
Optilab T-rEX User's Guide

(M1510 Rev. A)



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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.

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About the Optilab T-rEX

This chapter provides a general introduction to the Optilab T-rEX instrument and an overview of the sections in this manual.

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1.1 Introduction to the Optilab T-rEX

The Optilab T-rEX (refractometer with EXtended range) is a second generation refractometer that utilizes the pioneering design first used by Wyatt Technology's Optilab rEX. The Optilab T-rEX is a deflection-based instrument that uses an array of light sensitive detectors in the measurement of dRI. It has a linear array of 512 photodiodes, rather than the traditional two photodiodes used by other deflection-based instruments. This photodiode array allows for unprecedented sensitivity and range.

With most RI instruments, users must choose a gain setting, which essentially means choosing between good sensitivity over a limited range, reduced sensitivity over a large range, or some compromise between the two. The Optilab T-rEX has no gain settings; the maximum sensitivity exists over the entire range. Tiny signals and huge signals may be measured within the same data run. The ability to see both large and small signals may be quantified by the instrument's dynamic range (defined as range divided by noise). The dynamic range of the Optilab T-rEX is 6.27 million, literally orders of magnitude greater than any other RI instrument on the market today.

In addition to the photodiode array, the Optilab T-rEX contains a flow cell design that allows the measurement of a fluid's absolute Refractive Index (aRI) in addition to the dRI between two fluids. The aRI of a fluid is vital for characterizing a system and is a necessary input parameter for light scattering measurements that determine the molecular mass of molecules in solution.

1.2 Using This Manual

The organization of this manual is as follows:

- **Chapter 2, “Instrument Description”:** Describes the Optilab T-rEX instrument’s control interfaces and connections.
- **Chapter 3, “Quick Start”:** Contains a summary of instructions for setting up the Optilab T-rEX quickly and with minimal instruction.
- **Chapter 4, “Installation and Setup”:** Provides detailed connection information for installation and setup.
- **Chapter 5, “Using the LCD Display”:** Provides detailed information on how to use the tabs on the LCD display to control, configure, and monitor the Optilab T-rEX.
- **Chapter 6, “Off-Line Measurements”:** Describes how to make static measurements with the Optilab T-rEX, including instrument calibration, off-line dn/dc measurements, and absolute refractive index measurements.
- **Chapter 7, “In-Line HPLC Detection”:** Describes using the Optilab T-rEX as an in-line concentration sensitive detector for HPLC.
- **Chapter 8, “Service and Maintenance”:** Provides maintenance and service instructions.
- **Chapter 9, “Troubleshooting”:** Provides troubleshooting tips.
- **Appendix A, “Acronyms and Abbreviations List”:** Contains a list of acronyms and abbreviations used in this manual.
- **Appendix B, “Operating Principles and Theory”:** Provides a basic and theoretical description of how the Optilab T-rEX operates.
- **Appendix C, “Optilab T-rEX Specifications”:** Provides the specifications of the Optilab T-rEX.
- **Appendix D, “Instrument Connectivity”:** Provides details about connecting the Optilab T-rEX to a computer.
- **Appendix E, “Warnings”:** Lists warnings to be aware of when using the Optilab T-rEX.
- **Index:** Provides lookup assistance.

1.3 Contacting Wyatt Technology

We solicit and encourage questions and comments about this manual and the Optilab T-rEX instrument. Please contact:

Technical Support Department
Wyatt Technology Corporation
6300 Hollister Avenue
Santa Barbara, CA 93117

Telephone: (805) 681-9009
FAX: (805) 681-0123
E-mail: support@wyatt.com

2

Instrument Description

This chapter provides an overview of the Optilab T-rEX features.

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2.1 Overview

The Optilab T-rEX measures the dRI between the current liquid sample stream and a liquid (usually clean solvent) previously stored as a reference. The dRI is measured at a fixed wavelength determined by a Light Emitting Diode (LED)/fiber light source. The light source consists of a high-powered LED coupled to a special optical fiber. This “pigtail” configuration allows you to easily replace the light source. If you want to change the measurement wavelength, installing a “pigtail” with a different wavelength accomplishes this.

The dRI data can be read from the LCD display, the analog output channel, or digitally through the Ethernet port. Since the dRI of liquids depends critically on temperature, the instrument has a system that precisely regulates the temperature of both the flow cell and the optics.

In addition to the dRI between two liquids, with the same liquid present in the sample and reference chambers, the Optilab T-rEX measures the aRI of that liquid.

For a more detailed theoretical description, see **Appendix B, “Operating Principles and Theory”**.

2.2 Front Panel

The front panel, see Figure 2-1, provides fluid connections for the Optilab T-rEX and all of the necessary functions and displays for operating the instrument and monitoring data.

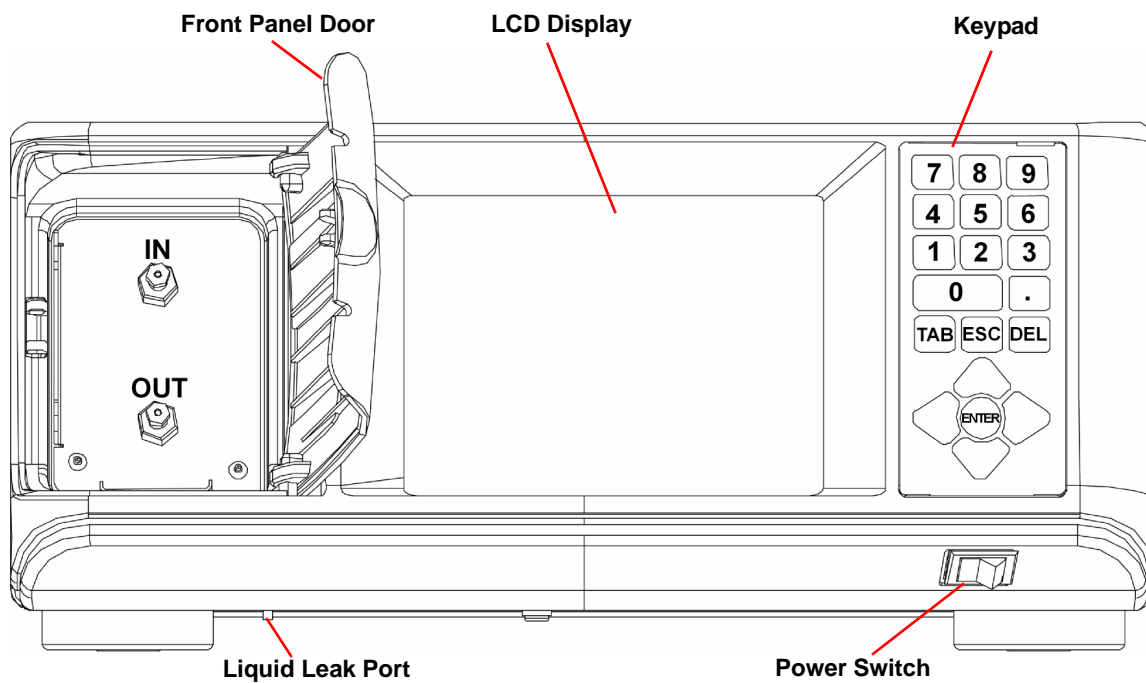


Figure 2-1: Front panel

Front Panel Door: The front panel door opens to provide access to the IN and OUT fluid connections for the Optilab T-rEX.

LCD Display: The LCD display provides a full-color, high-resolution user interface to the Optilab T-rEX. It allows you to monitor, control and configure the instrument. **Chapter 5, “Using the LCD Display”** describes all of the tabs available on the LCD display and their functions.

Keypad: The keypad allows you to control the LCD display. “Navigating the Optilab T-rEX Tabs” on page 5-2 describes how to use the keypad to navigate through the LCD display tabs.

IN/OUT Fluid Connectors: Fluid comes into the Optilab T-rEX through the IN port, and exits through the OUT port. The Optilab T-rEX should be connected *last* in line if other detectors are connected in series.

Liquid Leak Port: The liquid leak port provides an exit for any liquid that leaks from internal or external fittings. The Teflon tubing provided may be fit over this tubing and directed to a fluid waste bottle. When a liquid leak occurs, an internal liquid sensor activates an alarm shown on the LCD display (see “Alarms Tab” on page 5-7), and activates an Alarm Out signal that may be used to shut off a pump.

Power Switch: The power switch turns instrument power On and Off.

2.3 Rear Panel

The rear panel, see Figure 2-2, provides access for power, signal connections, the cooling fan, air filter, the exhaust fan, and a nitrogen purge port.

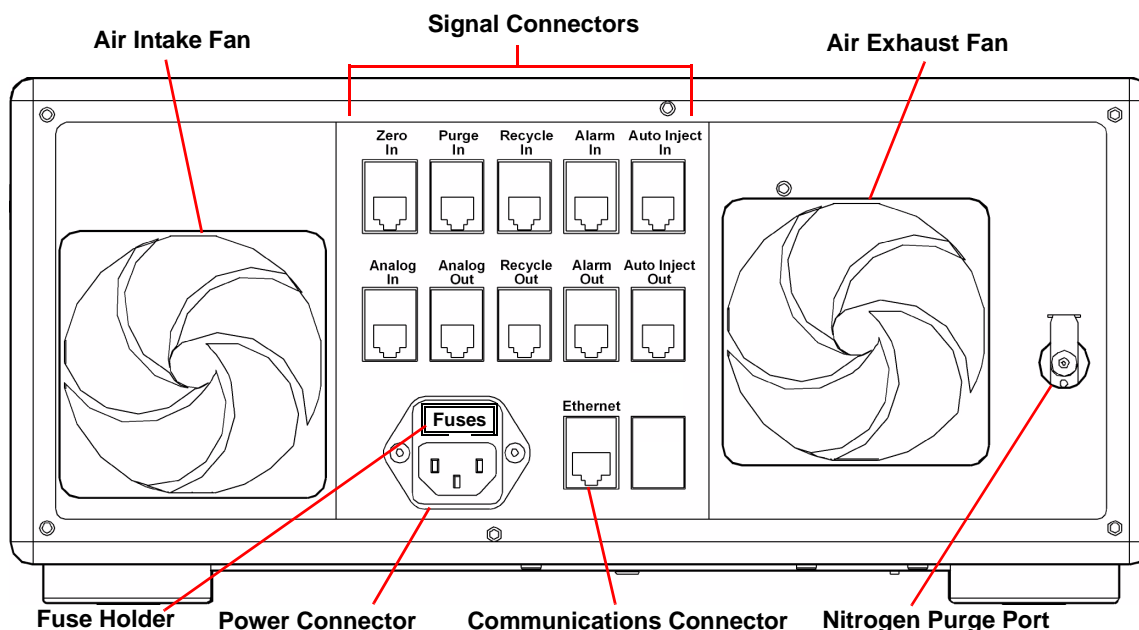


Figure 2-2: Rear panel

Air Intake and Exhaust Fans: The fans help to keep the instrument electronics at a constant temperature.

Signal Connectors: There are 10 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 “Power and Signal Connections” on page 4-2.

Power Connector: Provides power to the instrument. See “Power and Signal Connections” on page 4-2 for a complete description of the Optilab T-rEX’s power requirements.

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

Nitrogen Purge Port: Provides the connection to purge the instrument with dry nitrogen in order to prevent condensation on the optical and measurement systems. For detailed information regarding the nitrogen purge port, see “Dry Nitrogen Connection” on page 4-8.

3

Quick Start

This chapter provides rudimentary setup procedures for first-time or experienced users who want to start the Optilab T-rEX installation process before reading the entire manual.

Detailed descriptions and important use instructions concerning the Optilab T-rEX are provided in the chapters that follow.

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3.1 Setting Up the Optilab T-rEX

This section describes basic setup procedures for those who want to begin using the Optilab T-rEX as soon as possible.

More details about setting up the Optilab T-rEX are provided in **Chapter 4, “Installation and Setup”**. (You may notice that the setup sequence in this chapter differs a bit from that in Chapter 4. The sequence in this chapter allows you to begin stabilizing the temperature earlier in the sequence.)

See **Chapter 2, “Instrument Description”** for descriptions of the front and rear panel controls and connections.

For instructions on navigating the LCD display tabs, see “Navigating the Optilab T-rEX Tabs” on page 5-2.

Note:	The Optilab T-rEX can be safely stacked above and below other instruments.
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1. **Connect the power cable.** The power outlet is located on the Optilab T-rEX rear panel (see Figure 2-2). The power supply is universal, for immediate use with 100–120 V or 220–240 V power at 50-60 Hz. (See also “Power and Signal Connections” on page 4-2.)
2. **Power ON the Optilab T-rEX.** Time to full warm-up is approximately 30 minutes. (See also “Power On and Warm Up” on page 4-9.)
3. **Set temperature.** The Optilab T-rEX default temperature is 25° C, with a range of 4° C to 50° C. (If you are operating below 25° C, it is important that you see “Dry Nitrogen Connection” on page 4-8 before cooling the instrument.)
4. **Make fluid connections.** Standard HPLC fittings: 1/16 in. OD tubing, 10-32 threads. The Optilab T-rEX should be connected *last* in line if other detectors are to be connected in series. (See also “Fluid Connections” on page 4-7.) The IN and OUT ports are located behind a door on the front panel (see Figure 2-1).

Tubing recommendations are:

- 0.010 in. (0.254 mm) ID to the Optilab T-rEX IN port.
 - 0.020 in. (0.508 mm) or larger ID from the Optilab T-rEX OUT port to a waste bottle or solvent recycle valve.
5. **Flush the Optilab T-rEX in purge mode.** Use 20 mL of co-miscible solvents stepwise as necessary to prepare the cell for data collection. (See also “Purging the Optilab T-rEX” on page 4-9.) Tab to the **Purge On/Off** button using the front panel arrow keys (see Figure 2-1).

The Optilab T-rEX is shipped with Ethanol in the flow cell. A typical series of solvents (from polar to nonpolar) is shown below. Salt solutions should be considered separate steps from pure solvents.

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

6. **Zero the instrument.** Activate the Zero selection on the Optilab T-rEX LCD display. (See also “Main Tab” on page 5-3)
7. **Install software.**
 - a. For in-line use with chromatography, install ASTRA or equivalent chromatography software. See the appropriate software manual for installation instructions.
 - b. For off-line use (e.g., dn/dc determinations), install ASTRA V software. See the appropriate software manual for installation instructions.
8. **Connect communication cable.** (See also “Power and Signal Connections” on page 4-2.)
 - a. If data is to be collected using the ASTRA V software, connect the Optilab T-rEX directly to a computer or local area network (LAN) using the Ethernet cable provided.
 - b. If data is to be collected using ASTRA 4.X, DNDC, or other software, connect the Optilab T-rEX analog output to a DAWN or miniDAWN instrument’s analog input AUX1 port using the analog cable provided.

4

Installation and Setup

This chapter describes the fluid, power, and signal connections used in setting up the Optilab T-rEX.

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4.1 Power and Signal Connections

All power and signal connections to the Optilab T-rEX are made using the interfaces on the rear panel of the instrument. Refer to Figure 4-1 to view the connectors on the rear panel of the Optilab T-rEX.

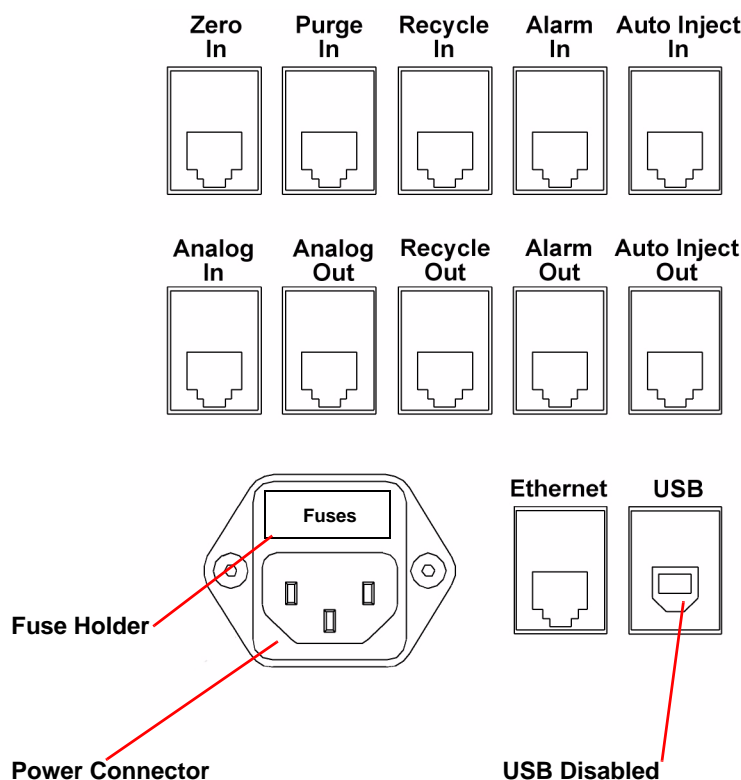


Figure 4-1: Rear panel power and signal connections

4.1.1 Power Connector

The power input accepts 100–250 VAC at 50–60 Hz. The power input module contains two fuses, each rated at 4 Amps: one for the line voltage in, and the other for the line voltage return.

4.1.2 Signal Connectors

The instrument rear panel contains 10 RJ-12 connectors for TTL inputs and outputs, analog input and output, auto inject input and output, and a solenoid drive output.

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 4-2 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 4-2.

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

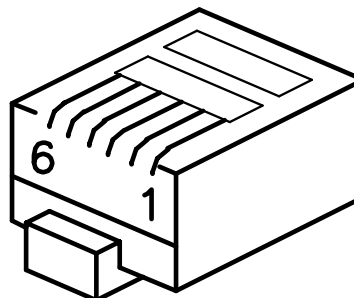


Figure 4-2: RJ-12 Connector and Pinouts

Several general-purpose cables are supplied for TTL inputs and outputs, analog inputs and outputs, auto inject input and output, and solenoid drive output.

Earlier revisions of the general-purpose cables have different wire colors than those listed in Figure 4-2. If your Wyatt Technology supplied general-purpose cables do not have the same colors listed in Figure 4-2, refer to the wire colors listed in Appendix D, “Wire Colors”.



Figure 4-3: General-purpose cable

An analog out cable is supplied to connect to the Optilab T-rEX analog output to a DAWN or miniDAWN AUX analog input.

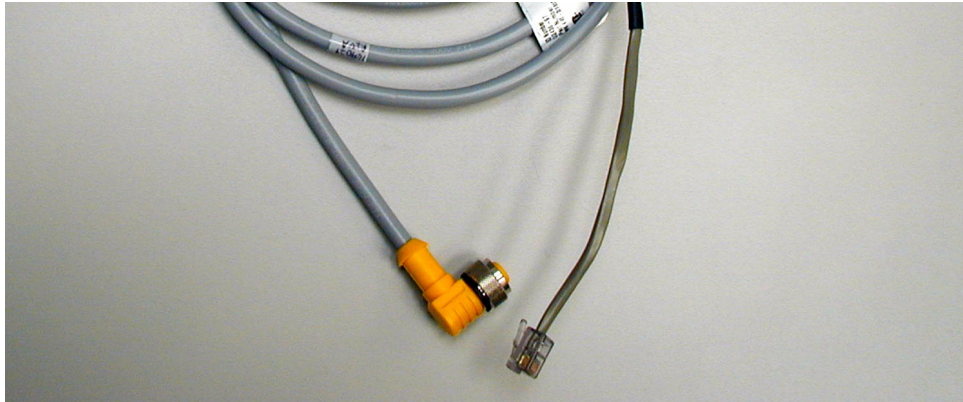


Figure 4-4: Analog Optilab T-rEX to DAWN or miniDAWN cable

When you connect your Optilab T-rEX to other instruments, see Table 4-1 for RJ-12 connector signal information.

Table 4-1: RJ-12 Signal Function

Optilab T-rEX Connector	RJ-12 Connector Wire Signal					
	White	Black	Red	Green	Yellow	Blue
Zero In			Signal	Signal Ground		
Purge In			Signal	Signal Ground		
Recycle In			Signal	Signal Ground		
Alarm In			Signal	Signal Ground		
Auto Inject In			Signal	Signal Ground		
Analog In			Positive	Negative		Ground
Analog Out	Positive	Negative				Ground
Recycle Out	Signal	Signal Ground				
Alarm Out	Signal	Signal Ground				
Auto Inject Out	Contact	Contact				

The Zero In, Purge In, Recycle In, Alarm In, and Alarm Out signals all follow the TTL voltage convention. In the TTL convention, 5 V on the signal line is interpreted as a High signal, and 0 V on the signal line is interpreted as a Low signal. Input signals to the Optilab T-rEX must have a duration of at least 100 msec in order to be reliably detected.

Zero In: TTL input on red (signal) and green (signal ground). When the signal on this line transitions from 0 V to 5 V, the last measured value of dRI is considered to be zero value, and is subtracted from the dRI data before it is output. This is exactly equivalent to activating the **Zero** button on the LCD display **Main** tab.

Purge In: TTL input on red (signal) and green (signal ground). When the signal on this line transitions from 0 V to 5 V, the internal purge valve is actuated, purging the reference chamber. While the signal on this line is held at 5 V, the purge valve remains actuated. When the signal on this line transitions from 5 V to 0 V, the purge valve is de-actuated. This is exactly equivalent to activating the **Purge** button on the LCD display **Main** tab (see “Main Tab” on page 5-3).

Recycle In: TTL input on red (signal) and green (signal ground). When the signal on this line transitions from 0 V to 5 V, the instrument actuates an external solenoid valve by supplying power to the Recycle Out connector.

Alarm In: TTL input on red (signal) and green (signal ground). On the instrument LCD display **Alarms** tab (see “Alarms Tab” on page 5-7), you may select the active state of this input. If you select “Active High”, the instrument considers a transition on this line from 0 V to 5 V to be an Alarm In event. If you select “Active Low”, the instrument considers an Alarm In event to occur when the signal on this line transitions from 5 V to 0 V. When an Alarm In event occurs, the Alarm signal flashes on the LCD display, and an Alarm Out signal is transmitted (see Alarm Out).

Auto Inject In: TTL input *or* contact closure input between wires red (TTL signal) and green (TTL signal ground). The red signal line is internally held at 5 V. When the red signal line is brought to 0 V, the instrument receives an Auto Inject signal. The signal line may be brought to 0 V by simply connecting the red and green wires together (hence the term contact closure). Most auto injectors supply a contact closure signal, which simply brings the two wires into contact with each other.

In general, the same auto injector contact closure signal cannot be sent to multiple instruments, since different instruments may be trying to maintain different internal voltages to sense the contact closure, and the instruments could be in conflict with each other.

The Optilab T-rEX provides an Auto Inject Out signal that simply retransmits the Auto Inject In signal as a contact closure output (see Auto Inject Out), allowing you to bring an auto injector contact closure signal to the Optilab T-rEX, and then send the signal to another device. The receipt of this signal by the Optilab T-rEX causes a mark to appear in the data on the LCD display **Main** tab, changes the state reported on the LCD display **System** tab **Auto Inject input** field, and causes a signal to be sent with the digital data stream indicating the time of receipt of the signal.

Analog In: Analog input Positive on red, Negative on green, and Ground on blue. The Optilab T-rEX digitizes an analog input in the range of ± 10 V with a resolution of 0.31 mV (16 bits).

Analog Out: Analog output Positive on white, Negative on black, and Ground on blue. The Optilab T-rEX supplies an analog output in the range of ± 10 V with a resolution of 0.31 mV (16 bits). In the **Constants** dialog, you may set the instrument to use only a subset of the possible voltage range (i.e., -1 V to $+1$ V), but the minimum voltage step remains at 0.31 mV. In the same dialog, you may set the dRI range to be presented over that voltage range, which determines the analog out calibration constant in Refractive Index Units (RIU)/Volt.

Recycle Out: The solenoid valve drives current on the white and black wires (the current direction is irrelevant for the solenoid). This signal may be connected to a user-supplied solenoid valve or a Wyatt Technology Recycle unit, which contains an internal solenoid valve that switches between waste and recycle. When this connector is actuated (via the **System** tab or the Recycle In input), the connector supplies current to drive a 12 V solenoid valve. The valve is actuated with 12 V (up to 1 Amp, depending upon resistance of the solenoid), held for 0.1 second, and then dropped down with 12 V across an internal 51 Ohm resistor.

Alarm Out: TTL output on white (signal) and black (signal ground). On the instrument LCD display **Alarms** tab (see “Alarms Tab” on page 5-7), you may select the active state of this output. If “Active High” is selected, the instrument keeps this signal at 0 V for no alarm state, and brings the signal to 5 V in the event of an alarm state. If “Active Low” is selected, the instrument keeps this signal at 5 V for no alarm state, and brings the signal to 0 V in the event of an alarm state. In this context, an alarm state occurs if the internal liquid leak sensor detects liquid, or the internal vapor alarm detects organic solvent vapors, or the rear panel connector Alarm In signal is active (see Alarm In).

Auto Inject Out: Contact closure output between wires white and black. This signal is a retransmit of Auto Inject In (see Auto Inject In). Internal to the instrument, the white and black wires are normally not in contact with one another. Upon actuation of this contact closure retransmit, the white and black wires are brought into contact with each other inside the instrument.

4.1.3 Communication Connector

The rear panel has one Ethernet connector, allowing digital communication to an external computer.

Ethernet: Standard Ethernet cable for connecting the instrument to an Ethernet network, see Figure 4-5.



Figure 4-5: Ethernet cable

Detailed instrument digital connectivity instructions are given in **Appendix D, “Instrument Connectivity”**.

4.2 Fluid Connections

The tubing recommendations for the Optilab T-rEX are:

- 0.010 in. (0.254 mm) ID to the Optilab T-rEX inlet
- 0.020 in. (0.508 mm) or larger ID from the Optilab T-rEX outlet to a waste bottle or solvent recycle valve.

The Optilab T-rEX accepts standard HPLC fittings: 1/16 in. outer diameter tubing with 10-32 threads.

Note:	The Optilab T-rEX should be connected <i>last</i> in line if other detectors are connected in series.
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The reasons for connecting the Optilab T-rEX last in line are:

- The seals on the quartz flow cell inside the Optilab T-rEX can withstand fluid pressures up to 100 psi (7 bar). However, the solenoid valve inside the Optilab T-rEX will no longer function properly, and may leak, with fluid pressures above 30 psi (2 bar). The back pressure generated by some instruments can exceed 30 psi or even 100 psi with only moderate flow rates, and so *the Optilab T-rEX may be damaged if it is placed upstream of another instrument.*
- Pressure fluctuations, such as result from pump pulses, cause measurable changes in a fluid's refractive index. With the Optilab T-rEX plumbed upstream of another detector, any pump pressure pulses are magnified due to the backpressure from the downstream instrument, causing a larger pump pulse signal in the data stream from the Optilab T-rEX.

- The Optilab T-rEX contains internally 0.010 in. (0.254 mm) ID stainless steel tubing from the IN port to the input to the flow cell, and 0.030 in. (0.762 mm) ID from the outlet from the flow cell to the OUT port. The large diameter tubing from the flow cell to the OUT port is intended to give the best possible pressure reference to atmospheric pressure for the reasons discussed in the previous item. However, large diameter tubing causes a great deal of mixing of the sample, resulting in band broadening for any instrument placed downstream of the Optilab T-rEX. Large ID outlet tubing does not effect the broadening between an instrument *upstream* of the Optilab T-rEX and the Optilab T-rEX itself.

4.3 Dry Nitrogen Connection

When operating below ambient temperature, a dry nitrogen purge is essential to prevent condensation on the optical and measurement systems.

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

The pressure in the dry air or nitrogen line should be 20–80 psi (0.14–0.28 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 Optilab T-rEX for approximately 150 days.

The Optilab T-rEX 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 LCD display **Alarms** tab. If the dry gas pressure drops below 20 psi (0.14 MPa), then the temperature control set point cannot be set below 20.5° C. If the system temperature is set below 20° C and the dry gas pressure subsequently drops below 20 psi (for example, if a nitrogen tank is fully depleted), the temperature control set point is automatically changed to 20.5° C.

4.4 Power On and Warm Up

The power switch for the Optilab T-rEX is located on the front of the instrument in the lower right hand corner (see Figure 2-1). Start by powering on the instrument using this switch.

After powering on, set the temperature control set point from the LCD display **Main** tab as described in “Main Tab” on page 5-3. The Optilab T-rEX has a temperature range from 4° C to 50° C. Subambient temperature operations require the flow of dry gas into the temperature controlled environment, as described in “Dry Nitrogen Connection” on page 4-8.

After setting the temperature control set point, wait approximately 30 minutes for the instrument to stabilize. If the temperature set point is quite different from room temperature, the instrument stability continues to improve for several hours. Flushing the instrument, as described in “Purging the Optilab T-rEX” on page 4-9, may be performed during this warm up period.

If the LCD display hangs (freezes) or crashes, see “Microsoft Windows Encounters Problems” on page 9-6 for information about rebooting.

4.5 Purging the Optilab T-rEX

Navigate to the **Purge** button on the **Main** tab of the LCD display, and enable the purge valve. If the purge valve is enabled, the button is yellow and reads “Purge On”. If the purge valve is disabled, the button is green in color and reads “Purge Off”.

With the purge valve disabled, liquid flows from the IN port, through the flow cell sample chamber, and then through the OUT port, bypassing the flow cell reference chamber. If the purge valve is enabled, then liquid flows from the IN port, through the flow cell sample chamber, through the flow cell reference chamber, and finally to the OUT port, flushing liquid through both the sample and reference chambers. Flush the Optilab T-rEX in purge mode with 20 mL of co-miscible solvents stepwise as necessary to prepare the cell for data collection. The Optilab T-rEX is shipped with Ethanol in the flow cell. A typical series of solvents (from polar to nonpolar) is shown below.

Note:	Salt solutions should be considered separate steps from pure solvents.
--------------	--

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

When the solvent required for data collection has been thoroughly flushed through both the sample and reference chambers, disable the purge valve. The fluid in the reference chamber is now captive, and the refractive index difference between this captive reference fluid and the fluid stream directed through the sample chamber is reported as the differential refractive index (dRI). Navigate to the **Zero** button on the LCD display **Main** tab and press Enter to cause the current state to be considered the “zero” differential refractive index.

4.6 Instrument Stability Check

To perform an instrument stability check, do the following:

1. Purge the Optilab T-rEX as described in “Purging the Optilab T-rEX” on page 4-9.
2. Un-plumb the instrument from any fluid lines.
3. Insert a plug (shipped with the Optilab T-rEX) into the OUT port (see Figure 2-1) and leave the IN port open to the atmosphere.
4. From the LCD display **Main** tab, set the temperature set point to 25° C and wait approximately 30 minutes for the instrument to stabilize. The instrument stability continues to improve for several hours after the temperature set point has been changed.
5. Navigate to the LCD display **System** tab, press Enter on the **Constants** button, and set the **Collection interval** to 2.0 seconds (equivalent to a 4 second time constant).
6. Navigate back to the LCD display **Main** tab and select **View: dRI**.
7. Observe the dRI signal through time.

Short term noise should be seen to be less than $\pm 7.5 \times 10^{-10}$ RIU, when measured as per ASTM-E1303-95 (2000) using a 2 second Collection interval (equivalent to a 4 second time constant).

5

Using the LCD Display

This section describes the keypad controls and the tabbed interface on the LCD display on the front panel of the instrument. These tabs allow you to monitor, configure, and control the function and operation of the Optilab T-rEX.

CONTENTS	PAGE
5.1 Navigating the Optilab T-rEX Tabs	5-2
5.2 Main Tab	5-3
5.3 aRI Tab	5-6
5.4 Alarms Tab.....	5-7
5.5 System Tab.....	5-10
5.5.1 Constants Dialog	5-12
5.6 Communications Tab	5-14

5.1 Navigating the Optilab T-rEX Tabs

The keypad consists of a number, Tab, Esc, Del, Enter, and arrow keys.

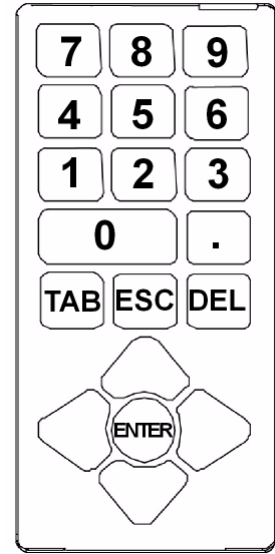
You use these keys to move through the tabs on the LCD display on the instrument front panel and to enter values where applicable.

To select a tab on the LCD display:

1. Press the Tab key until the cursor is positioned on a tab title at the top of the screen
2. Use an arrow key to move to the desired tab. Each tab is displayed as you use arrow keys to move to another tab.

Once the desired tab is displayed, use the keys as follows:

- **Tab key:** Move forward to the next field within a tab.
- **Esc key:** Exit a pull-down list or to cancel an entry.
- **Del key:** Remove the previous character from a numeric entry field. (Same as Backspace on a regular keyboard.)
- **Enter key:** Save an entry made using the number buttons, “click” a button, select an option, or choose an item in a pull-down list.
- **Up and Down Arrow keys:** Move to a different selection within a pull-down list or radio field.
- **Right and Left Arrow keys:** Move to another tab, or move the cursor within a numeric field.



Note: The Tab key *only* moves in the forward direction.

The sections that follow describe the Optilab T-rEX LCD display tabs.

5.2 Main Tab

The **Main** tab contains the most commonly used Optilab T-rEX functions. The display shows graphical representations of two of the data streams collected by the instrument. One data stream is displayed in red on the right axis and other is displayed in blue on the left axis.

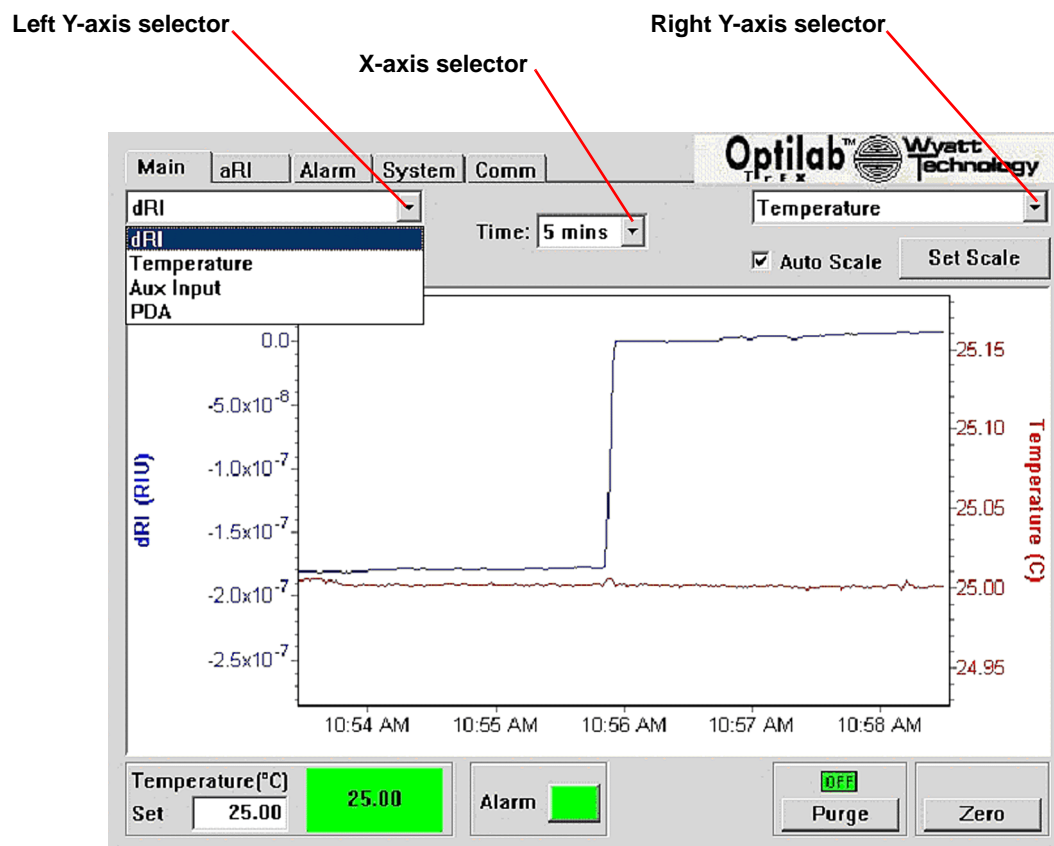


Figure 5-1: Main tab

Left and Right Y-axis Selectors

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

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

X-axis Selector

The X-axis selector sets the time range from 10 minutes to 2 hours. To change the time, see the **Set Time** field under “System Tab” on page 5-10

Adjusting the Display Range

You can adjust the range displayed in the graph in a variety of ways.

Tab to here
to display
the zoom/pan
buttons

Tab to here
to display
the zoom/pan
buttons

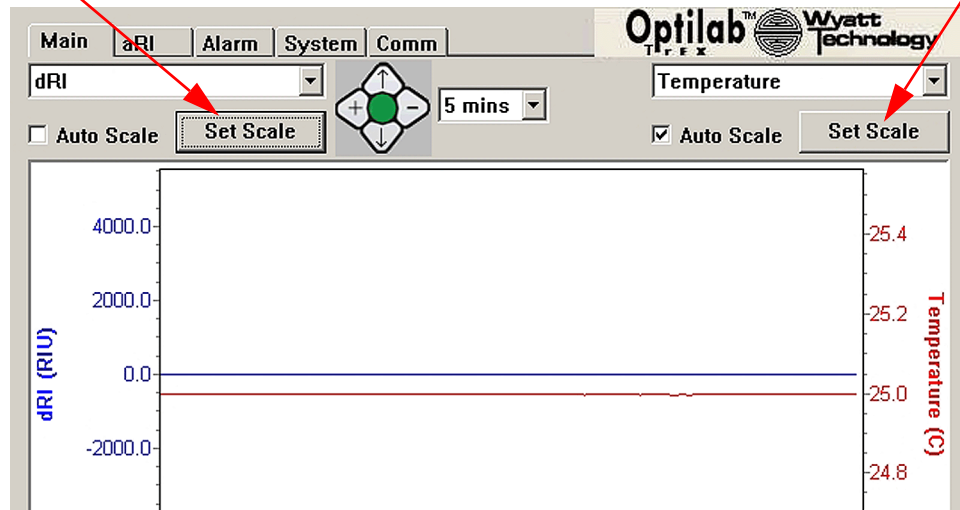
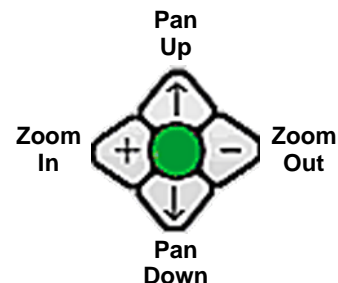


Figure 5-2: Zoom and Pan Buttons

To use the zoom and pan buttons:

1. Tab to the **Set Scale** button.
The zoom/pan buttons are displayed.
2. Press the left arrow to zoom in.
3. Press the right arrow to zoom out.
4. Press the up arrow to pan up.
5. Press the down arrow to pan down.



To change the scale numerically:

1. Tab to the **Set Scale** button.
2. Press **Enter**.
The Set Scale dialog is displayed.
3. To toggle the **Max** or **Min** sign, tab to the +/- button, press **Enter**.
4. To change values, tab to the **Max** or **Min** field and enter a value.
5. Press **Enter**.

Figure 5-3: Manual Scale Setting

Auto Scale: Changes the scaling so the display fills the window.

Temperature (° C) Set: Enter the value for the temperature that the instrument is to maintain. The value shown in the box to the right displays the current internal temperature of the instrument. The box displays as yellow until the temperature stabilizes. When the temperature has remained within $\pm 0.05^{\circ}\text{C}$ of the Temperature (° C) Set value for a minimum period of 15 minutes, the box is green.

Alarm: Flashes red if an instrument alarm is active, and remains solid green if no alarms are active. If any of the alarm conditions are detected, the **Alarm** button flashes on the **Main** tab, the **aRI** tab, and the **Alarms** tab. Navigate to the **Alarms** tab to determine the nature of the alarm.

Purge Off: This is a toggle button that turns the purge valve On or Off. In the ON state, the purge valve purges the reference chamber. If the purge valve is OFF, then fluid flows from the IN port, through the flow cell sample chamber, and then through the OUT port. If the purge valve is ON, then fluid flows from the IN port, through the flow cell sample chamber, through the flow cell reference chamber, and finally to the OUT port.

Zero: Press Enter on this button to cause the current value of dRI to be considered the “zero” value.

5.3 aRI Tab

The **aRI** tab is used to view the aRI value of the solvent in the flow cell.

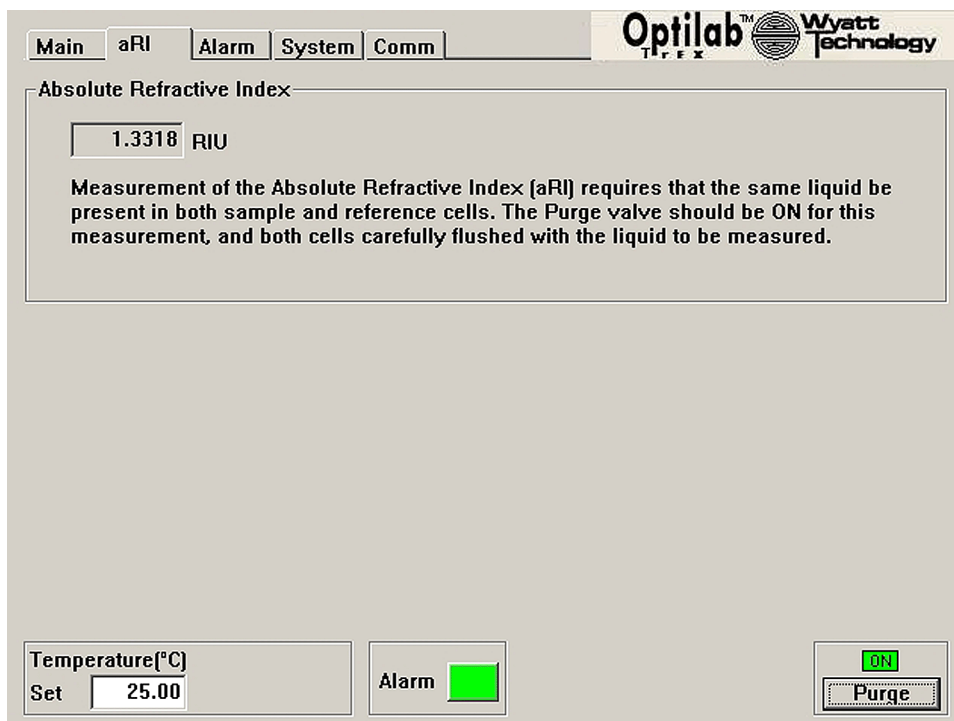


Figure 5-4: aRI tab

In order to measure the aRI, the same liquid must be present in both the sample and reference chambers. The purge valve should be ON and both chambers should be carefully flushed with the liquid to be measured.

RIU: With the purge valve ON, displays the aRI of the liquid in the flow cell.

5.4 Alarms Tab

The **Alarms** tab allows you to view the current state of Optilab T-rEX alarms, and to set the voltage state of external alarms. If any alarm is detected, the **Alarm** button flashes on the **Main**, **aRI**, and **Alarms** tabs.

If no alarm conditions are detected, the status buttons display green. If an alarm condition is detected, the status buttons display yellow. An alarm history of the last few alarms is shown and the time at which they occurred.

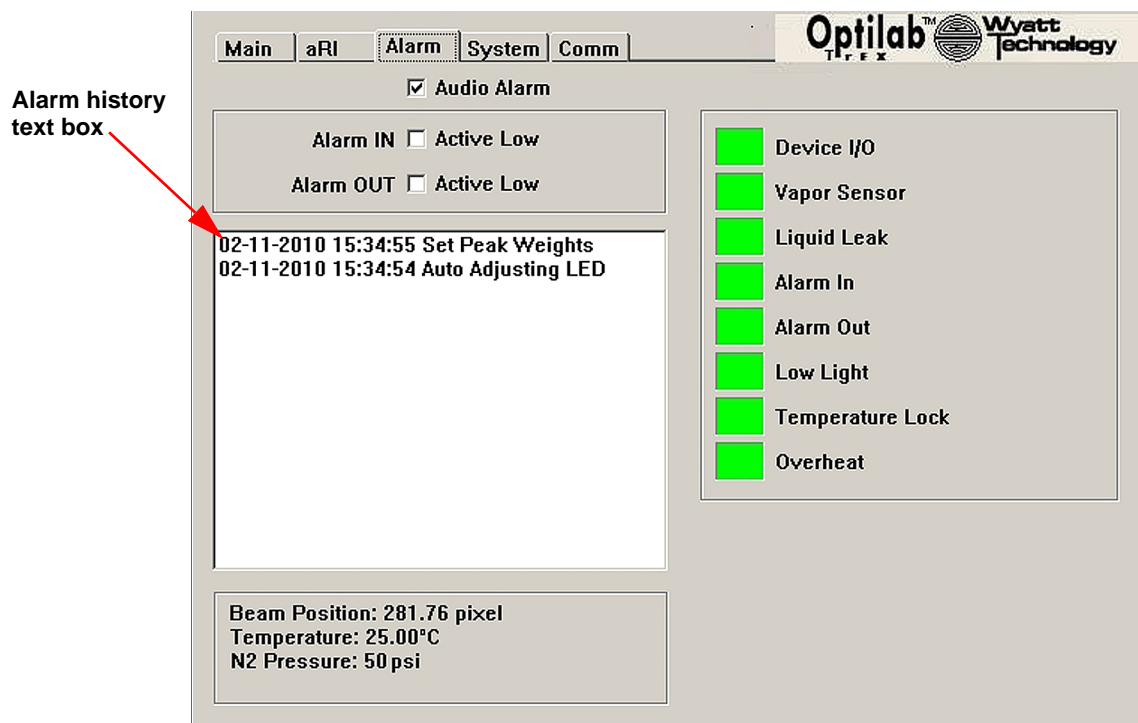


Figure 5-5: Alarms tab

External alarm settings are used when alarms from additional instruments or systems are daisy chained through the Optilab T-rEX. Connections are made at the rear panel using the Alarm In or Alarm Out connectors.

Audio Alarm: Navigate to this button and press Enter to toggle the Audio Alarm radio button.

Alarm IN: The external Alarm IN is an input used to capture alarm signals from additional devices, and then retransmit that alarm signal as an external Alarm OUT.

For example, multiple instruments in a chromatographic system typically have liquid leak detectors. It is desirable that the solvent pump be shut off in the event of a liquid leak in any one of those instruments. Since the alarm outputs from each instrument cannot all be connected to the same pump shut-off signal line, the alarm outputs from the instruments need to

be logically ORed with each other. The external alarm IN is logically ORed with the Optilab T-rEX internal vapor and liquid leak alarms, and then retransmitted as the external alarm Out.

Different devices have different conventions for alarm states. Some maintain a 5 V state on a wire to indicate no alarm, and bring the voltage to 0 V to indicate an alarm condition. This is called the “Active Low” convention. Others use 5 V to indicate an alarm condition; this is the “Active High” convention.

Select High or Low depending on the voltage output convention of the connecting device. If the connecting device expects to receive a low voltage to signal an alarm, select Low. Voltage signal ranges are from 0 V to 5 V.

Alarm OUT: This output signal is the logical OR of the external alarm In, the Vapor alarm, and the Liquid leak alarm. If connecting a device to the Optilab T-rEX external alarm Out, select High or Low depending on the voltage input of the external device. If the connecting device expects to receive a low voltage to signal an alarm, select Low. Voltage signal ranges are from 0 V to 5 V.

Beam Position: Displays the current position of the LED beam on the photodiode array

Temperature: Displays the current temperature in degrees Centigrade (°C) inside the Optilab T-rEX.

N2 Pressure: Displays the gas pressure in psi of the gas line attached to the back panel nitrogen purge port (see Figure 2-2). For sub-ambient operation, this pressure must be greater than 20 psi (0.14 MPa).

Alarm status area: This area displays red or yellow for the following items if the alarm is active. Otherwise, it shows green for each item.

- **Device I/O:** Alarm is displayed if the Optilab T-rEX software daemon stops running.
- **Vapor Sensor:** Alarm is displayed when organic solvent vapors are present in the instrument chassis.
- **Liquid Leak:** Alarm is displayed if the sensor located inside of the Optilab T-rEX comes in contact with any liquids.
- **Alarm In:** Alarm is displayed if the instrument detects a voltage signal on the rear panel Alarm In connector from an external device as configured by the **Alarm IN Active Low** radio button.
- **Alarm Out:** Alarm is displayed if the instrument is sending an alarm out through the rear panel Alarm Out connector. The voltage state of the Alarm Out signal is configured by the **Alarm OUT Active Low** radio button.
- **Low light:** Alarm is displayed if sufficient light is not being transmitted through the flow cell. Possible causes are bubbles in the flow cell, extreme refractive index differences between the sample and reference

cells (as occurs when switching solvents or when the sample concentration is well beyond the instrument's maximum range), or a faulty light source. See "Low Light Alarm" on page 9-2.

- **Temperature Lock:** Alarm turns green to indicate has reached within $\pm 0.05^{\circ}\text{C}$ of the Temperature Set value for a minimum period of 15 minutes.
- **Overheat:** Alarm is displayed if the temperature rises above 65°C . If this condition occurs, the heater/cooler is disabled and cannot be re-enabled until the power to the instrument has been cycled off and then on.

5.5 System Tab

The **System** tab allows you to configure a waste or recycle activation, set the time of day and the wavelength of the LED/fiber light source, view the instrument's serial number and the Auto Inject input status, turn the LED/fiber light source on or off, and modify calibration constants and analog out parameters.

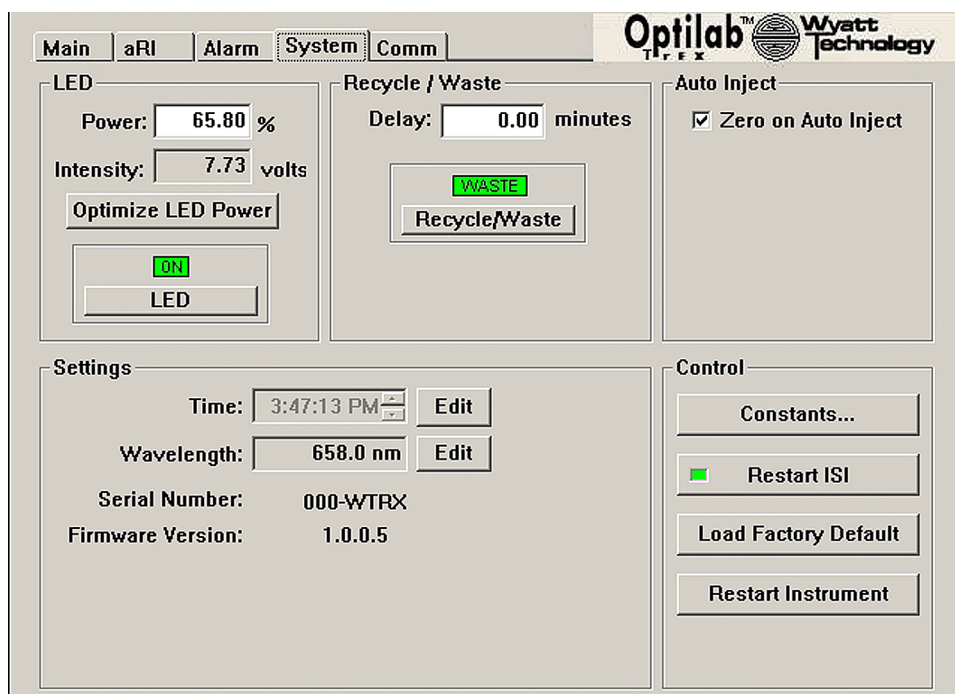


Figure 5-6: System tab

LED:

- **Percent Power:** Sets the power to the LED/fiber light source. See “Setting the Percent Max Power to Zero” on page 8-2 for instructions regarding setting this field to 0% when changing the light source.
- **Intensity:** Displays the current signal in Volts from the photodetector, which is proportional to the intensity of light detected by the photodetector. For optimal performance, this value should be between 7.6 V - 7.8 V.

The photodetector saturates slightly above 8 V, at which point an increase in light intensity no longer results in an increase in the signal voltage from the photodetector. If the light intensity is saturating the detector, the instrument firmware will automatically reduce the light intensity to an optimal setting between 7.6 V and 7.8 V.

Instrument performance degrades significantly if the photodetector voltage is above 8 V, and very slightly as the photodetector signal drops below the optimal value. When this voltage drops below 5 V, a Low Light alarm is activated. See “Low Light Alarm” on page 9-2.

- **Optimize LED Power:** Automatically determines the optimal percent of maximum power to send to the LED/fiber light source and sets the LED Power to bring the Intensity to its optimal value. The optimal light intensity value for all wavelengths is between 7.6 - 7.8 V.
- **LED:** Turn the power to the LED/fiber light source on or off.

Recycle/Waste: Modifies the state of the external solenoid valve output signal on the rear panel Recycle Out connector. Possible choices are Immediate activation or Delayed activation. If Delayed activation is selected, enter the number of minutes to delay activation in the **Delay** field. If an activation is pending, a countdown in the **Delay** field shows the time left before activation.

Zero on Auto Inject: When this item is selected and an Auto Inject signal is received, the last measured value of dRI is considered to be a zero value. That value is subtracted from the dRI data before it is output.

Settings:

- **Time:** Displays the current time of day used by the instrument. To change the time of day, navigate to the **Edit** button and enter the new values.
- **Wavelength (nm):** Displays the current value of the wavelength of the LED/fiber light source, which is stored in the instrument's on-board memory. This value should not be changed while a light source is connected. See "Changing the Wavelength Setting" on page 8-8 for instructions regarding changing the wavelength.
- **Serial number:** Displays the serial number of the instrument.
- **Firmware version:** Displays the version number of the firmware running on the Optilab T-rEX. Visit www.wyatt.com to obtain the latest firmware version and instructions for installing new firmware.

Control:

- **Constants...:** Refer to "Constants Dialog" on page 5-12.
- **Restart ISI:** This button is used to restart or reset the instrument communication in the event that a remote client, such as ASTRA or the Diagnostic manager, loses its communication to the instrument.
- **Load Factory Defaults:** this button is used to reset the instrument to the settings installed when the instrument was shipped.
- **Restart Instrument:** This button turns off the Optilab T-rEX and then turns it back on.

5.5.1 Constants Dialog

The **Constants** dialog opens if you select the **Constants** button in the **System** tab. This dialog displays current calibration constants and allows you to edit those constants.

System constants are loaded during manufacturer's quality control and should be checked periodically by running known samples. Refer to **Chapter 6, “Off-Line Measurements”** for more information regarding dRI and aRI calibration.

Figure 5-7: Constants dialog

Note: To change the + and – signs, tab to the box that contains the sign value and press the Enter key to toggle between the + and – values.

System Constants: Values of various instrument calibration constants and the measurement time constant.

- **dRI (differential Refractive Index) calibration constant:** This value relates light beam movement to the dRI measurement.
- **dRI offset:** This value represents the light beam position that corresponds to zero dRI. Zeroing the instrument (from the front panel **Main** tab) updates this value.
- **aRI calibration constant:** This values relates light beam movement to aRI.
- **aRI offset:** A second calibration constant associated with the aRI measurement.

- **Collection interval:** The time in seconds between data slices sent as a digital output. The minimum collection interval is 0.105 seconds, and the maximum is 2.53 seconds. The time over which data is averaged (the measurement time constant) is automatically set to be 2x the collection interval. This parameter is managed using the ASTRA V software Basic collection procedure, when the Optilab T-rEX is connected digitally to a PC via an Ethernet connection.

Differential Refractive Index Analog Output: The voltage output presented on the rear panel Analog Out connector may be adjusted by setting this parameter.

Table 5-1: dRI Analog Out Range Settings

dRI Analog Out Range Setting	Voltage (V)		dRI (RIU)		dRI Analog Out Calibration Constant (RIU/V)
	Min	Max	Min	Max	
Narrow	-10	+10	-2x10 ⁻⁵	+2x10 ⁻⁵	2x10 ⁻⁶
Standard	-10	+10	-2x10 ⁻⁴	+2x10 ⁻⁴	2x10 ⁻⁵
Wide	-10	+10	-1x10 ⁻³	+1x10 ⁻³	1x10 ⁻⁵
Full	-10	+10	-4.7x10 ⁻³	+4.7x10 ⁻³	4.7x10 ⁻⁴
Manual	User defined	User defined	User defined	User defined	User selected values

Note: The instrument's dynamic range exceeds 22 bits of resolution (more than 6×10^6 discrete steps). The 22-bit full range and sensitivity may be simultaneously accessed only via digital communication. The Digital/Analog converter has 18 bits of resolution (262,143 discrete steps) between -10 V and +10 V. If the entire dRI range is selected for analog output, the smallest observable signal on the analog output is set by the 18 bit Digital/Analog resolution, effectively reducing sensitivity. You may select any subset of the instrument range to be output within ± 10 V.

dRI analog out calibration constant: Relates the dRI change to voltage presented on the analog out signal. The dRI analog out calibration constant is calculated as:

$$(dRI_{MAX} - dRI_{MIN}) / (Voltage_{MAX} - Voltage_{MIN})$$

If you set the dRI analog out range parameter to Narrow, Standard, Wide, or Full, the Min and Max output Voltages and dRI values are set to preset values as in Table 5-1.

If you set the dRI analog out range parameter to Manual, you may set the Min and Max output Voltages to any value between -10 V and +10 V. You may also set the Min and Max dRI values to any value between -4.7x10⁻³ RIU and 4.7x10⁻³ RIU.

If you set the dRI analog out range parameter to Standard, the voltage response from a BSA solution (dn/dc at 660 nm = 0.185 RIU/g/mL) of concentration 1 mg/mL in the flow cell will remain barely below 10 V.

OK: Save changes made in this dialog and return to the **System** tab.

5.6 Communications Tab

The **Communications** tab allows you to set parameters relating to communication with computers. The Optilab T-rEX is equipped for Ethernet communication. Ethernet communication allows the instrument to be presented to any computer on a local area network (LAN). See **Appendix D, “Instrument Connectivity”** for instructions regarding network communications setup.

Figure 5-8: Communications tab

Instrument: This area displays current communication settings for the Optilab T-rEX.

- **Instrument Name:** Displays the instrument name as it would appear on a LAN.
- **IP Address / Subnet Mask:** These fields display the currently assigned IP address and subnet mask values for the instrument.
- **Mode:** Displays whether the Ethernet settings are set to automatic (DHCP) or manual (Static).

Configure Ethernet: This panel allows you to change IP preferences:

- **Obtain an IP address automatically:** If this option is selected, the Ethernet server automatically assigns an IP address to the instrument (DHCP).
- **Use the following IP address:** Selecting this option allows you to assign static IP Address and Subnet Mask values manually. You may need assistance from a network administrator to obtain valid static IP address and subnet mask values.

6

Off-Line Measurements

This chapter provides general information for instrument calibration, sample preparation, dn/dc measurement, and aRI measurement.

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6.1 Instrument Calibration for dRI

The Optilab T-rEX dRI measurement can be calibrated as described in this section. This procedure is also summarized in the *ASTRA V User's Guide* section on "RI Calibration."

6.1.1 General Information and Sample Preparation

The Optilab T-rEX dRI measurement performance should be checked regularly against a standard with a known dn/dc value. Changing solvents does *not* affect the Optilab T-rEX calibration constant.

Note:	For use with certain chromatography systems and the ASTRA software, the dRI calibration may be performed by injecting a suitable standard sample and verifying that the injected and calculated masses are equal. This option is often much more efficient than the off-line approach described below. Contact Wyatt Technical Support and/or consult the <i>ASTRA User's Guide</i> for more information.
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The off-line dRI calibration may be performed using any substance with a well-characterized dn/dc value. The recommended standard is anhydrous sodium chloride dissolved in pure deionized water. This solution has a dn/dc of 0.172 mL/g at a wavelength of 690 nm (0.181 mL/g at 488 nm, 0.174 mL/g at 633 nm or 658nm, and 0.170 mL/g at 900 nm).

Appropriate concentrations should be prepared using pure distilled water and *clean* glassware. The lowest and highest recommended concentrations are ~0.1 mg/mL and ~29 mg/mL respectively, although 0.1mg/mL to 5mg/mL is the most relevant range for Optilab T-rEX dRI calibration. Use six or more concentrations prepared within 1% or better accuracy to increase the precision of the final determination. Wyatt Technology provides validated, pre-mixed NaCl solutions in the ideal concentration range for this purpose. See below for details.

The Optilab T-rEX can differentiate between solvent that has been saturated with ambient gases and those that have been degassed. It is possible to detect concentration differences in the 1 ppm range. Consequently, standard samples and solvent blanks must be prepared from the same solvent stock. After preparing the standard solutions, fill two or three extra containers with water from the same solvent flask to use for "blank" analyses.

Samples should be kept well-sealed to prevent evaporation. Some solvents (such as water) keep fairly well for a number of months, but concentration changes may occur over time due to evaporation or contamination.

Single-use sodium chloride solutions are available for purchase from Wyatt Technology Corporation. Part Number: P8400; NaCl Solutions Kit. NaCl concentrations are as follows: 0.0 (blank), 0.1, 0.5, 1.0, 1.2, 2.0, 3.0, 4.0, and 5.0 mg/mL in nanopure water.

6.1.2 HPLC Pump with Injector

This arrangement is shown in Figure 6-1. Use 0.01 in. ID tubing between the injector and the Optilab T-rEX, and 0.02 in. ID tubing elsewhere. A flow rate of 0.5–1.0 mL/minute is ideal. Use a large sample loop, 0.5–1.0 mL to ensure that each injection produces a clear plateau and not a peak as it passes through the Optilab T-rEX.

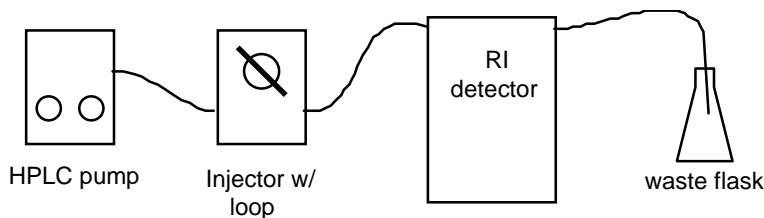


Figure 6-1: Setting up an HPLC pump and injector for calibration

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

A syringe pump fitted with a large syringe may be used instead of the HPLC pump.

6.1.3 Syringe Pump Infusion

Another option is to connect the Optilab T-rEX as shown in Figure 6-2, using a syringe pump. Use 0.02 in. ID tubing for both connections. A recommended flow rate is 0.1–0.2 mL/minute. 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 injected air may cause an unstable baseline for several minutes.

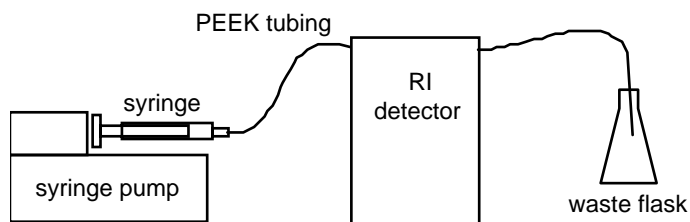


Figure 6-2: Setting up a syringe pump for calibration

6.1.4 Collecting Data for Optilab T-rEX dRI Calibration

This procedure is also summarized in the *ASTRA V User's Guide* section on “dRI Calibration.”

Data Collection

The sample and reference cells should be flushed with high purity water (first solvent to be analyzed) for several minutes before making measurements. Confirm that the temperature is stable at 25°C.

On the Optilab T-rEX **Main** tab, press Enter on the **Purge** button to turn the Purge on (The Purge indicator is yellow on the **Main** tab when the Purge is on). Wait until the baseline is stable, then press Enter on the **Purge** button a second time to turn the purge off (the Purge indicator is green on the **Main** tab when the Purge is off). Monitor the baseline for a few minutes to ensure that the baseline is still stable. Finally, zero the Optilab T-rEX by pressing Enter on the **Zero** button on the **Main** tab. After the Optilab T-rEX has been zeroed, it is ready to collect data and should not be zeroed again until the dRI Calibration procedure is complete.

Collect data using ASTRA V software. In ASTRA version 5.3.4 the dRI Calibration data collection procedure is as follows (see the *ASTRA V User's Guide* for more information):

Start the ASTRA V software and select **File->New->Experiment from Template**. In the dialog that opens, select the **System Templates** folder, then the **RI Measurement** folder, and finally open the **RI calibration** template. Expand the **Configuration** node and verify that the proper experimental parameters have been entered. Expand the **Procedures** node of the template. Click on the **Basic collection** procedure and enter appropriate values for each of the fields. Close the pane by clicking the **OK** button, and run the experiment.

Introduce pure solvent (blank) into the reference cell, making sure the solvent flows through the system at a constant rate.

dRI detectors do not react favorably to sudden changes in flow as the detector drift would overwhelm the signal. Thus, it is important to maintain a nearly constant flow through the instrument while measuring the solutions.

While ASTRA V collects data, begin to introduce the series of prepared standards into the sample cell of the detector. Introduce each standard solution one at a time, *beginning with the lowest concentration*.

Wait for the signal to stabilize (allow the signal to reach a plateau), which may take several minutes, while the previous solution is completely rinsed out of the cell. After all the standards have been injected, reinject a pure solvent sample (blank) to re-establish the baseline.

Once a good baseline signal is acquired, stop the ASTRA V data collection.

ASTRA Calculations

In ASTRA version 5.3.4 the dRI Calibration calculation procedure is as follows (see the *ASTRA V User's Guide* for more information):

After the data collection has ended, ASTRA V may produce a message box informing you that some parameters necessary to perform the calculations are missing. Click OK, and the software will expand the **Procedures** section of the experiment and open the **Baselines** procedure. Otherwise click the **Run** button to proceed.

In the **Define baselines** window define the baseline using the solvent blanks that were injected before and after the standard solutions. Click and drag from one solvent plateau to the other, such that a baseline is drawn under the sample solution plateaus. Click OK to close the Define baselines window.

In the **Define peaks** window define a peak for each of the flat plateau regions associated with each injected standard. Click and drag over the maximum flat region for each sample injection. Do not set peak regions for the “blank” injections.

As peak regions are defined, a table beneath the Define peaks graph shows a new column for each peak. In these columns, for the Refractive index, enter the known concentration for each standard in the row titled Concentration (g/mL). Once all the peaks have been assigned with their respective concentrations, click on the **OK** button at the bottom to close the pane.

Open the **RI Calibration** procedure. Enter the known dn/dc into the row titled **Known dn/dc (mL/g)** and press Enter. The value listed in the **Calibration** row should update once a known value is entered.

Expand the **Results** node and double click the **Report (summary)** line. The report will display the new **Calibration constant**, a correction factor. A value of 1.0000 would indicate that the dRI calibration constant has remained unchanged. If the value is 1.0120, the dRI calibration constant has increased by a factor of 1.2%. Multiply the old dRI calibration constant by the correction factor to determine the new dRI calibration constant. The old dRI calibration constant is viewable in the **Constants** dialog on the Optilab T-rEX front panel.

For example:

(old dRI calibration constant $3.3828\text{e-}3$) x (ASTRA V correction factor 1.012) =
new dRI calibration constant = $3.4234\text{e-}3$

Note: The new dRI calibration constant must be calculated manually, using the old dRI calibration constant and the correction factor provided by ASTRA.

Go to the **System** tab on the Optilab T-rEX front panel, open the **Constants** dialog, and enter the new **dRI calibration constant**. Tab to the **Apply** button and press Enter.

6.2 dn/dc Measurement

The dn/dc value is the change in solution refractive index with change in solute concentration, expressed in units of mL/g. It is possible to calculate dn/dc from Optilab T-rEX data if the Optilab T-rEX dRI calibration constant and sample solution concentrations are known.

Note: For use with certain chromatography systems and the ASTRA software, dn/dc calculations may be performed by injecting the unknown sample once, and assuming that the injected and calculated masses are equal. This option is often much more efficient than the off-line approach. Contact Wyatt Technical Support and/or consult the *ASTRA User's Guide* for more information.

The ideal sample concentration range for dn/dc measurements will vary depending on the dn/dc value itself. In general, a set of 5-6 concentrations over a range of an order of magnitude in concentration, such as 0.1 mg/mL to 1 mg/mL, is appropriate.

Use the general procedures described in “Instrument Calibration for dRI” on page 6-2 for sample preparation, injection, and data collection, using the ASTRA template named “**Batch (determine dn/dc)**.”

After data collection define a peak for each of the flat plateau regions associated with each injected sample solution, in the **Define peaks** window. Click and drag over the maximum flat region for each sample injection. Do not set peak regions for the “blank” injections.

As peak regions are defined, a table beneath the Define peaks graph shows a new column for each respective. In these columns, under the **Refractive index** node, enter the known concentration for each sample solution in the row titled **Concentration (g/mL)**. Once all the peaks have been assigned with their respective concentrations, click the **OK** button to close the window. The sample dn/dc value will be reported in the reports available in the Results section of the Experiment tree.

6.3 Instrument Calibration for aRI

The Optilab T-rEX aRI measurement can be calibrated as described in this section. This procedure is also summarized in the *ASTRA V User's Guide* section on "aRI Calibration."

We recommend that you verify and/or calibrate the absolute Refractive Index (aRI) measurement before operating the instrument in aRI mode for the first time, and it is recommended that you check these values for accuracy on a periodic basis.

6.3.1 General Information

Optilab T-rEX aRI measurement performance should be checked against one or more standards. For a complete aRI calibration, at least 3 pure solvents with known aRI values (which are specific for the operating wavelength of the Optilab T-rEX) should be used. See Table 6-1 for recommendations. It is imperative that each solvent infused into the Optilab T-rEX must be miscible with the solvent that it replaces.

The Optilab T-rEX aRI is factory-calibrated using the following four solvents in the following order:

1. High Purity Water (NANOpure water with a final 0.2 micron filter)
2. Methanol (HPLC-Grade Fisher A452-4)
3. Tetrahydrofuran (GPC-Grade w/BHT Burdick & Jackson Cat. 341-4)
4. Toluene HPLC Grade (HPLC-Grade Burdick & Jackson Cat. 347-4)

Table 6-1: Known aRI Values for Various Solvents at Various Wavelengths

Solvent	685nm	658nm	633nm	532nm	514nm	488nm
Water	1.3303	1.3309	1.3316	1.3347	1.3354	1.3364
Methanol	1.3242	1.3247	1.3253	1.3282	1.3289	1.3300
Tetrahydrofuran	1.4015	1.4022	1.4029	1.4069	1.4079	1.4094
Toluene	1.4882	1.4896	1.4910	1.4995	1.5017	1.5053

Note: It is imperative that each solvent be miscible with the solvent it replaces anytime the Optilab T-rEX is flushed with a new solvent.

6.3.2 Collecting Data for Optilab T-rEX aRI Calibration

1. The Optilab T-rEX should be purged (flow liquid with the Purge on) with high-purity water for several minutes before beginning the aRI calibration procedure. Confirm that the temperature is stable at 25°C, and operate the Optilab T-rEX with the Purge ON for all data collection (the Purge indicator will be yellow on the **Main** tab).

2. On the Optilab T-rEX front panel, navigate to the **System** tab, and open the **Constants** dialog. Record the values listed for dRI calibration constant, dRI offset, aRI calibration constant, and aRI offset.
3. Collect data using the ASTRA V software. As of the time of publication of this user's guide, the ASTRA collection procedure is as follows (refer to the current *ASTRA V User's Guide* for more information):
 - a. Start the ASTRA V software and select **File->New->Experiment from Template**.
 - b. In the dialog that opens, select the **System Templates** folder, then the **RI Measurement** folder, then the **Optilab rEX Specific** folder, and finally the **absolute RI calibration** template.
 - c. Expand the **Configuration** node and verify that the proper experimental parameters have been entered.
 - d. Expand the **Procedures** node of the template. Click on the **Basic collection** procedure and enter appropriate values for each of the fields. Close the pane by clicking the **OK** button.
 - e. Run the experiment.

Introduce Solvents

1. Flow the pure solvents directly into the Optilab T-rEX at a constant flow rate, using a syringe pump or an HPLC pump set in the flow rate range of 0.5mL/min to 1.0mL/min.
2. After flowing roughly 4-5 mL of solvent, toggle the purge valve to **PURGE OFF** for 15 seconds, and then toggle it back to **PURGE ON** for 15 seconds. Repeat this cycle for 2-3 minutes. Cycling the purge valve in this manner creates just enough agitation in the flow path to displace air bubbles and thoroughly remove the previous solvent.
3. On the Optilab T-rEX **System** tab, go to the **LED** button and press Enter. Adjust the **Percent max power** setting so that the **Light intensity** is close to, but not above, 7.8 Volts. Close the LED Intensity dialog and return to either the **Main** or **aRI** tab.
4. Complete the solvent introduction process by leaving the purge valve in the **PURGE ON** state for roughly one minute, and then turn the syringe pump off. After three minutes of stopped flow the Optilab T-rEX is stable. During this time ASTRA V will record the stable, flat no-flow region. Note the time of this no-flow period, which will be referenced during data processing.
5. Repeat this solvent introduction procedure (beginning with step 1 of this list) for each remaining solvent.

ASTRA Calculations

Follow these steps in ASTRA V:

1. After data collection, open the **Procedures** section of the experiment, and click **Define peaks**. On the graph, identify the flat no-flow regions associated with each solvent. Define a peak for each solvent by selecting a plateau of approximately 30 seconds located near the end of the flat region.
2. Beneath the graph a table is shown with a column for each defined solvent. Expand the **Refractive index** node. A row will now be visible titled **Real**. In each solvent's column, enter the known aRI value. Make sure that it is the correct aRI value for the Optilab T-rEX's operating wavelength. Table 6-1 lists aRI values for several solvents at various wavelengths. Click the **OK** button to close the window.
3. Click on the **Absolute RI Calibration** procedure to open its settings pane. Enter the **dRI calibration constant**, **dRI offset**, **old aRI calibration constant** and **old aRI offset**. These are the numbers recorded in Step 2 on page 6-8. Click **OK** to close the window.
4. Click the Run experiment icon on the icon toolbar at the top of the window. The template completes its data processing.
5. Expand the **Results** node and double click the **Report** line. The report will display the new aRI calibration constant and new aRI offset. Save the ASTRA V file. You may also want to print the report.
6. Go to the **System** tab on the Optilab T-rEX, tab to the **Constants** button and press Enter. Enter the new values into the Optilab T-rEX in the Constants dialog. Tab to the **Apply** button and press Enter. Tab to the **Close** button and press Enter. The Optilab T-rEX aRI is now recalibrated.
7. Measure and record the aRI value for pure ethanol, which should be near 1.35 after the correct constants are in place.

6.4 Absolute Refractive Index Measurement

In order to measure the absolute refractive index (aRI) of a liquid, the liquid must be present in both the sample and reference chambers of the Optilab T-rEX. The purge valve should be ON and both chambers should be carefully flushed with the liquid to be measured. See the previous section (aRI Calibration) for a detailed description of the recommended solvent introduction procedure for aRI measurements.

With the purge valve ON, the aRI of the liquid in the flow cell is displayed in the **aRI** tab on the Optilab T-rEX front panel.

7

In-Line HPLC Detection

This chapter provides details about preparing samples for optimal in-line operation and measurement.

CONTENTS	PAGE
7.1 Conditions for In-Line Operation.....	7-2
7.2 Sample Preparation.....	7-3
7.3 Measurement.....	7-3

7.1 Conditions for In-Line Operation

In order to use the high sensitivity Optilab T-rEX 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. The solvent should be degassed by vacuum, sonication, or boiling, and blanketed with an inert gas—such as helium or argon—that has a low solubility in the solvent. An in-line degasser may be used effectively. 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.
- 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)
 - the Optilab T-rEX itself

“Main Tab” on page 5-3 outlines how to control the Optilab T-rEX temperature. The Optilab T-rEX is extremely stable thermally, so unless the ambient temperature fluctuates considerably, the Optilab T-rEX should be able to achieve a stable temperature.

- A pump with a constant flow rate is required, and regularly checking the pressure is an important routine in HPLC. 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
 - air or gas in the system

These problems often create an unstable baseline due to an unstable column environment. The instabilities result in fluctuating solution density, hence a changing RI, which the Optilab T-rEX refractometer measures faithfully! The result is a signal with oscillations, spikes, or drift.

- When changing solvents, special care should be taken to avoid leaving residues of the old solvent in the chromatography system. It is best to work with pre-conditioned columns and to change solvents with the columns by-passed. The Optilab T-rEX may be used as a monitor

during this process, to ensure that solvents are thoroughly flushed through the detector. “Purging the Optilab T-rEX” on page 4-9 describes solvent change procedures.

The pump pressure should be noted regularly, and the system should be checked for any short-term variations in flow rate, eluent, or operating temperature. If variations are discovered, appropriate action should be taken as recommended in the user’s manual for the questionable component.

For further information regarding injection procedures, pumps, thermostats, etc., refer to the various instruction manuals for these devices and to the broad literature on HPLC.

7.2 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 (0.2–0.5 μm filter) and the use of a guard column is highly recommended.
- Degassing improves the quality of the solvent/sample analysis.

7.3 Measurement

1. Prior to injecting a sample, confirm that the baseline is stable.
2. Zero the instrument by navigating to the LCD display **Main** tab and pressing Enter on the **Zero** button.

8

Service and Maintenance

This chapter provides detailed instructions on minor maintenance tasks that can be performed by the user on the Optilab T-rEX.

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8.1 Replacing the LED/Fiber Light Source	8-2
8.2 Cleaning After a Fluid Leak	8-10
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8.1 Replacing the LED/Fiber Light Source

The Optilab T-rEX contains a light source consisting of a Light Emitting Diode (LED) coupled to an optical fiber. The wavelength of the light emitted from the LED defines the measurement wavelength. You can change the measurement wavelength of the Optilab T-rEX by changing the LED/fiber light source. If the replacement LED/fiber light source has a different wavelength than the current light source, you will need to change the wavelength setting on the Optilab T-rEX front panel.

It may also be advisable to change the LED/fiber light source if the optical power output from the LED/fiber has significantly diminished and the instrument performance is being compromised. Refer to **Chapter 9, “Troubleshooting”** to determine if the LED/fiber light source needs to be replaced.

Use the steps in the subsections that follow to change the Optilab T-rEX LED/fiber light source.

Items required:

- 1.5 mm hex driver (provided with the instrument)
- 2.0 mm hex driver (provided with the instrument)
- 2.5 mm hex driver (provided with the instrument)
- Replacement LED/fiber light source

Setting the Percent Max Power to Zero

1. On the Optilab T-rEX front panel, go to the **System** tab. Press Tab to move to the **LED** Power field, set the value to 0, and press Enter.

2. Tab to the **LED** button and turn the LED Off.

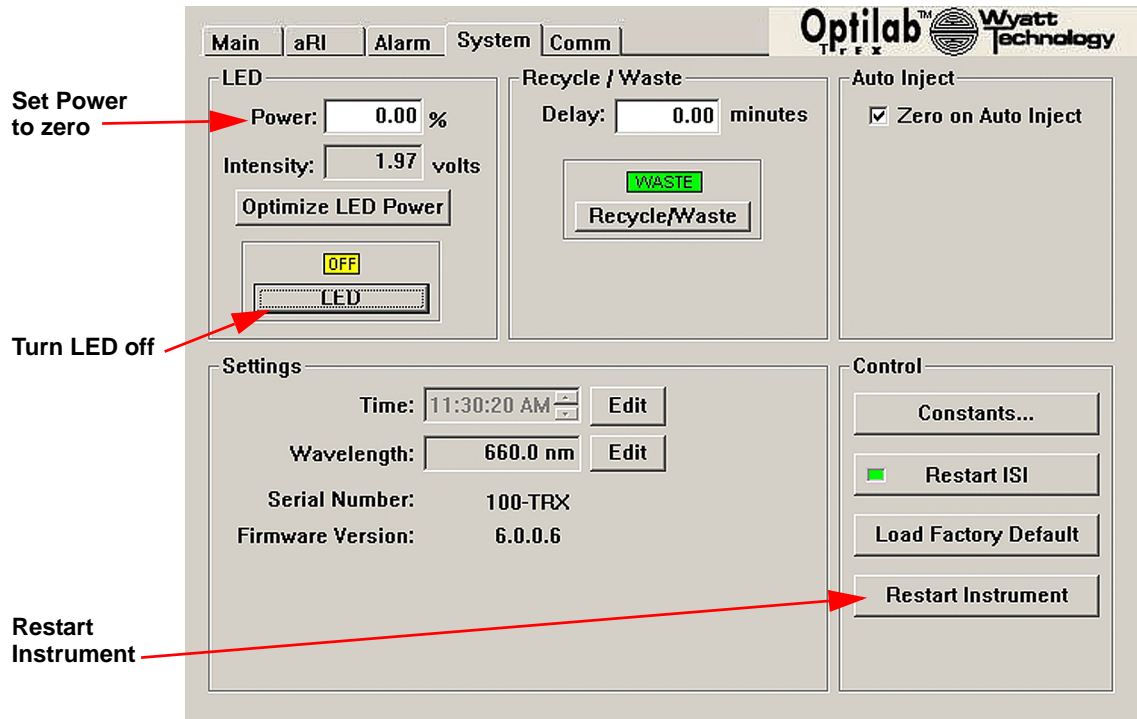


Figure 8-1: LED button locations

Note that the Optilab T-rEX Low Light Alarm is activated when you set the LED percent max power to 0.

3. Tab to the **Restart Instrument** button (also in the **System** tab), press Enter and allow the Optilab T-rEX to shutdown and restart. This ensures that the LED max power setting is saved at 0%.
4. After the instrument has fully restarted, power off the Optilab T-rEX and **disconnect the power cord from the instrument**.

Removing the Old LED/Fiber Light Source

CAUTION: It is strongly recommended that you wear an anti-static wristband whenever you open the Optilab T-rEX to help prevent potential damage to the instrumentation from electrostatic discharge.

WARNING: Be sure that the power cord is disconnected from the instrument before removing the cover.

1. Using a 2 mm hex driver, remove the four screws securing the Optilab T-rEX cover to the chassis. Then, remove the cover.

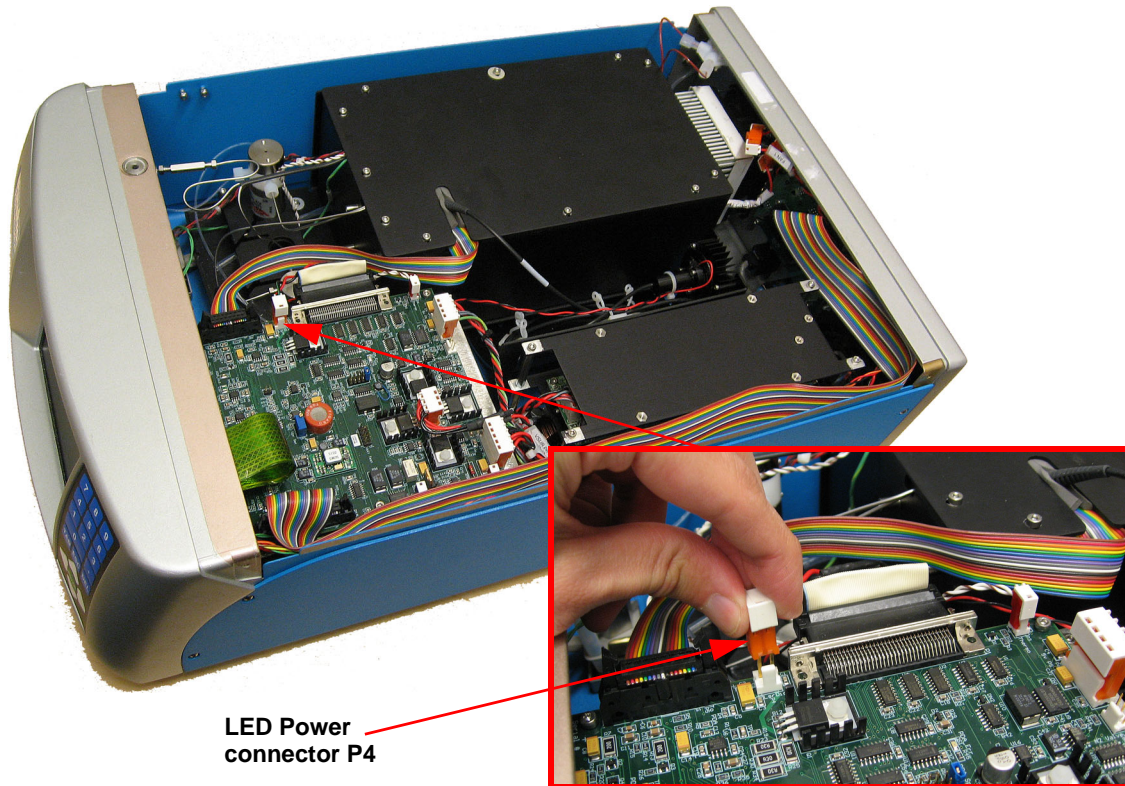


Figure 8-2: Cover removed

2. Gently disconnect the LED/fiber light source two-pin connector from the utility board at the spot labeled P4.

3. Using a 2.5 mm hex driver, unscrew all eleven screws on the thermal housing top plate. Remove the top plate and set it aside.

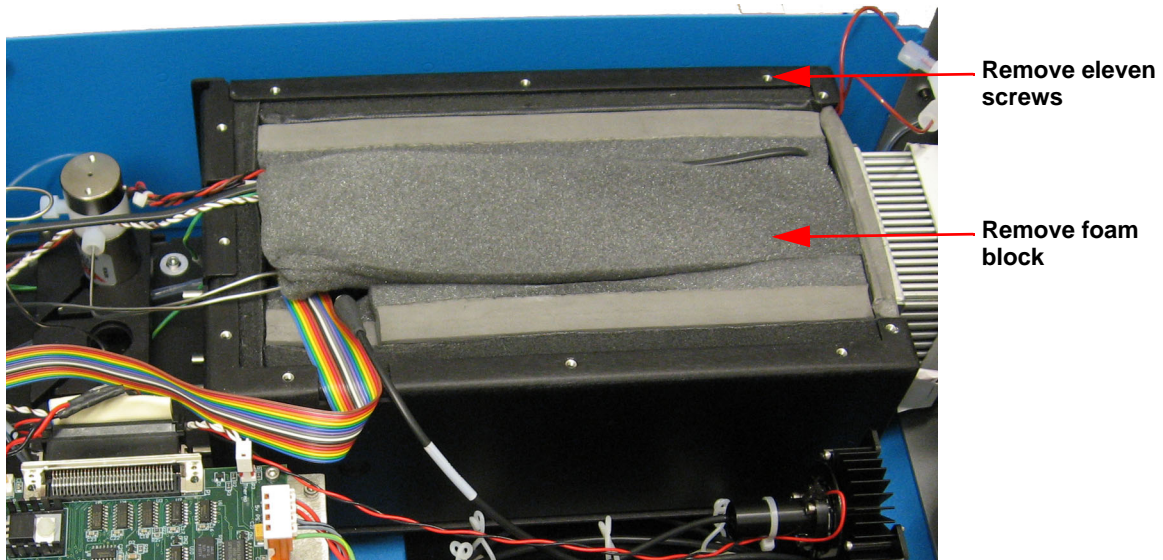


Figure 8-3: Thermal housing top plate removed

4. Remove the top most block of thermal foam. Set aside for reuse. This exposes the optical fiber connection port.

Optical fiber connection port

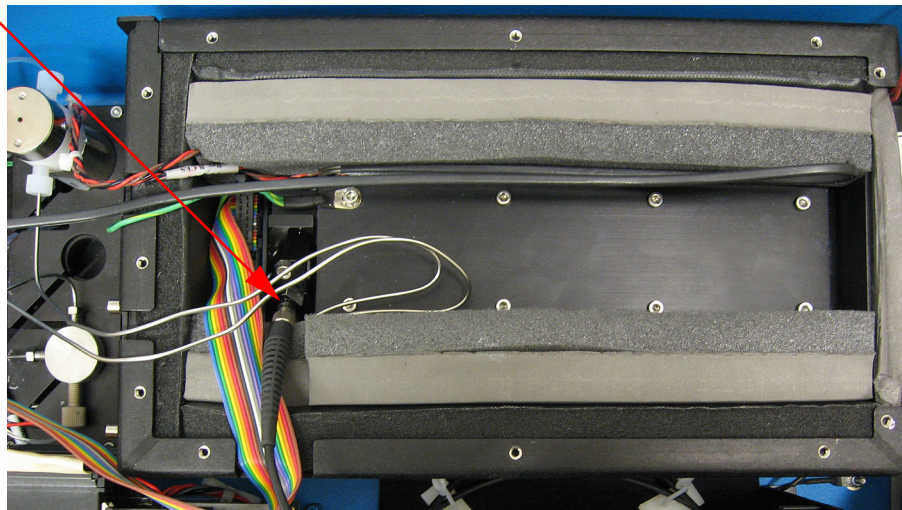


Figure 8-4: Optical fiber connection port exposed

5. By hand, gently unscrew the optical fiber at the optical fiber connection port. Once it is fully unscrewed, slide the optical fiber out of the thermal housing.

**Unscrew and remove
from housing**

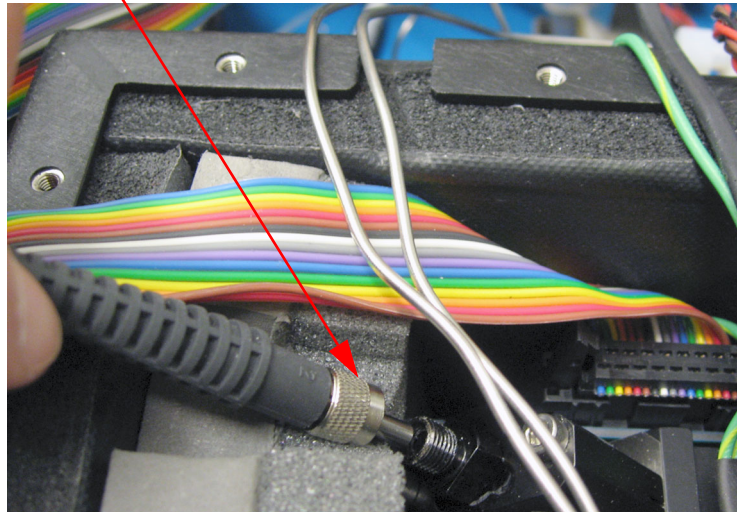


Figure 8-5: Removing light source

6. Open fiber holders and gently unravel light fiber from spool.

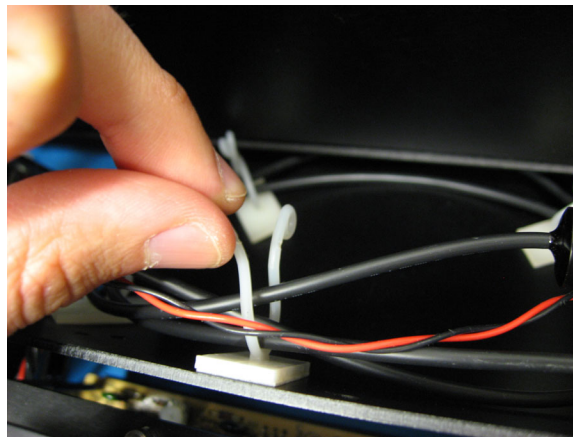


Figure 8-6: Fiber holder

7. Using a 1.5mm hex driver remove all three screws holding the LED/fiber heat sink to the fiber spool.

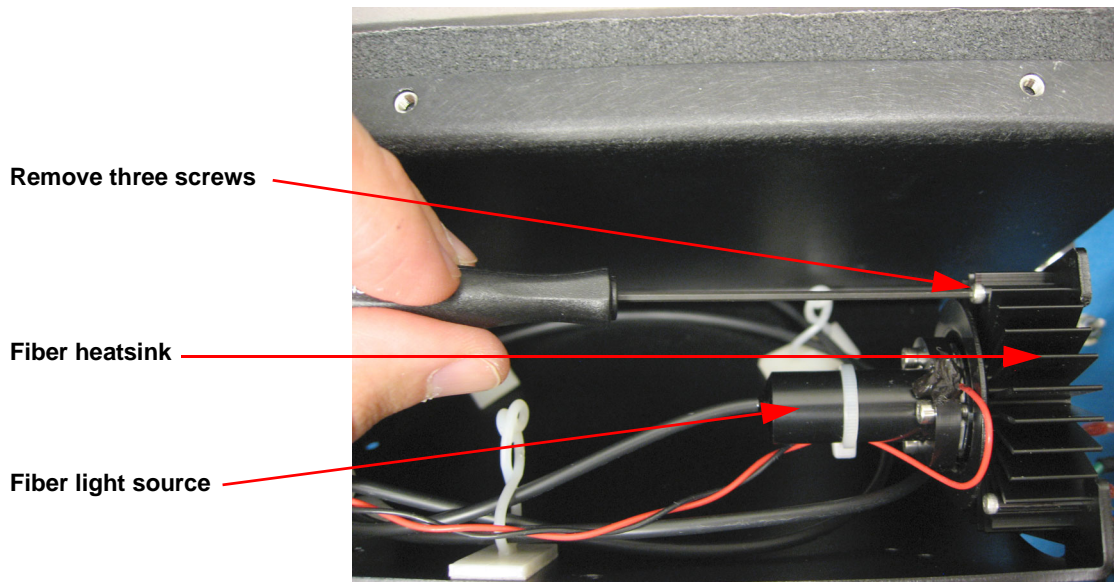


Figure 8-7: Removing light source from fiber spool

8. Remove the LED/fiber light source from the Optilab T-rEX.

CAUTION: Unless you are replacing a damaged LED/fiber light source, you should store the removed fiber in a safe place. Take care to ensure that extra curvature is not introduced into the fiber during storage; this helps prolong the LED/fiber light source lifetime.

Installing the New LED/Fiber Light Source

1. Using the 1.5mm hex driver attach the new LED/fiber heat sink to the fiber spool.
2. Gently coil the fiber onto the fiber spool using the attached fiber holders. Be careful not to introduce any additional curvature to the optical fiber during the process.

For proper coiling position see Figure 8-8.

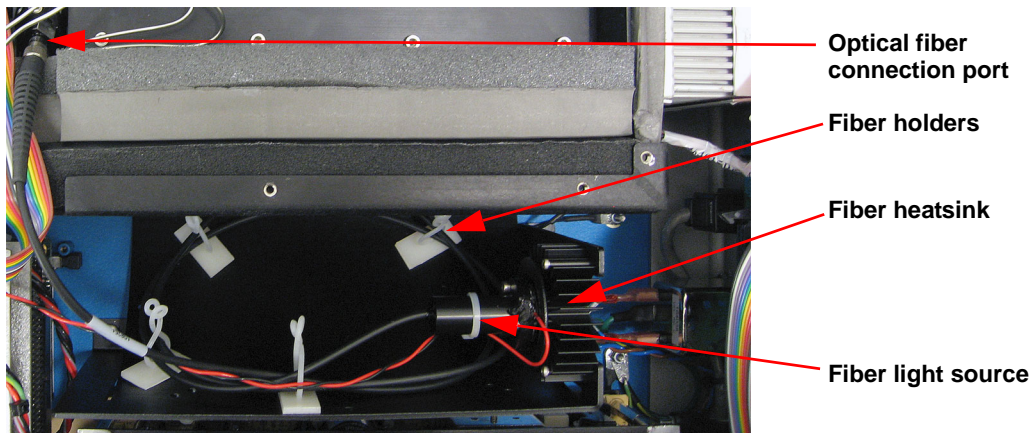


Figure 8-8: Properly installed light fiber

3. Slide the new optical fiber connector into the connection port; the fiber should slide easily into place.
4. Gently screw the new fiber into the connection port (clockwise). Ensure a tight connection by hand, but do not over-tighten. The connector threads should meet very little resistance while being screwed into the connection port.
When secured, no connection threads should be visible.
5. Connect the two-pin LED/fiber light source connector to the circuit board at the position marked P4. (See Figure 8-2 for location.)
6. Replace the foam block removed earlier back on top of the Optilab T-rEX optical bench.
7. Align the tubing, cables, and ribbon cable with their respective slots in the thermal housing. Replace the thermal housing top plate on top of the thermal housing. Using a 2.5 mm hex driver, secure all 11 screws into the thermal housing. Be careful not to pinch any cables or tubing.
8. Replace the Optilab T-rEX cover and seal it with the four screws.
9. Connect the power cord, and switch the power on.

CAUTION: Do not adjust the LED percent max power before changing the wavelength setting (if necessary) as described in the following paragraphs.

Changing the Wavelength Setting

If the new LED/fiber light source has a different wavelength than the previous light source, you need to change the wavelength setting on the Optilab T-rEX front panel.

1. Navigate to the **System** tab on the Optilab T-rEX front panel. Tab to the **Wavelength Edit** button and press Enter.

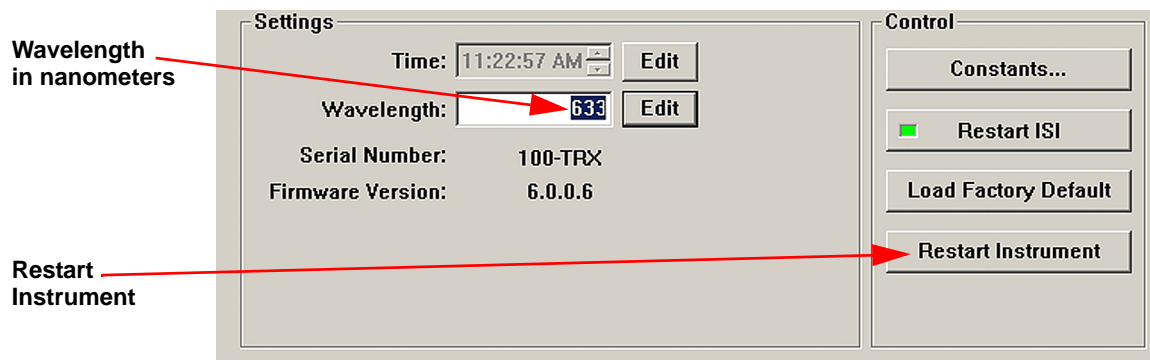


Figure 8-9: Wavelength Edit button location

2. In the **Wavelength Edit** box, enter the new wavelength in nm, then press **Enter**. The new wavelength will be shown in the **Wavelength** field of the **System** tab.
3. To ensure that the LED **Wavelength** setting is saved, restart the device by navigating to the **Restart Instrument** button in the **System** tab and allowing the Optilab T-rEX to shutdown and restart.

Optimizing the LED/Fiber Light Source

Note: If the Optilab T-rEX was disconnected from flow during the replacement procedure, flush the flow cell with your mobile phase before running the optimization procedure. The LED Optimization procedure fails if there is a bubble in the flow cell.

1. Navigate to the **System** tab on the Optilab T-rEX front panel. Tab to the **Optimize LED Power** button and press Enter.

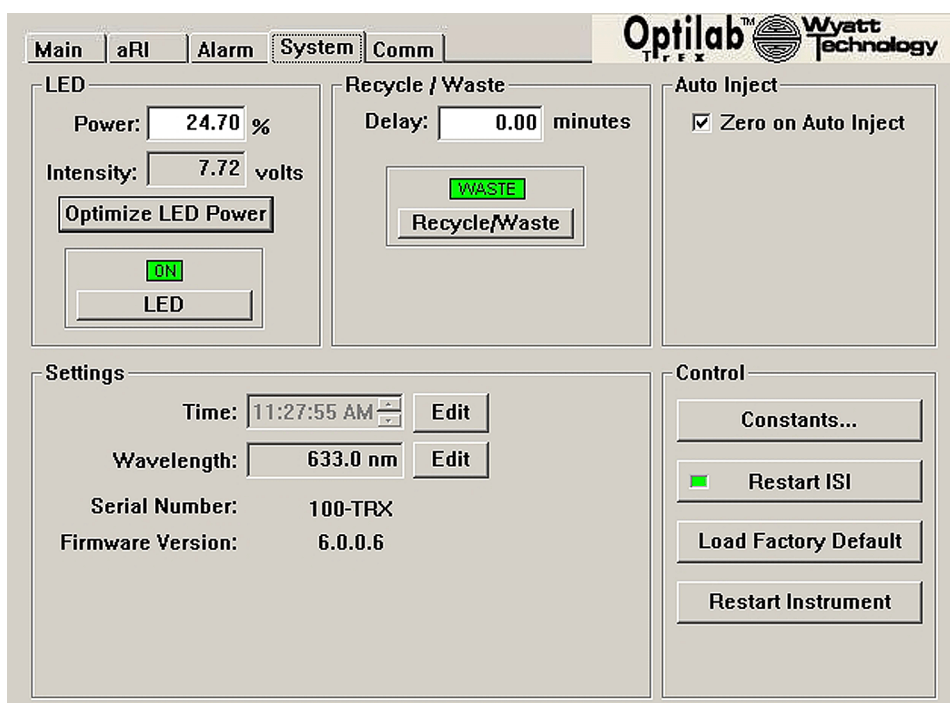


Figure 8-10: Optimize button location

The optimization process iteratively evaluates your system's optimum LED settings.

8.2 Cleaning After a Fluid Leak

Liquid from leaks of internal or external fittings is directed to a liquid leak well inside the instrument. If a liquid leak occurs, an optical liquid sensor in that well senses the presence of the liquid and activates an alarm that may be seen on the LCD display (see “Alarms Tab” on page 5-7 for a description of the **Alarms** tab), and activates an Alarm Out signal that may be used to disable a pump.

The well in which the liquid collects has two chambers. The chamber directly beneath the liquid leak detector, which is also the one that is more difficult to access, is the chamber that needs to be emptied and cleaned (see Figure 8-11).

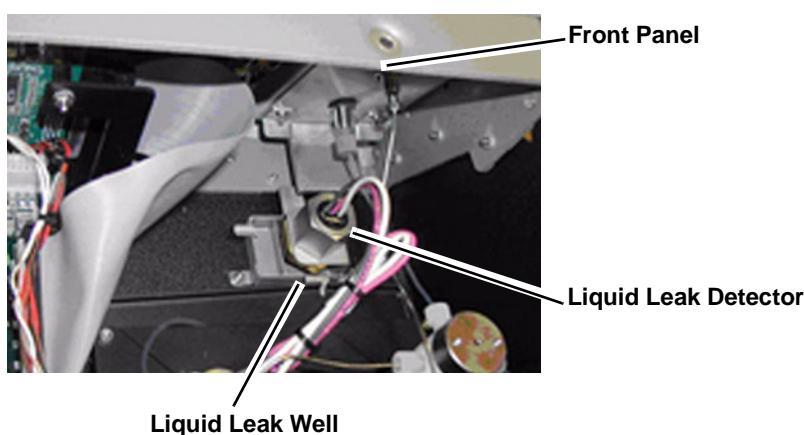


Figure 8-11: Liquid Leak Chamber

To clean a liquid leak, follow these steps:

1. Disable the pump or otherwise prevent additional liquid from being sent into the Optilab T-rEX.
2. Disconnect the power cord from the instrument.
3. Using a 2mm ball driver, remove the four screws that secure the cover to the instrument.
4. Lift the cover at the front and pull forward to remove.

CAUTION: Be sure to wear gloves and other protection appropriate for handling the solvent that has leaked.

5. Using absorbent paper or cotton, soak up as much as possible from the liquid leak well.

When the liquid leak well has been emptied, the liquid leak alarm should turn off. A slight wetting of the well should not be sufficient to set off the liquid leak alarm.

6. If the liquid leak well contains residue from the leak, use a syringe to flush the liquid leak well with a pure solvent that is co-miscible with the solvent that has leaked (see “Purging the Optilab T-rEX” on page 4-9 for a list of solvent miscibilities).
7. Repeat step 5 to remove any pure solvent you used to clean the liquid leak well.
8. Install the cover and secure it with the four screws you removed in step 3.

8.3 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 on the left (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 panel of the instrument.

8.4 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 AC Power module using a small blade screwdriver or similar tool.
3. Replace the burned out fuse(s). Refer to paragraph 4.1.1 for fuse specifications. Both fuses must be installed for the instrument to operate correctly.
4. Replace the cover of the AC Power module and reconnect the power cord.

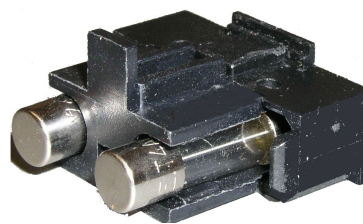


Figure 8-12: Fuseholder and Fuses

8.5 Optilab T-rEX Firmware Upgrades

For peak performance and stability of your Optilab T-rEX, check regularly for the most recent firmware. The rEX firmware upgrades are available at the wyatt.com support center. You will be required to log in. For help logging in, visit the www.wyatt.com support center signup page or send email to info@wyatt.com.

9

Troubleshooting

This chapter provides details about troubleshooting procedures for the Optilab T-rEX.

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9.1 Low Light Alarm

A low light alarm occurs if the light intensity on the photodiode array drops too low. Such a situation occurs any time the refractive index difference between the sample and reference fluids is large enough to cause the light beam to move off the end of the photodiode array.

This happens in the following situations:

- You are changing solvents.
- The sample fluid refractive index is very different from the reference fluid refractive index.
- The sample refractive index is changing dramatically, such as when a very concentrated solution is injected directly into the Optilab T-rEX. When injecting a very concentrated solution directly into the Optilab T-rEX, the low light alarm may sometimes be observed at the leading and trailing edge of the injection. A low light condition during the leading and trailing edge of an injection does not observably effect the accuracy of the measurement.

The low light alarm activates if the voltage measured from the photodiode array, which is proportional to the light intensity on the photodiode array, drops below a threshold value of 5 V for 660 nm light sources.

With the light source off, the voltage measured from the photodiode array is about 2 V. The maximum (saturation) voltage from the photodiode array is about 8 V, regardless of the intensity of the light on the array.

The voltage measured from the photodiode array may be seen by navigating to **System** tab, and observing the value in the Intensity box. The value of this voltage can give an indication of the problem causing a low light alarm.

In addition to the expected low light condition that arises when changing solvents or injecting a concentration that is beyond the capabilities of the Optilab T-rEX, other reasons the light intensity on the photodiode array may be too low are as follows:

- **LED Off:** On the **System** tab, the **LED** button allows you to turn the LED/fiber light source On and Off. Check that the LED/fiber light source is On.
- **Bubble in the Flow Cell:** If the LED light source is on, but the voltage read from the photodiode array is low, there may be a bubble in the flow cell. Typically a bubble in the flow cell causes intermittent episodes of low light and large noise. To prevent air bubbles from forming in the flow cell, be sure to use degassed solvents.

To dissolve air bubbles present in the flow cell, flow degassed solvent through the Optilab T-rEX in purge mode. Purge in this state for several hours to be sure that the bubble is 100% dissolved. Temporarily changing to a different solvent is another strategy for removing an air

bubble, particularly when troubleshooting bubbles in aqueous solvents. Introducing an alcohol, for example, breaks the surface tension of a bubble.

- **Light source dimmed with time:** If the LED light source is on, and the voltage read from the photodiode array is stable but slightly below a threshold value, the light source may simply have dimmed somewhat with time. Increasing the power to the light source increases the intensity of light on the photodiode array. When the voltage read from the photodiode array rises above the threshold value, the Low Light alarm deactivates. On the **System** tab, press the **Optimize LED Power** button to have the instrument automatically set the optimal power to the LED/fiber light source. Changing the power to the light source does not effect the instrument calibration constant.
- **Light source burned out:** If the LED light source is On and the system has been carefully purged with degassed solvent, but the voltage read from the photodiode array is still near 2 V, then the light source may be burned out. The light source is easily replaceable, taking about 15 minutes from start to finish. Contact Wyatt Technology Corporation for a replacement LED/fiber light source.

9.2 Temperature Will Not Set Below 20 C

For sub-ambient operation, dry gas must be slowly flowed into the cool thermal environment to prevent water vapor from condensing on the electronics or optics.

The Optilab T-rEX contains a gas pressure sensor to measure the pressure of the dry gas supplied to it. It will not set the temperature set point below 20 °C unless at least 20 psi of pressure is sensed. If dry gas of sufficient pressure is supplied to the Optilab T-rEX and the temperature is set below 20 °C, but the gas pressure subsequently drops below 20 psi, then the temperature set point is automatically set to 20.5 °C.

9.3 Noisy Baseline

If the baseline noise of the instrument seems excessive, try the following:

1. Check that the LED light source is On, and that no Low Light Alarm exists as described in “Low Light Alarm” on page 9-2.
2. Un-plumb the Optilab T-rEX from any flow system. Place a plug in the OUT port, and leave the IN port open to the atmosphere. See if the noise still exists.

If the noise no longer is present, then the noise could have its origin in the chromatographic system, or could be due to a small bubble in the flow cell that is forced into the measurement region only under flow conditions. Use a syringe to inject solvent by hand into the Optilab T-rEX. If the noise is still not present, then the noise probably has its origins in the chromatographic system. Refer to “Conditions for In-Line Operation” on page 7-2 for ways to reduce noise in chromatographic systems.

If the noise is intermittent during flow conditions, or remains in the absence of flow, the noise may be due to a bubble in the flow system that is small enough to not block light through the system, but large enough to significantly effect the measurement. Follow the procedure for eliminating bubbles outline in “Low Light Alarm” on page 9-2.

If the baseline noise persists, contact Wyatt Technology Corporation.

9.4 Unexpected RI trace

The Optilab T-rEX LED beam image must be measured and fitted properly in order for the recorded data to be accurately interpreted over the full range of measurement. If the LED intensity is too strong or too weak, a proper beam image will not be discernible by the internal optical detection device. The “low light” alarm was discussed in a previous section. An overly powerful LED will cause sudden jumps in an RI chromatogram, especially as the signal moves up and down along the sides of an eluting peak. Such data is not valid for quantitative analysis. An obscured beam (due to a heavily soiled flow cell) may result in a scattered, irregular trace, but this effect is rarely seen.

If unexpected data traces are observed, the LED power should be checked as described in “Optimizing the LED/Fiber Light Source” on page 8-9, and the Optilab T-rEX flow cell should be flush-cleaned with a solution such as Contrad. Optilab T-rEX firmware features automatic optimization of the LED power for improved reliability, making it essentially impossible to overpower an Optilab T-rEX LED.

The goodness of the rEX measurements may be assessed by viewing the “peak lock” and “fit peak lock” traces in an ASTRA data collection. In ASTRA, click Experiment | Graph | Add Custom Plot, and add the peak lock and fit peak lock traces from the “Original Data” category. See the ASTRA user guide for additional instructions for creating a custom graph, if needed. If a good rEX optical beam image is found for a given measurement slice, peak lock will be 1.00. If the components of the beam image are each successfully fit, then fit peak lock is also 1.00. If values lower than 1 are obtained for peak lock or fit peak lock in a given data slice, then the LED is possibly too bright, too dim, or the optics, cell, or cell contents are obscuring the beam.

9.5 High Pressure

A short length of 0.007"i.d. (inner diameter) tubing is connected to the inlet union inside the Optilab T-rEX chassis. This relatively narrow tubing, in the instrument by design, is a likely place for a blockage to form. The expected pressure generated by an Optilab T-rEX is roughly 50psi in water or aqueous buffer flowing at 1mL/min and 25C. This value will be higher or lower depending on the solvent, flow rate, and temperature used.

If a blockage is suspected, a good first troubleshooting step is to replace this 0.007"i.d. tubing. If high pressure persists, contact Wyatt Customer Support for assistance.

Caution: Never flush the Optilab T-rEX in reverse direction! Liquid should only be pumped into the Optilab T-rEX **IN** port while the Optilab T-rEX is powered on and fully initialized. Pumping liquid into the **OUT** port may cause serious damage to the Optilab T-rEX.

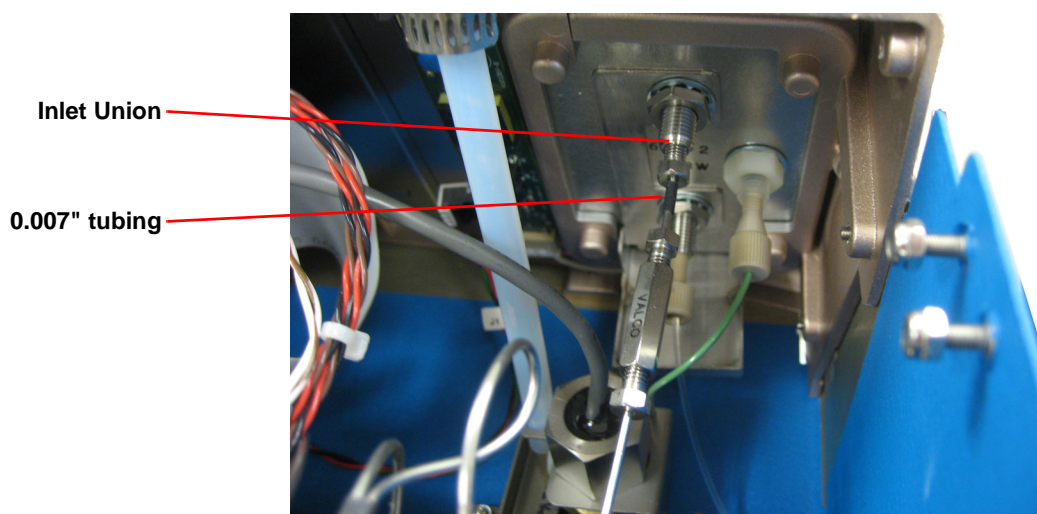


Figure 9-1: Optilab T-rEX with Top Cover Removed

9.6 Microsoft Windows Encounters Problems

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

Experiments in progress when the operating system crashes must be restarted.

A Acronyms and Abbreviations List

This appendix contains a list of acronyms and abbreviations used in this manual.

CONTENTS	PAGE
A.1 Acronyms and Abbreviations.....	A-2

A.1 Acronyms and Abbreviations

The following acronyms and abbreviations are used in this manual:

Item	Meaning
AC	alternating current
Amp	Ampere
aRI	absolute refractive index
° C	Centigrade
cm	centimeters
DC	direct current
DHCP	Dynamic Host Configuration Protocol
dRI	differential refractive index
g	gram
GPa	Gigapascal
HPLC	high pressure liquid chromatography
Hz	Hertz
ID	inside diameter
in.	inch
IP	Internet Protocol
LAN	local area network
LED	light emitting diode
mg	milligram
MHz	Megahertz
MPa	Megapascal
μL	microliter
μm	micrometer
mL	milliliter
mm	millimeter
mV	millivolt
nm	nanometer
NMOS	n-doped metal/oxide semiconductor
OD	outside diameter
ppm	parts per million
psi	pounds per square inch
RI	refractive index
RIU	refractive index unit
STP	standard temperature and pressure = 0 ° C and 1 atmosphere of pressure
TTL	transistor-transistor logic
V	Volt
VAC	Volts alternating current
VDC	Volts direct current

B Operating Principles and Theory

This appendix provides a basic overview of the theory and operation of the Optilab T-rEX.

CONTENTS	PAGE
B.1 Basic Principles	B-2
B.1.1 Conventional Flow Cell	B-2
B.1.2 Optilab T-rEX Flow Cell	B-3
B.2 Measurement of the Beam Deflection Angle	B-4
B.2.1 Conventional Split Photodiode Based Instrument	B-4
B.2.2 Wyatt Technology Corporation Beam Positioning Technology ..	B-5

B.1 Basic Principles

B.1.1 Conventional Flow Cell

The Optilab T-rEX is a special variation of the deflection-type differential refractometer. For this type of differential refractometer, a light beam passes through a flow cell containing reference and sample fluids, and undergoes an angular deflection based upon the refractive index difference between the two fluids. A conventional deflection type differential refractometer flow cell is shown in Figure B-1.

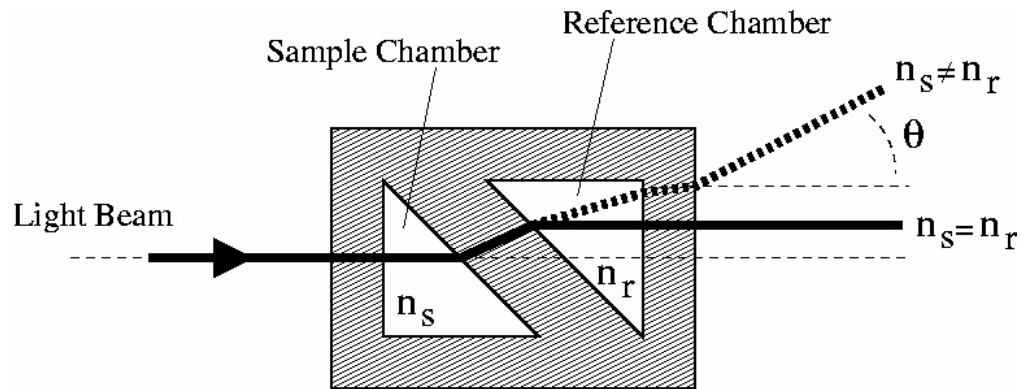


Figure B-1: Conventional deflection type flow cell

For this conventional flow cell, if the sample refractive index n_s and reference fluid refractive index n_r are identical, then the light beam exits the cell in a direction parallel to the incoming beam. If n_s is not equal to n_r , then the light beam exits the cell at some angle θ to the incoming beam. To the first order, this angle is proportional to the refractive index difference between n_s and n_r . In addition to any angular deflection, the light beam undergoes a slight translation due to passage through the material comprising the body of the flow cell (i.e., the window between the sample and reference fluid chambers). The optical system inside the differential refractometer must separate angular deflections from translation of the light beam.

To measure the refractive index difference between two fluids, both sample and reference chambers are filled with the reference fluid, and the starting position of the light beam is noted. This is considered the “zero” position. The reference chamber is then capped, trapping the reference fluid inside. Fluid is then directed through the sample chamber, and changes in the refractive index of that fluid cause an angular deflection of the light beam. Measurement of the light beam angular deflection yields the refractive index difference between the sample fluid and the reference fluid.

B.1.2 Optilab T-rEX Flow Cell

The Optilab T-rEX differs from the conventional design because its flow cell contains a novel feature to allow, under certain conditions, the measurement of the *absolute refractive index* (aRI) of a fluid, as well as the differential refractive index (dRI) between two fluids. The flow cell in the Optilab T-rEX is shown in Figure B-2. The reference chamber has a non-90 angle on its exit face.

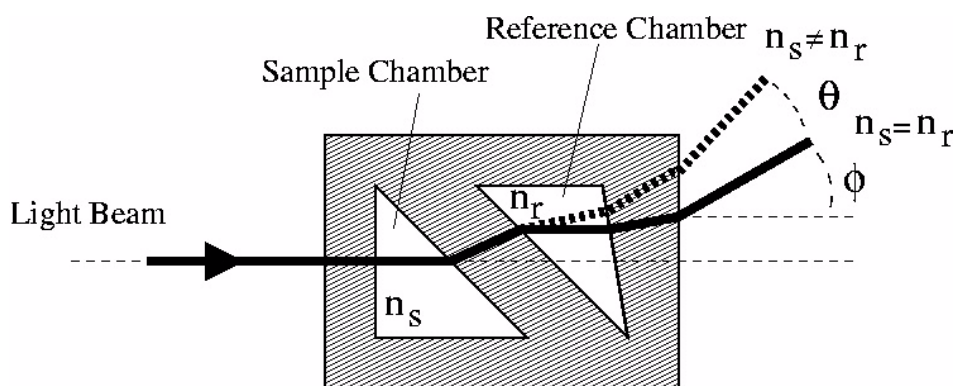


Figure B-2: Optilab T-rEX differential and absolute refractive index flow cell

This unique flow cell has an extra internal wedge in its reference chamber. The extra wedge lends an additional angular deflection ϕ to the light beam. The value of the angle ϕ depends upon the wedge angle and the refractive index difference between the reference fluid and the quartz body of the Optilab T-rEX flow cell. Knowing the wedge angle and the refractive index of the quartz, a measurement of ϕ yields the refractive index n_r of the reference fluid. In practice, the wedge angle is determined by measuring the angular deflection ϕ for a few fluids of known refractive index.

The total angular deflection of the light beam depends upon both the refractive index difference between the sample and reference fluids and the aRI of the reference fluid. To determine the aRI of a fluid, it is required that the *same fluid be present in both the sample and reference chambers*. With the same fluid in the sample and reference chambers, the refractive index difference is zero, and any angular deflection of the light beam is due solely to the aRI of the reference fluid.

Measurement of the refractive index difference between two fluids is accomplished by noting the angular deflection ϕ of the light beam at the beginning of a data run, and considering this the “zero” position of the light beam.

B.2 Measurement of the Beam Deflection Angle

B.2.1 Conventional Split Photodiode Based Instrument

Measurement of the deflection of the beam of light after it has passed through the flow cell is the basic measurement made in a deflection type refractometer. This deflection is proportional to the dRI of the two fluids being measured. Conventionally, a split photodiode is used to measure the light beam angular deflection, as pictured in the illustration below.

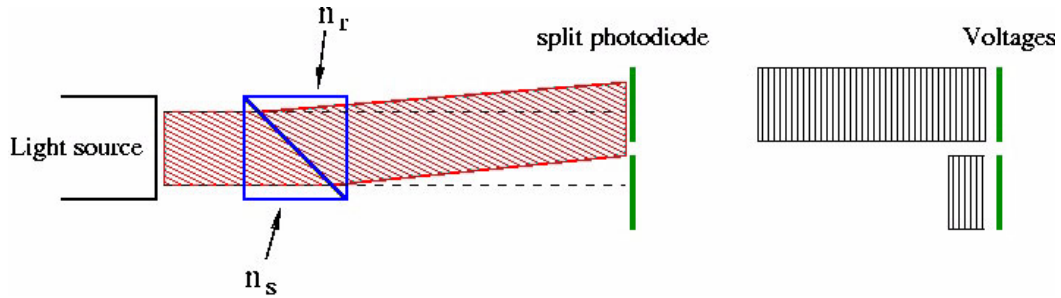


Figure B-3: Light beam angular deflection

The split photodiode contains two photodiode light detectors placed side to side. As the light beam changes angle, the beam moves away from one photodetector and onto the other. The voltage difference between the two detectors gives a measure of the light beam position, which is proportional to the light beam angular deflection, which is in turn proportional to the differential refractive index.

The precision of a split photodiode detector based instrument is limited by the precision with which the voltages may be read from the two photodetectors and the intensity stability of the light source. The maximum signal a split photodiode detector may observe depends upon the width of the light beam and the width of the photodetectors. Once the light beam has walked entirely off of one photodetector and onto the other, there is no way to determine the beam position.

B.2.2 Wyatt Technology Corporation Beam Positioning Technology

Rather than using two photodetectors, the Optilab T-rEX contains 512 light measuring elements in one photodiode array. Each element of the photodiode array, called a pixel, precisely measures the intensity of light impacting upon it.

The Optilab T-rEX records voltages from each of the 512 elements of the photodiode array 10–20 times per second. To obtain the most precise possible measurement, the signal from each element is measured many times and the results averaged together. Hundreds of thousands of voltage measurements per second are required. The data from the 512 pixels are analyzed using mathematical algorithms to determine the position of the light beam on the array.

The computational power to perform these mathematically intensive operations at such rates was unavailable 15 years ago, but now fits into the small Pentium-class processor inside your Optilab T-rEX. Using a photodiode array, high-speed electronics, and advanced mathematical analysis techniques, the light beam position may be measured to less than 10 Angstroms. The photodiode array is 1.3 cm long, and so the beam may move more than a centimeter before it slides off the end of the photodiode array.

C Optilab T-rEX Specifications

This appendix provides specification details for the Optilab T-rEX instrument.

CONTENTS	PAGE
C.1 Specifications.....	C-2

C.1 Specifications

Differential Refractive Index (dRI) Range and Sensitivity:	
Range (RIU)	– 0.0047 to + 0.0047
Peak-to-Peak Noise (RIU)	$\pm 7.5 \times 10^{-10}$ (see note ^a)
Absolute Refractive Index (aRI) Range and Sensitivity:	
Range (RIU)	1.2 to 1.8 RIU
Sensitivity (RIU)	± 0.002 RIU
Fluid Volumes	
Flow Cell:	7.4 μ L
Inlet tubing (0.010" ID):	44 μ L
Outlet tubing (0.030" ID):	370 μ L
Power Requirements:	AC 100/115/220/240 V, 50/60 Hz
Power Rating:	480 volt-amperes, 150 volt-amperes
Dynamic Range:	21+ bits only via digital communication
Response Time:	0.1 to 5.0 seconds, user selectable
Light Source:	660 nm (± 10 nm) LED light source, standard
Sample Temperature Control Range:	4° C to 50° C, temperature regulated
Temperature Regulation:	± 0.005 °C
Maximum System Pressure:	30 psi (2 bar)
Safety Sensors:	Vapor and liquid (leak)
CPU:	2 GHz VIA C7 Processor
Display / User Interface:	6.4" 16-bit color high resolution LCD display
Inputs and Outputs:	
Analog In:	16 bit ± 10 V Analog/Digital for auxiliary user data
Analog Out:	16 bit Digital/Analog for dRI data
Inputs:	Zero, Purge, Recycle, Auto Inject, Alarm In
Output:	Auto Inject Retransmit, Alarm Out External, Solenoid Recycle Valve. 12 VDC solenoid valve (sold separately or user-supplied) to switch automatically between waste and recycle
Digital Communication:	Ethernet
Dimensions:	168 mm x 357 mm x 595 mm (H x W x D)
Weight:	11.5 kg

- a. Short term peak-to-peak noise is measured as per ASTM-E1303-95(2000) using a 4-second time constant and temperature controlling at 25 °C.

D Instrument Connectivity

This appendix provides instrument connectivity instructions are current as of January, 2005. Please visit the www.wyatt.com support center for current information. Login access is required. For login help, visit the www.wyatt.com support center signup page or send email to info@wyatt.com.

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D.5 Connecting via Ethernet When Not on a LAN	D-16
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D.1 Introduction

These instructions contain a pictorial overview for connecting your Optilab T-rEX to a computer for data collection. The instructions are divided into the following topics:

- Components to be used for instrument connectivity (page D-3). All users should read this section.
- Instructions for connecting instruments to a computer via a Local Area Network (LAN) (page D-9). Read this section or the next section depending on your configuration.
- Instructions for connecting instruments to a computer via USB (page D-13).
- Instructions for connecting instruments to a computer that is not on the LAN (page D-16).
- Instrument front panel network settings (page D-18). All users should read this section.
- Accessing instruments via ASTRA V (page D-19). Read this section if you use ASTRA V.
- Trouble shooting and diagnostics (page D-20). Read this section if you experience problems connecting to your instrument.

This appendix provides instrument connectivity instructions are current as of January, 2005. Please visit the www.wyatt.com support center for current information. Login access is required. For login help, visit the www.wyatt.com support center signup page or send email to info@wyatt.com.

D.2 Components Used

D.2.1 Instrument Connections

Figure D-1 shows a detail of the instrument back panel. The yellow arrow points to the Ethernet port, which is to be used for all connections in these instructions.

The built-in USB port exhibits technical problems on some computers, and Wyatt Technology recommends using the Ethernet port instead. Please see “Connecting via USB” on page D-13 for instructions on establishing a USB connection.

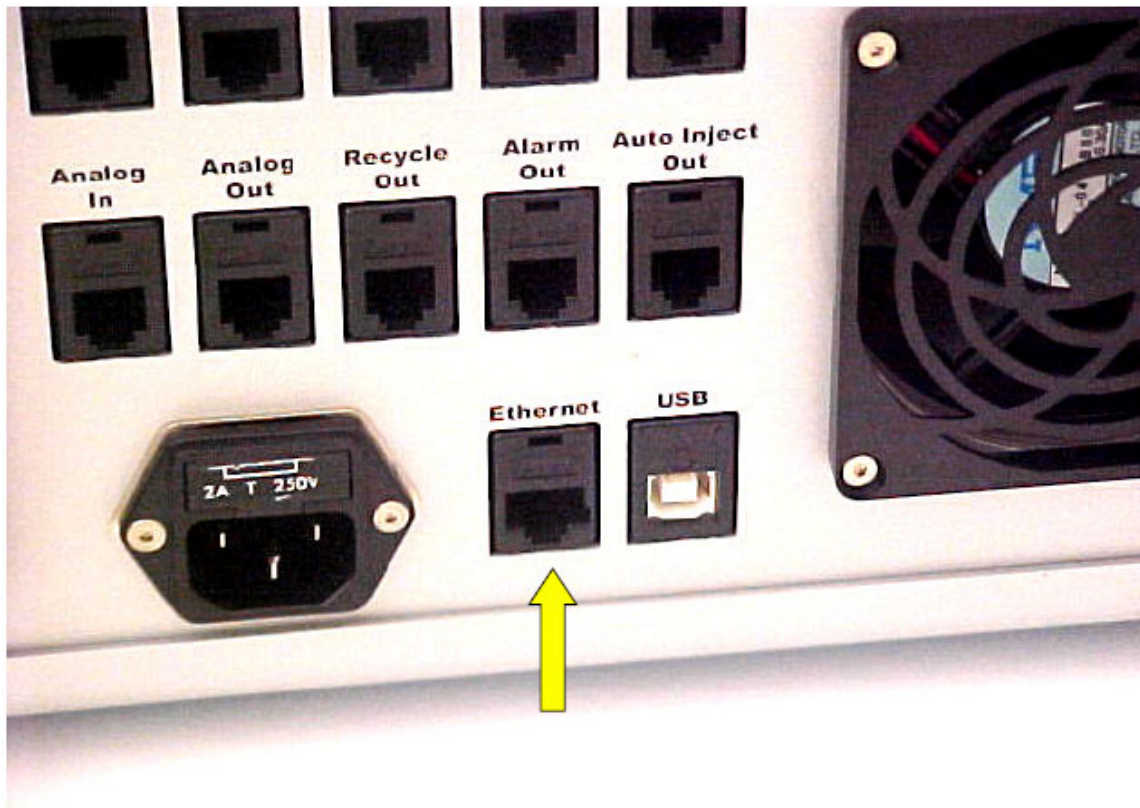


Figure D-1: Detail of the instrument back panel

D.2.2 LAN Connection

Figure D-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.

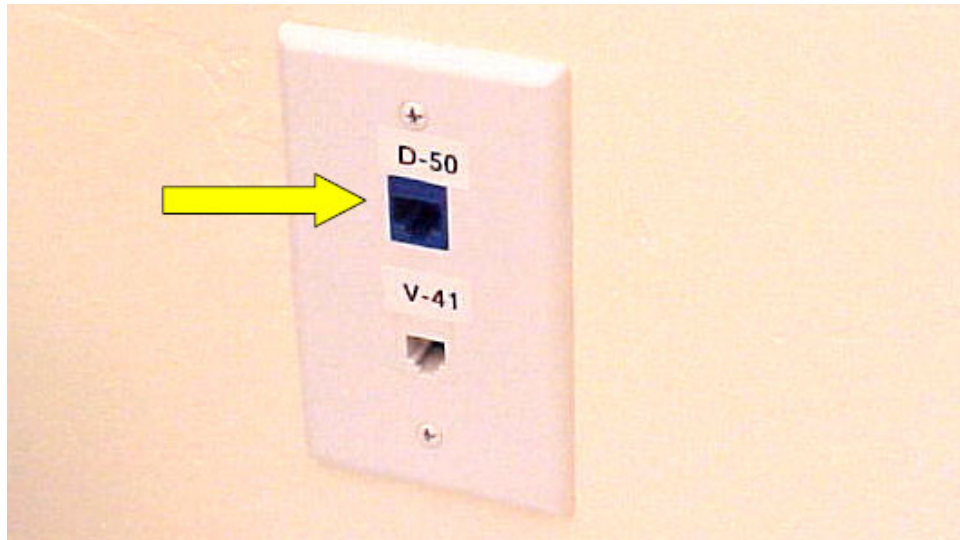


Figure D-2: Wall socket LAN connection

D.2.3 Computer Connections

Computer connections can be made via either an Ethernet or USB port on the computer. “Connecting to a LAN” on page D-9 and “Connecting via Ethernet When Not on a LAN” on page D-16 describe instrument connections made via the Ethernet port. “Connecting via USB” on page D-13 describes connections made via the USB port.

Figure D-3 shows the Ethernet (yellow arrow) and USB (green arrow) ports on a standard laptop computer.

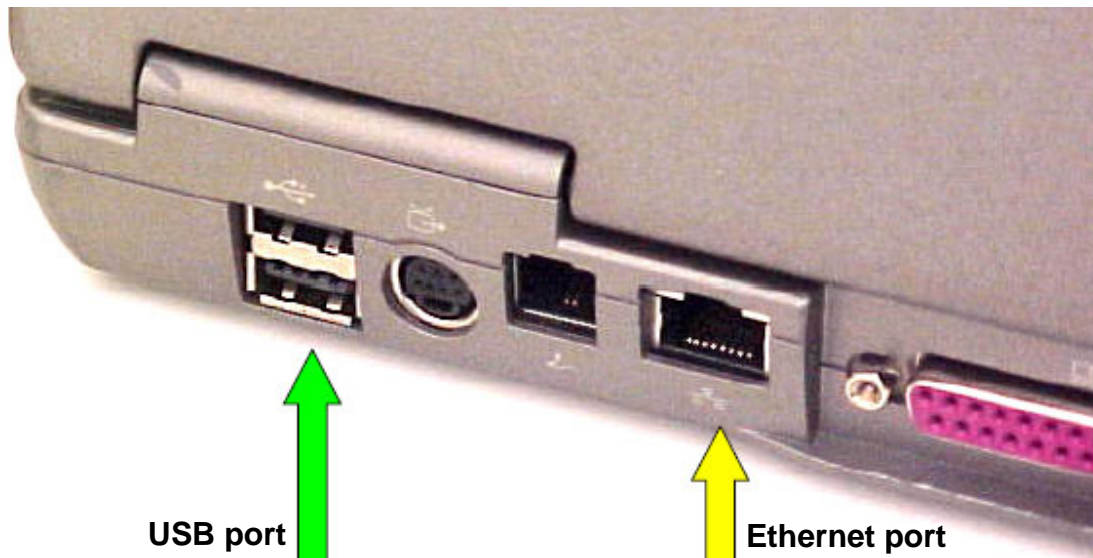


Figure D-3: Ethernet and USB ports on the computer

D.2.4 Crossover Cable

A crossover cable can be used to make a direct connection from the instrument to an Ethernet port on a computer or to an Ethernet to USB adapter.

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 D-4: Ethernet crossover cable shipped by Wyatt Technology

D.2.5 Ethernet Cable

A standard Ethernet cable is sometimes referred to as a patch cable, or a straight-through cable to distinguish it from the crossover cable in Figure D-4. Standard 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.

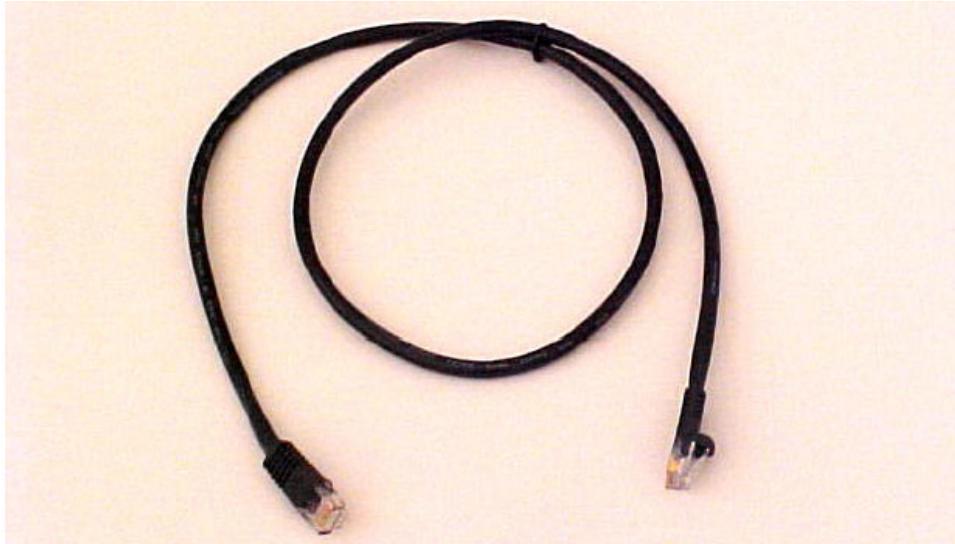


Figure D-5: Standard Ethernet cable

D.2.6 Ethernet to USB Adapter

This device can be used to connect an Ethernet cable to a USB port on the computer. Using this adapter, it is possible to have the computer connected to a LAN via the computer's Ethernet port, and the instruments connected to the computer via USB. The Ethernet to USB adapter supplied by Wyatt Technology will look similar to the one in Figure D-6.

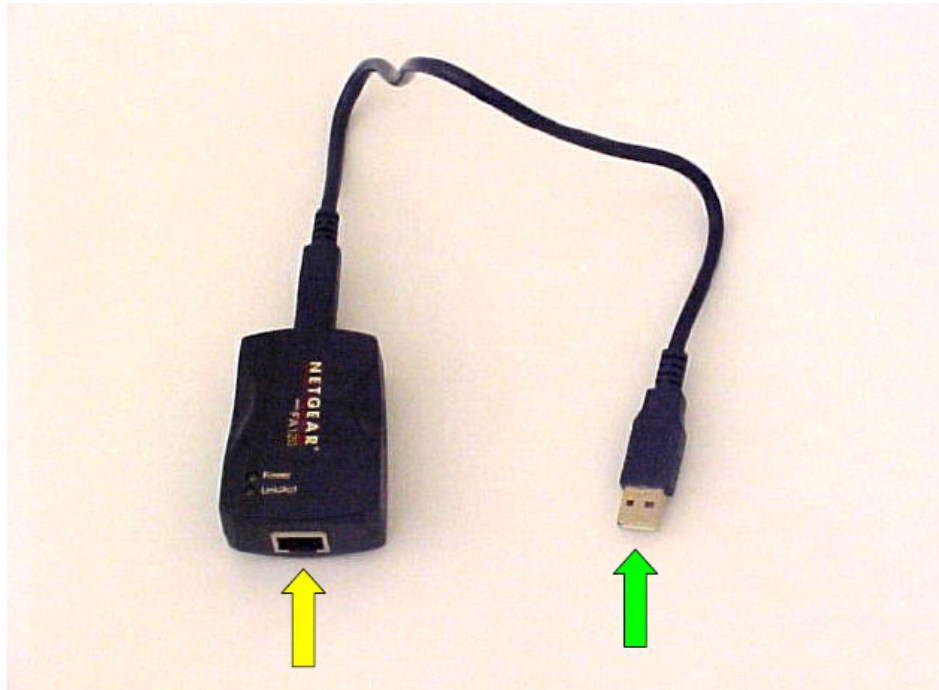


Figure D-6: Standard Ethernet to USB adapter

The Ethernet cable is plugged into the port with the yellow arrow, and the USB plug (green arrow) is plugged into a USB port on the computer.

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.

D.2.7 Ethernet Switch

Ethernet switches are used to connect several Ethernet cables to one resource, such as the LAN socket in Figure D-2.

The Ethernet switch supplied by Wyatt Technology will look similar to the five port switch shown below. Note that Ethernet cables can be connected to the switch in any order or position. Also, the switch has an external AC adapter (not shown) to provide power to the switch.



Figure D-7: Five-port Ethernet switch

D.3 Connecting to a LAN

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

The following sections describe various configurations for connecting one or more instruments to a LAN and possibly to a computer also. (For example, to a computer running ASTRA V.)

These sections use a LAN wall socket like the one in Figure D-2.

D.3.1 One Instrument to LAN

Plug the instrument into a LAN wall socket using a standard Ethernet cable (Figure D-5). The computer that is to communicate with the instrument must be on the same LAN.

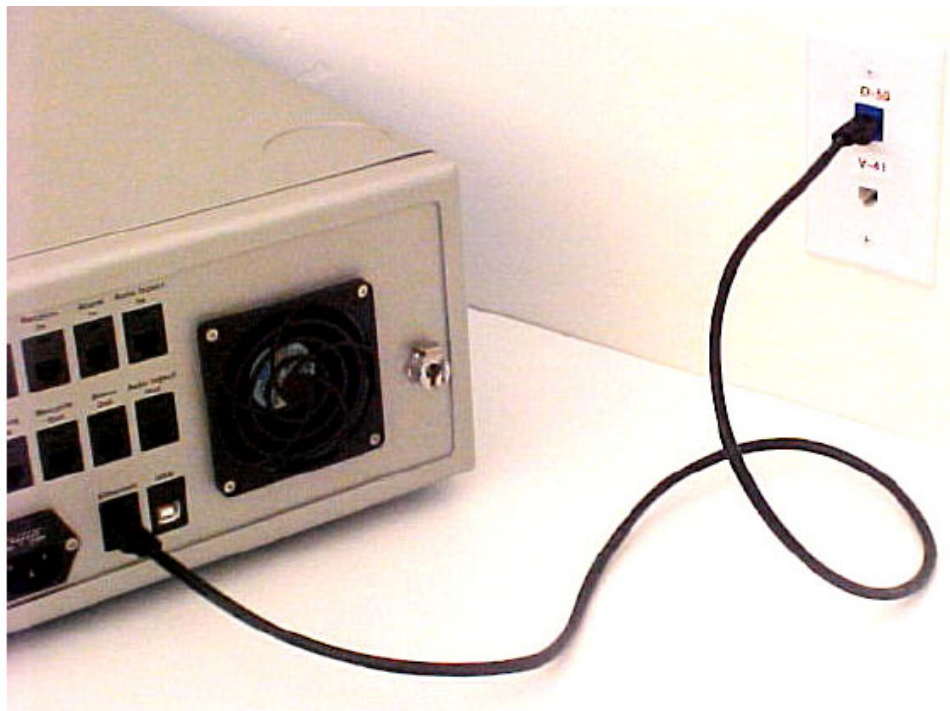


Figure D-8: Connection for one instrument to LAN

D.3.2 One Instrument and Computer to LAN

If there is only one LAN wall socket available for both the instrument and computer, it is necessary to use an Ethernet switch (Figure D-7) 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 D-9: Instrument and computer connected to LAN via Ethernet switch

D.3.3 Multiple Instruments to LAN

If there is only one LAN wall socket available, two or more instruments can be connected to the LAN via an Ethernet switch (Figure D-7). The instruments can be accessed via any computer on the LAN.

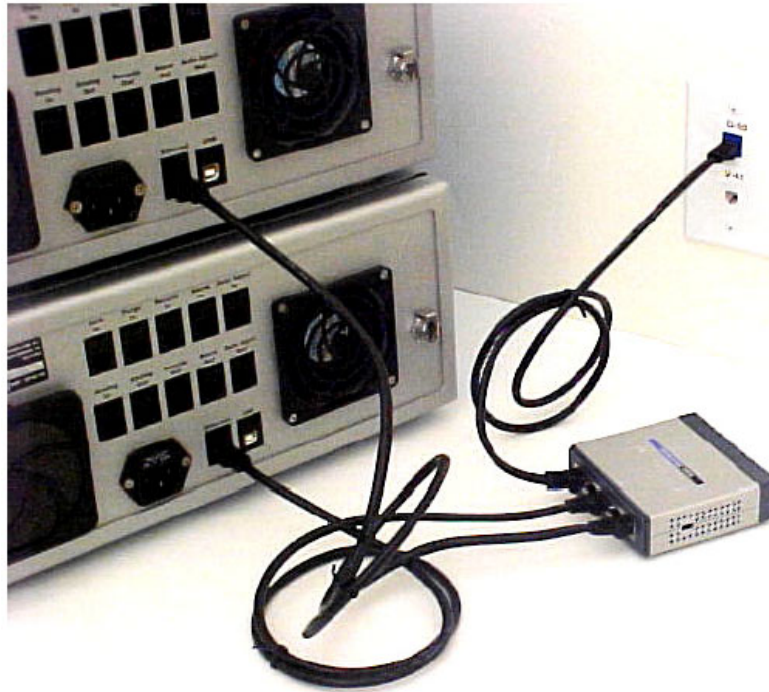


Figure D-10: Two instruments connected to LAN via an Ethernet switch

D.3.4 Multiple Instruments and Computer to LAN

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

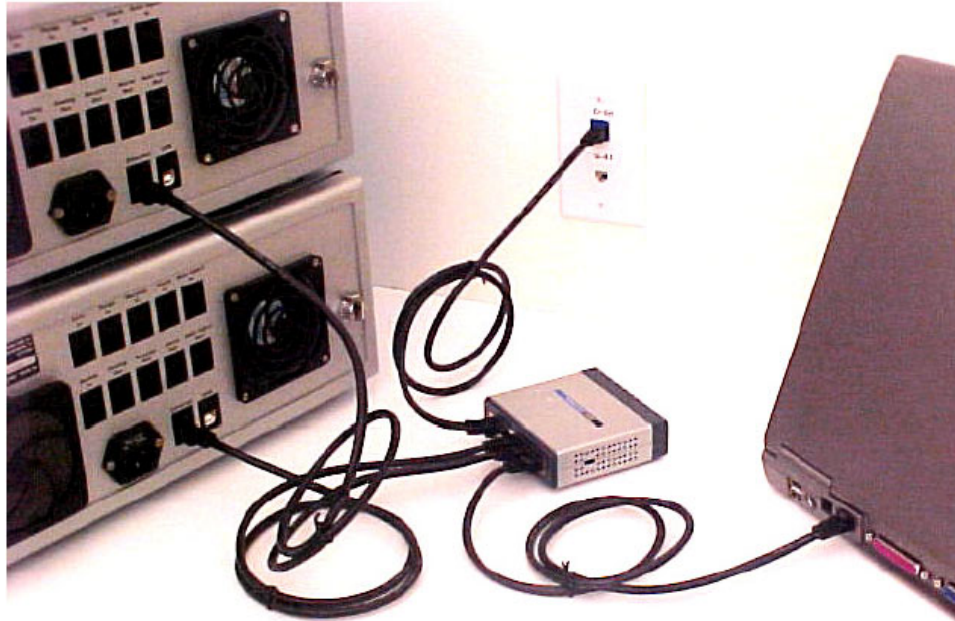


Figure D-11: Two instruments and computer to LAN via an Ethernet switch

D.4 Connecting via USB

If it is not possible or desired to have the instruments connected to a LAN, you can 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.

D.4.1 One Instrument to USB via a Crossover Cable

Connect the yellow crossover cable (Figure D-4) from the instrument to the Ethernet to USB adapter (Figure D-6). Plug the Ethernet to USB adapter into an available USB port on the computer.

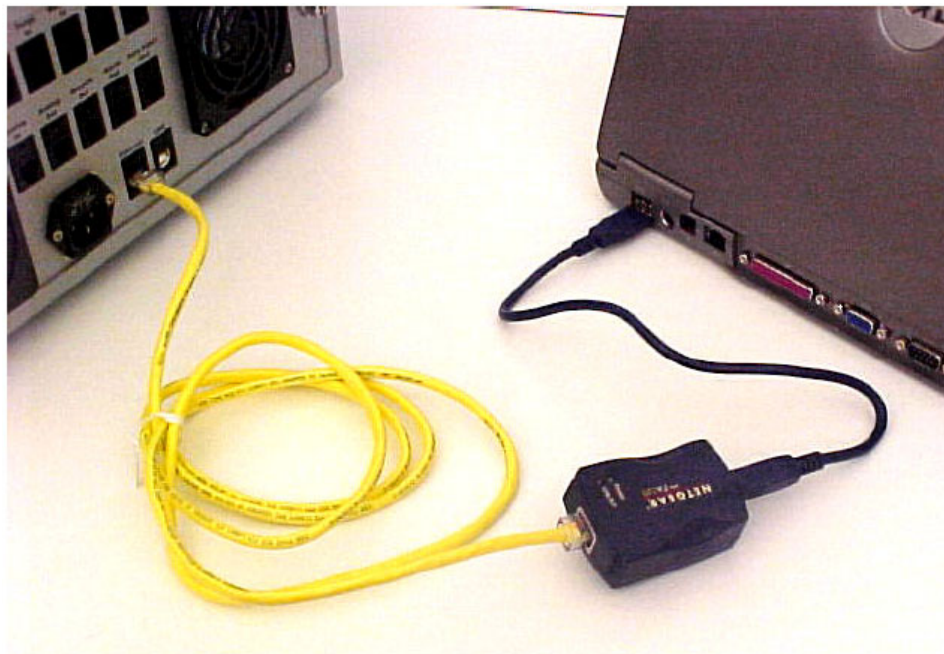


Figure D-12: One instrument to USB via yellow crossover cable

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.

D.4.2 One Instrument to USB Using an Ethernet Switch

Connect the instrument to the Ethernet switch using a standard Ethernet cable (Figure D-5). 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.

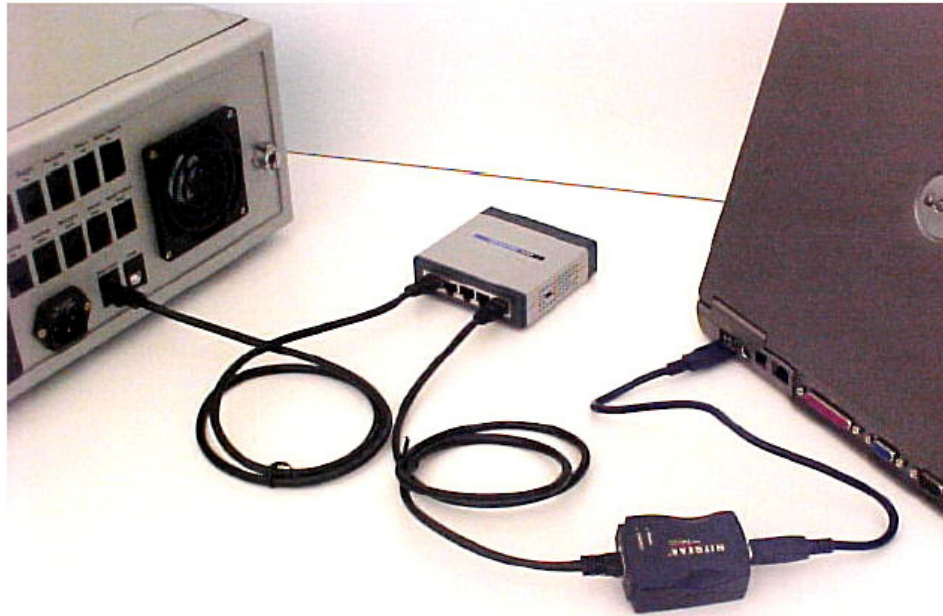


Figure D-13: Connecting one instrument to USB using an Ethernet switch

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.

D.4.3 Multiple Instruments to USB

Two or more instruments can be connected to USB using an Ethernet switch. Use a standard Ethernet cable to plug each instrument into the Ethernet switch. Then connect the Ethernet switch to the Ethernet to USB adapter using a standard Ethernet cable. Plug the Ethernet to USB adapter into an available USB port on the computer. Plug the Ethernet to USB adapter into an available USB port on the computer.



Figure D-14: Connecting two instruments to USB via Ethernet switch and adapter

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.

D.5 Connecting via Ethernet When Not on a LAN

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

D.5.1 One Instrument to Computer Using Crossover Cable

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

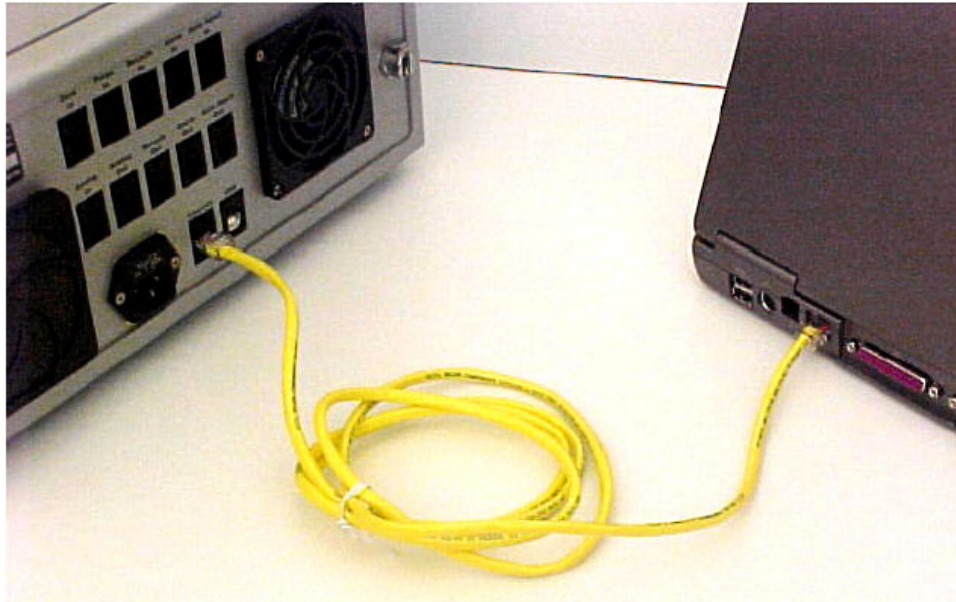


Figure D-15: Connecting one instrument to computer via yellow crossover cable

D.5.2 One Instrument to Computer 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.

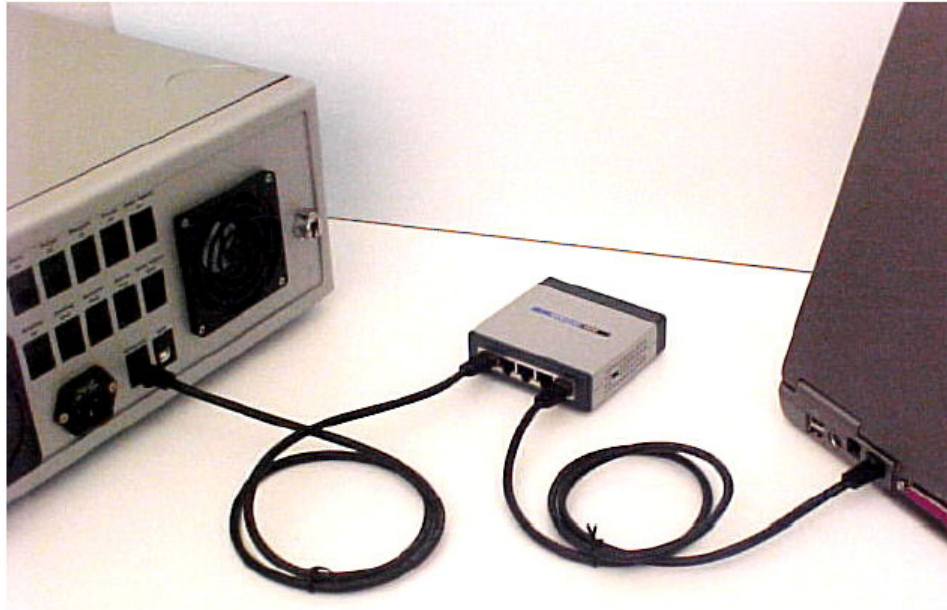


Figure D-16: Connecting instrument to computer via an Ethernet switch

D.5.3 Multiple Instruments to Computer Using an Ethernet Switch

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



Figure D-17: Connecting multiple instruments to computer via Ethernet switch

D.6 Instrument Network Settings

Figure D-18 shows an example of settings on the **Communications** tab of the instrument front panel that will work with all of the connection schemes described in this appendix.

The screenshot shows the front panel of an Optilab T-rEX instrument. At the top, there are tabs for 'Main', 'aRI', 'Alarm', 'System', and 'Comm'. The 'Comm' tab is selected. Below the tabs, the 'Instrument' section displays the following settings:

Name:	wyatt-100-trx
IP address:	192.168.254.10
Subnet mask:	255.255.255.0
Mode:	Static

Below the 'Instrument' section is the 'Configure Ethernet' section. It contains two radio buttons: 'Obtain an IP address automatically' (which is unselected) and 'Use the following IP address:' (which is selected). Under the selected option, there are two input fields:

IP Address:	192 . 168 . 254 . 10
Subnet Mask:	255 . 255 . 255 . 0

At the bottom right of the 'Configure Ethernet' section, there are two buttons: 'Apply' and 'Revert'.

Figure D-18: Standard instrument connectivity settings on front panel

On this tab, there is a choice between **Obtain an IP address automatically** and **Use the following IP address**.

With the first choice, once the instrument is connected to a computer or LAN, the IP address and subnet mask will be assigned automatically. This will work even with the USB connections described in “Connecting via USB” on page D-13.

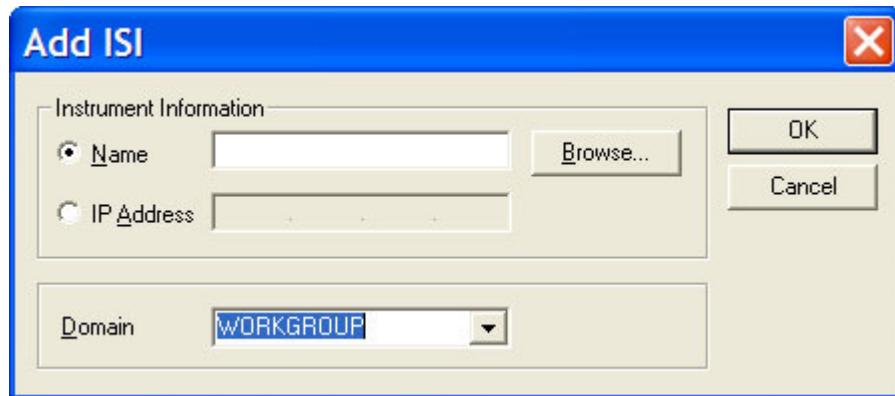
When obtaining an IP address automatically, it might take several minutes for the IP address to be assigned. During this time, the IP address and subnet mask on this tab will read 0.0.0.0. Once the IP address and subnet mask have been assigned, both will be automatically updated and should no longer read 0.0.0.0. At this point, it should be possible to connect to the instrument from the computer.

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

D.7 Accessing Instruments with ASTRA V

To access an instrument with ASTRA V after you have connected it as described in this chapter, follow these steps:

1. From the main menu of ASTRA V, select **System–ISI**.
2. In the ISI dialog, click **Add**. You will see the Add ISI dialog:



3. Do one of the following depending on how your instrument is connected and whether you are using a static IP address:
 - *If the instrument is connected to a LAN and you are not using a static IP address:* Click the **Browse** button in the Add ISI dialog, and then navigate to the instrument on the network. The instrument will be located under **Network Neighborhood–Entire Network–Microsoft Windows Network–WORKGROUP**. The instrument name will match the name on the System tab of the instrument front panel. It may take several minutes for the instrument name to appear after it has first been plugged into the LAN. Select the instrument and then click **OK**.
 - *If the instrument is not connected to the LAN or you are using a static IP address:* Type the IP address from the instrument front panel directly into the IP Address field in the Add ISI dialog. For Domain, select **WORKGROUP**.
4. Click **OK** to close the Add ISI dialog, then close the ISI dialog.
5. From the main menu, select **System–Instruments**. The instrument you selected should be shown in the Instruments dialog.
6. Select the instrument by clicking its icon, then click the **View** button. The Diagnostic Manager application should launch, which can be used to verify the instrument connection.

If it is not possible to connect to an instrument after following these instructions, read “Troubleshooting and Diagnostics” on page D-20.

D.8 Troubleshooting and Diagnostics

If you are experiencing instrument connectivity problems, please read and follow these steps. If you still cannot connect to your instrument after using this section, please contact Wyatt Technology for assistance.

D.8.1 Windows XP Professional Service Pack 2

If your computer has Windows XP Professional Service Pack 2 installed, you need to change some default configuration settings on your computer before you can connect to an instrument. Please log in to the Support Center on the Wyatt Technology web site (www.wyatt.com) and download the configuration instructions under **Software—Computer Info**, or contact customer service at Wyatt Technology to obtain the instructions.

D.8.2 ASTRA V 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 and select “WORKGROUP” for the domain as described in “Accessing Instruments with ASTRA V” on page D-19.

If either of these is not correct, from the main menu of ASTRA V select **System—ISI**, then first delete the incorrect ISI entry. Then click **Add** and repeat the steps, verifying that you have typed in the correct IP address and selected “WORKGROUP” as the domain.

D.8.3 Verifying Instrument Connections

If the previous suggestions fail, please verify that the instrument is communicating with the computer.

Open a Windows Command Prompt window on the computer. On the command line, type “ping” plus the IP address of the instrument as shown on the instrument front panel (see Figure D-18). If the instrument is connected properly, the result should be similar to that shown in Figure D-19.

```
C:\>ping 172.20.1.244

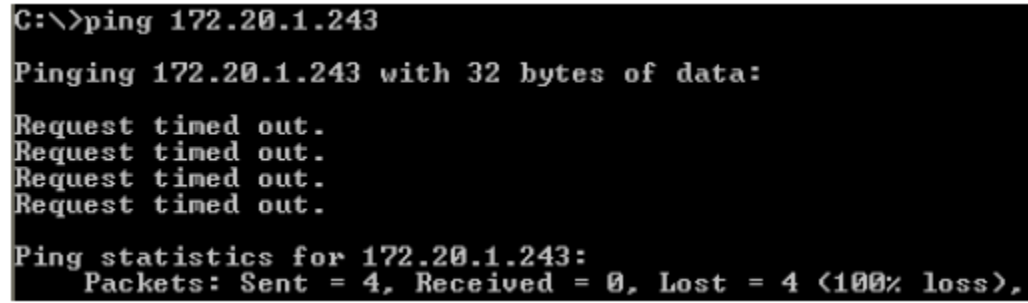
Pinging 172.20.1.244 with 32 bytes of data:

Reply from 172.20.1.244: bytes=32 time<1ms TTL=128
Reply from 172.20.1.244: bytes=32 time<1ms TTL=128
Reply from 172.20.1.244: bytes=32 time<1ms TTL=128
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 D-19: Using ping to verify the instrument connection

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



```
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 D-20: Failure to connect to instrument using ping

Warnings

This appendix provides a list of warnings that should be read and understood when using the Optilab T-rEX.

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E.1 List of Warnings



WARNING

If the power cord is connected, line voltages of 120-240 VAC, 50-60 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 Optilab T-rEX contains a “Class 1 LED Product per IEC 60825-1”, which is a non-lasing LED coupled to an optical fiber. The LED in this LED/fiber unit is mounted to a thermal heat sink located near the back panel of the Optilab T-rEX. The fiber brings the light from the LED into the optical system. With the instrument off and the power cable disconnected, it is safe to replace the LED/fiber unit. The LED/fiber unit should not be altered or modified in any way.



WARNING

The instrument contains fluid. Under normal operating conditions, the system may contain up to 0.5 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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