Hardware Manual for the DAWN® HELEOS™II Light Scattering Instrument

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M3200 Rev A

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Table of Contents

1 Introduction

When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meager and unsatisfactory kind: it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of science.

—Lord Kelvin

William Thomson Kelvin, the 19th century physicist and mathematician who wrote that paragraph, would have been very comfortable with the DAWN HELEOS II Enhanced Optical System laser photometer and software. The DAWN HELEOS II system, measuring scattered light at different angles simultaneously, can determine the molar masses of polymers and biopolymers from a few hundred to hundreds of millions of daltons. The DAWN HELEOS II measures 18 angles. Options permit temperature control of the flow cell, the use of the Wyatt COMET flow cell cleaning system, and QELS dynamic light scattering. The flexibility, versatility, and built-in redundancy of the DAWN HELEOS II instruments make them exceptional measuring systems.

Read on to learn more about the DAWN HELEOS II line of laser photometers.

CONTENTS **PAGE**

1.1 Overview

1.1.1 The Instruments

The DAWN HELEOS II combines the proven features of photometers, nephelometers, turbidimeters and "goniometers" into a single light scattering instrument. It can be used as a detector for continuous-flow (GPC/SEC/HPSEC) detection or as a stand-alone unit in a batch or micro-batch mode.

The read head and the laser system anchor to the base plate, and the flow cell and manifolds are mounted directly into the read head to provide a single, stable optical bench.

The laser beam is aimed into the flow cell, and passes in the same direction as the flowing stream. The windows that let light pass through the flow cell are recessed in the manifolds, minimizing sample volumes and stray light.

In the DAWN HELEOS II, 18 discrete photodetectors are spaced around the flow cell enabling simultaneous measurements over a range of angles (typically 15°-160°, depending on solvent/glass refractive indices).

Each photodetector has its own DSP (Digital Signal Processor) chip for processing the analog signal. In addition, four auxiliary analog inputs (with their own DSP chips) enable interfacing to external detectors such as differential refractive index and ultra-violet absorption detectors or differential viscometers. Electronic filters within the DSP chips and within the embedded computer process each light scattering and auxiliary signal for the greatest possible noise rejection without peak distortion. Since the analog-to-digital conversion is performed on-board, low light scattering signals are not prone to environmental "noise" or pickup. The digital output transmits to your computer through its Ethernet port, or USB (with the supplied ethernet-to-USB converter).

The DAWN HELEOS II system uses a 130mW laser operating at 658 nm which after intensity stabilization delivers 100mW to the sample. The DAWN HELEOS II also includes a state-of-the-art electronics package with an embedded microprocessor and a graphical user interface.

All functions are controlled by the microprocessor.

Instrument Options

- **COMET:** The Wyatt COMET flow cell cleaning option works by creating resonate sound waves in the flow cell bore, suspending dirt in the solution which is then flushed out by the flowing mobile phase.

- **QELS:** Quasi-elastic or dynamic light scattering is an internally installed option that measures time-dependent fluctuations in the scattered light signal using a fast photon counter. QELS measurements can determine the hydrodynamic radius of macromolecules or particles. This option is described in Appendix A.

The DAWN HELEOS II temperature control options are entirely air cooled, eliminating the need for an external water bath. The following on-board heating and cooling options are available:

- **Ambient:** Operates at room temperature only. This is the base model described in Chapters 1 through 4.
- **Ultra-High Temperature:** The read head may be heated from approximately 10° above ambient temperature to 210°C. Temperature can be controlled to within 0.01°C and is accurate to ±1°C. This option is described in Appendix B.
- **Peltier Heated/Cooled:** The read head may be cooled down to -30°C or heated up to 150°C. Temperature can be controlled to within 0.01°C and is accurate to ±1°C. This option is described in Appendix C.

  A nitrogen purge connector is included on all models of the DAWN HELEOS II. In addition to preventing condensation in a cooled instrument's read head, the nitrogen purge keeps the flow cell and read head cleaner at all temperatures.

  The nitrogen pressure is monitored by the microprocessor to insure that even when the nitrogen tank runs out, no water will condense on the read head.

1.1.2 The Software

Wyatt Technology offers the ASTRA® for Windows software package for collecting and analyzing data from the DAWN HELEOS II instrument:

ASTRA V for Windows collects and processes chromatography data from dilute polymer solutions. From polymers fractionated by size or molecular weight, ASTRA V calculates the molecular weight moments (number, weight, and z-average) along with the rms radius moments of the molecules in solution. From unfractionated polymers, ASTRA V displays Zimm, Debye, or Berry plots.

1.2 About This Manual

The DAWN HELEOS II *Hardware Manual* describes how to set up and use the DAWN HELEOS II laser photometer. Please see the *ASTRA V for Windows User's Guide* for details on data analysis.

1.2.1 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* means the same thing. You will see both terms used in this manual.

1.2.2 How the Manual Is Organized

The chapters and appendices in this manual are organized as follows:

Chapter 1, "Introduction" introduces the DAWN HELEOS II and this manual, and describes the support options available from Wyatt Technology.

Chapter 2, "Installing the DAWN HELEOS II" takes you through the necessary first steps for unpacking, connecting, and testing the instrument.

Chapter 3, "DAWN HELEOS II Components" takes you on a guided tour of the instrument.

Chapter 4, "Using the Display Window" shows you how to navigate and change settings in the DAWN HELEOS II Display Window or from a remote terminal.

Chapter 5, "DAWN HELEOS II Maintenance" has procedures for keeping the instrument in good working order, and includes flow cell cleaning and conversion to batch mode.

Appendix A, "Using QELS"describes procedures for using the QELS option.

Appendix B, "Ultra-High Temperature Option" describes the Ultra-High Temperature version of the DAWN HELEOS II and its operation.

Appendix C, "Peltier Heated/Cooled Option" describes the Heated/Cooled version of the DAWN HELEOS II and its operation.

Appendix D, "Polarization Option" tells about the installation and use of polarization filters.

Appendix E, "Interference Filter Option" describes the use of interference filters for keeping non-laser wavelengths from reaching the photodiodes.

Appendix F, "Laser Specifications" supplies the electrical, optical, and environmental specifications for the GaAs laser head.

Appendix G, "Flow Cell Properties" lists thermal and chemical properties, refractive indices, and scattering angles of solvents for the K5 and F2 flow cells.

Appendix H, "Connecting to Network or PC" covers connecting the DAWN HELEOS II to either a network through the ethernet, or to a host PC through the ethernet-to-USB converter.

1.3 How to Contact Wyatt Technology Corporation

If you have a question about your DAWN HELEOS II, first look in this manual or consult the online help that comes with ASTRA V for Windows. If you cannot find an answer, please contact Wyatt Technology Technical Support.

1.3.1 Corporate Headquarters

Wyatt Technology Corporation
6300 Hollister Ave.
Santa Barbara, CA, 93117
USA

1.3.2 Sales Department

Wyatt Technology Corporation Sales Hours are 8:30 A.M. to 5:00 P.M. Pacific Time.

Sales Phone: (805) 681-9009

Sales Fax: (805) 681-0123

1.3.3 Technical Support

Wyatt Technology Corporation offers a variety of support options to help you get the most from your DAWN HELEOS II.

You can also contact the Wyatt Technology Distributor in the country where you bought your product.

Before contacting technical support, try to resolve any problems through the ASTRA V for Windows on-line help system and this manual.

Internet

You can use the Internet to ask questions and receive answers via e-mail, as well as visit Wyatt Technology's world-wide-web site.

World-Wide-Web URL: http://www.wyatt.com

Electronic mail address: support@wyatt.com

FAX

You can fax your questions or comments to us at any time.

Wyatt Technology Corporation Technical Support Fax: (805) 681-0123

Telephone

You can reach the voice mail for Wyatt Technology Corporation Technical Support at any time. To speak to our support personnel directly, please call between 8:30 A.M. and 5:00 P.M. Pacific Time, Monday through Friday. When you call you should be at your instrument and have the documentation at hand. Please be prepared to provide the following information:

- Instrument serial number (located on the back panel).
- If the problem is software related: Microsoft Windows version number; ASTRA V version number; exact wording of any messages that appear on your computer screen. The software version number is located on the original distribution diskette(s), or you can view it by selecting About from the Help menu.
- The type of computer hardware you are using.
- What you were doing when the problem occurred.
- How you tried to solve the problem.

Wyatt Technology Corporation Technical Support Phone Number:

(805) 681-9009

1.4 Where to Go from Here

Continue to **Chapter 2, "Installing the DAWN HELEOS II"** to check out your shipment and make some necessary initial checks and adjustments.

If you have purchased special options, you will also want to read the appropriate appendix for a description and instructions for setting up and working with those options.

2 Installing the DAWN HELEOS II

This chapter helps you get the DAWN HELEOS II unpacked, tested, and connected. You will also make some first time adjustments.

CONTENTS **PAGE**

2.1 Unpacking the Instrument

Please read the shipping parts list (packing slip) included with your instrument shipment and check that everything arrived in good condition.

1. Carefully examine the shipping container. If it is damaged or shows signs of mishandling, CONTACT WYATT TECHNICAL SUPPORT IMMEDIATELY.
2. Unpack the instrument.
3. Place the DAWN HELEOS II on a level surface and inspect the cabinet for damage. If you see any damage, CONTACT WYATT TECHNICAL SUPPORT IMMEDIATELY.
4. Check that the boxes contain all of the items listed as included with your instrument shipment in addition to the instrument (the packing slip sent with the instrument contains the most up-to-date list).

2.2 Installing the Instrument

The installation procedure for the DAWN HELEOS II involves some initial tests to see that everything is working properly.

To install the DAWN HELEOS II, do the following:

1. Place the instrument on a flat, clean surface, standing on its feet and positioned to allow air flow through the back to keep its electronics cool. (See **Chapter 5, "DAWN HELEOS II Maintenance"** for more information about the required environment and how to keep the DAWN HELEOS II in peak condition.)

   The DAWN HELEOS II is designed to stack with the ViscoStar or rEX. It can be installed either at the top or bottom of the stack. If the optional batch conversion is to be used, we recommend that the DAWN HELEOS II be installed on the top of the stack so that the batch holder is easily accessible through the top cover.

2. Make sure the supplied power plug is correct for the local power outlet. The DAWN HELEOS II is equipped with a universal power supply, which operates anywhere in the world. It accepts inlet voltages between 90-250V and line frequencies from 50-60Hz.

3. Connect one end of the supplied ethernet cable to the ethernet port on the back of the DAWN HELEOS II and the other end to your local area network. Alternatively, you can use the supplied ethernet-to-USB converter and connect to the USB port on the host computer.

   When the DAWN HELEOS II is on the local area network, it may be accessed and controlled from any machine on the network. When using the USB converter, it can be accessed only by the host computer. See Appendix H for more details about implications for network security from the two different configurations.

4. Switch on the instrument and let it warm up for 30 minutes before beginning step 6. The power switch is on the front panel.

5. **For Nitrogen Purge Option for Peltier Cooled Instruments Only** (this step is not necessary for ambient or heated instruments):

   While the instrument is warming up, attach a filtered dry air or chromatographic grade nitrogen line to the Nitrogen Purge connector on the back of the DAWN HELEOS II. Use the 90-degree fitting and the 10-inch Polyethylene tubing provided. The dry gas will flow into the cell cavity and will minimize the amount of dust in the cell cavity. The pressure in the dry air or nitrogen line should be 20 psi or less. (If you are using a Peltier Heated/Cooled DAWN HELEOS II and operating below ambient temperature, it is particularly important to use the nitrogen purge line to prevent condensation. At ambient or high temperatures, the nitrogen purge line is not required, but may be used to exclude dust from the instrument by creating positive pressure inside the cell.)

6. The DAWN HELEOS II has been shipped with chromatography-grade toluene in the flow cell that can be used to verify that the instrument was not damaged during shipping. Cycle through the light scattering graphs and check that the solvent offsets are consistent with the Certificate of Performance (COP) supplied with each instrument.

   Sometimes when the instrument has been in storage or been subjected to extreme temperatures during transit, the cell will have bubbles. If this is the case, fill the cell with fresh toluene before checking the solvent offsets against the COP. Use a glass syringe with a 0.02 µm filter and inject toluene directly into the flow cell through the "in" port. You may wish to use a syringe pump to drive the syringe and to help prevent introducing bubbles into the flow cell.

7. Using the supplied ASTRA V software, perform the appropriate steps to configure the instrument to communicate with the software.

Note: The laser in the DAWN HELEOS II is software controlled and can be turned on and off from the main page.

8. After establishing communications, wait at least 30 minutes for the laser to warm up and stabilize.

9. Start the Diagnostic Manager and select the numeric real-time channel values (see the *ASTRA V for Windows User's Guide* for details).

10. Compare the channel values with the solvent offsets on the Certificate of Performance.

- If the readings for the detectors are different from those on the Certificate of Performance, check your laboratory temperature. The dark offsets for the detectors may differ from the Certificate of Performance by as much as 10 mV per ° C. For example, if your laboratory temperature is 20 ° C and the QC laboratory temperature was at 23 ° C, your current dark offsets may be 30 mV different. If you see a greater difference, monitor the dark offsets for a few days to see if they remain stable at this voltage. If they do not, contact Wyatt Technology Technical Support.
- The laser and forward monitors are set at the factory to have a scale of 0-100%. The laser monitor measures the intensity of the laser before the beam enters the cell. The laser's intensity is controlled via a feedback loop based on the laser monitor signal (see "System Panel" on page 4-12). The forward monitor measures laser intensity after the beam has passed through the cell. This value will be affected by absorption of the sample as well as reflection losses from the cell windows. Since the beam passes through many optical surfaces and approximately 3cm of fluid, the forward monitor is not nearly as stable as laser monitor and therefore is used primarily as a diagnostic signal. For example, when performing batch measurements, the forward monitor is used to detect the presence of bubbles or foreign matter in

the cell. It is only used as an analytical signal when used to correct for the attenuation of the beam due to absorbing samples (see Astra absorption correction).

Before shipment, the solvent offsets were measured with toluene and the flow cell was filled with toluene and capped, so the solvent offsets you see should be very close to those on the Certificate of Performance. More than 200 µV difference between your values and those on the Certificate of Performance may indicate air bubbles in the manifolds, in which case you will need to flush the cell with filtered toluene and recheck the solvent offsets. If your dark offsets differed from the Certificate of Performance in step 6, the solvent offsets should differ by the same amount.

11. Calibrate the DAWN HELEOS II using the ASTRA V software.

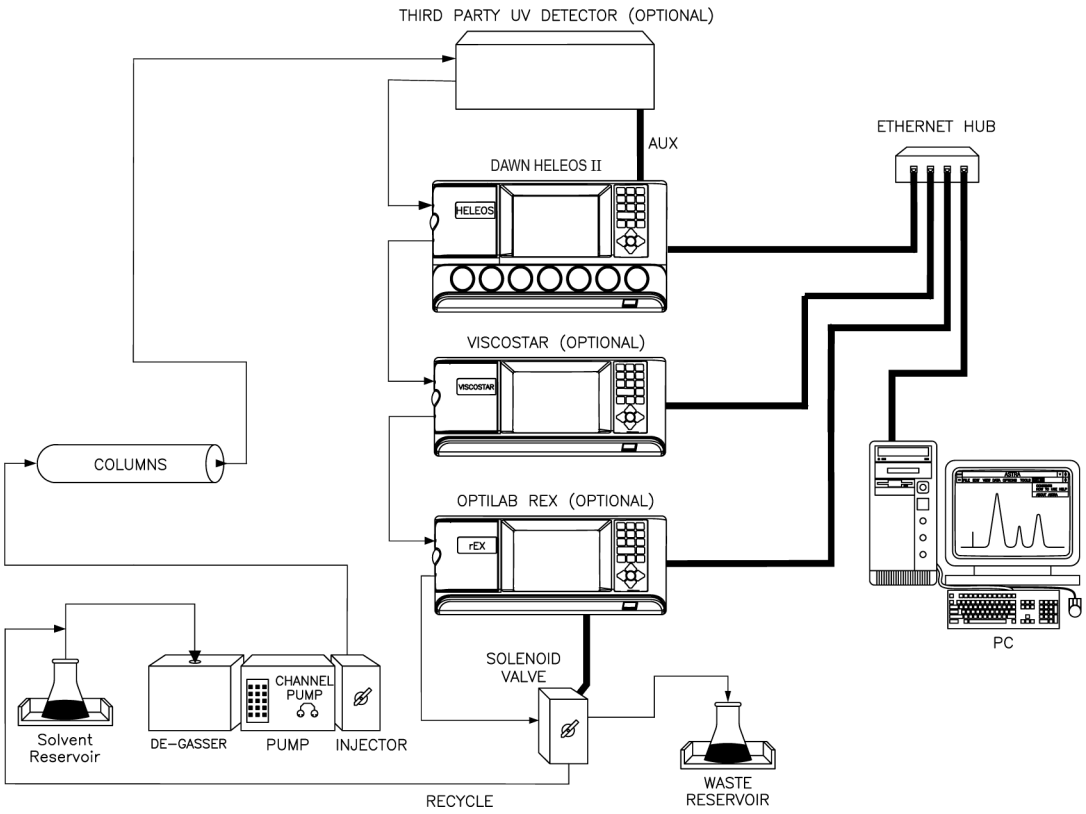
See the software user's guide for instructions to configure communication with the instrument and perform the calibration measurement.

12. Compare your calibration result with the value from the Certificate of Performance.

Your calibration result should be within 5% of the value on the Certificate of Performance.

13. When you have confirmed that the instrument is in good working order, connect the DAWN HELEOS II to any other devices for your application. (Auxiliary cable connection is described in the next section.)

The *ASTRA V for Windows User's Guide* describes how to connect the DAWN HELEOS II to your chromatography system.



This configuration requires the ASTRA V software. The dark black lines denote the electrical/data connections. The thin black lines show the fluid connections. Several optional instruments are shown. If not connecting one of the optional instruments, the fluid connections are bypassed. The (optional) recycle valve can be controlled by either the DAWN HELEOS II, ViscoStar, or Optilab rEX, but is always plumbed after the last instrument in the chain.

Figure 2-1: The DAWN HELEOS II in-line with a chromatography system

2.3 Connecting Auxiliary Devices

You can connect the DAWN HELEOS II to various other devices using the connectors on the back panel. Five cables are provided for such connections. These cables have a DAWN HELEOS II connector on one end and four wires on the other end. Because devices have a variety of connector types, you will need to attach these wires to the connector used by your devices. All auxiliary device connectors on the back panel of the DAWN HELEOS II are current limited to protect the internal circuitry.

The auxiliary device connectors on the back of the DAWN HELEOS II are:

- Aux 1, Aux 2, Aux 3, and Aux 4: You can connect the DAWN HELEOS II to up to four external detectors. These are usually concentration sensitive detectors. Aux 1 and Aux 2 are in one connector and Aux 3 and Aux 4 are wired in a second connector.
- Analog Out: You can use this connector to send two analog output signals to your existing data collection system or to a chart recorder. Analog Out 1 and Analog Out 2 are in one connector.
- AUTO INJECT: You can use this connector to sense an injection from an auto injector. This signal is then monitored by the ASTRA V software.

2.3.1 Attaching Auxiliary Device Connectors

The AUX input signals can accept an input range of -10V to 10V with a 1 µV resolution. Typically when the time constant is set to 1 sec, a noise level of less than 10µV is observed.

To attach an Auxiliary connector, do the following:

1. Attach a cable to the appropriate port on the rear panel of the DAWN HELEOS II. Aux 1 and 2 are on one connector; Aux 3 and 4 are on another.
2. Connect the wires of the cable to your other device as shown in Table 2-1. Usually when connecting the AUX channels, one need only connect AUX+ and AUX- to the signal source. If there is unacceptable noise pickup, you can connect the GND connection to either the Chassis or the AUX- connector of the source instrument.
3. You may need to connect the wires to a connector provided with your device or to the device directly. The following list contains additional information for various other connectors:

- **Auto Inject**: The auto inject input expects a contact closure. Most autosamplers and many manual injection valves incorporate such a contact closure. The auto inject input can be tested by simply touching the red and green wires together. When an auto inject signal is recorded, the graph on the DAWN HELEOS II main page will display a green line. Some injectors require programming in order for the closure to happen. Make sure that an injection closes the circuit.

- **Alarm In**: TTL input on red (signal) and green (signal ground). The TTL input signal is the voltage measured between the signal (red) and the signal ground (green). TTL voltage levels are +5V (logic 1) or 0V (logic 0). On the instrument display Alarm panel, you may select which of these indicates on or off (see "Alarm Panel" on page 4-10).
- **Alarm Out**: TTL output on white (signal) and black (signal ground). On the instrument display Alarm panel, you may select which of these indicates on or off (see "Alarm Panel" on page 4-10).
- **Recycle In**: TTL input on red (signal) and green (signal ground). When the signal on this line transitions from 0 V to 5 V, the instrument actuates an external solenoid valve by supplying power to the Recycle Out connector. When the signal transitions from 5V to 0V, the Recycle valve is de-actuated. This channel can be used by a third-party instrument to control the recycle valve.
- **Recycle Out**: The solenoid valve drives current on the white and black wires (the current direction is irrelevant for the solenoid). This signal may be connected to a user-supplied solenoid valve or a Wyatt Technology Recycle unit, which contains an internal solenoid valve that switches between waste and recycle. When this connector is actuated (via the System tab or the Recycle In input), the connector supplies current to drive a 12 V solenoid valve. The valve is actuated with 12 V (up to 1 Amp, depending upon resistance of the solenoid), held for 0.1 second, and then dropped down with 12 V across an internal 51 Ohm resistor.
- **Ethernet**: Ethernet connection for connecting the instrument to an Ethernet network. This connector is a standard RJ-45 wiring for a 10Base-T/100Base-TX connection.

Table 2-1: Back Panel Wiring

Connector Label	Pin #	Color	Signal	Comments
Aux 1 & 2	1	White	Aux1+	
	2	Black	Aux1-	
	3	Red	Aux2+	
	4	Green	Aux2-	
	5	Yellow	Aux1_GND	
	6	Blue	Aux2_GND	
Aux 3 & 4	1	White	Aux3+	
	2	Black	Aux3-	
	3	Red	Aux4+	
	4	Green	Aux4-	
	5	Yellow	Aux3_GND	
	6	Blue	Aux4_GND	

Table 2-1: Back Panel Wiring (Continued)

Connector Label	Pin #	Color	Signal	Comments
Analog Out 1 & 2	1	White	Analog Out1+	
	2	Black	Analog Out1-	
	3	Red	Analog Out2+	
	4	Green	Analog Out2-	
	5	Yellow	Analog Out1_GND	
	6	Blue	Analog Out2_GND	
Auto Inject In	1	White	NC	
	2	Black	NC	
	3	Red	Inject_In+	
	4	Green	Inject_In-	
	5	Yellow	NC	
	6	Blue	NC	
Auto Inject Out	1	White	Inject_Out+	
	2	Black	Inject_Out-	
	3	Red	NC	
	4	Green	NC	
	5	Yellow	NC	
	6	Blue	NC	
Alarm In	1	White	NC	
	2	Black	NC	
	3	Red	Alarm_In+	
	4	Green	Alarm_In-	
	5	Yellow	NC	
	6	Blue	NC	
Alarm Out	1	White	Alarm_Out	
	2	Black	Alarm_Out-R	
	3	Red	NC	
	4	Green	NC	
	5	Yellow	NC	
	6	Blue	NC	

Table 2-1: Back Panel Wiring (Continued)

Connector Label	Pin #	Color	Signal	Comments
Recycle In	1	White	NC	
	2	Black	NC	
	3	Red	Recycle_In	
	4	Green	Recycle_In_Rtn	
	5	Yellow	NC	
	6	Blue	NC	
Recycle Out	1	White	FV-12VDC	
	2	Black	FV-RTN	
	3	Red	NC	
	4	Green	NC	
	5	Yellow	NC	
	6	Blue	NC	
Ethernet	1	White/ Orange	Transmit+	Standard RJ45 wiring of 10Base-T/100Base-TX Ethernet
	2	Orange	Transmit-	
	3	White/ Green	Receive+	
	4	Blue	NC	
	5	White/Blue	NC	
	6	Green	Receive-	
	7	White/ Brown	NC	
	8	Brown	NC	

3 DAWN HELEOS II Components

This chapter gives you a guided tour of the DAWN HELEOS II components. If you have just installed the DAWN HELEOS II, read this chapter to become familiar with the various instrument parts and their functions.

CONTENTS **PAGE**

3.1 Front Panel View

The front panel (see Figure 3-1) contains the main power switch (On/Off), provides fluid connections for the DAWN HELEOS II, along with the display window and display controls for operating the instrument and monitoring data.

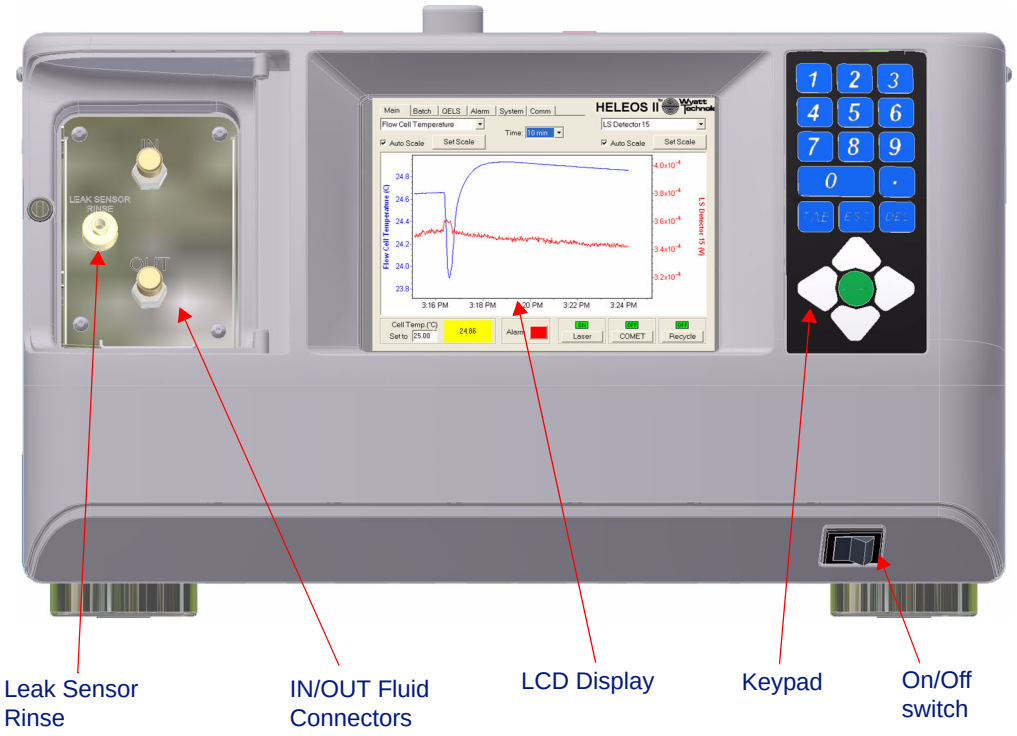


Figure 3-1: DAWN HELEOS II Front Panel

LCD Display: The LCD display allows you to monitor, control, and configure the DAWN HELEOS II. **Chapter 4, "Using the Display Window"** describes the functions of the tabs available on the LCD display.

Keypad: The keypad allows you to control the LCD display. "Navigating the Display Panels" on page 4-2 describes how to use the keypad.

IN/OUT Fluid Connectors: Fluid comes into the DAWN HELEOS II through the IN port, and exits through the OUT port. If the DAWN HELEOS II is stacked on top of the Optilab rEX, the drain system is designed to cascade so that only a single drain tube needs to be connected at the bottom of the instrument stack.

Note:	The fittings used by Wyatt instruments are standard 10-32 chromatography fittings as supplied by Parker, Upchurch, or Valco. Fittings supplied by Waters Corporation will seal but may cause a gap within the fitting, giving rise to excessive mixing. Waters fittings are not recommended.

LEAK SENSOR RINSE: Use the leak sensor rinse port to empty the leak sensor reservoir after a leak alarm. Connect a luer-lock syringe to the LEAK SENSOR RINSE port and draw out the fluid.

If you are using a mobile phase with salt, the salt can dry on the leak sensor causing it to malfunction by reporting a leak when no leak is present. In that case, water can be injected in and out of the leak sensor reservoir through the LEAK SENSOR RINSE port. After several rinse cycles, any dried salt should removed.

3.2 Back Panel View

The back panel contains the AC power module, auxiliary and serial connectors, heated line connector, nitrogen purge connector, and cooling fan. The main power fuses are located in the AC power module and are described below.

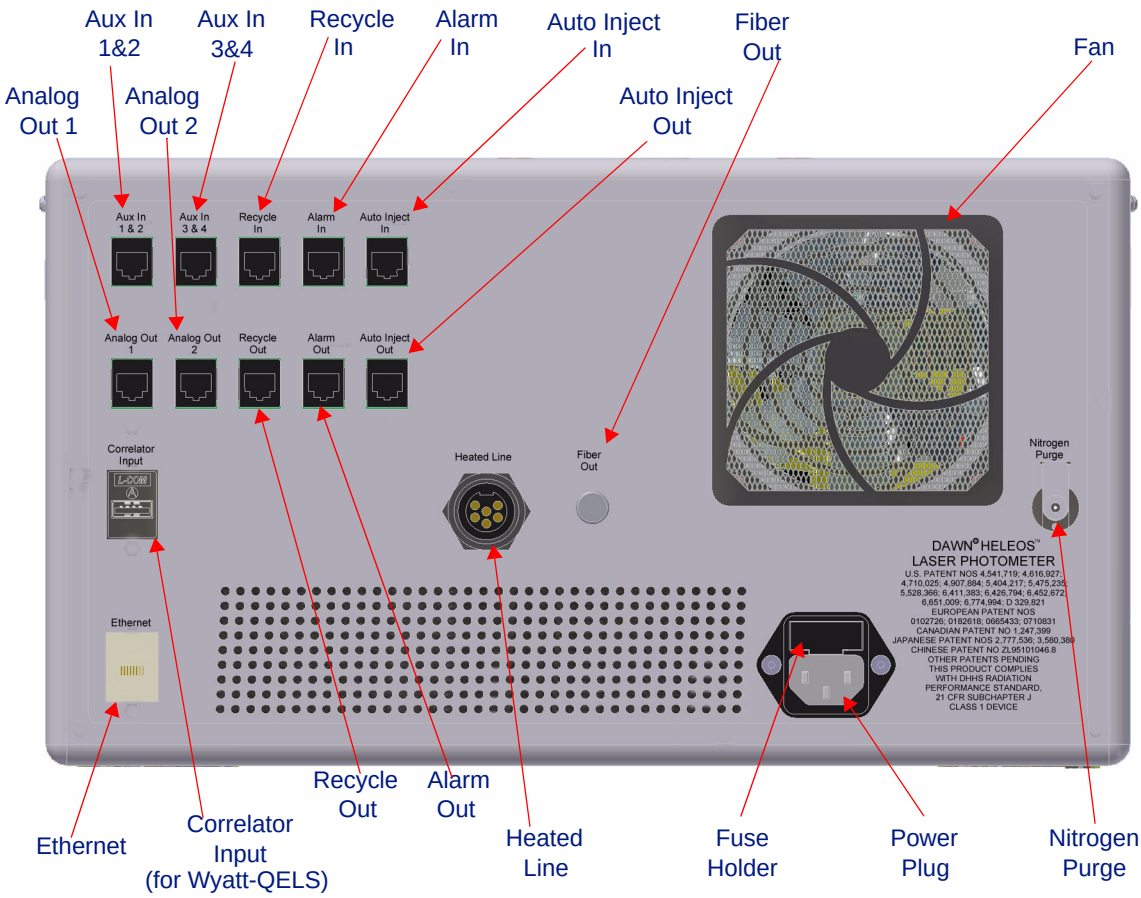


Figure 3-2: Back panel

3.2.1 Changing a Fuse

What you need to change a fuse:

- Tool for prying the AC Power module cover off, such as a small-bladed screwdriver.
- Fuses from the spares supplied in the accessory kit.

To replace the fuses, do the following:

1. Disconnect the power cord.
2. Open the cover of the AC Power module using a small blade screwdriver or similar tool.
3. Replace the burned out fuse with a 4 amp, 250V slow blow fuse. The fuse block contains two fuses. Both of them must be installed for the instrument to operate correctly.
4. Replace the cover of the AC Power module and reconnect the power cord.

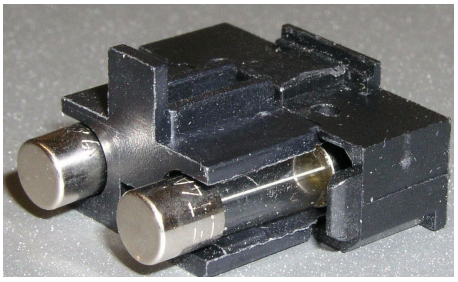


Figure 3-3: Fuseholder and Fuses

3.3 Top Cover

There are two covers. The standard cover, for chromatography applications, has no openings. If the customer has purchased the optional flow to batch conversion kit, or a heated transfer line, a new cover is included that has removable pieces. These pieces can be removed to allow access to the read head assembly and for introducing scintillation vials or the MicroCuvette when the batch measurement manifold is installed.

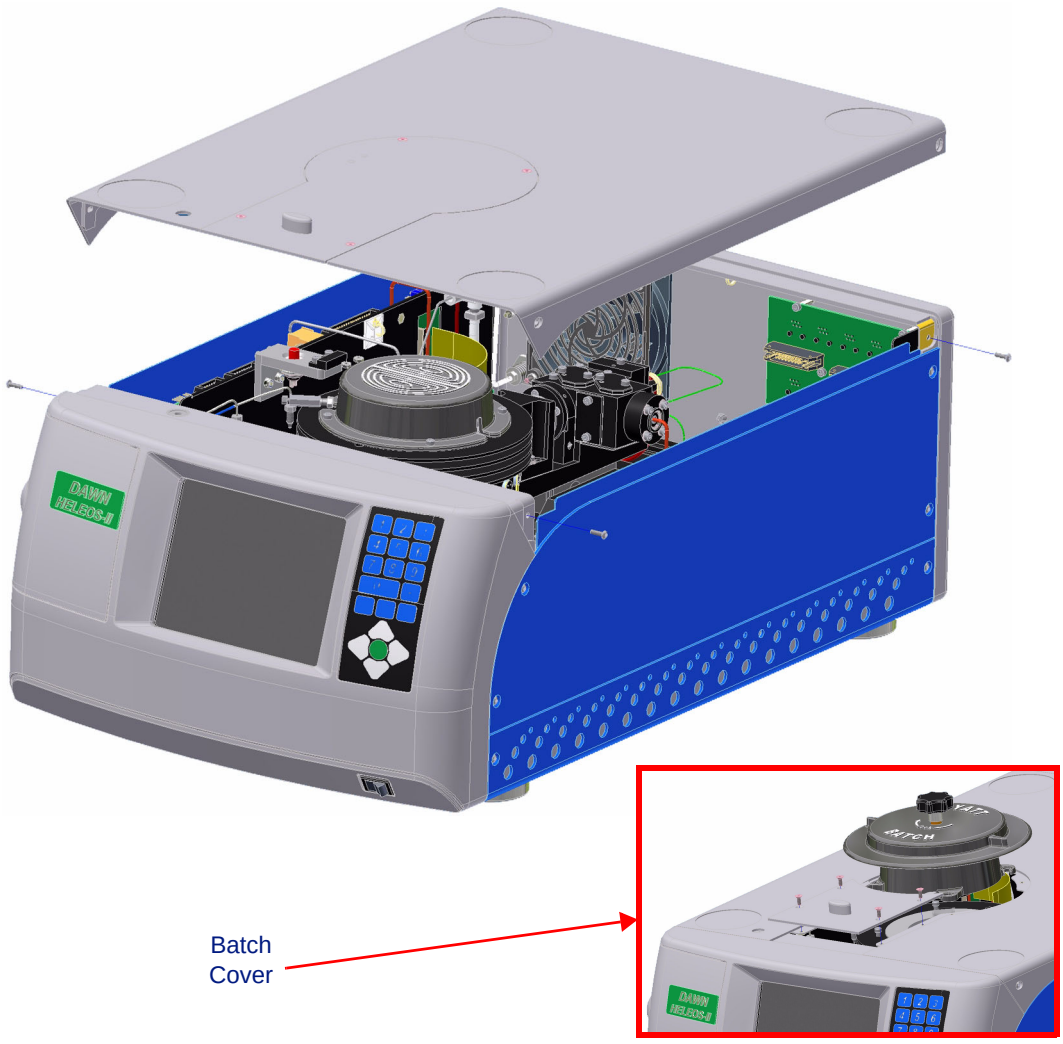


Figure 3-4: DAWN HELEOS II Top Cover

3.3.1 Removing the Cover

For normal operation and maintenance, you should not need to open the top cover. If you do need to open the top cover, to install the COMET or QELS options (see Appendix C, “User Installed Options”), follow these instructions.

What you need to remove the cover:

- 2.0 mm Ball driver

To remove the cover, do the following:

1. Make sure the DAWN HELEOS II has enough space above it to lift up the cover.
2. Disconnect the power cord.
3. Remove the four screws that fasten the top cover to the instrument using the 2.0 mm Ball driver. The screws are shown in Figure 3-4.
4. Slide the cover up to remove it. You can now see the components, as shown in Figure 3-5.

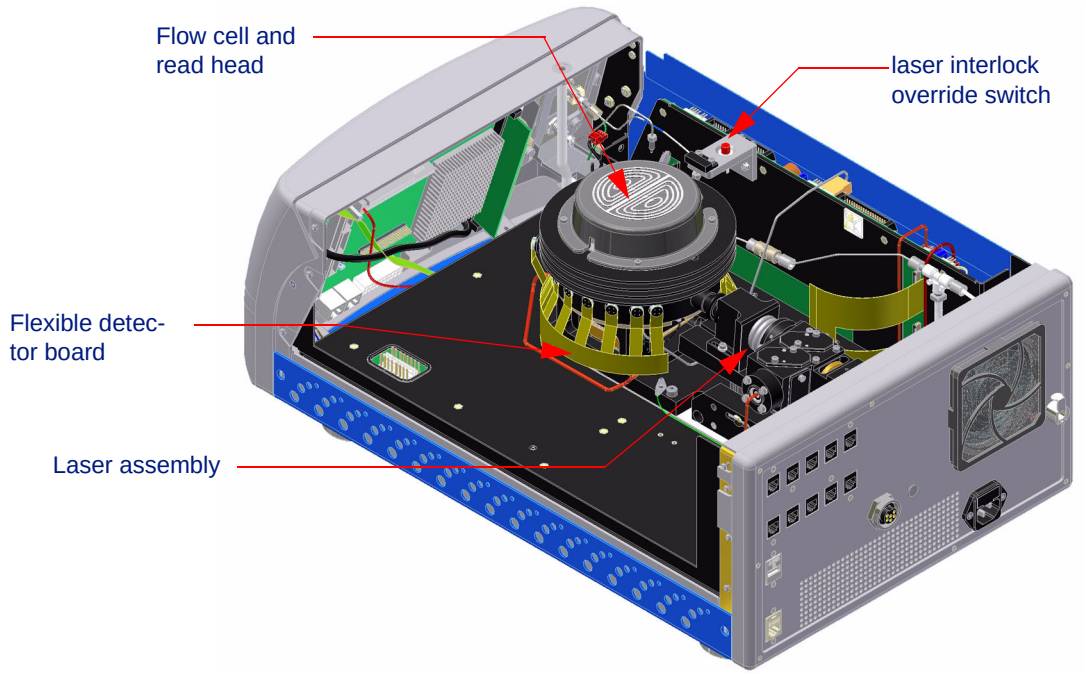


Figure 3-5: The DAWN HELEOS II uncovered

3.4 Laser

The 130 mW linearly polarized GaAs (gallium arsenide) laser provides the light source for the system. The laser system provides very high power density at the illuminated sample by means of a narrow beam diameter (the $1/e^2$ diameter of the Gaussian beam profile is 0.08 mm). This small beam diameter also helps reduce the noise contributions of larger particulate contaminants (such as dust). The laser is oriented so that the incident beam is vertically polarized. A beam monitor (laser monitor) is incorporated into the laser assembly. The output of this monitor can be displayed on the Main panel in the display window.

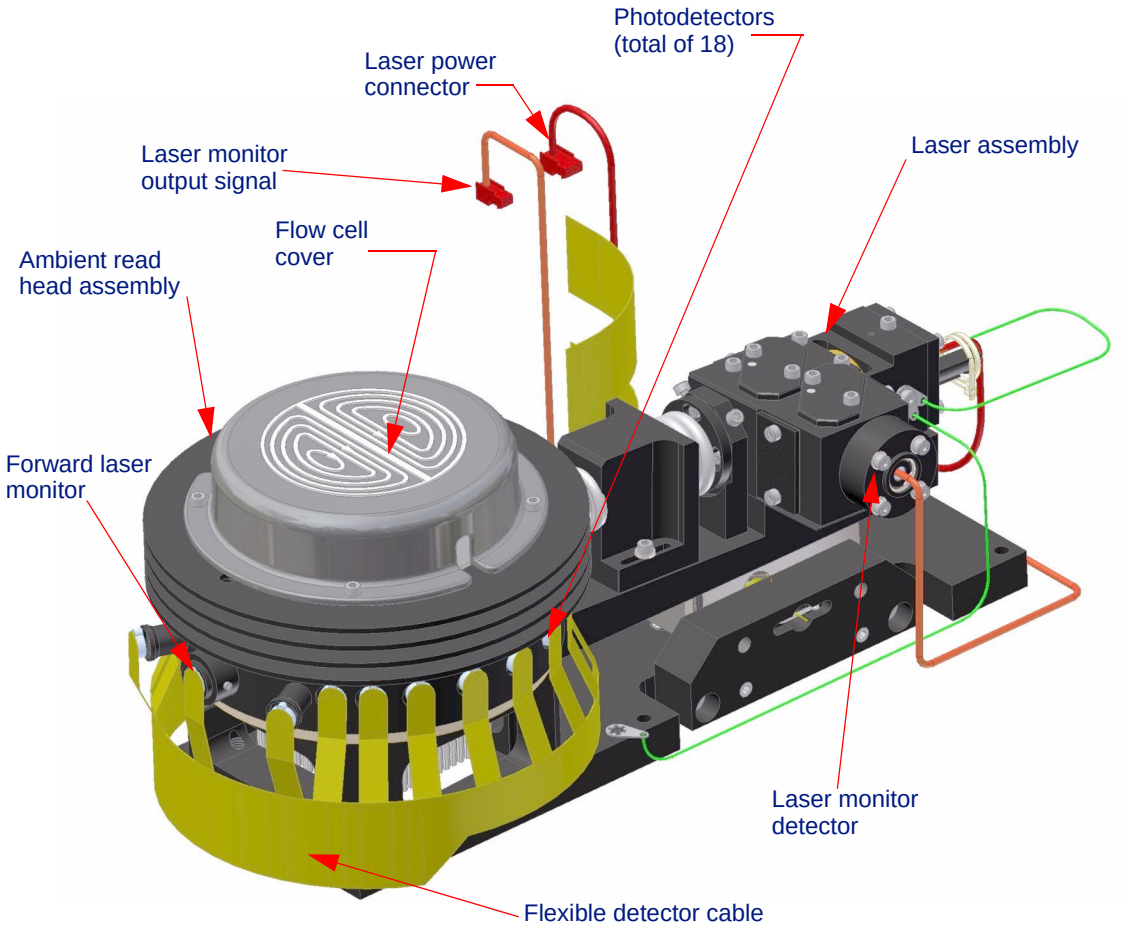


Figure 3-6: Read head and laser assemblies

3.4.1 Laser Beam Warning

Under normal operating conditions the laser beam is entirely contained within the read head. A laser interlock ensures that when the instrument top cover or batch vial cover is removed, the laser is deactivated. Pressing the laser interlock over-ride button, as shown in Figure 3-5, temporarily reactivates the laser.

Some operations, such as aligning the QELS fiber (see "Aligning the Optical Fiber" on page A-6) require the laser to be active. Pressing the over-ride button activates the laser. Take caution not to introduce a finger or mirror into the cell cavity while the over-ride button is pressed.

It is good laboratory practice with any laser source, irrespective of its power, to AVOID LOOKING INTO THE BEAM. Figure 3-7 shows the warning label affixed to the read head. Appendix F gives the laser specifications.

Figure 3-7: Laser beam warning label

3.4.2 Laser Monitors

The software uses the laser monitor signals to normalize the scattering signals relative to incident laser beam power. The method involves splitting the beam at its source and dividing background corrected values by the split signal. The normalization factor I_0—the incident intensity, is proportional to the beam emitted from the front of the laser and is obtained from the beam splitter on the laser assembly.

- The **Laser Monitor** measures the intensity of the beam before it enters the cell.
- The **Forward Monitor** enables the DAWN HELEOS II to measure transmitted light through the flow cell and sample. This signal is useful for measuring absorbing samples, which attenuate the beam intensity. The forward monitor measures the attenuation and can be used to determine the actual intensity at the center of the cell, where the scatter is measured.
- **Laser Current** signal is used to gauge the lifetime of the laser. As the laser ages, the current required to provide a constant intensity slowly increases. The laser current is measured in mA and its initial value is recorded on the Certificate of Performance (COP) delivered with the instrument. When the current reaches a value of 30% higher than the initial value, the DAWN HELEOS II will switch from an intensity mode, to a constant current mode. In the constant current mode, the laser intensity will begin to decrease and the signal to noise ratio will begin to degrade. The instrument will still provide accurate data, but it indicates that the laser is nearing its maximum usable lifetime and the instrument should be serviced. The DAWN HELEOS II also measures Laser Voltage, which is a diagnostic that Wyatt Technical Service can use to track laser ageing.
- The **Laser Monitor** and the **Forward Monitor** signals are displayed as a percentage of intensity. Zero percent means no light is detected. If the Laser Monitor signal differs from the Laser Power set point by more than 10% the Laser Monitor alarm will activate. The laser may have reached the end of its useful lifetime.

3.5 Read Head and Detectors

The next major assembly is the read head (Figure 3-8). Here, the sample cell is held precisely, scattered light is collimated, and the detectors are aligned in their proper angular positions.

3.5.1 Read Head Structure

The read head structure holds the 18 hybrid trans-impedance photo detectors, limits the sample field of view at each detector, and minimizes stray light effects by means of its special structure. Since each detector's field of view is limited by its own collimator, only the center of the illuminated sample scatters light into a given detector. A heavy aluminum mounting plate supports both the laser and the read head and is attached to the instrument sub-chassis, providing a single, stable optical bench.

The optics have been aligned at the factory and should need no adjustment. The detectors are connected via a flex cable to the HELEOS Electronics Module (HEM) which converts the analog signals to digital values with individual 24-bit analog to digital converters. Note that the instrument's major components are mounted on the steel sub-chassis, which also contains all power supplies (laser, meters, electronics) and fan assembly.

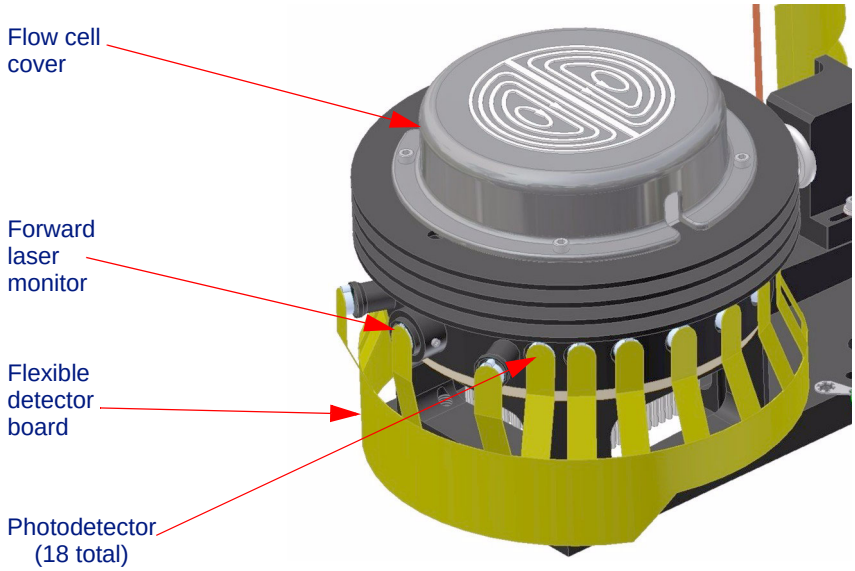


Figure 3-8: Ambient read head

With the read head covers removed to reveal the flow cell assembly, you can view the cell bore through an opening in the cell manifolds (Figure 3-10).

3.5.2 Detector Placement

The 18 detectors are placed as shown in Figure 3-9. Channel #1 is available only during Batch measurements (using scintillation vials).

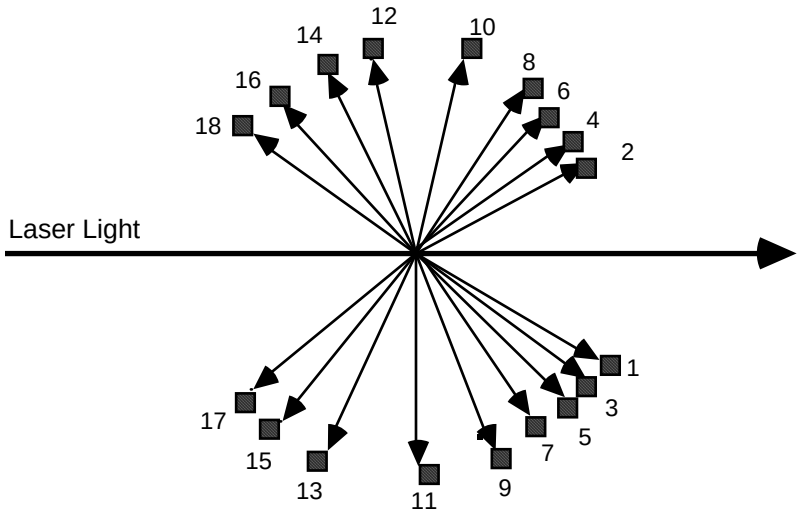


Figure 3-9: Detector locations

The angles are measured with respect to the direction of the laser beam. Since the observed angle changes with solvent refractive index, small scattering angle measurements are possible. To include at least some small scattering angles for all solvents, we have chosen the set of fixed detector angles, θ'. (See "Flow Cell" on page 3-13 in this chapter.)

Table 3-1: Positions of the 18 detectors relative to the incident laser beam

Channel #	(fixed detector angles)	Channel #	(fixed detector angles)
1	22.5°	10	81.0°
2	28.0°	11	90.0°
3	32.0°	12	99.0°
4	38.0°	13	108.0°
5	44.0°	14	117.0°
6	50.0°	15	126.0°
7	57.0°	16	134.0°
8	64.0°	17	141.0°
9	72.0°	18	147.0°

3.6 Flow Cell

3.6.1 Flow Cell Design

The patented flow cell is at the heart of the DAWN HELEOS II, and is critical to the instrument's unique measuring capabilities.

In many applications, such as chromatography, the ability to measure small samples is crucial, so cell volumes must be minimal. The total volume of the cell from the manifold inlet to the manifold outlet is about 70 µL. The actual scattering volume—the illuminated part of the sample that is viewed by the detectors—is less than 1 µL.

In other light scattering instruments, the cell walls are so close to the detected sample that the light scattered from the cell walls often overwhelms the small scattering signals from the sample. The DAWN HELEOS II' flow cell design resolves this dilemma. Because the windows are recessed in the manifolds, away from the scattering volume, any stray light from the air/glass/solvent interfaces is not seen by the detectors. As a result, the detectors measure scattering only from the sample and not from the cell.

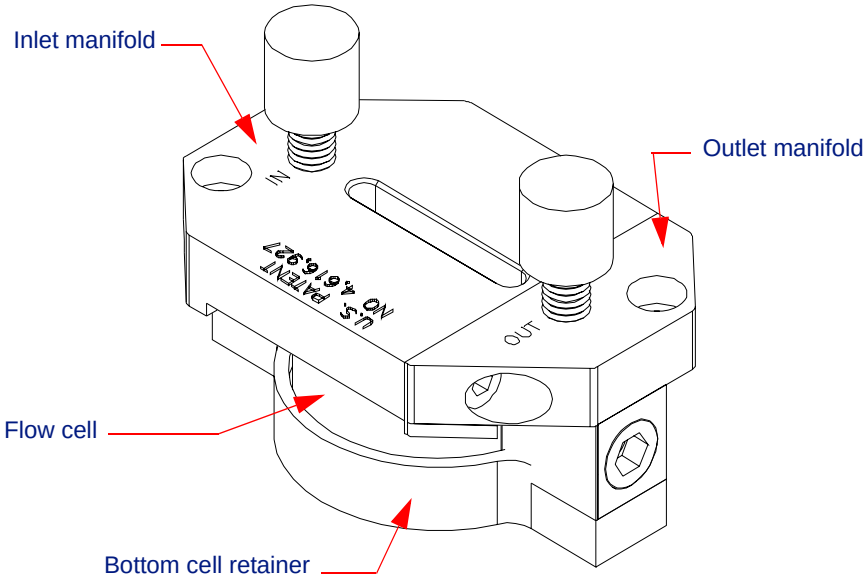


Figure 3-10: Flow cell assembly

3.6.2 Laser Beam Orientation

Another critical element of the DAWN HELEOS II flow cell is the laser beam's orientation: The laser passes in the same direction as the flowing stream. This helps to minimize beam/cell interface problems by keeping the cell and its interfaces clean of precipitates.

3.6.3 Cell Windows

The cell's windows protrude into the flowing stream at the entrance and exit manifolds. These miniature rods of glass are designed to minimize debris buildup on their flat ends, and, for the same reason, have no recessed rims or edges.

Note:	The large surface facing out from the cell has been coated to minimize reflections. Be careful not to scratch the coating during cleaning and do not use acids.

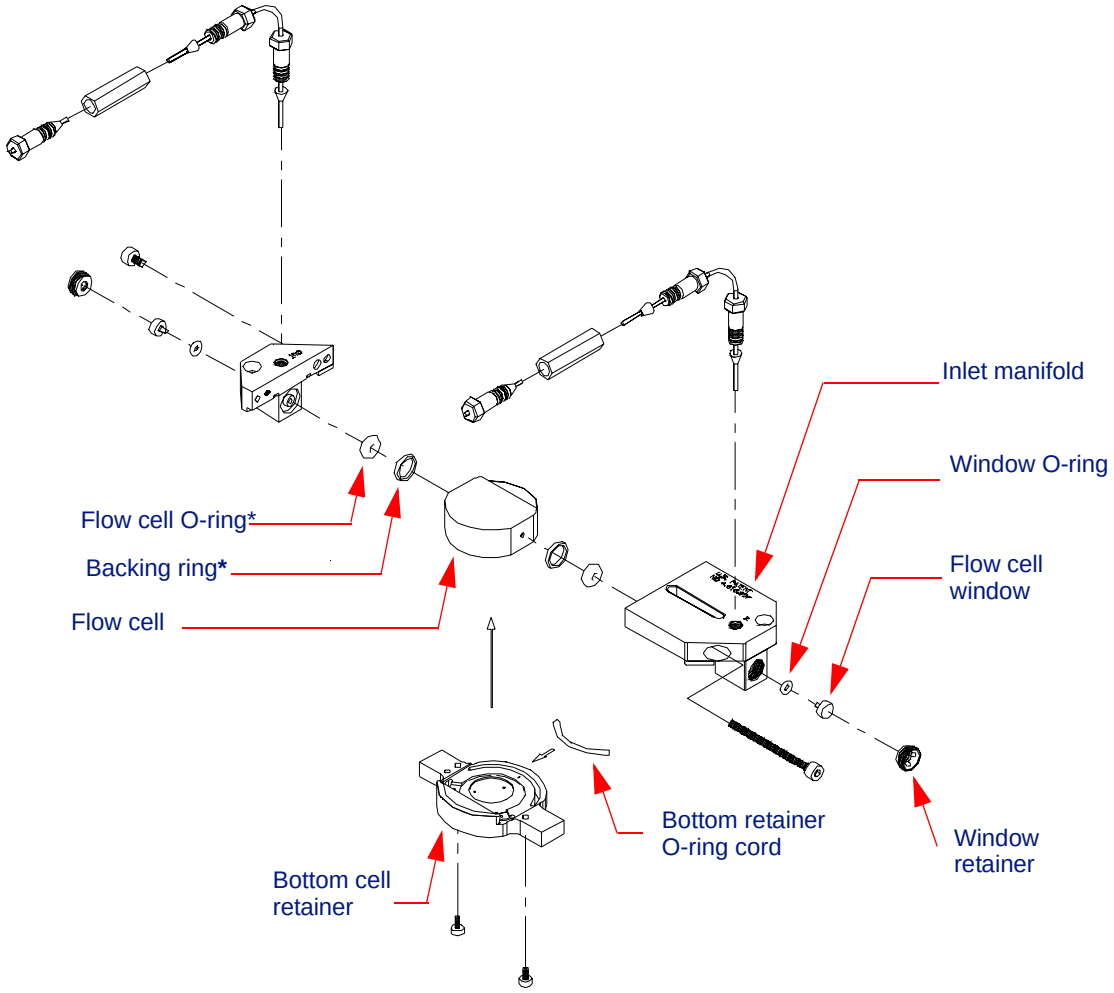


Figure 3-11: Exploded view of the flow cell assembly (–30°C to +80°C configuration)

Caution:	If you intend to operate your instrument above 80°C, the flow cell must use the 9 mm O-rings instead of the 6 mm O-ring and backing ring combination used at lower temperature. With the high temperature O-ring configuration, you may run the instrument over the entire temperature range, however, the dead volume at low temperatures will be increased. If the instrument temperature is set above 80°C, a warning message will appear on the front panel that the correct O-rings must be installed. If your instrument is configured for temperatures below 80°C and you decide to operate at temperatures above 80°C, you must change the O-ring configuration. Failure to do so may cause the flow cell glass to crack.

3.6.4 Refractive Index Differences—Liquid vs. Glass

The difference in refractive index between the solvent and the surrounding glass cell results in some of the most important features of the flow cell design. As long as the refractive index of the solvent is less than that of the cell glass, it will be possible to obtain measurements of light scattered at relatively small angles, with minimized background contributions. Figure 3-12 shows a detail of the liquid/glass interface and rays scattering from the laser-illuminated sample.

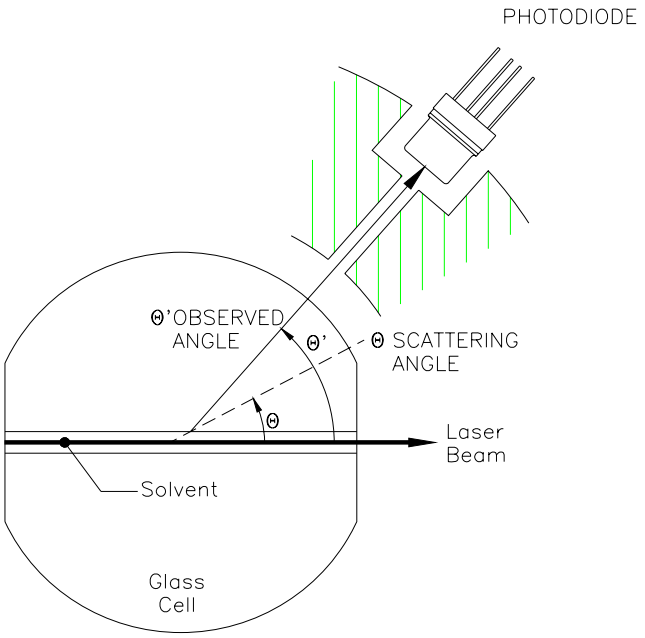


Figure 3-12: Flow cell refractions

Applying Snell's Law, the refraction of a ray scattering at angle θ may be determined from

(1) $$n_{liquid}\sin\left(\pi/2-\theta\right)=n_{glass}\sin\left(\pi/2-\theta'\right)$$

where the angle of incidence is $\pi/2-\theta$ and the angle of refraction is $\pi/2-\theta'$. Expanding the sine functions in Equation (1) results in

(2) $$n_{liquid}\cos(\theta)=n_{glass}\cos\left(\theta'\right)$$

The detectors are set to detect light at an angle θ, collimated to be centered in the cell. As a result of refraction, the light detected is the light scattered at an angle θ. In this way a greater angular range of scattered light can be detected. Table G-3 in Appendix G lists the flow cell scattering angles.

3.6.5 Accessible Available Detectors

Because of the refraction of scattered light passing from the solvent into the glass cell, some fixed detector angles are inaccessible. Consider water, for example. With a refractive index of 1.330 and the K5 cell refractive index of 1.51876 (at 690 nm), the smallest scattering angle must be $\theta = 0°$. Substituting into Snell's Law Equation (2) we find the smallest fixed detector angle, viz.

$$1.332\cos(0°) = 1.52064\cos(\theta')$$

$$\therefore \theta' = \cos^{-1}(1.332/1.52064) = 28.8°$$

We see that for water the first accessible detector is the third, corresponding to $\theta' = 32.0°$. Since this is the lowest detector for a water solvent system, theoretically no scattered light should enter Channels 1 and 2 (note that Channel 1 is actually blocked by the flow cell). Although there may be signals on these channels, their source is not light scattered from the sample, but rather stray light outside the range of the experiment. The ASTRA V software will select the appropriate detectors based on these considerations. See Table G-3 in Appendix G for more examples.

3.7 Alarms

The DAWN HELEOS II will sound an audible alarm when a potential hazard is reported. Hazards include:

- vapor or liquid leak sensor activates
- cell protection thermostat activates (indicating an over temperature condition)
- external alarm input activates

When a potential hazard is detected, the alarm output on the back panel also activates. This is so that this signal can be used to control other instruments. For example, this signal can be used to turn off the chromatography pump.

3.7.1 Vapor Sensor

The DAWN HELEOS II has a vapor sensor to aid in the safe operation of the instrument, especially at high temperatures. The vapor sensor is not intended as a protection device but as a convenience to alert the operator to the possibility of flammable liquid or vapor inside the instrument.

The alarm activates within 15 to 30 seconds after vapor is present. The alarm should reset within 30 seconds after all solvent disappears from the flow cell cavity. The sensitivity of the vapor sensing device is different for each solvent. The sensor is set to a sensitivity level that works for both toluene and tetrahydrofuran.

You can use the Alarm Out connector to shut down the pump system or activate an external alarm if a leak is detected. See “Attaching Auxiliary Device Connectors” on page 6.

3.7.2 Liquid Level Leak Sensor

The DAWN HELEOS II also has a liquid level leak sensor. The vapor sensor is not sensitive to aqueous solvents, but the liquid level leak sensor is sensitive to both aqueous and organic solvents. However approximately 2ml of liquid must leak into the reservoir before the liquid level leak sensor will activate, and therefore the liquid level leak sensor is much less sensitive to small leaks than is the vapor sensor.

The alarm will only reset after all liquid is removed from the leak sensor reservoir.

3.7.3 Turning Off the Alarm

When either the vapor or liquid sensor activates, there is an audible alarm and the alarm button on the main page (and the alarm page) turns red. When this occurs, you can turn off the audible alarm, but the red indicator will remain lit.

Note:	Even when the audible alarm is turned off, the back panel alarm output will remain active.

The DAWN HELEOS II has six internal printed circuit boards (PCBs):

- Front panel computer and display board
- Flexible detector board (cable)
- EOS instrument controller board (EIC)
- Utility board (on the subchassis)
- Vapor sensor board
- Nitrogen sensor board.

3.7.4 EIC Flexible Detector Board

The flexible detector board is the cable that wraps around the read head and connects to all of the light scattering detectors. This PCB sends signals from the detectors to the EOS instrument controller for amplification and digitizing.

3.7.5 EOS Instrument Controller Board

The EOS instrument controller (EIC) board contains 48Mhz microprocessor and 29 channels of amplifiers and 24-bit analog to digital converters. It also contains provisions for temperature measurement and control, as well as the laser driver feedback circuitry.

3.7.6 Utility board

The utility board is responsible for DC power distribution and control of the Peltier temperature control system. It also contains circuitry to process the keypad and send the resulting signals to the front panel computer.

3.7.7 Vapor sensor board

The Vapor sensor board drives the vapor sensor transducer and the liquid leak sensor. It has two LEDs which indicate the status of the two detectors.

3.7.8 Nitrogen sensor board

The Nitrogen sensor board measures the pressure of the N2 port. This is used to determine if a source of dry gas is connected for operation below ambient, or if the gas cylinder has emptied.

4 Using the Display Window

This chapter describes how to navigate and change settings in the DAWN HELEOS II Display Window.

CONTENTS

4.1 Navigating the Display Panels

You navigate through the Display Panels using the buttons to the right of the Display Window.

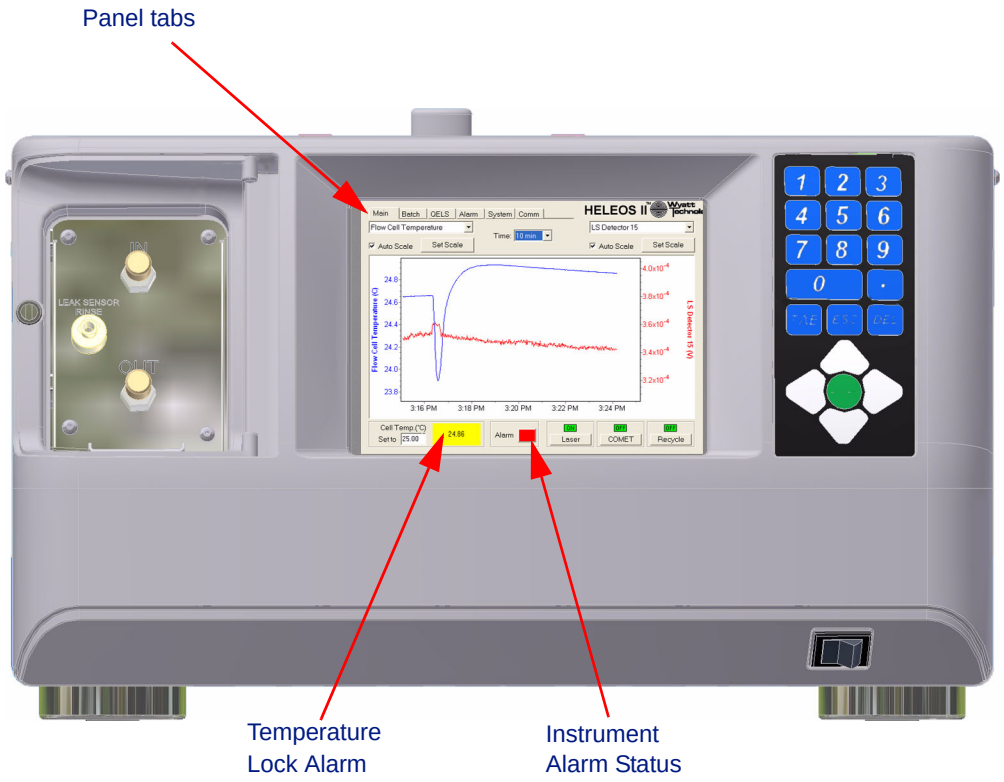


Figure 4-1: Main display panel

4.1.1 Front panel button description

Esc-left and **right arrows** navigate through the Panel tabs.

Esc and the number of the Panel tab (1 through 5) selects the first field in that Panel.

Tab cycles through various fields in the current Panel.

Esc-Tab selects the first field in the current Panel.

Enter displays the options of the selected field with the current option selected. Use the arrow keys to change the option, and then Enter to select.

If the field is a check box, **Enter** toggles the option.

Tip:	If you miss a field, press Esc and restart Tabbing through the fields.

4.2 Warning Lights and Alarms

Color	Meaning
Yellow	Not ready
Green	Ready
Red	Hazard

4.2.1 Hazards

The DAWN HELEOS II will sound an audible alarm when a potential hazard is detected. Hazards include:

- vapor or liquid leak is detected
- an over temperature condition is detected
- external alarm input is activated (signal from associated equipment)

4.2.2 Audio Alarm

Note:	Even when the audible alarm is turned off, the back panel alarm output will remain active.

To turn off the audible alarm:

- Display the Alarm panel. Tab to the Audio Alarm checkbox and press Enter to uncheck the Audio Alarm box.

To enable the audible alarm:

- Press Enter again to check the Audio Alarm box.

4.3 Main Panel

The Main panel contains the most commonly used DAWN HELEOS II functions. The display shows graphical representations of two of the data streams collected by the instrument. One data stream is displayed in red on the right axis and other is displayed in blue on the left axis.

4.3.1 Selecting Display Settings for the X, Y Axes

You can select the data channel you want displayed in each axis.

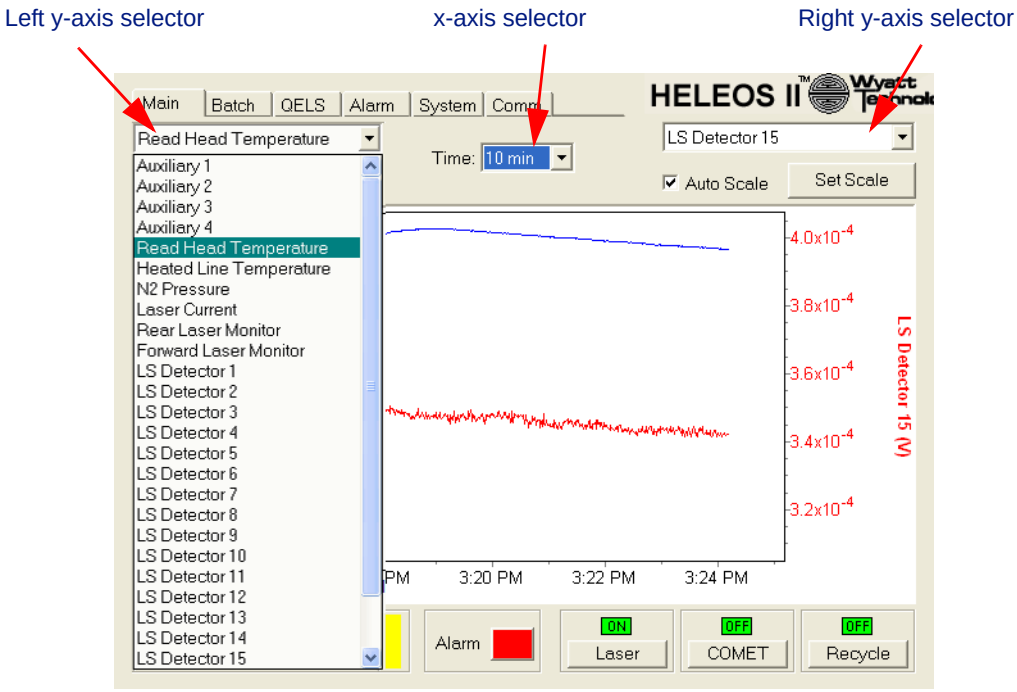


Figure 4-2: Main panel

Left and Right Y-axis Selectors

- Tab to the field and press Enter to display the data channels.
- Use the up and down arrow keys to scroll through the parameters. Press Enter to select.

The left Y-axis data channel displays in blue; the right Y-axis data channel displays in red.

X-axis Selector

The X-axis selector sets the time range from 10 minutes to 2 hours. To change the time, see the Set Time field under “System Panel” on page 4-12.

4.3.2 Adjusting the Display Range

You can adjust the range displayed in the graph in a variety of ways. This also applies to the Set Scale button in the Batch Panel.

To use the zoom and pan buttons:

1. Tab to the **Set Scale** button.

   The zoom/pan buttons are displayed.

2. Press the left arrow to zoom in.
3. Press the right arrow to zoom out.
4. Press the up arrow to pan up.
5. Press the down arrow to pan down.

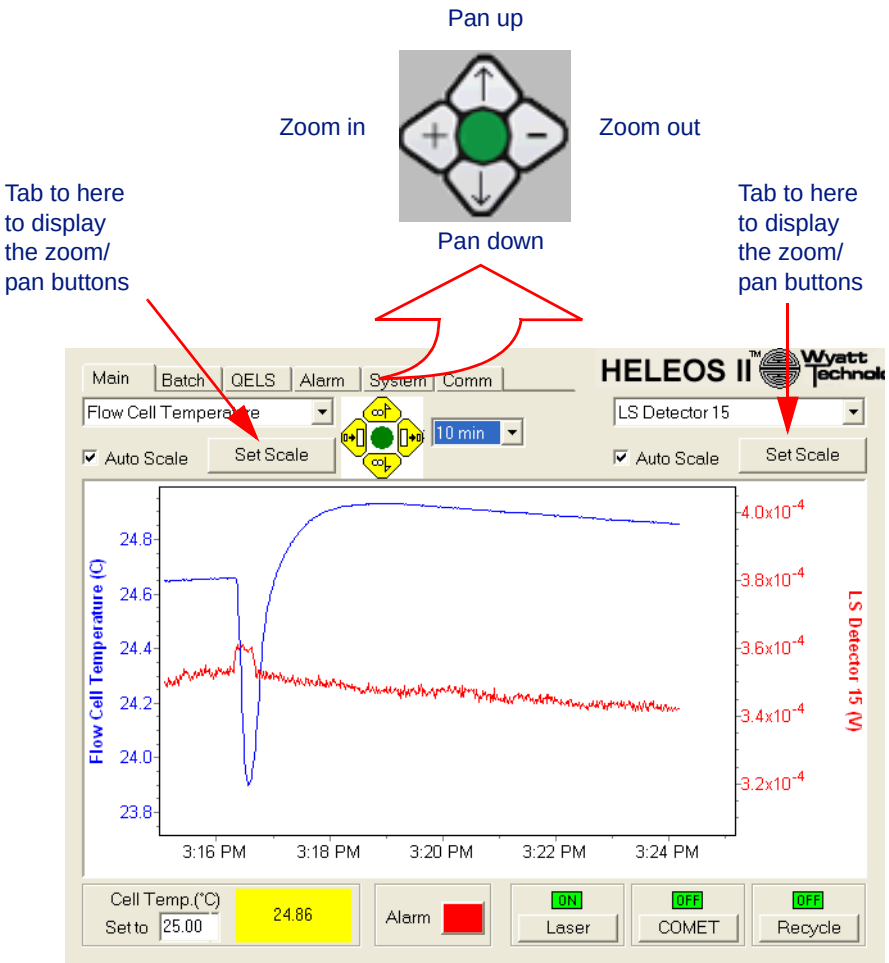


Figure 4-3: Zoom and pan buttons

To change the scale numerically:

1. Tab to the **Set Scale** button.
2. Press Enter.

   The Set Scale window is displayed.
3. To toggle positive and negative, tab to the **+/-** button and press Enter.
4. To change values, tab to the **Max** field and enter a value. Tab to the **Min** field and enter a value. Press Enter.

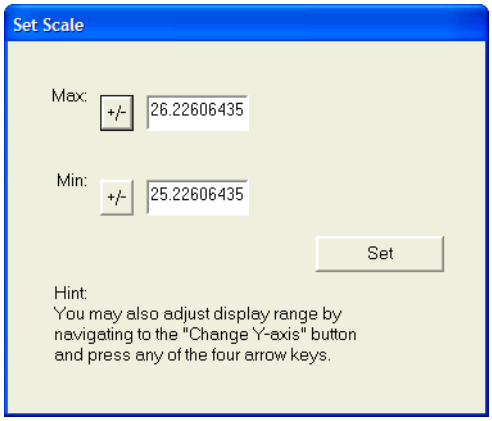


Figure 4-4: Setting the scale numerically

Autoscale

Changes the scaling so the display fills the window.

4.3.3 Setting Cell Temperature

You set the cell temperature by using the numeric keypad to enter the value. The ↑ up arrow key and ↓down arrow key toggle between positive and negative. The DAWN HELEOS II adjusts to within one-tenth of a degree of the set temperature.

4.3.4 Laser

Sets the laser to on or off. When the laser is off, the button is yellow with the word **OFF** to denote that the system is not ready to take data. When the laser is on, the button is green with the word **ON** to denote normal operation

4.3.5 Comet

Comet is an internally installed option that applies a radio frequency ultrasonic field which loosens particles that may have adhered to the cell walls and removes them on a daily basis. When used on a regular basis, the need to remove the flow cell for cleaning may be postponed indefinitely. In addition, periodically activating the device prevents new particles from adhering.

4.3.6 Recycle

On the back panel is a connector for driving a 12V solenoid valve that can be plumbed to divert the flow from recycle to waste. You can turn it on or off. A timer setting on the Systems Panel allows it to be programmed for delayed activation, see “Recycle” on page 4-13 for programming the delay.

4.4 Batch Panel

In the Batch panel, you can choose to display raw data or normalized data.

- Raw Data—This is the data gathered by the data collection procedure. For a light-scattering experiment, this is the detector voltages.
- Normalized Data—For a light-scattering experiment, this is the detector voltages multiplied by the empirically determined normalization coefficients for the particular solvent being used.

The Batch panel displays information and helps you set baseline and normalization coefficients while using prepared cuvettes of known sample concentrations. Each LS detector has a slightly different sensitivity, and views a slightly different illuminated volume. LS detectors at low and high angles look along the beam and see a larger illuminated volume, while the intermediate LS detectors look across the beam and see a smaller illuminated volume (see Figure 4-5).

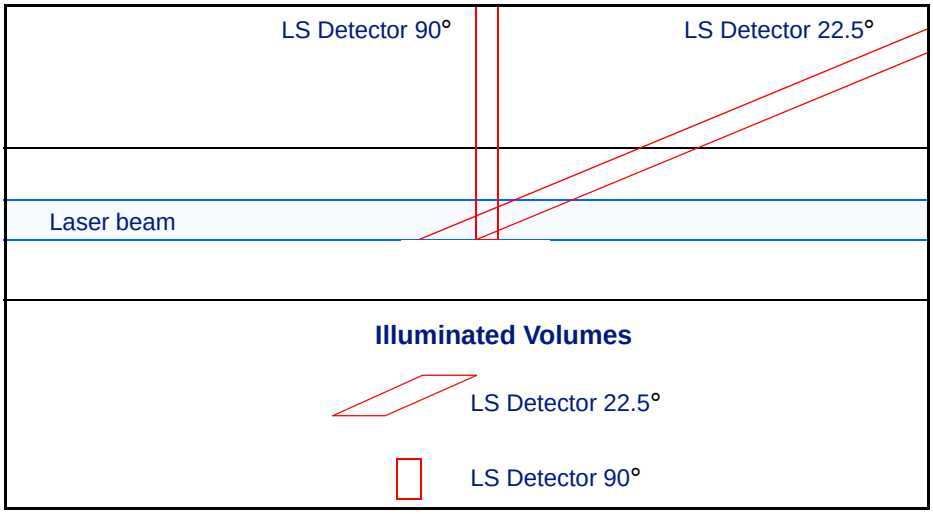


Figure 4-5: LS detector angles

In order to compensate for these inherent differences, you must set a baseline using a cuvette of pure solvent, then you normalize using a "normalization standard" (any sample, in the same solvent, which is small compared to 10nm). Small samples scatter light isotropically (the same in all directions). Normalizing in this way, allows the instrument to adjust the response of each detector so that they are all on the same scale as the 90 degree detector.

4.4.1 Setting the Baseline and Normalization Coefficients

Note: For each sample, rotate the cuvette to find a clean spot on the glass. When you have found a clean spot, the signal will be minimized.

1. **Press Esc 2**, select **Raw Data** and press **Enter**.
2. Insert a cuvette containing pure solvent.
3. Rotate the cuvette until the LS detector signal is minimized.
4. Tab to the **Set Baseline** button and press **Enter**.

   This allows the instrument to subsequently display only the additional scattering due to the sample.
5. Insert a cuvette with a normalization standard for the solvent you are using.
6. Rotate the cuvette until the LS detector signal is minimized.
7. Tab to the **Normalize** button, and press **Enter**.

Note: Use the check boxes next to the **Normalization Coefficients** to turn off (uncheck) any associated detector in the plot. When working with samples that have dust in them, the forward angles are often contaminated with extra light. Turning them off in the plot makes it easier to view the plot.

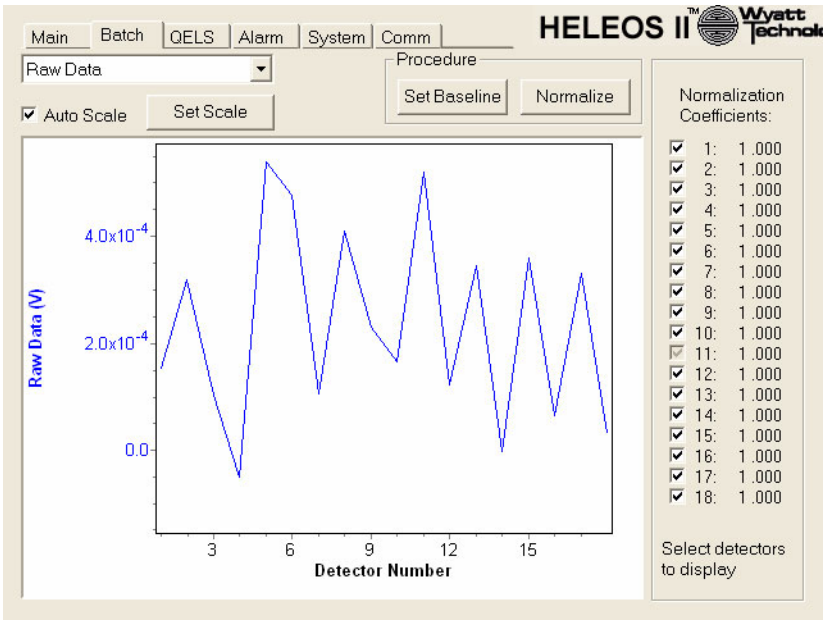


Figure 4-6: Raw data

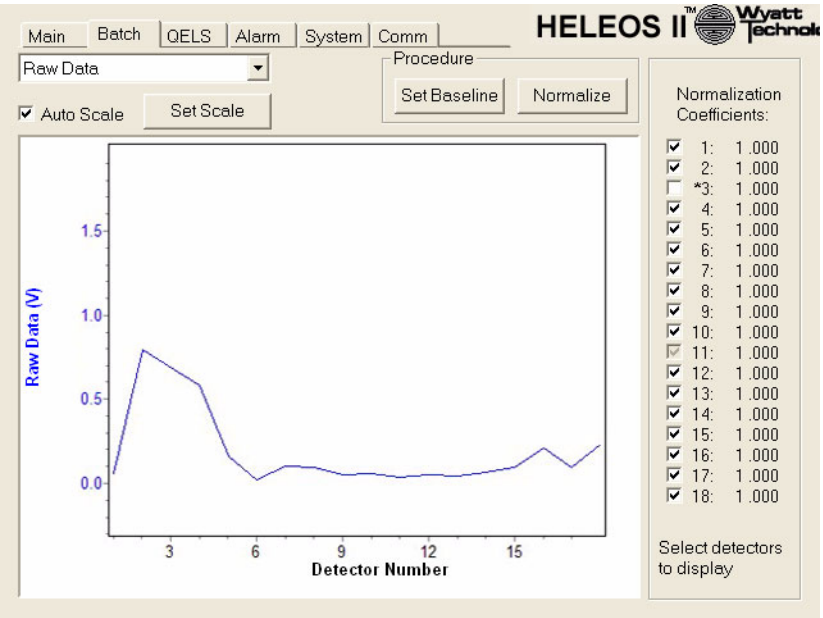


Figure 4-7: Normalized data

4.4.2 Adjusting the Display Range

Adjusting the display range is the same as on the main page. See "Adjusting the Display Range" on page 4-5 for more details.

4.5 QELS panel

For information on the QELS option, see **Appendix A, "Using QELS"**."

4.6 Alarm Panel

The Alarm panel displays sensor information and lets you adjust alarm settings. An alarm history is shown of the last few alarms and the time at which they occurred.

Some alarms are not visible for all instruments.

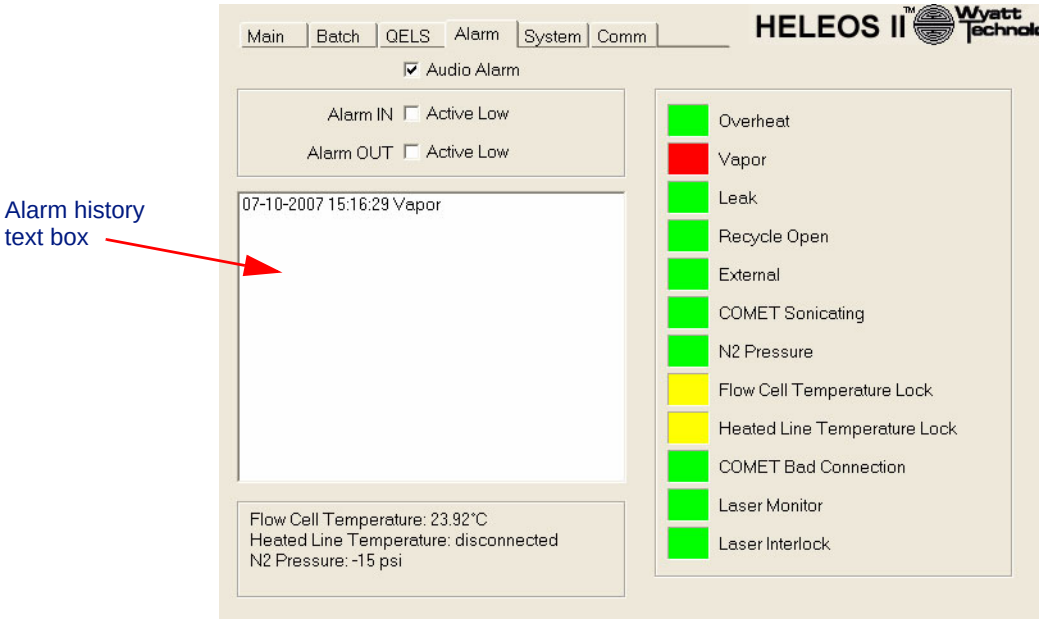


Figure 4-8: Alarm panel

4.6.1 Alarm Signal State

Select whether alarm input and output signals are active low.

Alarm IN: If you select **Active Low**, the instrument considers an Alarm In event to occur when the signal on this line transitions from 5 V to 0 V. When an Alarm In event occurs, the Alarm signal flashes on the LCD display, and an Alarm Out signal is transmitted (see Alarm Out). If you don't select **Active Low**, the instrument considers a transition on this line from 0 V to 5 V to be an Alarm In event.

Alarm OUT: If **Active Low** is selected, the instrument keeps this signal at 5 V for no alarm state, and brings the signal to 0 V in the event of an alarm state. In this context, an alarm state occurs if the internal liquid leak sensor detects liquid, or the internal vapor alarm detects organic solvent vapors, or the rear panel connector Alarm In signal is active (see Alarm In). If **Active Low** is not selected, the instrument keeps this signal at 0 V for no alarm state, and brings the signal to 5 V in the event of an alarm state.

4.6.2 Audio Alarm

To turn off, enable, or disable the audio alarm, display the Alarm Panel, Tab to the Audio Alarm field, then Enter to toggle the option.

Note:	Even when the audible alarm is turned off, the back panel alarm output will remain active.

Here is a list of the alarms and their meanings:

- **Overheat** (not in Ambient version) is triggered if the read head ever exceeds 220°C
- **Vapor**: Vapor sensor detected a leak.
- **Leak**: Liquid sensor detected a leak.
- **Recycle Open**: Back panel recycle valve input requested the recycle valve be actuated
- **External**: Back panel external alarm input is activated.
- **Comet Sonicating**: This error indicates that the COMET was activated, but the COMET electronics detected an error. This error will occur if the COMET is not installed and the COMET button is activated.
- **Flow Cell temperature lock** shows if the flow cell temperature is locked
- **Heated Line temperature lock** shows if the heated line temperature is locked
- **COMET Bad Connection**: This error indicates a problem with the transducer assembly. It can occur when the cable is disconnected, or if the transducer needs replacement.
- **Laser Monitor**: If the Laser Monitor signal differs from the Laser Power set point by more than 10% the Laser Monitor alarm will activate. The laser may have reached the end of its useful lifetime.
- **Laser Interlock**: The laser interlock switch is activated indicating that the cover is open.
- **N2 pressure** (not in Ambient version) is triggered when the temperature is set to less than 20°C, but the nitrogen pressure is less than 20psi. In this case, the alarm activates and resets the system temperature to 20.5°C. This prevents condensation from damaging the optics if the nitrogen connection is not made, or if the tank runs empty.

4.7 System Panel

The System panel contains additional options for some of the selections on the Main panel.

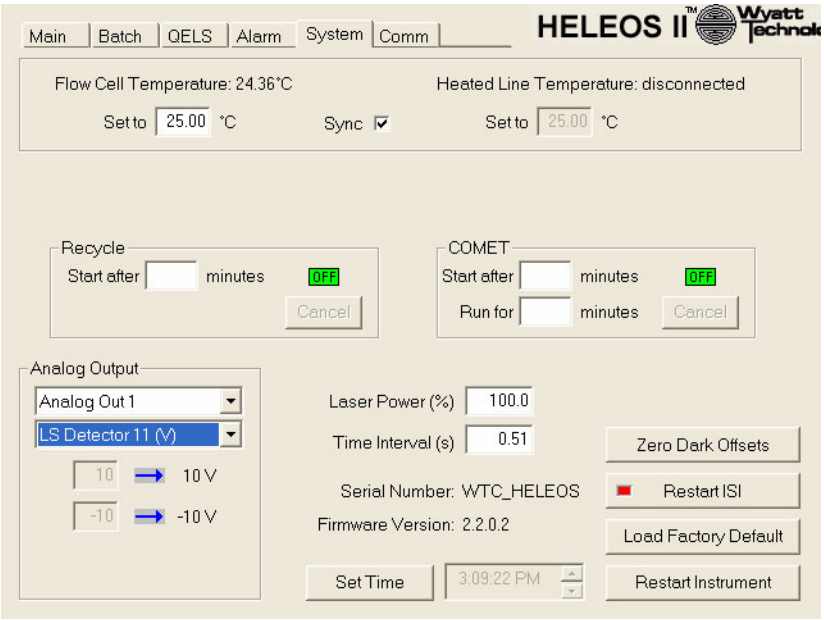


Figure 4-9: System

4.7.1 Flow Cell Temperature and Heated Line Temperature

The flow cell temperature is displayed for all instrument configurations. However, the ambient instrument configuration does not actively control the temperature and has no provisions for attaching a heated transfer line.

Flow Cell Temperature and **Heated Line Temperature** (not in Ambient version) displays the measured temperature of the flow cell for all instrument configurations and the heated lines. However, the ambient instrument does not actively control the temperature and has no provisions for attaching a heated transfer line. If you have these options, you can set these temperatures by selecting the **Set to** field, typing the desired value, then Enter.

The thermocontrollers are programmed to change the temperature at a rate of 1°C per minute to ensure that the flow cell glass does not crack due to thermal stresses. For example, if you wish to operate your system at 150°C, and your system is initially at 25°C, it will take about two hours for the temperature to reach 150°C

If you are using the Peltier Heated/Cooled model, the flow cell can be cooled or heated, but the heated lines can only be heated. Using a setpoint below ambient temperature will cool only the flow cell—it won't cool the lines.

Sync

Click the **Sync** button to synchronize the Flow Cell Temperature and Heated Line Temperature.

This means that changing the flow cell temperature automatically changes the heated line temperature to match. When Sync is unchecked, you can set the cell and heated line temperatures independently.

4.7.2 Recycle

A timer setting on the Systems Panel lets you program it for delayed activation. Enter the time delay in minutes in the **Start After** field.

4.7.3 Comet

You can set the start time and run time for the Comet option. This is useful when setting the COMET to run at the end of the day. Use the **Start after** field to provide delayed activation so the COMET activates after the last data run completes. Alternatively, you can schedule the COMET activation as part of an ASTRA V sample set (see the *ASTRA V for Windows User's Guide* for more details).

The **Run for** field sets the time for which the COMET runs once activated. Typically, you set the COMET to run for an hour or two after the last data run completes.

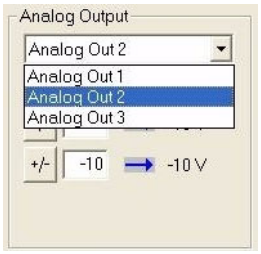
4.7.4 Set Time

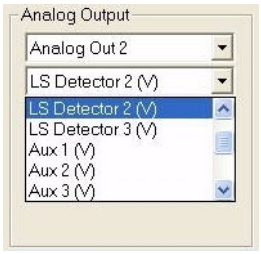
Set Time sets the time displayed in the X-axis of the graph.

4.7.5 Restart Instrument

Restart Instrument turns off the DAWN HELEOS II and turns it back on. This is typically used only when installing a firmware update.

4.7.6 Analog Outputs





The analog outputs on the back panel can be used to transmit up to three data channels to a third party instrument. Select the data channel from the drop down menu and type in the scale settings. The output is always -10 to 10 V. The two fields show which analog value will be mapped to each back panel output. If the upper field is set to 1 and the lower field is set to -1, then the output will be 10x the data channel signal. Note that if laser power control is enabled (internal jumper control), **Analog Out 3** is not available at the back panel, but is used internally for **Laser Power (%)**.

4.7.7 Laser Power (%)

You can control the laser power if this function is enabled (internal jumper control). This control is greyed out if the function is not enabled.

4.7.8 Time Interval

Time Interval specifies the samples per second. The default is 0.5 seconds.

4.7.9 Language

You can set the language of the user interface to English or other supported languages.

4.7.10 Zero Dark Offsets

The DAWN HELEOS II measures the dark offsets of the detectors, the Laser Monitor, and the Forward Monitor. It does this by turning the laser off for 10 seconds, measuring the dark offsets, and readjusting the offset for each detector so that dark measures as 0.

4.7.11 Restart ISI

Restart ISI is used to restart or reset the instrument communication in the event that a remote client, such as Astra V or the Diagnostic manager, crashes.

4.7.12 Load Factory Defaults

Load Factory Defaults is used to reset the instrument to the settings installed when the instrument was shipped.

4.8 Comm Panel

The Comm panel allows you to connect to a computer network.

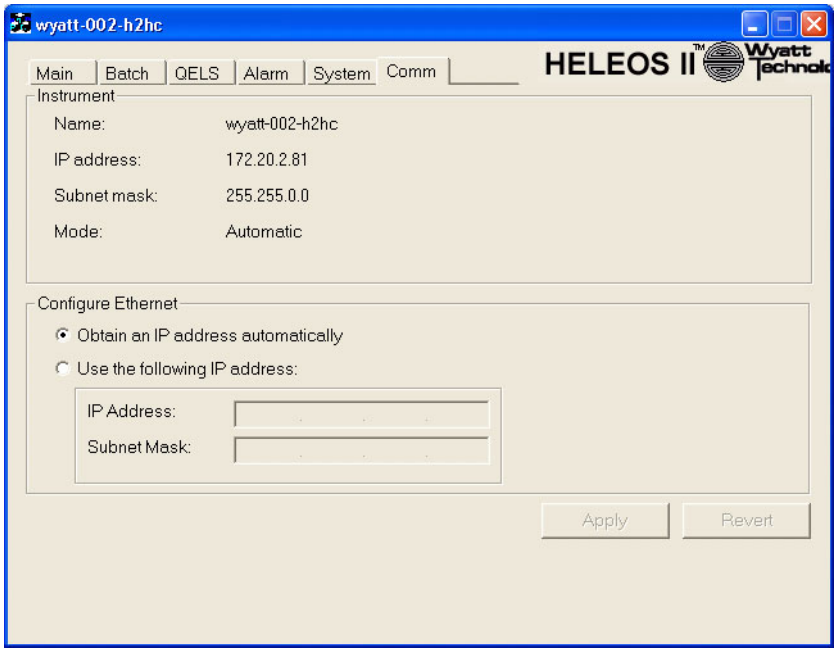


Figure 4-10: Comm

Obtain an IP address automatically - Once the instrument is connected to a computer or LAN, the IP address and subnet mask will be assigned automatically. This option requires that the network has a DHCP server. When using DHCP, it may take several minutes for the IP address to be assigned. During this time, the IP address and subnet mask will read 0.0.0.0. Once the IP address and subnet mask have been assigned, both will be automatically updated, and should no longer read 0.0.0.0. At this point, it should be possible to connect to the instrument from the computer.

Use the following IP address: - If you wish to use a static IP address and subnet mask, please contact your IT department to obtain a valid address and mask. Enter the information into the IP address and subnet mask fields.

5 DAWN HELEOS II Maintenance

The DAWN HELEOS II photometer requires little maintenance. When you remove parts for cleaning (or convert between flow and batch modes), you will find they are easy to access and disassemble. This chapter gives guidelines for keeping the instrument clean and in good working order. It also has the procedures for replacing the COMET sonicator plunger assembly and converting from the flow cell to scintillation vial batch mode measurements. Refer to the *MicroCuvette Measurement Accessory Option Guide* for the procedure for converting your DAWN HELEOS II from flow cell to MicroCuvette batch mode measurements.

CONTENTS **PAGE**

5.1 General Maintenance

For general maintenance, we suggest you do the following:

- Keep the DAWN HELEOS II on a flat, clean surface, with space behind and standing on its feet to allow proper air ventilation.
- Keep the case clean. Use a cloth dampened with water to clean it.
- Allow the instrument to warm up for 30 minutes before taking measurements.
- Keep the instrument cover on at all times with the bib installed.
- Keep the cell inlet and outlet sealed when not in use to prevent solvent evaporation or introduction of particles.
- Check the air filter every month or so. When the air filter gets dusty, pull the air filter cover off and remove the filter. Then gently clean it with warm soapy water, dry, and replace. You can also order replacement filters from www.wyatt.com

  If you are in a dusty environment, clean the filter more often than monthly. Failure to keep the air filter clean will cause the instrument to heat up and will decrease the ability of the fan to blow dust particles out of the instrument.

In addition, you will need to follow certain procedures for keeping the flow cell clean, described next.

Note:	For instructions on connecting the DAWN HELEOS II to an HPLC system, see the *ASTRA V for Windows User's Guide.*

5.2 Flow Cell Maintenance

The flow cell structure is critical to the operation of the DAWN HELEOS II. If you follow the guidelines here, you may never need to delve deeper into the instrument.

If the flow cell is not cared for properly, you will need to remove it from the read head for cleaning (described in the next section). This is a procedure that, while not complicated, can be circumvented if you can successfully clear contaminants, such as particles, from the bore of the flow cell while it is still installed.

5.2.1 On-line Cleaning

To keep the flow cell free of contaminants, we recommend regular maintenance as described here.

At All Times

- Use solvents, including water, that are HPLC grade and filtered through a 0.02 µm filter.
- If the instrument is connected to a chromatography system, keep pure, filtered solvent pumping continuously through the cell.
- If the instrument is in stand-alone mode (batch setup), store the flow cell filled with filtered solvent.
- When you do not plan to use the DAWN HELEOS II for some time, check the solvent in the cell about once a month. Add more filtered solvent as needed.

Before and After Completing Experiments

- With the flow cell still in place, disconnect the DAWN HELEOS II from your HPLC system. Inject pure, filtered (0.02 µm) solvent to flush the cell. We recommend that filtered ethanol or isopropanol be left in the cell.
- *Do not* flush the cell from Outlet to Inlet. Backflushing the cell can cause particles to become lodged in the inlet tube, which has a smaller inside diameter than the outlet tube.
- A mild detergent solution may also help clean the flow cell, and may be kept in it overnight when the instrument is not in use, then purged in the morning.
- There are two extra sets of inlet and outlet tubes in your hardware kit. One set consists of 4 pieces of color-code tubing (white for inlet and blue for outlet). This set of tubes is for use with the unions to make it easy to remove the flow cell for cleaning without breaking the seal at the manifolds. The second set of color-code tubes is for use without the unions.

With either set, you will need to bend the tubes in order to install them in the instrument. The bend radius should not be less than the bend radius of tubing that comes installed in your DAWN HELEOS II. To avoid introducing particles into the flow cell, flush the tubes after bending them and before installation.

COMET Option

We also recommend the use of the Wyatt COMET option. The COMET is a permanently installed ultrasonic flow cell cleaning system which operates on a different principle than traditional immersion bath-based cleaners. Traditional ultrasonic cleaners operate around 50 kHz and clean by creating cavitation bubbles in the solvent which scrub surfaces when they collapse. This can damage the fine polish on the optical surfaces of the flow cell. It is not recommended that you clean either the flow cell, or the windows in traditional ultrasonic baths.

The COMET, by contrast, operates between 600-900kHz and avoids cavitation completely. It works by creating resonate sound waves in the flow cell bore. These sound waves help suspend dirt in the solution which is then flushed out by the flowing mobile phase. Since it is permanently installed, you can activate it as needed. Many customers operate it every night as part of a standard cleaning regimen. Others schedule COMET activation between runs in an autosampler collection. It is intended to be operated while the mobile phase is flowing through the flow cell. You can also use it in conjunction with detergents for more effective cleaning.

Protease Cocktail

Some users have found that a simple protease “cocktail” rinse is effective in removing protein deposits from glass flow cell surfaces. You might be able to use this rinsing treatment rather than disassembling the flow cell:

Ingredients for 3 ml of protease cleaning solution:

All enzymes are sequencing grade preparations from either Boerhringer Manheim or Roche.

- Trypsin, modified—25 µg, lyophilized
- Chymotrypsin—25 µg, lyophilized
- Pepsin—25 µg, lyophilized

Note:	You might be able to get away with just pepsin alone, as it's so non-specific.

Procedure:

1. Reconstitute each with 1 mL of PBS (25 mM Na phosphate / 150 mM NaCl, pH 7.25
2. Mix the three solutions and vortex, load syringe fitted with 0.02 µm filter for LS detector
3. Flush detector with 20mL pure water, then infuse ~ 1 mL of cocktail via syringe pump
4. Stop flow, turn on Comet (if you have one) and leave it for a few hours or overnight
5. The following morning, remove syringe, flush with fresh 20 mL of water, then mobile phase

5.2.2 Particles in the Cell

Here is a list of symptoms of particles in the cell and what you can do to dislodge them.

Some Symptoms of Particles in the Cell

- Bright stationary spots when viewing the cell bore from above.
- An increase in baseline voltage at all angles.
- Unstable, fluctuating baselines.
- Distorted chromatography peaks (dips below baseline, shoulders on low angle peaks).

Some Suggestions for how to Dislodge Particles

- Change to a solvent with a different polarity.
- Try injecting a small air bubble. If the particle(s) move, repeat until they are flushed out.
- Flush the cell with 0.02 µm filtered HPLC grade water. Fill a syringe with a few mL of 6 M nitric acid, inject and leave the acid in the cell for 10 minutes, then flush with 0.02 µm filtered HPLC grade water again.

5.3 Cleaning the Flow Cell and Windows

When the flow cell is dirty, light scatters excessively, which shows up as high voltage, unstable baselines, and distorted chromatography peaks. The flow cell cleaning procedure can be broken down into five major steps:

Step 1—Removing the flow cell

Step 2—Disassembling the flow cell

Step 3—Cleaning the flow cell and windows

Step 4—Reassembling the flow cell

Step 5—Reinstalling the flow cell

What you will need for flow cell cleaning:

- A sheet of clean white paper taped down to your work surface
- Anti-static wrist strap
- Ball drivers: 1.5 mm and 2.5 mm
- Aperture installation tool (WTC #119033)
- Two ¼" Crescent wrenches for disconnecting the in-line unions
- Lens tissue. Fold several pieces in finger-width strips for handling the cell and cleaning.
- Lint-free gloves
- Oral-B SuperFloss
- Inert dusting gas. (Photographic supply stores carry this. At Wyatt Technology we use "Tech Spray" from Com-Kyl distributors in Santa Barbara, (805) 520-1731.)
- Filtered methanol, ethanol, or isopropanol
- Tweezers
- Optional: Sonicating bath (to clean stainless steel flow cell manifolds)
- Optional: UV light

Caution: The flow cell you are about to remove constitutes a substantial amount of the purchase price of the DAWN HELEOS II. Its parts are carefully machined and are expensive. If you have any doubts whatsoever about the safest procedure for handling the cell structure, do not hesitate to call Wyatt Technology.

We also offer a flow cell cleaning service for those who do not wish to clean the cell themselves. Contact Wyatt customer service for details.

5.3.1 Step 1—Removing the Flow Cell Assembly

In this first step you will remove the cell assembly from the read head.

1. Turn off the system power to the DAWN HELEOS II.
2. Put on the anti-static wrist strap.

   This is an important step. The strap keeps the flow cell glass and windows from building up a static charge and attracting particles while being handled.
3. Slide open the batch door to expose the read head.

If the COMET option is Installed, see Figure 5-1.

4. Disconnect the COMET coax cable.
5. Using the 2.5 mm Ball driver, remove the four M3 hex-head screws securing the COMET assembly then slide the assembly away and out of the instrument.

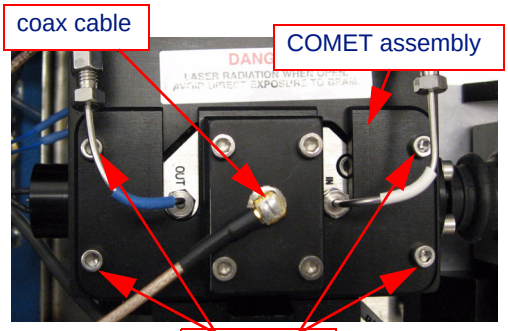


Figure 5-1: Flow Cell with COMET Option

If the COMET option is not installed, see Figure 5-2.

6. Using the 2.5 mm Ball driver, remove the four M3 hex-head screws and lift off the read head cover plates.

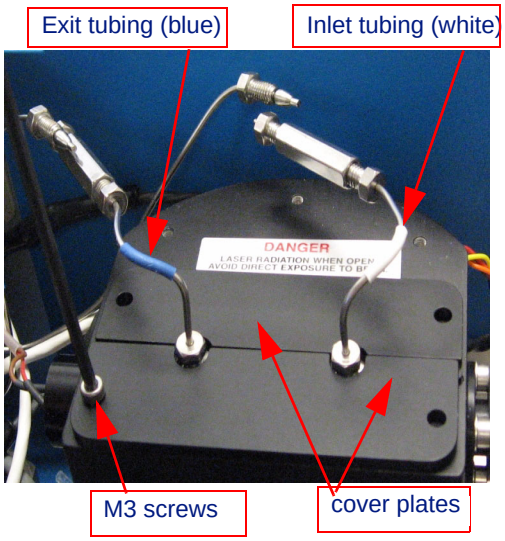


Figure 5-2: Flow Cell without COMET Option

The flow cell assembly is now visible.

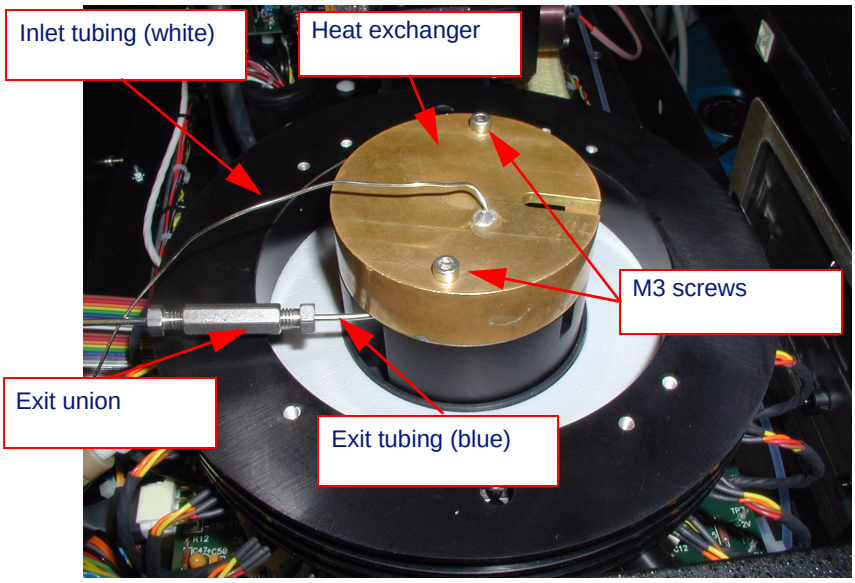


Figure 5-3: Flow cell assembly after insulating cover has been removed

7. Remove two M3 screws.

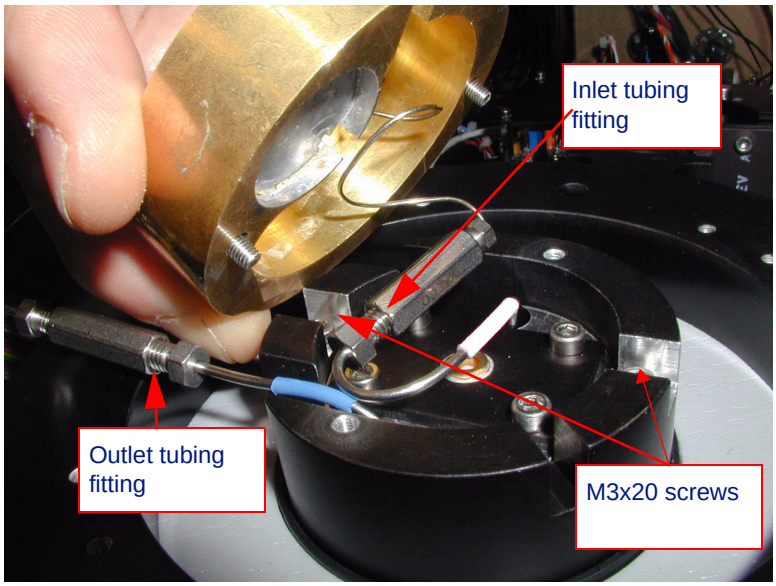


Figure 5-4: Removing the heat exchanger

8. Using the 1/4" crescent wrenches, remove the inlet tubing and outlet tubing at the fittings as shown in Figure 5-2. The flow cell does not need to be unplumbed at this point if you are simply going to inspect the cell for dirt.

9. gently tilt the heat exchanger so that it can be placed aside, or if unplumbed in the previous step, remove the heat exchanger completely. Slide the COMET away to the right.

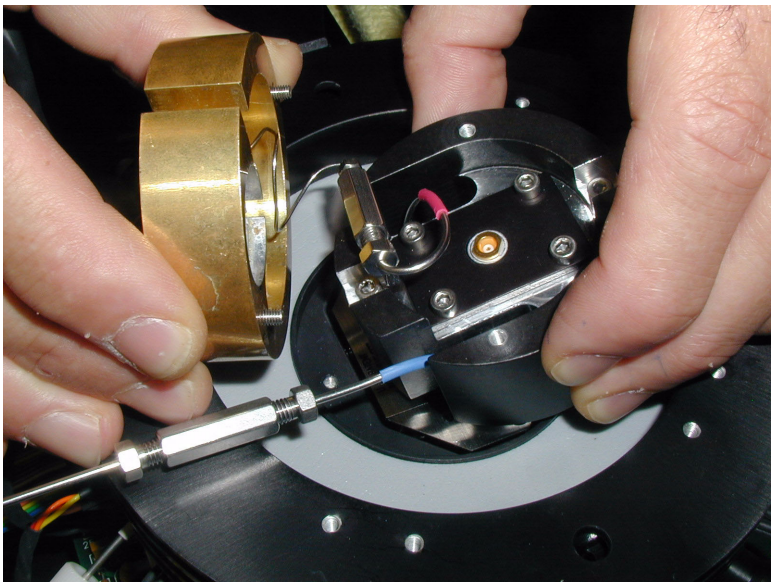


Figure 5-5: Removing the COMET assembly

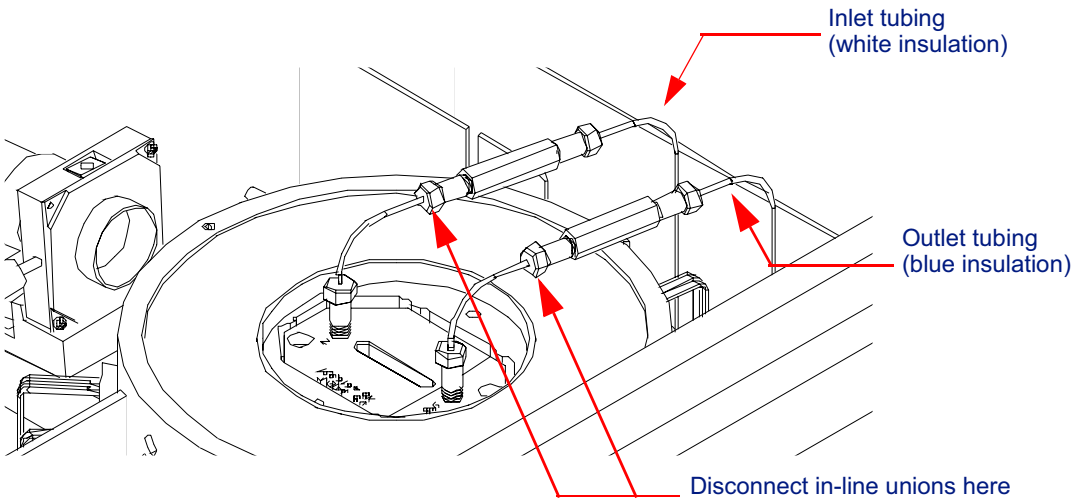


Figure 5-6: Flow cell tubing and unions

10. Use the 2.5 mm Ball driver to remove the two M3 screws, then lift the cell assembly up and out of the read head using the tubing. See Figure 5-7.

IMPORTANT: DO NOT PRY THE CELL OUT WITH A SCREW DRIVER OR ANY OTHER TOOL!

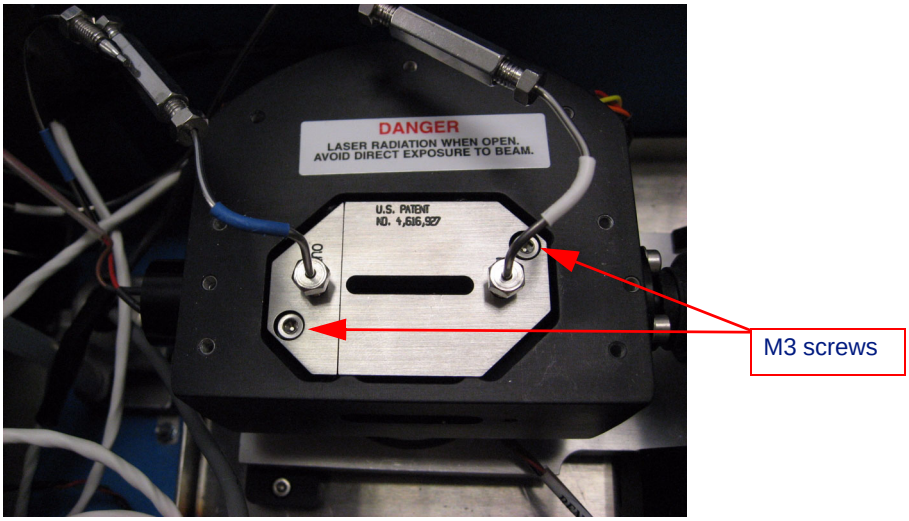


Figure 5-7: Flow Cell

11. Remove the short stainless steel tubing from the manifolds before proceeding with the disassembly and cleaning. The inlet tube has white insulation and an interior diameter of 0.005". The outlet tube has blue insulation and an interior diameter of 0.010".

5.3.2 Step 2—Disassembling the Flow Cell

The different parts that make up the flow cell assembly are shown in Figure 5-8.

1. Separate the stainless steel manifolds from the flow cell:

a. Use the 1.5 mm Ball driver to unscrew the two M2 screws holding the bottom cell retainer in place. Remove the bottom cell retainer taking care not to lose the two tiny screws and the bottom retainer O-ring and cord.

b. Use the 2.5 mm Ball driver to remove the M3 screws that connect the two manifolds.

c. Gently pull apart the manifolds, taking care not to drop the glass cell or touch its curved optical surfaces.

d. Place everything on your paper-covered work surface, taking care not to lose the O-rings sealing the manifolds to the cell.

There is a backing ring outside each 6 mm flow cell O-ring. If the DAWN HELEOS II is configured for use at or above 80°C, there is a 9 mm flow cell O-ring (but no backing ring) on each side.

2. Use the Aperture Installation tool (WTC #119033, shown at the right) to remove one window retainer at a time.

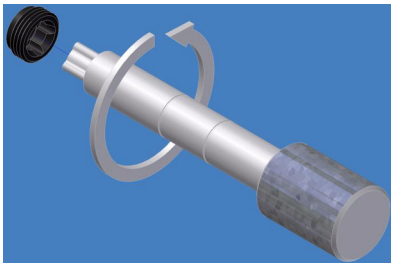


   Figure 5-8 #9 illustrates the window-mount and how it is housed in the manifold.

3. Lightly tap the assembly ONCE against a flat clean surface. The cell window and O-ring should fall out if the cell is dry.

   If the window does not fall out easily, you could carefully apply a very mild burst of pressurized air to dislodge it or you could try gently pushing it out from the opposite side with a small piece of Teflon tubing. If necessary, put some filtered alcohol in all the manifold openings and soak overnight.

4. Repeat Step 2 and Step 3 for the other window.

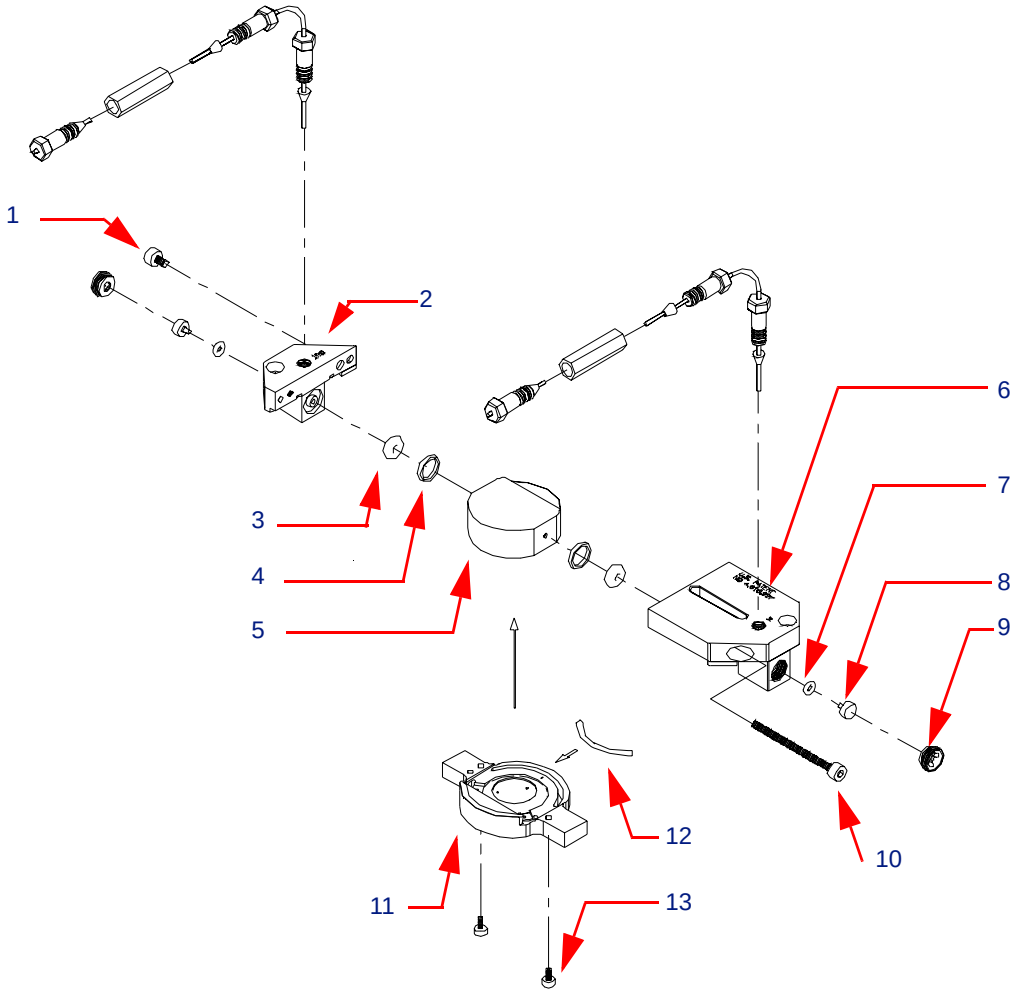


Figure 5-8: Flow cell assembly, exploded view

Table 5-1: Flow cell assembly, parts list

Item	P/N	Description
1	S5002-3004	M3 screw
2	200694	Manifold, out
3	P6504-2006	Flow cell O-ring (2) (P6504-2009 if DAWN HELEOS II is configured for use above 80°C)
4	200609	Backing ring (2) (not used if DAWN HELEOS II is configured for use above 80°C)
5	212095	Flow cell
6	200690	Manifold, in
7	P6504-2004	Window O-ring (2)
8	116007	Flow cell window (2)
9	212073	Window retainer (2)
10	S5002-3030	M3 screw
11	211048	Bottom flow cell retainer
12	S6501	Bottom retainer O-ring cord
13	S5002-2006	M2 screw (2)

5.3.3 Step 3—Cleaning the Flow Cell and Windows

From here on, you must be fastidious in your handling and cleaning of the flow cell parts. The smallest particle on the flow cell window or inside the bore can introduce stray light and distort your measurements.

1. Clean your hands thoroughly or wear clean lint-free gloves.

   When you disassemble the cell, be careful not to handle the glass cell's curved optical surfaces (the sides).

2. Clean the cell through-bore.

   a. Cut a ½" strip of lens tissue and roll it into a thin wick. Or, you may use "Oral-B SuperFloss", which is available in most pharmacies. The floss is a better tool, as it cannot leave any fibers behind.

   b. Insert the wick all the way through the cell bore, then moisten it with a small amount of filtered alcohol.

   c. While the wick is in the cell bore, untwist it slightly, move it back and forth to clean the cell, then pull it out.

   d. Immediately flush the bore with a stream of alcohol for 10–15 seconds.

      The alcohol stream flushes out any fibers that may have been left behind by the tissue wick.

   e. Blow out the alcohol for 10–15 seconds with inert dusting gas or let the glass bore drain in a vertical position.

   f. Examine the bore with a magnifying loupe.

      Look through the bore, focusing on the bore exit. Repeat from the opposite side. (See the **Note** at the end of this section.)

3. Clean the outside of the cell. (See Figure 5-9.)

   a. Pick up the cell with a folded lens tissue; touch only the flat surfaces.

   b. Wipe the curved optical surfaces with another folded lens tissue moistened with alcohol.

   c. If needed, wipe the alcohol off the optical surfaces with dry lens tissue. **Do not** repetitively rub the surfaces since this creates static electricity which attracts particles.

   d. Using a magnifying loupe, examine the optical surfaces for any dust. (See the **Note** at the end of this section.)

   e. Also, check the bottom and top surfaces for dust and finger marks.

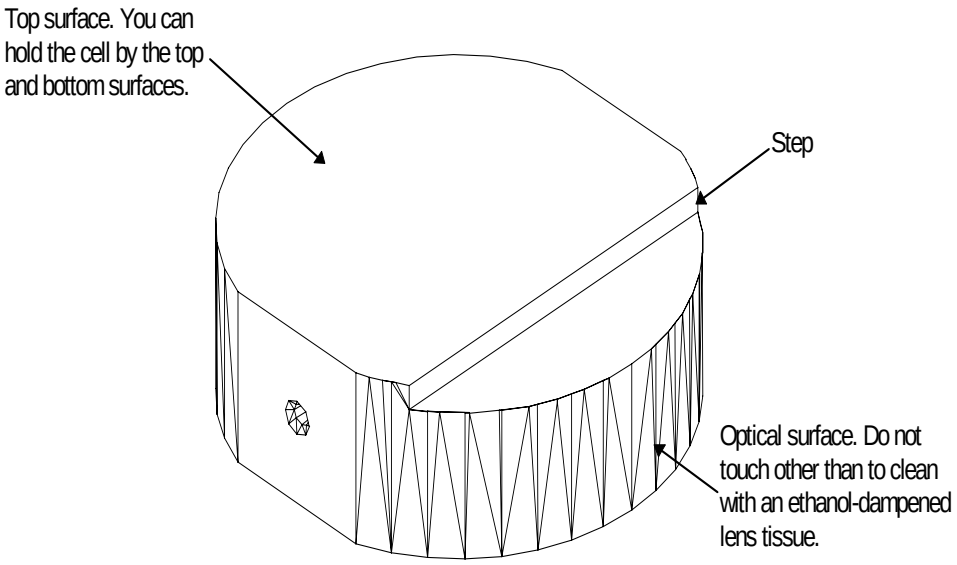


Figure 5-9: Flow cell

4. Clean the window faces.

This is the most important step in cell cleaning. Even the smallest particle left on the window faces will induce stray light and cause signal distortion, especially at low angles.

a. Take a folded lens tissue moistened with a couple of drops of alcohol and hold it against the side of your forefinger with your thumb and third finger.

b. Pick up the window with the tweezers. Hold the window by the sides, not by the window faces.

c. Smoothly wipe both window faces across the tissue.

d. Carefully examine both ends of the cleaned window for any particles.

With the loupe look straight through the window from end-to-end. (See the **Note** at the end of this section.)

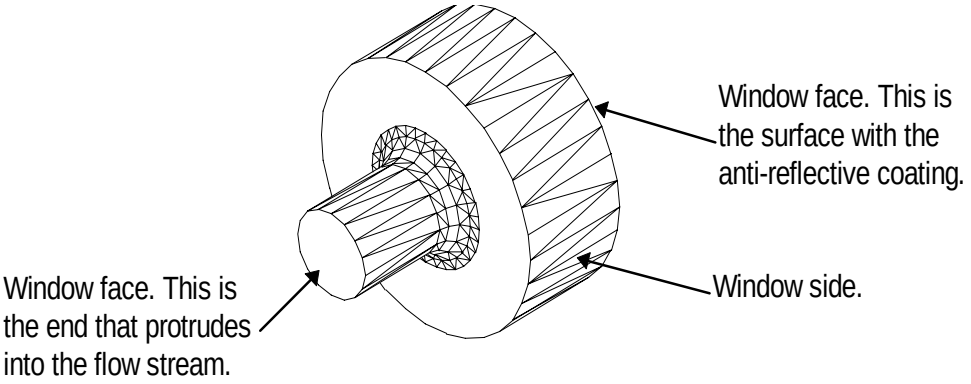


Figure 5-10: Cell window

Note: By examining the flow cell through-bore and the windows using a bright light you can, with some practice, easily find where any residue has accumulated. Examine them with a jeweler's loupe while back-lighting the glass at a slight angle. The area next to the light should be dark to provide good contrast. The bright light will illuminate any particles on the glass which, when viewed against the dark background, will show up clearly. If you have a UV light, you may shine it on surfaces at a slight angle to make certain types of dust particles, especially clothing fibers, more visible.

Since fingerprints on the glass cell circumference will alter the light scattering characteristics of a sample significantly, we urge you to use great care when handling the cell. Its role is vital in the measurement process and you must be certain to wipe it clean with high quality lens tissue before inserting it again in the cell assembly.

5.3.4 Step 4—Reassembling the Flow Cell

As you reassemble the flow cell you will clean the washers and O-rings.

Note:	Assemble the flow cell in a laminar flow hood if there is one available.

1. Carefully replace the windows with their O-rings, washers and retainers so that the windows are not chipped or over-tightened. (See Figure 5-12.)
   - **a.** Holding the window O-ring with the tweezers, put a drop of alcohol on it, then dry with a burst of pressurized air. Check for particles with the loupe.
   - **b.** Insert the O-ring into the manifold.
   - **c.** Lift the window with the tweezers. (Pick up the window near its back edge as shown in Figure 5-11.)

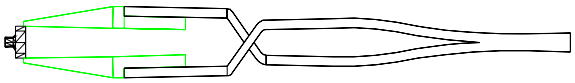


Figure 5-11: Holding the cell window for reinsertion into the manifold

   - **d.** Holding the manifold and window square with one another, gently push the window into the O-ring.
   - **e.** Let go of the window with the tweezers, pivoting them before you lift them out of the manifold.

     The fit is tight enough that you could dislodge the window if you were to just lift the tweezers straight up.
   - **f.** Inspect the tip of the Aperture Installation tool (WTC #119033) with the loupe for any particles, and, if necessary, clean with an alcohol-moistened lens tissue before proceeding.
   - **g.** Clean the window retainer with alcohol and pressurized air, then place it in the manifold and tighten with the Aperture Installation tool.
   - **h.** Inspect the window mount with the loupe.

     If any particles appear on the window, you need to remove it and its seals and clean again.
   - **i.** Repeat steps 1a) through 1h) for the second window.

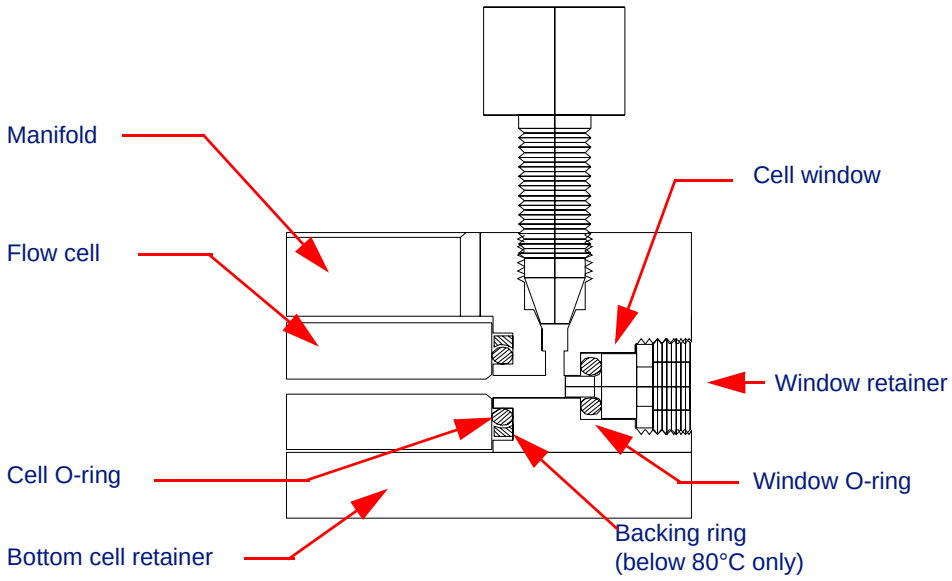


Figure 5-12: Window mount detail

2. Install the cell in the manifolds.
   a. Insert the cell O-rings followed by the backing rings if they were removed in Step 2 on page 10.

      If you have an ambient DAWN HELEOS II, and will only be operating below 80°C, install both the 6 mm O-rings and the backing rings.

      If you will be operating at or above 80°C, use only the 9 mm O-rings and do **NOT** use the backing rings. The cell O-rings need room to expand when heated above this temperature. Using backing rings at high temperatures could cause the glass to crack.

   b. Holding the cell with lens tissue, place it in the inlet manifold (the larger manifold).

      A step is machined into the top surface of the glass cell; the manifold has two pins to help align the cell properly.

   c. Push the cell step against the manifold pins.

   d. Make sure that the glass step and manifold pins are matched up well.

   e. Place the outlet manifold next to the inlet manifold and push them firmly together.

3. Insert the short M3 screw into the outlet manifold and tighten with the 2.5 mm Ball driver. Then, insert the long M3 screw into the inlet manifold and tighten.

4. View the O-rings through the bottom glass surface (make sure the surface is clean) and confirm that the bore is centered in each O-ring.

Also examine the alignment pins to make sure they touch the cell glass on each side.

5. Inspect the sides of the cell and apply a burst of air if you see any particles.
6. Replace the bottom cell retainer cord and O-ring and attach the bottom cell retainer to the manifolds using the 1.5 mm Ball driver.
7. Clean the fittings that will connect the inlet and outlet tubes to the unions. Use the same method for cleaning as you did for the window O-rings—a couple of drops of alcohol on lens tissue, then dry with a burst of air.
8. Reinstall the blue and white coated tubing in the correct holes. The inlet tubing has white insulation and an interior diameter of 0.005". The outlet tubing has blue insulation and an interior diameter of 0.010". Do not reverse the inlet and outlet tubing.

5.3.5 Step 5—Reinstalling the Flow Cell (Ambient Version)

Important The following steps apply to the DAWN HELEOS II Ambient version only. See “Step 5—Reinstalling the Flow Cell (Heated/Cooled Version)” on page 5-21 for the procedure for the DAWN HELEOS II Heated/Cooled version.

Note: If you are not careful, the cell could be reversed:
Make sure that the INlet manifold is in the rear position and the OUTlet manifold is in the forward position.

1. Replace the cell assembly in the read head, insert the two M3X10 screws and tighten with the 2.5 mm Ball driver. Loosely tighten both screws, and then alternately tighten them evenly until they are secure.
2. Reconnect the short pieces of stainless steel tubing to the in-line unions using the two ¼" Crescent wrenches.
3. Plug in the power cord and turn on the DAWN HELEOS II.
4. Connect the cell to your HPLC system and make sure the cell does not leak.

   Make certain the fittings are tight and leak free. Whenever you pump solvent through the cell, check the fittings at least twice during the first hour. Use a piece of tissue and touch the top of the fitting where the tubing emerges; no solvent should be visible on the tissue.

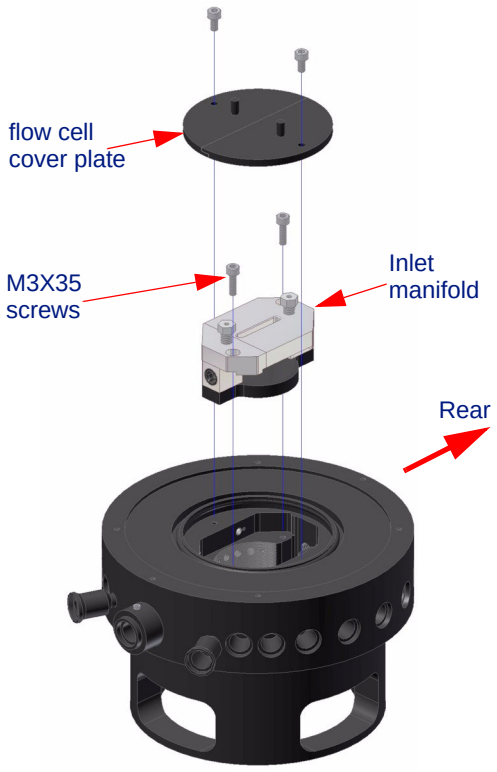


Figure 5-13: Ambient Flow Cell Installation

5. Replace both flow cell cover plates. Tighten the four Allen-head screws with the 2.5 mm Ball driver.
6. Replace the instrument cover bib.

5.3.6 Step 5—Reinstalling the Flow Cell (Heated/Cooled Version)

Important The following steps apply to the DAWN HELEOS II heated/cooled version only. See "Step 5—Reinstalling the Flow Cell (Ambient Version)" on page 5-20 for the procedure for the ambient version.

Note: If you are not careful, the cell could be reversed:
Make sure that the INlet manifold is in the rear position
and the OUTlet manifold is in the forward position.

1. Replace the cell assembly in the read head, insert the two M3 screws and tighten with the 2.5 mm Ball driver. Loosely tighten both screws, and then alternately tighten them evenly until they are secure.
2. Reconnect the blue exit tubing with two 1/4" crescent wrenches.
3. Connect the white inlet tubing to the adapter union inside the heat exchanger. (See Figure 5-5, above). Then slide either the COMET assembly or the spacer between the heat exchanger and the read head and bolt it into place with 2 M3x20 screws using the 2.5mm hex driver.
4. Connect to the HPLC system and check for leaks.
5. Bolt the heat exchanger onto the top of the COMET, making sure that all of the tubing is contained within the heat exchanger cavity and it is not pinched between the heat exchanger and the COMET.
6. Replace the insulating cover with two M3x8 screws.
7. Replace the instrument cover.

5.4 Replacing the COMET Sonicator Plunger

COMET Bad Connection: This error indicates a problem with the transducer (sonicator plunger assembly). It can occur when the cable is disconnected, or if the transducer needs replacement. After checking all COMET connections you may want to order a replacement sonicator plunger assembly (WTC #110025). The expected lifetime of this part is about two years. Refer to "Technical Support" on page 1-5 for information on how you can contact Wyatt Technology Corporation.

What you need to replace the sonicator plunger assembly:

- sonicator plunger assembly WTC #110025
- 2.5 mm Ball driver
- Lint-free gloves

1. Turn off the system power to the DAWN HELEOS II.
2. Slide open the batch door to expose the read head.
3. Disconnect the COMET coax cable.
4. Using the 2.5 mm Ball driver, remove the four M3 hex-head screws securing the COMET assembly then slide the COMET sonicator assembly away and out of the instrument.
5. Move to your workbench and place the assembly on it's side being careful of the protruding plunger blade.

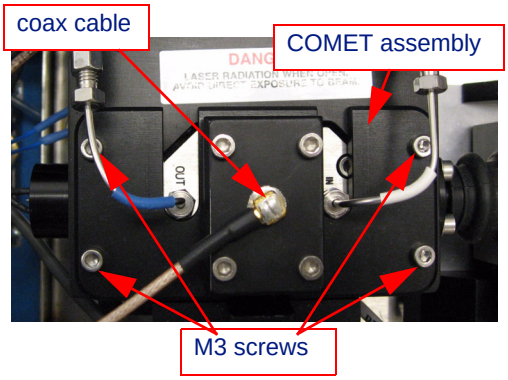


Figure 5-14: Flow Cell with COMET Option

6. Wearing your lint-free gloves, disassemble the sonicator by first removing the four M3X8 screws.
7. Carefully remove the cover assembly which has several soldered parts.

8. Remove the old plunger assembly and replace with sonicator plunger assembly WTC #110025, reinstalling the stack as shown in Figure 5-15.
9. Install the cover assembly and tighten the four M3X8 screws.
10. To install the sonicator assembly into your read head, first turn the protruding plunger blade so that it will fit into the viewing window of the flow cell.
11. Slide the COMET sonicator assembly under the flow lines and insert the plunger blade in the viewing window.

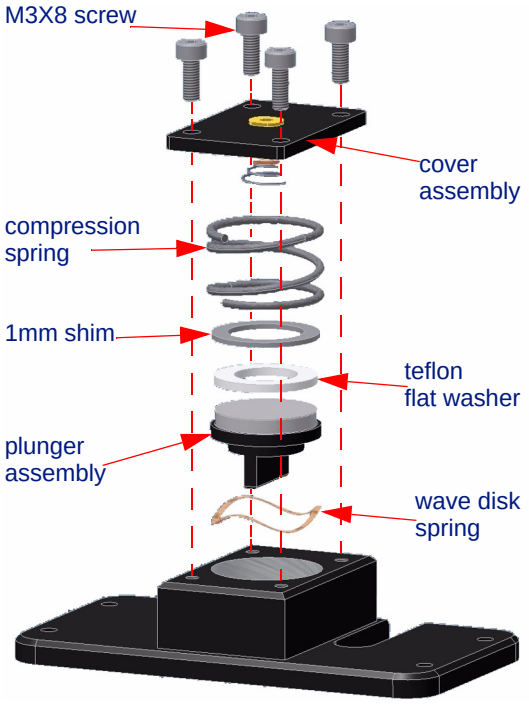


Figure 5-15: COMET Sonicator Assembly

12. Using the 2.5 mm Ball driver, install the four M3 hex-head screws securing the COMET assembly. Refer back to Figure 5-14.
13. Connect the COMET coax cable.
14.

5.5 Flow-to-Batch Conversion

You can easily alter the DAWN HELEOS II read head to take measurements from a 20 mL scintillation vial. The conversion procedure takes a minute or two.

The batch configuration permits a variety of uses that would not be possible with the flow configuration. Among these are the ability to store and analyze sealed samples, and to perform long term time-dependent studies, bioassays, depolarization and aggregation studies. As well, if you believe the sample may contaminate the flow cell it can be measured in a disposable vial.

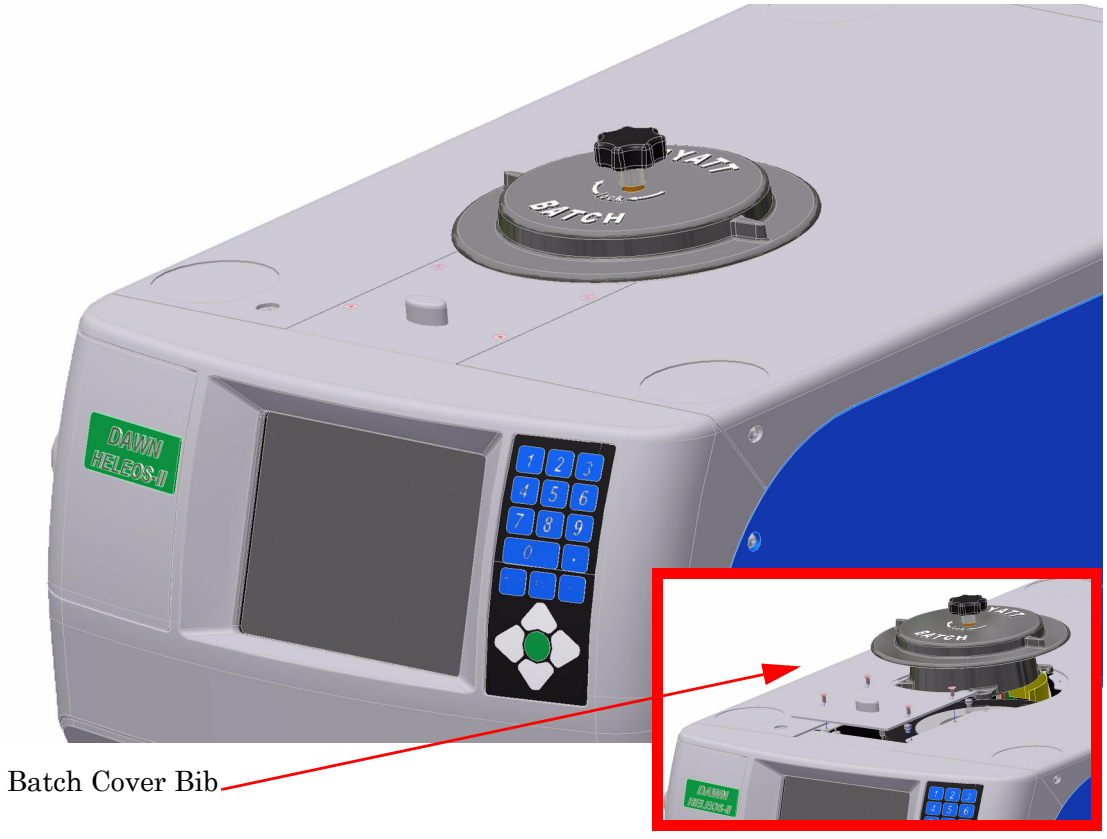
Because sample measurements can be repeated easily and rapidly, the batch method is often the fastest for determining molecular weight and radius of gyration from a static sample.

What you need for flow-to-batch conversion:

- 2.5 mm Ball driver
- Two ¼" Crescent wrenches for disconnecting the in-line unions
- Ambient Batch Conversion Kit.

To convert from flow-to-batch operation, do the following:

1. Remove the bib from the top cover of the instrument.



2. Remove the read head cover and the flow cell assembly from the instrument.

   If you need instructions, follow Step 1 of flow cell cleaning, described in "Cleaning the Flow Cell and Windows" on page 5-6 in this chapter).

3. Insert the batch spacer plate into the bottom of the read head cavity and secure it with the two M3x12 screws.
4. Insert the batch manifold and secure it with the two M3x12 screws.
5. Insert the batch vial spacer and secure it with the two M3x12 screws.
6. Put a sample scintillation vial in the batch manifold cavity.

Note: The vial should fit snugly in its mount but still be able to rotate. If it does not, slightly loosen or tighten the ball plungers as required.

7. Install 4 shoulder bolts into the batch spacer plate.
8. Install the insulating cover onto the read head by pressing it in place and rotating it until it engages the shoulder bolts.
9. Replace the instrument cover bib with the batch cover bib.

Figure 5-16: Flow-to-batch conversion kit, exploded

To replace the flow cell, reverse the previous process.

Note: When making measurements with scintillation vials, take great care to keep the outside of the vials clean and free of fingerprints, scratches, etc., as this can severely distort the measurement. We also advise you rotate the vial in the read head to find the position where the laser beam enters the cell with the minimum amount of scattering at the air/glass interface.

The batch cover includes a mechanism to rotate the scintillation vial in place until the signal is minimized. Simply press down on the knob on the top of the batch cover and turn to the right, while monitoring the results on the Batch page of the Display window. The *ASTRA V for Windows User's Guide* has additional information.

A Using QELS

QELS (Quasi-elastic or dynamic light scattering) is an internally installed option that measures time-dependent fluctuations in the scattered light signal using a fast photon counter. QELS measurements can determine the hydrodynamic radius of macromolecules or particles.

CONTENTS

A.1 QELS Panel

This section describes the QELS Panel in the DAWN HELEOS II display window.

A.1.1 Count Rate

The Count Rate contains the raw signals for each of the light-scattering detectors and the photon count rate for the QELS detector.

Time

The Time field sets the time range of the X-axis.

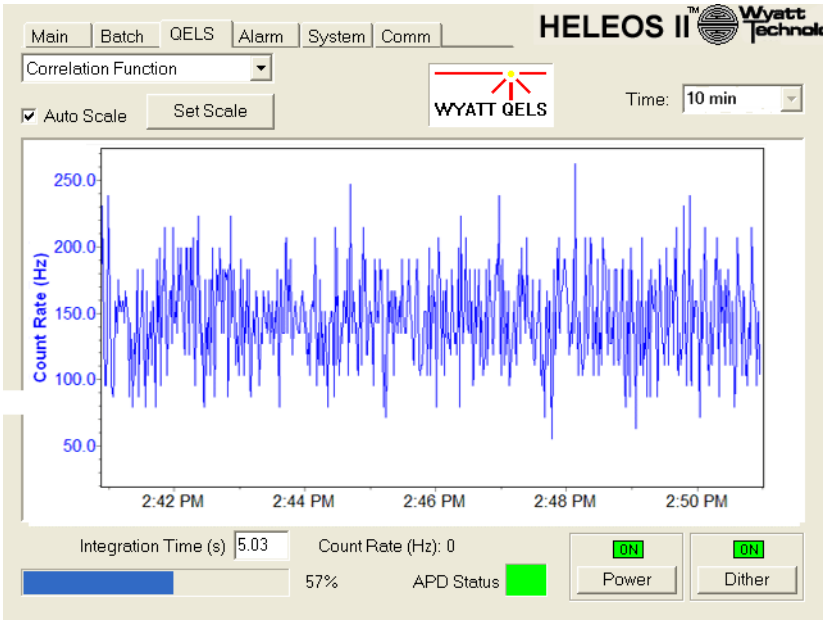


Figure A-1: Count Rate

A.1.2 Correlation Function

The Correlation Function displays the intensity correlation curve for a single slice of QELS data, which is the raw dynamic light scattering data from which the hydrodynamics properties are derived.

The QELS measures the correlation function, which is a statistical measurement of how the scattered intensity fluctuates. It is a function of τ, which is a time difference. For large values of τ, the correlation function approaches 1.0, indicating that the light intensity at time t is uncorrelated to the intensity at time $t + \tau$. For smaller values of τ, the correlation function increases, indicating that the scattered intensity is correlated.

The time difference at which the correlation function transitions from being correlated to being uncorrelated is related to the molecular diffusion coefficient. Small particles diffuse rapidly giving to rapid fluctuations of the scattered light which will have a short correlation time. Correspondingly, large particles diffuse slowly and have a long correlation time.

See the *ASTRA V for Windows User's Guide* for a more detailed explanation of the physics of QELS.

A.1.3 Integration Time

Integration Time is the QELS sample rate, in seconds, of each QELS measurement. The integration time can be set in increments of the minimum time of 0.105 seconds. Integration times of up to 3600 seconds can be set, but are rarely used. Typical values range up to 10 sec. The instrument will round off the set time to the nearest multiple of 0.105 sec. The collection rate depends on the sample concentration, the flow rate, and molecule size. In general, the value chosen should be proportional to expected size, times the concentration, divided by the flow rate. If one has a concentrated sample, a slow flow rate, and a small size, one should choose a sample rate of 1 second. Otherwise, longer sample times should be chosen to improve the measurement statistics.

The integration time selects the time for each measurement. The correlation function measurement is averaged for a time equal to the integration time. The longer the integration time, the more accurate is the result. However, there are a couple of caveats. If the sample is flowing through the cell, as in chromatography, the integration time cannot be made too long or one will get an average over the changing composition of the sample. Also, if one sets a long integration time, the probability of the measurement being contaminated by dust increases.

As an aid to setting the integration time, intermediate results are displayed in red every one second. They get progressively more accurate (less noisy) as time progresses. After the measurement is complete, it is plotted in blue, and the new intermediates are plotted. The slider on the bottom shows the percent complete of the measurement.

Delay Time

The delay time is the horizontal axis of the correlation function graph. It is always less than the integration time.

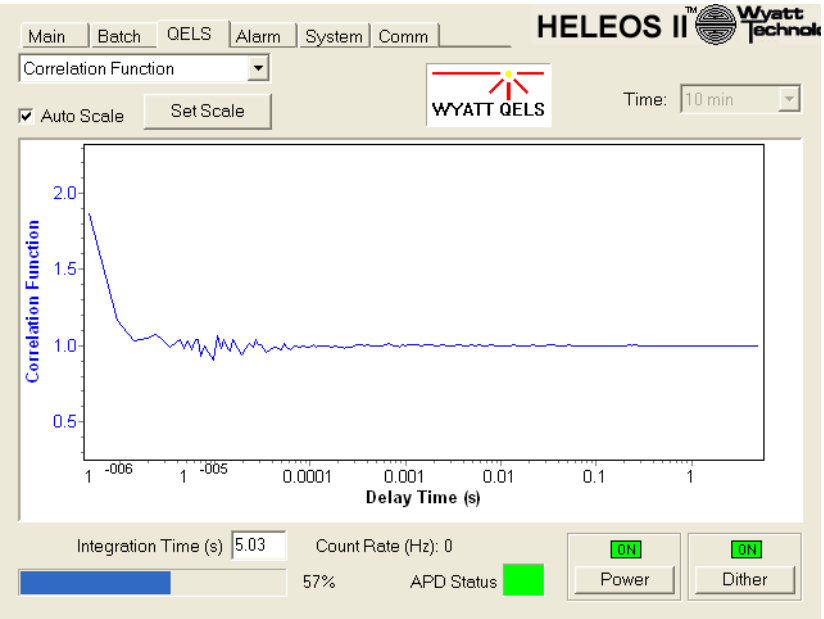


Figure A-2: Correlation function

A.1.4 APD Status

The avalanche photodiode (APD) contains an internal Peltier cooler that cools the active element to provide improved performance. When it is first powered on, the detector is especially susceptible to damage from over-illumination.

The APD is extremely sensitive to light and must be protected at all times. **Never** expose it to room light with the power on. It must either have the dust cover or light fiber connected to it at all times. The Wyatt QELS is equipped with a protection circuit that will shut off the APD in the event of over illumination, but it is intended as an emergency shutoff.

A.1.5 Power

The power switch on the QELS panel turns off the power to the QELS option. There is no external switch. This is included in case you are not using the QELS for some time, or if you want to open the flow cell to look inside to determine if there is dust or dirt.

CAUTION: Room light can damage the QELS detector, so it is important to power it off if there is the potential to expose it to room light.

When QELS power is turned on, the DAWN HELEOS II will turn off the laser for 30 seconds while the APD Peltier cools the sensor. Then it will turn the laser back on. If the laser was already off when the QELS is turned on, the laser will remain off after the 30 second cool down period.

The QELS hardware includes an APD protection system. The correlator hardware continuously monitors the count rate of the APD. If, at any point, it exceeds 10MHz, for more than 1 msec, it will shut down the detector to prevent damage. This is referred to as an APD alert. It will automatically restart during the next measurement.

A.1.6 Dither

The Wyatt light scattering instruments include a patented laser intensity stability algorithm that imposes a small dither onto the laser drive current. The frequency of this "dither" is 150Hz which is fast enough to be filtered out when performing MALS measurements, and slow enough that it does not affect the accuracy of the QELS results. It does however, cause a small artifact in the baseline of the QELS signal near 7msec. The "dither" button allows the user to turn off the dither to eliminate this artifact at the expense of slightly less stable MALS baselines. When the QELS is powered off (or not installed), the dither is always on.

A.2 Aligning the Optical Fiber

Before any measurements can be taken, the optical fiber must be aligned to the laser beam. The alignment is set at the factory, but may change during shipping.

1. Turn off the system power to the DAWN HELEOS II.
2. Remove the top cover of the DAWN HELEOS II instrument. Make sure that the flow cell is filled and has no bubbles in it.
3. Turn on the unit.
4. Navigate to the QELS panel of the GUI. Set the collection interval to 1 second. By default the QELS fiber is installed in detector #12.
5. Using a 2.0 mm hex driver, rotate the adjustment screw on the top of the QELS fiber mount (see Figure A-3). This drives the fiber up and down so that its field of view sweeps past the laser beam. The fiber is positioned correctly when the count rate is maximized. This is accomplished by rotating the fiber counter-clockwise until the fiber is at the top of the mount. Stop when the adjuster screw is about 3mm above the top of the mount. If turned too far, the adjuster screw will come out. If this happens, simply screw it back into place, taking care not to cross the threads.

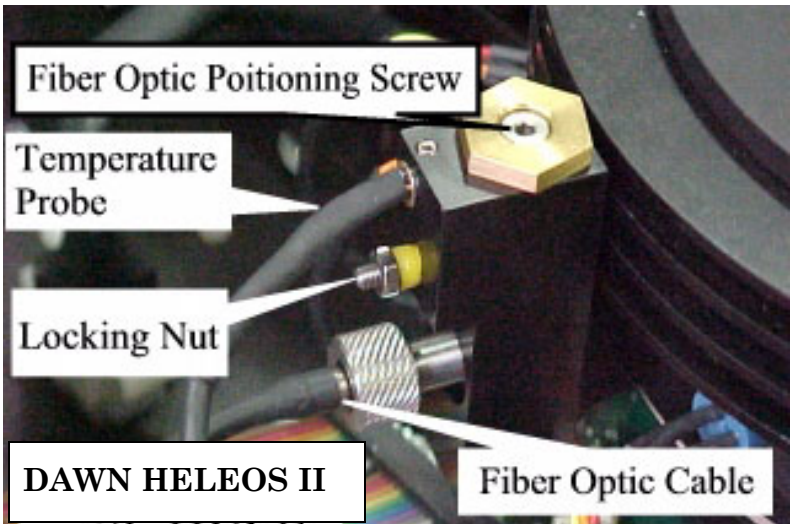


Figure A-3: Adjusting the fiber positioner for the DAWN HELEOS II QELS

6. Once the screw has been raised to its maximum position, slowly rotate the hex driver clockwise while monitoring the count rate on the computer display. As the fiber's field of view passes the beam, the count rate should grow, reach a maximum, and then decay. After passing through the peak once, again raise the mount by rotating counter-clockwise.

7. Repeat the above procedure, but stop when the count rate reaches a maximum. The fiber is then aligned. Do not adjust the locking nut on the side of the mount. The tension is adjusted at the factory and should not be changed.

A.3 Removing and Installing the Optical Fiber Receiver

The DAWN HELEOS II optical fiber receiver can be removed and re-installed in any detector location. By default, it is mounted in detector 12, which is a scattering angle of 100.3 degrees for water, when using the K5 cell.

A.3.1 To remove and reinstall the optical fiber receiver

1. Turn off the DAWN HELEOS II system power.
2. Remove the top cover by removing the four M3x6 button head screws in the four corners.
3. Unscrew the fiber collet (see Figure A-4).

   The fiber collet grips the optical fiber and holds it in the receiver assembly. The optical fiber will have a tendency to turn with the collet as it is removed or installed. This is normal. Take care to rotate the rest of the fiber to prevent it from becoming twisted.

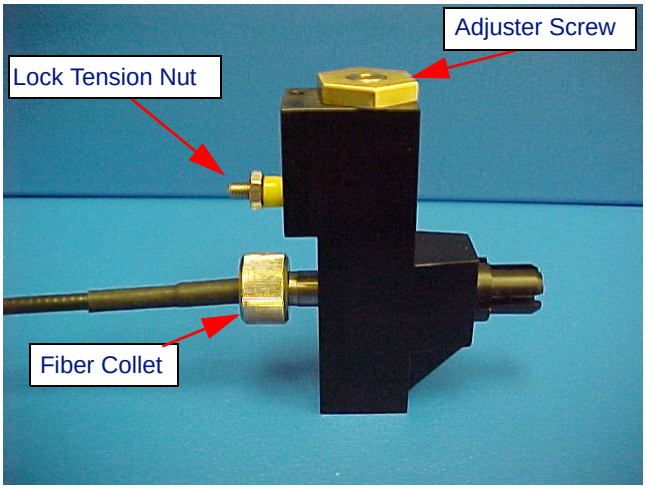


Figure A-4: Optical fiber receiver and positioner

4. Once the fiber collet has been unscrewed all the way, the optical fiber and collet will detach from the mount (see Figure A-5).
5. The fiber will usually not need to be removed from the collet to clean or inspect it. However, if desired, you may gently remove it by twisting it and drawing it out.

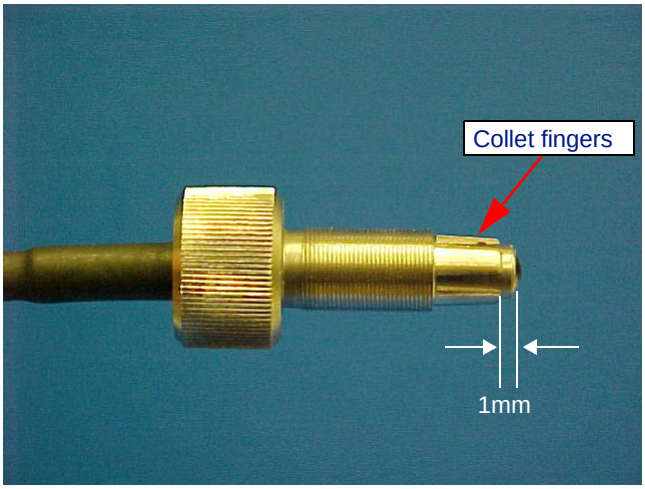


Figure A-5: Optical fiber in collet.
The end of the fiber should protrude about 1mm from the end of the collet.

6. Check that the fiber slides freely into the fiber collet. If it does not slide freely, gently bend the collet fingers outward until it does.
7. Thread the collet back into position until it begins to "grab." Do not over tighten or the collet fingers will bend inward and the fiber will not fit.
8. Install the fiber into the collet and press it all the way until it reaches its stop. If it does not go in all the way, remove the collet and repeat.
9. Gently tighten the collet until it clamps onto the fiber. Again, do not over tighten.

   When the collet is tight, gently pull on the fiber. It should not come loose.

A.3.2 To place the QELS fiber positioner in a different detector location

1. Turn off the DAWN HELEOS II system power.
2. Remove the QELS fiber receiver as described above.
3. Remove the photodetector from the new detector location
   a. The detector is held into the read head with a rubber O-ring and is connected on the back side to a socket on the flexible detector board.
   b. Gently remove the detector from the read head by grasping it on the sides and sliding it out of the read head. You do not need to remove the detector from the flex board. If it does come out of its socket, simply reinsert it.Typically the detector will come out of the read head with the O-ring around the side of its can.

c. The MALS detectors will not be damaged from room light, and can be left on the flex board after the QELS detector has been installed. Simply bend the flex board aside when installing the QELS fiber.

4. Remove the fiber mount from the read head by inserting a 4mm hex driver into the mount and loosening the interior screw as shown in Figure A-6.

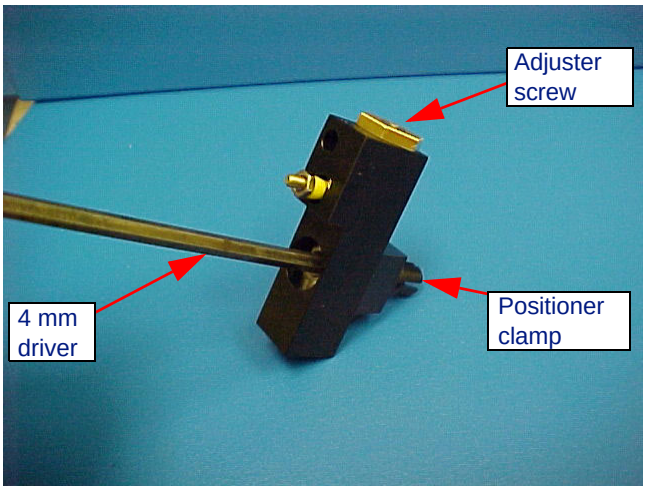


Figure A-6: Adjusting the EOS fiber positioner clamp.

5. Adjust the top adjuster screw to the center of its range to allow the 4mm driver to engage the nut on the positioner clamp. This is when the top of the adjuster screw is nearly flush with the top of the fiber positioner.

6. Use the 4mm driver to loosen the positioner clamp. It should slide free from the DAWN HELEOS II detector hole.

7. Move the fiber positioner to the new detector location and press it firmly into the detector hole.

8. Use the 4mm driver to tighten the positioner clamp. The clamp fingers will expand to grip the detector hole. The fiber positioner can rotate in the detector hole. Make sure to keep it vertical as the positioner clamp is tightened. The clamp should hold the positioner firmly in place.

Install the fiber collet and fiber and tension until firm. Do not over tighten. When installed, tug gently. If the fiber is properly seated, it will not slide out. Align the fiber as described above.Reinstall the MALS photodetector into the position vacated by the QELS detector. First remove the small O-ring from around the photodetector. Moisten it slightly with some water and place it in the detector hole. Press the detector into the O-ring.

B Ultra-High Temperature Option

The Ultra-High Temperature option for the DAWN HELEOS II has some differences from the ambient DAWN HELEOS II. This appendix describes those differences and supplies instructions for making adjustments and operating the Ultra-High Temperature DAWN HELEOS II.

CONTENTS **PAGE**

B.1 Overview

With the Ultra-High Temperature option, the read head may be heated from approximately 10°C above ambient temperature to 210°C. The temperature can be controlled to within 0.01°C and is accurate to ±1°C.

The temperature-controlled read head is comprised of three distinct shells of material:

- The outer aluminum detector ring, which contains the photodiode detectors.
- A layer composed of two insulating materials that keep the flow cell at a stable temperature while at the same time keeping the photodiodes as close to ambient temperature as possible.
- The innermost shell is the aluminum flow cell cavity.

The heater cartridges are located inside the read head. Directly underneath the flow cell is a platinum temperature sensor.

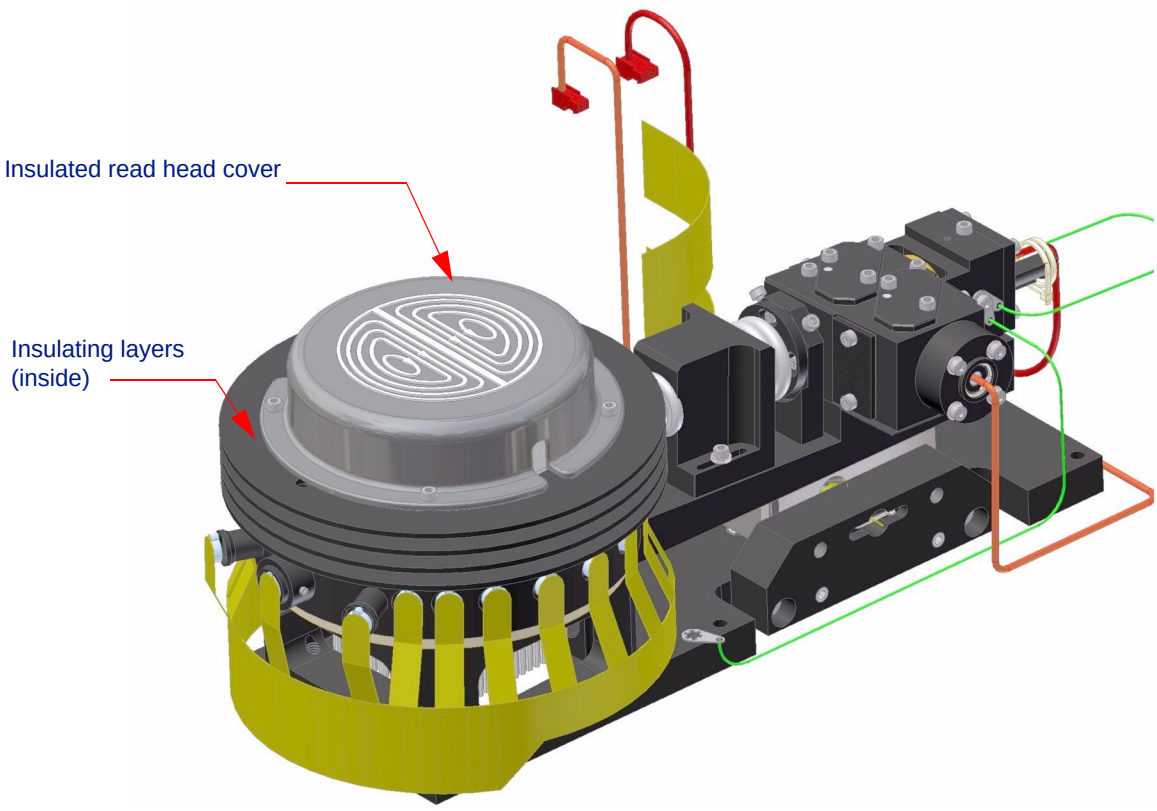


Figure B-1: Ultra-High Temperature read head and laser assemblies

B.2 Heating the Cell

The HELEOS flow cell is designed to operate at temperatures up to 210°C with the Ultra-High Temperature option. The high temperature cell is designed around two cartridge heaters. Temperature regulation is digitally controlled by the front panel computer. The resolution of the controllers is 0.01°C and the accuracy is ± 1°C.

B.2.1 About the Thermocontrollers

There are independent controllers for the read head and for the heated line (if installed). One controller controls the read head temperature. If you purchased the optional heated lines, the second controller controls the temperature of these heated lines.

Typically the heated line controller is "slaved" to the cell controller. That is, when the temperature of the cell is changed, the temperature of the heated line is changed in sync. On the System panel, it is possible to break this slave relationship by unchecking the **Sync** button. Then you can set the temperature of the heated line independently of the cell.

These controllers are designed to give the best possible temperature regulation. They use a Proportional Integral Derivative (PID) control loop, which measures the difference between the setpoint (the temperature you desire) and the process (the temperature of your system).

B.2.2 Setting the Operating Temperature

You can set the temperature of the cell on the Main display panel. Alternatively, you can navigate to the System panel and set the temperature of the cell and heated line separately.

1. Navigate to the associated field and type in the new temperature. Press Enter to select. The system will ramp the temperature at 1°C per minute. This is to ensure that differential thermal expansion of the cell materials do not cause damage.
2. Allow the read head temperature to ramp to the setpoint temperature.

   For example, if you wish to operate your system at 150°C, and your system is initially at 25°C, it will take about two hours for the temperature to reach 150°C, since the ramp rate is limited to 1°C per minute.

Note: If you want to perform temperature ramping experiments, contact Wyatt Technology for instructions on how to reprogram the ramp rate. It can be changed programatically to as slow a rate as required.

B.2.3 Heated Lines

The optional HELEOS heated lines can maintain temperatures up to 210 °C in the inlet and outlet lines if the HELEOS is connected to other high-temperature instruments and detectors.

The heated lines consist of two pieces of steel tubing that are insulated and contain a temperature sensor and a heater. One of them is marked at both ends with a piece of metal tape so that you can distinguish the tubings as they protrude from the insulation.

Typically the heated line controller is “slaved” to the cell controller. That is, when the temperature of the cell is changed, the temperature of the heated line is changed in sync. On the System panel, it is possible to break this slave relationship by unchecking the **Sync** button. Then you can set the temperature of the heated line independently of the cell.

If you are using the Peltier Heated/Cooled model, the read head can be cooled or heated, but the heated lines can only be heated. Using a setpoint below ambient temperature will only cool the read head—it won’t cool the lines.

B.2.4 Operating Precautions

Keep in mind these important points:

- Always have the insulating cover plate locked in place when bringing the cell up to temperature, or cooling it down.
- If possible, keep the instrument at operating temperature at all times.
- Replace the cell O-rings whenever the instrument is brought down from an elevated temperature! They conform to the geometry of the cell and, when brought down from an elevated temperature, may not seal reliably.
- The system will issue a warning if a heated/cooled instrument is set to a temperature above 80°C. This is to remind you that the O-rings must be changed to the high temperature configuration before setting any temperature above 80°C. No such warning is issued on the ultra-high temperature instrument since it is typically configured with the high temperature O-rings. However, if the O-ring set is ever changed to the low temperature setting, the same precaution must be obeyed.
- When heating the cell above 80°C, double check the fittings for leaks as thermal expansion can open fittings that were otherwise sealed at room temperature.

B.3 Removing the Cell Assembly

The Ultra-High Temperature cell assembly is the same as the ambient cell assembly, but with several added components for insulation. These instructions are for those instances when you need to remove the cell assembly—typically to clean the flow cell or to convert to batch mode.

What you will need to remove the cell assembly:

- Two ¼" Crescent wrenches
- 2.0 mm Ball driver
- 2.5 mm Ball driver

To remove the cell assembly, do the following:

1. Set the temperature of the cell and heated lines to 25°C and wait for the system to stabilize
2. Remove the bib from the cover of the instrument.
3. Using the 2.0 mm Ball driver, remove the read-head cover plate by removing the four M3x10 screws holding it in place.
4. Remove the heat exchanger by unscrewing the two M3x12 screws.
5. Disconnect the short pieces of 1.6mm OD stainless steel tubing from the in-line unions (shown in figure B-4) using the two ¼" Crescent wrenches.

   The adapter union connecting the brass heat exchanger to the flow cell tube on the inlet side has 1.6mm OD tubing on one side and 0.8mm OD tubing on the other. Always disconnect the larger of the two tubes since it is more mechanically robust and less likely to be damaged from over-tensioning when reinstalled.

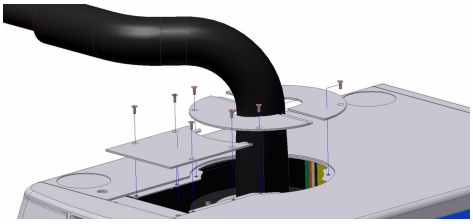


Figure B-2: Heated Lines Bib

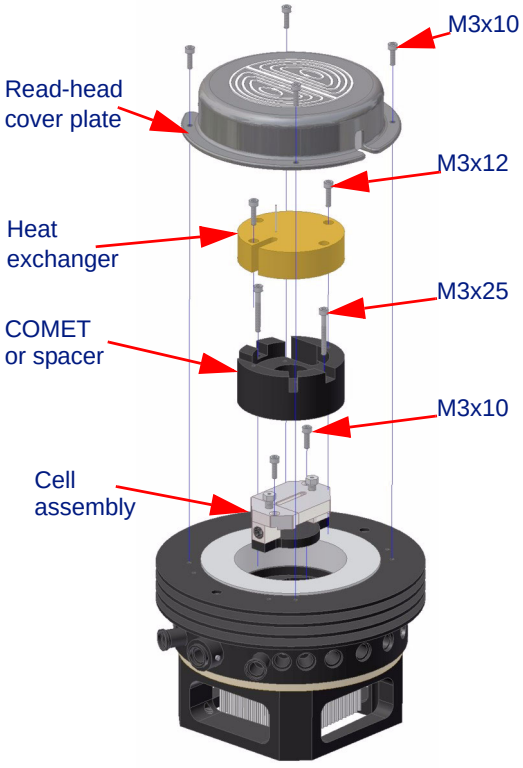


Figure B-3: Heated Lines Flow Cell Assembly

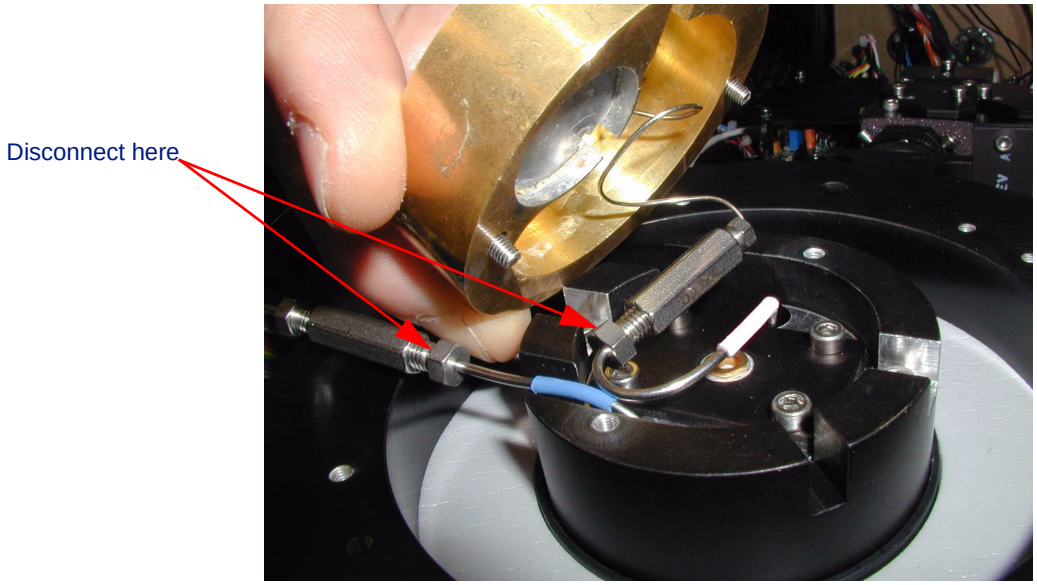


Figure B-4: In-Line Unions

Note	There should be two short lengths of 1.6mm OD tubing plumbed into the cell assembly ports when this procedure is completed.

6. Use the 2.5 mm Ball driver to remove the two M3x10 screws, then lift the cell assembly up and out of the read head.

Lift the assembly out using the connecting tubing. The cell assembly is the same as described in Chapter 3 under “Flow Cell Design.” Cell disassembly and cleaning is described in Chapter 5 under “Cleaning the Flow Cell and Windows."

Note:	Whenever you clean the flow cell, you should replace the O-rings. They become brittle when heated.

B.4 Using the HELEOS with an Oven

It typically takes two days to install a heated HELEOS in an oven such as the Waters 150C, Waters GPC2000, or the Polymer Labs 210. The internal plumbing of the oven should be done beforehand with the HELEOS heated lines connected between the columns and the RI detector.

This list summarizes the installation process (assuming the oven remains at operating temperature). The following pages provide more details about these steps.

DAY 1:

1. Calibrate the HELEOS using toluene.
2. Connect the heated lines to the HELEOS flow cell.
3. Set the pump rate to 0.1 mL/min.
4. Install the flow cell cover plate and the insulated read head cover plate.
5. Bring the HELEOS to 135 ° C (about 2 hours).
6. Slowly bring the flow rate to 1 mL/min (or other desired rate).
7. Check for leaks.
8. Check the calibration using TCB.
9. Measure the flow rate.
10. Prepare standards for the next day.

DAY 2:

1. Inject a narrow polystyrene standard (we suggest 400,000 Dalton); calculate the inter-detector delay volume.
2. Inject a low molecular weight polyethylene (we suggest 32,000 Dalton); normalize the HELEOS and calibrate the refractometer.
3. Inject two or three standard polymers.
4. Process the data.
5. Check normalization and calibration.

The *ASTRA V for Windows User's Guide* provides more details on calibration, normalization, and determination of the delay volume.

B.4.1 General Setup Procedure

Calibrate the instrument

Calibrate the instrument with toluene before connecting it to the oven. Once at temperature, and if the baseline signal is stable and free of particulate noise (typically less than 20 µV at detector 11), you can check the calibration with the mobile phase (if using 1,2,4-trichlorobenzene (TCB) near 135°C).

Place the HELEOS in-line between the columns and RI detector

The HELEOS has to be placed in-line between the columns and the RI detector in the oven. Therefore, it is necessary to take the output line from the columns out of the oven, through the HELEOS, and back into the RI detector. Use the heated lines provided with the HELEOS and additional stainless steel tubing inside the oven, if needed.

The HELEOS can be placed either on the right or left hand side of most ovens. Newer Waters 150C instruments have a pre-drilled hole in the left side. If you have an older Waters 150C, you can drill a hole yourself on either side of the 150C at the level of the columns.

Make sure there are no cold spots where the heated lines connect to the oven; the point of connection should be inside the injector or column compartments to ensure this.

The RI and autoinject cables should be attached to the RI detector integrator output and the autoinject terminals. On the Waters 150C, these are on the left side and are clearly marked.

B.4.2 Connecting the Heated Lines and Heating the HELEOS

DANGER: The HEATED LINE electrical connector on the side of the HELEOS contains live 48V DC pins. Keep the dust cap on this connector whenever the temperature controller is on and the heated lines are not connected.

If the oven is at operating temperature (such as 135°C), leave it connected to the oven, even when servicing the read cell assembly.

What you will need to connect the heated lines:

- Two ¼" Crescent wrenches
- Stainless steel nuts and ferrules

To connect the heated lines and heat the HELEOS, do the following:

1. Make sure the HELEOS is switched off using the switch on the front panel of the instrument.
2. Remove the cover bib from the top of the HELEOS.
3. On the HELEOS end of the heated lines, connect the two pieces of tubing in series using a short piece of stainless steel tubing and two unions. This effectively bypasses the cell and allows you to keep a small flow through the chromatography columns, while the cell is being serviced.

   Make sure you first seat the ferrules using the HELEOS flow cell manifolds. Shorten the exposed stainless steel tubing if needed.
4. Connect the heated lines to the column outlet using the tubing marked with metal tape.

   Which one you connect depends on whether the HELEOS is placed to the right or the left of the oven.

5. Run the pump at 0.1 mL/min until the lines are completely filled with solvent, then attach the unmarked tubing of the heated lines to the RI inlet.
6. Turn the pump off and disconnect the short piece of tubing at the HELEOS side of the heated lines.
7. Connect the tubing from the columns to the rear inlet of the HELEOS cell, and the other tubing to the front outlet of the cell.
8. Run the pump at 0.1 mL/min and replace the insulated read head cover (see "Heating the Cell," earlier).
9. Remove the dust cap from the HEATED LINE connector on the side panel of the HELEOS. Be careful not to touch the electrical contact.
10. Connect the electrical connector on the heated line to the HEATED LINE connector.
11. Turn the HELEOS on and adjust the read head temperature setpoint. This will increase the temperature of both the read head and the heated lines by 1°C per minute. Check for leaks around the connections every 30 minutes or so.

B.4.3 Operating the HELEOS with an Oven

Always increase the flow rate slowly (0.1 mL/min increases every 1–10 minutes, initially slower).

Watch the baseline of detector 11 in ASTRA V for changes whenever you increase the flow rate.

Check for leaks at all connections. You will need to remove the insulated read head cover temporarily to perform this check.

If everything is working well, the baseline noise on the HELEOS detectors (with booster board jumpers removed) should be random and less than 20 µV. If the baselines have regular oscillations, check the pump. You may also want to try further insulating the lines next to the read head.

To calculate the inter-detector delay volume and to normalize the HELEOS, follow the instructions in the *ASTRA V for Windows User's Guide*, "The First Chromatography Run," but use a 400,000 Dalton narrow polystyrene standard and a 32,000 Dalton polyethylene standard, respectively.

B.4.4 Potential Problems

Excessive baseline noise could have several sources:

Particulates and/or air bubbles in the solvent

Use only degassed high-purity HPLC solvents. Always filter your solvent using a 0.2 µm filter or smaller. With TCB, 0.2 µm is the smallest practical filter size. With other solvents it may be possible to use smaller pore filters. An in-line filter after the pump, but before the injector, may help.

Particulates from the columns

With time (several days under operating conditions) the noise should decrease; if it does not, choose another type of column. Take care not to change temperature or pressure too rapidly.

Pump not operating properly

Ensure that the pump is operating properly. If spikes corresponding to the pump strokes are observed in the baseline, the pump should be rebuilt with new check valves and new seals. Operating the pump with sufficient back pressure is important; to achieve this, always work with at least two columns in-line or place a restrictor immediately after the pump. Also, we strongly recommend a pulse dampener after the pump.

Problem with temperature regulation

Check that the temperature output on the oven is calibrated within 1°C. Heating and cooling effects between the two instruments could ruin the baseline stability. You may check the reading from the oven by placing a temperature probe under the top lid. Make sure the probe is not in contact with any metal objects within the oven.

If noise spikes appear in the RI signal after each injection, look for insulation problems at the heated line connections. If further insulation does not remove the spikes, try heating the HELEOS another five degrees. The spikes are likely due to partial polymer precipitation, and a higher temperature may be helpful.

Alternatively, you can break the “sync” connection between the heated line and the read head so that you can run the heated line at 5 degrees higher than the read head.

B.4.5 Disconnecting the HELEOS from an Oven

When you decide to disconnect the HELEOS from the oven, be aware that you must replace all the O-rings in the flow cell assembly after the instrument has cooled down. If this is not done, the flow cell may leak upon reheating.

To disconnect the HELEOS, do the following:

1. Decrease the pump speed slowly to 0.1 mL/minute.
2. Set the read head temperature setpoint on the HELEOS to room temperature.

   The temperature will slowly decrease to room temperature (2–3 hours).
3. When the cell is close to room temperature, turn off both the HELEOS and the pump.
4. Unscrew the bolts holding on the heated line spacer.
5. Slide the heated line up on the capillaries by several centimeters.
6. Remove the heated line spacer.

7. Disconnect the heated line electrical connector from the HEATED LINE connector and replace the dust cap on the HEATED LINE connector.

DANGER: The HEATED LINE fitting contains live 48 VDC pins. Keep the dust cap on this connector whenever the heated lines are not connected.

8. Disconnect the heated lines from the flow cell.
9. Remove the insulating read head cover from the HELEOS.
10. Flush the cell with a suitable solvent, such as toluene.
11. Continue flushing with methanol/ethanol and then cap the cell.
12. Remove the cell assembly from the read head, disassemble the cell, clean it and replace all O-ring seals.
13. If the disconnection is only temporary, do the following:
    a. Leave the heated lines connected to the oven.
    b. Attach the small piece of tubing with the unions at the HELEOS side of the lines.

B.5 Temperature Controlled Flow-to-Batch Conversion

The Heated HELEOS Flow-to-Batch conversion differs slightly from the ambient model because of the extra cover plate for the read head, an additional vial insulation ring, and an insulated cap.

What you need for flow-to-batch conversion:

- Two ¼" Crescent wrenches
- 2.5 mm Ball driver
- Phillips screw driver
- Heated Batch Conversion kit

To convert from flow to batch operation, do the following:

1. Remove the bib from the cover of the instrument.
2. Remove the read head cover, the flow cell cover plate, and the cell assembly from the instrument. (See the section on "Removing the Cell Assembly" on page B-5 in this chapter.)
3. Insert the batch manifold and secure it with the two M3x20 screws.
4. Install the batch spacer plate with four M3x20 shoulder bolts (ambient only)
5. Install the Batch vial spacer with two M3x10
6. Install the insulated batch cap.
7. Heat the read head to the required temperature, then put the pre-heated sample scintillation vial in the read head cavity and cover it with the insulated cap.

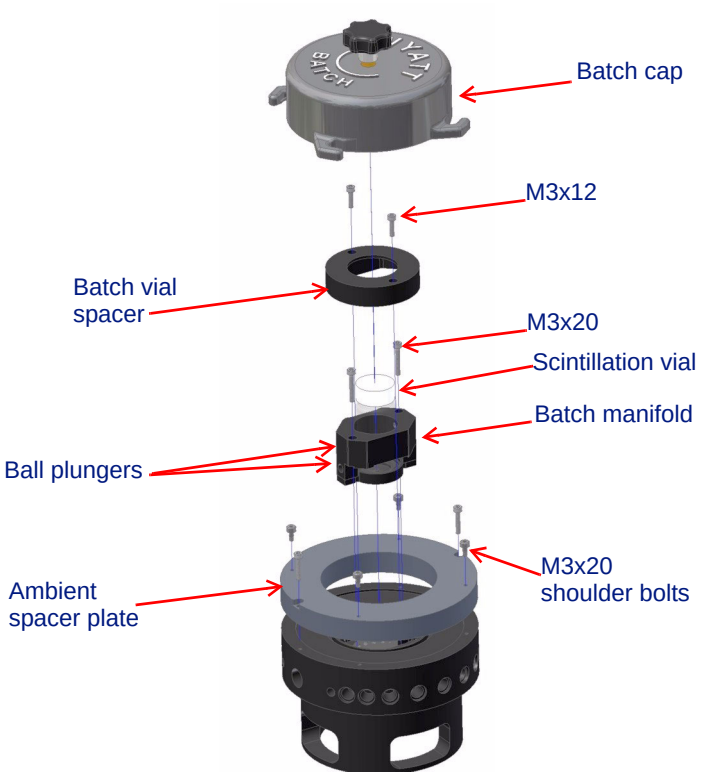


Figure B-5: Temperature Controlled flow-to-batch conversion kit, exploded

To replace the flow cell, cool down the read head then reverse the previous process.

Notes:

The sample should be initially heated in an oven then filtered and transferred to the HELEOS. Wait 15–20 minutes before taking a measurement.

When making measurements with scintillation vials, take great care to keep the outside of the vials clean and free of fingerprints, scratches, etc., as this can severely distort the measurement. We also advise you to rotate the vial in the read head to find the position where the laser beam enters the cell with the least amount of scattering at the air/glass interface. See the *ASTRA V for Windows User's Guide* for further instructions.

C Peltier Heated/Cooled Option

The Peltier Heated/Cooled HELEOS option has some differences from the ambient HELEOS. This appendix describes those differences and supplies instructions for making adjustments and operating this version of the instrument.

CONTENTS **PAGE**

C.1 Overview

The read head on the Peltier Heated/Cooled HELEOS can be heated up to 150°C or cooled down to –30°C. The Heated/Cooled HELEOS uses a solid-state Peltier device and a cartridge heater to operate over the whole temperature range.

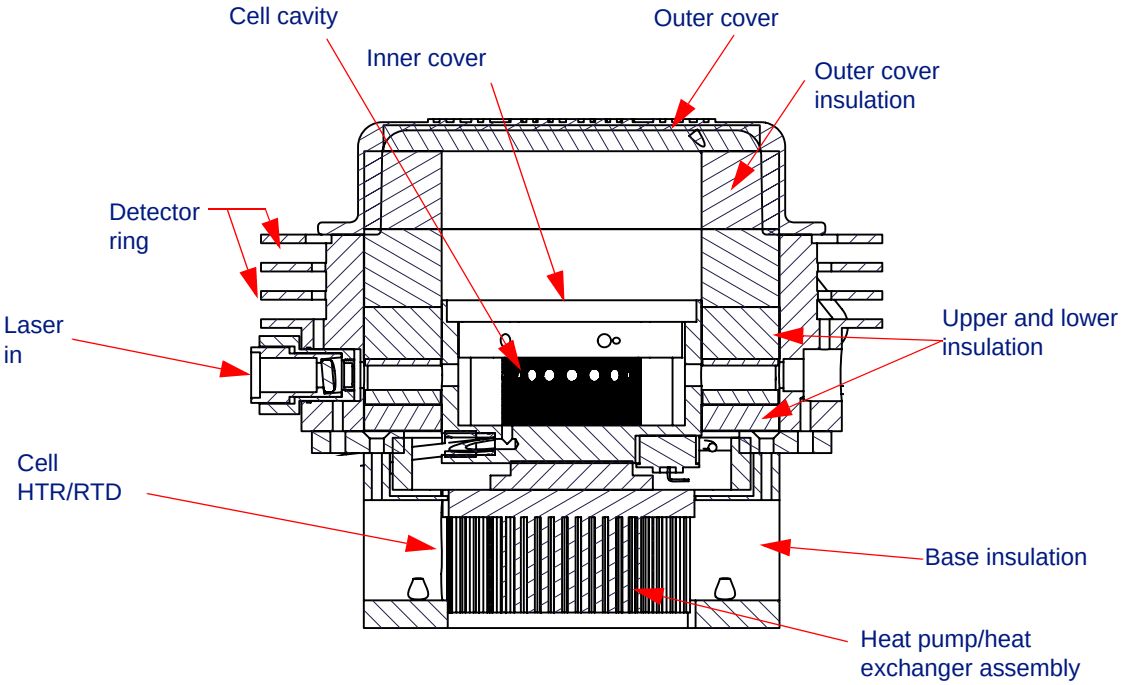


Figure C-1: Heated/cooled HELEOS read head, cross-section

The temperature-controlled read head is comprised of three distinct shells of material:

- The outer aluminum detector ring, which contains the photodiode detectors.
- A layer composed of two insulating materials that keep the flow cell at a stable temperature while at the same time keeping the photodiodes as close to ambient temperature as possible.
- The innermost shell is the aluminum flow cell cavity. Mounted directly underneath the cell cavity, between the read head and the circuit board, is a Peltier heat pump.
- The temperatures of the cell and optional heated lines are controlled by the front panel computer to a stability of 0.01°C and the accuracy is ± 1°C.

C.2 Recommended Procedures

- Be sure all chosen operating temperatures are between the freezing point and boiling point of the solvent you are using!
- Make sure the solvent is close to the same temperature as the flow cell.

  The heated cooled instrument has an integrated heat exchanger to bring the sample fluid to the same temperature as the flow cell. However, the closer the fluid is to the cell temperature, the more stable the results will be.
- Always have the insulating cover plate locked in place before heating or cooling the flow cell.
- Check for leaks each time the HELEOS has been heated above 80 °C.
- Replace the cell O-rings whenever the instrument is brought down from an elevated temperature! They conform to the geometry of the cell and, when brought down from an elevated temperature, may not seal reliably.
- The flow cell is initially configured for use at the temperature you indicate you will be using when you purchase the instrument. If you decide to operate at a different temperature, you may need to reconfigure the flow cell O-rings.

  If your instrument is configured to operate at or below 80 °C and you decide to operate above 80 °C, you must remove the backing rings and install the 9 mm flow cell O-rings instead of the 6 mm O-rings. Above 80°C the O-rings expand enough to crack the flow cell glass if the backing ring is installed.

  If your instrument is configured to operate above 80 °C and you decide to operate below 80 °C, install the backing rings and the 6 mm flow cell O-rings. This minimizes dead volume. If dead volume is not an issue, you may choose to use the high temperature O-ring set over the entire temperature range.
- When operating below ambient temperature, be sure to connect a dry air or nitrogen source to the HELEOS' Nitrogen Purge connector. Light scattered from condensed water ruins your measurements. It is a good idea to use dry air or nitrogen even at ambient or higher temperatures to minimize the amount of dust within the instrument.
- The temperature controller for the flow cell will not allow you to set a temperature below 20.5 °C unless it detects at least 20psi of gas pressure on the nitrogen port. This prevents accidental condensation on the flow cell and read head. If for some reason, you need to open the flow cell, heat it to at least 20.5C before opening the flow cell.

D Polarization Option

The polarization option consists of a special grooved cell retainer which holds two strips of Polaroid film around the sides of the flow cell. The vertically polarized strip has vertical notches which can still be seen when installed in the flow cell assembly. These strips detect the presence of depolarizing molecules or particles.

CONTENTS **PAGE**

D.1 Installation

What you need to install the polarization filters:

- 2.0 mm Ball driver
- 2.5 mm Ball driver
- Two ¼" Crescent wrenches
- 1.5 mm Hex driver

To install polarization filters, do the following:

1. Remove the bib from the cover of the instrument.
2. Remove the cell assembly from the read head See “Removing the Cell Assembly” on page 5.
3. Use the 1.5 mm Hex driver to unscrew the two M2 screws holding the bottom cell retainer in place, then remove the cell retainer.
4. Install a vertically polarized strip facing the odd detectors (left side), including detector 11 at 90°, and a horizontally polarized strip facing the even detectors (right side).

   The small notches at the edges of the Polaroid film indicate its horizontal or vertical orientation.
5. Reinstall the special polarizer bottom cell retainer.
6. Reinstall the flow cell assembly using two M3 screws.
7. Reconnect the tubing to the in-line unions.
8. Reinstall the flow cell cover plate.
9. Replace the instrument cover bib.

D.2 Normalization and Calibration with Polarization Strips Installed

D.2.1 Normalization

To normalize the HELEOS, it is necessary for each detector to receive light from an isotropic scatterer. With the polarization strips in, however, half of the detectors are receiving light that has passed through a horizontal polarizer, while the other half are receiving light that has passed through a vertical polarizer. In the case where the normalization standard does not depolarize the scattered light, the detectors with the horizontal polarizer will receive no scattered light at all. Therefore, it is not possible to normalize the HELEOS with the Polarization strips installed. Normalization should be performed without the Polarization strips. Then install the Polarization strips after normalization.

D.2.2 Calibration

For calibration, the 90 degree detector must receive a known amount of light. Typically, toluene is used as the calibration standard. However, toluene depolarizes the scattered light, so that there are horizontally and vertically polarized components that reach the detector. Therefore, installing a vertically polarized filter in front of the 90 degree detector blocks some of the horizontally polarized scattered light that is necessary for an accurate calibration. It might seem necessary, therefore, to remove the polarization strips before calibrating. The strips, however, attenuate some of the light, regardless of their polarizing properties, so it is necessary to take into account this attenuation.

There are two strategies to deal with calibration with the polarization strips.

Calibrate using toluene with the polarization strips installed. Then correct the calibration constant by using the Cabannes factor for toluene at the wavelength of the laser light.

Calibrate without the polarization strips installed. Then make a measurement of the scattered light on the 90 degree detector for a known sample that does not depolarize the scattered light (e.g. polystyrene, or any other random coil). Install the polarization strips, then measure the amount of scattered light on the 90 degree detector using the same sample *at the same concentration*. The ratio of the measurements with the polarization strips installed and absent gives the attenuation factor of the polarization strips. Use this factor to correct the calibration constant measured without the strips.

Either strategy should work. Contact Wyatt Technology Corporation if you have further questions.

E Interference Filter Option

Interference filters may be used to prevent light of wavelengths other than the laser's to reach the photodiodes. This can be useful when the sample fluoresces. Without these filters, a molecular weight is obtained as result of both the scattered light and the fluorescence being captured by the detectors.

CONTENTS **PAGE**

E.1 Installing Interference Filters

What you will need to install interference filters:

- Anti-static wrist strap
- Tweezers
- Nine interference filters with O-rings
- Wooden or plastic spatula (or similar tool)

To install interference filters, do the following:

1. Put on the anti-static wrist strap.
2. Switch off the power to the instrument and laser, then remove the instrument cover.
3. Ground yourself to the chassis and gently remove a photodiode from the read head using a pair of tweezers.

   Be careful not to stress the solder connection of the lead to the PCB. Also, make sure the leads do not touch one another to cause a short circuit.
4. Carefully insert an interference filter into the diode hole using a wooden or plastic spatula. **Touch the outer edge of the interference filter only**. The mirrored side of the filter should face out towards the photodiode; the colored side of the filter should face in towards the cell.
5. Insert the small O-ring and push it firmly against the filter. This holds the filter in place.
6. Remove the black O-ring from the photodiode and push it into the shoulder of the hole.
7. Moisten the O-ring, then push the photodiode through the O-ring, into its hole.

   Moistening the O-ring ensures that the photodiode slides easily into place.
8. Repeat steps 3 to 7 for the other interference filters.

   Installing a filter on every other diode should be sufficient. For example, you might install filters on the odd numbered detectors only.
9. Replace the instrument cover and switch the instrument and laser back on.
10. Repeat the calibration (if you installed a filter on detector 11), normalization and, for Batch mode, solvent offset measurements.

F Laser Specifications

The DAWN HELEOS II contains a GaAs laser operating at a nominal wavelength of 658nm.

The GaAs laser is a single transverse mode heterojunction that emits light at 658nm at a power of 100 mW delivered to the flow cell. Typically diode lasers undergo periodic mode hops between different longitudinal modes which have slightly different efficiencies giving rise to sudden changes in intensity, however Wyatt Technology utilizes a patented intensity stabilization method which achieves a typical long term intensity stability of 0.1%.

It is suggested that the laser be allowed at least 30 minutes to warm up before taking data. Note that the instrument must be connected to a computer running the ASTRA V software for laser emission to occur.

CONTENTS **PAGE**

F.1 Electrical and Optical Specifications

Table F-1: Electrical and optical specifications

	GaAs
Power Output	130 mW
Laser Operating Wavelength	658 nm
Vertical Beam $1.0/e^2$ Intensity Diameter	80 µm
Horizontal Beam $1.0/e^2$ Intensity Diameter	52 µm
Polarization Ratio	> 100:1
Max Power Stability	< 0.5%
Typical Optical Noise	0.1%
Typical Operating Voltage	2.4 VDC
Typical Operating Current	100 mA

F.2 Environmental Specifications and Safety Notes

Table F-2: Laser Environmental specifications

	GaAs Operating	GaAs Non-Operating
Temperature	-40 to +85°C	15 to +50°C
Relative Humidity	0-95%	10-85%
Shock	1500 G – 0.5 ms	1500 G – 0.5 ms
Vibration (5 to 500Hz sinusoidal)	2.0 G	2.0 G

The lasers used in the DAWN HELEOS II are Class IIIb lasers. However the DAWN HELEOS II itself is classified as a Class 1 Laser Product according to IEC60825-1:1993+A1+A2 and CFR Title 21 Subchapter J. Note these environmental specifications apply to the laser subsystem and not to the instrument as a whole. This means that under normal operation, no laser radiation should escape from the instrument, and no protective equipment must be worn. However the follow warning applies:

Caution:	Use of controls or adjustment or performance of procedures other than specified herein may result in hazardous radiation exposure.

The instrument also bears the following warning label:

Danger:	Laser Radiation when open. Avoid direct exposure to beam.

Note:	Laser safety labels are in English. If you need safely labels in a language other than English, please contact Wyatt Technology

G Flow Cell Properties

This appendix contains tables listing thermal and chemical properties of the two types of flow cells, and their refractive indices. Except for Table G-4, all data and descriptions are from the Schott Glass *Optical Glass Catalog*.

CONTENTS **PAGE**

G.1 Thermal Properties

Glass Classification	Thermal Expansion		Transformation Temperature	Specific Heat $c_p = (J/g \times K)$
	-30 to 70 °C	20 to 300 °C		
K5	8.2 x 10^{-6} /K	9.6 x 10^{-6} /K	543 °C	0.783
F2	8.2 x 10^{-6} /K	9.3 x 10^{-6} /K	432 °C	0.557

G.2 Refractive Indices

Glass Classification	Refractive Index $\lambda = 633nm$
K5	1.51876
F2	1.61311

G.3 Chemical Properties

To interpret the CR, FR, SR and AR values, see "Definition of Terms".

Glass Classification	Bubble Class	CR	FR	SR	AR
K5	0-1	1	0	1	1.0
F2	0	1	0	1	2.3

G.4 Definition of Terms

Transformation Temperature

Temperature at which deformation of precision finished surfaces and a change in the refractive index can occur.

Climate Resistance (CR 1-4)

Class CR 1; after 180 hours of exposure the glasses exhibit no or only slight signs of deterioration due to changing climatic conditions. Under normal humidity conditions that prevail during the processing and storage of optical glasses, no surface deterioration of class CR1 glasses is to be expected.

Resistance to Staining (FR 0–5)

Class FR 0; after exposure to a standard acetate solution (pH=4.6) for over 100 hours, no interference color staining is observed.

Resistance to Acids (SR 1–4)

Class SR 1; after a 100 hour exposure to an aggressive solution of 0.3n nitric acid (pH=0.3), the smallest visible detectable thickness, 0.1 micrometer, is not dissolved.

Resistance to Alkalis (AR 1–4)

A two-digit figure is used to express resistance to alkalis. The digit after the decimal point indicates what surface changes are visible to the naked eye after alkaline exposure. The alkaline resistance class indicates the time in minutes required to decompose a 0.1 micrometer layer of glass in an alkaline solution at 90°C (sodium hydroxide, pH=10).

Table G-1: Flow cell alkaline resistance classes

Alkaline Resistance	Time (in minutes)
1	>120
2	120–30
3	30–7.5
4	<7.5

Table G-2: Flow cell alkaline resistance visible surface changes

Visible Surface Changes	Description
0.0	No change
0.1	Scarred surface but no visible coatings (color change)
0.2	Interference colors
0.3	Whitish staining
0.4	White coating (thick layers)

G.5 Scattering Angles

The table below shows the scattering angles for two different flow cells in four different solvents at a wavelength of 658 nm. The table entries for TCB are at a temperature of 135°C, all others are at 25°C. Note that for a K5 cell in water, the first two detectors are not available; for an F2 cell in water, the first three detectors are not available.

The mathematics behind these changes in scattering angles are discussed in the "Flow Cell" section of Chapter 3.

Table G-3: Flow cell scattering angles (part A)

	K5				F2			
	water	**THF**	**toluene**	**TCB (135°C)**	**water**	**THF**	**toluene**	**TCB (135°C)**
n_g	1.51876	1.51876	1.51876	1.51876	1.61311	1.61311	1.61311	1.61311
n_s	1.330	1.401	1.488	1.500	1.330	1.401	1.488	1.500

Table G-4: Flow cell scattering angles (part B)

Det	**read head angle**	K5				F2			
		water	**THF**	**toluene**	**TCB (135°C)**	**water**	**THF**	**toluene**	**TCB (135°C)**
1	22.500	batch	batch	batch	batch	batch	batch	batch	batch
2	28.000	N/A	16.831	25.684	26.621	N/A	N/A	16.827	18.281
3	32.000	14.440	23.172	30.051	30.834	N/A	12.461	23.169	24.217
4	38.000	25.862	31.323	36.457	37.073	17.108	24.863	31.321	32.067
5	44.000	34.772	38.757	42.759	43.253	29.254	34.081	38.756	39.323
6	50.000	42.776	45.828	48.999	49.396	38.775	42.260	45.827	46.270
7	57.000	51.542	53.813	56.227	56.533	48.656	51.164	53.812	54.147
8	64.000	59.961	61.626	63.421	63.650	57.881	59.686	61.626	61.873
9	72.000	69.337	70.428	71.615	71.767	67.988	69.157	70.428	70.590
10	81.000	79.710	80.236	80.812	80.886	79.063	79.623	80.236	80.315
11	90.000	90.000	90.000	90.000	90.000	90.000	90.000	90.000	90.000
12	99.000	100.29	99.764	99.188	99.114	100.94	100.38	99.764	99.685
13	108.00	110.66	109.57	108.39	108.23	112.01	110.84	109.57	109.41

Table G-4: Flow cell scattering angles (part B)

Det	read head angle	K5				F2			
		water	THF	toluene	TCB (135°C)	water	THF	toluene	TCB (135°C)
14	117.00	121.23	119.48	117.61	117.37	123.41	121.52	119.48	119.22
15	126.00	132.16	129.58	126.87	126.52	135.47	132.59	129.58	129.21
16	134.00	142.49	138.86	135.16	134.70	147.41	143.11	138.86	138.33
17	141.00	152.55	147.40	142.49	141.89	160.49	153.48	147.40	146.69
18	147.00	163.28	155.39	148.87	148.12	180.00	164.94	155.39	154.41
A/C	81.000	79.710	80.236	80.812	80.886	79.063	79.623	80.236	80.315

H Connecting to Network or PC

These instructions contain a pictorial overview for connecting your DAWN HELEOS II to a computer for data collection. The instructions are divided into seven sections:

CONTENTS **PAGE**

Please read over Section H.1 to gain an understanding of the components to be used. Then read over either Section H.2, H.3, or H.4 depending on your configuration. Finally, read over Section H.5 for instrument settings.

Please read Section H.6 for instructions on accessing instruments via ASTRA V. Finally, if you experienced problems connecting to your instrument, please read Section H.7 for diagnostics and trouble-shooting.

H.1 Components

H.1.1 Instrument connections:

Figure H-1 is a detail of the instrument back panel. The Ethernet port, designated with a yellow arrow, is to be used for all connections in these instructions. Please see Section H.3 for instructions on establishing a USB connection.

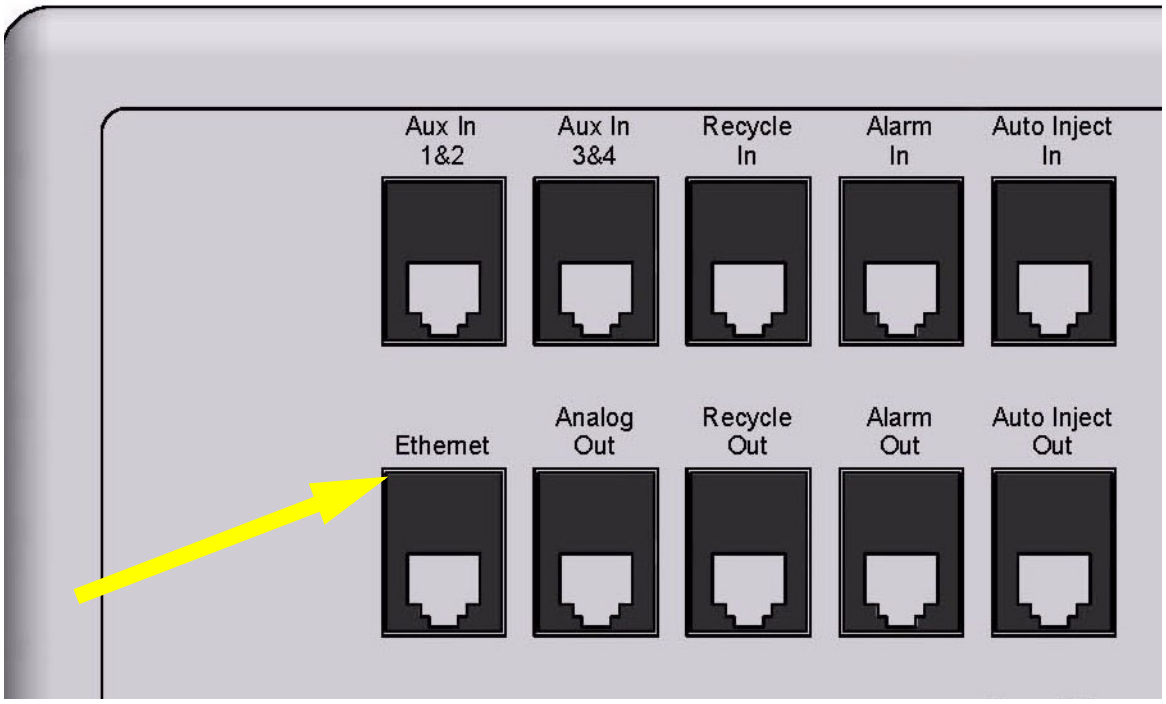


Figure H-1: Detail of the back panel of the DAWN HELEOS II. The yellow arrow designates the Ethernet port.

H.1.2 LAN connection:

Figure H-2 shows a typical wall socket connection to a Local Area Network (LAN). If you are going to connect the instrument to a LAN, you will need access to this type of socket.

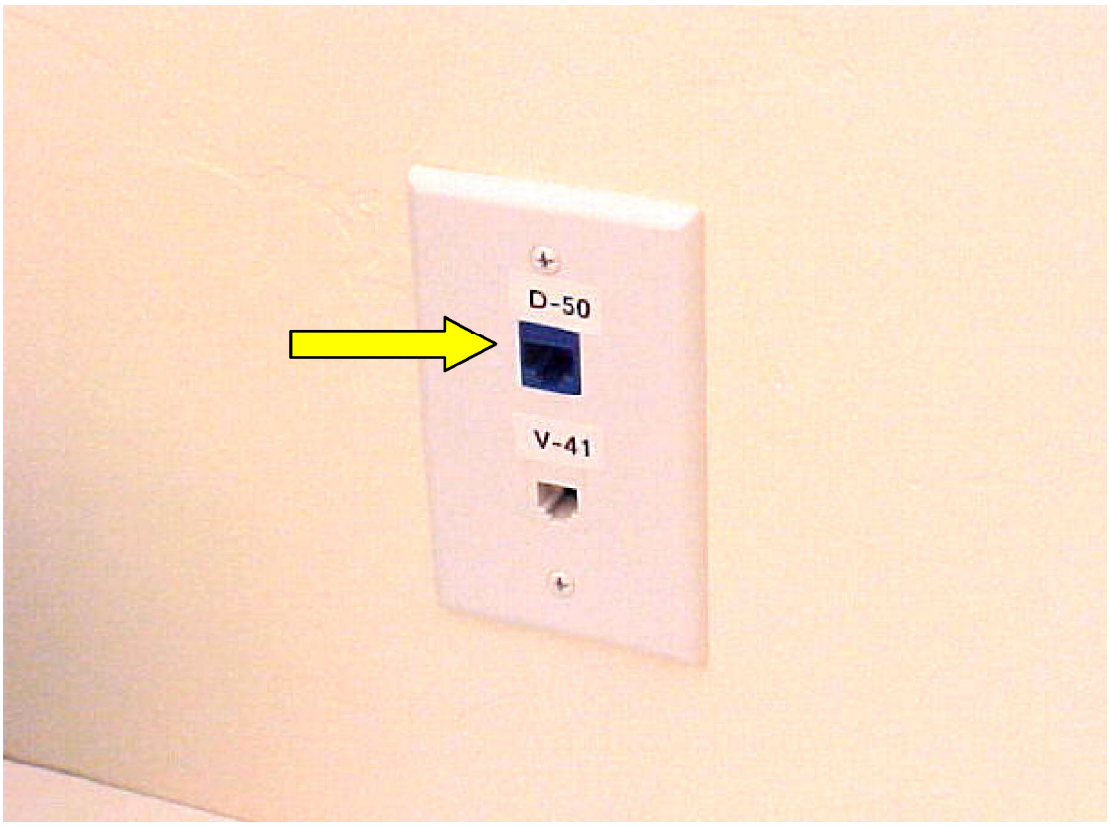


Figure H-2: Wall socket LAN connection indicated by yellow arrow.

H.1.3 Computer connections:

Computer connections can be made via either the Ethernet or USB port. Figure H-3 shows these ports on a standard laptop computer. Sections H.2 and H.4 describe instrument connections made via the Ethernet port. Section H.3 describes connections made via the USB port.

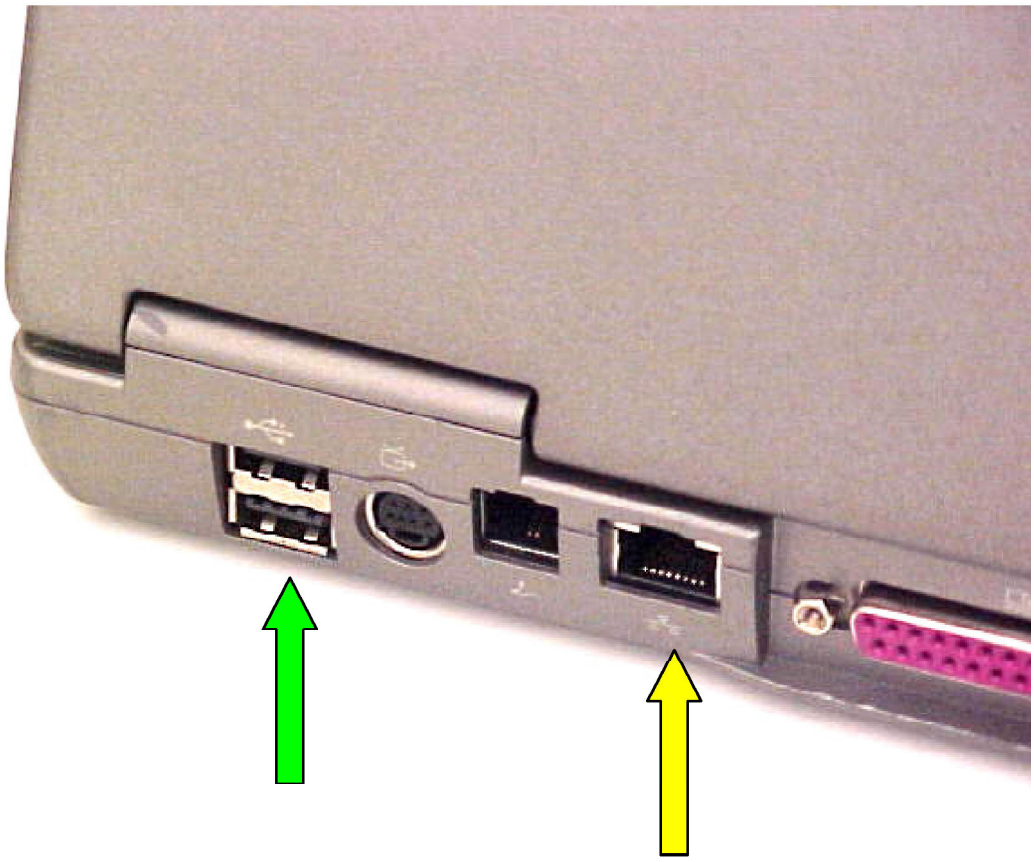


Figure H-3: Ethernet and USB ports on the computer.

The USB ports are designated by a green arrow, and the Ethernet port is designated by a yellow arrow.

H.1.4 Crossover cable:

A crossover cable can be used to make a direct connection from the instrument to an Ethernet port on a computer or to an Ethernet to USB adapter. Please note that the crossover cable shipped with Wyatt Technology instruments is yellow to distinguish it from a standard Ethernet cable. Please be careful to only use the yellow crossover cable where indicated.

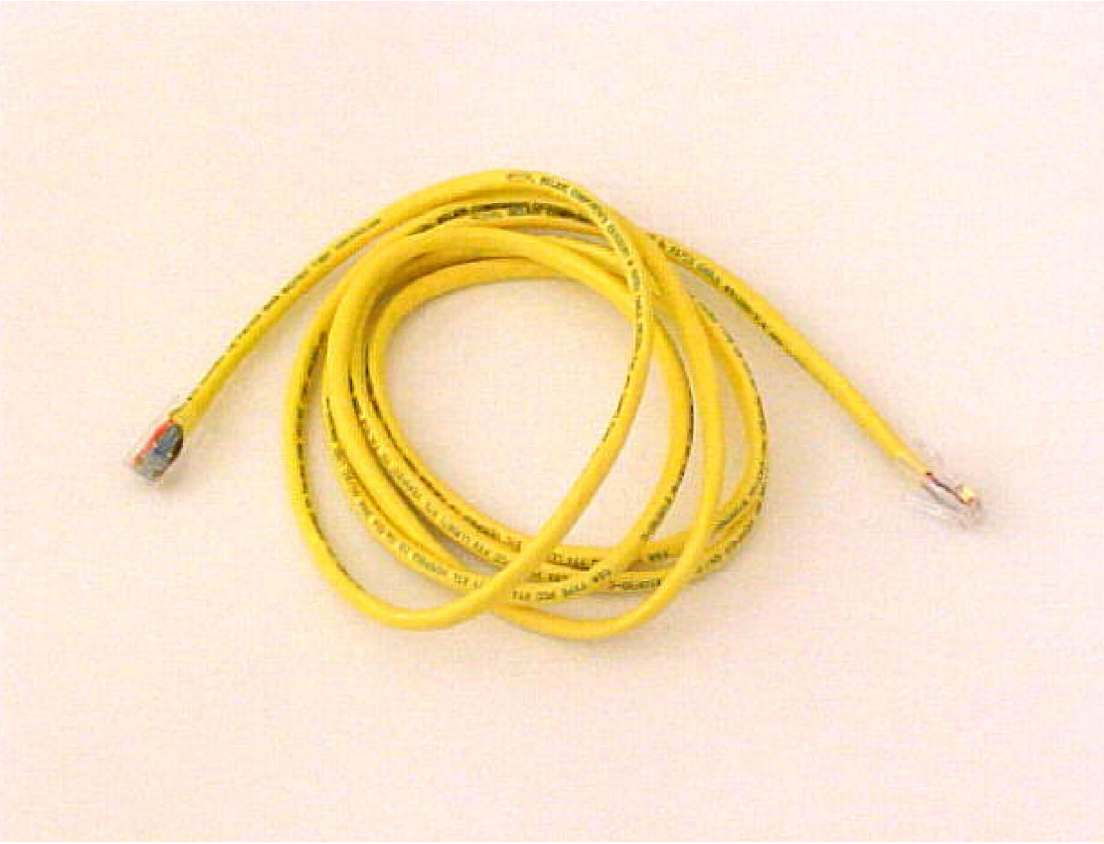


Figure H-4: The Ethernet crossover cable shipped by Wyatt Technology is yellow.

H.1.5 Ethernet cable:

A standard Ethernet cable is sometimes referred to as a patch cable, or a straight-through cable to distinguish it from the crossover cable in Section H.1.4. Ethernet cables provided by Wyatt Technology are black, blue, white, or gray, but never yellow (yellow is reserved for the crossover cable). For these instructions, the Ethernet cable will always be black.

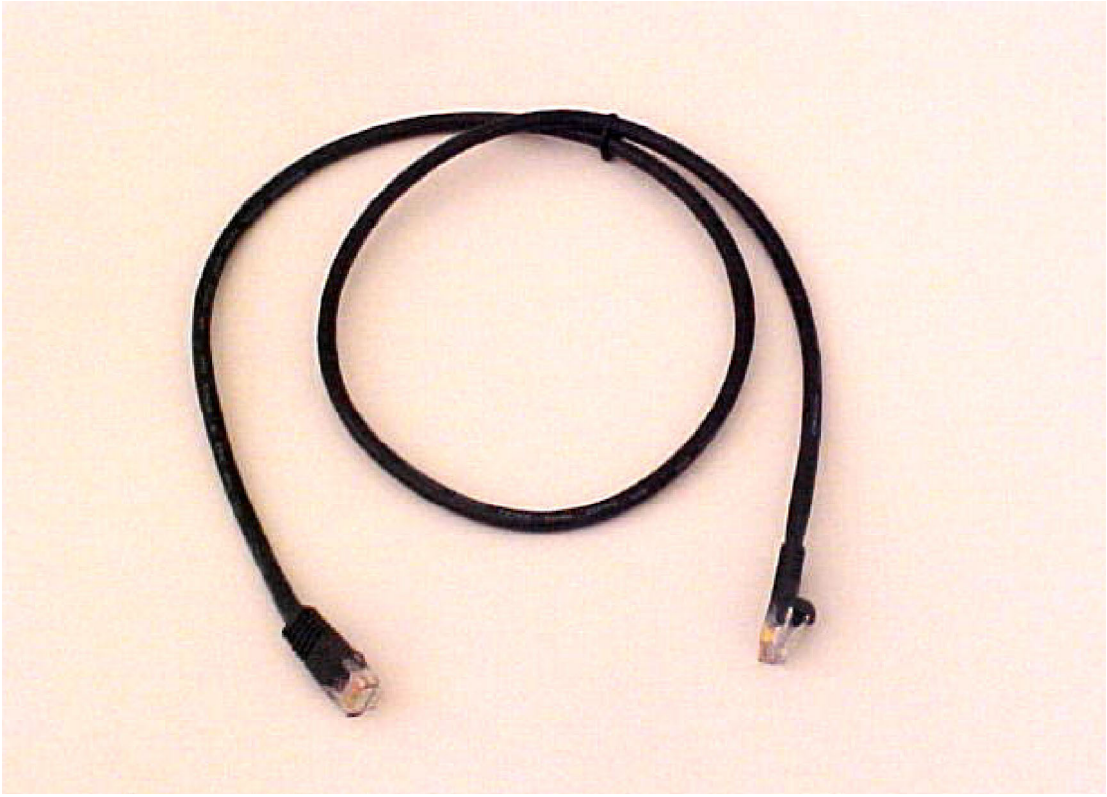


Figure H-5: Standard Ethernet cable.
For these instructions, the standard cable is always black.

H.1.6 Ethernet to USB adapter:

This device can be used to connect an Ethernet cable to a USB port on the computer. Using this adapter, it is possible to have the computer connected to a LAN via the computer's Ethernet port, and the instruments connected to the computer via USB. The Ethernet to USB adapter supplied by Wyatt Technology will look similar to this. The first time you connect an Ethernet to USB adapter to your computer, you may be prompted to install USB drivers for the device. To do so, use the CD supplied with the Ethernet to USB adapter, and follow the Microsoft Windows instructions.

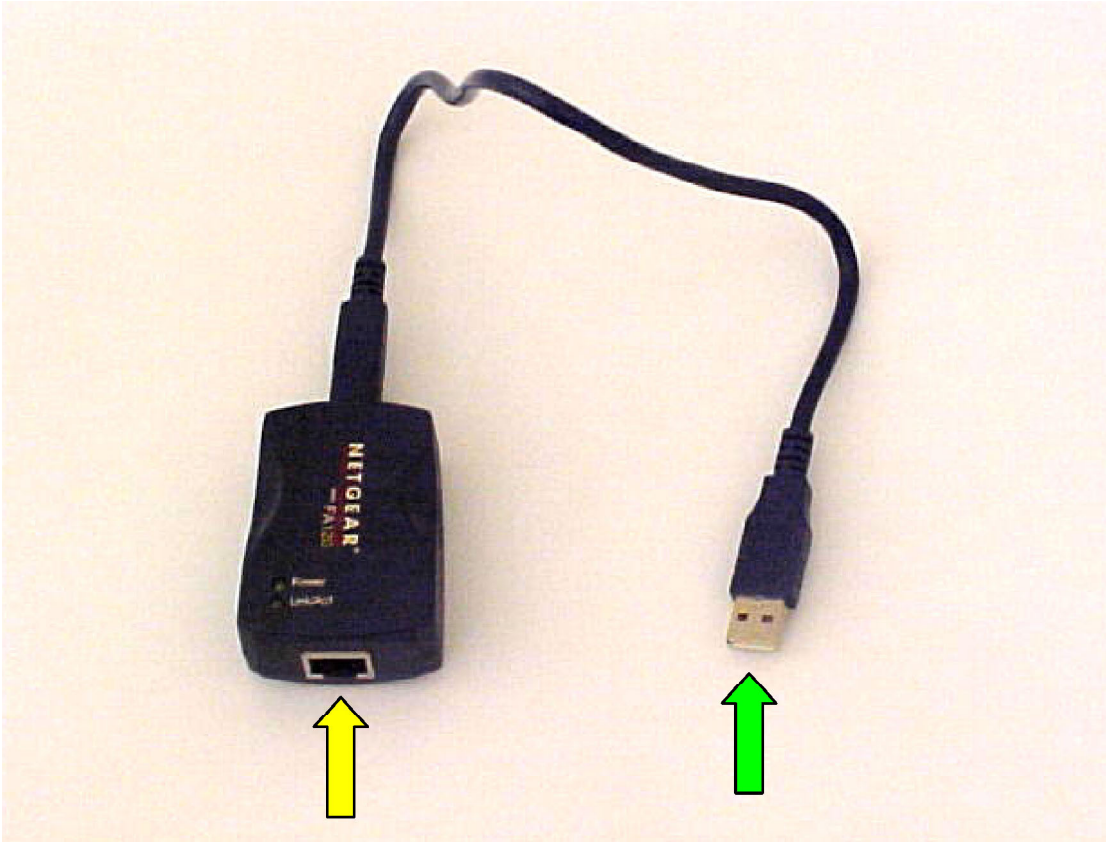


Figure H-6: Standard Ethernet to USB adapter. The Ethernet cable is plugged into the port with the yellow arrow, and the USB plug (green arrow) is plugged into a USB port on the computer.

H.1.7 Ethernet switch:

Ethernet switches are used to connect several Ethernet cables to one resource, such as the LAN socket in Figure H-2. The Ethernet switch supplied by Wyatt Technology will look similar to the five port switch shown below. Please note that Ethernet cables can be connected to the switch in any order or position. Also, the switch has an external AC adapter (not shown) to provide power to the switch.

Figure H-7: Five-port Ethernet switch.

H.2 Connecting to a LAN

If an instrument is connected to a LAN, it can be accessed by any computer plugged into the same LAN.

H.2.1 One instrument to LAN:

Plug the instrument into a LAN wall socket using a standard Ethernet cable. The computer that is to communicate with the instrument must be on the same LAN.

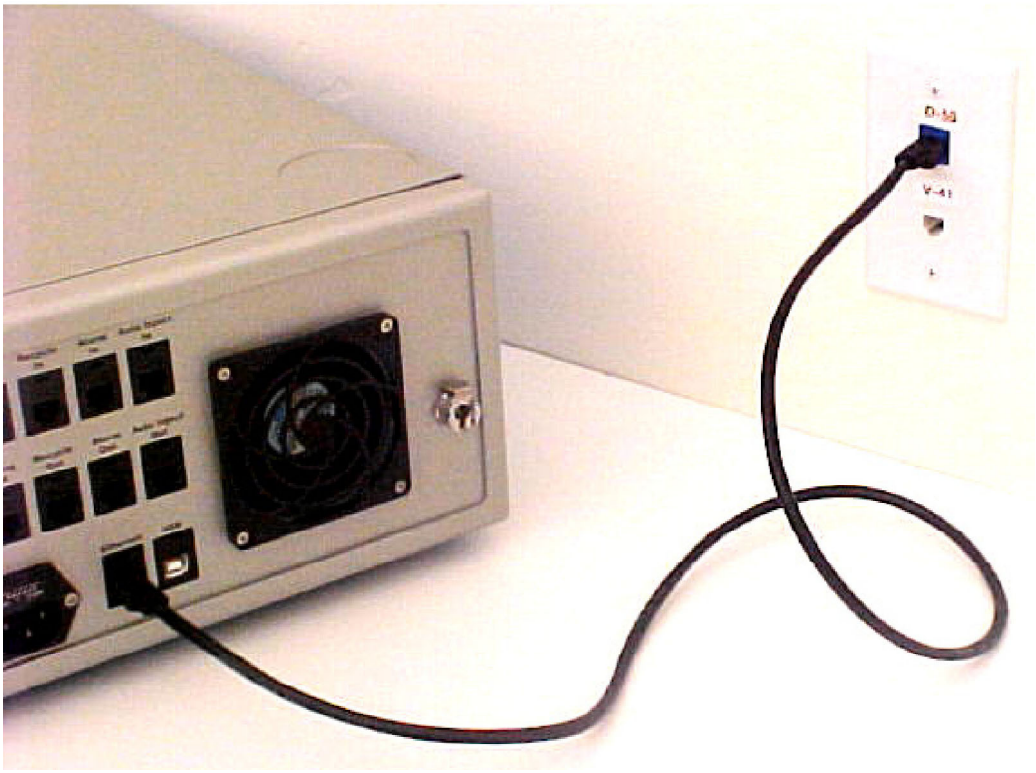


Figure H-8: Connection for one instrument to LAN.

Note:	This view is the back panel of the ViscoStar, but the same connection method is used by the DAWN HELEOS II.

H.2.2 One instrument and computer to LAN:

If there is only one LAN wall socket available for both the instrument and computer, it is necessary to use an Ethernet switch to connect both the computer and instrument to the LAN. In this configuration, the computer can access the LAN and the instrument, and the instrument can be accessed from any other computer on the LAN.

Figure H-9: One instrument and a computer can both be connected to the LAN using an Ethernet switch.

H.2.3 Multiple instruments to LAN:

If there is only one LAN wall socket available, two or more instruments can be connected to the LAN via an Ethernet switch. The instruments can be accessed via any computer on the LAN.

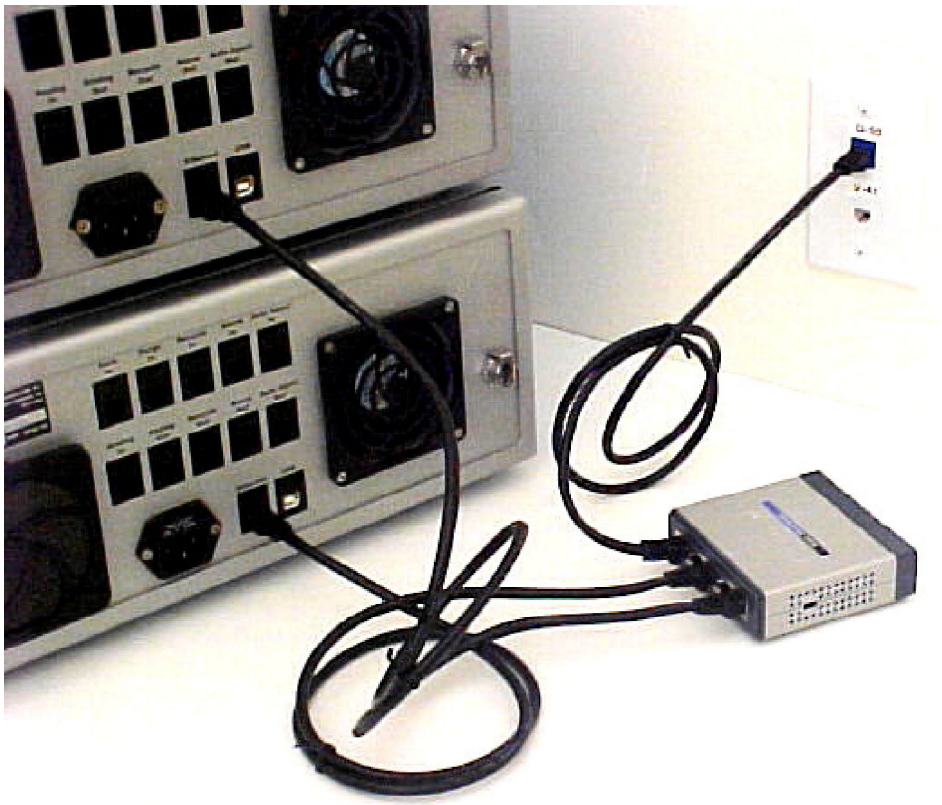


Figure H-10: Two instruments connected to the LAN via an Ethernet switch.

H.2.4 Multiple instruments and computer to LAN:

If there is only one LAN wall socket available for multiple instruments and a computer, it is necessary to use an Ethernet switch to connect both the computer and instruments to the LAN. In this configuration, the computer can access the LAN and the instruments, and the instruments can be accessed from any other computer on the LAN.

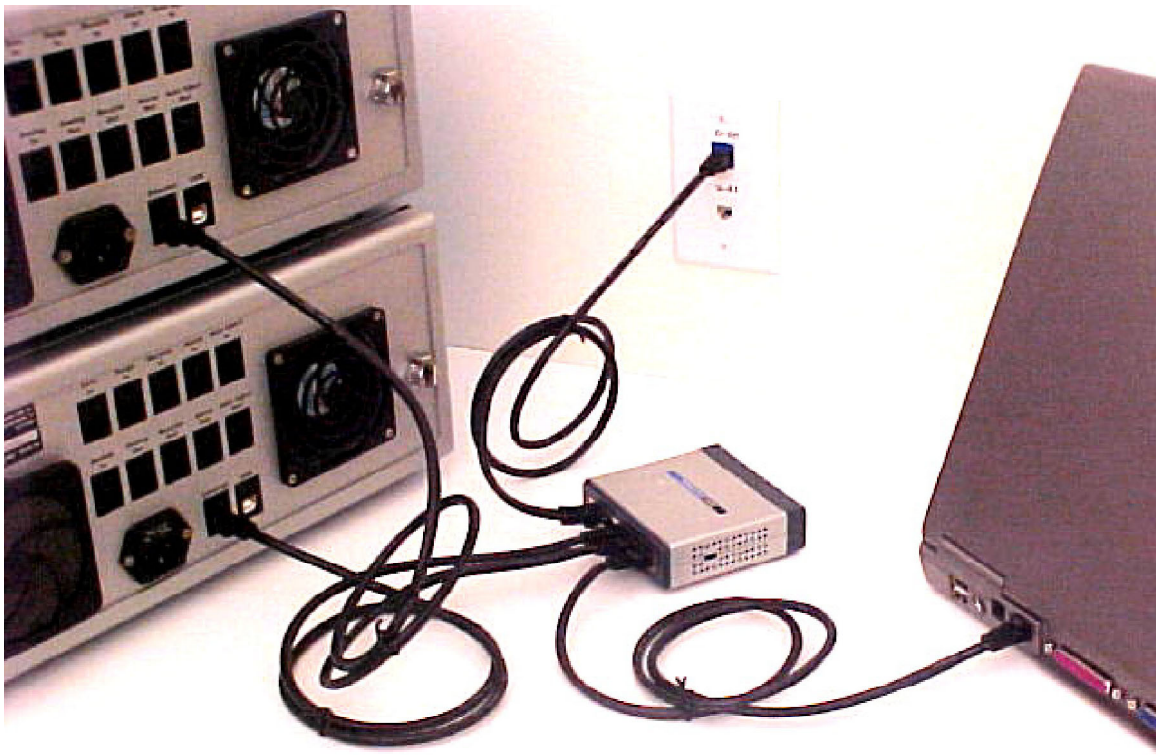


Figure H-11: Two instruments and a computer connected to the LAN via an Ethernet switch.

H.3 Connecting via USB

If it is not possible or desired to have the instruments connected to a LAN, it is possible to connect to the instruments via USB. In this way, the instruments can be isolated from the LAN, even while the computer maintains its own Ethernet connection with the LAN.

H.3.1 One instrument to USB via a crossover cable:

Connect the yellow crossover cable from the instrument to the Ethernet to USB adapter. Plug the Ethernet to USB adapter into an available USB port on the computer. You may be prompted to install drivers for the Ethernet to USB adapter the first time it is plugged into the computer. To install the drivers, insert the CD that came with the adapter and follow the Windows instructions.

Please note that the network communication setting in the Communications tab of the instrument display is Ethernet for this configuration, and not USB Virtual Ethernet.

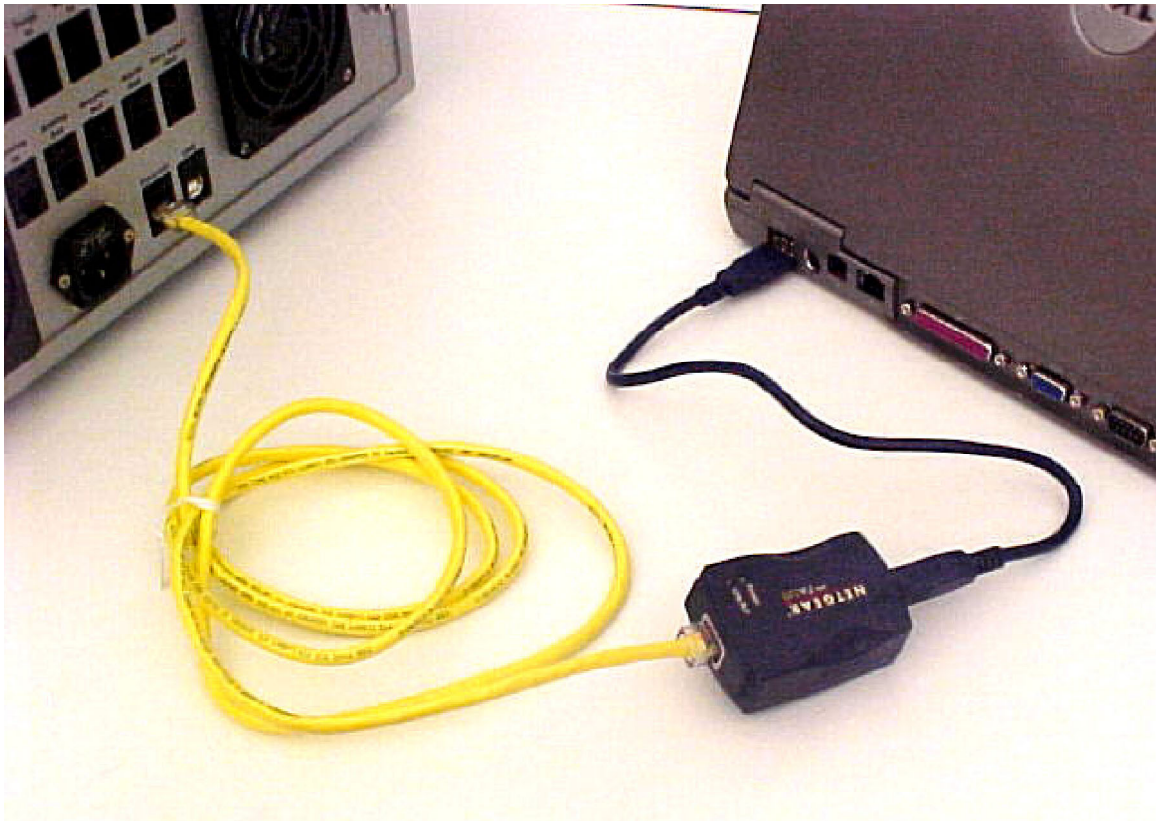


Figure H-12: One instrument to USB via yellow crossover cable.

H.3.2 One instrument to USB using an Ethernet switch:

Connect the instrument to the Ethernet switch using a standard Ethernet cable. Then connect the Ethernet switch to the Ethernet to USB adapter using a standard Ethernet cable. Plug the Ethernet to USB adapter into an available USB port on the computer. You may be prompted to install drivers for the Ethernet to USB adapter the first time it is plugged into the computer. To install the drivers, insert the CD that came with the adapter and follow the Windows instructions.

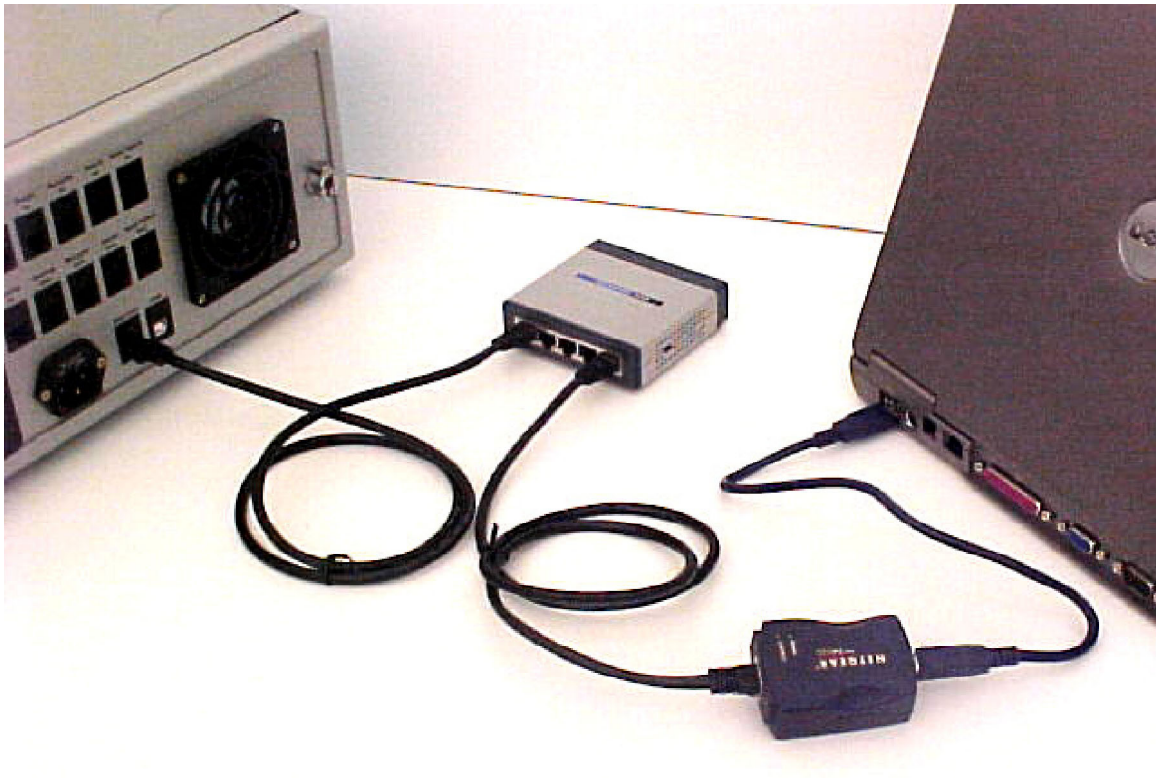


Figure H-13: Connecting one instrument to USB using an Ethernet switch.

H.3.3 Multiple instruments to USB:

Two or more instruments can be connected to USB using an Ethernet switch. Use a standard Ethernet cable to plug each instrument into the Ethernet switch. Then connect the Ethernet switch to the Ethernet to USB adapter using a standard Ethernet cable. Plug the Ethernet to USB adapter into an available USB port on the computer. You may be prompted to install drivers for the Ethernet to USB adapter the first time it is plugged into the computer. To install the drivers, insert the CD that came with the adapter and follow the Windows instructions.

Figure H-14: Connecting two or more instruments to USB using an Ethernet switch and Ethernet to USB adapter.

H.4 Connecting via Ethernet when not on a LAN.

If the computer is not on the LAN, it is possible to use the Ethernet port directly to connect to the instruments.

H.4.1 One instrument to computer not on LAN using crossover cable:

Connect the yellow crossover cable from the instrument directly to the Ethernet port on the computer.

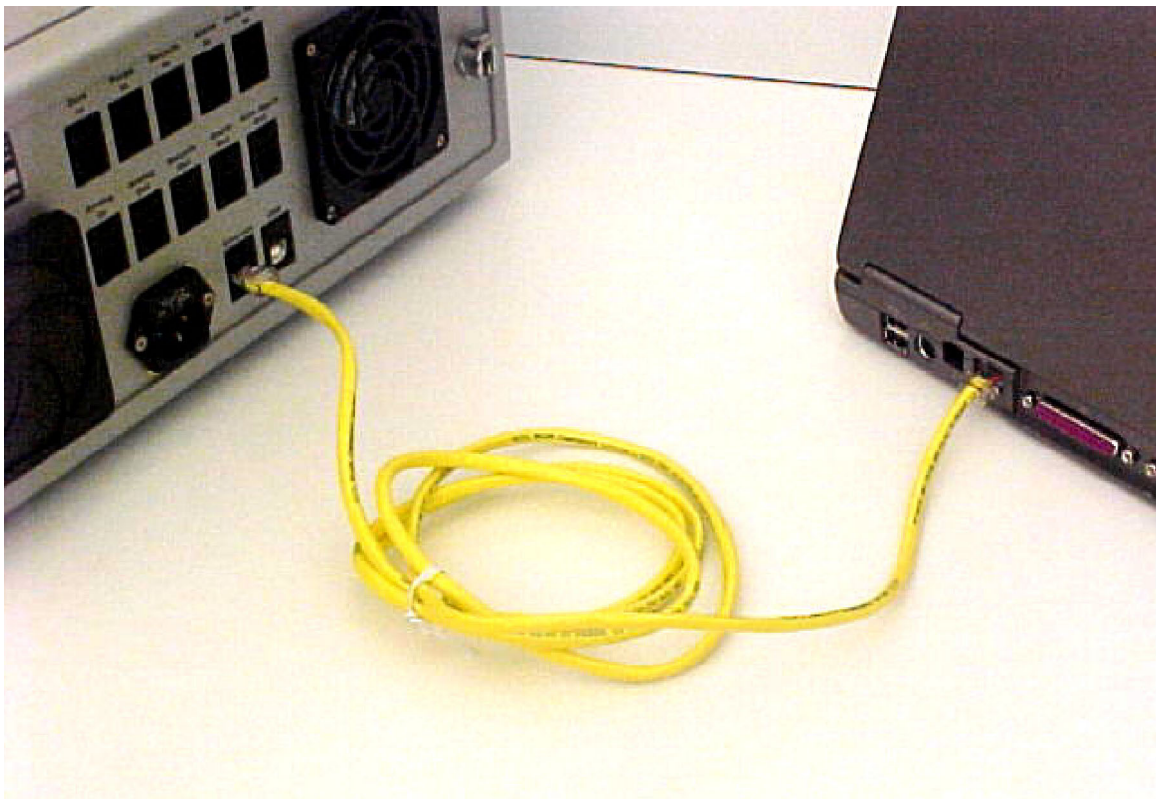


Figure H-15: Connecting one instrument directly to a computer that is not on the LAN using the yellow crossover cable.

H.4.2 One instrument to computer not on LAN using an Ethernet switch:

Connect the instrument to the Ethernet switch using a standard Ethernet cable. Then connect the switch to the computer Ethernet port using a standard Ethernet cable.

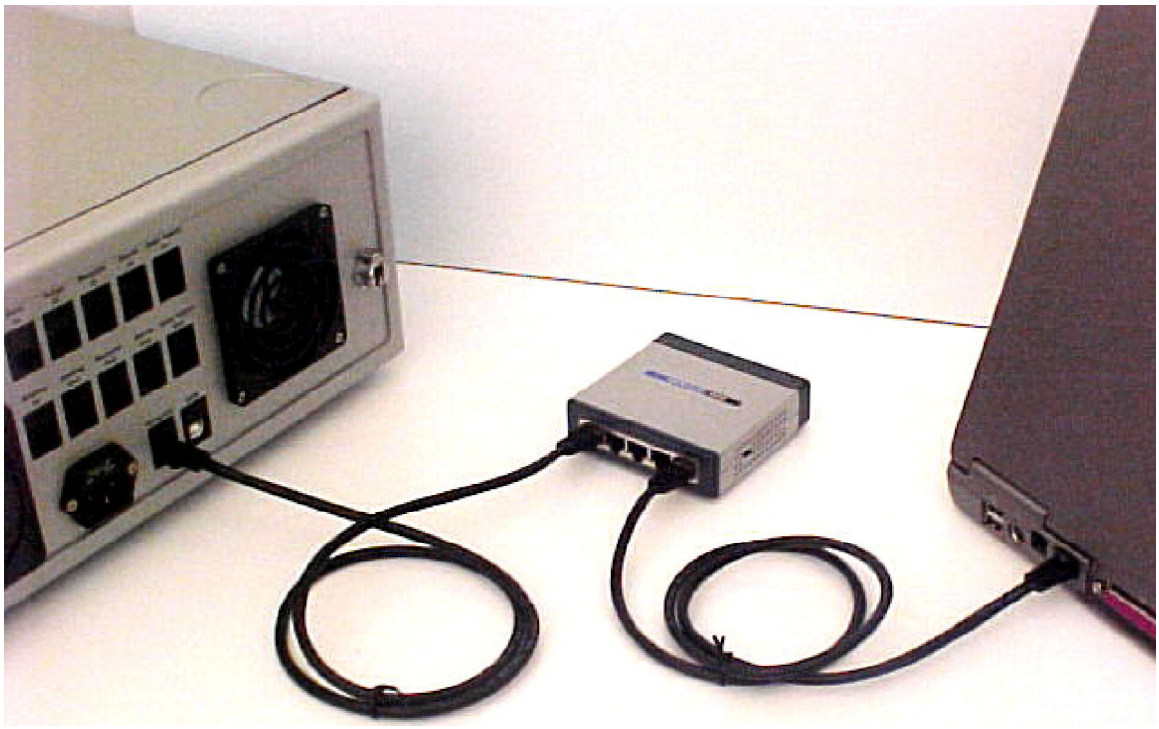


Figure H-16: Connecting one instrument to the computer using an Ethernet switch.

H.4.3 Multiple instruments to computer not on LAN using an Ethernet switch:

Connect each instrument to the Ethernet switch using a standard Ethernet cable. Then connect the switch to the computer Ethernet port using a standard Ethernet cable.

Figure H-17: Connecting multiple instruments to a computer not on the LAN using an Ethernet switch.

H.5 Instrument Network Settings

Figure H-18 shows the standard settings on the instrument front panel that will work with all of the above connection schemes.

As shown in Figure H-18, to set the IP address there is a choice of **Obtain an IP address automatically**, to use an associated DHCP server, or **Use the following IP address:** to set a static IP address. In general, this setting can be left to DHCP. With DHCP, once the instrument is connected to a computer or LAN, the IP address and subnet mask will be assigned automatically. This will even work with the USB connections described in "Connecting via USB" on page H-13. When using DHCP, it might take several minutes for the IP address to be assigned. During this time, the IP address and subnet mask will read 0.0.0.0. Once the IP address and subnet mask have been assigned, both will be automatically updated, and should no longer read 0.0.0.0. At this point, it should be possible to connect to the instrument from the computer.

If you wish to use a static IP address and subnet mask, please contact your IT department to obtain a valid address and mask.

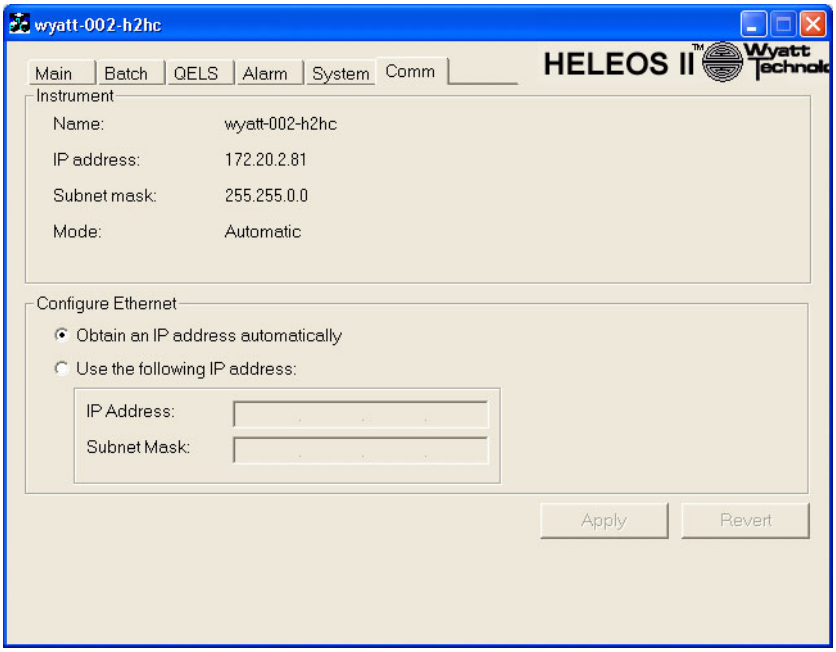


Figure H-18: Standard settings on instrument front panel for instrument connectivity.

H.6 Accessing instruments with ASTRA V

To access an instrument connected via a LAN, USB, or via Ethernet when not on a LAN using ASTRA V, please refer to the *ASTRA V for Windows User's Guide*.

H.7 Trouble-shooting and diagnostics

If you are experiencing instrument connectivity problems, please go over these steps. If you still cannot connect to your instrument after going over this section, please contact Wyatt Technology for assistance or visit www.wyatt.com for the latest troubleshooting guides.

H.7.1 Verifying instrument connections

Please verify that the instrument is communicating with the computer. Open a Windows cmd prompt, as shown in Figure H-19. At the command line, type "ping" plus the IP address of the instrument as shown on the instrument front panel (see Figure H-18). If the instrument is connected properly, the result should be similar to that shown in Figure H-19.

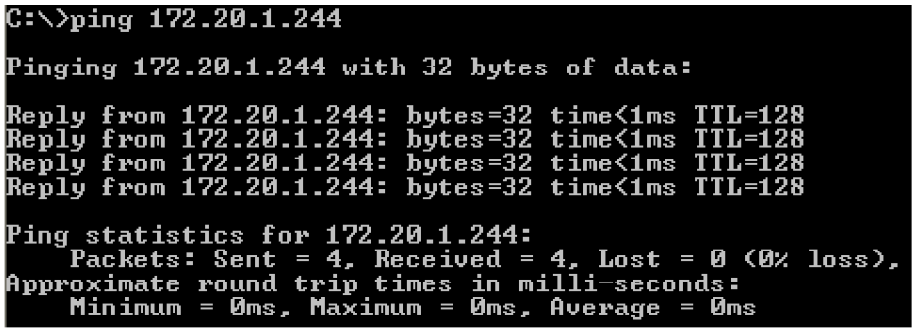


Figure H-19: Using ping to verify the instrument connection.

If the instrument is not connected properly, the result should be similar to that shown in Figure H-20.

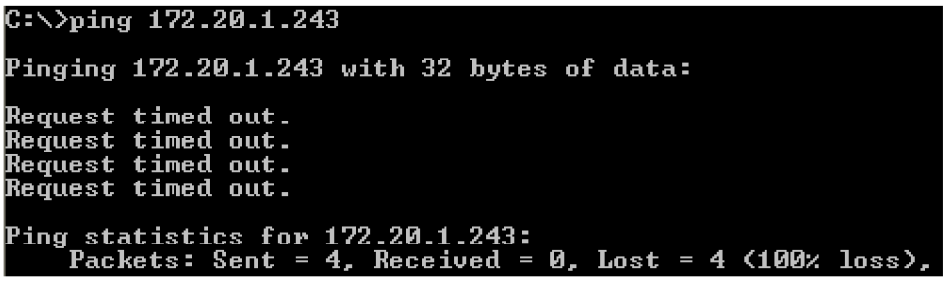


Figure H-20: Failure to connect to instrument using ping.

Index