**Introduction to ASTRA**

**Objectives**:

This seminar is a general introduction to the ASTRA software, covering

1. Software installation and activation
2. Connecting to instruments
3. Collecting data and calibrating – the “first” collection

**Topics**:

1. Software installation and activation
   1. Using the installation disk
   2. Upgrading and applying patches  
      ***Note:*** You can check for available upgrades in ASTRA 6 (**Help** → **Check for Upgrades**)
   3. Installed software and supporting materials
   4. Activating base ASTRA functionality and optional modules in ASTRA (**System** **→ Feature Activation**)
   5. Licensing:
      1. For data collection, one license required for each light scattering instrument.
      2. For the purpose of data processing (by the researcher collecting the data), ASTRA can be installed on additional computers under the same license: back in the office, your laptop, at home, whatever.
2. Connecting to networked instruments with the Configuration Wizard (DAWN HELEOS, miniDAWN TREOS, Viscostar, Optilab (T)rEX)

Networked instruments can be either connected using the Configuration Wizard (preferred) or manually (the ASTRA 5 way). In the following the use of the Configuration Wizard is described. When using the Configuration Wizard, it is easy to create either an *online (chromatography) experiment*, *batch experiment*, or *light scattering calibration experiment*.

Manual connection and manual setup of a calibration run is described in Appendix 2. Users of older generation instruments, such as the DAWN EOS or miniDAWN Tristar, will need to follow the steps described in Appendix 2 to connect to their instruments and run a calibration.

|  |  |
| --- | --- |
| 1. Go to **System → Configuration Wizard** |  |
| 1. In the **Select Solvent** dialog, make sure that **toluene** is chosen as the **Solvent** and **Calibration** for the **Experiment Mode**. Click on **Next**. |  |
| 1. In the **Select Instruments** dialog, choose your **Light Scattering Instrument** (e.g., “*DAWN HHELEOS @Wyatt-688-H2HC*”).  If you only have one instrument on your network, this field will be automatically populated.  Click on **Next**. |  |
| 1. In the **Name Configuration** dialog, choose a name for your set of instruments, e.g., “*Calibration\_* Heleos693”. Click on **Finish**. |  |
| 1. Click on the Instrument button at the bottom of your toolbar. This will display the instrument. |  |
| 1. Viewing and monitoring your instrument in the Diagnostic Manager:   Double-click on **DAWN HELEOS**. This will open the **Diagnostic Manager** Window. Click on **Start Monitoring** to see live data collection. You can also send commands to the instrument on the commands tab. |  |

**Networking issues:**

If you are using either an Optilab rEX or ViscoStar in ASTRA 6 and are unable to connect to your instruments as described above, you may need to configure your Firewall and/or your DCOM settings. Please see the document “**Instructions for configuring Windows XP Professional Service Pack.pptx”** in Section 3 of your USB memory drive for a guide.

Note: HELEOS, TREOS, T-rEX, and QELS instruments no longer require the user to adjust DCOM security settings or to open up the firewall for the DCOM protocol in ASTRA 6.

**Please refer to the Documents:**

* ReadMe - ASTRA Firewall Configuration (6001 Rev B).pdf
* ReadMe - Windows XP SP2 & Vista Configuration (M6006 Rev B).pdf
* ReadMe - Multiple Network Adapter Configuration.pdf

If you are using ASTRA 5 with Security Pack, the following documents may be helpful to configure the SQL server:

* ReadMe - SQL Server 2002 Configuration (M6003 Rev B).pdf
* ReadMe - SQL Server 2005 Express Automating Database Maintenance (M6005 Rev B).pdf
* ReadMe - SQL Server 2005 Express Configuration (M6004 Rev B).pdf

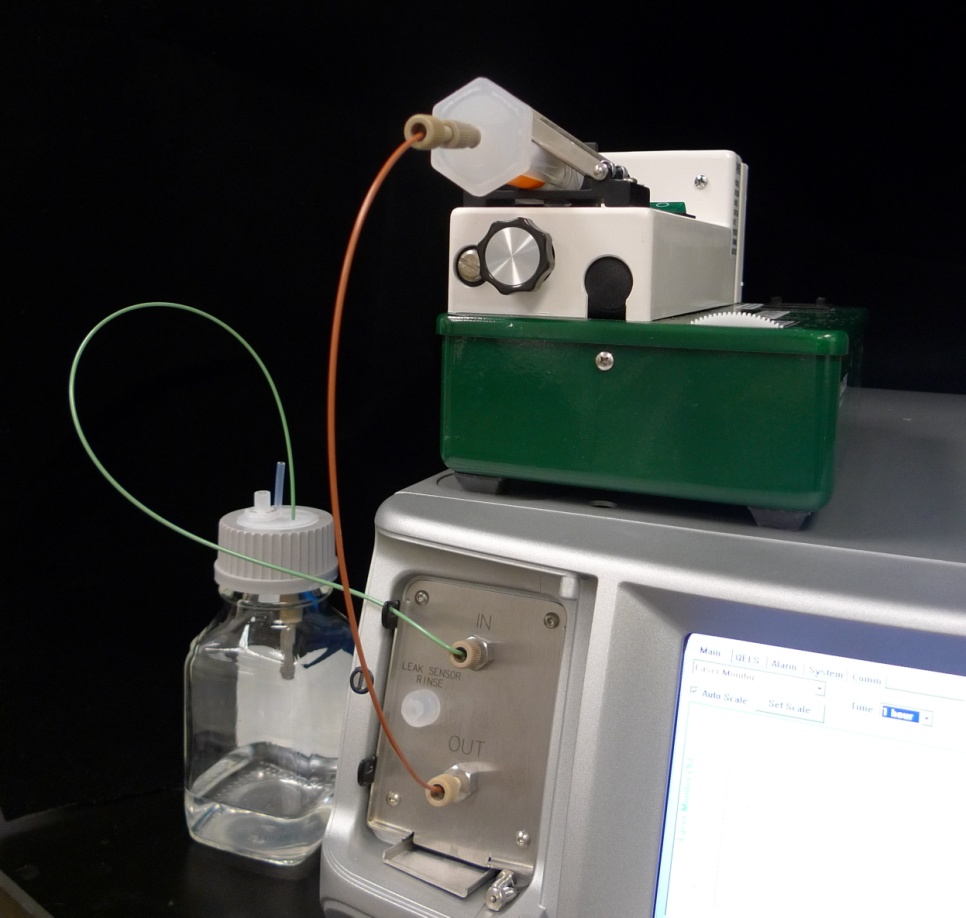
☺ You can find these documents on your USB memory drive in ***\Wyatt Hardware & Software Manuals\Software*** or on any computer with ASTRA installed in: ***C:\Program Files\WTC\Astra 6\ReadMe Files.*** ☺

Performing a Toluene Calibration of your Light Scattering Instrument

* 1. Make sure your light scattering instrument is powered on and connected to the computer as described above.
  2. If your flow cell does not already contain toluene or contains an air bubble (a *Forward Monitor* value of 0% generally indicates the presence of an air bubble), use a syringe pump and syringe containing with syringe tip filter to infuse the flow cell with toluene as shown below. Make sure that the solvent in the flow cell is compatible with toluene. If you are using an aqueous salt buffer, flush first with 10-20 mL of water, then 10-20 mL of alcohol and then change to toluene.

Connect a 10 mL syringe with luer connection and 0.02” tubing to the OUT port of your MALS instrument. Reverse flow will help create back pressure.

10 mL syringe with HPLC grade toluene and syringe tip filter (use smallest pore size available).

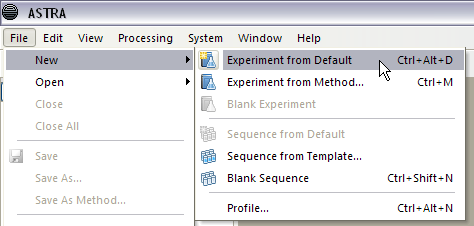


Syringe pump with flow rate ~ 0.5 mL/min.

Connect 0.03” tubing to the IN port of your MALS instrument and a waste reservoir,

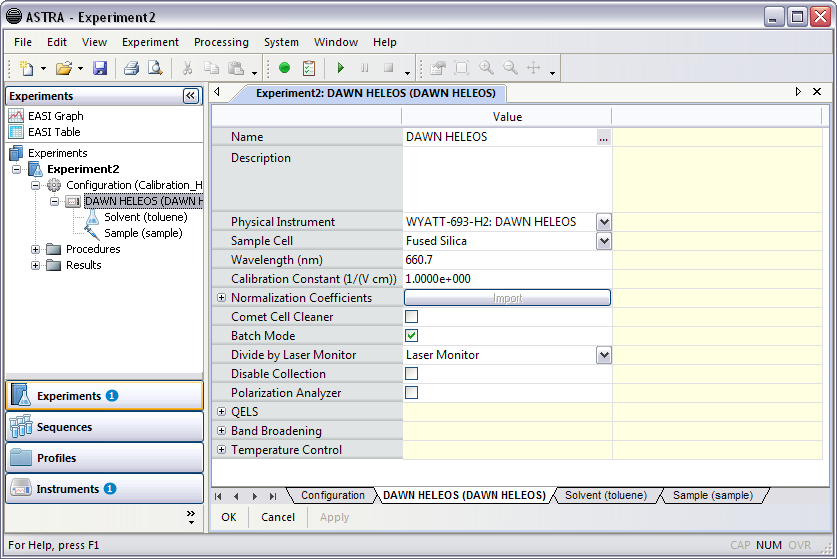
ASTRA data collection for Calibration

Select **File** → **New** → **Experiment from Default**. This will open a new file called **Experiment 1** using the Calibration method that you’ve created in Section 2 above.



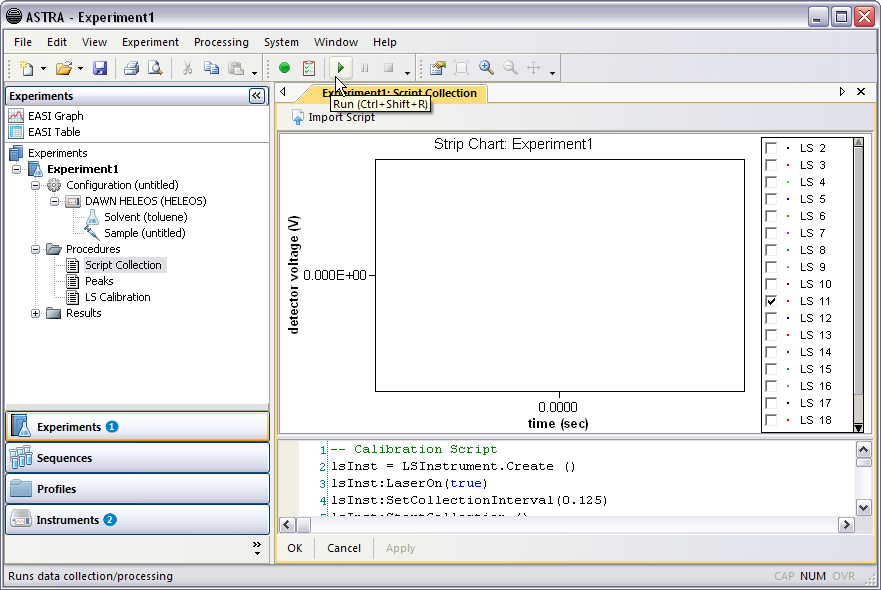
You’ve just created a new experiment named **HELEOS (from Experiment1).**

Expand the “**+**” sign next to **HELEOS (from Experiment1)** and in the **Configuration**. Double-click on **DAWN HELEOS** to see the instrument configuration parameters. Confirm that you have the correct **Sample Cell** selected. You can find this information on your *Certificate of Performance*; the flow cell type is **Fused Silica** for newer instruments and **K5** for older instruments.

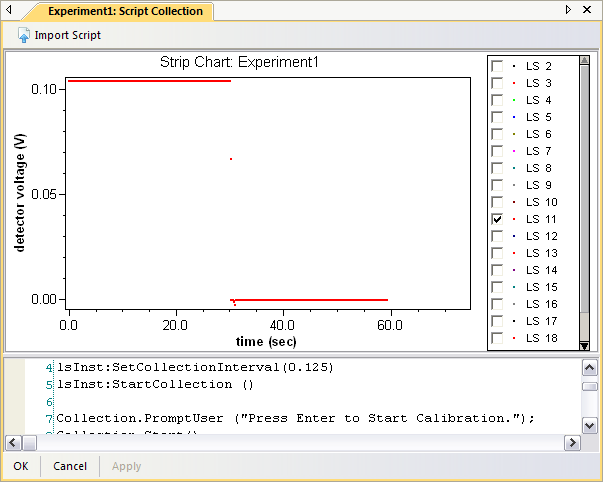


Click on **OK** if you have made any changes to your configuration to save your changes and close the window.

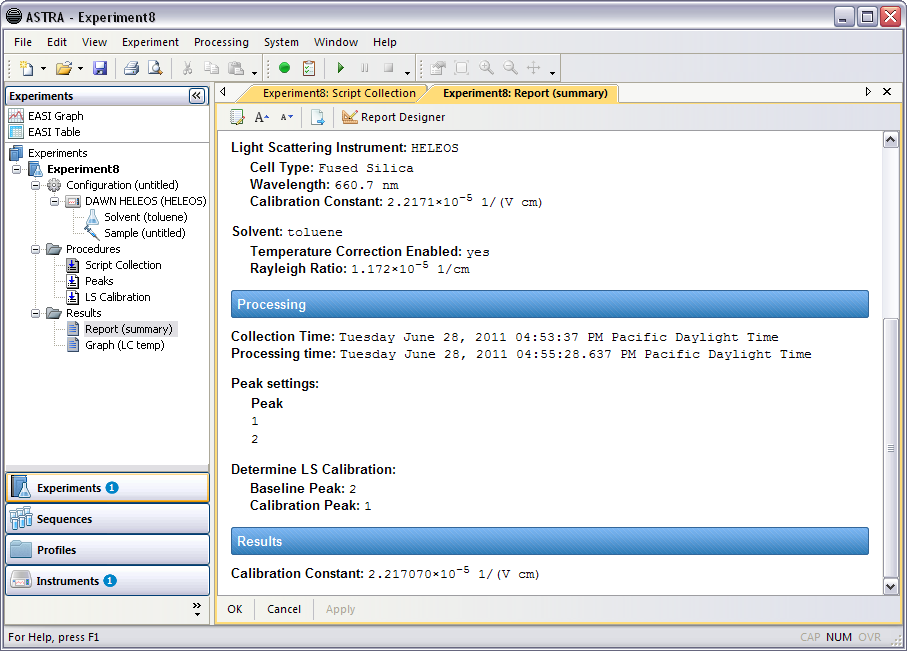
Double-click on **Script Collection** in the **Procedures** folder to open the Script Collection Window. Click on the Run Icon (green arrow) and press **OK** in the upcoming dialog window to start calibration.



Strip Chart after collection:



After the calibration is complete, you can view the calibration constant in the **Report**. Expand the **Results** node and select **Report**.



When installing a brand new instrument or recently serviced instrument, the **Calibration Constant** should be **within 5%** of the value reported to you by Wyatt Technology on your **Certificate of Performance**. If the calibration constant is different, it is possible that there is an air bubble in the cell. Refill the flow cell with toluene and repeat the calibration.

After calibration is complete, you will be asked whether you like to transfer the new calibration constant to your light scattering instrument. Select “No” if you are installing a brand new detector or your instrument came back from its annual service. In this case, we recommend using the Wyatt factory calibration constant instead. Otherwise, select “Yes”.

Your instrument is now ready to go.

***Remember to flush your flow cell with 20 mL of ALCOHOL and then 20 mL of WATER before going to an aqueous buffer, since***

***TOLUENE + WATER = A MESS!***

***Use only HPLC grade solvents and filter all solvents through a 0.02 μm syringe tip filter!***

**Appendix 1:   
Why are the units of the toluene calibration constant [V-1cm-1]?**

The absolute calibration of the Wyatt Light Scattering detectors employs a solvent that scatters strongly, such as toluene, with the well known Rayleigh Ratio @ 658.0 nm of 1.1931x10-5 cm-1. The Rayleigh Ratio is a measure for the amount of light that will be scattered from a point source at a fixed distance and angle, can be defined as:

 *Equation (1)*

Where:

***Rθ***= the Rayleigh ratio at angle θ; units of inverse Centimeters (cm-1);

***I***= the intensity of the incident radiation; units of Volts (V);

***i*θ** = the intensity of scattered radiation observed at an angle θ; units of Volts (V);

***r*** = the distance from the point of scattering; units of Centimeters (cm);

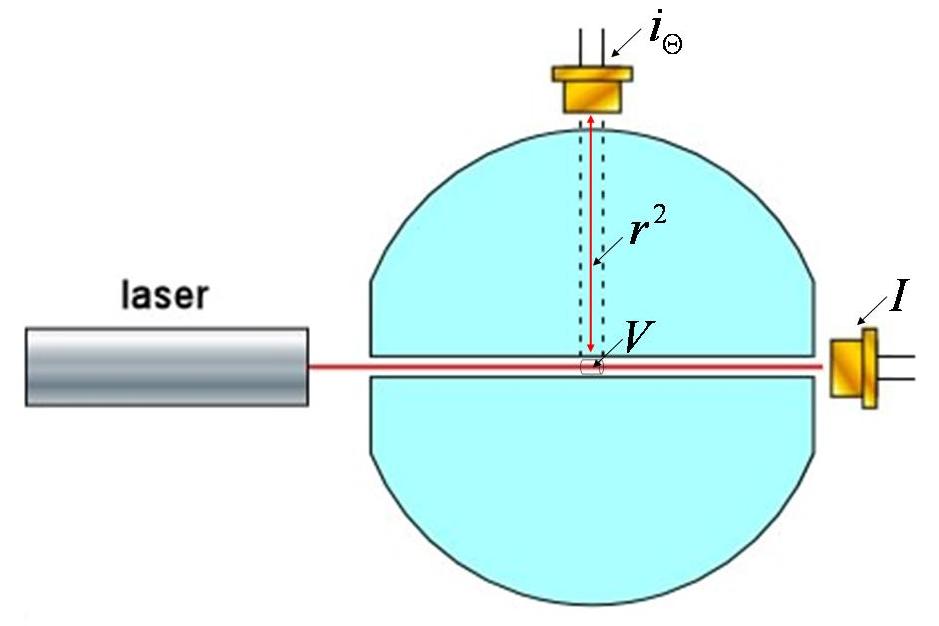
***V*** = the total scattering volume; units of Centimeters cubed (cm3);

***f*** = is a unit-less factor that takes account of polarization phenomena, for Wyatt detectors which use vertically polarized light, so the value of *f* = 1.

The units cancel out leaving inverse centimeters, which are the units for Rayleigh Ratio, refer to Equation 2. An image depicting the important variables in Equation 1 is shown in Figure 1, below.

 *Equation (2)*

In order to use Equation 1 to quantify light scattering intensity, the detectors must be calibrated because the quantities we measure directly are detector voltages and not light intensities, however because the voltages measured by the photodiodes are linearly proportional to light intensities, we can calibrate their response, this is done by deriving a calibration factor which gives the correct value for a known scattering standard and using a pure solvent as the scattering standard makes the calibration completely independent of any polymer sample.



**Figure 1:** Schematic image of the important variables used for the toluene calibration experiment.

Appendix 2: Manually connecting to your instruments and running a Calibration

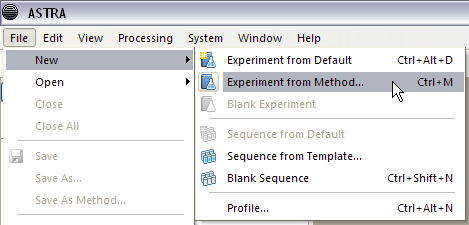
|  |  |
| --- | --- |
| 1. Go to **System** → **Instruments…** or **c**lick on the **Add Instrument** button in the workspace (the latter will take you directly to Step 3). |  |
| 1. In the **Instruments** window, choose **Add**. This will launch the **Add Instrument** dialog. |  |
| 1. In the **Add Instrument** dialog, click the **Search** button. This will show all available instruments on your network. 2. Select your instrument(e.g. WYATT-171-TS),and click **Add**. This will open the **Instruments** window. |  |
| 1. In the **Instruments** window select your instrument. |  |
| 1. Choose **View** to launch the **Diagnostic Manager** to view live data from your instrument. Click on **Start Monitoring** to view data collection. |  |

**b)** **Via serial connection (DAWN EOS, miniDAWN Tristar)**

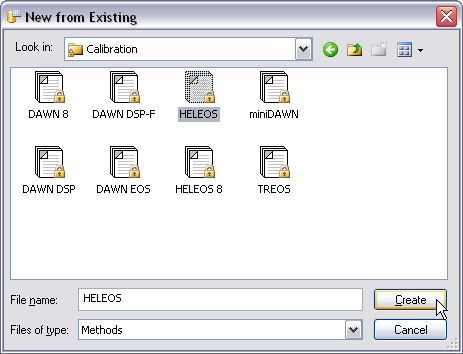
|  |  |
| --- | --- |
| 1. Go to **System → Instruments…** (see above). 2. In the **Instruments** window choose **Add** (see above). 3. In the **Add Instrument** dialog choose the **Manual** option in the lower half of the window. 4. The name of your Computer will automatically appear in the Name field. (here: LABQC5). |  |
| 1. Click the **Add** button at the bottom of the window. 2. Your instrument will appear in the Instruments window, e.g. “miniDAWN on COM1”. |  |
| 1. In the **Instruments** window, select your instrument and choose **View** to launch the Diagnostic Manager to view the instrument.  Click on **Start Monitoring** to view data collection. |  |

Running a Calibration after Connecting Manually

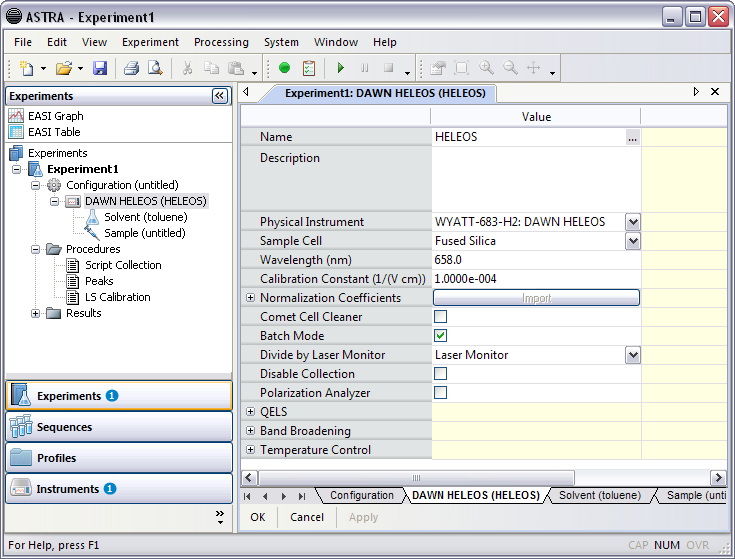
In ASTRA, experiments are created from a method. Select **File → New → Experiment from Method**.



In the New from Existing Window, navigate to the **System** → **Methods** → **Light Scattering** → **Calibration folder** and select your instrument (HELEOS, TREOS, miniDAWN, DAWN EOS, …). Click on Create – this will create a new Experiment in the workspace.



In the workspace, expand the **Configuration** node and then double click on the node for the light scattering instrument (here: **DAWN HELEOS**). Check that your **Physical Instrument**, **Sample Cell** and **Wavelength** are set correctly in instrument dialog. Note that the most commonly flow cells are **Fused Silica** in newer instruments or **K5** in older instruments. Click **OK** to close the tab and save your changes (if you’ve made any).



Then follow the instructions on pages 5, 7 and 8 to run your calibration.