

DAWN® User's Guide

M3220 Rev. B



WYATT
TECHNOLOGY

Wyatt Technology Corporation
6330 Hollister Avenue
Santa Barbara, CA 93117
Tel: +1 (805) 681-9009
Web: www.wyatt.com

Notices

DAWN User's Guide

M3220 Rev. B

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

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

Using this Manual

This user's guide describes how to set up and use the DAWN® multi-angle light scattering (MALS) laser photometer system hardware. Please refer to the *ASTRA® User's Guide* for details on data analysis.

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

Manual Conventions

The IUPAC Definition Committee specifies the term molar mass for the sum of the atomic weights of all atoms in a mole of molecules. The term molecular weight is often used in the literature. The term molar mass will be used in this manual.

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Preface

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Wyatt Technology Corporation

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

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

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

If you have a question about your DAWN, please refer to this manual or consult the *ASTRA User's Guide*. For additional assistance, please contact Wyatt Technology or take advantage of the online technical support options available to our customers in the following section.

Wyatt Technical Support

Wyatt Technical Support offers a variety of support options for maximizing the utility of your DAWN.

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

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

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

Wyatt Technology Technical Support Contact Information

Electronic mail address: support@wyatt.com

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

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

European customers can reach Wyatt Technology Europe support at support@wyatt.eu. Our Wyatt Technology UK office can be contacted directly at WTUKsupport@wyatt.com.

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

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

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

Sales Support

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

Sales Phone: (805) 681-9009

Sales email: sales@wyatt.com

1

About the DAWN Instrument

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 the above paragraph, would have strongly approved of the DAWN laser photometer, which is capable of quantifying the intensity of light scattered at 18 different angles simultaneously in a variety of configurations.

The DAWN is capable of making measurements over a wide range of molar mass, size, and concentration for the rapid characterization of macromolecules and particles from as little as 10 nm to 500 nm; though this can be extended to 1000 nm with specific conformation models.

The DAWN may be used in-line with HPLC for SEC-MALS or related chromatography techniques or field-flow fractionation to assess distributions of molar mass and size. When used without chromatography, the DAWN may be coupled to a Calypso® for analysis of molecular interactions, or in stand-alone (batch) mode to determine average values of molar mass and size. Add-on modules include embedded dynamic light scattering with the WyattQELS™ module.

In the following section, the DAWN will be introduced, the theory behind its operation expanded, and its options and software outlined. It will conclude with an overview of options and accessories, the software, and conventions when using this manual.

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Introduction to the DAWN

The DAWN Multi-Angle Light Scattering (MALS) detector performs absolute characterization of the molar mass and size of macromolecules and nanoparticles in solution, offering superb sensitivity over a wide range of molecular weight, size and concentration.



The DAWN measures the intensity of the scattered light simultaneously at 18 angles, typically between 15° and 160° depending on solvent/glass refractive indices. The DAWN can measure root mean square (RMS) radii between 10 nm and 500 nm, though this can be extended up to 1000 nm with specific conformation models. As a result, the molar mass values that can be measured range from 200 Da to 1 GDa as defined by the radius limit. Furthermore, precise measurements can be achieved with a sensitivity as low as 0.2 µg/mL, as measured on bovine serum albumin (BSA), a 66.4 kDa macromolecule.

The DAWN is available as an ambient model, a standard Peltier heated/cooled model (-15°C up to 150°C), and an ultra-high temperature model (ambient up to 210°C).

The standard 658 nm red laser may be replaced in the factory with an infrared (IR) laser operating at 785 nm. The IR laser minimizes or prevents sample fluorescence to ensure correct molar mass and size measurements on samples that fluoresce under 658 nm excitation. Narrow bandpass fluorescence-blocking filters are also available.

A DAWN 8 model is available that measures the intensity of the scattered light simultaneously at 8 angles, typically between 21° and 150° depending on solvent/glass refractive indices. The DAWN 8 is available as an ambient or heated/cooled model.

Two types of light scattering measurements are available in the DAWN:

- **Classical Light Scattering / Static Light Scattering (SLS)/ Multi-Angle Light Scattering (MALS)**

In a MALS measurement, the time-average intensity of the scattered light is measured as a function of angle. In the context of macromolecules, this is often called Rayleigh scattering and can be analyzed to yield the molar mass and RMS radius. Measurements of scattered intensity as a function of angle as well as concentration determine the second virial coefficient (A_2) or equilibrium dissociation constant (K_D). For certain classes of nanoparticles, classical light scattering can yield size, shape, and structure.

- **Dynamic Light Scattering (DLS) or Quasi-elastic Light Scattering (QELS) with the optional embedded WyattQELS module**

In a DLS measurement, time-dependent fluctuations in the scattered light signal are measured using a fast photon counter. DLS measurements determine the translational diffusion coefficient, from which it is possible to calculate the hydrodynamic radius of macromolecules or particles.

Light scattering can be applied in either batch or chromatography mode. Each technique has its advantages and applications. In either instance the sample may be recovered at the end of the measurement.

To utilize all the functions of the DAWN, you will need a copy of the ASTRA software, a versatile software package for the analysis of light scattering data.

For information about Wyatt HPLC Service, please refer to the *Wyatt HPLC Service User's Guide* (M1040). For information about ASTRA, please refer to the *ASTRA User's Guide* (M1006).

Understanding Static Light Scattering

The fundamental equations describing light and its interaction with matter were laid out by James Clerk Maxwell in 1865. These were simplified by John William Strutt (Lord Rayleigh) for the case of particles very small compared to the wavelength of the incident light. The extension of Rayleigh's theory to describe the scattering of light by larger macromolecules in solution is called the Rayleigh-Gans-Debye (RGD) theory of light scattering.

In a typical Rayleigh scattering experiment, a well collimated, single frequency, polarized light beam (for example, from a laser) is used to illuminate a solution containing a sample (macromolecule or nanoparticle) of interest. The electric field of the polarized light beam is preferably produced perpendicular to the plane in which the intensity and angular dependence of the subsequently scattered light is to be measured (by

convention, the polarization direction is denoted ‘vertical’ and the measurement plane ‘horizontal’). The overall intensity carries information about the molar mass, while the angular dependence within the horizontal plane carries information about the size of the macromolecule.

Intensity and Molar Mass

When a laser illuminates a macromolecule, the oscillating electric field of the light induces an oscillating dipole within it. This oscillating dipole re-radiates light. The intensity of the radiated light depends on the magnitude of the dipole induced in the macromolecule. The more polarizable the macromolecule, the larger the induced dipole, and hence, the greater the intensity of the scattered light.

Therefore, to analyze scattering from a solution of such macromolecules, it is necessary to know their polarizability relative to the surrounding medium (the solvent). This may be determined from a measurement of the change, Δn , of the solution's refractive index n with the molecular concentration change, Δc , by measuring the dn/dc (equal to $\Delta n/\Delta c$) value using a differential refractometer such as the Optilab[®] refractometer.

When many macromolecules are in solution, each macromolecule scatters light via the aforementioned induced dipole mechanism. Hence, the intensity of the scattered light is proportional to the concentration of the macromolecules in solution; twice as many molecules scatter twice as much light.

Consider the important case where two monomers aggregate to form a dimer in solution (Figure 1-2). The initially separate two monomers are constantly buffeted by solvent molecules, and undergo random motion known as Brownian motion. This results in randomness to the phase of the scattered light such that the light from the two separate monomers is incoherent. Averaged over time, the scattered intensities add as one expects from two independently moving macromolecules: $1 + 1 = 2$.

However, once monomers form a dimer, the two monomers move together (Figure 1-3). The scattered light from one monomer now has a definite phase relationship with the scattered light from the other. In other words, the scattering is coherent. The net result is that the

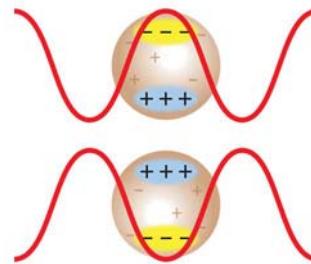


Figure 1-1: Oscillating dipole

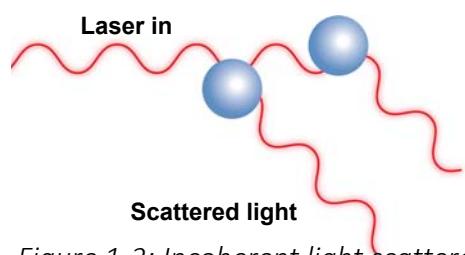


Figure 1-2: Incoherent light scatterers

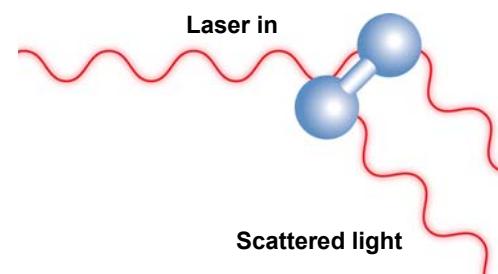


Figure 1-3: Coherent light scatterers

light scattered from the dimer is twice as intense as the total light scattered from two independent monomers. Simply by doubling the molar mass, even while keeping the concentration the same, the intensity of the scattered light doubles. The intensity of light scattered by a molecule, measured by means of a multi-angle light scattering (MALS) detector such as the DAWN, is directly proportional to the molar mass. Light scattering thus represents a powerful technique for determining the molar mass of macromolecules in solution as well as monitoring the presence and formation of aggregates.

Angular Dependence and Size Measurements

Macromolecules much smaller than the wavelength of the incident light can be treated as though they were essentially point scatterers. For such very small molecules, the intensity of light scattered into the plane perpendicular to the polarization of the incident light is independent of scattering angle. It is the same at every scattering angle—the macromolecule scatters light isotropically.

For larger macromolecules, however, light scattered from different parts of the macromolecule reach the detector with different phases (Figure 1-4). This can lead to the destructive or constructive interference of the overall light wave at the detector. The net result is that the intensity of light scattered varies with angle; light scattered at large scattering angles (further away from the direction of the laser beam propagation) is reduced relative to that at small scattering angles. This case is described by the RGD theory mentioned earlier.

If the angular dependence of the scattered light is measured in the horizontal plane, it is possible to determine the size of the molecule. This size is known as the root mean square (RMS) radius, or sometimes the “radius of gyration”, R_g . The RMS radius is a measure of its size weighted by the mass distribution about its center of mass (Figure 1-5). If the molecule's conformation is determined to belong to a specific class (for example,

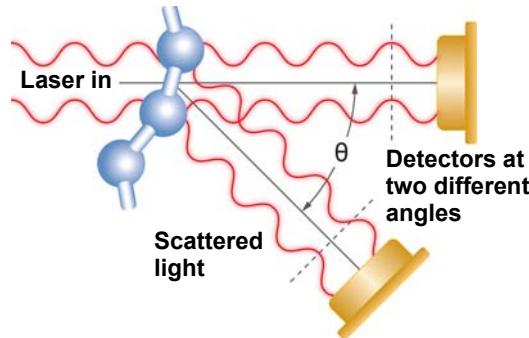


Figure 1-4: Variation of intensity from different scattering angles

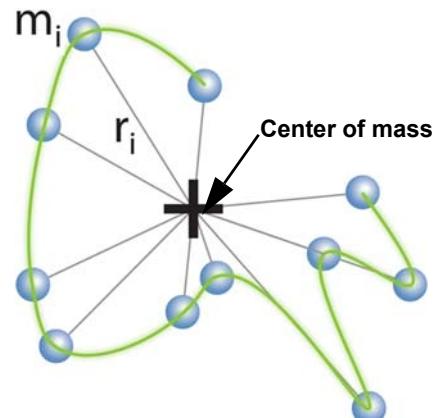


Figure 1-5: Mass distribution around a center of mass to determine RMS radius

random coil, sphere, or rod), the RMS radius can be related to its geometrical dimensions. A sample containing a broad distribution of molecular masses may be separated by SEC or GPC, and light scattering data acquired at each elution volume to determine molar mass and RMS radius. Measured RMS radius may be plotted against the correspondingly measured molar mass to determine the sample's conformation.

Instrument Options and Accessories

The DAWN has two laser options. The standard 658 nm red laser may be replaced in the factory with an infrared (IR) laser operating at 785 nm. The IR laser minimizes or prevents sample fluorescence to ensure correct molar mass and size measurements on samples that fluoresce under 658 nm excitation. Narrow bandpass fluorescence-blocking filters are also available.

The DAWN 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.
- **Ultra-High Temperature (UHT)** – The read head may be heated from approximately 10 °C above ambient temperature to 210 °C. Temperature can be controlled to within 0.01 °C and is accurate to ±1 °C. See [Ultra-High Temperature Option on page 158](#).
- **Peltier Heated/Cooled (HC)** – The read head may be cooled down to –15 °C or heated up to 150 °C. Temperature can be controlled to within 0.01 °C and is accurate to ±1 °C. A dry gas line is required for operating below 20 °C. Additionally, a temperature regulated (TR) model is available with a temperature range from approximately 20 °C to 70 °C. See [Temperature Controlled Options on page 154](#).

A dry gas purge connector is located on the back of the HC model of the DAWN to prevent condensation on an instrument's read head when operating below ambient temperature.

Note:	See Preventing Condensation (at Lower Temperatures) on page 124 , TN9001: <i>Operating Wyatt Instruments in a Cold Room</i> and TN9006: <i>Preventing Condensation in Wyatt Detectors</i> for additional information regarding safe operation of the instruments at sub-ambient temperatures.
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The **Wyatt COMET™** is an embedded module that comes installed with all DAWN instruments for cleaning the flow cell in situ. The COMET contains an ultrasonic transducer that cleans the flow cell automatically by agitating the fluid and loosening particles from the wetted glass surfaces. The COMET operation may be automated via ASTRA software. The use of the COMET is described in this manual.

The DAWN 8 model measures the intensity of the scattered light simultaneously at 8 angles, typically between 21° and 150° depending on solvent/glass refractive indices. The DAWN 8 is available as an ambient or heated/cooled model.

The following are add-on modules and accessories for the DAWN:

- **WyattQELS—Dynamic Light Scattering (DLS)** – The WyattQELS is an embedded module that measures dynamic light scattering signal (DLS) is also known as quasi-elastic light scattering, QELS). Time-dependent fluctuations of scattered light are measurements from which the hydrodynamic radius of macromolecules or nanoparticles can be derived. This is described in more detail in [DLS Using the WyattQELS on page 142](#).
- **microCuvette Kit** – The microCuvette™ accessory is designed for making batch static and dynamic light scattering measurements with as low as 30 µL sample volume. The use of the microCuvette is described in [microCuvette Accessory Kit on page 81](#).
- **Wyatt Orbit™** – The Wyatt Orbit Recycling system allows the eluent from the HPLC system to be directed either to the waste reservoir or back into the solvent reservoir. It is fully programmable through the ASTRA software. In conjunction with the Calypso, the Orbit is used to programmatically select between two wash solvents during automated post-experiment clean-up. The use of the Orbit is described in the *Orbit User's Guide (M1020)*.
- **WISH™ High-Pressure Injection System** – This accessory is ideal as a manual injector for chromatography work or for flowing batch (unfractionated) samples with the DAWN. Standard loop sizes are 10 µL, 100 µL, and 1 mL. The use of the WISH injector is described in the *WISH Injector User's Guide (M1010)*.
- **Microbatch Kit** – This kit provides finger-tight PEEK fittings, PEEK tubing, Luer adapters, filters, and disposable syringes for injecting samples directly into the flow cell of a DAWN in stop-flow mode. This technique requires a minimum volume of 300 µL and can also be used for Zimm plots, dn/dc determination, or calibration of an Optilab RI detector.
- **DLS Compatibility Kit** – This kit lets you connect your DynaPro NanoStar® or the Mobius® to a DAWN or miniDAWN® instrument for online DLS measurements. The DLS Compatibility kit is described in the *NanoStar User's Guide (M3300)* or the *Mobius User's Guide (M3001)*.
- **Solaris™** – This solvent protection system is ideal for aqueous applications by preventing microbial growth in the solvent reservoir via UV sterilization. Compatible with 1-5L amber bottles with GL-thread,

this easy-to-use system is an alternative to commonly utilized sodium azide or other preservatives when potential salt interactions are possible.

- **Inline Filter Kits** – When operating a DAWN in a SEC-MALS or FFF-MALS configuration, inline filters are critical to ensuring particle-free mobile phase and optimal light scattering measurements. Both aqueous and organic inline filters are available from Wyatt and are designed to be placed between an HPLC pump and the injector valve. Please see TN3504: *Wyatt Inline Filter Installation and Use* for more information.

Table 1-1: Instrument add-on modules and accessories

Item	Part Number
COMET	WIC
WyattQELS	WIQ
microCuvette Kit	WMCW
Wyatt Orbit	WORB
WISH High Pressure Injection Kit	WISH
Microbatch Kit	900003
DLS Compatibility Kit	WDPC-107
NanoFilter Kit	WNF-00
SOLARIS Solvent Protection System	WS
In-Line Filter Kit aqueous	900002-1
In-Line Filter Kit PVDF, not for DMF, DMAc, DMSO, or Acetone	900002-2
In-Line Filter Kit PTFE, not for benzene, toluene, xylene, or halogenated solvents	900002-3

The Software

Data collection and analysis are performed with the ASTRA software. ASTRA analyzes light scattering data from the DAWN to calculate molecular properties such as molar mass and molar mass distributions, root-mean-square (RMS) radius, and second virial coefficient A_2 . In addition, ASTRA provides advanced calculations, such as Protein Conjugate and Copolymer Analysis, Particles and Number Density Calculations, as well as Molecular Conformation and Branching Analysis. If the WyattQELS DLS module has been installed in the DAWN, ASTRA also analyzes the dynamic light scattering data to determine hydrodynamic radius and radius distributions.

Note: ASTRA 7.3 or higher is required for data collection from your DAWN instrument.

Please see the *ASTRA User's Guide* for information about the software and details of data collection and analysis.

Using this Manual

This User's Guide describes how to set up and use the DAWN laser photometer. See the *ASTRA User's Guide* for details on data analysis.

Manual Conventions

The IUPAC Definition Committee specifies the term *molar mass* for the sum of the atomic weights of all atoms in a mole of molecules. The term *molecular weight* is often used in the literature. You will see both terms used in this manual.

How the Manual Is Organized

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

[Chapter 1, About the DAWN Instrument](#) introduces the DAWN and describes the support options available from Wyatt Technology.

[Chapter 2, Instrument Description](#), gives you a tour of the instrument.

[Chapter 3, Quick Start](#), contains a summary of instructions for setting up the DAWN quickly and with minimal instruction.

[Chapter 4, Installation and Setup](#) takes you through the necessary first steps for unpacking, connecting, and testing the instrument.

[Chapter 5, Using the Front Panel Display](#) shows you how to navigate and change settings in the DAWN Display Window or from a remote terminal.

[Chapter 6, In-Line Operation with HPLC](#), describes using the DAWN as an in-line detector for HPLC.

[Chapter 7, Off-Line Operation](#), describes how to make batch measurements with the DAWN.

[Chapter 8, Service and Maintenance](#) has procedures for keeping the instrument in good working order, and includes flow cell cleaning.

[Chapter 9, Troubleshooting](#), provides details about troubleshooting procedures for the DAWN.

[Appendix A, Acronyms and Abbreviations List](#), contains a list of acronyms and abbreviations used in this manual.

[Appendix B, DLS Using the WyattQELS](#), describes procedures for using the DLS option.

[Appendix C, Temperature Controlled Options](#), describes the Heated/Cooled version of the DAWN and its operation.

[Appendix D, Ultra-High Temperature Option](#), describes the Ultra-High Temperature version of the DAWN and its operation.

[Appendix E, Interference Filter Option](#), describes the use of interference filters for keeping non-laser wavelengths from reaching the photodiodes.

[Appendix F, DAWN Specifications](#), provides the electrical, optical, and environmental specifications for the DAWN and lists thermal and chemical properties, refractive indices, and scattering angles of solvents for the fused silica and F2 flow cells.

[Appendix G, Instrument Connectivity](#), covers connecting the DAWN to either a network through the Ethernet, or to a host PC through the Ethernet-to-USB converter.

[Appendix H, List of Warnings](#) lists laser and power warnings to be aware of when using the DAWN.

2

Instrument Description

This chapter provides an overview of the DAWN features.

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Front Panel

The front panel (see Figure 2-1) contains the main power button (On/Off), the fluid connections for the DAWN, and the display window and controls for operating the instrument and monitoring data.



Figure 2-1: DAWN Front Panel

In/Out Fluid Connectors—The front panel fluid connections provides both inlet and outlet connections compatible with standard 10-32 chromatography fittings.

Multi-touch Display—The LCD panel allows you to monitor, control, and configure the DAWN. [Using the Front Panel Display on page 51](#) describes how to use the front panel display.

On/Off Button—The instrument has a front-facing on/off power button. When the instrument is powered on, the button is illuminated. Pressing the switch while off powers on the instrument. Pressing the switch while on opens a confirmation window for turning off the instrument. Pressing and holding the power button forces the instrument to shut down.

Liquid Leak Port—The liquid leak port on the bottom of the instrument provides an exit for any liquid that leaks from internal or external fittings. When a liquid leak occurs, an internal liquid sensor activates an alarm shown on the front panel display (see [Alarm Section on page 61](#)), and activates an Alarm Out signal that may be used to shut off a pump.

The Wyatt instrument stack is designed to direct leaks toward the bottom instrument of the stack, where a drain connector can be installed to direct leaks to a waste reservoir. An adapter and Versilon® tubing are provided in the hardware kit to direct any fluid to a waste bottle. For more information, see [Attaching the Drain Port Connector on page 50](#).

Follow the instructions for cleaning after a fluid leak in the service and maintenance section of this manual. See [Leak Sensors and Cleaning After a Fluid Leak on page 135](#).

Rear Panel

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

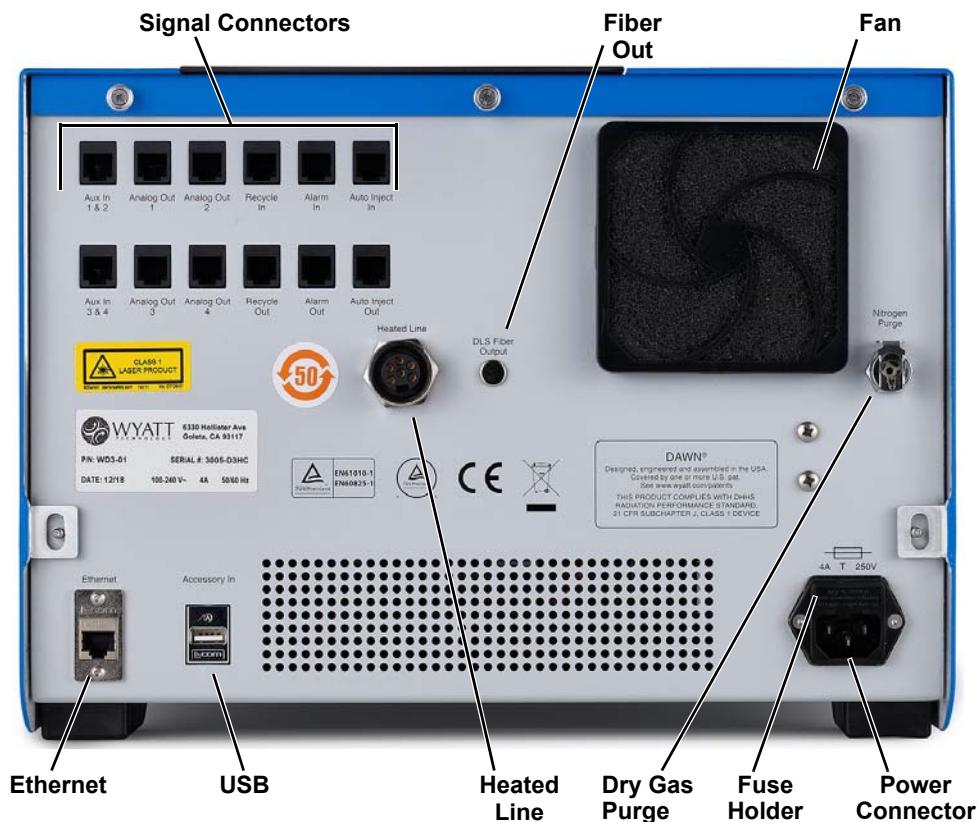


Figure 2-2: Rear panel

Fan—The fan helps to keep the instrument electronics at a constant temperature. To capture airborne particulates the fan comes with a mesh filter that should be replaced annually.

Fiber Out—This port is used to connect the DLS fiber of the DLS Compatibility Kit to the DynaPro NanoStar or Mobius.

Heated Line—A heated line connector is installed in the heated/cooled and ultra-high temperature DAWN to connect a heated line to the instrument.

Dry Gas Purge—a dry gas purge connector is available in heated/cooled DAWN to connect a dry gas source to the instrument to prevent condensation when operating below ambient temperature. Refer to [Preventing Condensation \(at Lower Temperatures\) on page 124](#) and [Temperature Controlled Options on page 154](#). Refer to TN9001: *Operating Wyatt Instruments in a Cold Room* and TN9006: *Preventing Condensation in Wyatt Detectors* for additional information regarding safe operation of the instruments at sub-ambient temperatures.

Signal Connectors—There are 12 RJ-12 connectors that provide connections to Transistor-Transistor Logic (TTL) inputs and outputs, Analog input and output, Auto Inject input and output, and a solenoid drive output. For a description of the signal connectors, see [Connecting Auxiliary Devices on page 43](#).

Power Connector—Provides power to the instrument. The power supply is universal, for immediate use with 100 V to 120 V or 220 V to 240 V power at 50 Hz to 60 Hz. The power input module contains two fuses, each rated at 4 A (one for the line voltage in and the other for the line voltage return).

Communications Connector—The Ethernet connector allows you to connect to a computer or an Ethernet network.

Accessory In USB port—This port is reserved for future use and is intended to be used to connect an optional USB accessory.

Laser

The 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.

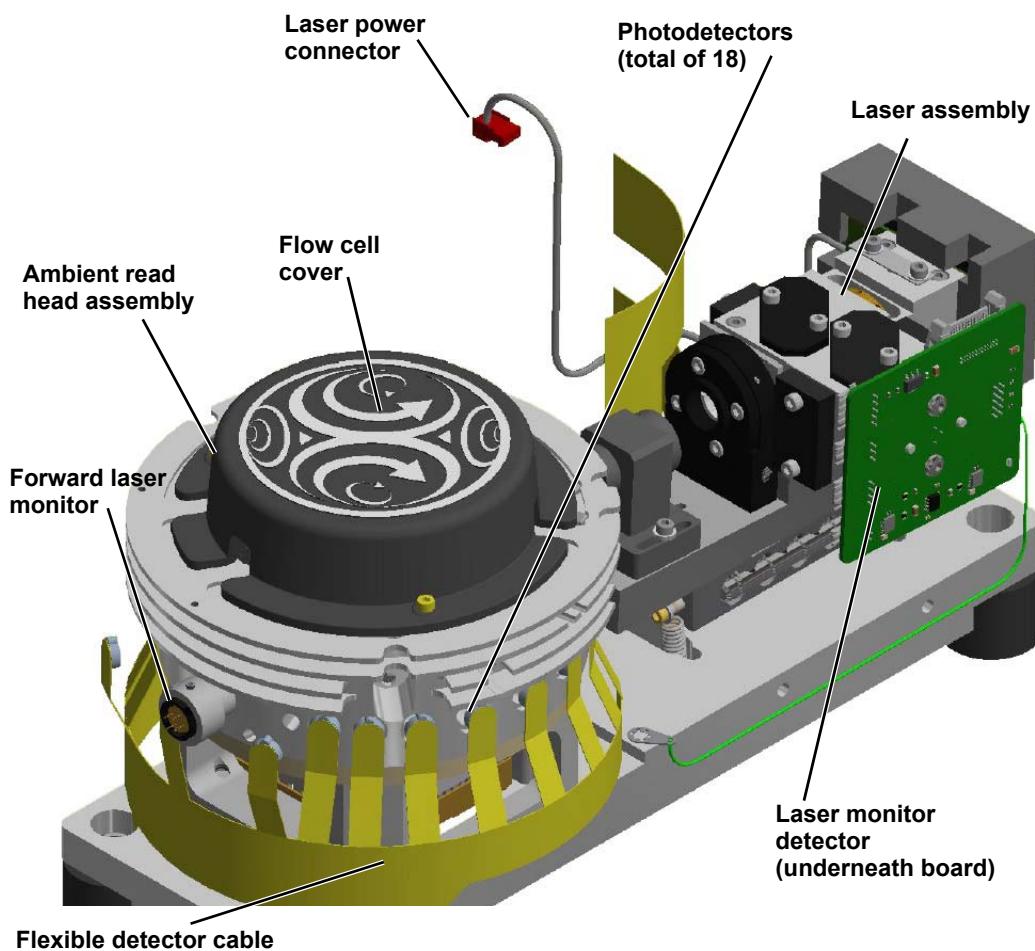


Figure 2-3: Read head and laser assemblies

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.

For additional laser specifications, refer to [Laser Specifications on page 184](#).

Laser Beam Warning

The DAWN is a Class 1 Laser Product, because the laser beam is contained within the optical bench, which itself is contained within the instrument chassis. Equipped with a laser safety interlock system to ensure the laser is off whenever the flow cell cover is removed, this instrument will emit no laser radiation and no laser radiation protective equipment must be worn under normal operation.

The interlock switches should never be defeated, and it is good laboratory practice with any laser source, irrespective of its power, to AVOID LOOKING INTO THE BEAM. Figure 2-4 shows the warning label affixed to the read head. Appendix F gives the laser specifications.

DANGER
LASER RADIATION WHEN OPEN.
AVOID DIRECT EXPOSURE TO BEAM.

Figure 2-4: Laser beam warning label

Laser Monitors

The software uses the laser monitor signal 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** is a photodiode with a low gain amplifier that measures the intensity of the beam before it enters the cell.
- The **Forward Monitor** is a photodiode with a low gain amplifier that measures the intensity of 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.
- The **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 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.

Read Head and Detectors

The read head (Figure 2-5) integrates the sample cell and the photodetectors.

Read Head Structure

The read head structure contains the 18 hybrid trans-impedance photodetectors (8 photodetectors in the DAWN 8) to measure scattered light plus one photodiode with low gain amplifier for the forward monitor. It limits the sample field of view at each detector and minimizes stray light effects. Since each photodiode's field of view is limited by its own collimator, only the center of the illuminated sample scatters light into a given detector. A solid aluminum mounting plate supports both the laser and the read head providing a single, stable optical bench.

The optics have been aligned at the factory and do not need user adjustment. The detectors are connected to an electronics module, which converts the analog signals to digital values with individual 24-bit analog to digital converters.

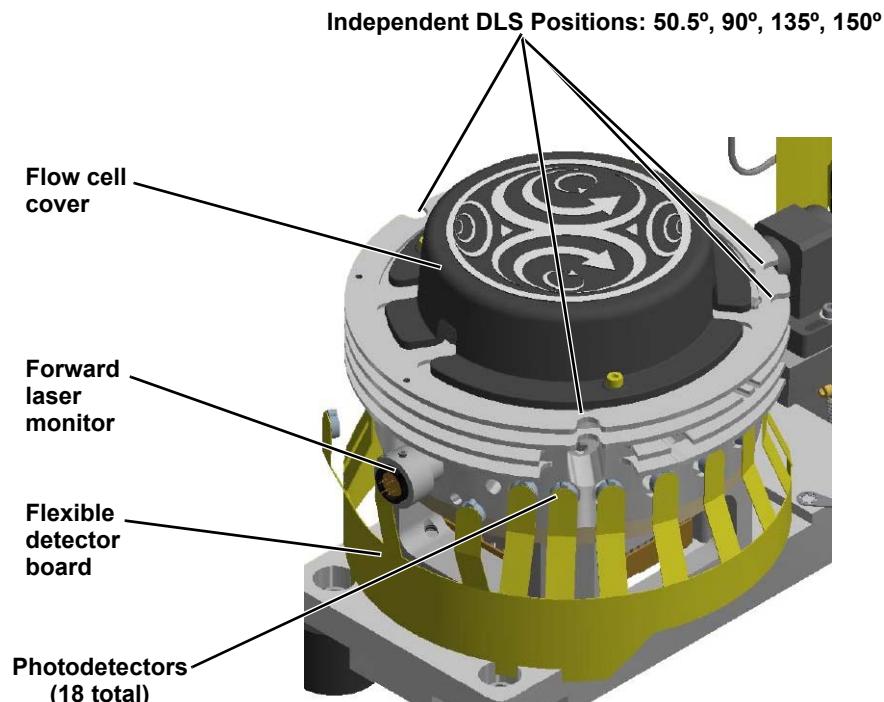


Figure 2-5: Ambient read head

With the read head covers removed to reveal the flow cell assembly and the COMET module removed, you can view the cell bore through an opening in the cell manifold (Figure 2-8).

Detector Placement

DAWN

The 18 detectors are placed at angles relative to the laser beam as listed in Table 2-1 and shown in Figure 2-6. Channel 1 is available only with mobile phases that have a refractive index greater than ~ 1.40 , such as toluene.

Table 2-1: Positions of DAWN detectors relative to laser beam

Channel Number	Fixed detector angles	Channel Number	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°

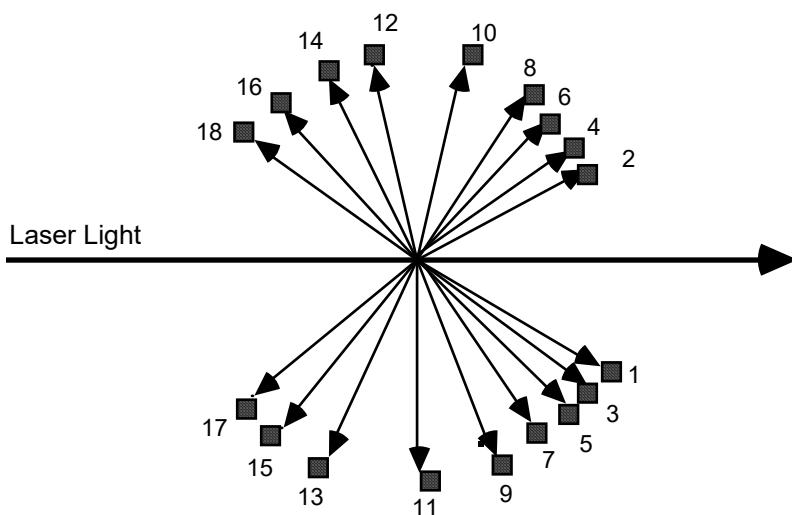


Figure 2-6: Detector locations in the DAWN

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' . For the placement of the optional DLS fiber assembly, which can be placed in 4 unique positions independent of the SLS, see [Moving the DLS Fiber to a Different Location on page 151](#).

DAWN 8

In the DAWN 8, all detectors are placed on one side of the read head as described in Table 2-2 and shown in Figure 2-8.

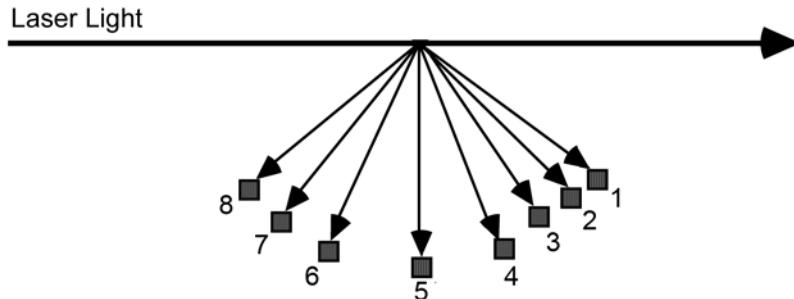


Figure 2-7: Detector locations in the DAWN 8

Table 2-2: Positions of DAWN 8 detectors relative to laser beam

Channel Number	Fixed detector angles	Channel Number	Fixed detector angles
1	32.0°	5	90.0°
2	44.0°	6	108.0°
3	57.0°	7	126.0°
4	72.0°	8	141.0°

Flow Cell

Flow Cell Design

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 approximately 70 μL . The actual scattering volume—the illuminated part of the sample that is viewed by the detectors—is approximately 0.02 μL .

The laser beam is aligned along the flow cell bore. Because the windows are recessed in the manifolds, away from the scattering volume, any scattering from stray light from the air/glass/solvent interfaces is minimized.

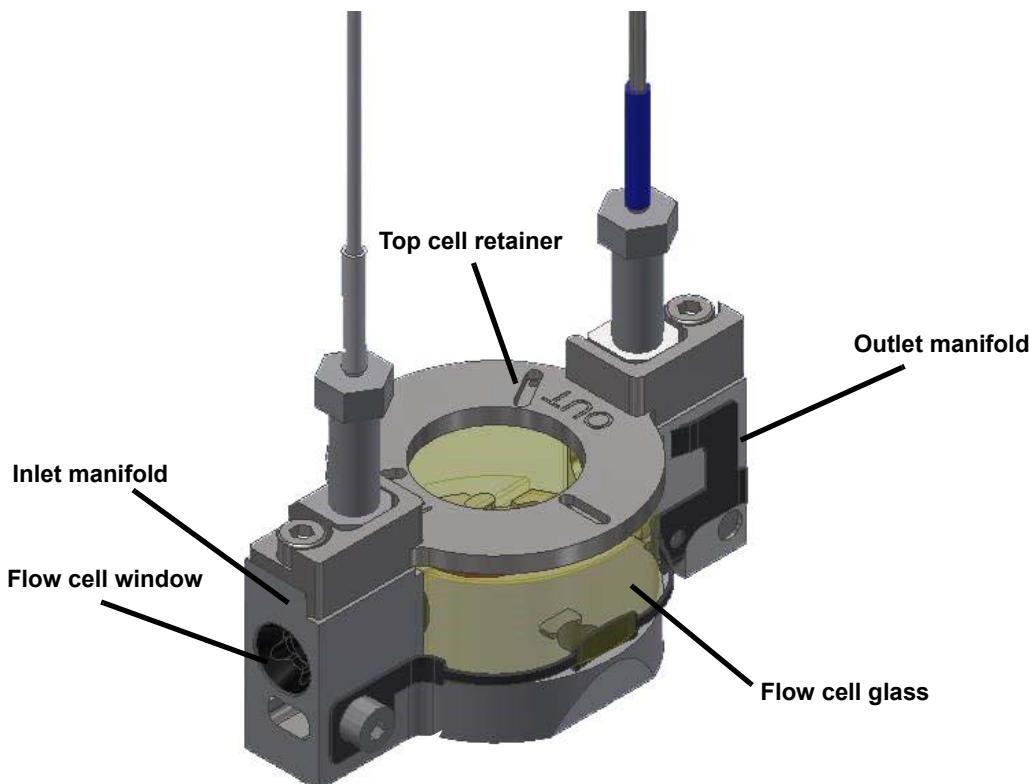


Figure 2-8: Flow cell assembly

Cell Windows

The flow cell windows protrude into the flowing stream at the inlet and outlet 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 cell window surface facing out from the cell has been coated to minimize reflections. Be careful not to scratch or expose the coating to acids after taking out the flow cell and disassembling the cell for cleaning. See [Flow Cell Maintenance on page 93](#) or refer to TN3100: *Removing the Flow Cell from a Wyatt MALS Instrument* for additional information. Also, see the video tutorial on our [Support Center](#).
-

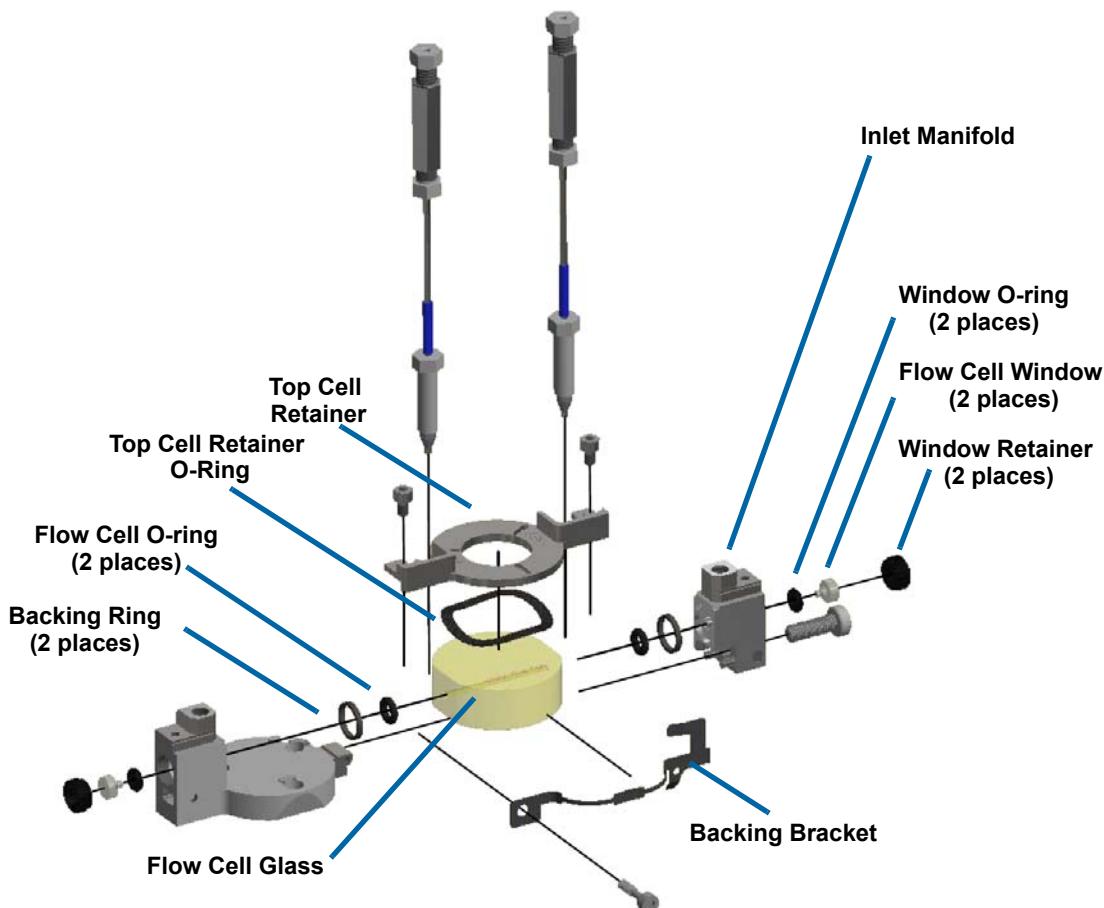


Figure 2-9: Exploded view of the flow cell assembly
(-15 °C to +80 °C configuration)

WARNING: 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. See [Appendix C, Temperature Controlled Options](#) or [Appendix D, Ultra-High Temperature Option](#) for information regarding flow cell manifold configurations for high temperature applications.

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 over a wider range of angles than the fixed read head detector angles. Figure 2-10 shows a detail of the liquid/glass interface and rays scattering from the laser-illuminated sample.

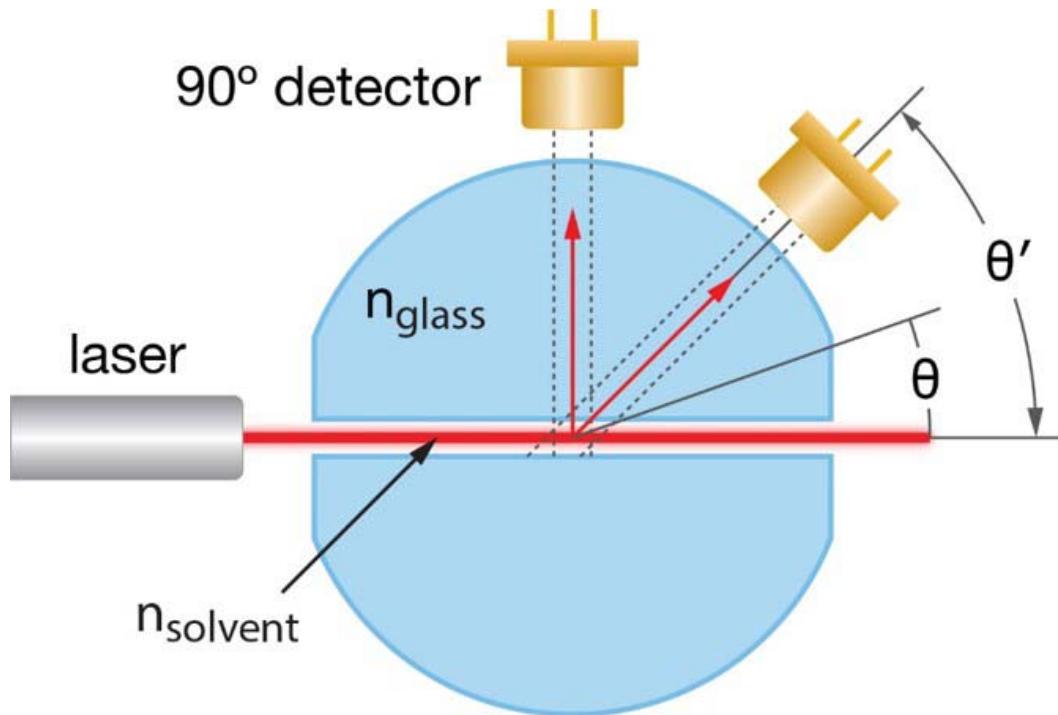


Figure 2-10: Flow cell refractions

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

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

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

$$(2) \quad n_{\text{liquid}} \cos(\theta) = n_{\text{glass}} \cos(\theta')$$

As a result of refraction, the light detected by the photodiode positioned at θ' is in fact the light scattered at an angle θ . By virtue of the flow cell design a greater angular range of scattered light can be detected than would be possible for a simple concentric flow cell design. The flow cell glass and flow cell windows are made of either fused silica or F2 glass. See [Flow Cell Properties on page 187](#) for additional information and properties of each glass type.

Accessible Available Detectors

Because of the refraction of scattered light passing from the solvent into the glass cell, some fixed detector angles are inaccessible. For example, in water using a fused silica flow cell and at a laser wavelength of 660 nm, detector 2 measures at an angle of 13.0°, while the read head detector itself is positioned at an angle of 28° in the instrument.

-
- Note:** Detector 1 in the DAWN is only available when performing measurements in solvents with a refractive index greater than ~1.40, such as toluene. In the DAWN 8 the detectors correspond to detectors 3, 5, 7, 9, 11, 13, 15, and 17 of the DAWN. Therefore all detectors are available in all solvents.
-

The ASTRA software will select the appropriate detectors based on these considerations and the solvent specified. See [Flow Cell Properties on page 187](#) for additional information and properties of each glass type.

3

Quick Start

This chapter provides basic setup procedures for experienced users who want to start the DAWN installation process before reading the entire manual.

Detailed descriptions and important use instructions concerning the DAWN are provided in the chapters that follow.

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Quick Start Setup Guide for the DAWN

This section outlines the setup procedures for those who are already familiar with the operation of the instrument and want to begin using the DAWN as soon as possible. More details about setting up the DAWN are provided in [Installation and Setup on page 38](#).

See [Instrument Description on page 22](#) for descriptions of the front and rear panel controls and connections.

See [Using the Front Panel Display on page 51](#) for details about the display interface.

Note: The DAWN can be safely stacked above or below other Wyatt instruments.

- 1. Connect the power cable**—The power outlet is located on the DAWN rear panel (see Figure 2-2). The power supply is universal, for immediate use with 100 V to 120 V or 220 V to 240 V power at 50 Hz to 60 Hz.
- 2. Establish network communications**—Connect the DAWN directly to a computer or local area network (LAN) using the provided Ethernet cable. (See [Instrument Connectivity on page 190](#).)
- 3. Install software:**
 - a.** For in-line use with chromatography, install ASTRA and either the Wyatt HPLC Service (see the *TN1501 HPLC Service Quick Guide* for more information) or the appropriate chromatography software for your HPLC. See the appropriate software manual for installation instructions.
 - b.** For off-line use (for example, batch MALS measurements or calibrating the instrument), install ASTRA. See your *ASTRA User's Guide* for complete installation instructions. The DAWN is compatible with ASTRA 7.3 or higher.
- 4. Power ON the DAWN**—Allow at least 30 minutes for the instrument electronics to warm up completely.
- 5. Calibrate with toluene**—Make fluid connections to the IN and OUT ports that are located behind the door on the front panel of your instrument (see [DAWN Front Panel on page 23](#) and details in [Syringe Pump Infusion on page 77](#)). The DAWN is shipped with chromatography-grade toluene in the flow cell that can be used for calibration. Ensure that the forward monitor is at 100 % to indicate that no air bubbles are present. If not, the calibration should be undertaken with fresh solvent.

If the WyattQELS module has been installed, align the DLS optical fiber to the maximum count rate as provided on the COP prior to performing a toluene calibration of the static light scattering detector. See [Aligning the Optical Fiber on page 149](#).

Inject 0.02 μm filtered HPLC-grade toluene into the flow cell and observe the forward monitor graph on the front panel display to ensure no air is present. Follow the steps in [Off-Line Operation on page 75](#) to run the calibration method in ASTRA and check that the calculated calibration constant is within 5 % of the value on the COP.

- 6. Flush the DAWN.** Use 20 mL of co-miscible solvents stepwise as necessary to prepare the cell for data collection in the desired solvent/mobile phase. The DAWN is compatible with most common mobile phases between pH 1 and pH 10. See [Flow Cell Properties on page 187](#) for more details.

The DAWN is shipped with toluene in the flow cell. A typical series of solvents (from polar to nonpolar) is shown below. Salt solutions are considered separate steps from pure solvents.

Water
Methanol, Ethanol
Isopropanol, Acetone
Tetrahydrofuran
Ethylacetate, Chloroform, Methylene chloride
Toluene, Carbon disulfide
Hexane, Petroleum ether

- 7. Make fluid connections for chromatography**—Standard HPLC fittings—1/16 in. OD tubing, 10-32 threads. Inlet and outlet tubing for DAWN is 0.010 in. (0.254 mm) ID. The typical flow path goes from the HPLC to UV detector (if used), to the DAWN, to a differential viscometer (if used), to a differential refractometer (if used), then to waste or a Wyatt Orbit recycling system (if used). (See also Figure 4-1.)

4

Installation and Setup

This chapter helps you get the DAWN unpacked, tested, and connected to the ASTRA software and HPLC equipment or injection system.

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Attaching Auxiliary Device Connectors	44
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Flushing the DAWN	49
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Unpacking the Instrument

Please check the shipping parts list 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. If Wyatt Technology Corporation is installing the instrument for you, leave the instrument boxed. Otherwise, unpack the instrument.
3. Place the DAWN on a level surface and inspect the instrument exterior for damage. If you see any damage, **contact Wyatt Technical Support immediately.**

Installing the Instrument

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

To install the DAWN, 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 [Service and Maintenance on page 90](#) for more information about the required environment and how to keep the DAWN in optimal condition.)

The DAWN is designed to stack with other Wyatt detectors (for example, Optilab, microOptilab™, ViscoStar®, and Eclipse™ instruments). It can be installed either at the top or bottom of the stack. For easy access to the flow cell, installing the microCuvette holder, or adjusting the DLS fiber, we recommend that the DAWN be installed on the top of the stack.

2. Make sure the supplied power plug is correct for the local power outlet. The DAWN is equipped with a universal power supply, which operates anywhere in the world. It accepts inlet voltages between 100 V to 120 V or 220 V to 240 V at 50 Hz to 60 Hz.
3. Connect one end of the supplied Ethernet cable to the Ethernet port on the back of the DAWN (Figure 2-2) 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 is on the local area network, it may be accessed and controlled from any computer on the network. When using the USB converter, it can be accessed only by the host computer. See [Instrument Connectivity on page 190](#) for more details about implications for network security of the two different configurations.

4. **For Dry Gas Purge Option for Peltier Cooled Instruments:** This step is not necessary for ambient or heated instruments, but when operating Peltier-cooled instruments below ambient temperature (20 °C), a dry gas purge is essential to prevent condensation on the optical and measurement systems.

To prevent condensation, attach a dry air or nitrogen line to the Dry Gas Purge fitting on the rear panel of the DAWN (see Figure 2-2). Use a right-angle male connector and a 10-foot length of Polyethylene tubing, provided with the DAWN, to connect to the Dry Gas Purge fitting.

The pressure in the dry gas line should be 20 psi to 80 psi (0.14 MPa to 0.55 MPa). With a pressure of 25 psi, the dry gas flows into the temperature regulated box at a rate of approximately 38 mL/minute. A standard high pressure gas tank contains about 8,600 L of gas at STP, and so a tank should supply a single DAWN for approximately 150 days.

The DAWN has an internal pressure sensor that measures the pressure of the dry gas being supplied. The measured dry gas pressure may be viewed on the front panel LCD Alarms page. If the dry gas pressure drops below 20 psi (0.14 MPa), then the temperature control set point cannot be set below 20 °C. If the system temperature is set below 20 °C and the dry gas pressure subsequently drops below 20 psi (for example, if a tank is fully depleted), the temperature control set point is automatically changed to 20 °C.

5. Switch on the instrument and let it warm up for 30 minutes before proceeding with step (7). The power switch is on the front panel.
6. Install the ASTRA software. For off-line use, batch measurements or performing instrument calibration, ASTRA is the only required software for both measuring and processing data. For in-line use with chromatography, you may need to install the appropriate chromatography software. Establish communication with ASTRA as described in the *ASTRA User's Guide*. The DAWN is compatible with ASTRA 7.3 or higher.
7. The DAWN has been shipped with chromatography-grade toluene in the flow cell that can be used to verify the instrument calibration constant and performance. Cycle through the light scattering graphs on the front panel of the instrument or the Diagnostic Manager in ASTRA and check that the solvent offsets are consistent with the Certificate of Performance (COP) supplied with your instrument. If the values displayed on the front panel or in the Diagnostic Manager are more than 10 % different than those of the COP, contact Wyatt Technical Support.

Sometimes when the instrument has been in storage for some time or been subjected to temperature fluctuations during transit, the flow cell will contain air bubbles. A Forward Monitor reading of 0 % is a strong indicator of air bubbles being present. If this is the case, flush the flow cell with HPLC grade, filtered toluene before checking the solvent offsets against the COP. Use either a glass syringe or a 100 % Polypropylene/Polyethylene (PP/PE) plastic syringe with a 0.02 µm syringe tip filter and inject toluene via a short piece of tubing with

fittings directly into the flow cell. See [Instrument Calibration on page 78](#) for details. Do not use a plastic syringe with a black rubber tipped plunger as the rubber will dissolve in toluene. Using a syringe pump to drive the syringe is highly recommended to help prevent the introduction of bubbles into the flow cell.

The laser and forward monitor signals are set at the factory to have a scale of 0 % to 100 % (0 V to 1 V in the ASTRA software). 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 [Laser Settings on page 67](#)). 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 the chromatography fluid, the forward monitor is not nearly as stable as the 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. However, the forward monitor voltage may be used as an analytical signal when used to correct for the attenuation of the beam due to absorbing samples in the ASTRA software.

Note: The laser in the DAWN is software programmable from 10 % to 100 % and can be set either from the Settings tab or the ASTRA software. The laser can be turned on and off from the **Dashboard** tab.

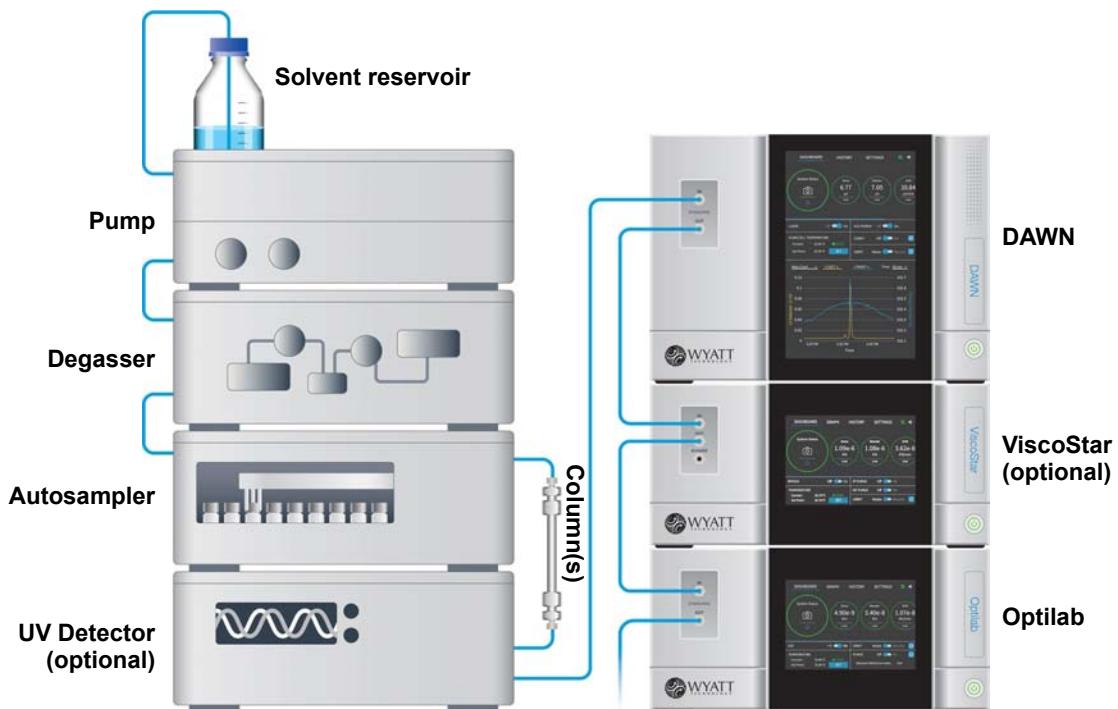
8. Calibrate the DAWN using the ASTRA software.

Note: If the WyattQELS module has been installed, align the DLS optical fiber in toluene and compare to the count rate with those specified in the QELS Certificate of Performance shipped with the instrument. See [Aligning the Optical Fiber on page 149](#).

9. Compare your calibration result with the calibration constant from the COP and confirm that the result is within 5 % of the factory calibration constant. If the results differ by more than 5 %, there may be an air bubble or dust in the flow cell. Flush with HPLC grade, 0.02 μm filtered toluene and repeat the experiment. If the issue persists, contact Wyatt Technical Support for assistance.

10. When you have confirmed that the instrument is in good working order, connect the DAWN to any other devices for your application. See Figure 4-1 for cable connections and fluid connections described in the next sections.

The ASTRA User's Guide contains detailed information on how to connect the DAWN to your chromatography system and set up methods for data collection.



An optional Orbit solvent recycler may be plumbed after the last instrument in the chain.

Figure 4-1: The DAWN in-line with a chromatography system

Connecting Auxiliary Devices

The DAWN can be connected to a variety of auxiliary devices via the ports on the back panel (Figure 4-2). Several general-purpose cables are supplied for transistor-transistor logic (TTL) inputs and outputs, analog inputs and outputs, auto inject input and output, and solenoid drive output. These cables have an RJ-12 connector on one end and six wires on the other end (Figure 4-4). Because devices have a variety of port types, you will need to attach these wires to the connector used by your devices. All auxiliary device I/O ports on the back panel of the DAWN are current-limited to protect the internal circuitry.

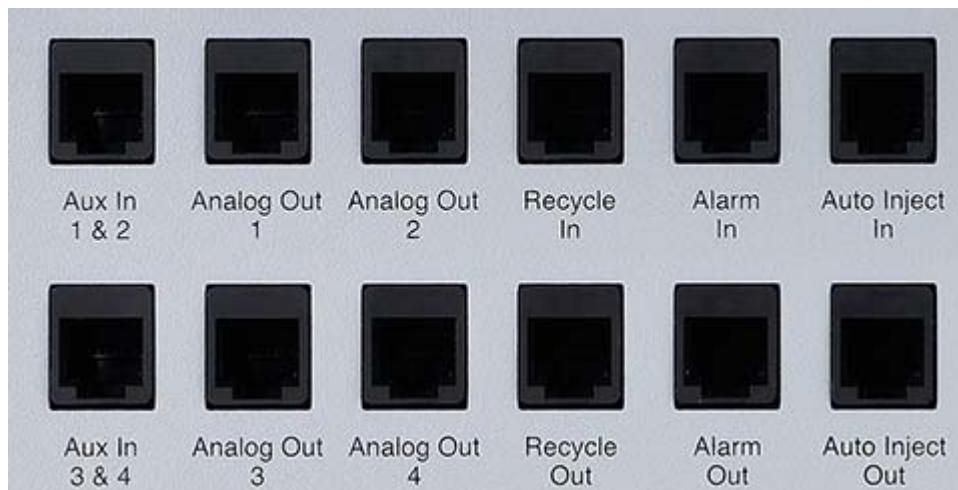


Figure 4-2: DAWN I/O Ports

Cables not supplied by Wyatt Technology may use a different color code scheme or have a different correspondence between color and pin number. If you are using non-Wyatt Technology supplied cables, refer to Figure 4-3 and Table 4-1 to determine the correct pin number and wire usage. We recommend using only cables supplied by Wyatt Technology; wiring the connection incorrectly could damage your instrument.

Pinouts for the RJ-12 connectors used in Wyatt Technology-supplied cables are shown in Figure 4-3.

Pin	Wire Color	
1	White	
2	Black	
3	Red	
4	Green	
5	Yellow	
6	Blue	

Figure 4-3: RJ-12 Connector and Pinouts for Wyatt Technology cables



Figure 4-4: General-purpose RJ-12 cable with flying leads

The auxiliary device ports on the back of the DAWN are:

- Aux 1, Aux 2, Aux 3, and Aux 4. You can connect the DAWN to up to four external instruments, typically concentration sensitive detectors. Aux 1 and Aux 2 are in one port and Aux 3 and Aux 4 are wired in a second port.
- Analog Out—You can use these four ports to deliver analog output signals from the DAWN to an existing data collection system or chart recorder. Each Analog Out port is wired separately.
- Alarm In—You can use this connector to sense an external Alarm on the DAWN.
- Alarm Out—You can send out an alarm to an external device, such as a pump whenever an alarm condition is recorded on the DAWN.
- Auto Inject In—You can use this port to sense an injection from an auto injector. This signal is then monitored by the ASTRA software.
- Auto Inject Out—You can use this connector to transmit a contact closure signal to trigger an auto injection of another device.

Attaching Auxiliary Device Connectors

The AUX input signals can accept an input range of -10 V to 10 V with a 1 μ V resolution. Typically when the time constant is set to 1 s, a noise level of less than 10 μ V 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. Aux 1 and 2 are on one port; Aux 3 and 4 are on another.
2. Connect the wires of the cable to your other device as shown in Table 4-1. Typically, only the AUX+ and AUX- wires need to be connected to the signal source when connecting AUX channels; however, if there is unacceptable noise pickup, connect the ground (GND) wire connection to either the instrument chassis or the AUX- connector of the source instrument.

3. It may be necessary to connect to another device via a connector that is directly situated on or provided with the device. The following list contains additional information for various other connectors:
 - **Auto Inject**—The auto inject input requires 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 detected by the DAWN, the graph on the Main page of the front panel will show a vertical green line. Some injectors require programming in order for the closure to happen. Ensure that an injection closes the circuit.
 - **Alarm In**—TTL input on red (Alarm_In+ signal) and green (Alarm_In- signal ground). The TTL input signal is the voltage measured between the signal (red) and the signal ground (green). TTL voltage levels are +5 V (logic 1) or 0 V (logic 0). On the instrument display Alarm tab, you may select which of these indicates on or off (see [Alarm Settings on page 69](#)).
 - **Alarm Out**—TTL output on white (Alarm_Out signal) and black (Alarm_Out-R signal ground). The TTL out signal is the voltage measured between the signal (white) and the signal ground (black). TTL voltage levels are +5 V (logic 1) or 0 V (logic 0). On the instrument display Alarm tab, you may select which of these indicates on or off (see [Alarm Settings on page 69](#)).
 - **Recycle In**—TTL input on red (Recycle_In signal) and green (Recycle_In_Rtn 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 port. When the signal transitions from 5 V to 0 V, the Recycle valve is de-actuated. These ports can be used by a third-party instrument to control the recycle valve.
 - **Recycle Out**—The solenoid valve drives current on the white (FV-12 VDC) and black (FV-RTN) wires (the current direction is irrelevant for the solenoid). This signal may be connected to a user-supplied solenoid valve or a Wyatt Technology Orbit unit, which contains an internal solenoid valve that switches between waste and recycle. When actuated (via the Dashboard tab or the Recycle In input), the port supplies current to drive a 12 V solenoid valve. The valve is actuated with 12 V (up to 1 A, depending upon resistance of the solenoid), held for 0.1 s, and then dropped down with 12 V across an internal 51 Ohm resistor.
 - **Ethernet**—Ethernet port for connecting the instrument to an Ethernet network. This port is a standard RJ-45 wiring for a 10Base-T/100Base-TX connection.

Table 4-1: Back Panel Wiring

Port Label	Connector Pin Number	Wire Color	Signal	Comments
Aux 1 & 2	1	White	Aux1+	
	2	Black	Aux1-	
	3	Red	Aux2+	
	4	Green	Aux2-	
	5	Yellow	Aux1_GND	Used when unacceptable noise is present in Aux 1.
	6	Blue	Aux2_GND	Used when unacceptable noise is present in Aux 2.
Aux 3 & 4	1	White	Aux3+	
	2	Black	Aux3-	
	3	Red	Aux4+	
	4	Green	Aux4-	
	5	Yellow	Aux3_GND	Used when unacceptable noise is present in Aux 3.
	6	Blue	Aux4_GND	Used when unacceptable noise is present in Aux 4.
Analog Out 1	1	White	Analog Out1+	
	2	Black	Analog_GND	
	3	Red	NC	
	4	Green	NC	
	5	Yellow	Analog_GND	
	6	Blue	NC	
Analog Out 2	1	White	Analog Out2+	
	2	Black	Analog_GND	
	3	Red	NC	
	4	Green	NC	
	5	Yellow	Analog_GND	
	6	Blue	NC	
Analog Out 3	1	White	Analog Out3+	
	2	Black	Analog_GND	
	3	Red	NC	
	4	Green	NC	
	5	Yellow	Analog_GND	
	6	Blue	NC	

Table 4-1: Back Panel Wiring (Continued)

Port Label	Connector Pin Number	Wire Color	Signal	Comments
Analog Out 4	1	White	Analog Out4+	
	2	Black	Analog_GND	
	3	Red	NC	
	4	Green	NC	
	5	Yellow	Analog_GND	
	6	Blue	NC	
Auto Inject In	1	White	NC	
	2	Black	NC	
	3	Red	Inject_In+	
	4	Green	Inject_In-	Contact closure required (for auto 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 4-1: Back Panel Wiring (Continued)

Port Label	Connector Pin Number	Wire 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	

Fluid Connections

Inlet and outlet tubing for DAWN is 0.010 in. (0.254 mm) ID.

The DAWN accepts standard HPLC fittings: 1/16 in. OD tubing with 10-32 threads. 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.

Depending on what other detectors are used for in-line SEC-MALS analysis, the DAWN is commonly placed after a UV detector and before a viscometer and/or differential refractometer. See Figure 4-1 for a schematic.

Flushing the DAWN

Flow solvent into the DAWN through the In port, and out to waste from the Out port. Flush the instrument with 20 mL of co-miscible solvents stepwise as necessary to prepare the cell for data collection. The DAWN is compatible with most common mobile phases between pH 1 and pH 10. See [Flow Cell Properties on page 187](#) for more details. The DAWN is shipped with toluene in the flow cell and will require co-miscible solvent flushes to be ready for aqueous measurements.

A typical series of solvents (from polar to nonpolar) is shown below. Salt solutions are considered separate steps from pure solvents. For example, if converting a system from toluene to a buffer solution, it is recommended to first flush out the toluene with an alcohol, then flush the system with water before finally introducing the buffer solution.

- Water
- Methanol, Ethanol
- Isopropanol, Acetone
- Tetrahydrofuran
- Ethylacetate, Chloroform, Methylene chloride
- Toluene, Carbon disulfide
- Hexane, Petroleum ether

Attaching the Drain Port Connector

If the DAWN is the instrument at the bottom of a stack of instruments, tubing needs to be connected to the drain port on the underside of the instrument to direct any liquid originating within the instrument or cascading from an instrument further up to a waste container.

The Wyatt instrument stack is designed to direct leaks toward the bottom instrument of the stack, where a drain connector can be installed to direct leaks to a waste reservoir. An elbow drain (Wyatt p/n 165397) is included in your hardware kit.

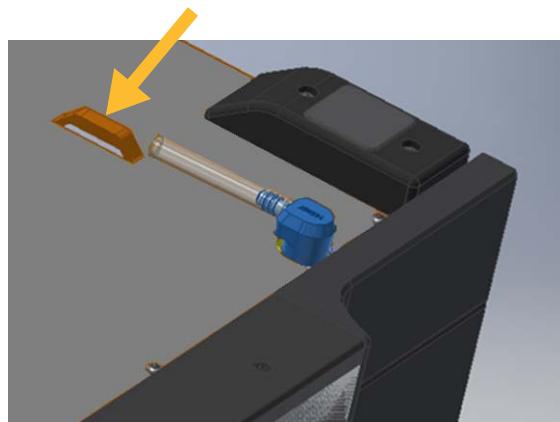
This drain is made of acetal and can be connected to supplied tubing made of Versilon.



Note:	The Wyatt-supplied leak drain plumbing contains Versilon and acetal components. These are suitable for most solvents. If your mobile phase is incompatible with these materials, please contact Wyatt Customer Support for help in selecting a suitable alternative.
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To install the elbow drain connector:

1. You may connect the Versilon tubing to the elbow drain connector.
2. Press the elbow drain connector firmly into place on the underside of the instrument to secure it to the underside of the instrument.
3. The DAWN instrument comes with a built-in guide on the underside of the instrument for zip-tying the tubing. This prevents the instrument feet from crushing the tubing. Connect the tubing to the connector shown above and use the loop to guide the tubing to a nearby waste reservoir.



5

Using the Front Panel Display

This section describes the multi-touch controls and display interface on the front panel of the instrument. This interface and its options allow you to monitor, configure, and control the function and operation of the DAWN.

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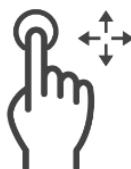
Using the Multi-Touch Controls

You can tap, swipe, pinch, and spread your fingers to perform useful actions on the screen.



Tap to click

Tap with one finger to select an interface option. You can tap to view drop-down lists and tap to select commands such as OK or Cancel.



Slide to scroll

Press and drag with one finger on the screen to scroll through the interface. Press and slide up to scroll down through the Settings tab, or press and slide left to scroll right through the Status Indicators on the Dashboard tab, or vice-versa. You can also press and drag the blue scroll bar shown on the Dashboard and Settings tabs to scroll.



Press and pinch or spread to zoom

Two fingers are needed to zoom in or out on display graphs. Using your thumb and pointer finger, press the display and spread two fingers apart to zoom in on a graph. Move two fingers together to zoom out on a graph.

Front Panel Overview

The top ribbon on the display provides the **Dashboard**, **History**, and **Settings** tabs, an alarm indicator, an alarm mute/unmute button, and a touchscreen disable button.



Select a tab by tapping it. A blue underline indicates which tab is currently shown. The tabs provide real-time data, instrument control, and setting control. These tabs are explained in the sections that follow.



A green checkmark indicates there are no warnings or alarms. A yellow caution icon means an issue requires attention. A red warning icon means the system is not ready or an alarm is active. Tapping these icons provides a shortcut to the Alarms section on the **History** tab (see [History Tab on page 61](#)).



The Vapor alarm and all types of Leak alarms trigger both visual and audible alarms. To mute alarms, tap the sound button on the top ribbon. Even if the audible alarm is turned off, the back panel alarm output remains active.



The front panel can be locked to prevent accidental actions. When locked, the display is dimmed. Tap the lock icon to unlock the display.

Dashboard Tab

The **Dashboard** tab provides most of the commonly used DAWN functions as well as real-time monitoring of the system. Basic functions are located on the Dashboard while more advanced settings, such as instrument communication settings, are provided on the **Settings** tab. The DAWN Dashboard tab also includes graphs.

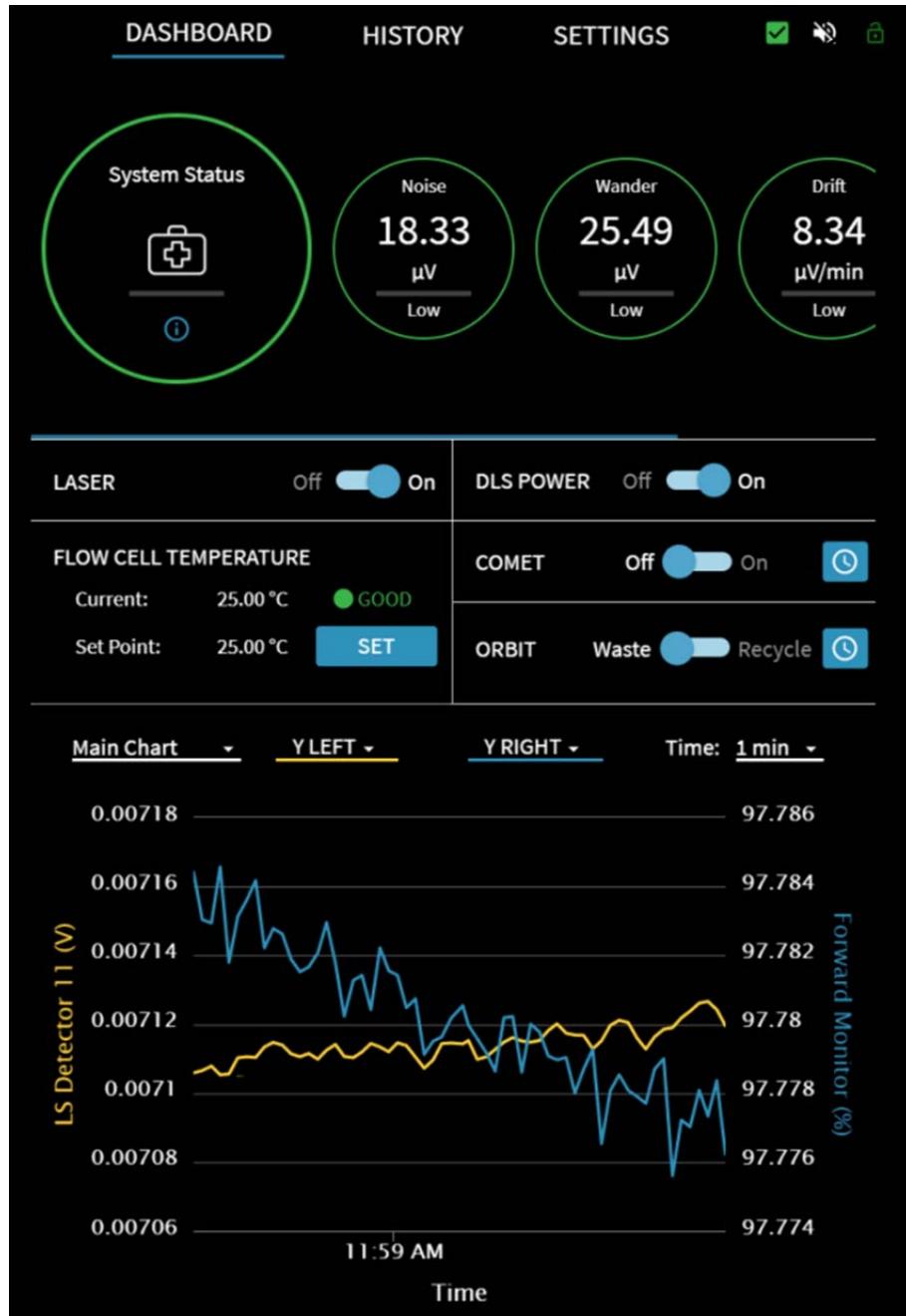


Figure 5-1: Dashboard tab

System Health Indicators

The system health indicators on the Dashboard are designed to provide an overview of the system and its suitability for data collection.

The **Dashboard** tab provides real-time instrument status information in the form of Health Indicators. There are five DAWN indicators: a summary indicator, light scattering noise, wander, drift, and forward monitor. These are measured and updated in real-time. Clicking on a Health Indicator's blue info icon opens a description of the parameter and potential solutions.

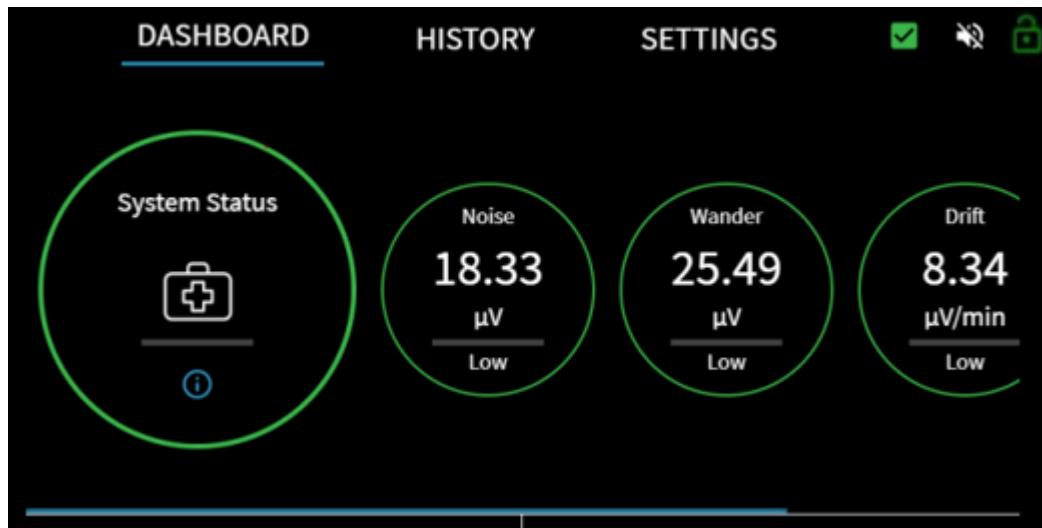


Figure 5-2: System Health Indicators on the Dashboard tab

Scroll right to view all five system health indicators.

The system status indicators can be green, yellow, or red.

- **Green** indicates a ready state and that the system is ready for data collection.
- **Yellow** indicates a cautionary state that noise, drift, or forward monitor levels are not ideal. Although you can proceed with data collection, it may be recommended to address the system health prior to data collection.
- **Red** indicates a warning state that noise, drift, or forward monitor levels are not at recommended specifications and data quality may be negatively affected. It is strongly recommended to address the system health prior to data collection.

To learn more about any of the health indicators, tap the circle. This expands the circle, and you can tap the information icon to display additional information as well as troubleshooting guidance.



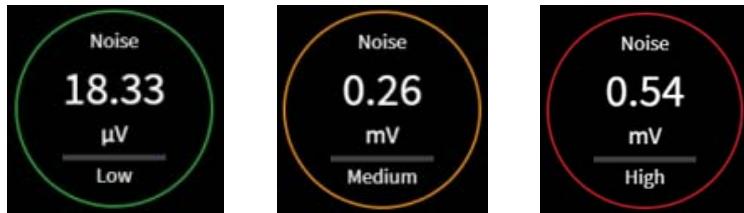
See [Health Indicator Settings on page 68](#) to control the stringency of the states or to disable the health indicators.

System Status

This is a live indicator of the ready state of the DAWN. If the system status indicator is yellow or red, it suggests an instrument alarm or health indicator should be addressed.

Detector Noise

This is a live indicator of the short-term 90° detector light scattering noise, measured in volts.



Detector Wander

This is a live indicator of the long-term 90° detector light scattering noise, measured in volts.

Detector Drift

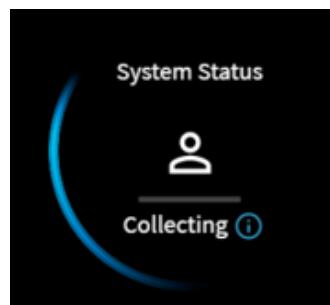
This is a live indicator of the long-term 90° detector light scattering signal stability, measured in volts per minute.

Forward Monitor Health

This is a live indicator of the forward monitor signal. Problems with this signal indicate a flow cell obstruction or sample absorption. If you suspect your sample of absorbing light at the laser wavelength, refer to TN1010: *Correcting for Absorbance at the Laser Wavelength* for strategies to address absorbance of the laser wavelength.

Collection in Progress

When data collection is in progress, the health indicators are replaced by a system status icon to reflect collection.



Dashboard Control Options

The **Dashboard** tab also contains commonly used controls for your instrument.



Laser Control

Toggles the laser on or off.

See [Laser Settings on page 67](#) for information about the laser and how to control the laser power.

When **Idle Mode** is enabled in the **Settings** tab, the laser power is reduced to 70 % after an hour with no data collection. The idle laser power can be customized in the **Settings** tab; see [Laser Settings on page 67](#) for details.

When Idle Mode is enabled, the **Laser** toggle switches to Idle / On (as shown below) instead of Off / On, and a yellow caution icon is displayed.



Note that all signals—including the forward monitor, DLS count rates, and detector voltages—will be reduced from their normal values when in Idle Mode.

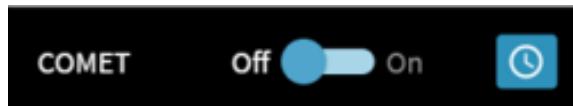
DLS Power

If the WyattQELS option is present, the DLS Power option allows you to turn this feature on and off. See [Accessing DLS Options on the Front Panel on page 144](#). for details.



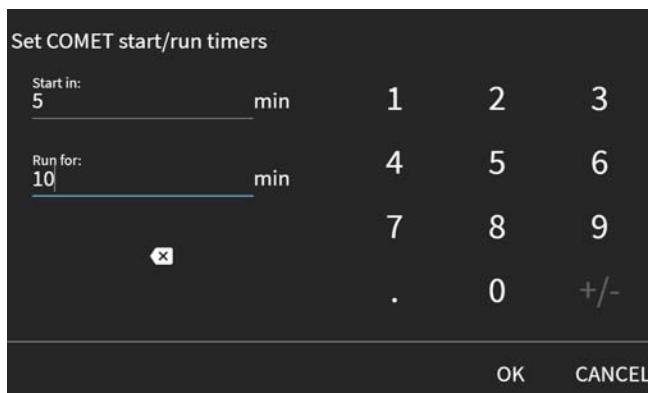
COMET Control

The COMET module, which is included with each DAWN for ultrasonic radio frequency flow cell cleaning, can be toggled On or Off on the front panel or via the ASTRA software.



The COMET applies an ultrasonic radio frequency field to loosen particles that may adhere to the flow cell walls. When used on a daily basis, new particles can be prevented from adhering to the flow cell and the need to remove the flow cell for cleaning may be postponed. Typically, it is recommended to run the COMET for an hour or two after the last data run completes.

Click the clock icon to program the COMET to start in a specified number of minutes and run for a specified amount of time. Use the touch screen to type in values and tap **OK**.



Alternatively, you can use ASTRA to cause the COMET to run at the end of an experiment or at a certain time. This is useful when setting the COMET to run at the end of the day.

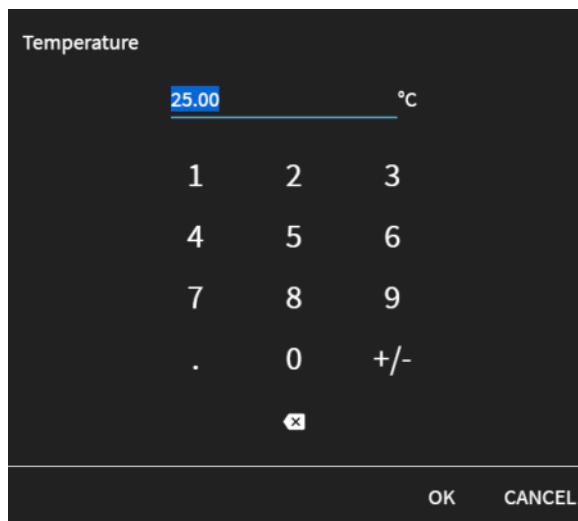
Flow Cell Temperature

All DAWN instruments measure the temperature of the flow cell. If your DAWN has temperature control options, you can also control the temperature. The **Dashboard** tab shows these temperatures.



An indicator shows whether temperature has reached the setpoint or is ramping.

Tap **Set** to change the temperature set point in °C.



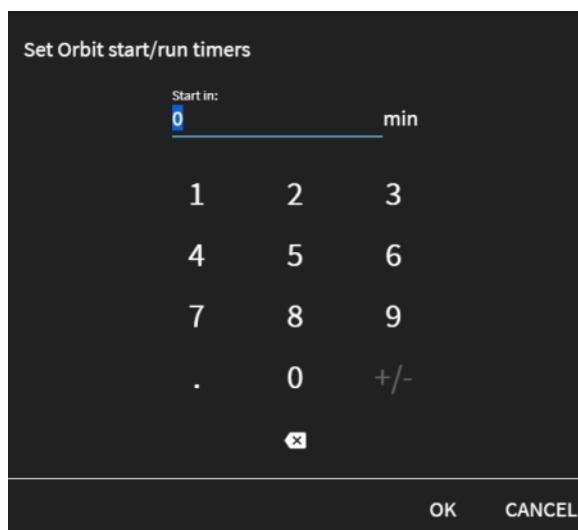
ORBIT Control

The optional Wyatt Orbit or an energized solenoid valve can be controlled via the Orbit option to direct flow to waste or recycle. The Dashboard allows you to toggle the Wyatt Orbit (if one is connected) between Waste or Recycle.



This option only functions when the back panel of the DAWN is connected to a 12V solenoid valve, such as the Wyatt Orbit solvent recycling system, which can be plumbed to divert the flow from recycle to waste. This accessory can be controlled in ASTRA or via the front panel.

Click the clock icon to program the Orbit to switch from Waste to Recycle after a specified amount of time. Use the touch screen to type in values and tap **OK**.

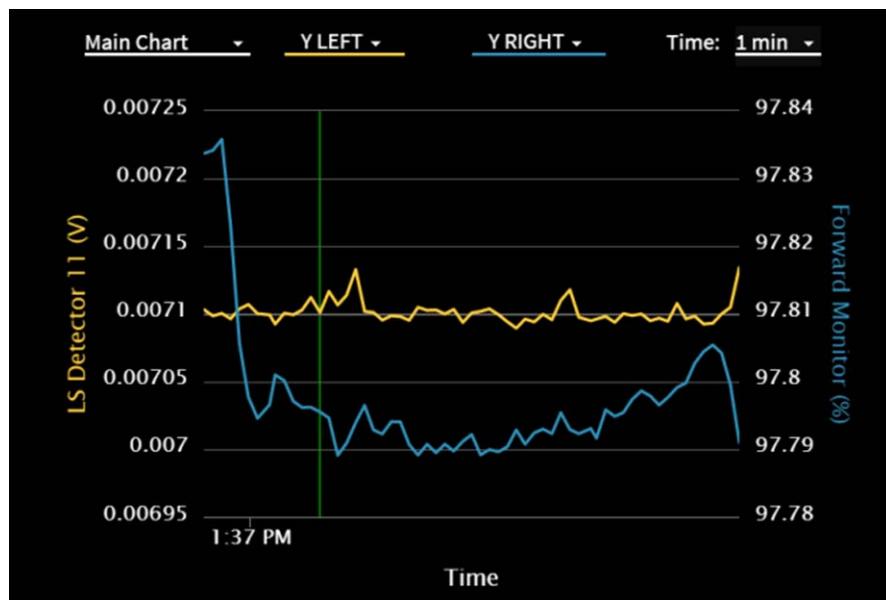


When the Orbit is directing flow to recycle, a caution icon is displayed. During an experiment run, you can use ASTRA or the front panel display to direct flow to either the waste reservoir or to be recycled.

You can use the **Settings** tab to hide the Orbit toggle for instruments not equipped with the Orbit. See [Instrument Information Settings on page 65](#).

Graph Display

The display shows real-time data that assists in observing experiment progress and current system performance. It also allows you to see when your instrument has received an auto-inject signal from an autosampler or manual injector, after which a vertical green line is shown on the graph as shown below.



Selecting a Chart

The default graph is the **Main Chart**.

If the optional WyattQELS module is installed, tap the drop-down list in the upper-left to access the autocorrelation function (**ACF**) and **Count Rate** chart options. See [Accessing DLS Options on the Front Panel on page 144](#) for additional information about these graphs.

Selecting the Display Axes

You can select a data channel to display in the graph for each y-axis.

The y-axis on the left corresponds to the yellow trace. The default is to show the output from Detector 11. You can select another data channel by tapping the **Y LEFT** drop-down.

The y-axis on the right corresponds to the blue trace. The default is to show the output from the Forward Monitor. You can select another data channel by tapping the **Y RIGHT** drop-down.

The options for the y-axes include all of the light scattering detectors, the auxiliary channels, forward monitor, laser monitor, laser drive current, dry gas pressure, and flow cell temperature.

If you have the UHT DAWN, you can also view the heated line temperature.

By default, the left and right y-axes scale to fit the real-time data streams you choose to display. In the window for selecting a display axis, you can customize the scale by tapping the **Set Scale** button.

Y Left	
<input type="radio"/> LS Detector 1	<input type="radio"/> LS Detector 15
<input type="radio"/> LS Detector 2	<input type="radio"/> LS Detector 16
<input type="radio"/> LS Detector 3	<input type="radio"/> LS Detector 17
<input type="radio"/> LS Detector 4	<input type="radio"/> LS Detector 18
<input type="radio"/> LS Detector 5	<input type="radio"/> Aux Input 1
<input type="radio"/> LS Detector 6	<input type="radio"/> Aux Input 2
<input type="radio"/> LS Detector 7	<input type="radio"/> Aux Input 3
<input type="radio"/> LS Detector 8	<input type="radio"/> Aux Input 4
<input type="radio"/> LS Detector 9	<input type="radio"/> Forward Monitor
<input type="radio"/> LS Detector 10	<input type="radio"/> Laser Monitor
<input checked="" type="radio"/> LS Detector 11	<input type="radio"/> Laser Drive Current
<input type="radio"/> LS Detector 12	<input type="radio"/> Flow Cell Temperature
<input type="radio"/> LS Detector 13	<input type="radio"/> Heated Line Temperature
<input type="radio"/> LS Detector 14	<input type="radio"/> Dry Gas Pressure

Traces

Auto Scale

Set Scale

Selecting the Time Axis

The x-axis always display time, but the time scale can be set using the drop-down options for **Time**.

You can zoom in or out on the chart by pinching or spreading your fingers as discussed in [Using the Multi-Touch Controls on page 52](#).

History Tab

The **History** tab displays information about the alarm status for the DAWN and the history of the system. This includes alarms from the DAWN instrument itself and external alarms that are collected through the back panel.



The Vapor alarm and all types of Leak alarms trigger both visual and audible alarms. To mute alarms, tap the sound button on the top ribbon.

Alarm Section

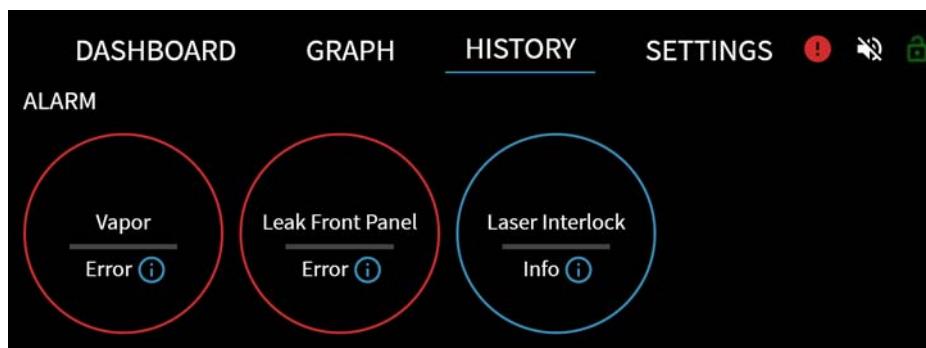
The **Alarm** section displays current alarms.

To learn more about an alarm, tap the information icon to display additional information.



The alarms are color-coded for severity:

- **Yellow** alarms are cautionary and suggest the instrument is not ready for operation (for example, a temperature lock has not been achieved).
- **Red** alarms suggest a hazard or severe warning, such as an external alarm, vapor, or liquid leak.
- **Blue** alarms refer to an information notice, such as the laser being disabled for safety reasons when the top cover of the instrument was removed.
- Alarms that are not active are not shown, so there are no green alarms.



The Vapor alarm and all types of Leak alarms also cause audible alarms unless alarms are muted (see [Front Panel Overview on page 52](#)).

History Section

The **History** section contains a list of events tracked by the instrument. This includes the type of alarm, severity, and the date/time of the alarm. Tap the information icon next to an alarm to display additional information.



HISTORY			
	Alarm	▼Date	
	Inject Start	13:00:26	01-23-19
	Inject Start	9:21:08	01-23-19
	Inject Start	17:28:17	01-22-19
	Temp Stabilizing Flow Cell	14:07:46	01-22-19

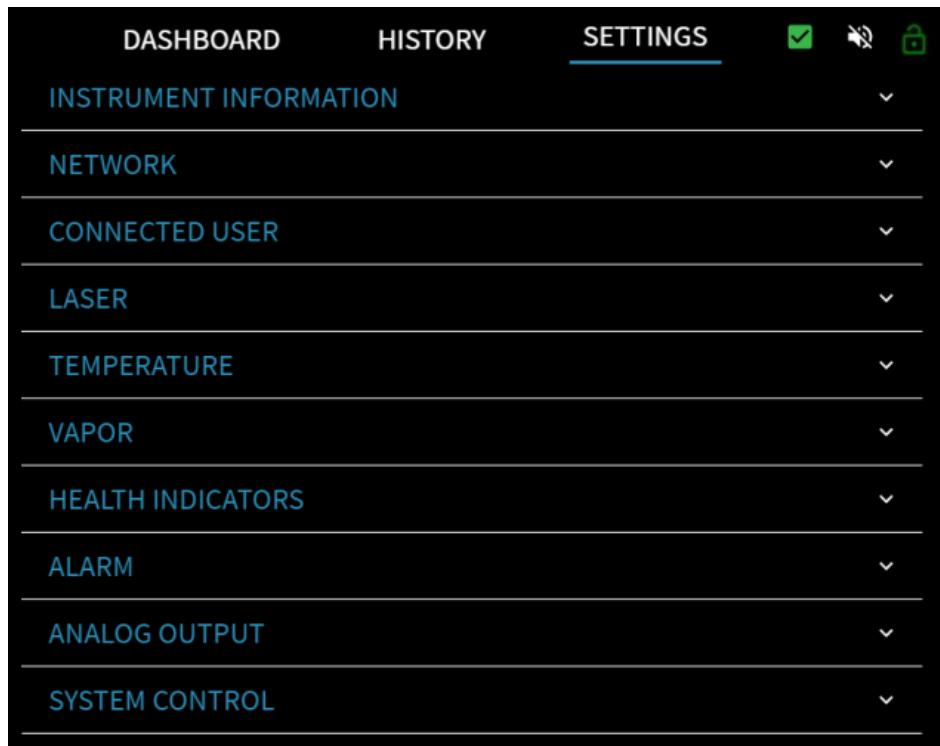
The following events or alarms can be recorded by the DAWN:

- **APD Alert:** An error has occurred with the APD (with WyattQELS). Please contact Wyatt Support.
- **COMET Error:** An error occurred while activating the COMET. Contact Wyatt Technical Support.
- **COMET Transducer Error:** There was an error with the COMET transducer assembly. There may be a connection problem or the transducer may need replacing. Contact Wyatt Technical Support.
- **Device Error:** Instrument error. Restart the instrument, and if the alarm persists contact Wyatt Technical Support.
- **External:** If an external alarm cable is connected to the Alarm IN port on the rear panel, the DAWN can receive a voltage signal to trigger an alarm state. By default, an input signal of 0 V is considered a ready (non-alarm) state, and an input signal of 5 V is considered an alarm state. If **Alarm In** is set to **Active Low** (see [Alarm Settings on page 69](#)), this is reversed—an input signal of 5 V is considered a non-alarm state, and an input signal of 0 V is considered an alarm state.
- **Flex Cable Connection:** A flex cable connection error has occurred. Please contact Wyatt Technical Support.
- **Inject Start:** An auto-inject signal was received or manual data collection has begun.
- **Laser Interlock:** The instrument top cover was removed, and the laser was disabled for your protection.

- **Laser Monitor:** Laser monitor signals differs from the set laser power by more than 20 %. There are several possible causes for this, one of which may be the laser reaching the end of its useful lifetime. Please contact Wyatt Technical Support.
- **Leak Flow Cell:** A leak was detected in the optical bench. This triggers an audible alarm. Follow the instructions in [Leak Sensors and Cleaning After a Fluid Leak on page 135](#) to remove the flow cell, check for leaks, and soak up any liquid. Contact Wyatt Technical Support if you need assistance.
- **Leak Flow Path:** A leak was detected in the instrument internal flow path. This triggers an audible alarm. Follow the instructions to check the connections for leaks or clogs. Contact Wyatt Technical Support if you need assistance.
- **Leak Front Panel:** A leak was detected at the front panel. This triggers an audible alarm. Make sure the inlet and outlet tubing are secure and that no spills or leaks have occurred. Clean and dry the leak sensor, as appropriate. See [Leak Sensors and Cleaning After a Fluid Leak on page 135](#) for more information.
- **Low Dry Gas Pressure:** Temperatures below 20 °C require at least 20 psi dry gas, such as dry air or nitrogen, to prevent condensation. The temperature is automatically set to 20.5 °C when this alarm occurs. Check your gas supply and ensure there is sufficient flow. This prevents condensation from damaging the optics if the dry gas connection is not made or if the tank runs empty.
- **Overheat:** (Not present in an ambient DAWN.) The instrument temperature exceeds the maximum limit. Contact Wyatt Technical Support.
- **Recycle In:** The instrument received a request from an external device to toggle the Orbit recycle valve.
- **Temp Stabilizing Flow Cell:** The flow cell temperature has not yet stabilized in a temperature-controlled DAWN.
- **Temp Stabilizing Heated Line:** The heated line temperature has not yet stabilized in a UHT DAWN with a heated-line connection.
- **Vapor:** The voltage measured from the organic vapor sensor is higher than the set threshold voltage. That threshold is settable for this instrument. This triggers an audible alarm. See [Vapor Settings on page 68](#).

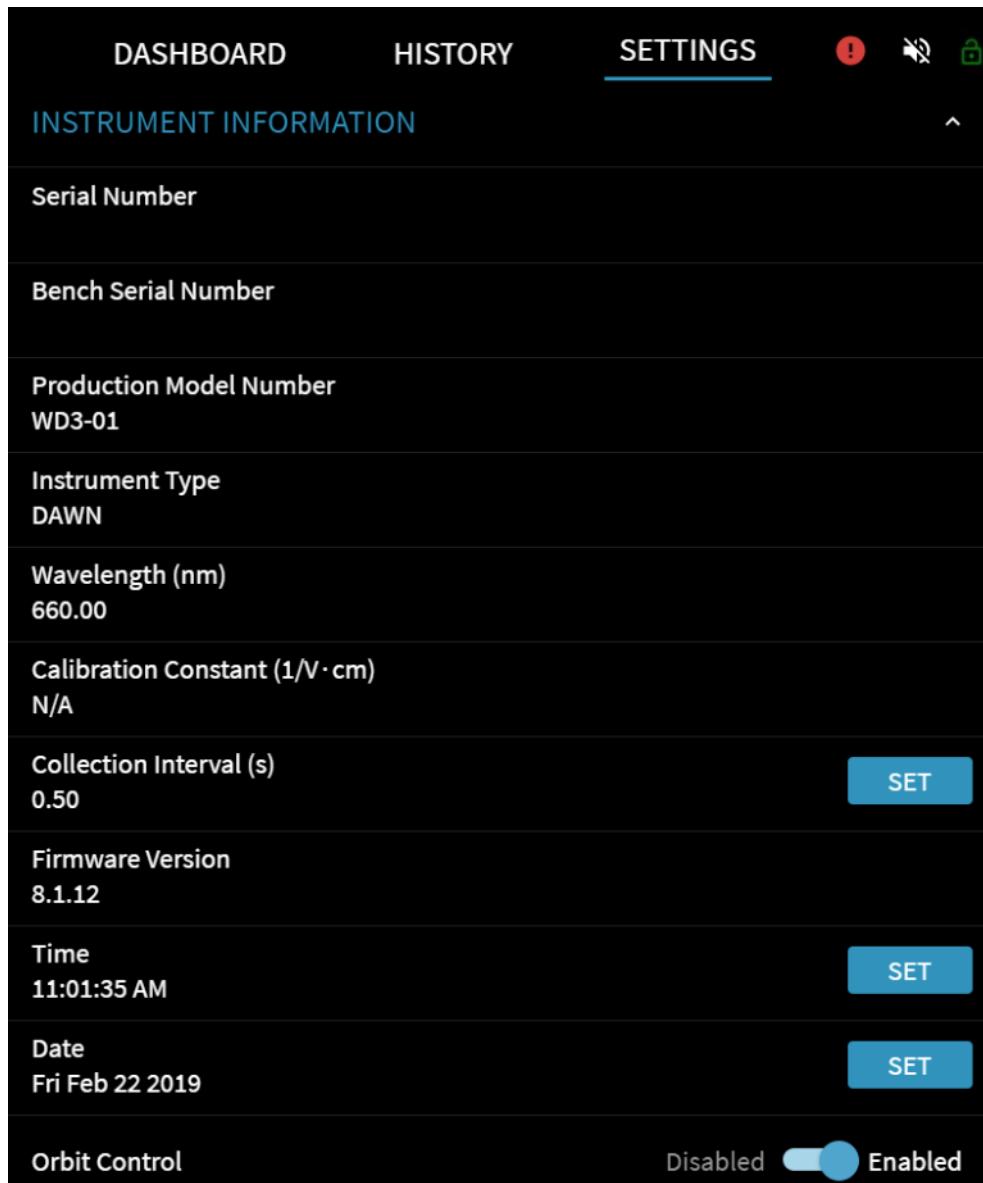
Settings Tab

The **Settings** tab provides external alarm, temperature, network, connected user, vapor, detector noise, analog output, system control, and instrument information. It is organized into several collapsible sections. Tap a drop-down icon to expand the relevant section.



Instrument Information Settings

Expand the **Instrument Information** section to see information about the instrument, such as serial numbers, model number, instrument type, firmware version, laser wavelength, calibration constant, collection interval, and date and time settings. The serial number and firmware version are useful to have when contacting Wyatt Technical Support.



You can modify the following settings:

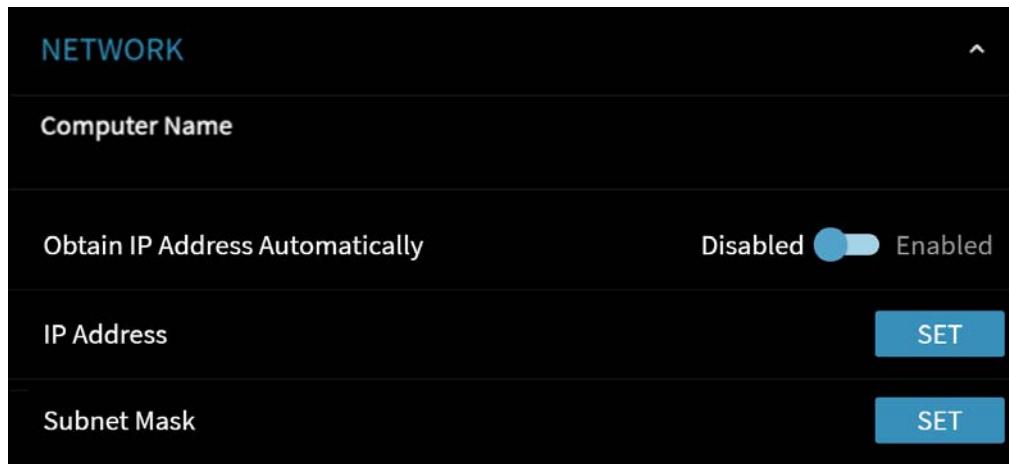
- **Collection Interval.** This specifies the time between each data slice displayed on the front panel; it is the inverse of the frequency of data slices. The default is 0.50 seconds. The time over which data are averaged (the measurement time constant) is automatically set to be 2x the collection interval. This interval can also be controlled by ASTRA.

- **Time and Date.** Set the instrument time by rotating the dials to select the hour, minute, and whether the time is AM or PM.
- **Orbit Control.** You can disable display of the Orbit in the front panel by toggling the Orbit Control. See [ORBIT Control on page 58](#).

Network Settings

Expand the **Network** section to see the network name of the instrument as it would appear on a LAN, whether the IP address should be obtained automatically (using DHCP) or be set manually (static IP).

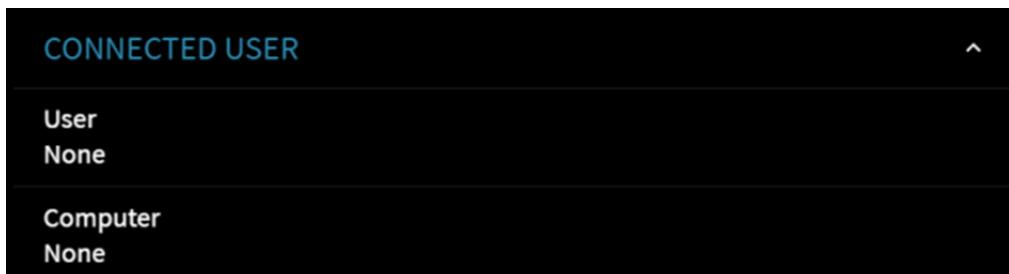
If you disable obtaining the IP address automatically (as shown below), you can set the **IP Address** and **Subnet Mask**.



See [Instrument Connectivity on page 190](#) for instructions regarding network communications setup.

Connected User Settings

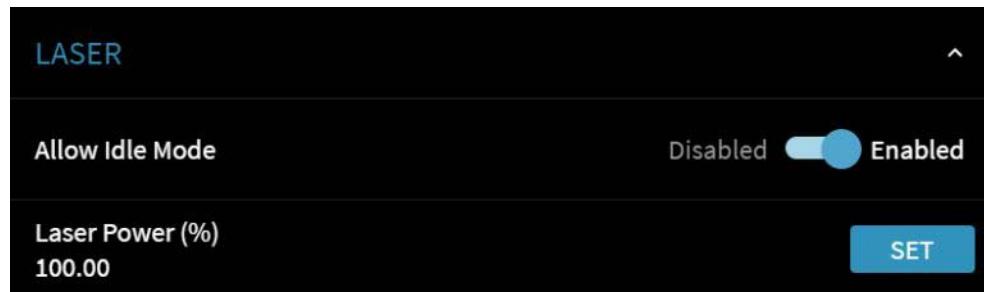
Expand the **Connected User** section to see if any computers are currently connected to the instrument via ASTRA. If a computer is connected, this section shows the user name and the computer name currently controlling the instrument via ASTRA.



Laser Settings

Expand the **Laser** section to see options for enabling or disabling the laser idle mode as well as setting the laser power.

If you enable **Idle Mode** here and the instrument has not been collecting data for an hour, the laser reduces its power to prolong laser lifetime. The **Dashboard** tab shows when the laser is in Idle Mode and allows you to toggle Idle Mode on and off (see [Laser Control on page 56](#)).



Temperature Settings

Expand the **Temperature** section to see the flow cell temperature and the system temperature for all DAWN instruments.

For Heated/Cooled DAWN instruments, the flow cell temperature can be set here or via the **Dashboard** tab. (See [Flow Cell Temperature on page 57](#).)

For Ultra-High Temperature DAWN instruments, the heated-line temperature can also be set here. When a heated line is detected, options to **Sync** the heated line and flow cell temperatures are also available.



The thermocontrollers 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. However, 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.

Vapor Settings

Expand the **Vapor** section to see the vapor sensitivity level. This sensitivity level adjusts the threshold for when organic solvent vapors trigger the vapor alarm. A low sensitivity level reduces the likelihood of vapor alarms. Alcohols and organic solvents can trigger the vapor alarm.

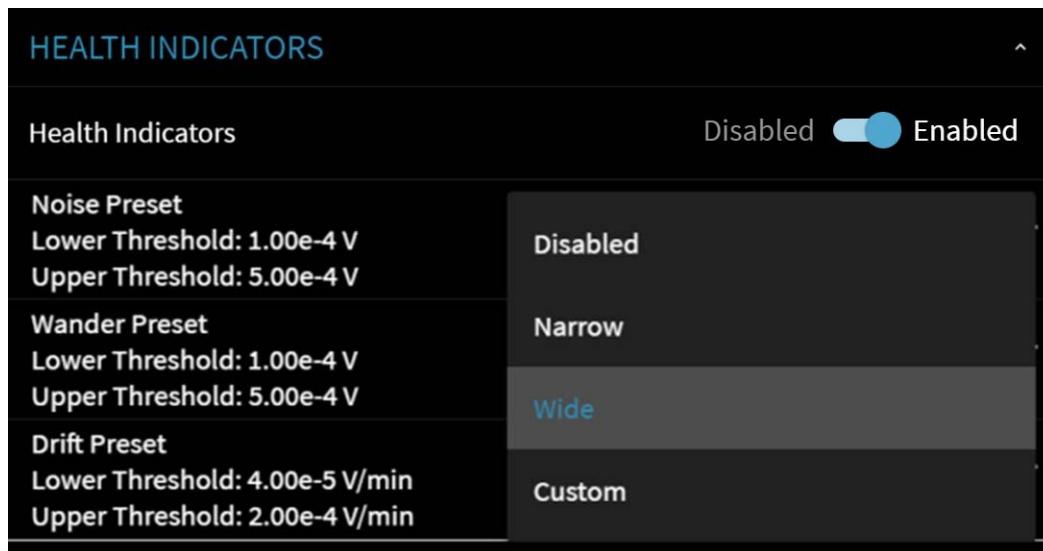


Health Indicator Settings

Expand the **Health Indicators** section to see and control the noise, wander, and drift values that trigger a health indicator. See [System Health Indicators on page 54](#) for information about viewing health indicators.

The default setting is **Wide**, which is appropriate for a wide range of systems. Use the **Narrow** preset if very low noise levels are required, for example when working with materials with a lower signal-to-noise ratio due to low dn/dc or molar mass.

The indicator is green below the lower threshold, yellow if between the lower and upper, and red if above the upper threshold. You can set **Custom** values for the lower and upper thresholds. If you disable the Health Indicators, they will no longer appear on the Dashboard.

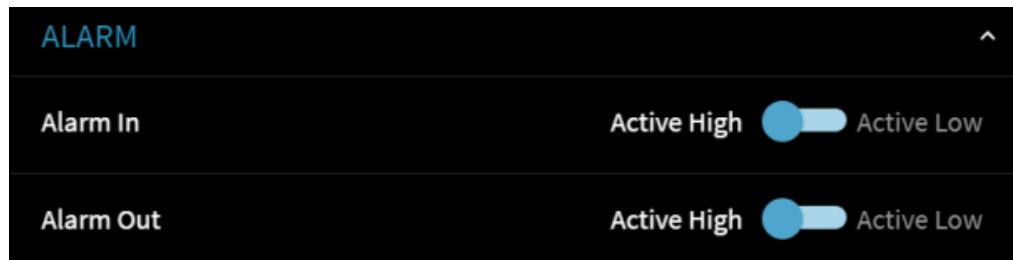


Alarm Settings

Expand the **Alarm** section to see and control the settings for alarms based on the **Alarm In** and **Alarm Out** ports.

If an external alarm cable is connected to the Alarm Out port on the rear panel, the DAWN can send out a voltage signal to an external destination in the event of an alarm being triggered. In the default configuration (**Active High**), the Alarm OUT port is kept at 0 V during a ready (non-alarm) state, and increases to 5 V in the event a serious (red) alarm is triggered.

If you select **Active Low**, the Alarm OUT port is kept at 5 V during a ready (non-alarm) state, and decreases to 0 V in the event a serious (red) alarm is triggered.



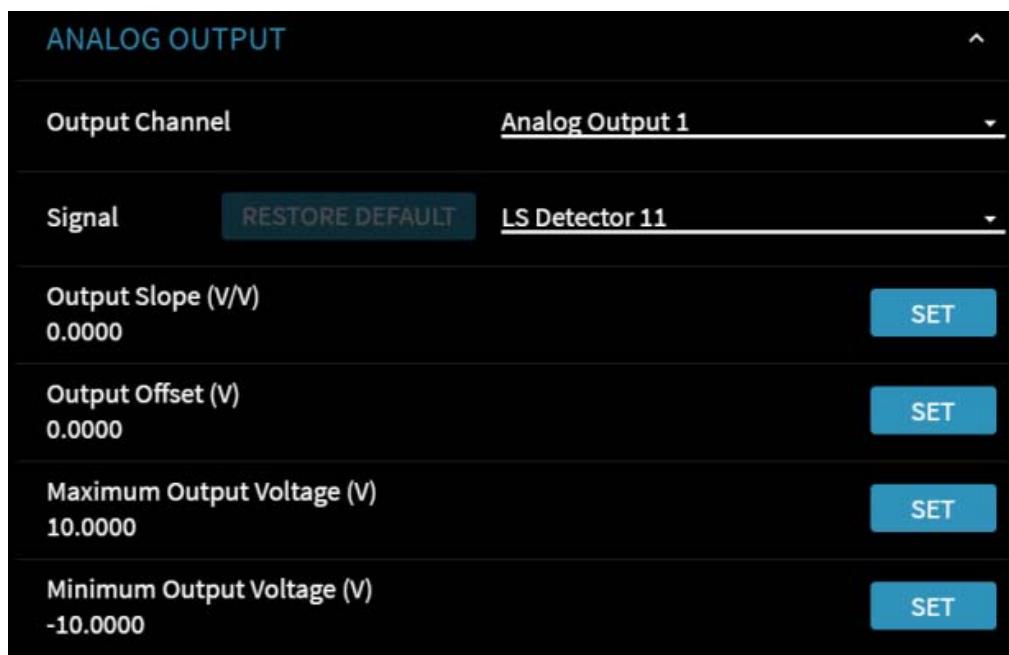
See [Connecting Auxiliary Devices on page 43](#) and [History Section on page 62](#).

Analog Output Settings

Expand the **Analog Output** section to see and control settings for the analog outputs on the back panel. These outputs can be used to transmit data channels to a third-party instrument. (See [Connecting Auxiliary Devices on page 43](#).)

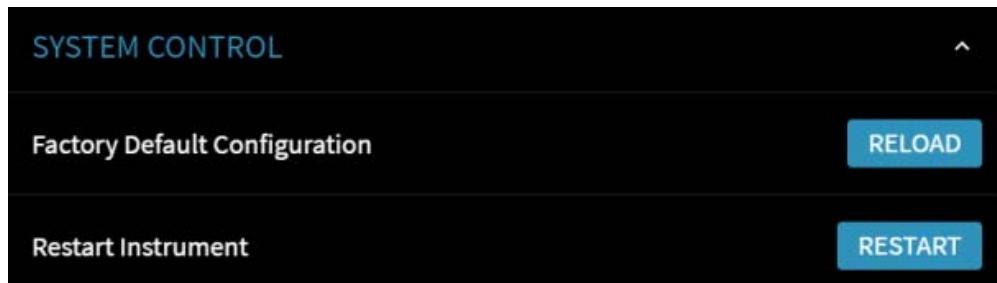
Select one of the four analog connectors from the **Output Channel** drop-down menu. Then select the data channel to transmit for that channel from the **Signal** drop-down menu.

By default, the output range is -10 V to 10 V. Click **Set** to modify any of the output signal parameters.



System Control Settings

Expand the **System Control** section if you want to reset the instrument to its **Factory Default Configuration** or **Restart** the instrument. You can also restart the instrument by pressing the On/Off button on the front panel and selecting the **Restart** option. The instrument can be powered down by holding the On/Off button.



You should restart the instrument after installing a firmware update.

6

In-Line Operation with HPLC

This chapter provides details about preparing samples for optimal in-line operation and measurement. There are HPLC connection guides for various HPLC manufacturers available online on the Support Center at www.wyatt.com/support.

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Conditions for In-Line Operation

In order to use the high sensitivity DAWN successfully, the following are recommended:

- Solvents should be pre-mixed and stored in a covered reservoir. For the lowest possible baseline noise, a stirrer should be used in the solvent flask to ensure no concentration gradients are present in the reservoir. The presence of bubbles or dissolved gases in the solvent can influence the results. An in-line degasser in your HPLC system is effective for removing bubbles and some dissolved gas. The solvent can be degassed by vacuum, sonication, or boiling and then subsequently blanketed with an inert gas such as argon that has low solubility in the solvent. The use of two or more pumps and a mixing chamber is not recommended since even a one part per million deviation in the mixed solvent ratio causes large deviations in the dRI signal, and there are no known mixing techniques that are able to maintain a one part per million level of stability.
- Solvent reservoirs should be well-maintained. For organic systems, only HPLC-grade solvents should be utilized. Solvents like THF and ether can form peroxides over time if not stabilized or even after several months when stabilized. Additionally, some chlorinated solvents can slowly decompose with moisture to form hydrochloric acid. For aqueous systems, bacterial growth can readily occur with buffer solutions. Additives like sodium azide and alcohol can prevent growth but it is still recommended to refresh buffer solutions regularly. If the instrument will not be utilized for an extended period of time, it is recommended that either an organic solvent or a water/alcohol mixture be recycled through the instrument.
- Thermal control and/or insulation of the system components are important in order to obtain good results, particularly in:
 - the column(s)
 - the eluent before the column(s) and tubing between detectors
 - the DAWN itself
- If the DAWN is not thermally regulated, the temperature stability will be determined by the surrounding environment. If this is the case, it is particularly important to place the DAWN in a location that has stable ambient temperatures. Avoid air ducts, heating systems, and air conditioning units. However, always ensure adequate space around the DAWN for air flow in order to keep the instrument electronics adequately ventilated.

- A pump that can output a constant flow rate is required and regularly checking the pressure of your HPLC and UHPLC system is critically important. A slowly increasing or unstable pressure is an indication that the chromatography conditions are changing, most often due to:
 - blockage in the solvent reservoir filter
 - blockage in the in-line filter after the pump
 - blockage in the column
 - malfunctioning pump
 - leak in the system
 - air or gas in the system

Such problems may create unstable baselines due to unstable column environments. The result is a signal with oscillations, spikes, or drift.

- SEC columns can occasionally shed packing material into downstream detectors resulting in column bleed. While the concentration of these shed particles is low, so they are not detected by concentration detectors, the size of the particles is very high and as such produces a high intensity of scattered light that the DAWN will measure. This will result in increased noise and impact the reliability of results. A clean system and quiet column should produce a baseline RMS noise of < 100 μV for any mobile phase.

The following are recommendations to minimize column bleed:

- Always change flow rates with a gradual ramp rather than abruptly. Use a maximum ramp rate of 0.1 mL/min per minute.
- Equilibrate SEC columns at the desired experimental flow rate for at least 24 hours prior to collecting data. Do not stop flow or change flow rate between beginning equilibration and the end of data acquisition.
- New SEC columns may require equilibration periods longer than 24 hours to reach acceptable noise levels. Always flush new columns to waste for at least 24 hours before connecting to the MALS detector.
- When changing solvents, take special care to avoid leaving residues of old solvent in the chromatography system. It is best to work with pre-conditioned columns and change solvents with the columns by-passed.

Check the pump pressure regularly, and check the system for any short-term variations in flow rate, eluent, or operating temperature. If variations are discovered, take appropriate action as recommended in the user's manual for the questionable component.

For further information regarding injection procedures, pumps, thermostats, and the like, refer to the instruction manuals for these specific components and consult the literature and manuals of your HPLC and UHPLC.

Sample Preparation

To avoid contamination of the column and obtain the best possible separation, it is important to have a clean sample solution.

Additionally, the following guidelines may be helpful in sample preparation:

- Solvent used for sample preparation should have the same composition as the eluent solution. Only HPLC-grade solvents should be used.
- Sample dissolution may be encouraged by shaking or swirling, homogenizing, or sonication. Care should be taken to avoid precipitation from excessively high concentrations. Several hours of dissolving time may be required for the sample to fully dissolve.
- Particle removal by filtration (0.2 μm to 0.5 μm filter) and the use of a guard column is highly recommended.
- The use of an in-line filter between pump and autosampler or injector is highly recommended.
- Degassing the HPLC solvent improves the quality of the solvent/sample analysis.

For information about creating and running an experiment that specifies the sample and solvent used, see the *ASTRA User's Guide*.

7

Off-Line Operation

This chapter provides general information regarding hardware setup and configuration for analyzing samples that have not been fractionated by an online HPLC system (for example, by SEC or FFF). For information on how to collect and analyze data for these experiments, see the appropriate sections of the *ASTRA User's Guide*.

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General Information and Sample Preparation

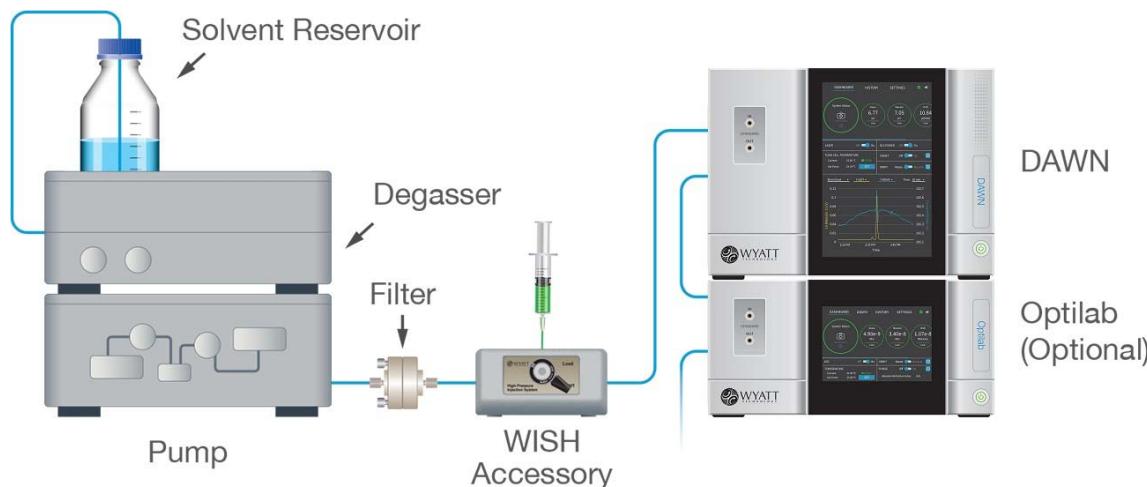
Offline light scattering measurements can be used to determine the weight-average molar mass, z-average RMS radius, and second virial coefficient (A_2) of an unfractionated sample.

Appropriate concentrations should be prepared using HPLC grade solvents or pre-filtered buffers and clean glassware. Since no concentration detector will be used, it is important that precise concentrations are known. It is recommended to prepare solutions by weight rather than volume as this tends to be more accurate. Use the same stock of solvent/buffer to prepare all sample solutions for a given experiment. Different stocks can introduce variation between samples in a given experiment.

Samples should be filtered appropriately according to their size to remove potential particle contaminants that will impact light scattering measurements, but not affect the concentration of the analyte of interest. Vials should be kept well-sealed to prevent evaporation. Once the solutions are prepared, seal one or two vials with solvent/buffer from the same stock to be used for the “blank” analyses.

HPLC Pump with Injector

Samples may be injected into the DAWN by using an HPLC pump, inline filter (optional), and a manual injector such as the WISH.



When using this setup,

- Use 0.010 in. (0.254 mm) ID tubing between the injector and the DAWN to minimize band broadening.
- Use 0.020 in. (0.508 mm) ID tubing elsewhere.

- Reverse the inlet and outlet fluid connections on the DAWN. That is, flow from the injector into the outlet port and from the inlet port out to waste. The DAWN has a narrow ID inlet tubing that will increase back pressure on the system to prevent air bubbles from forming when performing batch measurements with manual injections.
- Set the flow rate of the pump between 0.2 mL/min to 1.0 mL/min.
- Use a large sample loop, 0.5 mL to 1.0 mL to ensure that each injection produces a flat plateau and not a peak as it passes through the DAWN.

The objective is to inject known concentrations into the detector. If a peak with a rounded top and no flat plateau is obtained, the concentration at the top of the peak will be unknown; flat-topped plateaus indicate that the cell has been completely flushed with sample solution and a constant concentration has been reached.

Syringe Pump Infusion

Another option is to connect the DAWN as shown in Figure 7-1, using a syringe pump. Use 0.020 in. (0.508 mm) ID tubing to connect the syringe to the instrument and 0.030 in. (0.762 mm) ID tubing to connect the instrument to waste. Reverse the inlet and outlet fluid connections on the DAWN as described in the above section. Appropriate flow rates range from 0.1 mL/min to 0.5 mL/min. When using a non-disposable syringe, the syringe must be rinsed and dried thoroughly or replaced between samples to avoid contamination of one concentration by the next sample. When the syringe is disconnected to change samples, the pressure change and possible injected air may cause an unstable baseline for several minutes.

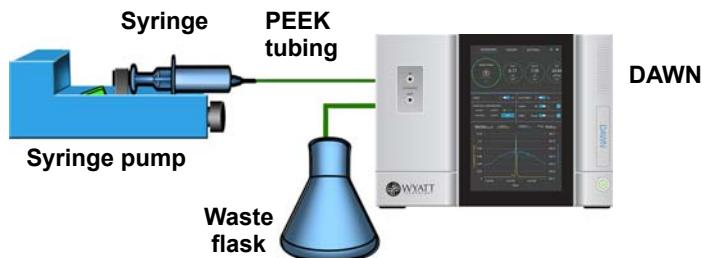


Figure 7-1: Setting up a syringe pump for calibration

Instrument Calibration

The DAWN instrument needs to be calibrated to enable ASTRA to convert voltage signals to Rayleigh ratios. A calibration constant must be determined before using ASTRA to calculate absolute molar masses. Calibration should be performed in batch mode; that is, before connecting the DAWN to a fractionation system.

When to Calibrate

Wyatt Technology calibrates each DAWN during manufacture and includes the calibration constant on the Certificate of Performance (COP) shipped with the instrument. We recommend that you check the calibration of the instrument in your own lab and compare the value obtained with the value on the COP report to verify that the instrument was received in good working order and no internal damage occurred during shipment.

The instrument should be recalibrated for changes that may affect the value of the scattering signal at the 90° detector. Calibrate when you:

- Disassemble and reassemble the flow cell after cleaning.
- Change sample cells (e.g. remove the flow cell and install the microCuvette, or vice versa).
- Have not calibrated your instruments in 1 year. Calibration constants can slowly drift over time.
- Change the 90° detector photo diode.

Setup for Instrument Calibration

1. Make sure your light scattering instrument is powered on and connected to the computer as described above. Additional information for connecting your DAWN to a computer can be found in [Instrument Connectivity on page 190](#).
2. If your flow cell does not already contain toluene or it contains an air bubble (a forward monitor value of 0 % generally indicates the presence of an air bubble), use a syringe pump and a 10 mL syringe with a syringe tip filter to infuse the flow cell with HPLC-grade toluene as shown below. Make sure that the solvent in the flow cell is co-miscible with toluene. If the instrument contains an aqueous salt buffer, flush first with 10 mL to 20 mL of water, then 10 mL to 20 mL of alcohol and then change to toluene. See [Flushing the DAWN on page 49](#).

Note: If a syringe pump is not available the solvent can be injected manually using a 1 mL or 3 mL syringe. Extra care should be taken to avoid introducing air when pausing during manual injection.

Connect a 10 mL syringe with luer connector and 0.02 in. tubing to the OUT port of your MALS instrument. Reversing the flow direction helps create back pressure since the inlet tubing has a smaller diameter than the outlet tubing.

Connect 0.03 in. tubing to the IN port of your MALS instrument and a waste reservoir.

10 mL syringe with HPLC grade toluene and 0.02 μm or 0.1 μm syringe tip filter (use the smallest pore size available).

Syringe pump with flow rate of approximately 0.1 mL/min to 0.5 mL/min.

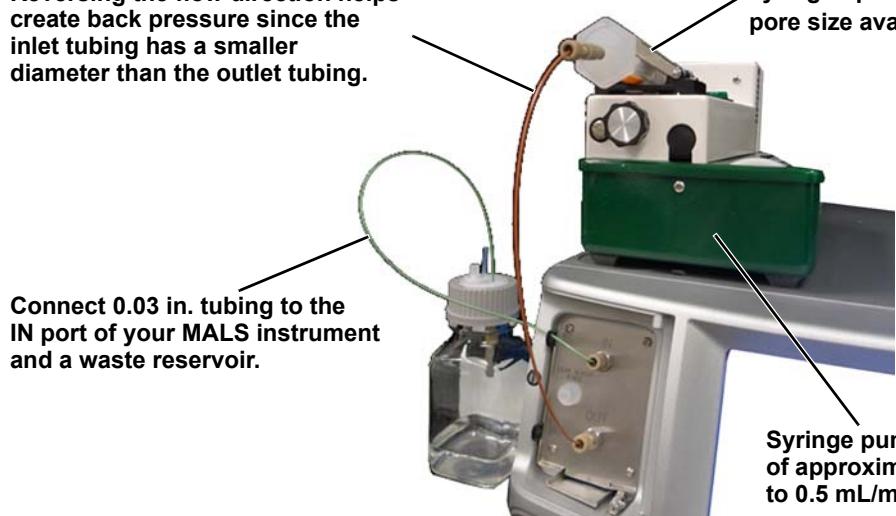


Figure 7-2: Syringe pump setup for calibration or sample batch injection

How to Calibrate

Calibration is performed in the ASTRA software. To perform a calibration experiment, follow these steps. See the *ASTRA User's Guide* for additional details:

1. Ensure the instrument has been switched on and warmed up for at least 30 min.
2. Set up the DAWN for a batch (non-flow) experiment with the selected syringe configuration as described above.
3. Create a new calibration experiment using ASTRA's Method Builder or by navigating to **File→New→Experiment From Method**.
4. In the **New from Existing** dialog, open the folder, **System→Methods→Light Scattering→Calibration** and select DAWN or DAWN 8 depending on your instrument.
5. Check the experiment configuration and make any necessary changes. Confirm connectivity with the physical instrument, the sample cell type (Fused Silica or F2), and the QELS option. It is not necessary to modify the Normalization Coefficients or Calibration Constant.

6. The default solvent is toluene. We recommend calibrating with HPLC grade toluene for the following reasons:
 - a. It has a high and accurately determined Rayleigh ratio.
 - b. It is a standard chromatography solvent that can be obtained in high purity.
 - c. Its refractive index is very similar to that of the flow cell windows.

If you would like to use a different solvent, navigate to Solvent (toluene) under the instrument option within Configuration, and click the “...” button for the Name property and open the System Solvents folder. Then select your solvent from the list of solvent profiles and click Select. You should calibrate the instrument using a highly pure solvent with a well characterized Rayleigh ratio.

7. Inject the filtered HPLC grade toluene into the flow cell using any of the configurations as described above when calibrating the flow cell. Use a pipette when dispensing toluene into the microCuvette for calibrations, taking care not to introduce any air bubbles. The solvent must be pure and free of particulates; we recommend using a 0.02 μm filter, though pore sizes up to 0.1 μm are acceptable if smaller sizes are not available.
8. Wait until solvent is flowing through the cell and/or the front panel display for the 90° detector stabilizes (detector 11 in the DAWN; detector 5 in the DAWN 8). The signal should be within 10 % of the toluene offset specified on the Certificate of Performance, generally around 0.1 V to 0.2 V.
9. Choose **Processing→Run** to begin running the calibration experiment. This method takes about one minute to run, sampling 30 seconds of data with the laser on and 30 seconds with the laser off. The calibration constant is automatically calculated and written to the LS Calibration Procedure and the final calibration report.
10. Use the calibration constant in other experiment configurations in either of the following ways:
 - Type the calibration constant in the Configuration for the instrument in other experiments.
 - Upload the calibration constant to the instrument. It will then be read into any new experiment for data collection in ASTRA. See the *ASTRA User’s Guide* for details.

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

microCuvette Accessory Kit

The microCuvette measurement accessory kit for the DAWN is designed for making batch light scattering measurements with sample volumes as small as 30 µL. In particular, it is an ideal substitute for more accurate flow cell measurements when sample volume is limited, sample recovery is necessary, or time-dependent studies are required.

The microCuvette accessory for making simultaneous dynamic and static light scattering measurements is easily installed in the instrument. In the DAWN (and DAWN 8), dynamic and static light scattering at four different angles can be measured. With the microCuvette measurement accessory, accurate molar mass and size can be determined for sample volumes that have traditionally been too low for the standard flow cell.

Productive use of the microCuvette requires careful adherence to the instructions. In particular, it is absolutely necessary to follow the guidelines for cleanliness in order to obtain consistently accurate results. Read the instructions carefully before using the microCuvette.

Items needed for installation

- 2.5 mm ball driver
 - Two 1/4" crescent wrenches for disconnecting the in-line unions
 - Anti-static wrist strap at a grounded work station
 - Anti-static bag for storing the flow cell
 - microCuvette measurement accessory kit
- See Figure 7-3 for an exploded view

Procedure

1. Make sure the instrument power is off.

CAUTION: Wear an anti-static wristband at a grounded workstation whenever you handle the flow cell. This keeps the flow cell glass and windows from building up a static charge and attracting particles while being handled.

2. Perform the steps in [Step 1—Accessing and Removing the Flow Cell Assembly on page 99](#) to remove the flow cell assembly.
3. In order to keep the flow cell assembly clean and dust free, place it in an anti-static bag for storage.
4. Insert the batch manifold and secure it with the three M3 screws. Note the orientation of the manifold. The manifold is keyed such that it is not possible to put it in backwards.
5. The DAWN is now ready for use with the microCuvette. Please read the rest of the instructions before introducing a sample into the microCuvette or inserting the microCuvette into the instrument.

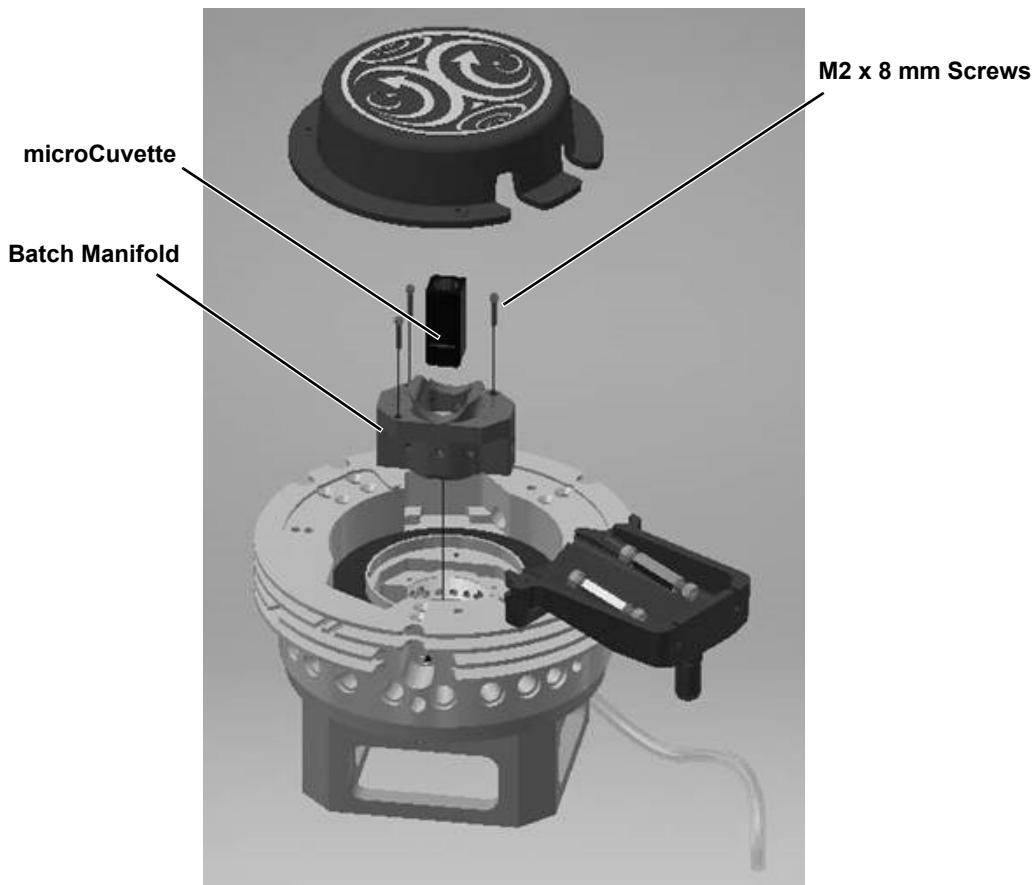


Figure 7-3: DAWN microCuvette Kit assembly

Handling

The microCuvette is made of fused Suprasil quartz. It is a precision optical component, and should be treated as such. Wearing gloves is recommended when handling the microCuvette; avoid touching the lower windows so as not to scratch or dirty them. In addition, the microCuvette should not be subjected to extreme shocks, either mechanical or thermal.

Cleaning the microCuvette

Prior to each measurement, the quartz microCuvette must be thoroughly cleaned.

Note:	See TN9000: <i>Quartz Cuvette Cleaning Protocol</i> for additional information regarding cleaning the microCuvette.
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The cleaning procedures described in this section are based on the assumption of an aqueous sample. For samples in organic solvents, the microCuvette must first be rinsed with a miscible intermediate solvent before using aqueous-based cleaning agents. For example, you could proceed from toluene to ethanol to water.

Whenever handling the microCuvette, make sure to touch only the upper part of the microCuvette to avoid smudges on or near the optical surfaces.

microCuvette Cleaning Materials

The materials listed below are required for a standard microCuvette cleaning. If more aggressive cleaning is needed, the microCuvette should be cleaned with 1 % Hellmanex, 10 % nitric acid, aqua regia, or an appropriate organic solvent prior to the standard cleaning described below.

- Distilled water
- Liquinox cleaning detergent for general cleaning or Tergazyme cleaning detergent for biological samples. Both detergents are prepared in a 1 % solution.
- Polyurethane foam swabs (from Hardware or microCuvette kit) for the interior reservoir. Lint-free lens cleaning paper (premium grade optical paper) may be used on the exterior for tough-to-remove smudges.
- Reagent alcohol (ethanol)
- Squeeze bottles or plastic transfer pipettes
- Filtered dry gas or compressed air

Procedure

1. Rinse microCuvette thoroughly with distilled or deionized water 3 to 5 times. With each rinse, grasp the microCuvette tightly and with a “flicking” motion, shake the water out of the microCuvette into the sink.
2. Rinse the inside and outside of the microCuvette with Liquinox (or Tergazyme for biological samples) solution 3 to 5 times.
3. To aid in the removal of any residue, use the polyurethane foam swabs to gently wipe the inside of the microCuvette when using the cleaning solutions above.
4. Fully rinse the microCuvette with distilled water 3 to 5 times while flicking the water out of the microCuvette after each rinse.

CAUTION: Ultrasonic cleaners can operate at frequencies that are resonant with the microCuvette, which can cause it to break. Wyatt Technology Corporation does not recommend the use of ultrasonic cleaners, and will not warranty a microCuvette that has been cleaned in ultrasonic cleaners.

5. With the microCuvette in hand, rinse the inside and outside with ethanol 3 to 5 times, shaking out the ethanol after each rinse.

6. If the microCuvette will be used immediately after cleaning, turn it upside down and dry with filtered dry air (or nitrogen). Dry the outside first, directing the gas flow from the bottom of the microCuvette toward the top. Dry the inside of the microCuvette by flushing with filtered air using a slight swirling motion for approximately 5 seconds.

Hint: Blowing a quartz microCuvette with filtered air can cause it to be electrically charged and attract dust. Using an ionizing air gun (if available) helps keep it dust-free.

7. If the microCuvette *will not* immediately be used, carefully place it upside down on a drying rack, taking care not to touch the optical surfaces. Prior to use, inspect the outer surfaces to ensure that no smudges are present. If necessary, rinse once more with an even spray of ethanol over the entire outer surface and allow it to air dry at least 15 min before use.

A jeweler's loupe may be used to inspect the cleanliness of the optical surfaces of the microCuvette. Streaks and residue on the outside or inside of the microCuvette indicate that the cleaning detergent has not been fully removed. In this case, more thorough rinsing with distilled water is advised.

microCuvette Cap Cleaning

If the sample cap is dirty, clean with detergent, followed by a copious water rinse, alcohol rinse, and blow dry with nitrogen or dry air.

ASTRA Settings and Angles

Data collection in ASTRA with the microCuvette is similar to batch measurements directly in a flow cell. See the *ASTRA User's Guide*. It is only necessary to choose the microCuvette sample cell type. In the DAWN Configuration, choose microCuvette as the Sample Cell.

Calibrating

Follow the usual calibration procedures in the *ASTRA User's Guide*. Note, however, that if more than one microCuvette is to be used, each microCuvette may have a different calibration constant. Calibrate each microCuvette, and record the calibration constant for each one. Each microCuvette has a unique serial number to help in identification.

Normalizing

Follow the usual normalization procedure, choosing an isotropic scatterer in the solvent to be used. Make sure that microCuvette is selected as the sample cell in the instrument configuration. See the *ASTRA User's Guide* for more information on normalization.

DLS Data Collection

The DAWN comes with four DLS positions that are independent of the light scattering positions. When collecting DLS data with the microCuvette, the DLS must be situated at the 90° position. ASTRA recognizes the DLS position automatically. See [Moving the DLS Fiber to a Different Location on page 151](#) for instructions when changing the position. In batch DLS analysis, sizes from 0.5 nm up to 1 μm can be measured.

If a significant drop in count rate is observed when going from the flow cell to the microCuvette, it might be necessary to re-optimize the DLS fiber position. See [Aligning the Optical Fiber on page 149](#).

Static Light Scattering and Analysis

Determining molar mass and size using static light scattering is identical to the methods for batch and micro-batch analysis described in the *ASTRA User's Guide*.

Making Measurements with Multiple Angles

For the DAWN, it is possible to use detectors 7, 9, 11, and 13 (3 through 6 in the DAWN 8) to make angular measurements of the light scattering in order to determine the RMS radius. To do this, take great care when removing and reinserting the microCuvette to ensure that it returns to its proper position. This can be done by observing the forward monitor when inserting the microCuvette, and making sure that the forward monitor signal returns to its previous value.

When using a microCuvette, you can measure molar masses from 200 Da up to 1 MDa for polymers or 10 MDa for proteins. RMS radii (or radii of gyration) from 10 nm to 50 nm (and up to 150 nm for shape-specific models) can be measured.

Also, normalization can be aided by first recording a baseline with solvent alone, and then adding the normalization standard into the microCuvette without removing the microCuvette. The resulting normalization constants in the **Settings** tab (see [Settings Tab on page 64](#)) on the front panel can then be used to verify that the signal levels of the various detectors return to their previous values when removing and reinserting the microCuvette, and that the correct ratios of signals are observed when measuring the actual sample.

In general, for samples with an RMS radius less than 10 nm, it is often sufficient to only use the 90° detector. For larger radii, all detectors must be used to accurately determine molar mass and RMS radius. It is a good idea to practice measurements with a known standard, for example polystyrene or dextran, before measuring an unknown sample.

Notes on Dust and Signal Stability

The presence of dirt and dust in a sample can lead to fluctuating signals. The static light scattering detectors are far more susceptible to this than the DLS measurements, since the static detectors view a greater illuminated sample volume and measuring the accurate absolute light scattering intensity is crucial. If fluctuating signals are observed, the simplest solution is to wait a few minutes before taking data. It is often the case that waiting will give time for any particulates to settle out of the illuminated scattering volume. Another solution is to recover and refilter the sample. However, the best solution is to avoid contamination of sample, solvents, and the microCuvette from the start. Be meticulous in keeping the microCuvette clean and dust free, and use proven methods to obtain clean, filtered sample.

Sample Preparation

The light scattered from a solution of particles is very sensitive to sample impurities and dust. It is important to develop routine procedures for preparing samples and collecting measurements. The information in this section describes some options for sample preparation. Before beginning your sample preparation, always work on a clean bench that has been thoroughly wiped with ethanol. In order to prevent contaminants from being transferred to the microCuvette, be sure to properly store the microCuvette with the cap when it is not being used.

Described below are a few suggested procedures for removing dust or other large particle contaminants from your samples. We recommend filtering, but if this is not possible, centrifuging samples is also effective.

Filtering Samples

In order to remove large particles, we recommend using a filter that is appropriate for the size of your molecule. For example, for the BSA monomer, a 0.02 μm filter would be effective for separating out large particles from the sample itself. It is important to remove large non-sample contaminants, because the upper size limit for DLS measurements is 1 μm radius. The presence of contaminant particles of this size or larger may cause inaccuracies in your measurements.

When working with samples with a radius approaching 1 μm , care must be taken to prevent the sample from settling during the measurement.

When you work with large particles, such as gold and silver colloids, macromolecular assemblies, and (some) polymers, the scattering from the sample itself is often sufficient to counter any scattering contribution from dust particles (if reasonable care is taken in sample preparation, such as using filtered solvent). In this case, number fluctuations due to dust are less likely to be seen, since the scattering contribution is negligible.

Filtering Options

If you are filtering your sample, you have several options:

If you have less than 300 μL sample to use for a measurement or are limited by sample volume, then you can use the Wyatt NanoFilter Kit (part number WNF-00), which has a dead volume of less than 5 μL . This minimizes the loss of precious sample. In addition, the Wyatt NanoFilter Kit has been designed to simplify sample recovery and refiltration.

If you are not limited by sample volume and have more than 300 μL of sample, use a traditional syringe filter. Make sure that the filter material used is compatible with your sample. If you are measuring BSA, the hydrodynamic radius of the monomer is 3.5 nm. This means that even oligomers of BSA are well below 20 nm radius. The average R_h may be larger if there are aggregates present in your sample. You may use the BSA standard and syringe filters that came with your instrument shipment, and compare the R_h value to the BSA Certificate of Analysis.

Centrifuging Samples

Rather than filtering your samples, you may prefer to centrifuge them. As a consequence of centripetal force, larger dust or contaminant particles migrate to the bottom of the centrifuge tube, thereby eliminating the need to remove the larger particles via standard filtration techniques.

Recommended spin rates and times vary with the sample. However, 10 to 15 min at 1000 to 5000 $\times g$ is typical. When removing the sample for loading, remember that only the top portion of the sample is dust-free.

Because of the need for volume control, a volumetric pipette works best for transferring sample from the centrifuge tube to the microCuvette. Dust can be removed from the pipette tip with a quick blast of filtered compressed air.

Sample Loading

The sample is loaded into the microCuvette by placing the needle or pipette tip all the way to the bottom of the microCuvette and dispensing the specified volume of sample. Be careful not to scratch the microCuvette window when placing the needle into the microCuvette.

Load the microCuvette with at least 30 μL of sample volume for best results and to avoid forming a meniscus in the optical cell. You may also use a small quantity of silicone oil to cover the sample, as long as it is less dense than your chosen sample solvent. This can also help prevent evaporation of your sample.

- If you encounter bubble problems, try slowly pulling the needle tip upwards as the sample is dispensed. If a few μL of sample loss is acceptable, avoid depressing your micropipette or syringe completely when dispensing your sample to avoid introducing air.

- A 100 μL pipettor with capillary tips also works well for loading a microCuvette, especially if you choose to centrifuge, rather than filter your sample. If you use a pipettor, it is recommended that you blow any dust out of the tip with compressed air, prior to filling with sample.

Always clean or rinse the microCuvette before use and make sure that it is capped during measurements.

Sample Preparation Troubleshooting

Air bubbles are a common issue with a low-volume microCuvette. Try tipping the needle into one of the corners of the microCuvette window. Also, insert the needle or pipette tip all the way to the bottom of the microCuvette and slowly inject the sample. This bottom up approach helps avoid trapping air bubbles. Many times, that will *pop* the bubble. Tapping the microCuvette gently against the tabletop can also help dislodge bubbles. Care must be taken not to damage the microCuvette or to “splash” the sample. If a few μL of sample loss is acceptable, avoid depressing your micropipette or syringe completely when dispensing your sample to avoid introducing air.

Also avoid dripping the sample down the side of the ground glass inlet section of the microCuvette, as this can sometimes introduce dust into an otherwise clean sample. Bubbles are often a sign of a cell that is not scrupulously clean. If they are persistent, try cleaning the cell with Hellmanex. See [Cleaning the microCuvette on page 82](#).

Inserting the microCuvette in the Instrument

Prior to loading the microCuvette into the DAWN, wipe any fingerprints or dust from the microCuvette, being careful to avoid scratching the surface. Lint-free optical-grade lens paper is recommended. When loading the microCuvette into the DAWN, you will note that there is one flattened corner on the microCuvette. This corner of the microCuvette must be positioned to the left front side of the optics block.

WARNING: There is only one correct orientation for inserting the microCuvette in the holder. The 45° beveled corner should be in contact with the ball plungers. Trying to force the microCuvette into the holder in any other orientation may damage the microCuvette.

As shown in Figure 7-4, the batch manifold has ball plungers that push the microCuvette against two reference surfaces for accurate positioning. The bottom of the manifold is the third reference surface, setting the height of the microCuvette. When inserting the microCuvette, position the microCuvette so that the 45° beveled corner is in contact with the ball plungers, then gently push the microCuvette into the manifold until it rests firmly on the bottom. Be careful not to insert the microCuvette too forcefully to avoid chipping or damaging the microCuvette.

The forward monitor is used to determine the microCuvette position. With the laser on, note the forward monitor reading without the microCuvette in position. Then insert the microCuvette. If a bubble-free sample is in the viewing volume of the microCuvette, the forward monitor should decrease by no more than a few percent when the microCuvette is correctly inserted. A decrease of more than 15 % indicates the sample is not in the viewing volume (for example, the microCuvette is not properly filled, the sample is turbid or absorbing, or the microCuvette has not been properly cleaned). A forward monitor reading close to 0 % indicates there is a bubble in the sample or the microCuvette is not properly positioned. Check for bubbles and reinsert the microCuvette, making sure that the microCuvette rests firmly against the reference surfaces. If alignment problems persist, verify that the batch manifold is installed correctly.

Once the microCuvette is properly inserted, use the batch cap or close the batch access on the cover to eliminate stray light. The sample in the microCuvette is now ready for data collection.

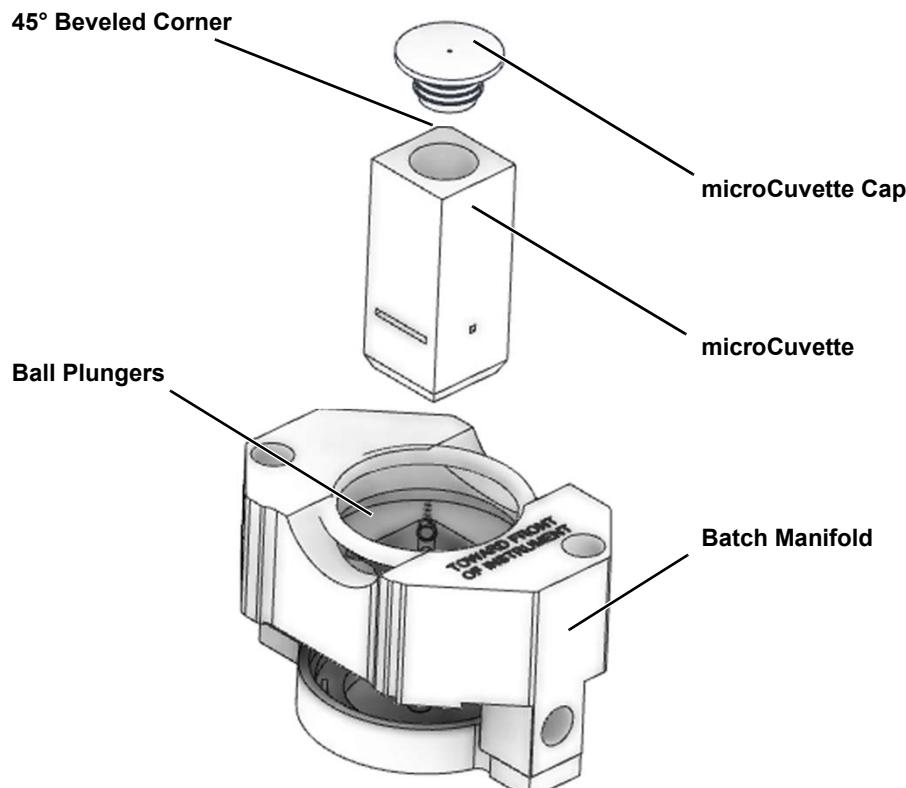


Figure 7-4: microCuvette insertion in batch manifold

Sample Recovery

Remove the sealing cap on the microCuvette, tip it slightly, and insert the recovery needle fully into the trough to remove the sample. Be careful not to scratch the inner surfaces of the optical windows. Make sure to clean the microCuvette thoroughly before storing. See [Cleaning the microCuvette on page 82](#) or [TN9000: Quartz Cuvette Cleaning Protocol](#) for additional information.

8

Service and Maintenance

This chapter gives guidelines for keeping the instrument clean and in good working order. See [microCuvette Accessory Kit on page 81](#) for the procedure for converting your DAWN from flow cell to batch mode measurements using a microCuvette.

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- Note:** Only trained personnel are authorized to perform work inside of the instrument. These authorized individuals are the individuals or group responsible for the safe use and maintenance of the equipment. Authorized personnel must have been trained by a Wyatt representative. Training may be achieved at LIGHT SCATTERING UNIVERSITY®, as part of on-site instruction during installation or on-site visit, or other type of instruction provided by a Wyatt representative. Please contact Wyatt Technology at support@wyatt.com for any questions.
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General Maintenance

For general maintenance, we suggest you do the following:

- Keep the instrument on a flat, clean surface, and standing on its feet to allow proper air ventilation. Allow a minimum of 10 cm (4 in) of space on all sides and a minimum of 15 cm (6 in) of space at the back.
- 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.
- Restart the instrument at least once a week by turning it off and on.
- Keep the instrument cover on at all times with the hatch closed.

CAUTION: The DAWN contains electrostatic discharge (ESD) sensitive parts. Wear an anti-static wristband whenever you open the DAWN to help prevent potential ESD damage to the instrument.

- Ensure that the power is turned off prior to servicing the instrument with the top cover removed. Wear an anti-static wrist strap that is properly grounded to the instrument chassis (if the instrument is still plugged in) or other suitable grounding site. A disposable anti-static wrist strap is included in the instrument hardware kit.

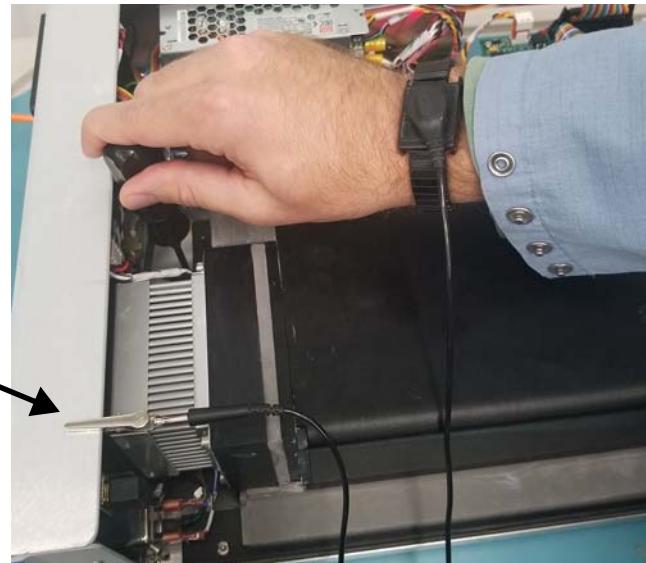


Figure 8-1: Anti-static Wristband

- When operating a temperature-controlled DAWN at sub-ambient temperatures, important considerations for minimizing condensation on the optical bench and electronics are needed. Please refer to [Preventing Condensation \(at Lower Temperatures\)](#) on page 124.

Daily Maintenance

Monitor baseline noise levels. The DAWN can achieve less than 100 µV rms noise for the 90° detector (less than 200 µV for the low and high angle detectors) depending on the cleanliness of the chromatography system. Higher rms noise (100 µV to 500 µV) can be tolerated for samples with high signal-to-noise. If noise increases beyond these values for the 90° detector, identify where the noise is coming from and take measures to reduce it. See [Troubleshooting on page 126](#) for details.

Turn laser off when instrument is not in use to prolong the lifetime of the laser. This can be done manually via the dashboard on the multi-touch display or can be programmed to be done automatically after the last experiment of the day has been completed via commands in ASTRA. See the *ASTRA User's Guide* for details.

Note:	The laser automatically powers down to 70 % intensity if the instrument is idle (not collecting data) for 1 hour as long as the Allow Idle Mode setting is enabled (see Laser Settings on page 67).
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Run the COMET for 5 minutes to 10 minutes after the last experiment of the day has been completed to maintain flow cell cleanliness. This can be done manually via the dashboard on the multi-touch display or can be programmed to be done automatically after the last experiment of the day has been completed via commands in ASTRA. See the *ASTRA User's Guide* for details.

Monthly Maintenance

Check the air filters on the rear panel fans for dust (Figure 2-2). Clean with compressed air or warm soapy water as needed. See [Cleaning the Air Intake Filter on page 124](#). If the filters are damaged, contact Wyatt Technical Support to order replacement filters.

Run appropriate validation standards (BSA, 30 kDa PS, etc) to check system constants such as normalization coefficients, alignment, and band broadening to confirm the DAWN is providing accurate results. See the *ASTRA User's Guide* for details.

Every 12 Months

Measure the calibration constant with filtered toluene. Calibration constants will slowly drift over time; this is perfectly normal. Calibration constants should not drift by more than 5 % every 12 months unless hardware has been replaced. If your DAWN calibration constant is drifting by more than 5 % within a 12-month period, contact Wyatt Technical Support.

Flow Cell Maintenance

The flow cell itself is critical to the operation of the DAWN. If the flow cell has become severely contaminated, you will need to remove it from the read head and disassemble it for cleaning as described in [Cleaning the Flow Cell and Windows on page 97](#). This is a procedure that, while not complicated, can be avoided by regular on-line cleaning as described below.

On-line Cleaning

To keep the flow cell free of contaminants, we recommend regular maintenance as follows:

At All Times

Use solvents, including water, that are HPLC grade and filtered through a 0.02 µm pore size filter.

If the instrument is connected to a chromatography system, keep pure, filtered solvent pumping continuously through the flow cell even when not actively collecting data. If an aqueous chromatography system and DAWN are not used for extended periods of time, recycle a 20 % or greater alcohol (EtOH, MeOH, IPA) solution through the system continuously. See [TN9004: Shutdown and Storage of Wyatt Detectors](#) for additional information regarding long-term storage.

To prevent bacterial fouling when using aqueous buffers either include an antimicrobial preservative such as 250 µg/mL sodium azide, actively use a Wyatt SOLARIS UV sterilization device, or replace daily with freshly prepared buffer.

If the instrument is in stand-alone mode (batch setup), store the flow cell filled with filtered solvent containing at least 20 % alcohol or an antimicrobial preservative to prevent bacterial growth.

For long term storage, flush the flow cell with organic solvent or 20 % or greater alcohol (EtOH, MeOH, or IPA) in water to prevent bacterial growth. Check the solvent in the cell about once a month. Add more filtered solvent as needed.

Off-Line Cleaning Before and After Completing Experiments

With the flow cell still in place, disconnect the DAWN from your HPLC system. Inject pure, filtered (0.02 µm pore size) solvent to flush the cell. We recommend that filtered ethanol or isopropanol (or other pure, organic solvent) be left in the cell.

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. See [TN3103: In-Situ Cleaning Protocol for a Wyatt MALS Instrument](#) for additional information.

COMET

We also recommend the use of the Wyatt COMET. 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 kHz to 900 kHz 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. It is recommended to operate it every night as part of a standard cleaning regimen. Alternately you can 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.

Replacing Inlet and Outlet tubing

Sometimes the source of excessive light scattering noise and high back pressure are caused by contamination or partial blocking of the tubing in the instrument. In this case, the inlet and/or outlet tubing of the flow cell may be replaced.

CAUTION: Never swage or tighten the fittings with a wrench when the flow cell manifold is in the read head, as this may damage the glass. Remove the flow cell manifold from the read head to tighten or swage fittings. Avoid touching the optical surface of the glass.

There are two extra sets of inlet and outlet tubes in your hardware kit. One set consists of four pieces of color-coded tubing, where the inlet tube has white insulation and an interior diameter of 0.005 in. and the outlet tube has blue insulation and an interior diameter of 0.010 in. 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-coded 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. To avoid introducing particles into the flow cell, flush the tubes after bending them and before installation with filtered (0.02 μm pore size) HPLC-grade solvent.

10 % Nitric Acid Cleaning

The DAWN flow cell and tubing are compatible with 10 % nitric acid, which can be flushed through the flow cell to clean dirty systems. The DAWN can be taken offline to perform nitric acid cleaning. Do not run nitric acid through a chromatography column and consult your HPLC manufacturer for compatibility with nitric acid. For organic systems, use the co-miscible solvents to convert the system from an organic medium to a pure alcohol followed by pure water before flushing with 10 % nitric acid. Conversely, after cleaning, flush with plenty of water until the pH is neutral, then use alcohol before converting your system back to its organic mobile phase. For aqueous systems, use pure water to remove any salt solutions before introducing 10 % nitric acid.

Protease Cocktail

For aqueous systems, 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 Boehringer Manheim or Roche.

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

Note: Using pepsin alone may be sufficient, as it is non-specific.

Procedure

1. Reconstitute each with 1 mL of PBS (25 mM Na₃PO₄ / 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 20 mL 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 20 mL of HPLC grade, filtered water, then mobile phase.

Particles in the Cell

Here are symptoms of particles in the cell and strategies to dislodge them.

Some Symptoms of Particles in the Cell

- 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 HPLC grade, 0.02 μm filtered water. For organic systems, first prepare the system for water by flushing out organic solvents with co-miscible solvents (for example, alcohols) before introducing water. Fill a syringe with a few mL of 10 % nitric acid, inject and leave the acid in the cell for 10 minutes, then flush with HPLC grade, 0.02 μm filtered water again.

Cleaning the Flow Cell and Windows

When the flow cell is dirty, light scatters excessively, which results in high and unstable light scattering baselines as well as distorted chromatography peaks.

The flow cell cleaning procedure can be broken down into the following major steps:

- Step 1—Accessing and Removing the Flow Cell Assembly on page 99
- Step 2—Disassembling the Flow Cell Manifold on page 104
- Step 3—Removing the Flow Cell Windows on page 110
- Step 4—Cleaning the Flow Cell Manifolds on page 111
- Step 5—Cleaning the Flow Cell Windows on page 113
- Step 6—Reassembling the Cell Windows on page 114
- Step 7—Cleaning the Flow Cell Glass on page 115
- Step 8—Reassembling the Flow Cell Manifold on page 116
- Step 9—Reinstalling the Flow Cell Manifold on page 119

Wyatt Technology offers a flow cell cleaning service for those who prefer not to clean the flow cell themselves. Contact Wyatt Technical Support for details.

CAUTION: The flow cell is a precision machined and expensive component part in your DAWN. If you have any questions about the safest procedure for handling the flow cell, contact Wyatt Technical Support.

Required Tools & Supplies

Note: Wyatt Technology offers flow cell cleaning kits (900001-21 for the ambient DAWN, 900001-22 for the temperature controlled DAWN) that contains the necessary supplies for flow cell cleaning. This kit is highly recommended. Contact support@wyatt.com for more information.

- Flathead screwdriver (to remove glass flow cell access cover)
- Tools from the instrument ship kit:
 - 2.5 mm hex driver (to remove 3 screws securing flow cell cover)
 - 1.5 mm hex driver (to remove top bracket and adjusting manifold)
 - 2.0 mm hex driver (to slide manifold)
 - Two open-ended $\frac{1}{4}$ " wrenches (to loosen unions and fittings)
 - Jeweler's loupe (to inspect cleanliness)
 - Flow cell window remover tool

- Flow cell cleaning kit (Wyatt P/N 900001-21)
 - Aperture installation tool for flow cell window retainer removal and installation
 - Replacement O-rings
 - Optical-grade lens paper
 - Floss
 - 10 mL disposal syringe (non-rubber plunger compatible with organic solvents)
 - Anotop syringe tip filter (0.02 µm)
 - Reverse action tweezers (soft tip)
 - Anti-static wrist strap at a grounded work station
 - Miniature Nylon brush
- Squirt bottle of alcohol (preferably IPA for rinsing clean the flow cell and manifold). A chemically-resistant squeeze bottle is recommended.
- Squirt bottle of detergent or cleaning solution (Contrad 70, Liquinox, etc.). A chemically-resistant squeeze bottle is recommended.
- Clean work space for performing the flow cell cleaning
- Powder-free gloves
- UV light (optional)
- Dry gas spray gun with filter for drying (optional)

Note: Canned dusting air is *not* recommended; it often contains chemical residues and can ionize the flow cell, attracting airborne particles. If filtered air or nitrogen is not available, the flow cell should be air-dried.

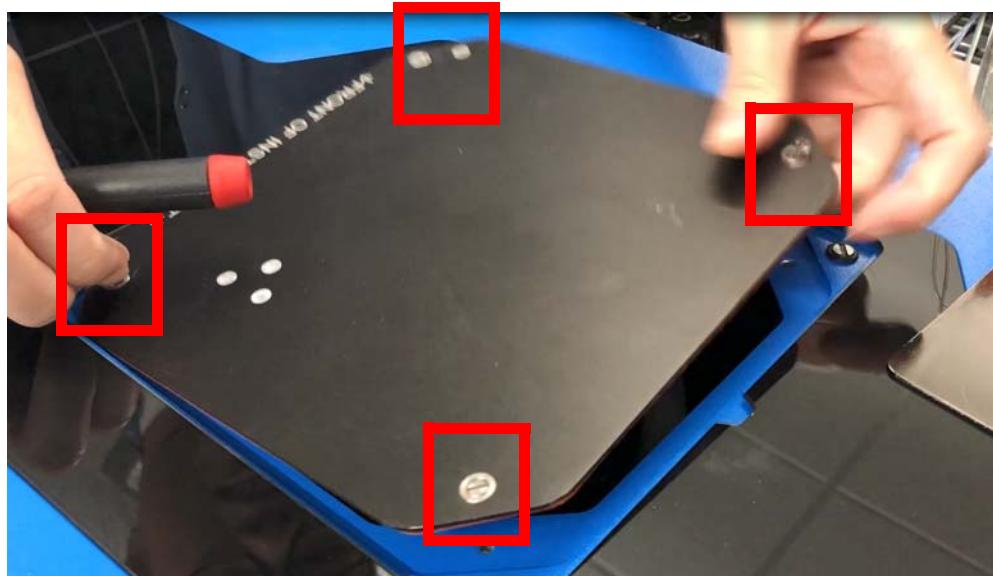
Step 1—Accessing and Removing the Flow Cell Assembly

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

1. Turn off the power to the DAWN instrument.
2. The top cover glass panel is held in place magnetically. Remove it by hand or with a flathead screwdriver.

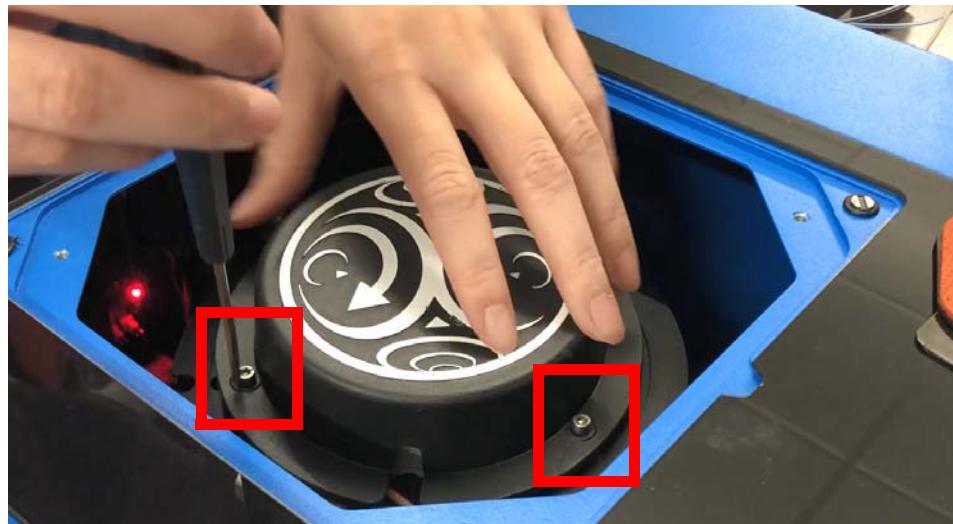


3. Beneath the glass panel is the flow cell access cover. It is held in place by four captive screws. Loosen the screws with a flathead screwdriver.



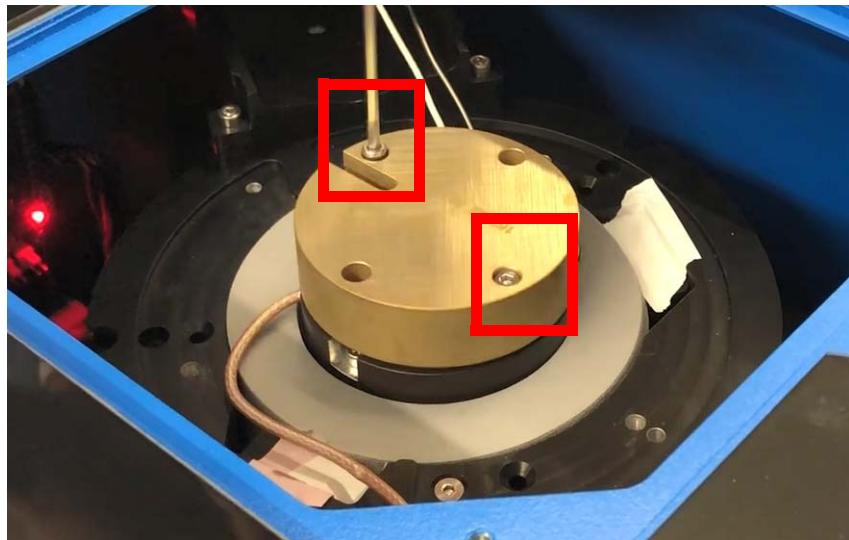
CAUTION: Wear an anti-static wristband at a grounded workstation whenever you handle the flow cell. This keeps the flow cell glass and windows from building up a static charge and attracting particles while being handled.

4. The flow cell manifold cover is secured to the read head with three screws (two shown below). Remove these screws using a 2.5 mm hex driver to gain access to the flow cell and the heat exchanger.



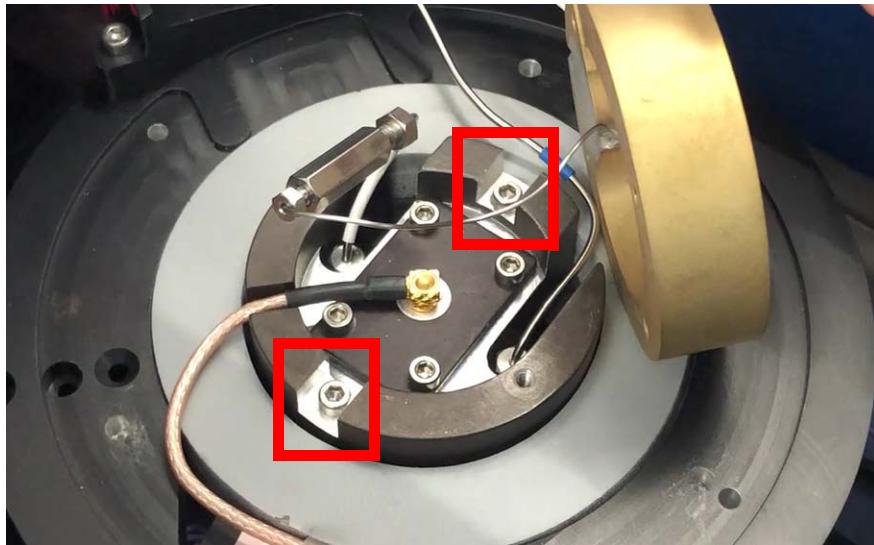
Note: If your DAWN is an ambient model, you will not have a heat exchanger as shown below. You can skip to Step 6.

5. The heat exchanger is secured by two screws. Remove them with the 2.5 mm hex driver,

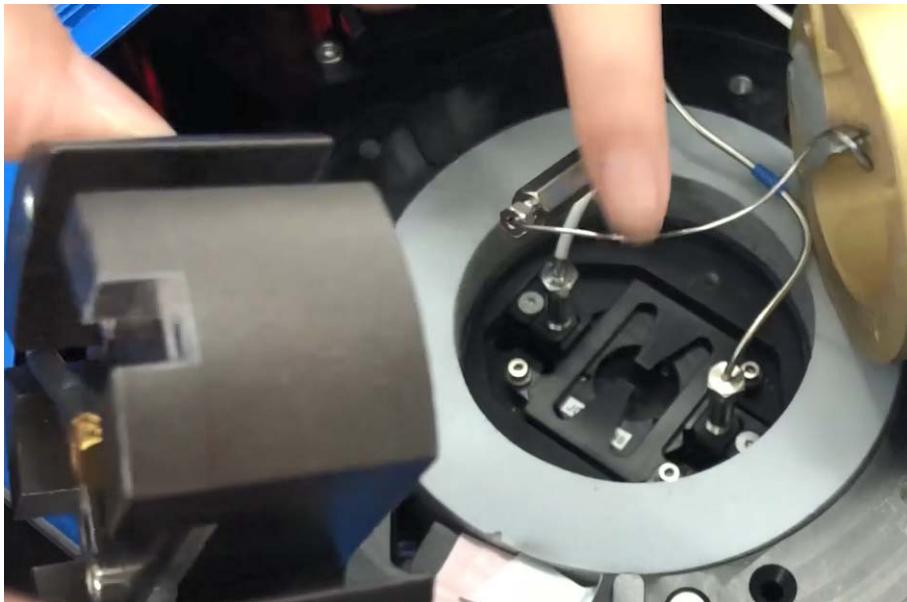


The inlet and outlet tubing into the flow cell, the COMET module, and the bracket holding the flow cell in place are now visible.

6. Use a 2.5 mm hex driver to remove the two screws that secure the COMET assembly to the flow cell manifold. You may need to gently move the tubing aside to access these screws.



7. Carefully slide the COMET assembly out, leaving the inlet and outlet tubing in place.

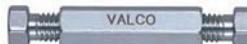


The **inlet** tubing, identified by white insulation is nearest the front of the read head whereas the **outlet** tubing, identified by blue insulation, is positioned closer to the rear of the instrument. The inlet tubing has a smaller inner diameter (0.005") than the outlet (0.01").

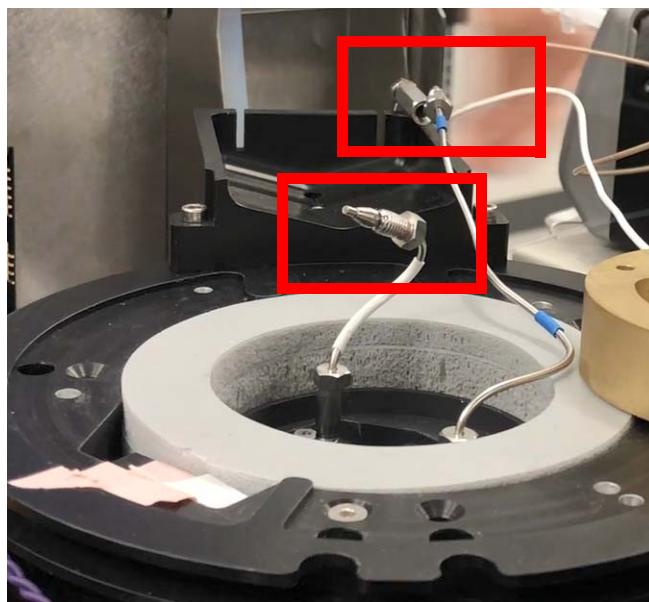
8. Use two $\frac{1}{4}$ inch wrenches to disconnect the fittings going into the unions from the flow cell. The unions will remain in the instrument, whereas the flow cell manifold will be removed as described in the steps that follow.



The 2-port 0.010 in. ID 1/16 in. Valco unions (shown below) include one stainless steel union, two stainless steel fittings, and two stainless steel ferrules. The union may be also purchased directly from the manufacturer (Valco part number ZU1C).

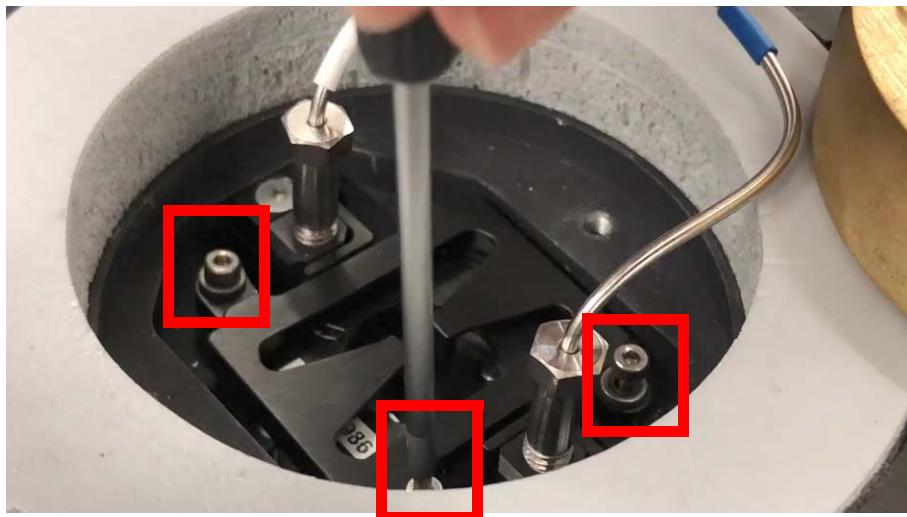


After removing the fittings connected to the unions, the flow cell connections will appear as below.



9. After you disconnect the unions from the fittings at the ends of the tubing connected to the flow cells, you may plug the unions to prevent evaporation from the lines.
10. Use a 1.5 mm hex driver to remove the two or three captive screws of the bracket that has secured the flow cell in place.

Note: Some newer instruments have a bracket with two screws on one end of the bracket. Remove such brackets by unscrewing the two screws and then sliding the other end out of the slot in the read head.



11. Lift the bracket out.

- 12.** Remove the flow cell from the read head by pulling vertically on the fittings to the inlet and outlet ports of the flow cell manifold. The optical glass surface will be exposed after removing the flow cell. Use care to avoid damaging the flow cell glass.

CAUTION: Do not pry the cell out with a screw driver or any other tool.



Once the flow cell is removed, the alignment bosses should be visible in the read head. You have successfully removed the flow cell from the read head and may now disassemble the manifold for manual cleaning or contact Wyatt Technology Support about shipping the entire flow cell manifold for professional cleaning.

Step 2—Disassembling the Flow Cell Manifold

Once you have removed the flow cell manifold from the read head, the manifold itself can be disassembled for cleaning or the entire manifold with flow cell glass can be sent to Wyatt Technology for cleaning.

CAUTION: Use care when handling the flow cell glass to avoid damaging it.

The parts that make up the flow cell assembly are shown in the following figure. Part numbers are listed in Table 8-1.

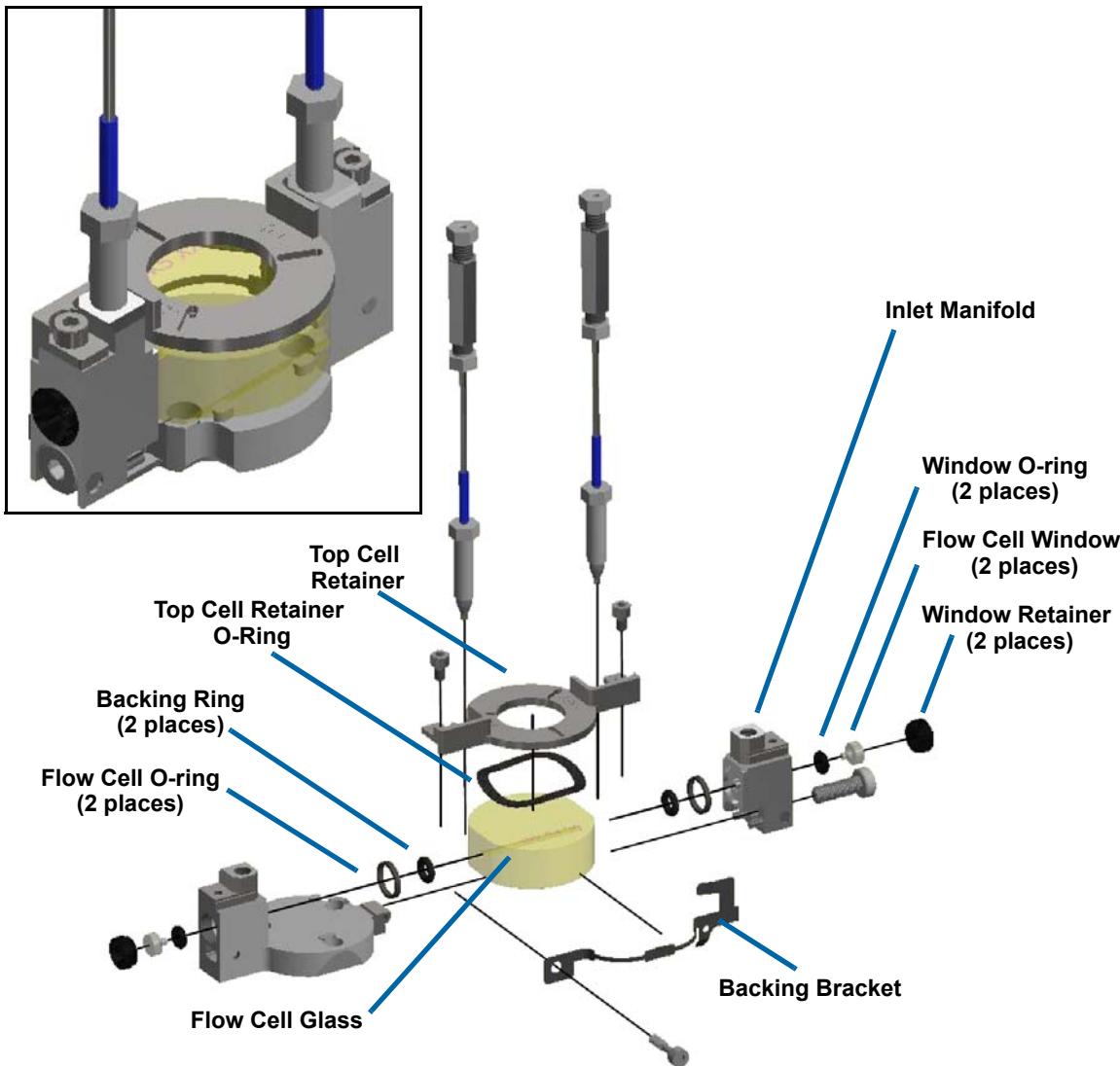


Table 8-1: Flow cell assembly, parts list

Flow Cell Part	Wyatt Part Number
Assy, Replaceable Glass Manifold	161744-01 [ambient], -02 [h/c], -03 [UH]
Inlet tubing 0.005" x 5"	212053-0559 [ambient]
Inlet tubing, 0.005" x 3.25"	212053-0539 [h/c]
Inlet tubing, 0.010" x 7"	212053-1076
Manifold set	165074 (2 pieces)
Horizontal spring	165069
Flow cell clamp assy	165702
Window (2)	163753-2

Table 8-1: Flow cell assembly, parts list

Flow Cell Part	Wyatt Part Number
Backup ring (2)	200609 [not used in UH]
Retainer for windows (2)	212073-1
Flow cell	212095-02 [fused silica], 212095-03 [F2]
Ferrule	P6455-12
Fitting, 1/16" tubing for 10-32 SS	P6405-310
Union, 0.010" ID	P6427-31010 [2 for ambient, 1 for heated/cooled]
Reducing union	P6430-31006101 [1 for heated/cooled]
O-ring, Kalrez 7075, size 2-004	P6504-20047075
O-ring, Kalrez 7075, size 2-006	P6504-20067075
Screw, SHC SS M2.5 x 4	S5002-2504
Screw, SHC SS M2.5 x 10 captive	S5002-2510I
Screw, button head SS M1.6x0.35x3	S5004-16003

Follow these steps to disassembling the flow cell manifold:

1. The first step in the flow cell disassembly process is to loosen the flow cell windows. If the instrument has been in use for some time, the windows will have become sealed to the 4 mm O-ring inside the manifold. Using force to remove the windows may damage them.

There are two ways the windows can be loosened from the O-rings.

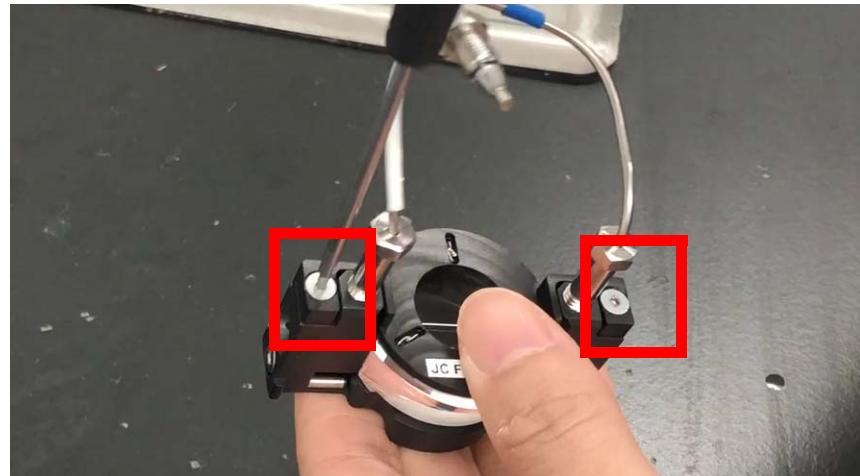
The first method is to schedule cell cleaning so that you can leave your cell overnight filled with IPA and with the window retainers loosened using the Cell Aperture tool (Wyatt part number 119033, shown in Figure 8-2). The square end of the aperture tool can be inserted into the window to loosen the retainer. This overnight soak should loosen the window/O-ring seal so the windows simply fall out the next day.



Figure 8-2: Aperture Tool

The second method will loosen the windows in about 15 minutes using a syringe pump. Use a 10 mL syringe and a Luer fitting to flow IPA into the outlet tube of the cell. Attach a waste line to the 0.005 in. inlet tube and start flowing IPA at a flow rate of 1 mL/min. The 0.005 in. tube acts as a flow restriction and will build a small amount of pressure in the cell. Use the Cell Aperture tool to loosen one window retainer at a time. Loosen one window retainer and you should see IPA begin to flow out around the retainer. Loosen and tighten the retainer several times. This compresses and relaxes the O-ring and helps loosen the manifold/O-ring/window seal. Tighten the first retainer and repeat this procedure for the other window. The pressure inside the cell forces IPA past the window/O-ring seal and allows easier and safer window removal.

2. Begin the cell disassembly after the windows are loosened as described above. Use a clean, hard tabletop or lab bench as a work area. Wipe the area with soap and water and then alcohol and dry thoroughly.
Do not work on paper towels or a sheet of paper as these have particles and fibers that can transfer to your clean parts.
If the bench top cannot be suitably cleaned, use optical grade lint-free lens paper or spec-wipe as a protective surface.
3. Next, assemble the tools you will need and lay them out at the top of the table. These include the 1.5 mm, 2 mm, and 2.5 mm ball drivers, the tweezers, and the Cell Aperture removal tool.
4. Use a 1.5 mm hex driver to remove the two screws on the top of the manifold. Then remove the top of the manifold. This exposes the top of the flow cell glass:



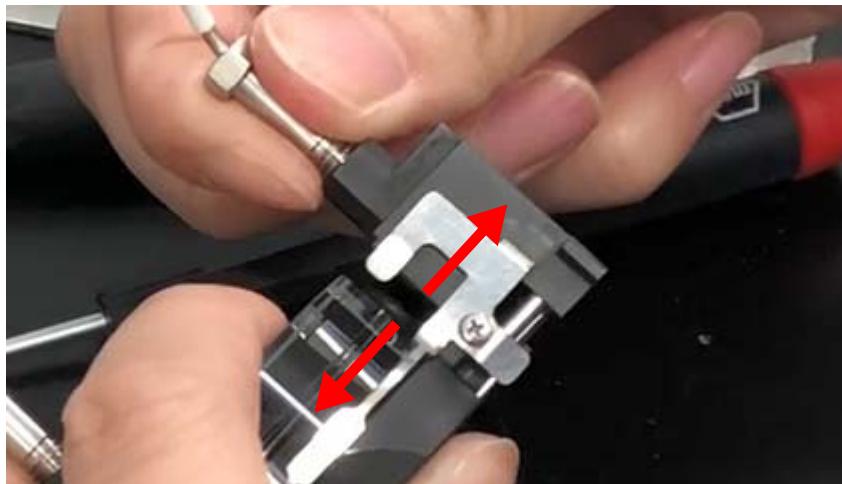
5. Use a 2 mm hex driver to loosen (not remove) the metal backing bracket that secures the ends of the manifold to each other and keeps the flow cell glass in place.



6. Use a 2.5 mm hex driver to loosen (not remove) the screw securing the two halves of the manifold together.



7. Once loosened, pull the side of the manifold away from the flow cell without separating the manifold into two pieces. It is possible that salt deposits may make it difficult to separate the manifold. In this case, it is recommended that you submerge the flow cell in water or alcohol to help loosen the manifold.



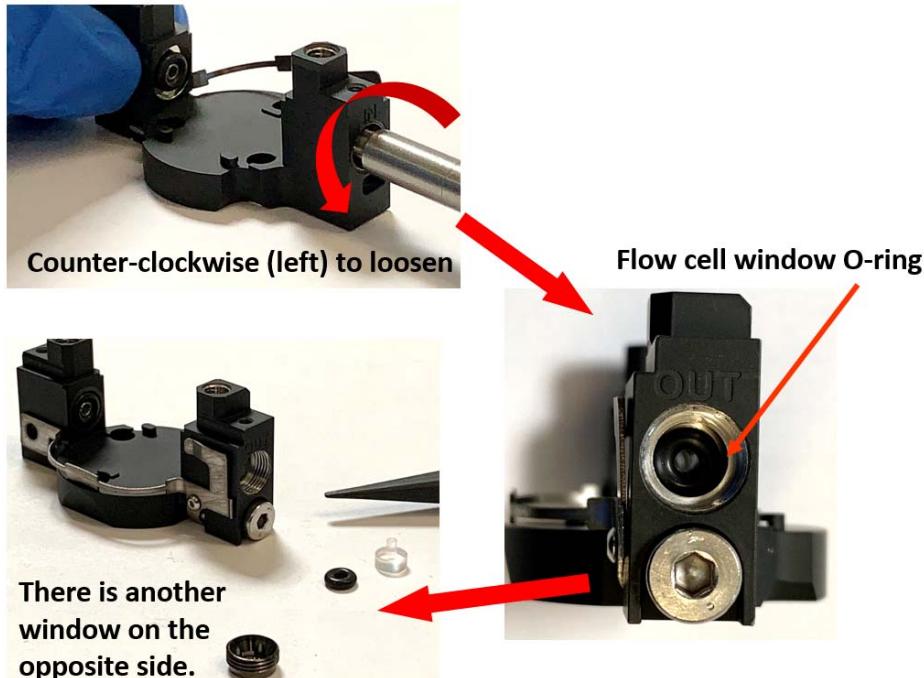
8. With both the backing metal brace and the manifold loosened, remove the flow cell from the manifold.



The next section describes how to clean this flow cell glass as well as how to remove and clean the flow cell windows.

Step 3—Removing the Flow Cell Windows

1. Remove each window retainer (using the Cell Aperture removal tool). Use a short piece of PEEK tubing to push the window out of the manifold. Stubborn cases may require overnight soaking in IPA to release the window.



2. The 4 mm O-ring may come out with the window but it usually remains inside the manifold. To remove it, use the 1.5 mm ball driver to gently pry it up from the center.
3. The 6 mm O-rings and the 9 mm backing rings on the inner section of the flow cell manifold can be left in place unless they are damaged or have a build-up of material between them. The 9 mm backup ring can be damaged during removal and can cause leaks if it becomes deformed. To remove, soak with Liquinox and then gently blow on them with a jet of air.

Step 4—Cleaning the Flow Cell Manifolds

The next step is to clean the manifold. Cells used with aqueous mobile phase systems often show a small amount of white salt build-up on the 6 mm O-rings in the area above the bore.

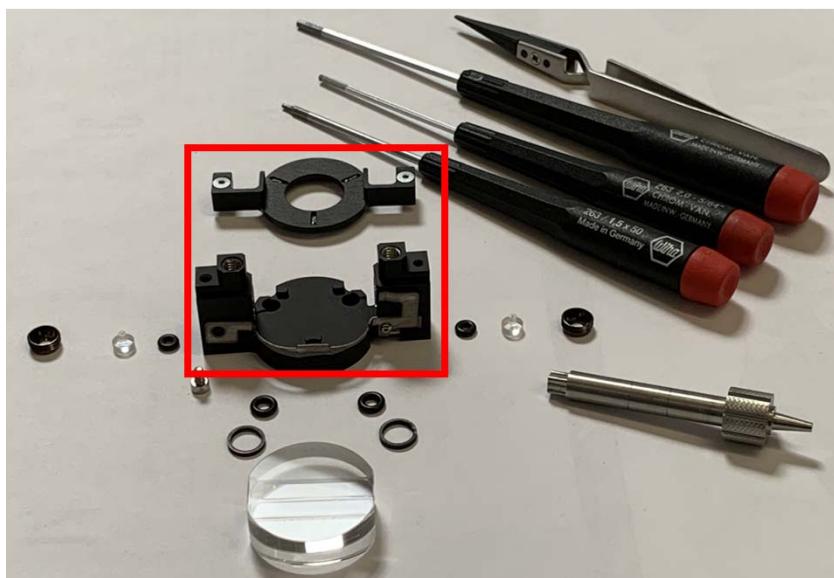


Figure 8-3: Disassembled flow cell with manifolds in red box

1. Take the manifold to a sink and rinse with water. If available, use a de-ionized water spigot at a lab sink. Rinse with Liquinox and rub the entire manifold with your gloved fingers. Take the 1/16 in. brush and rub the face of the 6 mm O-ring. Now run the brush through the bore of the manifold and clean the threads for the window retainer. Finally, carefully run the brush into the fluid connection port to clean the channel and spin the brush to clean the threads. Rinse thoroughly with water. Those labs without a source of running de-ionized water may simply use HPLC water in a squeeze bottle. Now rinse the manifold thoroughly with IPA from a squeeze bottle, flushing the water from all openings and surfaces. The squeeze bottle of clean IPA is a very important component of this cell cleaning procedure.

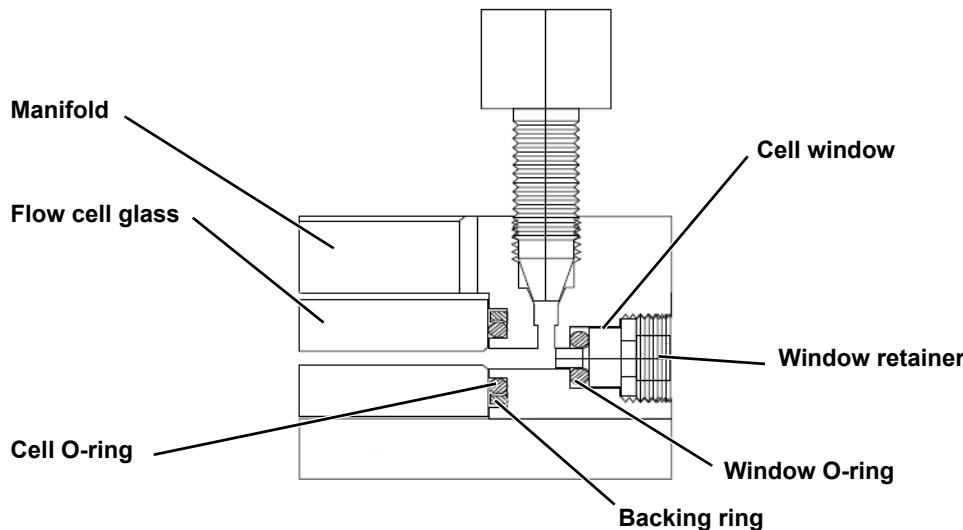


Figure 8-4: Manifold and flow cell window mount detail

2. Complete the cleaning of the manifold by blowing the IPA off with air until manifold is completely dry. Use the jeweler's loupe to inspect the O-rings for damage, particles, or residual deposits. The O-rings should have a uniformly smooth, flat black finish when clean. Set the dry manifold in the center of your clean area and repeat for the other manifold.
3. The 9 mm back up ring or the 6 mm O-ring may have come out during the cleaning process; or if you took them out, rinse them off, dry and replace them in the manifold. They may fit loosely or it may be a tight fit. To insert them, simply lay them in place and use the tip of the tweezers to push into the manifold. If they are deformed or damaged, replace them with spares from the hardware kit you received with your instrument.
4. Now take the two 4 mm O-rings to the sink, slide them onto the 1/16 in. brush, wet with Liquinox and spin them up and down the brush with your gloved fingers. Rinse with de-ionized water, remove them from the brush, grip both with the tweezers and rinse with de-ionized water again. Now rinse with IPA and blow dry. Again, use the jeweler's loupe to inspect the O-rings. At your clean work area, drop an O-ring into each manifold making sure it is lying flat on the bottom of the window recess.

Step 5—Cleaning the Flow Cell Windows

1. The next step is to clean the windows.

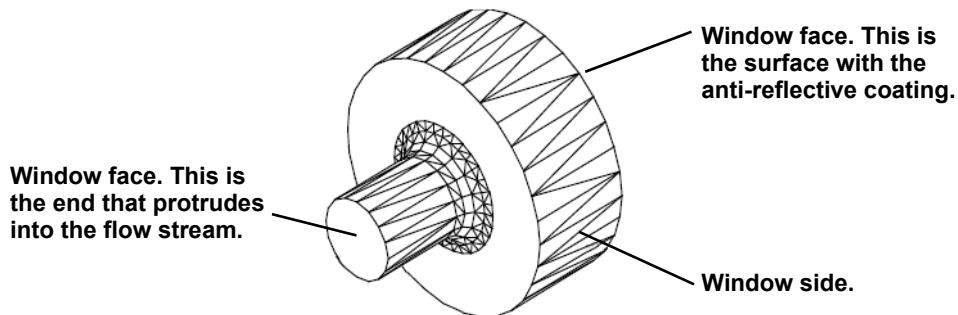


Figure 8-5: Cell window

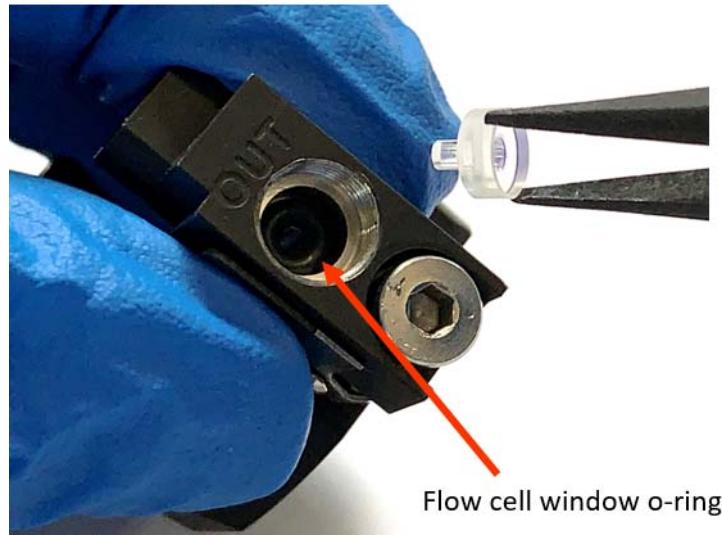
- The simplest method of cleaning is to use the tweezers to hold the window by the stem and then wipe it on a lens tissue that has been wrapped around your finger and moistened with HPLC ethanol.



- For more thorough cleaning, hold the stem of the window by the tip of the tweezers, soak with Liquinox, and rub between gloved fingers. Rub gently to prevent damage to the anti-reflection coating on the larger optical surface. Now rinse with water, then IPA, and blow-dry. Spray with a fairly strong jet of IPA, hitting both optical surfaces a couple times. Hold the tweezers upright so that all the IPA drains down and away from the window. Liquinox was developed specifically for cleaning lab glassware and does a very good job cleaning deposits on windows. The smaller optical surface may need more than one cleaning attempt to remove all deposits. Never use acids to clean the exterior cell window, as these may remove the anti-reflective coating.
2. While holding the window by the stem and looking toward a ceiling light, use the jeweler's loupe to examine the window for any particles or deposits on the optical surfaces. The ceiling adjacent to the light will be relatively dark so use this as a background for viewing the window. Against this dark background, any particles illuminated by the ceiling light will stand out as bright specks on the window surfaces. Windows should not have any cracks or chips but one or two very small particles are acceptable.

Step 6—Reassembling the Cell Windows

1. When you are satisfied that your window is clean, set it down on the table with the large optical surface pointed to the right (if you are right-handed). Approach the window from the right side with the tweezers and grasp the window with the tweezers tips about half way down the thick portion of the window.
2. Pick up the manifold, and, holding it almost vertically, insert the smaller window face through the 4 mm O-ring and gently release. Pull the tweezers out cleanly. The window should fit easily through the hole in the O-ring. If it doesn't, remove the O-ring and push it down onto the O-ring expanding side of the aperture tool to expand it. A drop of IPA on the tweezers will help the O-ring slide down the tip. If you grasp the window more than half way down the thick portion of the window, it will be very difficult to release the window because the tweezers tips will become wedged against the threads in the manifold.



3. Now examine the installed window for particles the same way you viewed the window earlier. If the surfaces look clean, you can screw in the window retainer. To clean the retainers, hold both retainers with the tweezers and rinse them with IPA and blow-dry them. Set them down in your clean area and take the top one and start threading it into the manifold. Use the window retainer tool to hand-tighten the window retainer until it stops; by design you cannot over-tighten the retainer. Once the stop has been reached, the retainer has bottomed out and formed a leak-proof seal between the window, O-ring, and manifold. Make one last inspection of windows for particles. Repeat for the other manifold.

4. With the flow cell windows re-installed on both ends of the manifold, place them on the tabletop with the larger one nearest you and with the 6 mm O-rings facing you. Fold two or three sheets of lens tissue twice in half the long way and then once the short way and lay them in the clean area of the tabletop.
5. The final cleaning step is one last wipe of the optical surfaces. Fold a lens tissue in half three times the long way and twice the short way. Put two or three drops of IPA on the fold. With a smooth, deliberate motion, wipe the length of the optical surface, being careful not to let your finger touch the glass as you finish. Now take a fresh, folded tissue moistened with IPA and use the stiff corner of the tissue to wipe the seam of the optical surfaces at the very ends between glass and manifold. Wipe both sides in this manner. You may have to repeat once or twice to achieve a smear-free, particle-free surface.
6. Connect the inlet and outlet tubing to the manifold.

Step 7—Cleaning the Flow Cell Glass

1. The next step is to clean the flow cell glass and re-assemble the cell. Take the cell to the sink or wherever you are able to rinse with de-ionized water. Flush the cell with Liquinox and gently roll/rub it with a gloved hand. Grasp the flow cell with the left hand with the glass-step to your right and facing away from you. Run the fuzzy end of two strands of Oral-B Super Floss dental floss together up from the bottom and flush the cell with Liquinox so that the floss is soaked. Pull the floss back and forth three or four times and then pull it back out the bottom of the cell. Rinse thoroughly with de-ionized water as you pull the floss back-and-forth a couple more times. Then rinse thoroughly with clean IPA from the squeeze bottle. Direct a strong jet down the bore of the cell for several seconds. Rinsing the bore with a jet of clean IPA is probably the most important part of the entire procedure. Make sure the entire surface of the cell is thoroughly rinsed.

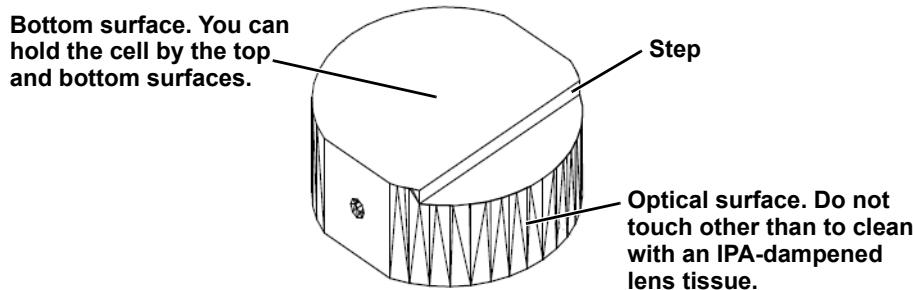


Figure 8-6: Flow cell

When holding the cell with the glass step to your right and facing away from you, the entrance or inlet to the cell is the opening of the cell bore nearest to you. Always flush or blow into this opening and never flush or blow up from the bottom.

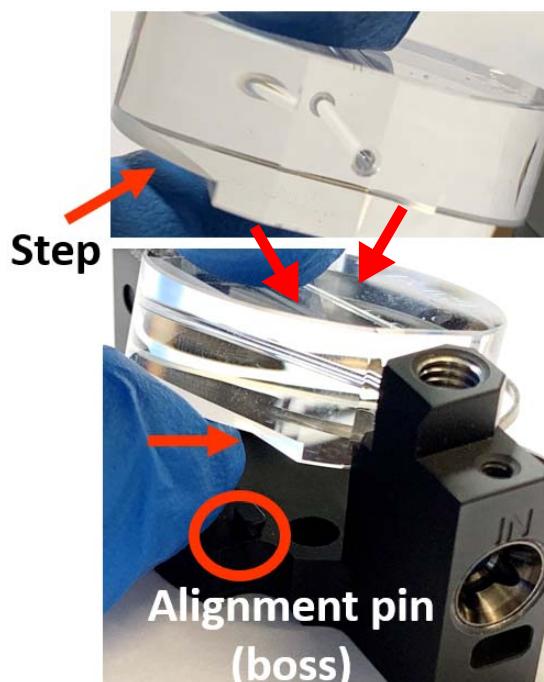
2. Immediately blow-dry the cell. Again, start the flow of air and bring the entrance of the cell bore into the flow stream. After a few seconds, move the stream away from the cell bore and try to blow off the upper and lower cell surfaces. Do not at any time stop the flow of air while drying the cell. Under these conditions the drying procedure takes about five or six seconds. It is fine if a little fluid remains on the cell; this will be wiped away in the next step. A filter-tipped nozzle at a pressure of 40 psi to dispense dried compressed air is recommended. Avoid using canned air, which may leave chemical residue on your flow cell window or ionize the flow cell, which will attract airborne particulates.

Step 8—Reassembling the Flow Cell Manifold

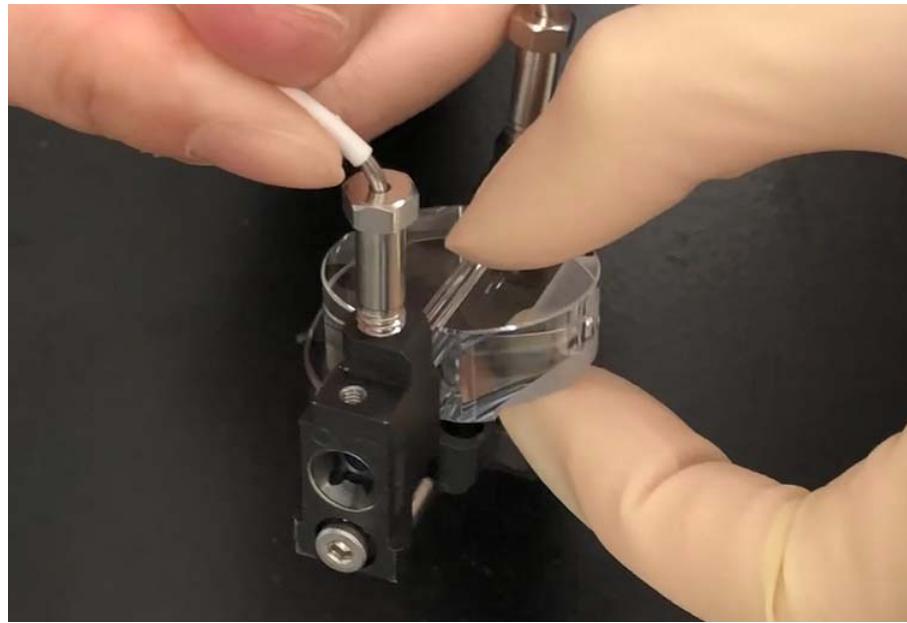
Once you have cleaned the flow cell glass and flow cell windows and re-installed the flow cell windows, reassemble the flow cell manifold by following these steps:

Note:	Avoid touching the optical surface (the sides) of the flow cell glass--apply pressure from the top of the glass instead of the side. You may also fold a piece of optical-grade lens paper to assist with handling the flow cell glass.
--------------	---

1. With the windows in place, reinsert the flow cell glass into the manifold. Identify the precision cut inset (step) in the flow cell glass and the alignment pin (boss) on the manifold that aligns the flow cell.



2. Slide the flow cell glass back into place so it sits flat on the bottom of the manifold.



3. While applying a gentle force against the bosses to keep the flow cell aligned, use the 1.5 mm hex driver to tighten the metal backing bracket slightly.



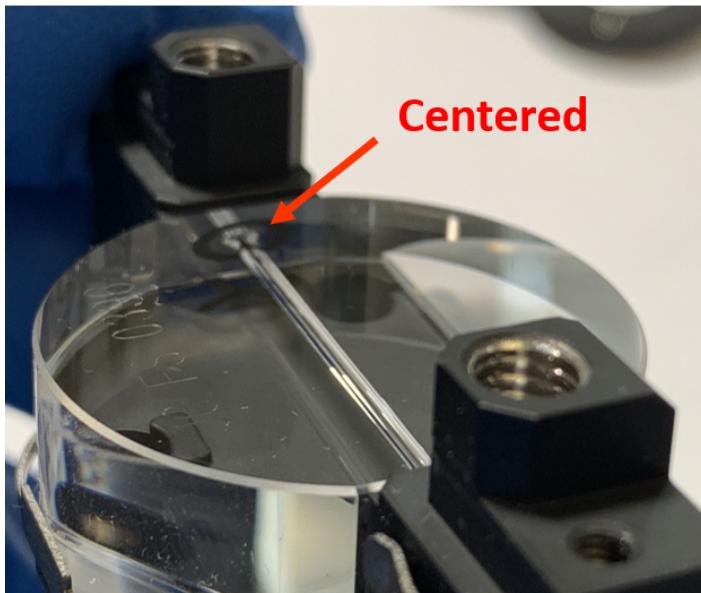
4. Adjust the metal backing bracket to ensure it is resting along the flow cell glass and tighten further. The screw to tighten is indicated below.



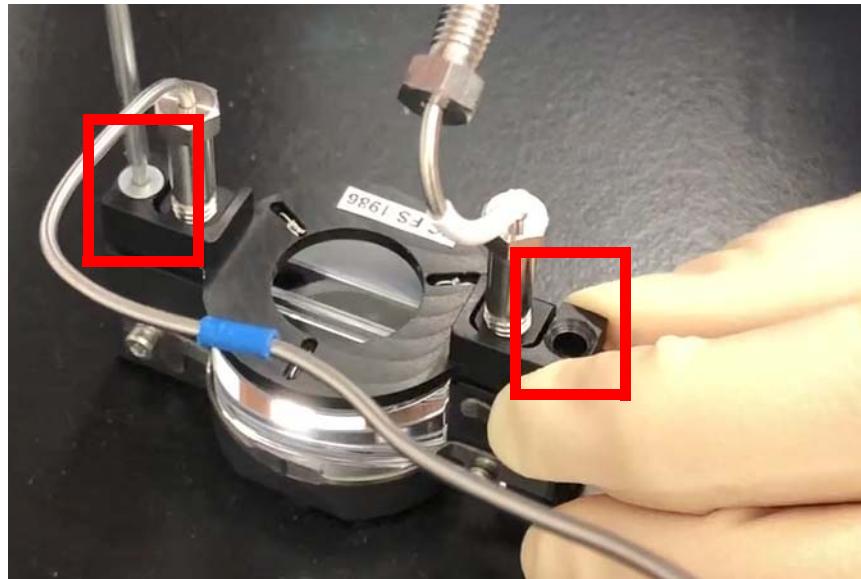
5. While still applying a gentle force to keep the flow cell aligned against the bosses, tighten the screw securing the two halves of the flow cell manifold with a 2 mm hex driver.



6. Alternate between tightening the screw that secures the two halves and the bracket screw to secure firmly. The flow cell glass should now be secure within the manifold.
7. Inspect the bore to ensure it is centered in the flow cell. When looking down the flow cell bore, it should appear concentric as shown in the “GOOD” view in the following image.



8. Insert the top cover of the manifold around the stainless steel fittings and re-secure it using a 1.5 mm hex driver.



Step 9—Reinstalling the Flow Cell Manifold

Note: Confirm that you are grounded before replacing the cell assembly.

1. The recess for the flow cell manifold contains 3 alignment pins that orient the flow cell manifold in the read head.



2. Use the fittings to hold the flow cell manifold, being sure to not touch the flow cell glass, to lower the flow cell manifold into the read head.



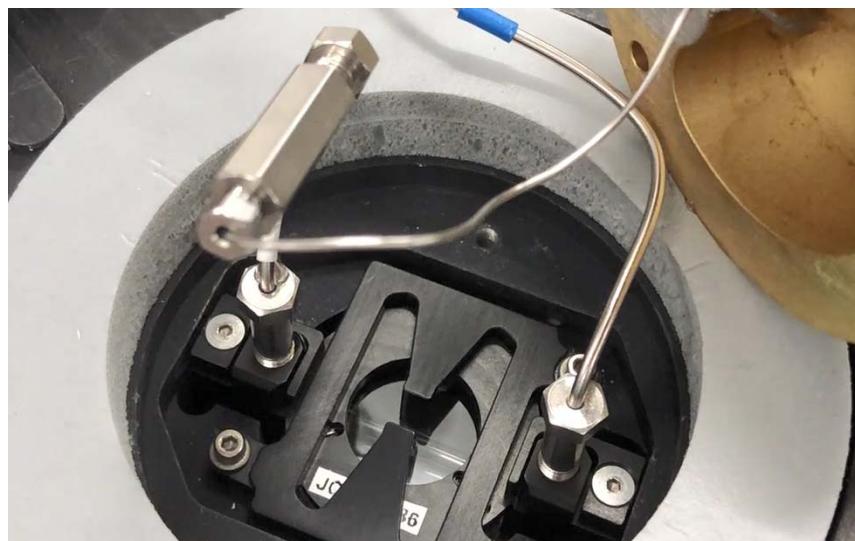
3. Ensure that the flow cell is level and flat.
4. Secure the top bracket by replacing the two or three screws using a 2.5 mm hex driver. (For some newer instruments, the brackets have only two screws. Such brackets are secured by first inserting the end of the bracket without screws into a slot in the read head and then tightening the screws.) Take turns between which screw you are tightening to ensure the bracket is secured evenly.



Note: Your bracket may be designed with only two screws instead of the three screws shown in the previous picture. That bracket type has an end that slides into a slot in the read head to secure the flow cell.

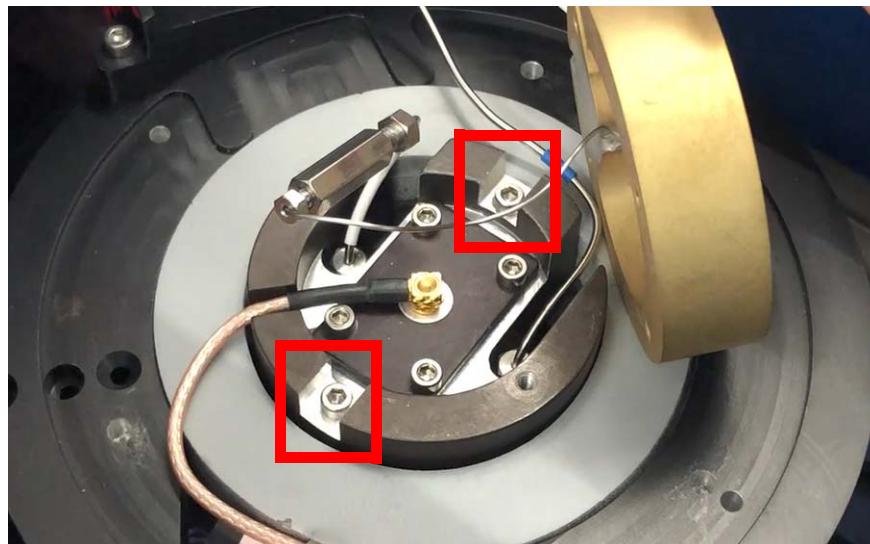
5. Reconnect the inlet and outlet fittings on the manifold to the unions in the instrument. Ensure that the stainless steel fitting is hand-tightened a couple rotations before tightening with a wrench to ensure the ferrule does not become permanently wedged into the manifold.

Remember, the **inlet** tubing, identified by white insulation is nearest the front of the read head whereas the **outlet** tubing, identified by blue insulation, is closer to the rear of the instrument. The inlet tubing has a smaller inner diameter (0.005") than the outlet (0.01").



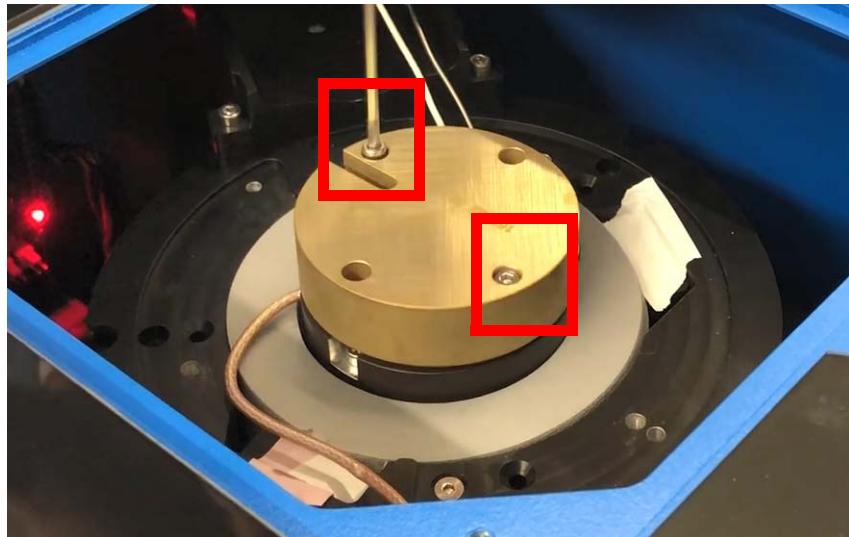
Be **absolutely certain** that you have flushed the inlet and outlet tubes with clean mobile phase before you reattach them to your clean cell. We recommend rinsing and blow drying all fluid fittings before connecting, especially those connections made to the flow cell.

6. Use the 2.5 mm hex driver to replace the two screws that secure the COMET assembly.

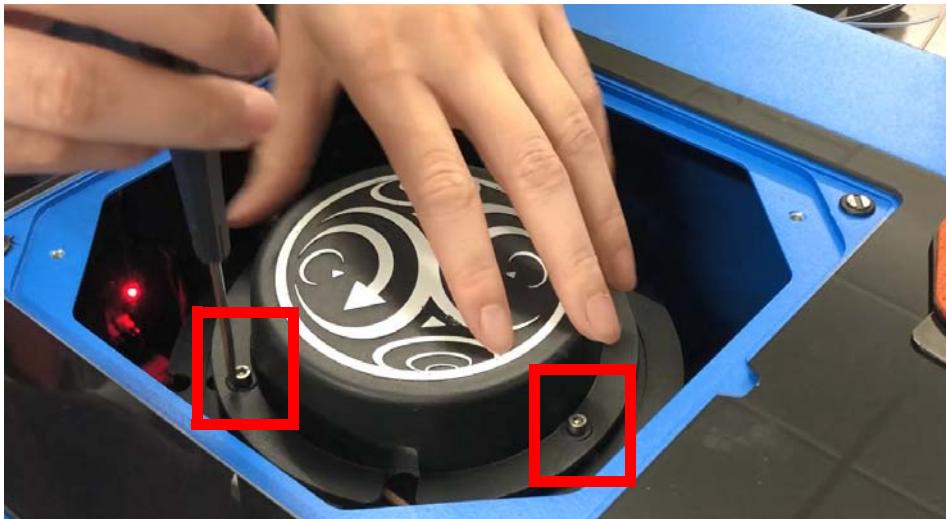


Note: If your DAWN is an ambient model, you will not have a heat exchanger as shown below. You can skip to Step 8.

7. Use the 2.5 mm hex driver to replace the two screws that secure the heat exchanger (if included).



8. Use the 2.5 mm hex driver to replace the three screws that secure the flow cell manifold cover (two screws shown below).



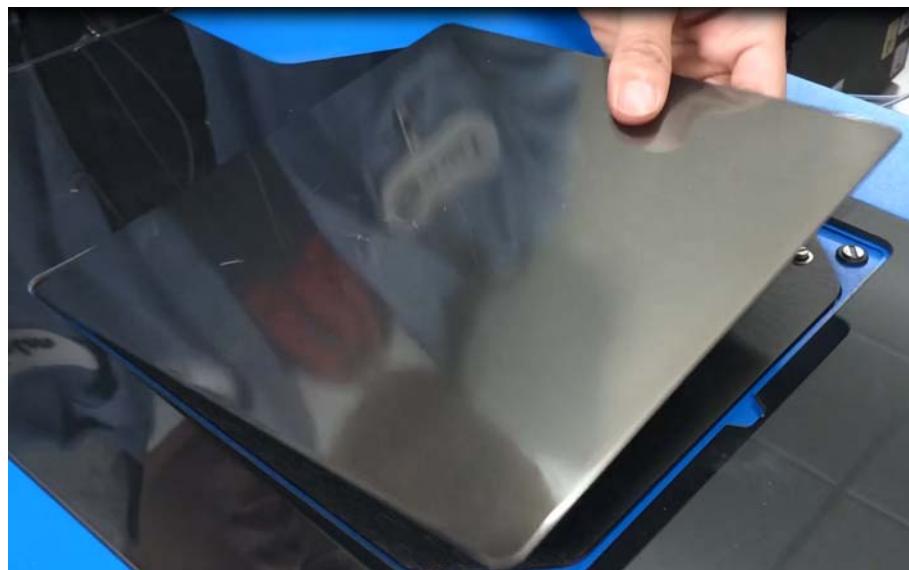
9. Plug in the power cord and turn on the DAWN.
10. Connect the cell to your HPLC system and make sure the cell does not leak.

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.

- 11.** Replace the flow cell access cover and tighten the four captive screws with a flathead screwdriver.



- 12.** Replace the top cover glass panel.



Cleaning the Air Intake Filter

Periodically remove and clean the foam filter for the instrument's air intake fan. The air intake fan is located on the rear panel of the instrument next to the power connector (see Figure 2-2).

1. Lift the filter bracket off of the rear panel of the instrument and remove the air filter from the filter bracket.
2. Wash the air filter with mild soap and water.
3. When dry, place the air filter back into the filter bracket.
4. Attach the filter bracket with filter back onto the rear instrument panel.

Preventing Condensation (at Lower Temperatures)

If operating temperature-controlled Wyatt instruments at sub-ambient temperatures (such as 5 °C), a dry gas (nitrogen or other inert gas) purge is essential to prevent condensation. A minimum pressure of 20 psi is necessary when using a temperature control set point below 20 °C. See [Installing the Instrument on page 39](#) for how to connect a dry gas line.

CAUTION: Instruments operated below ambient temperature must reach ambient temperature before they can be switched off. Fans and the dry gas purge valves do not work if the unit is powered off, so it is crucial that the instrument is brought up in temperature with fans operating and dry gas flowing to avoid condensation.

Failure to follow the steps below may result in electronic failures and corrosion damage to the instrument.

- Set the temperature to 25 °C after a low-temperature measurement has been completed. This is best done be from the **Dashboard** tab on the instrument's front panel (see [Flow Cell Temperature on page 57](#)).
- Leave the instrument powered on for at least 12 hours (overnight) after ambient temperature has been reached to ensure that any residual moisture has been removed.
- Let the instrument warm up to 25 °C at least every 4 weeks.

Note: Control the instrument temperature from the front panel of the DAWN. Configuring the "Temperature Control" in ASTRA does not set the instrument temperature.

5. See [Temperature Controlled Options on page 154](#) for more about temperature-controlled operation. For recommendations on how to operate Wyatt instruments in a cold room, see TN9001: *Operating Wyatt Instruments in a Cold Room* and TN9006: *Preventing Condensation in Wyatt Detectors*.

DAWN Firmware Upgrades

For peak performance and stability of your DAWN, check regularly for the most recent firmware. The DAWN firmware upgrades are available on the Wyatt web page. Visit www.wyatt.com/firmware and look for the appropriate update.

Instructions for installing the latest version of DAWN firmware for optimal performance of your instrument are also provided through the Wyatt Technology Corporation website. Note that you will need to run the executable firmware updater from a computer that communicates with your Wyatt instrument. Once installed, the Firmware Updater will automatically restart the instrument to complete the firmware update process for firmware. If the instrument does not restart itself, tap the **Restart** button in the **System Control** settings (see [System Control Settings on page 70](#)).

When performing a firmware update, do not use the on/off switch on the front of the instrument to restart it. After the installer has finished running on your instrument, confirm on the **Settings** tab that the newest firmware version has been installed (see [Instrument Information Settings on page 65](#)).

Please refer to TN2500: *Wyatt Instrument Firmware Update Guide* or TN2501: *Firmware Updater Connection Troubleshooting* for additional information for updating the firmware.

9

Troubleshooting

This chapter provides details about troubleshooting procedures for the DAWN. Prior to troubleshooting any issues, ensure that the instrument firmware is updated to the current version. See [DAWN Firmware Upgrades on page 125](#) for instructions on how to do this.

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Noisy Baselines

The DAWN is an extremely sensitive detector and will generate a response signal even if low concentrations of particle contaminants are present. Contaminants frequently come from the upstream column or HPLC system itself. Your DAWN will only perform as well as your HPLC system does!

A clean system should produce RMS baseline noise levels < 100 µV for the 90° detector photodiode detector regardless of what solvent is being used. If noise exceeds 100 µV there are several possible causes:

- The SEC column is shedding particles.
- The HPLC pump or other HPLC components are dirty.
- The mobile phase is dirty or the inline filter membrane needs to be replaced.
- The flow cell is dirty
- One or more photodiodes are malfunctioning

To test if the SEC column is shedding particles, disconnect the column and replace with a union such that mobile phase is flowing from the pump directly into the DAWN. If the noise level decreases, the column is the source of that noise. Try equilibrating the column at the experimental flow rate for longer (may require up to 72 hours for some columns), or replace the column.

If the column is not the source of the noise, test if the mobile phase or HPLC pump are dirty. Disconnect the DAWN from the flow path. Then remove a 5 mL to 10 mL aliquot from the HPLC reservoir and use a syringe pump to directly inject into the DAWN. A flow rate of 0.5 mL/min will suffice. If the noise decreases, the HPLC pump is dirty. If the noise level remains high, the mobile phase is likely contaminated. Other sources of HPLC noise are the autosampler or injector or UV (if present). Consult the HPLC manufacturer for suggestions on how to clean the HPLC components, and replace seals and frits as necessary. If the mobile phase is dirty, thoroughly clean and dry the reservoir, prepare fresh mobile phase, and filter with a 0.2 µm filter. Installing an inline filter between the pump and the injector is strongly recommended.

If noise remains high after eliminating the upstream components as sources of contamination, test for malfunctioning photodiodes. Disconnect the DAWN from the flow path and use a syringe pump to inject 10 mL comiscible solvents directly into the detector to exchange the system to alcohol (MeOH, EtOH, or IPA). Use a 0.02 µm syringe tip filter to ensure the solvents are clean. If the noise decreases when alcohol is injected, the flow cell is clean and the photodiodes are working properly.

For information on diagnosing and cleaning a dirty flow cell, see [Flow Cell Maintenance on page 93](#).

Wavy Baselines

The ambient DAWN has no temperature regulation for the optical bench. If the ambient temperature fluctuates significantly during data collection, this will generate fluctuations in the MALS baseline signal. To minimize this, maintain the ambient temperature of the lab to be as stable as possible. Do not place the DAWN close to heating or air conditioning vents, or near open windows and other sources of draft. Ensure sufficient ventilation space around the DAWN as described in [General Maintenance on page 91](#).

Wavy baselines could also be caused by a dirty flow cell and/or dirty windows. Refer to [Flow Cell Maintenance on page 93](#) and [Cleaning the Flow Cell and Windows on page 97](#).

Unexpected Peaks

When using the DAWN in online (flow) mode with a column, a peak may be visible in the scattering trace at the void volume of the column while no peak is present in the concentration trace. Since intensity of scattered light is dependent on both size, and concentration, larger particles can still produce a light scattering signal response at concentrations that are too low to produce a response in the concentration detector trace.

During the injection sequence a short pressure shock is delivered to the column which may loosen some column packing material. These particles exceed the separation range of the column and will elute in the void volume. Since the concentration is extremely low, no peak will be generated in the concentration detector trace. However, since the particles are large, a peak will be produced in the light scattering trace.

If you experience such a system peak, try equilibrating the column at the experimental flow rate for longer (this may require up to 72 hours for some columns). You can also try using a smaller injection loop on the autosampler or manual injector box, or reducing the mobile phase flow rate. If none of these options works, and the system peak interferes with sample peaks, you may need to use a different column or use an injector that produces less pressure change during the injection.

Low Forward Monitor

The forward monitor signal (FM) is adjusted to read 1.0 V (100 % as displayed on the front panel) when the laser is properly aligned and at maximum power. If the forward monitor drops more than a few percent below 100 % (this is solvent dependent) there are several possible causes:

- There is an air bubble in the flow cell
- The sample absorbs light at 658 nm
- The flow cell windows are dirty and/or misaligned
- The laser is misaligned
- The photodiode is malfunctioning

If an air bubble is in the flow cell, the FM signal will be at or close to zero. This is normal if the mobile phase is being changed. However, if the FM signal does not increase back to 100 % after flushing with 10 mL of mobile phase, the bubble is likely lodged. If installed, run the COMET for 5–10 min while flushing with mobile phase. If this does not work, use comiscible solvents to exchange the flow cell into alcohol (MeOH, EtOH, or IPA). Alcohol has a surface tension much lower than water, and bubbles will collapse rapidly once the flush is complete. Once the FM signal returns to normal, use comiscible solvents to flush back to your chosen mobile phase.

If your sample absorbs light at the wavelength of the laser, the FM signal will produce an inverse peak as the sample elutes. This is expected and minor absorption can be corrected for in the ASTRA software. For more information, contact Wyatt Technical Support for details.

If the FM signal is consistently low regardless of sample elution profiles, it is possible that the flow cell windows are either dirty or misaligned. Cleaning and/or replacing the flow cell windows will be necessary. See [Cleaning the Flow Cell and Windows on page 97](#) for instructions.

If cleaning and/or replacing the flow cell windows does not resolve the issue, it is possible that the laser has become misaligned or the forward monitor photo diode is malfunctioning. Please contact Wyatt Technical Support if you suspect this.

Microsoft Windows Encounters Problems

If the Microsoft Windows operating system on the multi-touch display encounters a problem that causes it to freeze or crash, the DAWN reboots the operating system within one minute. You can use the power switch on the front panel to turn the DAWN off and on if you want to reboot the instrument yourself.

Experiments in progress when the operating system crashes must be restarted. A weekly reboot of your instrument is recommended.

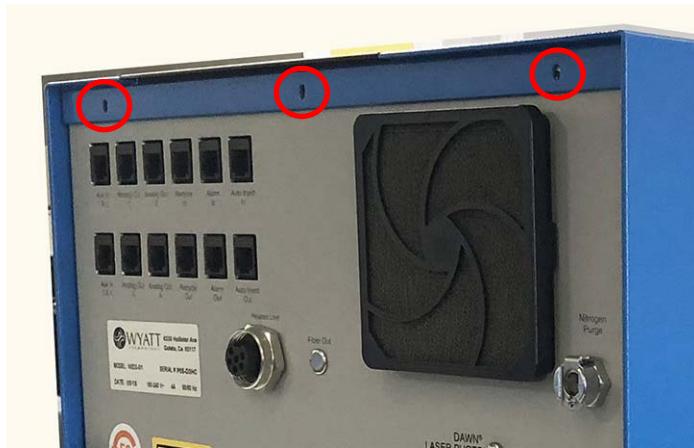
Removing the Top Cover of the DAWN

To replace components or work inside the DAWN, it may be necessary to remove the entire top cover when the flow cell access hatch does not provide enough accessibility to the internal components.

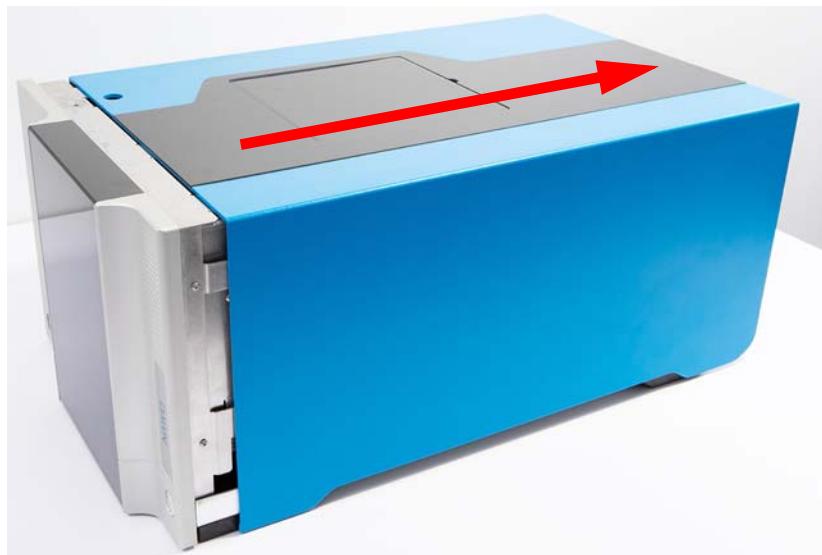
To remove the top cover, follow these steps:

CAUTION: Be sure to always wear an anti-static wrist strap when working inside the instrument.

1. Loosen the three captive screws on the rear of the instrument, indicated by the red circles in the following image.



2. Slide the entire top cover back a few inches. This reveals a gap between the front panel of the instrument and the top cover.



3. After the top cover has been pushed back a few inches, remove the top cover by pulling upward. You may need to pull the sides of the cover slightly apart if there is resistance lifting the lid up. The top cover is one continuous piece that includes the sides and glass cover.



4. Once the cover has been removed, you can access the inside of the instrument, including the internal stainless-steel tubing and the photodiodes.



5. Please refer to the sections that follow for information regarding troubleshooting inside the instrument.

High Pressure

If the system is experiencing high back pressure and you have isolated the source of the back pressure to the DAWN, there is likely an obstruction in the fluid lines. The white insulated flow cell inlet tubing has a narrower ID (0.005 in.) than the rest of the DAWN tubing (0.010 in.). This relatively narrow tubing, in the instrument by design, is a likely place for a blockage to form. The expected pressure generated by a DAWN is roughly 36 psi in water or aqueous buffer flowing at 1.0 mL/min at room temperature ($\sim 20^\circ\text{C}$).

If a blockage is suspected, a good first troubleshooting step is to replace this narrow tubing. See [Replacing Inlet and Outlet tubing on page 94](#). Also refer to [TN3102: Troubleshooting a Clog in a Wyatt MALS Instrument](#). If high pressure persists, contact Wyatt Technical Support for assistance.

Replacing a Photodiode

If a photodiode is malfunctioning it will need to be replaced. A malfunctioning diode will display a very high or very low voltage as compared to the other photodiodes, erratic voltage jumps, or sudden increases or decreases in output voltage rather than a smooth trace, even when flushing 0.02 μm filtered mobile phase through a clean flow cell.

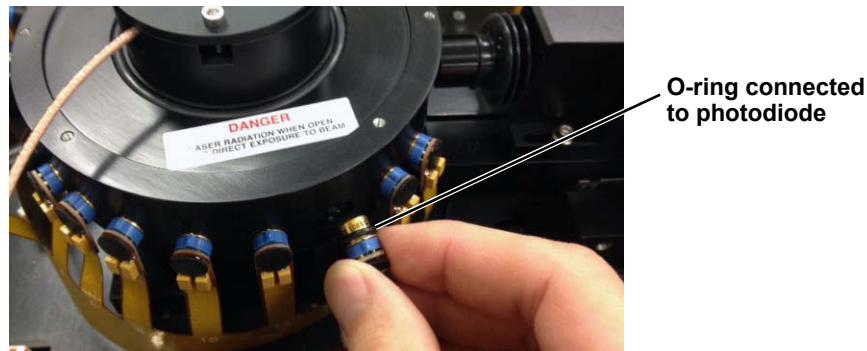
Tools needed:

- DAWN/miniDAWN® Photodiode replacement kit (900097) which contains the photodiode (212096-1), Photodiode O-ring (S6503-2011), and photodiode Light Termination (200590).
- Small flat-head screwdriver to assist with prying out the malfunctioning photodetector.
- Anti-static wrist strap

Procedure:

1. Ground yourself with the anti-static wrist strap as described in [General Maintenance on page 91](#).
2. With the instrument powered off, remove the top cover.

3. Using your fingers, grasp the photodiode that needs replacing and gently pull it directly out from the read head.



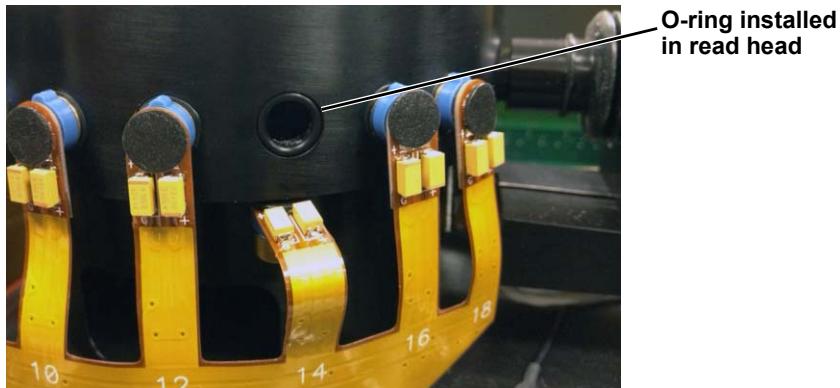
4. If the O-ring is still connected to the photodiode cylinder, remove it.
5. Using a fingernail or small flat-head screwdriver, pry the photodiode away from the blue mounting socket.



6. Connect the new photodiode onto the blue mounting socket, lining up the tab on the top side of the photodiode with the tab on the top side of the socket. Insert the 4 prongs into the appropriate plugs. Take care to not touch the photodiode surface. If the photodiode and socket are misaligned, the voltage output polarities will be reversed, so ensure that the tabs are lined up as shown in the picture below.



7. Moisten the O-ring with alcohol (methanol, ethanol, or IPA) and place it flush into the read head at the appropriate detector location. If the existing O-ring is damaged, replace it with a new one.



8. Place the photodiode directly in the center of the O-ring and gently press it straight into the read head until it is fully inserted.



9. Replace the top cover.

Changing a Fuse

The fuses are located in the power connection socket (see Figure 2-2). In order to change fuses, you need a tool for prying the AC Power module cover off, such as a small-bladed screwdriver, and fuses from the spares supplied in the accessory kit.

To replace a fuse, follow these steps:

1. Disconnect the power cord.
2. Open the cover of the Power module using a small blade screwdriver or similar tool.
3. Replace the burned out fuse(s) with a 4 A, 250 V slow burn fuse. A glass fuse can be visually inspected to see if it is burned or not. For ceramic fuses, a multimeter or ohmmeter can be used to check whether the fuse is blown and no longer provides a value (O.L) for resistance. Both fuses must be installed for the instrument to operate correctly.
4. Replace the cover of the Power module and reconnect the power cord.

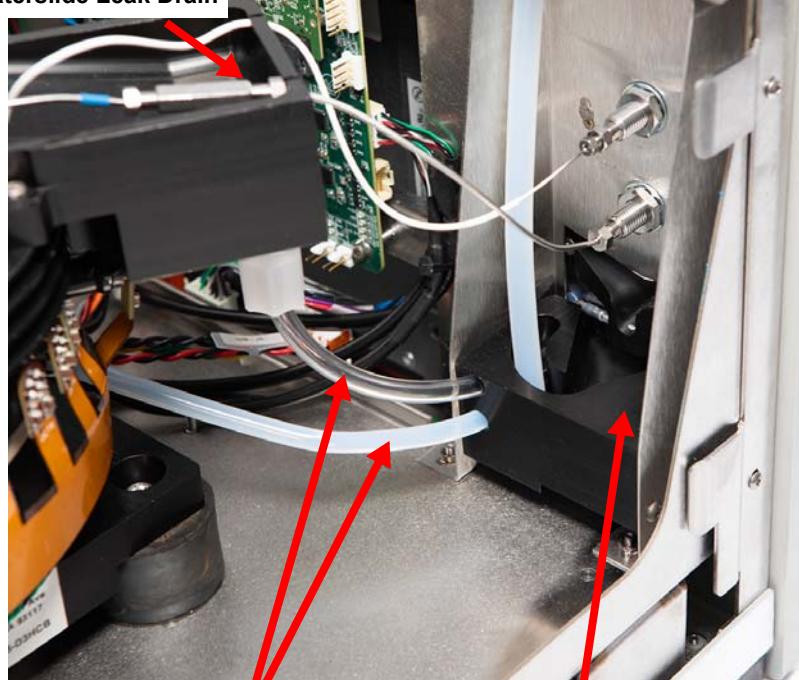


Leak Sensors and Cleaning After a Fluid Leak

Liquid from leaks of internal and external fittings is directed into a well inside the instrument. If a liquid leak occurs, a sensor activates an alarm. For more information about alarms, see [History Tab on page 61](#).



Waterslide Leak Drain



Drain Tubing from Waterslide & Manifold

Drip Tray

Leaks trigger alarms on the front panel display and drain into the drip tray, from which fluid can be drained to waste through the leak port on the underside of the instrument.

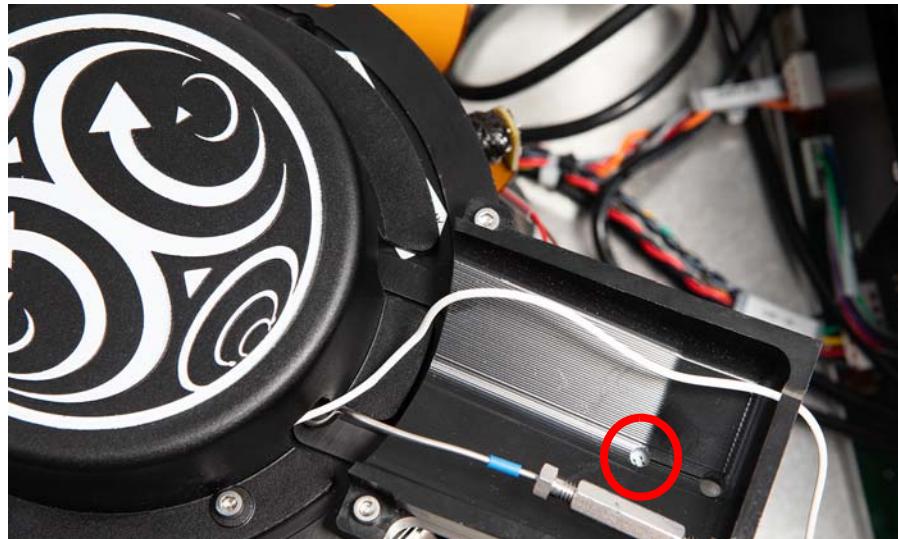
To clean up after a leak, follow these steps:

CAUTION: Be sure to wear gloves and other protection appropriate for handling the solvent that has leaked. Always use absorbent paper or cotton to absorb as much of the leak as possible before cleaning the leak sensor.

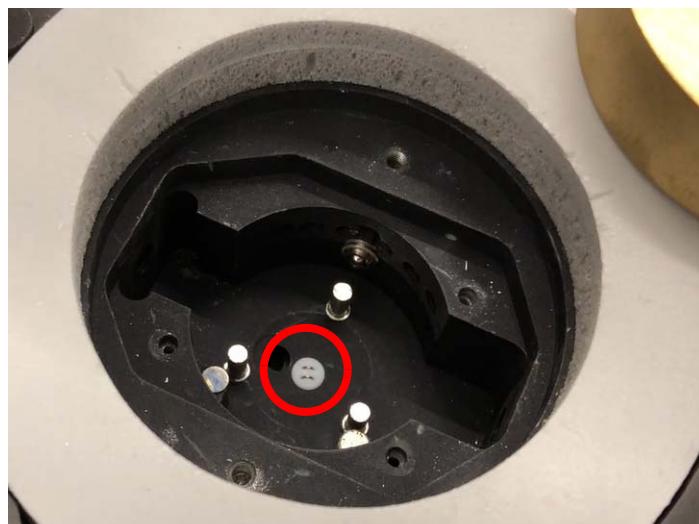
1. Disable the pump or otherwise prevent additional liquid from being sent into the DAWN.
2. Disconnect the power cord from the instrument.
3. **Front Panel Leak Sensor:** This sensor can be cleaned entirely from the front panel without removing the cover. Squirt some clean deionized water or alcohol (or a solvent that is co-miscible with the leaked solvent) into the drip tray from the front of the instrument and clean the sensor with a cotton swab (provided in the hardware kit). Rinsing with clean water can help remove salt deposits that may activate the alarm.



4. **Waterslide (Fluid Connections):** This sensor (circled in red below) can be cleaned by removing the top manifold access hatch. See [Step 1—Accessing and Removing the Flow Cell Assembly on page 99](#) for how to remove the top panel. Squirt some clean water or alcohol (or a solvent that is co-miscible with the leaked solvent) on the sensor and clean it with a cotton swab.



5. **Flow Cell Leak Sensor:** This sensor (circled in red below) is located inside the read head directly below the manifold. Refer to [Step 1—Accessing and Removing the Flow Cell Assembly on page 99](#) for procedures for removing the flow cell manifold. Clean the sensor with a cotton swab and clean water or alcohol (or a solvent that is co-miscible with the leaked solvent).



6. Install and secure the cover with the screws you removed.

If the alarm continues after cleaning the sensor surface, contact Wyatt Technical Support for assistance.

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 [Wyatt Technical Support on page 10](#) 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

Procedure:

1. Turn off the system power to the DAWN.
2. Remove the top cover of the instrument.
3. Using the 2.5 mm Ball driver, remove the M3 hex-head screws and lift off the read head cover plates. See Figure 9-1. then slide the COMET sonicator assembly away and out of the instrument.
4. Disconnect the COMET coax cable.
5. Move to your workbench and place the assembly on its side being careful of the protruding plunger blade.
6. Wearing your lint-free gloves, disassemble the sonicator by first removing the four M3X8 screws.

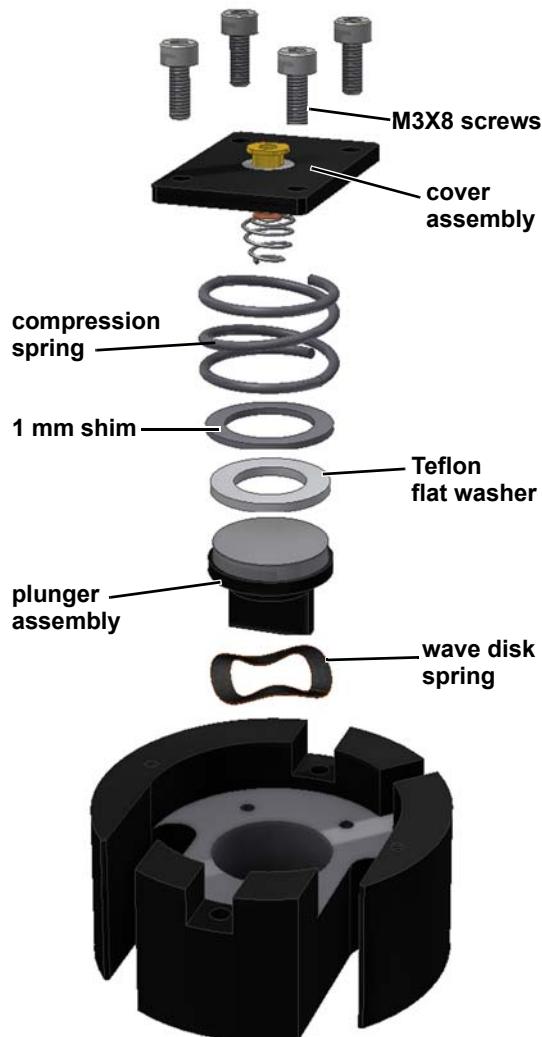
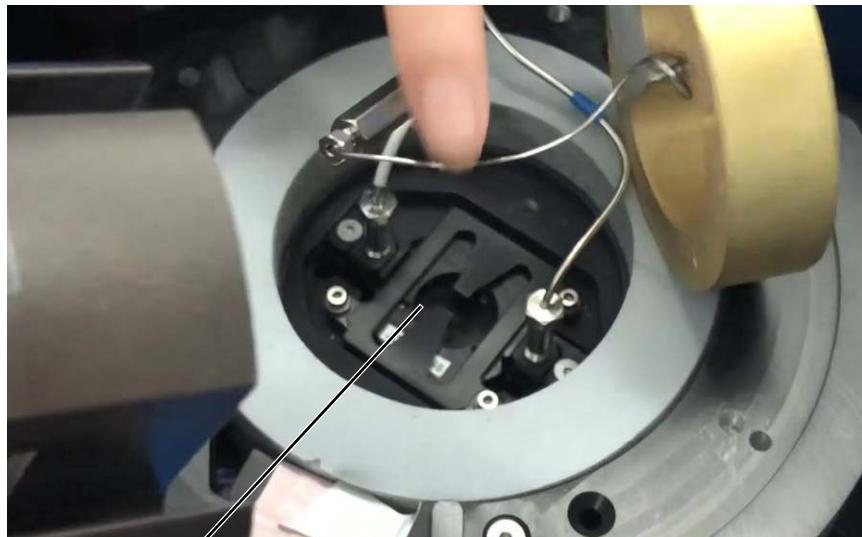


Figure 9-1: COMET Sonicator Assembly

7. Carefully remove the cover assembly. Take extra caution when removing the heat exchanger in the heated/cooled DAWN to avoid damage to the soldered parts.
8. Remove the old plunger assembly and replace with sonicator plunger assembly WTC 110025, reinstalling the stack as shown in Figure 9-1.
9. Install the cover assembly and tighten the four M3X8 screws.
10. To install the sonicator assembly into your read head, first turn the plunger blade so that it will fit into the plunger blade insertion slot in the flow cell.
11. Slide the COMET sonicator assembly under the flow lines and insert the plunger blade into the insertion slot.



**COMET plunger
can be inserted here**

12. Connect the COMET coax cable.
13. Using the 2.5 mm Ball driver, install the M3 hex-head screws securing the read head cover plates and COMET assembly.
14. Replace the instrument cover.

A

Acronyms and Abbreviations List

The following acronyms and abbreviations are used in this manual:

Item	Meaning
°C	degree celsius
A	ampere
A ₂	Second Virial Coefficient
ac	alternating current
APD	Avalanche Photodiode
aRI	absolute refractive index
cm	centimeter
COP	Certificate of Performance
dc	direct current
DHCP	dynamic host configuration protocol
DLS	dynamic light scattering
dn/dc	refractive index increment
dRI	differential refractive index
D _t	Translational diffusion coefficient
FFF	Field Flow Fractionation
g	gram
GND	ground
GPa	gigapascal
GPC	Gel Permeation Chromatography
HPLC	high pressure liquid chromatography
Hz	hertz
ID	inside diameter
in.	inch
IP	internet protocol
IPA	isopropanol (Isopropyl Alcohol)
K _D	dissociation constant
L	liter
LAN	local area network

Item	Meaning
LED	light emitting diode
LS	light scattering
MALS	Multi-Angle Light Scattering
mg	milligram
MHz	megahertz
μL	microliter
mL	milliliter
μm	micrometer
mm	millimeter
MPa	megapascal
mV	millivolt
N ₂	nitrogen
nm	nanometer
OD	outside diameter
PID	proportional integral derivative
ppm	parts per million
PPE	personal protective equipment
PP/PE	polypropylene/polyethylene
psi	pounds per square inch
QELS	quasi-elastic light scattering
R _h	hydrodynamic radius
RI	refractive index
RIU	refractive index unit
RMS radius	root-mean-square radius
SEC	Size-Exclusion Chromatography
SPCM	Single Photon Counting Module
STP	standard temperature and pressure = 0 °C and 1 atmosphere of pressure
TCB	trichlorobenzene
TTL	transistor-transistor logic
V	volt
Vac	volts alternating current
Vdc	volts direct current

B

DLS Using the WyattQELS

The WyattQELS (dynamic light scattering, DLS or quasi-elastic light scattering, QELS) is an internally installed accessory that measures time-dependent fluctuations in the scattered light signal using a fast photon counter. DLS measurements can determine the hydrodynamic radius of macromolecules or particles. While the lower limit of detection for light scattering is 10 nm (RMS radius), the lower limit for DLS measurements can be as low as ~ 0.5 nm (hydrodynamic radius).

The light scattered by the sample is collected and guided via a fiber optic cable to an actively quenched, solid state Single Photon Counting Module (SPCM), which contains an Avalanche Photodiode (APD). The photons are then converted to electrical pulses which are sent to a multi-tau hardware correlator.

The WyattQELS accessory analyzes the time scale of the scattered light intensity fluctuations by a mathematical process called auto correlation. To perform the very fast data manipulation necessary to obtain results in real time, the WyattQELS uses the latest generation of correlator running multiple tau algorithms. The translational diffusion coefficient (D_t) of the molecules in the sample is determined from the decay of the intensity auto correlation data. The hydrodynamic radius (R_h) of the sample is then derived from D_t using the Stokes-Einstein equation,

$$R_h = \frac{k_b T}{6\pi\eta D_t}$$

where: k_b is the Boltzmann constant, T is the temperature in degrees K, and η is the solvent viscosity.

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Flow Cell Options for Online DLS Measurements

The standard DAWN flow cell has a 1.2 mm inner diameter (I.D.) bore that is optimized for macromolecules and small particles with an Rh up to 30 nm at flow rates of 0.5 mL/min. The default DLS position for this 1.2 mm I.D. bore is 135°. With lower flow rates (0.3 mL/min), sizing can be achieved up to an Rh of 50 nm.

If you are measuring samples via online DLS at flow rates higher than 0.5 mL/min or for macromolecules larger than 30 nm in radius, you may consider the 3 mm I.D. bore DAWN flow cell option. The wider bore flow cell, due to its larger inner diameter, reduces mean flow velocity to improve DLS analysis while reducing non-ideal transit and shear effects. At flow rates of 0.5 mL/min with the DLS at the 90° angle, sizing can potentially be achieved up to an Rh of 300 nm.

When working with smaller macromolecules, proteins, or nanoparticles, it is recommended to use the standard 1.2 mm I.D. bore to improve molar mass accuracy and dispersion. The accuracy of molar mass measurements with the 3 mm I.D. wide bore is 10 % compared to 5 % with the standard 1.2 mm I.D. bore.

Standard 1.2 mm I.D. Bore

For the standard flow cell, the DLS angle of 135° is recommended. This configuration with a flow rate of 0.5 mL/min can size up to around an Rh of 30 nm. Decreasing the flow rate to 0.3 mL/min can extend that range to an Rh of 50 nm. Flow rates lower than that are not recommended.

Wide 3 mm I.D. Bore

For the wide-bore flow cell, the DLS angle of 90° is recommended, as it is better suited for minimizing stray light and is recommended for the largest range of particles (Rh > 150 nm). On the other hand, the DLS 135° angle is suited for mixtures of small and large particles in batch mode, or for large particles (Rh > 150 nm) in solvents with a viscosity greater than 2 cP.

Data Analysis in ASTRA

While the calibration constant may shift slightly with the wider bore, all data processing and analysis in ASTRA (including calibration, normalization, alignment, and band broadening procedures) are the same. It is important to re-determine these system parameters with an appropriate standard, because alignment and band broadening will change with the increase bore volume.

Additionally, for DLS analysis, setting a maximum ACF fit delay time of 0.02 seconds in the ASTRA method can help minimize the impact of number fluctuations while still being sufficiently long enough to use all available data.

If you have any further questions, contact Wyatt Technology Support.

Accessing DLS Options on the Front Panel

This section describes the graphs and options available on the front panel display when using an embedded WyattQELS module. For navigating the DAWN display, see [Using the Multi-Touch Controls on page 52](#).

If you are using a DLS Compatibility Kit to connect your NanoStar (M3300) or Mobius (M3001) to your DAWN instrument for acquiring DLS data, refer to the NanoStar or Mobius user's guide for front panel options.

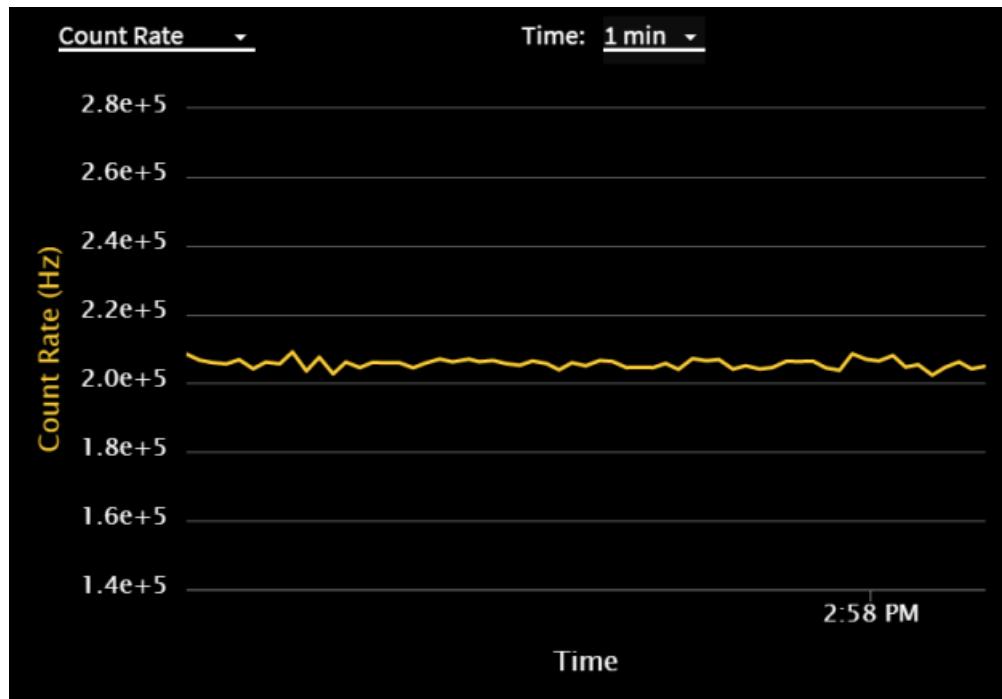
When an embedded WyattQELS module is installed, additional chart options are present on the **Dashboard**, including **ACF** and **Count Rate**.



Count Rate Graph

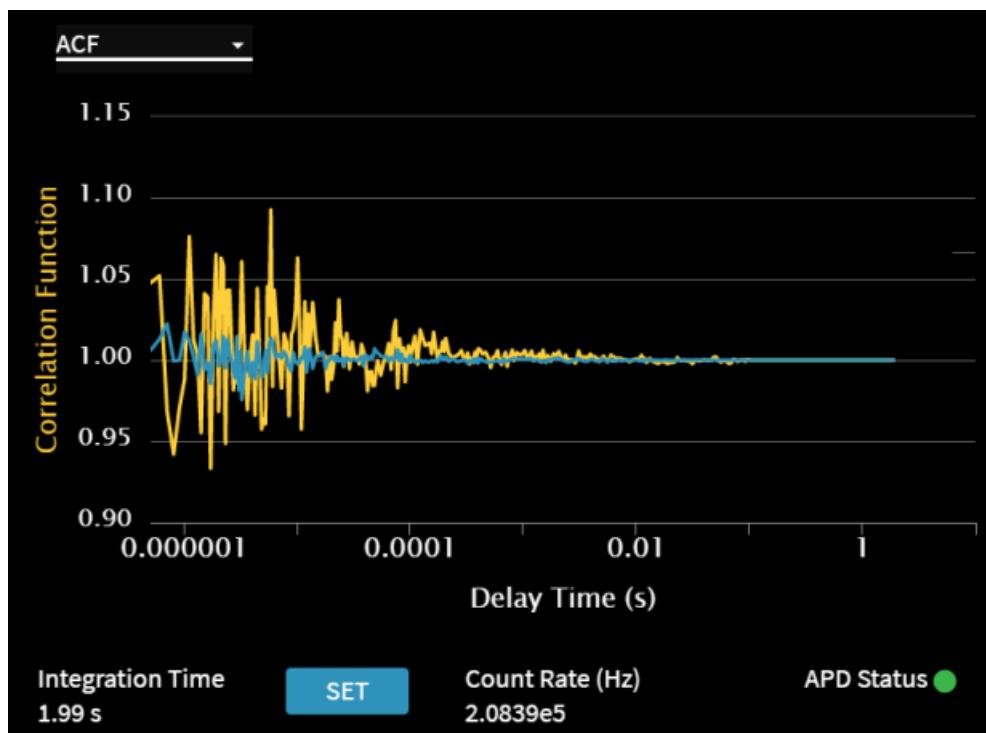
The **Count Rate** graph displays the photon count rate for the DLS detector on the Y axis vs. time on the X axis.

Tap the **Time** drop-down menu to set the time range of the X axis.



Correlation Function Graph

The **Correlation Function** graph displays the intensity correlation curve for a single slice of DLS data, which is the raw dynamic light scattering data from which the hydrodynamic radius is derived.



The WyattQELS measures the correlation function, a statistical measurement of how the scattered intensity fluctuates. The intensity fluctuation function is shifted along the time axis by a time τ and the correlation between the shifted and unshifted function is calculated. The correlation function represents the decay in correlation as τ is increased.

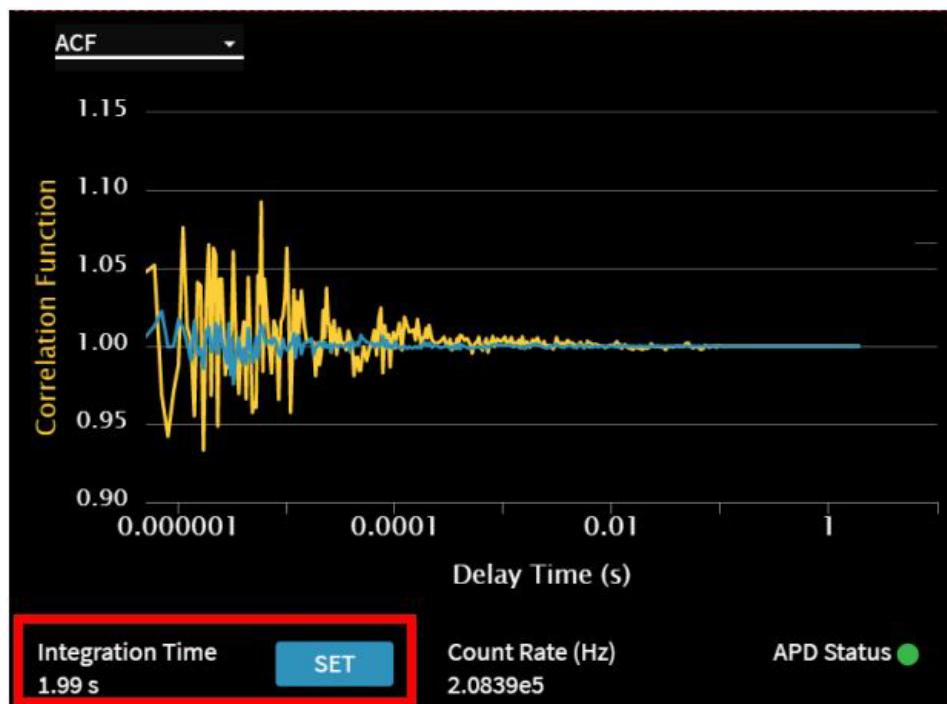
- For large values of τ , the correlation function approaches 1.0, indicating that the light intensity at time t is uncorrelated to the intensity at time $t + \tau$.
- For smaller values of τ , the correlation function increases, indicating that the scattered intensity is correlated.

The time difference at which the correlation function transitions from being correlated to being uncorrelated is related to the translational diffusion coefficient. Small particles diffuse rapidly resulting in 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 User's Guide* for a more detailed explanation of the background of DLS.

Integration Time

Integration Time is the DLS acquisition time, in seconds, of each DLS measurement. The integration time can be set in increments of the minimum time of 0.105 s. Integration times of up to 3600 s can be set, but typical values range between 2 s and 5 s. The instrument rounds off the set time to the nearest multiple of 0.105 s.



The integration time depends on the sample concentration, the flow rate when using chromatography, and molecule size. Generally, longer integration times should be chosen to improve the measurement statistics for less concentrated samples.

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

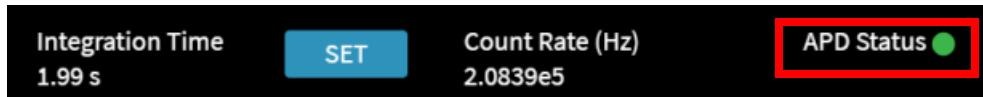
As an aid to setting the integration time, intermediate results are displayed in blue every 1 s. They get progressively more accurate (less noisy) as time progresses. After the measurement is complete, it is plotted in red, and the new intermediates are plotted. The slider on the bottom shows the percent completion 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.

APD Status

The **APD Status** is shown in the lower-right below the ACF graph.



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 WyattQELS is equipped with a protection circuit that will shut off the APD in the event of over illumination, but it is only intended as an emergency shutoff.

Power

You can turn on or off **DLS Power** on the Dashboard. (See [Dashboard Tab on page 53](#).)



This turns on or off the power to the WyattQELS module. There is no external switch. This is included in case you are not using the WyattQELS 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 SPCM module, so it is important to power it off if there is the potential to expose it to room light.

When the WyattQELS power is turned on, the DAWN turns off the laser for 30 s while the APD Peltier cools the sensor. Then it turns the laser back on. If the laser was already off when the WyattQELS power is turned on, the laser will remain off after the 30 s cool down period.

The WyattQELS hardware includes an APD protection system. The correlator hardware continuously monitors the count rate of the APD. If, at any point, it exceeds 20 MHz for more than 1 ms, 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.

Aligning the Optical Fiber

The WyattQELS module can be placed at four independent positions around the flow cell.

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. Follow the steps below to align the optical fiber.

Note: Align the DLS optical fiber to maximum count rate before performing a toluene calibration of the static light scattering detector.

1. Turn on the system power to the DAWN, if not already powered on.
2. Remove the top access hatch cover of the DAWN instrument. Make sure that the flow cell is filled and has no bubbles in it.
3. Choose the DLS count rate to be displayed in the front panel interface of the multi-touch display.

Note: For changing the DLS fiber location, see [Moving the DLS Fiber to a Different Location on page 151](#).

4. Rotate the brass locking nut by hand counter-clockwise to loosen it. This enables the fiber to be adjusted up and down for alignment.
5. Using a 2.0 mm hex driver, rotate the fiber optic positioning screw on the top of the DLS fiber mount to maximize the count rate recorded in the Count Rate graph (see [Count Rate Graph on page 145](#)) as specified by the WyattQELS Certificate of Performance.

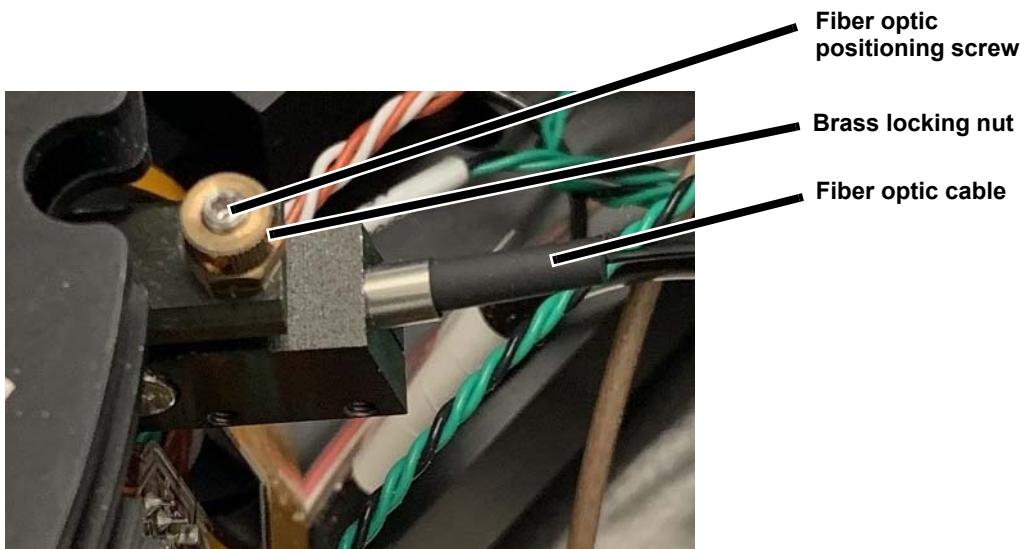


Figure B-1: Adjusting the fiber positioner for the WyattQELS

Rotating the screw moves 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.

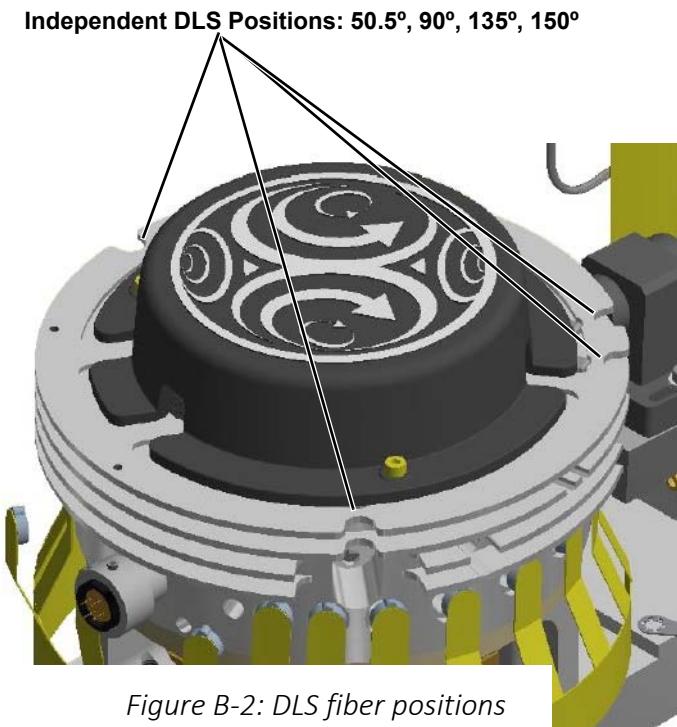
This is accomplished by rotating the fiber positioning screw counter-clockwise until the fiber is at the top of the mount. Stop when the adjuster screw is about 3 mm above the top of the mount. If turned too far, the adjuster screw will come out. If this happens, simply screw it back into place, taking care not to cross the threads.

Note:	The DLS (QELS) Certificate of Performance will indicate what the maximum count rate of the DLS unit was either at the time of manufacturing, or the last factory service of the instrument.
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6. 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 increase, reach a maximum, and then decrease. After passing through the peak once, rotate the fiber positioning screw counter-clockwise.
7. Stop when the count rate reaches a maximum again. 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.

Moving the DLS Fiber to a Different Location

The WyattQELS or DLS optical fiber (with the NanoStar or Mobius compatibility kit) may be moved and installed in any of the four available DLS detector locations. By default, the DLS fiber is installed in the 135° position. The other three positions are the 50.5°, 90°, and 150° physical angles.



The DLS attachment consists of a fiber mount to hold the DLS fiber, a fiber collet, a locking screw, and an adjuster screw. The mount is secured to the read head by two screws.

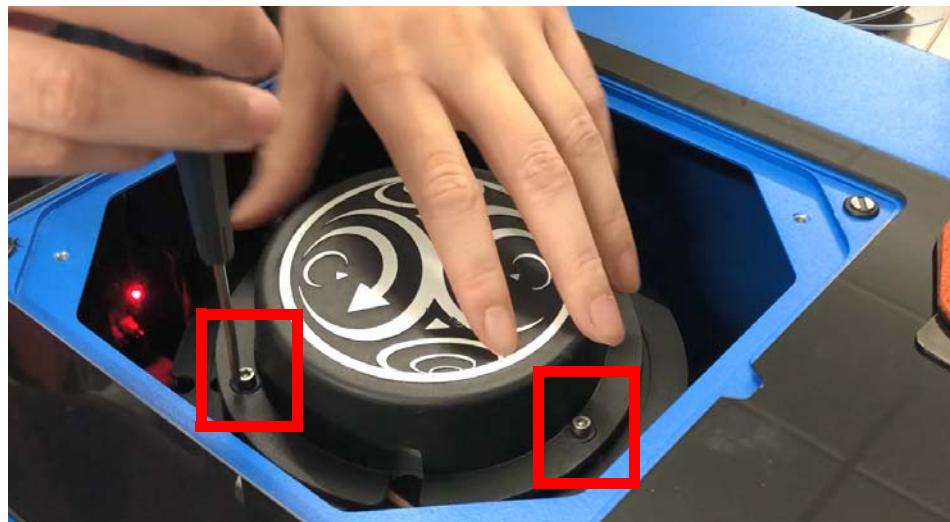
Materials needed to move the Optical Fiber

- Anti-static wrist strap
- 2 mm hex driver

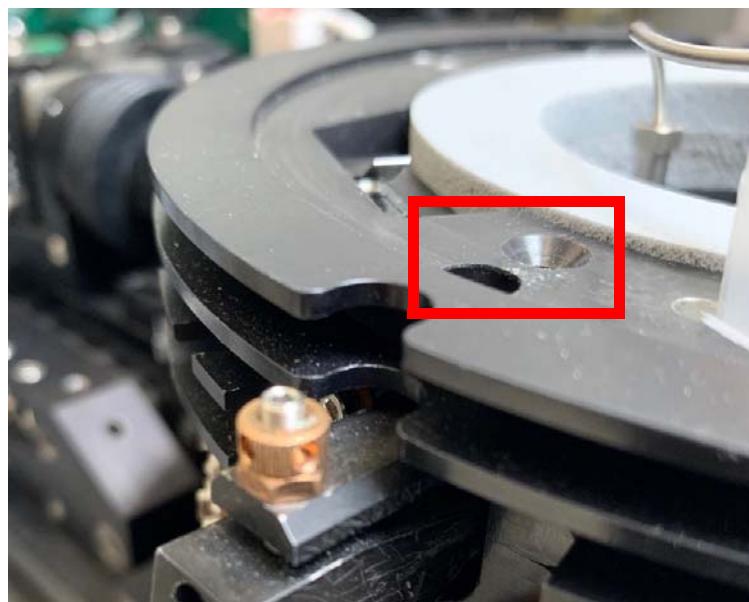
CAUTION: The DAWN contains electrostatic discharge (ESD) sensitive parts. Wear an anti-static wristband grounded to the instrument chassis if it is plugged in or other suitable grounding site whenever you open the DAWN to help prevent potential damage to the instrument from ESD. A disposable anti-static wrist strap is included in the instrument hardware kit.

Removing and Reinstalling the Optical Fiber Receiver

1. Turn off the DAWN system power.
2. Ensure you are properly grounded.
3. Remove the top cover hatch. See [Step 1—Accessing and Removing the Flow Cell Assembly on page 99](#).
4. The flow cell manifold cover is secured to the read head with three screws (two shown below). Remove these screws using a 2.5 mm hex driver to gain access to the flow cell and the heat exchanger.



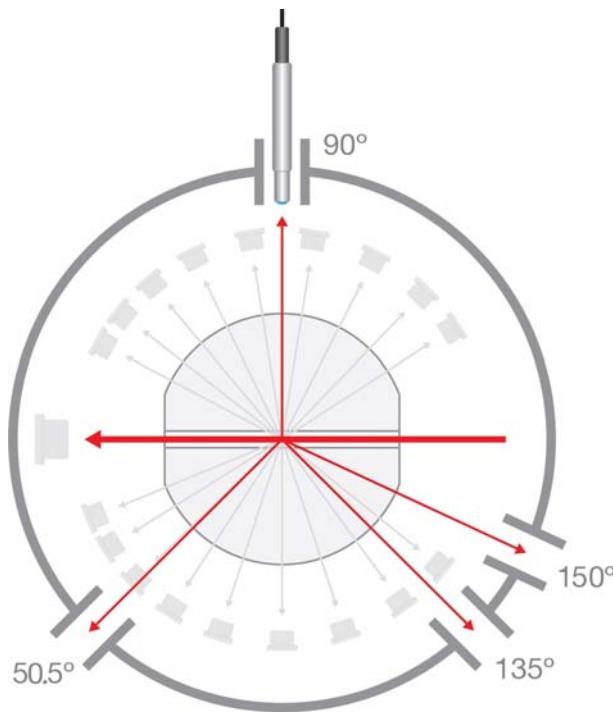
5. Use a 2 mm hex driver to remove the two screws that secure the DLS mount to the read head. The two screws may be identical and interchangeable in some models.



6. Remove the entire fiber mount from the read head by pulling the mount out of the DLS location. You may need to move the DLS fiber out of alignment to remove the holder. This can be done by loosening (unscrewing counter-clockwise) the alignment screw in the center of the brass locking nut.



7. Re-insert the fiber mount in any of the independent DLS positions.



8. Secure the fiber mount with the two screws on top of the read head.
9. Once it is re-installed, the DLS fiber will need to be aligned again as described in [Aligning the Optical Fiber on page 149](#).
10. Replace the top cover on the instrument.
11. ASTRA should automatically detect the DLS position in your method when using ASTRA 7.3 or above. See the *ASTRA User's Guide* for details.

C

Temperature Controlled Options

The DAWN temperature controlled options, Heated/Cooled (HC) and Temperature Regulated (TR), have some differences from the ambient DAWN. This appendix describes those differences and supplies instructions for making adjustments and operating these versions of the instrument.

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Overview

The read head on the HC DAWN can be heated up to 150 °C or cooled down to –15 °C. The TR DAWN can be controlled from approximately 20 °C to 70 °C. The DAWN temperature-controlled models use a solid-state Peltier device and a cartridge heater to operate over the whole temperature range.

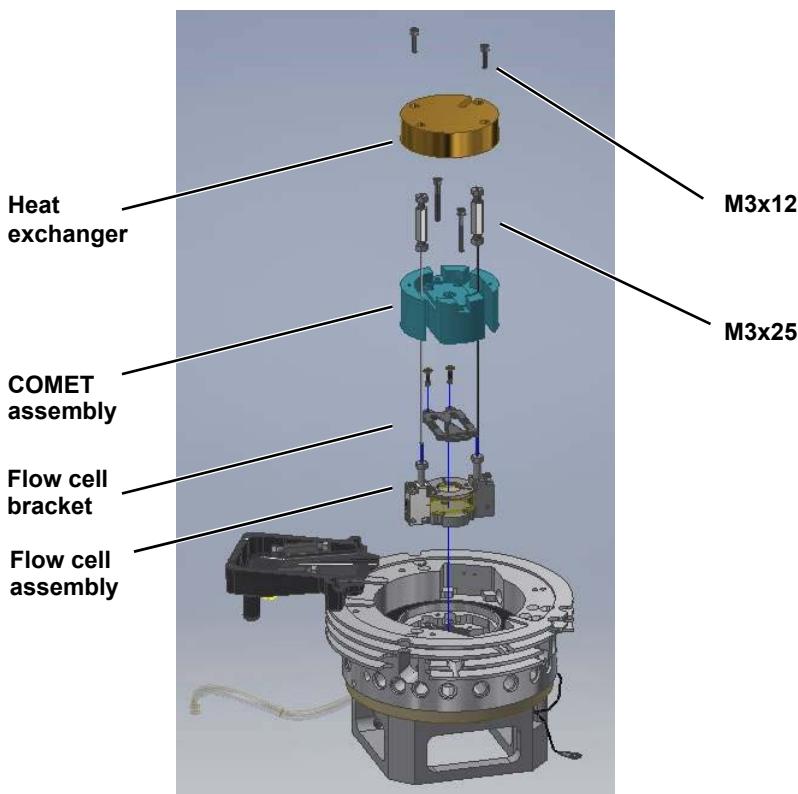


Figure C-1: Temperature controlled DAWN read head

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 an accuracy of ± 1 °C at the sensor.

To remove the heat exchanger and COMET module, follow the steps in [Step 1—Accessing and Removing the Flow Cell Assembly on page 99](#).

Safe Operation Requirements

- Be sure all chosen operating temperatures are between the freezing point and boiling point of the solvent you are using.
- Always lock the insulating cover plate in place before heating or cooling the flow cell.
- The flow cell is initially configured for use at the temperature you indicated you would be using when you purchased 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.

- Replace 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 elevated temperatures, may not seal reliably.
- Check for leaks each time the DAWN has been heated above 80 °C.
- 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.
- When operating below ambient temperature, be sure to connect a dry air or nitrogen source to the DAWN dry gas purge connector. Light scattered from condensed water ruins measurements. Use dry air or nitrogen even at ambient or higher temperatures to minimize the amount of dust within the instrument.

CAUTION: See [Preventing Condensation \(at Lower Temperatures\) on page 124](#) for safe sub-ambient operation. Failure to follow those requirements may result in electronic failures and corrosion damage to the instrument. For further details, see *TN9001: Operating Wyatt Instruments in a Cold Room* and *TN9006: Preventing Condensation in Wyatt Detectors*.

- The temperature controller for the flow cell will not allow you to set a temperature below 20 °C unless it detects at least 20 psi of gas pressure on the dry gas port. This prevents accidental condensation of moisture on the flow cell and read head. If you need to open the flow cell, heat the flow cell to at least 20.5 °C before removing the top cover.
- If you are using a Peltier Heated/Cooled DAWN 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 will not cool the lines.

D

Ultra-High Temperature Option

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

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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.

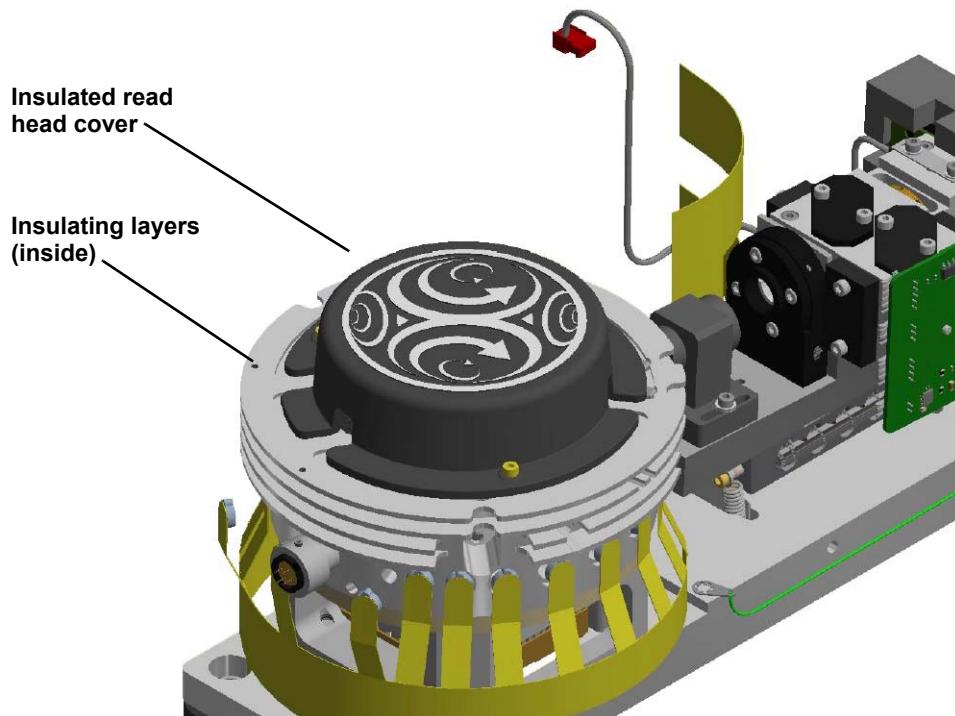


Figure D-1: Ultra-High Temperature read head and laser assemblies

Heating the Cell

The DAWN 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 an accuracy of ± 1 °C at the sensor. Along the entire heated line, the temperature accuracy is within ± 7 °C of the setpoint.

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 synchronized with the cell controller. That is, when the temperature of the cell is changed, the temperature of the heated line is changed to the same value. On the System tab, it is possible to break this synchronized 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).

Setting the Operating Temperature

You can set the temperature of the cell on the dashboard. From the Settings tab, you can set the temperature of the cell and heated line separately (after unchecking the Sync button in the System tab).

1. Connect the heated-line assembly communications cable into the Heated Line connector on the back panel of the instrument.
2. Navigate to the associated field, type in the new temperature, and press Enter. The system will ramp the temperature at 1 °C/min. This ramp rate ensures that the differential thermal expansion of the cell materials does not cause damage.
3. 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 approximately two hours for the system to reach 150 °C, since the ramp rate is limited to 1 °C/min.

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 programmatically to as slow a rate as required.
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Heated Lines

The optional DAWN heated lines can maintain temperatures up to 210 °C in the inlet and outlet lines if the DAWN 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.



Figure D-2: Heated-line assembly communications cable connected to back of DAWN

If you are using a Peltier Heated/Cooled DAWN 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 will not cool the lines.

Operating Precautions

- 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 9 mm flow cell O-rings whenever the instrument is brought down below 80 °C from an elevated temperature! They conform to the geometry of the cell and, when brought down from an elevated temperature, may become brittle and not seal reliably.
- The system will issue a warning if a heated/cooled instrument is set to a temperature above 80 °C. The warning 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 9 mm O-rings at the factory. However, if the 6mm O-rings are installed for reduced delay volume and compatibility with systems below 80 °C, the same precaution must be obeyed.

- When heating the cell above 80 °C, double check the fittings for leaks as thermal expansion can cause fittings to leak at higher temperature, even if they were sealed at room temperature.
- Because UHT DAWN frequently operates above the boiling point of toluene, the calibration of this flow cell should be performed in trichlorobenzene (TCB).

Configuring ASTRA

Follow the instructions in the *ASTRA User's Guide* to determine normalization coefficients, interdetector delay volumes and band broadening coefficients.

- Use an isotropic scatterer for normalization, for example, a 32,000 g/mol polyethylene standard.
- Use a monodisperse polymer for determining interdetector delay volumes and band broadening coefficients, for example, a 200,000 g/mol polystyrene standard.

The following dn/dc values are helpful for concentration determination as well as dRI calibration constant determination:

- Polystyrene in trichlorobenzene; dn/dc = 0.047 mL/g
- Polyethylene in trichlorobenzene; dn/dc = -0.108 mL/g

UHT DAWN Heated-lines Installation

Note that the ultra-high temperature DAWN is shipped with all parts necessary to operate as a standard heated/cooled instrument (insulated read head cap, brass heat exchanger, flow cell inlet and outlet SS tubing and unions) but these parts will not be used in the heated-line configuration described in this section. See [Temperature Controlled Options on page 154](#) for converting the system to the standard heated/cooled configuration. Refer to [Instrument Calibration on page 78](#) for detailed information on how to calibrate the DAWN. When operating at high temperatures, calibrate the instrument in 1,2,4-trichlorobenzene (TCB).

Tools needed

- All items contained in the heated-line ship kit
- 2.0 mm hex driver (L-shape hex key recommended)
- 2.5 mm hex driver (L-shape hex key recommended)
- 3/16" hex driver (or appropriate driver) to secure heated line to GPC. Please check with your GPC oven vendor for the appropriate size.

- Two 1/4 in. Crescent wrenches
- Appropriately size wrench to fit the GPC column
- 1/2 in. SAE stainless steel washer for securing heated line inside oven compartment (optional)
- Suitable standard sample of known molar mass (isotropic scatterer for normalization, monodisperse sample for detector alignment and band broadening)
- Thermally insulated gloves (for example, terrycloth or Kevlar)

Preparing the DAWN:

1. Unpack the DAWN and place the instrument on a flat, clean surface, standing on its feet about 20 cm to the side of the access port for the heated lines on the GPC oven.
2. Make sure the supplied power plug is correct for the local power outlet. The DAWN is equipped with a universal power supply, which operates anywhere in the world. It accepts inlet voltages between 100 V to 120 V or 220 V to 240 V at 50 Hz to 60 Hz.
3. Connect one end of the supplied Ethernet cable to the Ethernet port on the back of the DAWN 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 is on the local area network, it may be accessed and controlled from any computer on the network. When using the USB converter, it can be accessed only by the host computer. See [Instrument Connectivity on page 190](#) for more details about implications for network security of the two different configurations.
4. Install the ASTRA software.
5. Check the DAWN calibration constant in toluene. If the forward monitor is low, flush the flow cell with 0.02 µm filtered toluene and re-check the calibration constant. See [Instrument Calibration on page 78](#) for details.

Note:	For heated-line instruments there is no tubing connected between the inlet/outlet fittings on the front of the instrument and the flow cell. <i>Do not connect fluid lines to these fittings.</i> Instead, remove the DAWN top cover and insulated read head cap, and connect necessary fluid lines directly to the 2-way unions at the flow cell assembly itself.
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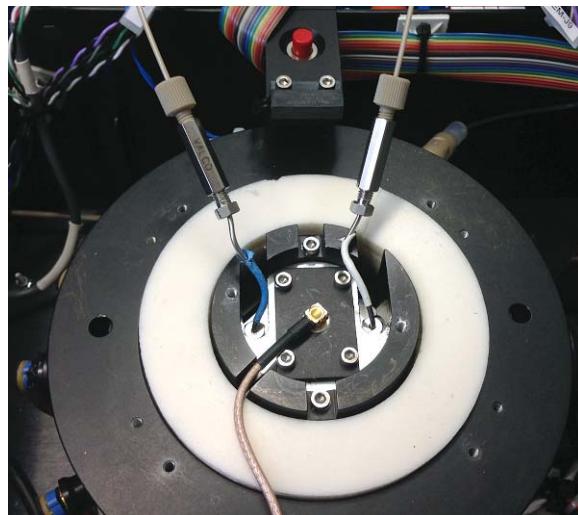


Figure D-3: Flushing the flow cell with additional toluene for calibration.

6. Connect the DAWN to any other devices for your application. See [Connecting Auxiliary Devices on page 43](#).
7. Remove the DAWN hatch cover and set aside.
8. Remove the insulated cap from the DAWN read head and store for future use in case the DAWN will be used in the standard heated/cooled configuration.
9. Remove the COMET from the read head and set aside. Remove the 2-way unions and then remove the manifold from the read head while being careful not to touch the optical surface of the flow cell glass. Then remove the short inlet and outlet tubing from the flow cell manifold and store for future use in case the DAWN will be used in the standard heated/cooled configuration in the future. Never use wrenches on the manifold while it is installed in the read head, as this may chip the glass.

Note: If you are experiencing problems removing the inlet or outlet tubing fitting from the flow cell manifold, bend the stainless steel tubing upward to become more vertical with respect to the flow cell to allow for the free rotation and upward motion of the fittings.

10. If the GPC mobile phase is co-miscible with toluene, proceed directly to [Connecting the Flexible Heated-line Assembly to the Instrument on page 165](#).
If the mobile phase is not co-miscible with toluene, use a co-miscible intermediary solvent to perform a stepwise solvent exchange from toluene into the mobile phase. See [Flushing the DAWN on page 49](#) for more information.
11. It is recommended to recalibrate the instrument in trichlorobenzene (TCB) when operating at high temperatures.

Connecting the Flexible Heated-line Assembly to the Instrument

This section provides instructions on installing the UHT DAWN with a flexible heated-line assembly light scattering detector.

The flexible heated-line assembly directions below are fully compatible with GPC ovens such as the Agilent PL-GPC 220 System or Polymer Char GPC-IR System. Contact Wyatt Technical Support for information about compatibility or instructions for installing this heated-line with other oven systems, such as a TOSOH oven.

Note:	If this DAWN has been formerly used at temperatures below 80 °C, check the flow cell configuration to ensure the backing rings have been removed and the 9 mm O-rings have been installed. New UHT DAWN instruments will have a flow cell already configured with the 9 mm O-rings.
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1. Unpack the flexible heated-line. There should be a heated-line as shown below in Figure D-4, two M3x35 socket head screws, an attached communications cable, and a foam ring. Within the heated-line should be two lengths of 0.020" ID stainless steel tubing. To allow for adjusting the length of the tubing inside the oven, they do not come pre-swaged.

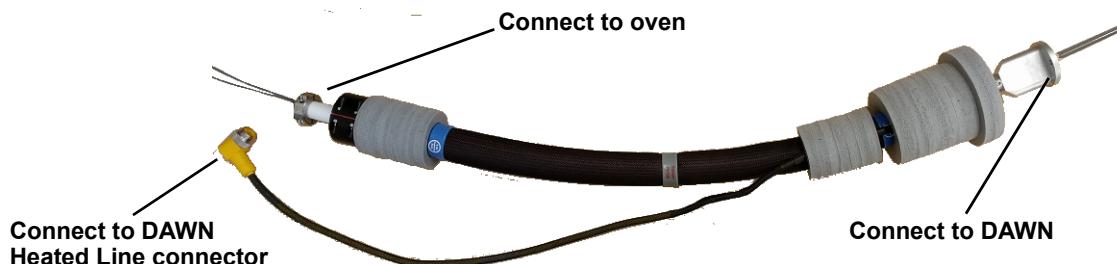


Figure D-4: Flexible heated-line assembly

2. See [Step 1—Accessing and Removing the Flow Cell Assembly on page 99](#) (steps 1 through 3 only) for how to remove the top access hatch.
3. If your DAWN has been operating in the standard heated/cooled configuration, remove the 2-way unions and the short inlet and outlet tubing from the flow cell manifold as described in the previous section. The flexible heated-line assembly will connect directly to the flow cell manifold.

CAUTION:	Never swage or tighten the fittings with a wrench when the flow cell manifold is in the read head, as this may damage the glass. Remove the flow cell manifold from the read head to tighten or swage fittings. Avoid touching the optical surface of the glass.
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4. On the end of the heated-line assembly to be connected to the DAWN, the stainless steel tubing will need to be swaged. To swage the tubing to the DAWN, ensure that the ends of the stainless steel tubing are flat and smooth, with no burrs or other irregularities. Thread a stainless steel fitting and ferrule onto the tubing ends that will connect to the flow cell manifold. Swage the fittings directly into the flow cell manifold inlet and outlet ports.

Note: Never swage to any other fitting (i.e. a union) as the manifold fittings are unique and swaging other fittings will result in leaks. Additional information about swaging stainless steel tubing can be found in technical note TN3101: *Swaging Stainless Steel Tubing*.

5. Remove the fittings and check for a proper swage. Although the tubing is interchangeable, it is recommended that you identify which lines are for the outlet and inlet on the end for connecting to the oven by pulling the stainless steel tubing without having it fully enter the heated-line assembly.
6. Place the circular metal insert on the heated-line assembly—beneath the foam but above the copper heat fixture.
7. Make sure the foam ring is positioned as shown in Figure D-5. The figure shows the original inlet and outlet tubing that should be removed and replaced with the newly swaged (from Step 4) tubing from the heated-line assembly.

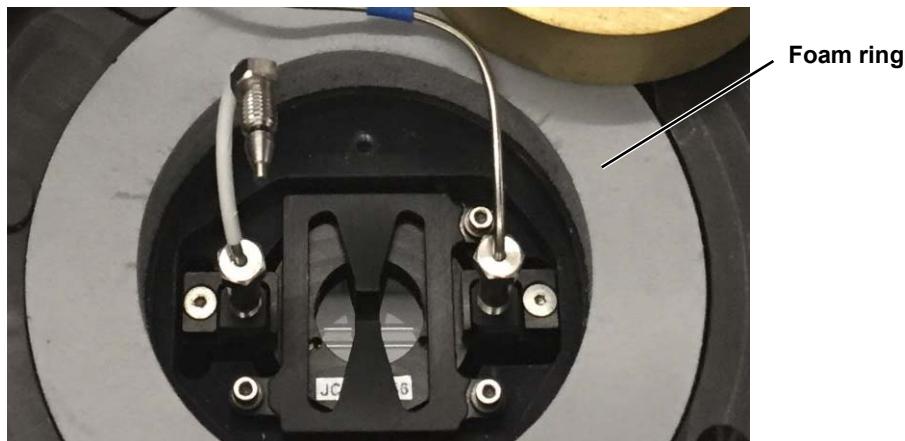


Figure D-5: foam ring installed. Although not shown in this figure, at this stage the long heated-line stainless steel tubing should already be installed, replacing the default inlet/outlet tubing shown in this image.

8. Connect the tubes to the manifold using the fittings you swaged in Step 4. Flowing solvent slowly through the stainless steel tubing in the flexible heated-line assembly can prevent air bubbles from getting into the UHT DAWN flow cell. The heated line needs to be laid beside or on the instrument for the tubes to reach the manifold. The tube should not be pulled out of the heated-line so much that it is no longer visible on the column oven side of the heated line.
9. Check to ensure there are no leaks in the flow cell manifold by running toluene or your intended solvent (if its low temperature viscosity permits) and checking for leaks. Depending on your solvent, it may be possible to plug in the heated-line assembly communications cable into the back of the DAWN and heat the system to 80 °C and then 135 °C (or the desired operating temperature) to ensure no leaks are present.

CAUTION: The instrument is extremely hot! Wear heat resistant gloves whenever handling heated parts and take care not to get burned.

10. If no leaks are detected, return the system to room temperature and place the COMET on the read head as shown in Figure D-6. Do not screw down the COMET yet.

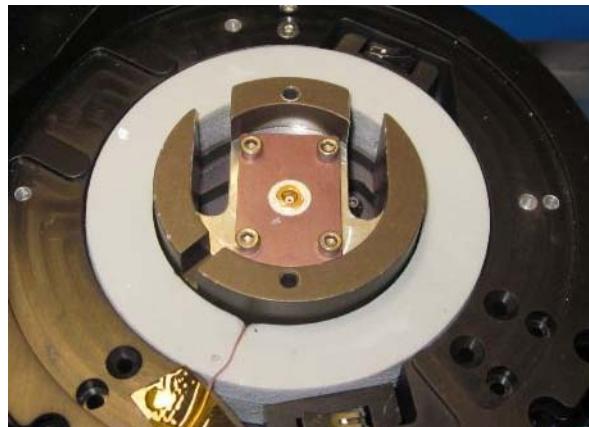


Figure D-6: COMET installed in read head. Although not shown in this figure, at this stage the long heated-line stainless steel tubing should already be installed.

- 11.** Put the copper heat fixture in place. Secure the heat fixture and COMET to the read head using two M3x35 screws (included in the hardware kit). The screw locations are shown in Figure D-7.

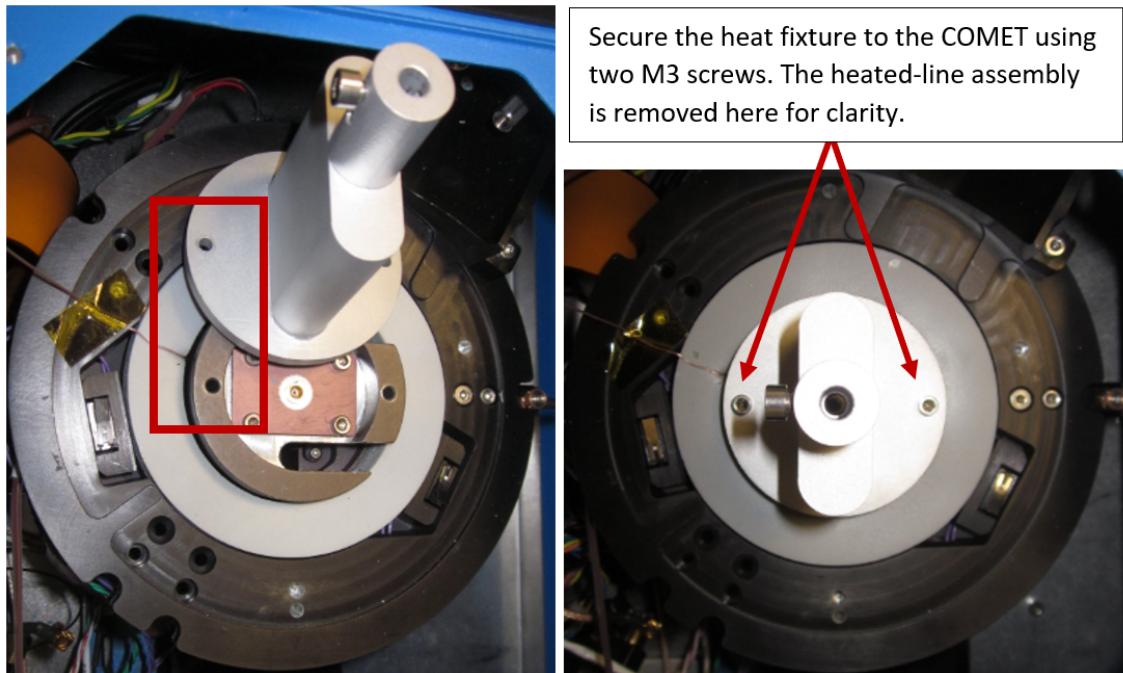


Figure D-7: Secure the heated-line assembly to the read head through the COMET. Although not shown in this figure, at this stage the long heated-line stainless steel tubing should already be installed through the heat fixture.

- 12.** Slide the foam down as far as possible. There should not be any gaps between the foam pieces.

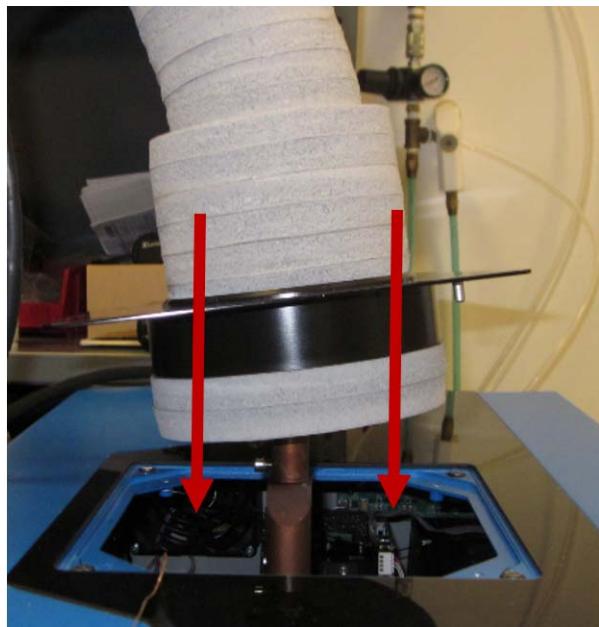


Figure D-8: Installing the insulating foam rings

- 13.** Attach the replacement access hatch with four screws (see Figure D-9). Once secured, use the front panel display to confirm that the laser interlock has been satisfied by the foam assembly.

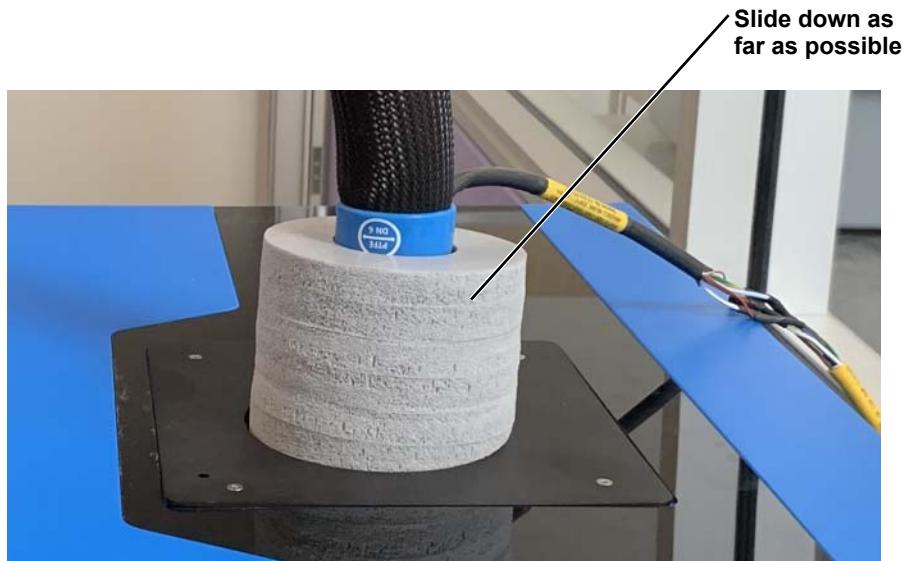


Figure D-9: Final assembly of the DAWN heated-line assembly

- 14.** To install the heated-line assembly to the oven wall, slide off the two-piece shaft collar on the oven side of the heated-line assembly and carefully reposition the DAWN until the heated-line assembly is near the GPC oven wall. The instructions for installing the heated-line assembly to the oven may differ for your specific oven. Refer to the sections that follow for installing the heated line on an Agilent PL-GPC 220 system and on a Polymer Char GPC-IR system.

Installing the Flexible Heated Line to a Polymer Char GPC-IR System

1. Insert the heated line into the oven wall until the assembly is flush with the oven wall as shown in Figure D-10.

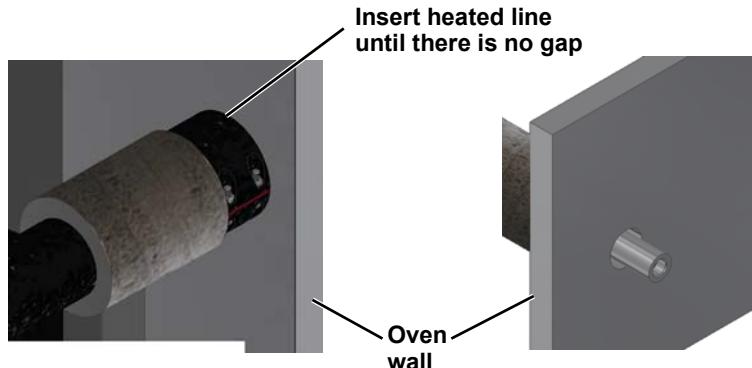


Figure D-10: Installing the oven-side of the heated-line assembly

2. Lock the heated line to the oven wall by reattaching the two-piece shaft collar on the inside of the oven wall as shown in Figure D-11.

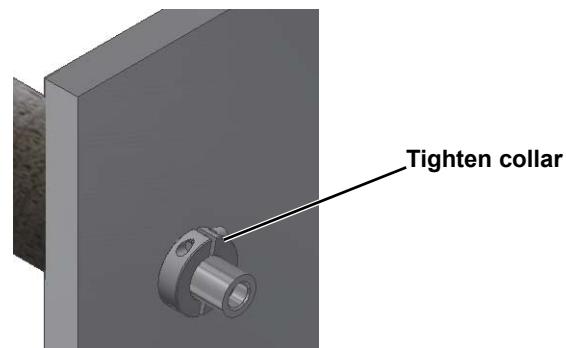


Figure D-11: Secure the heated-line assembly to the oven wall with the two-piece shaft collar.

Installing the Flexible Heated Line to an Agilent System

Note: This section describes installation of the flexible heated line for use with Agilent PL-GPC 220 and Agilent Infinity II High-Temperature GPC systems.

The heated-line assembly for this oven contains a detachable foam insert and a longer heated line as shown in Figure D-12.

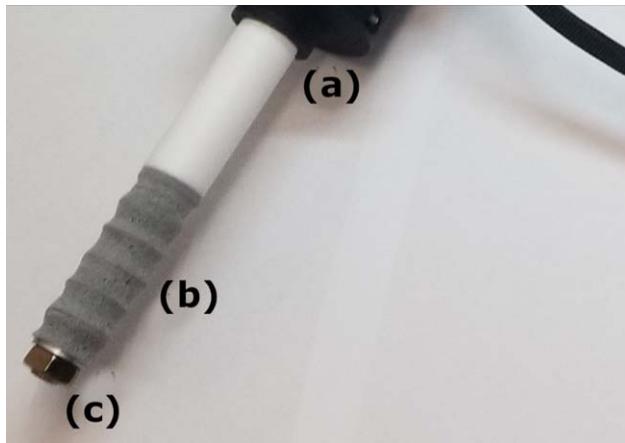


Figure D-12: Flexible heated-line assembly for the Agilent PL-GPC 220 or Agilent 1260 Infinity II High-Temperature GPC System. Note locations of (a) flange on the clamp, (b) foam insulation, and (c) nut and washers for securing the heated line.

1. Unscrew the nut and remove the washers (c) from the heated-line adapter.
2. Slide the adapter with foam through the oven hole until the flange on the clamp (a) fully goes into the oven wall. Cut the foam to adjust length as necessary (b). See Figure D-13.

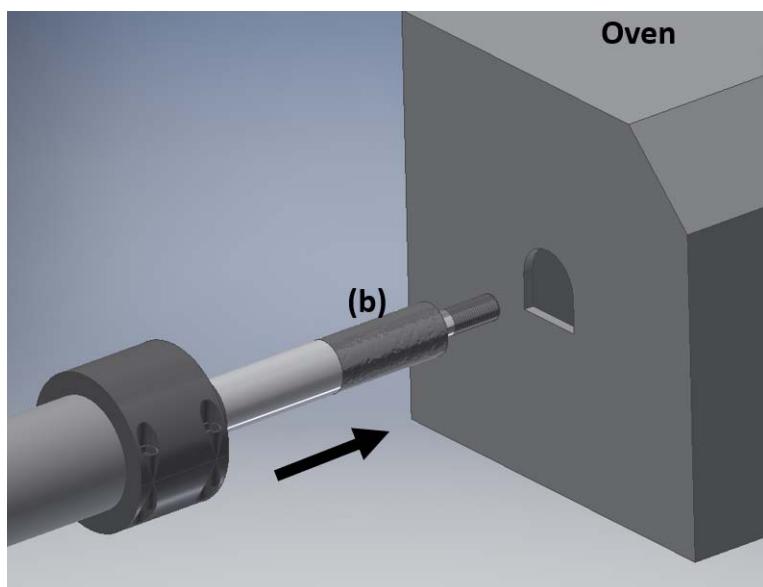


Figure D-13: Inserting heated-line assembly with foam into oven

3. Inside the oven, install the washer and nut as shown in Figure D-14. Secure the nut finger-tight, and then use the wrench to rotate a quarter (1/4) turn. Be careful to not over-tighten. If the end of the thread is too far, extra washers are provided in the shipment.



Figure D-14: Interior view of oven showing washer and nut

Installing the Flexible Heated Line to a Tosoh EcoSEC HT GPC System

To install the flexible heated-line to a Tosoh oven, please follow the instructions in this section. The early steps describe how to attach a Wyatt-supplied clamp to the Tosoh-supplied heated-line. The later steps describe how to connect the Wyatt heated-line to the Tosoh heated-line.

An adapter designed specifically for each oven is provided.

1. Feed the Tosoh heated-line through the oven wall so that it protrudes out. The Tosoh heated-line should extend at least 14 cm from the oven as shown on the left image in Figure D-15.
2. Slide the foam insulation (P/N 165323, supplied in the Wyatt heated-line kit) over the Tosoh heated-line until it is flush with the oven.

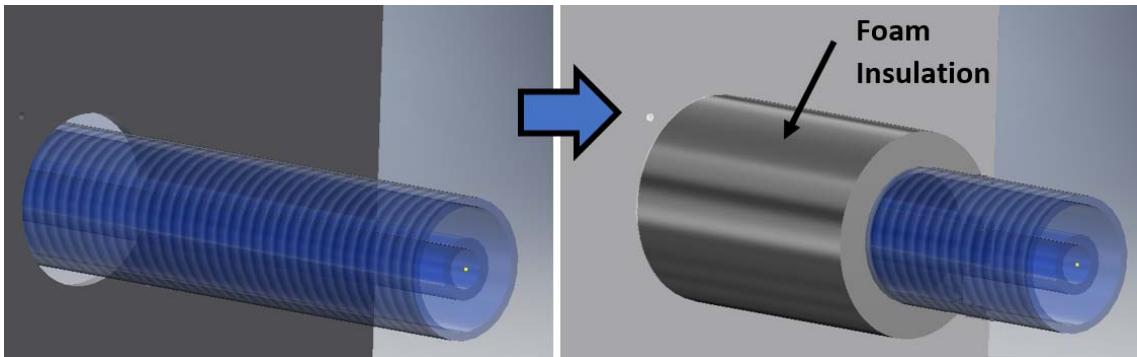


Figure D-15: Tosoh-supplied heated-line protruding out of the oven (left) and with foam insulation installed (right)

3. Secure the Tosoh heated-line with a 2-piece clamp (P/N 165494) as shown on the left side of Figure D-16. The clamp is supplied in the Wyatt ship kit with appropriate screws. This 2-piece clamp can be oriented in any way. It is held together with two M4x30 screws and two M4 nuts. Use an appropriate Phillips screwdriver, which is not supplied in the ship kit.

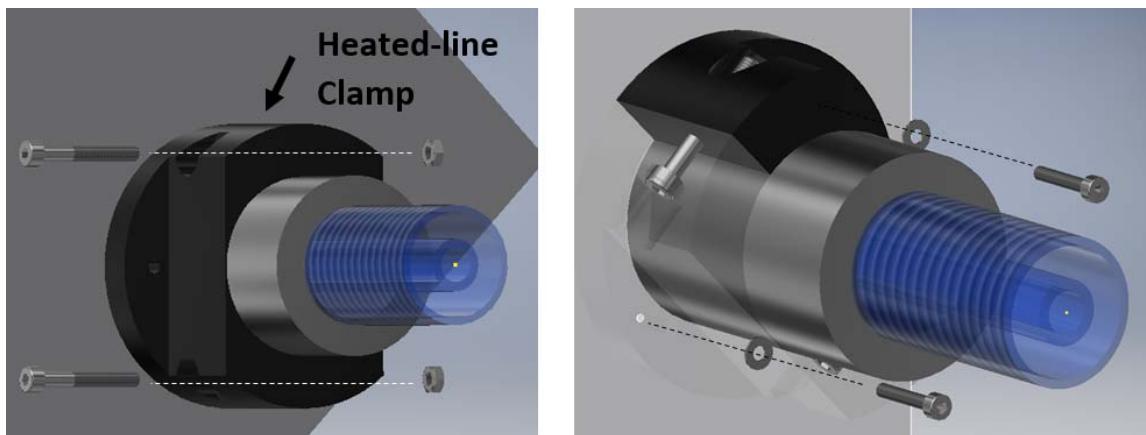


Figure D-16: Tosoh heated-line secured via clamp supplied by Wyatt (left) and secured to the oven via screws that connect the heated-line to the clamp (right)

4. Rotate the Tosoh heated-line and clamp (installed in the previous step) so that the heated-line and clamp can be secured as shown on the right side of Figure D-16. These may be secured with two M3x14 screws and two M3 washers. Because the Tosoh heated-line may have variations in threading and length, the Wyatt kit also provides M3x16, M2.5x14, and M2x16 screws as alternatives.
5. With the heated-line secured, pull the two stainless steel chromatography tubes through from the Tosoh oven and the Tosoh heated-line. Swage the tubing to Valco unions (P/N P6427-31020), which are supplied in the Wyatt ship kit. Stagger the two unions as shown in Figure D-17 to minimize the width they occupy. The unions will later be pushed back into the Tosoh heated-line.

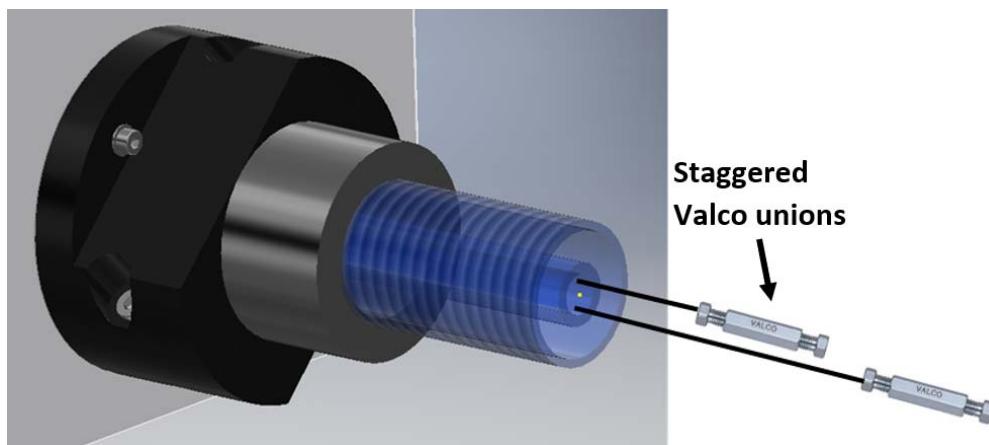


Figure D-17: Chromatography tubing pulled from the oven and swaged to Valco unions

6. Swage the opposite end of the Valco unions to the stainless steel tubing provided with the Wyatt heated-line assembly as shown in Figure D-18. See technical note TN3101: *Swaging Stainless Steel Tubing* for help swaging the stainless steel tubing to the Valco unions.

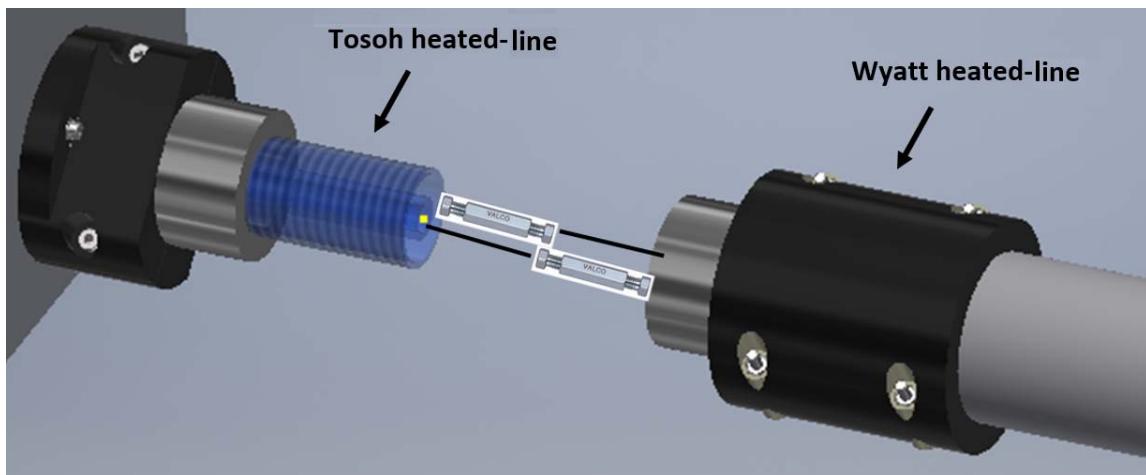


Figure D-18: Two chromatography lines (inlet and outlet from the flow cell) swaged to a Valco union to connect the two heated-line assemblies.

7. Loosen the four M4 screws on the Wyatt heated-line adapter indicated in Figure D-19. This allows the foam tube (P/N 165323) to slide.

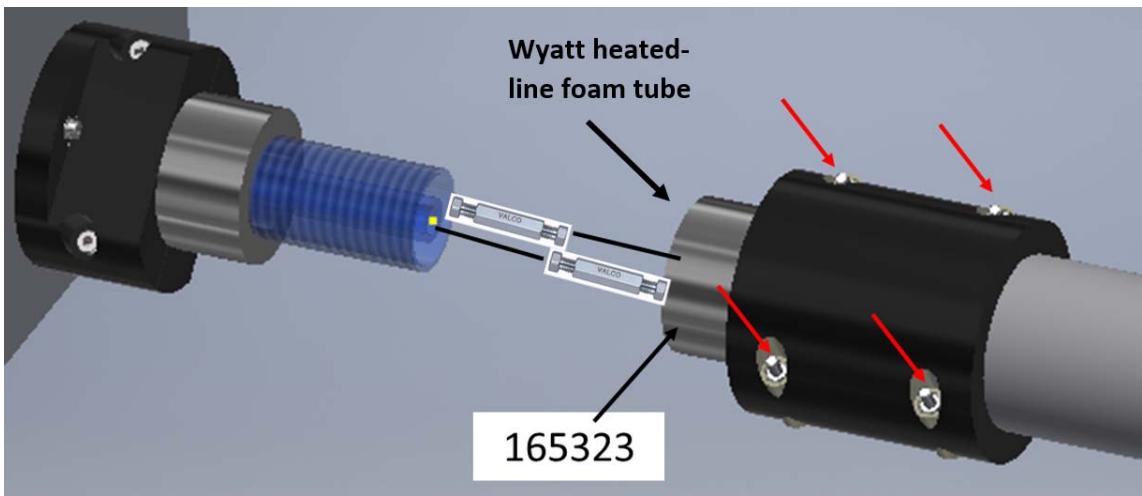


Figure D-19: Four M4 screws to loosen indicated by red arrows

8. Slide the foam over the chromatography tubing and on to the Tosoh heated-line. Make it flush with the heated-line as shown in Figure D-20.

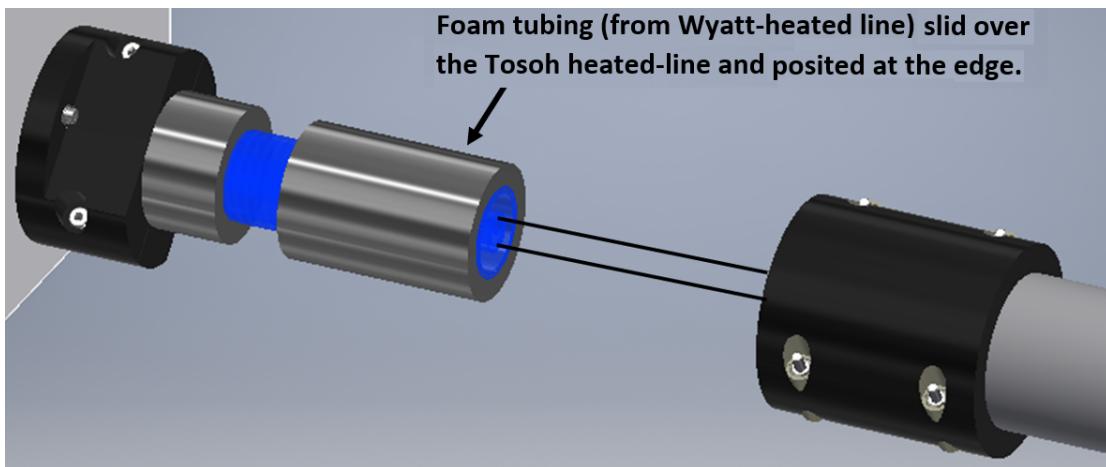


Figure D-20: Foam tube from the Wyatt heated-line assembly slid over the Tosoh heated-line assembly

9. Remove the black adapter from the Wyatt heated-line. Slide the Valco unions and the Wyatt heated-line adapter as far into the Tosoh heated-line as possible, as shown in Figure D-21. The foam tube should remain in place at the edge of the Tosoh heated-line.

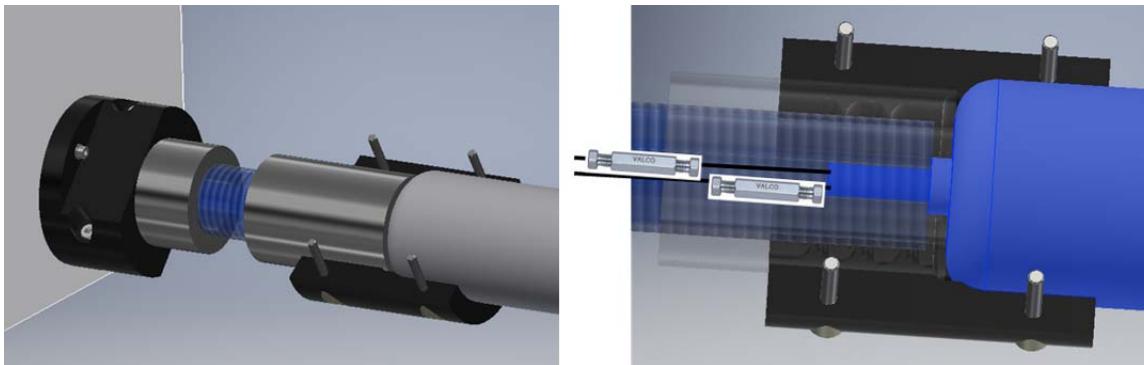


Figure D-21: Remove the Wyatt adapter and slide both the chromatography fittings and Wyatt heated-line into the Tosoh heated-line.

10. Re-assemble the adapter from the Wyatt heated-line by re-attaching the M4 screws as shown in Figure D-22. The ribs of the adapter should grip the foam tube, not the Wyatt heated-line. The heated-line installation is now complete.

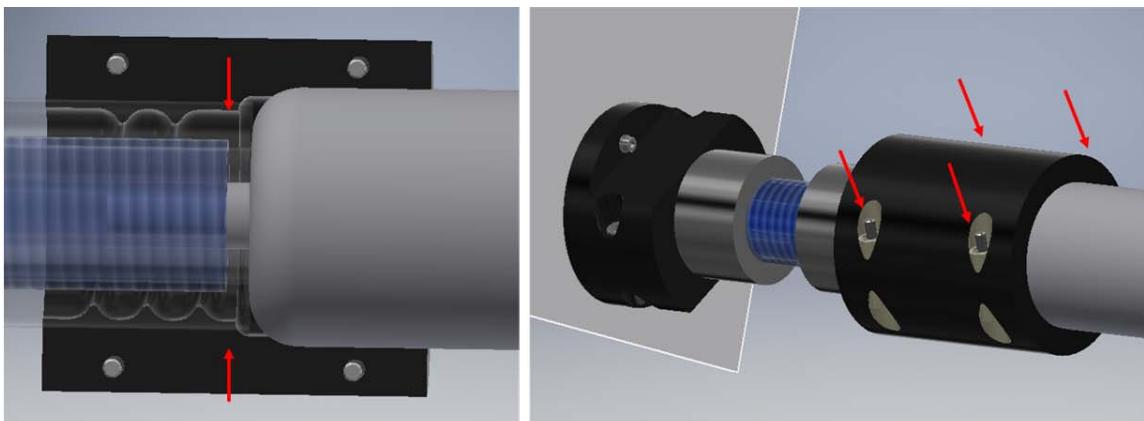


Figure D-22: Final assembly of the heated-line. The Wyatt heated-line adapter secures the foam tube on the Tosoh heated-line and secures both heated-lines to each other.

Removing the UHT Flow Cell Assembly

These instructions are for those instances when you need to remove the UHT heated-line assembly—typically to clean the flow cell or to convert to batch mode.

What you will need to remove the cell assembly:

- Two 1/4 in. 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. For protection of the flow cell assembly, the temperature will ramp down at 1 °C per minute.
2. Remove the top cover hatch by unscrewing the four screws and sliding the foam up along the heated-line insulation.



Figure D-23: Heated-line assembly, top cover hatch secured by four screws

3. Remove the two M3x35 screws holding the copper baseplate and the COMET to the read head and slide the copper baseplate and COMET out. The metal insert on the read head should come out along with the copper baseplate.
4. Use the 2.5 mm Ball driver to remove the two M3 screws.
5. Lift the cell assembly up and out of the read head using the connecting tubing. The cell assembly is the same as described in [Flow Cell on page 31](#). Cell disassembly and cleaning is described in [Cleaning the Flow Cell and Windows on page 97](#).

Note:	Whenever you clean the flow cell of an UHT DAWN that has come down from elevated temperatures, you should replace the 9 mm O-rings. They become brittle when heated and then cooled.
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Interference Filter Option

If a sample fluoresces when illuminated by laser light at either 660 nm or 785 nm, fluorescent light will reach the detectors since photodiodes detect light over a wide range of wavelengths. Interference filters with a narrow bandwidth may be used to filter out light of a different wavelength than the laser wavelength to reach the photodiode detectors. Without interference filters the molecular weight measurements of a fluorescing sample will be inaccurate due to the additional contribution of fluorescence to the scattered light of the sample.

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Installing Interference Filters	179

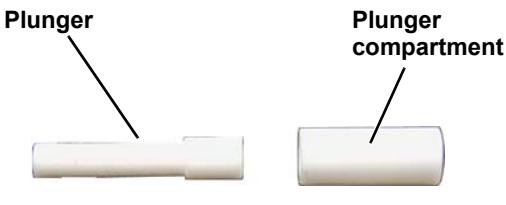
Installing Interference Filters

What you will need to install/remove interference filters:

- Anti-static wrist strap (part number P9012)
- Tweezers (soft tip, reversible action, part number P9011)
- Set of interference filters and 9 mm O-rings (A variety of interference filters are available. Two examples are shown in Figure E-1.)
- Plastic plunger (part number 169205) supplied with your interference filter shipment. Plunger parts are shown in Figure E-2.



Figure E-1: Filters



Assembled Plunger

Figure E-2: Plunger assembly

- Sticky tape (for removal of filters)
- Dental pick (for removal of filters)

To install interference filters, do the following:

1. Put on an 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 (if the instrument is still plugged in, otherwise ground yourself to a suitable grounding station) and gently remove a photodiode and its O-ring from the read head. The O-ring should remain attached to the photodiode
4. Place a 9 mm O-ring into plunger compartment, then place the interference filter on top of the O-ring (see Figure E-3).

Touch the outer edge of the interference filter only. If the filter has an arrow on its side, then place the filter so that the arrow is pointing down toward the plunger (and the O-ring). The arrow, once the filter is installed, should point in the direction the scattered light will propagate from the flow cell to the photodiode.

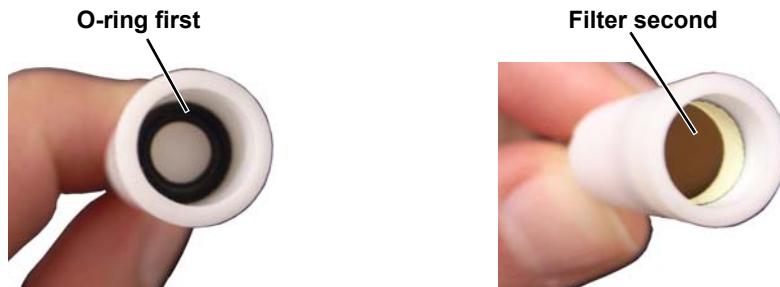


Figure E-3: O-ring and Filter Inserted into Plunger

If the interference filter does not have an arrow, then place the filter so that the side that is mirrored (has no depth when looking down through it as shown in Figure E-4) is facing up—away from the 9 mm O-ring.



Figure E-4: Filter orientation

5. Ensure that there is no space between the 9 mm O-ring and the interference filter inside the plunger compartment by placing the opening of the plunger compartment down facing a clean surface and pushing the plunger to push the O-ring and interference filter to the edge of the plunger compartment and remove any space between the O-ring and plunger.
6. Insert the plunger tool into the read head photodiode hole and push the plunger to install the O-ring and filter (see Figure E-5).

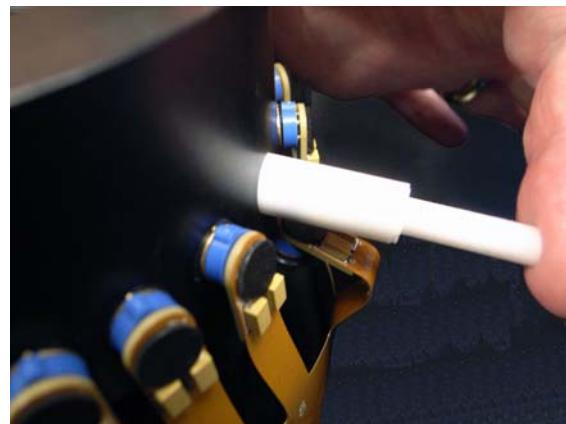


Figure E-5: Installing the Interference filter

7. Remove the O-ring from the photodiode and push it into the shoulder of the hole.
8. Moisten the O-ring with alcohol, then push the photodiode through the O-ring, into its hole. Moistening the O-ring ensures that the photodiode slides easily into place.

9. Repeat steps (3) to (8) for the other interference filters. In the 18-angle DAWN, installing a filter on every other diode should be sufficient. For example, you might install filters on the even numbered detectors only. In the 8-angle DAWN 8, interference filters are typically installed on all detectors.
10. Replace the instrument cover and switch the instrument and laser back on.
11. Repeat the calibration (if you installed a filter on the 90° detector), normalization and, for Batch mode, solvent offset measurements.

Note: Interference filters are not installed with the DLS fiber holders. Contact Wyatt Technical Support if your experiments require installing interference filters for DLS measurements.

To remove installed 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.
4. Remove the 9 mm O-ring installed with the interference filter gently using a dental pick.
5. Cover the end of the plunger with sticky tape. Insert the plunger into the read head photodiode hole and remove the interference filter by allowing it to stick to the edge of the plunger.
6. Remove the O-ring from the photodiode and push it into the shoulder of the hole.
7. Moisten the O-ring with alcohol, 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.
9. Replace the instrument cover and switch the instrument and laser back on.
10. Repeat the calibration (if you removed a filter on the 90° detector), normalization and, for Batch mode, solvent offset measurements.
11. Clean the filters with lens tissue moistened with a little IPA.



DAWN Specifications

This appendix provides technical specifications for the DAWN instrument.

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Wetted Materials	185
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General Specifications

Measurements

Molar Mass Range ^a	200 Da to 1 GDa
Molecular Size Range (R_g) ^{a, b}	10 nm to 500 nm, up to 1000 nm with shape-specific models
Molecular Size Range ^{a, c} (R_h , with WyattQELS DLS module or DynaPro NanoStar)	Depends on scattering angle and flow rate: In flow mode, ~0.5 nm to 300 nm ^d ; In batch mode, 0.5 nm to 1 μ m
Sensitivity	0.2 μ g/mL BSA; 0.1 μ g BSA typical HPLC loading

Fluidics

Mobile Phase Compatibility ^e	All-solvent compatible (aqueous and organic). Wetted materials are 316 stainless steel, fused silica, flow cell glass, and Kalrez®. Aqueous pH range from pH 1 to 10.
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Detectors

MALS Detectors	High-gain, high dynamic range, static light scattering detectors at 18 angular positions
Auxiliary Detectors	Laser monitor for stabilization feedback; forward transmission monitor to correct signals for absorbing samples and to assess data quality.
A/D Resolution	24 bit (detector dynamic range > 16,000,000)

DLS Detector (optional)	WyattQELS module installs directly inside the instrument chassis. Alternatively, the optical fiber pickup of the DynaPro NanoStar cuvette-based DLS instrument or the Mobius instrument to connect the respective instrument's DLS correlator for processing DLS data may be installed in the DAWN.
-------------------------	---

Laser Properties^e

Laser Wavelength	658 nm (nominal). Each instrument is individually characterized. The standard 658 nm red laser may be replaced during production in the factory with an infrared (IR) laser operating at 785 nm.
Laser Power Control	Programmable 10 % to 100 %

Flow Cells^e

Fused Silica	optimal for solvent refractive index < 1.50
F2	optimal for solvent refractive index > 1.50

Temperature Options

Ambient	No temperature control; +15 °C to +50 °C environment
Heated Cooled (HC)	-15 °C to +150 °C
Temperature Regulated (TR)	+20 °C to +70 °C
Ultra-High Temperature (UHT)	Ambient to +210 °C

Electronics

Analog Inputs	4 differential analog inputs with 24 bit resolution. Input range -10 V to +10 V
Analog Outputs	4 single-ended analog outputs from user selectable measurement channels -10 V to +10 V
Other Inputs/Outputs	Alarm In, Alarm retransmit, Auto-Inject in, Auto-inject contact closure, Recycle In, Recycle valve power
Computer Interface	Ethernet
Processed Data Transmission Rate	31.25 Hz to 0.0001 Hz
Front Panel Display	10", 1280x800, high-resolution projected-capacitive (PCAP) touch screen

Physical

Dimensions	58 cm (D) x 36 cm (W) x 26 cm (H)
Weight	21.2 kg

- a. Typical—Actual range depends on dn/dc, sample concentration, sample conformation and chromatography conditions.
- b. Typical—Actual range depends on the sample.
- c. Configuration and flow-rate dependent.
- d. Typical size range for base configuration is 0.5 nm to 50 nm.
- e. See below for details.

Laser Specifications

The DAWN contains a linearly polarized GaAs laser operating at a nominal wavelength of 658 nm.

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 %. Although the laser can warm up within minutes, it is recommended to warm up instrument hardware for 30 minutes prior to collecting data.

Table F-1: GaAs Laser electrical and optical specifications

Laser Operating Wavelength	658 nm ± 10 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	~150-170 mA

The lasers utilized in the DAWN are Class IIIb lasers but overall the DAWN itself is classified as a Class 1 Laser Product because the laser beam is contained within the optical bench, which itself is contained within the instrument chassis. Equipped with a laser safety interlock system to ensure the laser is off whenever the cover hatch is opened or the cover is removed, this instrument will emit no laser radiation and no laser radiation protective equipment must be worn under normal operation.

However, the following warnings still apply:

-
- CAUTION:** Modification from the controls or performance parameters specified here may result in hazardous radiation exposure.
Avoid direct exposure to the beam.
Laser safety labels are provided in English. For safety labels in a language other than English, please contact Wyatt Technology Corporation.
-

Table F-2: Laser environmental specifications

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

Wetted Materials/Cell Properties

This section contains information about the wetted materials, and thermal and chemical properties of the two types of flow cells, and their refractive indices. Except for [Scattering Angles on page 188](#), all data and descriptions are from the Schott Glass *Optical Glass Catalog*.

Wetted Materials

The wetted materials are 316 stainless steel, Kalrez (perfluoroelastomer), flow cell glass, and fused silica.

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 Alkalies (AR 1–4)

A two-digit figure is used to express resistance to alkalies. The first digit lists the alkali resistance class. 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).

Flow cell alkaline resistance classes

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

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)

Flow Cell Properties

Thermal Properties

Glass Classification	Thermal Expansion		Transformation Temperature	Specific Heat $C_p = (J/g \times K)$
	-30 to 70 °C	20 to 300 °C		
F2	$8.2 \times 10^{-6} /K$	$9.3 \times 10^{-6} /K$	432 °C	0.557
Fused silica	$4.7 \times 10^{-7} /K$	$4.7 \times 10^{-7} /K$	970 °C	0.749

Refractive Indices

Glass Classification	Refractive Index $\lambda = 633nm$
F2	1.61311
Fused silica	1.457055

Chemical Properties

To interpret the CR, FR, SR and AR values, see [Definition of Terms on page 185](#).

Glass Classification	Bubble Class	CR	FR	SR	AR
F2	0	1	0	1	2.3
Fused silica Acceptable mobile phase pH range: 1- 11	0	1	0	1	no data

Scattering Angles

The tables below show the scattering angles for different flow cells in four different solvents at a wavelength of 658 nm. The table entries for 1,2,4-trichlorobenzene (TCB) are at a temperature of 135 °C, all others are at 25 °C. Note that for an F2 cell in water, the first three detectors are not available.

The mathematics behind these changes in scattering angles are discussed in [Refractive Index Differences—Liquid vs. Glass on page 33](#).

Table F-3: Scattering Angles for Fused Silica Flow Cell

Detector	Read Head Angle	water ($n_s = 1.330$)	THF ($n_s = 1.401$)	toluene ($n_s = 1.488$)	TCB (135 °C) ($n_s = 1.5524$)
Fused silica ($n_g = 1.45706$)					
1	22.5	N/A	N/A	24.4	29.1
2	28.0	13.0	22.4	29.5	33.4
3	32.0	20.7	27.4	33.3	36.7
4	38.0	29.6	34.4	39.0	41.8
5	44.0	37.5	41.1	44.8	47.1
6	50.0	44.8	47.7	50.7	52.5
7	57.0	53.1	55.2	57.5	59.0
8	64.0	61.1	62.7	64.4	65.5
9	72.0	70.1	71.1	72.3	73.0
10	81.0	80.1	80.6	81.1	81.5
11	90.0	90.0	90.0	90.0	90.0
12	99.0	99.9	99.4	98.9	98.5
13	108.0	109.9	108.9	107.7	107.0
14	117.0	120.1	118.4	116.6	115.4
15	126.0	130.4	128.0	125.4	123.8
16	134.0	140.0	136.7	133.2	131.1
17	141.0	149.0	144.5	140.0	137.3
18	147.0	157.7	151.4	145.8	142.5

Table F-4: Scattering Angles for F2 Glass Flow Cell

Detector	Read Head Angle	water ($n_s = 1.330$)	THF ($n_s = 1.401$)	toluene ($n_s = 1.488$)	TCB (135 °C) ($n_s = 1.5524$)
F2 ($n_g = 1.61311$)					
1	22.5	N/A	N/A	N/A	16.0
2	28.0	N/A	N/A	16.8	23.3
3	32.0	N/A	12.4	23.2	28.1
4	38.0	17.0	24.8	31.3	34.9
5	44.0	29.2	34.1	38.7	41.6
6	50.0	38.8	42.3	45.8	48.0
7	57.0	48.6	51.2	53.8	55.5
8	64.0	57.9	59.7	61.6	62.9
9	72.0	68.0	69.2	70.4	71.2
10	81.0	79.1	79.6	80.2	80.6
11	90.0	90.0	90.0	90.0	90.0
12	99.0	100.9	100.4	99.8	99.4
13	108.0	112.0	110.8	109.6	108.8
14	117.0	123.4	121.5	119.5	118.2
15	126.0	135.5	132.6	129.6	127.7
16	134.0	147.4	143.1	138.9	136.3
17	141.0	160.5	153.5	147.4	143.9
18	147.0	n/a	165.0	155.4	150.7



Instrument Connectivity

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

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Please read over [Components](#) to gain an understanding of the components to be used. Then read over either [Connecting to a LAN](#), [Connecting via USB](#), or [Connecting via Ethernet when not on a LAN](#) depending on your configuration. Finally, read over [Instrument Network Settings](#) for instrument settings.

Read [Accessing Instruments with ASTRA](#) for instructions on accessing instruments via ASTRA. Finally, if you experienced problems connecting to your instrument, read [Troubleshooting and Diagnostics](#).

Components

Instrument Connections

Figure G-1 shows the instrument back panel. The Ethernet port is to be used for all connections in these instructions. See [Connecting via USB on page 197](#) for instructions on establishing a USB connection.



Figure G-1: Instrument back panel

LAN Connection

Figure G-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.

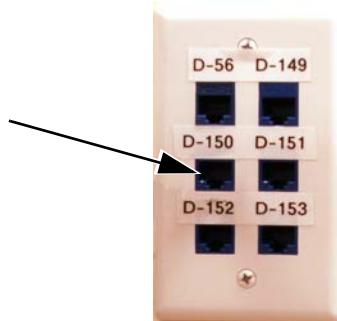


Figure G-2: Wall socket LAN connection indicated by arrow

Computer Connections

Computer connections can be made via either the Ethernet or USB port. Figure G-3 shows these ports on a standard laptop computer. [Connecting to a LAN on page 194](#) and [Connecting via Ethernet when not on a LAN on page 199](#) describe instrument connections made via the Ethernet port. [Connecting via USB on page 197](#) describes connections made via the USB port.

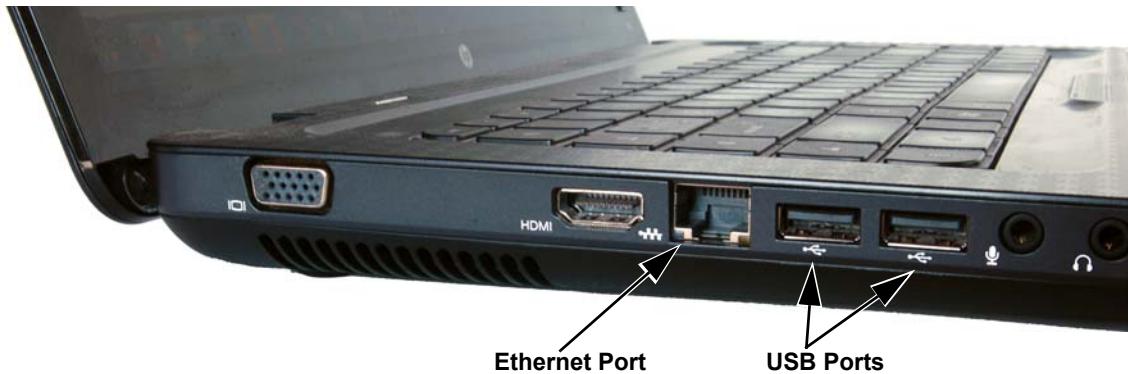


Figure G-3: Ethernet and USB ports on the computer

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. Note that the crossover cable shipped with Wyatt Technology instruments is yellow to distinguish it from a standard Ethernet cable. Be careful to only use the yellow crossover cable where indicated.



Figure G-4: Ethernet crossover cable shipped by Wyatt Technology

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. 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.

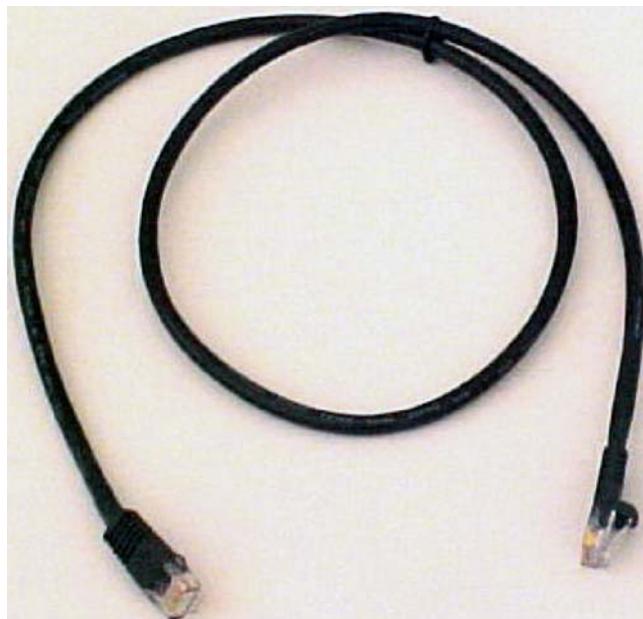


Figure G-5: Standard Ethernet cable

While the pictured cable is black, they may also come in blue, white, or gray; however, yellow is reserved exclusively for crossover cables.

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 G-6: Standard Ethernet to USB adapter.

The Ethernet cable is plugged into the Ethernet port (arrow), and the USB connector is plugged into a USB port on the computer.

Ethernet Switch

Ethernet switches are used to connect several Ethernet cables to one resource, such as the LAN socket in Figure G-2. The Ethernet switch supplied by Wyatt Technology will look similar to the five port switch shown below. Note that although Ethernet cables can be connected to the switch in any order or position, best practice is to use the Uplink port to connect to a LAN while leaving port 8 empty. Also, the switch has an external AC adapter (not shown) to provide power to the switch.



Figure G-7: Eight-port Ethernet switch

Connecting to a LAN

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

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 G-8: Connection for one instrument to LAN

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 G-9: Instrument and computer, both connected to LAN via Ethernet switch

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 G-10: Two instruments connected to the LAN via an Ethernet switch

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 G-11: Two instruments and computer connected to LAN via Ethernet switch

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.

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.



Figure G-12: One instrument to USB via yellow

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 G-13: Connecting one instrument to USB using an

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 G-14: Connecting two or more instruments to

Connecting via Ethernet when not on a LAN

If the computer is not on the LAN, it is possible to use either the USB port described above or the Ethernet port directly to connect to the instruments.

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 G-15: Connecting one instrument to a computer not on the LAN using

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 G-16: Connecting one instrument to the computer using an

Multiple Instruments to Computer Not on LAN with 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 G-17: Connecting multiple instruments to computer using Ethernet switch

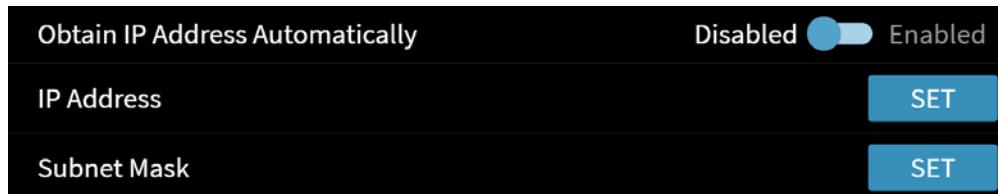
Instrument Network Settings

In the **Network** section of the **Settings** tab on the front panel display ([Network Settings on page 66](#)), there is a choice to enable **Obtain IP Address Automatically**, which will use an associated DHCP server. If this is disabled, you can set a static IP address.

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 197](#).

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 are automatically updated. At this point, it should be possible to connect to the instrument from the computer.

In many environments, setting a private, static IP address onto the instrument is the optimal method of communication to avoid interference from a company network, which can be subject to interruption. Your IT department may require a specific IP address for the computer. The IP address assigned to the Wyatt instruments should match for the first three sets of numbers from the computer IP and then be different for the last set of numbers. The subnet mask must be identical for the computer and the instruments. Please see TN1018- Instrument Connection Guide for ASTRA for additional details on this configuration.



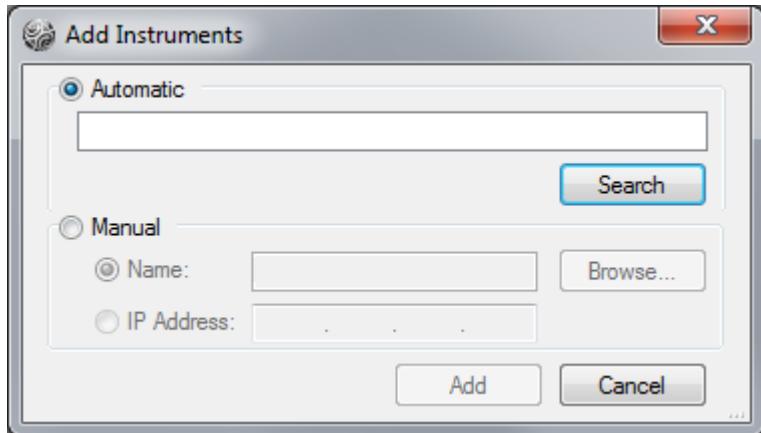
Accessing Instruments with ASTRA

To access an instrument with ASTRA after you have connected it as described in this chapter, you may use either the Method Builder or add the instruments via the **System** drop down menu.

To use the Method Builder, select **System**→**Method Builder Wizard** and follow the prompts for selecting an appropriate method, adding your instruments, and inputting parameters. More information about the Method Builder can be found in the *ASTRA User's Guide*.

To add your instruments without the Method Builder, follow these steps:

1. From the main menu of ASTRA, select **System→Instruments**.
2. In the Instruments dialog, click **Add**, which opens the Add Instruments dialog.



3. Select either **Automatic** or **Manual**.
 - If using **Automatic**, click the **Search** button. Any detectable Wyatt instruments will be listed. Highlight the desired instrument and click **Add**. The selected instrument will then appear in the Workgroup list in the Instruments dialog.
 - If using **Manual**, add the instrument by name or IP address.
4. To add the instrument by name, type the instrument's computer name and click **Add**. The instrument's computer name can be found on the front panel, in the **Settings** tab under **Network**.
5. To add the instrument by IP address, type the IP address of the desired instrument and click **Add**.
6. It may take several minutes for ASTRA to identify instruments on the network and several minutes more for a selected instrument to appear in the Workgroup list. Once it is there, select the instrument by clicking on it, then click the **View** button. The Diagnostic Manager application should launch, and can be used to verify the instrument connection.

Troubleshooting and Diagnostics

If you are experiencing instrument connectivity challenges, please go over the following steps and consult TN1018: *Instrument Connection Guide for ASTRA* in Wyatt Technology's [Customer Support Center](#). If you still cannot connect to your instrument, please contact Wyatt Technology Support at support@wyatt.com for assistance.

Computer Configuration

See Wyatt Technology's website for current software and hardware specifications, such as Supported Microsoft Operating Systems, System Requirements, and Computer Connections.

ASTRA Settings

If the instrument is not connected to the LAN **or** you are using a static IP address, it is critical that you type in the correct IP address.

Verifying Instrument Connections

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

```
C:\>ping 172.20.1.244
Pinging 172.20.1.244 with 32 bytes of data:
Reply from 172.20.1.244: bytes=32 time<1ms TTL=128

Ping statistics for 172.20.1.244:
    Packets: Sent = 4, Received = 4, Lost = 0 (0% loss),
    Approximate round trip times in milli-seconds:
        Minimum = 0ms, Maximum = 0ms, Average = 0ms
```

Figure G-18: Using ping to verify the instrument connection

If the instrument is not connected properly, the result should be similar to that shown in Figure G-19.

```
C:\>ping 172.20.1.243
Pinging 172.20.1.243 with 32 bytes of data:
Request timed out.
Request timed out.
Request timed out.
Request timed out.

Ping statistics for 172.20.1.243:
    Packets: Sent = 4, Received = 0, Lost = 4 (100% loss),
```

Figure G-19: Failure to connect to instrument using ping

H

List of Warnings

This appendix provides a list of warnings that should be read and understood when using the DAWN.

-
- Note:** Laser safety labels are in English. If you need safely labels in a language other than English, please contact Wyatt Technology Support at support@wyatt.com.
-



The instrument contains a Class IIIb laser. However the DAWN itself is classified as a Class 1 Laser Product according to IEC60825-1:1993+A1+A2 and CFR Title 21 Subchapter J. 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 the cover hatch is removed, the laser is deactivated. The specifications listed apply to the laser subsystem and not to the instrument as a whole. Under normal operation, no laser radiation should escape from the instrument, and no protective equipment must be worn.

However the following 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:

-
- CAUTION:** Laser Radiation when open. Avoid direct exposure to beam.
-

Warning:

If the power cord is connected, line voltages of 120 Vac to 240 Vac, 50 Hz to 60 Hz are present within the system even when the power switch is off. Always disconnect the power cord before opening the instrument cover.



Warning:

High voltage is stored in the instrument power supplies for some time (hours) after the instrument is switched off and the power cord is disconnected. Under no circumstances should the power supplies be accessed by unqualified personnel.

Warning:



The instrument contains fluid. Under normal operating conditions, the system may contain up to 0.01 mL of fluid introduced into the system by the user. If a leak develops internal to the instrument, additional fluid may be present in the system. The system has been designed to bring fluid leaks to standard locations within the instrument, but there always exists the possibility of fluid present in non-standard locations. Fluid introduced into the instrument by the user or its vapors may be hazardous. Safety precautions appropriate to the fluid within the system should be taken under the assumptions that the fluid may not reside in standard locations within the instrument, and may not be contained by the instrument.

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