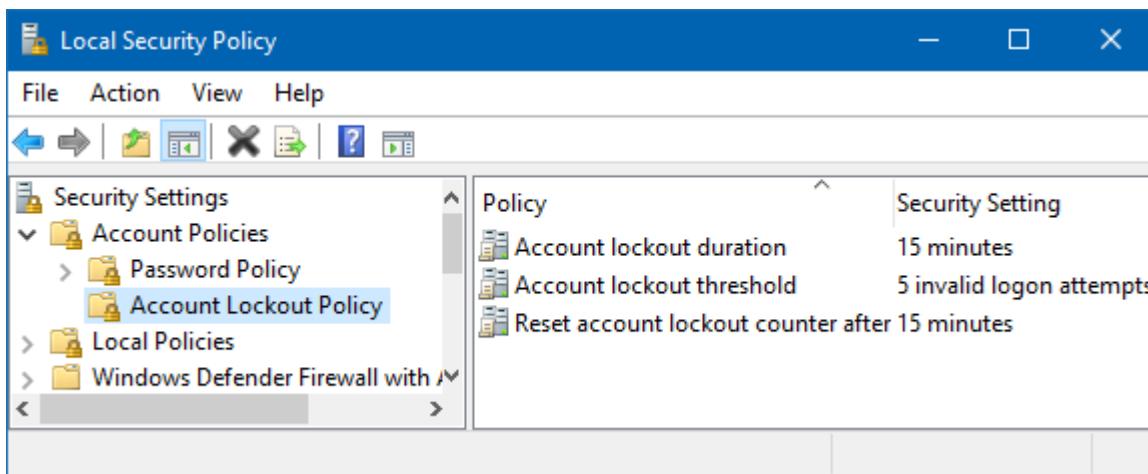
Another user may login to ASTRA when it is locked so long as that user has a privilege level greater than or equal to the previous user.

To manually lock the interface when you will be away from the computer, press **Ctrl+Alt+L** or choose **System→Lock ASTRA** from the menus.

Automatic interface idle locking is off by default. Users with the ASTRAAdministrators privilege can enable or disable the idle lock and configure the idle lock time interval. Choose **System→Security** and select the **Logon** tab. Type the desired **Idle lock interval** in minutes. To disable interface locking, type 0 (zero) as the interval.



ASTRA uses Microsoft Windows to manage its user accounts. The Lockout Policy Duration, Observation Window, and Threshold can be modified using the Local Security Policy (secpol.msc) or Group Policy Editor (gpedit.msc) application provided by Windows.



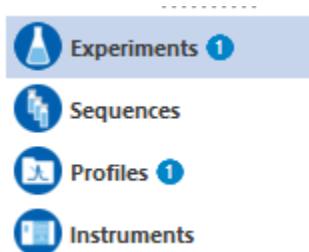
For details about the Account Validation Source portion of this dialog, see [Setting a Validation Domain for User Accounts and Groups on page 36](#).

## 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. The buttons show the number of currently open items of that type.



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 icons instead of buttons for one or more navigation panes by clicking the >> arrows in the lower toolbar and selecting **Show Fewer Buttons**.



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 used 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.

You can print logs using the **System→Log→Print** command or the **View→Logs→<log type>→Print** commands.

## 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.

<b>Note:</b>	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.

## Reporting Issues

If you encounter an issue with ASTRA, please report the problem by choosing **Help→Report Software Issue** from the menus.

You will see a dialog that allows you to describe the problem in detail and to provide your contact information. The information you provide is sent to Wyatt along with important ASTRA diagnostic information.

## Exiting 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.

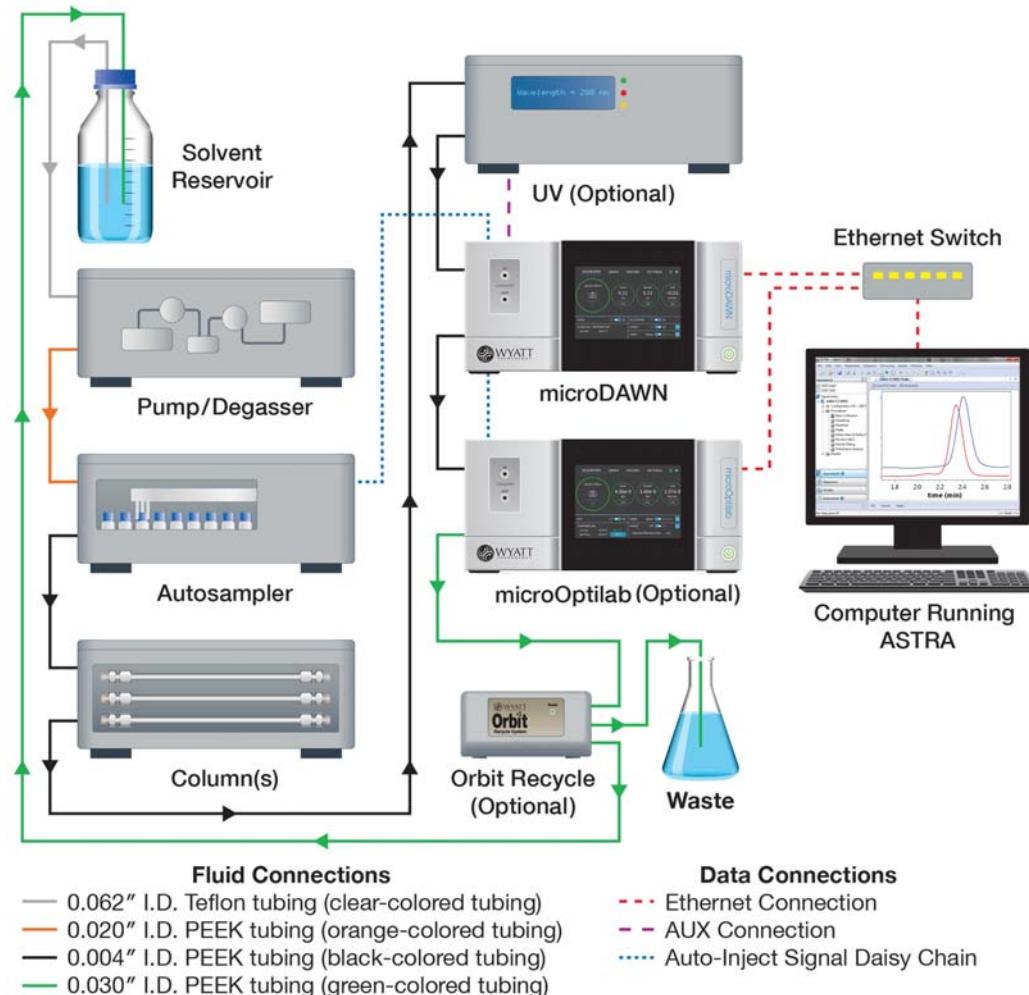
## Hardware Connections for Autoinject Signals

In general, you should refer to the user's guide for your Wyatt instruments when setting up the fluid and AUX connections between instruments.

If you are using an autoinjector, this section describes the recommended "daisy chain" setup, which allows all instruments to receive the autoinject signal. This connection setup is recommended instead of earlier setups that sent the autoinject signal to the MALS instrument only.

The autoinject signal from the HPLC system should be sent to the DAWN, miniDAWN, or microDAWN via the **Autoinject In** port on the back of the instrument. From there, an autoinject crossover cable should be used to connect the DAWN/miniDAWN/or microDAWN to the Optilab/microOptilab instrument. ViscoStar instruments should be connected as well, if present. The crossover cable connects from the **Autoinject Out** port on the first instrument to the **Autoinject In** port on the second instrument. Contact Wyatt Technology for the appropriate autoinject crossover cable.

The following figure shows the recommended connections. The example shows a microDAWN, but a DAWN or miniDAWN could be used instead.



The autoinject line, typically coming from an HPLC system, is connected to the lead Wyatt instrument in the stack. The Autoinject Out port of the lead instrument is then connected to the Autoinject In port of the next instrument. This pattern continues for all of the Wyatt instruments, each being connected to the one above it.

In systems using multiple instruments, the autoinject trigger is the most effective way to provide reliable, precision alignment between instruments.

---

<b>Note:</b>	The daisy chain setup is required for effective microDAWN and microOptilab use. For other instrument combinations it is optional, but recommended.
--------------	--

---

There are three setup options, depending on how the instruments are connected and whether an autoinject signal is present:

- **Daisy chain:** If the system is connected using the “daisy chain” setup described earlier, the experiment starts automatically when the autoinject signal is received, and all instrument data will be synchronized using the autoinject signal.
- **No daisy chain.** Any instruments that do not receive an autoinject signal will be aligned using *network synchronization*, which uses network timing information to synchronize data across instruments. This method is less precise than the method using the autoinject daisy chain. If two instruments receive the autoinject signal but the third instrument does not, then the first two will be aligned using the external signal and the third will be aligned using the network method.
- **No external autoinject signal:** All Wyatt instruments may be connected with autoinject crossover cables even if there is no external autoinject trigger. This is typically the case when starting experiments manually. In this case, ASTRA commands the lead Wyatt instrument to send a synthetic autoinject signal to be received by the other Wyatt instruments, providing the signal for synchronization.

# 5

## ASTRA Administration

This chapter explains how to administer the ASTRA 8 experiment databases. Such databases are used in ASTRA with Security Pack.

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## 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 ensure 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.
- **Improved Trend Analysis:** Results can be “mined” to find historical trends or to compare analytical techniques.
- **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/downloads/RegulatoryInformation/Guidances/ucm125125.pdf>.

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

## 21 CFR Part 11 Support in ASTRA 8



ASTRA 21 CFR Part 11 compliance features related to user accounts and logging activities are available only in ASTRA with Security Pack. The icon to the left identifies information that is specific to ASTRA 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 72](#)
- [Managing User Accounts on page 75](#)
- [Using the Logs on page 77](#)
- [Deleting Experiments on page 79](#)

ASTRA features related to the use of an experiment database to store experiments and sequences are available in ASTRA with Security Pack. The icon to the left above identifies information that applies to that software version.

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## Connecting to a Database

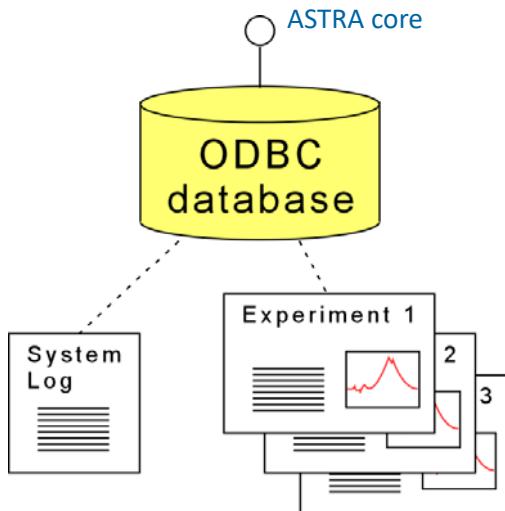
ASTRA 8 uses two databases:



- **System database:** This database stores experiment methods, sequence templates, profiles, solvents, and molecular standards. A system database is used by both ASTRA Basic and ASTRA with Security Pack. Each installation of ASTRA 8 uses its own, local system database. You cannot specify a different database to use as the system database. See [Importing a System Database on page 30](#) for information about updating the system database.
- **Experiment database:** This database stores information about experiments, database actions, warnings, and more. This database logs all activities performed with ASTRA that must be logged for 21 CFR Part 11 compliance. An experiment database is used *only* in ASTRA with Security Pack.

The rest of this section describes 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 SQL Server databases.



By default, the experiment database is a Microsoft SQL Server database.

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. SQL Server 2012 is recommended.

For more information, see the “ReadMe Files” directory of your ASTRA installation, which contains information about database and network issues.

Each experiment run with ASTRA uses an average of about 2 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.

ASTRA is capable of dealing with very large database archives without significant reduction in performance.

Database access is fully concurrent. Multiple users can interact with the same database instance simultaneously.

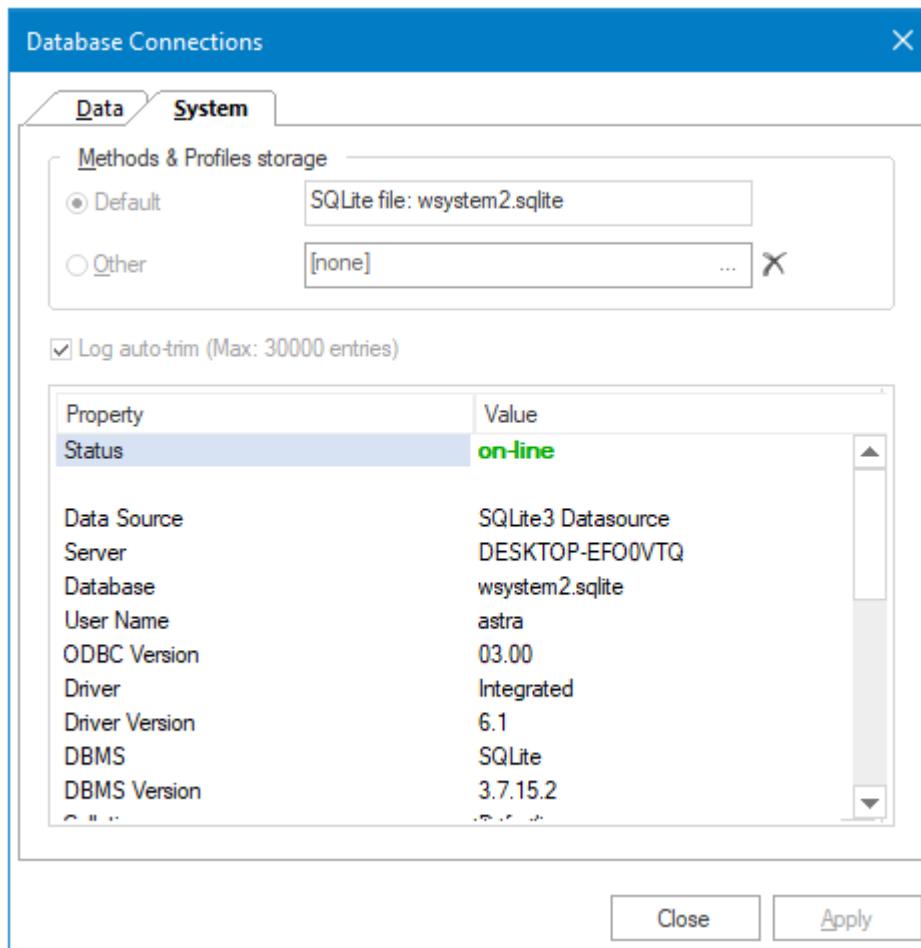
## Viewing Current Databases and Selecting an Experiment Database

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

1. Log in to ASTRA using an account with AstraAdministrators access.

2. Choose **System**→**Database Administration**→**Connections**.

Information about the size and location of the system database is shown in the **System** tab. You cannot change the system database location.



3. If you are using ASTRA with Security Pack, use the **Data** tab to see information about the current experiment database. You can use this dialog to change the experiment database. See the “ReadMe- SQL Server Configuration” PDF file provided in the “ReadMe Files” directory of the ASTRA installation for details.

## Creating a Microsoft SQL Server Experiment Database



If you are using ASTRA with Security Pack, configuring a SQL Server database to serve as the experiment database involves different steps depending on the version of SQL Server you have. See the “ReadMe- SQL Server Configuration” PDF file provided in the “ReadMe Files” directory of the ASTRA installation for detailed instructions. You will need to perform the following procedures:

1. set up user accounts for ASTRA access.
2. Set up a SQL Server database. (Creating a separate database for use with ASTRA is recommended to make maintenance easier.)
3. Configure the database security.
4. Enable discovery of the SQL Service.
5. Set up an ODBC connection to the database.
6. Configure ASTRA to use the ODBC connection.

## Managing User Accounts



User accounts in ASTRA 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:

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

The permissions granted to members of these groups are as follows:

*Table 5-1: Permissions granted by user group*

Access Rights	Administrators	Researchers	Technicians	Guests
Configuration and setup	✓	✗	✗	✗
Manage audit trails (clear logs)	✓	✗	✗	✗
Manage database connection and maintenance (delete files)	✓	✗	✗	✗

Table 5-1: Permissions granted by user group

Access Rights	Administrators	Researchers	Technicians	Guests
Configure instruments	✓	✓	✗	✗
Create sequence templates and experiment methods	✓	✓	✗	✗
Lock/unlock experiments (via electronic signature)	✓	✓	✗	✗
Export sequences and experiments	✓	✓	✗	✗
Modify and save sequences and experiments	✓	✓	✓	✗
Connect to instruments and run sequences and experiments	✓	✓	✓	✗
Collect data, change analysis processing parameters, and save data	✓	✓	✓	✗
Sign experiments (electronic signature)	✓	✓	✓	✗
Save imported sequences and experiments to the database	✓	✓	✓	✗
Open sequences and experiments from database or import from file system	✓	✓	✓	✓
Review system and database audit trail	✓	✓	✓	✓
Review sequence and experiment audit trail	✓	✓	✓	✓

With ASTRA Basic, 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 34](#) since that portion of ASTRA administration needed to be performed 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 Logs

ASTRA logs information about actions performed and the user who performs each action. The following types of logs are used by ASTRA:

- **System log:** Tracks global actions such as connections to the system and experiment databases. This log is stored in the system database, and all versions of ASTRA have a system log.
- **Data log:** Tracks connections to the experiment database and actions that open, save, or close an experiment. The data log is stored in the experiment database, which is used only by ASTRA with Security Pack.
- **Experiment logs:** Tracks actions on a particular experiment, including procedures, data set definitions, reports, methods, and more. These logs show when the procedures that make up an experiment were run and by whom. Experiment logs are stored as part of the ASTRA experiment (either in the experiment database for ASTRA with Security Pack or in the experiment file for ASTRA Basic). All versions of ASTRA can view experiment logs.
- **Sequence logs:** Tracks actions on a particular sequence. Sequence logs are stored as part of the ASTRA sequence (either in the experiment database for ASTRA with Security Pack or in the sequence file for ASTRA Basic). All versions of ASTRA can view sequence logs.

### Viewing the System Log

The system log tracks connections to the system and experiment databases.

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

Type	Date	Time	Category	Event	User
Information	6/19/2020	9:14:01 AM	Experiment	Create	WYATT\mra
Information	6/18/2020	6:07:32 PM	Experiment	Open	WYATT\mra
Information	6/18/2020	6:07:25 PM	Database	Database	WYATT\mra
Security	6/18/2020	6:07:16 PM	System	Login	WYATT\mra
Warning	6/18/2020	6:07:08 PM	System	Login	WYATT\mra
Security	6/18/2020	6:06:54 PM	Database	Connect (data)	WYATT\mra

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**.

To save the system log to a CSV or text file, choose **System→Log→Save As** or **View→Logs→System→Save As**.

To print the system log, choose **System**→**Log**→**Print** or **View**→**Logs**→**System**→**Print**.

## Viewing the Data Log



The data log tracks connections to the experiment database and actions that open, save, or close an experiment.

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

If you open, save, or close an experiment while this log is displayed, you can view the latest log entries by choosing **System**→**Database Administration**→**Log**→**Refresh** or **View**→**Logs**→**Database**→**Refresh**.

To save the entire data log to a CSV or text file, choose **System**→**Database Administration**→**Log**→**Save As** or **View**→**Logs**→**Database**→**Save As**.

To print the entire data log, choose **System**→**Database Administration**→**Log**→**Print** or **View**→**Logs**→**Database**→**Print**.

## Viewing Experiment Logs



Experiment logs show changes to an individual experiment.

To view the log for the current experiment, select the experiment and choose **Experiment**→**Log**→**Open**.

To save an individual experiment log to a CSV or text file, choose **Experiment**→**Log**→**Save As**.

To print an individual experiment log, choose **Experiment**→**Log**→**Print**.

## Viewing Sequence Logs



Sequence logs show changes to an individual sequence.

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 10, Using Sequences](#).

To save a sequence log to a CSV or text file, choose **Sequence**→**Log**→**Save As**.

To print a sequence log, choose **Sequence**→**Log**→**Print**.

## Working with Logs



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.

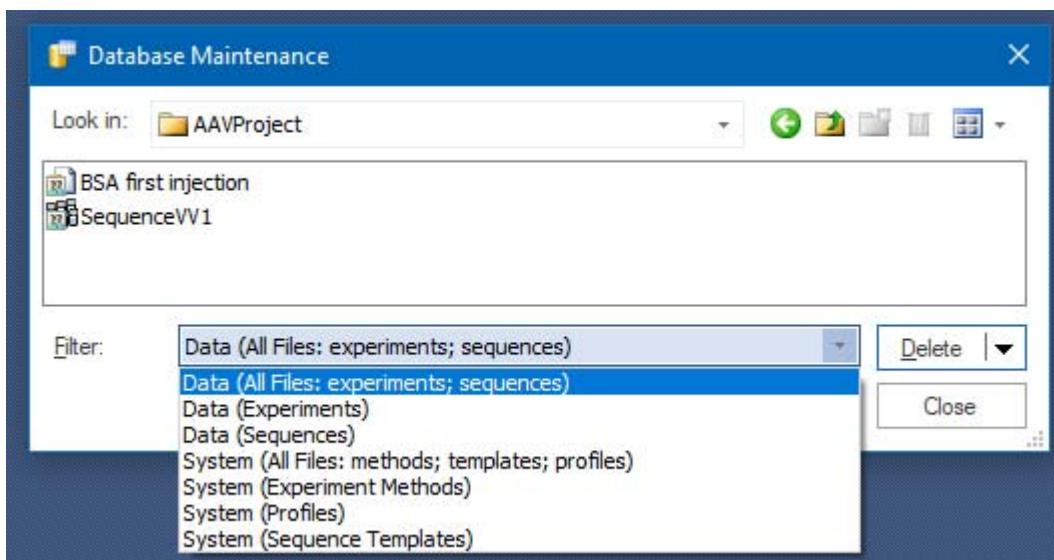
## Deleting Experiments



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.
2. Use the **Filter** drop-down list to select the type of information you would like to see. Choose a **Data** option for the Data Database, which stores experiments and sequences. Choose a **System** option for the System Database, which stores experiment methods, sequence templates, and profiles.)



3. Highlight the experiment you wish to delete in the list.
4. Click **Delete**.
5. 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 327](#), [Deleting a Profile on page 365](#), and [Deleting a Method on page 119](#).

## Performing Database Maintenance



### Security

Your IT department should set up a schedule for backing up your experiment database located in SQL Server and the system database located on the local computer running ASTRA.

## Upgrading ASTRA with Security Pack



### Security

Before upgrading ASTRA with Security Pack, back up your current experiment database. After the upgrade completes, log in to ASTRA using the ASTRAAdministrators privilege.

For upgrades from previous ASTRA versions (including ASTRA V, ASTRA 6, or ASTRA 7) to the latest ASTRA 8 version, you will need to create a new database and then import files from your existing database into the new database using ASTRA's bulk import feature. For instructions on how to create a new database refer to the "ReadMe – SQL Server Configuration" document in the "ReadMe Files" directory of the ASTRA installation. See [Importing Multiple Data Files on page 111](#) of this User's Guide for instructions on importing files from an existing database into the new database.

---

**CAUTION:** Make sure that all users who access the experiment database are upgraded at the same time. Using different versions of ASTRA with the same experiment database can lead to database corruption.

---

# 6

## Setup for HPLC Functionality

ASTRA can be set up for use with HPLC functionality via Wyatt's HPLC CONNECT software. Currently Agilent HPLC hardware is supported. This chapter guides you through the installation and configuration of the Wyatt HPLC CONNECT software components.

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Setting Up Wyatt HPLC CONNECT .....	82
Using HPLC DASHBOARD .....	90

### Introduction to HPLC CONNECT



Wyatt's HPLC CONNECT software enables ASTRA to control your HPLC as well as providing a dashboard for monitoring and control.

To enable HPLC functionality in ASTRA, there are two main software components to install and configure. Perform these installations on the PC that is connected to the HPLC hardware by a physical Ethernet connection.

You can install these components in any order.

- **ASTRA:** ASTRA provides configuration options for controlling the HPLC system. Install ASTRA as described in [Chapter 2, Installing and Setting Up ASTRA](#) to enable the HPLC features within ASTRA. Then, see [Configuring Instrument Profiles for Use with HPLC on page 177](#) for further details.
- **Wyatt HPLC CONNECT:** This software component is installed in conjunction with ASTRA. See [Using HPLC DASHBOARD on page 90](#). HPLC CONNECT has the following interfaces:
  - **HPLC DASHBOARD™:** This interface serves as a dashboard to monitor HPLC system parameters such as flow rate and pressure.
  - **System tray icon:** Use the icon in the Windows system tray to configure HPLC CONNECT to connect to the Agilent hardware.

## Setting Up Wyatt HPLC CONNECT

If you do not have the HPLC CONNECT installer and would like to obtain it, please contact [support@wyatt.com](mailto:support@wyatt.com).

In order to use Wyatt's HPLC CONNECT, you must have the following:

- **Supported Third-Party HPLC Hardware:** For a list of supported hardware, see the *ReadMe - HPLC CONNECT Supported Hardware (M1045).pdf* file, which is installed in the “Documents” folder of the HPLC CONNECT installation.
- **Local Area Network (LAN) connection:** Your HPLC hardware must be connected via a LAN to the computer where you will install HPLC CONNECT. An add-on LAN Interface Card may be necessary to connect the HPLC Instrument stack to a computer.

### Installing Wyatt HPLC CONNECT



Use the Wyatt HPLC CONNECT installer to install the HPLC CONNECT components: HPLC CONNECT and the HPLC DASHBOARD application.

Follow these steps to complete the installation successfully:

1. Double-click on the `HPLC_Connect_#_#_#_#_Setup.exe` installer to begin the setup process. Depending on the Windows user account restrictions, you may need administrator privileges.
2. Click **Next** when the main installer window appears.
3. Accept the license agreement and click **Next**.
4. Make sure that a **Complete** installation is selected and click **Next**.
5. Click **Install** to begin the installation.
6. When the “Agilent Instrument Control Framework” window appears, click **Next**.
7. Accept the terms of the agreement and click **Next**.
8. The defaults on the next screen are correct for most users; click **Next**.
9. Click **Install** to begin installing the Agilent ICF.
10. When the ICF installation is complete, click **Finish**.
11. When the Agilent LC Drivers window appears, click **Next**.
12. Accept the terms of the agreement and click **Next**.
13. The defaults on the next screen are correct for most users; click **Next**.
14. Click **Install** to begin installing the Agilent LC Drivers.
15. When the LC Driver installation is complete, click **Finish**.
16. When the installation is complete, click **Finish**.

## Activating the HPLC DASHBOARD

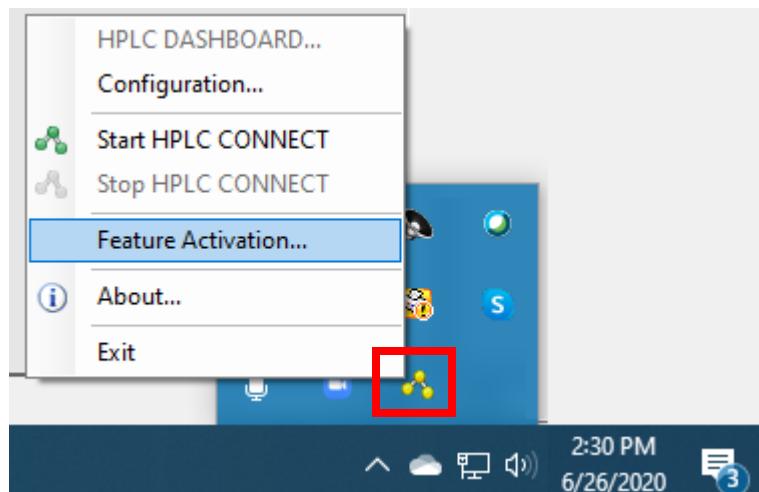
The HPLC CONNECT software requires a feature activation key. If you start the HPLC DASHBOARD before entering the key, you will see an error message that says the key was not found.

To enter the activation key, follow these steps:

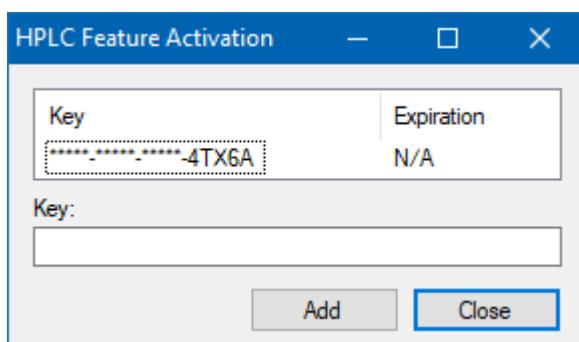
1. Find the Wyatt HPLC CONNECT icon in the Windows system tray, which is located in the bottom right of your task bar. It may be in a hidden dock with other tray icons. The icon shows a small molecule. The icon's color indicates the state of HPLC CONNECT and the connection; before the service is started, the molecule is white.



2. Right click on the HPLC CONNECT system tray icon and select **Feature Activation**.



3. Type or paste your HPLC CONNECT key in the feature activation dialog and click **Add**.



4. Right click on the HPLC CONNECT system tray icon and select **Start HPLC CONNECT**.

## Configuring HPLC CONNECT

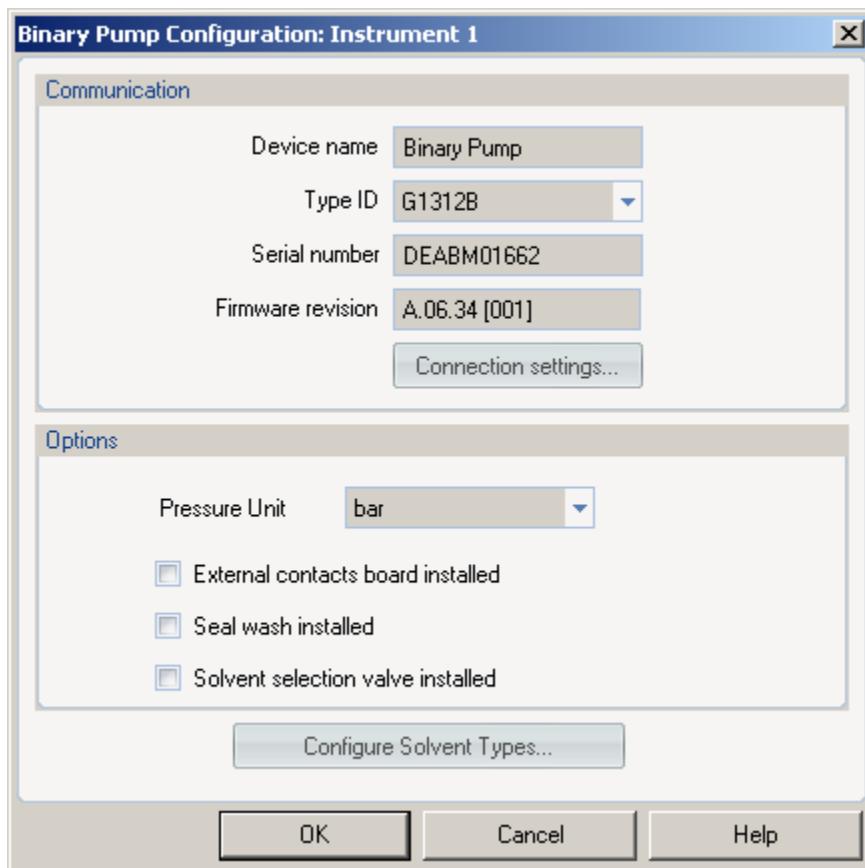
Once the Wyatt HPLC components have been installed and the service has been started, the next step is to configure the HPLC hardware.

### Configuring Agilent Hardware

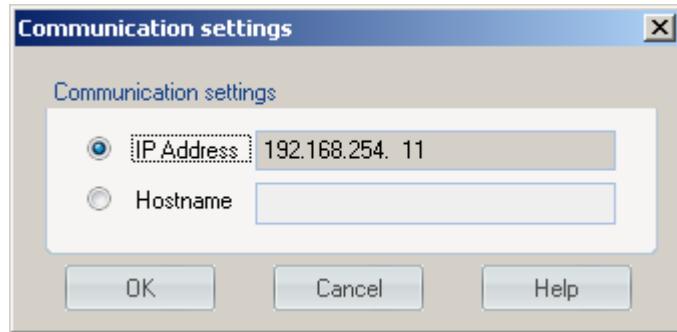
1. Find the IP address for your Agilent hardware. Write down the IP address, and save it for later. To find your IP address, follow the steps for the software interface of your Agilent system:

#### Chemstation or OpenLAB:

- a. Navigate to the **Instrument** menu in the top ribbon, and select **Instrument Configuration**.
- b. Select any Agilent LC Module and click the **Configure** button.



- c. Click **Connection Settings**. The Communication Settings window will display the IP address.

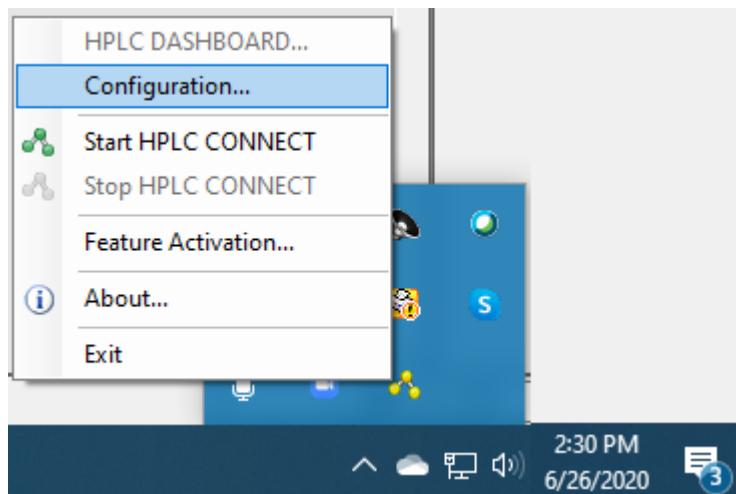


**Handheld controller (Instant Pilot):** If you have a built in network card, you can view the IP address on the Instant Pilot. If you are on the welcome front page of the Instant Pilot, select the **Details** button on the right side. This brings up the system information page, which contains the IP address information.

2. Find the HPLC CONNECT system tray icon, which is located in the bottom right of your task bar. Once you start the service, the tray icon should be yellow to indicate that the service is started, but not yet connected.

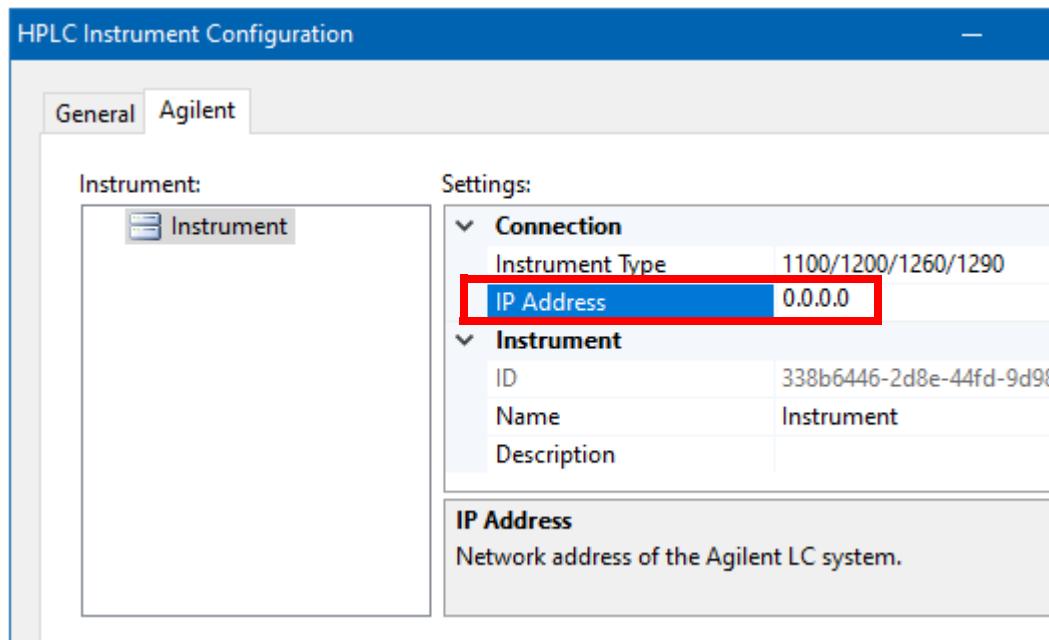


3. Right-click on the HPLC CONNECT system tray icon and select **Configuration**.

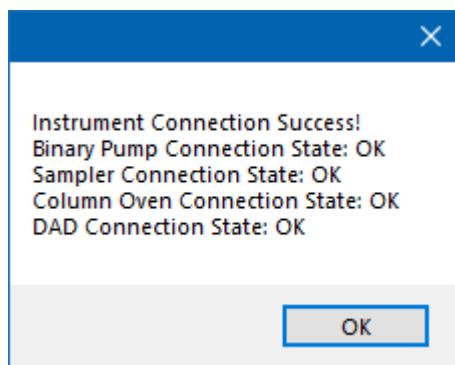


4. The HPLC Instrument Configuration window appears. The **General** tab lists the HPLC instrument series for which communication is supported. At this time, only Agilent instruments are supported.

5. Select the **Agilent** tab.



6. This window allows you to configure Wyatt HPLC CONNECT to be able to communicate with the HPLC hardware. In the Connection area in the right pane next to **IP Address**, type the IP address you found for the Agilent hardware.
7. After you type the IP address, it is shown in bold. Press the **Enter** key.
8. HPLC CONNECT attempts to communicate with the Agilent hardware. This may take a few minutes. If the communication attempt is successful, a message appears indicating success and the state of the devices in the Agilent system.



- 9.** The HPLC CONNECT system tray icon should now be green. The color of the icon indicates the state of HPLC CONNECT and the connection:

	Stopped
	Connecting, starting, or stopping
	Started, but not connected
	Started and connected

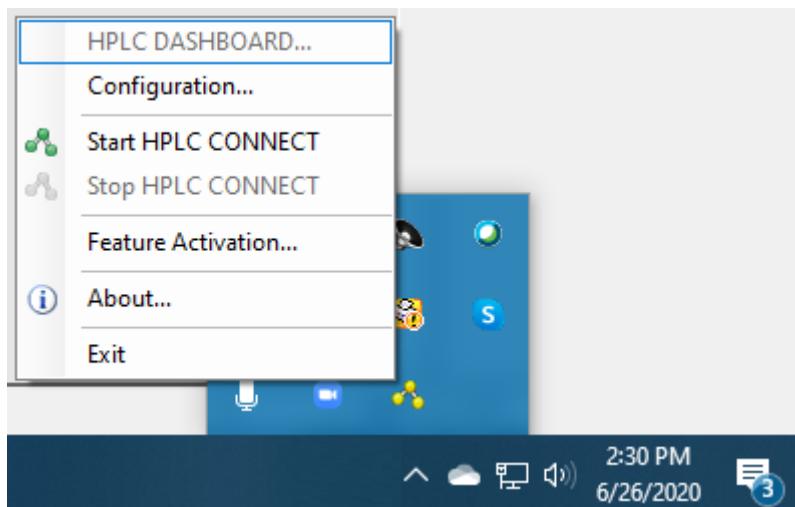
- 10.** After successfully connecting the instrument, edit the **Name** and **Description** fields of the instrument in the Instrument area of the HPLC Instrument Configuration window. The other fields in the Instrument section are automatically filled in.

- 11.** Click **OK**.

## Testing HPLC CONNECT

After you connect to an Agilent instrument, test the connection as follows:

1. Launch the Wyatt HPLC DASHBOARD by right-clicking the HPLC CONNECT system tray icon and selecting **HPLC DASHBOARD**.

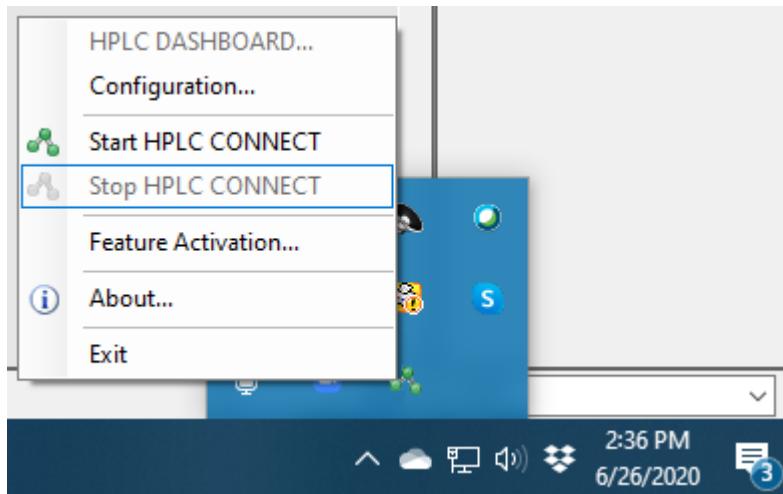


You can also choose **System→HPLC DASHBOARD** from the ASTRA menus to launch the HPLC DASHBOARD.

2. The HPLC DASHBOARD displays the current parameters for the HPLC devices at the top of the **General** tab and a graph at the bottom of the tab. The graph shows the flow rate over time.
3. Confirm that the parameter value matches the current HPLC hardware state. For details see [Using HPLC DASHBOARD on page 90](#).

## Disconnecting, Connecting, and Stopping HPLC CONNECT

To disconnect HPLC CONNECT, right-click the HPLC CONNECT system tray icon, then select **Disconnect HPLC**. This command releases control of the HPLC to allow other HPLC software to be able to connect to the HPLC. Administrator privileges are not required.



To connect HPLC CONNECT using the previously entered IP address, right-click the HPLC CONNECT system tray icon, then select **Connect HPLC**.

To close HPLC CONNECT, right-click the HPLC CONNECT system tray icon, then select **Service→Stop**. This command requires administrator privileges.

## Troubleshooting

If the connection attempt fails, there are several possible causes:

- **IP address:** Make sure the correct IP address for the Agilent hardware was entered.
- **Other clients:** Make sure no other clients are connected to the HPLC hardware. If ChemStation or OpenLAB are connected to the HPLC hardware, Wyatt's HPLC CONNECT will not be able to connect.
- **Network connection:** Make sure the hardware is on a network that can be reached from the workstation you are using. If the HPLC hardware is on a local network connected to a different machine, then it may not be reachable.

### Connection Conflicts

If HPLC CONNECT is connected to the HPLC hardware, it prevents all other clients from connecting to the hardware. If HPLC CONNECT is active, applications such as ChemStation and OpenLAB will not be able to connect.

When you are not using HPLC CONNECT, you can disconnect temporarily as shown in [Disconnecting, Connecting, and Stopping HPLC CONNECT on page 88](#).

## Firmware Compatibility

If the connection to the Agilent hardware fails, one possible reason is that the firmware on the Agilent devices (pump, autosampler, DAD/VWD, etc.) is incompatible with HPLC CONNECT. Make sure the latest HPLC firmware is installed on the Agilent devices.

HPLC CONNECT uses the Instrument Control Framework (ICF), which is the bridge between ASTRA and the Agilent instruments.

Wyatt's HPLC CONNECT 2.0 installs version A.02.05 of the ICF software and version A.02.18 of the Agilent LC and CE drivers. If more recent versions of the framework and drivers have been previously installed, these versions will not be installed, as that would be a downgrade.

The HPLC firmware dependencies for A.02.18 are provided in the *Agilent Instrument Control Framework for Agilent LC/CE and GC instruments in Non-Agilent Chromatography Data Systems A.02.18* document, which may be obtained from Agilent. The most recent such document is available [on the Agilent website for A.02.18](#).

In general, HPLC CONNECT will work with the most recent Agilent firmware.

For firmware installation instructions and downloads, visit the following Agilent pages:

- Firmware Downloads:  
<http://www.agilent.com/en-us/firmwareDownload?whid=69761>
- Instructions:  
<http://www.agilent.com/en-us/firmwareDownload?whid=83974>
- Adjusting the LAN card to set an IP address for G1369C:  
[https://www.agilent.com/cs/library/usermanuals/public/G1369C\\_LAN-InterfaceCard.pdf](https://www.agilent.com/cs/library/usermanuals/public/G1369C_LAN-InterfaceCard.pdf)

## Service Start/Stop Windows Settings

Administrator privileges are required to start or stop HPLC CONNECT or change the service settings.

The HPLC CONNECT system tray icon can be used to start and stop HPLC CONNECT. By default, HPLC CONNECT is started automatically when it is installed and each time the computer restarts.

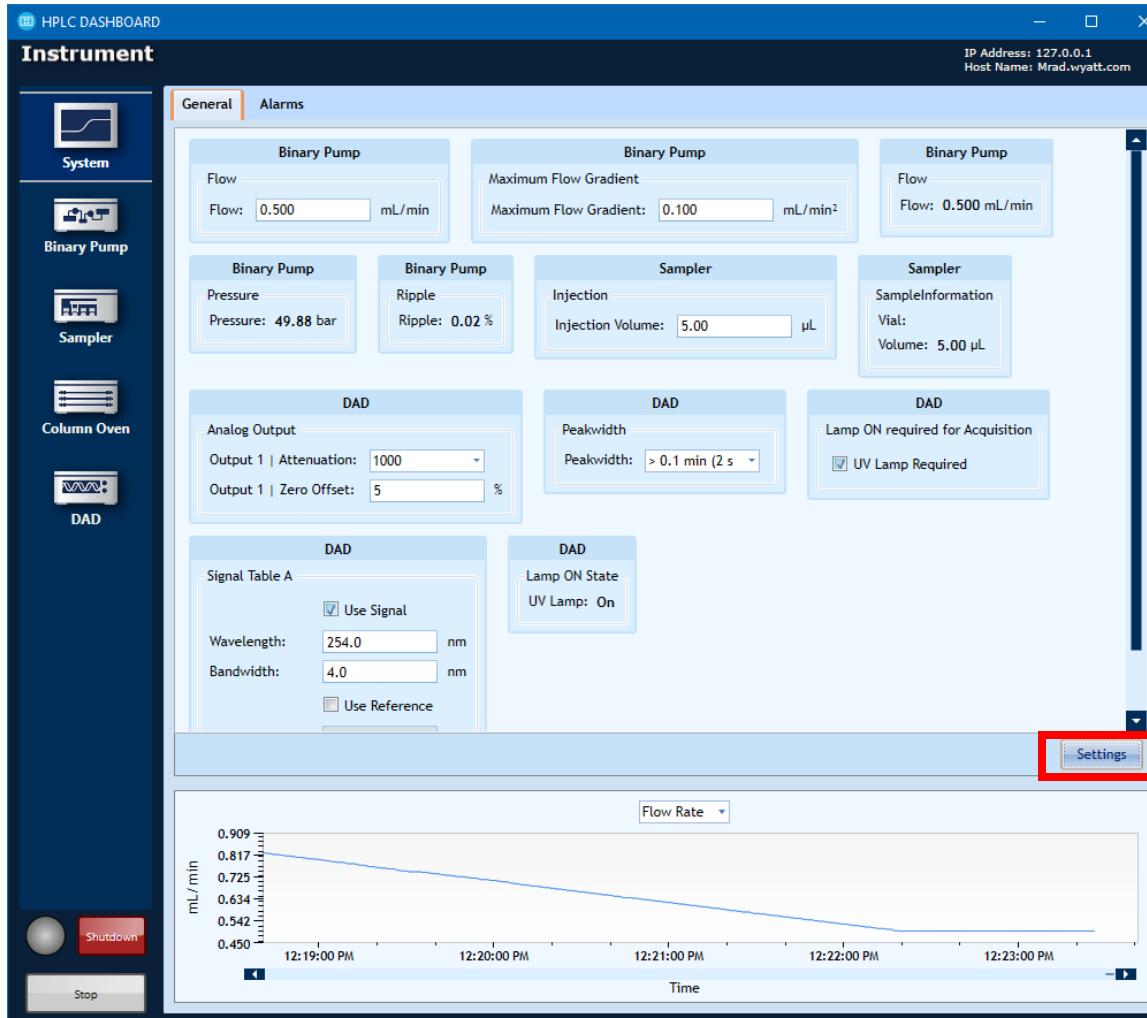
See the Microsoft Windows documentation for information about starting a service.

## Using HPLC DASHBOARD



The Wyatt HPLC DASHBOARD is an HPLC controller that allows you to change HPLC system parameters such as the pump flow rate, UV wavelength, and pump flow gradient.

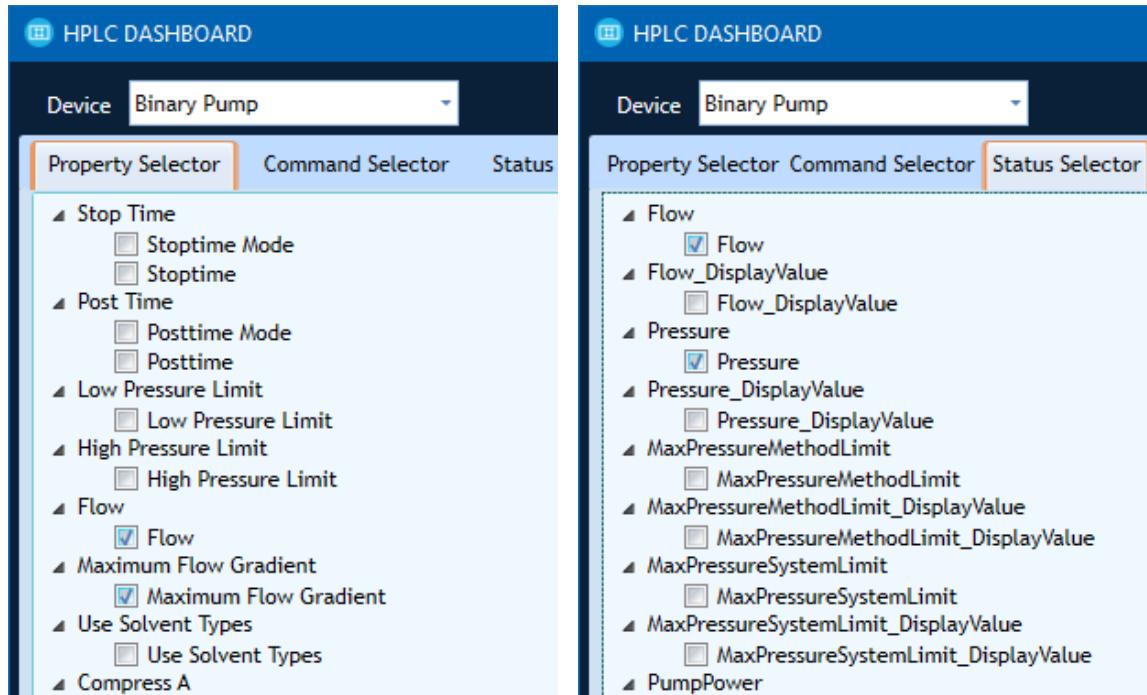
In the General tab, the HPLC system's status can be monitored in real-time. Parameters such as pump pressure and pump ripple can be viewed in the General tab parameters and graph.



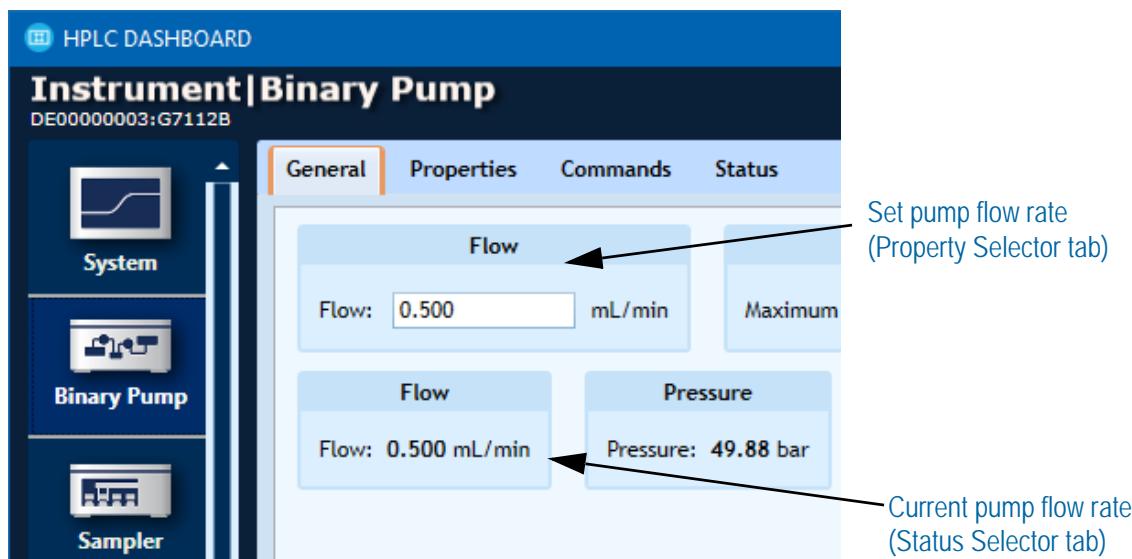
You can customize the view for the System and each of the HPLC modules. To customize the fields displayed, click the **Settings** button in the right side of the window below the parameters.

For each settable parameter, such as flow rate, there are two fields:

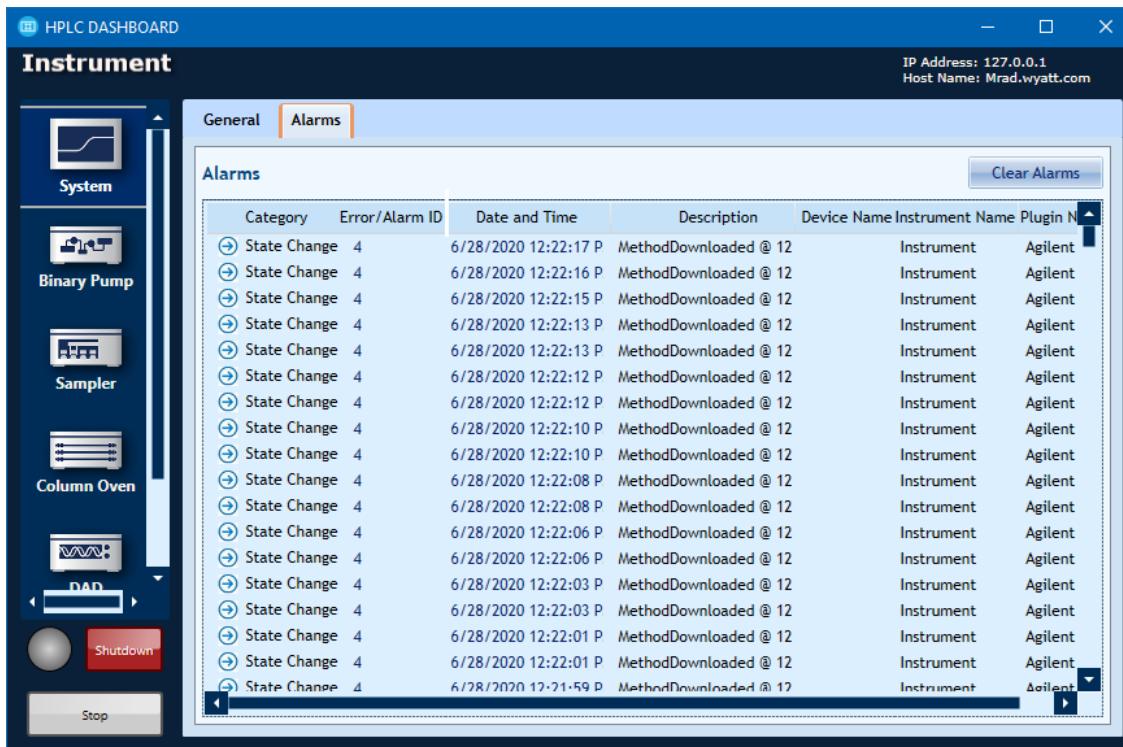
- **Property Selector:** This field displays the value that has been set. For example, the desired flow rate.
- **Status Selector tab:** This field displays the current value on the instrument. For example, the actual current flow rate.



For example, the following figure shows the two items for **Flow**: one field is the current set value and the other is the status.



The **Alarms** tab displays any HPLC alarm notices—such as a system leak—and details such as the Date, Time, and Description. You can clear all alarms by clicking the **Clear Alarms** button in the top right corner.



To shut down the HPLC system, click the **Shutdown** button on the bottom left corner of the HPLC DASHBOARD window. The **Shutdown** button sets the flow rate to 0 mL/min.

**Note:** The Wyatt HPLC DASHBOARD can run simultaneously with ASTRA, but cannot run simultaneously with ChemStation. The Wyatt HPLC DASHBOARD cannot run single or sequence injections. ASTRA with HPLC CONNECT added to a method can be used to run single or multiple injections. See [Configuring Instrument Profiles for Use with HPLC](#) on page 177 for details.

# 7

## Creating & Running Experiments

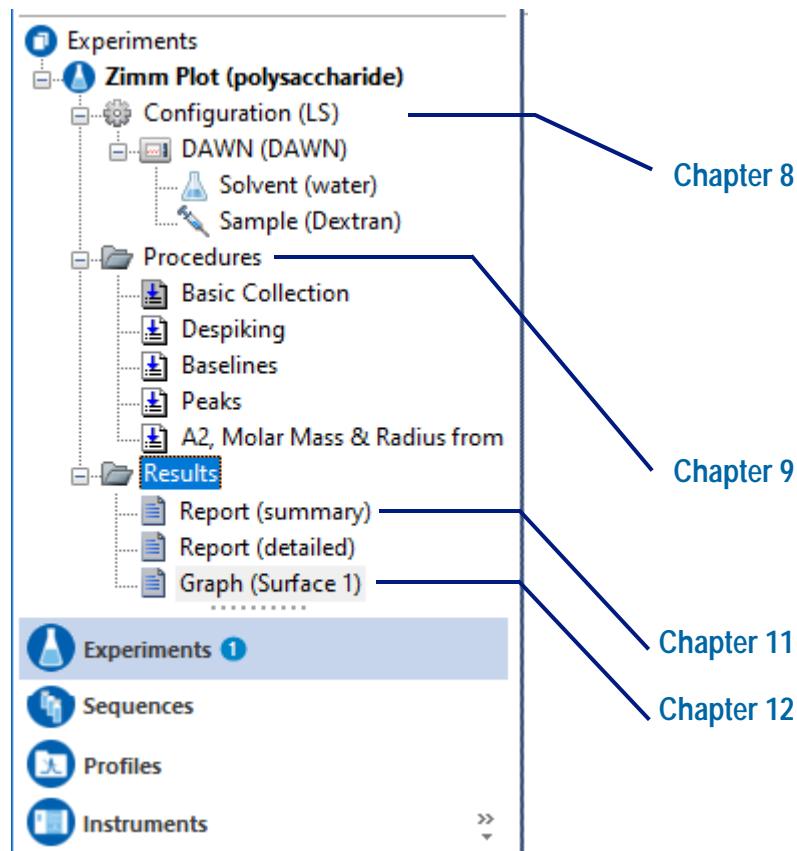
This chapter tells how to work with ASTRA experiments. The details of items contained in an experiment are covered in other chapters. This chapter describes actions you perform with the entire experiment, such as creating a new one, saving it, running it, or exporting it.

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## 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.

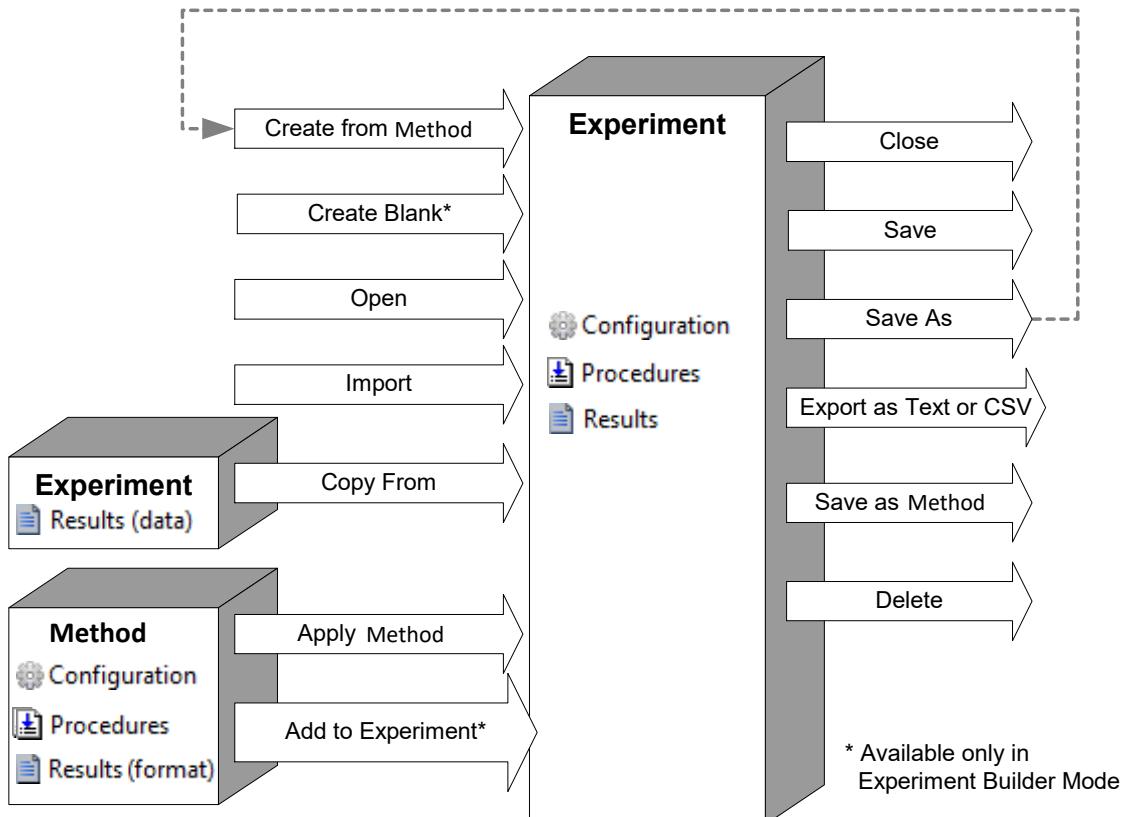


You can expand or collapse the three sections of an experiment as desired. Each experiment contains the following sections:

- **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 8, Configuring Experiments](#).
- **Procedures:** The actions to be performed in order when the experiment is run. There are collection, transformation, analysis, and administrative procedures. For details on all types of procedures, see [Chapter 9, Editing Procedures](#).

- **Results:** The reports and graphs to be produced after the experiment procedure has been run. For details, see [Chapter 11, Working with Reports](#) and [Chapter 12, 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 Basic or a version that uses an experiment database (ASTRA with Security Pack).

Table 7-1: 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 97
Create From Method	Make a new experiment based on a configuration, procedure, and results method.	page 97
Create Blank	Make a new, empty experiment with no predefined configuration, procedure, or results.	page 98

Table 7-1: Actions to Perform on Experiments

Action	Description	See
Open	Basic: Open an experiment from a file. This may include experiments saved with previous versions of Wyatt software. Security: Open an experiment from the experiment database.	page 108 and page 108
Import	Security: Open an experiment saved in a folder. This may include experiments saved with previous versions of Wyatt software.	page 110
Export	Save an experiment to a file, or save experiment data to a tab-delimited or comma-separated values file.	page 107
Close	Close the current experiment.	page 122
Save	Basic: Save an experiment to a file. Security: Save an experiment to the experiment database.	page 106 and page 105
Save As	Basic: Save an experiment to a file with a different name. Security: Save experiment to the database with different name.	page 106 and page 105
Export as Text or CSV	Create a text or comma-separated file containing experiment data.	page 107
Save As Method	Save the configuration, procedure, and results formats so that they can be used as the basis for future experiments.	page 117
Delete	Delete the experiment from the experiment database.	page 114
Run	Run the experiment procedure.	page 99
Run Indefinitely	Run the experiment procedure ignoring the Duration.	page 99
Copy From	Copy results data from one experiment to another.	page 116
Apply Method	Create a copy of the experiment with the analysis procedures and result presentation from the selected method.	page 120
Add to Experiment	Add items to the configuration, procedure, or reports. (Experiment Builder mode only.)	page 122
Editing Configuration	Add, remove, or change items in the experiment configuration.	page 127

## Creating New Experiments

The recommended way to create an experiment is from a method generated by the Method Builder wizard (see page 58). Experiments can also be created using the System methods described below. Experiment Builders may choose to create and build 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 118), 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 **Run Default** button. The experiment is created and begins data collection automatically. See page 118 for information on setting 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.



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+M.

Click the down-arrow next to the **File New** icon in the standard toolbar.

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.
  - **User folders:** You may have saved your own methods in a folder you created, such as **My Methods**. See [Creating a Method on page 117](#).

If you read about an experiment method you want to use, but don't see it in the New from Existing dialog, see [Importing a System Database on page 30](#) 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 is automatically set to match instruments of the appropriate type that are in your instrument list. However, note that the configuration might not match the desired configuration. For example, ASTRA defaults to a DAWN for a MALS instrument and includes a UV detector for online methods. If you need to change the configuration, refer to [Replacing an Experiment Configuration or Item on page 185](#).

## 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 choose **File→New→Blank Experiment**.

**Shortcuts:** Use any of the following shortcuts:

Press Ctrl+Shift+B.

Click the down-arrow next to the icon.

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

## 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

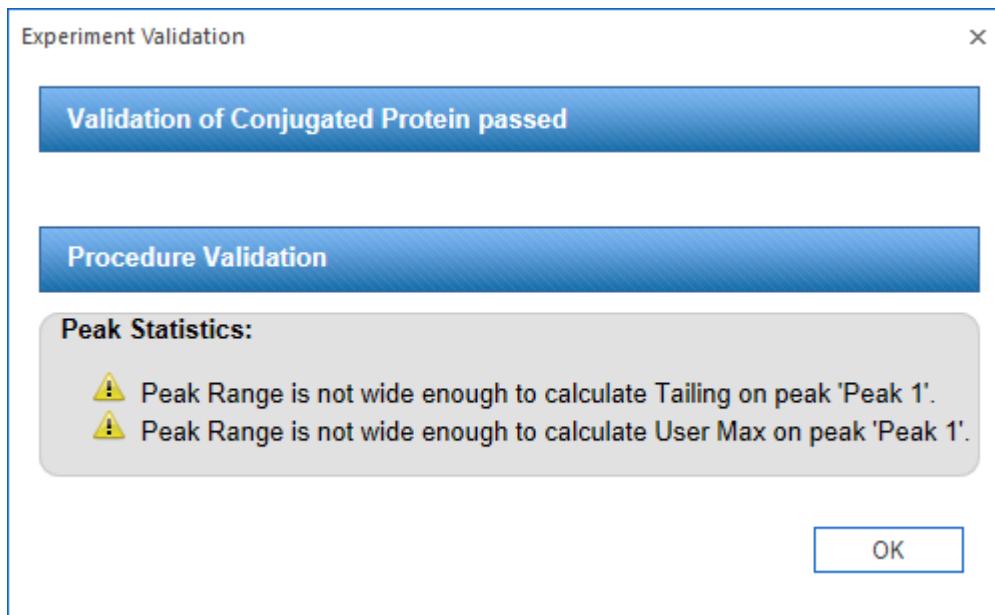
You can validate an experiment's procedure list and configuration by choosing **Processing→Validate**.

**Shortcuts:** Press Ctrl+Shift+V.

Validation makes a number of checks, including:

- Checks the procedure order for conflicts.
- If the experiment collects data, validation also checks that the necessary instruments are connected and available.
- Checks to make sure light scattering instruments are normalized.
- Checks to make sure the experiment configuration contains a solvent and a sample.
- 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.
- Checks the dn/dc to make sure it is in the range of -0.3 to 0.3.
- Checks for calibration constants greater than 1.0 or less than 1.0e-8.
- Checks for UV extinction coefficients that are negative or greater than 100.
- Checks for concentrations that are negative or greater than 1.0 g/mL.
- If the “100% Mass Recovery” feature is used, checks for a supplied injected mass in the peak.
- Checks for injected masses that are negative or greater than 1.0 g.
- Checks the collection script if you write a custom script.

The results of the experiment validation are shown in an Experiment Validation dialog. For example:



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 [Changing the Procedure Order on page 196](#) or revise the experiment configuration to include the appropriate instruments.

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 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 instruments.
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, you will be prompted to click **OK** and then inject the sample.
4. The spinning bar icon on the experiment node in the workspace shows that the experiment is running. The data collected is displayed in real time. The checkboxes next to the graph legend on the right of the window can be used to turn on or off data traces from various sources. Light scattering data (for the detector at the 90 degree angle) is red, RI data is blue, UV data is green, DLS data is magenta, and viscosity data is black. A green vertical line marker is displayed to indicate any autoinjection signals.
5. Once data collection is complete, 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.

You can quickly create and start running an experiment using the default method by choosing **Processing→Run Default** or clicking the **Run Default** button. The experiment is created and begins data collection automatically. See page 118 for information on creating default methods.

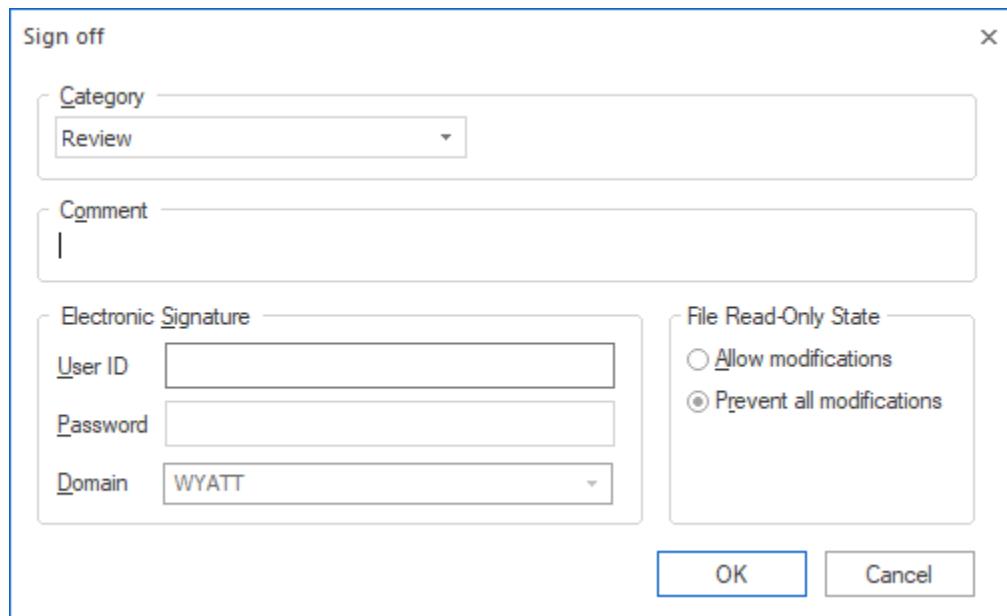
## Signing Off on an Experiment



If you are using ASTRA with Security Pack, you can sign off to indicate the status of the experiment and who verified that status. This sign off meets 21 CFR Part 11 standards for electronic records and electronic signatures.

To sign off, follow these steps:

1. Choose **Experiment→Sign Off** from the menus.

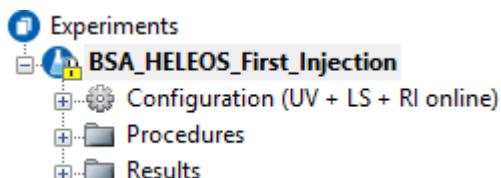


2. In the Sign off dialog, choose a sign off **Category** from the list. The categories are as follows:

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 **Comment** 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 56](#). 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**.

6. A user with ASTRA Administrator or ASTRA Researcher privileges can choose to **Prevent all modifications** to an experiment file after signing. Once an experiment is “locked” a lock icon  appears next to the experiment name when open in ASTRA or browsing in the database.



No other modifications to the experiment can be made except for the addition of signatures by users with ASTRA Technician, ASTRA Researcher, or ASTRA Administrator privilege. ASTRA Researchers and ASTRA Administrators can unlock an experiment by re-signing the experiment and choosing “allow modifications.”

Electronic signatures can be executed for any data collected in ASTRA. The electronic signature is saved in the associated log and is shown 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.

## Pausing an Experiment

You may temporarily pause data collection by choosing **Processing→Pause**. Choosing **Pause** again resumes data collection. This option is useful for batch experiments between sample injections.

## 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 51](#) 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 two crash recovery files in your public documents folder. This is usually

C:\Users\Public\Documents\ASTRA 8 Recovered Files. The files have an extension of \*.afr8. For each data file being collected, there are two files:

- *date\_time\_experimentFilename\_data.afr8* contains the slice data for the experiment. For example,  
08232019\_161446935\_Experiment1\_data.afr8.
- *date\_time\_experimentFilename.afr8* contains the configuration information about the experiment. For example,  
08232019\_161446935\_Experiment1.afr8.

Both files are required for successful crash recovery. When data collection is complete and data is successfully saved, these files are 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 files are retained.

Each time ASTRA starts, it checks for crash recovery files in the public document's location. If an .afr8 file set is present, ASTRA combines the files into an ASTRA experiment file (\*.afe8) and asks if you want to see the recovered data file.

## Saving an Experiment to a File

**Basic**

It is a good idea to save experiments frequently.

If you are using ASTRA Basic, experiments are stored in files with an extension of \*.afe8.

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 \*.afe8. You can choose an older version of ASTRA if you like.

**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



If you use ASTRA with Security Pack, experiments are saved in the ASTRA database. To save experiments in separate files, see [Exporting an Experiment on page 107](#).

It is a good idea to save experiments frequently.

---

**Note:** 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.

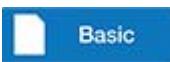
Right-click the experiment name in the tree and choose **Save**.

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 117](#).
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

**Basic**

If you are using ASTRA Basic, this command allows you to export experiments as tab-delimited and comma-delimited files. To save an experiment to a different \*.afe8 file in the current ASTRA format, use the Save As command instead.

**Security**

You must have at least Researcher access to export an experiment. The export command allows you to export experiments to tab-delimited and comma-delimited files. In addition, an experiment can be saved under a different name and in a folder other than the experiment database.

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.
3. In the File Name field, type a file name for the experiment.
4. In the Save As Type field, select a type. These formats are available:

Type	Description
.afe8	ASTRA 8 file that can be imported by ASTRA 8 on this or another computer. (Use Save As instead for ASTRA 8 Basic.)
.afe7	ASTRA 7 file that can be imported by ASTRA 8 on this or another computer.
.afe6	ASTRA 6 file that can be imported by ASTRA 8 on this or another computer.
.vaf	ASTRA V file. Choose an ASTRA version for compatibility with earlier versions.
.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. Both interpolated and non-interpolated (instrument) formats are supported. The interpolated format puts all measurements on the same time scale, allowing for easy display in software such as Excel.
.csv	Comma-delimited text file for exporting a data set defined by the selected data set definition. This format is easily imported into most spreadsheets. Both interpolated and non-interpolated (instrument) formats are supported. The interpolated format puts all measurements 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 344](#) for information about data set definitions.

6. Click **Save**.

If the organization of the tab-delimited or comma-delimited output is not useful, try the output described in [Exporting Data on page 357](#).

## Opening an Experiment from a File


**Basic**

You can open and work with any experiment you have saved with ASTRA V, ASTRA 6, ASTRA 7, or ASTRA 8. If you are using ASTRA Basic, experiments are stored in separate files with an extension of \*.afe8.

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
.afe8	Experiment file saved or exported by ASTRA 8.
.afe7	Experiment file saved or exported by ASTRA 7.
.afe6	Experiment file saved or exported by ASTRA 6.
.vaf	Experiment file saved or exported by ASTRA V.
.afr8	ASTRA 8 crash recovery file. (See page 104.)
.afr7	ASTRA 7 crash recovery file. (See page 104.)
.afr6	ASTRA 6 crash recovery file. (See page 104.)
.vrf	ASTRA V crash recovery file. (See page 104.)

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

## Opening an Experiment from the Database


**Security**

If you are using ASTRA 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 earlier ASTRA versions), see [Importing an Experiment from a File on page 110](#).

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

**Note:**

There are no access level restrictions on opening an experiment.

To open an experiment, follow these steps:

**1. Choose File→Open→Experiment.**

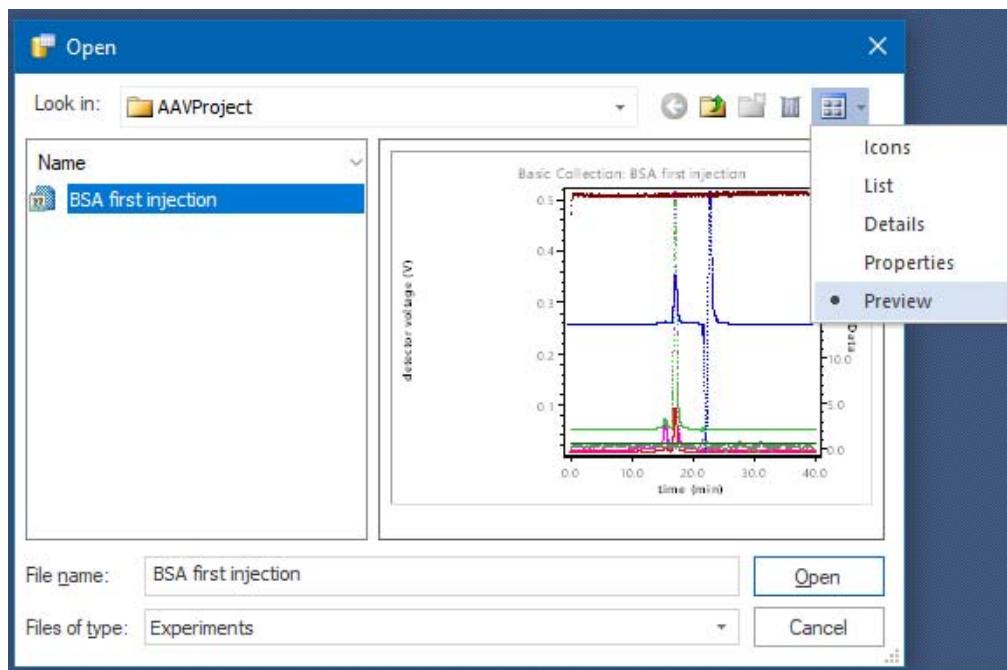
**Shortcuts:** Press Ctrl+O.

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. To see information about an experiment, select **Properties** in the view type selector; see page 115 for more about experiment properties. If data has been collected for the experiment, you can see the Collection View for that experiment by selecting **Preview** in the view type selector.**



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

**3. 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.



If you are using ASTRA with Security Pack, the results that were computed when you last saved the file are compared to the results computed when you open the file. If there are any changes—for example, due to an ASTRA software update—you will be notified about the change.

## Importing an Experiment from a File



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



If you are using ASTRA with Security Pack, you can import experiments stored in folders other than the experiment database. This includes experiments saved with ASTRA V, ASTRA 6, ASTRA 7, and ASTRA 8. It also includes exported experiments.

**Note:** You must have at least Technician 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 from Windows Explorer to ASTRA.

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
.afe8	Experiment file saved or exported by ASTRA 8.
.afe7	Experiment file saved or exported by ASTRA 7.
.afe6	Experiment file saved or exported by ASTRA 6.
*.vaf	Experiment file saved or exported by ASTRA V.
.afs8	ASTRA 8 sequence file.
.afs7	ASTRA 7 sequence file.
.afs6	ASTRA 6 sequence file.
.vsf	ASTRA V sequence file.
.afr8	ASTRA 8 crash recovery file. (See page 104.)
.afr7	ASTRA 7 crash recovery file. (See page 104.)
.afr6	ASTRA 6 crash recovery file. (See page 104.)
.vrf	ASTRA V crash recovery file. (See page 104.)

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

## Importing Multiple Data Files



This item is disabled in ASTRA Basic since there is no experiment database. See page 108.



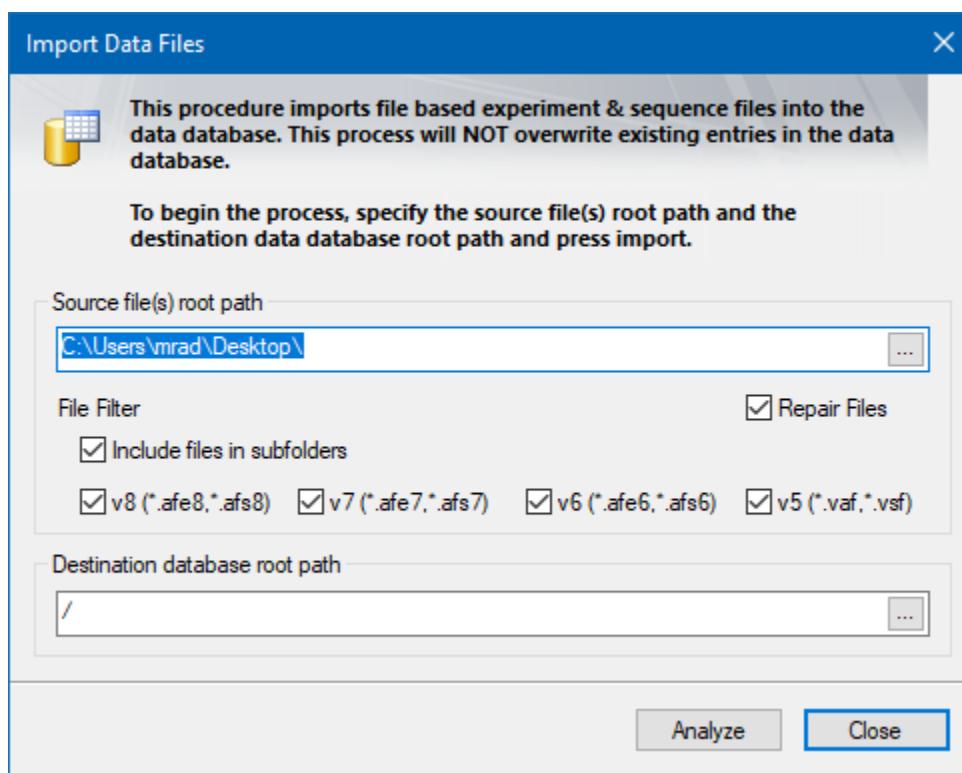
If you are using ASTRA with Security Pack, you can import multiple experiments stored in data files at once. This includes experiments saved or exported with ASTRA V, ASTRA 6, ASTRA 7, and ASTRA 8.

**Note:**

ASTRA Technicians and ASTRA Researchers can import single data files. However, you must be an ASTRA Administrator to bulk import experiments into the database.

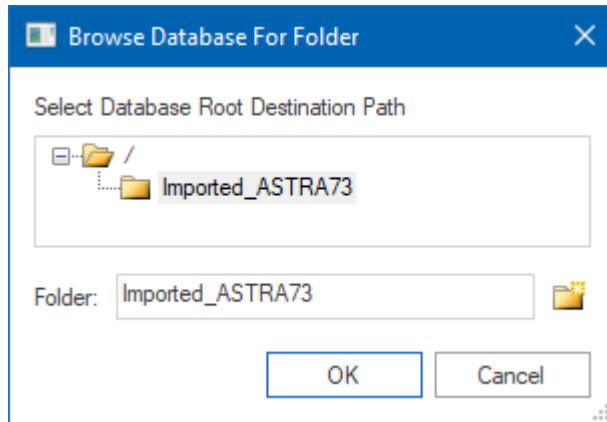
Follow these steps:

1. Choose **System**→**Database Administration**→**Import Data Files**.



2. In the **Source file(s) root path** field, browse (by clicking the ... button) for the folder that contains the data files you want to import.
3. Uncheck the **Include files in subfolders** box if you only want to import data files in the specific folder you selected.
4. Uncheck any boxes for ASTRA versions whose file types you do not want to import. The file extensions used by the various versions of ASTRA are shown.

5. In the **Destination database root path** field, you can browse the database (by clicking the ... button) for the folder where you want to store the imported files. To create a folder, select a location to contain the folder, type a **Folder** name, and click the folder icon.



6. Click **Analyze** in the Import Data Files dialog to search for source files. You will see a status message about how many files and folders were found.
7. Click **Import** to import those files into the experiment database. The import process may take several minutes, especially if there are many data files or if the files are large.

## Importing from Another Experiment Database



This item is disabled in ASTRA Basic since there is no experiment database. See page 108.



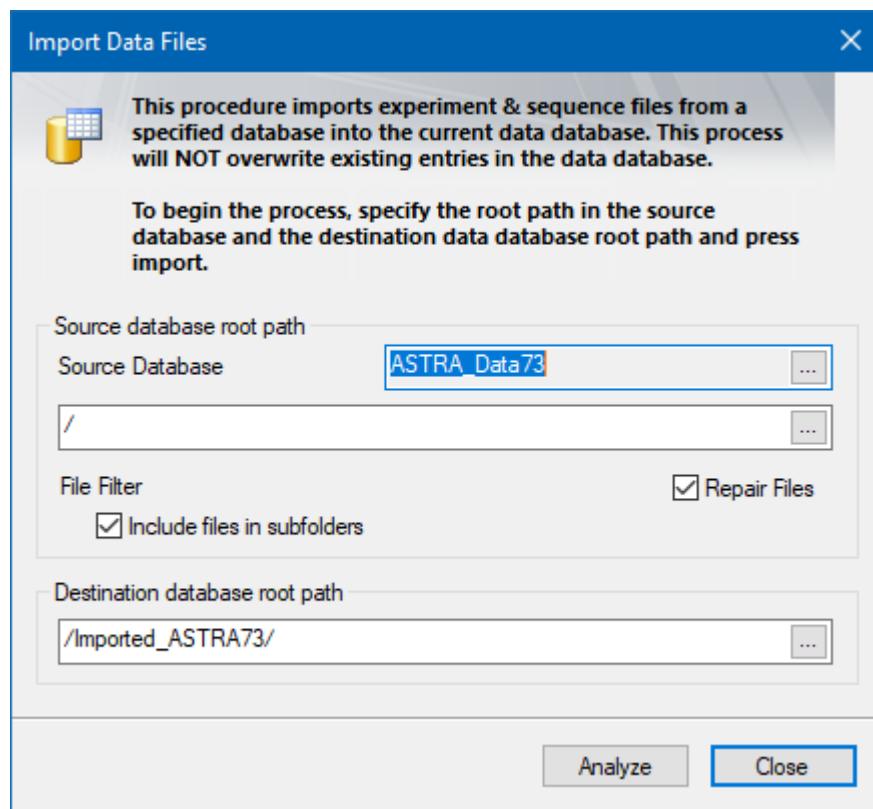
If you are using ASTRA with Security Pack, you can import the contents of ASTRA V, ASTRA 6, and ASTRA 7 experiment databases into your current ASTRA 8 database.

**Note:**

You must be an ASTRA Administrator to import experiments from another database.

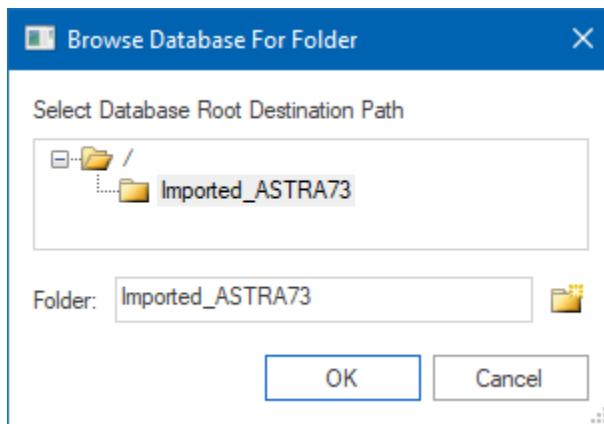
Follow these steps:

1. Choose **System**→**Database Administration**→**Import From Database**.



2. For the **Source database root path**, click the ... button to browse for the ODBC data source that contains the old experiment database. The storage path for the selected database is shown below this field.
3. If you select a Microsoft Access database, you may be prompted to browse for the file in which the database is stored.
4. Click the ... button in the Source Database area to browse for the folder in the database from which you want to import experiments.
5. You can uncheck the **Include files in subfolders** box if you do not want to import experiments in database subfolders.

6. Click the ... button for the **Destination database root path** field to browse the database for the folder where you want to store the imported experiments. To create a folder, select a location to contain the folder, type a **Folder** name, and click the folder icon.



7. Click **Analyze** in the Import Data Files dialog to search for source files. You will see a status message about how many files and folders were found.
8. Click **Import** to import those files into the experiment database. The import process may take several minutes, especially if there are many experiments or if the experiments are large.

---

## Deleting an Experiment



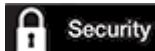
If you are using ASTRA with Security Pack, experiments can only be deleted by an ASTRA administrator. See [Deleting Experiments on page 79](#) for details.



If you are using ASTRA Basic, any user may delete an experiment by deleting the \*.afe8 file that contains the experiment.

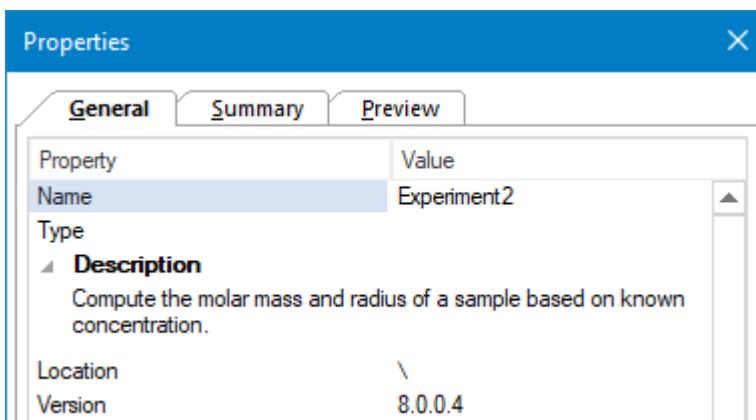
## Viewing Experiment Properties

You can view and change information about the current experiment by choosing **File→Properties**. Similar information is available for profiles and sequences.

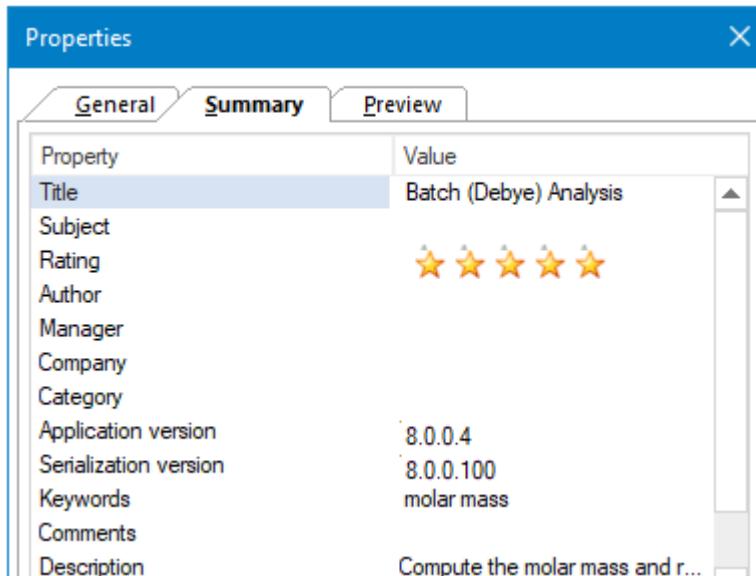


You can also see experiment properties when you choose **File→Open** or **File→Save As** when using ASTRA with Security Pack.

The **General** tab of the Properties dialog shows information like the location, version of ASTRA used, and creation and access dates for the experiment. You can change the **Description** in the Experiment Configuration (page 147). The other information in the General tab is set automatically.



The **Summary** tab lets you enter information about the experiment, such as a title, subject, 1-to-5 star rating, author, manager, company, category keywords, comments, and description.



The **Preview** tab shows a Collection View for the experiment if data has already been collected.

## Copying Data

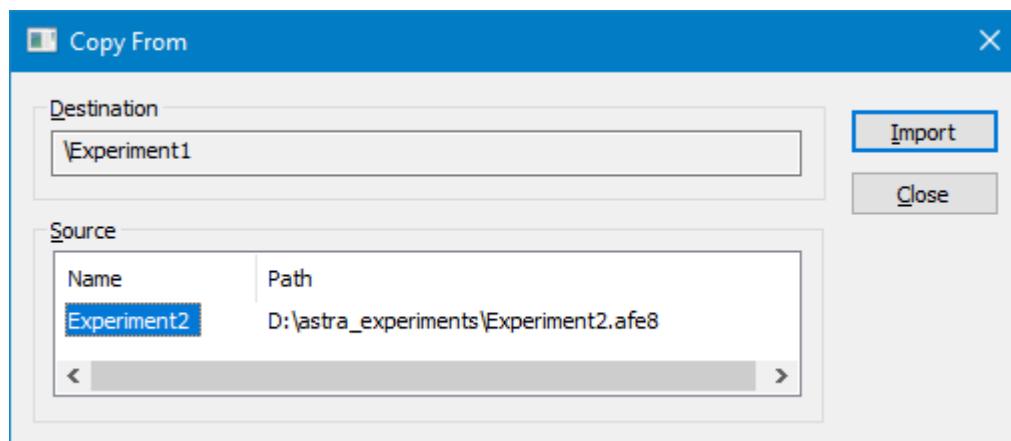
You can copy data generated for one experiment into another experiment. You might do this to create combined plots of results from several experiments, or to use the data in a procedure such as branching.

The copied data is available in the Data for Custom Plot window and for importing. Copied data will not appear in the Basic Collection procedure.

Note that the procedure described below is rarely needed. ASTRA provides buttons for importing data in various procedure pages for which it is common to want to combine data. For example, see the **Import Linear** button described in [Branching Procedure on page 283](#).

To copy data into an experiment, follow these steps:

1. Open both source and destination experiments. The source experiment contains data you want to copy. You will copy 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 344](#) for details on 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 destination experiment. The data set definition allows you to display the data in graphs. In addition, the data is available for procedures such as branching.

## Creating a Method

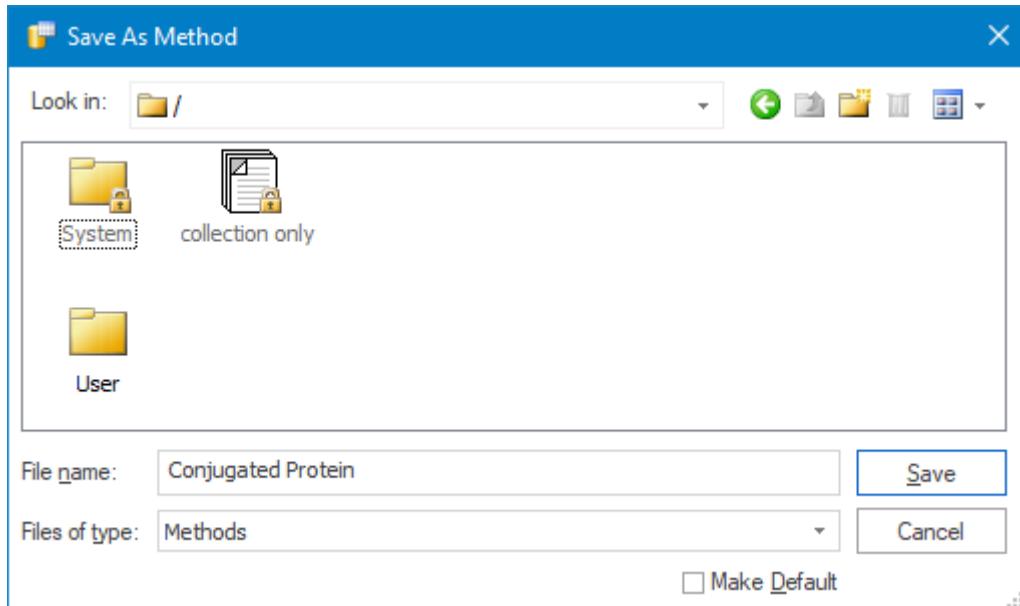
After you modify an experiment, you may want to save it as a method for other experiments. The saved method includes the configuration, procedure, and result formats.



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**.

---

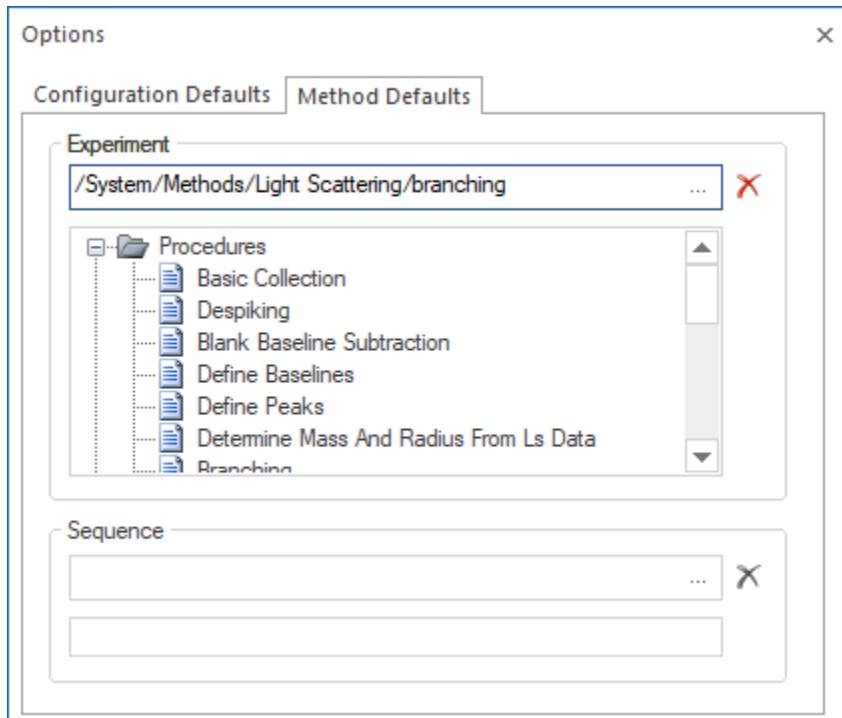
**Note:** Methods are saved in the system database. This database is separate from the experiment database.

## 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 Method Builder Wizard opens and you can use it to define your default method. See [Using the Method Builder Wizard on page 129](#) for details.

You can also specify the default experiment method as follows:

1. Choose **System→ Preferences→Options**.
2. Select the **Method Defaults** tab. The **Experiment** area shows the path to the current default method, if one is selected.



3. To change the default method, click the “...” button and browse for the method you want to use as the default. The area below shows the procedures and configuration in the selected method.
4. If you choose **File→New→Experiment from Default**, the default method is 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.
5. To remove a default experiment or sequence, click the “x” button next to the “...” button.

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 316](#) for information about setting a default sequence and [Setting a Default Configuration on page 128](#) for information about setting configuration defaults.

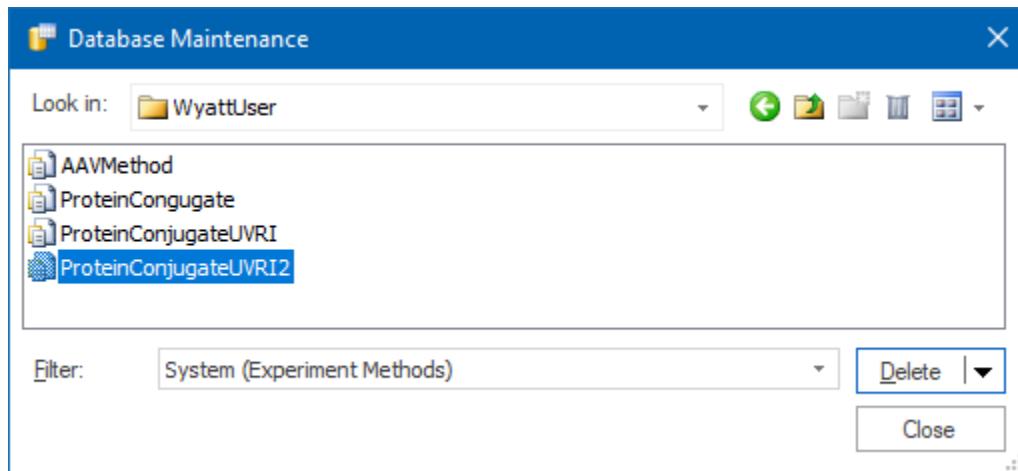
## Deleting a Method



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. Choose System (Experiment Methods) from the **Filter** drop-down list to see only system methods. Navigate to the appropriate folder. Methods are generally stored under “Method Builder” in folders with names specific to users or projects. 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.

## 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 121](#).)

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.

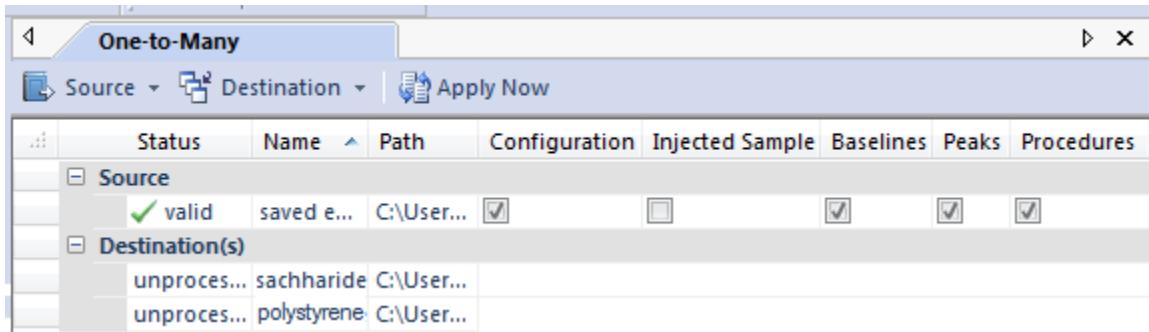
To apply a method, follow these steps:

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.

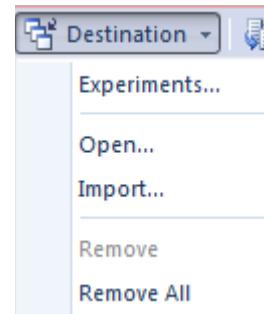
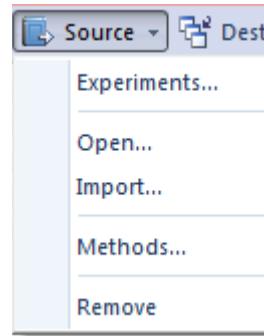
## Applying a Method to Multiple Experiments

The One-to-Many feature allows you to apply a method (or some aspect of a method) to multiple experiments. This feature is useful for applying a parameter set for a standard sample such as BSA to sample files.

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



3. Click the **Source** button at the top of the One-to-Many area. Then select a method or experiment as the source of the method you want to apply to other experiments. If you choose **Experiments**, you can select from the currently open experiments. If you choose **Methods**, you can browse the system database for a saved method. You can also choose **Open** to open an experiment, or in Security Pack mode you can use **Import** to open an experiment stored in a folder other than the experiment database.
4. Click the **Destination** button and select one or more experiments to which you want to apply the source method. Choose **Experiments** to choose currently open experiments. You can also choose **Open** to open an experiment, or when in Security Pack mode you can use **Import** to open an experiment stored in a folder that is not the experiment database.
5. If you want to remove experiments from the destination list, click the **Destination** 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.
7. Click the **Apply Now** button at the top of the One-to-Many area. The progress column will show which experiment is being processed.



## Adding Elements to an Experiment



In most cases, you can add items to an experiment only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

To add instruments, connections, and other items to an experiment, see [Adding Instruments and Connections on page 184](#).

To add a procedure to an experiment, see [Advanced Procedure Editing on page 195](#).

To add a data set definition to an experiment, see [Creating Data Set Definitions on page 344](#).

To add a report to an experiment, see [Adding a Report on page 335](#).

To add a graph to an experiment, see [Creating Custom Plots on page 347](#).



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

---

## 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.

# 8

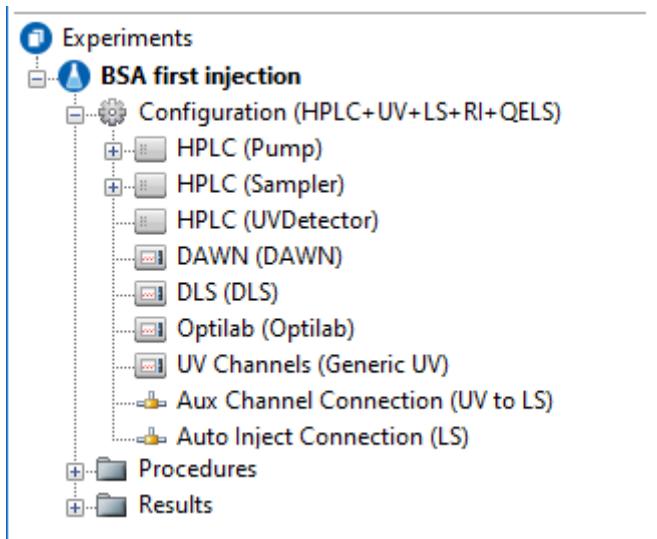
## Configuring Experiments

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 8 configurations and profiles.

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## About Configurations and Profiles

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 configurations provided with ASTRA include most instruments and connections you use in a typical experiment.



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 8 breaks up the different parts of the experiment into logical units called *profiles*.

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.

The *configuration* of an experiment is defined by the *profiles* in it. In addition to profiles in configurations, free-standing profiles can be created which can be copied into experiments as needed.

This chapter focuses on using profiles in configurations. Experiment methods provided with ASTRA contain commonly used configurations. However, as you gain more experience using ASTRA, you may want to use more or other profiles with your experiments. See [Chapter 13, 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, close and reopen it after enabling Experiment Builder mode.



You need at least Researcher access to modify 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 here.

Experiment1: DAWN (DAWN (NEON))	
	Value
Name	DAWN (NEON) ...
Description	
Physical Instrument	None
Sample Cell	Fused Silica
Wavelength (nm)	658.0
Calibration Constant (1/(V cm))	1.0000e+00
▷ Normalization Coefficients	Import
Comet Cell Cleaner	<input checked="" type="checkbox"/>
Divide by Laser Monitor	Laser Monitor
Polarization Analyzer	<input type="checkbox"/>
▷ DLS	
▷ Band Broadening	
▷ Temperature Control	
▷ Auxiliary Channel Gains	

## Configuration Example

A configuration for an online light-scattering experiment is set up as shown in Figure 8-1. The same experiment is shown in the schematic of Figure 8-2. Comparing these two figures highlights the logical structure of the configuration and its constituent profile elements in the workspace.

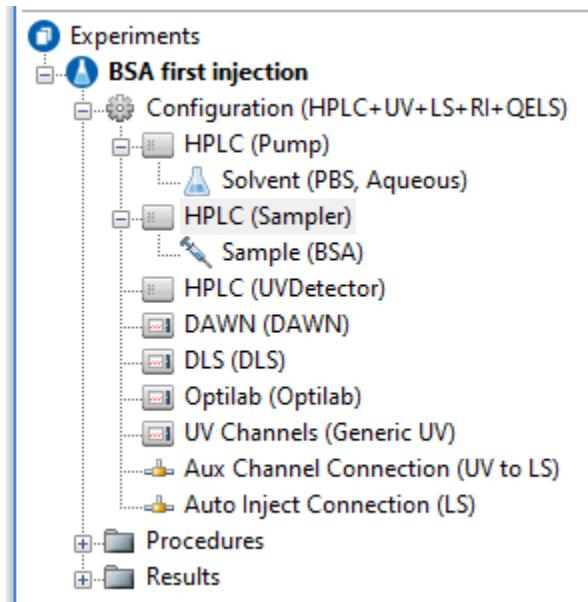


Figure 8-1: Configuration for an LS Online Experiment

The hardware setup for the configuration in Figure 8-1 would be organized similar to Figure 8-2:

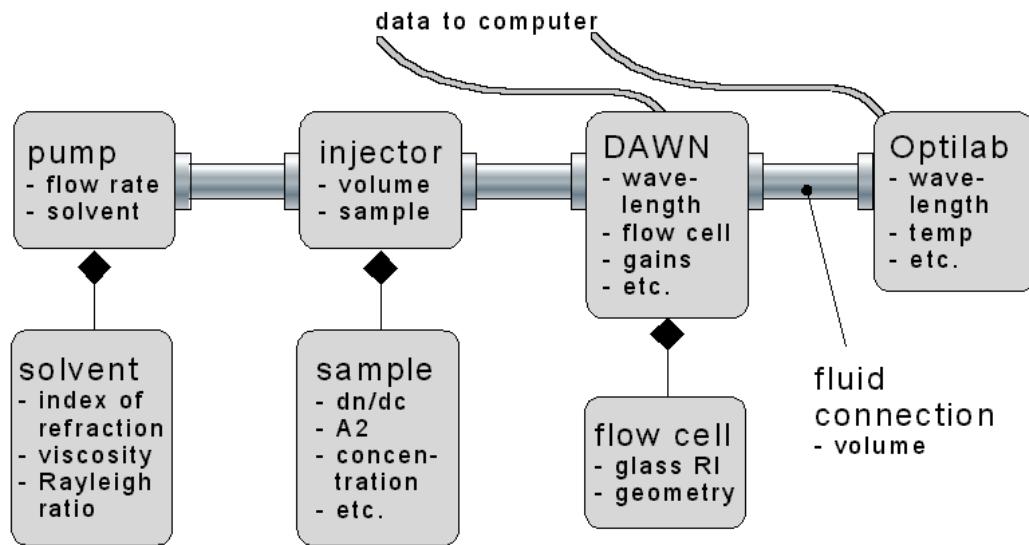


Figure 8-2: Hardware Connections for LS Online Experiment Setup

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**Note:** See page 177 for information about configuring an experiment that uses ASTRA to control HPLC instruments.

---

## Using Configurations

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

---



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.

---

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 8-1 for an example.  
You can expand or hide lists of related properties if there is a + or - sign next to a property.
2. Set properties by typing, selecting from a list, or checking a box.
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.

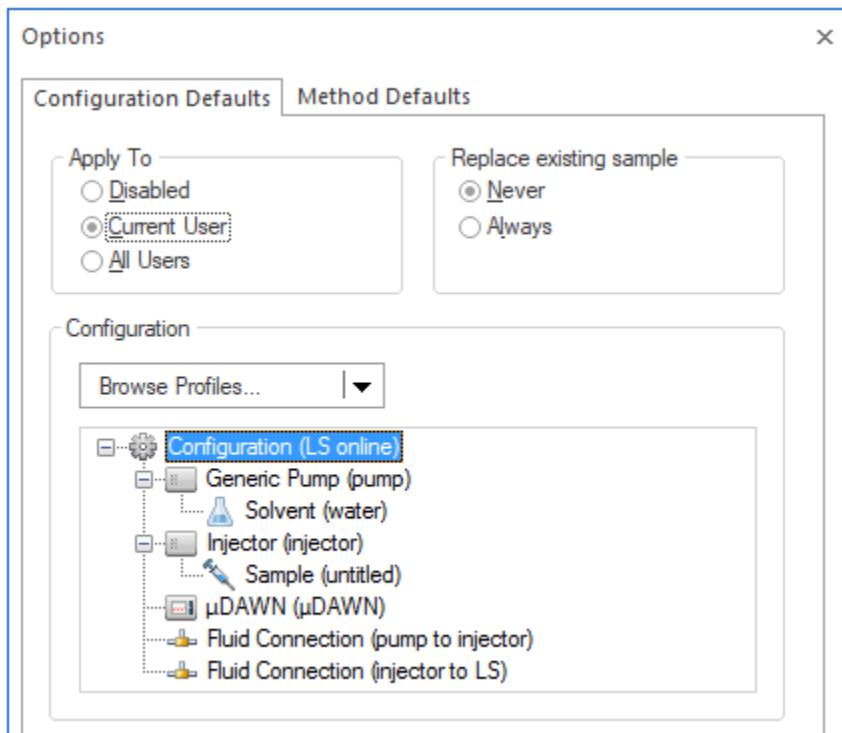
5. Click **Apply** or **OK** to make the changes.

For more advanced ways of editing configurations, see [Advanced Configuration Editing on page 184](#).

## 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.

To set the default configuration for new experiments, choose **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 described in [Creating Profiles on page 361](#).

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 118](#) for information about setting method defaults and [Setting a Default Sequence on page 316](#) for information about setting a default sequence.

## Using the Method Builder Wizard

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

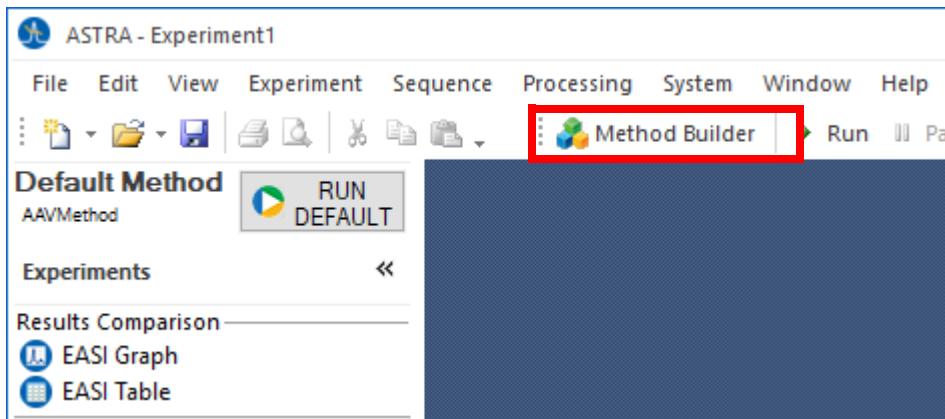
---

This section provides details about using the Method Builder Wizard. For a quick introduction, see [Performing a SEC-MALS Experiment on page 58](#).

The Method Builder Wizard guides you through the experiment setup process. This multi-paged wizard creates an experiment appropriate for collecting data from your instruments and devices, analyzing the collected data, and reporting the results. The wizard allows you to set up experiments for flow separation, batch analysis, or instrument calibration. Additionally, a method can be designated as the default method and run with one click from the main ASTRA window.

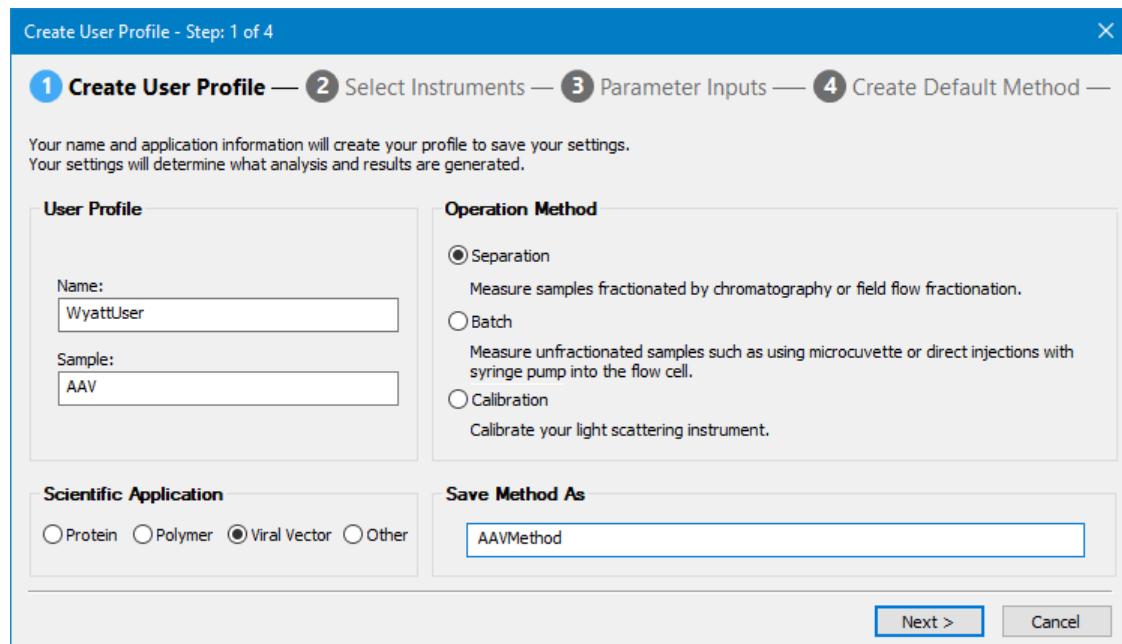
The Method Builder Wizard starts automatically when you first launch of ASTRA after the initial installation.

To manually launch the wizard, click the **Method Builder** button in the toolbar. You can also launch the Wizard by selecting **System→Method Builder** from the menus.



## Method Builder Details: Step 1 of 4

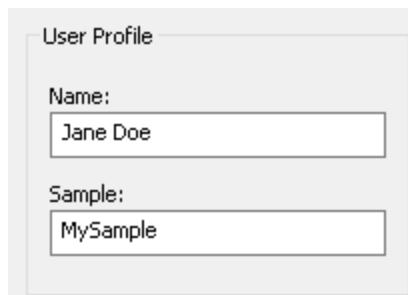
The first page of the wizard creates a user profile or links to an existing profile. The Scientific Application and Operation Method fields determine the analysis most likely required for this method.



Specify values for the fields in this step as follows:

- **Name:** (required) Type a name to identify the user who is creating the method or a project name. This name will be used to create a folder in the ASTRA system database, in which the method created by the wizard will be stored. You can reuse this name to store additional methods in the same folder of the ASTRA system database.
- **Sample:** (required) Type a name for the sample. This name will be used to create a subfolder under the Name folder.

The following example creates a “Jane Doe\MySample” folder in the ASTRA system database and stores the generated method in that folder:

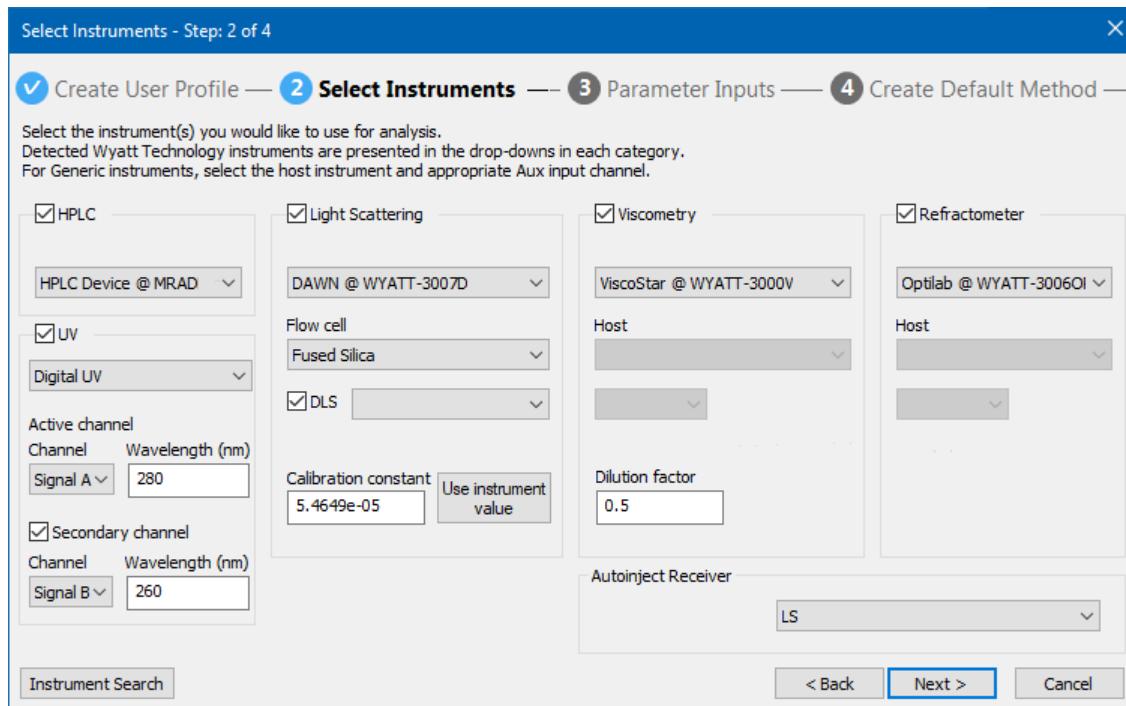


If you reuse a Name/Sample combination, the methods will be stored in the same folder of the ASTRA system database. This allows you to organize your methods for easier selection when running experiments.

- **Save Method As:** (required) Type a name for the method. This method name must be unique within the methods defined for the Name/Sample combination. Duplicate method names are not permitted in the same Sample folder.  
This name will be used as the default method name shown in the upper-left corner of the ASTRA workspace.
- **Scientific Application:** Select the application that best fits your sample. This choice determines which data analysis and reports to include in the method. The options are: Protein, Polymer, Viral Vector, and Other. Viral Vector will be grayed out if the license key for this procedure is not present.
- **Operation Method:** Select the method of operation to be used for this experiment.
  - **Separation:** Select this method for analyzing samples fractionated using SEC or FFF.
  - **Batch:** Select this method for unfractionated samples.
  - **Calibration:** Select this method to measure the calibration constant of a MALS instrument. For calibration methods, you can select only a MALS instrument in Step 2. Calibration methods are not set as the default method.

## Method Builder Details: Step 2 of 4

Step 2 of the wizard specifies the instruments used during data collection. An instrument search for available devices is performed when this page opens. Some instruments may not be available depending on the Operation Method you selected in Step 1.



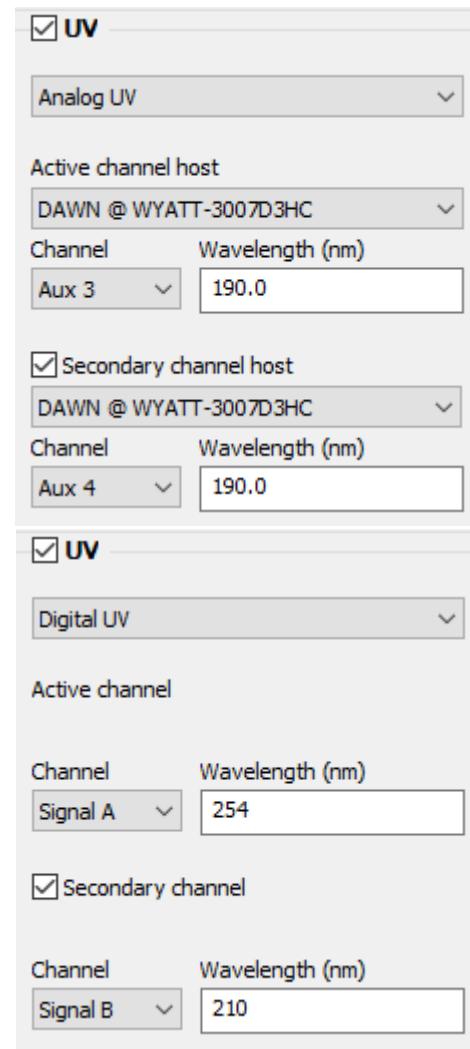
Check the boxes for the types of instruments to use in this method. If your instruments do not automatically appear, click the **Instrument Search** button in the lower left corner to find any connected instruments. Specify values for the fields in this step as follows:



- **HPLC:** From the drop-down list, select the Agilent HPLC Instrument if available. (See [Setting Up Wyatt HPLC CONNECT on page 82](#) for information about activating the Wyatt HPLC DASHBOARD.)

- **UV:** Check this box to use third-party ultraviolet detector(s) in line with Wyatt instruments. The incoming signal can be handled as either analog or digital input (depending on the limitations of your HPLC device). The Method Builder supports only one or two UV channels. To specify additional wavelengths to collect, add them to the **UV Channels** configuration in an experiment; then save the experiment as a method prior to collection.
  - **Analog UV:** (Default) Choose **Channel Host(s)** and auxiliary **Channel(s)** for the UV signal. To determine which auxiliary channel is receiving the signal, refer to the hardware manual of the corresponding Wyatt instrument. Enter the **Wavelengths** here for record-keeping purposes, and configure the wavelengths in the HPLC software.
  - **Digital UV:** If you have HPLC CONNECT, you can choose to import a digital signal by choosing **Digital UV** in the drop-down list. For a list of Agilent UV detectors supported for Digital UV, see the *ReadMe - HPLC CONNECT Supported Hardware (M1045).pdf* file, which is installed in the “Documents” folder of the HPLC CONNECT installation.

Select the **Channel(s)** that receive the UV signal and enter the **Wavelength(s)**. This tells the HPLC UV detector which digital signals to export to ASTRA.
- **Light Scattering:** Select the MALS instrument this method should use from the drop-down list. Only Wyatt light scattering instruments are detected.
- **Flow cell:** Select the type of flow cell used in the MALS instrument. Fused Silica is a typical flow cell type. F2 is a typical flow cell for high temperature applications. For microDAWN users, the Vertical flow



cell is typical. Scintillation vials and Microcuvettes are used when Batch mode is selected in Step 1 of the wizard. Note that DAWN (NEON) instruments do not support Scintillation vials.

- **DLS:** The box is automatically checked if a Wyatt dynamic light scattering module (WyattQELS) is detected inside the MALS instrument. DAWN (NEON) instruments automatically detect the location of the DLS fiber.

If you have a DAWN HELEOS® instrument, specify the position of the DLS detector with values from 1 to 18. The factory default is 12.

- **Calibration Constant:** (required) Type the calibration constant of the selected MALS instrument as found on the Certificate of Performance or as determined from a calibration measurement.

The **Use Instrument Value** button automatically fills in the last calibration constant stored on the instrument.

- **Viscometry:** Select your ViscoStar instrument from the drop-down list. If a third party viscometer is used, select Generic VI.
  - **Host:** Select the Wyatt instrument that receives the DP signal if you are using a Generic VI.
  - **AUX:** Select the auxiliary channel that receives the DP signal if you are using a Generic VI.
  - **Dilution factor:** Type the dilution factor for the instrument as listed on the Certificate of Performance or as measured.
- **Refractometer:** Select the Optilab instrument from the drop-down list. If a third party refractometer is used, select Generic RI.
  - **Host:** Select the Wyatt instrument that receives the RI signal if you are using a Generic RI.
  - **AUX:** Select the auxiliary channel that receives the RI signal if you are using a Generic RI.

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<b>Note:</b>	If you use multiple generic instruments (for example, generic UV and RI detectors), different Aux channels must be used for different instruments.
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- **Autoinjector Receiver:** Select the instrument that will receive an auto-injection signal from the HPLC sampler to trigger data collection to begin when a run is started. If you are using HPLC CONNECT, ASTRA will obtain the autoinject signal directly from the HPLC instrument. However, for optimal timing between the instruments, we recommend connecting an autoinject cable between the HPLC Sampler and a Wyatt detector (usually the MALS detector) and using a daisy chain to send the signal to any other Wyatt instruments. Choose the instrument with the autoinject cable connection as the Autoinject Receiver. (See [Hardware Connections for Autoinject Signals on page 68.](#))

## Method Builder Details: Step 3 of 4

Step 3 shows different fields depending on the Scientific Application, Operation Method, and instruments you selected on the prior pages.

**Sample Information**

Name:	AAV
Concentration (mg/mL)	0
Protein dn/dc (mL/g)	0.185
Nucleic acid dn/dc (mL/g)	0.17

**UV Extinction Coefficient (mL/(mg cm))**

Protein at 280 nm	Nucleic acid at 280 nm
0.000	0.000
Protein at 260 nm	Nucleic acid at 260 nm
0.000	0.000

**Expected Molar Mass (MDa)**

Protein	Nucleic acid
0.000	0.000

**Collection Parameters**

Solvent	HPLC injection volume (µL)
water	0
Duration (min)	HPLC Vial number
0	Vial 1
HPLC flow rate (mL/min)	
0	

**Data Processing**

Manual  
Manually define baselines and select peaks.

Automatic  
Automatically define baselines for each detector and select all peaks.  
You can manually adjust baseline and peak selections if needed.

The following list includes fields that may or may not be visible depending on your selections for Scientific Application, Operation Method, and instruments.

### Sample Information for Protein or Polymer Scientific Applications:

- **Is this a conjugate protein?** If you selected Protein as the Scientific Application in Step 1, indicate whether you are analyzing a conjugate molecule composed of two components. For example, select Yes for a glycosylated protein.
- **Is this a copolymer?** If you selected Polymer in Step 1, indicate whether you are analyzing a conjugate molecule composed of two components. For example, select Yes for a copolymer composed of two monomers.
- **Protein (or Polymer) dn/dc (mL/g):** Specify this value regardless of whether an RI detector is used for concentration.
- **Protein (or Polymer) extinction coefficient (mL/(mg cm)):** This field is shown if you selected a UV detector. This value will be used to calculate the concentration from the UV signal.
- **Modifier dn/dc:** Type the dn/dc of the second component conjugated to your protein. Available only if you selected yes for protein conjugate or copolymer.

- **Modifier extinction coefficient:** Type the extinction coefficient of the second component conjugated to your protein. Available only if you selected yes for protein conjugate or copolymer.
- **Concentration (mg/mL):** Type the concentration of the injected sample. In batch mode, this will be the concentration used to determine the molar mass. In separation mode, the concentration is used to calculate the injected mass (when an injected volume is entered) and factors into the percent recovered mass calculation.

### Sample Information for Viral Vector Scientific Applications:

<b>Note:</b>	For guidance in determining conditions and parameters for Viral Vector Analysis, contact Wyatt Support.
<ul style="list-style-type: none"><li>• <b>Concentration (mg/mL):</b> Type the concentration of the injected sample. In batch mode, this will be the concentration used to determine the molar mass. In separation mode, the concentration is used to calculate the injected mass (when an injected volume is entered) and factors into the percent recovered mass calculation.</li><li>• <b>Protein dn/dc (mL/g):</b> Specify the dn/dc for the protein capsid regardless of whether an RI detector is used for concentration.</li><li>• <b>Nucleic acid dn/dc (mL/g):</b> Specify the dn/dc for the genetic payload regardless of whether an RI detector is used for concentration.</li><li>• <b>Protein extinction coefficient (mL/(mg cm)): </b>These values are used to calculate the concentration from the UV signal. For Viral Vectors, enter this for the protein capsid at the wavelength(s) chosen in Step 2.</li><li>• <b>Nucleic acid extinction coefficient (mL/(mg cm)): </b>These values are used to calculate the concentration from the UV signal. For Viral Vectors, enter this for the genetic payload at the wavelength(s) chosen in Step 2.</li><li>• <b>Protein Expected Molar Mass (MDa):</b> Type the expected molar mass of the protein capsid.</li><li>• <b>Nucleic Acid Expected Molar Mass (MDa):</b> Type the expected molar mass of the genetic payload.</li></ul>	

### Collection Parameters:

- **Solvent:** Select the solvent from the drop down list. If a custom solvent has been created (see [Solvent Profiles on page 165](#)), it will be included in that list.
- **Duration:** Duration of the experiment run in minutes.



- **If HPLC was selected in Step 2:**
  - **HPLC flow rate (mL/min):** Enter the flow rate desired for the sample run. This is shown if Separation mode was selected in Step 1.
  - **HPLC injection volume ( $\mu\text{L}$ ):** Enter the volume you would like the HPLC sampler to inject.
  - **HPLC Vial number:** Enter the vial location of the sample to specify the vial to the HPLC autosampler.
- **If no HPLC option was selected in Step 2:**
  - **Flow rate (mL/min):** Enter the flow rate of your HPLC pump. For FFF users, enter the detector flow rate. The flow rate is used by ASTRA to convert abscissa units from time to volume. This field is shown if Separation mode was selected in Step 1.
  - **Injection volume ( $\mu\text{L}$ ):** Enter the volume injected by the HPLC sampler. The injected volume is used to calculate the injected mass (when a concentration is entered) and factors into the percent recovered mass calculation. This field is shown if Separation mode was selected in Step 1.

#### Data Processing:

- **Manual:** Select to manually define baselines and selects peaks. After the data collection is complete, the user will need to set baselines and select peaks manually in the Baseline and Peaks procedures.
- **Automatic:** Select to automatically define baselines for each detector and select all peaks. You can later manually adjust baseline and peak selection as needed. After the data collection is complete, ASTRA sets baselines for all signals automatically. ASTRA also detects peaks and assigns them automatically. See [Baselines Procedure on page 240](#) and [Peaks Procedure on page 247](#) for more information.

### Method Builder Details: Step 4 of 4

Step 4, the last step of the wizard, creates and saves the method. This step sets the created method to be the default method.

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<b>Note:</b>	Calibration methods are not set to be the default method.
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Click **Run now** to close the wizard, create a new experiment using the newly created method, and immediately start a collection.

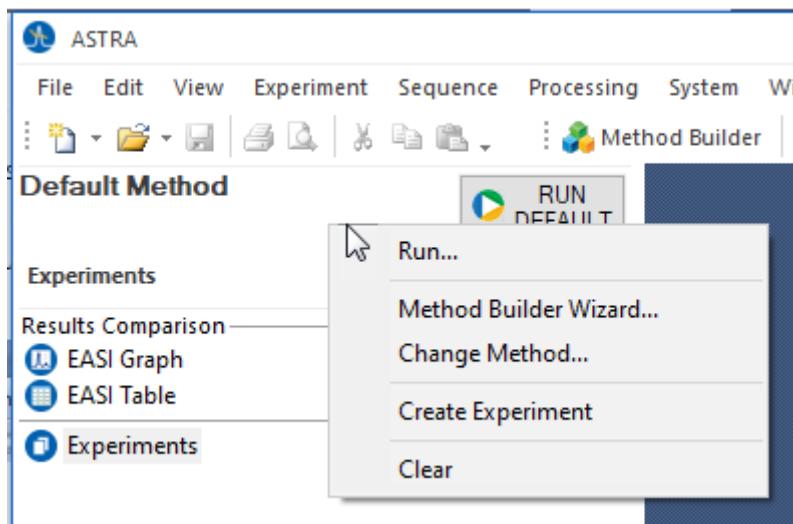
Click **Run later** to close the wizard and store the method for future use.

## Running Methods

Once the default method is created, the name of the method is shown in the upper-left corner of the ASTRA workspace. By clicking the **Run Default** button, a new experiment is created and run using the default method.

The default method can be quickly run from the main ASTRA window.

A context menu for the default method can be accessed by right-clicking in the Default Method area.



- **Run:** Creates a new experiment from the default method and starts the run.
- **Create Experiment:** Creates a new Experiment from the default method but does not start the run.
- **Method Builder Wizard:** Launches the Method Builder Wizard to create a new method to replace your current default method.
- **Change Method:** Opens a dialog box that allows you to select a different existing method to be used as the default method.
- **Clear:** Removes the default method selection and resets the default method to “none”.

## Creating an Experiment Outside the Wizard



You must use an account with AstraResearchers or AstraAdministrators access to follow the steps in this section.

### Creating an Experiment Manually

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:

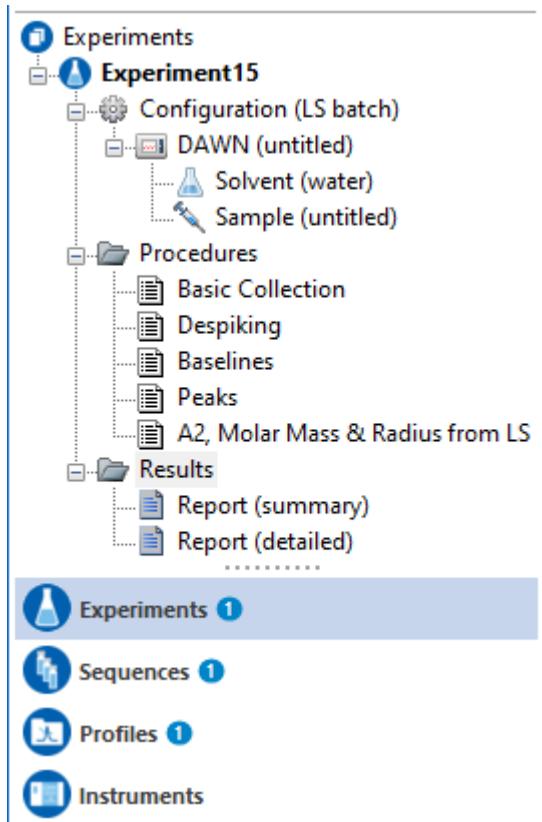
1. Choose **File→New→Experiment From Method** (Ctrl+M). Appendix B, [System Methods](#) lists methods provided with ASTRA.
2. In the “New from Existing” dialog, open the “System” folder, then the “Methods” folder.
3. Open the “Light Scattering” folder.
4. Select a “batch” method and click **Create**.

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, MALS, Optilab, and data connections. For details

on all types of items that may be configured and their properties, see [Chapter 8, Configuring Experiments](#).



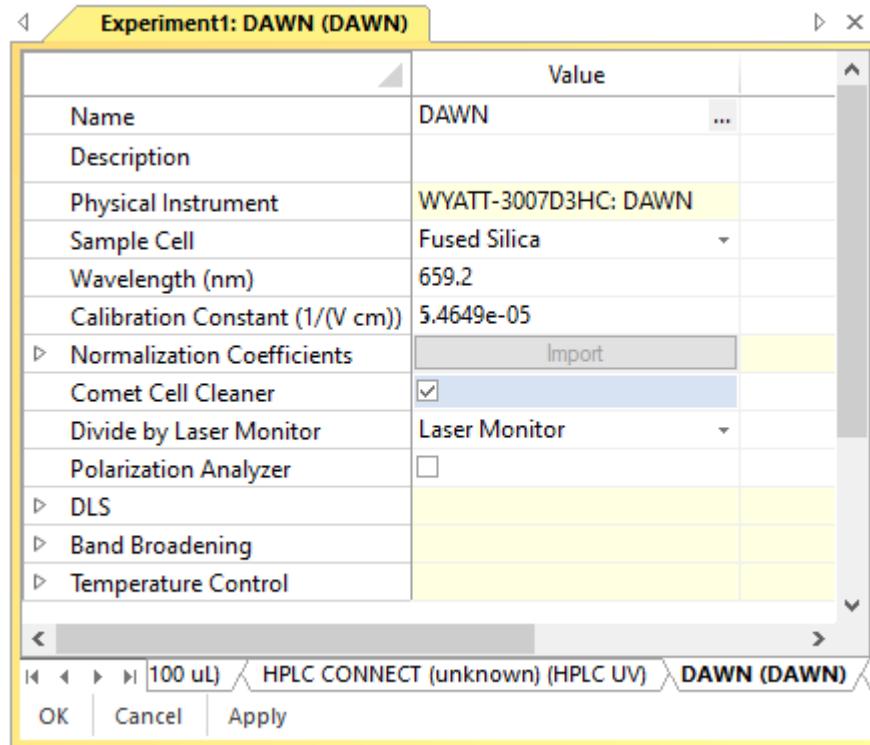
- **Procedures:** Actions to be performed in order when the experiment is run. There are configuration, collection, transformation, analysis, and administrative procedures. For details on all types of procedures, see [Chapter 9, Editing Procedures](#).
- **Results:** Reports and graphs to be produced after the experiment has been run. For details, see [Chapter 11, Working with Reports](#).

## Modifying the Configuration

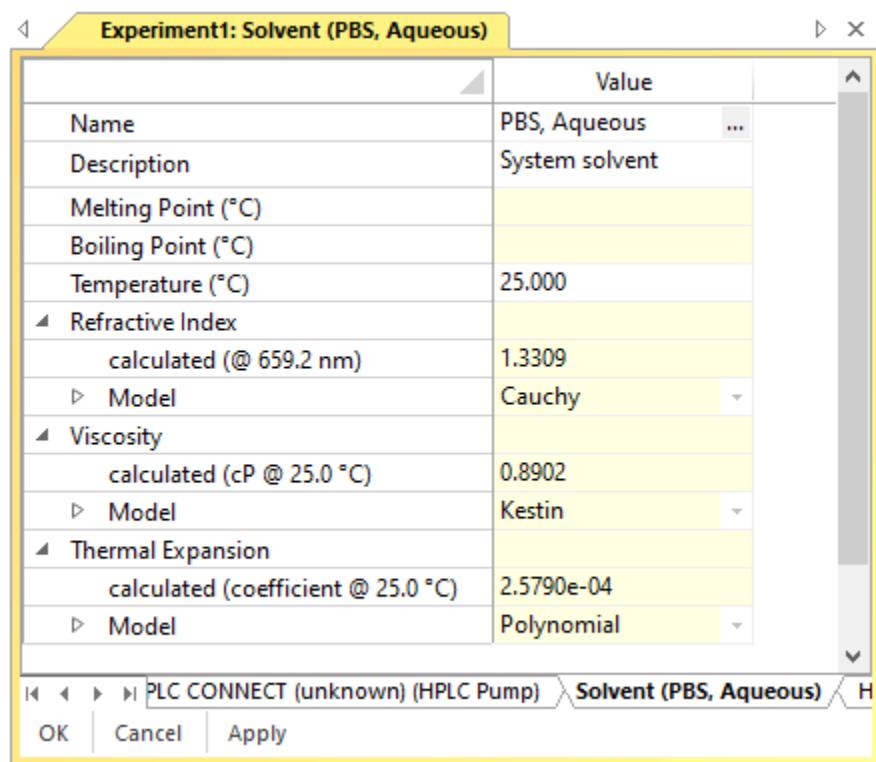
The experiment method sets most of the properties to values you are likely to use. There are just a few properties you typically need to set.

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.

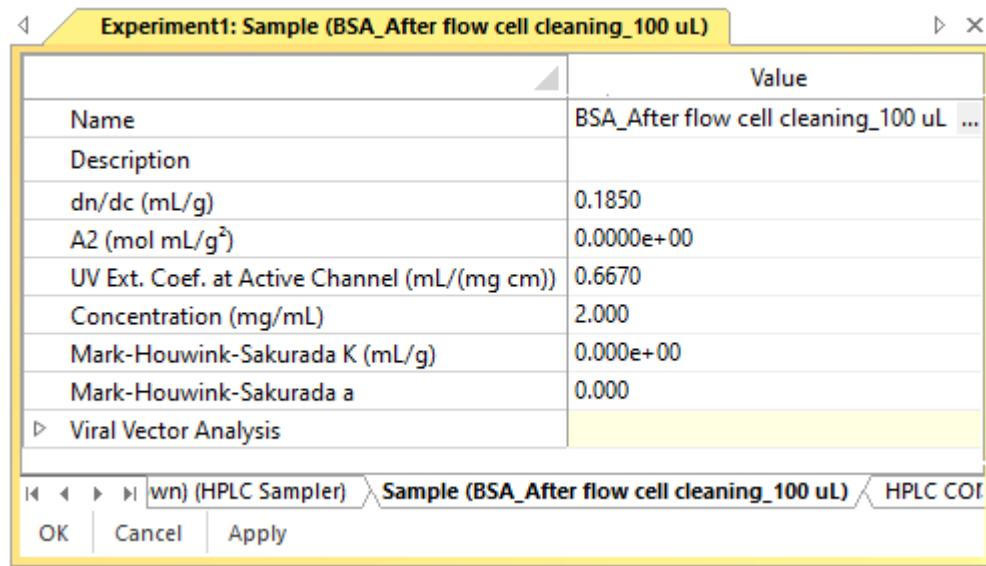


4. In the Physical Instrument row, make sure the correct instrument is selected. (When you create an experiment from a method, ASTRA automatically sets the Physical Instrument value to match instruments of the appropriate type in your instrument list.)
5. Select the **Solvent** tab from the tabs at the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



6. In the Name row, click the “...” button on the far right.
7. In the Select a Profile dialog, open the System > Solvents folder and select the solvent you are using. For example, you may be using toluene. Then click **Select**. 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.



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.

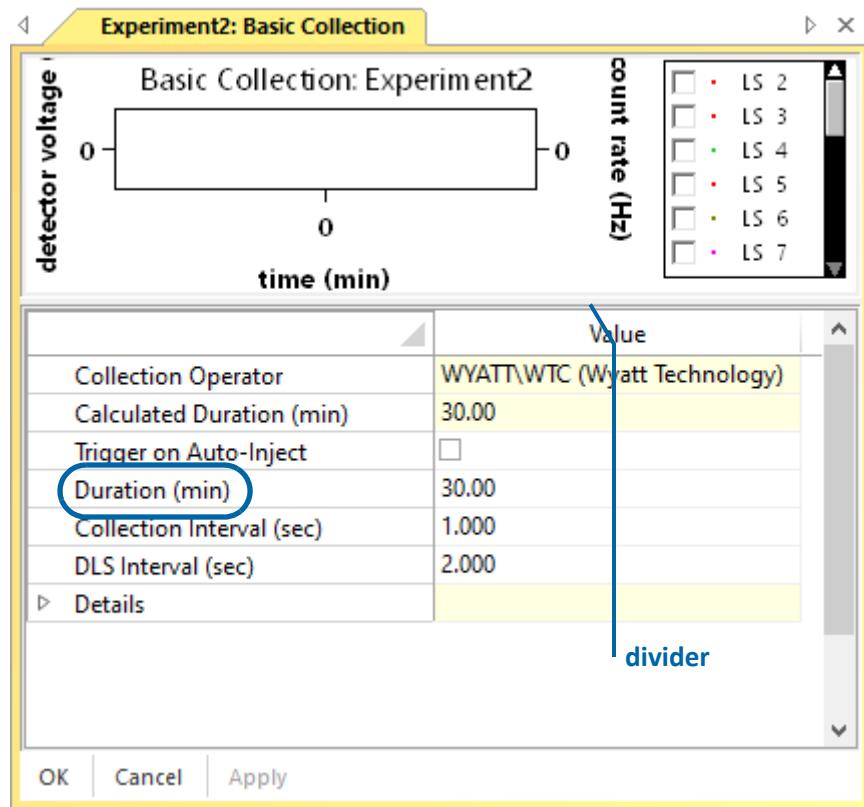
You can set other properties for the experiment if you like. Configurations are described in detail in [Chapter 8, Configuring Experiments](#).

## 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).

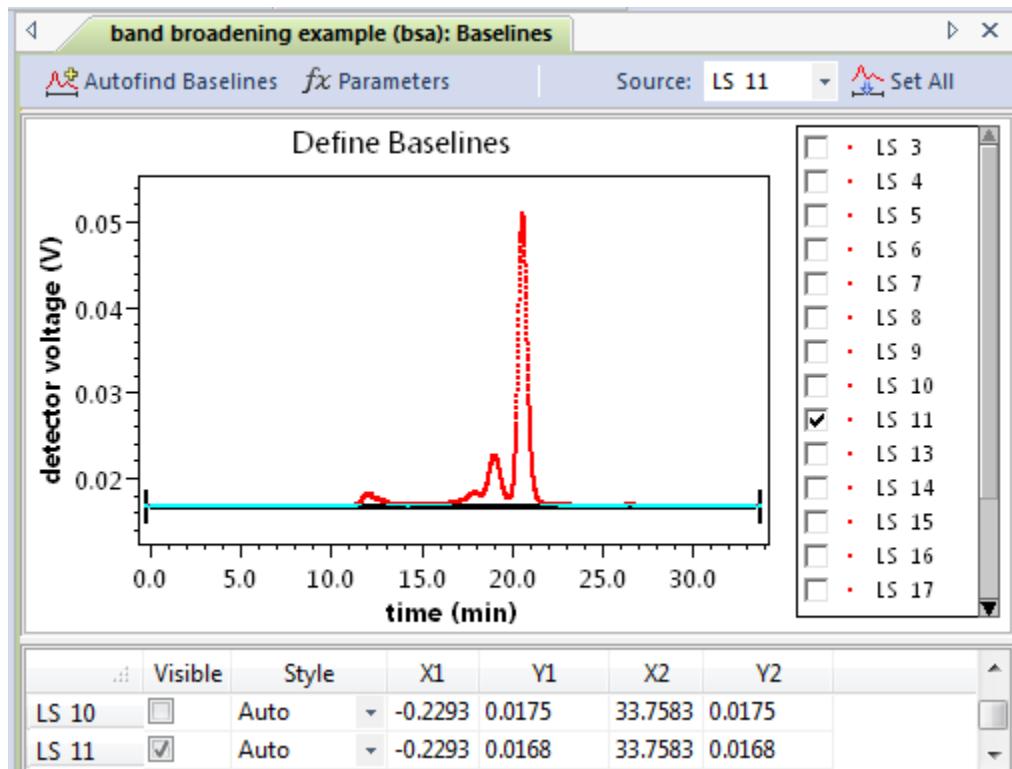
**Note:** If you are using ASTRA without access to a light-scattering instrument, you can open an experiment with pre-collected data by choosing **File→Open→Experiment** (Ctrl+O) and opening the “Sample Data” folder, then “Practice Experiments”, then “batch processing example.afe8”. Skip to step 4.

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.

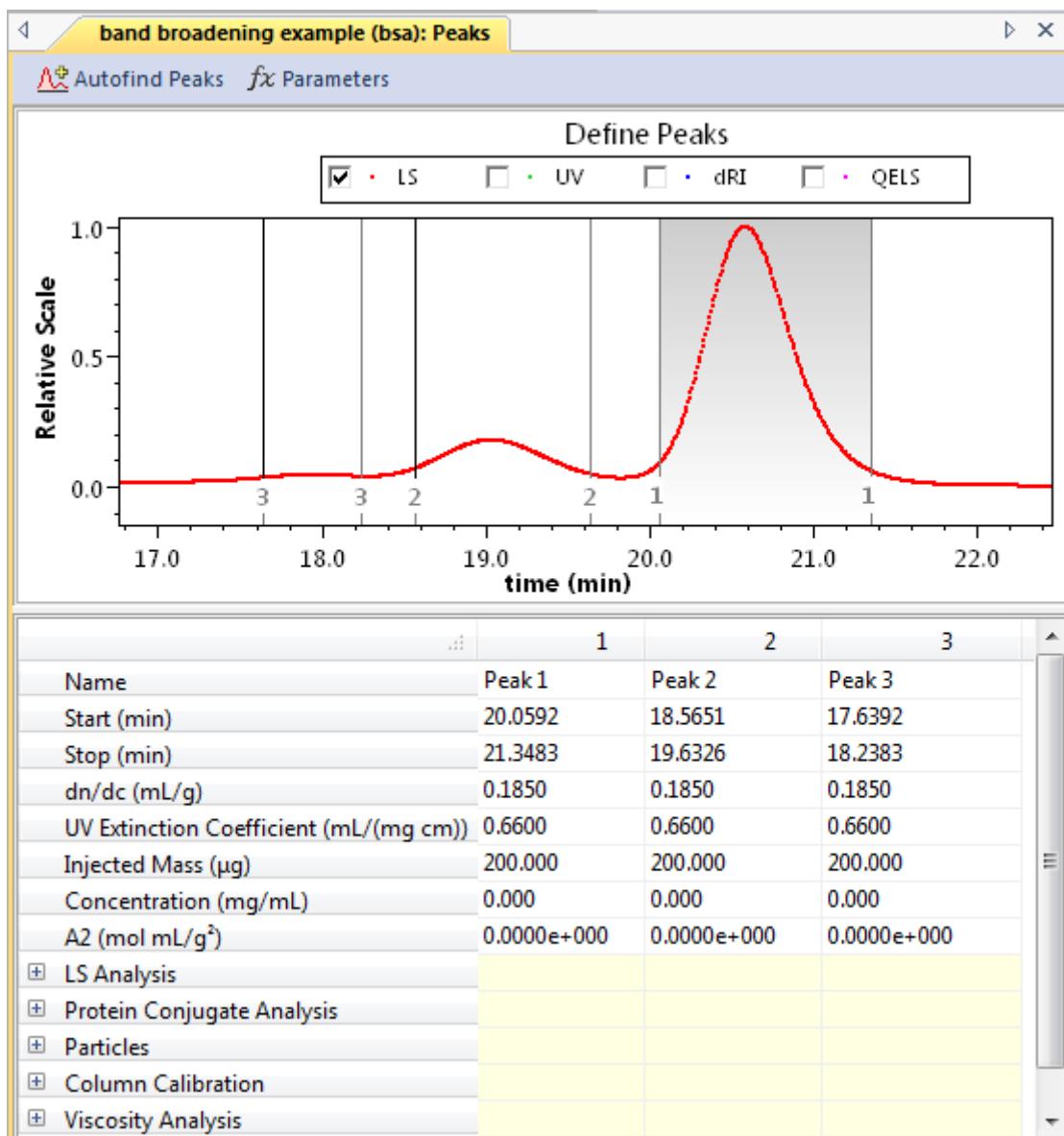
3. Inject samples and/or start pumps as needed to run the experiment. After data is collected, you see a message that says a baseline needs to be set. Set a baseline by following these steps:

- 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 Procedure on page 240](#).
  - 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:
- Click **OK** to open the page for setting peaks.



- 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.

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**Note:** You can use the **Autofind Peaks** button only if you are running an online (fractionated) experiment. See [Peaks Procedure on page 247](#) for more about peak detection.

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- 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 in an invalid location.

For more about running experiments, see [Chapter 7, Creating & Running Experiments](#).

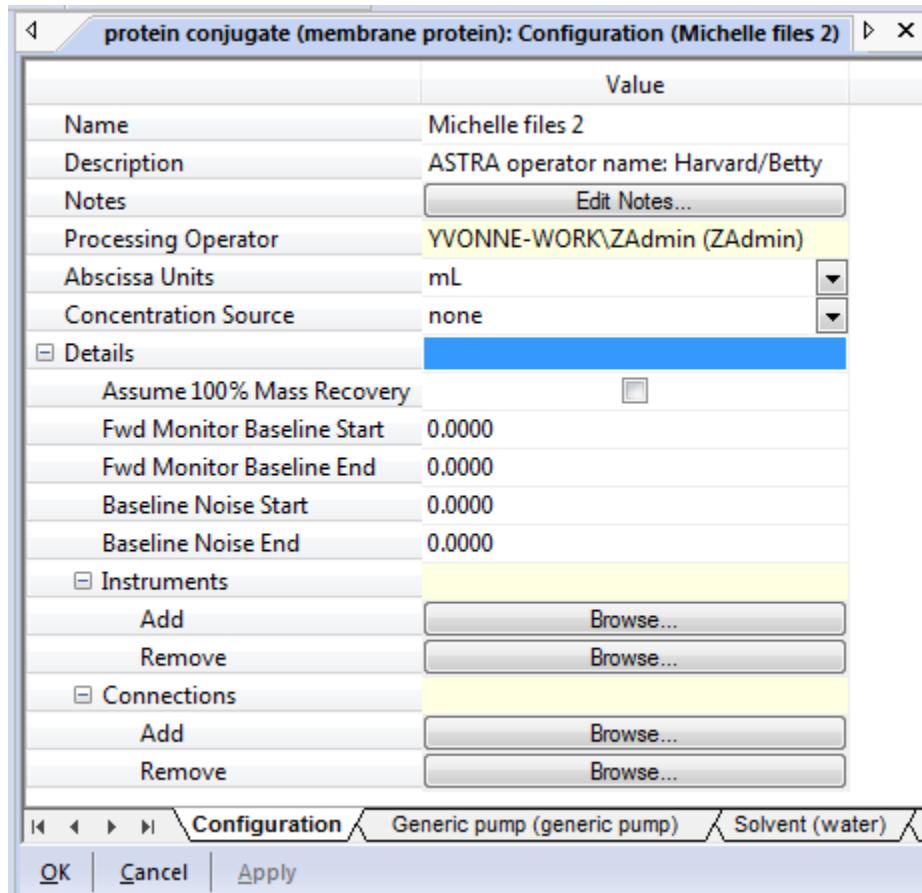
## Viewing Reports

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 11, Working with Reports](#).

## 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 97](#).



You can set the following properties for an experiment configuration:

*Table 8-1: 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.

Table 8-1: Experiment Configuration Properties (continued)

Field	Description
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 Basic, you can edit this field to show the name you want listed in a report. See <a href="#">Operator Names in Reports on page 329</a> for details.
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 <a href="#">Concentration Calculation Methods on page 435</a>.</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 150), 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.

Table 8-1: Experiment Configuration Properties (continued)

Field	Description
>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 <b>Browse</b> to select an instrument profile to add to the experiment. This property is available only in Experiment Builder Mode.
Remove Instruments	Click <b>Browse</b> to select an instrument profile to remove from the experiment. This property is available only in Experiment Builder Mode.
Add Connections	Click <b>Browse</b> to select a connection profile to add to the experiment. This property is available only in Experiment Builder Mode.
Remove Connections	Click <b>Browse</b> to select a connection profile to remove from the experiment. This property is available only in Experiment Builder Mode.



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.

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**Note:** See page 177 for information about configuring an experiment that uses ASTRA to control HPLC instruments.

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## 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 and DAWN 8 Profiles

You can set the following properties for a DAWN instrument. This includes DAWN (NEON), DAWN 8 (NEON), ultraDAWN™, DAWN HELEOS, and DAWN HELEOS 8. The DAWN is the default light scattering instrument in most light scattering experiment methods.

Table 8-2: DAWN 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 <a href="#">Accessing and Viewing Hardware on page 41</a> .)
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	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 <a href="#">LS Calibration Procedure on page 203</a> 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 213) 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.

Table 8-2: DAWN Properties (continued)

Field	Description
COMET Cell Cleaner	Check this box if a COMET™ cell cleaner is to be used with the MALS instrument. Please see the COMET hardware manual for more information about the COMET cell cleaner.
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"> <li>• “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.</li> <li>• “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. The forward monitor signal must be above 0.02 V to use this option.</li> <li>• “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.</li> </ul>
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the DAWN hardware manual for details.
DLS > DLS Option	Check this box if the MALS instrument contains a WyattQELS fiber.
DLS > Detector Angle or DLS > Replaced Detector	If DLS is enabled, the location of the DLS fiber is automatically detected for DAWN (NEON) instruments. For DAWN HELEOS instruments, enter the number of the detector that the DLS fiber replaces.
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 <a href="#">Band Broadening Procedure on page 208</a> . If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See <a href="#">Band Broadening Procedure on page 208</a> 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.

## miniDAWN and microDAWN Profiles

You can set the following properties for miniDAWN (NEON), microDAWN (NEON), miniDAWN TREOS®, and µDAWN® instruments:

*Table 8-3: microDAWN and miniDAWN 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 <a href="#">Accessing and Viewing Hardware on page 41</a> .)
Sample Cell	Type of sample cell used during data collection. The only option for the microDAWN is Vertical. The options for the miniDAWN 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	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 <a href="#">LS Calibration Procedure on page 203</a> 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 (1-3)	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.

Table 8-3: microDAWN and miniDAWN Instrument Properties (continued)

Field	Description
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"> <li>“Laser Monitor”: The light scattering signals are divided by the laser monitor, which corrects for fluctuations in the laser intensity due to power fluctuations.</li> <li>“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.</li> <li>“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.</li> </ul>
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the hardware manual for details.
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 208). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the mixing term. The units are in microliters.

## WyattQELS Profiles

A WyattQELS device is a Quasi-Elastic Light Scattering device, also known as dynamic light scattering (DLS). You can set the following properties for a WyattQELS instrument:

Table 8-4: 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 <a href="#">Accessing and Viewing Hardware on page 41</a> .)
Model	The instrument model. Currently only the WyattQELS is supported.
Details > Use DLS 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 DLS 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 $r_h$ results. 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 Profiles

You can set the following properties for Optilab (NEON), microOptilab (NEON), Optilab T-rEX™, Optilab UT-rEX™, and Optilab rEX™ instruments:

Table 8-5: Optilab 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 <a href="#">Accessing and Viewing Hardware on page 41</a> .)
Model	The Optilab model used for the measurement: Optilab (NEON), microOptilab (NEON), T-rEX, UT-rEX, or rEX.
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 retrieves the configured temperature from the instrument and stores it here.
dRI Calibration Constant	When data collection begins, ASTRA retrieves the configured calibration constant from the instrument and stores it here.
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 208). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the mixing term. The units are in microliters.

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

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

## 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 8 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 175](#) for details.

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

*Table 8-6: 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).
Temperature Control > Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.
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 208). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See <a href="#">Band Broadening Procedure on page 208</a> 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 H, 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 or microViscoStar™ instrument:

*Table 8-7: 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 <a href="#">Accessing and Viewing Hardware on page 41</a> .)
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 <a href="#">Measuring the Dilution Factor on page 158</a> 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 should be set to zero. The capillary volume is now accounted for in the algorithm and should not be changed from zero.
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.
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 208). If band broadening has been enabled, you can disable it using this check box.

Table 8-7: ViscoStar Profile Fields (continued)

Field	Description
Band Broadening > Instrumental Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See <a href="#">Band Broadening Procedure on page 208</a> 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.

See [Dilution Factor Procedure on page 235](#) for steps to measure the dilution factor.

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 8-8: 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 instrument, which typically contains more information than the Name.

Table 8-8: Generic Viscometer Profile Fields (continued)

Field	Description
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 <a href="#">Measuring the Dilution Factor on page 158</a> 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.
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 208). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See <a href="#">Band Broadening Procedure on page 208</a> 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 8 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 175](#) for details.

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<b>Note:</b>	See page 177 for information about configuring an experiment that uses ASTRA to control HPLC instruments.
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You can set the following properties for a generic UV instrument:

*Table 8-9: 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 175) 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.

Table 8-9: Generic UV Instrument Profile Fields (continued)

Field	Description
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.
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 208). If band broadening has been enabled, you can disable it using this check box.
Band Broadening > Instrumental Term	See <a href="#">Band Broadening Procedure on page 208</a> for an explanation of the instrumental term. The units are in microliters.
Band Broadening > Mixing Term	See <a href="#">Band Broadening Procedure on page 208</a> 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 8-10: 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.

An injector configuration always has a sample configuration associated with it in a profile. See [Sample Profile Properties on page 162](#).

## Sample Profiles

A *sample* is the substance being tested. It is dissolved in the solvent, forming a solution. The solution is placed in the sample vial or flows through it after injection. A sample 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 Profile Properties

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

You can set the following properties for a sample:

Table 8-11: 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 <sup>2</sup> . 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.

Table 8-11: Sample Properties (continued)

Field	Description
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: $[\eta] = KM^a$
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 161](#).

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 8-11 for each sample vial. See [Autoinject Connection Profiles on page 176](#).

## 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 8-12: 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 viral coefficient value associated with the sample. (mol mL/g <sup>2</sup> )
Molar Mass	Molar mass value associated with the sample. (g/mol)

Table 8-12: Molecular Standard Properties (continued)

Field	Description
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 radius, rms, or hydrodynamic.
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 361](#).

## 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 8-13: 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 165](#).

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**Note:** See page 177 for information about configuring an experiment that uses ASTRA to control HPLC instruments.

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## 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 values in existing solvent profiles. You can build custom profiles for any other solvents you use. Solvent Models used for the calculations may be displayed in the Report.

You can set the following properties for a custom solvent:

Table 8-14: 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.
Melting Point (°C)	The melting point in °C of the solvent (at 1 atm).
Boiling Point (°C)	The boiling point in °C of the solvent (at 1 atm).
Temperature (°C)	The temperature at which this experiment is to be run.
Refractive Index	
Refractive Index > Calculated	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.
Refractive Index > Model	The model used to compute the refractive index. May be <b>Fixed</b> , <b>Cauchy</b> , or <b>Rational Polynomial</b> . Set the model type before setting the parameters required for that model. <ul style="list-style-type: none"> <li>• If <b>Fixed</b>, specify the Reference Refractive Index and Reference Temp.</li> <li>• If <b>Cauchy</b>, specify parameters 1-4 and the Reference Temp.</li> <li>• If <b>Rational Polynomial</b>, specify the numerator and denominator parameters (1-3 for each).</li> </ul>
Refractive Index > Reference Refractive Index	Specify the refractive index of the solvent at the wavelength and temperature you will use. This property is required for both <b>Fixed</b> and <b>Cauchy</b> models.

Table 8-14: Solvent Properties (continued)

Field	Description
Refractive Index > Model Parameters	If the Refractive Index model is <b>Fixed</b> , the Reference Refractive Index is used. If the Refractive Index model is <b>Cauchy</b> , set parameters 1-4 using the following polynomial model, which is used to compute the Refractive Index as a function of wavelength. Note that this is a modification of Cauchy's equation, with an added temperature adjustment term. $n(\lambda) = P_0 + \frac{P_1}{\lambda^2} + \frac{P_2}{\lambda^4} + \frac{P_3}{\lambda^6} - P_4 T_0$ If the Refractive Index model is <b>Rational Polynomial</b> , set the numerator and denominator parameters using the following polynomial model, which is used to compute the Refractive Index: $\frac{a+b\lambda+c\lambda^2}{1+d\lambda+e\lambda^2+f\lambda^3}$
Refractive Index > Reference Temp.	If the Refractive Index model is <b>Fixed</b> or <b>Cauchy</b> , set the Reference temperature (T in the previous equation) (°C) for which this fit is valid.
Viscosity	
Viscosity > Calculated	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. When editing a solvent profile that is not part of an experiment configuration, this value is not displayed.
Viscosity > Model	The model used to compute the viscosity of the solvent. May be <b>Fixed</b> , <b>Rational Polynomial</b> , <b>Linear</b> , <b>Exponential</b> , <b>Andrade Organic</b> <sup>a</sup> , or <b>Kestin</b> <sup>b</sup> . Set the model type before setting the parameters required for that model. The Andrade Organic model is used for most organic solvents in the provided solvent profiles. The Kestin model is used for water and aqueous solutions in the provided solvent profiles.
Viscosity > Reference Viscosity	If the Viscosity Model is <b>Fixed</b> , specify the viscosity of the solvent in cP (centipoise) at the temperature you will use. If the Viscosity Model is <b>Linear</b> , <b>Exponential</b> , or <b>Kestin</b> , specify the viscosity of the solvent in cP (centipoise) at the reference temperature.

Table 8-14: Solvent Properties (continued)

Field	Description
Viscosity > Model Parameters	<p>If the Viscosity model is <b>Fixed</b>, the Reference Viscosity is used.</p> <p>If the Viscosity model is <b>Rational Polynomial</b>, set the numerator and denominator parameters using the following polynomial model, which is used to compute the viscosity, where T is the temperature as determined by the appropriate device's temperature probe in °C:</p> $\frac{a+bT+cT^2}{1+dT+eT^2+fT^3}$ <p>If the Viscosity model is <b>Linear</b>, set the parameters using the following model, where <math>\eta(T)</math> is the viscosity as a function of temperature.</p> $\eta(T) = P_1 + P_2(T - P_3)$ <p>and the parameters are defined as follows:</p> <ul style="list-style-type: none"> <li>• <math>P_1</math> is the viscosity in P at the reference temperature.</li> <li>• <math>P_2</math> is linear temperature dependence of the viscosity (cP/°C).</li> <li>• <math>P_3</math> is the reference temperature for the model in °C.</li> <li>• <math>T</math> is the temperature as determined by the device in °C.</li> </ul> <p>If the Viscosity model is <b>Exponential</b>, set the parameters using the following model.</p> $\eta = \eta_0 e^{(-a)/T} + o$ <p>and the parameters are defined as follows:</p> <ul style="list-style-type: none"> <li>• <math>\eta_0</math> is the reference viscosity</li> <li>• <math>a</math> is the exponential temperature dependence in 1 / °C</li> <li>• <math>T</math> is the reference temperature for the model in °C</li> <li>• <math>o</math> is the offset term</li> </ul> <p>If the Viscosity model is <b>Andrade Organic</b>, enter parameters A and B for use in the following equation.</p> $\ln(\eta) = A + \frac{B}{T}$ <p>If the Viscosity model is <b>Kestin</b>, enter parameters A-E and the viscosity (<math>\eta_0</math>) at a specified reference temperature (<math>T_0</math>) for use in the following equation.</p> $\log\left(\frac{\eta}{\eta_0}\right) = \frac{T_0 - T}{T + A} [B - C(T_0 - T) + D(T_0 - T)^2 + E(T_0 - T)^3]$
Viscosity > Reference Temp.	If the Viscosity model is <b>Fixed</b> , <b>Linear</b> , <b>Exponential</b> , or <b>Kestin</b> , set the Reference temperature (°C) for which the reference viscosity is valid.

Table 8-14: Solvent Properties (continued)

Field	Description
Rayleigh Ratio	Note: Rayleigh ratio properties are shown only if you are using Experiment Builder mode.
Rayleigh Ratio > Calculated	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.
Rayleigh Ratio > Model	The model used to specify the Rayleigh ratio of the solvent. May be <b>Fixed</b> , <b>Rational Polynomial</b> , or <b>Corrected Lambda^4</b> . Set the model type before setting the parameters required for that model.
Rayleigh Ratio > Reference Rayleigh Ratio	If the Rayleigh Ratio Model is <b>Fixed</b> , specify the Rayleigh ratio of the solvent in 1 / (cm) at the wavelength and temperature you will use. If the Rayleigh Ratio Model is <b>Corrected Lambda^4</b> , specify the Rayleigh ratio of the solvent at the reference wavelength in 1 / (cm).
Rayleigh Ratio > Reference Wavelength	If the Rayleigh Ratio Model is <b>Corrected Lambda^4</b> , set the reference wavelength in $\mu\text{m}$ . $R(\lambda)$ is the calculated solvent Rayleigh ratio using this formula:
	$R(\lambda) = R_0 \left( \frac{\lambda_0}{\lambda} \right)^4 \frac{n(\lambda)}{n(\lambda_0)} \frac{n(\lambda) - 1}{n(\lambda_0) - 1}$ <ul style="list-style-type: none"> <li>• <math>R_0</math> is the Reference Rayleigh Ratio of the solvent</li> <li>• <math>\lambda_0</math> is the Reference Wavelength in <math>\mu\text{m}</math></li> <li>• <math>\lambda</math> is the wavelength in <math>\mu\text{m}</math> 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.</li> <li>• <math>n(\lambda)</math> 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.</li> <li>• <math>n(\lambda_0)</math> is the refractive index of the solvent at <math>\lambda_0</math> (the reference wavelength). This is calculated using the formula specified for the Polynomial Refractive Index Model.</li> </ul>

Table 8-14: Solvent Properties (continued)

Field	Description
Rayleigh Ratio > Model Parameters	If the Rayleigh Ratio model is <b>Rational Polynomial</b> , set the numerator and denominator parameters using the following polynomial model, which is used to compute the Rayleigh ratio, where T is the temperature as determined by the appropriate device's temperature probe in °C:
$\frac{a+bT+cT^2}{1+dT+eT^2+fT^3}$	
Thermal Expansion	
Thermal Expansion > Calculated	Displays the computed Thermal Expansion of the solvent at the wavelength used in the experiment. This property is shown only if this profile is part of an experiment configuration. (Thermal Expansion parameters are available only if you are using Experiment Builder mode.)
Thermal Expansion > Model	The model to use for the thermal expansion of the solvent. May be <b>Fixed</b> , <b>Polynomial</b> , <b>Rational Polynomial</b> , or <b>Knovel<sup>c</sup></b> . Set the model type before setting the parameters required for that model. The Thermal Expansion Coefficient (TEC) is used to correct the refractive index, concentration, and dn/dc as a function of temperature.
Thermal Expansion > Ref. Thermal Exp.	If the Thermal Expansion model is <b>Fixed</b> , specify the known value for the thermal expansion of the solvent as the percent per °C of change.
Thermal Expansion > Reference Temp.	If the Thermal Expansion model is <b>Fixed</b> , set the temperature at which the specified Reference Thermal Expansion is valid.

Table 8-14: Solvent Properties (continued)

Field	Description
Thermal Expansion > Parameters	<p>If the Thermal Expansion model is <b>Polynomial</b>, set the parameters according to the following equation, where <math>T</math> is the temperature in °C and the parameter values are based on a regression analysis of experimental data:</p> $\text{TEC}(T) = (a + bT + cT^2 + dT^3) \times 10^{-6}$ <p>If the Thermal Expansion model is <b>Rational Polynomial</b>, set the numerator and denominator parameters using the following polynomial model, which is used to compute the Thermal Expansion:</p> $\frac{a+bT+cT^2}{1+dT+eT^2+fT^3}$ <p>If the Thermal Expansion model is <b>Knovel</b> (for organic solvents), set the parameters according to the following equation, where <math>a</math> is the pre-factor, <math>T_c</math> is the critical temperature in °C, <math>T</math> is the experimental temperature in °C, and <math>m</math> is the exponent.:</p> $\text{TEC} = a \left(1 - \frac{T}{T_c}\right)^m$

- a. Andrade, Edward Neville da Costa, *Nature* 125: 309 (1930). Cited in *The Properties of Gases and Liquids*, 5th edition.
- b. Kestin, Joseph, et al., "Viscosity of Liquid Water in the range -8 °C to 150 °C" *J Phys Chem Ref Data* (1978) 7:941-948.
- c. Yaws, Carl L., *Yaws' Handbook of Thermodynamic and Physical Properties of Chemical Compounds*, Knovel (2003).

## Column Profiles

Columns are used in size-exclusion chromatography (SEC) to fractionate a mixture of sample 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 227). You can set the following properties for a generic column:

Table 8-15: 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.

Table 8-15: Generic Column Profile Fields (continued)

Field	Description
Asymmetry Factor	<p>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.</p> <p>The asymmetry factor describes the shape of peaks generated by the column. It is calculated as the tailing width divided by the leading width at 10% of the maximum height of the peak. (See <a href="#">About the Asymmetry and Tailing Factors on page 289</a>.)</p> <p>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.</p>
Resolution	<p>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.</p> <p>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 <math>R_s = 2(V_2 - V_1)/(W_2 + W_1)</math>, where <math>V</math> is the elution volume for each species and <math>W</math> is the width of each peak at the baseline.</p>
Calibration Technique	The type of column calibration performed. The options are: Absolute Molar Mass by MALS, Conventional, Universal with Viscometer Data, and Universal without Viscometer Data. This property can also be specified in the Molar Mass from Column Calibration procedure. See page 308.
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 8-15: Generic Column Profile Fields (continued)

Field	Description
Conventional Calibration Function	<p>Expanding this row shows the <math>A_i</math> coefficients of the following linear regression equation, where <math>M</math> is the polymer molar mass and <math>V</math> is the elution volume:</p> $\log(M) = A_0 + A_1V + A_2V^2 + A_3V^3 + \dots$ <p>Note that a column profile can contain results both for a conventional and universal calibration.</p>
Universal Calibration Function	<p>Expanding this row shows the <math>A_i</math> coefficients of the following linear regression equation, where <math>M</math> is the polymer molar mass, <math>\eta</math> is the intrinsic viscosity, and <math>V</math> is the elution volume:</p> $\log(M[\eta]) = A_0 + A_1V + A_2V^2 + A_3V^3 + \dots$ <p>Note that a column profile can contain results both for a conventional and universal calibration.</p>
Length	Specifies the column length in mm. This parameter is necessary to calculate selectivity.
Inner Diameter	Specifies the inner diameter of the column in mm. This parameter is necessary to calculate selectivity.

## 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 8-1 in [Configuration Example on page 126](#) 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 the Experiment tree 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 8-16: 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.

Table 8-16: Fluid Connection Properties (continued)

Field	Description
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 8-17: 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.
Source AUX Channel	The source AUX channel number on the UV instrument. If you are using a UV instrument with multiple channels, check your UV instrument's user's manual for details about the analog output connections.
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.

Table 8-17: AUX Connection Properties (continued)

Field	Description
Destination AUX Channel	The input AUX channel number on the destination instrument. If you are using a UV instrument with multiple channels, see <a href="#">Generic UV Detector Profiles on page 159</a> 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 <a href="#">RI Calibration Procedure on page 218</a> .

## 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 8-18: 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, typically containing 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 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 instrument profile types available for a connection.

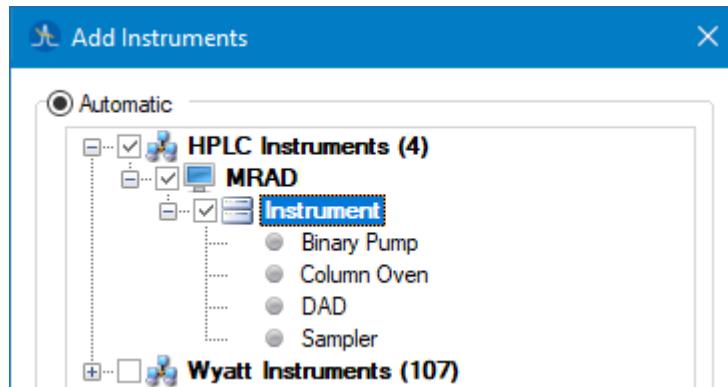
When a collection is running, the collection graph shows markers for autoinjection signals.



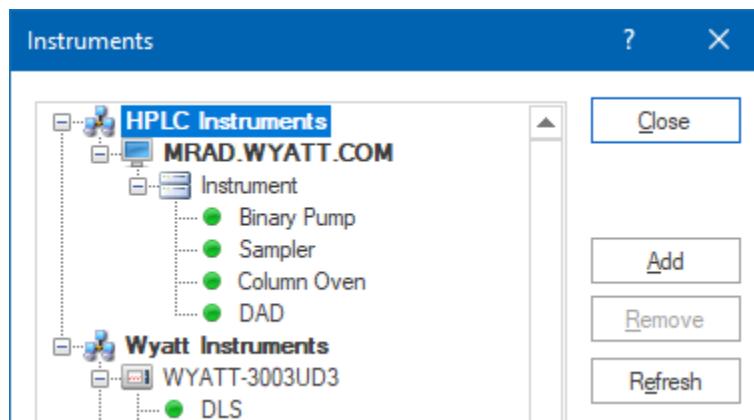
Install the latest version of ASTRA as described in [Chapter 2, Installing and Setting Up ASTRA](#). The default ASTRA installation enables the HPLC features within ASTRA.

## Configuring ASTRA for Use with HPLC

1. Within ASTRA, select **System**→**Instruments** from the menus.
2. In the dialog that appears, click the **Add** button.
3. In the Add Instruments dialog, click **Search**. You see instruments in two categories: **HPLC Instruments** and **Wyatt Instruments**.
4. Expand the **HPLC Instruments** list and the computer on which HPLC CONNECT is running.
5. Check the box next to the HPLC Instrument you wish to add, and click the **Add** button at the bottom of the dialog.



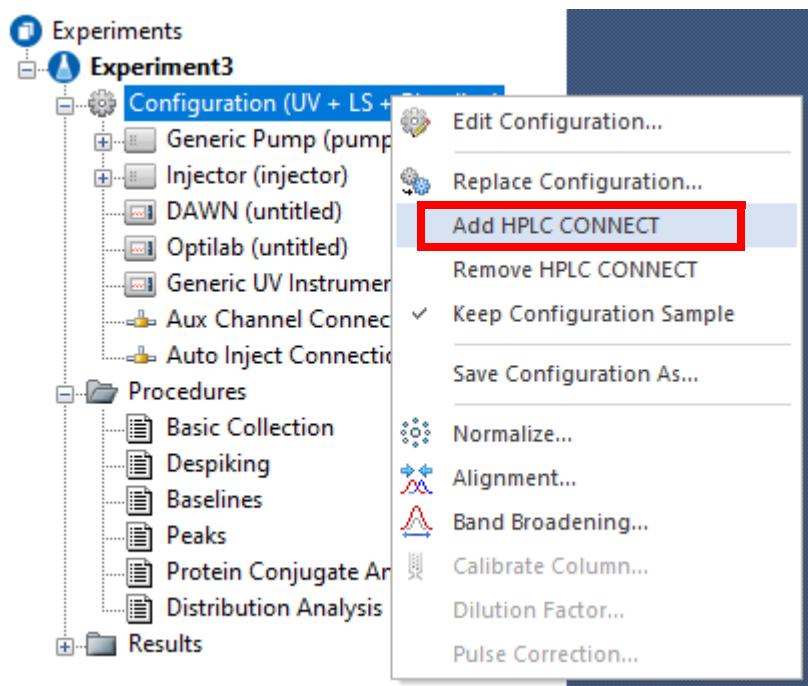
6. ASTRA communicates with the HPLC hardware for a minute. Once it has made the connection, the HPLC hardware is added to the Instruments list. Successful connections are indicated by a green circle.



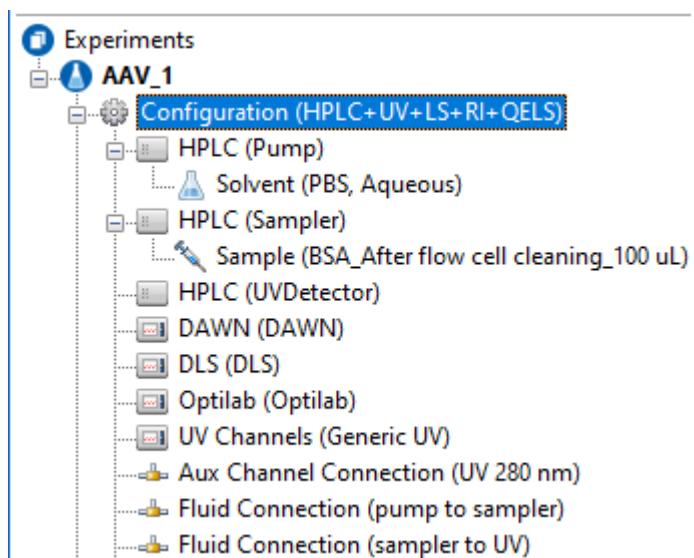
If you click **View** while an HPLC component is selected in the Instruments dialog, ASTRA opens the HPLC DASHBOARD.

7. Click the **Close** button.

8. Create a new experiment. Refer to [Creating New Experiments on page 97](#) for how to create new experiments. Make sure all desired Wyatt hardware is present in the configuration before adding HPLC CONNECT to it.
9. Right click on the **Configuration** node of the experiment, and select **Add HPLC CONNECT** to update the experiment configuration to use HPLC CONNECT.



10. Expand the experiment configuration and make sure the new HPLC Devices have been added. Each HPLC Device in your Agilent system will have a corresponding device in the ASTRA configuration.



- 11.** To edit the properties of a device, double-click on the HPLC Device you wish to edit. The Solvent parameters can be set under the HPLC Pump device (if any), and the Sample parameters can be set under the HPLC Sampler device (if any). For example, double-clicking on **HPLC Device (HPLC Pump)** displays the following parameters:

	Value
Name	HPLC Pump ...
Description	
Physical Instrument	SWTESTER-7: Instrument
Physical Device	Quat. Pump
Model	G1311A
▷ Stop Time	
▷ Post Time	
Low Pressure Limit (bar)	0.00
High Pressure Limit (bar)	400.00
Flow (mL/min)	1.000
Maximum Flow Gradient (mL/min <sup>2</sup> )	0.100
Primary Channel	Automatic
▷ Compress A	
▷ Stroke A	
▷ Solvent Composition 1	
▷ Solvent Composition 2	
▷ Solvent Composition 3	
▷ Solvent Composition 4	

Any parameter that is available on the hardware can be configured through this interface. Click **OK** or **Apply** to save your changes.

- 12.** Although ASTRA controls the HPLC UV device (if any), a physical AUX cable connection to your Wyatt instruments is still necessary to in order to collect the UV signal. To fully configure the VWD/DAD detectors, you will also need to set parameters for the UV-related items in the ASTRA experiment configuration.
- 13.** Set parameters for the Agilent UV detector in the **HPLC Device (HPLC UV)** item in the experiment configuration. The parameters available will depend on your specific detector model. However, some important parameters to set are:
- **Wavelength(s)**
  - **Analog attenuation:** typically set to 1000 mAU
  - **Analog Zero Offset (%):** typically set to 5%
  - **Scan Range Step:** typically set to 2nm

- 14.** Set parameters related to the analog UV signal in the **UV Channels (UV)** item. The parameters to set are:
- **Cell length:** Enter the path length of the UV flow cell.
  - **Channels:** Enable the channels received from the UV detector. Each channel must correspond to a physical cable connection.
  - **Active Channel:** Select the channel to be used to determine the concentration.
  - **Secondary Channel:** If you are using the Viral Vector procedure, select a second channel to use for calculations (see [page 298](#)).
  - **UV response:** (For each channel) This factor is related to the Analog Attenuation and defines the relationship between the received voltage signal and absorbance units (typically is 1.000 AU/V).

The screenshot shows the ASTRA software interface with the following details:

- Default Method:** AAV\_1
- Experiments:** AAV\_1 is selected.
- Configuration (HPLC+UV+LS+P):**
  - HPLC (Pump)
  - HPLC (Sampler)
  - HPLC (UVDetector)** (highlighted with a red box)
  - DAWN (DAWN)
  - DLS (DLS)
  - Optilab (Optilab)
  - UV Channels (Generic UV)** (highlighted with a red box)
  - Aux Channel Connection (U)
  - Fluid Connection (pump to)
- AAV\_1: UV Channels (Generic UV) Configuration Table:**

Name	Value		
Description	Generic UV		
Cell Length (cm)	1.000		
Channels	enable		
Channel 1	<input checked="" type="checkbox"/>	280.000	1.000
Channel 2	<input checked="" type="checkbox"/>	260.000	1.000
Channel 3	<input type="checkbox"/>	0.000	1.000
Channel 4	<input type="checkbox"/>	0.000	1.000
Active Channel	Channel 1		
Secondary Channel	Channel 2		
Temperature Control			
Batch Mode	<input type="checkbox"/>		
Band Broadening			

## Configuring and Running a Basic Collection



1. Expand the **Procedures** node of the experiment tree and double-click on **Basic Collection**.
2. Expand the **HPLC Details** node.

If an autosampler module is part of the HPLC configuration, enter the **Vial Number** for the injection and the **Injection Volume**.

If a UV module is part of the HPLC configuration, enter the desired state of the lamp at the end of the collection. Choose **Lamp Off** to turn the lamp off at the end of a single experiment. Choose **Lamp On** if this method will be used in a sequence. (To turn the lamp off at the end of a sequence, use the HPLC UV Lamp Off utility. See [Utilities on page 397](#).)

	Value
Collection Operator	ADEKE\wtc (Wyatt_User)
Calculated Duration (min)	40.00
Trigger on Auto-Inject	<input checked="" type="checkbox"/>
Duration (min)	40.00
Collection Interval (sec)	0.500
DLS Interval (sec)	2.000
▲ HPLC Details	
Vial Number	Vial 1
Injection Volume ( $\mu$ L)	100.00
Post-collection UV Lamp	Lamp On
Post-collection Vis Lamp	Lamp On

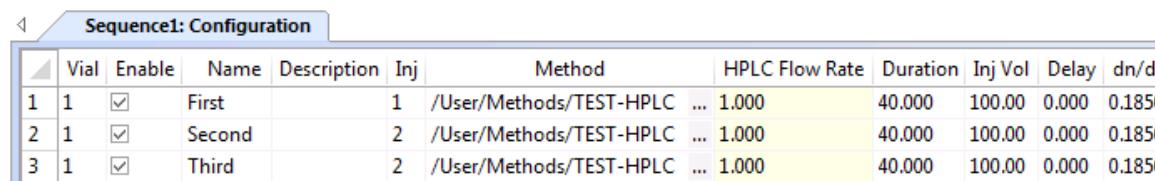
**Note:** The injection volume entered in the Basic Collection parameters overrides the value set in the HPLC Device configuration for the HPLC Sampler.

3. Make sure the **Trigger on Auto-Inject** checkbox is checked.
4. Save your new HPLC-enabled experiment as a method. (See [Creating a Method on page 117](#).)
5. Start the collection by clicking the green Run icon in the toolbar. You can monitor the pump flow rate, pressure, and ripple by enabling the relevant traces from the plot legend on the left side of the window. You can also monitor these traces in the HPLC DASHBOARD.

## Configuring a Sequence for HPLC



1. Create a new sequence. (See [Creating New Sequences on page 315](#).)
2. The **Vial** column in the sequence indicates the vial position for the autosampler. Adjust the **Duration** and **Injection Volume** for each sample as needed. The Injection Volume value in the sequence table overrides the value set in the HPLC-enabled method. Read-only parameters, such as flow rate, are indicated by a yellow background.

Sequence1: Configuration

	Vial	Enable	Name	Description	Inj	Method	HPLC Flow Rate	Duration	Inj Vol	Delay	dn/d
1	1	<input checked="" type="checkbox"/>	First		1	/User/Methods/TEST-HPLC ...	1.000	40.000	100.00	0.000	0.185
2	1	<input checked="" type="checkbox"/>	Second		2	/User/Methods/TEST-HPLC ...	1.000	40.000	100.00	0.000	0.185
3	1	<input checked="" type="checkbox"/>	Third		2	/User/Methods/TEST-HPLC ...	1.000	40.000	100.00	0.000	0.185

## Setting the Vial Position



To set the vial position for an injection, use the **Vial Number** for a single experiment or the use the **Vial** column in a sequence. Use the following formats depending on your autosampler type:

### Single Tray Autosamplers

Use the following format. (The Vial prefix is optional.)

Vial 1, Vial 2, . . . max vial count

### Autosamplers with a Tray for Two Well Plates

Use the following format depending on which side of the drawer the plate is in:

Left Plate: Vial 1, Vial 2, Vial 3 . . . max vial count  
(The Vial prefix is optional.)

Right Plate: Vial 101, Vial 102, Vial 103 . . . max vial count  
(The Vial prefix optional)

### Multi Drawer Autosamplers

Multi drawer autosamplers use the format PX-YZ (or PX-Y-Z with an optional hyphen), where:

- PX is a prefix that indicates the drawer location and whether it is the front or rear plate:

	Front	Rear
Drawer 1	P1	P2
Drawer 2	P3	P4
Drawer 3	P5	P6
Drawer 4	P7	P8

- YZ is the location of the row and column of the vial on the plate:

A10	B10	C10	D10	E10	F10
A9	B9	C9	D9	E9	F9
A8	B8	C8	D8	E8	F8
A7	B7	C7	D7	E7	F7
A6	B6	C6	D6	E6	F6
A5	B5	C5	D5	E5	F5
A4	B4	C4	D4	E4	F4
A3	B3	C3	D3	E3	F3
A2	B2	C2	D2	E2	F2
A1	B1	C1	D1	E1	F1

Figure 8-3: The configuration of a plate with 6 rows A-F, and 10 columns 1-10. A1 is located in the front left of the HPLC.

In the Basic Collection example below, P1-A1 references the sample located in the first drawer, front plate in row A, column 1



In the sequence example below, the injections will be from the first drawer in row A, columns 1-3 and then back to column 1.

Vial	Enable	Name	Descrip...	Inj	Method		HPLC Flow Rate...	Duration...	Inj Vol
1	P1-A1	<input checked="" type="checkbox"/>	BSA		1	/User/Methods/50ul...	0.500	70.000	50.00
2	P1-A2	<input checked="" type="checkbox"/>	Protein 1		1	/User/Methods/50ul...	0.500	70.000	50.00
3	P1-A3	<input checked="" type="checkbox"/>	Protein 2		1	/User/Methods/50ul...	0.500	70.000	50.00
4	P1-A1	<input checked="" type="checkbox"/>	BSA		1	/User/Methods/50ul...	0.500	70.000	50.00

## Advanced Configuration Editing

This section describes more advanced ways of editing configurations than simply setting properties (page 127) and setting a default configuration (page 128).

### Adding Instruments and Connections



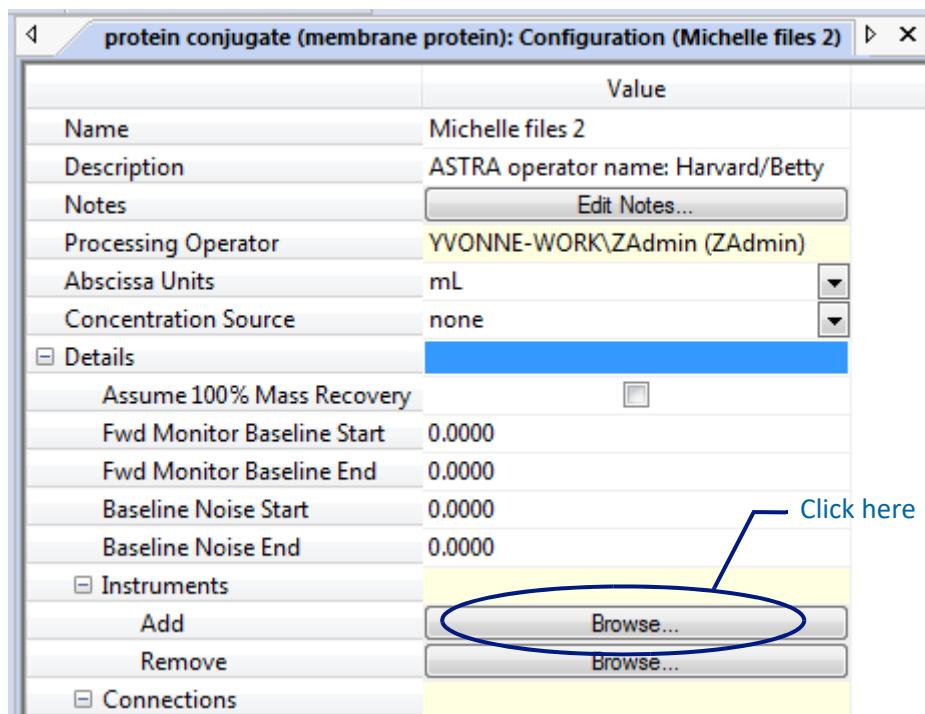
You can add items to a configuration only if you enable Experiment Builder mode by choosing **System**→**Preferences**→**Experiment Builder Mode**. If you have already opened the Configuration properties page, close and reopen it after enabling Experiment Builder mode.

- 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 362](#).

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 configuration.

- Shortcuts:** Double-click the Configuration node 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 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 361](#) or a profile available in the Systems folder. For example, you might navigate to a “My Profiles” folder if you have created one to select a custom profile. Or, 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 362](#).

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.

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.

## 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 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 361](#). 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.

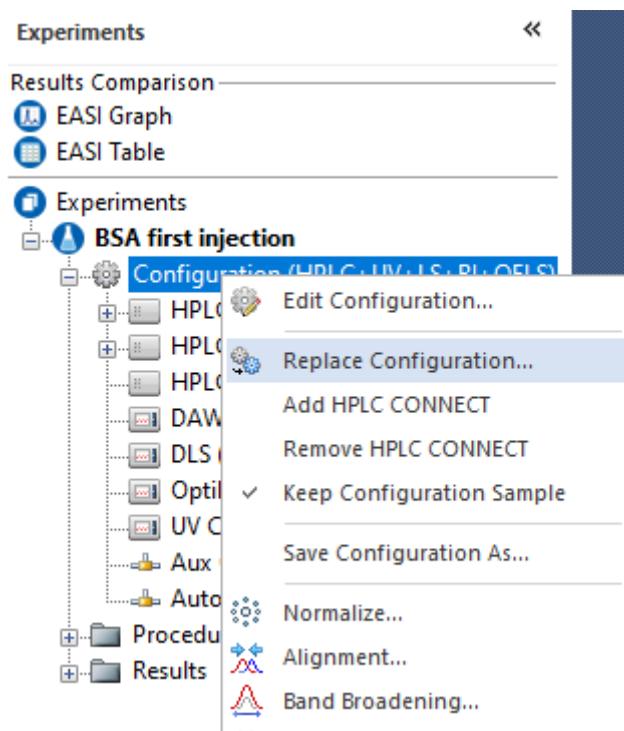
See [Chapter 13, Working with Profiles](#) for more about profiles.

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 checked.
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.

**Note:** See page 177 for information about configuring an experiment that uses ASTRA to control HPLC instruments.

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 362](#).

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 13, Working with Profiles](#) for more about using a profile you export.

# 9

## Editing Procedures

This chapter explains how to set up your experiment in ASTRA 8 to collect and analyze data. This is done using ASTRA 8 procedures.

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Analysis Procedures .....	259

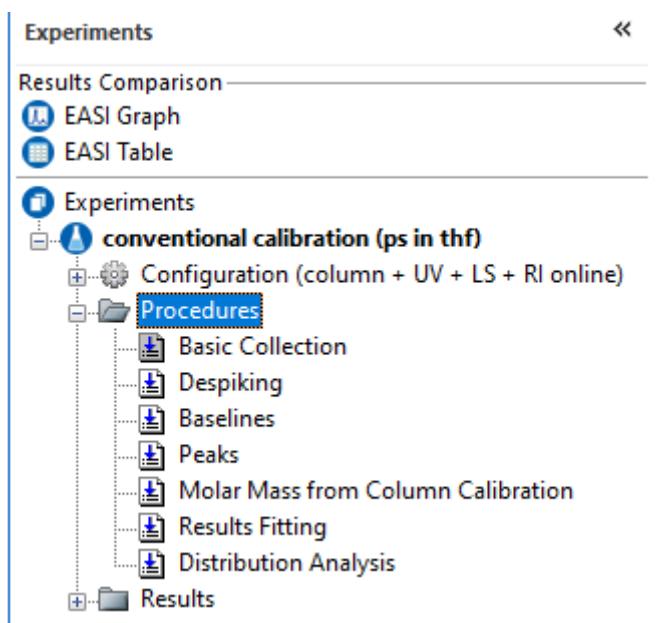
## About Procedures

Data collection and analysis in an experiment are broken down into a logical set of units called *procedures* in ASTRA 8. 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.

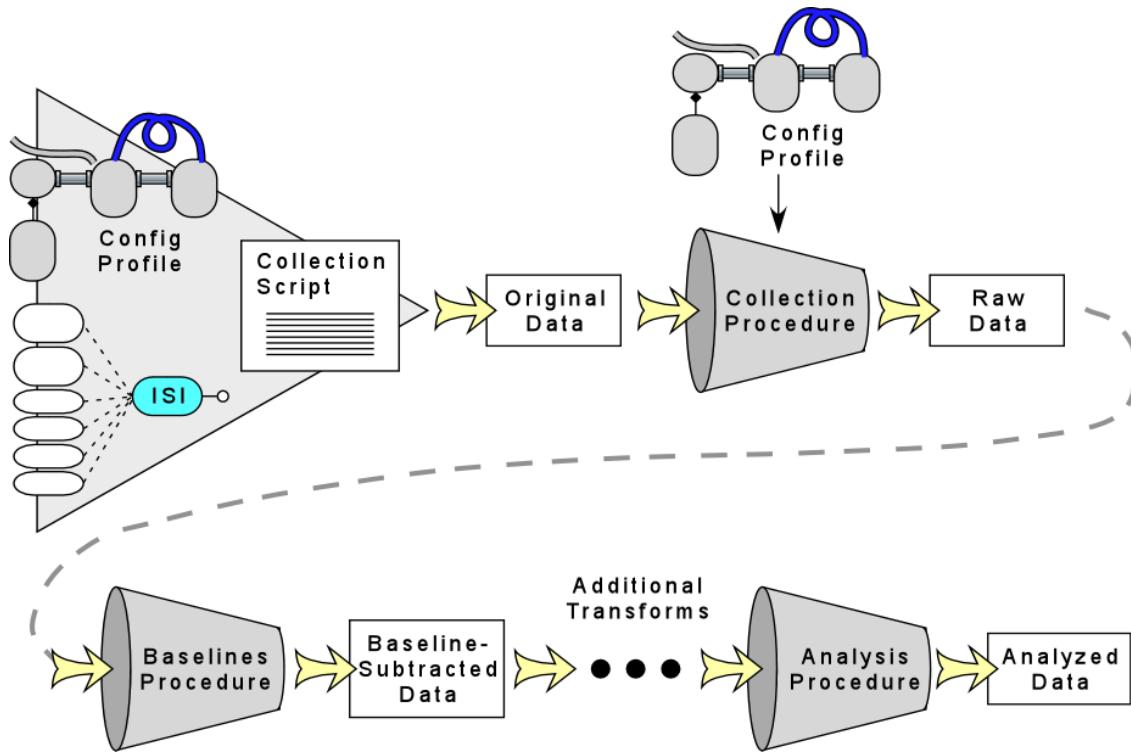


A procedure's status is indicated by its icon, as follows.

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	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 from the 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 original data is a data set composed of all signal collected from all the instruments in the configuration.

ASTRA then performs preprocessing on this original data based on the configuration to create what is called the *raw data*. The raw data is a set of data that contains the relevant signals for the analysis procedures.

For example, the original data contains all the AUX channel traces received by a Wyatt instrument, but the raw data displays the relevant AUX channel and relates it to the appropriate generic instrument as specified by the AUX connection configuration profile. The alignment (if values are available) is also applied to the signals when the raw data is generated. The raw data is displayed in the Basic Collection procedure. Both the raw data and original data sets can be graphed. See [Using Custom Plots and Data Set Definitions on page 344](#).

Transformation procedures (such as the baseline procedure) are then applied to the raw data to perform the analysis procedures. Some transformation procedures are only viewable in Experiment Builder mode.

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. The analysis methods (such as protein conjugate or branching, etc.) differ depending on the method chosen and on the data collected.

## Working with Procedures

Most users will not need to add, remove, or change the order of the procedures. The methods provided with ASTRA 8 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 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 the Experiment tree if you do not need to interact with them. All procedures are shown in Experiment Builder mode.



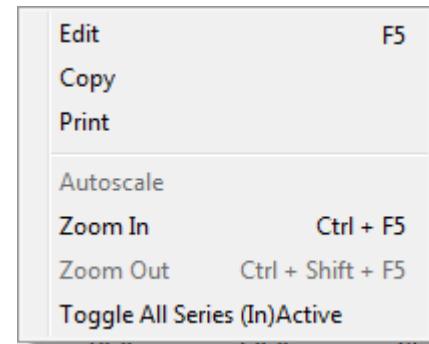
You can open any set of procedure pages if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

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

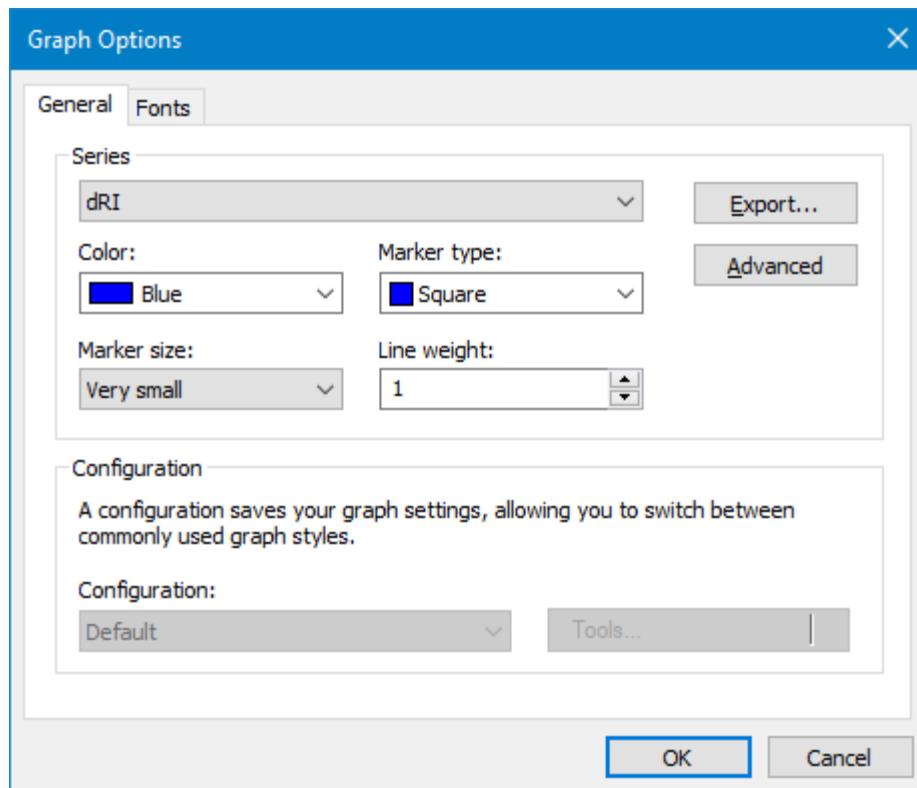
A number of procedure pages contain graphs. You can manipulate these graphs in the following ways:

- **View Data Values:** Hover the mouse over 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 354](#) 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.
- **Fit to Peaks:** In the Peaks procedure, you can right-click and choose **Fit to Peaks** to zoom in to see only the portion of the graph where peaks are marked.
- **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 9-1: Graph Options 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 to use in the graph. The default is square.
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 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 351](#).

## 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 8 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 and Reordering Procedures within an Experiment



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

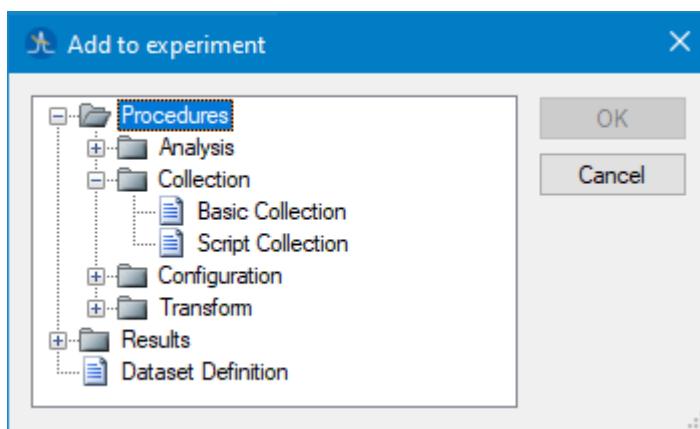
To add a procedure to an experiment, follow these steps:

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.

Right-click on experiment name in the experiment tree and choose **Add to Experiment**.



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. If the procedure you added has a red X on its icon, the procedure is in an invalid location in the list (or it requires data from an instrument that is not in the configuration). See [Changing the Procedure Order on page 196](#).

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 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**.

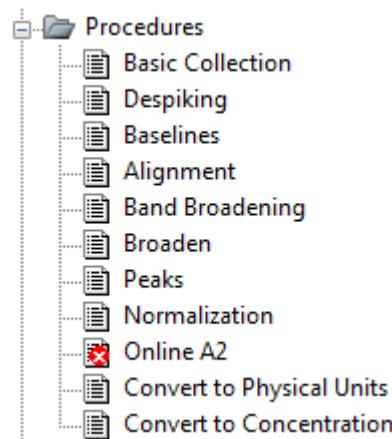
To delete a procedure from an experiment, select the procedure and press the **Delete** key.

## Changing the 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.

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

## Validating 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 99](#) 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 the Procedure Order on page 196](#) 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 Procedure on page 198](#)
- [Script Collection Procedure on page 201](#)

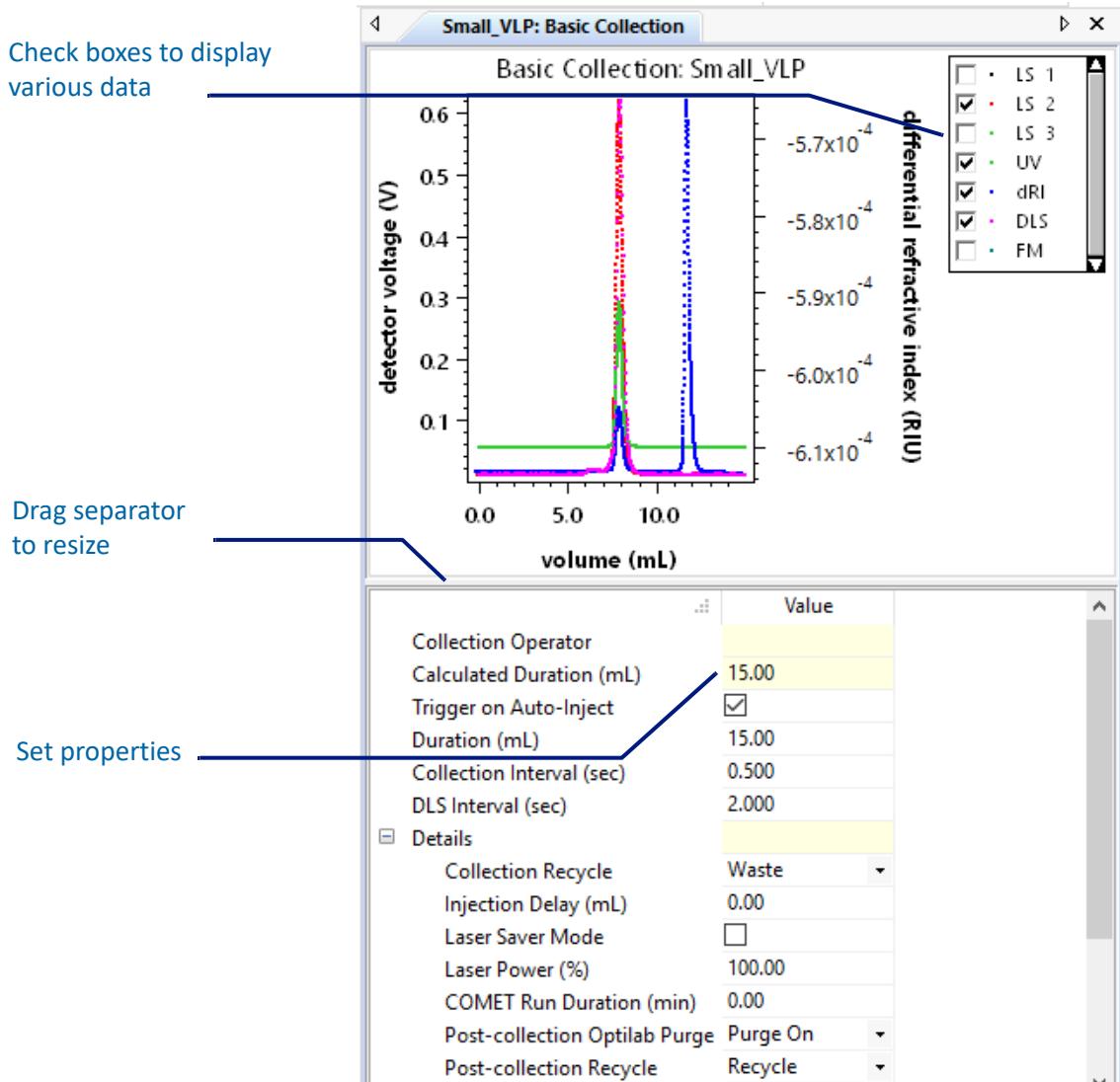
### Basic Collection Procedure

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. 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, DLS 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 147). By default, the units are minutes.

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 9-2: Basic Collection Properties

Field	Description
Collection Operator	The user at the time data collection is started. See <a href="#">Operator Names in Reports</a> on page 329 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.

Table 9-2: Basic Collection Properties (continued)

Field	Description
Collection Interval (sec)	How often the light-scattering or other instrument should collect data. The default is every 0.5 seconds for HPLC methods and 0.05 for UHPLC methods. This interval may be set to a multiple of 0.125 seconds for instruments used in HPLC (DAWN and miniDAWN) and 0.014 seconds for the UHPLC applications (microDAWN and microOptilab). This interval is used to set the collection interval for Wyatt LS instruments, ViscoStar instruments, and/or the Optilab.
DLS Interval (sec)	How often the WyattQELS 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.
Details	
>Collection Recycle	Controls the Recycle valve position during the collection. This may be set to “waste” or “recycle”. The default is “waste”. If you are using this collection as part of a sequence, keep this property set to “waste” and use one of the system method utilities at the end of the sequence to turn the Orbit™ (“recycle”) on. See <a href="#">Utilities (Collection Methods) on page 389</a> .
>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 utility methods as the last experiment method in the sequence. See <a href="#">Utilities (Collection Methods) on page 389</a> .
>Laser Power (%)	The percentage of full laser power that the instrument will be set to prior to collecting data.
>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. Do not set the COMET to Run if this collection will be used as part of a sequence. Instead use one of the system method utilities at the end of the sequence to turn the COMET on. See <a href="#">Utilities (Collection Methods) on page 389</a> .

Table 9-2: Basic Collection Properties (continued)

Field	Description
>Post-collection Optilab Purge	Change to Purge On to purge the Optilab at the end of a run. If you are using this collection as part of a sequence, set this property to Purge Off and use a utility method to purge the Optilab at the end of the sequence. See <a href="#">Utilities (Collection Methods) on page 389</a> .
>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 increase the Duration while an experiment is running. However, you cannot change the collection interval or auto-inject signals after collection has started.

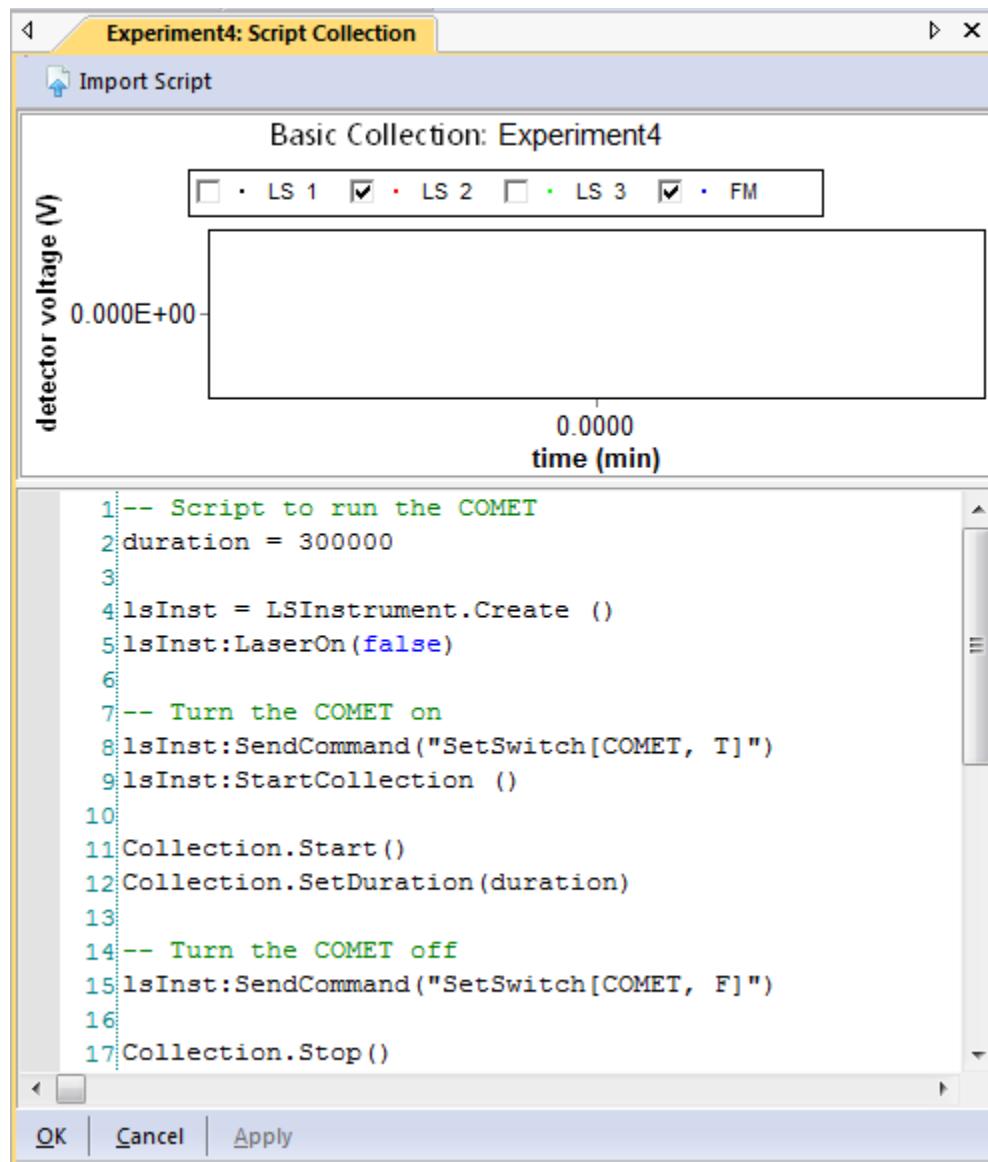
The “Basic Collection” procedure automatically closes all purge valves, switches recycle valves to waste (by default), and deactivates the COMET feature prior to data collection. The exception to the closing of purge valves is when absolute RI analysis is conducted, the Optilab purge valve is left open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

## Script Collection Procedure

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 8, 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 **Import Script** 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 box, 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 Procedure on page 203](#)
- [Alignment \(Interdetector Delay\) Procedure on page 206](#)
- [Band Broadening Procedure on page 208](#)
- [Normalization Procedure on page 213](#)
- [RI Calibration Procedure on page 218](#)
- [Absolute RI Calibration Procedure on page 223](#)
- [Calibrate Column Procedure on page 227](#)
- [Pulse Correction Procedure on page 232](#)
- [Dilution Factor Procedure on page 235](#)

### LS Calibration Procedure

A MALS instrument needs to be calibrated to enable ASTRA to convert its signals to Rayleigh ratios. This script collects the LS signal for 30 seconds, then turns off the laser and collects dark counts for 30 seconds. 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 MALS instrument 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 procedures 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 check the calibration constant for the MALS instrument in your own lab and compare the value you obtain with the value on the QC report to verify that the MALS instrument is in good working order.

A MALS instrument 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 photodiode.
- Have not calibrated in the last 6 months to 1 year.

See [Measured Quantities and Calibration](#) on page 428 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 **Select**.

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:

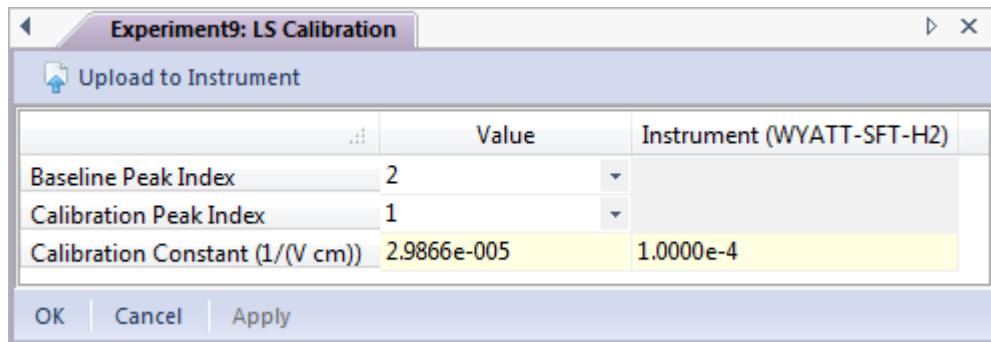
- It has a high and accurately determined Rayleigh ratio.
  - It is readily available in high purity.
  - Its refractive index is very similar to that of the flow cell windows.
9. Inject the solvent into the flow cell using a syringe pump. The solvent must be pure and free of particulates; we recommend that you use a 0.02  $\mu\text{m}$  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 the DAWN, detector 5 on a DAWN 8, and detector 2 on the miniDAWN or microDAWN) is stable. The variation in the signal should be 20  $\mu\text{V}$  or less.
  11. Choose **Processing→Run** to begin running the calibration experiment. The calibration constant is calculated and written to the MALS 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 MALS instrument in other experiments.

- Export the calibrated MALS profile as a profile, then import the profile whenever you create a new experiment. See [Chapter 13, Working with Profiles](#) for details.

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

### 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:



This procedure has the following properties:

*Table 9-3: 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. (DAWN, DAWN 8, microDAWN, and miniDAWN 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.

The calibration constant loaded on the instrument can be viewed with the **Experiment→Log** command. Double-click the first instrument (status) event.

## Alignment (Interdetector Delay) Procedure

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. This operation shifts the traces in time to align the data. As a result, the traces will no longer start and stop at the same time when viewed in a plot.

### 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.

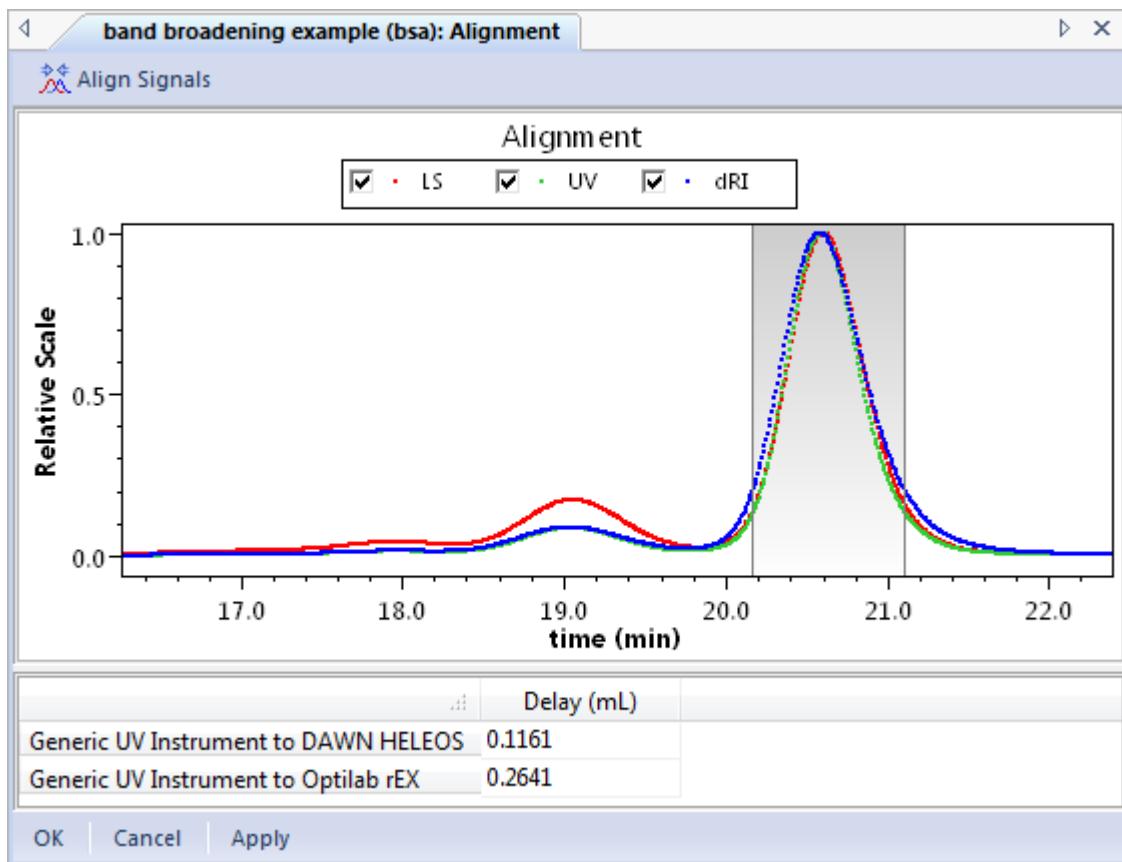
### How to Determine the Delay

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 for HPLC applications and 0.03-0.05 seconds for UHPLC applications. Intervals below these ranges are too noisy to provide good resolution.

3. The page for this procedure looks as follows.



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

4. Use your mouse to select a range that contains the peaks you want to use to align the detector delays.
5. Click the **Align Signals** button at the top of the procedure page. The delays between instruments are automatically calculated. This procedure stores the interdetector delays in the properties of the fluid connections in the experiment configuration.
6. 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 9-4: 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 fluid volume between the two instruments. The units are in mL.
Align Signals	Click this button at the top of the procedure display 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 208), 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.



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, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

## Band Broadening Procedure

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 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.

The current ASTRA band broadening model is used for all newer experiments. If you open an ASTRA V file, it will use the legacy ASTRA V band broadening model.

### **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.

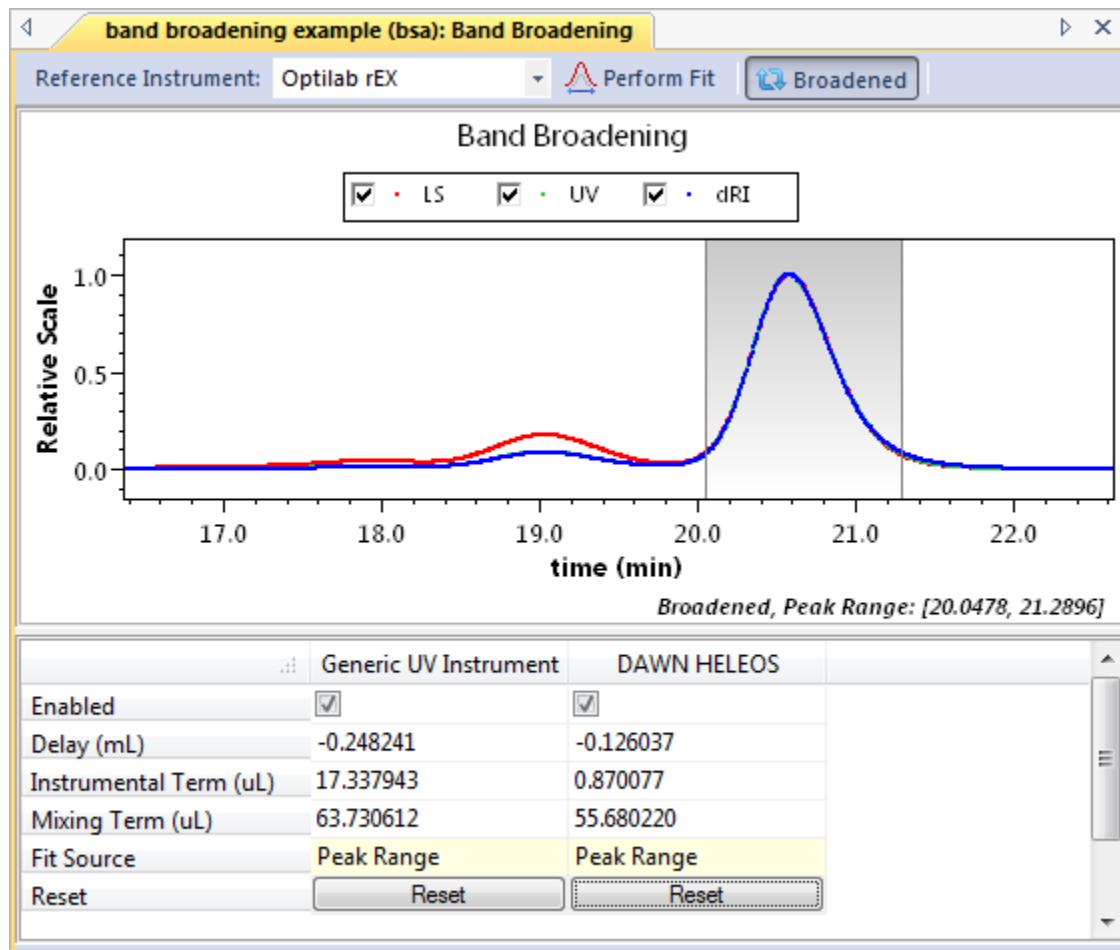
### **How to Determine Band Broadening**

Collect data from a narrow, monodisperse sample to determine 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 **Reference 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.
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 monodisperse 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 on the left edge of the peak (above the baseline) to the right edge of the peak (also above 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. 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 ( $1 \mu\text{l}$ ).
  - **No Viscometer and Despiking or Smoothing Performed:** The value of the instrumental term will generally be larger than  $1 \mu\text{l}$  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.
7. 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  $1 \mu\text{l}$  for the instrumental term and  $50 \mu\text{l}$  for the mixing term when using a DAWN or mini-DAWN instrument, or  $1 \mu\text{l}$  and  $5 \mu\text{L}$  when using a microDAWN.
  - **Viscometer Used:** Use seed values of  $20 \mu\text{l}$  for the instrumental term and  $20 \mu\text{l}$  for the mixing term.
8. Click **Perform Fit** again.
9. 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.
10. 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 9-5: Band Broadening Properties*

Field	Description
Reference Instrument	The instrument with the largest degree of broadening in the instrument series. This is typically the last instrument in the series.
Perform Fit	Click <b>Perform Fit</b> 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.

Table 9-5: Band Broadening Properties (continued)

Field	Description
Delay (mL)	The interdetector volume (or time) between the reference instrument and the instrument to broaden. 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.
Fit Source	Describes how the Instrumental Term and Mixing Term have been determined. Values are either “Manual” if default or manually entered parameters were used, “Peak Range” if a peak was used to determine Band Broadening parameters, or “Configuration” if the values were obtained from previously saved values in the configuration (for example, if the method used to collect the data has values set).
Reset	Click <b>Reset</b> to change the instrumental and mixing terms back to 1 $\mu\text{L}$ and 50 $\mu\text{L}$ (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**.

### Typical Band Broadening Values

Typical band broadening values for most instruments are as follows:

- Instrumental Term: 1
- Mixing Term: 50

Typical band broadening values for low-volume instruments, such as the microDAWN and microOptilab, are as follows:

- Instrumental Term: 1
- Mixing Term: 5

## Normalization Procedure

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, detector 2 on a miniDAWN or microDAWN, and detector 11 on all other DAWN instruments.) 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.
- Whenever you reinstall the DAWN flow cell.

See [Normalization on page 432](#) 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 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 MALS detector (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 not good practice to normalize with a broad distribution (polydispersity greater than 1.2) or unknown sample. Always use a relatively narrow distribution, low molar mass sample for normalization.

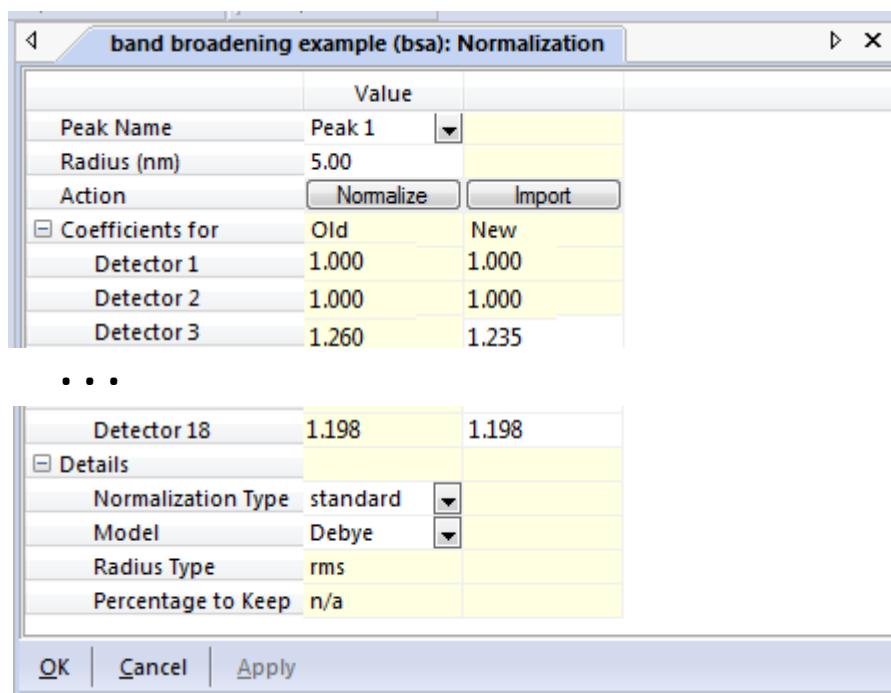
It is usually appropriate to use the same sample for normalization that you used to determine the delay volumes between the instruments. If there are absolutely no narrow standards available in the solvent you are using, you may be able to normalize properly by setting the peak region to include only 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 30,000 g/mol polystyrene in toluene or THF, or 10,000 g/mol dextran, pullulan or PEO in water, works well. All of these have rms radii of about 5 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.



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, the radius is a “radius” instead of an rms 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, miniDAWN, or microDAWN.

## 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 the Experiment tree 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 9-6: Normalization Properties*

Field	Description
Peak Name	Select the peak that corresponds to your normalization standard.
Radius	The radius of the normalization standard in nm.
Action	Click the <b>Normalize</b> button in this row when you are ready. Click the <b>Import</b> button to import normalization coefficients from another experiment.
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"> <li>“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.</li> <li>“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.</li> </ul>
Model	Displays the fitting model being used.
Radius Type	The type of radius specified. Options are radius, rms, and hydrodynamic. If you are using a Lorenz-Mie or sphere model for the sample, the radius is a “radius” instead of an rms 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 261](#)) 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 260) 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.

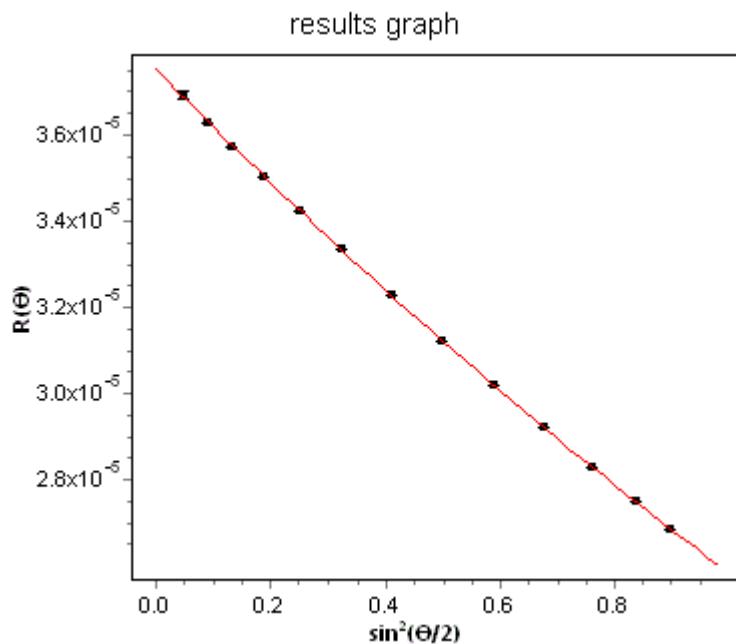


Figure 9-1: Analysis plot illustrating accurate normalization coefficients for all angles

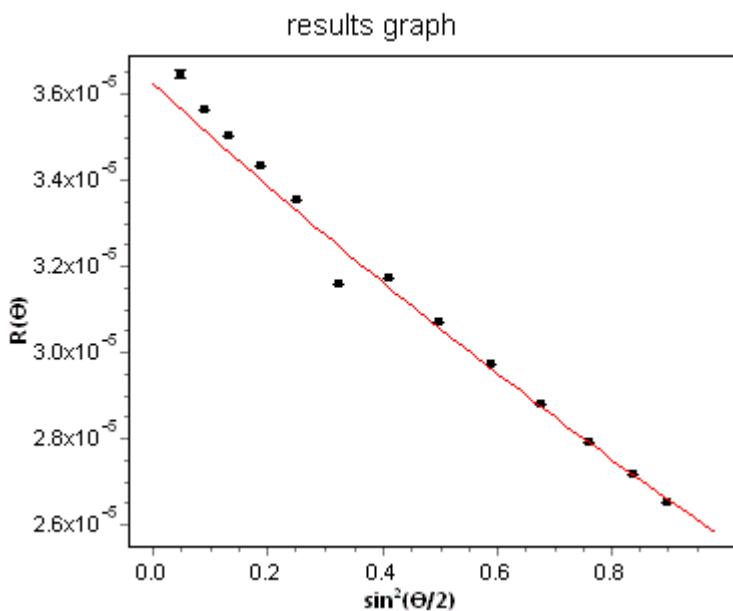


Figure 9-2: Analysis plot illustrating incorrect normalization coefficient for detector 9

## RI Calibration Procedure

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  $dn/dc$ .

The Wyatt Optilab is intended to communicate digitally with ASTRA in refractive index units. As such the Optilab dRI calibration constant is an internal constant stored on-board the Optilab itself.

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.

### When to Calibrate

Optilab instruments manufactured by Wyatt Technology 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 recommended 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. Setting up and performing a dRI calibration is described in detail in your instrument user's guide.

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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 <a href="#">RI Calibration from Peak Procedure on page 279</a> for more information.
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### Collecting Data

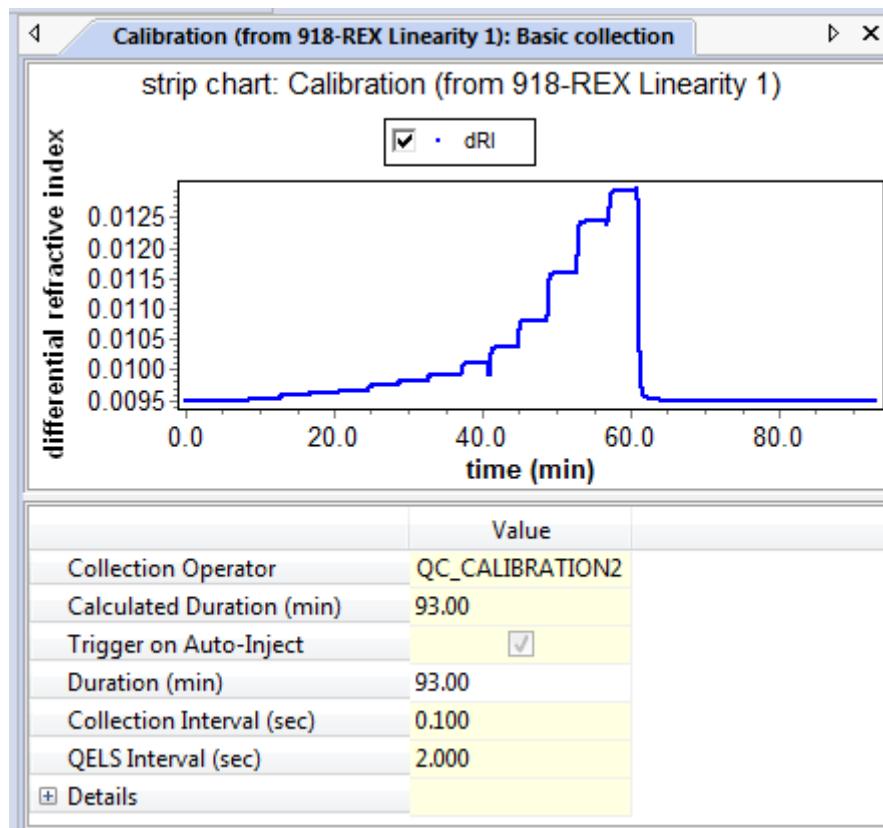
The dRI instrument's sample and reference cells should be flushed with high purity water (first solvent to be analyzed) for several minutes before making measurements. Confirm that the temperature is stable, and activate the instrument's purge feature while flowing pure water for several minutes. Deactivate the purge, flow with water for several more minutes, then zero the instrument. Please see your instrument user's guide for detailed information on how to set up a dRI calibration experiment.

Collect data using ASTRA 8 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.

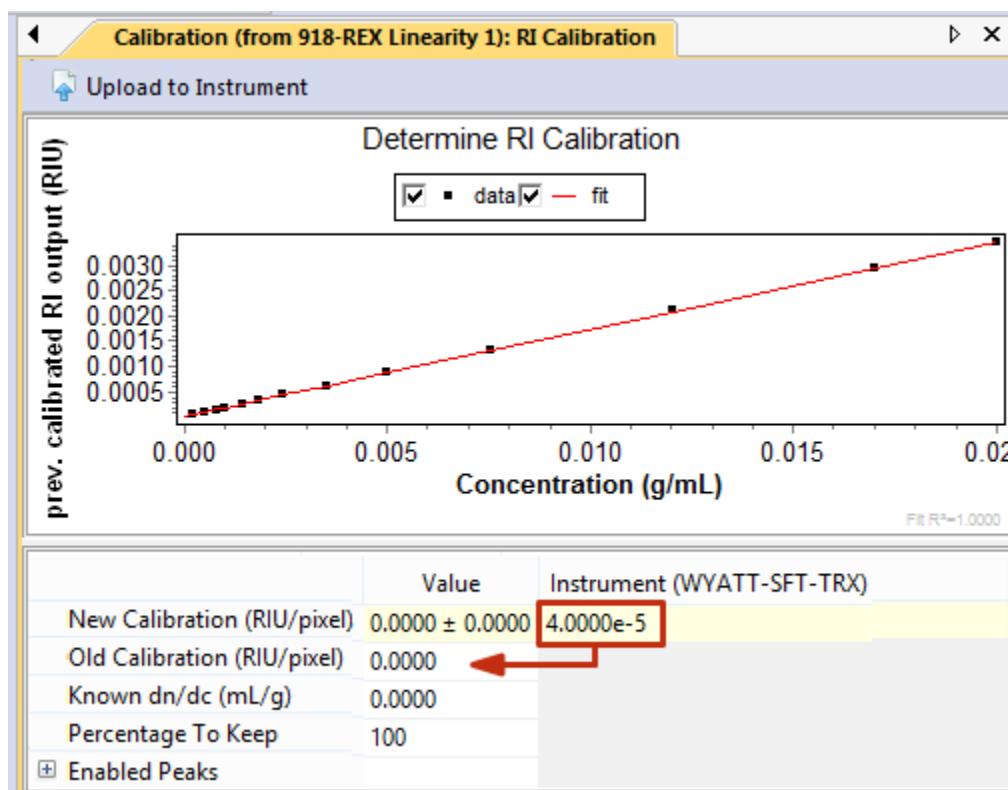


## Data Processing

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 9-7: RI Calibration Properties

Field	Description
New Calibration	The resulting calibration constant.
Old Calibration	The previous calibration constant.
Known dn/dc	The known dn/dc value for the calibration standard used.
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. Copy the value in the Instrument column of the **New Calibration** row to the Value column in the **Old Calibration** row as shown in the previous figure. This value is also available on the front panel of the instrument.
7. Enter the known *dn/dc* into the row titled Known dn/dc (mL/g) and click **Apply**.
8. 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. (Optilab instruments only.)
9. Expand the **Results** node of the experiment and double-click the **Report (summary)** line. The report displays the new Calibration constant.

### 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 Procedure

You can calibrate the Optilab aRI measurement as described in this section. This procedure is also summarized in the *Optilab 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 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) should be used. See Table 9-8 for recommendations. It is imperative that each solvent infused into the Optilab must be miscible with the solvent that it replaces.

The Optilab 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 9-8: 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
Tetrahydro-furan	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 is flushed with a new solvent.

### Collecting Data for Optilab aRI Calibration

- 1.** The Optilab 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 with the Purge ON for all data collection (the Purge indicator will be yellow on the **Main** tab).
- 2.** Collect data using the ASTRA software as follows:
  - a.** Start the ASTRA software and select **File**→**New**→**Experiment from Method**.

- b. In the dialog that opens, select the **System > Methods > RI Measurement > Calibration > 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. In the Absolute RI calibration procedure in ASTRA, copy the numbers from the second column in the **New Abs. RI Calibration** and **New Abs. RI Offset** rows to the first column in the **Old Abs. RI Calibration** and **Old Abs. RI Offset** rows as indicated in Figure 9-3. (Because ASTRA's calibration calculation is an adjustment, it needs the instrument's current pre-calibration constants.) These numbers are also shown on the Optilab instrument's front panel in Constants on the System tab. Click **OK** to close the window.

**Note:** You must perform this step (e) before collecting data; you will not be able to edit the calibration constant after collection, which would result in incorrect new calibration constants.

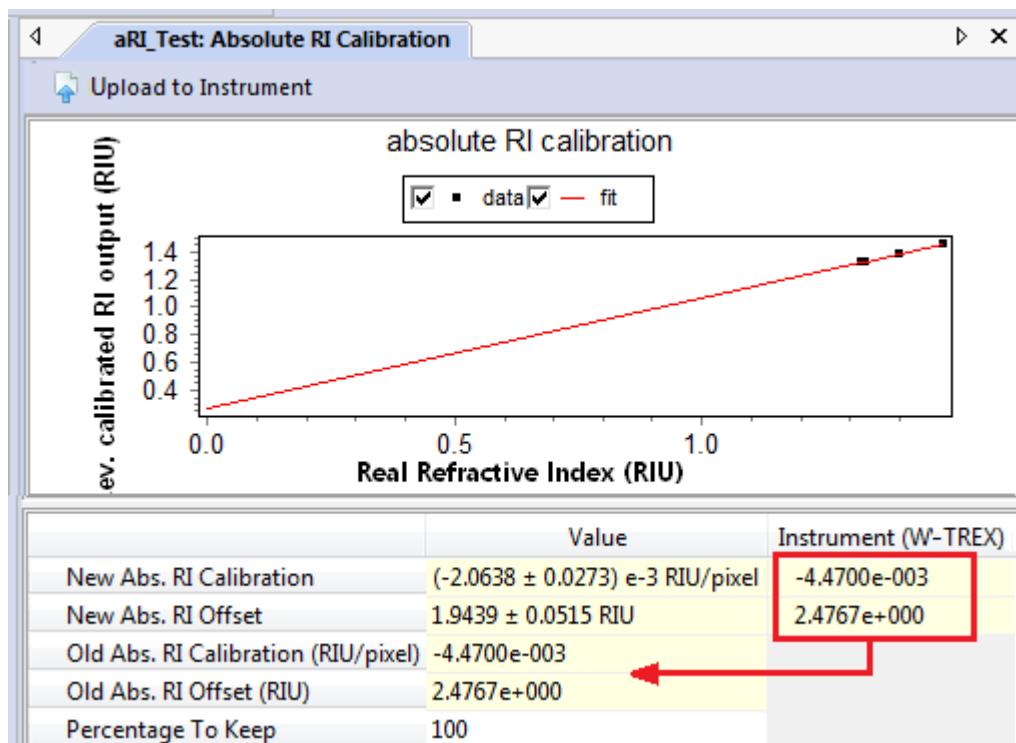


Figure 9-3: Copying New RI Values to Old Value Fields

- f. Run the experiment.

## Introduce Solvents

1. Flow the pure solvents directly into the Optilab 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 **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 V. 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 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. Open the **Absolute RI Calibration** procedure. Enter the known aRI value for each solvent peak in the **Peaks Real RI** list. Make sure that you specify the correct aRI value for the Optilab's operating wavelength. Table 9-8 lists aRI values for several solvents at various wavelengths.
3. In the **Absolute RI Calibration** procedure, confirm that the instrument's values from the second column in the **New Abs. RI Calibration** and **New Abs. RI Offset** rows match the first column in the **Old Abs. RI Calibration** and **Old Abs. RI Offset** rows as indicated in Figure 9-3. Click **Apply**.
4. If you are satisfied with the new calibration constant, you can click the **Upload to Instrument** button above the graph to update the instrument with the newly computed absolute RI calibration constant. (Optilab 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, tab to the **Constants** button and press Enter. Enter the new values into the Optilab in the Constants dialog. Tab to the **Apply** or **OK** button and press Enter. Tab to the **Close** button and press Enter. The Optilab 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 Optilab with ethanol as described in the section [Introduce Solvents on page 225](#).

### Setting Absolute RI Calibration Properties

This procedure has the following properties:

*Table 9-9: 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. The right column displays the current value on the instrument if it is connected.
New Abs. RI Offset	The resulting absolute RI offset in RIU. The right column displays the current value on the instrument if it is connected.
Old Abs. RI Calibration	The previously used absolute RI calibration constant from the Optilab.
Old Abs. RI Offset	The previously used absolute RI offset from the Optilab.
Diff. RI Calibration	The differential RI calibration constant from which the absolute value was determined (from the Optilab).
Diff. RI Offset	The differential RI offset from which the absolute value was determined (from the Optilab).
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.
Peaks Real RI	If you have multiple peaks, you can expand this list to see and modify the known aRI values for the solvents. See Table 9-8 for a list of the aRI values of common solvents at various wavelengths.
Peaks Enabled	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. (Optilab 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 Security Pack), and open the “absolute RI calibration.afe8” experiment in the Sample Data > Analyzed Experiments folder.

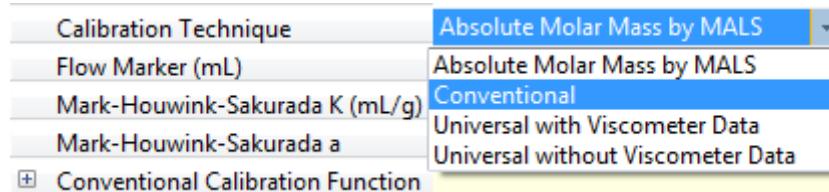
You can place this procedure with other analysis procedures and after all the transformation procedures. A procedure list can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine the dn/dc or RI calibration in a procedure, only the first one is valid.

## Calibrate Column Procedure

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 171).

Two main column calibration techniques are available and can be selected in the Generic Column configuration. “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.




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**Note:** Select “Absolute Molar Mass by MALS” as the calibration technique to have ASTRA determine absolute molar masses from light-scattering without creating a new method.

---

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 Security Pack) and opening the conventional calibration or universal calibration \*.afe8 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.

### 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.



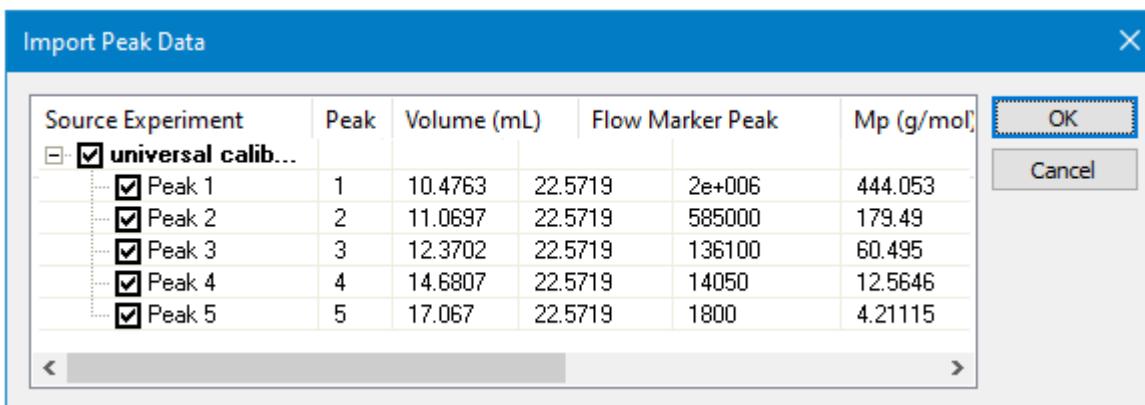
In an experiment, this procedure must come after the Baselines and Peaks procedures. If multiple detectors are used, this procedure must also come after the Alignment and Band Broadening procedures.

### 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**.



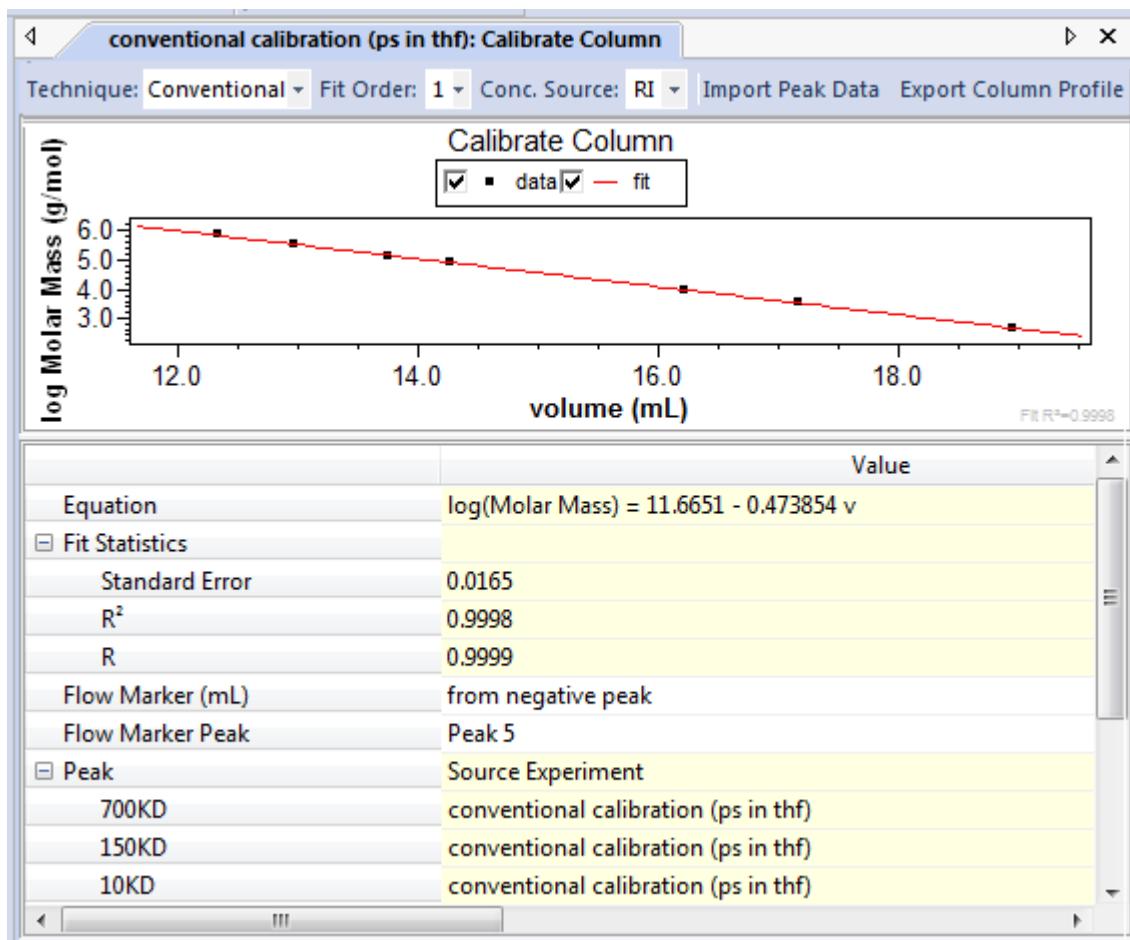
- 10.** Confirm that the peaks have been added to the list and adjust the curve fit order. Then, click **Apply** to store the imported peaks.
- 11.** Click **Export** in the Calibrate Column page to open the Save Calibrated Column Profile dialog. (You can click **Export** only after you have clicked **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 171).

## 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 9-10: Column Calibration Properties*

Field	Description
Technique	Above the graph, select the type of column calibration to perform. 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 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.
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 <a href="#">Experiment Configuration on page 147</a> .
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 <sup>2</sup>	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.

Table 9-10: Column Calibration Properties (continued)

Field	Description
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"> <li>- None- no flow marker is to be used. The flow marker value is set to zero.</li> <li>- Enter value- enter the flow marker value in mL in the cell to the right of the drop-down.</li> <li>- 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.</li> <li>- 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.</li> </ul>
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.

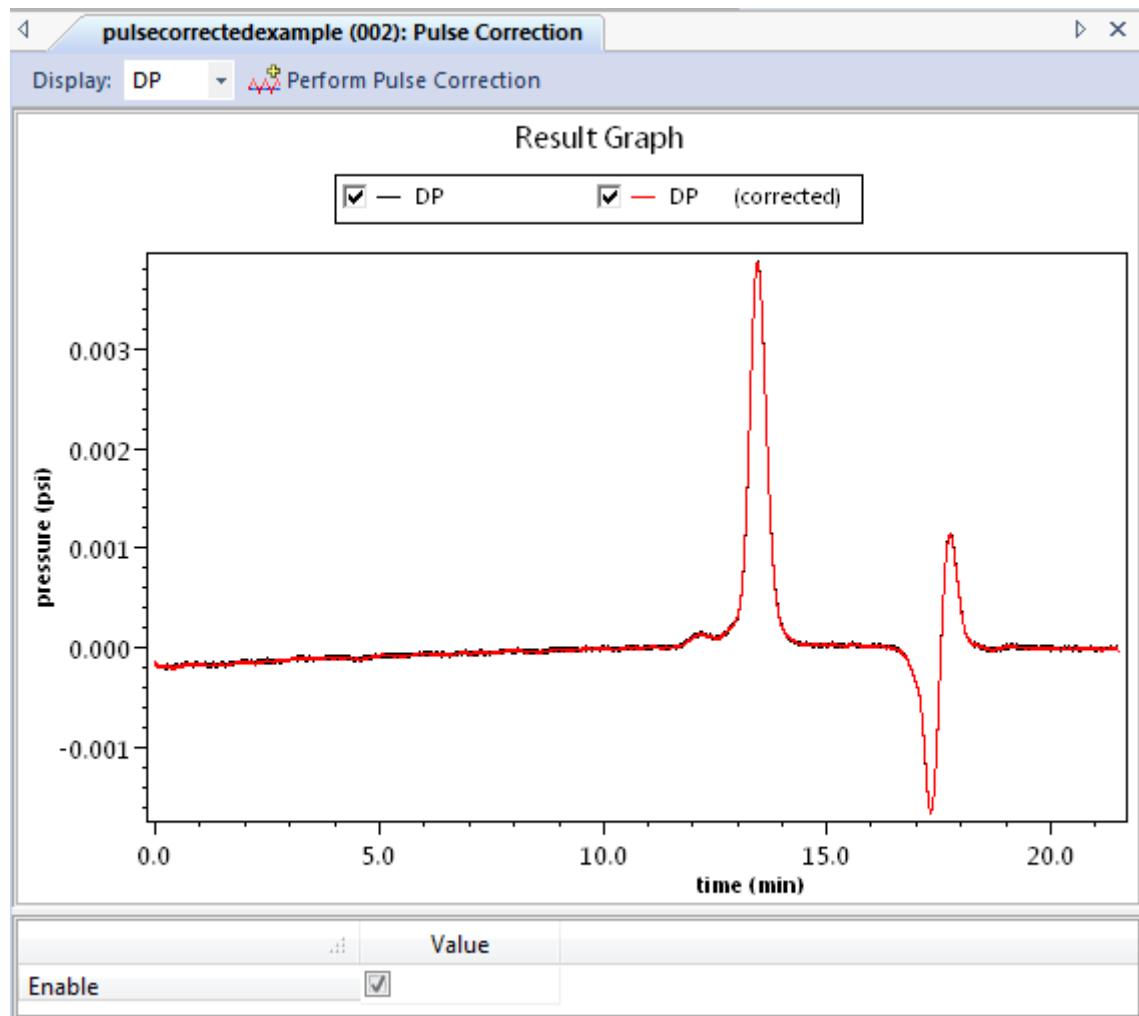
## Pulse Correction Procedure

This procedure identifies fluctuations in the signals caused by pump pulses based on the pump pulsation observed in the IP trace (from a ViscoStar instrument only). It removes these pulses from every data trace.

To apply this procedure if you are *not* using Experiment Builder Mode, follow these steps:

1. Right-click on the **Configuration** node and select **Pulse Correction**.
2. Click **Perform Pulse Correction** at the top of the Pulse Correction window.

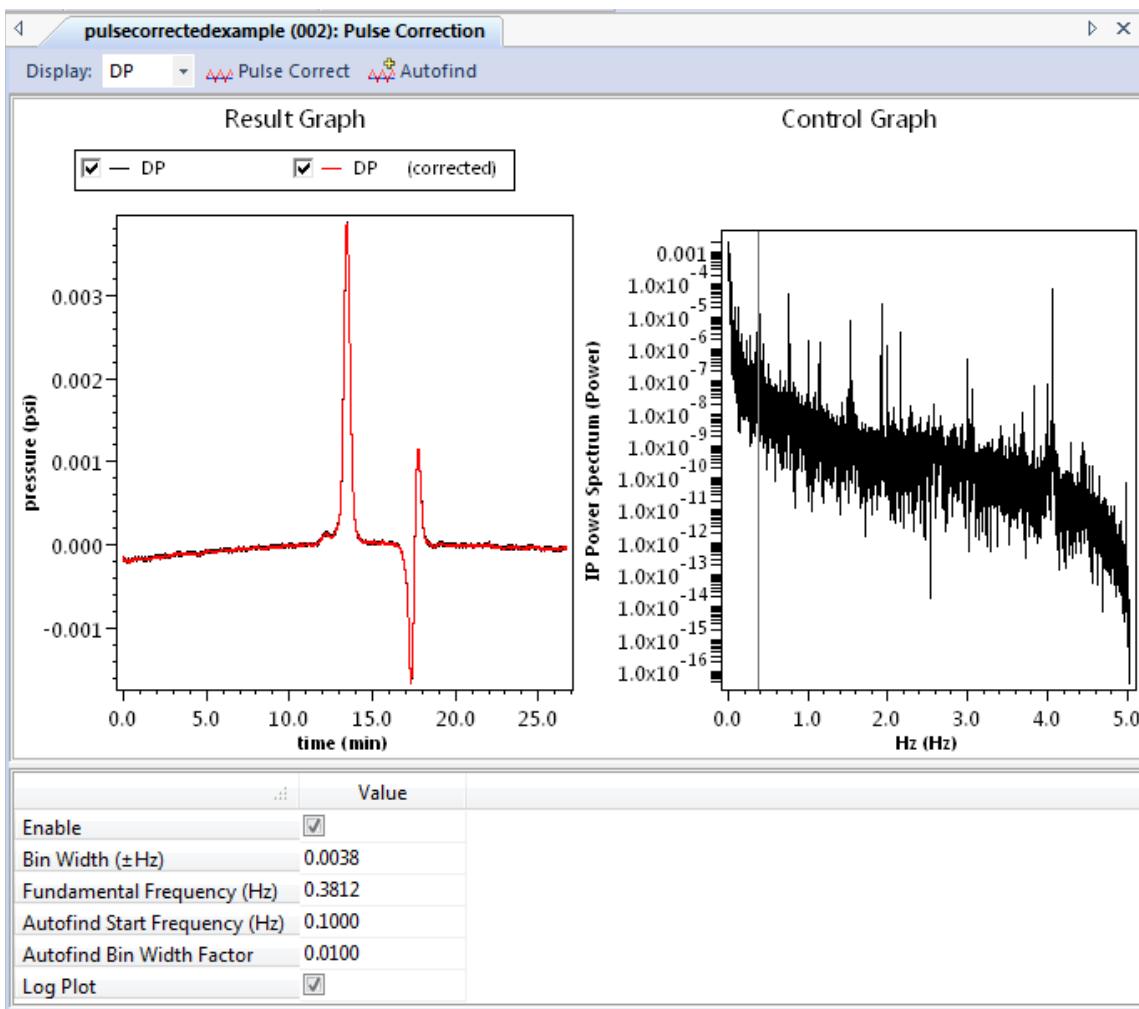
3. Use the **Display** drop-down list to select which signal you would like to display. The Result Graph displays both the corrected and uncorrected traces for comparison.
4. Use the **Enable** checkbox below the graph to toggle whether the pulse correction should be applied to the data processing. Checking or unchecking the box also enables or disables pulse correction in the pump configuration.



Additional parameters are shown if you are using Experiment Builder Mode (see [User Modes on page 62](#)). Follow these steps:

1. In Experiment Builder Mode, the Pulse Correction procedure is visible in the Procedures list for the experiment.
2. Use the **Display** drop-down list to select which signal you would like to display in the Results Graph (left graph).

3. The Control Graph (right graph) shows the power spectrum of the IP signal. Use this graph to select the peak of the fundamental frequency of the pump pulsation. Select the peak manually or click **Autofind** in the top toolbar. Setting the frequency sets the parameters described in Table 9-11.
4. Click **Pulse Correct** in the top toolbar after setting the fundamental frequency.
5. Use the **Enable** checkbox below the graph to toggle whether the pulse correction should be applied to the data processing. Checking or unchecking the box also enables or disables pulse correction in the pump configuration.




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**Note:** Changing the pump flow rate changes the fundamental frequency and requires that it be recalculated. A banner at the top of the window notifies you if the flow rate has changed since the fundamental frequency was set.

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<b>Note:</b>	We recommend that you collect at least 15 minutes of baseline data in order to provide enough data for this calculation. This is a recommendation, not a requirement.
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This procedure has the following properties in Experiment Builder Mode:

Table 9-11: Pulse Correction Properties

Field	Description
Bin Width (+/- Hz)	The width of the bin around the fundamental frequency that will be removed. This bin is centered around the fundamental frequency. The default Bin Width is 1% of the Fundamental Frequency value. You can modify this property.
Fundamental Frequency (Hz)	The frequency with the maximum power within the defined peak boundaries. You can set this by typing a value in Hz or by clicking and dragging in the Control Graph. ASTRA finds the peak automatically if you click Autofind.
Autofind Start Frequency	This parameter affects where the autofind algorithm begins its search for the fundamental frequency in the Control Graph. You can change this value if Autofind consistently fails to determine a fundamental frequency.
Autofind Bin Width Factor	The Bin Width is determined by multiplying this factor by the fundamental frequency. The default Bin Width is 1% of the Fundamental Frequency value. You can change the default percentage here.

## Dilution Factor Procedure

This procedure calculates the dilution factor for a viscometer by taking ratio of the area under the sample peak ( $a_1$ ) and the area under the breakthrough peak ( $a_2$ , the peak corresponding to the sample that goes through the delay columns) as follows:

$$\text{Dilution factor} = a_1 / (a_1 + a_2)$$

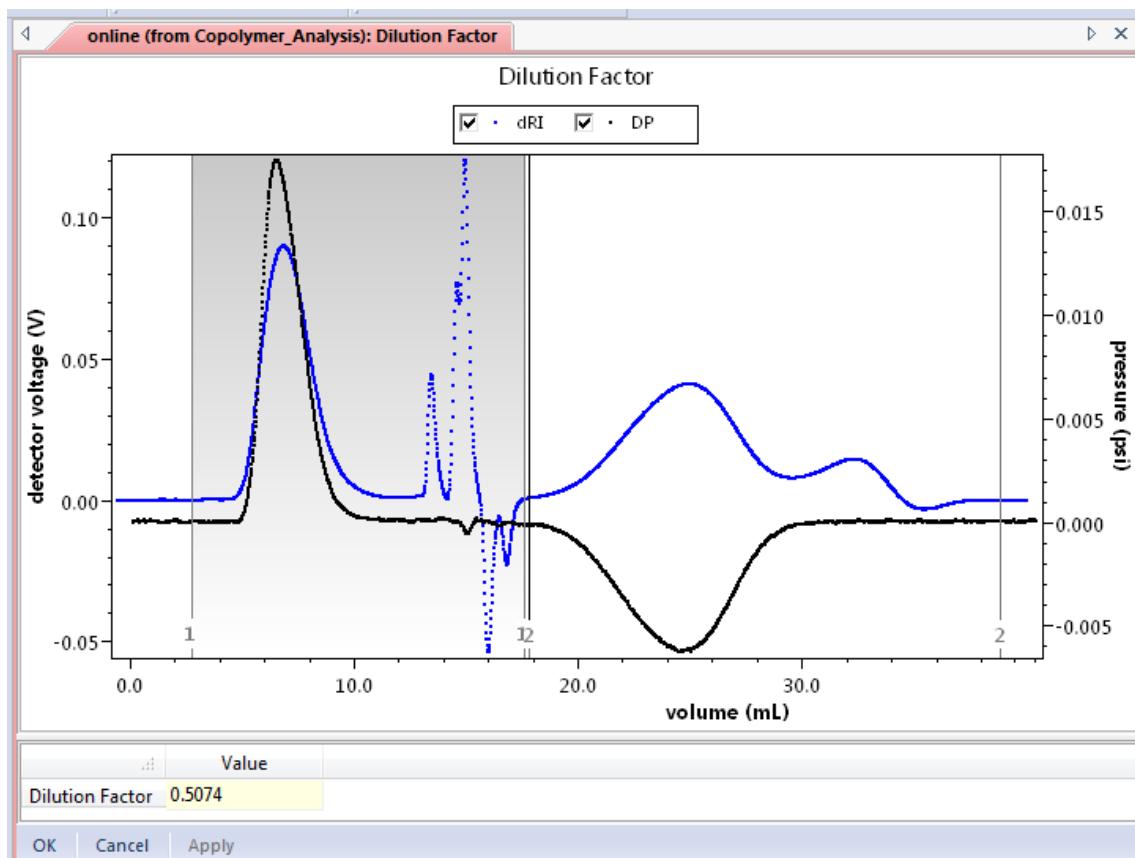
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<b>Note:</b>	To use this procedure, the concentration detector <i>must</i> be plumbed downstream of the ViscoStar.
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To apply this procedure, follow these steps:

1. Right-click on the experiment's **Configuration** node and select **Dilution Factor**. The graph for this procedure shows both the concentration source signal (that is, the dRI or UV signal) and the DP signal.
2. Draw two peaks only. ASTRA determines which peak is which by assuming the most upstream peak is the sample peak.
  - **Sample peak:** This peak should encompass the entire sample peak and the solvent peak as seen in the concentration source signal (such as the RI signal).
  - **Breakthrough peak:** This peak should encompass the entire concentration source signal corresponding to the negative peak in the DP signal. The breakthrough peak indicates when the sample and solvent went through the delay columns in the ViscoStar.



3. After you define two peaks, a dilution factor is calculated and displayed in the properties below the graph.
4. Click **Apply**. The experiment will be reprocessed using the new dilution factor. The Dilution Factor will be stored in the viscometer's configuration profile.

## 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 Procedure on page 237](#)
- [Smoothing Procedure on page 238](#)
- [Baselines Procedure on page 240](#)
- [Blank Baseline Subtraction Procedure on page 244](#)
- [Peaks Procedure on page 247](#)
- [Broaden Procedure on page 255](#)
- [Convert to Physical Units Procedure on page 255](#)
- [Convert to Concentration Procedure on page 256](#)
- [Convert Specific to Intrinsic Viscosity Procedure on page 256](#)
- [Results Fitting Procedure on page 257](#)

### Despiking Procedure

The Despiking procedure removes spurious noise spikes from the collected data. Such spikes are likely caused by dust in the solvent.

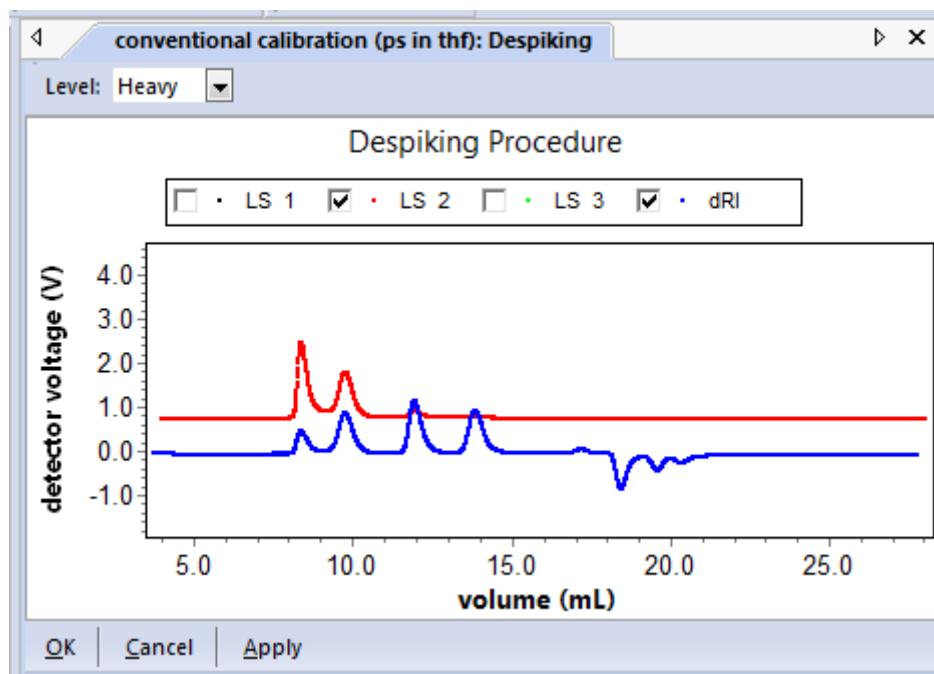
You can set the property for this procedure before running the experiment, or you can modify it after running the experiment and re-run the experiment to see the effects of changing the setting.

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 (page 389) 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 9-12: Despiking Properties*

Field	Description
Level	Choose the degree of despiking. The options are None, Normal, and Heavy. The default is Normal.

### Smoothing Procedure

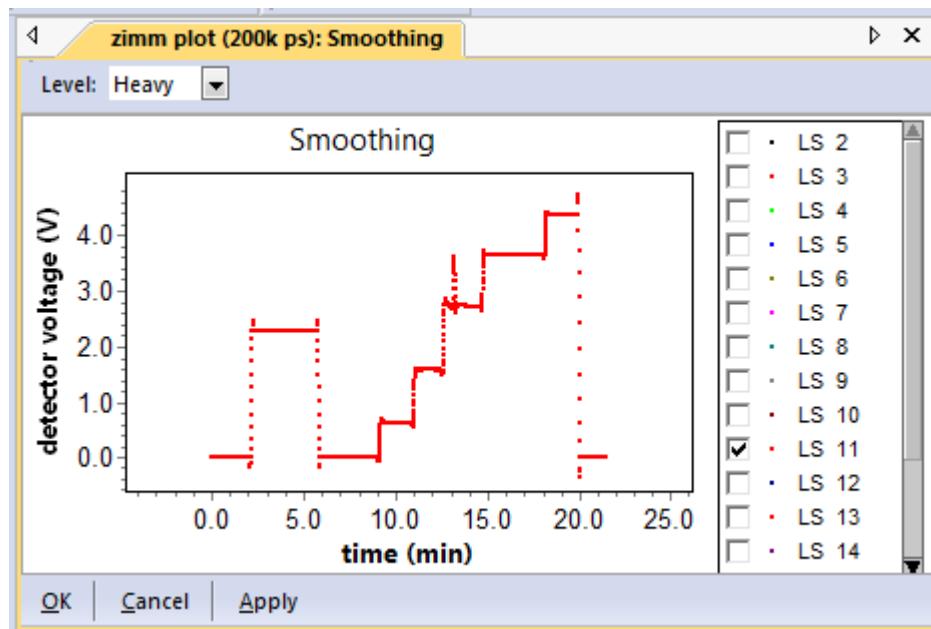
The Smoothing procedure smooths noisy data. Smoothing can be useful in certain circumstances, but in general it is better to remove the source of the noise, such as particulates in the mobile phase and pump pulsations, rather than to smooth the data.

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 9-13: 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 molar mass 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 sample.

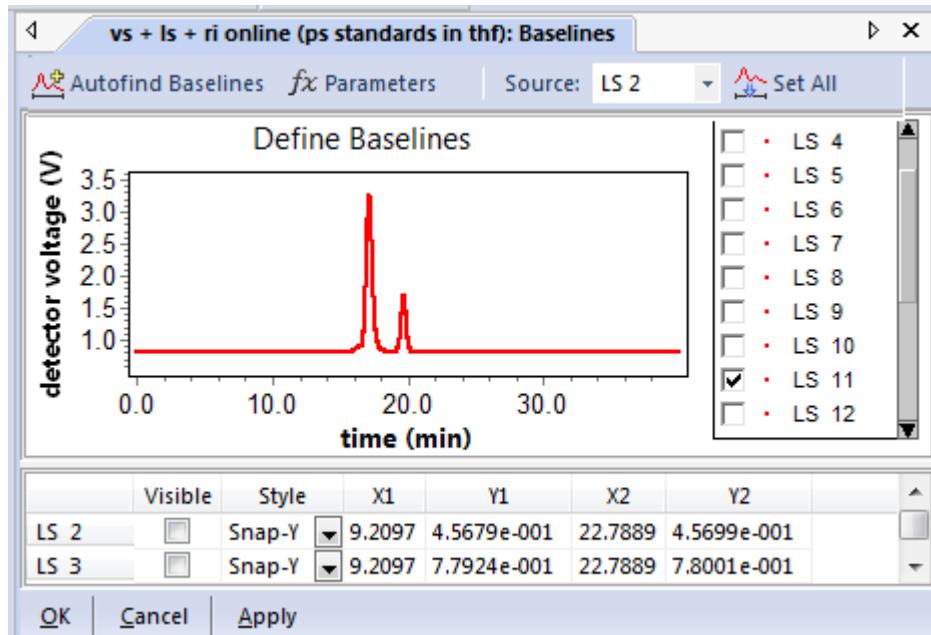
## Baselines Procedure

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.



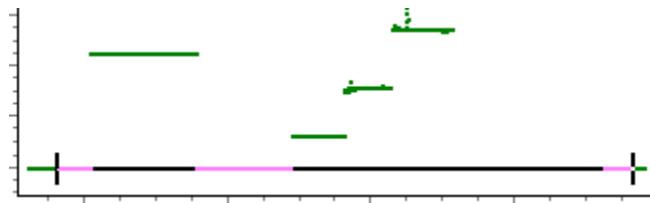
2. Click the **Autofind Baselines** **Autofind Baselines** button above the graph. Clicking this button automatically detects and applies the baseline for each of the source signals.
3. If you want finer control over how baselines are detected by the **Find Baselines** button, click the **Parameters** **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 243](#).

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.

---

<b>Note:</b>	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.
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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:

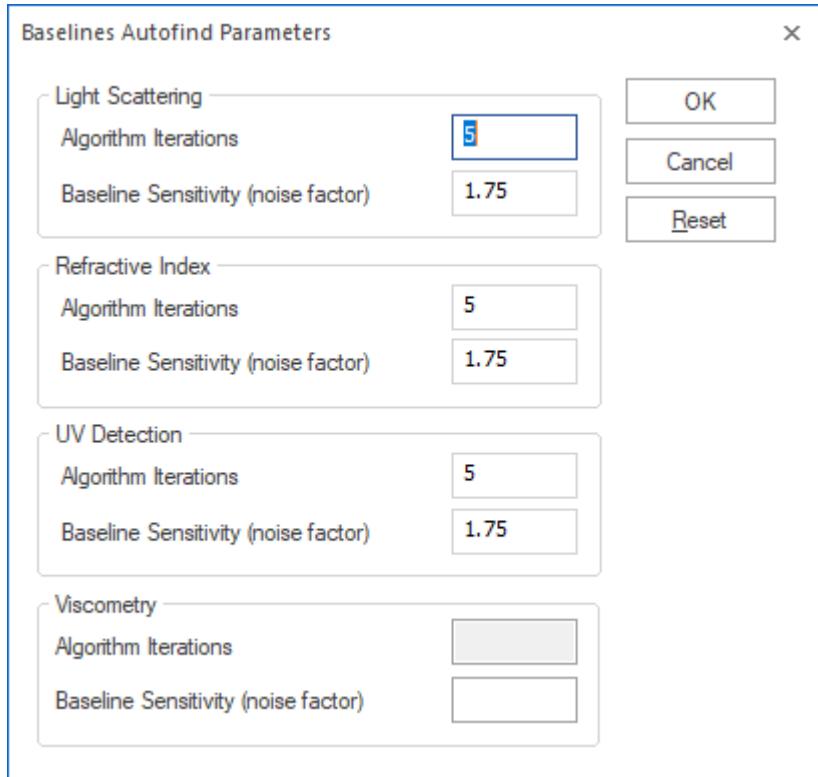
*Table 9-14: Baseline Properties*

Field	Description
Visible	This box is checked if the data from this detector is shown in the graph.
Style	“None” indicates 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.
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 (page 389) reports baseline detector noise and drift.

## Parameters for Finding Baselines

If you want finer control over how baselines are detected, click the **fx Parameters** **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:

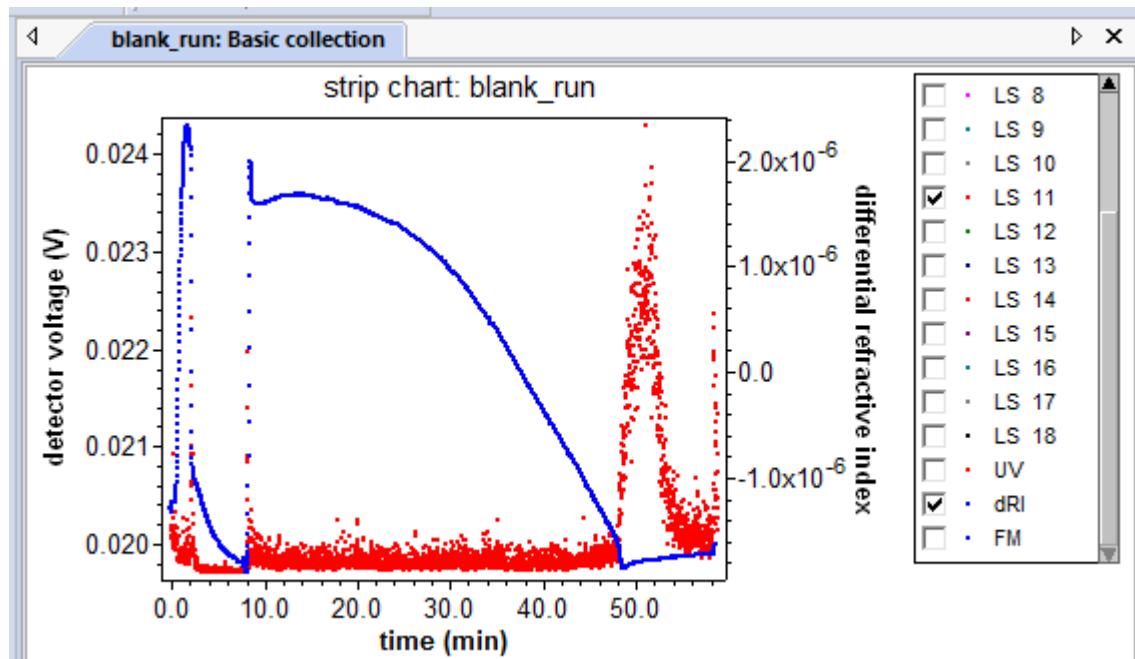
- **Baseline Sensitivity:** Adjusting this parameter allows you to tune how ASTRA differentiates between outliers or peak regions and actual baselines. The value is the maximum distance from the mean y-axis value that a point can have and still be considered part of the “baseline”. The units of this 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.

## Blank Baseline Subtraction Procedure

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. This feature is most commonly used with FFF-MALS applications.

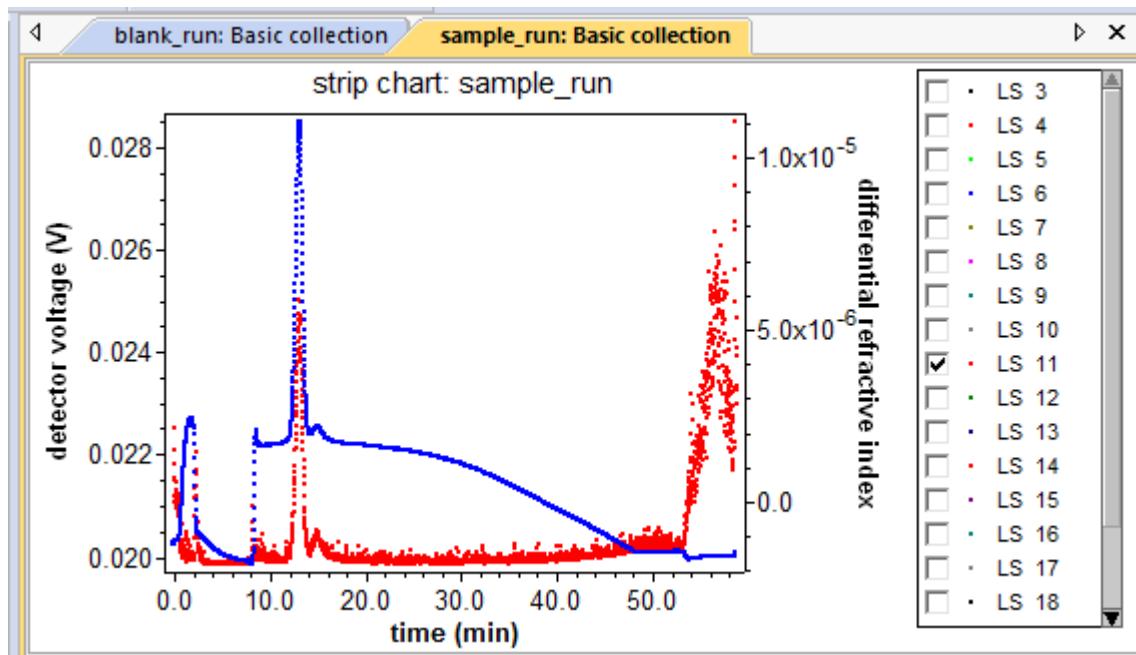
When the baseline changes are caused by a repeatable set of conditions, such as Eclipse instrument flow adjustments, a set of “blank” data can be collected that will model these effects. By subtracting this “blank” run, we can generate well-behaved result data.

1. Collect sample “blank” data for the experiment by running a solvent 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):

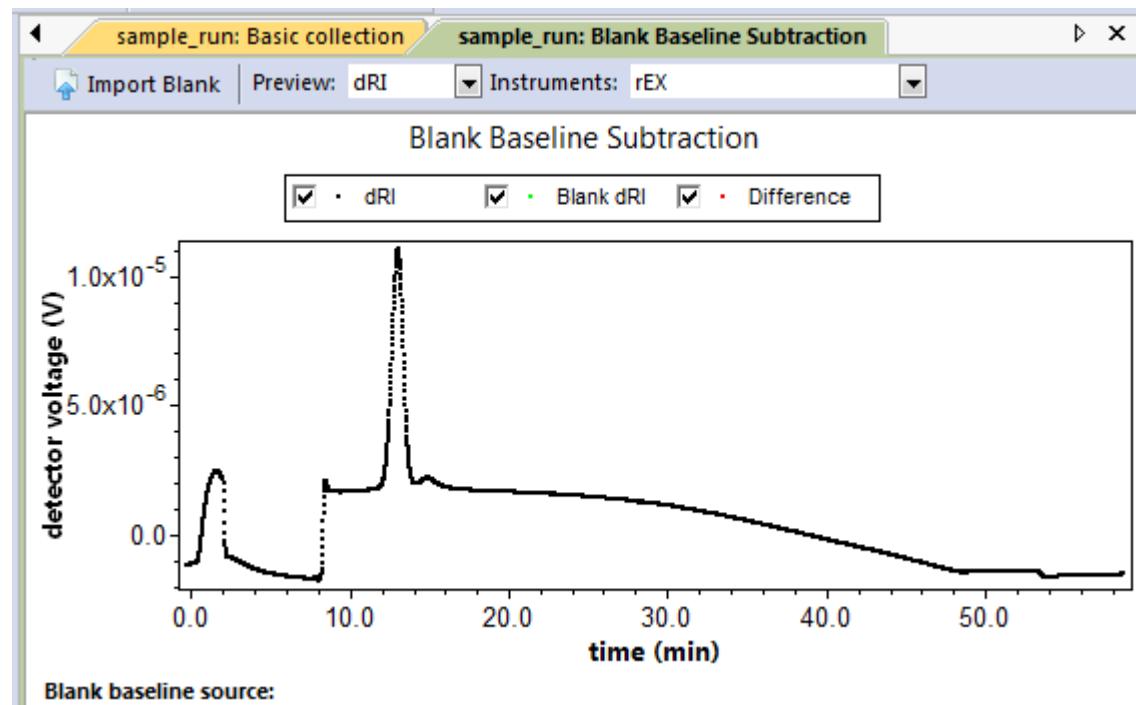


2. Choose **File→New→Experiment from Method**, and use the “online” experiment in the System > Methods > Light Scattering > Baseline Subtraction folder to create a new experiment.

3. Run a data collection for the samples.

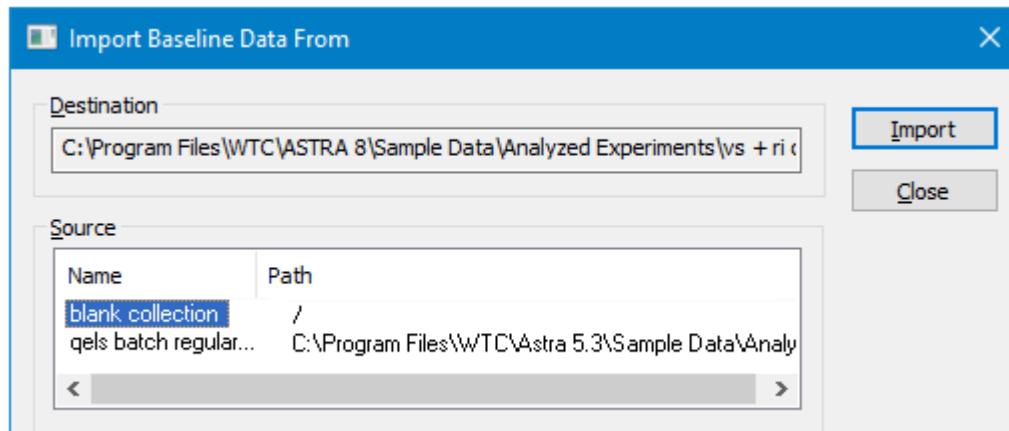


4. Keep both the “blank” and “sample” experiments open in ASTRA.
5. In the “sample” experiment, open the “Blank Baseline Subtraction” procedure.

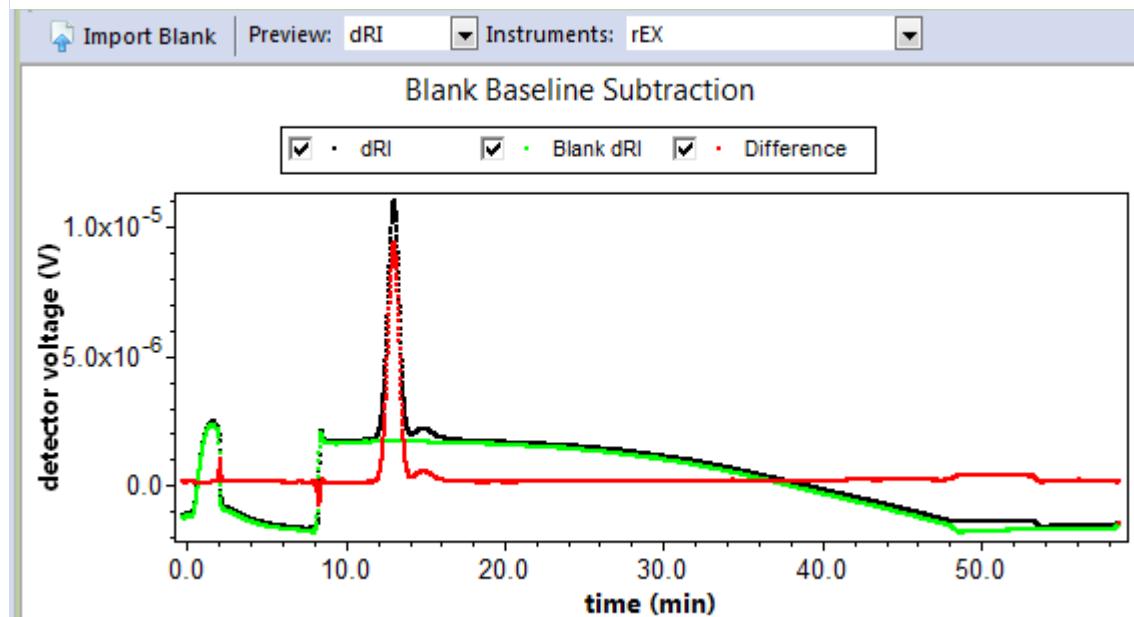


6. Click the **Import Blank** button above the graph to open the Import Baseline Data From dialog.

- In the Source list, select the “blank” experiment and click **Import**. A compatible data set from the experiment you select is imported.



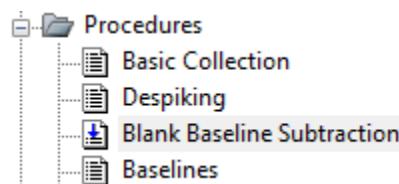
- You can view the results of the subtraction in the Blank Baseline Subtraction procedure view. Use the **Preview** drop-down to see how the blank subtraction affects a particular signal.



- 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.

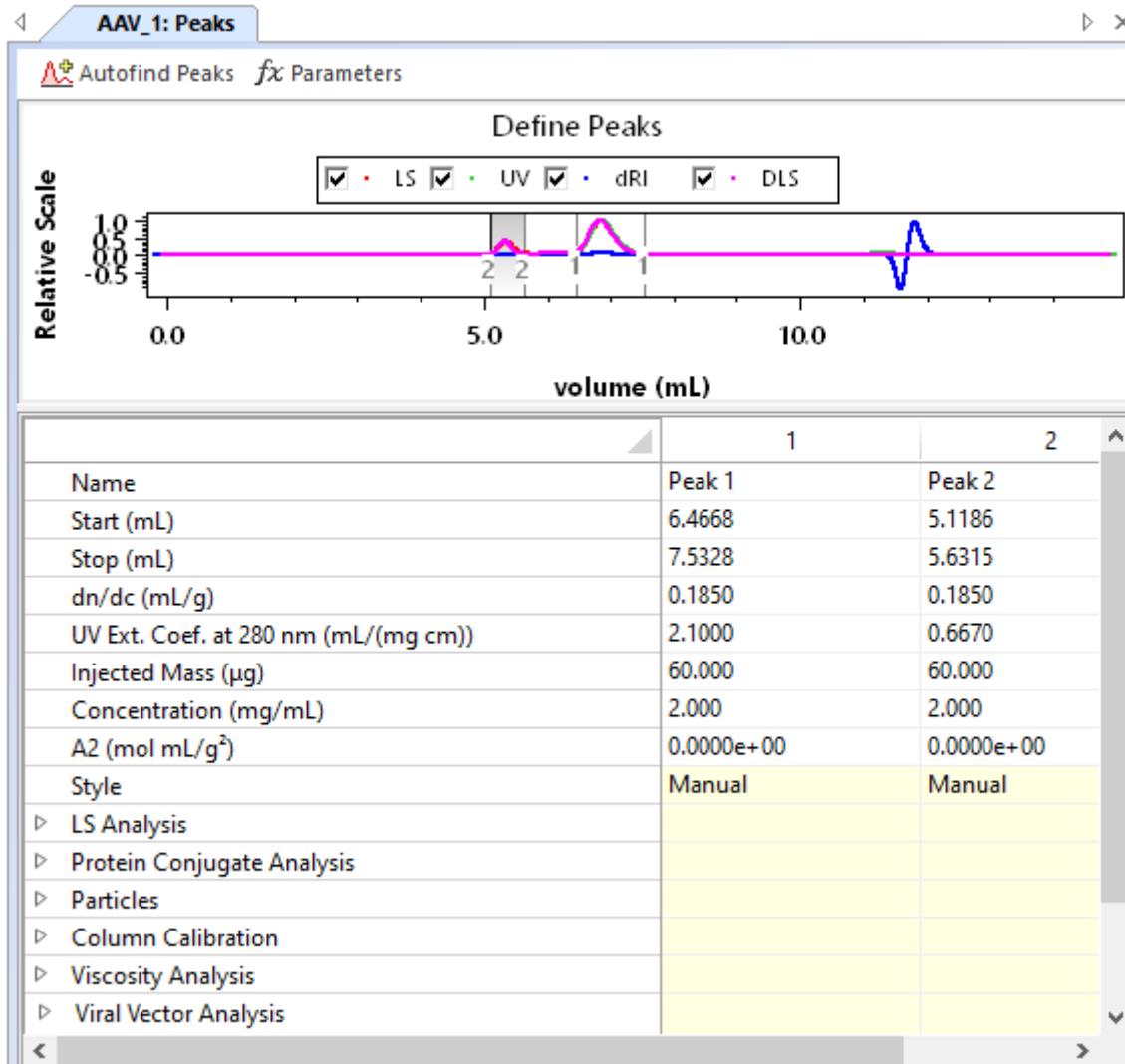


## Peaks Procedure

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.



2. Click the **Autofind Peaks** button above the graph. Clicking this button automatically detects and applies peaks to the data.

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**Note:** **Autofind Peaks** can only be used with online (that is, fractionated) experiments. It does not work for batch experiments.

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3. If you want finer control over how peaks are detected, click the **fx Parameters** **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 254](#).

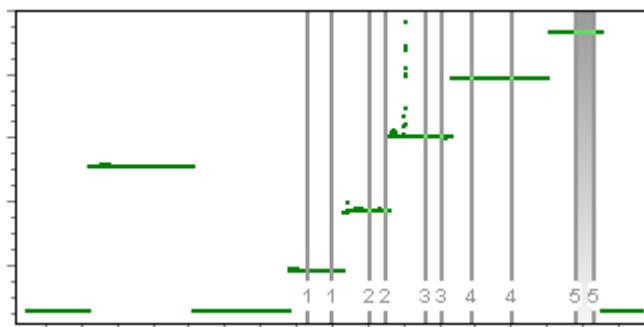
If you are running a batch experiment or do not want to use the automatic peak detection, you can manually set peaks as follows:

1. 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 blue, UV data is green, viscosity data is gray, and DLS data is magenta. Multiple detector angles use colors assigned by the graphing system.
2. Click on the graph to add a peak range to the collected data.
3. 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).

4. 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 to 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. Right-click and choose **Fit to Peaks** to zoom to see only the portion of the graph where peaks are marked.

5. If you selected “Forward Monitor” in the Divide by Laser Monitor field or the configuration for your light scattering instrument (see page 150), you should create a “peak” for pure solvent and specify the number of this peak in the [Experiment Configuration on page 147](#). 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 9-15 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 9-15: Peaks 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. Alternatively, 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 Procedure RI Calibration Procedure UV Extinction from RI Procedure Protein Conjugate Analysis Procedure Viral Vector Analysis Procedure (for the protein capsid) 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 Procedure Protein Conjugate Analysis Procedure Viral Vector Analysis (for protein capsid at active wavelength) If you are performing a Protein Conjugate Analysis, this parameter corresponds to the extinction value for the protein.

Table 9-15: Peaks Properties (continued)

Field	Description
Injected Mass ( $\mu\text{g}$ )	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 Procedure UV Extinction from Peak Procedure
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 Procedure Dn/dc from RI Procedure Molar Mass and Radius from LS Procedure (batch mode with no concentration detector)
A2	This parameter can be set to 0 for most chromatography conditions. For additional information, see <a href="#">TN9102: Effect of Nonspecific Interactions</a> .
Style	This is set to "Manual" if you create or modify a peak manually. It is set to "Automatic" when you click <b>Autofind Peaks</b> .
<b>LS Analysis</b>	
>Model	Set this parameter when you perform any of the following analysis procedures: Radius from LS Procedure Molar Mass and Radius from LS Procedure Number from LS Procedure Protein Conjugate Analysis Procedure Viral Vector Analysis Procedure  The available models are: Zimm (the default), Debye, Berry, random coil, sphere, Lorenz-Mie, coated sphere, and rod. See <a href="#">Choosing a Fit Model on page 252</a> 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 <a href="#">Choosing a Fit Model on page 252</a> for details.

Table 9-15: Peaks Properties (continued)

Field	Description
<b>Protein Conjugate Analysis</b>	
>Modifier dn/dc	If you are using the Protein Conjugate Analysis Procedure 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 procedure, specify the extinction coefficient in mL/(mg cm) for the modifier protein.
<b>Particles</b>	
>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 Procedure Radius from LS Procedure (using Lorenz-Mie, sphere, or coated sphere) Number from LS Procedure
>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.
>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.
<b>Column Calibration</b>	
>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.

Table 9-15: Peaks Properties (continued)

Field	Description
>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.
<b>Viscosity Analysis</b>	
>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 <a href="#">Appendix H, Viscosity Theory</a> .
>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.
<b>Viral Vector Analysis</b>	
>Nucleic Acid dn/dc (mL/g)	If you are using the Viral Vector Analysis procedure, specify the dn/dc value in mL/g for the nucleic acid payload. (The dn/dc for the protein capsid is near the top of the Peaks parameter list.)
>Nucleic Acid UV Ext. Coef. (mL/(mg cm))	If you are using the Viral Vector Analysis procedure, specify the extinction coefficient in mL/(mg cm) for the nucleic acid payload for the specified UV wavelength.
>Protein UV Ext. Coef. (mL/(mg cm))	If you are using the Viral Vector Analysis procedure, specify the extinction coefficient in mL/(mg cm) for the protein capsid at the secondary UV wavelength. (The UV extinction for the protein capsid at the active wavelength is near the top of the Peaks parameter list.)
>Expected Protein Molar Mass (MDa)	If you are using the Viral Vector Analysis procedure, specify the expected molar mass for the protein capsid in MDa.
>Expected Nucleic Acid Molar Mass (MDa)	If you are using the Viral Vector Analysis procedure, specify the expected molar mass for the nucleic acid payload in MDa.

6. Click **OK** to continue running the experiment.

### Choosing a Fit Model

The light-scattering fit models are as follows:

- **Zimm model:** uses the  $K^*c/R_\theta$  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

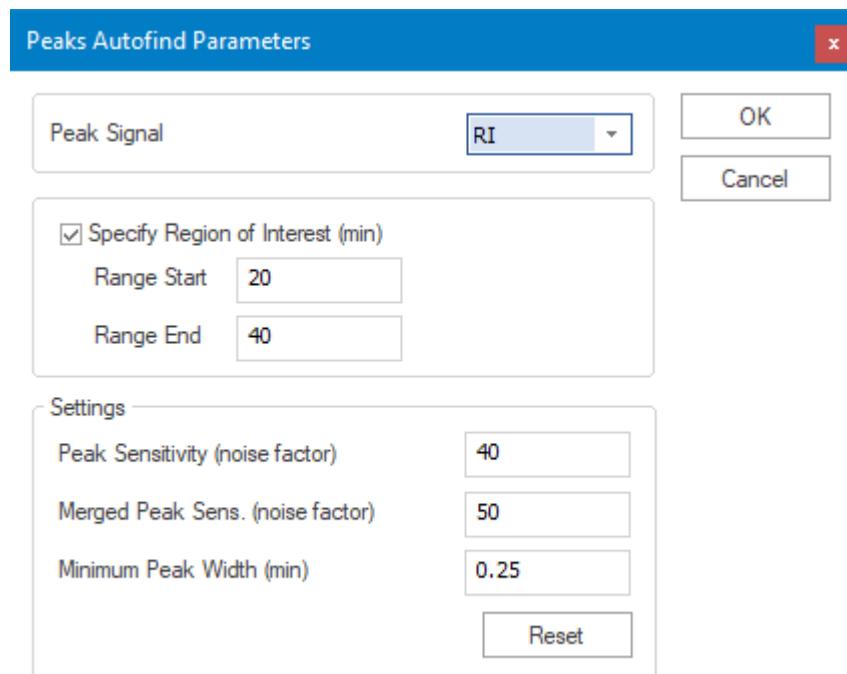
often requires a lower fit degree for the same size molecule. For large ( $>50$  nm) molecules, the Zimm model often produces a negative molar mass and should not be used.

- **Debye model:** Uses the  $R_\theta/K^*c$  formalism. It gives better results over a wider range of molar mass, including the very large (greater than  $\sim 10^6$  Daltons or  $\sim 100$  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.
- **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.
- **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.
- **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.

See the [Determination of Molar Mass and Sizes on page 438](#) for a discussion of the fit models.

## Parameters for Finding Peaks

If you want finer control over how peaks are detected, click the **fx Parameters** **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 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 **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 Procedure



This procedure is hidden in the Experiment tree 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 Security Pack), and open the “band broadening example (BSA).afe8 experiment in the Sample Data > Analyzed Experiments folder.

## Convert to Physical Units Procedure

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 the Experiment tree 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 Procedure

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 147) 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  $d\eta/dc$  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 the Experiment tree 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 Procedure

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 Procedure on page 256](#) 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 Procedure on page 247](#) 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 the Experiment tree 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**.

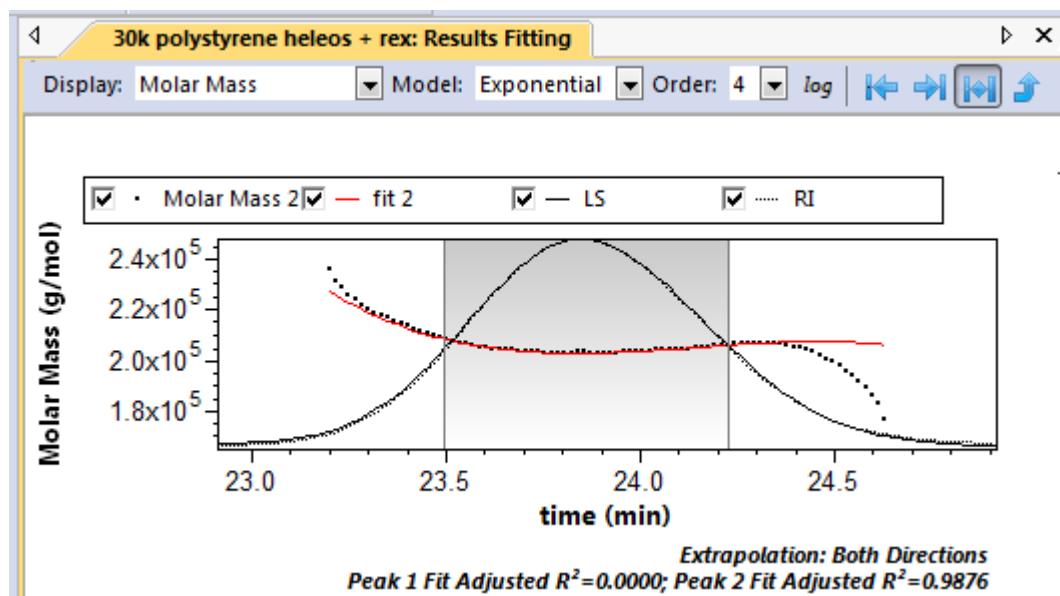
## Results Fitting Procedure

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:

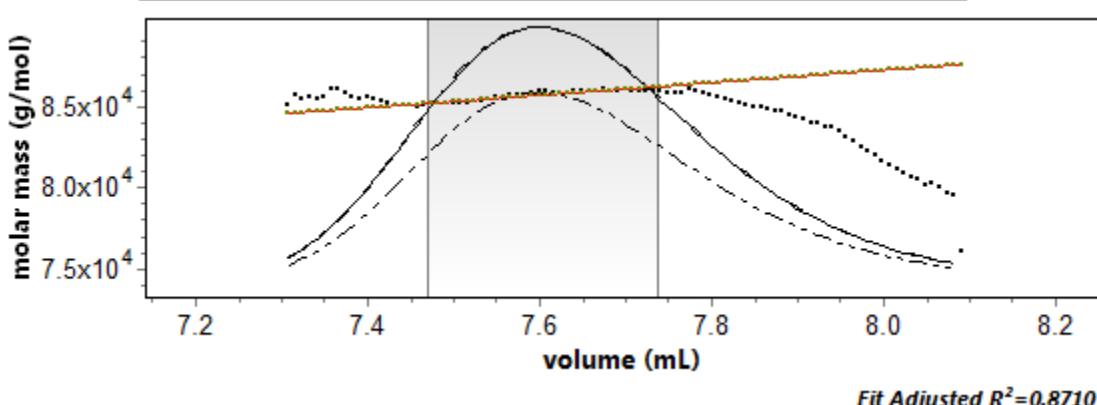
Table 9-16: Results Fitting Properties

Field	Description
Display	Choose the data to display and fit. The options depend on the data available in your experiment. For example, molar mass, radius, rms radius, hydrodynamic 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.

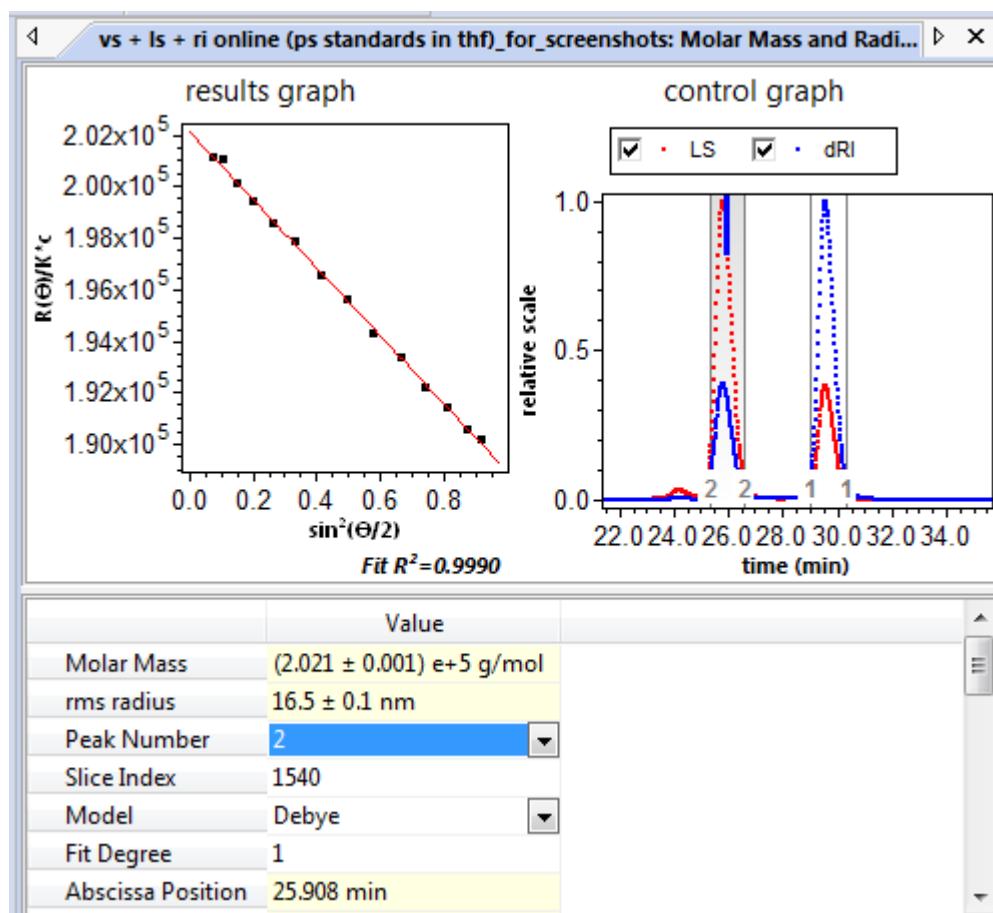
The following analysis procedure types are available:

- A2, Molar Mass, and Radius from LS Procedure on page 264
- Branching Procedure on page 283
- Copolymer Analysis Procedure on page 294
- Cumulants Procedure on page 305
- Distribution Analysis Procedure on page 292
- Distributions and Moments Procedure on page 275
- Dn/dc from Peak Procedure on page 278
- Dn/dc from RI Procedure on page 277
- Dn/dc from UV Procedure on page 278
- Mark-Houwink-Sakurada Procedure on page 290
- Molar Mass and Radius from LS Procedure on page 260
- Molar Mass from Column Calibration Procedure on page 308
- Molar Mass from VS Procedure on page 271
- Moments Procedure on page 276
- Number from LS Procedure on page 272
- Online A2 Procedure on page 267
- Parametric Plot Procedure on page 287
- Peak Statistics Procedure on page 287
- Protein Conjugate Analysis Procedure on page 295
- Radius from LS Procedure on page 274
- Regularization Procedure on page 301
- rh from DLS Procedure on page 280
- rh from VS Procedure on page 283
- RI Calibration from Peak Procedure on page 279
- UV Extinction from Peak Procedure on page 279
- UV Extinction from RI Procedure on page 280
- Viral Vector Analysis Procedure on page 298

## Molar Mass and Radius from LS Procedure

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.

The procedure has the following page:



The left graph shows the results in a Debye graph. See [About Analysis Plots on page 261](#).

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 9-17: Molar Mass and Radius from LS Properties*

Field	Description
Molar Mass	Shows the calculated molar mass. This field is display only.
Radius, rms radius, or Rod Length	Shows the calculated radius or rod length for the currently selected peak. The value displayed depends on the LS fit model specified for the peak.
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.
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 Procedure on page 260](#)
- [Number from LS Procedure on page 272](#)
- [Radius from LS Procedure on page 274](#)

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.

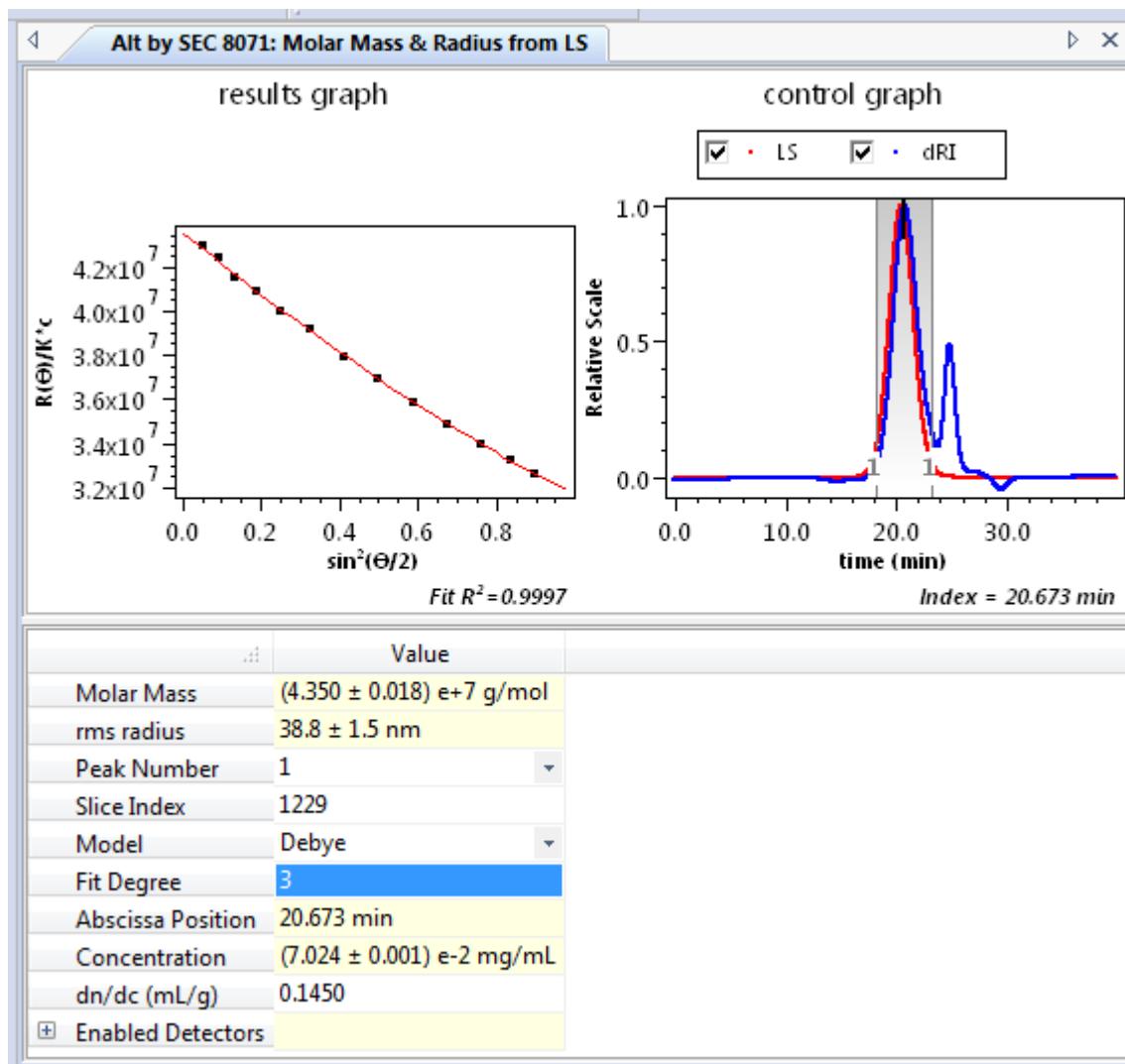


Figure 9-4: Analysis plot with fit degree of 3

### 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 9-4 with that of Figure 9-5. 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.

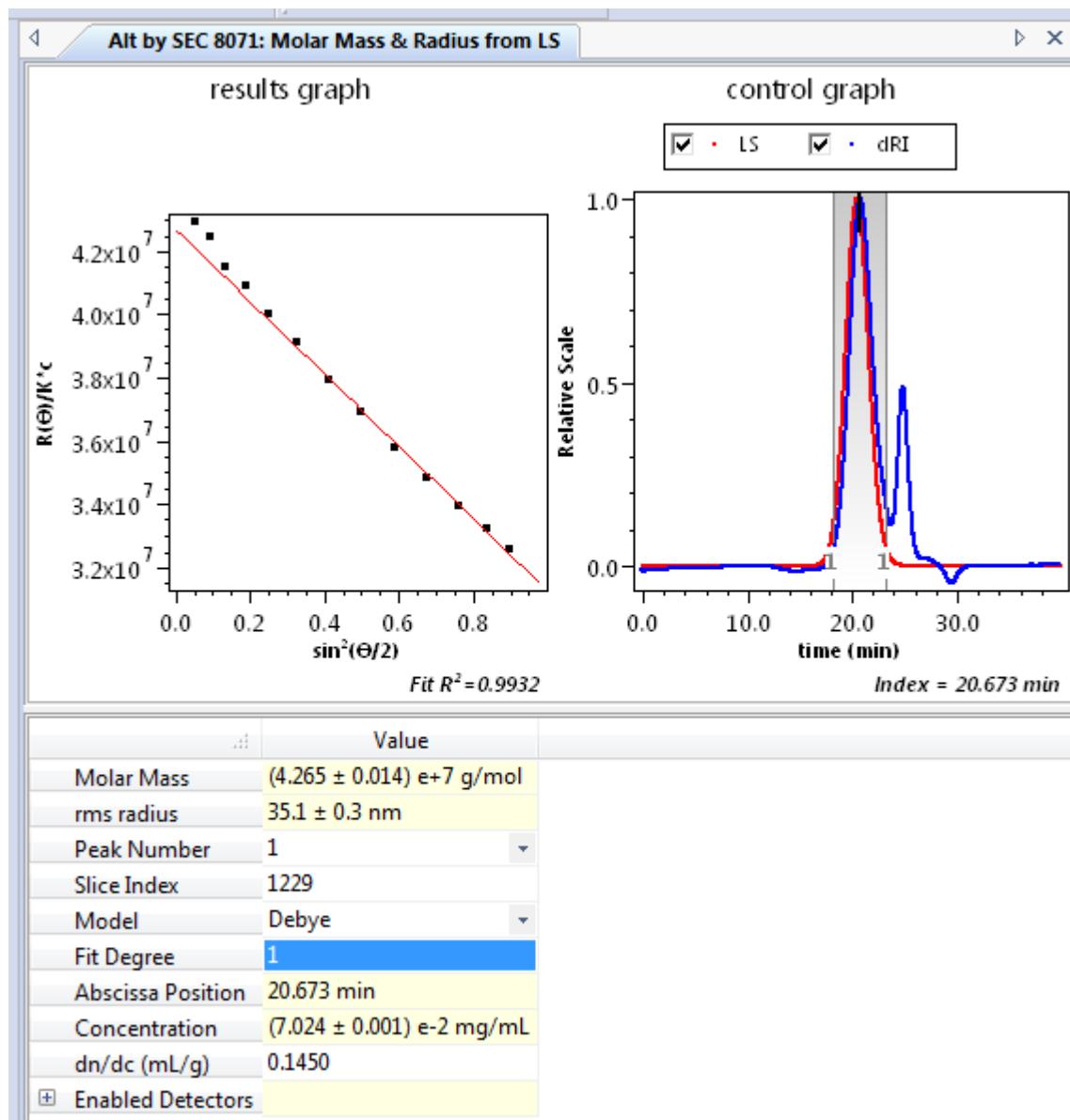


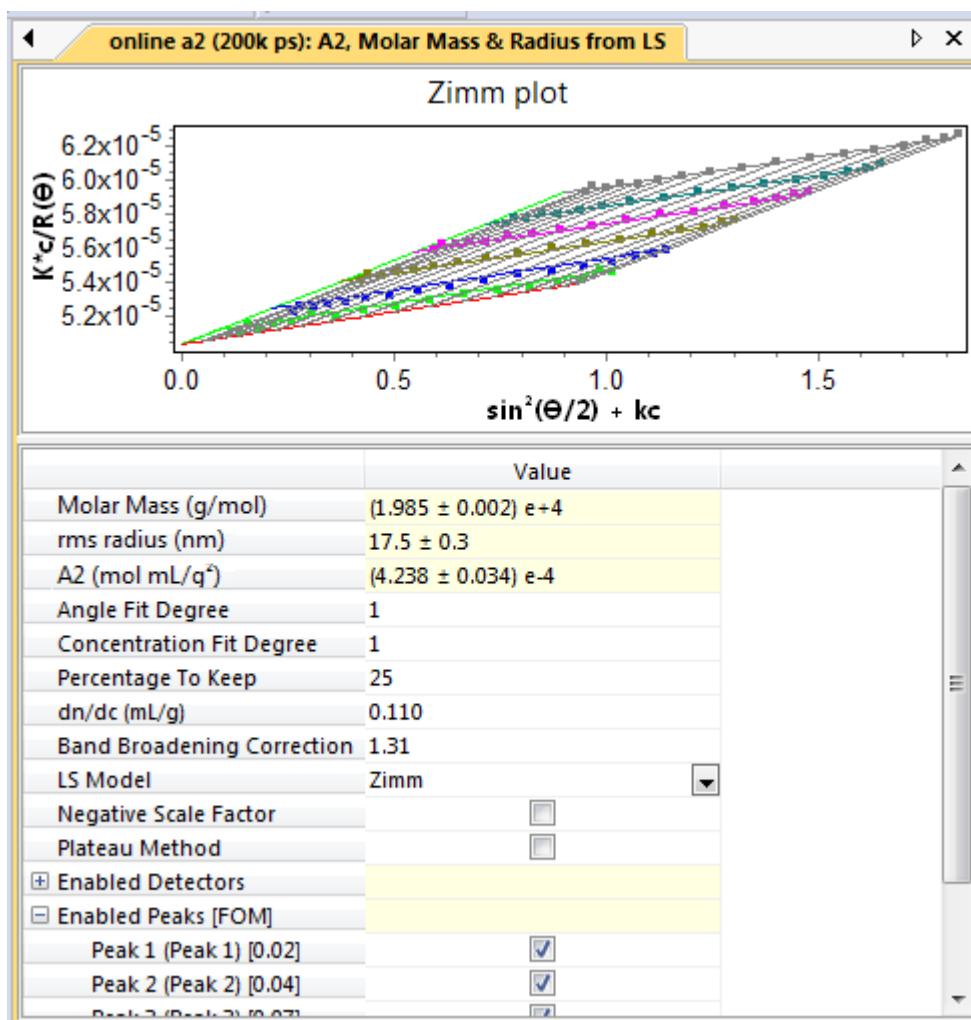
Figure 9-5: Analysis plot with fit degree of 1

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 Procedure

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 Procedure on page 267](#).

The procedure has the following page:



By default, the graph shows the results in a Zimm plot style, although ASTRA 8 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<sub>2</sub> measurements could only be done in batch (plateau) mode. ASTRA now supports A<sub>2</sub> measurement in online mode through use of the Plateau calculation method. Use the “online A2” experiment method in the System > Methods > Light Scattering folder for online A<sub>2</sub> measurement. A completed “online A2” example experiment is also available for importing.

The properties for this procedure are as follows:

*Table 9-18: A2, Molar Mass, and Radius from LS Properties*

Field	Description
Molar Mass	Shows the calculated molar mass. This field is display only.
Radius	Shows the calculated radius. The type of radius (radius or rms) displayed depends upon the LS fit model specified for the peak. This field is display only.
A2 (mol mL/g <sup>2</sup> )	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 A2 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.

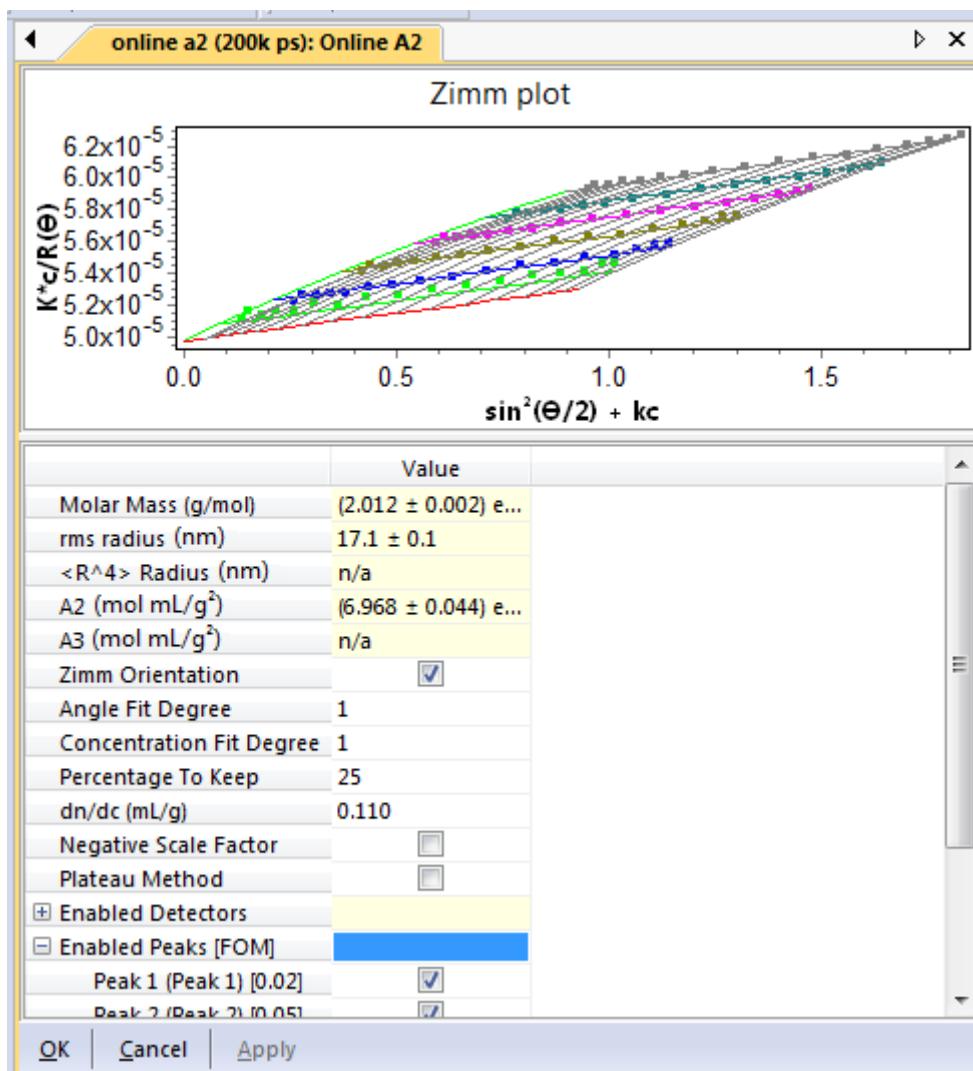
Table 9-18: A2, Molar Mass, and Radius from LS Properties (continued)

Field	Description
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 [A2 FOM] > 1 to n	You can omit peaks from the plot by removing the checkmark next to the peak number. The [A2 FOM] value shown for each peak is a “Figure of Merit,” which is a unitless value that reflects the ability to measure A2 accurately. If the A2 FOM for a peak is less than 1 or slightly greater than 1, then the peak will help measure A2 accurately.

## Online A2 Procedure

This procedure supports the analysis of rapid injections of small volumes of a sample, ideally prepared in an autodilution process in an autosampler. The online A2 analysis can retrieve A<sub>2</sub> (and A<sub>3</sub>) using a fraction of the sample needed for traditional measurements, and supports automation and high-throughput studies.

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.

The properties for this procedure are as follows:

Table 9-19: Online A2 Properties

Field	Description
Molar Mass	Shows the calculated molar mass. This field is display only.
Radius	Shows the calculated radius. The type of radius (radius or rms) displayed depends upon the LS fit model specified for the peak. This field is display only.
R <sup>4</sup> Radius	Shows the calculated R <sup>4</sup> radius. This is a physical quantity derived from the Zimm series expansion. It is similar to the radius or rms radius, except that it is the “fourth-root-mean-fourth-power.” This field is display only.
A <sub>2</sub> (mol mL/g <sup>2</sup> )	Shows the calculated second virial coefficient. This field is display only.
A <sub>3</sub> (mol mL/g <sup>3</sup> )	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 <sub>2</sub> 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).

Table 9-19: Online A2 Properties (continued)

Field	Description
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 [A2 FOM] > 1 to n	You can omit peaks from the plot by removing the checkmark next to the peak number. The [A2 FOM] value shown for each peak is a "Figure of Merit," which is a unitless value that reflects the ability to measure A2 accurately. If the A2 FOM for a peak is less than 1 or slightly greater than 1, then the peak will help measure A2 accurately.

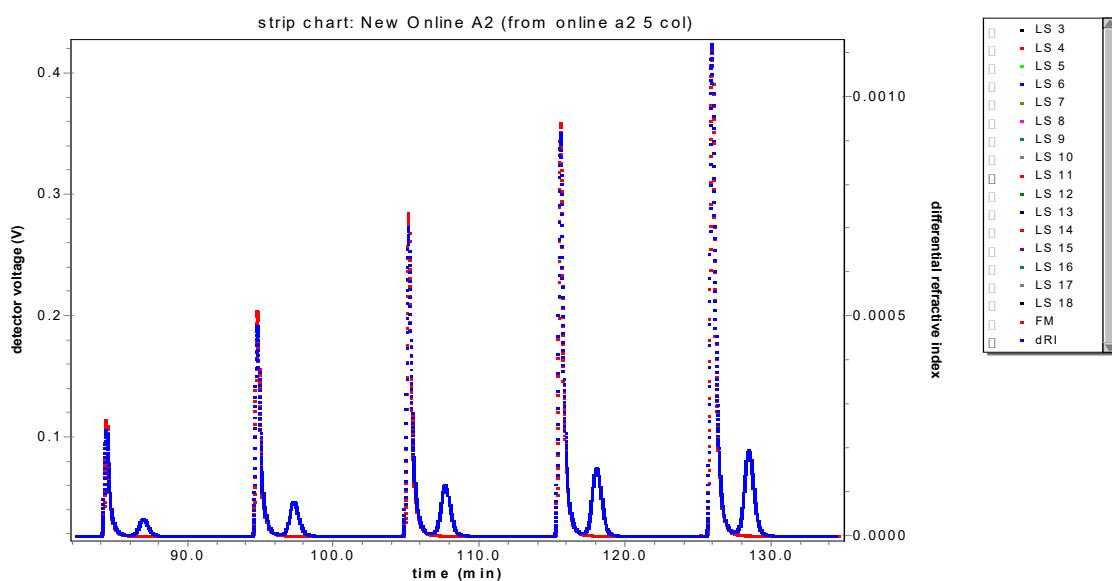
The Online A2 model is based on a series expansion of the Zimm model in concentration. It is best suited for small molecules for which other models are not applicable. You cannot specify the light scattering fit model for this analysis.

### Example: 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:

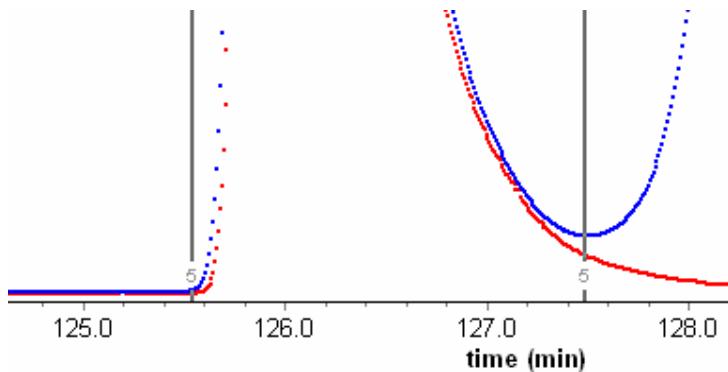
1. Create a new experiment using the System > Methods > Light Scattering folder > online A2 method.
2. Adjust the configuration to reflect your light scattering and concentration detectors (the default is a DAWN and an Optilab).
3. Run a data collection consisting of a series of injections of varying 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 9-20: Online A2 Results for Various Fit Degrees

Angle Fit Degree ( $\sin^2(\theta/2)$ )	Concentration Fit Degree	Results Excluded
0		R^4 Radius, rms Radius
1		R^4 Radius
2		None
	0	A2, A3
	1	A3
	2	None

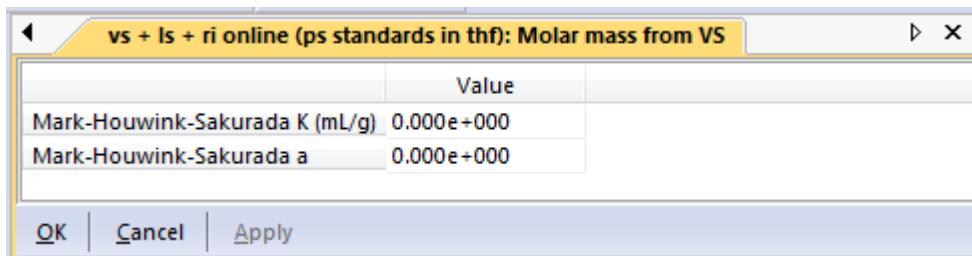
## Molar Mass from VS Procedure

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 H, Viscosity Theory](#) in this manual.

You can use the Mark-Houwink-Sakurada Procedure on page 290 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:



The properties for this procedure are as follows:

Table 9-21: Molar Mass from VS Data Properties

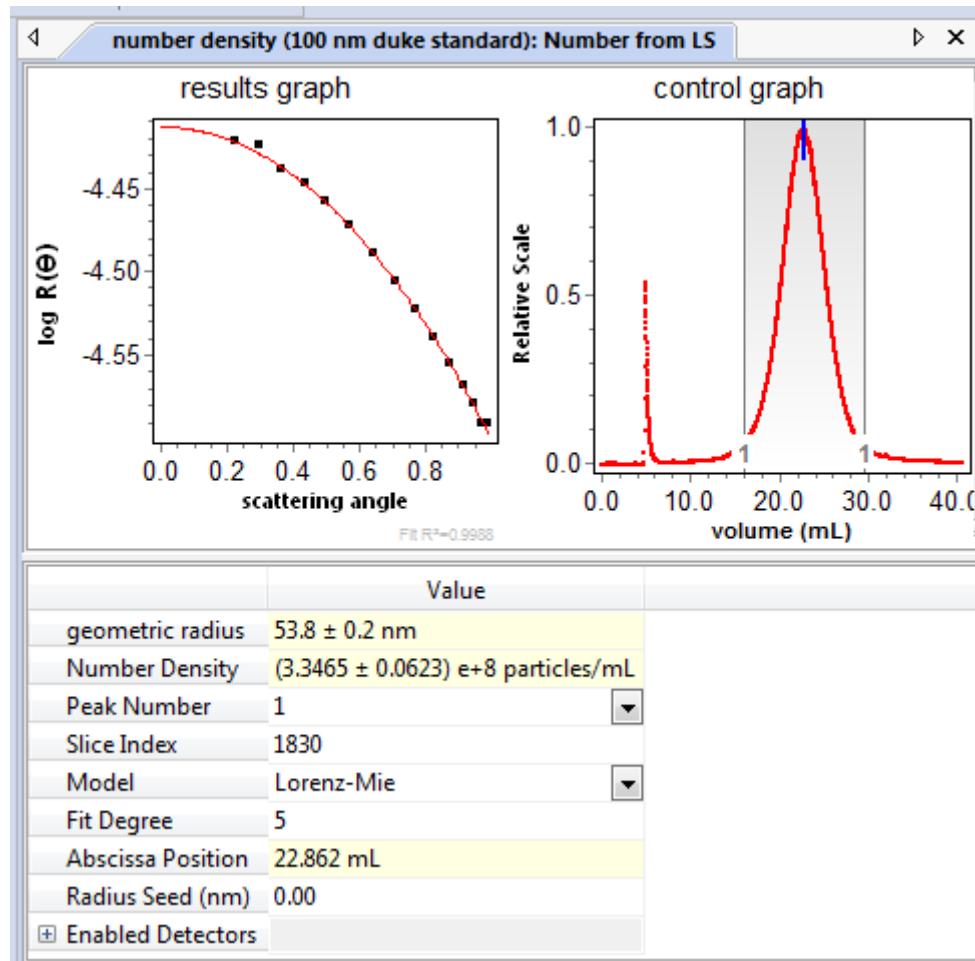
Field	Description
Mark-Houwink-Sakurada K	The "K" fit parameter for the Mark-Houwink-Sakurada analysis.
Mark-Houwink-Sakurada a	The "a" fit parameter for the Mark-Houwink-Sakurada analysis.

## Number from LS Procedure

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.

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:

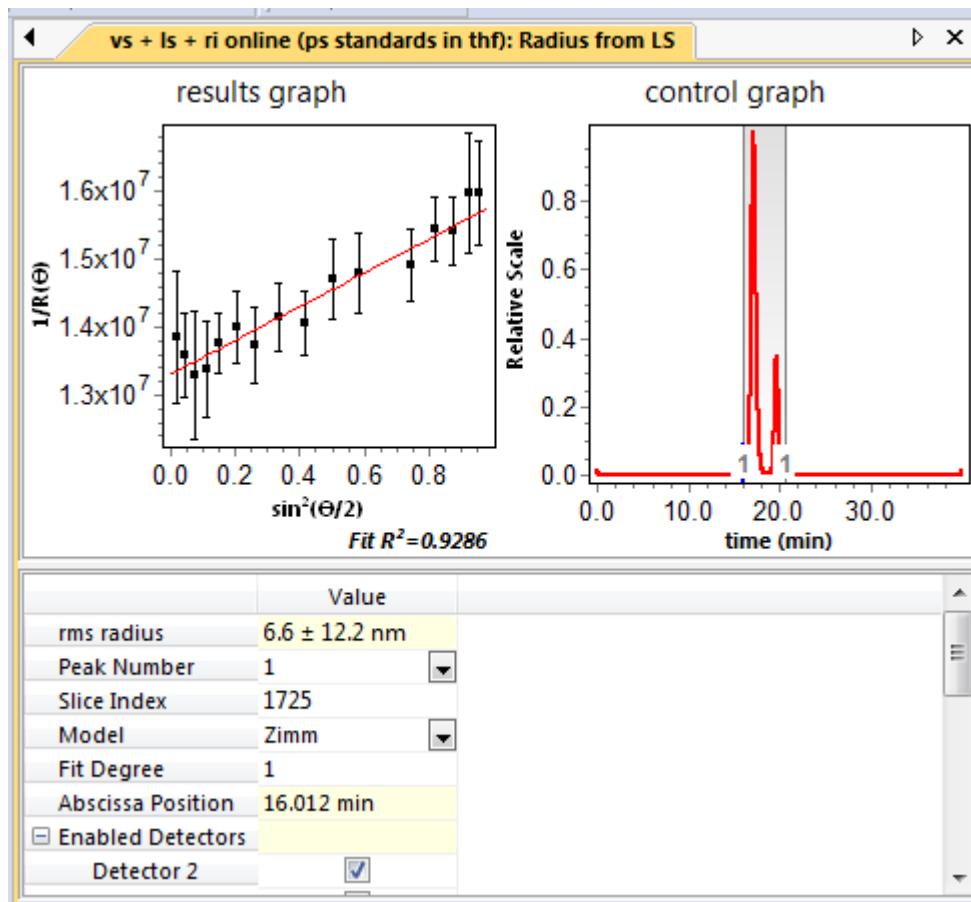
*Table 9-22: Number from LS Data Properties*

Field	Description
Radius, rms radius, or Rod Length	Shows the calculated radius or rod length for the currently selected peak. The value displayed depends on the LS fit model specified for the peak.
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 <a href="#">Choosing a Fit Model on page 252</a> for details.
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 Procedure

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 9-23: Radius from LS Data Properties

Field	Description
Radius, rms radius, or Rod Length	Shows the calculated radius or rod length for the currently selected peak. The value displayed depends on the LS fit model specified for the peak.
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.

Table 9-23: Radius from LS Data Properties (continued)

Field	Description
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 Procedure

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 Procedure on page 292](#).

You can place this procedure with other analysis procedures, and after all the transformation procedures.



This procedure is hidden in the Experiment tree 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:

	Value
Data Analysis Range	99.75
Smoothing Level	Normal

The property for this procedure is as follows:

*Table 9-24: 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.
Smoothing Level	Tunes the smoothing level used when calculating the differential weight and number distribution. Choose one of these levels: Least, Less, Normal, More, Most. The default is Normal.

## Moments Procedure

The Moments procedure provides statistics (mean and standard deviation) for certain data sets, such as UV, RI, aRI, Rayleigh ratios, and viscosity. It may be useful for batch experiments in which the value of the data set is approximately constant within a peak.

You can place this procedure with other analysis procedures, and after all the analysis procedures.

The properties for this procedure are as follows:

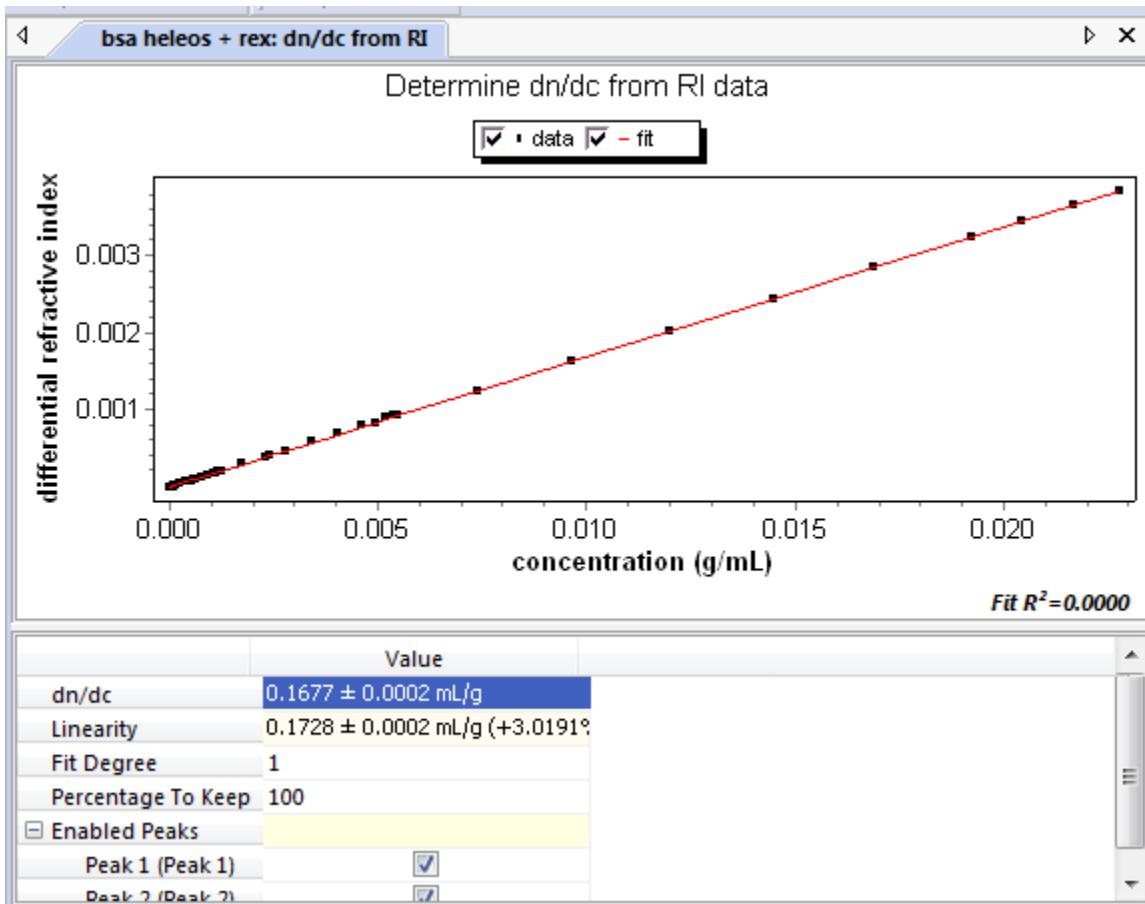
*Table 9-25: Moments Properties*

Field	Description
Display	Choose which data set you want to display in the graph and use for calculations.
Name	Shows the peak numbers.
Start	The start time (in minutes by default) of the peak.
Stop	The end time (in minutes by default) of the peak.
Mean	The mean value on the y-axis within the peak.
Standard Deviation	The standard deviation of y-axis values within the peak.

## Dn/dc from RI Procedure

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.

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 9-26: 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.

Table 9-26: dn/dc from RI Properties (continued)

Field	Description
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.

## Dn/dc from Peak Procedure

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 the Experiment tree 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.

## Dn/dc from UV Procedure

This procedure calculates the refractive index increment dn/dc using UV 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 refractive index increment. If you place multiple methods that determine dn/dc 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 the Experiment tree 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**.

## RI Calibration from Peak Procedure

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 the Experiment tree 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 Procedure

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 the Experiment tree 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 Procedure

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.



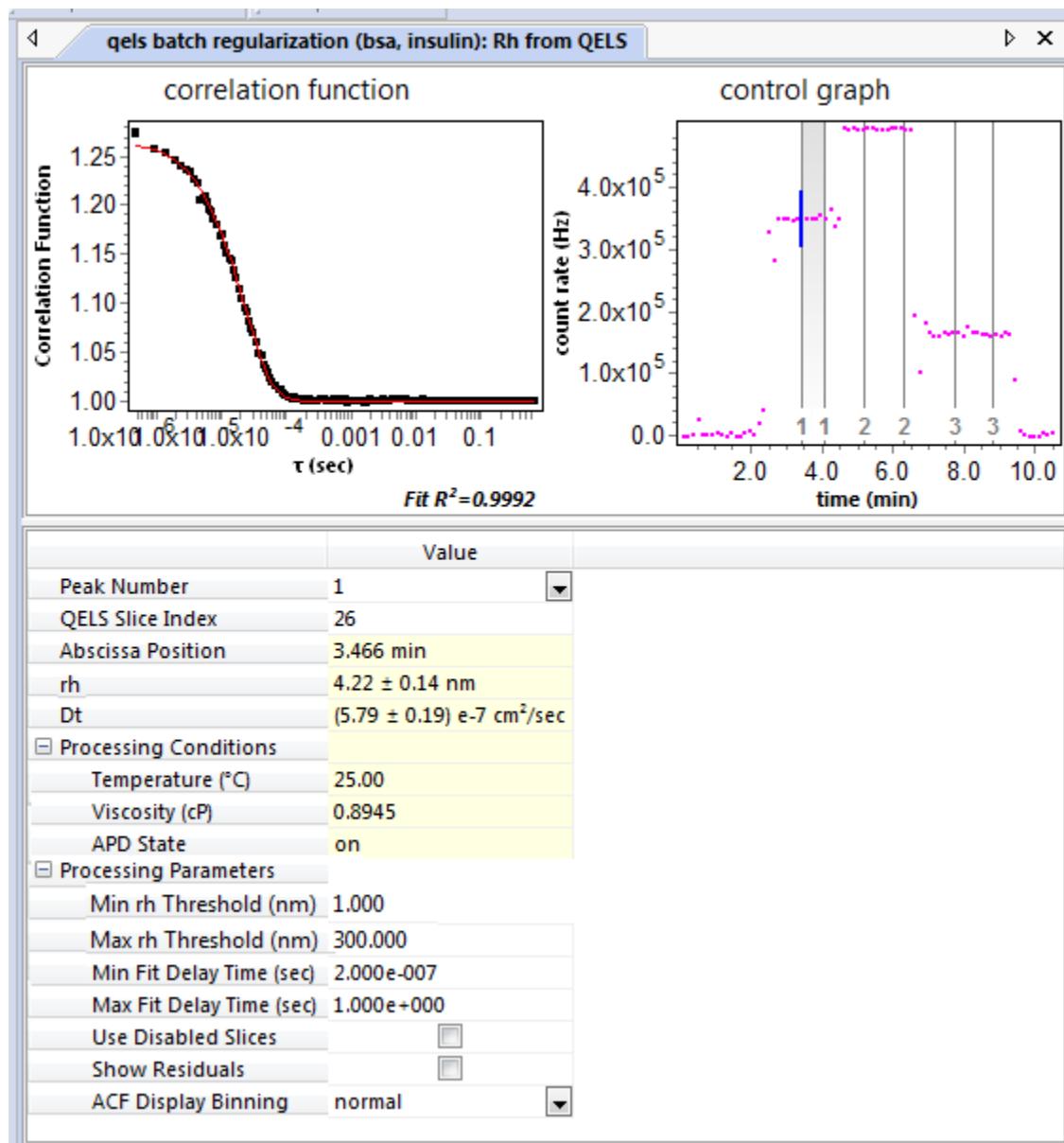
This procedure is hidden in the Experiment tree in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

## rh from DLS Procedure

This procedure calculates the translational diffusion ( $D_t$ ) and hydrodynamic radius ( $r_h$ ) using DLS data. You can use this procedure if your experiment configuration uses the WyattQELS option in online mode. If you use DLS in batch mode, see [Regularization Procedure on page 301](#).

For an experiment method, choose **File→New→Experiment From Method** to open a method in the System > Methods > Light Scattering > With DLS folder.

The procedure has the following page:



The graph on the left shows the autocorrelation function and the fit results for the peak and slice you select in the graph on the right. 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 more than one procedure that determines  $r_h$ . The results identify  $r_h$  values calculated using WyattQELS data with “ $r_h(Q)$ ”.

Data points excluded from the calculations by the max and min properties are shown in red. The DLS data collection and analysis tolerate gaps that may occur in DLS data collection due to instrument problems.

With DLS data,  $r_h$  can be determined for smaller particles ( $\sim 10$  nm) than with light scattering data. For example, if your experiment includes both the Number from LS and the  $r_h$  from DLS procedures, peaks for very small particles may have results for  $r_h$  but not the radius.

The properties for this procedure are as follows:

Table 9-27:  $r_h$  from DLS Data Properties

Field	Description
Peak Number	The peak number of the displayed correlation function. You can create additional peaks in this procedure.
DLS 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.
$r_h$	Shows the calculated hydrodynamic radius ( $r_h$ ) value. This field is display only.
$D_t$	Shows the calculated translational diffusion ( $D_t$ ) 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 photodiode (APD) in the WyattQELS detector. If the DLS 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.
Processing Parameters	
>Min $r_h$ Threshold	Fitted $r_h$ values with a lower radius than the value you type are not used in the analysis.
>Max $r_h$ Threshold	Fitted $r_h$ values with a higher radius than the value you type are not used in the analysis
>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.

Table 9-27: *rh* from DLS Data Properties (continued)

Field	Description
>Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photodiode (APD) is triggered or the delay time or $r_h$ 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.
>Show Residuals	Check this box if you want to show residuals from the fit to the DLS autocorrelation function.
>ACF Display Binning	Adjust the number of autocorrelation 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 Procedure

This procedure calculates the hydrodynamic radius ( $r_h$ ) 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 more than one procedure that determines  $r_h$ . The results identify  $r_h$  values calculated using viscosity with “ $r_h(v)$ ”.

There are no properties to set for this procedure. It runs without prompting for any values.



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, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

### Branching Procedure

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” ( $e$ ).

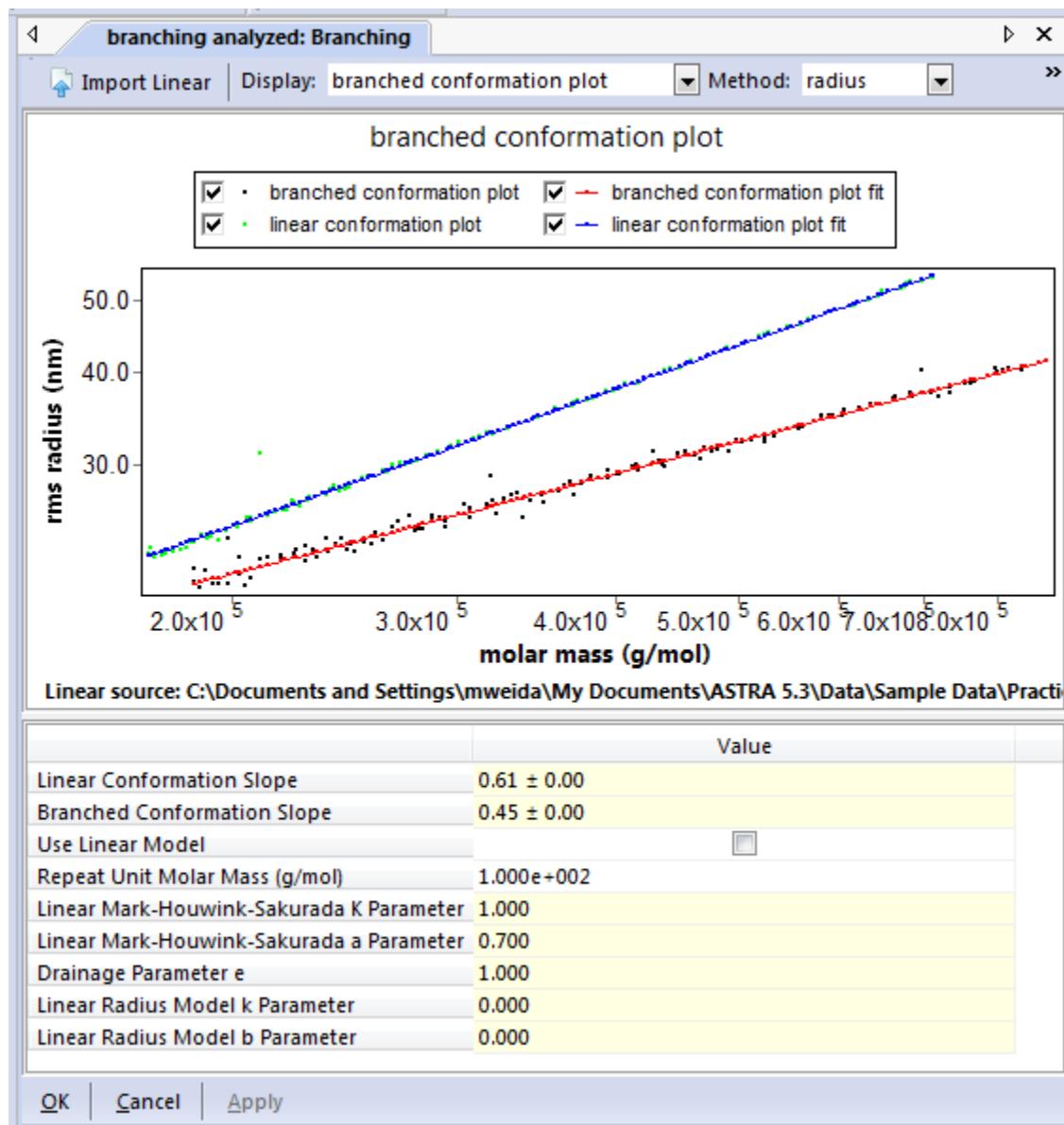
## Linear Models

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 conformation or Mark-Houwink-Sakurada plot were created, or from published values.

- **Radius Model:** The radius model is specified using an offset (k) and slope (b) for a conformation plot of the rms radius as a function of molar mass. The offset (k) is determined by taking 10 to the power of the rms conformation plot y-intercept. The slope (b) is the slope of the plot.
- **Viscosity Model:** The viscosity model is specified using the Mark-Houwink-Sakurada K and a parameters.

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 9-28: 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.

## Parametric Plot Procedure

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 9-29: 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 344](#) for information about the dialog that appears when you click the “...” button for either the X Data or the Y Data property.

## Peak Statistics Procedure

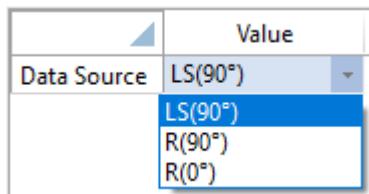
This procedure calculates the area under the defined peaks for all detectors. The values calculated by this procedure are peak area, peak height retention time, resolution, peak width at half-height, peak width at quarter-height, peak width at tenth-height, peak width at user-specified height, asymmetry factor, tailing factor, column plate count, mean, standard deviation, skew, %peak area, and selectivity.

You can place this procedure after the Peaks procedure.



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, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

Peak statistics can be based on one of the following data sources, which can be chosen by clicking on the Peak Statistics Procedure once it is visible (in Experiment Builder mode):



- **LS(90°)**: The raw LS voltage at 90° before conversion to a Rayleigh ratio. Units are volts (V). This is the default data source, because the signal at 90° usually has the highest signal-to-noise ratio, and using statistics before conversion to Rayleigh ratio eliminates a potential source of error.
- **R(90°)**: The Rayleigh ratio for the 90° detector. Units are cm-1. The Convert to Physical Units Procedure, which is only visible in Experiment Builder mode, must be present and prior to Peak Statistics for this calculation to be valid.
- **R(0°)**: The Rayleigh ratio for the 0° detector. Units are cm-1. This can be used as a proxy for Mw. The Molar Mass and Radius Procedure must be prior to the Peak Statistics Procedure for this calculation to be valid.

Legacy experiments open with the data source that was used previously. If a data source is not valid for calculating peak statistics, the ASTRA report shows “invalid” as the result. If the data source is valid but a result cannot be calculated by the algorithm, the report shows “n/a” as the result.

This procedure calculates the following values:

*Table 9-30: Peak Statistics Properties*

Field	Description
Peak Area	Reports the area under the peak for each detector used.
Peak Height	Reports the peak height of the peak for each detector used.
Retention Time	The measured elution time of the apex of the peak. This is t in the column plate count equation in <a href="#">About the Column Plate Count on page 290</a> .
Resolution	The resolution of a column, which is defined as 1.18 times the retention time of Peak A minus the retention time of Peak B divided by the difference of the peak width of Peak A at 50% maximum height and peak width of Peak B at 50% maximum height.
Peak Width at Half-Height	The measured peak width at the midpoint (50%) below the peak apex. For example, if the apex of the peak measures 2 V, the width of the peak is measured at the 1 V level. This is w in the column plate count equation in <a href="#">About the Column Plate Count on page 290</a> .
Peak Width at Quarter-Height	The width of the peak at 25% height.
Peak Width at Tenth-Height	The width of the peak at 10% height.
Peak Width at User-Specified-Height (4.4%)	The width of the peak at 4.4% height.

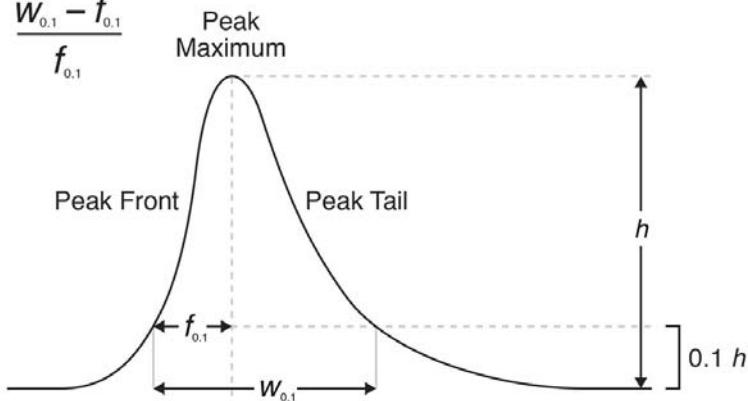
Table 9-30: Peak Statistics Properties (continued)

Field	Description
Asymmetry Factor	The tailing width divided by the leading width at 10% of the maximum height of the peak. See <a href="#">About the Asymmetry and Tailing Factors on page 289</a> .
Tailing Factor	The tailing factor, also known as the symmetry factor, is the sum of the leading width and the tailing width of the peak divided by twice the leading width; with the leading and tailing widths measured at 5% maximum height of the peak. See <a href="#">About the Asymmetry and Tailing Factors on page 289</a> .
Column Plate Count	The number of theoretical plates of the column. See <a href="#">About the Column Plate Count on page 290</a> for details.
Mean	Determines first moment (mean) of the peak distribution.
Standard Deviation	Determines second moment (standard deviation) of the peak distribution.
Skew	Determines third moment (skew) of the peak distribution
Peak Area (%)	Percent peak area for a single peak in the chromatogram.

### About the Asymmetry and Tailing Factors

Figure shows how the asymmetry factor and tailing factor are calculated.

$$\text{Asymmetry Factor} = \frac{W_{0.1} - f_{0.1}}{f_{0.1}}$$



$$\text{Tailing Factor} = \frac{W_{0.05}}{2f_{0.05}}$$

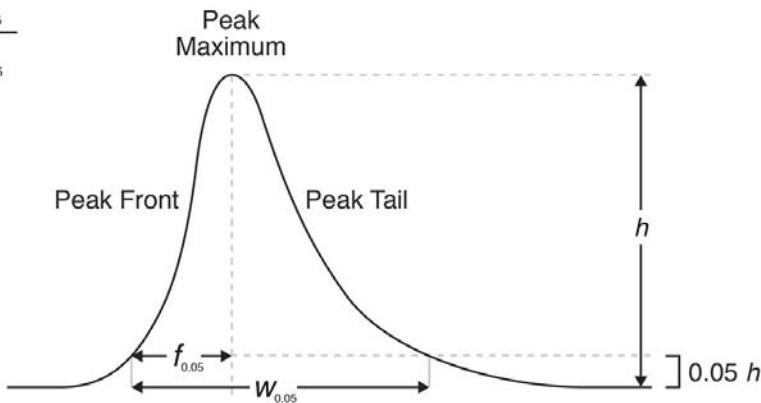


Figure 9-6: Peak Statistics Properties include the asymmetry factor and tailing factor.

### About the Column Plate Count

The column 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 calculates 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 equilibrations 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:

$$N = \frac{5.54t^2}{w^2}$$

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.

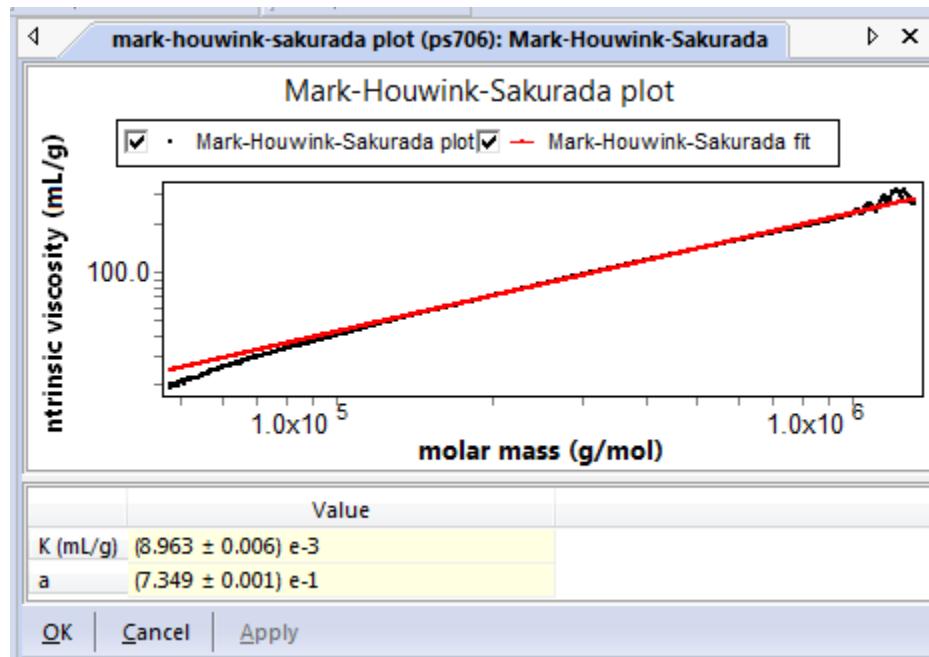
### Mark-Houwink-Sakurada Procedure

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 Security Pack) and open the “Mark-Houwink-Sakurada plot (PS706).afe8” 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 > Light Scattering > with Viscometry 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 9-31: Mark-Houwink-Sakurada Properties

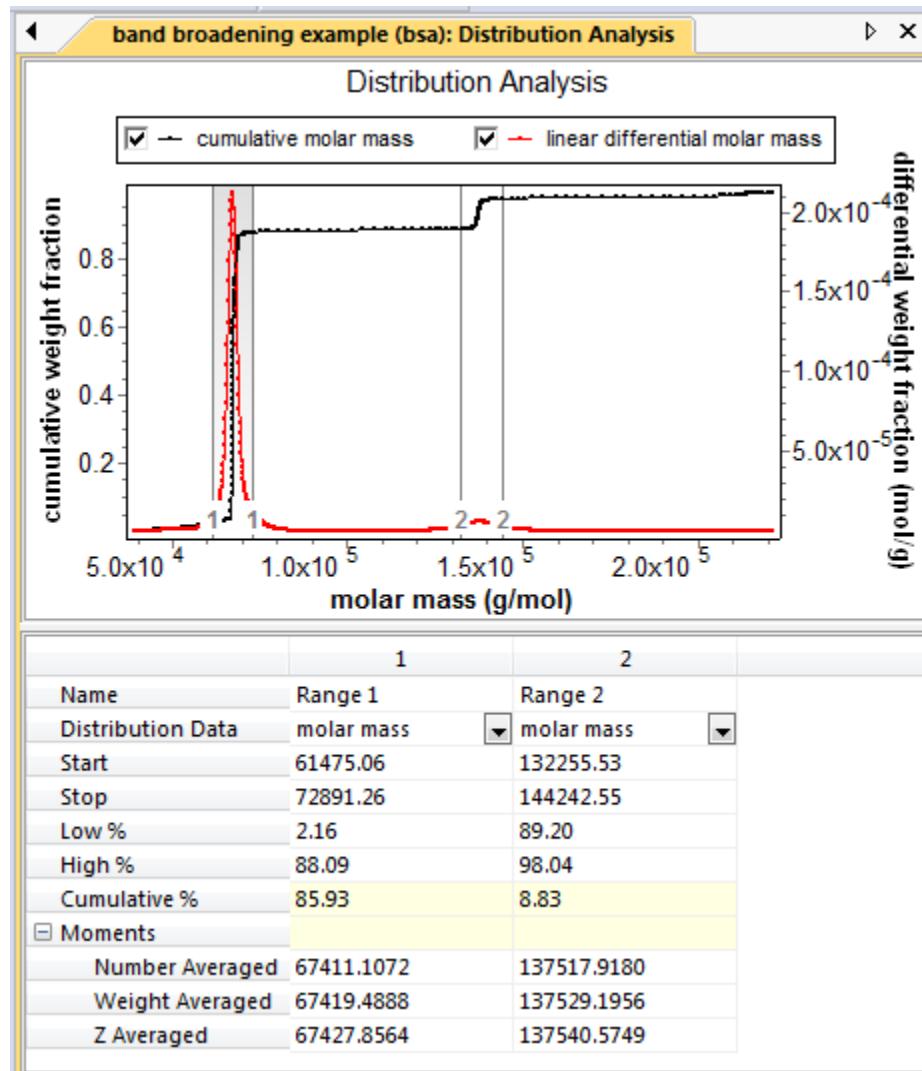
Field	Description
K	The resulting “K” fit parameter for the Mark-Houwink-Sakurada analysis.
a	The resulting “a” fit parameter for the Mark-Houwink-Sakurada analysis.

## Distribution Analysis Procedure

This procedure analyzes the distribution of the 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 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 Security Pack), and open the “band broadening example” experiment in the Sample Data > Analyzed Experiments folder.

The properties for this procedure are as follows:

*Table 9-32: 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.

## Copolymer Analysis Procedure

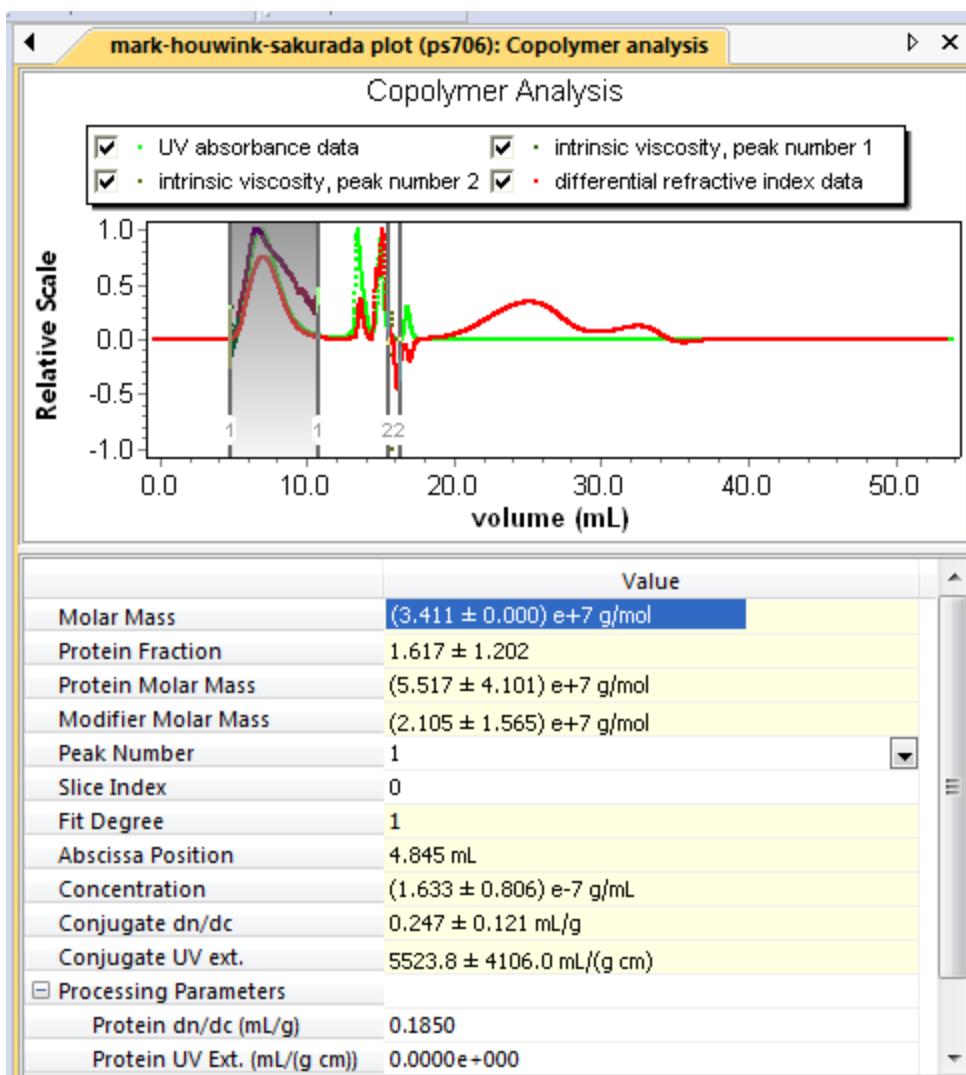
Like the [Protein Conjugate Analysis Procedure on page 295](#), this analysis technique allows you to differentiate between two polymers with the same molecular size. This analysis procedure requires use of a viscometer in conjunction with a UV and RI detector.

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 9-33: 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.
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 Procedure

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.

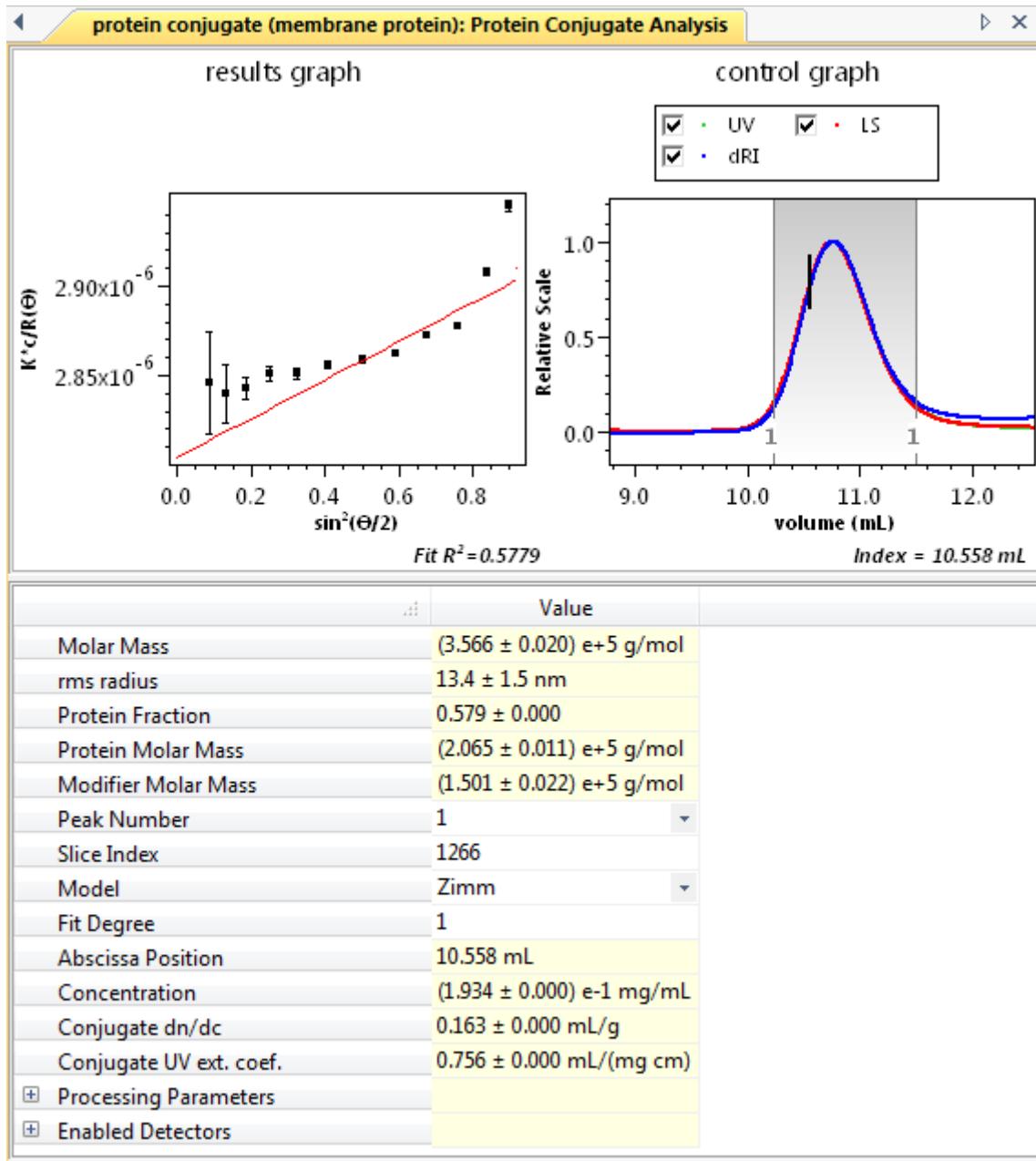
The Protein Conjugate Analysis page allows you to see the total molar mass and protein fraction on a slice by slice basis. ASTRA calculates the size of the complex, molar mass of the complex, and molar masses of the constituents, and the uncertainties for these values.

For an example experiment that performs a protein conjugate analysis, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA with Security Pack), 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 DLS folder.

This procedure should be placed in the list with the analysis procedures.

The procedure has the following page:



The left graph shows a Debye plot of the results. The right graph shows collection data and peaks.

The properties for this procedure are as follows:

*Table 9-34: Protein Conjugate Analysis Properties*

Field	Description
Molar Mass	Shows the total molar mass for the currently selected peak.
Radius, rms radius, or Rod Length	Shows the calculated radius or rod length for the currently selected peak. The value displayed depends on the LS fit model specified for the peak.
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.
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.
<b>Processing Parameters</b>	
>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.
Use Original Moments Calculations	Check this box to use the original moments calculations. We recommend keeping this checked, which is the default.
Enabled Detectors	The detectors to enable for this analysis.

## Viral Vector Analysis Procedure

This procedure calculates attributes of viral vectors comprised of a protein capsid and an encapsulated DNA payload, such as an adeno-associated virus (AAV) vector.

**Note:** For guidance in determining conditions and parameters for Viral Vector Analysis, contact Wyatt Support.

The method requires a light scattering detector in conjunction with two concentration detectors with differing responses to protein and nucleic acid constituents. This can be accomplished with either:

- A UV detector and an RI detector
- A single UV detector with two different UV wavelengths, such as 280 nm and 260 nm.

HPLC CONNECT, with its native support for recording digital UV data, makes it easy to capture multiple UV wavelengths and perform this calculation using two UV signals.

If you have both an RI and a UV detector, you may choose to collect data at two or more UV wavelengths in addition to collecting RI data. In this case, you must set one UV signal as the “active channel” and another as the “secondary channel.” In the Method Builder, you enter extinction coefficients for the protein and nucleic acid at two UV wavelengths. ASTRA can perform calculations using “RI & UV Active,” “RI & UV Secondary,” or “UV Active & UV Secondary” as the two concentration sources. You can compare results from these calculations without changing any sample or peak parameters by changing the **Concentration Source** setting in the Experiment configuration.

	Value
Name	HPLC+UV+LS+RI+QELS
Description	
Notes	<a href="#">Edit Notes...</a>
Processing Operator	WYATT\mrad
Abscissa Units	mL
Concentration Source	RI & UV Active
► Details	<div style="border: 1px solid #ccc; padding: 5px; display: inline-block;"> <span style="color: blue;">RI &amp; UV Active</span>  <span style="color: gray;">RI &amp; UV Secondary</span>  <span style="color: gray;">UV Active &amp; UV Secondary</span> </div>

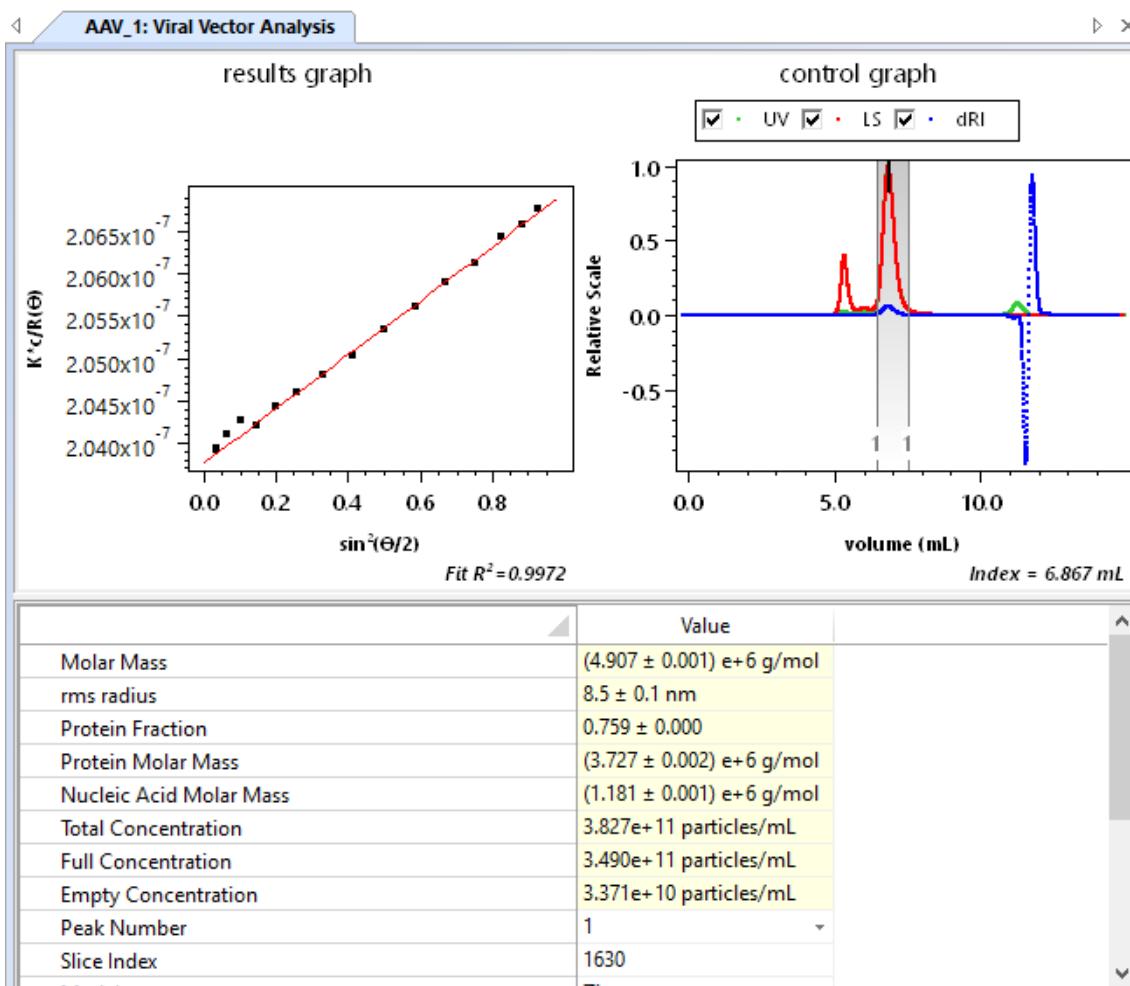
The Viral Vector Analysis procedure allows you to see the slice-by-slice protein molar mass, nucleic acid molar mass, and viral particle concentrations including full and empty capsid concentrations.

To create a Viral Vector Analysis experiment, we recommend using the Method Builder Wizard (see [page 129](#)). The Method Builder guides you through entering the HPLC and UV wavelength settings.

The Method Builder supports only one or two UV channels. To specify additional wavelengths to collect, add them to the **UV Channels** configuration in an experiment; then save the experiment as a method prior to collection.

If you have an existing experiment to which you would like to add the Viral Vector Analysis procedure, right-click on the experiment and choose **Apply Method**. Choose the “viral vector” method in the System > Methods > Light Scattering folder or the “viral vector” method in the System > Methods > Light Scattering > With DLS folder.

The procedure has the following page. The left graph shows a Debye plot of the results. The right graph shows collection data and peaks.



The properties for this procedure are as follows:

*Table 9-35: Viral Vector Analysis Properties*

Field	Description
Molar Mass (g/mol)	Shows the total molar mass for the currently selected peak.
Radius (nm)	Shows the calculated radius or rod length for the currently selected peak. The value displayed depends on the LS fit model specified for the peak.
Protein Fraction	Shows the protein fraction for the currently selected peak.
Protein Molar Mass (g/mol)	The molar mass for the protein capsid for the currently selected peak.
Nucleic Acid Molar Mass (g/mol)	The molar mass for the genetic payload for the currently selected peak.
Total Concentration (particles/mL)	Total particle concentration for the selected peak.
Full Concentration (particles/mL)	Total concentration of full capsids for the selected peak.
Empty Concentration (particles/mL)	Total concentration of empty capsids for 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.
Model	The model selected for this peak.
Fit Degree	The fit degree selected for this peak.
Abscissa Position (mL)	The value on the x-axis for the selected peak and slice.
Concentration (mg/mL)	The concentration at the selected slice.
Conjugate dn/dc (mL/g)	The dn/dc at the selected slice.
Conjugate UV Ext. Coef. (mL/(mg cm))	The UV extinction at the selected slice.
<b>Processing Parameters</b>	
Protein Capsid dn/dc (mL/g)	Specify the dn/dc of the protein capsid.
Nucleic Acid dn/dc (mL/g)	Specify the dn/dc of the nucleic acid.
Protein UV Ext. Coef. (mL/(mg cm))	Specify the UV Extinction coefficient of the protein capsid in mL/(mg cm) for wavelength specified.
Nucleic Acid UV Ext. Coef. (mL/(mg cm))	Specify the UV Extinction coefficient of the nucleic acid in mL/(mg cm) for the wavelength specified.

Table 9-35: Viral Vector Analysis Properties (continued)

Field	Description
Expected Protein Molar Mass (MDa)	Specify the expected molar mass of the protein capsid in MDa.
Expected Nucleic Acid Molar Mass (MDa)	Specify the expected molar mass of the nucleic acid in MDa.
Use Original Moments Calculations	Check this box to use the original moments calculations. We recommend keeping this checked, which is the default.
Enabled Detectors	The detectors to enable for this analysis.

For information about how to obtain the correct parameters and conditions for Viral Vector Analysis contact Wyatt Support.

## Regularization Procedure

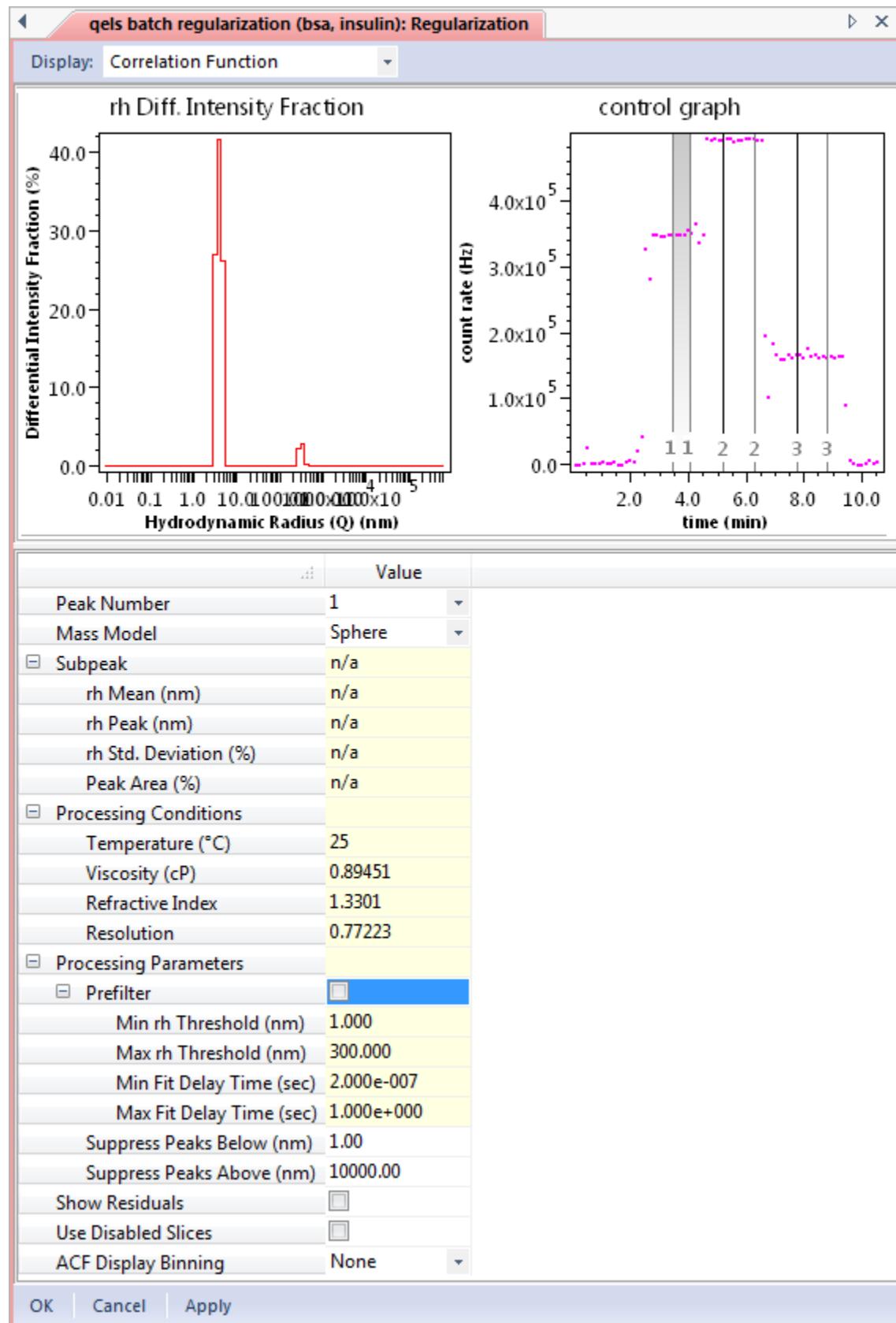
This procedure regularizes the results of a DLS batch experiment using the DYNALS regularization algorithm from ALANGO. See [Regularization Theory on page 470](#).

The Regularization procedure supports the reporting of 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 DLS data to identify the various  $r_h$  values of the mixture in the vial.

You can place this procedure at the end of a DLS 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 9-36: 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. Subpeaks are available when displaying rh or Dt distributions. 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 as a percentage.
>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 165).
>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.
Processing Parameters	Various values that govern the processing of the data. Users do not frequently need to change these values.

Table 9-36: Regularization Properties (continued)

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.
>>Min rh Threshold	Fitted $r_h$ values with a lower radius than the value you type are not used in the analysis. The Min and Max $r_h$ 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 $r_h$ Thresholds are ignored.
>>Max rh Threshold	Fitted $r_h$ values with a higher radius than the value you type are not used in the analysis.
>>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 Above	Type a size in nanometers above which peaks should be omitted from the regularization.
>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 it to omit residuals.
Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photodiode (APD) is triggered or the delay time or $r_h$ 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 <a href="#">rh from DLS Procedure on page 280</a> for a more about the APD State property.
ACF Display Binning	Adjust the number of autocorrelation 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.

- **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.

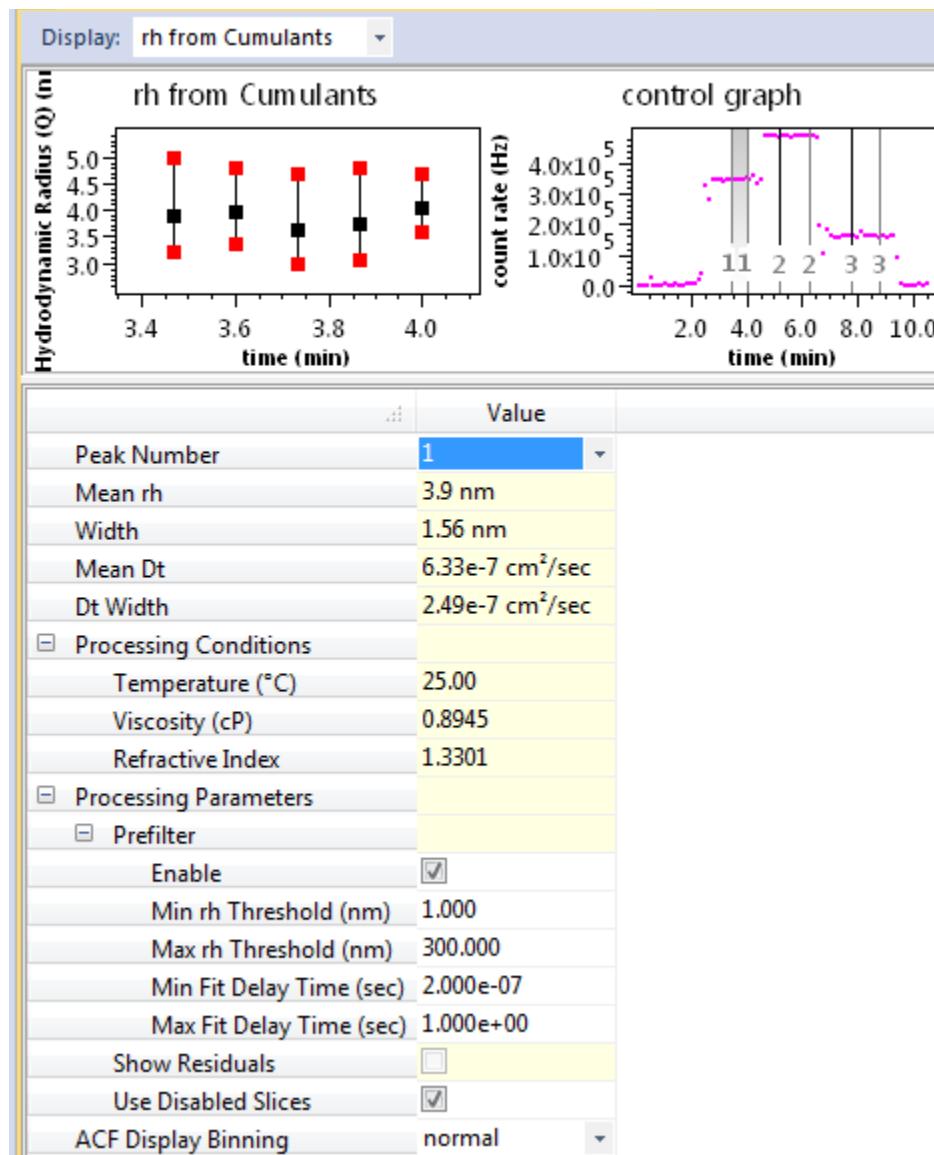
- **$r_h$  diff intensity fraction plot.** This graph gives an idea of the number of each type of species by showing the intensity fraction as a function of hydrodynamic radius. For peak 1, there seems to be a lot of particles with a radius of 4 nm and a much smaller number with a 300 nm size. Peak 2 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.
- **$r_h$  diff weight fraction plot.** This graph shows the distribution of  $r_h$  by weight fraction. In peaks 1 and 2 the larger particles don't even register on this view.
- **D<sub>t</sub> 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.
- **$r_h$  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.
- **$r_h$  cumulative weight fraction plot.** This graph shows the contribution to weight fraction for different sizes.
- **D<sub>t</sub> 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 Procedure

Cumulants analysis is used with DLS data in batch and online mode. It is a strategy for extracting information about the underlying size distribution of a polydisperse sample from the correlation function when combined with fractionation, as each data slice along the peak has an autocorrelation function that is fit. 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. See page 465 for details on cumulants analysis.

The experiment configuration for cumulants analysis must include a light-scattering instrument and WyattQELS.

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 Procedure](#) on [page 301](#). 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 show in the left graph. The options are Correlation Function, rh from Cumulants, and Dt from Cumulants.

The properties for this procedure are as follows:

Table 9-37: 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 ( $r_h$ ) for the peak.
Width	Shows the width in nm.
Mean Dt	Shows the mean translational diffusion (Dt) corresponding to the calculated $r_h$ value. The units are $\text{cm}^2 / \text{sec}$ .
Dt Width	Shows the width of the translational diffusion (Dt) in $\text{cm}^2 / \text{sec}$ .
Processing Conditions	Conditions (per peak) during the cumulants 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 165).
>Refractive Index	Refractive index of the sample for the given peak.
Processing Parameters	
>Prefilter	
>Enable	If the “Enable” 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.
>Min rh Threshold	Fitted $r_h$ 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 $r_h$ 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 $r_h$ Thresholds are ignored.
>Max rh Threshold	Fitted $r_h$ values with a higher radius than the value you type are not used in the analysis. The default is 300 nm.
>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.

Table 9-37: Cumulants Properties (continued)

Field	Description
>Show Residuals	Check this box if you want the correlation function graph to show residuals. The default it to omit residuals.
>Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photodiode (APD) is triggered or the delay time or $r_h$ 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 <a href="#">rh from DLS Procedure on page 280</a> for information about the APD State property.
ACF Display Binning	Adjust the number of autocorrelation 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 Procedure

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 Procedure on page 227](#) for details. This section shows the Column Calibration page and options, followed by a step-by-step example of how to use a Column Profile.

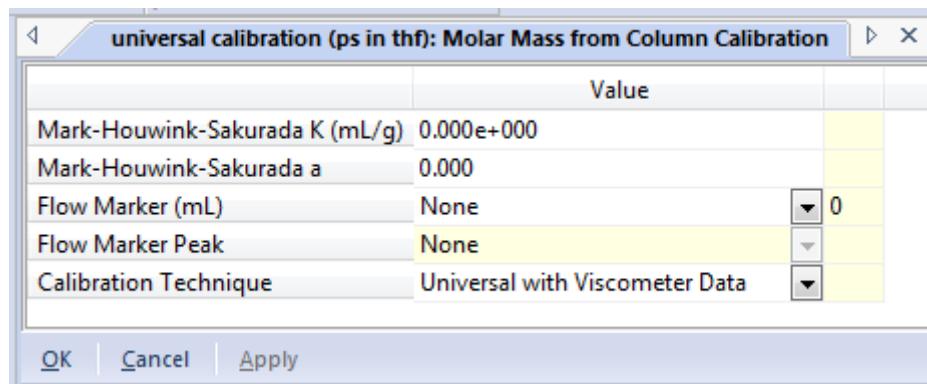
Two main types of column calibration are available. “Conventional calibration” and “universal calibration.” For conventional calibration, the polymer type of the analyzed polymer must be the same as the polymer type of the polymer used for calibration. For universal calibration, the polymers may be different.

For experiments that perform this procedure, choose **File→Open→Experiment** (or **File→Import→Experiment**) if you are using ASTRA with Security Pack), 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.

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 Procedure on page 227](#). If your experiment does not contain the Calibrate Column procedure, you should import the results of a column calibration using [Column Profiles on page 171](#).

This procedure has the following page:



The properties for this procedure are as follows:

*Table 9-38: 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"> <li>- None- no flow marker is to be used. The flow marker value is set to zero.</li> <li>- Enter value- enter the flow marker value in mL in the cell to the right of the drop-down.</li> <li>- 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.</li> <li>- 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.</li> </ul>

Table 9-38: Molar Mass from Column Calibration Properties (continued)

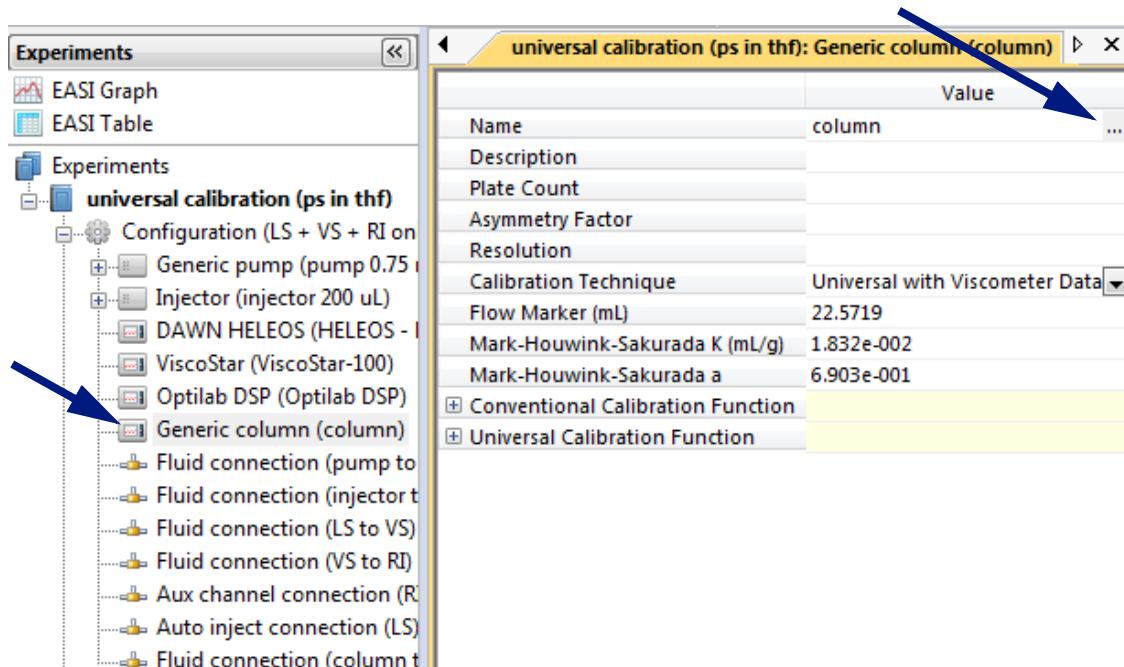
Field	Description
Flow Marker Peak	Select the peak for the flow marker if you used one.
Calibration Technique	The type of column calibration performed. The options are: none, Conventional, Universal with Viscometer Data, and Universal without Viscometer Data. This property can also be specified in the Column profile. See page 171.

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

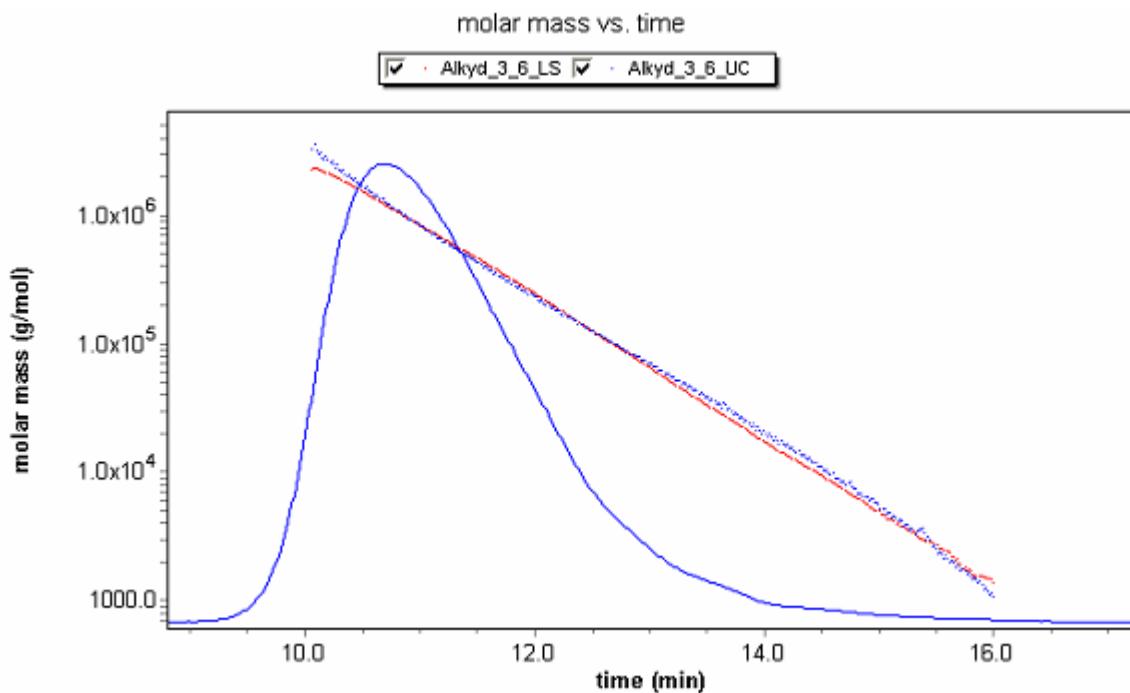
To use a column calibration to measure the characteristics of an unknown sample, follow these steps:

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.
4. Press the “...” button next to the Column name, and select a column calibration you performed earlier.

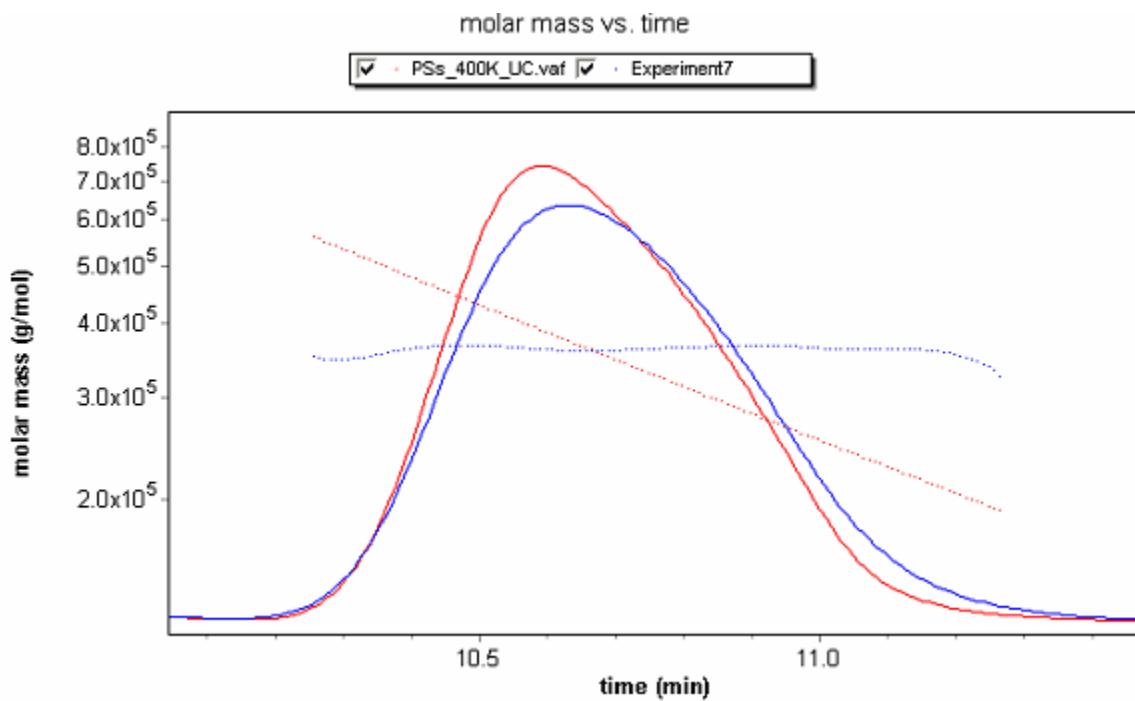


The column profile is updated with the coefficients from the known sample run.

5. Select the particular instruments used at your location, configure the alignment, and make any other experiment configuration changes you normally make.
6. Choose **Experiment→Configuration→Save As** to save this experiment as a method for later use.
7. 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.
8. 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:



# 10

## Using Sequences

This chapter explains how to configure and run sequences in ASTRA 8.

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Editing a Sequence .....	319
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## About Sequences

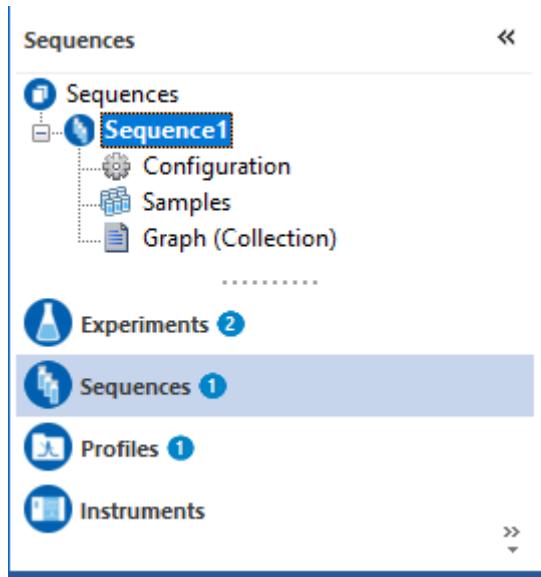
ASTRA sequences provide an easy way to manage experiment sequences with multiple samples. A sequence can be configured with a default experiment method, such that all samples are collected in the same fashion. Or, a different experiment method can be specified for each sample, making it possible to collect different types of data for each sample. For example, you might collect the DLS signal 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 predefined method for each injection, and then runs the experiment to collect and analyze the data. Therefore, a sequence can create many experiments.



You must have at least Researcher access to create new sequences. If you are a Technician, you can modify and run sequences created from a template or default. If you are a Guest, you have read-only access to sequences.

ASTRA 8 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 from a blank sequence. If you are a Technician, you can create a sequence from a sequence template.

### Creating Blank Sequences

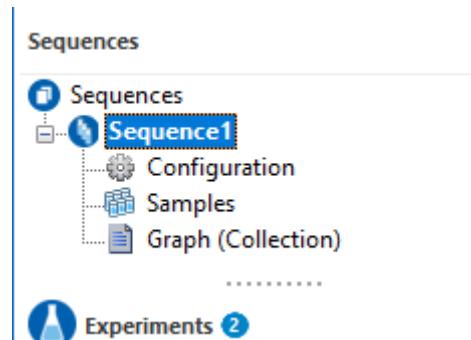
Create an empty sequence by following this step:

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 117 and page 118), that method is the default for all samples in the new blank sequence.

### 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 316](#)).

## 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.

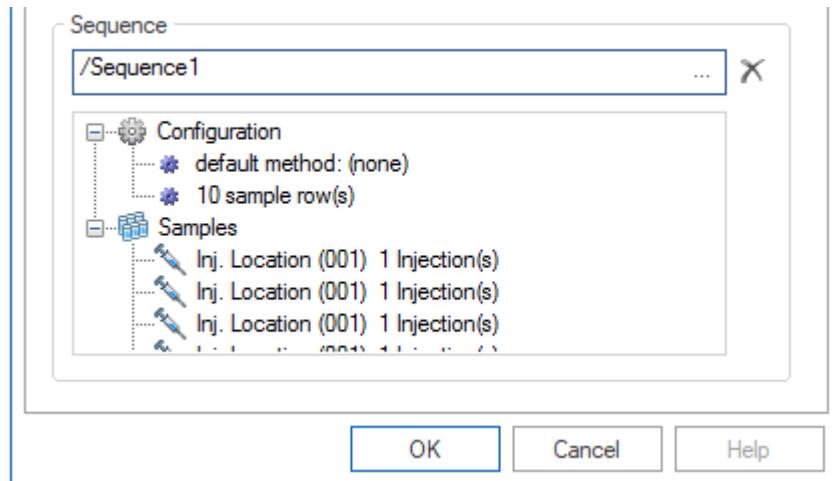
2. In the New from Existing dialog, browse to and select the sequence template you want to use. Sequence templates are available only if you have saved them as described in [Saving Sequences as Templates on page 326](#).

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** area shows the path to the current default sequence, if one is selected.

To change the default sequence, click the “...” button and browse for the sequence you want to use as the default. The area below shows the configuration and samples of the selected default sequence.

When you choose **File→New→Sequence from Default**, the default sequence is used to create a new sequence.

To remove a default sequence, click the “x” button next to the “...” button.

See [Setting a Default Method on page 118](#) for information about setting a default method and [Setting a Default Configuration on page 128](#) 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 with Security Pack, experiments are stored in the experiment database, and you open experiments from that database. If you are using ASTRA Basic, sequences are stored in separate files with an extension of \*.afs8.

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 Technician access to import sequences.

---

You can import ASTRA sequences saved with ASTRA 8, ASTRA 6, ASTRA 7, and ASTRA V. Sequences have the following file extensions:

File Extension	Description
.afs8	ASTRA 8 sequence file.
.afs7	ASTRA 7 sequence file.
.afs6	ASTRA 6 sequence file.
.vsf	ASTRA V sequence file.



This item is disabled in ASTRA 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

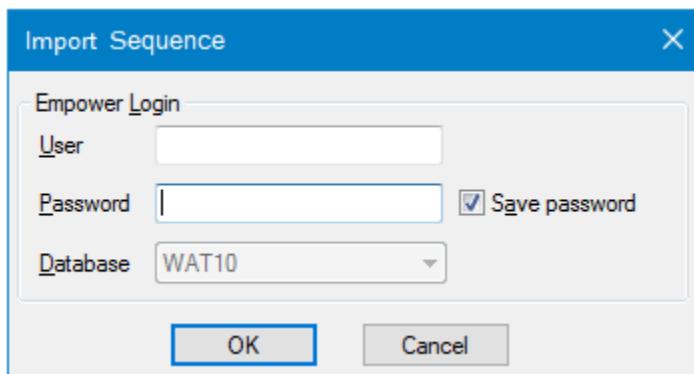


You must have at least Technician access to import sequences.

ASTRA (32-bit edition only) 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→New→Sequence from Empower**.
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**.

## Importing a ChemStation Sequence

---



You must have at least Technician access to import sequences.

---

ASTRA can import a sequence that you have exported from the Agilent ChemStation software.

To import a ChemStation sequence, follow these steps:

1. Use the **Print Output** feature of the ChemStation software to generate a text version of a sequence and save it to a text file.
2. Choose **File→New→Sequence from Chemstation**.
3. Browse to find the text file you saved and click **Open**. The sequence is shown in your Sequences navigation pane with the name of the sequence you imported.

ASTRA converts the ChemStation sequence to a full-featured ASTRA sequence. Sample names and identifying information are all brought over from ChemStation.

4. To save the sequence in your experiment database, choose **File→Save**.

---

## 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.

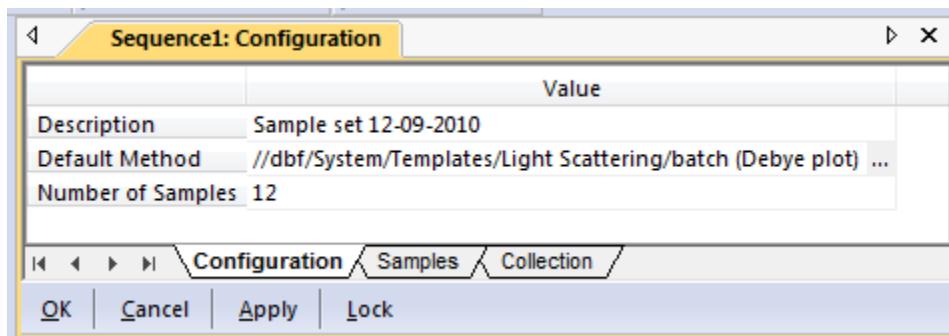
The remaining sections of this chapter contain details about the properties you can set in the various tabs.

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 vial 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 10-1: 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 <a href="#">Creating Experiments from Methods on page 97</a> . For information on creating experiment methods, see <a href="#">Creating a Method on page 117</a> .
Number of Samples	The number of samples in the sequence. This can also be changed in the Samples view after the initial configuration.

Click the **Apply** button to apply any changes you made.

Replacing configurations (page 185) 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.

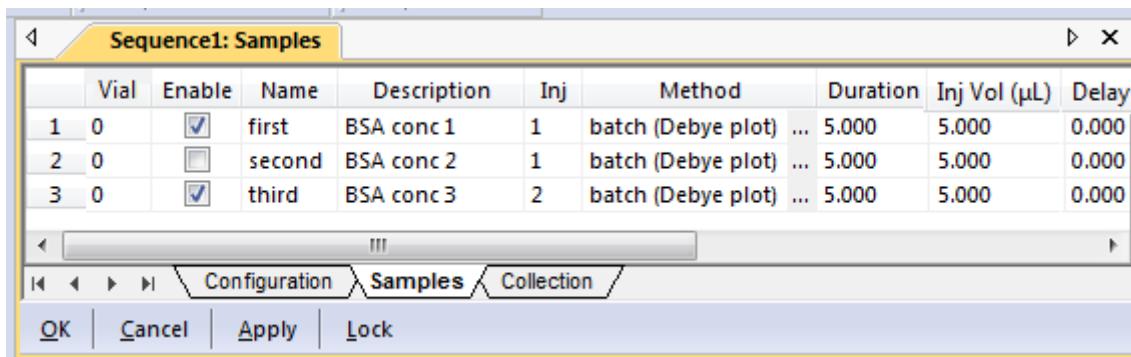
---

**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 checked.

---

## Samples Tab

The Samples tab of the Sequence property page looks like this.



While a sequence is running, you can change values for a sample that has not yet run. Once a particular sample has run, its fields are noneditable.

If you right-click on a sample row, the pop-up menu allows you to **Add** a sample to the end of the list, **Insert** a sample above the selected sample, and **Delete** the selected sample. These operations are all available even while the sequence is being run. You must click 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 10-2: Sample Properties

Field	Description
Seq #	The sequence number in the sequence. This field is non-editable.
Vial	The number of the injection vial in the sample tray. This is for informational purposes only to indicate a specific vial/well position in the autosampler. You need not use this field.
Enable	Check this box to include the sample in 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. Do not use any special characters in the name, such as / % * or \.
Description	Description of the sample, which typically contains more detail than the Name.
Inj	The number of injections to be made for the sample.

Table 10-2: Sample Properties (continued)

Field	Description
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 <a href="#">Creating Experiments from Methods on page 97</a> . For information on creating experiment methods, see <a href="#">Creating a Method on page 117</a> .
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. This time should match the run time specified for the sample in the chromatography software sequence that manages the actual injections. Caution: If the duration entered here is longer than that in the chromatography software sequence, a sample could be injected before ASTRA data collection starts.
Inj Vol ( $\mu\text{L}$ )	The injection volume of the sample in $\mu\text{L}$ . If provided, ASTRA uses this value in combination with the Conc value to calculate mass recovery.
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 (and typically recommended value) is zero.
dn/dc (mL/g)	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 in the profile is the default value when peaks are set for the data.
A2 (mol mL/g <sup>2</sup> )	Second viral coefficient value associated with the sample, if known, measured in mol mL/g <sup>2</sup> . The value set here is used as a default value for peaks set in the experiment. This value is used to correct for non-linear effects due to concentration. The value is often left as 0.0, because concentrations are typically too low to cause any effect.
UV Ext (mL/(mg cm))	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 (mg/mL)	The concentration of the sample in mg/mL. If provided, ASTRA uses this value in combination with the Vol value (below) to calculate mass recovery.

For HPLC Instrument collections, a Flow Rate column is also included. (See [Chapter 6, Setup for HPLC Functionality](#).)

The Inj, Inj Vol, and Vial number columns control the HPLC function. See [Configuring a Sequence for HPLC](#) on page 182 for more information.

In order to avoid cycling the laser frequently, which will shorten its life-span, 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.

---

<b>Note:</b>	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**.

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 97](#). For information about modifying the procedure sequence, see [Changing the Procedure Order on page 196](#).

### 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.

**Note:** Do not click the **Run Default** button. This button only collects data from a single injection using your default method.

3. In the Save window that appears, navigate to the location where you want to save the ASTRA data. Type a name for the sequence in the **File name** field and click **Save**. The data files generated by the sequence will have the format “Name[SequenceName].afe8”.
4. The message “Waiting for auto-inject signal” appears. Use your HPLC or UHPLC software to begin the sample injections.
5. 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 runs, a message says “Sequence run completed.”

**Note:** For μDawn users, ASTRA automatically performs a data quality check at the end of each collection in a sequence. If a collection is found to have a discontinuity in the data stream, the background color of that row in the sequence table is shaded yellow. A dialog box appears at the end of the collection to alert the user to the discontinuous data. The dialog indicates which rows should be investigated. The user is advised to examine the collected experiments closely, paying particular attention to time points highlighted by vertical red markers.

If you frequently observe discontinuities in your experiments, please contact Wyatt Technical Support.

---

## 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 51](#) for details.

## Viewing a Sequence Log



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 10, Using Sequences](#).

Double-click on a line in the log to see more details.

To save a sequence log to a CSV or text file, choose **Sequence→Log→Save As**.

To print a sequence log, choose **Sequence→Log→Print**.

## 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.



The Save As Type field shows that the file will be saved with an extension of \*.ASF8 if you are using ASTRA Basic.



If you are using ASTRA 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

To save a sequence as a template, right-click 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 316](#) for more information.

## Exporting Sequences



If you are using ASTRA with Security Pack, you can export a sequence from the experiment database to a file. 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 filename for the sequence.
4. Click **Save** to create the file.

## Deleting a Sequence

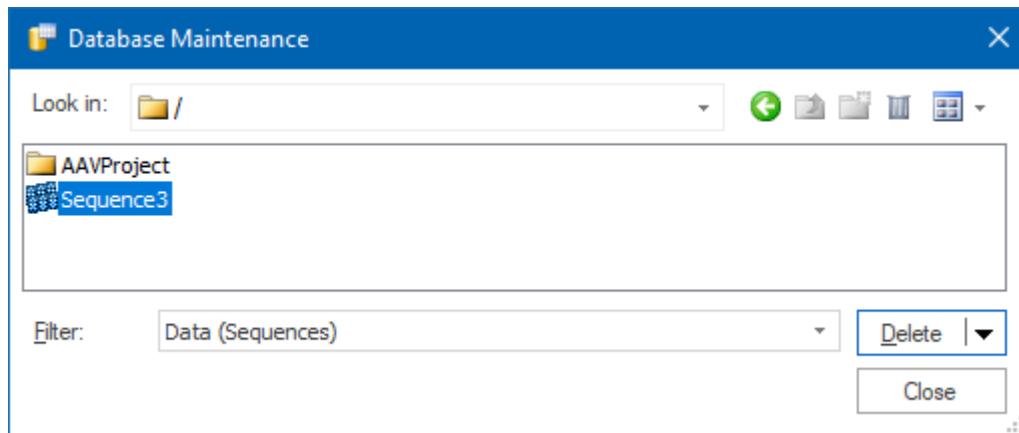
**Basic**

If you are using ASTRA Basic, you may delete a sequence by deleting the \*.ASF8 file that contains the sequence.

**Security**

If you are using ASTRA with Security Pack, delete a sequence by following these steps:

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



2. Sequences are stored in the Data Database. In the **Filter** drop-down list, choose **Data (Sequences)** to filter by sequences and navigate to the appropriate file folder.
3. Highlight the sequence you wish to delete in the list.
4. Click **Delete**.
5. Click **Close** when you have finished deleting sequences.

**Security**

If you are using ASTRA 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.

# 11

## Working with Reports

This chapter explains how to create, customize, and print reports with ASTRA.

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## About Reports

The methods provided with ASTRA 8 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



#### Basic

If you are using ASTRA 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.



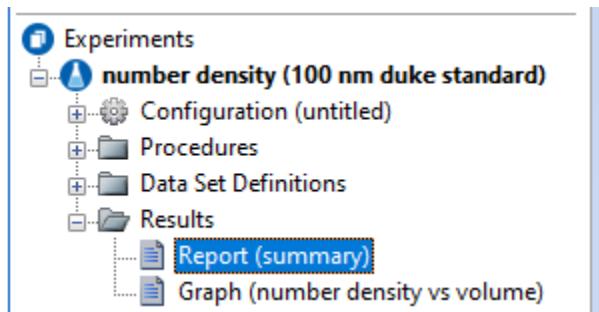
#### Security

If you use ASTRA 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.

ASTRA Report number density (100 nm duke standard)

WYATT TECHNOLOGY

Sample: sample

Configuration

Concentration Source: none  
Flow Rate: 1.500 mL/min

Light Scattering Instrument: DAWN DSP

Cell Type: K5  
Wavelength: 632.8 nm  
Calibration Constant:  $1.8620 \times 10^{-5}$  1/(V cm)

You can use this view as follows:

- If there are any problems with the report, you will see a warning message at the top of the report. Click **Details** for more information.
- Click the **Report Options** icon to open the Report Options dialog. See page 332 for details.
- Click a **Font Size** icons to make the text larger or smaller.
- Click the **Export** icon to save the report to an XML file. See page 334 for details.
- Click the **Report Designer** icon to open the Report Designer pane. See page 333 for details.
- Print the report as described on page 334.

## Abbreviations Used in Reports

The following are some of the abbreviations used in report results. See [Molar Mass and rms Radius Moments on page 444](#) 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. 43.
- **$M_p$ :** 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. 44.
- **$M_z$ :** z-average molar mass. This result is sensitive to fractions with high molar masses. See Eq. 45.
- **$M_{z+1}$ :** 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:

$$M_{z+1} = \frac{\sum_i c_i M_i^3}{\sum_i c_i M_i^2}$$

- **$M(\text{avg})$ :** uncertainty-weighted average molar mass.
- **$r_n$ :** Number-average mean square radius. See Eq. 50.
- **$r_w$ :** Weight-average mean square radius. See Eq. 51.
- **$r_z$ :** z-average mean square radius.
- **$r(\text{avg})$ :** Uncertainty-weighted average radius. See Eq. 52.
- **$[\eta]_n$ :** Number-average intrinsic viscosity. See Appendix H.
- **$[\eta]_w$ :** Weight-average intrinsic viscosity.
- **$[\eta]_z$ :** z-average intrinsic viscosity.
- **$[\eta](\text{avg})$ :** 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.
- **$r_h(\text{avg})$ :** uncertainty-weighted average hydrodynamic radius.

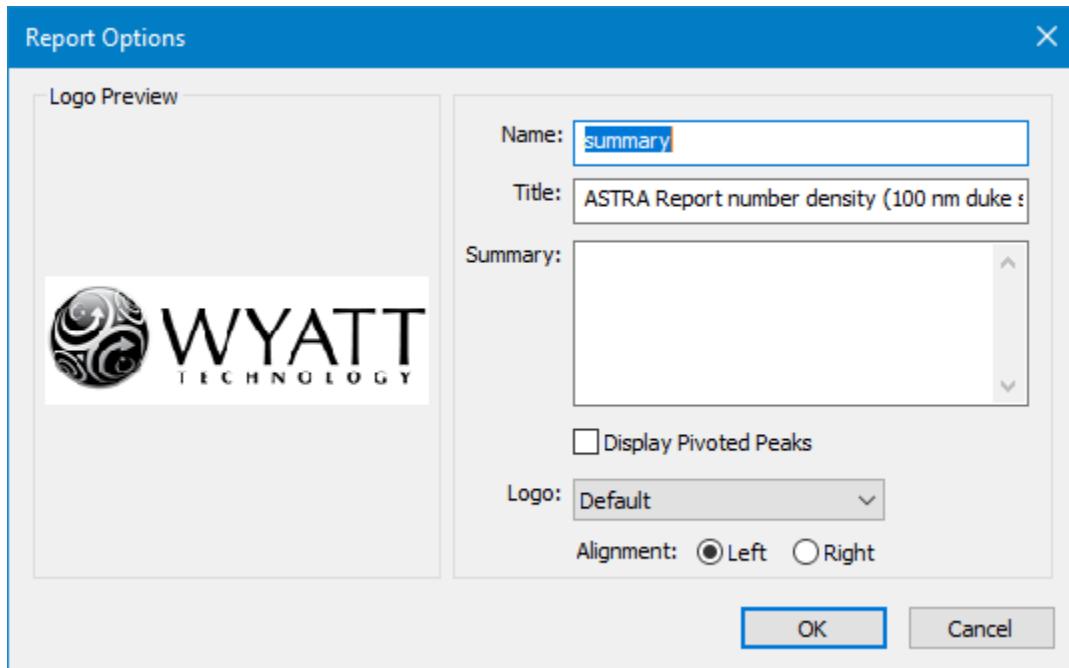
## Setting Report Options



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



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 351](#).
- **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.

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.

The screenshot shows the Report Designer pane with a tree view of report items. The items are categorized under 'Printing Operator', 'File Properties', 'Graphs', 'Configuration', 'Sample', 'Processing', 'Results', and 'Signatures'. Under 'Configuration', 'Flow Rate' is selected and highlighted with a blue border. In the bottom right corner, there is a detailed configuration panel for 'Flow Rate' with the following settings:

Decimal places	3
Scientific	False
Percent uncertai...	False

Below the table, a tooltip for 'Decimal places' states: 'Specify the number of decimal places to display.'

## 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 an .xml or .txt file.

To export experimental data only, see [Exporting Data on page 357](#).

## 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 332](#).
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 333](#).
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.

# 12

## Working with Graphs and Tables

This chapter describes how to create and use EASI graphs, data set definitions (DSDs), and DSD-based graphs.

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## 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 192](#) for information about these graphs.
- **EASI Graphs.** These graphs are flexible and easy to create. EASI Graphs allow you to display the results of multiple experiments in the same plot. 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 338](#) 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 341](#) 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 344](#) and [Creating Custom Plots on page 347](#) 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 Procedure on page 287](#).
- **Surface Plots.** You can create a 3D surface plot of detector data. See [Creating Surface Plots on page 349](#).

For all types of graphs, see the following sections:

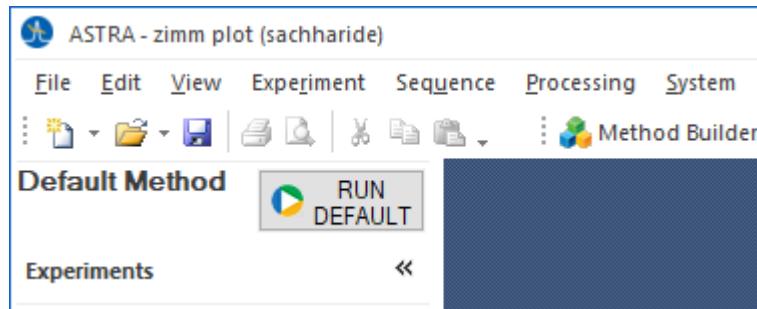
- [Viewing and Modifying Graphs on page 351](#) for information about how to zoom in and out and change the look of the graph.
- [Printing Graphs on page 354](#) for information about printing any type of graph.
- [Exporting Graphs on page 355](#) for information about saving graphs to image files.

## Using the EASI Graph

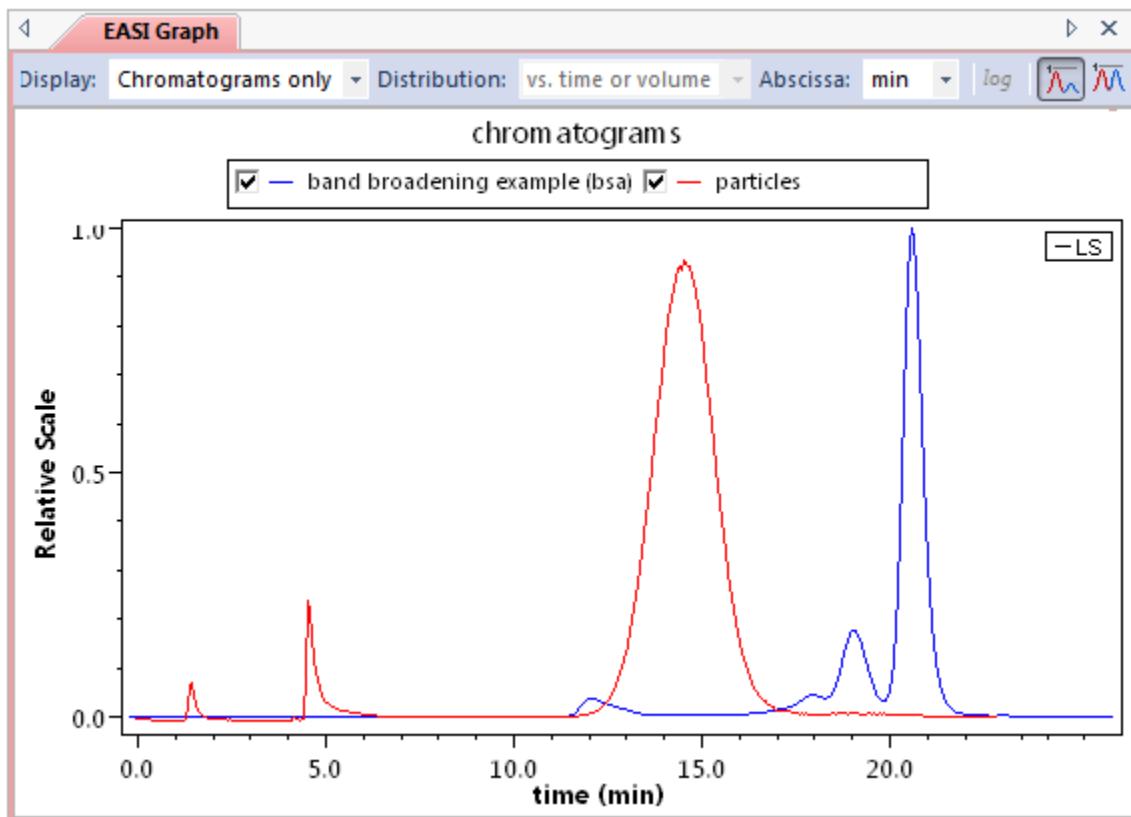
EASI graphs provide a way to quickly visualize results.

To view the EASI Graph, do one of the following:

- Choose **View→EASI Graph**, or
- Double-click **EASI Graph** at the top of the Experiments 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 use the following controls to modify the graph:

- **Display:** Choose the type of analysis you would like to display. The options are: chromatograms only, 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, specific viscosity, Mark-Houwink-Sakurada plot, radius, mean-square radius, rod, translational diffusion coefficient, branching ratio (g and g'), branch units per molecule, long-chain branching, protein conjugate, instrument voltages, log ( $M[\eta]$ ) plot, viral vector concentration, and viral vector molar mass. The plots that involve hydrodynamic radius ( $r_h$ ) can use DLS or viscometry data.
- **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.
- **Global Scaling:** Each chromatogram is scaled against the greatest magnitude across all chromatogram data.
- **Individual Scaling:** Each chromatogram is scaled against its own magnitude. Local maxima/minima are used when zooming in.
- **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 EASI Graph Options dialog, which has the following fields in the **General** tab:

Table 12-1: EASI 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.

Table 12-1: EASI Graph Fields (continued)

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.
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, DLS 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.
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.
Series	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 may also change font properties for text in the graph in the **Fonts** tab.

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 than is described in this manual. For help on settings in the Advanced dialog, move to a field and press F1.

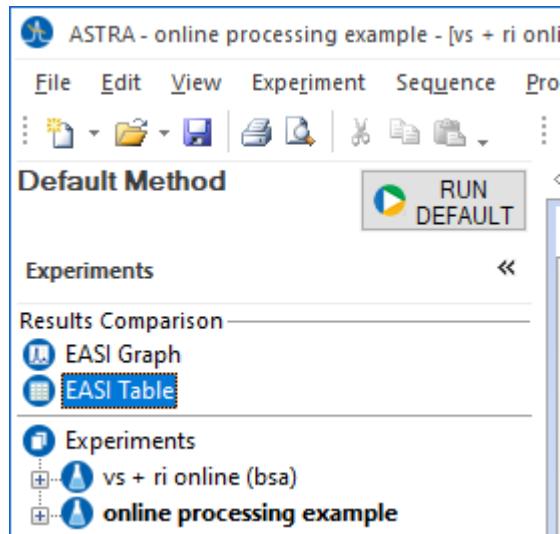
You can print an EASI graph by right-clicking on the graph and choosing **Print** from the pop-up menu. You can also copy the graph to your clipboard and paste it into another application, such as a document.

## 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 Table, do one of the following:

- Choose **View→EASI Table**, or
- 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.

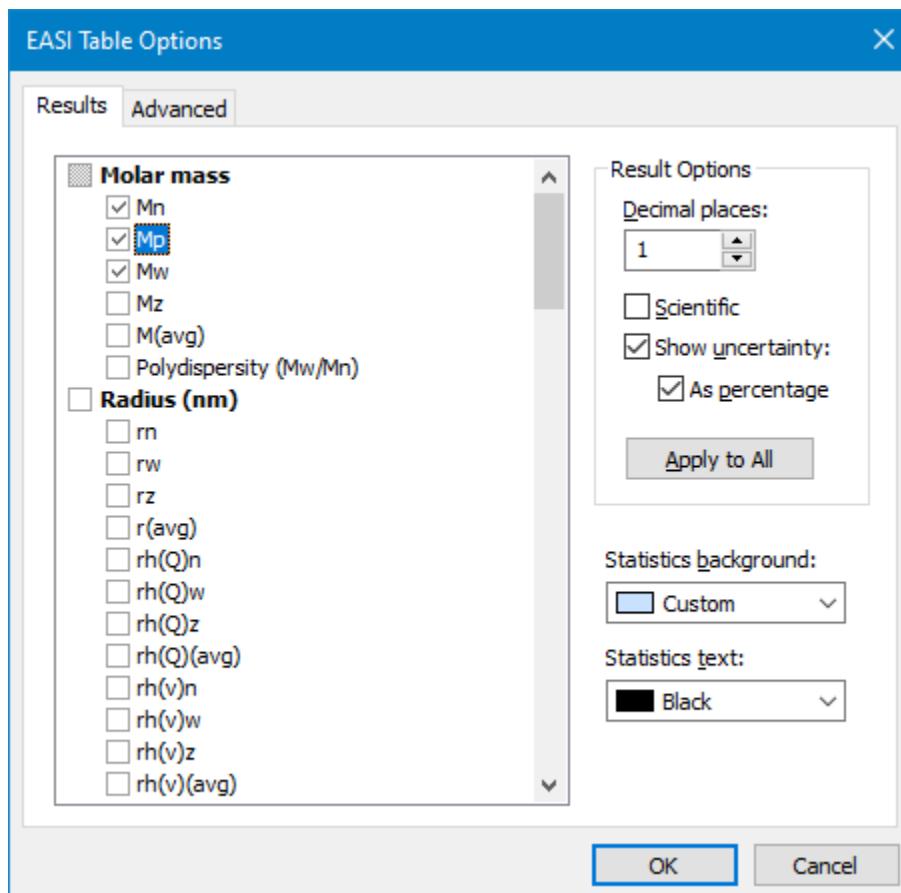
	General			Peak 1		
	Mw (kDa)	Rz rms (nm)	Rz geo	Injected mass	Calculated mass	Mr
online a2 (200k ps)	198.5 (±0.1%)	17.5 (±1.5%)		0.00e000	2.03e01	3.0
online a2 (bsa with desalting)	68.6 (±0.1%)	3.1 (±15.9%)	0.0	0.00e000	8.51e02	6.6
online a2 (Igg with column)	143.7 (±0.1%)	0.0		0.00e000	3.34e02	3.6
Average	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. You may remove scalars by either unchecking the boxes or by right-clicking the column header and selecting “Remove <scalar>”.
- **Abscissa:** Choose whether you want the x-axis values to be measures in milliseconds, seconds, minutes, or hours,

For each column in the table the minimum, maximum, average, and standard deviation values are shown.

To format data, right-click on the column header and select **Format** *column\_name* .



The EASI Table Options dialog appears. To format data, follow these steps:

1. Check the scalars in the **Results** list that you would like to display in the table.
2. In the **Results Options** area, modify how the selected column is displayed. You can adjust the number of decimal places shown and whether scientific notation is used, toggle whether the uncertainty is shown in a column, and toggle whether uncertainty is shown as a percentage or as a value.
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 the selected column.
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

ASTRA allows you to create custom plots using any set of data that ASTRA has collected. You can plot raw data or any of the analyzed or processed data sets.

ASTRA stores data in categories that are referred to as data sets. 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 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 group of data sets includes the results after each of the despiking, smoothing, baselines, peaks, and other procedures. 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**.

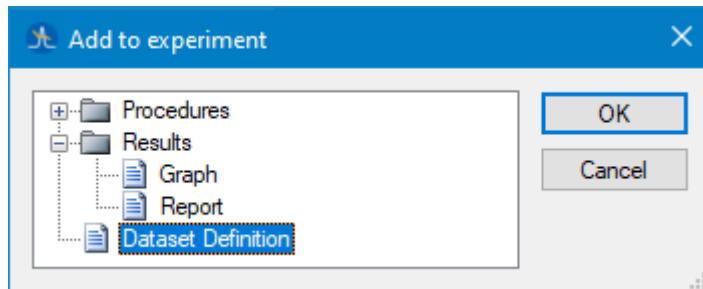
You can create a data set definition before or after you run an experiment.

To create a new data set definition, follow these steps:

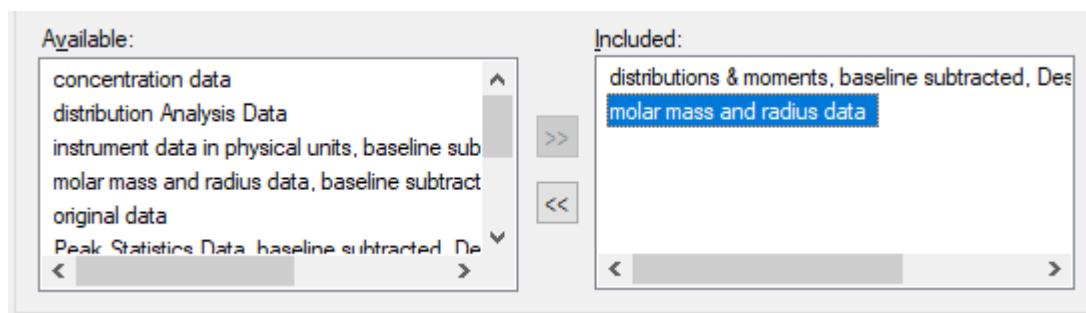
1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results nodes of the experiment.

**Shortcuts:** Press Ctrl+Shift+P.

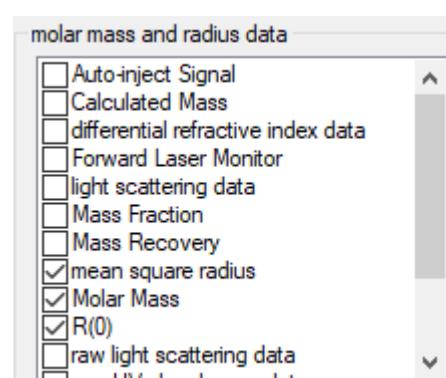
2. Unexpand or scroll past the Procedures node, select Data Set Definition, and click OK.



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 344](#).
6. Click the >> button to move the selected items to the Included list.

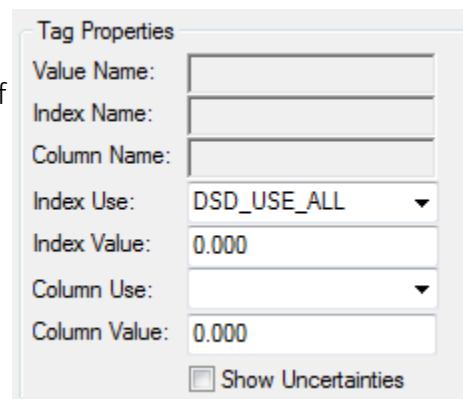


7. Select an item you have moved to the Included list. In the list of data in that stage, place checkmarks next to the data you want to graph. The first data set is graphed against the left y-axis. The second data set is graphed against the right y-axis.



**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 12-2: 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.
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.

Table 12-2: Data Set Definition Properties (continued)

Field	Description
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 116](#) 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 105](#) or [Exporting an Experiment on page 107](#).

## 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.

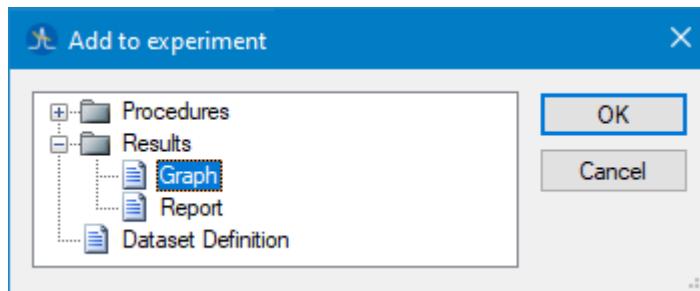


You can also add graphs as follows if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**:

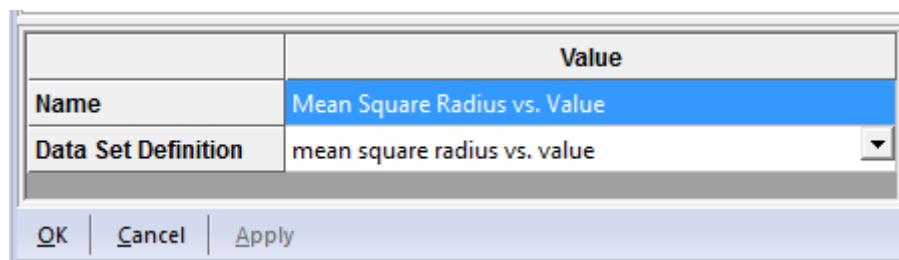
1. Create a data set definition for the data to be graphed as described in [Creating Data Set Definitions on page 344](#).
2. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results folders of 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.

You can return to the Data for Custom Plot dialog for a custom plot and make changes by right-clicking on a custom plot and choosing **Modify Chart Data** from the pop-up menu.

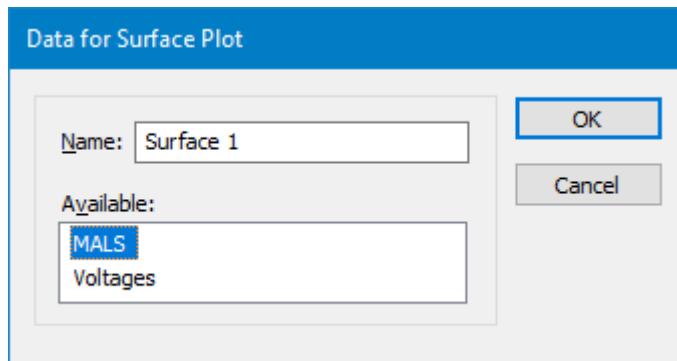
## Creating Surface Plots

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.
- 

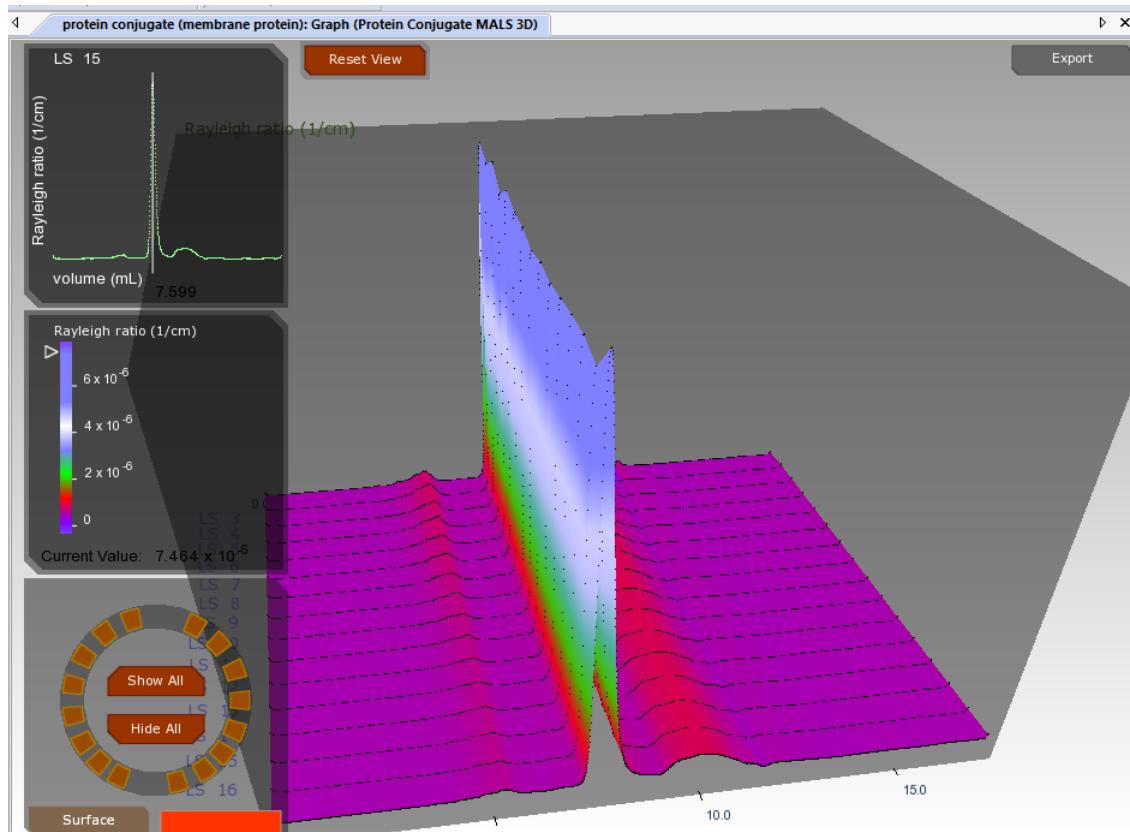
To create a 3D surface plot, follow these steps:

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

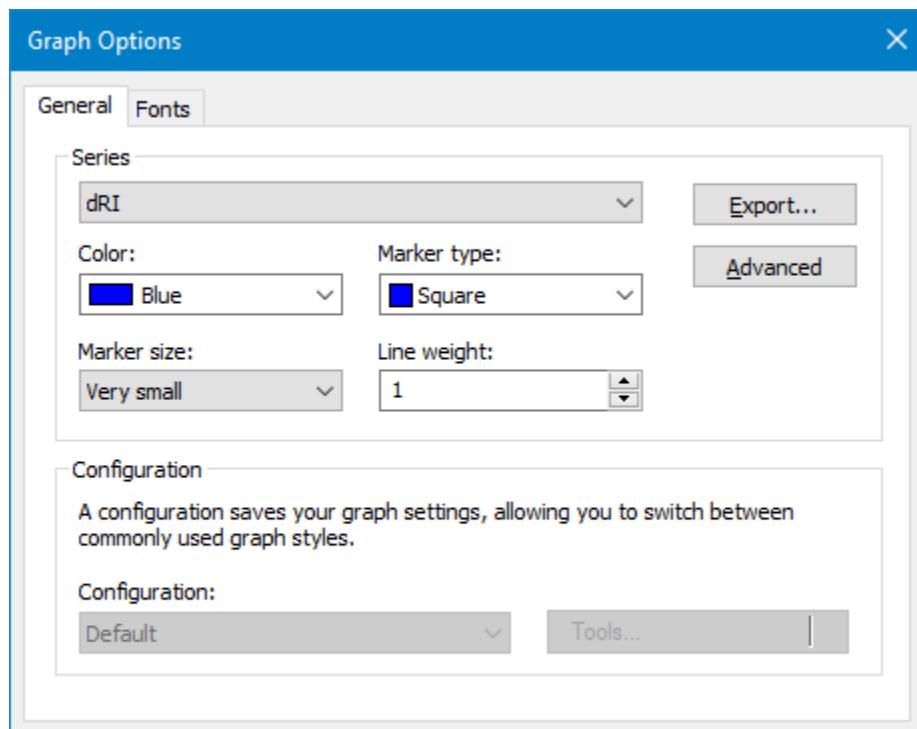
Graphs you generate can be manipulated in a number of ways.

### 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 plot style for a data set, first choose a data **Series**.

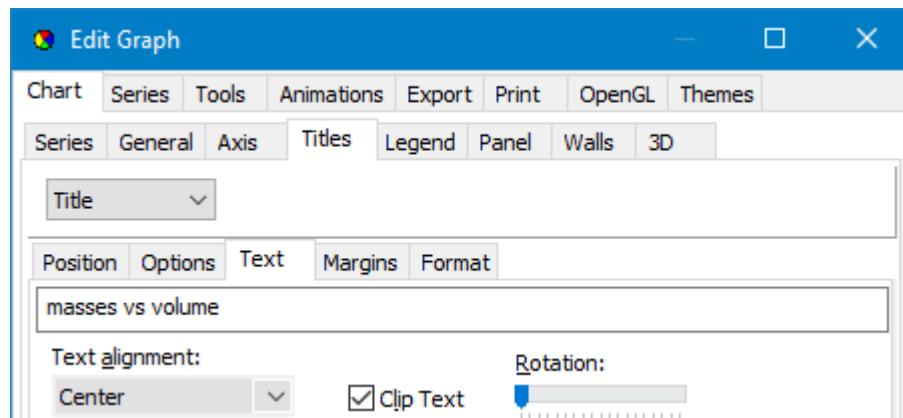
In the **Color** field, select a defined color or “Custom” to choose other colors. The **Marker Size**, **Marker Type**, and **Line Weight** fields let you set the size and shape of the data points or width of the line. Some lines are made up of individual data points and some are drawn as a line. Use 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.

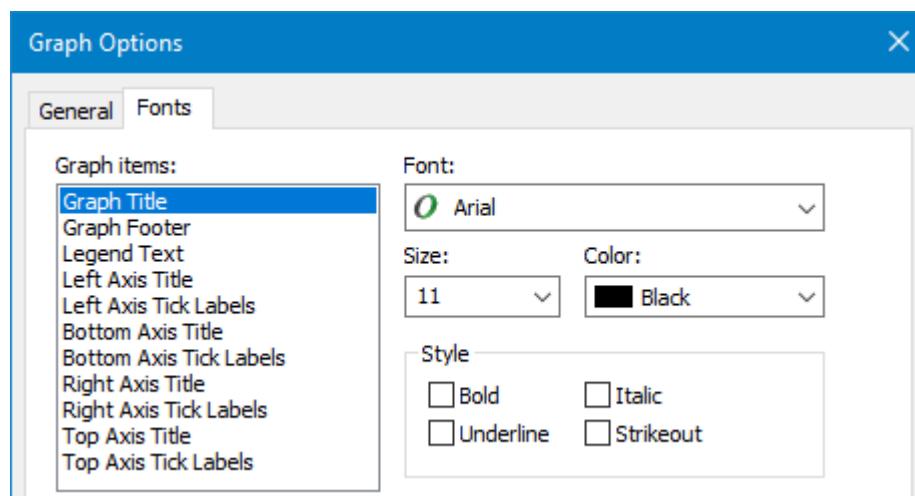
Graph customizations—such as line weight, color, marker style, and title changes—are saved for EASI graphs, custom plots, parametric plots, and surface plots. However, such graph customizations are not saved when you close procedure pages and reports that contain graphs.

When you make graph customizations, the configuration changes from the Default configuration to a new configuration called Custom (1). Once you click **OK**, these changes will be saved in the Custom (1) configuration. Use the pull-down options in the **Tools** menu next to the configuration name to clone, rename, or delete a configuration. The **Clone** option allows you to build on your current customizations to create new configurations. 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 than is described in this manual.



The **F**onts 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 339 for details about the EASI Graph version of the Graph Options 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. Right-click on a graph and choose **Edit** to open the Graph Options 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 Graph Options 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.

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

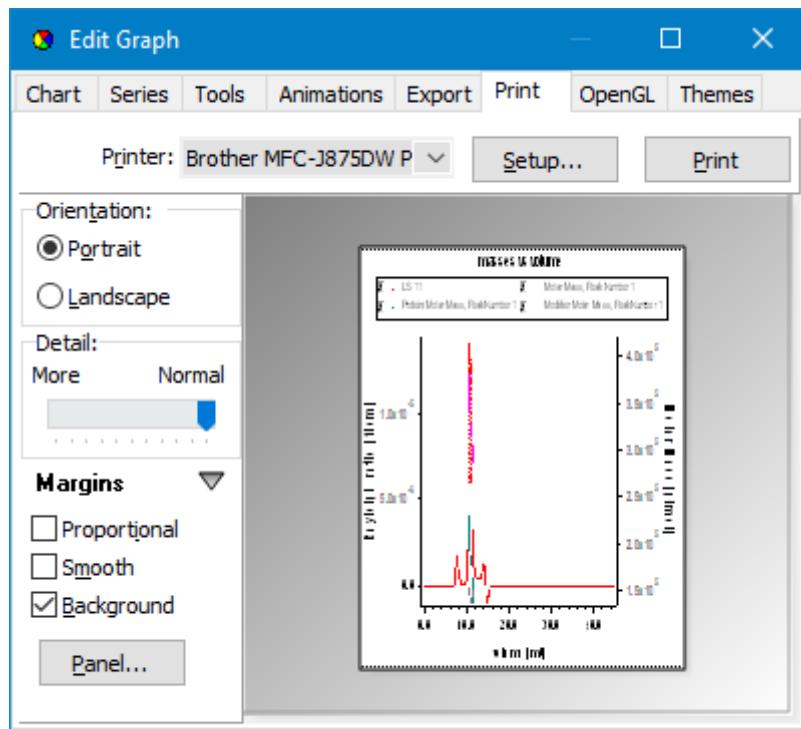
To print a graph, do either of the following:

- 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. Right-click on a graph and choose **Edit** to open the Graph Options 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.
5. Use the **Detail** slider to adjust the quality of the output and number of major tick marks on the axes.
6. Click the triangle next to **Margins** to show the margin options. Note that margins are specified as a percentage of the page size.
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**.

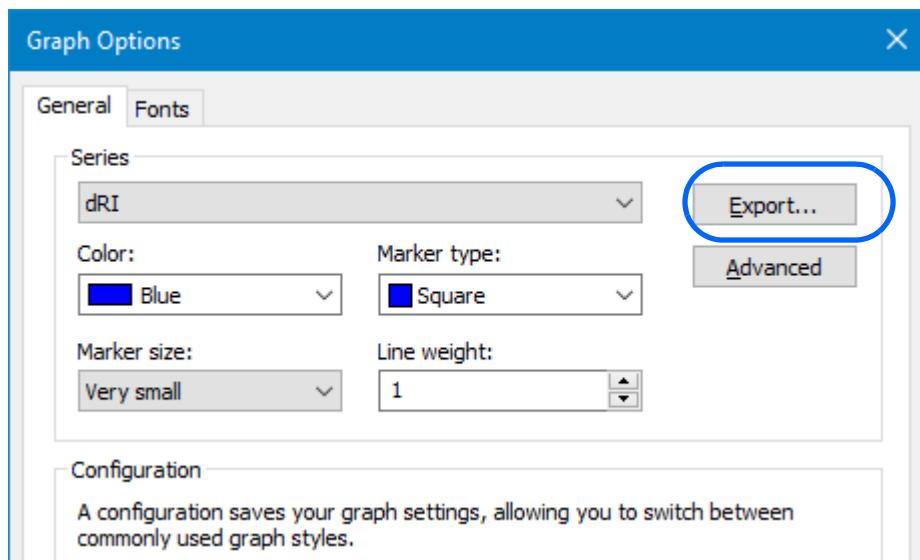
---

## 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 right-click on a graph and choose **Edit**.

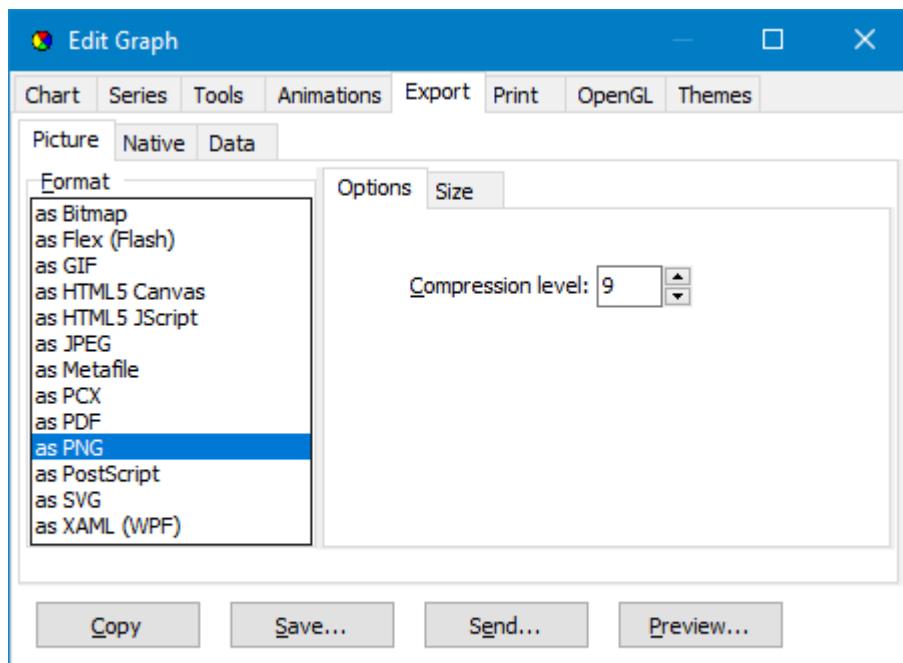
### Exporting Pictures

To quickly export a graph image, right-click on a graph and choose **Edit** to open the Graph Options dialog. Click the **Export** button and choose the JPEG output file type. See [Working with Procedure Graphs on page 192](#) for details.



Alternatively, you can export a graph to a graphics file with more control over the output by following these steps:

1. Right-click on a graph and choose **Edit**. In the Graph Options 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.

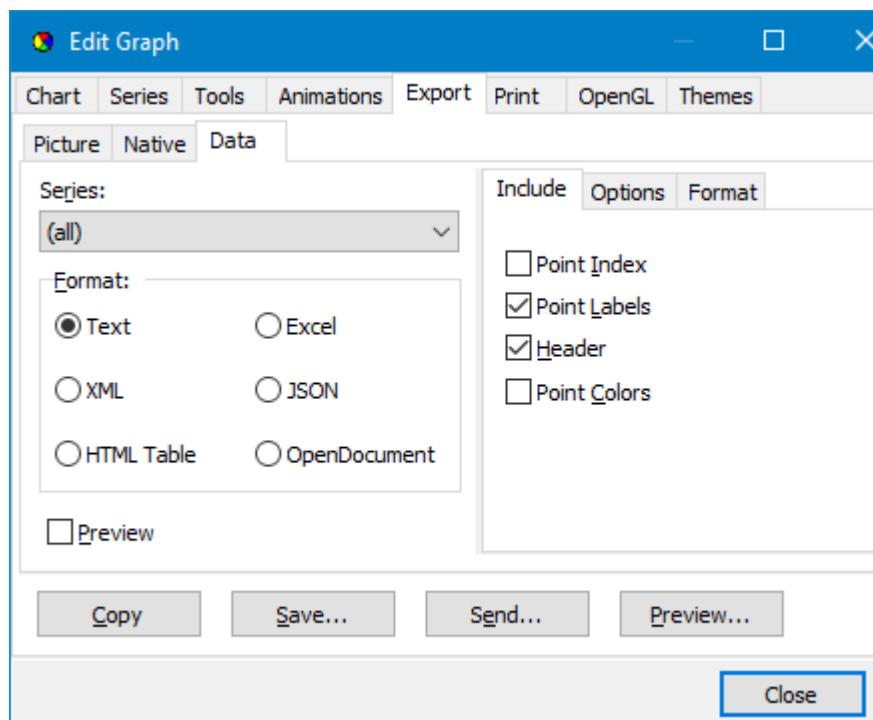
## Exporting Data

To quickly export graph data, right-click on a graph and choose **Edit** to open the Graph Options 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)
- Data saved as an HTML table (.htm)
- Data saved in JSON format (.json)
- Data saved in OpenDocument format (.ods)

Alternatively, you can export a graph to a data file with more control over the output by following these steps:

1. Right-click on a graph and choose **Edit** to open the Graph Options 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, Microsoft Excel, JSON, or OpenDocument.

5. Depending on the output format you select, you can control various options in the **Include**, **Options**, and **Format** tabs.  
For example, for Text files, you can select a Delimiter to separate the fields in the **Options** tab. 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. For JSON files, you can choose whether to format dates and times as timestamps or in the format used by JavaScript.
6. Select any other information you want to include in the data file, such as headers.
7. 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 Graph Options window.
  - **Send:** Send the data to an application such as Microsoft Outlook. This is typically used to email the data.
  - **Preview:** Send the output to an appropriate application.

If you do not find the organization of data output useful, try the output described in [Exporting an Experiment on page 107](#).

# 13

## Working with Profiles

This chapter explains how to create and use profiles in ASTRA 8.

<b>CONTENTS</b>	<b>PAGE</b>
About Profiles .....	360
Creating Profiles.....	361
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## About Profiles

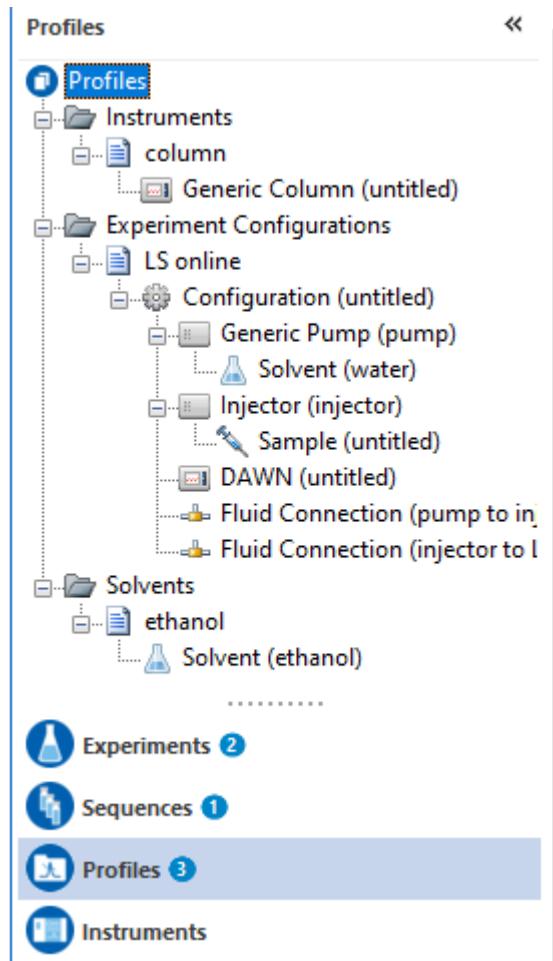
In an experiment, a set of instrument and connection profiles describes how an experiment is set up. This is called the *configuration*. You can also store *profiles* outside of experiments and copy them into experiments as needed. Profiles provided by ASTRA are called *system profiles*.

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 117](#).)

This chapter focuses on profiles. See [Chapter 8, 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

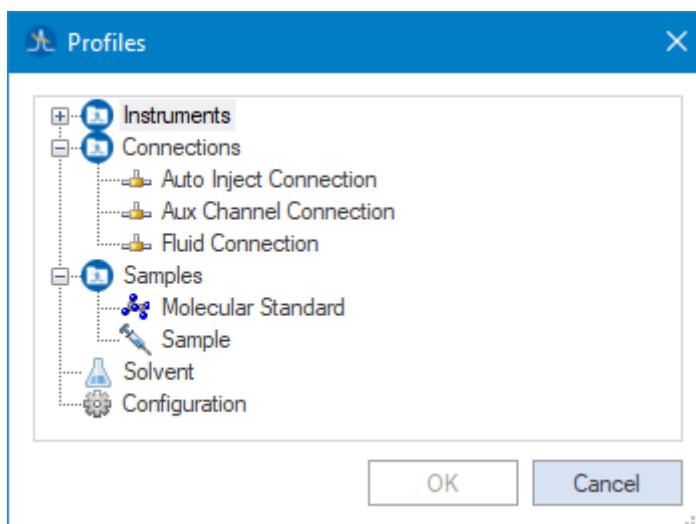
There are several ways to create profiles. These profiles are stored in the system database, rather than in separate files. Experiments use copies of profiles, but modifying the portion of an experiment configuration that came from a profile does not affect the profile itself.

### 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**  
or click the down-arrow next to the  icon.



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.

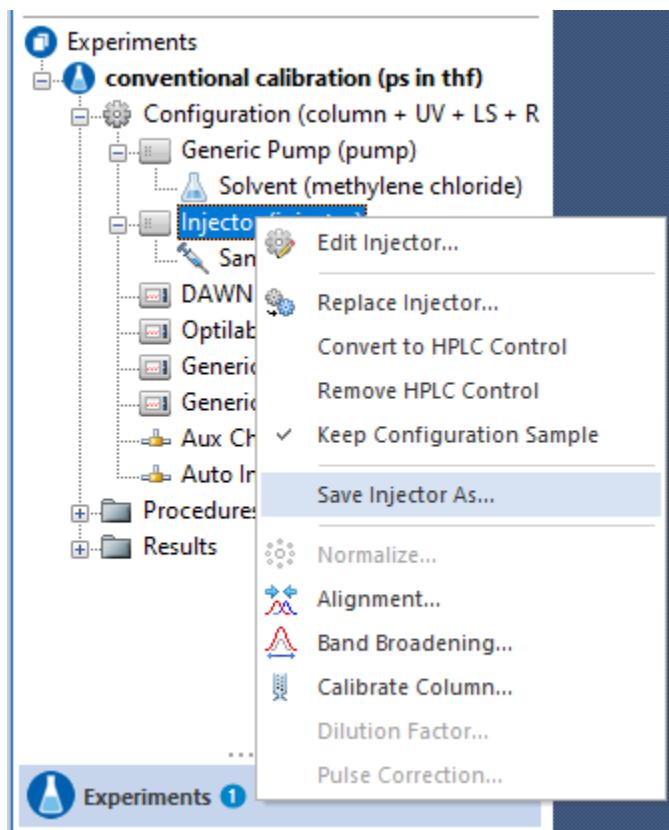
If you created a profile type that has multiple components, there will be a tab for each component. For example, a light scattering instrument in batch mode has a tab for a solvent and a sample. This information is stored with and imported with the instrument profile.

6. Edit the properties as needed. See [Chapter 8, 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 8, 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.

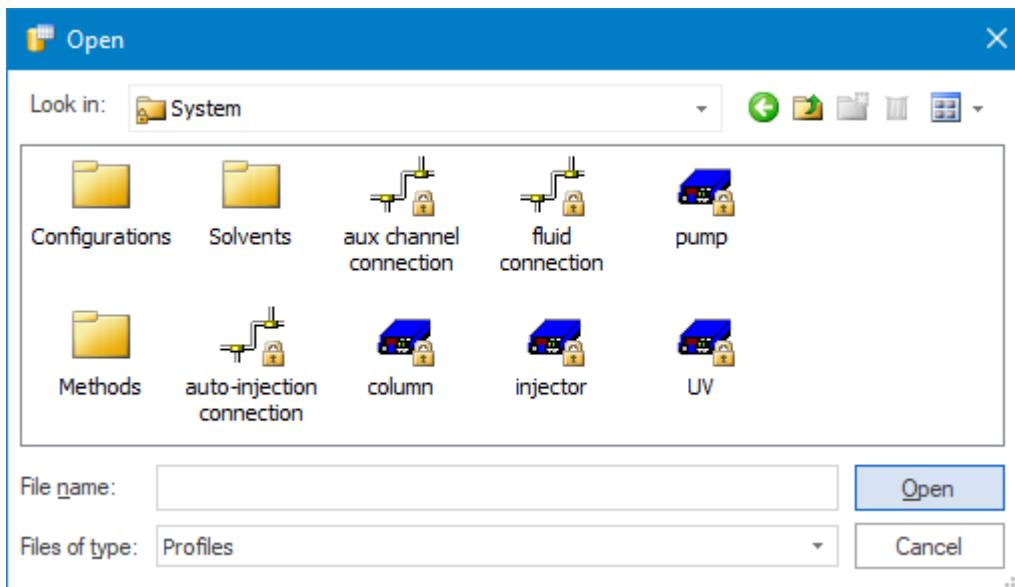
To open a profile, follow these steps:

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:

- **System > Configurations:** A set of complete configurations.
  - **System > Solvents:** A collection of solvent profiles.
  - **User:** A handy place to save profiles you create.
3. You can select the type of profile you want to find in the Files of type drop-down 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.

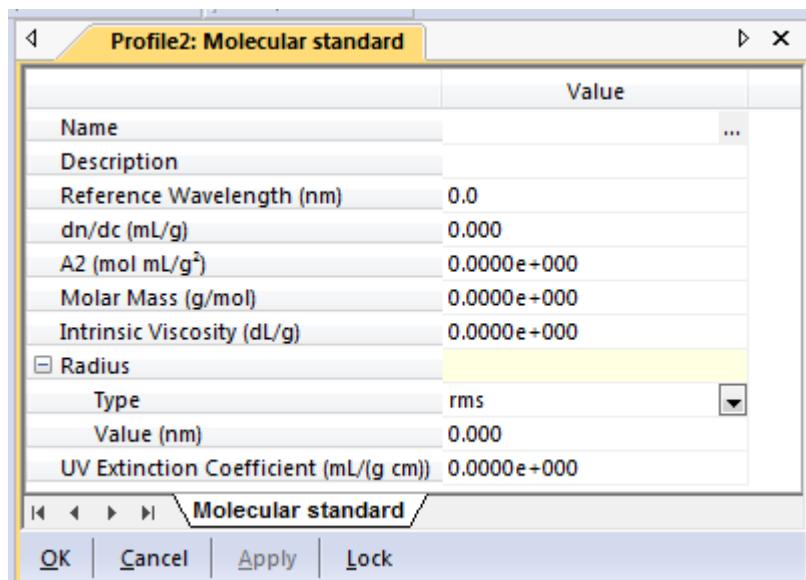
## Editing a Profile

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 8, Configuring Experiments](#) for details about the properties of all profile types.



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

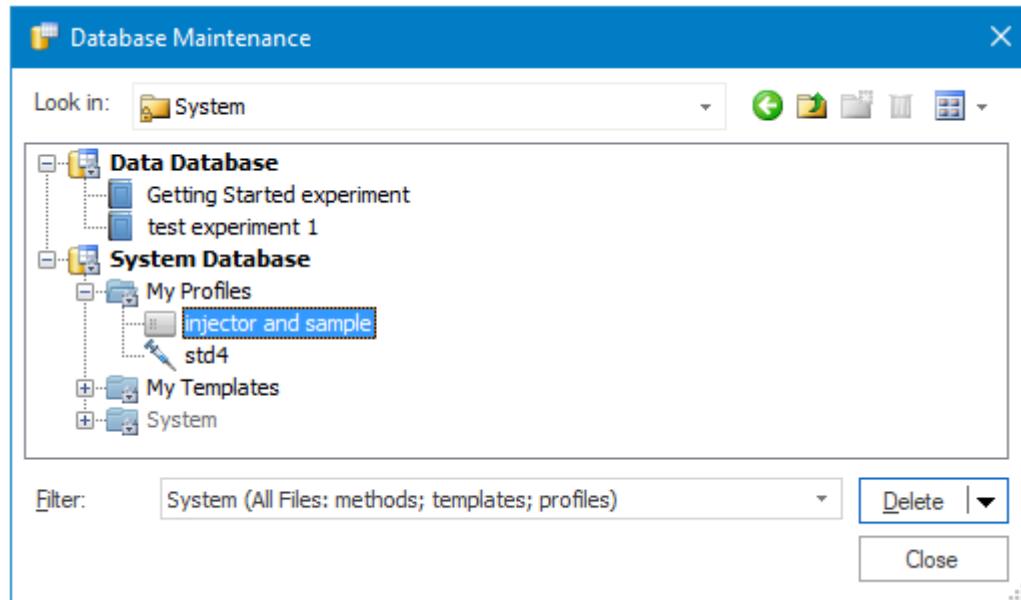
To close a profile, select the profile you want to close and choose **File→Close**. The profile is still available for use in the system database; it is simply not shown in the Profiles navigation pane.

**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 127](#).

### Adding an Item to a Configuration

You might want to add a component to an experiment. For example, you may want to add a UV instrument to an experiment.

To add an item to an experiment configuration using a profile, follow these steps:

- 1.** Enable **System**→**Preferences**→**Experiment Builder Mode**.
- 2.** Double-click the Configuration node.
- 3.** Expand the Details section.
- 4.** Under the Instruments section or the Connections section, click the **Browse** button next to the **Add** field.
- 5.** In the Add Instrument folder, navigate to the folder with the desired profile.
- 6.** When you find the profile, select it and click **Open**.

---

<b>Note:</b>	Be careful when adding an instrument to an existing configuration to also add fluid connections (visible only in Experiment builder mode) as needed.
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The item is added to your experiment. (If you want to import an entire configuration, see [Replacing an Entire Configuration on page 367](#).)

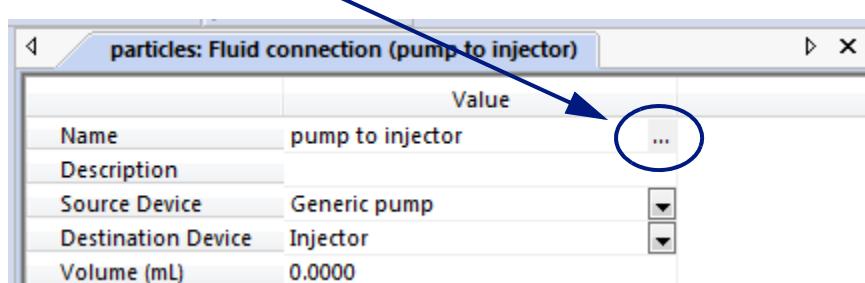
### Replacing a Single Configuration Item

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 Select a Profile dialog, find the profile you want to copy from. The Files Of Type field is automatically set to match only the type of item you are editing.
4. Click **Select**. 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 checked.
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 8 in the Configurations folder.
7. When you find a profile, select it and click **Open**.
8. 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 185](#).

# A

## Menu Quick Reference

This appendix contains a quick reference for ASTRA 8 menu commands and keyboard shortcuts.

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### Modes and User Levels

The following tables list the commands in the ASTRA 8 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.



**Security:** This command exists only when using ASTRA with Security Pack. The user levels apply only to ASTRA with Security Pack.

- **G:** Guest user level
- **T:** Technician user level
- **R:** Researcher user level
- **A:** Administrator user level

If a command is not limited to “Security”, it is always available in ASTRA Basic, even if user levels are listed.

## File Menu

The File menu contains the following commands:

Command	Keyboard Shortcut	Description	Modes
<b>File→New</b>			
→ <b>Experiment from Default</b>	Ctrl+N	Create an experiment from the default method. See page 97.	T, R, A
→ <b>Experiment from Method</b>	Ctrl+M	Create an experiment from a method. See page 97.	T, R, A
→ <b>Blank Experiment</b>	Ctrl+Shift+B	Create an empty skeleton of an experiment. See page 98.	Builder: R, A
→ <b>Sequence from Default</b>	Ctrl+D	Create a sequence from the default sequence template. See page 316.	T, R, A
→ <b>Sequence from Template</b>	Ctrl+T	Create a sequence from a sequence template. See page 316.	T, R, A
→ <b>Sequence from Empower</b>	Ctrl+Shift+E	Create a sequence from a Waters Empower sequence. See page 318.	T, R, A
→ <b>Sequence from Chemstation</b>	Ctrl+Shift+H	Create a sequence from a Agilent ChemStation sequence. See page 318.	T, R, A
→ <b>Blank Sequence</b>	Ctrl+Shift+N	Create an empty skeleton of a sequence. See page 315.	R, A
→ <b>Profile</b>	Ctrl+Alt+N	Create a profile. See page 361.	R, A
<b>File→Open</b>			
→ <b>Experiment</b>	Ctrl+O	Open an existing experiment. See page 108 and page 108.	All
→ <b>Sequence</b>	Ctrl+Shift+O	Open an existing sequence. See page 317.	All
→ <b>Profile</b>	Ctrl+Alt+O	Open an existing profile. See page 363.	All
<b>File→Import</b>			
→ <b>Experiment</b>	Ctrl+I	Import an experiment from a file. See page 110.	Security: T, R, A
→ <b>Sequence</b>	Ctrl+Shift+I	Import a sequence from a file. See page 317.	Security: T, R, A
<b>File→Export</b>		Save the selected experiment or sequence to a separate file. See page 107 and page 326.	R, A

Command	Keyboard Shortcut	Description	Modes
<b>File→Save</b>	Ctrl+S	Save the selection. See page 106 and page 105.	T, R, A
<b>File→Save As</b>	Ctrl+Alt+S	Save the selection with a new name. See page 106 and page 105.	T, R, A
<b>File→Save As Method</b>		Save the experiment as a method. See page 117.	R, A
<b>File→Save All</b>	Ctrl+Shift+S	Save all open items.	T, R, A
<b>File→Close</b>		Close the active item. See page 122 and page 365.	All
<b>File→Close All</b>		Close all items in the current tab. See page 122.	All
<b>File→One-to-Many</b>	Ctrl+B	Apply experiment method to multiple experiments. See page 121.	T, R, A
<b>File→Print</b>	Ctrl+P	Print the currently active view. See page 66.	All
<b>File→Print Preview</b>		Preview the currently active report. See page 334.	All
<b>File→Page Setup</b>	Ctrl+Alt+P	Set up paper size, orientation, and margins. See page 66.	All
<b>File→Print Setup</b>		Set up page formatting for printing. See page 66.	All
<b>File→Properties</b>		View the properties of the current experiment. See page 115.	All
<b>File→Recent Files</b>		Open a recently used experiment. See page 108 and page 108.	All
<b>File→Exit</b>		Close all windows and exit from ASTRA 8. See page 67.	All

## Edit Menu

The Edit menu contains the following commands:

Command	Keyboard Shortcut	Description	
<b>Edit→Undo</b>	Ctrl+Z	Undo the previous action. This command is currently disabled.	All
<b>Edit→Cut</b>	Ctrl+X	Cut the currently selected item and place it on the clipboard. This command is available only as appropriate.	All
<b>Edit→Copy</b>	Ctrl+C	Copy the currently selected item to the clipboard. If the current page displays two graphs, both are copied. This command is available only as appropriate.	All
<b>Edit→Copy Left</b>		If the current page displays two graphs, only the left one is copied.	All
<b>Edit→Copy Right</b>		If the current page displays two graphs, only the right one is copied.	All
<b>Edit→Copy as Image Settings</b>		Set the size of the image you want when you copy a graph. See page 354.	All
<b>Edit→Paste</b>	Ctrl+V	Paste the contents of the clipboard to the current location. This command is available only as appropriate.	All
<b>Edit→Delete</b>		Delete the currently selected item. This command is available only as appropriate.	All
<b>Edit→Select All</b>	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	
<b>View→EASI Graph</b>		Open a quick, customizable graph that can compare data across experiments. See page 338.	All
<b>View→EASI Table</b>		Add a customizable table that lists data from multiple experiments. See page 341.	All
<b>View→Experiments</b>		Select the Experiments navigation pane. See page 64.	All
<b>View→Sequences</b>		Select the Sequences navigation pane. See page 64.	All
<b>View→Profiles</b>		Select the Profiles navigation pane. See page 64.	All
<b>View→Instruments</b>		Select the Instruments navigation pane. See page 64.	All
<b>View→Toolbars</b>			All
→ <b>Standard Toolbar</b>		Hide and show toolbar with new, open, save, and print icons. See page 64.	All
→ <b>Processing Tool-bar</b>		Hide and show toolbar with begin, validate, run, pause, and stop icons. See page 64.	All
→ <b>Graph Toolbar</b>		Hide and show toolbar with icons for use with graphs. See page 64.	All
→ <b>Customize</b>		Open dialog to customize toolbars and keyboard shortcuts. See page 64.	All
<b>View→Status Bar</b>		Hide and show status bar at bottom of main window. See page 64.	All
<b>View→Visual Manager</b>		Open dialog to set Windows display options. See page 64.	All
<b>View→Full Screen</b>		Toggle between normal display and display that maximizes room for graph display. See page 64.	All
<b>View→Logs</b>			
→ <b>Experiment</b>		Display log for a single experiment. See page 78.	All

Command	Keyboard Shortcut	Description	
→Sequence		Display sequence log. See page 78 and page 325.	All
→System		Display, save, or print system log. See page 77.	All
→Database		Display, save, or print full data log. See page 78.	All

## Experiment Menu

The Experiment menu contains the following commands:

Command	Keyboard Shortcut	Description	
<b>Experiment→Configuration</b>			
→Edit		Open the property page for configuration of the currently selected experiment. See page 184.	All (view-only as Guest)
→Replace		Import a configuration item or entire configuration from a profile. See page 185 and page 362.	R, A
→Add HPLC CONNECT		Convert the experiment configuration to use HPLC CONNECT. See page 177.	HPLC: R, A
→Remove HPLC CONNECT		Convert the experiment configuration to not use HPLC CONNECT. See page 177.	HPLC: R, A
→Keep Sample		Toggle whether samples are retained when a configuration is imported. See page 185.	R, A
→Save As		Export a configuration item or an entire configuration to a profile. See page 187 and page 361.	R, A
→Alignment		Opens procedure page for determining the interdetector delay. See page 206.	R, A

Command	Keyboard Shortcut	Description	
→ <b>Band Broadening</b>		Opens procedure page for correcting effects of fluid mixing between instruments. See page 208.	R, A
→ <b>Normalize</b>		Opens procedure page for relating detector signals to the 90 degree detector signal and the instrument calibration constant. See page 213.	R, A
→ <b>Calibrate Column</b>		Perform a column calibration for a SEC column. See page 227.	R, A
→ <b>Dilution Factor</b>		Calculate the dilution factor. See page 235.	
→ <b>Pulse Correction</b>		Perform a pulse correction. See page 232.	
<b>Experiment</b> → <b>Add To Experiment</b>	Ctrl+Shift+P	Add a procedure, report, graph, or data set definition to an experiment. See page 195, page 335, and page 347.	Builder: R, A
<b>Experiment</b> → <b>Copy From</b>		Copy data from one experiment to the current experiment. See page 116.	R, A
<b>Experiment</b> → <b>Apply Method</b>		Apply procedures and results to the current experiment, creating a new experiment. See page 120.	T, R, A
<b>Experiment</b> → <b>Report</b>			
→ <b>Add Report</b>	Ctrl+Alt+E	Add a report to the experiment. See page 335.	R, A
→ <b>Export</b>	Ctrl+Shift+X	Export a report to a text file or a comma-separated values file. See page 334.	R, A
→ <b>Report Options</b>	Alt+T	Set name, title, summary, and logo for the current report. See page 332.	--
<b>Experiment</b> → <b>Graph</b>			
→ <b>Add Custom Plot</b>		Add a custom plot to the results. See page 347.	R, A
→ <b>Add Parametric Plot</b>		Add a parametric plot to the results. See page 287.	R, A

Command	Keyboard Shortcut	Description	
→Add Surface Plot		Add a surface plot to the results. See page 349.	R, A
Experiment→Sign Off		Allow user to sign off experiment for 21 CFR Part 11 compliance. See page 102.	T, R, A
Experiment→Log→Open		Display experiment log. See page 78.	All
Experiment→Log→Save As		Save experiment log to a file. See page 78.	All
Experiment→Log→Print		Print experiment log. See page 78.	All

## Sequence Menu

The Sequence menu contains the following commands:

Command	Keyboard Shortcut	Description	
Sequence→Edit		Open the property page for configuration of the selected sequence. See page 319.	All (view-only as Guest)
Sequence→Log→ Open		Display sequence log. See page 325.	All
Sequence→Log→ Save As		Save sequence log to a file. See page 325.	All
Sequence→Log→ Print		Print sequence log. See page 325.	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 97.	T, R, A
Processing→Validate	Ctrl+Shift+V	Validate the experiment procedure and instrument availability. See page 99. Also validates sequences. See page 324.	T, R, A

Command	Keyboard Shortcut	Description	
<b>Processing→Run</b>	Ctrl+R	Start the experiment or sequence run. See page 99 and page 324.	T, R, A
<b>Processing→Run Indefinitely</b>		Run the experiment data collection until manually stopped. See page 101.	T, R, A
<b>Processing→Pause</b>		Temporarily stop data collection.	T, R, A
<b>Processing→Stop</b>	Ctrl+Shift+S	Halt the experiment or sequence. See page 103 and page 325.	T, R, A

## System Menu

The System menu contains the following commands:

Command	Keyboard Shortcut	Description	
<b>System→Instruments</b>		View list of connected instruments. See page 41.	R, A
<b>System→Diagnostic Manager</b>		Open the Diagnostic Manager. See page 45.	All
<b>System→HPLC DASHBOARD</b>		Open the HPLC DASHBOARD. See page 87.	HPLC: All
<b>System→CheckPlus</b>		Open CheckPlus to report an issue. See page 54.	All
<b>System→Method Builder Wizard</b>		Open the Method Builder Wizard. See page 129.	R, A
<b>System→Feature Activation</b>		Activate features by providing activation key. See page 33.	Security: A
<b>System→Security</b>		Specify a domain to use for user authentication. See page 36.	Security: A
<b>System→Switch Active User</b>		Login with a different user account of equal or higher privilege. See page 62.	Security: All
<b>System→Lock ASTRA</b>	Ctrl+Alt+L	Manually lock the interface when you will be away from the computer. See page 63.	Security: All
<b>System→File Recovery</b>		Attempt to recover a file or database.	T, R, A
<b>System→Database Administration</b>			

Command	Keyboard Shortcut	Description	
→ <b>Connections</b>		Connect to a different experiment database. See page 74.	Security: A
→ <b>Maintenance</b>		Delete experiments, sequences, profiles, or methods from database. See page 79, page 119, page 327, and page 365.	Security: A
→ <b>Import Data Files</b>		Import data files saved Wyatt software into your experiment database. See page 111.	Security: A
→ <b>Import From Database</b>		Import from other ASTRA experiment databases into your current experiment database. See page 113.	Security: A
→ <b>Import System Database</b>		Update system database with latest ASTRA methods and profiles. See page 30.	Security: A
→ <b>Log</b> → <b>Open</b>		View data log. See page 78.	Security: All
→ <b>Log</b> → <b>Refresh</b>		Get latest entries for data log. See page 78.	Security: All
→ <b>Log</b> → <b>Save As</b>		Save data log to a file. See page 78.	Security: All
→ <b>Log</b> → <b>Print</b>		Print the data log. See page 78.	Security: All
<b>System</b> → <b>Preferences</b>			
→ <b>Options</b>		Set defaults for configurations and methods. See page 118 and page 128.	All
→ <b>Experiment Builder Mode</b>		Set to Experiment Builder mode. See page 62.	Security: R, A
→ <b>Show Desktop Alerts</b>		Show instrument alarms in Windows taskbar. See page 43.	All
→ <b>Reset Warnings</b>		This will reset the state of any “Do not show me again” flags that the user sets when prompted with a warning.	All
<b>System</b> → <b>Log</b> → <b>Open</b>		View system log. See page 77.	All
<b>System</b> → <b>Log</b> → <b>Refresh</b>		Get latest entries for system log. See page 77.	All

Command	Keyboard Shortcut	Description	
<b>System→Log→Save As</b>		Save system log to a file. See page 77.	All
<b>System→Log→Print</b>		Print system log. See page 77.	All

## Window Menu

The Window menu contains the following commands:

Command	Keyboard Shortcut	Description	
<b>Window→Close</b>	Ctrl+F4	Close current view. See page 65.	All
<b>Window→Close All</b>		Close all views. See page 65.	All
<b>Window→Next</b>	Ctrl+Tab	Move next view to front. See page 65.	All
<b>Window→Previous</b>	Ctrl+Shift+Tab	Move previous view to front. See page 65.	All
<b>Window→Cascade</b>		Arrange open views in cascading fashion. See page 65.	All
<b>Window→Tile Horizontal</b>		Arrange views in column (wide). See page 65.	All
<b>Window→Tile Vertical</b>		Arrange views in row (tall). See page 65.	All
<b>Window→Arrange Icons</b>		Line up minimized windows. See page 65.	All
<b>Window→Tab Groups</b>		Toggle between tabbed pages and separate windows in workspace. See page 65.	All
<b>Window→Windows</b>		Open a list of windows. See page 65.	All

## Help Menu

The Help menu contains the following commands:

Command	Keyboard Shortcut	Description	
<b>Help→Contents</b>	F1	Open help table of contents. See page 66.	All
<b>Help→Search</b>		Open search tab of online help.	All
<b>Help→Index</b>		Open index tab of online help.	All
<b>Help→What's New</b>		Display a window showing important changes in the current ASTRA version.	All
<b>Help→Wyatt Online</b>		Open Wyatt Support Center website.	All
<b>Help→Check for Updates</b>		Look for newer versions of ASTRA available for download. See page 28.	All
<b>Help→Report Software Issue</b>		Open a form that lets you send reports of problems in ASTRA to Wyatt. See <a href="#">Getting More Help on page 25</a> .	All
<b>Help→About ASTRA</b>		Show version and copyright information.	All

# B

## System Methods

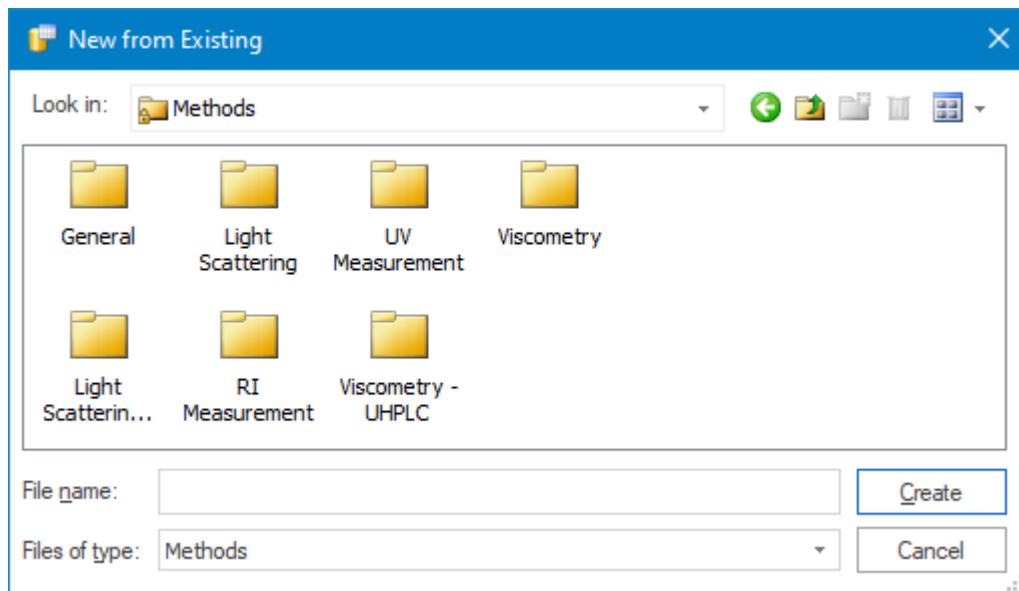
This appendix provides an overview of the ASTRA 8 System Methods

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## Overview of System Methods

This appendix provides an overview of the ASTRA 8 system methods. These methods are stored in five different folders: General, Light Scattering, Light Scattering- UHPLC, RI Measurement, UV Measurement, and Viscometry. 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.

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**Hint:** If you read about an experiment method you want to use, but don't see it in the New from Existing dialog, see [Importing a System Database on page 30](#) to update your system database so you have all the latest experiment methods and system profiles.

---

## General Methods

The methods in the sections that follow are provided in the General folder.

### Concentration Determination (Analysis Method)

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
<b>Concentration (mg/mL)</b>		
Average concentration (mg/mL)	0.094 ( $\pm 0.0\%$ )	0.019 ( $\pm 0.0\%$ )
<b>Masses (µg)</b>		
Calculated mass (µg)	167.75	28.76

### Enhanced Column Calibration (Analysis Method)

This method performs a specialized column calibration analysis and requires a key for feature activation.

### Peak Statistics (Analysis Method)

This method includes the Peak Statistics procedure (page 287) and reports data about the area under the selected peaks.

This procedure calculates the area under the defined peaks for all detectors. The values calculated by this procedure are peak area, peak height, retention time, resolution, peak width at half-height, peak width at quarter-height, peak width at tenth-height, peak width at user-specified height, asymmetry factor, tailing factor, column plate count, mean, standard deviation, skew, %peak area, and selectivity.

The units depend on the type of detector and the units you have chosen in the configuration for the abscissa unit.

## Light Scattering Methods

The methods and folders in the sections that follow are provided in the Light Scattering folder.

Similarly named utilities and methods for the microDAWN are located in the “Light Scattering- UHPLC” folder tree.

### Batch (Debye plot) (Analysis Method)

A simple method to collect and analyze LS data from a batch injection to determine the weight-average molar mass and the z-average RMS radius. The user enters the concentration in the Peaks procedure.

### Batch (Zimm plot) (Analysis Method)

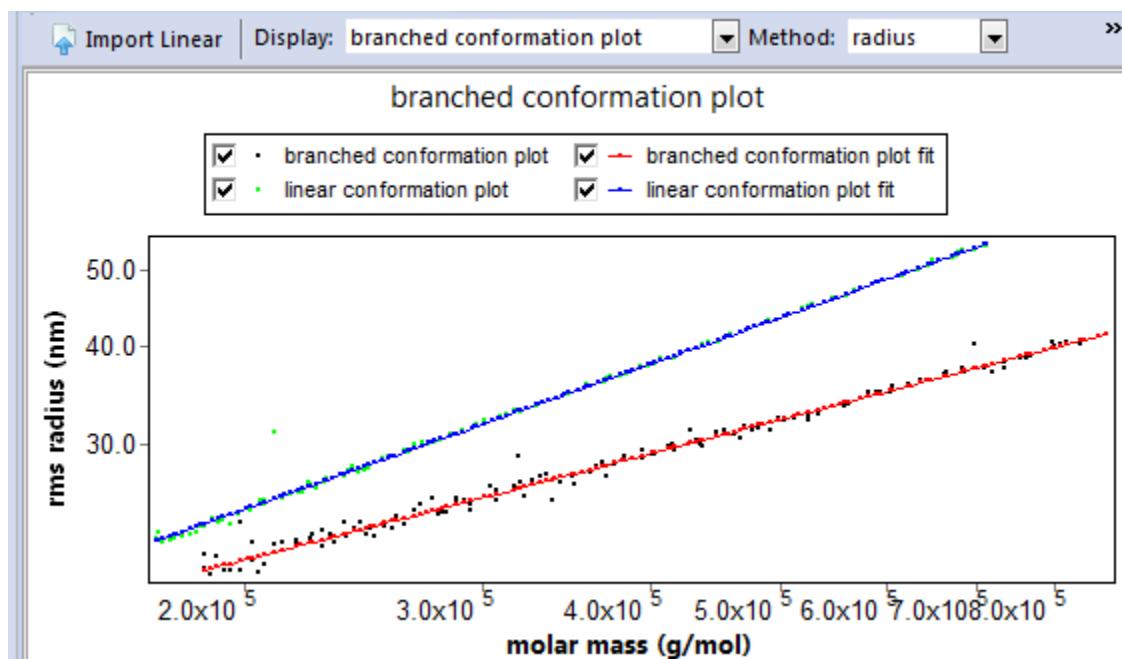
A method to collect LS data from batch injections of a series of concentrations of the same molecule. The concentrations are entered by the user into the concentration field in the Peaks procedure. The analysis provides the weight-average molar mass, the z-average RMS radius, and the second virial coefficient ( $A_2$ ) from fitting the generated Zimm plot.

### Branching (Analysis Method)

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. The equation shown here is used, where  $g$  is the branching ratio, and  $R$  is the RMS radius.

$$g = \left( \frac{R_{br}^2}{R_{lin}^2} \right)_M$$

The “Branching” procedure shows the branched conformation plot and the slope of the linear and branched polymer.

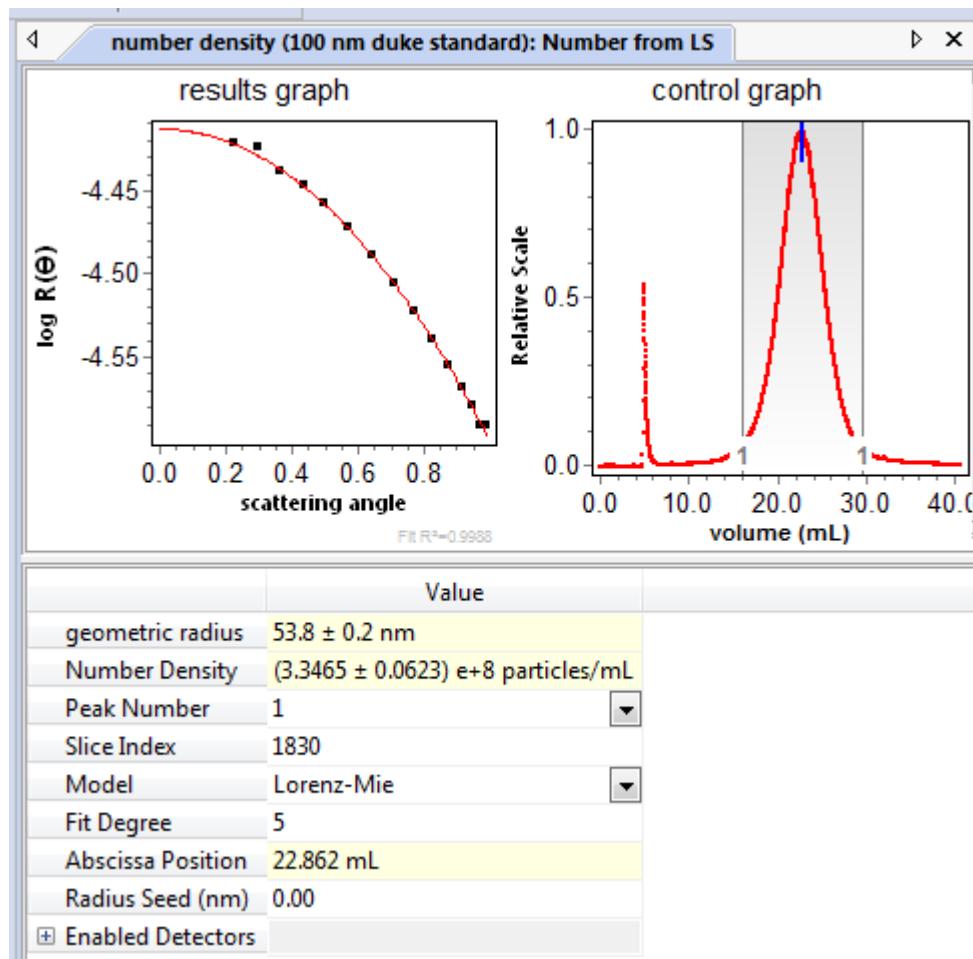


The branching ratio is displayed in the report.

## Number Density (Analysis Method)

This method provides a procedure to calculate number density, which is 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.



- **Model:** Shows (display only) the light-scattering model selected for this peak in the Define Peaks page.
- **Fit Degree:** Shows (display only) the fit degree selected for the peak.

### **Online (Collection Method) -- Basic Light Scattering**

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.

A similar method that also performs results fitting is provided in the "With Results Fitting" subfolder.

### **Online (Zimm plot) (Analysis Method)**

A method that collects LS data as well as RI or UV data from batch injections of a series of concentrations of the same molecule. The RI or UV signals are used to determine the concentration. The analysis provides the weight-average molar mass, the z-average RMS radius, and the second virial coefficient (A2) from fitting the generated Zimm plot.

### **Online A2 (Analysis Method)**

A method for collecting data from injections of a series of concentrations using an HPLC system (with analytical or desalting column). Both LS and RI or UV signals are collected. A Zimm plot is constructed by integrating under the peaks. See page 267 for details. The analysis provides the weight-average molar mass, the z-average RMS radius, and the second virial coefficient (A2) from fitting the generated Zimm plot.

### **Particles (Analysis Method)**

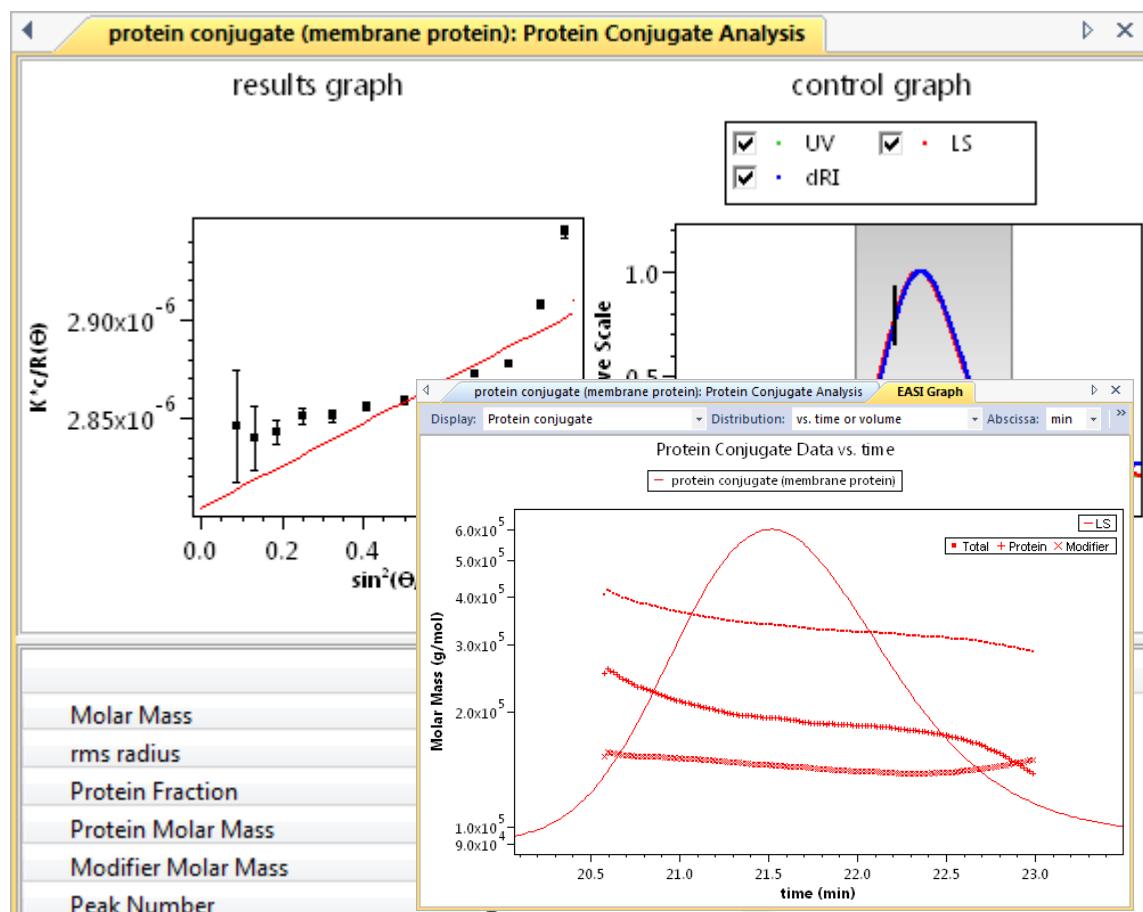
In particles mode, it is possible to measure the size (radius) and number density of a sample using just a light scattering detector without any concentration detector. Note that you won't be able to measure the molar mass using this method.

## Protein Conjugate (Analysis Method)

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.

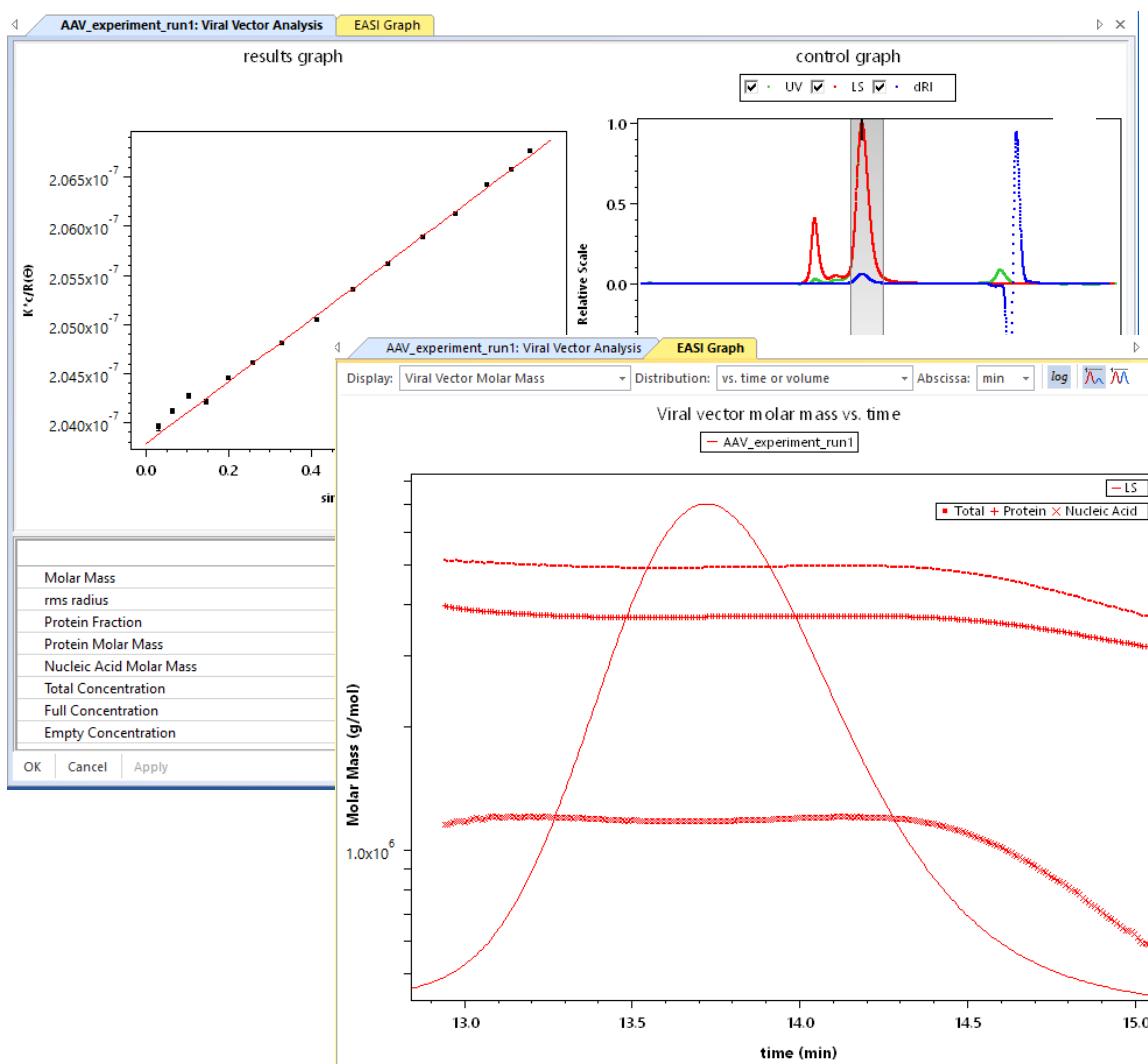


A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Viral Vector (Analysis Method)

This method allows you to determine molar mass, size, and relative protein-nucleic acid fractions of a viral vector using light scattering. All that is required are two additional detectors with differing sensitivities to the protein capsid and nucleic acid payload. The detectors can be a UV detector and an RI detector or a single UV detector with two different wavelengths.

You need to enter the dn/dc and UV extinction values for the protein capsid and nucleic acid payload. The mass and protein fraction on a slice-by-slide basis are displayed. ASTRA calculates the size of the viral vector, mass of the viral vector, and masses of the protein capsid and nucleic acid payload, displaying them with uncertainties. Particle concentrations are calculated for total complex concentration, full concentration, and empty concentration. Additionally, the complex concentration, dn/dc, and UV extinction coefficient are displayed.



A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Baseline Subtraction

The following methods are provided in the Light Scattering > Baseline Subtraction folder.

### Online (Collection Method)

Use this method to run an online light scattering experiment to measure the baseline for later baseline subtraction.

### Protein Conjugate (Analysis Method)

This method incorporates blank baseline subtraction into the Protein Conjugate method discussed in [Protein Conjugate \(Analysis Method\) on page 386](#).

### Viral Vector (Analysis Method)

This method incorporates blank baseline subtraction into the Viral Vector method discussed in [Viral Vector \(Analysis Method\) on page 387](#).

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<b>Note:</b>	Copies of the Online, Protein Conjugate, and Viral Vector methods for Baseline Subtraction that also perform results fitting are provided in the Light Scattering > Baseline Subtraction>With Results Fitting folder
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## Calibration (Collection Method)

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 8 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:**  $2.8445 \times 10^{-5}$  1/(V cm)

Methods are available for the following instruments:

- DAWN (NEON)
- DAWN 8 (NEON)
- HELEOS
- HELEOS 8
- TREOS
- microDAWN (in the “Light Scattering- UHPLC” folder tree)

## Utilities (Collection Methods)

The following methods are provided in the Light Scattering > Utilities folder. For microDAWN methods, look under Light Scattering - UHPLC > Utilities.

### COMET On (Collection Methods)

When you run a single experiment or sequence, the “COMET on” methods allow you to program the COMET cell cleaner for a certain amount of time (shown in the name of the method). Note that during these methods the laser is automatically turned off.

“COMET on” methods are provided for the DAWN, microDAWN, HELEOS, and TREOS. For each instrument, methods are provided that turn the COMET on for 5 minutes, 10 minutes, and 2 hours.

### Turn Laser Off (Collection methods)

The “Turn Laser off” methods can be used only with a sequence. Before using a “Laser off” method, you need to customize it. This means you should perform the physical instrument connection and save the resulting experiment as a method before running a sequence.

“Turn Laser off” methods are provided for the DAWN, microDAWN, HELEOS, and TREOS instruments.

### Orbit On (Collection Method)

The “Orbit on” methods place the Orbit device in Recycle mode for the DAWN, microDAWN, TREOS and HELEOS instruments.

### Turn APD Off (Collection Method)

The “Turn APD off” methods turn the APD off for the DAWN, microDAWN, TREOS and HELEOS instruments (with WyattQELS only).

## Diagnostics for Light Scattering

The following methods are provided in the Light Scattering > Diagnostics folder.

### Detector Overlay (Analysis Method)

This method allows you to visualize an overlay of the light scattering detectors. It is a useful tool for troubleshooting:

- 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 for a dirty cell. If the cell is dirty, the peaks are not the same shape for all detectors.

### View 2 (Overlay Test) (Collection Method)

This is similar to the Detector Overlay method.

### Noise (Analysis Method)

- DAWN (NEON) Noise
- miniDAWN (NEON) Noise
- HELEOS Noise
- TREOS Noise
- microDAWN Noise (in the “Light Scattering- UHPLC” folder tree)

The noise methods analyze the noise level of the light scattering detectors. This requires a stable baseline. Some criteria are already entered in the method in the “LS Noise” procedure.

	Value
Noise Interval (min)	0.500
RMS Noise Limit (V)	1.000e-005
Peak to Peak Noise Limit (V)	1.000e-003
Wander Interval (min)	5.000
RMS Wander Limit (V)	1.000e-005
Peak to Peak Wander Limit (V)	1.000e-003
Drift Limit (V/min)	4.000e-006
Peak Number	None

The LS Noise properties are defined as follows:

Table B-1: LS Noise Properties

Field	Description
Noise Interval (min)	Time interval to use when calculating noise values. Default value is 30 seconds.
rms Noise Limit (V)	Limit on detector rms values during the noise interval.
Peak to Peak Noise Limit (V)	Limit on the peak to peak detector rms values during the noise interval.
Wander Interval (min)	Time interval to use when calculating wander values. Default value is 5 minutes.
rms Wander Limit (V)	Limit on detector rms values during the wander interval.
Peak to Peak Wander Limit (V)	Limit on peak to peak detector rms values during the wander interval.
Drift Limit (V/min)	Absolute limit on the rate of change for the LS or RI detectors.
Peak Number	Peak selection for noise calculation.

The report indicates whether the measured noise levels are within specification.

## With DLS

### Batch (Collection Method)

Use this method to perform batch DLS measurements. Inject the sample directly into the light scattering detector.

The  $r_h$  from the autocorrelation function is displayed in the “rh from DLS” procedure.

### Online (Collection Method)

Use this method to perform online DLS measurements. Run the sample through the chromatography system and detectors.

The  $r_h$  from the autocorrelation function is displayed in the “rh from DLS” procedure.

### Regularization (Analysis Method)

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 (Analysis Method)

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 (Analysis Method)

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 DLS data.

### Protein Conjugate (Analysis Method)

This method permits measurement of  $r_h$  from DLS data as well as rms radius and molar mass using Protein Conjugate analysis.

### Viral Vector (Analysis Method)

This method permits measurement of  $r_h$  from DLS data as well as rms radius and molar mass using Viral Vector analysis.

### Temperature Ramp (Collection Method)

This method includes a script collection procedure that ramps the temperature during collection.

## With Viscometry

The following methods are provided in the Light Scattering > With Viscometry folder.

### **Branching (VS+LS) (Analysis Method)**

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 (Collection Method)**

This is the standard online method for use when you have a light scattering detector and a viscometer.

### **Copolymer Analysis (Analysis Method)**

This method is for use with viscometer data. It is the equivalent of the Protein Conjugate method, but includes additional analysis for viscometry data.

## RI Measurement Methods

The methods and folders in the sections that follow 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 (Analysis Method)**

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 (Collection Method)**

This method is used to calibrate any generic refractometer. The Optilab uses a special method described in the section that follows. 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.

### Calibration

The following methods are provided in the RI Measurement > Calibration folder.

#### **Absolute RI Calibration (Collection Method)**

The method is specifically for calibrating the Optilab for absolute refractive index measurements. Absolute measurements mean that there is no reference to a standard. For these analyses, the purge valve must be ON.

Inject at least three different solvents with known (and different) refractive indexes (for example, toluene, THF, and water).

### RI Calibration from Peak (Analysis Method)

This method is only used to calibrate the Optilab. For this method there is a pump, injector, and an Optilab. No other instruments are necessary.

To perform 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.

### RI Calibration (Analysis Method)

Use this method only with batch measurements. The experiment configuration contains only an Optilab.

Inject at least five concentration of sodium chloride, which has a well-known dn/dc. The slope allows ASTRA to determine the calibration constant.

## Diagnostics

The following methods are provided in the RI Measurement > Diagnostics folder.

### RI Noise (Collection Method)

These methods analyze the noise level, wander interval, and drift of the dRI signal. Standard criteria for the Optilab instrument models are already entered in the “RI Noise” procedure in these methods.

The RI Noise properties are defined as described for the LS Noise procedure (page 389), but with RI units instead of light scattering units.

The report indicates whether the measured noise levels are within specifications.

### Grimace (Analysis Method)

The Grimace method allows you to determine the mixing volume in the RI detector. Typically, this method is applied to the trailing edge of a plateau injection of a known standard with a known concentration.

## Utilities

The following methods are provided in the RI Measurement > Utilities folder.

### Zero dRI (Collection Method)

This method sets the dRI offset value to zero.

### Orbit On (Collection Method)

This method places the Orbit device in Recycle mode for the Optilab.

### Purge On (Collection Method)

We recommend that you purge the Optilab when not running samples; the “Purge On” method is a convenient way to automate this as part of a sequence.

### Purge Off (Collection Method)

You can use this method in combination with the “Purge On” method in a sequence to close the purge valves on an Optilab instrument.

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

### Optilab Flush (Collection Method)

These methods are for the Optilab (NEON) or Optilab (T-rEX).

This method cycles the purge valve during a flushing operation. This action is useful for ensuring that all internal wetted surfaces are flushed during a solvent change.

## Absolute RI measurement (Collection Method)

The method is specifically for collecting absolute refractive index (aRI) data. Absolute measurement means that there is no reference to a standard. For these analyses, the purge valve must be ON. This method will collect data and allow users to determine the mean and standard deviation of the aRI signal.

## Batch (Determine dn/dc) (Collection Method)

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.

## Conventional Calibration Curve (Collection Method)

This method is used to determine a universal calibration curve for analysis of a sample using conventional calibration using a concentration detector only. The response of a column to a set of standards with known molecular weights must be measured and a column profile determined and saved. This column profile may be used in the Conventional Calibration Analysis method.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Conventional Calibration Analysis (Collection Method)

To use this method, you must have acquired and saved a column profile using the Conventional Calibration Curve method.

In the Generic Column of the experiment configuration, select the corresponding column profile by clicking the “...” button.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Copolymer Analysis (Analysis Method)

This method is for experiments without a light scattering instrument. To use this method, a conventional 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.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## dn/dc from UV (Analysis Method)

This method calculates the dn/dc from the UV signal. If a UV signal is recorded and the extinction coefficient is known, the total injected mass of the sample under the peak can be calculated. The dn/dc can then be determined from the injected mass, and the area under the RI peak.

## RI Peak Areas (Analysis Method)

When this method is applied to an experiment, only the refractometer peak area for each selected peak is shown in the report.

## UV Measurement Methods

The method in the section that follows is provided in the UV Measurement folder.

### UV Extinction from RI Peak (Analysis Method)

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 a sample mass recovery of 100% is not 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.

### Utilities



If you are using HPLC CONNECT to control your UV detector, you can turn the lamp off at the end of a sequence using one of the methods in the UV Measurement > Utilities folder.

Depending on the lamp types on your detector, choose HPLC UV Lamp Off [UV] to turn off a UV lamp, HPLC UV Lamp Off [Vis] to turn off a visible light lamp, or HPLC UV Lamp Off [UV + Vis] to turn off both lamps if present.

## Viscometry Methods

The methods and folders in the sections that follow are provided in the Viscometry folder. If you have a microViscoStar instrument the methods are provided in the Viscometry- UHPLC folder.

### Diagnostics for Viscometry

The method is provided in the Viscometry > Diagnostics folder. For a microViscoStar instrument, the corresponding method is provided in the Viscometry- UHPLC > Diagnostics folder.

#### VS Noise (Collection Method)

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
Noise Interval (min)	0.500
DP RMS Noise Limit (psi)	2.200e-005
DP Peak to Peak Noise Limit (psi)	2.200e-005
IP RMS Noise Limit (psi)	1.000e-002
IP Peak to Peak Noise Limit (psi)	1.000e-002
Wander Interval (min)	5.000
DP RMS Wander Limit (psi)	1.000e-004
DP Peak to Peak Wander Limit (psi)	1.000e-004
IP RMS Wander Limit (psi)	5.000e-002
IP Peak to Peak Wander Limit (psi)	5.000e-002
DP Drift Limit (psi/min)	6.000e-006
IP Drift Limit (psi/min)	1.670e-003
Peak Number	None

The Viscometer Noise properties are defined as follows:

Table B-2: Viscometer Noise Properties

Field	Description
Noise Interval (min)	Time interval to use when calculating noise values. Default value is 30 seconds.
DP rms Noise Limit (psi)	Limit on the DP transducer rms values during the noise interval.
DP Peak to Peak Noise Limit (psi)	Limit on the peak to peak DP transducer rms values during the noise interval.

Table B-2: Viscometer Noise Properties (continued)

Field	Description
IP rms Noise Limit (psi)	Limit on the IP transducer rms values during the noise interval.
IP Peak to Peak Noise Limit (psi)	Limit on the peak to peak IP transducer rms values during the noise interval.
Wander Interval (min)	Time interval to use when calculating wander values. Default value is 5 minutes.
DP rms Wander Limit (psi)	Limit on the DP transducer rms values during the wander interval.
DP Peak to Peak Wander Limit (psi)	Limit on the peak to peak DP transducer rms values during the wander interval.
IP rms Wander Limit (psi)	Limit on the IP transducer rms values during the wander interval.
IP Peak to Peak Wander Limit (psi)	Limit on the peak to peak IP transducer rms values during the wander interval.
DP Drift Limit (psi/min)	Limit on the rate of change for the DP transducer signal.
IP Drift Limit (psi/min)	Limit on the rate of change for the IP transducer signal.
Peak Number	Peak selection for noise calculation.

## Utilities for Viscometry (ViscoStar (NEON), ViscoStar 3, and microViscoStar)

The following methods are provided in the following folders. Use only the utilities that match the version of your instrument.

- Viscometry > Utilities > ViscoStar (NEON)
- Viscometry > Utilities > ViscoStar 3
- Viscometry- UHPLC > Utilities (for the microViscoStar)

### Orbit On (Collection Method)

This method places the Orbit device in Recycle mode. Use this method when the Orbit is connected to the ViscoStar/microViscoStar.

### Complete Solvent Exchange (Collection Method)

This method cycles the IP and DP valves to flush the delay columns and compensation volume thoroughly from one mobile phase to another. This method is suitable for flow rates close to 0.5 mL/min and for miscible solvents with different viscosities. You should flush with solvent overnight (>8 hours for a ViscoStar or >5 hours for a microViscoStar) while running this method. The method sets the temperature of the ViscoStar/microViscoStar to 60 °C for the first 37 minutes and back to 25 °C

for the duration of the method. At the end of this method, the ViscoStar/microViscoStar will be in “Run Mode” and ready for sample injections.

### Quick Solvent Exchange (Collection Method)

This method cycles the IP and DP valves to flush the delay columns and compensation volume. This method is suitable for flow rates close to 1 mL/min and for miscible solvents with similar viscosities or compositions. You should flush with solvent (~4 hours for a ViscoStar or ~3 hours for a microViscoStar) while running this method. The method sets the temperature of the ViscoStar/microViscoStar to 60 °C for the first 37 minutes and back to 25 °C for the duration of the method. At the end of this method, the ViscoStar/microViscoStar will be in “Run Mode” and ready for sample injections.

## Utilities for Viscometry (ViscoStar 1 and 2)

The following methods are provided in the Viscometry > Utilities > ViscoStar 1 and 2 folders.

### Orbit On (Collection Method)

This method places the Orbit device in Recycle mode. Use this method when the Orbit is connected to the ViscoStar.

### Valve Conditioning (Collection Method)

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.

### IP DP Flush (Collection Method)

This method cycles both the IP and DP valves during a flushing operation. This is useful for ensuring that all internal wetted surfaces are flushed during a solvent change.

## Branching from Column Calibration (Analysis Method)

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.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Copolymer Analysis (Analysis Method)

This method is for use with viscometer data. It is the equivalent of the Protein Conjugate method, but include viscometry analysis.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Mass from VS Data (Analysis Method)

This method calculates the molar mass distribution all along the chromatogram based on the viscometer data.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Online (Collection Method) — Viscometry

This is the standard online method for use when you have only a viscometer and no light scattering instrument.

## Universal Calibration Curve (Analysis Method)

This method is used to determine a universal calibration curve for analysis of a sample using universal or conventional calibration. The response of a column to a set of standards with known molecular weights must be measured and a column profile determined and saved.

This column profile may be used in the Universal Calibration Analysis method.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

## Universal Calibration Analysis (Analysis Method)

To use this method, you must have acquired and saved a column profile using the Universal Calibration Curve method.

In the Generic Column of the experiment configuration, select the corresponding column profile by clicking the “...” button.

A similar method that also performs results fitting is provided in the “With Results Fitting” subfolder.

# C

## Data Collection with Scripts

This appendix describes the scripting language you can use for script-based data collection.

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## Introduction

ASTRA 8 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 Procedure on page 201](#). 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.
QELSInstrument.Create ()	Get a reference to the experiment configuration's dynamic light scattering instrument.
RIIInstrument.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 405](#).

For example, the following commands turn the recycle valve of an Orbit connected to 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.

Command	Effect
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 405](#).

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 of Collection Scripts

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 Ierusalimschy.

The following script collects data for three auto-injections:

```
lsInst = LSIInstrument.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 = LSIInstrument.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 users, there are several experiment methods in the **System > Methods > RI Measurement > Utilities** 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

## Querying an Experiment Database



This appendix describes some ways to query the data stored in the ASTRA experiment database used in the Security Pack tier.

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## Introduction



The ASTRA with Security Pack tier stores all data and settings in a Microsoft SQL Server instance. The raw instrument signals are stored in an encoded (compressed) form, and are therefore inaccessible to queries. However, ASTRA stores scalar result values as distinct entries in the database. This means that any results displayed in ASTRA reports can be queried directly from the database using Structured Query Language (SQL) commands.

The ability to directly access data across hundreds or thousands of data collections provides an extremely powerful tool for exploring trends and comparing new analysis methods.

---

<b>Warning:</b>	<b>Security Considerations:</b> The contents of the database are protected by encrypted checksums to detect modification. Consequently, executing SQL commands that modify data will cause any relevant checksums to fail, and ASTRA will mark the resulting item as corrupted. Therefore, it is important to exercise extreme caution when working directly with the database. A good safety measure is to create a read-only user in the SQL Server instance for performing queries so that you cannot accidentally corrupt your database.
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## Experiment Database Structure



The ASTRA experiment database's primary function is as a data store for the ASTRA application. It has been designed to maximize efficient storage and internal consistency. The database is normalized so that information is stored in as few places as possible. This facilitates future changes to the schema. While this design is good for program access to the database, it means that queries to find data are complex. Much of the related information a user might wish to see is spread across multiple tables, and must be combined using SQL JOIN operations to produce meaningful results.

The following section describes tables in the experiment database that you may want to query.

[Example SQL Queries on page 421](#) presents a few examples that retrieve particular kinds of data. You can use and modify these queries to retrieve other kinds of information.

## Database Table Summary



While the database contains over fifty tables, only a handful are necessary for most data queries. This section highlights the most important ones.

### WResultData

This table holds numerical values that appear in ASTRA reports. Each row of the table corresponds to an individual number displayed in the report. It contains the following columns:

groupID	This unique ID ties all results to a particular experiment.
m_nDataName	The ID for the data label (for example, "Mw").
m_nValueName	The ID for the value label (for example, "Molar Mass").
m_nValueUnits	The ID for the value units (for example, "kDa").
m_dValue	Floating point value of the data.
m_dHighUncertainty	Floating point value of the uncertainty.

### WExperimentConfigurationProfile

This table is useful for finding the name of the processing operator.

groupID	This unique ID ties the row to a particular experiment.
m_sProcessingOperatorName	The name of the operator who performed processing.

### WFileEntry

This table holds information about the data file, such as its name, modification time, and so forth. You would JOIN the m\_gEntryGroupID column against the groupID of a result to see what file the result came from.

m_gEntryGroupID	This unique ID ties the file entry to a particular experiment.
directory	The name of the directory holding the experiment.
fileName	The name of the experiment file.
m_TimeModified	The date and time of the last modification.

## WInjectedSampleProfile

This table holds information about the sample used in the experiment. Since many of its settings can be overridden at the Peak level, it is primary useful for finding the sample name.

groupID	This unique ID ties the sample to a particular experiment.
m_sName	The name of the sample.

## WPeakRange

This table holds information about an individual analytical peak range used in the experiment. It holds a great number of values related to analysis, such as dn/dc, UV extinction coefficient, LS model, and so forth.

groupID	This unique ID ties the sample to a particular experiment.
m_dDNDC	dn/dc
m_dUVExtinctionCoefficient	UV extinction coefficient
m_nLSModel	Integer representing choice of light scattering analysis.

---

## Extracting Meaningful Data



To perform queries, you need to be aware of the coding conventions used to represent the different types of data produced by ASTRA.

### Result Codes

Result values in the database are stored in the WResultData table, and are coded with an ID representing the label used for the data (for example, "Mw") in the m\_nDataName column, and an ID for the kind of data (for example, "Molar Mass") in the m\_nValueName column. The following table summarizes the different IDs used to code result data in the database.

ID	Label
2130	Calculated Mass
2140	RI Calibration Constant
2148	UV Calibration Constant
2159	Number of Particles
2200	Branched Conformation Slope
2201	Linear Conformation Slope
2207	Passed Short Term Noise Test

ID	Label
2208	Passed Drift Test
2209	Passed Wander Test
2215	short Term Noise
2216	Wander
2217	Drift
2219	Analyzed Interval
2239	Protein Molar Mass
2240	Modifier Molar Mass
2241	Protein Fraction
2255	New Absolute RI Calibration
2256	New Absolute RI Offset
2259	Peak Area
2269	Passed DP Short Term Noise Test
2270	Passed DP Drift Test
2271	Passed DP Wander Test
2272	Passed IP Short Term Noise Test
2273	Passed IP Drift Test
2274	Passed IP Wander Test
2281	DP Short Term Noise
2282	DP Wander
2283	DP Drift
2284	IP Short Term Noise
2285	IP Wander
2286	IP Drift
2300	LS Short Term Noise Test
2301	LS Drift Test
2302	LS Wander Test
2303	LS Short Term Noise
2304	LS Wander
2305	LS Drift
2315	Time Constant
2316	Amplitude
2317	Mixing Volume
2318	Start Time
2319	Final Amplitude
2321	Conjugate dn/dc

ID	Label
2322	Conjugate UV Ext. Coef.
2351	g' Branching Ratio
2370	Mass Recovery
2371	Mass Fraction
2375	RI Calibration R <sup>2</sup>
2376	UV Calibration R <sup>2</sup>
2377	New Absolute RI Calibration R <sup>2</sup>
2378	dn/dc R <sup>2</sup>
2379	UV Extinction R <sup>2</sup>
2380	Column Calibration R <sup>2</sup>
2381	Peak Area %
2382	Peak Height
2383	Retention Time
2385	LS Short Term Noise
2386	LS Wander
2387	LS Drift
3001	Aux Calibration Constant
3031	Calibration Constant
12008	A2
12010	scattering angle
12012	aux channel voltage
12013	Concentration
12015	detector voltage
12016	dn/dc
12017	differential refractive index
12018	laser monitor signal
12019	light scattering data
12020	Molar Mass
12021	raw aux input data
12022	raw light scattering data
12023	raw refractive index data
12024	Rayleigh ratio
12025	differential refractive index data
12026	Radius
12028	volume
12045	Injected Mass

ID	Label
12054	UV Ext. Coef.
12077	Abscissa Units
12099	DLS autocorrelation function
12108	laser monitor average
12109	raw UV absorbance data
12110	UV absorbance data
12111	raw specific viscosity data
12112	specific viscosity data
12113	absorbance
12120	Mn
12121	Mw
12122	Mz
12123	rn
12124	rw
12125	rz
12126	M(avg)
12127	r(avg)
12128	rms radius
12129	mean square radius
12151	radius
12152	rn (radius)
12153	rw (radius)
12154	rz (radius)
12155	r (avg) (radius)
12161	Mw/Mn
12162	Mz/Mn
12163	R(0)
12165	linear differential molar mass
12166	differential weight fraction
12167	cumulative molar mass
12168	cumulative weight fraction
12169	rms radius vs. molar mass
12170	linear differential rms radius
12171	cumulative rms radius
12172	radius vs. molar mass
12173	linear differential radius

ID	Label
12174	cumulative radius
12176	log differential molar mass
12177	log differential rms radius
12178	log differential radius
12182	fit of rms radius vs. molar mass
12183	rms conformation plot slope
12189	mass
12190	temperature
12191	count rate
12193	count rate at correlation function
12195	Correlation Function
12196	APD state
12198	Translational Diffusion Coefficient
12200	Hydrodynamic Radius (Q)
12204	Number Density
12207	number
12208	particles
12209	$rh(Q)n$
12210	$rh(Q)w$
12211	$rh(Q)z$
12212	$rh(Q)(avg)$
12213	$Dt(n)$
12214	$Dt(w)$
12215	$Dt(z)$
12216	$Dt(avg)$
12217	linear differential hydrodynamic radius
12218	$rh$ Diff. Weight Fraction
12219	$rh$ Cumulative Weight Fraction
12220	differential number fraction
12221	cumulative number fraction
12230	Specific Viscosity
12231	pressure
12235	Intrinsic Viscosity
12248	$F(avg)$
12250	$rh$ Diff. Intensity Fraction
12251	Differential Intensity Fraction

ID	Label
12252	rh Cumulative Intensity
12253	Cumulative Intensity Fraction
12254	Dt Diff. Intensity Fraction
12255	Dt Cumulative Intensity Fraction
12256	Regularization Resolution
12261	Mark-Houwink-Sakurada K
12263	$[\eta]_n$
12264	$[\eta]_w$
12265	$[\eta]_z$
12266	$[\eta](avg)$
12267	Linear Differential Intrinsic Viscosity
12268	log Differential Intrinsic Viscosity
12269	Cumulative Intrinsic Viscosity
12270	Linear Differential Translational Diffusion
12272	Intensity-averaged Hydrodynamic Radius
12285	Average concentration
12302	rms conformation plot
12303	laser current
12305	Forward Laser Monitor
12306	Read Head Temperature
12307	Heated Line Temperature
12308	A2 FOM
12321	Number Averaged Moment
12322	Weight Averaged Moment
12323	z-averaged Moment
12327	$F_n$
12328	$F_w$
12329	$F_z$
12330	Linear Differential Protein Fraction
12331	Cumulative Protein Fraction
12334	Mark-Houwink-Sakurada a
12342	Forward Monitor Average
12343	$M_p$
12345	$M_v$
12346	$g'(M)$
12347	number-averaged g branching ratio

ID	Label
12348	weight-averaged g branching ratio
12349	z-averaged g branching ratio
12350	average g branching ratio
12351	linear differential g branching ratio
12352	log differential g branching ratio
12353	cumulative g branching ratio
12356	number-averaged g' branching ratio
12357	weight-averaged g' branching ratio
12358	z-averaged g' branching ratio
12359	average g' branching ratio
12360	linear differential g' branching ratio
12361	log differential g' branching ratio
12362	cumulative g' branching ratio
12366	rh(Q) conformation plot slope
12374	Mn (protein)
12375	Mp (protein)
12376	Mv (protein)
12377	Mw (protein)
12378	Mz (protein)
12379	Mn (modifier)
12380	Mp (modifier)
12381	Mv (modifier)
12382	Mw (modifier)
12383	Mz (modifier)
12384	Mw/Mn (Protein)
12385	Mz/Mn (Protein)
12386	Mw/Mn (Modifier)
12387	Mz/Mn (Modifier)
12388	Average protein molar mass
12389	Average modifier molar mass
12390	Protein Fraction
12394	third virial coefficient
12395	laser voltage
12396	Protein mass
12397	Modifier mass
12402	Cumulants Width

ID	Label
12403	Mz+1
12418	First Moment
12419	Second Moment
12420	number-averaged molar mass
12421	concentration peak molar mass
12422	viscosity-averaged molar mass
12423	weight-averaged molar mass
12424	z-averaged molar mass
12425	z+1-averaged molar mass
12426	Average molar mass
12427	number-averaged rms radius
12428	weight-averaged rms radius
12429	z-averaged rms radius
12430	Average rms radius
12431	Number-averaged Hydrodynamic Radius
12432	Weight-averaged Hydrodynamic Radius
12433	Z-averaged Hydrodynamic Radius
12434	Average Hydrodynamic Radius
12435	number-averaged radius
12436	weight-averaged radius
12437	z-averaged radius
12438	average radius
12439	Number-averaged Translational Diffusion Coefficient
12440	Weight-averaged Translational Diffusion Coefficient
12441	Z-averaged Translational Diffusion Coefficient
12442	Average Translational Diffusion Coefficient
12443	Number-averaged Intrinsic Viscosity
12444	weight-averaged Intrinsic Viscosity
12445	z-averaged Intrinsic Viscosity
12446	Average Intrinsic Viscosity
12472	Column Plate Count
12473	Column Asymmetry Factor
12474	second virial coefficient
12475	rms Conformation Plot y-intercept
12476	rh(Q) Conformation Plot y-intercept
12478	rms Radius vs. rh(Q) Plot y-intercept

ID	Label
12479	Std Dev rn
12480	Std Dev rw
12481	Std Dev rn
12482	Std Dev rw
12483	Std Dev rh(Q)n
12484	Std Dev rh(Q)w
12487	rms Radius/rh(Q)
12488	absolute refractive index
12489	absolute refractive index data
12490	Mean
12491	Standard Deviation
12492	laser current average
12493	laser voltage average
12494	cumulative protein molar mass
12495	cumulative modifier molar mass
12496	log differential protein molar mass
12497	log differential modifier molar mass
12498	linear differential protein molar mass
12499	linear differential modifier molar mass
12500	Concentration at Protein Molar Mass
12501	Concentration at Modifier Molar Mass
12515	Hydrodynamic Radius (v)
12516	rh(v)n
12518	rh(v)w
12520	rh(v)z
12521	rh(v)(avg)
12532	rh(v) Conformation Plot y-intercept

## Unit Codes

Units are represented in the m\_nValueUnits and indexUnits columns of the WResultData table, and can be converted to human-readable values by performing a JOIN against the WExternalQuerySupport Table.

## Example SQL Queries



A discussion of the SQL language, database concepts, and data mining are beyond the scope of this appendix. This section demonstrates some of the possible ways to access the database, and is meant as a starting point for further exploration.

You can run the SQL queries in the following sections within the SQL Server Management Studio application. In the following examples, replace the example “APPLAB” database name in the “USE APPLAB;” declaration (at the start of the query) with the actual name of the database you want to query.

## Example 1: Historical DAWN HELEOS Calibration Constants



This query retrieves the calibration constants used by all experiments in the database that used a DAWN HELEOS instrument.

```
/* Look at Historical Calibration Constants */

USE APPLAB; /* Change this line to match the name of your database */

SELECT id.m_sComputerName AS Name
      ,h.m_sDescription AS Description
      ,h.m_dTemperature AS 'Set Temp'
      ,h.m_dWavelength AS Wavelength
      ,h.m_dCalibrationConstant AS 'Calibration Constant'
      ,fe.m_TimeCreated AS 'Run Date and Time'
FROM [dbo].[WDawn2Profile] h
JOIN [dbo].[WInstrumentDescriptor] id ON id.groupID = h.groupID
JOIN [dbo].[WFileEntry] fe ON fe.m_gEntryGroupID = h.groupID
WHERE id.m_nInstrumentType = 6 /* HELEOS instrument type */
ORDER BY fe.m_TimeCreated
```

This will return results similar to the following:

Name	Description	Set Temp	Wavelength	Calibration Constant	Run Date and Time
Wyatt-112-H		25	658	0.00014671	2006-04-11 02:00:55.7570000
Wyatt-112-H		25	658	0.00014671	2006-04-11 20:36:37.3010000
Wyatt-112-H		25	658	0.00014671	2006-04-12 21:49:02.2010000
WYATT-659-H2		25	660.87	2.88E-05	2011-10-07 21:08:53.9420000
WYATT-659-H2		25	660.87	2.9177E-05	2012-04-12 22:45:13.6740000
Wyatt-371-H2HC		25	658	4.25935E-05	2010-04-21 00:01:26.4360000
Wyatt-371-H2HC		25	658	4.25935E-05	2010-04-21 22:24:34.4010000

## Example 2: Mw Results for Pierce BSA



This query retrieves the Mw results for Peak 1 of all experiments with a sample that includes the name “Pierce” in the title.

```
/* Ask the database for the Mw values from all experiments */

USE APPLAB; /* Change this line to match the name of your database */

SELECT fe.directory AS Directory
    ,fe.fileName AS 'File Name'
    ,isp.m_sName AS 'Sample Name'
    ,rd.m_dValue AS 'Value'
    ,rd.m_dHighUncertainty AS 'Uncertainty'
    ,eqs.label AS 'Units'
FROM [dbo].[WResultData] rd
JOIN [dbo].[WExternalQuerySupport] eqs ON eqs.id = rd.m_nValueUnits
JOIN [dbo].[WFFileEntry] fe ON fe.m_gEntryGroupID = rd.groupID
JOIN [dbo].[WInjectedSampleProfile] isp ON isp.groupID = rd.groupID
WHERE rd.m_nDataName = 12121 /* ID for Mw */
    AND rd.m_bUnableToCalculate = 0
    AND isp.m_sName LIKE '%Pierce%' /* Change to other sample names*/
    AND rd.m_nPeak = 1
ORDER BY m_sName
```

This query returns results similar to the following:

Directory	File Name	Sample Name	Value	Uncertainty	Units
02202012\	Pierce BSA [02202012]	Pierce BSA	66745.20	54.08	g/mol
2_Day Runs 0629	10uL BSA	Pierce BSA	66224.99	891.29	g/mol
2_Day Runs 0630	25uL	Pierce BSA	68479.84	2082.77	g/mol
2008\LabX	Pierce BSA_1	Pierce BSA	66888.60	80.54	g/mol
09_22_08\	Pierce BSA WTC PBS injection_1	Pierce BSA in 0.9% NaCl	66760.40	273.06	g/mol

### Example 3: Finding Large Particles



The following query locates all cases where radius values calculated using the Sphere model generated values above 100 nm.

```
/* Find Rw (radius) values calculated with the Sphere model
Larger than 100 nm */

USE APPLAB; /* Change this line to match the name of your database */

SELECT (CAST (fe.directory AS nvarchar) + N'\\' +
       CAST (fe.fileName AS nvarchar)) AS 'File'
      ,isp.m_sName AS 'Sample Name'
      ,bcp.m_sCollectionOperatorName AS 'Collection User'
      ,ecp.m_sProcessingOperatorName AS 'Processing User'
      ,rd.m_dValue AS 'Value'
      ,rd.m_dHighUncertainty AS 'Uncertainty'
      ,eqs.label AS 'Units'
  FROM [dbo].[WResultData] rd
 JOIN [dbo].[WExternalQuerySupport] eqs ON eqs.id = rd.m_nValueUnits
 JOIN [dbo].[WFileEntry] fe ON fe.m_gEntryGroupID = rd.groupID
 JOIN [dbo].[WInjectedSampleProfile] isp ON isp.groupID = rd.groupID
 JOIN [dbo].[WBasicCollectionProcedure] bcp
   ON bcp.groupID = rd.groupID
 JOIN [dbo].[WExperimentConfigurationProfile] ecp
   ON ecp.groupID = rd.groupID
 JOIN [dbo].[WPeakRange] pr ON pr.groupID = rd.groupID
 WHERE rd.m_nDataName = 12153 /* ID for Rw (radius) */
       AND rd.m_bUnableToCalculate = 0
       AND pr.m_nLSModel = 4 /* Sphere */
       AND rd.m_dValue > 100.00
 ORDER BY isp.m_sName
```

This query returns results similar to the following:

File	Sample Name	Collection user	Processing user	Value	Uncertainty	Units
02202012\Test1	aTest 100_1000	BZimm	PWyatt	146.36	6.22	nm
02202012\Test2	aTest 100_1000	BZimm	PWyatt	164.80	6.23	nm
06152011\APC1	Latex Sphere	JDoe	JSmith	257.67	2.34	nm
07192010\Mixture1	Mix 6	JDoe	JSmith	136.07	0.44	nm
05122012\Spheres	100_Latex	JDoe	JSmith	100.85	0.26	nm



# 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.

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## 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  $\theta$  per unit solid angle subtended by said detector is called the Rayleigh ratio or  $R_\theta$ , and is defined as:

$$R_\theta = \frac{I_\theta r^2}{I_0 V} \quad (\text{Eq. #1})$$

where  $I_\theta$  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  $\theta$  collects light, and  $r$  is the distance between  $V$  and the detector.

Eq. 1 implies that the dimension of  $R_\theta$  is  $(\text{length})^{-1}$ .

---

**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,  $R_\theta$  usually refers to the excess scattering from a solution above that scattered by the solvent alone as follows:

$$R_\theta = \frac{(I_\theta - I_{\theta,\text{solvent}}) r^2}{I_0 V} \quad (\text{Eq. #2})$$

where  $I_{\theta,\text{solvent}}$  is the scattered intensity into angle  $\theta$  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  $R_\theta$  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  $R_\theta$ ? Photodetectors used in static light scattering instruments generate electrical voltages or currents that are proportional to the intensity of incident light. In order to relate the electrical signal to Rayleigh ratio, we must calibrate our detectors. This calibration is not difficult and will be explained shortly.

Furthermore, in the DAWN and miniDAWN instruments, 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 also contribute to differences in scattering volumes.

We correct for the slightly different scattering volumes and subtended angles observed by each detector by using the proportionality between  $R_\theta$  and  $I_\theta$  in Eq. 1 to derive a calibration factor which gives the correct value of  $R_\theta$  for a known scatterer. Several common solvents have been thoroughly studied, and their Rayleigh ratios are well known, allowing us to use a pure solvent as the calibration standard. Using pure solvent as the scattering standard makes the calibration completely independent of any particular sample.

The simplest and most robust calibration procedure utilizes scattering at 90° to the incident light beam. We combine the detector sensitivity with the geometric volume and solid angle factors into a single Configuration Specific Calibration Constant, called  $A_{CSCC}$ .<sup>1</sup> By using the proportionality between detector voltage and light intensity, Eq. 1 may be expressed as:

$$R_{90^\circ} = A_{CSCC} \left( \frac{V_{90^\circ} - V_{90^\circ, dark}}{V_{laser} - V_{laser, dark}} \right) \quad (\text{Eq. #3})$$

where  $V_{90^\circ}$  and  $V_{90^\circ, dark}$  are the 90° detector signal voltage and its dark offset voltage, respectively.  $V_{laser}$  and  $V_{laser, dark}$  are the laser monitor signal and its dark offset, respectively. Dark offsets are obtained by turning off the laser and measuring the detector signal. Division 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.<sup>2</sup> 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 implies

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.

$A_{CSCC} \approx 7.0 \times 10^{-5} \text{ cm}^{-1}$ . 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. Unfortunately this simplistic procedure ignores the geometric factors affecting the volume of scattering molecules seen by the 90° detector, as well as the solid angle it subtends and reflections at interfaces between different materials such as air-glass and solvent-glass. 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:

$$A_{CSCC} = A_{inst} (\text{Reflection correction})(\text{Geometry correction}) \quad (\text{Eq. #4})$$

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 is calculated from changes in 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:

$$T_{12} = \frac{4n_1 n_2}{(n_1 + n_2)^2} \quad (\text{Eq. #5})$$

If  $g$  represents the flow cell glass,  $s$  represents the solvent, and  $a$  represents air, then the complete reflection correction can be written as:

$$\text{Reflection correction} = \frac{1}{T_{sg}^2 T_{ga}^{N^*}} \quad (\text{Eq. #6})$$

where  $N^*$  is the number of uncoated glass-air interfaces the incident and scattered light traverse. It is assumed that the reflective losses at an antireflection coated interface (such as the air-glass laser window 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<sup>1,2</sup>, but there are no exact analytical expressions for practical cells. 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 the DAWN flow cell using the paraxial approximation, and for scintillation vials and cuvettes 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 DAWN flow cell, 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:

$$A_{\text{CSCC}} = A_{\text{inst}} \frac{n_s n_g}{T_{sg}^2 T_{ga}} \quad (\text{Eq. #7})$$

for the standard flow cell with an antireflection coated laser entrance and exit window and uncoated exit surface to the detector, 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:

- 
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).

$$A_{\text{CSCC}} = A_{\text{inst}} \frac{n_s^{1.797}}{T_{ga}^2} \quad (\text{Eq. #8})$$

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

$$A_{\text{CSCC}} = A_{\text{inst}} n_s^{1.983} \quad (\text{Eq. #9})$$

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  $A_{\text{inst}}$  via Eq. 7, Eq. 8, or Eq. 9 depending on the cell type. The  $A_{\text{inst}}$  value is reported as the “Calibration Constant” and is the value entered in the DAWN or miniDAWN profile.

If at some later time  $A_{\text{inst}}$  is changed in the instrument profile, ASTRA will recalculate  $A_{\text{CSCC}}$ . ASTRA also recalculates  $A_{\text{CSCC}}$  if the solvent or cell type is changed. This process readily enables one to calibrate with one solvent and make measurements with another, while the software efficiently handles all the details.

Changing the sample cell requires recalibration of the MALS detector with the specific sample cell intended to measure the sample of interest.

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

After performing the calibration procedure in toluene, 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  $R_\theta$  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 geometric 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 erroneous molar mass and size.

Therefore, we use a set of *normalization coefficients*  $N_\theta$  to relate each detector to the 90° detector. These coefficients must be determined using the *same temperature* and the *same solvent* that are used for the actual sample measurement, since the refractive index of the solvent changes the scattering angles and the geometric 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  $R_\theta$  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  $R_\theta$  is independent of  $\theta$ ) and compute a set of coefficients so that each detector gives the same  $R_\theta$  as the 90° detector when its signal is multiplied by its correct normalization coefficient. Expressed algebraically we have:

$$R_\theta = N_\theta A_{CSCC} \left( \frac{V_\theta - V_{\theta, dark}}{V_{laser} - V_{laser, dark}} \right) \quad (\text{Eq. #10})$$

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:

$$R_\theta = N_\theta A_{CSCC} \left( \frac{V_\theta - V_{\theta,baseline}}{V_{laser} - V_{laser,dark}} \right) \quad (\text{Eq. #11})$$

where  $R_\theta$  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] plus the detector dark offset. Eq. 11 is used by ASTRA.

## Implementation of Normalization

ASTRA provides two normalization techniques: “Area” normalization is the default used in ASTRA 6, ASTRA 7, and ASTRA 8. “Standard” normalization was used in older software versions of ASTRA.

### Area Normalization

When using Area Normalization, the results are calculated from the integration of the Rayleigh Ratio peak as follows, rather than just using the peak apex as in Standard Normalization:

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.

### 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.

$$y = a_0x + a_1x + a_2x^2 + a_3x^3 + a_4x^4 + a_5x^5 + a_6x^6 \quad (\text{Eq. #12})$$

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.

## Concentration Calculation Methods

The concentration of flowing samples may be determined via any online concentration detector, such as UV/Vis absorption, differential refractive index, or fluorescence detectors. In batch methods, where concentration detectors cannot be used, the concentration values must be entered manually into the appropriate fields.

### Optilab

ASTRA communicates digitally with the Optilab to obtain the difference in the refractive index of the sample and reference cells. Since the reference cell usually contains pure solvent, this difference reflects the change in refractive index (dRI) due to the solute, which can be related to its concentration via  $dn/dc$ , the refractive index increment of the specific solute in the specific solvent. The value of  $dn/dc$  may be found in the literature for many solutes and solvents, calculated from a mass average of  $dn/dc$  values of components of the solute, or measured empirically with an Optilab.

Even if the Optilab's reference cell does not contain pure solvent, the solute concentration is determined by subtracting the dRI measured in pure solvent from the dRI measured in the presence of solute, given by Eq. 13:

$$\Delta c = \frac{(d_{solute} - d_{solvent})}{dn/dc} \quad (\text{Eq. #13})$$

### Other Concentration Detectors

Using any other concentration detector requires connecting an analog output signal, which is proportional to the signal measured by the concentration detector, to an analog input (AUX) channel on one of the Wyatt detectors used in the measurement, such as the DAWN, miniDAWN, Optilab, or ViscoStar. A concentration calibration factor alpha, which relates the analog voltage difference on the AUX channel to concentration, must be determined.

There are several distinct options for configuring an analog concentration signal, such as UV and generic RI concentration detectors. These options are described in the following sections.

## Determining Concentration by Generic RI

A generic RI concentration detector can be used with either a known  $dn/dc$  value and a known AUX calibration constant or with only a known AUX calibration constant if you assume that 100% mass recovery occurs.

### Known $dn/dc$ and Known AUX Calibration Constant

The sample concentration for each data slice is determined by 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  $\alpha$  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  $\alpha$  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  $i^{\text{th}}$  slice, the change in refractive index compared to pure solvent is given by:

$$\Delta n_i = \alpha(V_i - V_{i,\text{baseline}}) \quad (\text{Eq. #14})$$

where  $V_i$  and  $V_{i,\text{baseline}}$  are the RI signal and baseline voltages, respectively. Dividing  $\Delta n_i$  by  $dn/dc$  gives the change in concentration of solute, compared to the baseline, for that slice:

$$\Delta c_i = \frac{\Delta n_i}{dn/dc} = \frac{\alpha(V_i - V_{i,\text{baseline}})}{dn/dc} \quad (\text{Eq. #15})$$

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:

$$w_i = c_i \Delta v_i \quad (\text{Eq. #16})$$

where  $\Delta v_i$  is the volume of the slice. Note that  $\Delta v_i$  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

$$W = \sum_{\text{peak}} w_i \quad (\text{Eq. #17})$$

where the sum is over the slices in the peak. When using this method, ASTRA calculates the peak mass according to Eq. 17 where the sum is over the slices in the peak within the limits set in the Peaks graph. Comparing

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,  $\alpha$ .
- 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. 15 and using Eq. 18:

$$dn / dc = \frac{\alpha}{W_{\text{injected peak}}} \sum \Delta v_i (V_i - V_{i,\text{baseline}}) \quad (\text{Eq. #18})$$

You must be sure that all the injected mass elutes in the peak area selected and that the flow rate is accurate.

## Determining Concentration by UV

This method is similar to determining concentration with a generic RI detector, except that the product of the extinction coefficient and the cell path length are used in place of  $dn/dc$ .

The value of alpha, called the UV response, may be calculated from the UV instrument’s AUFS (absorption units at full scale) parameters and the maximum analog output value.

For example, some UV detectors have a maximum full scale output of 2 V. If the AUFS parameter on the UV instrument is set to 0.1 a.u. then alpha is  $(2 \text{ V} / 0.1 \text{ a.u.}) = 20 \text{ V/a.u.}$  The calculated value should be entered into the Generic UV Instrument (UV) configuration field, as should the cell path length.

## Determination of Molar Mass and Sizes

Different calculations can be used to determine molar mass and size depending on whether the solution is dilute or semi-dilute.

### Dilute Solutions

At low concentrations typical of chromatographic separations, the distances between macromolecules are large enough that non-specific intermolecular interactions—otherwise known as “thermodynamic non-ideality”—may be ignored. This is generally true for concentrations below 0.1- 1.0 mg/mL, though the cutoff concentration depends on the specific volume of the macromolecule and any long-range interactions due to poorly shielded charges, for example, with polyelectrolytes in low-ionic-strength buffers.

In the dilute, or ideal, limit, the relationship between the scattered light intensity, molar mass and concentration are given by Eq. 19:

$$\frac{R_\theta}{K^*} = McP(\theta) \quad (\text{Eq. #19})$$

Where:

- $R_\theta$  is the excess Rayleigh ratio ( $\text{cm}^{-1}$ ).
- $K^*$  is an optical constant.  $K^* = 4\pi^2 n_0^2 (dn/dc)^2 / (\lambda_0^4 N_A)$ , where:
  - $n_0$  is the index of refraction of the solvent.
  - $dn/dc$  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).
  - $\lambda_0$  is the wavelength of the laser light in vacuum.
  - $N_A$  is Avogadro's number, equal to  $6.022 \times 10^{23} \text{ mol}^{-1}$ .
- $M$  is the weight average molar mass (g/mol).
- $c$  is the mass concentration of the solute molecules in the solvent (mg/mL).
- $P(\theta)$  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.

Eq. 19 is the basis for all molar mass and size calculations in ASTRA's chromatographic analyses when the Peaks view sets **A2** to zero and the **LS Analysis > Model** property to Debye. If the Zimm or Berry model is selected, then a variant of this equation is used (see page 439).

At each data slice, the angular signals are fit to this equation in order to determine  $M$  and  $r_g$ .  $P(\theta)$  is expanded as a polynomial in  $\sin^2(\theta/2)$  according to the value of **LS Analysis > Fit Degree** in the Peaks view.

## Semi-Dilute Solutions

At higher sample concentrations, non-specific intermolecular interactions begin to have a first-order effect on the light scattering intensity. Again depending on properties such as the specific volume and degree of ionic shielding, the impact may be described by a first-order virial expansion in the form of Eq. 20<sup>1</sup>:

$$\frac{K^* c}{R_\theta} = \frac{1}{MP(\theta)} + 2A_2 c \quad (\text{Eq. #20})$$

Where  $A_2$  is the second virial coefficient (mol mL / g<sup>2</sup>).

In a Zimm plot (in batch mode), multiple concentrations are measured and Eq. 20 is fit to the complete data set  $R_\theta(c)$  in order to obtain  $M$ ,  $r_g$ , and  $A_2$ . ASTRA's calculation linearizes  $1/P(\theta)$  as the following polynomial in  $\sin^2(\theta/2)$  in order to avoid numerical instabilities.

$$\frac{1}{P(\theta)} \sim 1 + 2\mu^2 \langle r_g^2 \rangle / 3! - \dots \quad (\text{Eq. #21})$$

In chromatographic mode where each data slice is considered individually, the value of  $A_2$  is assumed to be known (and entered into the sample parameters in the Peaks view), and Eq. 20 is fit to  $R_\theta$  in order to obtain  $M$  and  $r_g$ .

ASTRA offers other models for analyzing molar mass and size, as well as  $A_2$ . Each of these models presents a different approximation to Eq. 20, which is formally identical to the result of thermodynamic fluctuation theory.

The Debye model, represented in Eq. 22, is optimal for determining  $r_g$  when  $A_2$  may be ignored, while the Zimm model is optimal for determining  $A_2$  when  $r_g$  can be ignored.

$$\frac{R_\theta}{K^* c} = MP(\theta) - 2A_2 c M^2 P^2(\theta) \quad (\text{Eq. #22})$$

The Berry model, represented in Eq. 23, is implemented in ASTRA by linearizing  $1/\sqrt{P(\theta)}$  to avoid numerical instability. It is a compromise between Zimm and Debye and is useful for many larger macromolecules:

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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).

$$\sqrt{\frac{K^* c}{R_\theta}} = \frac{1}{\sqrt{M P(\theta)}} + A_2 c \sqrt{M P(\theta)} \quad (\text{Eq. #23})$$

In order to prevent ambiguous fitting results, ASTRA does not permit fitting to virial expansions beyond 2nd order.

## 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  $\theta_i$  where measurements are collected for angles  $i = 1, 2, \dots, m$ . From these fits, obtain the intercept,  $R_0 / (K^* c)$ , at  $\theta = 0$  and the slope at zero angle, given by:

$$s = d \left[ R_\theta / (K^* c) \right] / d \left[ \sin^2(\theta/2) \right]_{\theta \rightarrow 0} \quad (\text{Eq. #24})$$

Note that as  $\theta$  approaches zero, the form factor  $P(\theta)$  approaches unity. Therefore, Eq. 19 becomes:

$$\frac{R_0}{K^* c} = M - 2A_2 c M^2 \quad (\text{Eq. #25})$$

If  $A_2 = 0$ , then

$$M = \frac{R_0}{K^* c} \quad (\text{Eq. #26})$$

On the other hand, solving Eq. 25 for  $M$  yields:

$$M = \frac{2 \left( 1 - \sqrt{1 - 8A_2 c \left( \frac{R_0}{K^* c} \right)} \right)}{8A_2 c} \quad (\text{Eq. #27})$$

Note that only one of the two solutions of Eq. 25 is physically reasonable. For very small values of  $A_2$ , we may rewrite Eq. 27 as follows:

$$M = \frac{2 \left( \frac{R_0}{K^* c} \right)}{1 + \sqrt{1 - 8A_2 c \left( \frac{R_0}{K^* c} \right)}} \quad (\text{Eq. #28})$$

Eq. 28 is not susceptible to round-off error. Note that Eq. 28 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. 19 may be written approximately as:

$$\frac{R_\theta}{K^* c} = MP(\theta) - 2A_2 c M^2 P^2(\theta) \approx M \left[ 1 - 2\mu^2 \langle r_g^2 \rangle / 3! \right] - 2A_2 c M^2 \left[ 1 - 2\mu^2 \langle r_g^2 \rangle / 3! \right]^2 \quad (\text{Eq. #29})$$

$$\approx M \left[ 1 - 2\mu^2 \langle r_g^2 \rangle / 3! \right] - 2A_2 c M^2 \left[ 1 - 4\mu^2 \langle r_g^2 \rangle / 3! \right]$$

Eq. 24 may be written in terms of  $\mu^2 = (4\pi/\lambda)^2 \sin^2(\theta/2)$ . That is:

$$s = d \left[ R_\theta / (K^* c) \right] / d \left[ \sin^2(\theta/2) \right] = \frac{16\pi^2}{\lambda^2} d \left[ R_\theta / (K^* c) \right] / d \left[ \mu^2 \right] \quad (\text{Eq. #30})$$

$$= -\frac{16\pi^2}{\lambda^2} \frac{M \langle r_g^2 \rangle}{3} \{1 - 4A_2 c M\}$$

Therefore:

$$\langle r_g^2 \rangle = \frac{-3s\lambda^2}{16\pi^2 M (1 - 4A_2 c M)} \quad (\text{Eq. #31})$$

## 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. 19 to first order in  $c$ :

$$\frac{K^* c}{R_\theta} = \frac{1}{MP(\theta)} + 2A_2 c \quad (\text{Eq. #32})$$

By following the procedures described for the conventional plot, we obtain the following results:

$$M = \left( \frac{K^* c}{R_0} - 2A_2 c \right)^{-1} \quad (\text{Eq. #33})$$

and

$$\langle r_g^2 \rangle = \frac{3sM\lambda^2}{16\pi^2} \quad (\text{Eq. #34})$$

where:

$$s = d \left[ K^* c / R_\theta \right] / d \left[ \sin^2(\theta/2) \right]_{\theta \rightarrow 0} \quad (\text{Eq. #35})$$

## 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. 19 to first order in  $C$ :

$$\sqrt{\frac{K^* c}{R_\theta}} = \frac{1}{\sqrt{MP(\theta)}} + A_2 c \sqrt{MP(\theta)} \quad (\text{Eq. #36})$$

In this case the results are:

$$M = \frac{4}{\left( \sqrt{K^* c / R_0} + \sqrt{K^* c / R_0 - 4A_2 c} \right)^2} \quad (\text{Eq. #37})$$

and

$$\langle r_g^2 \rangle = \frac{3\lambda_s^2 s}{8\pi^2 \sqrt{M} (1/M - A_2 c)} \quad (\text{Eq. #38})$$

where:

$$s \equiv \frac{d \left[ \sqrt{K^* c / R_\theta} \right]}{d \left[ \sin^2(\theta/2) \right]_{\theta \rightarrow 0}} \quad (\text{Eq. #39})$$

## 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 [Number Density Calculation on page 461](#)). 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. 19, we replace the theoretical form factor  $P(\theta)$  by its exact form first derived by Debye<sup>1</sup>:

$$P(\theta) = \frac{2}{u^2} (e^{-u} - 1 + u) \quad (\text{Eq. #40})$$

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 247), 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  $R_\theta/K^*C$  vs.  $\sin(\theta/2)$ . As in the Conventional method, we insert into Eq. 19 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 Hulst<sup>1</sup>. Note that the sphere and coated sphere models yield radii, while the rod model produces a length.

### Spheres

$$P(\theta) = \frac{3}{u^3} (\sin u - u \cos u) \quad (\text{Eq. #41})$$

where  $u = (4\pi r/\lambda) \sin(\theta/2)$ .

### Rods

$$P(\theta) = \left(\frac{1}{u}\right) \int_0^{2u} \frac{\sin t}{t} dt - \frac{\sin^2 u}{u^2} \quad (\text{Eq. #42})$$

where  $u = [(2\pi n_o/\lambda_o) L \sin(\theta/2)]$ , and  $L$  is the rod length, which is assumed to be much greater than its negligible diameter.

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1. H.C. van de Hulst, *Light Scattering by Small Particles*, Wiley, New York (1957)

## Molar Mass and rms Radius Moments

In chromatographic mode, 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:

$$M_n = \frac{\sum_i n_i M_i}{\sum_i n_i} = \frac{\sum_i c_i}{\sum_i c_i / M_i} \quad (\text{Eq. #43})$$

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. 43 follow immediately.

### Weight-average molar mass:

$$M_w = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i} = \frac{\sum_i c_i M_i}{\sum_i c_i} \quad (\text{Eq. #44})$$

### z-average molar mass:

$$M_z = \frac{\sum_i n_i M_i^3}{\sum_i n_i M_i^2} = \frac{\sum_i c_i M_i^2}{\sum_i c_i M_i} \quad (\text{Eq. #45})$$

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:

$$\langle r_g^2 \rangle_{LS} = \frac{\sum_i c_i M_i \langle r_g^2 \rangle_i}{\sum_i c_i M_i} \quad (\text{Eq. #46})$$

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:

$$M = a \langle r_g^2 \rangle^{0.5} \text{ or } \langle r_g^2 \rangle \propto M^2 \quad (\text{Eq. #47})$$

where  $a$  is a constant. Substituting this value of  $\langle r_g^2 \rangle$  into Eq. 46 yields:

$$\langle r_g^2 \rangle_{LS} = \frac{\sum_i c_i M_i M_i^2}{a^2 \sum_i c_i M_i} = \frac{\sum_i c_i M_i^3}{a^2 \sum_i c_i M_i} \quad (\text{Eq. #48})$$

But this is identical (except for the constant  $a^2$ ) to Eq. 45, the so-called z-average molar mass. This is the origin of the light scattering derived value of the mean square radius, that is:

$$\langle r_g^2 \rangle_z \equiv \langle r_g^2 \rangle_{LS} \quad (\text{Eq. #49})$$

Defining Eq. 46 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 not clear.

#### **Number-average mean square radius:**

$$\langle r_g^2 \rangle_n = \frac{\sum_i \frac{c_i}{M_i} \langle r_g^2 \rangle_i}{\sum_i \frac{c_i}{M_i}} \quad (\text{Eq. #50})$$

#### **Weight-average mean square radius:**

$$\langle r_g^2 \rangle_w = \frac{\sum_i c_i \langle r_g^2 \rangle_i}{\sum_i c_i} \quad (\text{Eq. #51})$$

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:

$$M_{w-avg} = \frac{\sum_i M_i \frac{1}{\sigma_{M_i}^2}}{\sum_i \frac{1}{\sigma_{M_i}^2}} \quad (Eq. \#52)$$

$\sigma_{M_i}$  is the uncertainty in the value of  $M_i$ . The error in this calculation is defined as follows:

$$\sigma_{M_{w-avg}} = \sqrt{\frac{1}{\sum_i \frac{1}{\sigma_{M_i}^2}}} \quad (Eq. \#53)$$

## 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  $R_\theta$  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.

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## Differential Distribution Calculations

ASTRA 8 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 defined<sup>1</sup> as:

$$g_M = \left( \frac{\langle r^2 \rangle_{br}}{\langle r^2 \rangle_{lin}} \right)_M \quad (\text{Eq. #54})$$

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. molar mass data for both the linear and branched files. If a results fitting method has been selected, ASTRA uses the fitted data from molar mass 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. 54 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 Procedure on page 283](#).

### 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,<sup>2</sup> it can be shown that:

$$g_M = \left( \frac{M_{lin}}{M_{br}} \right)^{\frac{(a+1)}{e}}_V \quad (\text{Eq. #55})$$

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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.<sup>1</sup> 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. 55 is taken at constant elution volume  $V$ .

If no results fitting method has been selected, ASTRA uses the raw molar mass vs. volume data for both the linear and branched files. If a results fitting method has been selected, ASTRA uses the fitted data from molar mass 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.

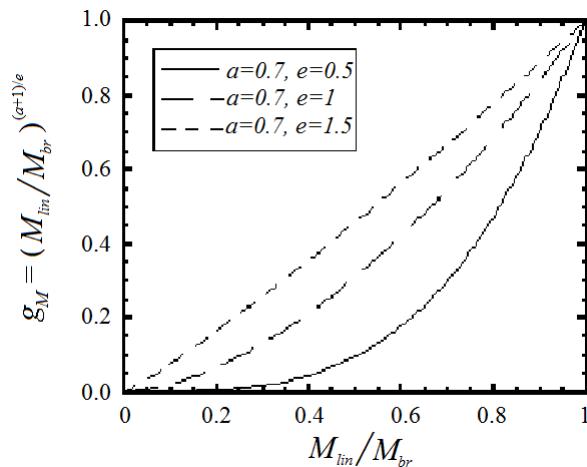


Figure E-1: Branching ratio  $g_M$  as a function of  $a$  and  $e$  for the Mass Method

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 Procedure on page 283](#).

## Branching Ratio: Viscosity Method

The branching ratio  $g'M$  is formally defined as:

$$g'(M) = \frac{[\eta(M)]_{br}}{[\eta(M)]_{lin}} \quad (\text{Eq. #56})$$

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).

where  $\eta_{br}$  and  $\eta_{lin}$  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.

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. molar mass data for both the linear and branched files. For a number of points (300 points per decade of molar mass), Eq. 56 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 Procedure on page 283](#).

## Branching Per Molecule

The number of branch points 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. You can also choose comb and star branching models, but they are only available for monodisperse distributions.

These formulas<sup>1</sup> relate  $g_M$  to  $B$  for randomly branched polymers:

### Trifunctional Branching

- Polydisperse:

$$g_M = \frac{6}{B_{3w}} \left\{ \frac{1}{2} \left( \frac{2 + B_{3w}}{B_{3w}} \right)^{\frac{1}{2}} \ln \left[ \frac{\left( 2 + B_{3w} \right)^{\frac{1}{2}} + B_{3w}^{\frac{1}{2}}}{\left( 2 + B_{3w} \right)^{\frac{1}{2}} - B_{3w}^{\frac{1}{2}}} \right] - 1 \right\} \quad (\text{Eq. #57})$$

- Monodisperse:

$$g_M = \left[ \left( 1 + \frac{B_{3n}}{7} \right)^{\frac{1}{2}} + \frac{4B_{3n}}{9\pi} \right]^{-\frac{1}{2}} \quad (\text{Eq. #58})$$

---

1. B.H. Zimm and W.H. Stockmayer, *ibid.*

### Tetrafunctional Branching

- Polydisperse:

$$g_M = \frac{\ln(1 + B_{4w})}{B_{4w}} \quad (\text{Eq. #59})$$

- Monodisperse:

$$g_M = \left[ \left( 1 + \frac{B_{4n}}{6} \right)^{\frac{1}{2}} + \frac{4B_{4n}}{3\pi} \right]^{-\frac{1}{2}} \quad (\text{Eq. #60})$$

### Star Branching

- Monodisperse<sup>1</sup>:

$$g_M = \frac{6B}{B^2 + 3B + 2} \quad (\text{Eq. #61})$$

### Comb Branching

- Monodisperse<sup>2</sup>:

$$g_M = \frac{2B^3 + 12B^2 + 10B + 3}{4B^3 + 12B^2 + 11B + 3} \quad (\text{Eq. #62})$$

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

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1. B.H. Zimm and W.H. Stockmayer, *ibid.*  
 2. W. Radke, A. H. E. Müller, “Synthesis and Characterization of Comb-Shaped Polymers by SEC with On-Line Light Scattering and Viscometry Detection”, *Macromolecules*, vol. 38, pp. 3957. (2005)

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.

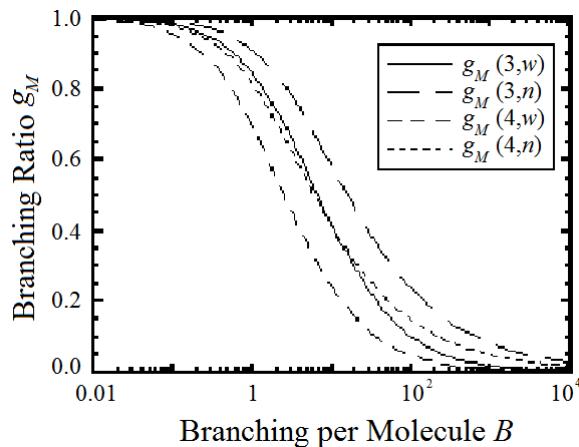


Figure E-2: Branching ratio  $g_M$  as a function of  $B$  for various branching options

### Long Chain Branching

The long chain branching per 1000 repeat units is defined for each slice as

$$\lambda = 1000B \frac{R}{M} \quad (\text{Eq. #63})$$

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.

# F

## Particles

Particles support is an add-on option for ASTRA. This option provides a procedure to calculate particle number densities (see [Number from LS Procedure on page 272](#)). Particles include colloidal particles such as polystyrene latex spheres, 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).

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## Determination of Sizes

As discussed in [Appendix E, 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 in order to determine size. It is assumed further that the particle concentrations are low enough that there are no interactions of consequence between them, 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, 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 457](#)) must satisfy the Rayleigh-Gans criteria:

$$\begin{aligned} |m - 1| &<< 1, \text{ where } m = n / n_0 \text{ and} \\ ka|m - 1| &<< 1, \text{ where } k = 2\pi / \lambda \end{aligned} \quad (\text{Eq. #1})$$

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  $R_\theta$  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 E.

$$\langle r_g^2 \rangle = \frac{-3s\lambda^2}{16\pi^2 R_0} \quad (\text{Eq. #2})$$

In the Zimm, Debye, and Berry models, the mean square radius  $r_g$  is determined by fitting Eq. 20, Eq. 22 or Eq. 23 respectively to the angular data.

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 438](#).

## Mean Square Radius Determination from an Assumed Random Coil

As discussed in [Determination of Molar Mass and Sizes on page 438](#), we insert the theoretical form factor  $P(\theta)$  for random coils into Eq. 19.

$$P(\theta) = \frac{2}{u^2} (e^{-u} - 1 + u) \quad (\text{Eq. #3})$$

$$\text{where } u = (4\pi/\lambda^2) \langle r_g^2 \rangle \sin^2(\theta/2)$$

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. For a polymer comprised of  $N$  segments of length  $a$ , the relationship between  $r_g$ ,  $N$ , and  $a$  of such a polymer in a theta solvent is given by:

$$\langle r_g^2 \rangle = Na^2 / 6 \quad (\text{Eq. #4})$$

## Size from a Known Structure

To derive a particle size based on a structure known *a priori*, we again plot  $R_\theta$  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 Hulst<sup>1</sup>. Note that the sphere and coated sphere models yield a radius, while the rod model produces a length.

### Sphere

$$P(\theta) = \left[ \frac{3}{u^3} (\sin u - u \cos u) \right]^2 \quad (\text{Eq. #5})$$

$$\text{where } u = 2ka \sin(\theta/2).$$

### Rod

$$P(\theta) = \left( \frac{1}{u} \right) \int_0^{2u} \frac{\sin t}{t} dt - \frac{\sin^2 u}{u^2} \quad (\text{Eq. #6})$$

where  $u = [(2\pi n_o / \lambda_o) L \sin(\theta/2)]$ , and  $L$  is the rod length, where  $L$  is assumed to be much greater than the rod diameter.

1. H.C. van de Hulst, *Light Scattering by Small Particles*, Wiley, New York (1957)

## 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  $R_\theta$  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. Kerker<sup>1</sup>.

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1. 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 Calculation

ASTRA calculates the mean square radius moments for each peak as discussed in [Molar Mass and rms Radius Moments on page 444](#). 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 (radius, rms radius, or hydrodynamic radius) depends on the type of analysis being performed. As in Appendix E, all summations are taken over one peak.

### rms Radius

#### LS Average

$$\langle r_g^2 \rangle_{LS} = \frac{\sum_i R_0 \langle r_g^2 \rangle_i}{\sum_i R_0} \equiv \langle r_g^2 \rangle_z \quad (\text{Eq. #7})$$

The quantities  $R_0$ , and  $\langle r_g^2 \rangle_i$  in Eq. 7 and  $V_i$  in the equations that follow are respectively the Rayleigh Ratio, mean square radius, and volume of the  $i^{\text{th}}$  slice. The root mean square (rms) radii are simply the square roots of the appropriate mean square radii.

#### Number Average

$$\langle r_g^2 \rangle_n = \frac{\sum_i \frac{R_0 \langle r_g^2 \rangle_i}{V_i^2}}{\sum_i \frac{R_0}{V_i^2}} \quad (\text{Eq. #8})$$

#### Weight Average

$$\langle r_g^2 \rangle_w = \frac{\sum_i \frac{R_0 \langle r_g^2 \rangle_i}{V_i}}{\sum_i \frac{R_0}{V_i}} \quad (\text{Eq. #9})$$

### Uncertainty Weighted Average

$$\langle r_g^2 \rangle_{avg} = \frac{\sum_i \langle r_g^2 \rangle_i \frac{1}{\sigma_{\langle r_g^2 \rangle_i}^2}}{\sum_i \frac{1}{\sigma_{\langle r_g^2 \rangle_i}^2}} \quad (Eq. \#10)$$

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:

$$\sigma_{\langle r_g^2 \rangle_{avg}}^2 = \frac{1}{\sum_i \frac{1}{\sigma_{\langle r_g^2 \rangle_i}^2}} \quad (Eq. \#11)$$

### Radius and Hydrodynamic Radius

The quantities  $R_0$ ,  $r_i$ , and  $V_i$  in these equations are respectively the Rayleigh Ratio, radius (either radius or hydrodynamic radius), and the volume of the  $i^{th}$  slice.

#### Number Average

$$R_n = \frac{\sum_i \frac{R_0 r_i}{V_i^2}}{\sum_i \frac{R_0}{V_i^2}} \quad (Eq. \#12)$$

#### Weight Average

$$R_w = \frac{\sum_i \frac{R_0 r_i}{V_i}}{\sum_i \frac{R_0}{V_i}} \quad (Eq. \#13)$$

**Z-Average**

$$R_z = \frac{\sum R_0 r_i}{\sum R_0} \quad (\text{Eq. #14})$$

**Uncertainty Weighted Average**

$$r_{avg} = \frac{\sum r_i \frac{1}{\sigma_{r_i}^2}}{\sum \frac{1}{\sigma_{r_i}^2}} \quad (\text{Eq. #15})$$

where  $r_i$  is as defined previously, and  $\sigma_{r_i}$  is the uncertainty in the radius measurement. The error in this calculation is defined as follows:

$$\sigma_{r_{avg}} = \frac{1}{\sqrt{\sum \sigma_{r_i}^2}} \quad (\text{Eq. #16})$$

## Number Density Calculation

This section discusses how the distribution plots are calculated and why a model is needed.

The mean square radius is given by Eq. 17, where the distances  $r_i$  are measured from the particle's center of mass to the mass element  $m_i$ .

$$\langle r^2 \rangle = \frac{\sum_i r_i^2 m_i}{\sum_i m_i} = \frac{1}{M} \int r^2 dm \quad (\text{Eq. #17})$$

Eq. 17 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.<sup>1</sup> 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):

$$\frac{K^* c}{R(\theta)} \approx \frac{1}{M_w P(\theta)} \quad (\text{Eq. #18})$$

can be re-written in the limit as  $\theta \rightarrow 0$ ,  $P(0) = 1$ , and we have:

$$R(0) = K^* c_i M_i = K^* n_i M_i^2 \quad (\text{Eq. #19})$$

since the concentration of mass in the  $i^{\text{th}}$  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  $i^{\text{th}}$  slice,  $n_i$ , is proportional to the extrapolated zero-angle Rayleigh ratio divided by the square of the particle's volume, i.e.:

$$n_i \propto R(0) / V_i^2 \quad (\text{Eq. #20})$$

Therefore, we can write the *number fraction* of particles within slice  $i$  as  $n_i/D$  where:

$$D = \sum_j n_j \quad (\text{Eq. #21})$$

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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,  $2\pi a[m-1] n_0/\lambda_0$ , 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. 19), 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.<sup>1</sup> The result is that values of  $R_0$  may be generated directly from the measurements if the particle's structure is known, or from the more general expansion of the form factor  $P(\theta)$ .<sup>2</sup>

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).

# G

## DLS Theory

This appendix gives a quick overview of the theory behind cumulants and regularization, which are analysis techniques used with DLS data from a WyattQELS module. This includes descriptions of the implementation in ASTRA and interpretation of results.

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## Cumulants Theory

The analysis of DLS 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 additional information, 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 DLS 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.

### Cumulants Method Computations

The result of a DLS measurement is a second order correlation function:

$$g^{(2)}(\tau) = \frac{\langle I(t)I(t + \tau) \rangle}{\langle I(t) \rangle^2} \quad (\text{Eq. #1})$$

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  $\tau$ , 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 G-1.

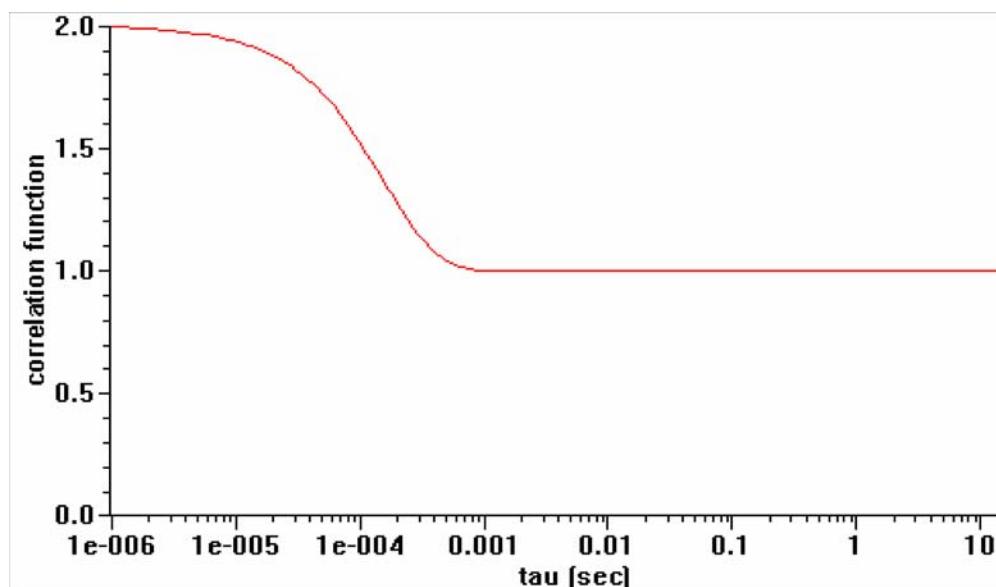


Figure G-1: Correlation function for a multi-tau correlator like that in WyattQELS

As described in various light scattering texts<sup>1</sup>, the correlation function for a monodisperse sample can be analyzed via the equation:

$$g^{(2)}(\tau) = B + \beta \exp(-2\Gamma\tau) \quad (\text{Eq. #2})$$

where  $B$  is the baseline of the correlation function at infinite delay,  $\beta$  is the correlation function amplitude at zero delay, and  $\Gamma$  is the decay time. A nonlinear least squares fitting algorithm can be applied to Eq. 2 to retrieve the correlation function decay time  $\Gamma$ . This is exactly what is done in the ASTRA QELS analysis.

From this point,  $\Gamma$  can be converted to the diffusion constant  $D$  for the particle via the relation:

$$D = \frac{\Gamma}{q^2} \quad (\text{Eq. #3})$$

Here,  $q$  is the magnitude of the scattering vector, and is given by

$$q = \frac{4\pi n}{\lambda_0} \sin(\theta/2) \quad (\text{Eq. #4})$$

where  $n$  is the solvent index of refraction,  $\lambda_0$  is the vacuum wavelength of the incident light, and  $\theta$  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:

$$r_h = \frac{kT}{6\pi\eta D} \quad (\text{Eq. #5})$$

where  $k$  is Boltzmann's constant and  $\eta$  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 Koppel<sup>2</sup>, 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:

$$g^{(2)}(\tau) = B + \beta \exp\left(-2\bar{\Gamma}\tau + \kappa_2\tau^2 - \frac{\kappa_3}{3}\tau^3 \dots\right) \quad (\text{Eq. #6})$$

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 Frisken<sup>1</sup>, and is given by:

$$g^{(2)}(\tau) = B + \beta \exp(-2\bar{\Gamma}\tau) \left(1 + \frac{\mu_2}{2!} \tau^2 - \frac{\mu_3}{3!} \tau^3 \dots\right)^2 \quad (\text{Eq. #7})$$

Here, the moments  $\mu_n$  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  $\tau$ , thus leading to a more robust analysis of the correlation function than has traditionally been obtained from the method of cumulants.

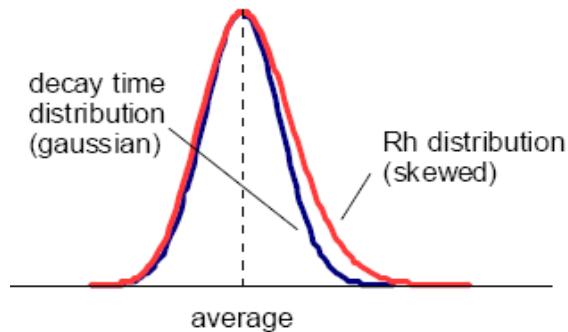
The results obtained from the fit in the DLS batch cumulant analysis are the first two moments,  $\bar{\Gamma}$  and  $\mu_2$  in Eq. 7, as well as the baseline  $B$  and amplitude  $\beta$ . The baseline and amplitude values are used in the data filtering algorithm to reject correlation functions after 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 G-2.



*Figure G-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

ASTRA presents 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 G-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 G-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 Theory

Whereas the method of cumulants is one of the simplest approaches to analyzing DLS 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.<sup>1</sup>

### Regularization Analysis Histograms

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 G-3 for a bimodal distribution consisting of widely separated sizes:

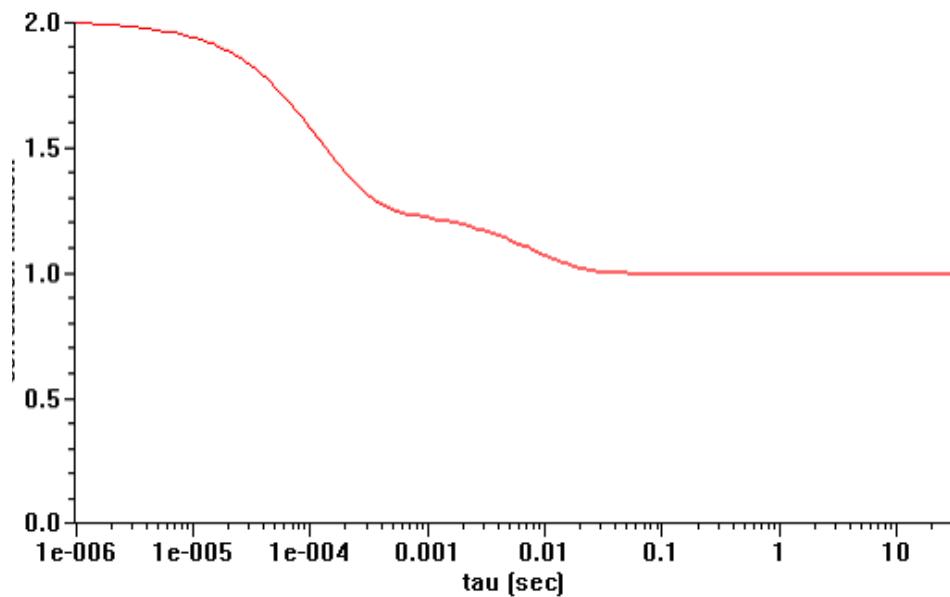


Figure G-3: Bimodal correlation function for mixture of 10 nm and 1  $\mu\text{m}$  particles.

Clearly, the correlation function in Figure G-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 G-4, that is, only two bins would have any intensity. The correlation function is then modeled by “adding”

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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.)

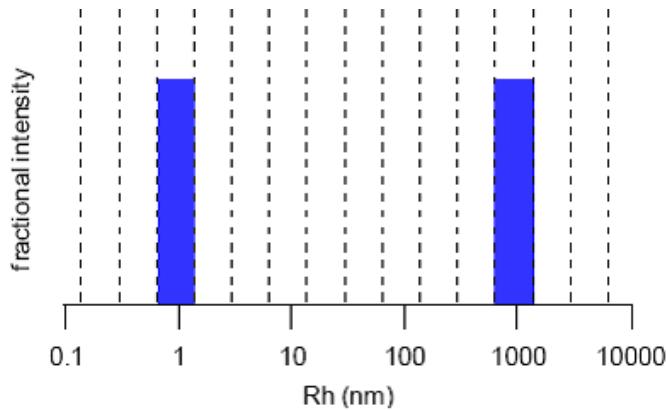


Figure B-2. Model histogram for bimodal size distribution.

Figure G-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.<sup>1</sup> 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 is

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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>.

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.

# H

## Viscosity Theory

This appendix reviews the theory of viscosity-related calculations.

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## Calculating Intrinsic Viscosity

ASTRA 8 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:

$$[\eta] = \lim_{c \rightarrow 0} \frac{\eta_{sp}}{c} \quad (\text{Eq. #1})$$

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 or a UV absorption detector.

### Huggins Equation

The Huggins equation is specified as:

$$\eta_{sp} = [\eta]c + k'[\eta]^2 c^2 + O(c^3) \quad (\text{Eq. #2})$$

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:

$$[\eta] = \frac{\eta_{sp}}{c} \quad (\text{Eq. #3})$$

Solving Eq. 2 for intrinsic viscosity yields:

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{\eta_{sp}^2 k'}{c} + O(\eta_{sp}^3) \quad (\text{Eq. #4})$$

Eq. 4 is simplified to the following for computation:

$$[\eta] \approx \frac{-1 + \sqrt{1 + 4\eta_{sp}k'}}{2k'c} \quad (\text{Eq. #5})$$

## Kraemer Equation

The Kraemer equation is:

$$\frac{\ln(\eta_{sp} + 1)}{c} \approx [\eta] + k''[\eta]^2 c \quad (\text{Eq. #6})$$

Solving Eq. 6 for intrinsic viscosity yields:

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{(1/2 + k'')\eta_{sp}^2}{c} + O(\eta_{sp}^3) \quad (\text{Eq. #7})$$

The expansion shows that for small values of specific viscosity, which is almost always the case for chromatography, the two formalisms are related:

$$k' = 1/2 + k'' \quad (\text{Eq. #8})$$

Eq. 7 is simplified to the following for computation:

$$[\eta] \approx \frac{-1 + \sqrt{1 + 4k'' \ln(1 + \eta_{sp})}}{2k'' c} \quad (\text{Eq. #9})$$

## Solomon-Gatesman Equation

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  $k' = 1/3$ .

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{\eta_{sp}^2}{3c} + O(\eta_{sp}^3) \quad (\text{Eq. #10})$$

Eq. 10 is simplified to the following for computation:

$$[\eta] \approx \frac{\sqrt{2\eta_{sp} - 2 \ln(\eta_{sp} + 1)}}{c} \quad (\text{Eq. #11})$$

## Intrinsic Viscosity and Molecular Parameters

The simplest model of the intrinsic viscosity is due to Einstein and Simha<sup>1</sup>. 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:

$$\eta = \eta_0(1 + \gamma\phi) \quad (\text{Eq. #12})$$

where  $\phi$  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:

$$[\eta] = \frac{\gamma N_A V_h}{M} \quad (\text{Eq. #13})$$

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:

$$r_h = \left[ \frac{3V}{4\pi} \right]^{1/3} \quad (\text{Eq. #14})$$

When defined in this way,  $r_h$  is the radius of a sphere with the same intrinsic viscosity as the molecule under study.

1. A. Einstein, "Eine neue Bestimmung der Molekildimensionen," *Ann.Physik*, vol. 19, pp. 289-306 (1906).  
A. Einstein, "Berichtigung zu meiner Arbeit: Eine neue Bestimmung der Molekildimensionen," *Ann.Physik*, vol. 34, p. 591-592 (1911).  
R. Simha, "The Influence of Brownian Movement on the Viscosity of Solutions," *J.Phys. Chem.*, vol. 44, pp. 25-34 (1940).  
J.W. Mehl, J.L. Oncley, and R. Simha, "Viscosity and the Shape of Protein Molecules," *Science*, vol. 92, pp. 132-133 (1940).

## Flory-Fox Relation

While the Einstein-Simha relation can be used to define the hydrodynamic radius for solid molecules with adsorbed solvation layers, it is 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:

$$[\eta] = \Phi \langle r^2 \rangle^{3/2} / M \quad (\text{Eq. #15})$$

where  $\langle r^2 \rangle$  is the mean squared end-to-end distance of the chain, and  $\Phi_0$  is a universal constant having the value  $2.87 \times 10^{23}$ . In practice, this constant varies somewhat from polymer to polymer with an experimental value closer to  $2.5 \times 10^{23}$ .

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:

$$[\eta] = \Phi(\epsilon) \langle r^2 \rangle^{3/2} / M \quad (\text{Eq. #16})$$

$$\Phi(\epsilon) = \Phi_0 (1 - 2.63\epsilon + 2.86\epsilon^2) \quad (\text{Eq. #17})$$

where  $\Phi$  is now a function of the polymer-solvent interaction parameter  $\epsilon$ , and  $\Phi_0$  is the Flory-Fox constant. When  $\epsilon = 0$ , it reduces to the theta solvent result.

The  $\epsilon$  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:

$$[\eta] = KM^a \quad (\text{Eq. #18})$$

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  $\log[\eta]$  vs.  $\log[M]$ . If the data is fit well, this should be a straight line. The slope parameter  $a$  is related to  $\epsilon$  by:

$$\epsilon = (2a - 1) / 3 \quad (\text{Eq. #19})$$

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