Hardware Manual for the miniDAWN Tristar Light Scattering Instrument
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A variety of U.S. and foreign patents have been issued and/or are pending on various aspects of the apparatus and methodology implemented by this instrumentation.
# Table of Contents

## Chapter 1: Introduction

- Overview ........................................................................................................... 1-2
- The Instrument .........................................................................................................1-2
- The Software ............................................................................................................1-3
- About This Manual ............................................................................................ 1-4
- Manual Conventions ................................................................................................1-4
- How the Manual Is Organized ..................................................................................1-4

## How to Contact Wyatt Technology Corporation ................................................ 1-5
- Corporate Headquarters ..........................................................................................1-5
- Sales Department ....................................................................................................1-5
- Technical Support .....................................................................................................1-5

## Chapter 2: Installing the miniDAWN Tristar

- Unpacking the Instrument ................................................................................. 2-2
- Installing the Instrument .................................................................................... 2-4
- Auxiliary Devices ............................................................................................... 2-7
  - Attaching Auxiliary Device Connectors .................................................................2-7
  - Adjusting the Auxiliary Gain .....................................................................................2-7

## Chapter 3: miniDAWN Tristar Components

- Front Panel View ............................................................................................... 3-2
- Back Panel View .....................................................................................................3-3
  - Changing a Fuse ......................................................................................................3-3
- Removing the Cover ............................................................................................ 3-3
  - Removing the Cover ................................................................................................3-3
- Laser ................................................................................................................. 3-5
  - Laser Beam Warning ..............................................................................................3-5
  - Laser Monitors .........................................................................................................3-5
- Read Head and Detectors ................................................................................. 3-6
- Flow Cell ........................................................................................................... 3-8
  - Flow Cell Design ......................................................................................................3-8
  - Refractive Index Differences—Liquid vs. Glass .......................................................3-9

## Chapter 4: Maintenance

- General Maintenance ........................................................................................ 4-2
- Flow Cell Maintenance ...................................................................................... 4-2
  - On-line Cleaning ....................................................................................................4-2
  - Particles in the Cell ..................................................................................................4-3
This chapter is the introduction to the miniDAWN Tristar® laser light scattering photometer. This chapter describes the miniDAWN and lists available support options available from Wyatt Technology Corporation (WTC).

**CONTENTS**

Overview ................................................................................................... 1-2
The Instrument .......................................................................................... 1-2
The Software ............................................................................................ 1-3
About This Manual .................................................................................... 1-4
Manual Conventions .............................................................................. 1-4
How the Manual Is Organized ................................................................ 1-4
How to Contact Wyatt Technology Corporation ........................................ 1-5
Corporate Headquarters ........................................................................ 1-5
Sales Department .................................................................................. 1-5
Technical Support ................................................................................ 1-5
Overview

Figure 1-1: The miniDAWN Tristar laser photometer

The Instrument

The miniDAWN Tristar is a multi-angle laser light scattering photometer which can be used as on line detector for liquid chromatography or as a stand-alone detector for batch measurements. The miniDAWN Tristar connects easily to any HPLC system to determine absolute molar masses from 200 to over a million g/mol (daltons), and root mean square ($\text{rms}$) radii from 10 nm to over 50 nm. It is an excellent tool for polymer or protein characterization in support of either research or product quality control.

The laser diode assembly and the flow cell both anchor to the read head, which is bolted to the optical bench thus providing and extremely stable platform.

The laser beam is directed through the flow cell in the same direction as the flowing stream. The flow cell windows are recessed in the flow cell manifolds thus minimizing both sample volume and stray light. The three discrete photodiode detectors spaced around the flow cell enable simultaneous measurements at three different scattering angles over a range of angles from approximately 45 to 135 degrees. The precise scattering angle measured depends upon the refractive index of the solvent. The analog signal from each photodiode is processed by its own DSP (Digital Signal Processor) integrated circuit with 16-bit digital conversion for high signal resolution. In addition, two auxiliary analog inputs (with their own DSP chips) enable interfacing to external instruments such as a differential refractive index detector, an ultra-violet detector, or a differential viscometer. A six-pole Gaussian filter in the DSP chip processes each light scattering and auxiliary signal for the greatest possible noise rejection without peak distortion. Since the analog to digital conversion is performed on-board inside the miniDAWN, low light scattering signals are not prone to environmental "noise" or pickup during transmission to the computer. The digital output is transmitted to the computer through an RS-232 serial port in the computer.
The Software

Wyatt Technology offers the following software package for collecting and analyzing data from the miniDAWN Tristar instrument:

- **ASTRA® for Windows**
  ASTRA for Windows collects and processes light scattering data as a function of time. If the sample is fractionated by size or molar mass using size exclusion chromatography (SEC), ASTRA computes the molar mass moments (number, weight and z-average) and the rms radius moments of the sample. A concentration sensitive detector is required for the molar mass calculations. For unfractionated polymers ASTRA computes the weight average molar mass, the z-average rms radius, and the second virial coefficient using a Zimm Plot.
About This Manual

This miniDAWN Tristar Hardware Manual describes how to set up and use the miniDAWN Tristar laser photometer. Please consult the ASTRA for Windows Software manual for guidelines in sample preparation and auxiliary hardware setup.

Manual Conventions

Whenever internal components are describe it is assumed the reader is facing the front of the instrument.

Normally either a hex wrench (Allen wrench) or a Ball driver can be used for the same task. In this manual the tool will be referred to as a Ball driver.

The IUPAC Definition Committee specifies the term molar mass for the sum of the atomic weights of all atoms in a mole of a molecule. This term can be used interchangeably with molecular weight.

How the Manual Is Organized

The chapters and appendices in the miniDAWN Tristar Hardware Manual are organized as follows:

Chapter 1—Introduction - offers a general overview of the miniDAWN Tristar instrument, this manual, and the support options available from Wyatt Technology.

Chapter 2—Installing the miniDAWN Tristar - describes the steps necessary for unpacking, connecting and initial testing of the miniDAWN Tristar.

Chapter 3—miniDAWN Tristar Components - is a description of the miniDAWN Tristar's exterior and interior.

Chapter 4—Maintenance - describes general maintenance guidelines and the procedure for cleaning the flow cell.

Appendix A—Accessories describes recommended accessories for various light scattering applications
How to Contact Wyatt Technology Corporation

For questions concerning the miniDAWN Tristar, first look in this manual or consult the online help that comes with the ASTRA software. If the answer cannot be found, please contact Wyatt Technology Technical Support.

Corporate Headquarters
Wyatt Technology Corporation
30 South La Patera Lane, B-7
Santa Barbara, CA 93117
USA

Sales Department
Wyatt Technology Corporation Sales Hours are 8:30 a.m. to 5:00 p.m. Pacific Time.

Sales Phone: (805) 681-9009
Sales Fax: (805) 681-0123

Technical Support
Wyatt Technology Corporation offers a variety of support options to help you get the most from your miniDAWN Tristar.

For users outside the United States, the Wyatt Technology distributor should be contacted for assistance.

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

Internet
Visit Wyatt Technology’s world-wide-web site to e-mail requests for assistance.

World-Wide-Web URL: http://www.wyatt.com
Electronic mail address: support@wyatt.com

FAX
Please fill in the answers to the items listed on page 1-6, and fax it to:
Wyatt Technology Corporation Technical Support Fax: (805) 681-0123

Questions or comments may be faxed at any time.

Mail
Please fill in the answers to the items listed on page 1-6, then mail it to our corporate headquarters.
Telephone

To speak to our support personnel directly, please call between 8:30 a.m. and 5:00 p.m. Pacific Time, Monday through Friday. Calls made during non-business hours will be handled by a voice mail system. When the call is placed please at the instrument and have the documentation at hand if possible. Please be prepared to provide the following information:

- The miniDAWN Tristar instrument serial number (located on the back panel).

If the problem is software related:

- The operating system name and version number.
- The WTC software version number. The software version number is located on the original distribution diskettes, or can be determined by selecting About from the software Help menu.
- The exact wording of any messages that appeared on the computer screen.

Then, for either a hardware or software problem:

- The type of computer hardware being used.
- Details of what was happening when the problem occurred.
- Efforts attempted to solve the problem.

Wyatt Technology Corporation Technical Support Phone Number:

(805) 681-9009
2 Installing the miniDAWN Tristar

This chapter guides the user through unpacking the miniDAWN Tristar and the setting up and testing of the instrument.

**CONTENTS**

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpacking the Instrument</td>
<td>2-2</td>
</tr>
<tr>
<td>Installing the Instrument</td>
<td>2-4</td>
</tr>
<tr>
<td>Auxiliary Devices</td>
<td>2-7</td>
</tr>
<tr>
<td>Attaching Auxiliary Device Connectors</td>
<td>2-7</td>
</tr>
<tr>
<td>Adjusting the Auxiliary Gain</td>
<td>2-7</td>
</tr>
</tbody>
</table>
Unpacking the Instrument

This section lists the accessories and spare parts that were shipped with the instrument. Please read this section and check that everything has arrived in good condition.

1. Carefully examine the shipping container. If it has been mishandled, CONTACT THE SHIPPING CARRIER IMMEDIATELY.

2. Unpack the instrument.

3. In addition to receiving the miniDAWN Tristar Laser Photometer, check that the boxes contain the following items (the packing slip sent with the instrument contains an up-to-date list)

Table 2-1: Parts List:

<table>
<thead>
<tr>
<th>Documentation:</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>This miniDAWN Tristar Hardware Manual</td>
<td></td>
</tr>
<tr>
<td>Software disks and manuals</td>
<td></td>
</tr>
</tbody>
</table>

| Cables:                                       |             |
| Interface cable adapter                      | P3796-0925  |
| RS-232 interface cable                        | P4045-03    |
| Main Power Cord                               | P4100-120 (or 220) |
| 4 Auxiliary Cables, 4 cond., Turk Fittings    | P4045-05    |

| Fuses:                                        |             |
| Spare fuses                                   | 3616-00500 (or 3616-00250) |

| Fluid Fittings and Tubing:                    |             |
| 2 Flow cell windows                           | 116007      |
| 2 Window O-rings                              | P6504-2004  |
| 2 Flow cell O-rings                           | P6504-2006  |
| 2 Backing rings                               | 200609      |
| 2 Stainless Steel Fittings and Ferrules       | P6406-10/P6455-10 |
| Tubing, inlet (pre-bent, 0.005" ID, red label)| 110101-005  |
| Tubing, outlet (pre-bent, 0.010" ID)          | 110100-2    |

| Tools:                                        |             |
| 1.5 mm Ball driver (for screws holding bottom cell retainer in place) | P9004 |
| 2.0 mm Ball driver (for the screws securing the instrument cover)    | P9015 |
| 2.5 mm Ball driver (the screws attaching the flow cell assembly and cover plates to the read head, and the M3 screws that hold the cell together) | P9005 |
| 4 mm Ball driver (for the window retainer screws)                     | P9008      |
| Installation Tool (for window retainer screws)                        | 110933     |
4. Place the miniDAWN Tristar on a level surface and inspect the instrument. If any damage is apparent, CONTACT THE CARRIER IMMEDIATELY.

<table>
<thead>
<tr>
<th>Item</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Open end wrenches (for loosening the In and Out bulk head unions)</td>
<td>P9014</td>
</tr>
<tr>
<td>Jeweler's loupe (to examine the laser beam in the flow cell and flow cell components)</td>
<td>P8404</td>
</tr>
<tr>
<td>Anti-static wrist strap</td>
<td>P9012</td>
</tr>
<tr>
<td>Molar Mass Standards Kit</td>
<td>P8401 (or P8402)</td>
</tr>
</tbody>
</table>
Installing the Instrument

The installation procedure for the miniDAWN Tristar involves some initial tests to verify that everything is working properly.

To install the miniDAWN Tristar:

1. Plug the power cord into its connector on the back panel. Plug the other end to an AC outlet.

2. Connect one end of the RS232 serial interface cable to its port on the instrument’s back panel. Plug the other end to the computer’s serial port.

   The RS232 serial interface cable is a 9 pin cable that connects the SERIAL DATA OUT connector on the back panel of the miniDAWN Tristar (see “miniDAWN Tristar back panel.” on page 3-2) to a serial port on the back panel of the computer. COM2 is recommend for most systems.

   Note: If interface cables other than the one supplied by WTC is used, make sure it is a 9 pin RS232 serial cable with all 9 wires wired straight through.

3. Switch on the instrument and let it warm up for 30 minutes before proceeding to the next step.

4. The main power switch is on the back panel, next to the power connector.

5. Press the SELECT button to switch the channels on the front panel display, and compare the voltages with the dark offsets in the miniDAWN Tristar Certificate of Performance.

<table>
<thead>
<tr>
<th>Channel Setting</th>
<th>Value Displayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3</td>
<td>Detector 1, 2, or 3 voltage</td>
</tr>
<tr>
<td>F</td>
<td>Forward laser monitor voltage</td>
</tr>
<tr>
<td>0P</td>
<td>Rear laser monitor voltage</td>
</tr>
<tr>
<td>0I</td>
<td>Laser current</td>
</tr>
</tbody>
</table>

If the voltage readings for detectors 1, 2 and 3 differ by more that 20 mV from the voltages listed on the Certificate of Performance, check the temperature in the laboratory. The dark offsets on the detectors may differ from the Certificate of Performance by as much as 10 mV per °C. For example, if the laboratory temperature is 20°C and the QC laboratory temperature was at 23°C, the dark offsets may be 30 mV different. If a greater difference is observed, monitor the dark offsets for a few days to see if they remain stable at this voltage. If they do not, contact Wyatt Technology Technical Support.

The dark offset forward laser monitor was set to 0 mV with the laser off at the factory and should be within ±50 mV of zero.
6. **Install the ASTRA software.** Using the ASTRA software perform the appropriate steps to configure the instrument to communicate with the software (see the ASTRA software user’s guide for instructions to configure communications with the instrument.)

7. With the laser turned on, wait at least 30 minutes for the laser to warm up and stabilize before proceeding. The laser is only on while ASTRA is open.

**Note:** The laser in the miniDAWN Tristar is software controlled and will automatically be turned on by the software once the communications have been established with the instrument.

8. Compare the channel voltages in the software with the solvent offsets listed on the Certificate of Performance.

**Note:** The voltages can be read from the miniDAWN front panel or in the ASTRA software. Refer to the ASTRA for Windows software manual. The F value and the laser current are only available on the front panel.

The solvent offsets were measured at the factory using toluene. The flow cell was filled with toluene and sealed before shipment so the solvent offsets should be very close to those on the Certificate of Performance. More than 20 mV difference between the measured values and those on the Certificate of Performance (for channels 1, 2, and 3) may indicate the presence of an air bubbles in the flow cell. To remove the air bubble flush the cell with filtered toluene and recheck the solvent offsets. If the dark offsets were different from the Certificate of Performance (see step 5) the solvent offsets should be off by the same amount.

9. Calibrate the miniDAWN Tristar using the WTC ASTRA software.

   See the ASTRA for Windows software manual for instructions on how to perform the calibration measurement.

10. Compare the calibration result with the value from the Certificate of Performance.

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

11. Once it has been confirmed that the instrument is in proper working order, the miniDAWN Tristar may be connected to any other devices for specific applications. (Auxiliary cable connection is described next.)

   The ASTRA for Windows User’s Guide describes how to connect the miniDAWN Tristar to a chromatography system.
This configuration requires the ASTRA software.

12. Keep the miniDAWN Tristar on a flat, clean surface, standing on its feet and positioned to allow air convection through the back of the instrument to keep its electronics cool.

See “Maintenance” on page 4-1 for more information about the miniDAWN Tristar environment and how to keep it in peak condition.
Auxiliary Devices

The Auxiliary cables are used in a chromatography setup. One or two concentration-sensitive detectors (DRI, UV, DAD, etc.) may be connected through the Auxiliary channels. The brown (+) and blue (-) wires of the AUXILIARY cables connect to the integrator (or recorder) analog output of the auxiliary detectors.

- The AUTO INJECT cable can be used to sense an injection from an auto injector. The brown and blue wires from the Auxiliary cable connect to the closure switch on the injector. This switch should be normally open.
- The 90 DEG Auxiliary cable can be used export the 90 DEG analog signal into existing data collection systems or a chart recorder. The 90 DEG output is a 0 to 10 V analog signal.

Attaching Auxiliary Device Connectors

To attach the Auxiliary connector and its cables:

1. Attach the Auxiliary cable to the AUXILIARY input/output port on the rear panel of the miniDAWN Tristar (see Figure 2-1).

2. Connect the appropriate Auxiliary cable to any other device(s) as follows:
   a. Brown wire to the positive terminal.
   b. Blue wire to the negative terminal.
   c. Black wire to the ground terminal (usually not needed).
   d. Bare (shielded) wire to the shield terminal (usually not needed).

3. If connecting the AUX1 or AUX2 cable to a concentration detector and electronic drift or noise is experienced, try connecting the ground wire and/or the shield wire.

4. If connecting the AUTO INJECT cable to an auto injector, ensure that an injection closes the circuit.

   The status of the auto inject circuit can be monitored in ASTRA. Some injectors require programming for this closure to happen.

Adjusting the Auxiliary Gain

The miniDAWN Tristar is shipped with an AUX gain settings of 1×. The AUX gain can be adjusted via the DIP switch on the main PCB.

To adjust the gain settings:

1. Remove the miniDAWN Tristar top cover (see “Removing the Cover” on page 3-3).

2. Locate the DIP switch at the front left corner of the main PCB (see “miniDAWN Tristar top view” on page 3-4).

   The switch settings should all be “ON” for a 1× gain.
3. Set the switch according to Table 2-2.

The gain adjustments for AUX1 and AUX2 are independent of one another, so, for example, the gain could be set at 1000 for AUX1 and at 10 for AUX2.

*Table 2-2: AUX gain settings*

<table>
<thead>
<tr>
<th>GAIN</th>
<th>SW1</th>
<th>SW2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ON</td>
<td>ON</td>
</tr>
<tr>
<td>10</td>
<td>ON</td>
<td>OFF</td>
</tr>
<tr>
<td>100</td>
<td>OFF</td>
<td>ON</td>
</tr>
<tr>
<td>1000</td>
<td>OFF</td>
<td>OFF</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GAIN</th>
<th>SW3</th>
<th>SW4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ON</td>
<td>ON</td>
</tr>
<tr>
<td>10</td>
<td>ON</td>
<td>OFF</td>
</tr>
<tr>
<td>100</td>
<td>OFF</td>
<td>ON</td>
</tr>
<tr>
<td>1000</td>
<td>OFF</td>
<td>OFF</td>
</tr>
</tbody>
</table>
This chapter describes the interior and exterior of the miniDAWN Tristar. Read it before making any measurements to become familiar with the various parts and their functions.

Under normal operating conditions there should no need access to the internal components other than to remove the flow cell for cleaning or to set the AUX gains.

**CONTENTS**

Front Panel View ....................................................................................... 3-2
Back Panel View ....................................................................................... 3-3
Changing a Fuse .................................................................................... 3-3
Removing the Cover .................................................................................3-3
Laser ......................................................................................................... 3-5
Laser Beam Warning ............................................................................. 3-5
Laser Monitors ....................................................................................... 3-5
Read Head and Detectors ........................................................................ 3-6
Flow Cell ................................................................................................... 3-8
Flow Cell Design .................................................................................... 3-8
Refractive Index Differences—Liquid vs. Glass ..................................... 3-9
Front Panel View

The channel voltage LED display and the SELECT button for switching channels are located on the front panel. As the SELECT is pushed, the voltages are displayed for the selected channel.

Table 3-1: Channel types

<table>
<thead>
<tr>
<th>Channel Number</th>
<th>Value Displayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3</td>
<td>Detectors 1, 2 or 3 voltage</td>
</tr>
<tr>
<td>A1, A2</td>
<td>Auxiliary detectors 1 or 2 voltage</td>
</tr>
<tr>
<td>F</td>
<td>Forward laser monitor voltage</td>
</tr>
<tr>
<td>0P</td>
<td>Rear laser monitor voltage</td>
</tr>
<tr>
<td>01</td>
<td>Laser current</td>
</tr>
</tbody>
</table>

Figure 3-1: miniDawn Tristar front panel.

Figure 3-2: miniDAWN Tristar back panel.
Back Panel View

The back panel contains the Main Power switch and sockets for the Main Power, Auxiliary Input/Output and Serial Data cables. The fuse holder is located next to the Main Power switch.

The back panel also contains information about the laser. No direct laser radiation is received by the user.

The miniDAWN Tristar is configured for the AC power listed on the instrument identification label, and may be used anywhere in the world without reconfiguration.

Changing a Fuse

To change a fuse, the following are required:

• Tool for prying the AC Power module cover off.
• Fuse from the spares supplied in the accessory kit.

To replace a fuse:

1. Disconnect the power cord.
2. Pry open the AC Input Module cover with a small screwdriver or similar tool.
3. Remove the fuse and insert one of the spare fuses supplied in the accessory kit:

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Amperes</th>
<th>Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>100/240</td>
<td>0.50</td>
<td>slow</td>
</tr>
</tbody>
</table>

4. Replace the cover of the AC Power module and reconnect the power cord.

Removing the Cover

Remove the top cover to inspect the internal components of the instrument. The only other reason to access the interior would be to set the AUX gains and to remove the flow cell for cleaning.

Removing the Cover

The DAWN has access covers for the flow cell, AC power module, and Amplifier Booster board. For normal operation and maintenance, you should not need to open the top cover. If you do need to open the top cover, follow these instructions.

Tools required to remove the cover:

• 2.0 mm Ball driver
To remove the cover:

1. Place the miniDAWN Tristar on a clean open bench space with plenty of room.

2. Disconnect the power cord.
   
   All AC power circuits are protected by safety shields. The miniDAWN, however, should be unplugged from the AC power source before opening the top cover as an additional safety measure.

3. Remove the four screws at the top of the miniDAWN using the Ball driver.

4. Lift off the top cover.

*Figure 3-3: miniDAWN Tristar top view*
Laser

The 30 mW semiconductor diode laser provides the exceptional light source for the system. The laser and optical system provides a very high power density in the flow cell due to the extremely narrow beam diameter. The laser is positioned so that the incident beam is vertically polarized.

Laser Beam Warning

It is good laboratory practice with any laser source, irrespective of its power, to **AVOID LOOKING INTO THE BEAM**. Figure 3-4 shows the warning label affixed to the read head.

*Figure 3-4: Laser beam warning label*

DANGER
LASER RADIATION WHEN OPEN
AVOID DIRECT EXPOSURE TO BEAM.

Laser Monitors

Two photodiodes in the miniDAWN Tristar monitor the laser beam intensity. A beam splitter diverts a small percentage of the laser beam into the rear laser monitor photodiode before the laser beam enters the flow cell. The ASTRA software uses this laser monitor (LM) voltage to compensate for any incident laser beam power fluctuations. All scattering voltages recorded in the ASTRA software have been corrected for any laser power fluctuations based upon the laser monitor voltage.

The rear laser monitor is also an integral component in a feedback loop circuit controlling the current to the laser. If the laser intensity were to change, the rear laser monitor would detect this change and increase or decrease the current to the laser to compensate. This feedback circuit ensures the laser intensity is constant.

The forward laser monitor provides the miniDAWN Tristar with the ability to measure transmitted light through the flow cell and sample. (The software does not use this voltage in any computation). It is useful for determining if the flow path has obstructions such as air bubbles, which may reduce the signal intensity to near zero volts. It is also useful for estimating how much light is absorbed by a sample containing chromophores which may absorb at 690 nm. The forward laser monitor voltage appears on the front panel as CHANNEL F and normally reads between 1 and 5 V with filtered air-free toluene in the flow cell. The forward laser monitor voltage can be recorded and stored in the ASTRA software by setting the toggle switch on the rear of the miniDAWN Tristar to the “FWD – AUX2 position”. When the toggle switch is in the “FWD-AUX2” position the forward laser monitor voltage is recorded and stored in the ASTRA AUX2 channel.
Read Head and Detectors

Figure 3-3 shows the read head assembly installed in the miniDAWN Tristar; and Figure 3-5 shows an exploded view of the read head assembly. The read head holds the sample cell precisely, collimates scattered light and aligns and holds the detectors in place. The read head structure holds four hybrid transimpedance photodiodes (three detectors and the forward laser monitor), limits the sample field of view at each detector, and minimizes stray light effects by means of its special structure. Since each detector's field of view is limited by its own collimator, only scattered light from a very small volume (the scattering volume) in the center of the bore in the center of the flow cell is measured by the detector photodiode thus minimizing the effect of stray light scattered from the flow cell windows. The read head is attached directly to the laser diode housing providing a single optical bench structure. The optical bench is solidly attached to the instrument chassis for maximum stability.

The photodiodes are mounted securely in the read head and require a special tool for removal. The three scattering detectors are arranged as shown in Figure 3-6. Like all high performance electronics, they are susceptible to damage from electrostatic discharge (ESD) and should not be handled unless the individual is electrically grounded.

The optics have been aligned at the factory and are not user adjustable. Under no circumstances should an attempt be made to alter the alignment settings; to do so will void the warranty and may cause damage to the instrument.

There are no internal fuses or circuit breakers.
The detectors are arranged around the read head to point to the center of the cell. The baser beam passes in the same direction as the flow. The two vertical lines in the bore mark the scattering volume--less than 1 µL.
Flow Cell

Flow Cell Design

The patented flow cell is at the heart of the miniDAWN Tristar, and is critical to the instrument’s unique measuring capabilities.

In many applications, such as chromatography, the ability to measure small samples is crucial, so cell volumes must be minimal. The total volume of the cell from the manifold inlet to the manifold outlet is about 70 \( \mu \text{L} \); the volume of the glass bore is about 30 \( \mu \text{L} \). The actual scattering volume—the illuminated part of the sample that is viewed by the detectors—is less than 1 \( \mu \text{L} \).

To minimize stray light, the laser passes in the same direction as the solvent/sample flow and the cell’s windows are recessed in the manifolds, away from the scattering volume. Any stray light from the air/window/solvent interfaces is therefore normally not seen by the detectors. The windows protrude into the flowing stream at the manifolds to minimize debris buildup on their flat ends. As a result, the detectors measure scattering only from the sample and not from the windows.

Figure 3-7: Flow cell assembly

- Inlet manifold
- Outlet manifold
- Flow cell
- Bottom cell retainer
Refractive Index Differences—Liquid vs. Glass

The difference in refractive index between the solvent and the surrounding glass cylindrical cell results in some of the most important features of the flow cell design. As long as the refractive index of the solvent is less than that of the cell glass, it will be possible to obtain measurements of light scattered at relatively small angles, with minimized background contributions. Figure 3-9 shows a detail of the liquid/glass interface and rays scattering from the laser-illuminated sample.
Figure 3-9: Flow cell refractions

The angles are measured with respect to the direction of the laser beam. The illustration shows detector #1.

Applying Snell’s Law, the refraction of a ray scattering at angle $\theta$ may be determined from

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

(1)

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

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

(2)

The detectors are set to detect light at an angle $\theta'$ collimated to be centered in the cell. As a result of refraction, the light detected is the light scattered at an angle $\theta$. In this way a greater angular range of scattered light can be detected. The miniDAWN Tristar's controlling software handles these calculations automatically. The refractive index of the solvent being used must be entered into the ASTRA software.

Table 3-2: Scattering angles for the three detectors in toluene, water, and THF

<table>
<thead>
<tr>
<th>Detector Number</th>
<th>Toluene</th>
<th>Water</th>
<th>THF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48.0°</td>
<td>41.5°</td>
<td>44.7°</td>
</tr>
<tr>
<td>2</td>
<td>90.0°</td>
<td>90.0°</td>
<td>90.0°</td>
</tr>
<tr>
<td>3</td>
<td>132.0°</td>
<td>138.5°</td>
<td>135.3°</td>
</tr>
</tbody>
</table>
The DAWN photometer requires little maintenance. When you remove parts for cleaning (or convert between flow and batch modes), you will find they are easy to access and disassemble. This chapter gives guidelines for keeping the instrument clean and in good working order. It also has the procedure for converting from the flow cell to Batch mode measurements with scintillation vials.

### CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Maintenance</td>
<td>4-2</td>
</tr>
<tr>
<td>Flow Cell Maintenance</td>
<td>4-2</td>
</tr>
<tr>
<td>On-line Cleaning</td>
<td>4-2</td>
</tr>
<tr>
<td>Particles in the Cell</td>
<td>4-3</td>
</tr>
<tr>
<td>Cleaning the Flow Cell and Windows</td>
<td>4-4</td>
</tr>
<tr>
<td>Step 1—Removing the Flow Cell Assembly</td>
<td>4-4</td>
</tr>
<tr>
<td>Step 2—Disassembling the Flow Cell</td>
<td>4-5</td>
</tr>
<tr>
<td>Step 3—Cleaning the Flow Cell and Windows</td>
<td>4-7</td>
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<tr>
<td>Step 4—Reassembling the Flow Cell</td>
<td>4-9</td>
</tr>
<tr>
<td>Step 5—Reinstalling the Flow Cell</td>
<td>4-11</td>
</tr>
</tbody>
</table>
### General Maintenance

General operation and maintenance guidelines for keeping the miniDAWN Tristar in good working order:

- Keep the miniDAWN Tristar on a flat, clean surface, with space behind it and standing on its feet to allow proper air ventilation.
- Keep the case clean. Use a cloth dampened with water to clean it.
- Allow the instrument and its laser to warm up for at least 30 minutes before taking measurements.
- Keep the top cover on the miniDAWN at all times.
- Keep the inlet and outlet sealed when not in use to prevent solvent evaporation or introduction of particles.

The next section describes procedures to help keep the flow cell clean.

---

**Note:** For instructions on connecting the miniDAWN Tristar to an HPLC system, see the *ASTRA for Windows User's Guide.*

### Flow Cell Maintenance

The quality of the light scattering results obtained from the miniDAWN Tristar depends critically on the cleanliness of the flow cell. If the following guidelines are followed, the flow cell should rarely need to be disassembled for cleaning.

Under most circumstances, the cell can be kept clean from contaminants using the following on-line cleaning methods. Eventually, most cells need to be removed from the read head and disassembled for cleaning of the individual parts (described in the next section).

#### On-line Cleaning

To keep the flow cell free of particles, the following regular maintenance is suggested:

**At All Times**

- Use well filtered solvents (including water) that are of HPLC grade.
- If the instrument is connected to a chromatography system, keep clean solvent pumping continuously through the cell.
- If the instrument is in stand-alone mode (batch setup), keep the cell filled with filtered solvent (such as ethanol).
- When the miniDAWN Tristar will not be used for long periods of time (weeks or months) fill the flow cell with pure, filtered solvent (such as ethanol) and plug the inlet and the outlet. Periodically check to ensure the cell still contains solvent. Add more filtered solvent as needed.
Before and After Completing Experiments

- With the flow cell still in place, disconnect the miniDAWN Tristar from the HPLC system. Inject a compatible, filtered (0.02 µm) solvent to flush the cell. Filtered ethanol or isopropanol may be left in the cell.

- Do not flush the cell from OUTLET to INLET since the inlet uses 0.005” ID tubing which is easily blocked.

- Depending on the type of sample analyzed and the solvent used, 6M nitric acid may be used to flush the cell before and after completing an experiment.

- 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 flushed with pure solvent in the morning.

Particles in the Cell

The following is a list of symptoms of particles in the cell and what can done to dislodge them.

Some Symptoms of Particles in the Cell

- Bright stationary spots when viewing the cell bore with the laser on.
- An increase in baseline voltage at all angles.
- Unstable, fluctuating baselines.
- Distorted chromatography peaks: Dips below baseline, shoulders on low angle peaks. The ASTRA for Windows User’s Guide has a more thorough description of these symptoms.

Some Suggestions for how to Dislodge Particles

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

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

- Step 1—Removing the flow cell
- Step 2—Disassembling the flow cell
- Step 3—Cleaning the flow cell and windows
- Step 4—Reassembling the flow cell
- Step 5—Reinstalling the flow cell

For flow cell cleaning the following are required:

- A sheet of clean white printer paper taped down to the work surface.
- Anti-static wrist strap.
- Ball drivers: 1.5 mm, 2.5 mm and 4 mm.
- 2 open end wrenches, 1/2” and 3/8”.
- Lens tissue. Fold several pieces in finger-width strips for handling and cleaning.
- Lint-free gloves.
- Oral-B SuperFloss.
- Inert dusting gas. (Photographic supply stores carry this. At Wyatt Technology we use "Tech Spray" from Com-Kyl distributors in Santa Barbara, (805) 520-1731.)
- Filtered methanol, ethanol, or isopropanol.
- Tweezers.
- Optional: Sonicating bath.

---

**Caution:** The flow cell constitutes a significant portion of the purchase price of the miniDAWN Tristar. Its parts are carefully machined and are expensive. If in doubt whatsoever about the safest procedure for handling the cell structure, do not hesitate to call Wyatt Technology. If preferred, the flow cell may be shipped to Wyatt Technology for cleaning.

---

**Step 1—Removing the Flow Cell Assembly**

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

1. Put on the anti-static wrist strap.
   
   This is an important first step. The strap keeps the flow cell glass parts from building up a static charge and attracting particles while being handled.

2. Disconnect the power cord and remove the cover of the instrument (see “Removing the Cover” on page 3-3).
Looking down at the read head, see the two halves of the read head cover plate (on which the laser warning label is affixed) held by four Allen-head screws.

3. Use the 2.5 mm Ball driver to remove the four M3 screws, then lift off both sections of the read head cover plate.

The flow cell assembly is now visible.

4. Use the 2.5 mm Ball driver to remove the two M3 screws.

5. Use the two open end wrenches to slightly loosen the two bulk head unions, then lift the cell assembly (including tubing and the side plate) up and out of the read head. See Figure 4-1.

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

6. Carefully remove the two connecting tubing from the flow cell taking care not to twist or bend them. Leave the other side of the tubing connected to the side plate.

*Figure 4-1: Removing the flow cell and side plate from the read head*

---

**Step 2—Disassembling the Flow Cell**

The flow cell assembly has 22 separate pieces, as shown in Figure 4-2.

1. Separate the stainless steel manifolds from the flow cell:
   a. Use the 1.5 mm Ball driver to unscrew the two M2 screws holding the bottom cell retainer in place. Remove the bottom cell retainer, taking care not to lose the two tiny screws and the bottom retainer O-ring and cord.

   b. Use the 2.5 mm Ball driver to remove the M3 screws that connect the two manifolds.
c. Gently pull apart the manifolds, taking care not to drop the glass cell. Try to avoid touching the curved surfaces of the glass cell.

d. Place everything on the paper-covered work surface.

2. Use the special Installation Tool (119033) to remove one window retainer at a time. Some flow cells may require a 4 mm Ball driver to remove the window retainers.

Figure 4-6 illustrates the window-mount and how it is housed in the manifold.

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

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

4. Repeat Step 3 for the other window.

*Figure 4-2: Flow cell assembly, exploded view*
Step 3—Cleaning the Flow Cell and Windows

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

**Tip:** For more thorough cleaning of the optical parts (glass cell and windows), we suggest the use of an ultrasonic cleaning unit. If used, place the parts in a small beaker and cover with filtered alcohol. Fill the ultrasonic unit with enough water to reach part way up the side of the beaker. Place the beaker in the unit and sonicate the parts for about five minutes. Let the flow cell dry on a piece of lens tissue with the bore in a vertical position.

1. Clean your hands thoroughly or wear lint-free gloves.
   Once disassembled, be careful not to handle the glass cell’s curved optical surfaces (the sides) of the cell.

2. Clean the cell through-bore.
   a. Cut a ⅛" strip of lens tissue and roll it into a thin wick or, use “Oral-B SuperFloss” available in most pharmacies.
   b. Insert the wick all the way through the cell bore, then moisten it with a small amount of filtered alcohol.
   c. While the wick is in the cell bore, untwist it slightly, move it back and forth to clean the cell, then pull it out.
   d. Immediately flush the bore with a stream of alcohol for 10–15 seconds.
      The alcohol stream flushes out any fibers that may have been left behind by the tissue wick.

---

Table 4-1: Flow cell assembly, parts list

<table>
<thead>
<tr>
<th>Item</th>
<th>P/N</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S5002-3004</td>
<td>M3 screw</td>
</tr>
<tr>
<td>2</td>
<td>200694</td>
<td>Manifold, out</td>
</tr>
<tr>
<td>3</td>
<td>P6504-2006</td>
<td>Flow cell O-ring (2)</td>
</tr>
<tr>
<td>4</td>
<td>200609</td>
<td>Backing ring (2)</td>
</tr>
<tr>
<td>5</td>
<td>212095</td>
<td>Flow cell</td>
</tr>
<tr>
<td>6</td>
<td>200690</td>
<td>Manifold, in</td>
</tr>
<tr>
<td>7</td>
<td>P6504-2004</td>
<td>Window O-ring (2)</td>
</tr>
<tr>
<td>8</td>
<td>116007</td>
<td>Flow cell window (2)</td>
</tr>
<tr>
<td>9</td>
<td>212073</td>
<td>Window retainer (2)</td>
</tr>
<tr>
<td>10</td>
<td>S5002-3030</td>
<td>M3 screw</td>
</tr>
<tr>
<td>11</td>
<td>211048</td>
<td>Bottom flow cell retainer</td>
</tr>
<tr>
<td>12</td>
<td>S6501</td>
<td>Bottom retainer O-ring cord</td>
</tr>
<tr>
<td>13</td>
<td>S5002-2006</td>
<td>M2 screw (2)</td>
</tr>
</tbody>
</table>
e. Blow out the alcohol for 10–15 seconds with inert dusting gas or let the glass bore drain in a vertical position.

f. Examine the bore with a magnifying loupe. (See the note on page 4-9.)

3. Clean the outside of the cell. (See Figure 4-3.)
   a. Pick up the cell with a folded lens tissue; touch only the flat surfaces.
   b. Wipe the curved optical surfaces with another folded lens tissue moistened with alcohol.
   c. If needed, wipe the alcohol off the optical surfaces with dry lens tissue. **Do not** repetitively rub the surfaces since this creates static electricity which attracts particles.
   d. Using a magnifying loupe, examine the optical surfaces for any dust. (See the note on page 4-9.)
   e. Also, check the bottom and top surfaces for dust and finger marks.

*Figure 4-3: Flow cell glass*

4. Clean the window faces.
   This is the most important step in cell cleaning. Even the smallest particle left on the window faces will induce stray light and cause signal distortion, especially at low angles.
   a. Take a folded lens tissue moistened with a couple of drops of alcohol and hold it against the side of your forefinger with your thumb and third finger.
   b. Pick up the window with the tweezers.
   c. Smoothly wipe both window faces across the tissue.
   d. Carefully examine both ends of the cleaned window for particles. With the loupe look straight through the window from end-to-end. (See the note on page 4-9.)
This tiny glass part is specially manufactured and is expensive.

**Note:** By examining the flow cell through-bore and the windows using a bright light one can, with some practice, easily find where any residue has accumulated. Examine them with a jeweler’s loupe while back-lighting the glass at a slight angle. The area next to the light should be dark to provide good contrast. The bright light will illuminate any particles on the glass which, when viewed against the dark background, will show up clearly. Since fingerprints on the glass cell circumference will alter the light scattering characteristics of a sample significantly, we urge great care when handling the cell. Its role is vital in the measurement process and must be wiped clean with high quality lens tissue before it is inserted in the cell assembly.

**Step 4—Reassembling the Flow Cell**

Before reassembling the flow cell clean the washers and O-rings.

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

1. Carefully replace the windows with their O-rings, washers and retainers so that the windows are not chipped or over-tightened. (See Figure 4-6.)
   a. Holding the window O-ring with the tweezers, put a drop of alcohol on it, then dry with a burst of pressurized air. Check for particles with the loupe.
   b. Insert the O-ring into the manifold.
   c. Lift the window with the tweezers (pick up the window near its back edge as shown in Figure 4-5.)

**Figure 4-5: Tweezers orientation for window insertion.**
d. Holding the manifold and window square with one another, gently push the window into the O-ring.

e. Let go of the window with the tweezers, pivoting them before you lift them out of the manifold.

   The fit is tight enough that the window could be dislodged if lifted straight up with the tweezers.

f. Inspect the special Installation Tool (or the 4 mm Ball driver) with the loupe for any particles, and, if necessary, clean with an alcohol-moistened lens tissue before proceeding.

g. Clean the window retainer with alcohol and pressurized air, then place it in the manifold and tighten with the 4 mm Ball driver.

   You may need to use your fingers to start the tightening of the retainer.

h. Inspect the window mount with the loupe.

   If any particles appear on the window, you need to remove it and its seals and clean again.

i. Repeat steps 1a) through 1i) for the second window.

Figure 4-6: Window mount detail

2. Install the cell in the manifolds.

   a. Insert the cell O-rings followed by the backing rings if they were removed during disassembly.

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

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

   c. Push the cell step against the manifold pins.
d. Make sure that the glass step and manifold pins are matched up well.

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

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

4. View the O-rings through the bottom glass surface (make sure the surface is clean) and confirm that the bore is centered in each O-ring. Also examine the alignment pins to make sure they touch the cell glass on each side.

5. Inspect the sides of the cell and apply a burst of air if you see any particles.

6. Replace the bottom cell retainer cord and O-ring and attach the bottom cell retainer to the manifolds using the 1.5 mm Ball driver.

**Step 5—Reinstalling the Flow Cell**

1. Reattach the inlet and outlet tubing to the cell (the side plate should still be attached to the tubing)

   If you are not careful, the cell could be reversed. Make sure that the manifold labels (IN and OUT) match the labels on the side plate. See Figure 4-1.

   After several cleanings the fittings may become worn or deformed, at which point the tubing and fittings should be replaced. The instrument is shipped with one spare set: The inlet tube (0.005” ID) is marked with a red label.

2. Replace the cell assembly in the read head while at the same time sliding the side plate into place (see Figure 4-1). Tighten the two bulk head unions using the two open end wrenches. Insert the two M3 screws into the top of the cell and tighten with the 2.5 mm Ball driver.

3. Plug in the power cord and turn on the miniDAWN Tristar.

4. Ascertain that the cell does not leak.

   Make certain the fittings are tight and leak free. Whenever solvent is pumped 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.

5. Replace both sections of the read head cover plate, insert the four M3 screws and tighten with the 2.5 mm Ball driver.

6. Replace the instrument cover.
This appendix lists recommended accessories for various light scattering applications. The WTC kits can be purchased directly from Wyatt Technology; other accessory parts can be purchased from the listed vendors.

**CONTENTS**

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wyatt Technology Kits</td>
<td>A-2</td>
</tr>
<tr>
<td>Accessory Parts</td>
<td>A-2</td>
</tr>
<tr>
<td>Syringe Filters</td>
<td>A-2</td>
</tr>
<tr>
<td>Syringes</td>
<td>A-2</td>
</tr>
<tr>
<td>Accessories for Microbatch Work with a Syringe</td>
<td>A-3</td>
</tr>
<tr>
<td>GPC In-Line High Pressure Filter</td>
<td>A-3</td>
</tr>
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Wyatt Technology Kits

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
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<tr>
<td>Cell Cleaning Kit</td>
<td>Tweezers, lens tissue, dusting gas, wrist strap</td>
<td>900001</td>
</tr>
<tr>
<td>Filter Kit A</td>
<td>In-line filter holder, fittings, filter membranes (aqueous or organic)</td>
<td>900002</td>
</tr>
<tr>
<td>Filter Kit B</td>
<td>Syringes, syringe filters (0.02 µm), fittings, PEEK tubing</td>
<td>900003</td>
</tr>
<tr>
<td>DNDC Kit</td>
<td>Injection valve, 1 mL injection loop, syringes, PEEK tubing, fittings</td>
<td>900004</td>
</tr>
</tbody>
</table>

Accessory Parts

The following accessories can be used with the miniDAWN Tristar. Contact the following vendors for the listed parts:

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<thead>
<tr>
<th>Vendors</th>
<th>Phone Number</th>
</tr>
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<tbody>
<tr>
<td>Alltech</td>
<td>800-255-8324</td>
</tr>
<tr>
<td>Millipore</td>
<td>800-645-5476</td>
</tr>
<tr>
<td>Upchurch</td>
<td>800-426-0191</td>
</tr>
<tr>
<td>VWR</td>
<td>800-932-5000</td>
</tr>
<tr>
<td>Air-Tite of Virginia</td>
<td>800-231-7762</td>
</tr>
<tr>
<td>RAZEL Scientific Instruments</td>
<td>203-324-9914</td>
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</table>

Syringe Filters

Inorganic media, good for aqueous or organic solvents.

<table>
<thead>
<tr>
<th>Name</th>
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<th>Alltech Part Number</th>
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<tr>
<td>Whatman Anotop10</td>
<td>10</td>
<td>0.02</td>
<td>2172</td>
</tr>
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<tr>
<td>Whatman Anotop25</td>
<td>25</td>
<td>0.20</td>
<td>2130</td>
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Syringes

10 mL, all polymer (good for organic and aqueous solvents)

<table>
<thead>
<tr>
<th>Importer</th>
<th>Available from VWR</th>
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<tr>
<td>Air-Tite of Virginia, Inc.</td>
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**Accessories for Microbatch Work with a Syringe**

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<tr>
<td>Female Luer adapter</td>
<td>from Luer to 10-32 threads</td>
<td>P-642</td>
</tr>
<tr>
<td>Finger Tight fittings, extra long</td>
<td>For 1/16” tubing, 10-32 threads</td>
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</tr>
<tr>
<td>PEEK tubing</td>
<td>0.010” x 1/16” x 5’</td>
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<tr>
<td>PEEK tubing</td>
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<tr>
<td>RAZEL syringe pump</td>
<td>Low cost basic pump</td>
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**GPC In-Line High Pressure Filter**

For 1/16” Lines

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<td>Stainless Steel</td>
<td></td>
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<td></td>
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<tr>
<td>Diameter = 25 mm</td>
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<td></td>
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<tr>
<td>Anodisc inorganic membrane filter</td>
<td>Alltech</td>
<td>2250 2268 2255</td>
<td>50/pk</td>
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<tr>
<td>Pore sizes 0.2, 0.1, or 0.02 µm</td>
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<tr>
<td>Diameter = 25 mm</td>
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<td></td>
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<tr>
<td>Durapore* membrane filter</td>
<td>Millipore</td>
<td>VVLP 025 00</td>
<td>100/pk</td>
</tr>
<tr>
<td>Pore size = 0.10 µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter = 25 mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF** membrane filter</td>
<td>Millipore</td>
<td>VSWP 025 00</td>
<td>100/pk</td>
</tr>
<tr>
<td>Pore size = 0.025 µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter = 25 mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stainless Steel fitting</td>
<td>Alltech</td>
<td>61038</td>
<td>1 each</td>
</tr>
<tr>
<td>1/8” to 1/8” MPT</td>
<td></td>
<td>(2 required)</td>
<td></td>
</tr>
<tr>
<td>Reducing ferrules</td>
<td>Alltech</td>
<td>RF-200/100T</td>
<td>10/pk</td>
</tr>
<tr>
<td>Teflon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/8” to 1/16”</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Hydrophilic Durapore - Aqueous and selected organic solvents (Toluene, THF, etc)

** MF Mixed Cellulose - Aqueous and selected organic solvents (Toluene, THF, etc)
The miniDAWN contains a GaAs laser operating at a nominal wavelength of 685nm. The GaAs laser is a single transverse mode heterojunction that emits light between 680nm and 690nm, where the exact wavelength varies from device to device. 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%.

**Contents**

<table>
<thead>
<tr>
<th>Electrical and Optical Specifications</th>
<th>B-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental Specifications and Safety Notes</td>
<td>B-2</td>
</tr>
</tbody>
</table>
Appendix B: Laser Specifications

**Electrical and Optical Specifications**

*Table B-1: Electrical and optical specifications*

<table>
<thead>
<tr>
<th>Specification</th>
<th>GaAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power Output</td>
<td>30 mW</td>
</tr>
<tr>
<td>Laser Operating Wavelength</td>
<td>680 nm – 690 nm</td>
</tr>
<tr>
<td>Vertical Beam $1.0/e^2$ Intensity Diameter</td>
<td>80 µm</td>
</tr>
<tr>
<td>Horizontal Beam $1.0/e^2$ Intensity Diameter</td>
<td>52 µm</td>
</tr>
<tr>
<td>Polarization Ratio</td>
<td>&gt; 100:1</td>
</tr>
<tr>
<td>Max Power Stability</td>
<td>&lt; 0.5%</td>
</tr>
<tr>
<td>Typical Optical Noise</td>
<td>0.1%</td>
</tr>
<tr>
<td>Typical Operating Voltage</td>
<td>2.4 VDC</td>
</tr>
<tr>
<td>Typical Operating Current</td>
<td>85 mA</td>
</tr>
</tbody>
</table>

**Environmental Specifications and Safety Notes**

*Table B-2: Environmental specifications*

<table>
<thead>
<tr>
<th>Specification</th>
<th>GaAs Operating</th>
<th>GaAs Non-Operating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>-10 to +60 °C</td>
<td>-40 to +85 °C</td>
</tr>
<tr>
<td>Relative Humidity</td>
<td>0-95%</td>
<td>0-95%</td>
</tr>
<tr>
<td>Shock</td>
<td>1500 G – 0.5 ms</td>
<td>1500 G – 0.5 ms</td>
</tr>
</tbody>
</table>

The lasers used in the miniDAWN are classified as Class 1 Laser Product according to IEC60825-1:1993+A1+A2. This means that under normal operation, no laser radiation should escape from the instrument, and no protective equipment must be worn. However the follow warning applies:

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

**A**  
accessory parts A-2  
autoinjector 2-7  
AUX 1-2  
cabling 2-5, 2-7  
auxiliary  
connectors 2-7  
device 2-7  
removing the assembly 4-4  
volume 3-8  
window 4-7  
front panel 3-2  
fuse  
location 3-3  
fuses 3-3  
replacing 3-3  

**B**  
back panel 3-3  
bib on cover 3-3  

**C**  
calibration 2-5  
cell window 4-6  
changing a fuse 3-3  
channels 3-5  
display 3-2  
cleaning  
flow cell and windows 4-4  
flow cell on-line 4-2  
communications, Internet 1-5  
concentration detector 2-7  
connectors 3-3  
auxiliary 2-7  
cover 3-3  
removing 3-3  
customer service 1-5  

**D**  
detector  
concentration detector 2-7  
position 3-6  
DIP switch 2-7  
dust, detecting 4-9  

**E**  
equipment list 2-2  

**F**  
flow cell 3-8  
cleaning 4-4  
maintenance 4-2  
particles 4-3  
refractive index 3-9  

**H**  
help  
technical support 1-5  
where to get 1-5  
high temperature option A-2  

**I**  
jeweler’s loupe 4-8, 4-9  

**J**  
laser  
description 3-5  
forward monitor 2-4, 3-5  
monitors 3-5  
specifications B-2  
warning 3-5  

**M**  
maintenance  
flow cell 4-2  
general 4-2  
manifold 4-5, 4-11  
manual conventions 1-4  

**O**  
options  
Wyatt Technology Kits A-2  
output  
auxiliary detector 2-7  
see serial port 1-2
overview 1-2

P
packing list 2-2
particles
dislodging 4-3
parts list 2-2
photodiode 1-2, 3-5, 3-6
power
cord 2-4
switch 2-4, 3-3

R
read head 3-6
refractive index 3-9
removing flow cell 4-4
temperature controlled A-2
removing the cover 3-3
RS-232 see serial port 1-2

S
serial number location 1-6
serial port
cabling 2-4
connecting 2-4
input/output 1-2
shipping list 2-2
Snell's Law 3-10
software
ASTRA for Windows 1-3
specifications
laser B-2
Syringe A-2

T
technical support 1-5
temperature control 1-3
option A-2

U
Ultra-High Temperature option A-2
unpacking the instrument 2-2
UV light 4-9

V
ventilation 4-2
voltage 3-3
volume of flow cell 3-8

W
warm up time 2-4
warning
laser 3-5
window 1-2
removing 4-6
retainer 4-6, 4-9

wrist strap 4-4
Wyatt Technology Corporation
corporate headquarters 1-5
Internet 1-5
sales department 1-5
technical support 1-5