LEDspec

Bio-Photometric Detection System

INSTRUCTION MANUAL

Serial No._____

021215

World Precision Instruments

LEDSPEC MODULE CONFIGURATION

Note the wavelength of your LED modules here.

1. _____

2. _____

3. _____

4. _____

5. _____

6. _____

7. _____

CONTENTS

ABOUT THIS MANUAL

The following symbols are used in this guide:

This symbol indicates a **CAUTION**. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

This symbol indicates a **WARNING**. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and **TIPS** contain helpful information.

Fig. 1—LEDspec

INTRODUCTION

LEDspec and **LEDspec^{UV}** are stand-alone LED-based bio-photometric detection systems designed to give you the information you want to see. Now you can conduct flow analysis and single shot applications with high precision over a large dynamic range.

LEDspec is a unique, highly precise photometer available in two variations, **LEDspec** (visible) or **LEDspec^{UV}** (ultraviolet). **LEDspec** passes light through a sample and measures the absorption of light at specific wavelengths which are defined by the wavelength modules installed in the **LEDspec** instrument. Its noise (< 0.1mAU peak to peak dynamic range) and drift performance (<0.5mAU/h) exceeds that of a CCD or photodiode array detection system.

Up to seven different wavelength modules can be installed, allowing a sample to be tested against up to seven different wavelengths. In addition, **LEDspec** is equipped with two to four channels so that up to four different samples can be examined simultaneously, greatly speeding the experimental process. The **LEDspec** comes with three wavelengths that are chosen at the time of purchase, but up to four additional ones can be purchased separately and installed, as needed.

Many biochemistry applications require information at specific, important wavelengths, instead of a full spectrum analysis. For example, the Bradford, BCA and Lowry assays for protein analysis rely on specific wavelengths. Because the unit examines only those specific wavelengths, no extraneous information is displayed, only what is required. **LEDspec** is ideally suited for oceanographic applications such as detecting nM concentrations of nitrite/nitrate, phosphate and iron using WPI's **LWCC** sample cells.

LEDspec uses a unique LED system of lights that are extremely durable, eliminating the need to replace costly lamps. **LEDspec** uses dual–beams to reduce light source drift. In contrast, conventional single beam spectrometers notice baseline drift caused by warm up, temperature stability and bulb aging. A reference channel in the LEDspec corrects for baseline while you make sample measurements.

Analysis of output data is a snap with **LEDspec**'s easy-to-use software. Chromatographs can be exported directly to a PC or laptop (via USB) in Microsoft® Excel format. The software provides:

- Full computer control of **LEDspec**
- Continuous flow or single-shot analysis of up to four independent channels simultaneously or sequentially.
- Immediate calibration and analysis (mean and standard deviation) of up to four channels

LEDspec

The standard **LEDspec** measures light absorbance, transmission and intensity of visible wavelengths of light. The following types of sample cells can be plugged into up to four channels:

- **LWCC**
- Fiber Optic Cuvette Holders
- **V-Vette**

LEDspec accepts the following wavelength modules:

- 400nm
- 450nm
- 540nm
- 560nm
- 600nm
- 650nm
- 700nm
- Some custom designed wavelengths. Contact WPI with your specifications to see what other options are available.

LEDspec can be used with the following applications:

- Environmental/Oceanography, including nitrite/nitrate at 540nm, phosphate at 700nm and Iron at 560nm
- Pharmaceutical Process Control
- Semiconductors - water purity, trace metal analysis (Fe, Pd, Cu, U)

LEDspec^{UV}

The **LEDspec^{UV}** measures visible and ultraviolet wavelengths of light and is capable of performing BSA, Lowry and Bradford assays. The following types of sample cells can be plugged into up to four channels:

- **LWCC**
- Fiber Optic Cuvette Holder
- **V-Vette**

LEDspec^{UV} accepts the following wavelength modules:

- 260nm
- 280nm
- 340nm
- 400nm
- 450nm
- 540nm
- 560nm
- 600nm
- 650nm
- 700nm

- Some custom designed wavelenths. Contact WPI with your specifications to see what other options are available.

LEDspec^{UV} can be used with the following applications:

- All **LEDspec** applications
- Biochemistry
  - DNA 0.5-1000ng/mL at 260nm (with WPI's **V-Vette**)
  - Protein: 0.1-30mg/mL at 280nm (with WPI's **V-Vette**)
- Pharmaceutical
  - Drug discovery
  - Dissolution testing

Parts List

After unpacking, verify that there is no visible damage to the instrument. Verify that all items are included:

(1) **LEDSpec-2**, **LEDspec-4**, **LEDspec-UV2** or **LEDspec-UV4** - Biophotometric detection device with three installed wavelength LED modules chosen at the time of ordering. ("-2" indicates a two-channel unit, and "-4" is a four-channel unit.)

(1) Power supply and cord

(1) Software installation CD

(1) USB cable

(1) 0.05" hex wrench

(1) Instruction Manual

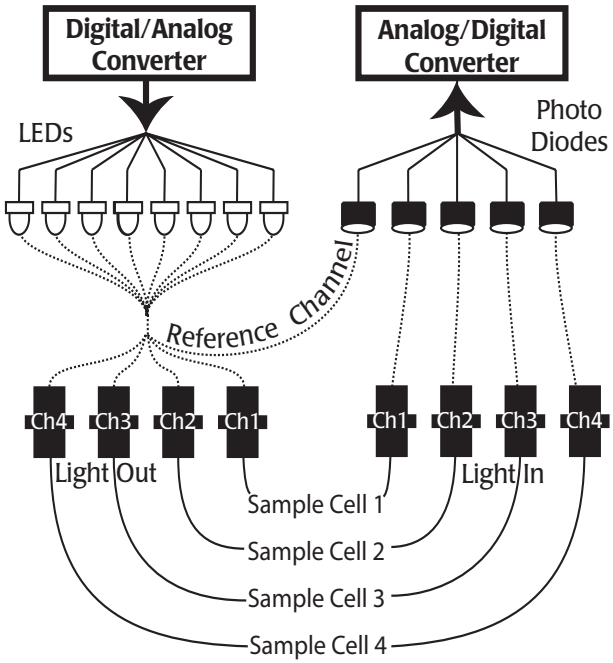
Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 43 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 43 of this manual.

INSTRUMENT DESCRIPTION

Hardware Description



LEDspec is setup with up to seven LED modules that each produce a unique wavelength of light that can be passes through all the LIGHT OUT ports and through the sample cells. In addition, a reference fiber optic cable which does not pass through any sample is connected directly with a photo detector. **Fig. 2** (left) shows this process.

Fig. 2—(Left) Schematic drawing of LEDspec workings. The two channel version has only two Light Out/Light In ports.

Current passes through a digital/analog converter (DAC) where it is turned into a signal that powers the LED modules. (Seven are the internal LED modules, the eighth is reserved for an external light source option that will be added in the future.) The fiber optic cables from the LED modules merge into one fiber optic cable which is split again into five cables that travel to each of the sample cells through the LIGHT OUT ports. The fifth fiber optic cable (the reference) connects directly to a photo receptor. From the sample cells, light travels through fiber optic cables back to LEDspec through the LIGHT IN ports. From there light travels to the photo receptors and then to an analog/digital converter (ADC) where the signal is converted back to a voltage that can be sent to a monitoring device.

Before taking measurements, LEDspec turns all LEDs off and measures the dark readings of the photo detectors to "cancel" out any background light accidentally introduced into the system. Then, the chosen LED modules illuminate, one at a time, and the photo detector readings for each LED are separately stored and averaged.

After measurements are taken, LEDspec calculates the intensity, absorbance or the transmission for each LED and photo detector according to the following formulas:

$$\text{Intensity} = (I_S)(I_{R0}/I_R)$$

$$\text{Absorbance} = \log_{10}\left(\frac{I_{S0} * I_R}{I_S * I_{R0}}\right)$$

$$\text{Transmission} = \frac{I_S * I_{R0}}{I_{S0} * I_R}$$

I_S = measured intensity of light of a particular LED after it passes through a sample

I_R = reference intensity of light not passing through a sample. There is only one reference.

I_{S0} = intensity value stored in memory after last zeroing

I_{R0} = reference value stored in memory after last zeroing

Front panel

Fig. 3—LEDspec front panel

The front panel has the following controls:

- Power (I/O): Use this toggle button to turn the power on and off.
- LCD Display: This display shows the menu options and readouts directly from the LED modules, depending on which menu is chosen. Use of these menu options is discussed in "LEDspec Control from Menu Display" on page 33.
- Key pad: The touch pad has four arrow keys for navigating through the menu options, **PREV MENU** and **NEXT MENU** buttons for selecting menus, an **ENTER** button for making a menu selection and a **CANCEL** button for returning to the previous field without making changes.
- SCAN button: The red LED above the SCAN button illuminates when LEDspec is taking measurements. LEDspec continually scans the attached sample cells taking measurements, even when the software is in single shot mode. To turn off the scanning, press the SCAN button. The red LED will go out.

Rear Panel

Fig. 4—LEDspec rear panel

The rear panel has the following controls:

- Digital I/O IN/OUT - These two nine-pin connections are reserved for future use.
- USB - A USB port connects the LEDspec with a PC or laptop to interface with the analytic software.
- ANALOG OUTPUTS - Two or four analog outputs are available, one for each channel. You can conduct flow mode analysis without a computer by connecting the channels (through these ports) to a data acquisition device like WPI's LAB-TRAX-4/16.
- POWER - The power port is used for connecting 15VDC power to the LEDspec. The power supply plugs into the mains wall outlet and into this port.

Side panel

Fig. 5—LEDspec side panel

The side panel has the following controls:

- LIGHT OUT/LIGHT IN - Two or four connection pairs are available for connecting up to four sample cells with LEDspec. Use the sample cell connection cables with SMA connectors to connect the LIGHT OUT ports and LIGHT IN ports with the sample cells. Each LIGHT OUT port is paired with the LIGHT IN port directly beneath it.
- HIGH POWER LED I/O - This nine-pin connection is reserved for future use.

Hardware Setup

LEDspec is fully assembled when it is shipped. Unless additional LED modules were ordered, LEDspec is manufactured with three LED modules. Additional LED modules may be added, as needed.

Use care when handling fiber optic cables, inside and outside the LEDspec case. Fiber optic cables are made of glass and will not tolerate rough handling. When handling LED modules, be careful of static electricity which can damage delicate circuitry.

The figure below shows a LEDspec unit with the top cover removed. Items discussed later are labeled. Take note of the LED module connections. The position numbers are indicated on the drawing. These are important when installing new LED modules.

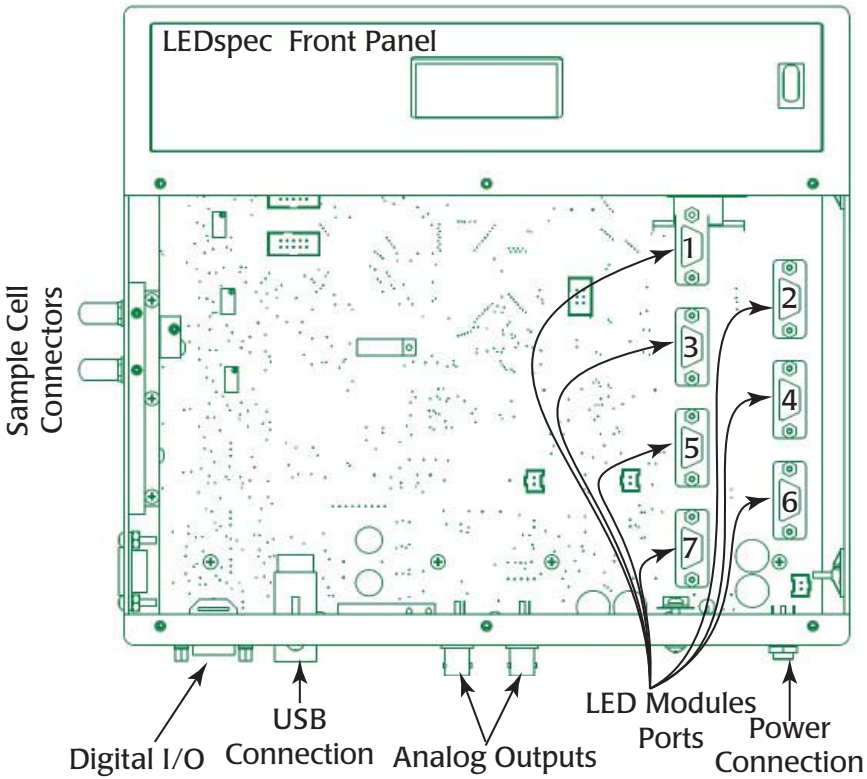


Fig. 6—LEDspec unit with the cover removed, as viewed from the top looking inside

Connecting a Fiber Optic Sample Cell

Depending on the LEDspec model, two or four sample cells may be connected. To connect a sample cell:

1. Insert the SMA connector on one end of a sample cell connection cable into a LIGHT OUT port on the side of the LEDspec and finger tighten it.
2. Insert the SMA connector on the other end of the cable into one side of the sample cell and finger tighten it.
3. In the same manner, connect a second sample cell connection cable to the LIGHT IN port directly beneath the chosen LIGHT OUT port and the other end of the sample cell. Finger tighten all the SMA connections.

Installing an LED Module

LED modules come in a variety of wavelengths, and up to seven may be installed at a time.

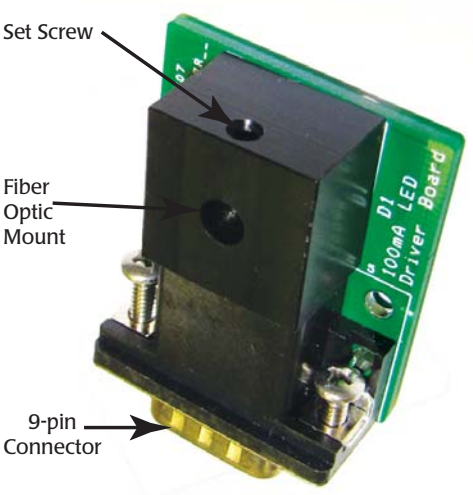
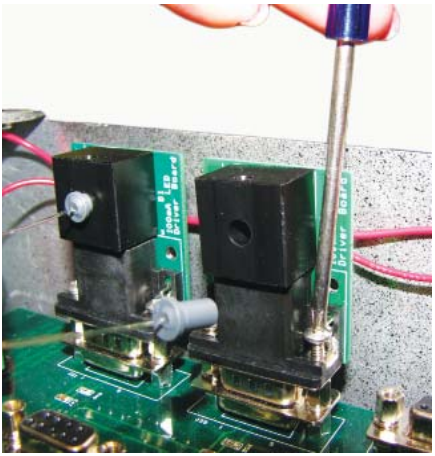


Fig. 7—The LED module is labeled.

To install an LED module:

1. Turn the LEDspec power off.
2. Using a Phillips-head screwdriver, remove the six screws on the top face of the LEDspec.
3. Lift the cover from the top of the LEDspec. The LED modules are located on the left side of the LEDspec. LED modules look similar to the one shown in **Fig. 7**. The seven module ports are numbered as shown in **Fig. 6**, page 8.
4. Locate an empty LED module slot. If necessary, remove an LED module. See "Removing an LED Module" on page 11.

5. Carefully align the LED module with the appropriate 9-pin connector and gently push the new LED module into place.
6. Insert and tighten the two screws on the sides of the LED module as shown in ***Fig. 8***.



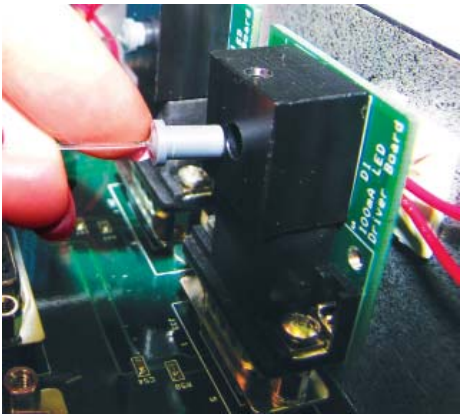


Fig. 8—(Left) Tighten the LED module screws

Fig. 9—(Right) Insert fiber optic ferrule into the mount

7. Insert the ferrule of the fiber optic cable into the fiber optic mount (hole) on the LED module (**Fig. 9**).
8. Using the included 0.05″ hex wrench, tighten the set screw on the top of the LED module to secure the fiber optic cable (**Fig. 10**).

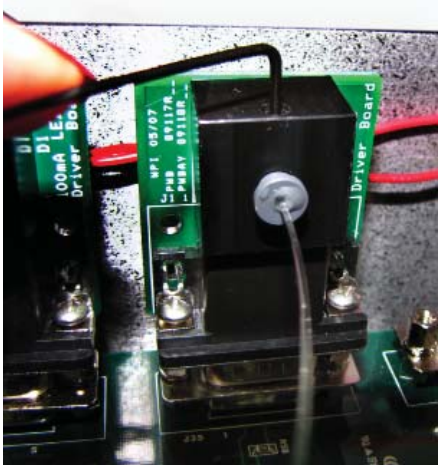


Fig. 10—Tighten the set screw

9. Make a note (on the inside cover of this manual) of wavelength of the LED module in each of the seven slots. These values must be entered in the LEDspec software configuration window, and the values entered in that window must correspond with the placement of the modules. Values only need to be entered in the software once, when modifications have been made to the LED module configuration. See **Fig. 11** to determine which LED module location was used. See "Entering LED Module Information" on page 17.

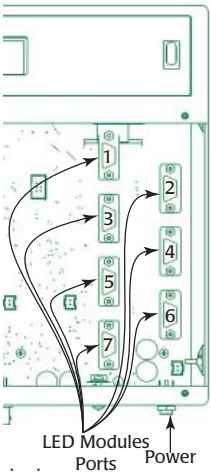


Fig. 11—LED Module addresses

Removing an LED Module

1. Turn the LEDspec power off.
2. Using a Phillips-head screwdriver, remove the six screws on the top face of the LEDspec.
3. Lift the cover from the top of the LEDspec. The LED modules are located on the left side of the LEDspec (**Fig. 6**, page 8).
4. Using the included 0.05" hex wrench, loosen the set screw on the top of the LED module to be removed.
5. Gently remove the ferrule of the fiber optic cable from the fiber optic mount (hole) on the back side of the LED module.
6. Insert the ferrule into an open fiber optic mount near the sample cell connectors and tighten its set screw with the supplied 0.05" hex wrench.
7. Remove the two screws on the sides of the LED module.
8. Pull the LED module out of the LEDspec case and store it in an anti-static bag or a plastic bag.

Connecting LEDspec with a PC

To connect LEDspec with a PC:

1. Install the LEDspec software. See "Installing LEDspec Software" on page 13.

NOTE: Install the software BEFORE connecting the LEDspec to the PC (via USB).

2. Verify that the LEDspec is connected with the power supply and powered on. The red LED above the **SCAN** button illuminates when LEDspec is powered on.
3. Then, insert the USB cable into the USB port on the back panel of the LEDspec. Plug the other end of the USB cable into an open USB port on the PC.
4. Start the LEDspec software by clicking on the Windows Start button, selecting **Programs**, choosing the **LEDspec** folder and clicking on **LEDspec** (

).
5. If the LEDspec software is receiving data from the hardware, the icon in the upper right corner shows a blue pipe (). Otherwise, the red X displays ().
6. To troubleshoot the connection, see "Choosing a COM Port" on page 17.

Connecting LEDspec with a Data Acquisition System

LEDspec uses the BNC analog output connectors on the back panel to connect directly with a data acquisition system like Lab-Trax that has ±10V. Each channel is connected individually.

1. Connect one end of the cable to the BNC connector to the CH1 Analog Output and the other end to channel 1 of the data acquisition system. Connect channels 2, 3 and 4 of the LEDspec in a similar fashion to the channels on the data acquisition system.
2. Use the LEDspec software or the display menu on the front panel of the LEDspec to configure how the analog output voltage is scaled. See "Analog Output to Data Recorder" on page 29 for instructions on using the LEDspec software to configure the analog output. See "Analog Output Data Setup Menu" on page 36 for instructions on using the display menu on the LEDspec front panel.
3. Follow the instructions in the data acquisition instruction manual for connecting the data acquisition system to a computer and working with the data.

Software Setup

Installing LEDspec Software

1. Insert the LEDspec CD. The setup should start automatically. If it does not start, click the Windows Start button and choose **Run** (**Fig. 12**). Click the **Browse** button and select **setup.exe** from the CD directory. The LEDspec installation window appears (**Fig. 13**).

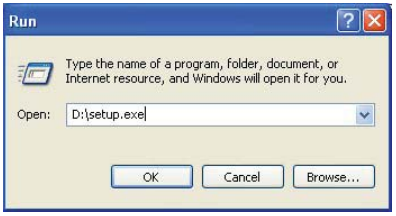


Fig. 12—Windows Run dialog box

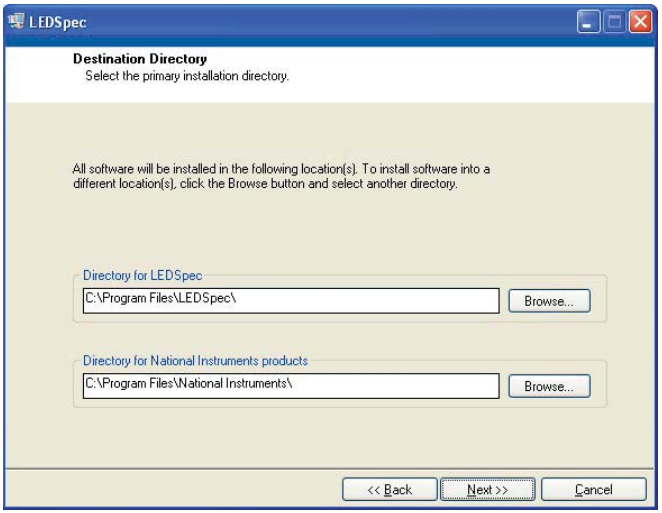


Fig. 13—LEDspec Installation window

2. Click each **Browse** button and navigate to the directories where the LEDspec programs are to be installed. Click the **Next** button. The two license agreements display (**Fig. 14**) individually.

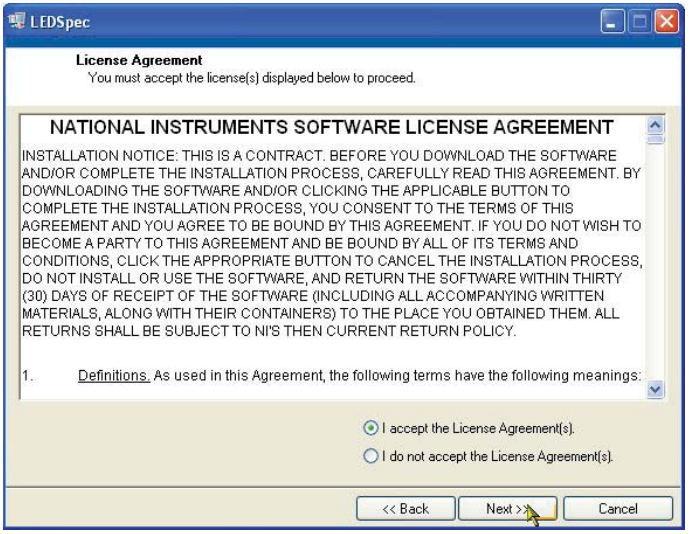


Fig. 14—National Instruments license agreement

3. Select the **I accept the License Agreement(s)** radio button and click the **Next** button.

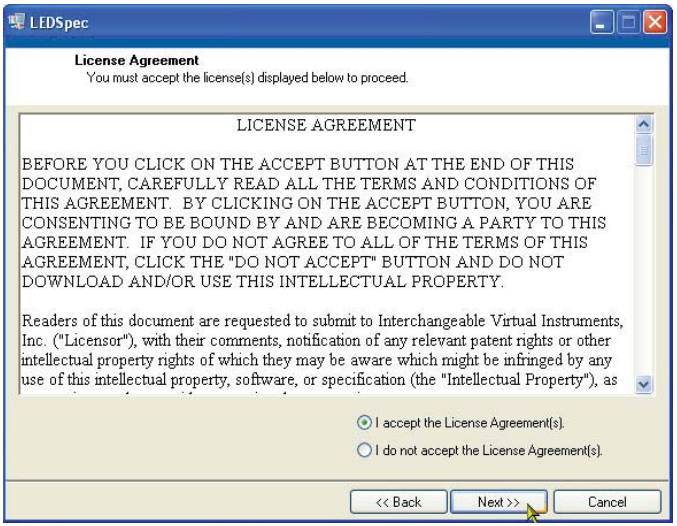


Fig. 15—LEDspec software license agreement

4. Select the **I accept the License Agreement(s)** radio button and click the **Next** button. The LEDspec Start installation window appears (**Fig. 16**).

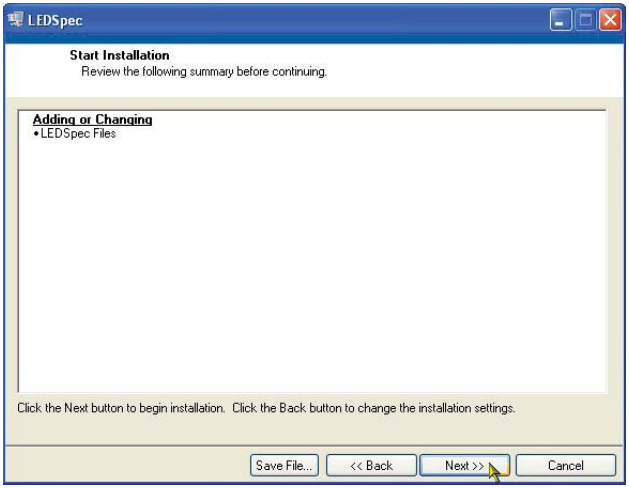


Fig. 16—Start Installation window

5. Click the **Next** button. The progress window displays (**Fig. 17**).

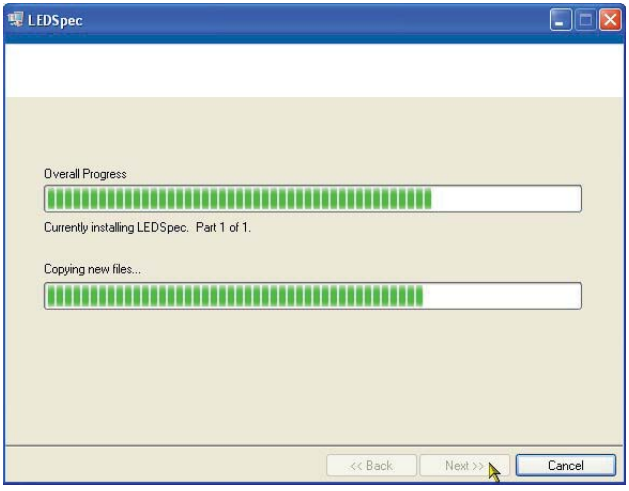


Fig. 17—Installation progress window

6. When the installation finishes, the Installation Complete window appears. Click the **Next** button (**Fig. 18**).

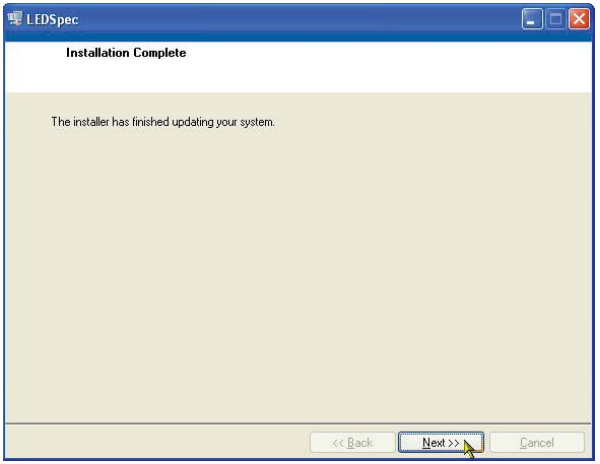


Fig. 18— Installation Complete window

7. To start the LEDspec software, click the Windows start button, select **Programs**, choose the **LEDspec** folder and click on **LEDspec (** 

).

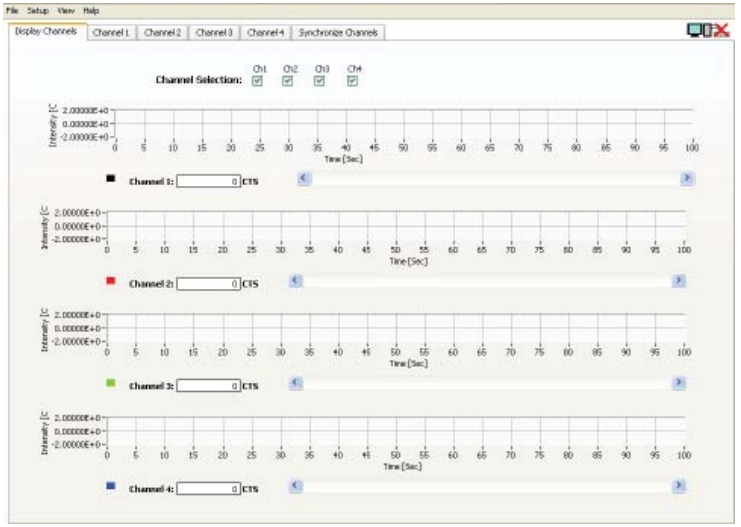


Fig. 19—LEDspec Software window.

Choosing a COM Port

When the LEDspec software is receiving data from the hardware, the icon in the upper right corner shows a blue pipe ( ). Otherwise, the red X displays ( ). If the software and hardware are not communicating, try selecting a different COM port.

1. Verify that the LEDspec is properly connected to the PC. See "Connecting LEDspec with a PC" on page 12.
2. From the **Setup** menu, select **Instrument**. The **Menu Com Port Select** window appears (**Fig. 20**).

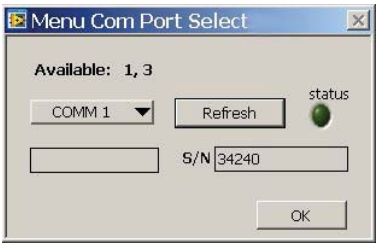


Fig. 20—Menu Com Port Select window when LEDspec software is not receiving data

3. Click the COMM1 dropdown list and choose another available COM port. Unavailable COM ports are grayed out. Click the **Refresh** button. When the Status light on the window glows neon green, then the hardware and software are communicating (**Fig. 21**.)

   NOTE: When they are communicating, this window also displays the model (2 Channels or 4 Channels) and serial number (S/N) of the LEDspec hardware.

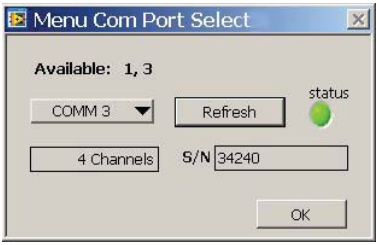


Fig. 21—Menu Com Port Select window when LEDspec software is receiving data

Entering LED Module Information

Use the LED Labels window when changes are made to the LED module configuration. For example, if an LED module is added or removed, edit the information in this window.

1. Click the **Setup** menu and choose **LED Wavelengths**. The LED Labels window displays (**Fig. 22**). If the LEDspec is not communicating with the hardware a warning message appears (**Fig. 23**). To correct the issue see "Choosing a COM Port" on page 17.

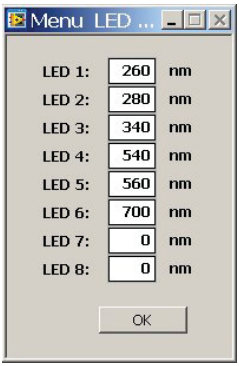


Fig. 22—(Left) LED Labels window

Fig. 23—(Right) Warning message

2. Enter the appropriate wavelength in each text field. LED 1 corresponds with the LED in the position 1 in the LEDspec chassis. See **Fig. 6** on page 8. LED 8 is reserved for future use; leave it blank.

3. Press the **OK** button. The information is uploaded to the LEDspec firmware. Each time the software program is started, it calls the information from the firmware. There is no need to update this information unless an LED module is added or removed.

Closing LEDspec Software

To close the LEDspec software window, from the **FIle** menu, select **Exit**.

OPERATING INSTRUCTIONS

While the software interface is easier to use, most of its functions can be performed using the menu display on the front panel of the LEDspec unit. This section discusses the operation of LEDspec using the software. For information on using the menu display on the front panel, see "LEDspec Control from Menu Display" on page 33.

LEDspec operates in two modes: Single shot and Time lapse (continuous flow). Single Shot mode is used primarily with biological applications. In this mode, measurements of the samples are only recorded when the **SCAN** button is pressed. In Time Lapse mode, sample reading are taken and recorded continuously.

When LEDspec software opens, a window appears asking for a mode selection (**Fig. 24**). Choose **Time Lapse** for continuous flow analysis or **Single Shot** for single readings of the samples. To change the mode of operation from the software when the software is already running, click the **Setup** menu and choose **Single Shot** or **Time Lapse**.

Fig. 24—Splash Screen for choosing Single Shot or Time Lapse mode

Single Shot Analysis

Single Shot analysis is a three part process. First, the application parameters must be established. Then the LEDspec must be calibrated. Finally, sample readings may be collected. The Single Shot window has three tabs that correspond with these steps. To open the window, click the **Setup** menu and choose **Single Shot.**

Application Setup

To establish application parameters, click on the **Application Setup** tab on the Single Shot window (**Fig. 25**).

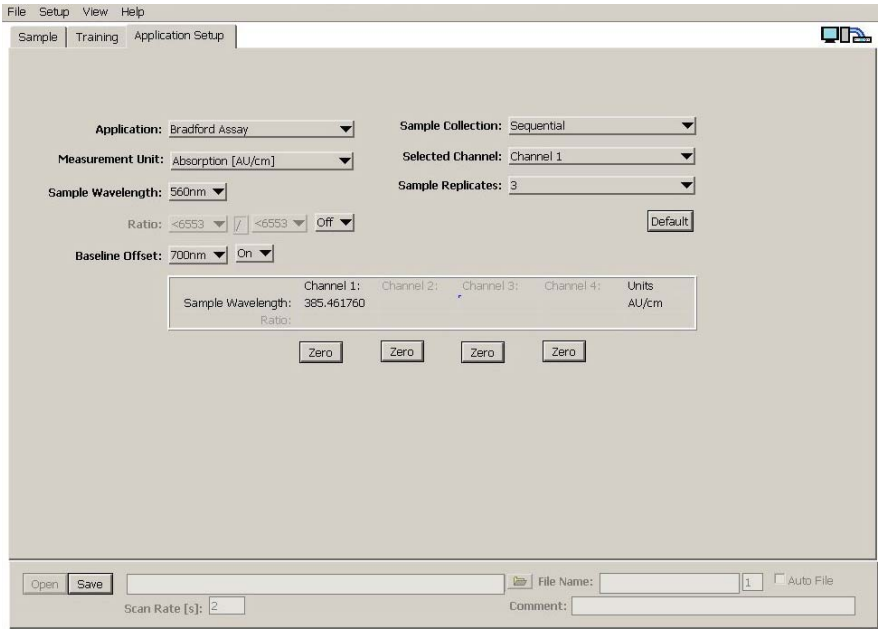


Fig. 25—Single Shot window, Application Setup tab

1. To use a previously saved setup, click the **Open** button, navigate to and select the desired file. Otherwise, choose the correct application from the **Application** drop-down list. Five options are pre-configured for the common applications. Choosing one of these five automatically sets the other fields on this window. The User Defined option allows for complete freedom in configuring the experimental setup. Options include: Nucleic Acid, Protein, Bradford Assay, BCA Assay, Lowry Assay and User Defined.
2. Select the desired unit for measuring the absorbance of the sample cells from the **Measurement Unit** drop-down list. Options include: Absorbance (AU), Absorption (AU/cm), Transmittance (%), Intensity (counts) and Raw Intensity (ADC value).
3. If necessary, change the sample wavelength of light that passes through the sample cells by choosing the appropriate value from the **Sample Wavelength** drop-down list. This selects the LED module that is used for the experiment. Only LED modules that have been installed and configured are available. See "Entering LED Module Information" on page 17.

4. To compare two wavelengths, turn the ratio option on by selecting **On** from the **Ratio** Off/On drop-down list. Then, select the two wavelengths to be compared from the other two drop-down lists.

5. If a baseline offset is required, select **On** from the **Baseline Offset** On/Off drop-down list and choose the wavelength to which the baseline offset is to be applied.

6. From the **Sample Collection** drop-down list, select **Sequential** or **Simultaneous**. **Sequential** takes a measurement from the one sample cell that is selected in the **Selected Channels** drop-down list. **Simultaneous** takes a reading from all the sample cells selected in the **Selected Channels** drop-down list at the same time. If **Sequential** is selected, choose the number of measurements to be made (1-4) from the **Sample Replicates** drop-down list.

7. Click **Default** to reset all the values to the default values for the application selected. For example, if **Bradford Assay** is selected, **Default** resets the **Measurement Unit** to Absorption, the **Sample Wavelength** to 560nm, the **Baseline Offset** to 700nm, sequential collection, with three replicates of channel 1.

8. The table in the center of the window shows the live readings and ratios of the sample cells in the selected channels. To set the current reading of a channel as the baseline, press the **Zero** button beneath that channel's data. A warning message appears (**Fig. 26**). Click the **OK** button to zero the channel or the **Cancel** button to abort the process.

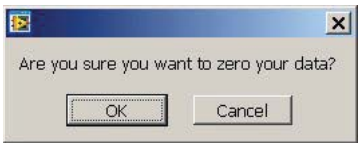


Fig. 26—Verification message when zeroing a channel

9. To save a user-defined application setup, enter a valid path (directory) in the first text box or select a path by pressing the folder icon. Then, enter the file name in the **File Name** text box and click the **Save** button. The file is saved in the selected directory in .txt format.

CAUTION: Choose a unique file name so that a previously saved file is not overwritten. Or, select the **Auto File** check box. **Auto File** saves the files with a unique name in the designated directory each time the **Save** button is pressed.

10. In the **Scan Rate** text box, enter the rate at which data is displayed. This value determines how long measurements are taken before they are averaged and displayed.

Calibrating the Software

To calibrate the LEDspec, click on the **Training** tab on the Single Shot window (**Fig. 27**).

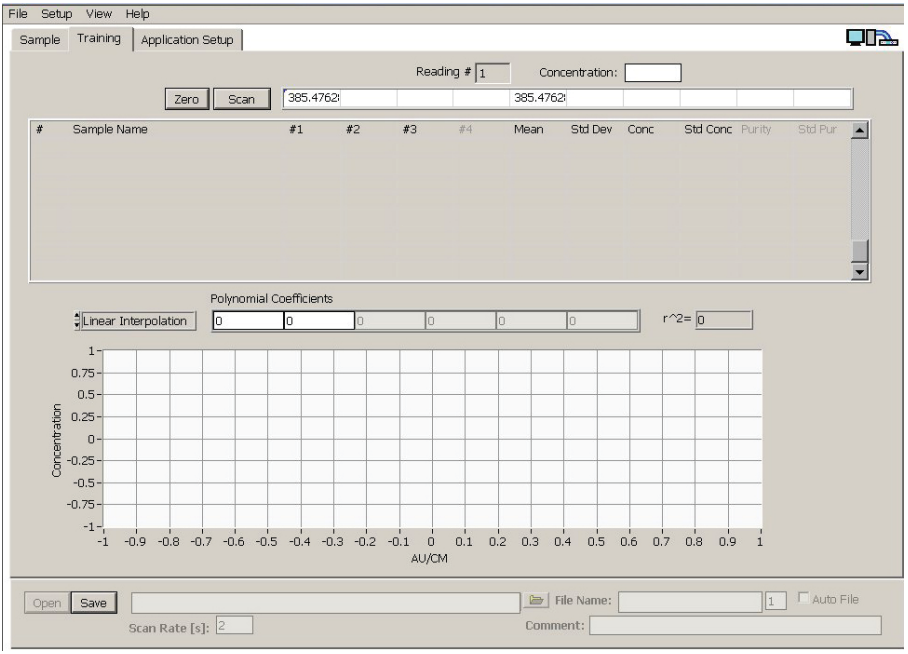


Fig. 27—Single Shot window, Training tab

Pressing the **Zero** button sets the current reading as the baseline. To take a measurement of the samples, press the **Scan** button. Each time the **Scan** button is pressed, it records the measurements of each sample cell, calculates the values in the table and plots the points on the graph at the bottom of the window.

1. To use a previous calibration matrix, click the **Open** button and navigate to the desired file. To begin a Fresh experiment, insert a blank buffer or standard solution into the sample cells being used.
2. Press the **Zero** button. This sets the current reading as the baseline. A warning message appears (**Fig. 28**). Click the **OK** button to zero the channel or the **Cancel** button to abort the process.

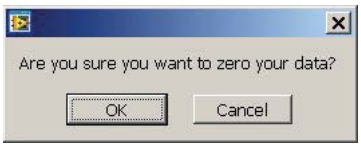


Fig. 28—Verification message when zeroing a channel

3. Insert the first set of calibration samples into the sample cells. These are samples of known concentration with known absorption values.
4. Enter the known concentration of the samples in the **Concentration** text field at the top of the window.
5. Press the **Scan** button on the top of the window. The measurement of the samples is recorded in the table, and the mean, standard deviation, concentration, standard concentration, purity and standard purity are calculated.

   TIP: To manipulate data in the table, right click in the table area. The pop-up menu allow you to insert or delete a row, clear the table or copy the table to the clipboard so it can be pasted into another program.
6. Repeat steps 3-5 for each set of calibration samples.
7. From the drop-down list select one of the following:
   - Linear Interpolation– This plots the absorption/cm against the concentration of the calibration data and extrapolates a straight line. The r^2 value of the line is shown in the **r^2=** field. A value of 1.0 represents a perfect calibration.
   - Polynomial Order 1-5– These options allow the reconciling of data points along a curve. A first order polynomial plots a straight line in the form of ax+b=y. The software computes the a and b coefficients that best represent the data points. Higher orders of polynomial plot the data along a curve using equations like $ax^2+bx+c=y$ (2nd order) or $ax^3+bx^2+cx+d=y$ (3rd order). The higher the order of polynomial chosen, the more coefficients are used and the more precise the calibration curve. The LEDspec software chooses the coefficients based on the calibration data set, and the coefficients define a function.
   - User Defined Fit– If the desired polynomial coefficients are already known, select this option and enter them into the appropriate text boxes. If the same experiment was run before, the coefficients may be copied from it.

   TIP: Right click on the graph to copy data, reveal controls (like plot legend, scale, magnifier, cursor legend, scroll bars, X and Y scales), clear the graph of data, make annotations, autoscale, smooth updates, autosize the plot legend and chose an optional plane to display (Nyquist, Nichols, S Plane or Z Plane).

   TIP: Choose **Visible Items>Graph Palette** to reveal the magnification controls and the hand icon that allows you to grab the graph and reposition it.

   TIP: To change the scale on the table, click on the first or last number on the scale and enter the desired value.
6. To save the calibration values, enter a valid path (directory) in the first text box or select a path by pressing the folder icon. Then, enter the file name in the **File Name** text box and click the **Save** button. The file is saved in the selected directory in .txt format.

CAUTION: Choose a unique file name so that a previously saved file is not overwritten. Or, select the **Auto File** check box. Select The **Auto File** checkbox to save the files with a unique name in the designated directory each time the **Save** button is pressed.

7. In the **Scan Rate** text box, enter the rate at which data is displayed. This value determines how long measurements are taken before they are averaged and displayed.

Making Measurements

To take measurements, click on the **Sample** tab on the Single Shot window (**Fig. 29**).

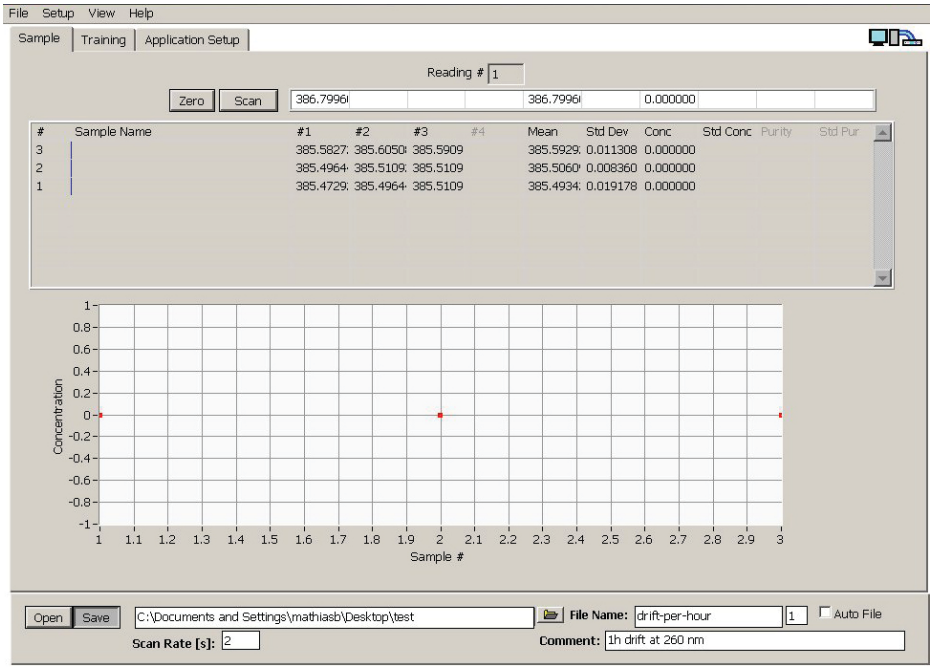


Fig. 29—Single Shot window, Sample tab

Pressing the **Zero** button sets the current reading as the baseline. To take a measurement of the samples, press the **Scan** button. Each time the **Scan** button is pressed, it records the measurements of each sample cell and calculates the values in the table and plots the points on the graph at the bottom of the window.

1. To view data from a previously saved experiment, click the **Open** button and navigate to the desired file. To begin taking new measurements, insert the first sample solution into the sample cells being used.

2. Press the **Zero** button. This sets the current reading as the baseline. A warning message appears (**Fig. 30**). Click the **OK** button to zero the channel or the **Cancel** button to abort the process.

Fig. 30—Verification message when zeroing a channel

3. To take measurements, press the **Scan** button. The measurement of the sample is recorded in the table, and the mean, standard deviation (Std Dev), concentration (Conc), standard concentration (Std Conc), purity of the sample and standard purity (Std Pur) are calculated.

   TIP: To manipulate data in the table, right click in the table area. The pop-up menu allow you to insert or delete a row, clear the table or copy the table to the clipboard so it can be pasted into another program.

4. Insert the next set of samples and press the **Scan** button. As the table is populated with data, the graph below plots the samples against their calculated concentrations. Repeat this step for each set of samples.

   TIP: Right click on the graph to copy data, reveal controls (like plot legend, scale, magnifier, cursor legend, scroll bars, X and Y scales), clear the graph of data, make annotations, autoscale, smooth updates, autosize the plot legend and chose an optional plane to display (Nyquist, Nichols, S Plane or Z Plane).

   TIP: Choose **Visible Items>Graph Palette** to reveal the magnification controls and the hand icon that allows you to grab the graph and reposition it.

   TIP: To change the scale on the table, click on the first or last number on the scale and enter the desired value.

5. To save the experimental data, enter a valid path (directory) in the first text box or select a path by pressing the folder icon. Then, enter the file name in the **File Name** text box and click the **Save** button. The file is saved in the selected directory in .txt format.

CAUTION: Choose a unique file name so that a previously saved file is not overwritten. Or, select the **Auto File** check box. Select The **Auto File** checkbox to save the files with a unique name in the designated directory each time the **Save** button is pressed.

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6. In the **Scan Rate** text box, enter the rate at which data is displayed. This value determines how long measurements are taken before they are averaged and displayed.

Continuous Flow Analysis

In a continuous flow experiment, the sample cells have a continuous stream of fluid pumped through them, and LEDspec takes continuous readings. To open the window, click the **Setup** menu and choose **Time Lapse**. The Continuous Flow Analysis window has six tabs.

The **Display Channels** tab (**Fig. 31**) shows the real time readings for up to four channels individually. Each channel has its own graphical display. Select the appropriate **Channel Selection** check boxes to display the channel(s) data. **Ch1** reveals the Channel 1 data, **Ch2** reveals the Channel 2 data, etc.

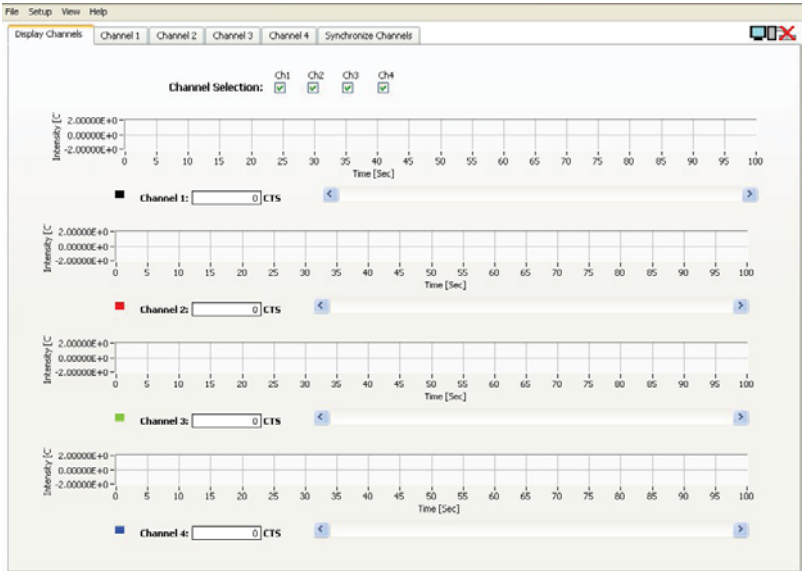

Fig. 31—Continuous Flow Analysis window, Display Channels tab

TIP: Right click on a graph to copy the data, annotate the date or autoscale the graph.

The **Channel 1**, **Channel 2**, **Channel 3** and **Channel 4** tabs (**FigFig. 32**) display data from a single channel.

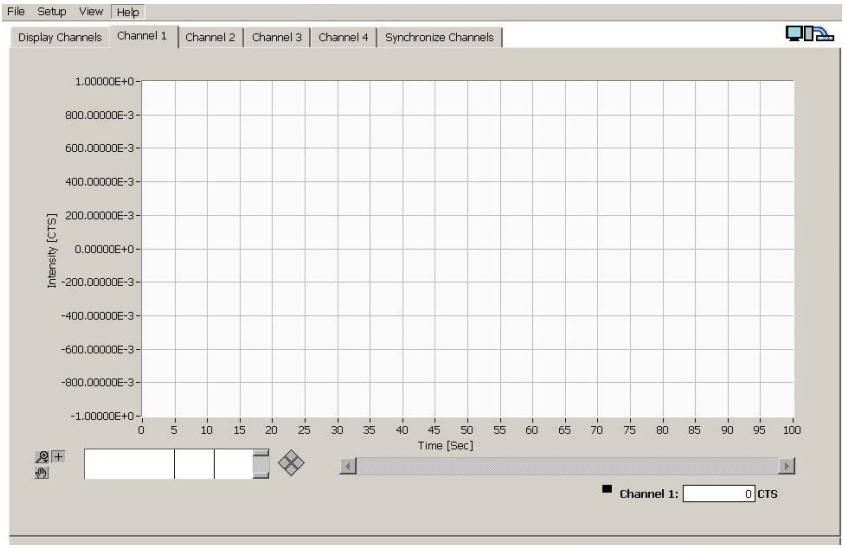

Fig. 32—Continuous Flow Analysis window, Channel 1 tab

TIP: Right click on the graph to reinitialize to the default value, cut or copy or paste data, reveal controls (like plot legend, scale, magnifier, cursor legend, scroll bars, X and Y scales), export the graph as an image, clear the graph of data, make annotations, autoscale or smooth updates.

TIP: Choose **Visible Items>Graph Palette** to reveal the magnification controls and the hand icon that allows you to grab the graph and reposition it.

TIP: To change the scale on the table, click on the first or last number on the scale and enter the desired value.

The **Synchronize Channels** tab (**Fig. 33**) displays the data of all the channels, or as many as desired, on a single graph. Select the appropriate **Channel Selection** check boxes to display the channel(s) data. **Ch1** reveals the Channel 1 data, **Ch2** reveals the Channel 2 data, etc.

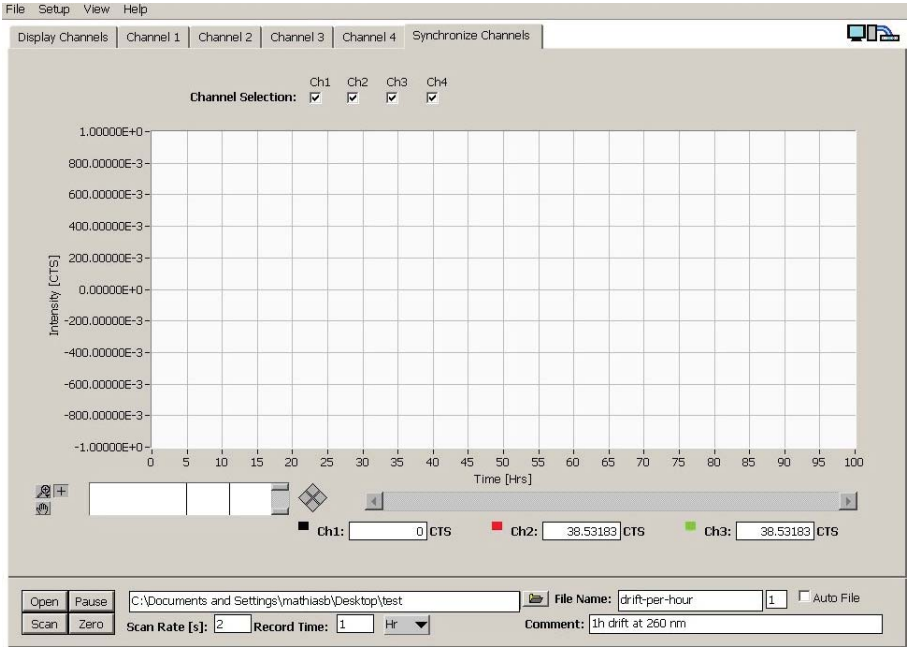

Fig. 33—Continuous Flow Analysis window, Synchronize Channels tab

TIP: Right click on the graph to reinitialize to the default value, cut or copy or paste data, reveal controls (like plot legend, scale, magnifier, cursor legend, scroll bars, X and Y scales), export the graph as an image, clear the graph of data, make annotations, autoscale or smooth updates.

TIP: Choose **Visible Items>Graph Palette** to reveal the magnification controls and the hand icon that allows you to grab the graph and reposition it.

TIP: To change the scale on the table, click on the first or last number on the scale and enter the desired value.

All six tabs of the **Continuous Flow Analysis** window display the real time readings from the LEDspec. To conduct an experiment:

1. Click on the **Synchronize Channels** tab.
2. Run the buffer or standard solution through the system till the graph shows a steady baseline reading. Click the **Zero** button at the bottom of the window to set the value as the baseline. The time it takes to establish a stable baseline depends on the medium used and the setup.

3. Add the sample to the buffer or standard solution until the graph reaches a new plateau. The values on the graph gradually increase as the buffer or standard solution is eliminated from the sample cell. When the sample cell is full of the sample, the reading comes to a stable high value. This usually takes about two minutes.
4. Remove the sample from the stream and flush the system with the buffer or standard solution till the baseline is reached again.
5. Repeat steps 3-4 for each set of samples.
6. To save the experimental data, enter a valid path (directory) in the first text box at the bottom of the window or select a path by pressing the folder icon. Then, enter the file name in the **File Name** text box and click the **Save** button. The file is saved in the selected directory in .txt format.

CAUTION: Choose a unique file name so that a previously saved file is not overwritten. Or, select the **Auto File** check box. Select The **Auto File** checkbox to save the files with a unique name in the designated directory each time the **Save** button is pressed.

---

7. In the **Scan Rate** text box, enter the rate at which data is displayed. This value determines how long measurements are taken before they are averaged and displayed.

Analog Output to Data Recorder

Use the **Display and Analog Out** window (**Fig. 35**) to setup the analog output of data to a data acquisition device like Lab-Trax. See "Connecting LEDspec with a Data Acquisition System" on page 12 for information on connecting LEDspec with a data acquisition system.

The top half of the window (Data Setup section) determines the data that gets exported through the analog output channels, and the bottom half of the window (Analog Out section) defines how data is processed and how it is scaled for the analog output channels.

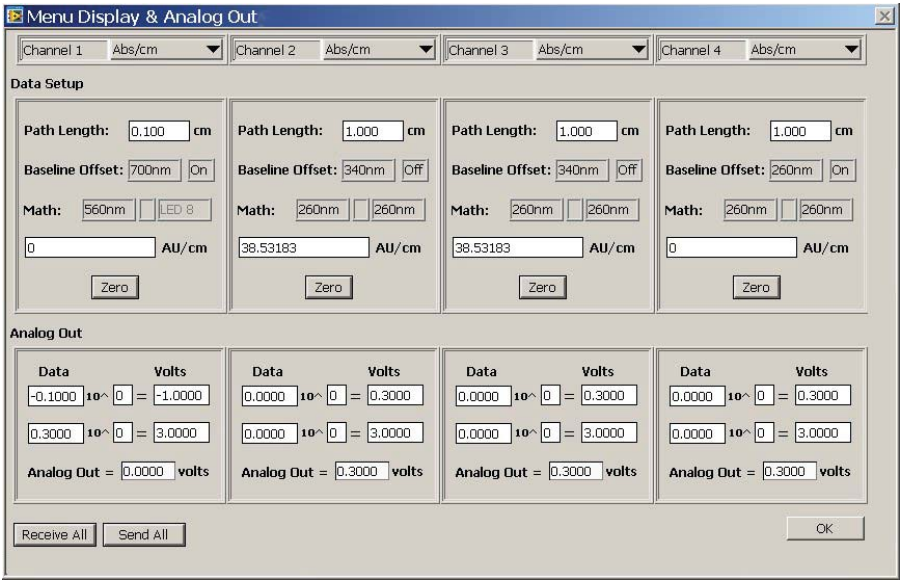

Fig. 34—Display and Analog Output window

1. From the **Setup** menu, select **Display and Analog Out**. If the LEDspec is not connected, a warning message appears (**Fig. 35**). Click the **OK** button and see "Connecting LEDspec with a PC" on page 12 or "Choosing a COM Port" on page 17 for help troubleshooting the connection problem.

Fig. 35—Warning message

2. For each channel, click the drop-down list at the top and select Intensity, Absorption, Transmission, Counts, Dark or Abs/cm to determine what information is exported for each channel.
3. In the **Path Length** text field, enter the value in centimeters of the path length for each channel. This is the distance that light travels through a sample cell.
4. If a baseline offset is to be used, select **On** from the second **Baseline Offset** drop-down list and select a wavelength value for each channel. If no baseline is to be used, select **Off** from the drop-down list.
5. The **Math** fields allow you to subtract one wavelength value from another or

divide one by another prior to export. For example, you might want to subtract a baseline reading or look at a ratio. The current reading displays in the selected units beneath the **Math** fields.

6. In the **Analog Out** section for each channel, set the scale for the output. This pairs specific LEDspec measurements with specific upper and lower scale voltage values. The live data is then converted to a voltage reading that is read by the data acquisition system. The data acquisition software is used to convert the voltage signal back into data that can be analyzed.

   For example, Data -0.100 10^0 could be set to -1.000 Volts for the lower limit, and Data +0.300 10^0 could be set to +3.000 Volts for the upper limit.

   NOTE: The maximum voltage output is 10V.

7. When finished, click the **OK** button to close the window.

LED Status

Use the **LED Status** window (**Fig. 36**) to set up LEDspec for an experiment. From here, you can turn LED modules off and on for each channel, set the experimental mode and sample rate and view the live reading from each channel.

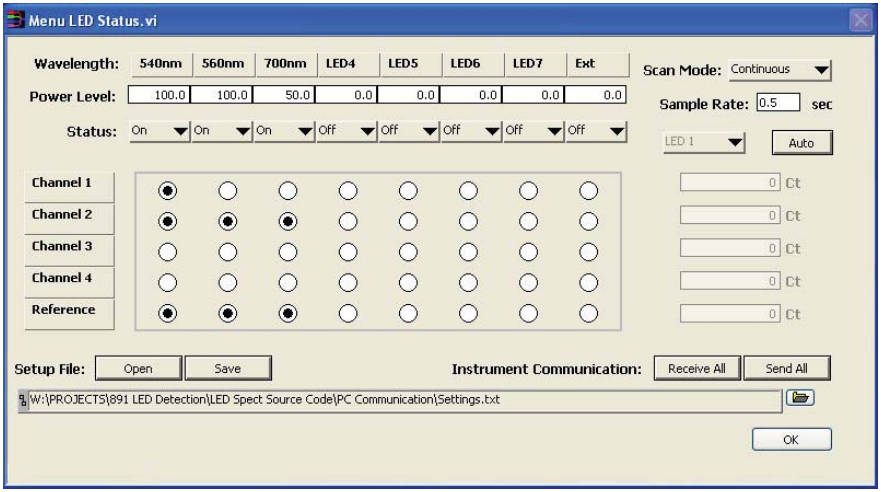

Fig. 36—LED Status window

1. From the **Setup** menu, select **LED Status**. If the LEDspec is not connected, a warning message appears (**Fig. 37**). Click the **OK** button and see "Connecting LEDspec with a PC" on page 12 or "Choosing a COM Port" on page 17 for help troubleshooting the connection problem.

Fig. 37— Warning message

2. The wavelengths of the modules display across the top. If no module is installed, the window displays LED# in that field.

3. To use a previously saved setup, click the **Open** button, navigate to and select the desired file. Otherwise, select the mode of operation from the **Scan Mode** drop-down list (Continuous or Single). If using the software for viewing data and taking measurements, always leave this set to **Continuous**. However, when using LEDspec hardware for taking single shot measurements, select **Single**.

4. Enter the number of times per second that a measurement is taken in the **Sample Rate** text field. The maximum sample rate is 5 times per second. However, the actual sample rate is slower, depending on the number of channels operating. Enter the desired value, and LEDspec configures itself to run at the highest sample rate equal to or below the desired value. The actual sample rate displays in this field after a few seconds.

5. In the **Power Level** text fields, enter the amount of power for each LED module. For example, if 75.0 is entered, the LED module runs on 75% of its power. It will not be as bright as when it runs on 100% power. Normally, power is set to about 80%.

   NOTE: To optimize the power for all the LED modules, click the **Auto** button.

6. Use the row of **Status** buttons to turn the LED modules on or off for each channel. For example, in Figure 40 Channel 1 only uses the 540nm LED, and Channel 2 uses all three. Whenever an LED module is set to on for any channel, the reference LED is also on.

7. The **Receive All** button uploads data from the LEDspec firmware, and the **Send All** button downloads data from the software to the LEDspec firmware.

   NOTE: To view the data for a particular LED module, select the LED module from the drop-down button under the **Sample Rate** field. The live data readings display dynamically below the drop-down button. The units displayed are the ones selected in the Single Shot Analysis window or the Continuous Flow window.

8. To save this data setup, enter a valid path and filename in the text box at the bottom of the window or select a path by pressing the folder icon. Click the **Save** button. The file is saved in the selected directory in .txt format.

CAUTION: Choose a unique file name so that a previously saved file is not overwritten.

9. When finished, click the **OK** button to close the window.

LEDspec Control from Menu Display

LEDspec has four menus that are accessed from the main display window on the front of the unit (**Fig. 38**). Use the touch pad on the front of the unit to set parameters and scroll through the data. The touch pad has four arrow keys for navigating through the menu options, **PREV MENU** and **NEXT MENU** buttons for selecting menus, an **ENTER** button for making a menu selection and a **CANCEL** button for returning to the previous field.

NOTE: When navigating through menu options, pressing the left and right arrow keys chooses the selected setting in the same way that pressing **ENTER** does.

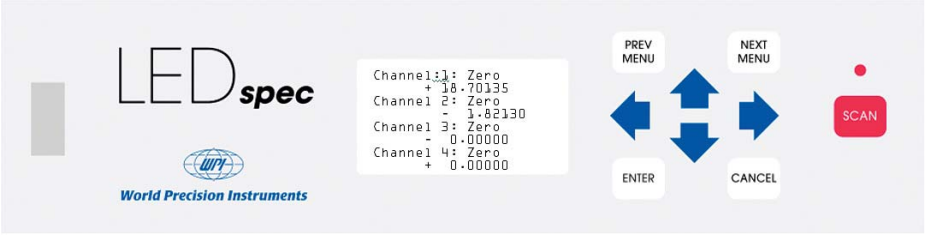

Fig. 38—Front panel of LEDspec

Measurement Menu

Use the **Measurement** menu (**Fig. 39**) to view the current readout of each channel or to zero a channel.

```
Channel 1: Zero
      + 18.70135
Channel 2: Zero
      -  1.82130
Channel 3: Zero
      -  0.00000
Channel 4: Zero
      +  0.00000
```

Fig. 39—Measurement menu

1. To set the current reading for a channel as the baseline, use the arrow keys to highlight the field **Zero** for that channel. Press the **ENTER** button. **Y/N** displays to confirm the action. To continue, highlight the **Y** and press the **ENTER** button. To abort, highlight the **N** and press the **ENTER** button or press the **CANCEL** button.
2. The current measurement for each channel displays under the **Zero** fields. In **Fig. 39** (above), Channel 1 is registering 18.70135 and Channel 2 is showing -1.82130.

LED Status Menu

Use the **LED Status** menu (**Fig. 40**) as you would use the LED Status window in the software to set up LEDspec for an experiment. From here, you can turn LED modules off and on for each channel, set the experimental mode and sample rate and view the live reading from each channel. See "LED Status" on page 31.

TIP: The software can be used to rapidly enter these experimental parameters, even if you want to use the LEDspec unit for making measurements and viewing live readings.

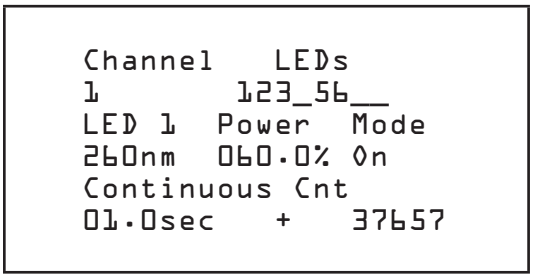

Fig. 40—LED Status menu

1. Highlight the **Channel** field and select the appropriate channel (1, 2, 3, 4 or reference).
2. In the **LEDs** field, choose which LED modules are on for the selected channel. Each position allows for the choice of a number or an underscore. The numbers indicate which LEDs are on. In the example above, LED modules 1, 2, 3, 5 and 6 are on for Channel 1. Since 4 and 7 display underscores, they are set to off for channel 1. The eighth character is reserved for future use.
3. In the **LED #** field, select the desired LED module. The wavelength value of the select LED module displays.
4. In the **POWER** field, enter the amount of power for the selected LED module. For example, if 75.0 is entered, the LED module runs on 75% of its power. It will not be as bright as when it runs on 100% power. Normally, power is set to about 80%.

5. In the **MODE** field, choose **Off**, **On**, **Auto** or **Test**.
   - Set to **On** or **Off** to turn power to the LED on or off.
   - Select **Auto** to allow LEDspec to select the optimal power level value.
   - Normally, the LED pulses at a constant interval, which makes it difficult to run diagnostics. To set the LED module to a continuous display so that it doesn't pulse, select **Test**. This allows for testing the LED module with a light meter or other measuring instrument.
6. Set the next field to **Continuous** for continuous flow analysis or **Single** for single shot analysis.
7. Choose the data value to be measured or calculated in the next field. Choices include Cnt (count), Tr (transmission), Abs (absorption), Int (intensity), Abs/cm (absorbance per centimeter) and Drk (dark).

   NOTE: Intensity and count are similar values. Count is the raw data with the dark reading subtracted out. The intensity is the count after zeroing. In other words, the baseline and the dark readings are subtracted from the raw data to produce the intensity measurement.
8. In the next field, set the sample rate. This is the number of times per second that a measurement is taken. The maximum sample rate is 5 times per second. However, the actual sample rate is slower, depending on the number of channels operating. Enter the desired value, and LEDspec configures itself to run at the highest sample rate equal to or below the desired value. The actual sample rate displays in this field after a few seconds.
9. The last field displays the live reading for the selected LED module.
10. Press the **ENTER** button when finished.

Analog Output Menu

Use the **Analog Output** menu (**Fig. 41**) as you would use the **Analog Out** section of the **Display and Analog Out** window. See "Analog Output to Data Recorder" on page 29. This window is used to configure the data and scaling information that is sent to a data acquisition system through the **Analog Output** ports on the back panel of the LEDspec unit.

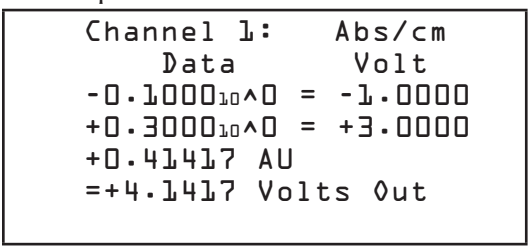

Fig. 41—Analog Out menu

1. Select the channel from the **Channel** field.
2. In the next field, choose the data to be output to the data acquisition system like Lab-Trax. Choices include Cnt (count), Tr (transmission), Abs (absorption), Int (intensity), Abs/cm (absorbance per centimeter) and Drk (dark).

   NOTE: Intensity and count are similar values. Count is the raw data with the dark reading subtracted out. The intensity is the count after zeroing. In other words, the baseline and the dark readings are subtracted from the raw data to produce the intensity measurement.
3. Use the **Data** and **Volts** fields to set the scale for the output. This pairs specific LEDspec measurements with specific upper and lower scale voltage values. The live data is then converted to a voltage reading that is read by the data acquisition system. The data acquisition software is used to convert the voltage signal back into data that can be analyzed.

   For example, Data -0.100 10^0 could be set to -1.000 Volts for the lower limit, and Data +0.300 10^0 could be set to +3.000 Volts for the upper limit.

   NOTE: The maximum voltage output is 10V.
4. Press the **ENTER** button when finished.

Analog Output Data Setup Menu

Use the **Analog Output Data Setup** menu (**Fig. 42**) as you would use the **Data Setup** section of the **Display and Analog Out** window. See "Analog Output to Data Recorder" on page 29. This window is used to establish the data that is sent to a data acquisition system through the **Analog Output** ports on the back panel of the LEDspec unit.

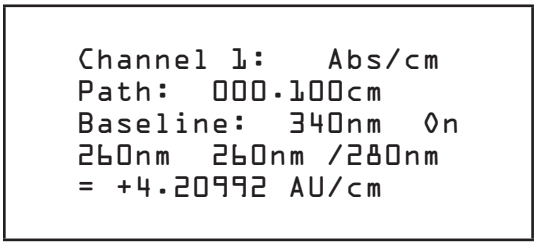

Fig. 42—Analog Output Data Setup menu

1. Select the channel from the **Channel** field.
2. In the next field, choose the data to be output to the data acquisition system like Lab-Trax. Choices include Cnt (count), Tr (transmission), Abs (absorption), Int (intensity), Abs/cm (absorbance per centimeter) and Drk (dark).

   NOTE: Intensity and count are similar values. Count is the raw data with the dark reading subtracted out. The intensity is the count after zeroing. In other

words, the baseline and the dark readings are subtracted from the raw data to produce the intensity measurement.

3. In the **Path** field, set the value in centimeters of the path length for each channel. This is the distance that light travels through a sample cell.

4. If a baseline offset is to be used, select the wavelength value of the offset from the **Baseline** field and set the next field to **On**. If no baseline is to be used, select **Off**.

5. The last set of fields allow you to subtract one wavelength value from another or divide one by another prior to export. For example, you might want to subtract a baseline reading or look at a ratio. The current reading for the selected channel displays on the last line of the menu.

6. Press the **ENTER** button when finished.

ACCESSORIES

Table 1: Accessories

Part Number	Description
89273	LED module, 260 NM
89272	LED module, 280 NM
89274	LED module, 340 NM
89245	LED module, 400 nm
89246	LED module, 450 NM
89247	LED module, 540 nm
89248	LED module, 560 nm
89275	LED module, 600 nm
89276	LED module, 650 nm
89249	LED module, 700 nm
LWCC-2200-LED	LEDSPEC UPGRADE: LWCC-2200,2 fibers, sample injector kit (58006), waveguide cleaning kit (501609)
LWCC-2050-LED	LEDSPEC UPGRADE: LWCC-2050,2 fibers, sample injector kit (58006), waveguide cleaning kit (501609)
LWCC-2100-LED	LEDSPEC UPGRADE: LWCC-2200,2 fibers, sample injector kit (58006), waveguide cleaning kit (501609)
PERIPRO-4L	Peri-Star Pro, 4-channel, low rate, small tubing
MINISTAR	Miniature Peristaltic Pump, 1-channel

TROUBLESHOOTING

LEDspec Software Not Receiving Data

If the LEDspec software is receiving data from the LEDspec unit, the icon in the upper right corner of the LEDspec software has a blue pipe (). If it is not connected, a red X displays ().

TIP: Hover the cursor over this icon to see the real-time data being transferred. If the LEDspec is not connected, it displays "No Data Available".

If the software is not receiving data:

1. Verify that the LEDspec unit is connected to a power supply and the unit is powered on. The red LED above the **SCAN** button should illuminate when the instrument is powered on.
2. Verify that the USB cable is connected securely to both the LEDspec and the computer.
3. In the software, from the **Setup** menu, select **Instrument**. When connected, the

Status button on this window glows neon green. Click on the COMM button and choose a different communications port and press the **Refresh** button.

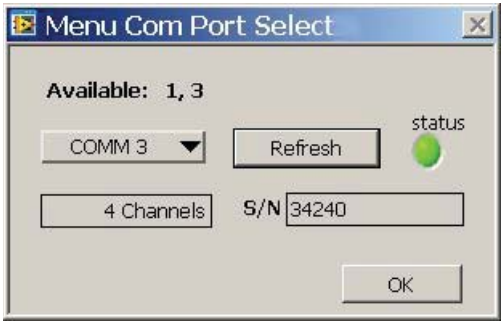

Fig. 43—LEDspec is communicating with the software when the status button glows green

TIP: If the LEDspec is powered on and properly connected but the software is still not receiving data, try connecting to a different USB port on the computer.

SPECIFICATIONS

The **LEDspec** conforms to the following specifications:

OPTICAL BASICS	LED-based multi-wavelength detector W/build-in reference channel
CHANNELS	2 or 4
DETECTOR	Photodiode
SPECTRAL BANDWIDTH (FWHM)	10 nm (LEDs >400nm)
	4 nm (260, 280, 340nm LEDs)
DYNAMIC RANGE	0-3 AU
DETECTOR RESOLUTION	24 Bit
NOISE (PEAK TO PEAK)	< 0.1 mAU
WARMUP TIME	Instant
FIBER OPTIC INPUT	600 µm
DRIFT	< 0.5 mAU/h
DIGITAL INPUTS AND OUTPUTS	8/8
ANALOG OUTPUT	+/- 10 V, scaleable output
DIMENSIONS (W*H*D)	290 x 80 x 250 mm (11.4″ x 3.2″ x 9.9″)
WEIGHT	2 kg (2.2 lbs.)
INTERFACE	USB
MAINS	100 – 240V/50 - 60Hz

INDEX

WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

World Precision Instruments, Inc.

USA

International Trade Center, 175 Sarasota Center Blvd., Sarasota FL 34240-9258

Tel: 941-371-1003 • Fax: 941-377-5428 • E-mail: sales@wpiinc.com

UK

1 Hunting Gate, Hitchin, Hertfordshire SG4 0TJ

Tel: 44 (0)1462 424700 • Fax: 44 (0)1462 424701 • E-mail: wpiuk@wpi-europe.com

Germany

Zossener Str. 55, 10961 Berlin

Tel: 030-6188845 • Fax: 030-6188670 • E-mail: wpide@wpi-europe.com

China & Hong Kong

WPI Shanghai Trading Co., Ltd.

Rm 20a, No8 Dong Fang Rd., Lu Jia Zui Financial District, Shanghai PRC

Tel: +86 688 85517 • E-mail:chinasales@china.wpiinc.com

Internet

www.wpiinc.com • www.wpi-europe.com • www.wpiinc.cn