Varian Analytical Instruments
2700 Mitchell Drive
Walnut Creek, CA 94598-1675/USA

ProStar 363

Fluorescence Detector

Operation Manual

QUALITY SYSTEM ISO 9001 CERTIFIED

03-914875-00: 1

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Introduction

General

Figure 1 ProStar 363 Fluorescence Detector

The ProStar 363 Fluorescence detector can be operated as a stand-alone HPLC detector or can be integrated into a Liquid Chromatography System controlled remotely from a Star Workstation.

In the stand-alone mode of operation, all user inputs are made on the front panel keypad. All operator inputs are displayed on a front panel display consisting of two lines of forty characters each. The detector measures the sample fluorescence at the user selected excitation and emission wavelengths. The fluorescence is output as an analog signal to an external data system, workstation, integrator, or recorder. This manual instructs the user in the installation and operation of the 363 Fluorescence detector for stand-alone applications.

Features of the ProStar 363 Fluorescence detector are:

- Stackable with the other ProStar modules
- High sensitivity at all wavelengths
- Scanning to determine the best excitation and emission wavelengths
- Time-programmable excitation and emission wavelengths
- Star Workstation control – Refer to the Star Chromatography Workstation Manuals.

Installation

General

The modular design of the ProStar 363 Detector enables you to locate it anywhere within the limitations imposed by the length of the power cord and signal cables. In order to keep liquid dead volume as low as possible, the detector should be positioned so that distance between the column outlet to the flow cell inlet is as short as is practical.

Flow cell fittings (inlet and outlet tubing) are located in the front panel.

All power and signal cable connections are made on the back panel. The power switch is located on the front panel of the unit. Prior to installation, please make preparatory arrangements for satisfying the installation requirements according to this manual. If relocation of this instrument becomes necessary after its initial installation, please notify your local Varian sales representative or nearest Varian service office.

Before unpacking, make sure that the bench top to be used is sturdy, level and can support the weight of the entire chromatographic system.

Receiving Inspection

Inspection and unpacking instructions have been provided in the ProStar pre-installation instructions prior to delivery. It is repeated here for future reference.

You will receive the instrument and the accessory package in a single shipping carton. Before you proceed with the unpacking, visually check the outside of the carton for any evidence of shipping damage, i.e., water stains, crushed corners, etc. If such evidence is present do not unpack the contents but report the condition to the carrier, and to:

Varian Chromatography Systems
2700 Mitchell Drive
Walnut Creek, California 94598-1675
Attention: Manager of Customer Service
Phone (925) 939-2400
or your local Varian Sales/Service Center

Unpacking

Avoid back strain or injury by following all precautions for lifting heavy

Carefully unpack the instrument and place it on the bench top.

After unpacking the system, carefully check the contents of each box against the packing list.

If any part is missing or damaged, contact the nearest Varian dealer.

Laboratory Requirements

The instrument should be installed in a laboratory that meets the following requirements:

Power Supply

Line voltage: 100 to 115 or 220 to 240 Vac ± 10% of the rated voltage.

Frequency: 50 or 60 Hz ± 4% of the rated frequency.

Power capacity:	360 VA for the detector. The detector is used as part of an HPLC system, therefore the power to be provided to the system is dependent on the configuration of the system. Ensure that the power supply is sufficient to meet the needs of the entire system.

Ground Terminal

WARNING:
SHOCK HAZARD

Ground Properly to Prevent Electric Shock Hazard!

- **Be sure to use the power cable supplied with the instrument. Use of a different power cable may result in an electric shock hazard.**
- **This instrument is classified as "1" in IEC1010-1 Annex H and "plug-connected type" in IEC1010-1, so connect the power cable to a grounded 3-wire outlet.**
- **If a grounded 3-wire outlet is not available be sure to provide proper grounding connection.**

Provide grounding connection having resistance of less than 100 ohms within a distance of three meters.

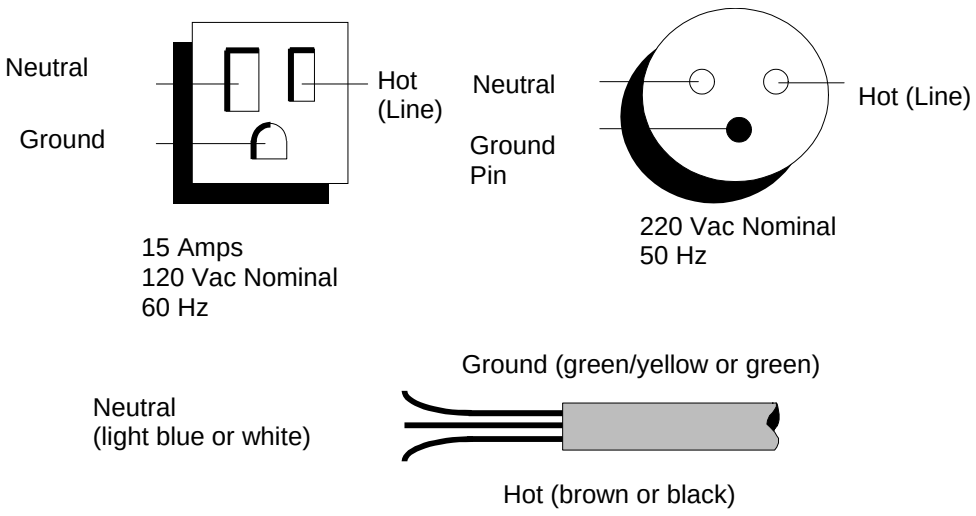


Figure 2 Power Receptacle Polarity

If your instrument fails to power up when the power switch is turned on, check that the power cord is properly connected, and check for power at the wall receptacle.

Location

The ProStar 363 Fluorescence Detector is a sensitive instrument and should always be handled with the degree of care appropriate to laboratory instrumentation. After unpacking, place the detector conveniently near your HPLC system. For normal operation, the detector should be located on a clean flat surface away from:

- heat sources (such as direct sunlight or a heater vent)
- drafts (such as an open doorway, window, or air-conditioner vent)
- corrosive or dusty atmosphere
- vibration
- potential liquid spills

Place the detector feet on a firm flat surface. Allow at least four inches of clear space at the back of the instrument so that the cooling fan intake is not impeded. The detector should be installed in a location with the following dimensions:

Minimum width = 450 mm

Minimum depth = 650 mm

The bench that is used for the instrument should be capable of supporting at least 50 kg.

Installation of Door Cap

If the ProStar 363 is the top module in the stack, the door cap should be installed prior to installing the door on the module. If the ProStar 363 is not at the top of the stack, do not install the cap and proceed to the next section describing door installation.

Take the cap from the accessory kit and remove the protective paper exposing the adhesive that will attach the cap to the door. Orient the door and cap as shown in Figure 3 with the door standing on a flat surface. Insert cap into door and press adhesive onto inside of door lip as shown in Figure 4. Be sure to keep door edges and cap edges flush.

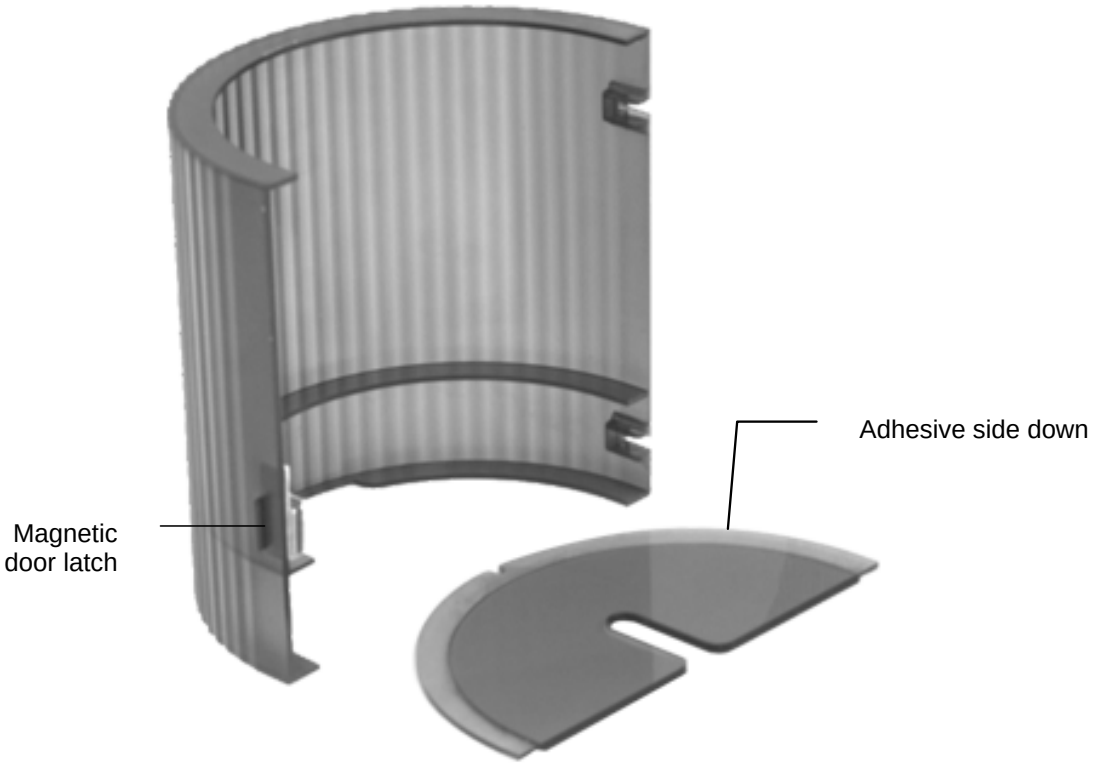


Figure 3 Door and Door Cap Orientation

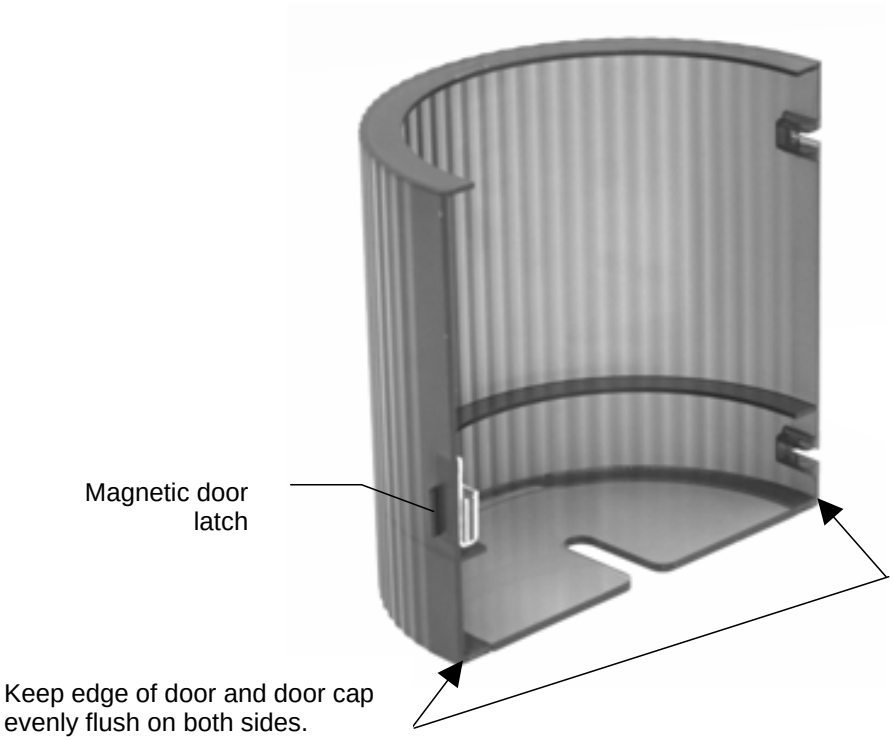


Figure 4 Door and Door Cap Assembly

Installation and Removal of Door

The module door may be attached to the front of the ProStar 363 to cover the tubing connections to the flow cell, see Figure 5.

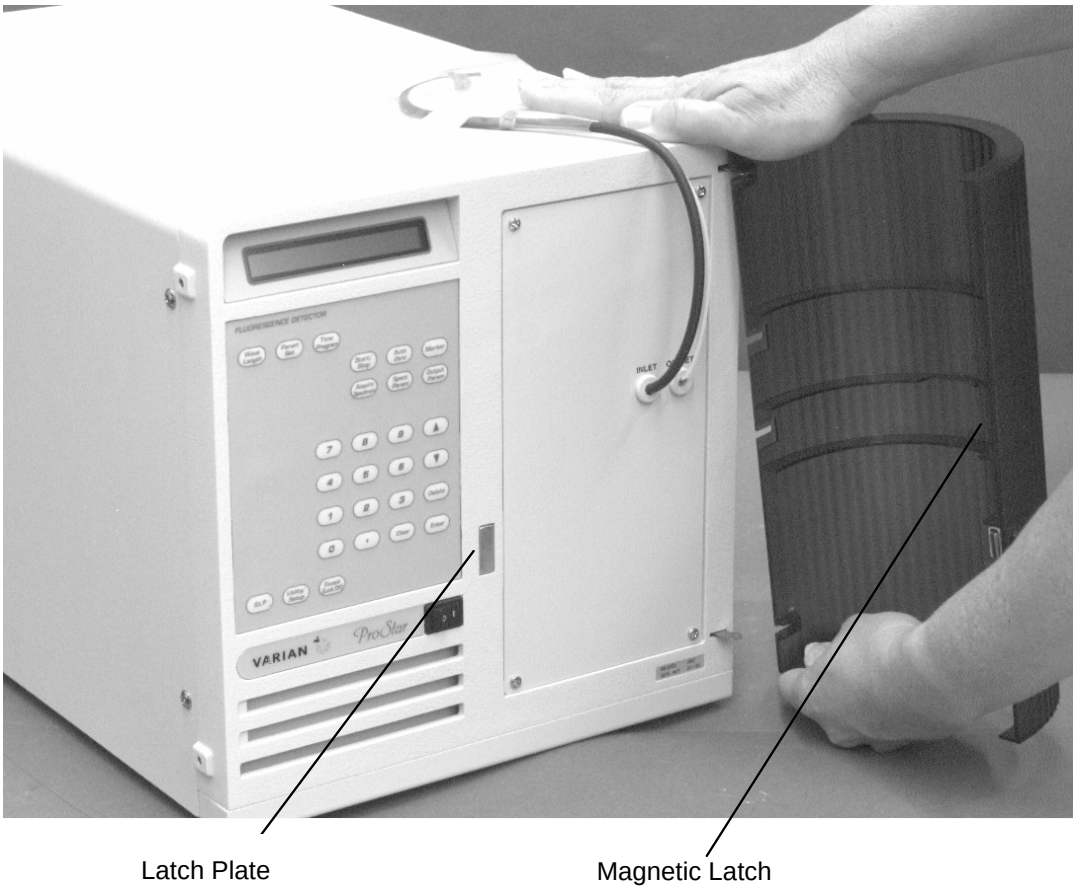


Figure 5 Door Installation

Orient the door so that the magnetic door latch lines up with the latch plate. Insert the top hinge pin into the top hinge pin into the top hinge. Gently press down on the top of the door (as shown in the figure) and slide the lower hinge pin into the lower hinge and stop pressing. The door should now pivot on the pins and close against the magnetic latch.

To remove the door, gently push down on the door and slide the lower hinge pin out of the lower hinge. Now lift and slide the door out.

Power and Signal Cable Connections (Rear Panel)

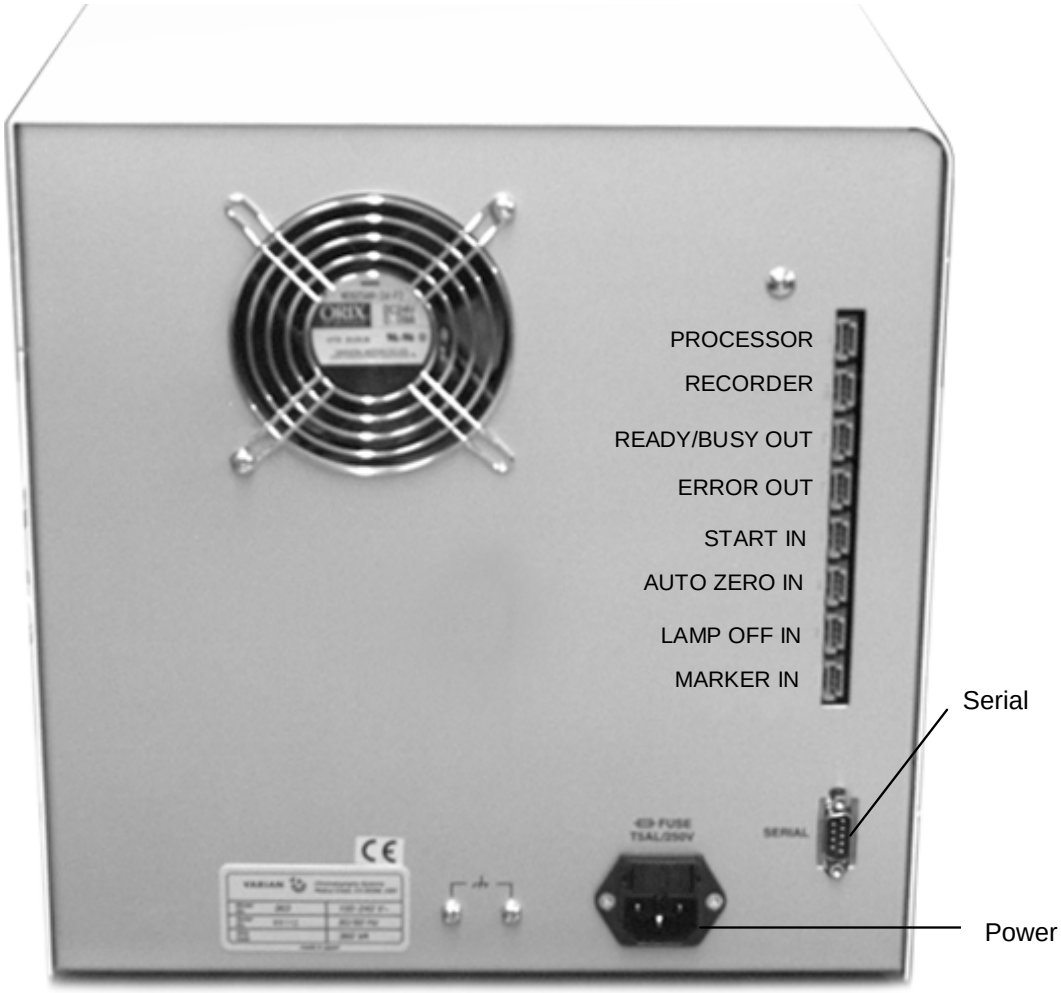


Figure 6 Rear Panel

Connect the power cord on the rear of the detector.

If the ProStar 363 will be controlled by the Star Workstation, connect the serial cable (03-935462-01) to the serial connector on the back of the ProStar 363 and to a serial port on the Star Workstation. This same cable can be connected to the ProStar AutoSamplers and/or the ProStar 210/215 or SD-1 pumps.

If the ProStar 363 is to send data to a Star Workstation, the Processor or Data System output must be connected either to a CIM box, CIM in a pump, or an ADC board. Connect the ProStar 363 to the ADC board with the Fluorescence detector interface cable (03-936156-01). Connect the ProStar 363 to the CIM input with the CIM to ProStar 363 cable (03-935922-01).

If you are going to connect the ProStar 363 to some other instrumentation, you can use the connections on the rear panel for synchronizing the module with any other HPLC system. The inputs and output are explained in the table below.

Table 1 Input/Output Terminals

Designation	Input/ Output	Function	Description
Data System/ Processor	Output	Analog output for processor, 1 volt full scale	The following signals can be output according to the setting. • Fluorescence intensity at each time point • Stored spectral data
Recorder	Output	Analog output for recorder, 10 mV full scale	The following signals can be output according to the setting. • Fluorescence intensity at each time point • Stored spectral data
Ready/ Busy Out	Output	Level change	Level goes high when detector is ready.
Error Out			Not used at this time.
Start In	Input	Contact closure	A contact closure here will start the program on the ProStar 363.
AutoZero In	Input	The auto zero function is turned on by the contact signal input.	The auto zero function is started when the terminals are shorted for at least one second.
Marker In	Input	The marker function is turned on by the contact signal input.	The marker function is started when the terminals are shorted for at least one second. A positive pulse is added to Recorder Output Signal.
Lamp Off In	Input	The xenon lamp is turned off by the contact signal input.	The lamp is turned off when the terminals are shorted for at least one second. An alarm sounds and an alert message is displayed. Pressing clear will stop alarm and re-light the lamp.

Table 2 Analog Signal Attenuation

	Fluorescence Intensity	***Spectrum***
Data System/Processor The output terminal for a data processor can output the present fluorescence intensity or the spectrum (full scale voltage of 1 V).	The fluorescence intensity at the currently set wavelength is output.	The spectral data is output according to SPECT RCD setting of RECORD
Recorder This output terminal is used for a recorder. The full scale voltage is 10 mV. The full scale fluorescence intensity can be set via the RCD RANGE from 1 to 1000 (in increments of 1) in output parameters menu. Two kinds of output signals can be selected. The output value is updated every 0.1 second.	The fluorescence intensity at the currently specified wavelength is output to the recorder.	The spectral data is output according to SPECT RCD setting of RECORD.

Hydraulic Connections

The only line installed by the user where dead volume and low holdup are critical is the line from the column exit to the flow cell inlet port. In all installations, this line should be as short as possible.

Detector Outlet Backpressure Terminator

A backpressure terminator assembly is included in the Standard Accessory Kit shipped with your ProStar 363 Detector. The back pressure terminator (03-919393-00) comes completely assembled and should be connected into the flow cell. The terminator applies about 40 psi back pressure to the flow cell to prevent outgassing and bubble formation in the light path which would cause an unstable baseline.

Note the arrow stamped on the terminator body. This arrow must point away from the flow cell outlet port, and toward the waste receiver. The threaded plastic fittings should be finger-tightened only enough to prevent leaks.

About 48" of 1/16" Teflon tubing is supplied at both the inlet and outlet of the terminator. The inlet tubing is provided with a 1/16" plastic tubing fitting for connecting to the flow cell. The inlet tubing can be removed from the terminator and the flow cell tubing can be connected directly. Either the outlet tubing can be directed to the waste container, or the tubing removed, and the terminator itself dropped to the bottom of the waste bottle.

The terminator pressure setting is not adjustable. If the terminator fails or becomes plugged, replace the existing cartridge with a new 40 psi replacement supplied in Kit 03-919239-90.

Installing and adjusting the Xenon lamp

The final part of installation is to install and align the xenon lamp. This is the same procedure that you will have to do when you replace a xenon lamp. Refer to procedures in *Replacing the Xenon Lamp* beginning on page 49.

System Description

General

The following paragraphs describe the ProStar 363 Detector as a stand-alone Fluorescence time-programmable detector controlled entirely from the local front panel keypad. When the instrument is being controlled from the Star Workstation, an [L] is displayed in the upper right hand corner of the two-line display. Information about controlling the detector from the Star Workstation is found in the Star Workstation manual.

Optics Hardware

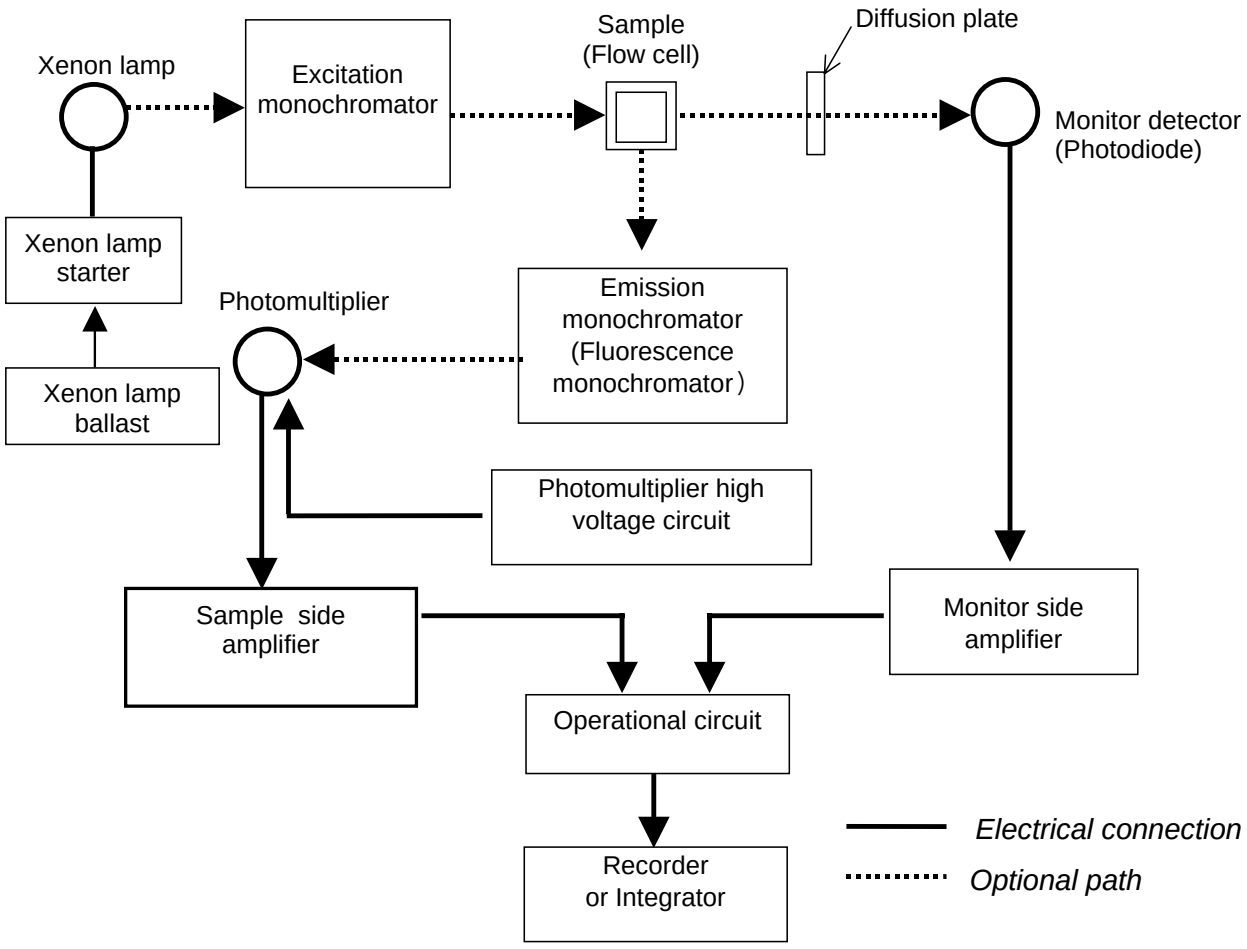


Figure 7 Functional Block Diagram

The components of the optical system are shown in Figure 7. The beam of the xenon lamp is converged by a mirror and enters the excitation monochromator where it is dispersed via a large aperture concave grating. Only light of specified wavelengths is allowed to pass through the exit slit. After the exit slit, the excitation light strikes the sample.

The fluorescence (emitted light) enters the emission monochromator, where it is dispersed by the monochromator.

The selected radiation strikes the measuring detector (photomultiplier).

The monitor detector (photodiode) measures the intensity of the excitation beam as a function of time and corrects for changes in the lamp intensity.

Keyboard and Display

All data entry is made via the keyboard on the front panel of the unit (see Figure 8). The role of each key is indicated in Table 3. For additional information about a key, see the detailed discussions in the Operation Section.

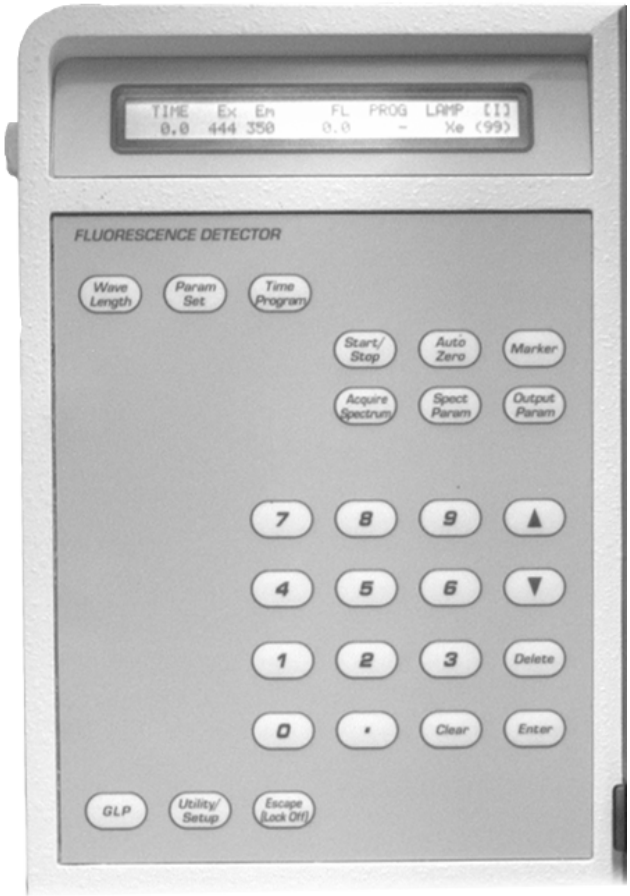


Figure 8 Keyboard

Table 3 Keyboard

Key	*Function*	*Key*	*Function*
Wave Length	Used to set the Excitation and Fluorescence Wavelengths.	●	Used for inputting a decimal point.
Start/ Stop	Starts (Stops) the time program. Starts (Stops) analog output of the stored spectrum.	**Clear**	Used to remove the present data input on the display before the **Enter** key is pressed (e.g., to erase incorrect data). Also used to reset system when an error condition is indicated.
Auto Zero	Sets the fluorescence intensity to zero at the indicated wavelength.	**Delete**	Used for deleting one step when editing a program with many lines.
Marker	Places a positive spike on the Recorder Output.	**Spect Param**	Used to set up excitation scanning range and fluorescence scanning range.
Time Program	Allows you to select a method by number and then edit that method.	**Acquire Spectrum**	Obtains spectrum over a specified wavelength range and stores the spectrum in memory. See Spect Param to specify scanning conditions.
Param Set	Used to select the time constant, pmt voltage, emission bandwidth, whether to use the time program or not, and to turn the lamp off or on.	**Output Param**	Used to specify the recorder full scale range, recorder output speed, and which stored spectra to output.
Utilty/ Setup	Sets offset value and accesses display mode. If the **Utility** key is pressed when the power switch is turned on, you can set the serial address for the module. This is used with Star Workstation communication. (SET UP)	**Escape (Lock Off)**	(1) Used to return from data input mode to the status screen. (2) Used to interrupt the recording of the spectrum. (3) Used to release the key lock when keys are locked.
GLP	Used for setting the key lock, checking lamp energy, and accessing the xenon lamp logbook.	**Enter**	Accepts the information that was entered.
▲ ▼	Accesses the next screen (or previous screen).	**0 to 9**	Used for entering numeric values.

Rear Panel

A complete description of the rear panel connectors and their functions is found in the Installation section.

Flow Cell

The flow cell is located on the right hand side of the detector. (See Figure 9.)

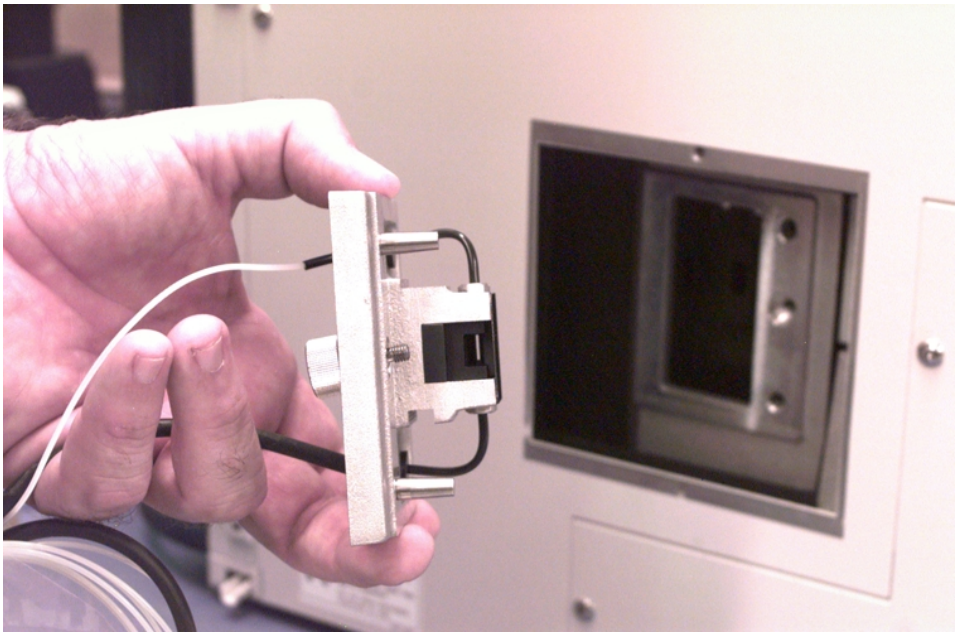


Figure 9 Flow Cell Connection

Connection to the flow cell is made though the front of the instrument behind the door. The flow cell provides a path for light to enter from the excitation monochromator and pass directly through to the monitor detector. There is also a slit in the flow cell which allows the fluorescent light to exit the flow cell and enter the emission monochromator. Flow through the flow cell is from the bottom to the top in order to minimize the likelihood that bubbles could be trapped in the flow cell. Also, a back pressure restrictor should be put on the end of the waste line to prevent bubble formation.

Xenon Lamp

The 150W xenon lamp is located on the right side of the instrument behind the flow cell as shown in Figure 10.

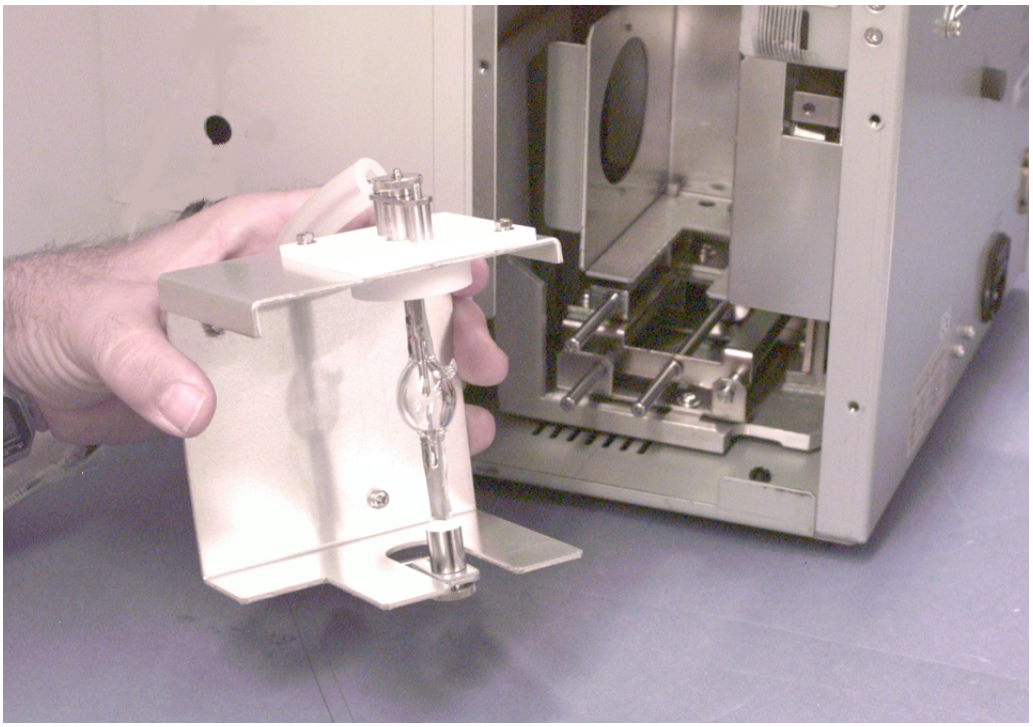


Figure 10 Xenon Lamp Location

The xenon lamp is mounted in a removable holder and adjusted with three screws located below at the bottom right of the lamp area. These screws adjust the lamp in three dimensions, up and down, right and left, and forward and back. Adjusting the lamp is important to give the highest light throughput and therefore the best sensitivity.

Operation

Turning on the Power

Turn on the **Power** switch of the detector.

An initialization test is carried out when the unit is powered up. During the initialization period, the lamp is ignited, the monochromators are initialized, and the output is autozeroed.

NOTE: The sequence of events described in this section are automatically performed when the instrument is powered up. If the status display screen does not appear on the LCD within three minutes after turning on the power, or if an error message appears on the LCD, refer to the Maintenance and Troubleshooting section.

Liquid Crystal Display (LCD)

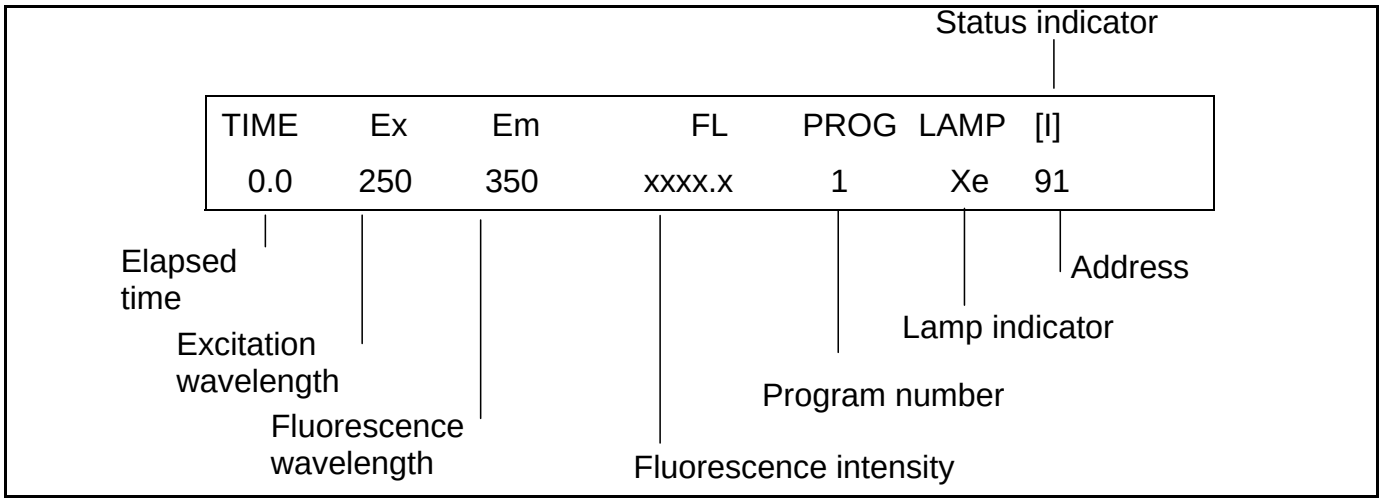
Varian ProStar 363 Fluorescence Detector Firmware P/N xxxxxxx-xx (Year)

After a short period of time, the monitor display presents:

WL DRIVE & LAMP INITIALIZING

The sequence of initializing the monochromator, turning on the lamp, and auto zeroing the instrument is performed during this period.

Normal Status screen (FL indicates Fluorescence output)



1. The currently selected program number is indicated.

2. 'Xe' is indicated when the xenon lamp is turned on, and 'OFF' is indicated when the lamp is turned off.

3. The address indicator shows the serial device indentifier of the ProStar 363. This identifier is used when communicating with the Star Workstation.

4. The status indicator describes the present status of the system.
   'I' is indicated when the detector is in the initial status, 'R' is indicated when it is in the time program running status, 'B' is indicated when it is in the busy status, and 'L' is indicated when it is in the key-locked status. The “L” status is displayed when the ProStar 363 is being controlled by the Star Workstation.

After the initialization process is complete, the unit should be allowed to warm up for approximately 30 minutes to meet specifications. It will operate immediately.

1. Use the **Numeric** keys and the **Enter** key to enter a value.
2. The **Clear** key is used to erase the present data entry (e.g., if you have made an error in entering data, but have not pressed the **Enter** key).
3. If the present settings are acceptable, simply press the **Enter** key or the ▼ key to access the next display. The ▲ or ▼ arrow keys are used to access the previous or next display.
4. After you have finished editing a display, use the **Escape** key to return to the status screen.
5. If the time program is not in use, spectral output can be performed at any time. If spectral output is started during data acquisition, the data is automatically directed to the integrator.

Normal Operation

Selecting the Serial Communications Channel

To access the Serial Communication set-up screen when powering up the instrument, press at the same time:

Power Switch | **Utility/ Setup**

The setup screen will be displayed.

Setup	Unit ID	
	0	<8E1 19200>

The Unit ID represents the address of the ProStar 363 on the serial bus. The addresses range from 90 to 99 so a unit ID of “0” means an address of 90, and a unit ID of 4 means an address of “4”. Every ProStar 363 on the same Star Workstation must have a different address.

Note that this full address number will be displayed in status as 90, 92, 92, 93.

To change the Unit ID, Press **Enter**. The screen below appears.

Unit ID (0-9)
0

At this point, you can enter any number from **0** through **9** with **0** being the default. The values 0-3 are recognized by the Star Workstation and translates to Device Identifiers 90-93.

Turn off the power to the system and then turn the power back on. The serial communication address is now configured.

Setting Up the Instrument Conditions

Param Set

Call up the instrument condition setup screen by pressing the **Param Set** key. The instrument parameters include:

- TIME CONSTANT
- PMT VOLTAGE (photomultiplier voltage)
- Em BANDWIDTH (emission bandwidth)
- USE TIME PROGRAM (selection of use/non-use of time program)
- LAMP MODE (selection of lamp turn-on/off)

Specify a time constant

TIME CONSTANT	(0.1s=1, 0.5s=2, 2.0s=3,
3	4.0s=4, 8.0s=5))

The current time constant value is displayed; the default value is **3**. Time constant value ranging from **1** to **5** can be entered using the Numeric keys. Select the desired value and press **Enter**.

The photomultiplier voltage setup screen will be presented. (If the indicated time constant is acceptable, simply press the ▼ key to go to the photomultiplier voltage setup screen, or press the ▼ key to go to the monitor screen.

As an alternative, press the **Escape** key to return to the monitor screen without changing the current setting.

Specify a Photomultiplier (PMT) voltage

```
PMT VOLTAGE  (HIGH=1,  MED=2,  LOW=3)
1
```

The current PMT output range value is displayed; the default value is **2**. An output range value of **1** to **3** can be entered using the Numeric keys.

Select the desired value and press **Enter**.

The Em bandwidth setting screen will be presented.

If the indicated PMT voltage is acceptable, simply press the ▼ key to go to the Em bandwidth setting screen, or press the ▲ key to go to the time constant screen. Press the **Escape** key to return to the monitor screen without changing the current setting.

Specify an emission bandwidth

```
Em BANDWIDTH  (STANDARD=1, WIDE=2)
1
```

The current bandwidth setting of the emission monochromator is displayed; the default value is **1**.

Input Value	Meaning
1	Emission monochromator bandwidth is standard (15 nm).
2	Emission monochromator bandwidth is wide (30 nm).

Select the desired value and press **Enter**. The time program screen will appear.

If the indicated setting is acceptable, simply press the ▼ key to go to the time program screen, or press the ▲ key to go to the photomultiplier voltage setup screen. Press the **Escape** key to return to the monitor screen without changing the current setting.

Indicate if the time program should be used.

```
USE TIME PROGRAM    (NO=0,   YES=1)
1
```

Select the desired value and press **Enter.**

The lamp select screen will be presented. (If the indicated time constant is acceptable, simply press the ▼ key or the **Enter** key to present the lamp select screen, or press the ▲ key to go to the Em bandwidth setting screen.

Press the **Escape** key to return to the monitor screen without changing the current setting.

Selecting the Lamp Mode.

```
LAMP MODE    (OFF=0,    ON=1)
1
```

The currently specified lamp mode value is displayed on the monitor screen; the default value is **1**.

Input Value	Meaning
0	The lamp is turned off.
1	The lamp is turned on.

Select the desired value and press **Enter.**

The monitor screen will be presented. If the indicated time constant is acceptable, press the ▼ key or the **Enter** key to present the monitor screen, or press the ▲ key to go to the time program screen.

Wavelength Range Setup Screen

Wave Length

Press the **Wave Length** key

Specify an excitation wavelength

Ex	Em	(200-850, 0) nm
250	350	

The currently specified excitation wavelength is displayed. (The default value is 250 nm.) The desired excitation wavelength (input value) can be entered (range = 200 to 850 nm).

Press the **Numeric** key for the desired excitation wavelength, press **Enter** → Fluorescence wavelength setup screen.

If the indicated excitation wavelength is acceptable, simply press the ▼ key to go to the fluorescence wavelength screen, or press the ▲ key to return to the monitor screen. As an alternative, press the **Escape** key to return to the monitor screen without changing the preset setting.

Specify a fluorescence wavelength

Ex	Em	(250-900, 0) nm
250	350	

The currently specified fluorescence wavelength is displayed; the default value is 350 nm. The desired fluorescence wavelength (input value) can be entered (range = 250 to 900 nm).

Press, **Numeric** key for the desired excitation wavelength, the **Enter** key → Monitor setup screen

If the indicated fluorescence wavelength is acceptable, simply press the ▼ key to go to the monitor screen, or press the ▲ key

to return to the excitation wavelength screen. As an alternative, press the **Escape** key to return to the monitor screen without changing the current setting.

To Adjust the Auto Zero

Auto Zero

Press the **Auto Zero** key to adjust the FL intensity to zero.

This will affect the reading on the monitor screen and the output signal level.

TIME	EX	EM	FL	PROG	LAMP	[I]
0.0	250	350	xxxx.x	1	Xe	(D1)

To correct for baseline drift or if an adjustment to the zero setting is desired, press the **Auto Zero** key to reset the output value to zero.

To Place a Marker on the Recorder

Marker

Press the **Marker** key to indicate a marker line on the recorder.

When the **Marker** key is pressed, a marker of approximately 0.6 mV is output on the recorder terminal signal. This marker can be used to indicate the time when the sample is injected or when a change in a present condition is made during measurement.

Time Program

Time Program

Press the **Time Program** key, select/setup screen.

Select a time program

A time program is used when the excitation and/or emission wavelength should be changed during the run. After the wavelength is changed, the baseline can be autozeroed or held as desired.

Up to 9 programs can be stored and up to 100 steps can be included in the time programs (e.g. one program can use 100 steps, or 9 programs can use 11 steps, etc.).

```
TIME PROGRAM NO.   (1-9)
1
```

The currently selected program number is displayed; the default value is 1. The acceptable range is from 1 to 9. The time programs are held in memory by battery backup when power to the system is turned off.

Numeric key → **Enter** key

Enter the number for the program to be created or modified. The relevant execution program number is changed at the same time.

To return to the monitor screen without changing the value, press the **Escape** key.

When a program is selected, it is necessary to specify whether it should be modified or a new program should be created.

```
MODIFY=0,  NEW=1
0
```

Numeric key → **Enter** key

Set up the time program

The first line of the time program is indicated below.

```
TIME    Ex    Em    BASE (A/Z=1, HOLD=2)
 0.0   250  350     1
```

Cursor movement

Enter the appropriate time (the time for the first line is fixed at 0.0) and press **Enter** to place the cursor in the Excitation Wavelength field. Enter the desired excitation wavelength and press **Enter** to place the cursor in the Fluorescence (Em) field.

After the fluorescence wavelength is selected, the cursor will appear in the BASE field. Enter **1** to indicate if the **Auto Zero** feature should be used to reset the baseline or enter **2** to indicate if the present level of the baseline should be maintained when the wavelength is changed.

NOTE 1: In the time program, the 'TIME' for the first step is 0.0, which cannot be changed or deleted. After this step has been edited, the time field for the second step can be accessed by using the ▼ key. At this point, any time up to 600.0 minutes can be used. Continue to enter lines. The time program can be carried out until the end-of-measurement time specified at the last step is reached.

NOTE 2: The time interval between lines should be at least 0.3 min.

The currently specified values for each setting are displayed.

- measurement time (TIME)
- excitation wavelength (Ex)
- fluorescence wavelength (Em)
- baseline processing (BASE)

a) Measurement time input range; the default value is 0.0. The range of the measurement time is from 0 to 600 minutes in increments of 0.1 minute.

b) Excitation wavelength input range; the default value is 250 nm. The range of the excitation wavelength is from 200 to 850 nm and 0 (zero order).

c) Fluorescence wavelength input range; the default value is 350 nm. The range of the fluorescence wavelength is from 250 to 900 nm and 0 (zero order).

d) Baseline processing input range; the default value is **1**.

Input Value	Operation	Baseline Processing
1	Auto Zero	**Auto Zero** setting should be performed after the wavelength is changed.
2	Hold	No adjustment is made to output signal.

- To erase a numeric value that was selected but not entered, use the **Clear** key.
- To remove a stored numeric value, use the **Delete** key. To go to the previous or next step, use the ▲ or ▼ keys.
- After the final step is set up, press the **Escape** key to return to the monitor screen.

Executing the Time Program

Start/ Stop

Start the time program by pressing the **Start/Stop** key. The status indicator “R” appears and indicates the program is running.

To stop execution of the time program, press the **Start/Stop** key.

Setting Up the Utilities

Utility/ Setup

To access the Utility/Setup setup screen, press the **Utility/ Setup** key.

The Utility screen provides access to the selection of a number of features including setting the signal offset value; if the lamp should be automatically shut off when the program ends, and also the selection of the active (displayed) parameter, and the selection of what is the displayed output (fluorescence, excitation, emission).

Note: the “turn off lamp by end signal” is not available or described.

Select the desired utility item for editing

UTILITY:	OFFSET=1, LAMP UTIL=2,
<1-3>1	DISPLAY MODE=3

Numeric key → **Enter** key

Settable items:

- Offset value
- Lamp turn-off enable/disable condition – not available
- Setting of monitor display mode

The currently specified values are displayed; the default value is **1**.

Input Value	Meaning
1	OFFSET Enter **1** to set the offset value.
2	LAMP UTIL (Lamp utility) - Not available Enter **2** to set the lamp turn-off enable/disable condition.
3	DISPLAY MODE (Signal mode) Enter **3** to set the monitor display mode.

Specify an offset value

```
OFFSET (0-1000)
_  0
```

Numeric key → **ENTER** key

The currently specified value is displayed; the default value is **0**.

The offset value adds a specified input signal value to the actual measured value on the output.

Offset setting range: 0 to 1000

The offset value can be specified in increments of 1.

Typically, the negative input limit or an integrator or data processor is approximately –10 mV. If the baseline decreases below this level, it becomes impossible to integrate the peaks in the chromatogram (see Figure 11a). In such a case, the offset function is used to adjust the shifted baseline to the allowable input signal range of the data processor (see Figure 11b).

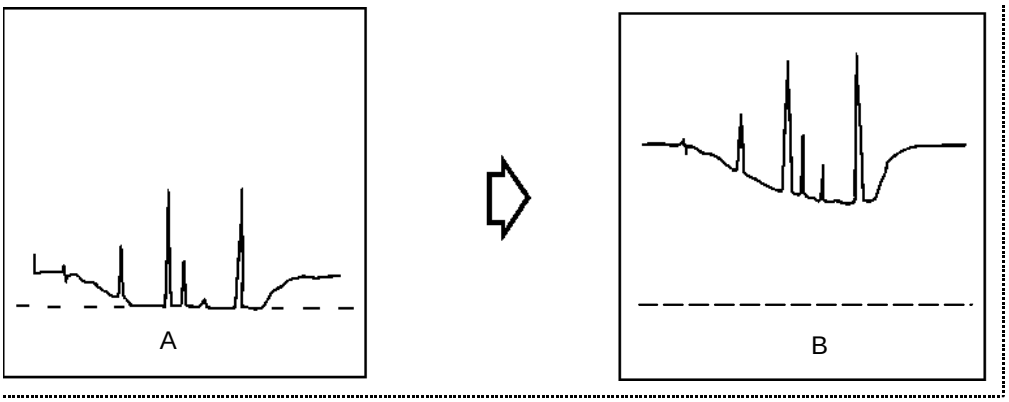


Figure 11 Example of Offsetting

Set the monitor display mode.

```
DISPLAY MODE  (FL=1,  Ex<M>=2,  Em<M>=3)
1          _
```

Numeric key → **Enter** key

The currently specified value is displayed; the default value is **1**.

Input Value	Meaning
1	FL mode Enter **1** for fluorescence intensity measurement. (Normal status screen)
2	Ex<M> mode Enter **2** for excitation energy check.
3	Em<M> mode Enter **3** for fluorescence energy check.

This option is used mainly for the wavelength accuracy check for monochromator during Operational Qualification.

Setting Up Spectrum Acquisition Parameters and Output

The spectrum Acquisition Parameters and Output window is used when an excitation or fluorescence spectrum is to be collected.

Spect Param

Set parameters for acquisition of the spectrum.

To set the excitation and fluorescence scan ranges (maximum and minimum value), press the **Spect Param** key.

The maximum wavelength scan range (= end wavelength - start wavelength) is 350 nm. Select an acquisition (scan) mode.

SCAN MODE (Ex=1, Em=2)
2

Numeric key → **Enter** key

The currently specified value is displayed; the default value is 2.

Input Value	Scan Mode	Meaning
1	Ex	Enter **1** to define the excitation scan range.
2	Em	Enter **2** to define the fluorescence scan range.

NOTE: The system will present either the Ex SCANNING WL RANGE screen or the Em SCANNING WL RANGE when **Enter** is selected.

Specify an excitation scan range.

Indicate the starting wavelength (minimum value). (The information in this section is not relevant in Em Scan Mode.)

Ex SCANNING WL RANGE (200-800) nm	
200 - 550	1 nm step

Enter the desired wavelength with the numeric keypad and press the **Enter** key to change the value. The currently specified value is displayed; the default value is 200. The allowable input range is from 200 to 800 nm.

The ▲ and ▼ key allow you to move back and forth between starting and ending wavelength.

Indicate the ending wavelength (maximum value)

Ex SCANNING WL RANGE (250-550) nm	
200 – 550	1 nm step

Enter the desired wavelength and press the **Enter** key to change the value. The currently specified value is displayed. (The default value is 550.) The allowable input range is “start wavelength + 50” to “start wavelength + 350”.

Specify a fluorescence (also called emission) scan range; the information in this section is not relevant in Ex Scan Mode.

Indicate the starting wavelength (minimum value)

Em SCANNING WL RANGE (250-850) nm	
250 - 600	1 nm step

Enter the desired wavelength and press the **Enter** key to change the value. The currently specified value is displayed; the default value is 250. The allowable input range is from 250 to 850 nm.

Use the ▲ or ▼ keys if the present value is acceptable.
The ▲ key returns to the monitor screen, while the ▼ key accesses the Em SCANNING WL RANGE end wavelength field.

Indicate the ending wavelength (maximum value)

Em SCANNING WL RANGE (300-600) nm	
250 - 600	1 nm step

Enter the desired wavelength and press the **Enter** key to change the value. The currently specified value is displayed; the default value is 600. The allowable input range is “start wavelength + 50” to “start wavelength + 350”.

The ▲ and ▼ key allow you to move back and forth between starting and ending wavelength.

Note that the maximum wavelength scan range (= end wavelength - start wavelength) for both excitation and emission is 350 nm.

For spectral acquisition in a range beyond 350 nm, two separate scans will have to be made.

Collecting the Spectra

Acquire Spectrum

When the **Acquire Spectrum** key is pressed, the appropriate Monochromator is scanned over the range which was set in the section, Setting Up Spectrum Acquisition Parameters and Output and the spectrum is stored using the indicated memory number. When collecting a spectrum, there should be a sample in the cell and the flow rate should be zero (0).

Specify a storage file.

SPECTRUM MEMORY NO. (1-8) MODE: Em
No.

Numeric key → **Enter** key

The scanning starts with the display below.

Up to eight spectra can be stored. The currently selected scan mode is displayed by the MODE field. The spectral memory numbers (input values) are used for identification of stored spectra. When a spectral memory number is specified, the spectrum in that memory location is deleted and the new spectrum data is stored.

While the unit is scanning, the monitor screen will present:

SCANNING SPECTRUM NO. x
Ex:xxx Em:xxx

The specified spectrum number and the current wavelength are displayed. After the spectrum has been stored, the status screen is presented. The stored spectral data is not retained when power is turned OFF.

NOTE: Stored spectral data is not retained when the power is turned OFF.

To cancel spectral data acquisition, press the **Escape** key.

NOTE: The spectrum will not be saved if the **Escape** key is pressed.

Setting the Recorder and Spectral Output Parameters

Output Param

Access the recorder/spectral parameter factor setup screen by pressing **Output Param**. The recorder/spectral output parameter setup screen will then appear. This screen is used to set:

- The recorder full scale range
- The recorder output speed
- The output data

The **Output Param** key is used to access several screens which are used to indicate information about the recorder and output of the spectrum.

```
RECORD:  RCD RANGE=1, RCD SPEED=2,
<1-3>  1 SPECT RCD=3
```

Numeric key → **Enter** key

The currently specified recorder full scale range is displayed; the default value is **1**.

Input Value	Meaning
1	RCD RANGE (Recorder range) Enter **1** to set recorder output full scale range.
2	RCD SPEED (Recorder speed) Enter **2** to set recorder output speed.
3	SPECT RCD (Spectral recorder) Enter **3** to set output data format.

```
RECORDER RANGE (1-1000)
1000
```

Numeric key → **Enter** key

The recorder range only affects the recorder output, not the processor output. The default value of 1000 means F.L. (Fluorescence units) will be full scale (10 mV) on the recorder output. If 100 was entered, 100 F.L. would be 10 mV. This is a means to expand the Fluorescence range to match the recorder range.

```
RCD SPEED (40 nm/min=1, 60 nm/min=2)
2
```

Indicate the output speed for the recorder.

Numeric key → **ENTER** key

The currently specified recorder speed is displayed; the default Recorder Speed value is 2.

Input Value	Meaning
1	40 nm/min=1
2	60 nm/min=2

RECORD SPECTRUM NO. (1-8)
1 –

Assign a spectral number to output data

Numeric key → **Enter** key

The currently specified value is displayed; the default value is **1**. A value ranging from **1** to **8** can be entered using the Numeric keys.

BACKGROUND SPECTRUM NO. (0-8)
0

Specify background data. The currently specified background is displayed; the default value is **0**. A value ranging from **0** to **8** can be entered using the Numeric keys. The background spectrum is used to compensate for fluorescence due to the solvent and/or the flow cell. To ensure that an appropriately corrected spectrum is used in analytical work, a new background spectrum should be collected whenever the solvent or wavelength range is changed.

Input Value	Meaning
0	The spectrum selected by RECORD SPECTRUM NO. is presented. No background correction is made.
1 to 8	The specified background is subtracted from the spectrum selected by RECORD SPECTRUM NO., and the resultant spectral data is presented.

Numeric key → **Enter** key

After the parameters are entered, the monitor screen presents;

RCD NO.	SPEED	WL-RANGE	MODE: Em
x	60	200-550	PRESS "START"

Start/ Stop

During collection of the spectrum, the monitor screen will present the Output monitor screen:

RCD NO.	Ex	Em	FL	SPEED	MODE
x	xxx	xxx	xxxx.x	60	Em

To cancel collection of the spectrum before it is presented, press the **Escape** key. To cancel it during output of spectral data, press the **Start/Stop** key.

When the detector is connected to the Star Workstation, spectral data can be collected in the Workstation by pressing the **Start** key on the ProStar 363 module in System Control.

For more information about scanning form the Star Workstation, see the ProStar 363 control information in the Star Workstation manual.

NOTE 1: In a fluorescence spectrum, scattered excitation radiation, Raman scattering, fluorescence due to solvent or impurities contained in the solvent, and second order (and third order) emission will be superimposed on the fluorescence of the analyte. Similarly, an excitation spectrum may be affected by scattered radiation at the fluorescence (emission) wavelength, Raman scattering, fluorescence due to solvent or impurities contained in it, and second order (and third order) scattered light of excitation beam. When selecting the optimum excitation and fluorescence wavelengths, make certain that these potential interferences are considered.

NOTE 2: The fluorescence spectrum presented by the ProStar 363 is not compensated for the wavelength dependent sensitivity of the photomultiplier or the gratings; thus an excitation spectrum/ fluorescence spectrum attained from the ProStar 363 will not directly correspond to the true spectrum of the analyte. This difference will be most noticeable at emission wavelengths below 300 nm.

Selecting the GLP Function

GLP

Access the GLP function setup screen. These functions are used to enter information about a variety of security functions and performance related issues.

Select the GLP function item to be edited.

```
CONFIDENCE:  KEY LOCK=1, CHECK=2,
<1-4>  1 LOGBOOK=3, LAMP CHANGE=4
```

Press the desired **Numeric** key and press the **Enter** key.

GLP function items:

a. Key locking/unlocking
b. Check - no longer used
c. Display of xenon lamp logbook data
d. Resetting of logbook function

The key-lock function can be used to deactivate the keypad.

```
KEY LOCK (NO=0, YES=1)
0
```

Numeric key → **Enter** key

To cancel Key Lock, press the **Escape** key.

If an error is observed by the system, the keypad is automatically unlocked

The logbook function is used to indicate when the lamp was last changed.

The display will present number of hours of use, the number of times the light was turned on, and the date that the lamp was last changed, as shown below:

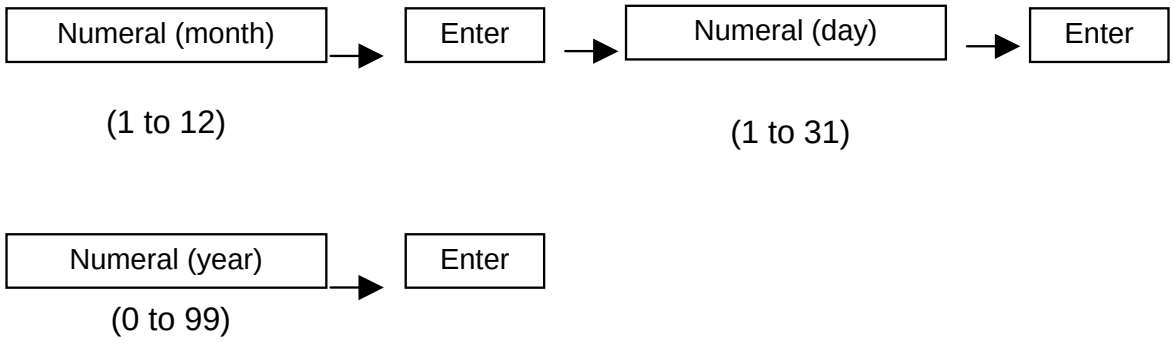
```
LIGHT-ON        SWITCHING     LAST CHANGED DATE
     xxxxh         xxxxtimes                1 1 1
```

The Lamp Change function is used to reset the logbook functions when the lamp is changed.

When Lamp Change is selected, the display presents:

```
LAMP CHANGE (MM DD YY)
_1 1 1
```

The date is entered in the format MM DD YY.



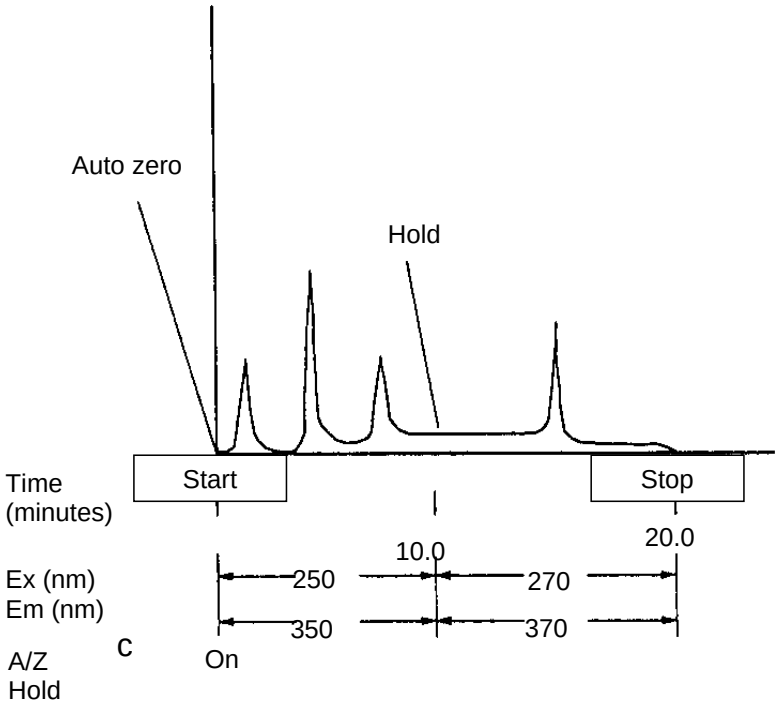
Enter the appropriate data.

NOTE:	The logbook data entry is reset when the **numeric** value representing 'year' and **Enter** are selected. It is necessary to enter the year, even if the lamp was last changed during the present calendar year. As an example, if the lamp was changed on 3-11-00 and it is being changed on 11-27-00, make certain that 00 is reentered before **Enter** is selected.

Example of Time Program Operation

This selection describes how a time-based program operates. The events indicated in the figure below will be used in the discussion.

Setting up a Time Program



In this example, a new time program for the activity described in the above figure will be generated.

1. At the start of the separation, the Ex monochromator is set to 250 nm, the Em monochromator is set to 350 nm and the autozero function is used to define the baseline.

2. After 10 minutes, the Ex monochromator is set to 270 nm and the Em monochromator is set to 370 nm. When the monochromators are changed, the baseline is maintained (held).

3. After 20 minutes, the measurement of fluorescence is completed.

NOTE 1: In time program setting, the initial 'TIME' for the first step must be 0.0. This initial value cannot be changed or deleted.
Be sure to specify the end time of measurement in the last step. The time program will be run until the end-of-measurement time specified at the last step is reached.

NOTE 2: In time program setting, provide an interval of at least 0.3 minute between steps.

NOTE 3: When a time program is selected, the display presents the operator with the opportunity to indicate if the present program should be modified or if a new program is to be entered.
If the 'MODIFY' setting is selected, the first line of the program is displayed for editing as described below.
If the 'NEW' setting is selected for a time program, the previous time program is deleted. The first step in the method will include the initial values indicated in the table below. This table also includes the allowable range for each parameter.

	Initial Value	Input Range
TIME	0.0	0.0 to 600.0
Ex		200 to 850, 0
Em		250 to 900, 0
BASE	1	1, 2

START STOP key

TIME PROGRAM NO. (1-9)
1

1 key → **Enter** key

MODIFY=0, NEW=1
0

1 key → **Enter** key

(**0** key →) **Enter** key →
(**2 5 0** key →) **Enter** key →
(**3 5 0** key →) **Enter** key →
(**1** key →) **Enter** key →

TIME	Ex	Em	BASE (A/Z=1, HOLD=2)
0.0	250	350	1

1 0 key → **Enter** key →
2 7 0 key → **Enter** key →
3 7 0 key → **Enter** key →
2 key → **Enter** key→

TIME	Ex	Em	BASE (A/Z=1, HOLD=2)
1.00	2.70	370	2

(**2 0** key →) **Enter** key → **Escape** key

TIME	Ex	Em	BASE (A/Z=1, HOLD=2)
20.0			

Monitor screen

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0	1	Xe	(90)

To indicate that the program should be stopped at a certain time, open a new step that is identical to the immediately previous step, except that the time is different. In this example, the line for 10 min (Ex 270 nm, Em 370 nm, Hold), should be repeated using a time of 20.0 min.

View a time program

To check the program that was just entered.

Press the **Time Program** key to access the Time Program screen:

TIME PROGRAM NO. (1-9)
1

Enter the desired program number and press the **Enter** key.

The display will present:

MODIFY=0, NEW=1
0

Enter **0** and press the **Enter** key.

The display will present:

TIME	Ex	Em	BASE(A/Z=1, HOLD=2)
_ 0.0	250	350	1

To access the next line, press the ▼ key

TIME	Ex	Em	BASE(A/Z=1, HOLD=2)
_ 10.0	270	370	2

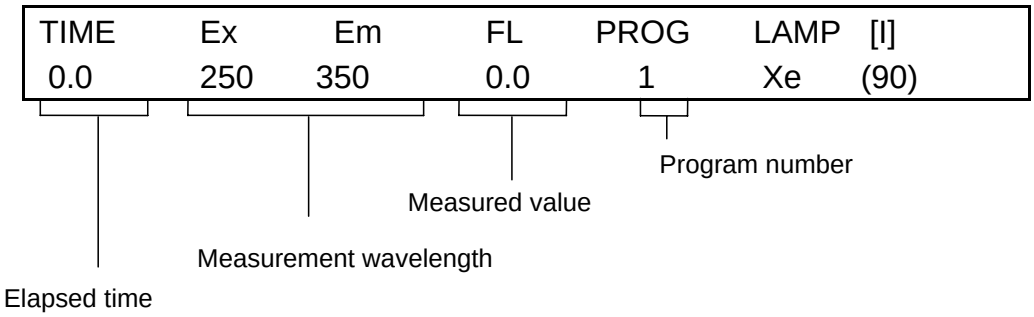
To access the next line, press the ▼ key.

TIME	Ex	Em	BASE(A/Z=1, HOLD=2)
_ 20.0	270	370	

To return to the first line of the program press the **Escape** key.

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0	1	Xe	(90)

To execute the Time Program press the Start/Stop key.



On completion of the time program, measurement is automatically terminated and the display returns to the status screen. At this point, the wavelength settings are those that were on the status screen before the time program was executed.

NOTE: It is advisable to set the wavelengths on the monitor screen to the same wavelengths as indicated in the Time = 0.0 of the time program.

Shutting Down the Detector

To turn the detector off, turn off the **Power** switch.

The set measurement conditions are retained by the memory when the power is turned off. When the power is turned on again, the conditions that were in place when the power was turned off will be restored.

Flushing the Flow Cell After Use

Be sure to flush the flow cell with distilled water after using any buffers or salts in the mobile phase. Failure to remove buffer or salt solutions may lead to corrosion and or the formation of salt deposits in the system.

Maintenance and Troubleshooting

Replacing the Xenon Lamp

The xenon lamp is too hot to touch immediately after the instrument is turned off. When replacing the xenon lamp, wait until the old lamp cools down completely as described below.

To Cool Down the Xenon Lamp:

Press the following keys:

Param Set key → **Enter** key → **Enter** key → **Enter** key → **Enter** key

The following screen will appear.

```
LAMP MODE  (OFF=0,  ON=1)
1
```

On this screen, press the **0** key, followed by the **Enter** key to turn off the xenon lamp. The fan will continue to run to cool down the xenon lamp. Leave the power to the instrument on and wait for approximately 30 minutes.

The xenon lamp must be removed from the detector prior to shipping the unit. Failure to follow this procedure will result in a broken lamp.

WARNING:
BURN HAZARD

- **The xenon lamp remains hot for a while even after power-off and can severely burn you if touched.**
- **Although the xenon lamp will be automatically extinguished by a safety mechanism when the light source cover is opened, before replacement of the lamp, turn power off and wait for about 2 hours until it cools down sufficiently.**

WARNING:
SHOCK HAZARD

- **Potentially Dangerous Voltages are Present within the Instrument.**
- **While the POWER switch is on, a voltage of approximately 90V is applied to the anode of xenon lamp. When the lamp goes on, a high voltage of approximately 30 kV is applied.**
- **To ensure safety, turn off the POWER switch and unplug the power cord from the outlet.**
- **Before removing the xenon lamp for replacement, turn power off and wait for more than two hours. Then, open the light source cover.**

WARNING:
EXPLOSION HAZARD

- **Explosion of the xenon lamp could cause serious injury. High-pressure gas at about 1 MPa is filled in the lamp at room temperature.**
- **Be careful not to apply force to the lamp bulb (glass part) when replacing the lamp.**
- **Wear protective goggles and gloves when handling the lamp**
- **When tightening the lamp clamp nut, hold the metal parts on the anode and cathode sides with fingers. Never hold the lamp bulb when securing it. Take care not to apply excessive force or shock to the lamp bulb. If the lamp bulb is contaminated with dust or fingerprints, wipe it using the furnished cleaning paper or gauze/absorbent cotton cloth moistened with high-quality alcohol. In this cleaning, be careful not to apply strong impact or shock to the lamp.**
- **Be sure to mount the lamp in the specified direction. If the mounting direction (polarity) is wrong, the cathode will be consumed significantly to disable turn-on of the lamp.**
- **Mount the lamp so that the '+' (anode) mark on it will be positioned at the support metal of the lamp holder.**
- **If a lamp with its cathode consumed excessively is used continuously, the pressure inside the lamp bulb may become too high, causing explosion. To prevent this, replace the lamp with a new one immediately if its cathode has been consumed significantly.**

WARNING:
EYE HAZARD

Gazing at Illuminated Xenon Lamp Could Cause Eye Damage!

- **The xenon lamp radiates intense ultraviolet light when it is on.**
- **Do not look at the xenon lamp directly when it is lit.**
- **Wear tinted safety glasses to prevent possible eye damage**

Lamp Replacement

After the xenon lamp is cooled down, turn off the **Power** switch. Then, remove the lamp holder (with the xenon lamp mounted) by reversing steps (1) to (6) beginning on page 53.

Remove the xenon lamp by reversing steps (3) and (4) beginning on page 53.

To place a new xenon lamp in the system, follow steps (1) to (6) beginning on page 53.

NOTE: After the lamp has been replaced, reset the Logbook display (which indicates the date that the lamp was replaced, the number of lamp starts and the period of time that the lamp has been on). When the lamp installation date is changed, the cumulative time and the number of starts are automatically re-set to zero. For details on setting the logbook, refer to Selecting GLP function, on page 42.
To set the data that the lamp was changed, use the sequence of keys indicated below (using the example of December 10, 1999). The data should be entered in the format of MM/DD/YY (e.g. 12/10/99).

GLP key → **4** key → **Enter** key → **1** key → **2** key → **Enter** key → **1** key → **0** key → **Enter** key → **9** key → **9** key → **Enter** key

Installing the Xenon Lamp

WARNING:
SHOCK HAZARD

- **Potentially Dangerous Voltages are Present within the Instrument.**
- **While the POWER switch is on, a voltage of approximately 90V is applied to the anode of xenon lamp. When the lamp goes on, a high voltage of approximately 30 kV is applied to it.**
- **To ensure safety, however, turn off the POWER switch and unplug the power cord from the outlet.**
- **Before removing the xenon lamp for replacement, turn power off and wait for at least 30 minutes. Then, open the light source cover.**

1. Make sure the **Power** switch is turned off before installing the xenon lamp. To remove the light source cover, first loosen the four mounting screws indicated in Figure 12.

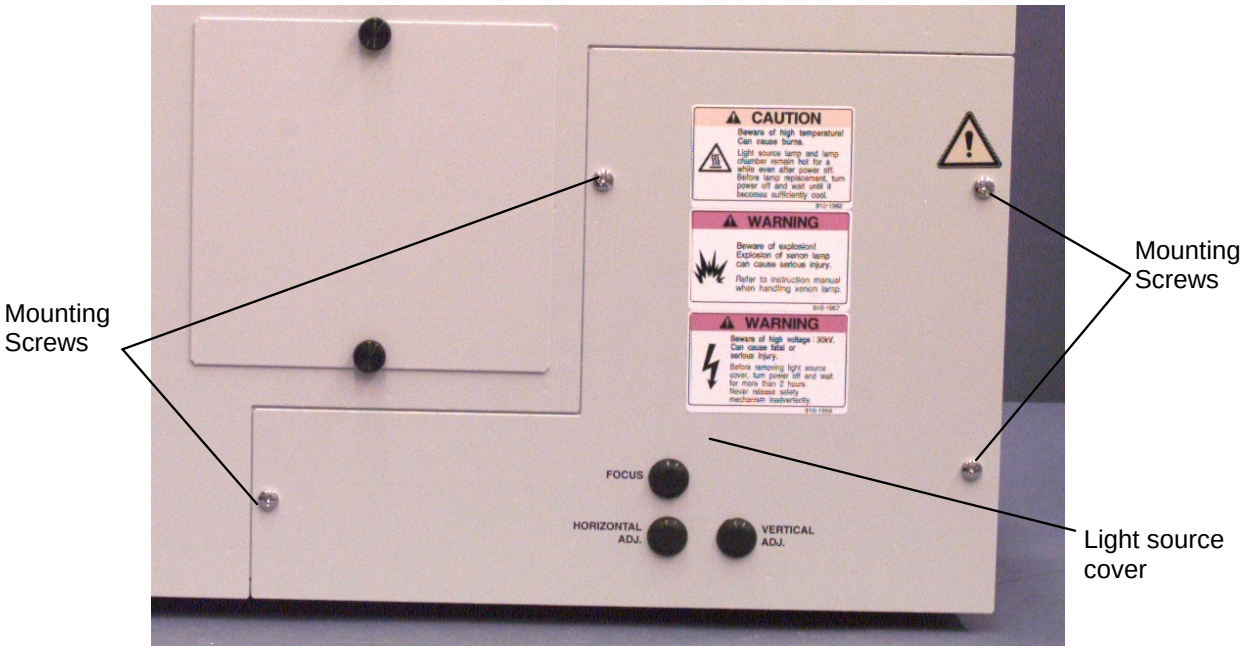


Figure 12 Removal of Light Source Cover

WARNING:
EXPLOSION HAZARD

WARNING:
EYE HAZARD

- **Explosion of the xenon lamp could cause serious injury.**
  High-pressure gas at about 1 MPa is filled in the lamp at room temperature.
- **Be careful not to apply force to the lamp bulb (glass part) when replacing the lamp.**
- **Wear protective goggles and gloves when handling the lamp.**

2. When placing the xenon lamp in the lamp holder, (Figure 13), put the anode terminal (marked '+') of the lamp into the metal fixture on the ceramic plate. Then, using the anode nut, mount the lamp so that the protruding part of the bulb is positioned as shown in Figure 14. Do not touch the quartz envelope while the lamp is being installed. Handle the lamp by holding the metal pieces on each end of the lamp.

   A thin metallic wire is attached on the center area of the bulb, as shown in Figure 14. This wire serves to ensure that the lamp is easily started. When handling the lamp, be careful not to break this wire. If the thin metallic wire is not located on the side of the lamp with the small

quartz protrusion, side the wire so it is properly positioned.

3. Attach the cathode lead plate to the cathode threaded part, and secure the lead plate with the cathode nut.

WARNING:
EXPLOSION HAZARD

- **When tightening the lamp clamp nut, hold the metal parts on the anode and cathode sides with fingers. Never hold the lamp bulb when securing it. Take care not to apply excessive force or shock to the lamp bulb.**

- **If the lamp bulb is contaminated with dust or fingerprints, wipe it using the furnished cleaning paper or gauze/absorbent cotton cloth moistened with high-quality alcohol. In this cleaning, be careful not to apply strong impact or shock to the lamp.**

- **Be sure to mount the lamp in the specified direction. If the mounting direction (polarity) is wrong, the cathode will be consumed significantly to disable turn-on of the lamp.**
  Mount the lamp so that the '+' (anode) mark on it will be positioned at the support metal of the lamp holder.

- **If a lamp with its cathode consumed excessively is used continuously, the pressure inside the lamp bulb may become too high, causing explosion. To prevent this, replace the lamp with a new one immediately if its cathode has been consumed significantly**

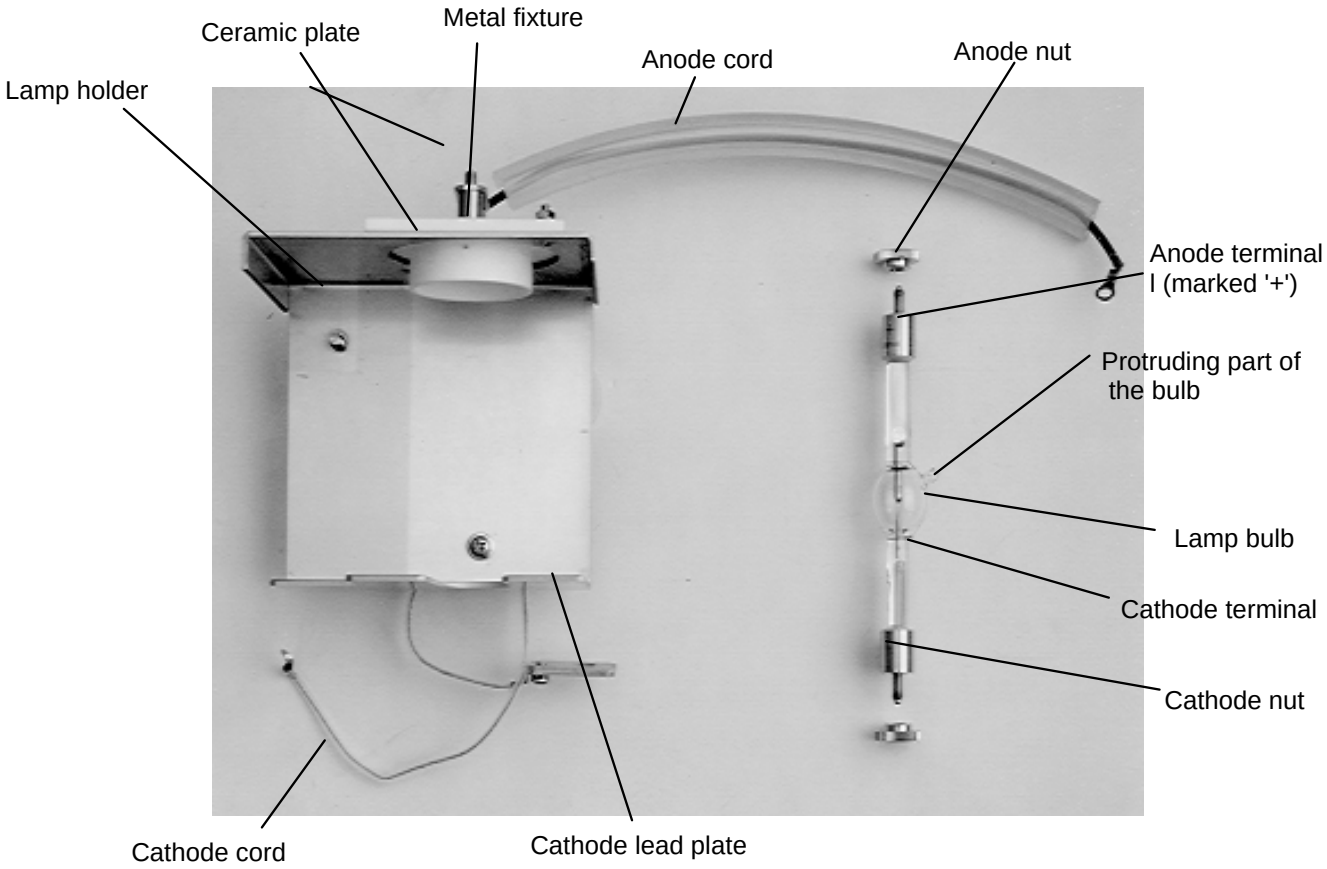


Figure 13 Xenon Lamp and Its Holder

Mount the lamp so that the protruding part of the bulb comes to the instrument front panel side (see Figure 14).

Mount the xenon lamp so that the protruding part of bulb is positioned on the same side as the anode cord.

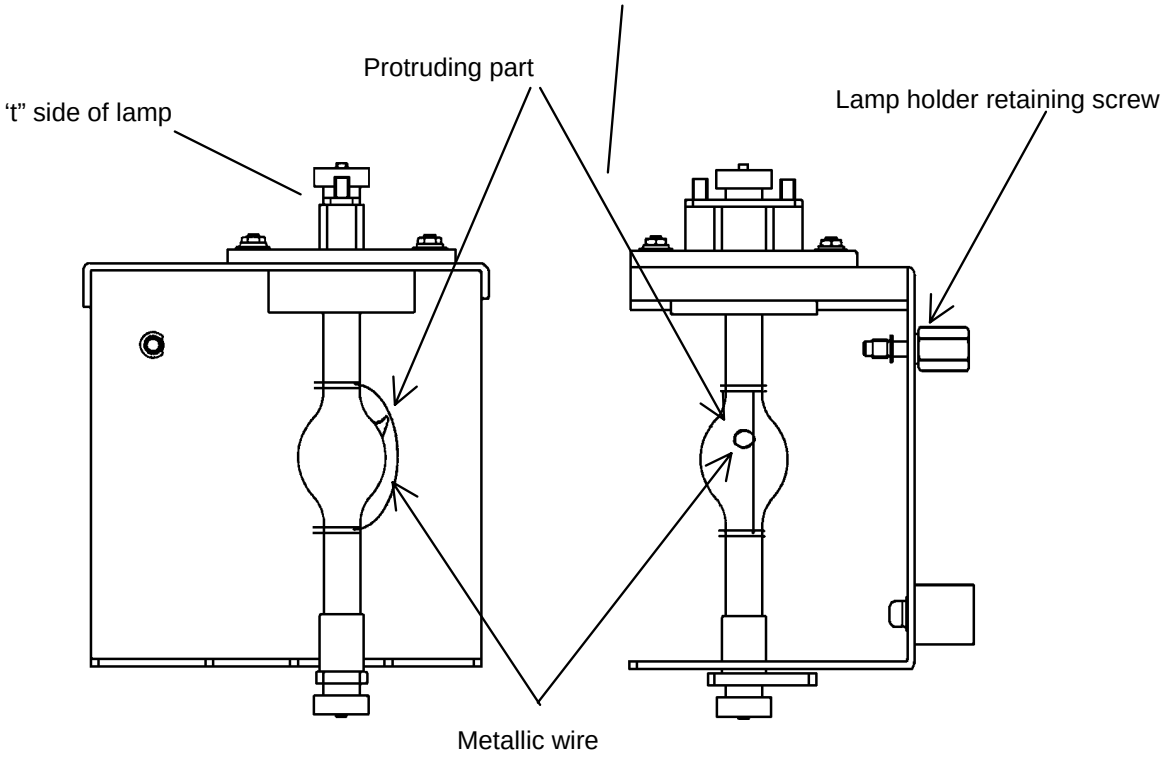


Figure 14 Protrusion of Lamp Bulb

NOTE:	Mount the xenon lamp so that the protruding part of the bulb comes to the instrument front panel side (see Figure 14). A mistake in the positioning may cause an increased light loss and preclude obtaining the desired performance from the instrument.

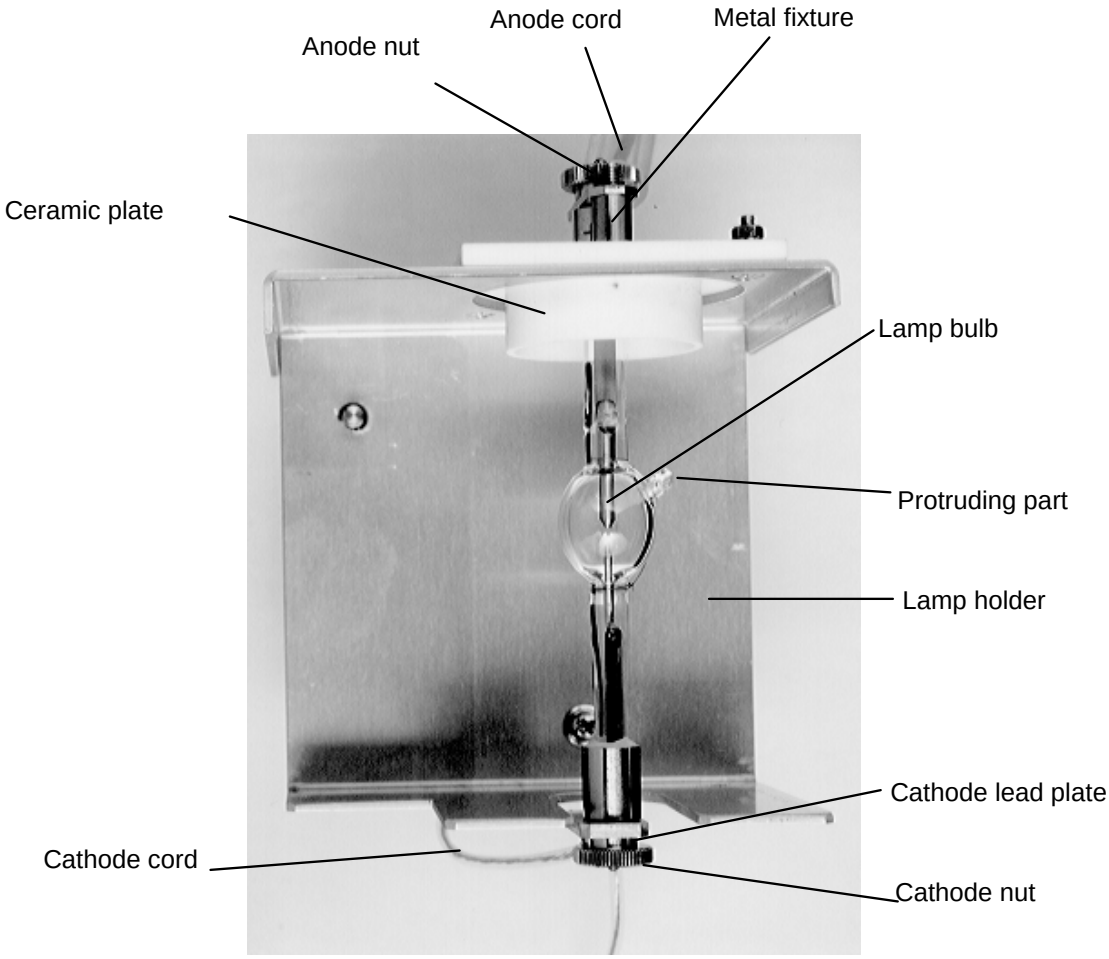


Figure 15 Xenon Lamp Mounted in Holder

WARNING:
EXPLOSION HAZARD

Be sure to secure the anode and cathode nuts when attaching the xenon lamp. The terminal part may heat up and explode while the lamp is lit if the nuts are loose

When tightening the lamp clamp nut, hold the metal parts on the anode and cathode sides with fingers. Never hold the lamp bulb when securing it. Take care not to apply excessive force or shock to the lamp bulb.

4. After mounting the xenon lamp in the holder, place the holder into the lamp housing. The holder should be secured with the lamp holder retaining screw.

5. To connect the cables for the lamp, first loosen the connecting terminals of the lamp igniter (Figure 17). The anode cable (red) is connected to the +HV terminal of the lamp power supply and the cathode cable is connected to the COM terminal.

NOTE: When removing the lamp holder, reverse steps 4 and 5.

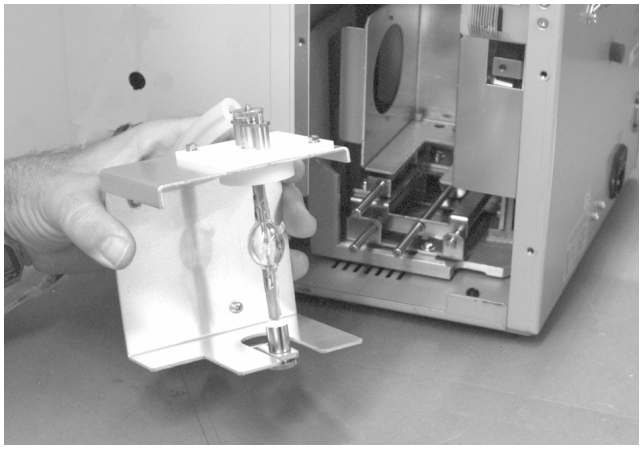


Figure 16 Mounting the Lamp Holder

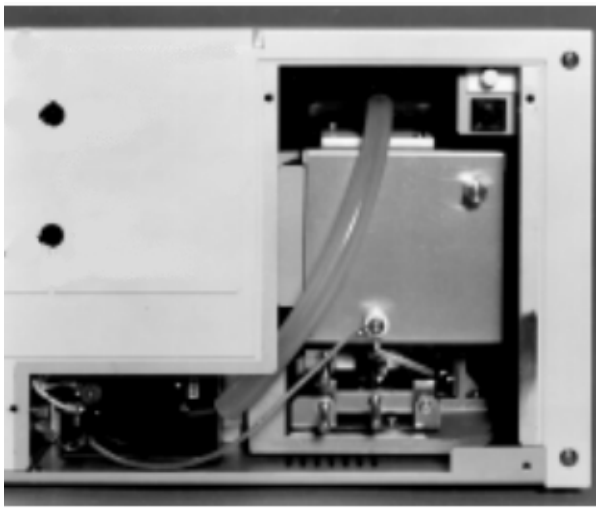


Figure 17 Connecting the Anode and Cathode Cables

6. After mounting the lamp holder, reattach the light source cover. Turn the power on and adjust the lamp as described in the following section.

Adjusting the Position of the Xenon Lamp

Beam position varies from one lamp to another, so it is necessary to adjust the position of the lamp when it is first installed. This adjustment will ensure that the optimum radiation from the lamp is focused into the optical system and will maximize the sensitivity.

To adjust the lamp position, use the three adjust screws located at the right side of the instrument.

HORIZONTAL ADJ	For adjustment in the horizontal direction.
VERTICAL ADJ	For adjustment in the vertical direction.
FOCUS	For adjustment of the focal point of light source.

Adjustment of the lamp position involves a coarse adjustment (in which the position of the light image is monitored) and a fine adjustment (in which the signal detected by the monitor photomultiplier is observed). The coarse adjustment is performed first, followed by the fine adjustment.

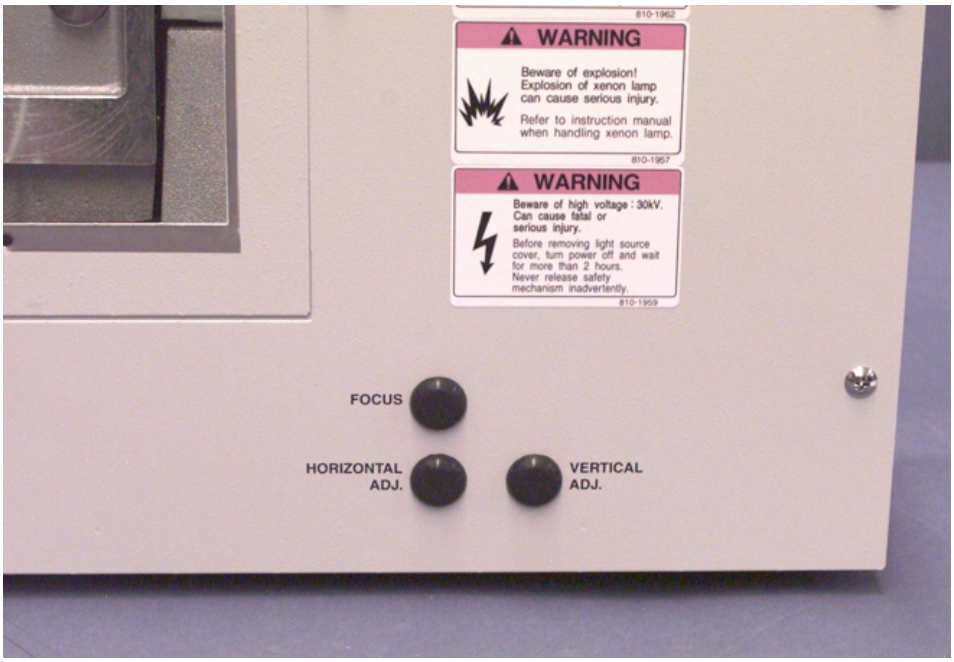


Figure 18 Adjusting the Lamp Position

Coarse Adjustment

For coarse adjustment of the lamp position, use the following procedure.

1. Turn on power to display the monitor screen.
   The monitor screen will appear as shown below.

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0	1	Xe	(90)

2. Remove the flow cell unit.
   The monitor screen will appear as shown below.

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	****	1	Xe	(90)

3. Set the Excitation Monochromator.
   Set the excitation wavelength to 550 nm on the screen by pressing the following keys.

   Wave Length → **5 5 0** → **Ente**r → **Enter**

   After the **Enter** key is pressed, the monitor will present;

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	550	350	****	1	Xe	(90)

4. Monitor the Excitation Radiation
   Place a paper strip at the excitation beam exit of the sample chamber as shown in Figure 19 and observe the condition of excitation beam. Using the three position adjusting screws mentioned before, adjust so that the excitation beam (rainbow color) becomes brightest. You may need to readjust all screws three or four times before obtaining the most intense light amount.

NOTE: Allow the lamp to stabilize for approximately 15 minutes after it has been turned on, before optimizing the position.

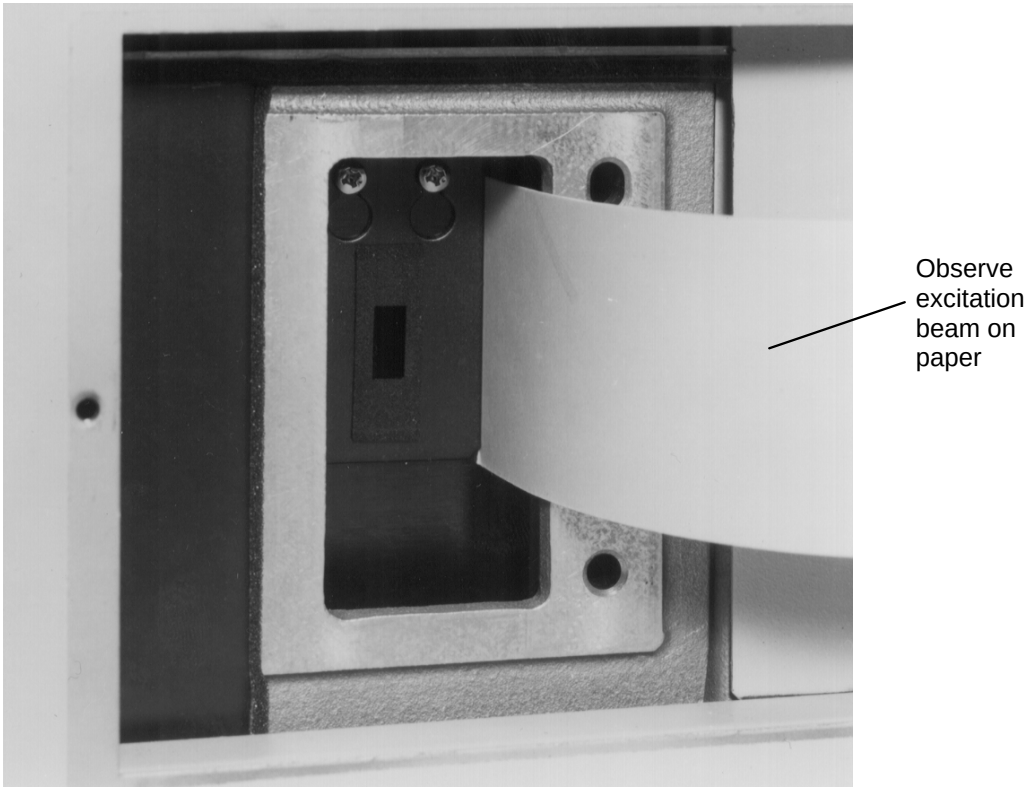


Figure 19 Observation of Excitation Beam

Fine Adjustment

1. Remount the flow cell unit. The flow cell should be flushed with MeOH and blown dry with clean air.

2. Change display mode:

   Utility/Setup → 3 (Display Mode) → Enter → 2 (Ex) → Enter

   Now the status display monitors Excitation energy.

3. Change wavelength and PMT

Wave Length

Ex	Em	(200-850, 0)	nm
250	350		

3 5 0 → Enter → Enter

Ex	Em	MONITOR(Ex)	LAMP	[I]
350	350	xxxx	Xe	(90)

Param Set → Enter

PMT VOLTAGE (HIGH=1, MED=2, LOW=3)
2

2 → Enter → Escape key

Ex	Em	MONITOR(Ex)	LAMP	[I]
350	350	xxxx	Xe	(90)

4. Perform the fine adjustment as described below.
   Turn the FOCUS, HORIZONTAL ADJ and VERTICAL ADJ screws gradually in a repetitive manner while observing the MONITOR value on the screen. Turn each screw on an iterative basis until the MONITOR value is maximized. At least three cycles should be used. When a maximum value is reached, fine adjustment of the lamp position is completed.

5. After lamp alignment is complete change display mode back to Fluorescence.

   Utility/Setup → 3 (Display Mode) → Enter 1 (FL) → Enter

Flow Cell Unit Maintenance

Checking and Cleaning the Flow Cell

If the inside of the flow cell is contaminated, it must be cleaned.

The following items are required for cleaning the flow cell.

- A glass syringe with capacity of approximately 10 mL
- Appropriate washing solution (step 5).

1. Loosen the clamp screw retaining the flow cell unit.
2. Remove the flow cell.
3. Check the inside of the cell to see if it is contaminated with any foreign substance (e.g. oils, salts or buffers).
4. Check the outside of the cell for leakage.
5. If any contamination is observed inside the cell, it must be cleaned with an appropriate solvent.
   Connect the syringe to the tip of drain tube, and inject the washing solvent into the cell for cleaning.
   If an aqueous mobile phase is used, water should be used.
   If an organic based mobile phase is used, methanol or acetonitrile should be used.
6. Replace the washing solvent with the eluent that is used for the separation and repeat the process indicated in step (5).
7. If air bubbles are observed in the cell, deliver mobile phase into the flow cell with the pump using a flow rate of less than 1.0 mL/min and a pressure of less than 150 psi (10 atm). While the liquid is being delivered to the flow cell, pinch the end of the drain tube for a few seconds to increase the pressure inside the cell. Repeat this procedure three or four times until all air bubbles have been removed.

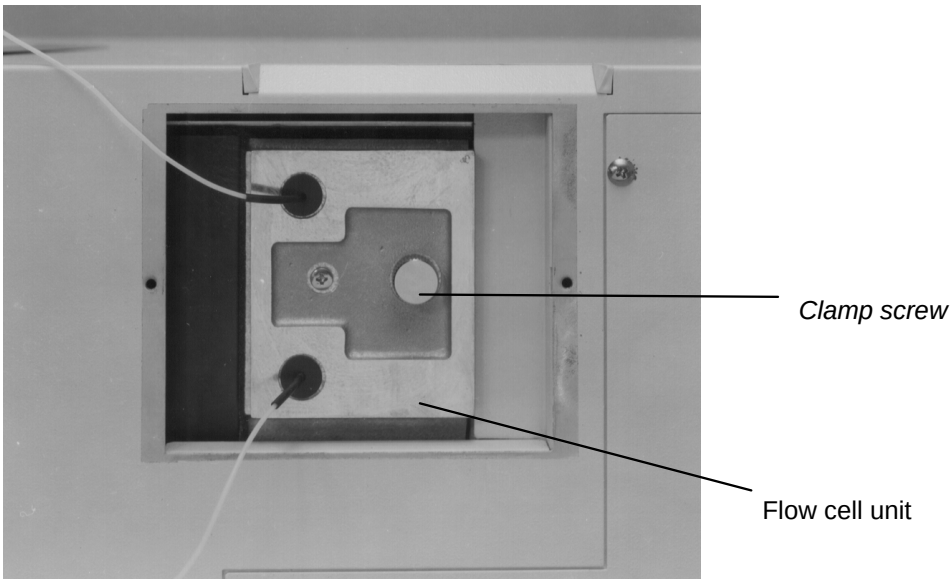
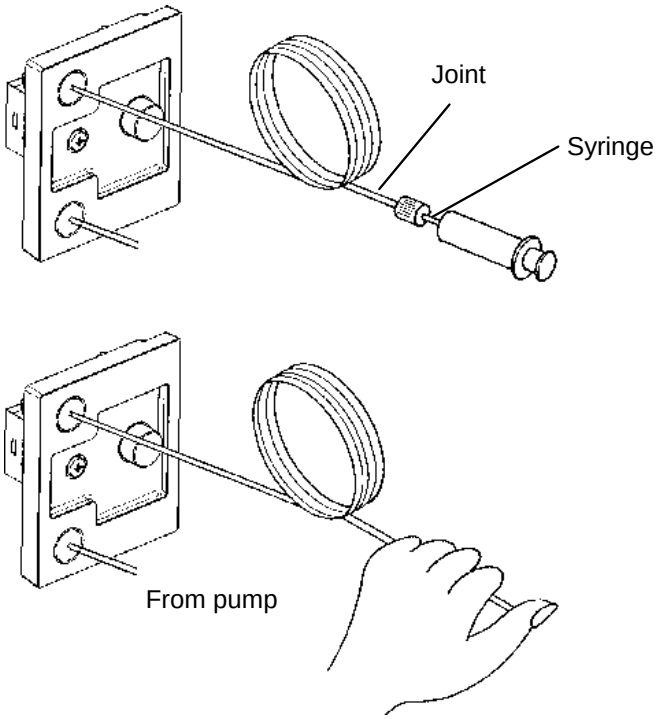


Figure 20 Flow Cell Cleaning



CAUTION

If pressure is too high, the flow cell may be damaged or broken. When removing air bubbles, set the pump pressure limit to 150 psi (10 atm) or less.

Disassembly of the Flow Cell

If the contamination in the flow cell cannot be removed by the aforementioned cleaning procedure, disassemble the cell and clean its parts as instructed below.

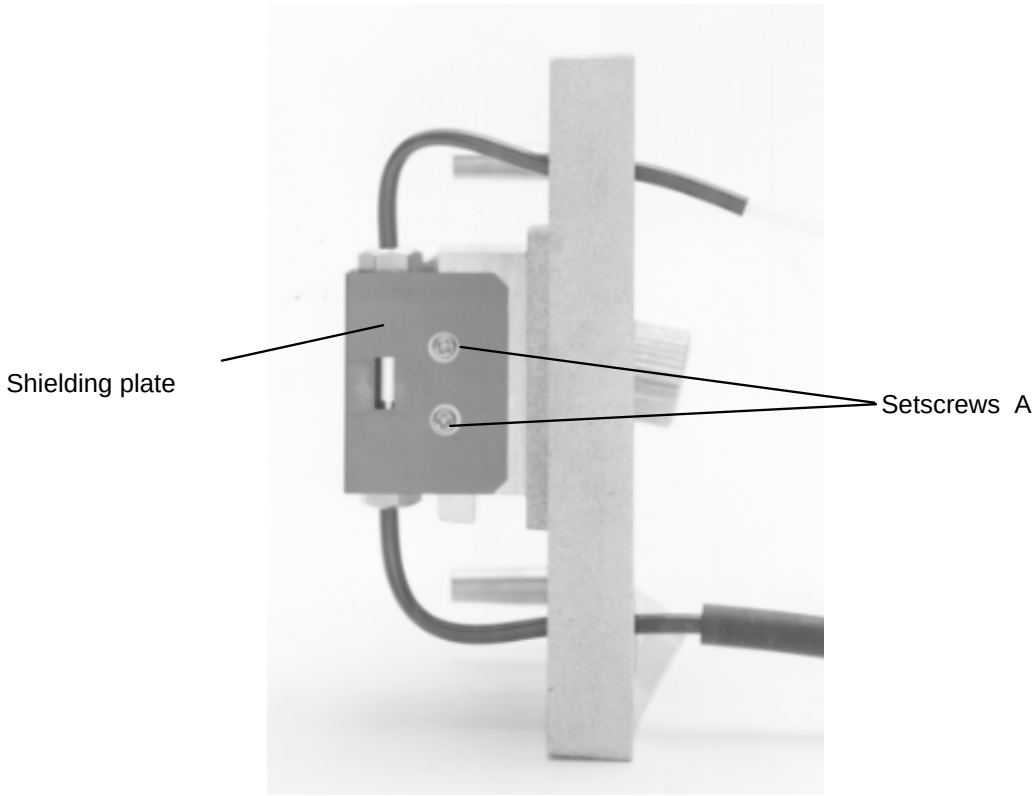


Figure 21 Flow Cell Unit

1. Loosen setscrews A and detach the shielding plate.
2. Detach the cell window.
3. Loosen the upper and lower retaining screws 1 and 2, see Figure 22.
4. Detach the cell clamp.
5. Take out the flow cell.

Cleaning of Flow Cell

1. Clean the flow cell in running water.
   If the contamination cannot be removed with running water, clean it with an ultrasonic cleaner. Be sure to put the flow cell in a paper or plastic container and then place it in the ultrasonic cleaner to avoid scratching the cell.

2. Wipe the surface of the flow cell with gauze moistened with acetone or ethanol.

CAUTION

Be careful not to scratch the flow cell surface when disassembling it.

Be sure to put the flow cell in a paper or plastic container when cleaning it. Using a glass container may scratch the cell or prevent obtaining the desired performance from the instrument.

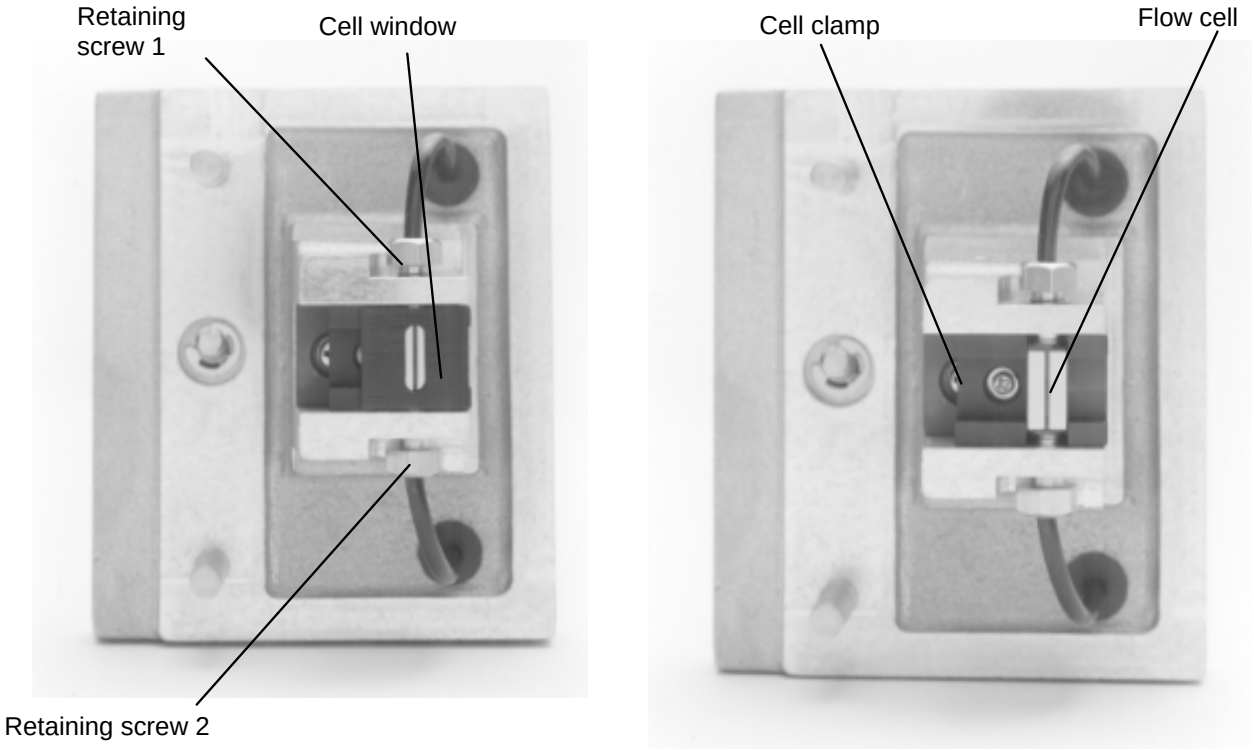


Figure 22 Disassembly of Flow Cell Unit

Assembly of Flow Cell

1. Assemble the flow cell into the cell holder in the orientation shown in Figure 23 and fix it with the cell clamp.

2. Tighten the upper and lower retaining screws.

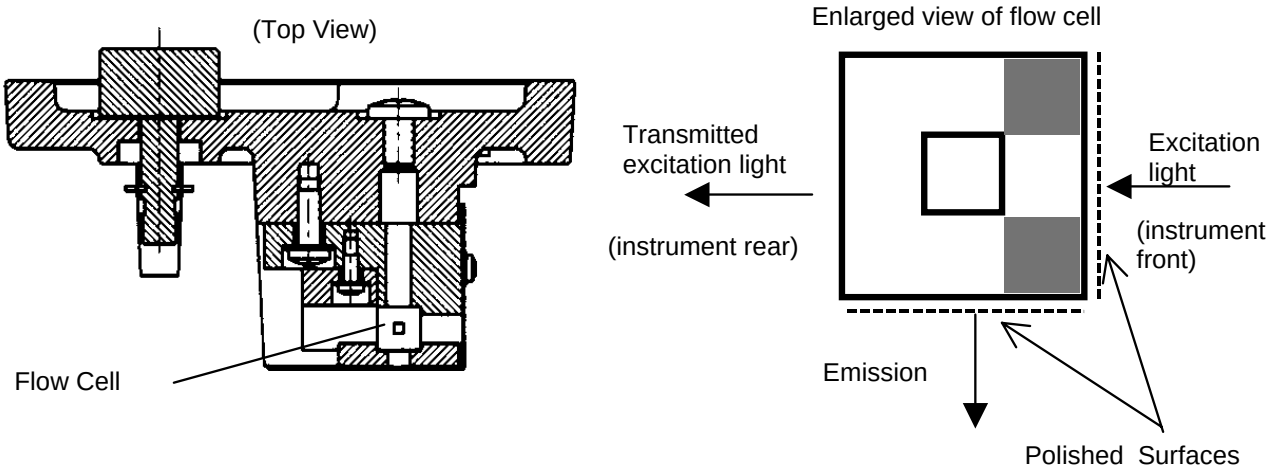


Figure 23 Assembly of Flow Cell Unit

3. Attach the cell window and shielding plate.

4. After assembling the flow cell in the order reverse to disassembly, flow a liquid through the unit and make sure there is no leakage.

NOTE 1: During assembly be careful not to scratch the flow cell surface.

NOTE 2: Be sure you properly position the flow cell when putting it into the cell holder. (See Figure 23.) A mistake in the orientation may preclude obtaining the proper performance from the instrument.

NOTE 3: Finger-tighten retaining screws 1 and 2 fully, then tighten another 1/3 turn or so with an open–end wrench.

Leakage of the Flow Cell

It is important to check that the flow cell does not leak before collecting analytical data. This check can be performed by monitoring the flow for a few seconds. If the cell leaks, stop the pump immediately. Use the following procedure to remedy the leak:

1. Using the pump, feed liquid to check if the flow through the cell is normal (e.g., does the fluid exit the cell and the drain tube to the waste receptacle).

2. If liquid does not come out of the outlet tube, the tube is clogged. Replace the outlet tube with a new one.

3. If leakage occurs and liquid comes out of the outlet tube normally, it is possible that the upper nipple may not be securely tightened. Ensure that the upper nipple is finger tight and then secure it using the wrench that is provided (turn it through approximately 10° to 20°).

4. Remove all liquid on the outside of the flow cell and inside the flow cell compartment.

5. Before replacing the flow cell, pump the mobile phase through the flow cell to make sure that the problem has been remedied.

NOTE 1: It is important to check the outlet tube of the flow cell first. If the upper fitting is loose and it is retightened with the wrench without checking the outlet tube, the flow cell may be damaged or broken when the fluid is pumped through the cell.

NOTE 2: If leakage is found in the sample chamber or on the optical base in it, remove liquid thoroughly and clean with a cloth moistened with water.

If liquid comes out of the drain port located at the lower right of the instrument during measurement, stop the pump immediately and turn off the **Power** switch. Then, follow steps (1) to (5) above.

Flow Cell Storage

If the flow cell will be left unused for less than a few days, clean the flow cell thoroughly by pumping distilled water or alcohol through it and then storing it in a safe place. If the period is more than a few days, the inside of the cell may be filled completely with a solvent like ethanol or acetonitrile and then stored.

Fuse Replacement

WARNING:
SHOCK HAZARD

Beware of electric shock!

Before replacing the fuse, be sure to turn OFF the power switch and unplug the power cord from the outlet.

1. Obtain a spare fuse (03-926131-12). Always use fuses of the correct rating and type.

2. Make sure that the **Power** switch of this instrument is turned off, and that the power cord is unplugged from the instrument.

3. Replace the fuse as follows.

   a) Unplug the power cord from the power socket on the rear panel.

   b) Remove the fuse holder by holding both knobs of its case.

4. Replace the existing fuse with a new one, and then push in the fuse holder securely.

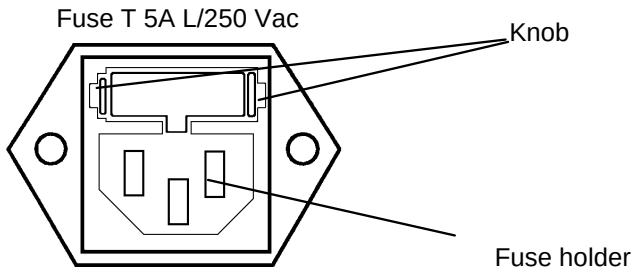


Figure 24 Fuse Replacement

Troubleshooting

WARNING:
SHOCK HAZARD

- **Beware of electric shock!**
- **Before replacing the fuse, be sure to turn OFF the power switch and unplug the power cord from the outlet.**

WARNING:
BURN HAZARD

- **Touching Hot Parts Could Result in Burns!**
- **The light source part (lamp mounting part) becomes extremely hot during operation. Never touch it with hands to prevent burns.**
- **The xenon lamp remains hot for a while even after power-off and can severely burn you if touched.**
- **Although the xenon lamp will be automatically extinguished by a safety mechanism when the light source cover is opened, before replacement of the lamp, turn power off and wait for at least 30 minutes until it cools down sufficiently.**

WARNING:
EYE HAZARD

- **Directly Gazing at Illuminated Xenon Lamp Could Cause Eye Damage!**
- **The xenon lamp radiates intense ultraviolet light when it is on.**
- **Do not look at the xenon lamp directly when it is lit. Be sure to wear tinted safety glasses to prevent possible eye damage.**

Table 4 Troubleshooting

Symptom	Cause	Check	Remedy
. Self-diagnosis (initialization) is not performed when the **Power** switch is turned on.	The power cord plug is not securely plugged in.	Visual check	Plug in the power cord securely.
	The fuse is blown.	Check the fuse for continuity.	Replace the fuse with a new one.
	The lamp cover is not properly attached. (The cover activates a safety interlock switch.)	Visual check	Retighten the retaining screws of the lamp cover.
2. The keyboard does not work.	The instrument is not ready for operation.	Check the monitor screen.	Press the **Escape** key. The READY status will be presented.
3. The **Auto Zero** or **Acquire/Spectrum** key does not work.	On the monitor screen, the data mode is not set to FL.	Check the monitor screen.	Using **Utility/Setup** Mode, setup Display Mode to FL .
4. The Time Program does not start.	The instrument is not ready to operate.	Check the monitor screen.	Press the **Escape** key. The READY status will be presented.
5. Excessive noise	The flow cell is contaminated.	Visual check	Clean the flow cell.
	The mobile phase is not sufficiently degassed.	A spike occurs.	Degas the mobile phase thoroughly.
	The mobile phase contains impurities.	Check the mobile phase.	Purify the mobile phase, or replace it with mobile phase of a higher quality.
	The gain level of the recorder is too high.	Check the gain level of the recorder.	Adjust the gain level of the recorder.
	The pump is faulty.	Check to ensure that the operation of the pump is smooth.	Check the pump.
		Check for noise when the pump is stopped.	

Symptom	Cause	Check	Remedy
	The xenon lamp has reached the end of its useful life.	The S/N is less than 120/1.	Replace the xenon lamp with a new one.
6. Excessive drift	The warm-up period is not sufficient.	—	Wait until the instrument becomes stable (at least ten minutes).
	Mobile phase is leaking from a fitting.	Check each fitting.	Retighten the fitting.
	An impurity (a strongly retained compound) is eluting from the column.	Stop feeding liquid from the pump.	Wait until elution is completed, or replace the column with a new one.
	There is a significant change in the ambient temperature.	—	Control the temperature variation.
	The mobile phase is not sufficiently pure.	Check the mobile phase solvent.	Purify the mobile phase, or replace it with one of a higher quality.
7. Poor S/N ratio	Flow cell contaminated	Visual check	Clean the flow cell.
	Xenon lamp off-position	Check lamp energy level	Adjust lamp position.
	Error in flow cell mounting	Visual check	Correctly mount flow cell.
	Flow cell surface scratched	Visual check	Replace flow cell.

Error Messages

Message	Description	Remedy
A/Z OVER RANGE PRESS **CLEAR** KEY TO CLEAR MESSAGE	The allowable auto zero range is exceeded.	Change the wavelength setting. Use a mobile phase with a lower level of fluorescing impurities. Clean the flow cell.
LAMP ERROR	The lamp goes off during analysis, the lamp does not turn on, or a momentary power interruption is encountered.	Press the **Clear** key to reset the system after a momentary power interruption. If the lamp does not start, turn the power off and turn it on again. If the lamp is at the end of its useful life time, replace it with a new one.
Ex(Em) SIDE OF WL DRIVE MECHANISM ERROR	The monochromator cannot properly set during the self-diagnosis test (initialization).	Turn power on again.Notify the nearest Varian service center.
ROM ERROR	Error in ROM	Notify the nearest Varian service center.
C-RAM ERROR	Error in RAM	Notify the nearest Varian service center.
CHECK TIME PROGRAM	The allowable maximum number of time program steps is exceeded.	Remove unnecessary steps from the time program.
OVER HEAT! LAMP WAS TURNED OFF	The fan has stopped and the temperature sensor was activated.	The fan is faulty. Notify the nearest Varian service center.
	The lamp becomes too hot. It is not cooled down by the fan after turn-off.	After the lamp cools down, turn on power on again.
LAMP WAS TURNED OFF BY LAMP COVER OPEN TURN OFF THE POWER	Lamp cover is open.	Close the lamp cover. Turn on power supply again.

Message	Description	Remedy
NO SPECTRA OR SPECTRA ARE MISMATCHED PRESS **CLEAR** KEY TO CLEAR MESSAGE	No spectral data, scan mode in-correct, fixed wavelength incorrect, background WL range doesn't include entire spectrum WL range, or background slit position differs from spectrum slit position.	Select correct parameters.
PARAMETER ERROR	Error in parameter data (data cannot be retained due to EEPROM error	Turn on power again. Contact your nearest Varian service center.

NOTICE: An input value stored in this instrument may be deleted if certain error messages are presented.
If this is the case, enter the relevant value again.
In the event of PARAMETER ERROR, the wavelength calibration value is deleted.
In this case, it is necessary to perform the wavelength calibration procedure.

Appendix

Specifications

Photometric principle	Ratio photometry; intensity of transmitted beam is monitored.
Light source	150 W xenon lamp
Excitation wavelength setting range	200 to 850 nm, and zero order
Emission wavelength setting range	250 to 900 nm, and zero order Note that photomultiplier must be changed at emission wavelengths greater than 731 nm.
Excitation side spectral bandwidth	15 nm
Emission side spectral bandwidth	15 or 30 nm
Wavelength setting	By keyboard or through Star Workstation
Wavelength accuracy	±3 nm
Wavelength repeatability	±0.5 nm
Recorder output	10 mV full scale Full scale range of 1 to 1000 is settable in increments of 1.
Data System output	1V full scale
Response	Changeable in 5 steps, corresponding to time constants of 0.1, 0.5, 2.0, 4.0, 8.0 seconds.
Auto zero range	0 to 1000
Offset	0 to 1000 (settable in steps of 1)
Spectrum memory	Up to 8 excitation or emission spectra can be stored.

Marker	Marker output of approx. 0.6 mV at recorder terminal (corresponding to about 6 graduations on chart)
Instrument parameter setting	Following parameters are settable and stored in battery-backed-up memory when power is turned off. Full scale range Spectrum measuring range Photomultiplier applied voltage Offset value
Time program	# of programs: 9 Settable time: up to 600 minutes in increments of 0.1 minute No. of steps storable: Up to 100 steps for a total of 9 files Programmable items: Excitation wavelength, emission wavelength, baseline processing
Display function	40 characters × 2 lines on backlighted LCD
Communication function	RS-422/485 serial communication
External output terminals	Analog output For processor : 1 V full scale For recorder : 10 mV full scale
External I/O contact terminals	Time program start, error input/output, busy output. Auto zero input, marker input, lamp off input
Flow cell capacity	Standard cell 12 µL (irradiated capacity)
Flow cell withstand pressure	29 ATM (425 psi)
Operating temperature range	4 to 35 °C
Operating humidity range	45 to 80% RH (non-condensing)
Power requirement	100-115, 220-240 V AC, 50/60 Hz
Power consumption	360 VA
Dimensions	292 mm W x 470 mm D × 305 mm H
Weight	Approx. 19 kg

Accessories

Part Number	Part Name	Remarks
∗03-926131-02	150 W xenon lamp	Service life: ~ 1200 hours
∗03-926131-03	Long-life xenon lamp (option)	Service life: ~ 2000 hours
03-926131-13	Fuse T 5A L 5x20 mm time lag fuse designed to IEC standard, 2 required.	Service life: ~ 1 year
03-926131-09	Flow cell (12 µL)	Cell only
03-926131-20	Nitoflon tube 0.33 ID (outlet)	Length: 2 m Outside diameter: 1.57 mm
03-926131-19	Nitoflon tube 0.25 ID (inlet)	Length: 2 m Outside diameter: 1.57 mm
03-926131-11	Standard photomultiplier	R3788
56-180002-00	Extended range photomultiplier	R924 red sensitive

∗Indicates a consumable item.

NOTICE: The intensity of an unused xenon lamp that has been stored for a long period of time (e.g., a year) will be less than that of a newly acquired lamp. It is suggested that the user maintain a small supply of spare lamps and reorder lamps as required, rather than purchasing a large number of lamps at one time.

Raman Scattering

When fluorescence is measured, two additional peaks may appear in the spectrum. The Rayleigh peak appears at the excitation wavelength and is due to scattered light, while the Raman peak appears at longer wavelength than the excitation. The position of the Raman band is dependent on the excitation wavelength, while the position of the fluorescence is independent of the excitation wavelength.

The Rayleigh peak and the Raman peak will occur even if the eluent does not contain any compound that fluoresces. If there is any doubt whether an observed peak is a Raman peak, a Rayleigh peak or a peak due to the fluorescence from the compound of interest, simply change the excitation wavelength slightly.

If the peak is due to the Raman effect or the Rayleigh effect, the observed wavelength will shift. If the peak is due to fluorescence from the compound of interest the wavelength will not change (although the peak height may change).

The Raman effect is moderately strong when water is the solvent, but is considerably weaker for other solvents that are commonly used in HPLC.

Table 5 presents the position of the Raman peak for a variety of excitation wavelengths.

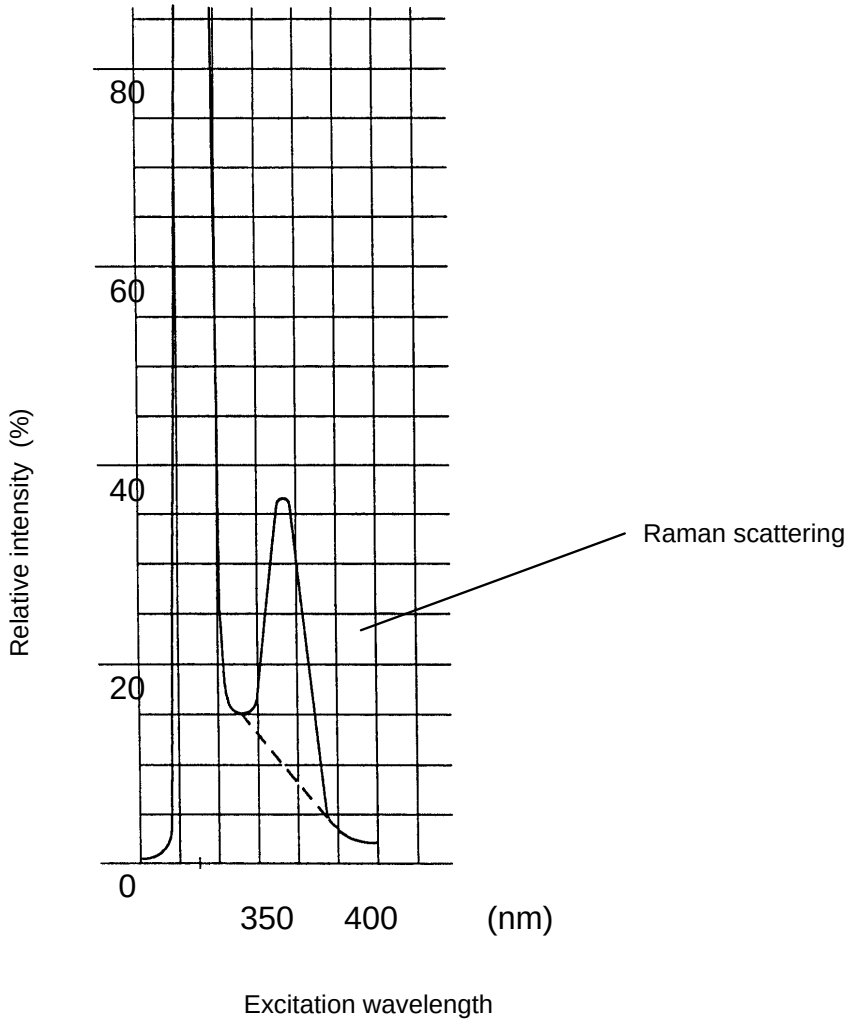


Figure 25 Raman Spectrum of Water

Table 5 Raman Spectral Peak Position at Each Excitation Wavelength

	(Excitation Wavelength)	Water	Ethanol	Cyclohexane	Carbon Tetrachloride	Chloroform
Excitation wavelength, and Raman peak position (nm)	248	271	267	267	—	—
	313	350	344	344	320	346
	365	416	405	408	375	410
	405	469	459	458	418	461
	436	511	500	499	450	502

Samples Containing High Concentration of the Compound of Interest

When the concentration of the compound of interest is relatively high, the concentration reported by fluorescence detection may be lower than the actual concentration. This phenomenon is due to the “Inner Filter effect” which is depicted in Figure 26.
In this example, the concentration of the compound of interest is sufficiently high that essentially all of the excitation radiation is absorbed by molecules that are close to the entrance slit. These molecules emit, but are not in the region that is defined by the emission slit so that the fluorescence is not observed. Molecules that are in the region defined by the emission (Fluorescence) slits are not excited by the incoming radiation (since it was already absorbed).

The example described above is an extreme situation. Typically if the absorbance of the sample is greater than 0.05, the inner filter effect may reduce the observed fluorescence intensity.
To determine if the inner filter effect is reducing the fluorescence intensity, it is suggested that the sample be diluted and the intensity measured again. If the reduction in the fluorescence intensity matches the dilution, the inner filter effect is not in effect.

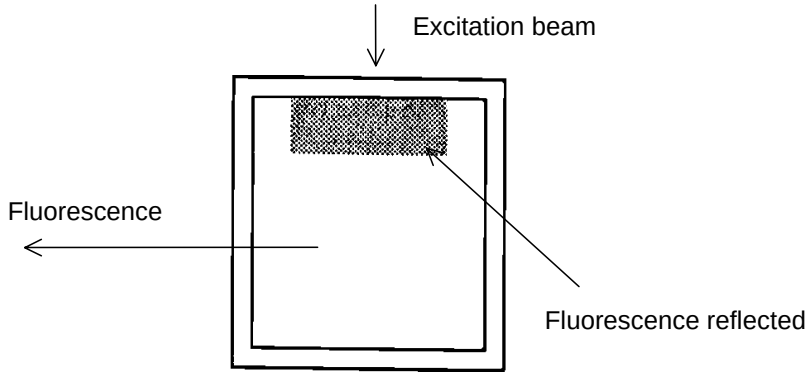


Figure 26 Sample Containing Extremely High Concentration

Another potential error that may occur when fluorescence detection is used is the re-absorption of fluorescence (self-absorption of fluorescence). An example of this phenomenon is shown in Figure 27. Re-absorption of fluorescence occurs when the tail of the shorter-wavelength side of fluorescence spectrum

overlaps the tail of the long-wavelength side of the excitation spectrum. When this phenomenon occurs, the fluorescence spectrum appears to be somewhat shifted toward the longer-wavelength.

This phenomenon will very rarely cause a substantial error in quantitation when fluorescence detection is used, and should not be a cause for concern.

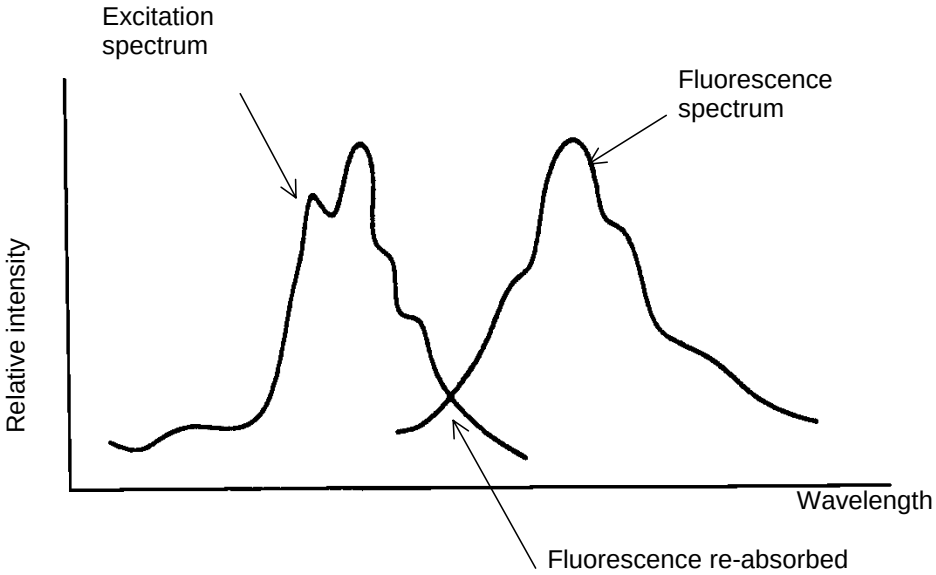


Figure 27 Re-absorption of Fluorescence

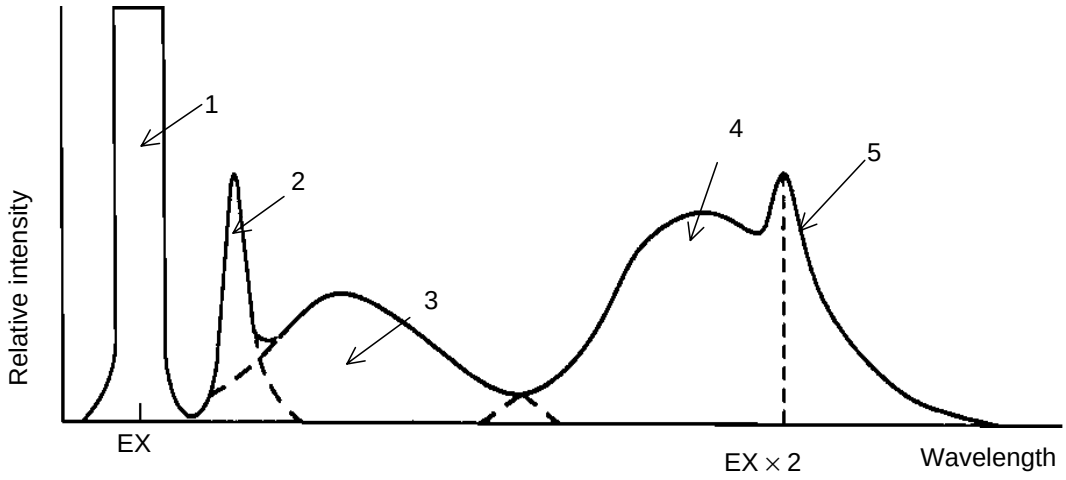
Second-order and Third-order Radiation

A diffraction grating that is set to transmit radiation of a specific wavelength (e.g., 250 nm) will also transmit multiples of the radiation (e.g., 500 nm). When a fluorescence spectrum is collected, the analyst should be aware of the second order (and third order) phenomena and take care that peaks are not mis-identified.

For instance, if the excitation wavelength is 240 nm, the second-order and third-order excitation occur at 480 and 720 nm, respectively. To eliminate second and third order radiation, it is necessary to place a short-wavelength cutoff filter in the path of fluorescing radiation (before the fluorescence monochromator).

An Example of a Fluorescence Spectrum

Figure 28 provides an example of a fluorescence spectrum which contains the various types of peaks indicated above. In most cases, the fluorescence of the compound of interest lies closer to the excitation than shown in Figure 28, and overlapping of peak of interest and the second order radiation is rarely a problem. Typically, the peak for the compound of interest might correspond to peak 3, and the analyst might have to correct for the Raman peak (e.g. use a shorter excitation wavelength).



1. Scattering of excitation beam
2. Raman scattering due to eluent
3. Fluorescence of compound of interest
4. Fluorescence of impurity
5. Second-order scattering of excitation beam

Figure 28 Measurement Example of Fluorescence Spectrum