206-93107L
Apr. 2008

Spectrofluorophotometer
RF-5301PC Series
INSTRUCTION MANUAL

Read the instruction manual thoroughly before you use the product. Keep this instruction manual for future reference.

SHIMADZU CORPORATION
KYOTO JAPAN
ANALYTICAL & MEASURING INSTRUMENTS DIVISION

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Introduction

Read this manual thoroughly before using the instrument.

Thank you for your purchase of the RF-5301PC spectrofluorophotometer.
The RF-5301PC allows high sensitivity analysis to be carried out, based on a unique optical system that involves a highly efficient BHG (Blazed Holographic Grating) as well as a low-noise circuitry that includes a digital filter.
For convenience and enhanced operability, the system is designed to run on an MS-Windows platform, providing an intuitive, graphical user interface. As a result, the operator can feel comfortable in a wide variety of applications raging from routine analysis to state-of-the-art research work.
For trouble-free, efficient use of this equipment, be sure to study this manual thoroughly.

IMPORTANT

- If the user or usage location changes, be sure this Instruction Manual is always kept together with the product.
- If this documentation or the warning labels on the instrument become lost or damaged, promptly obtain replacements from your Shimadzu representative.
- To ensure safe operation, read the Safety Instructions before using the instrument.
- To ensure safe operation, contact your Shimadzu representative if product installation, adjustment, or re-installation (after the product is moved) is required.

Safety Instructions

- To ensure safe operation of the instrument, read these Safety Instructions carefully before use.
- Observe all of the WARNINGS and CAUTIONS described in this section. They are extremely important for safety.

In this manual, warnings and cautions are indicated using the following conventions:

WARNING	Indicates a potentially hazardous situation which, if not avoided, could result in serious injury or possibly death.
CAUTION	Indicates a potentially hazardous situation which, if not avoided, may result in minor to moderate injury or equipment damage.
NOTE	Emphasizes additional information that is provided to ensure the proper use of this product.

Additionally, in this manual, safety symbols are stipulated as follows:

	Voltage is dangerously high.
	For operation and handling, refer to the instruction manual.

Installation Site Precautions

WARNING

When using flammable and toxic samples, be sure to install ventilation equipment at an installation site.

CAUTION

- The weight of this instrument is 43 kg. During installation, consider the entire weight combined with other instruments.

  The lab table on which this instrument is installed should be strong enough to support the total weight of this instrument. It should be level stable, and have depth of at least 800 mm.
  Otherwise, the instrument could tip over or fall off the table.

- Avoid installation sites that are exposed to corrosive gases or excessive dust.

  These adverse conditions may be detrimental to maintaining instrument performance and may shorten its service life.

Installation Precautions

To ensure safe operation, contact your Shimadzu representative if product installation, adjustment, or re-installation (after the product is moved) is required.

WARNING

- Take measures to prevent the instrument from falling in the event of earthquake or other disaster.

  Strong vibrations could cause the instrument to fall over, resulting in injury.

- The power supply voltage of the instrument is indicated on the label on the side of the instrument. Connect the instrument only to a power supply of the voltage indicated; otherwise, fire or electric shock could result. Check that the power supply voltage is stable and that its current capacity is sufficient to operate all the components of the system. If not, the instrument will not operate at its rated performance.
- Ground the instrument

  Grounding is necessary to prevent electric shock in the event of an accident or electrical discharge, and important for ensuring stable operation.

- Do not place heavy objects on the power cord, and keep any hot items away.

  The cord could be damaged, resulting in fire, electrical shock or malfunction. If the cord becomes damaged, contact your Shimadzu representative immediately.

- Do not modify the cord in any way. Do not bend it excessively or pull on it.

  The cord could be damaged, resulting in fire, electrical shock or malfunction. If the cord becomes damaged, contact your Shimadzu representative immediately.

Operation Precautions

WARNING

- Always wear protective gloves when handling any toxic or biologically infectious samples.
- Do not use flammable sprays (hair sprays, insecticide sprays, etc.) near the instrument.
  They could ignite and cause a fire.

CAUTION

- Do not use mobile phones near the instrument.
  They could damage data.

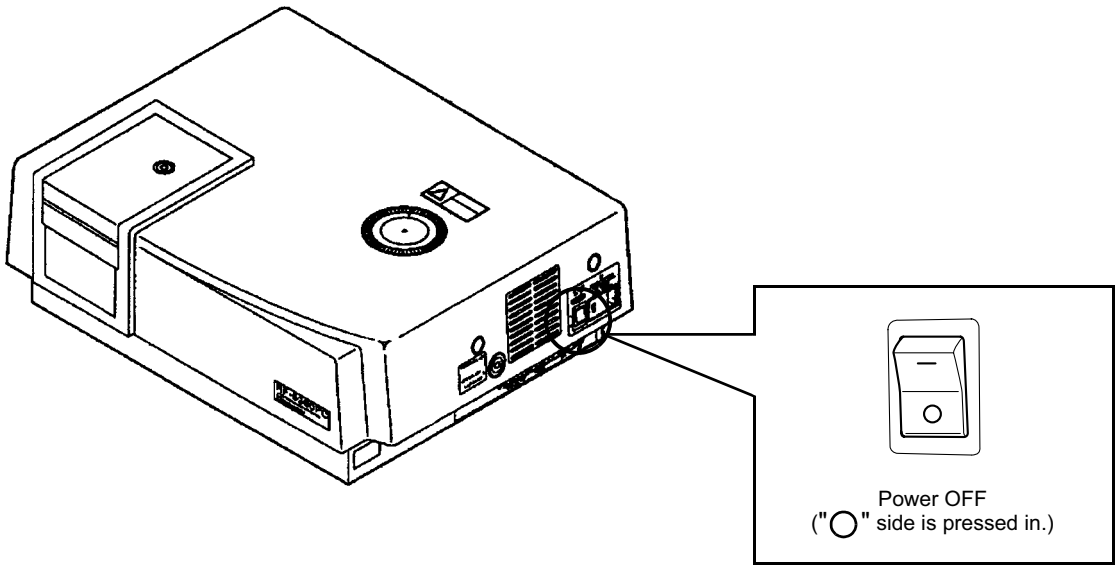
Precautions for Instrument Inspection, Maintenance, Adjustment and Care.

WARNING

- Unplug the instrument before inspection, maintenance, or parts replacement.
  Otherwise, electrical shock or short-circuit accidents could occur.
- Never remove the main cover.
  This may cause injury or malfunction of the instrument.
  The main cover does not need to be removed for routine maintenance, inspection and adjustment.
  Have your Shimadzu representative perform any repairs requiring removal of the main cover.
- If the power cord plug gets dusty, remove the plug from the power outlet and wipe away the dust with a dry cloth.
  If dust is allowed to accumulate, fire could result.
- Replacement parts must be of the specifications given in "1.1 Parts Inspection" and "8.8 Service Parts List".
  Use of any other parts may result in instrument damage and malfunction.
- If any water gets onto the instrument, wipe it away immediately to prevent rust. Never use alcohol or thinner solvents for cleaning the instrument.
  They could cause discoloration.
- Dispose waste liquid properly and in accordance with the instructions of your administrative department.

Emergency Operation

In an emergency situation, press the "◯" side of the power switch located on the right side bottom of the RF-5301PC to turn OFF the instrument.



Operation at Power Outage

In case of electrical failure, perform the following operations:

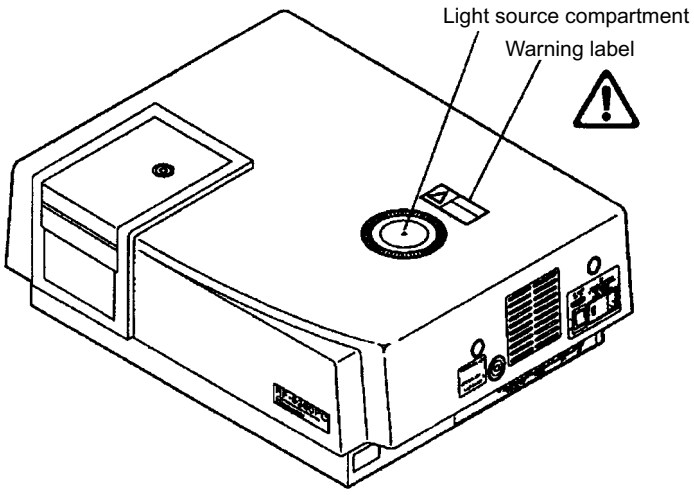
1. Press the "◯" side of the power switch located on the right side bottom of the RF-5301PC to turn OFF the instrument.
2. After the power comes back on, start up the RF-5301PC normally by following "Installation Precautions" and "Operation Precautions".

Warning Labels Indicated on the Unit

WARNING

HIGH TEMPERATURE
HIGH VOLTAGE
EXPLOSION OF LAMP

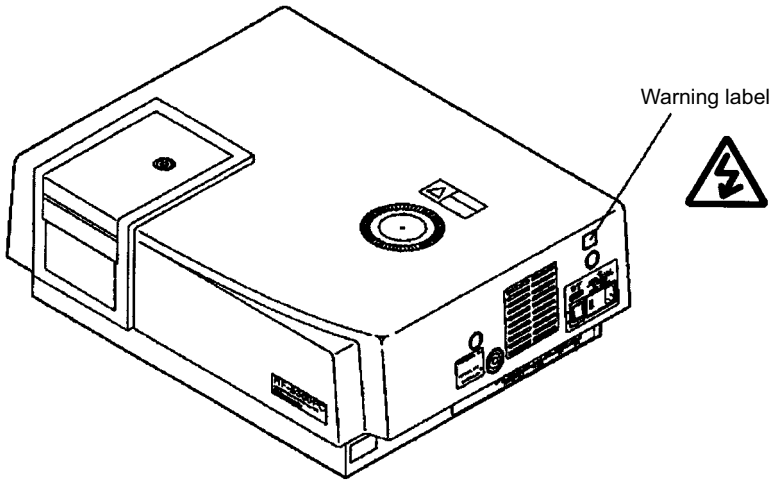
* The Xenon lamp is guaranteed against flicker within 500 hours of use. Once noise starts appearing significantly on measured data, be sure to replace the lamp. Using the lamp over 1000 hours may cause a lamp burst. Be sure to replace the lamp before the lighting time exceeds 1000 hours.
* When handling the Xenon lamp, be sure to wear protective gears, such as protective mask, long sleeve shirt of bulky material, and gloves. High pressure gas is contained in the Xenon lamp. The lamp, therefore, may burst and fragments of broken glass flying all over the floor, if any strong impact is given to the Xenon lamp, or surface of the glass portion is scratched.
* When installing or replacing the Xenon lamp, be sure to turn OFF the power switch and disconnect the power cable. When it is just after turning off the power switch, voltage is applied to the electrode by electric charge. Accordingly, leave the lamp five minutes or more after having turned off the power, then open the cover of light source compartment.
* The Xenon lamp is hot while it is lighting up. After turning off the power, leave the lamp one hour or more to cool the lamp, and then replace it with a new one.
* High voltage of approx. 30 kV is applied to the Xenon lamp when the lamp comes on. For your safety, be sure to install the lamp and close the cover of light source compartment before turning ON the power.



WARNING

RISK OF ELECTRONIC SHOCK

Before changing a fuse or the inlet voltage, turn off the power switch and disconnect the power cable.



Symbols Indicated on the Unit

Symbol	Contents
	Indicates current (a.c.).
	Indicates protective conductor terminal.
	Indicates power ON.
	Indicates power OFF.

Product Warranty

Our company provides a warranty on this product, as stated below.

Details

1. Warranty Period: Please consult your Shimadzu representative for information about the extent of the warranty.

2. Warranty Description: If failure occurs for reasons attributable to our company during the warranty period, our company will provide repairs or the replacement of parts without charge. However, we may not be able to provide identical products in the case of products such as PCs, and their peripherals and parts, which have a short lifespan in the market.

3. Warranty Exceptions: The failures caused by the following events are excluded from the warranty, even if they occur during the warranty period.

1) The product is handled in an improper way.
2) Repairs or modifications are performed by companies or people other than our company and our designated companies.
3) This product was used in combination with hardware or software other than those designated by our company.
4) Device failures and damage to data and software, including the basic software, that are caused by computer viruses.
5) Device failures and damage to data and software, including the basic software, that are caused by power failures, including power outages and sudden drops of voltage.
6) Device failures and damage to data and software, including the basic software, that are caused by powering off the device without the proper shutdown procedure.
7) Failures caused by reasons other than the device itself.
8) Failures caused by use in harsh environments, such as in high temperature or humidity, corrosive gas, or vibration.
9) Failures caused by fires and earthquakes or any other act of providence, contamination by radio active substances and hazardous substances, or any other force majeure event including wars, riots, and crimes.
10) Problems occur because the device is transferred or transported after installation.
11) Expendable items and parts
    Note: Recording media such as floppy disks and CD-ROMs are considered expendables.

* If there is a document such as a warranty attached to the product, or there is a separate contract agreed upon that includes warranty conditions, the rules stated in those documents shall be followed.
Warranty periods for products with special specifications and systems are provided separately.

After-Sales Service and Replacement Parts Availability

After-Sales Service

If any problem occurs with this instrument, inspect it and take appropriate corrective action as described in the Section "Troubleshooting". If the problem persists, or symptoms not covered in the Troubleshooting section occur, contact your Shimadzu representative.

Replacement Parts Availability

Replacement parts for this instrument will be available for a period of seven (7) years after the discontinuation of the product. Thereafter, such parts may cease to available. Note, however, that the availability of parts not manufactured by Shimadzu shall be determined by the relevant manufacturers.

Disposal Precautions

■ Disposal of RF-5301PC

When disposing of the RF-5301PC, contact your Shimadzu representative.
Otherwise, be sure to dispose of the product separately from general garbage, in compliance with the applicable laws or regulations in the country or region where it is used.

■ When disposing of Xenon lamp

When disposing of the Xenon lamp, be sure to follow the description in this manual "8.4 Safe Disposal of Xenon Lamp".

Action for Environment (WEEE)

To all users of Shimadzu equipment in the European Union:

WEEE Mark

Equipment marked with this symbol indicates that it was sold on or after 13th August 2005, which means it should not be disposed of with general household waste. Note that our equipment is for industrial/professional use only.

Contact Shimadzu service representative when the equipment has reached the end of its life. They will advise you regarding the equipment take-back.

With your co-operation we are aiming to reduce contamination from waste electronic and electrical equipment and preserve natural resource through reuse and recycling.
Do not hesitate to ask Shimadzu service representative, if you require further information.

Regulatory Information

For Europe:
The product complies with the requirements of the EMC Directive 89/336/EEC amended by 92/31/EEC, 93/68/EEC, and Low Voltage Directive 73/23/EEC amended by 93/68/EEC.

Product name	:	Spectrofluorophotometer
Model Name	:	RF-5301PC
Manufacturer	:	SHIMADZU CORPORATION ANALYTICAL & MEASURING INSTRUMENTS DIVISION
Address	:	1, NISHINOKYO-KUWABARACHO, NAKAGYO-KU, KYOTO, 604-8511, JAPAN
Authorized Representative in EU	:	Shimadzu Europa GmbH
Address	:	Albert-Hahn-Strasse 6-10, 47269 Duisburg, F.R. Germany

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Chapter 10 Basics of Fluorometric Analysis

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Chapter 1 Parts Inspection

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1.1 Parts Inspection

The RF-5301PC should include the following items. After unpacking the shipment, check to make sure that all the following items are included.

i) RF-5300PC 1 unit

ii) Standard accessories

No.	Type	Description	Part No.	Qty
①	Part	Power Cable (100/120 V) or	071-60814-01	1
		Power Cable (220/230/240 V)	071-60814-06	
②		I/F Cable	206-83579-91	1
③		RFPC Set-Up CD-ROM	206-26288-91	1
④	Consumables	150 W Xenon Lamp	200-81500-01	1
⑤	Replacement Part	Fuse 5 A (100/120 V) or	072-01663-14	2
		Fuse 3.15 A (220/230/240 V)	072-01663-12	
⑥	Tool	Phillips-head Screwdriver	200-94612	1
⑦		Sensitivity Adjustment Flat-blade Screwdriver	200-94613	1
⑧	Supplies	Instruction Manual	206-93107	1

Chapter 2 Installation

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2.1 Location

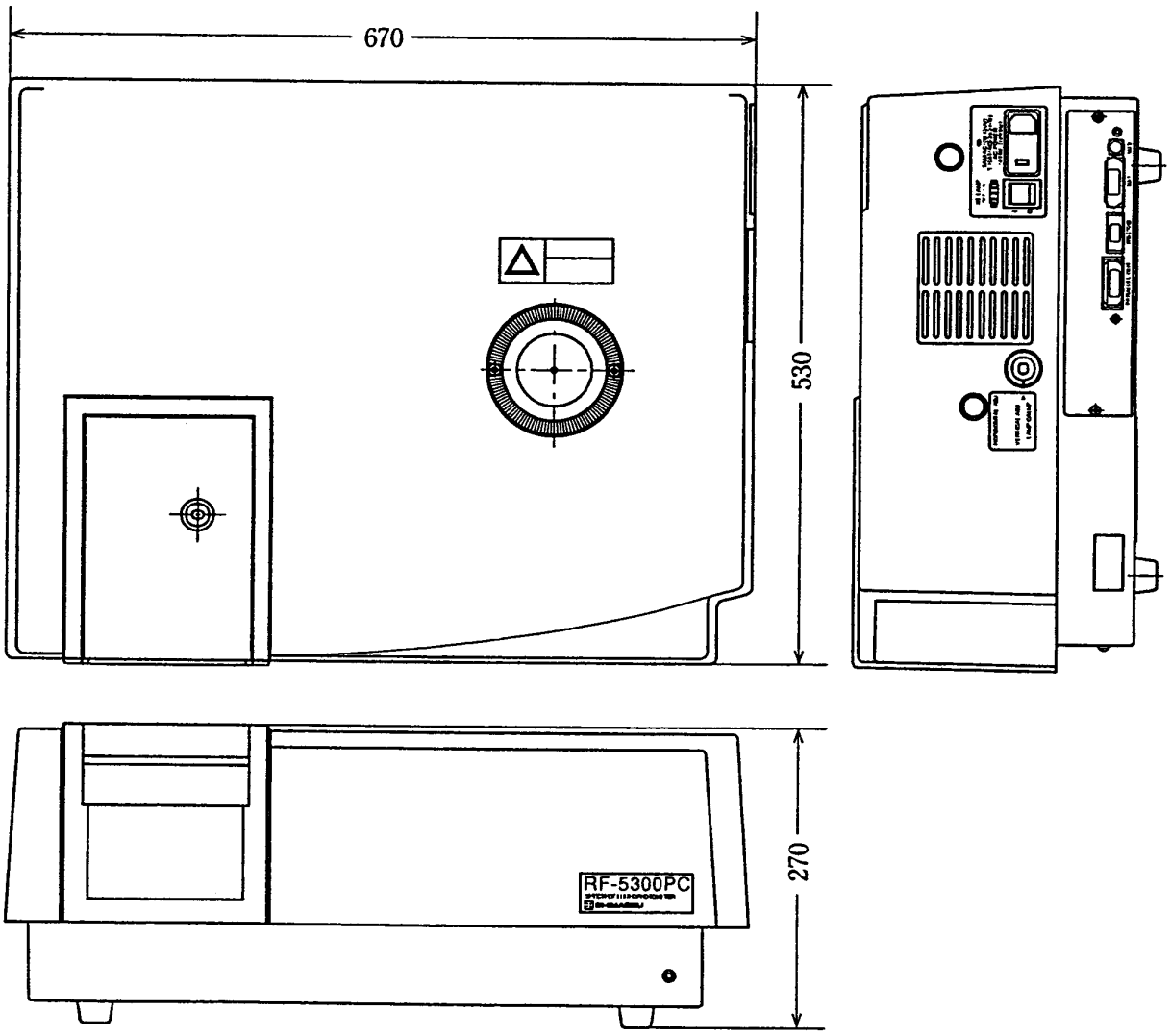
NOTE

To provide optimal performance of the instrument and to ensure a long trouble-free operating life, be sure to install the instrument in a location that satisfies the requirements listed below.
Note that Shimadzu Corporation shall not be responsible for any damages or costs arising from deteriorated performance or mechanical damage arising from the use of the instrument in a location that fails to satisfy the following requirements, even during the guarantee period.

- Room temperature within the range of 15 °C to 35 °C.
- A position not exposed to direct sunlight.
- A position not subject to strong vibration, or any continuous (even weak) vibration.
- A position free from strong magnetic or electromagnetic fields.
- Relative humidity within the range 45 % to 80 %. (If the room temperature is 30 °C or higher, the relative humidity must be no more than 70 %.)
- A location free from exposure to corrosive gas, or any organic or inorganic gas that has an absorption band in the UV region.
- A location substantially free from dirt or dust.

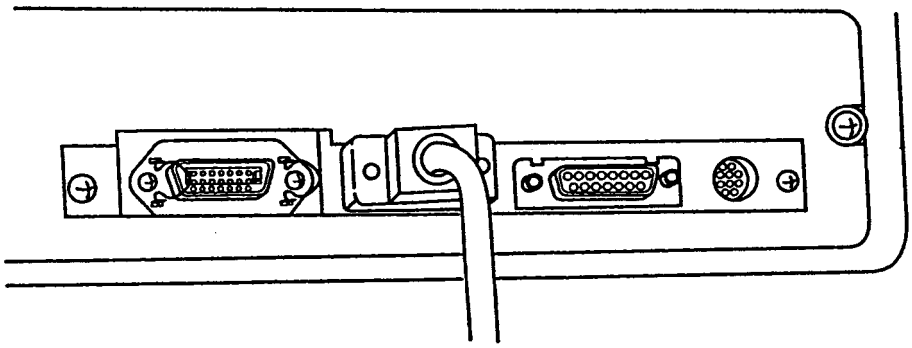
The dimensions of the RF-5301PC are 670 (w) × 530 (d) × 270 (h) mm. To install the RF-5301PC together with a personal computer that serves as an operating panel and monitor, a floor area of about 1500 (w) × 800 (d) mm is required (the area may vary depending on the particular personal computer used.)
The RF-5301PC weighs 43 kg. Make sure that the bench or table used to support the instrument is comfortably able to withstand this load.

Appearance and dimensions of the RF-5301PC



2.2 Connection with Personal Computer

Once the instrument is installed in position, the RF-5301PC unit must be connected to the personal computer (hereinafter called "computer") with the attached I/F cable.
First, connect the male end of the cable to the connector (marked "Serial") on the right-side face of the RF-5301PC unit (see the illustration below), and adjust the two screws to secure the connection. Next, connect the female end of the cable to the serial port (IBM-PC compatible computers usually has two serial ports; COM1 and COM2), and tighten the two screws to secure the connection.



2.3 Software Installation

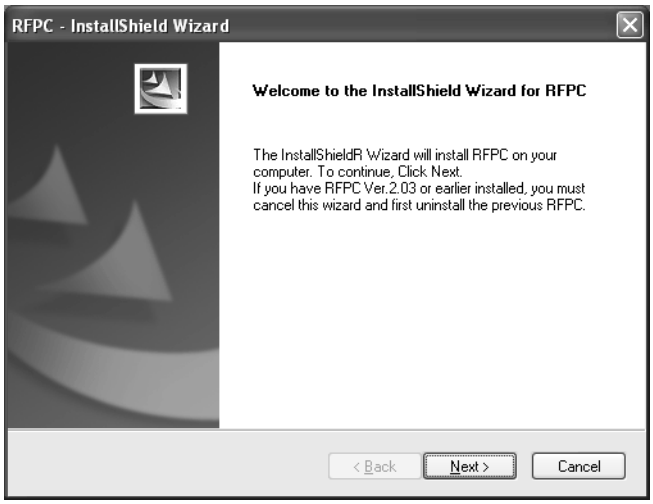
2.3.1 Installation Procedure

This section describes how to install the RFPC software to control the RF-5300PC.
To update the software, uninstall the old version of the software beforehand (see Sec. 2.3.2), and follow the procedure described in this section.

(1) Insert the RFPC Set-Up CD-ROM into the computer. The procedure to set up the software will start automatically.

> **NOTE**
> If the set-up program does not run automatically, double-click the AutoRun.exe icon in the CD-ROM window.

(2) The welcome screen for software set-up is displayed. Click the [Next] button.

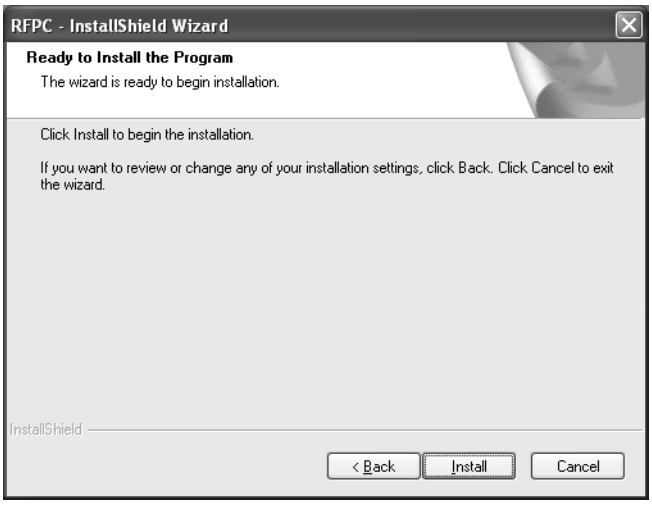


(3) Specify a destination folder to install the software.

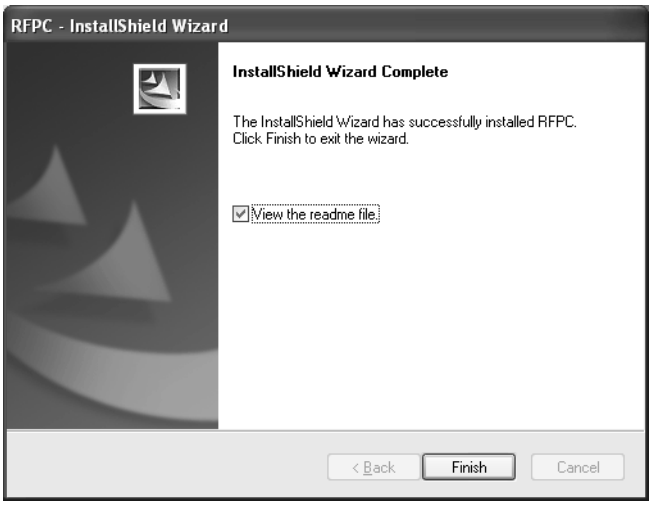
To install the software in the default folder, just click the [Next] button.

To change the destination, click the [Browse] button, select the desired folder, and click the [Next] button.

(4) When a window is displayed prompting you that installation is ready, click the [Install] button.



(5) After the installation process is complete, click the [Finish] button.



When the installation process is completed normally, the shortcut icon for the newly installed RFPC software will appear on the computer desktop.

2.3.2 Uninstallation

To uninstall the software, select the control panel from the operation system.

If the RFPC software is version 2.03 or earlier, first move necessary data files and parameter files (extension: ".cfg") from the installation folder of the RFPC software, then delete the entire folder.

NOTE

Even though the user deletes the installation folder, shortcut icons on the desktop and the start menu will not be deleted. Manually delete the shortcut icons from the older version of the software.

2.4 Mounting the Xenon Lamp

This section describes how to mount the Xenon lamp. Before starting the installation work, make sure the power switch is in the OFF position and disconnect the power cable from the power outlet to prevent accidental electrical shock.

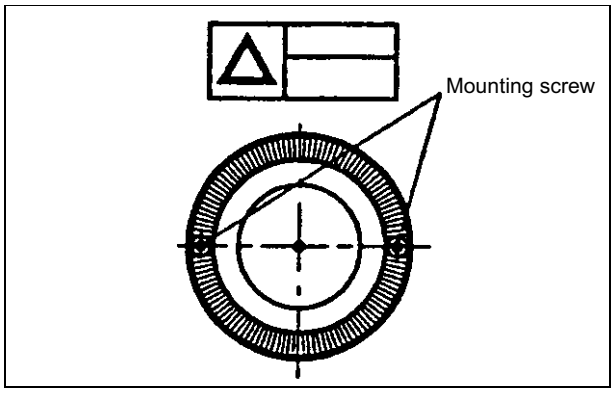
WARNING

* When handling the Xenon lamp, be sure to wear protective gears, such as protective mask, long sleeve shirt of bulky material, and gloves.
  High pressure gas is contained in the Xenon lamp. The lamp, therefore, may burst and fragments of broken glass flying all over the floor, if any strong impact is given to the Xenon lamp, or surface of the glass portion is scratched.
* When opening the cover of the light source compartment, be sure to turn OFF the power switch and disconnect the power cable. When the lamp comes on, high voltage of approx. 30 kV is applied to + pole of the Xenon lamp and this high voltage is dangerous.
  In addition, just after turning OFF the power switch, voltage is applied to the Xenon lamp by the electric charge. In order to prevent electric shock, leave the lamp five minutes or more after turning off the power, then open the cover of light source compartment.
* The Xenon lamp is excessively hot just after it was turned OFF. Cool the lamp completely before it is replaced. It takes about one hour to cool the Xenon lamp after turning off the power to the lamp. Or, it takes about 30 minutes if only the Xenon lamp was put out by using the ON/OFF switch at the right side of the unit when the power to the unit is supplied.

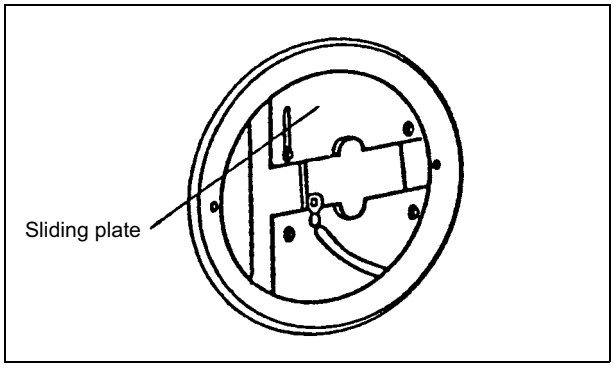
CAUTION

While installing the lamp, do not touch the bulb with bare fingers. If any fingerprints remain on the bulb, remove them with gauze moistened with ethyl alcohol or the special cleaning agent supplied with the lamp. Finger oil remaining on the bulb can become baked onto the bulb when the lamp is lit, possibly causing the lamp to burst.

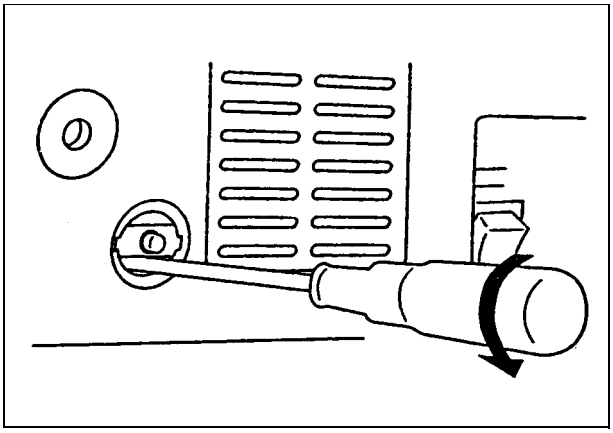
(1) Loosen the two mounting screws on the cover of the lamp housing, and remove the cover.



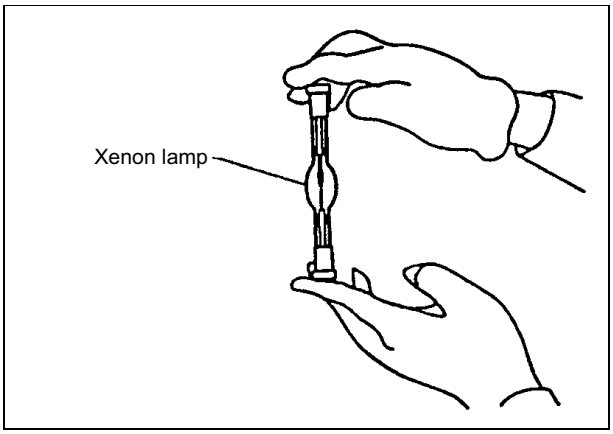
(2) Loosen the fixing screws on the slide plate. Shift the sliding plate to expose the inside of the lamp housing.



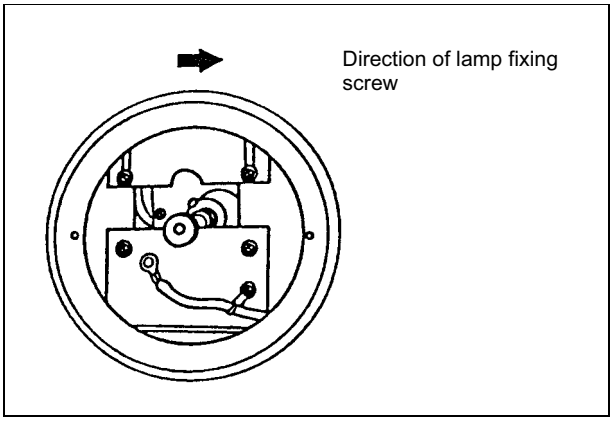
(3) Insert the screwdriver for fixing the lamp (standard accessory) into the opening provided for that purpose along the guide tube. Loosen the lamp fixing screw by turning it counterclockwise.



(4) Remove the Xenon lamp from its shipping case, and remove the knurled screws on the positive and negative sides.



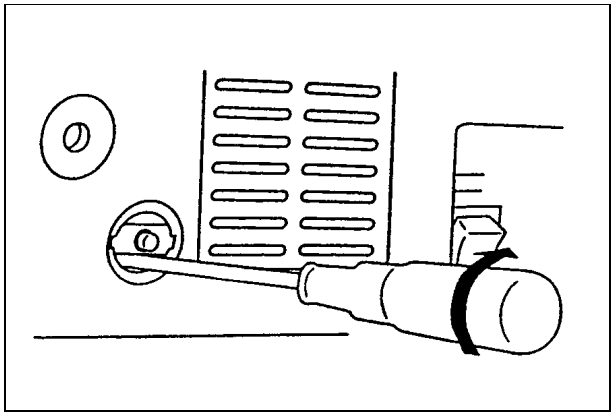
(5) Direct the black or red mark on the cement part of the negative side of the lamp toward the lamp fixing screw, and then insert the lamp into the light source compartment.



CAUTION

Do not confuse the positive side of the lamp with the negative side when installing the lamp.
If the instrument is powered ON with the lamp installed in the wrong direction, it will be damaged.

(6) Insert the screwdriver into the opening again, and turn the lamp fixing screw clockwise to secure the lamp in position.

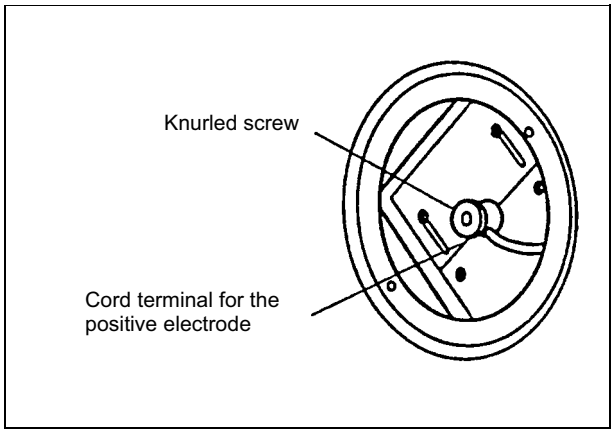


CAUTION

Pulling the positive electrode side with the power cord may damage the lamp while it is lighting up. When securing the terminal of the positive electrode with the knurled screw, make sure that the power cord at the positive side is a little slack.
Tighten the knurled screw by hand only. Using a tool such as a wrench may damage the lamp.

(7) Close the sliding plate, and secure it with the fixing screws.

(8) Connect the power cord terminal for the positive electrode with the positive end of the cord. Secure the terminal to the lamp with the knurled screw.



(9) Install the cover of the lamp housing. Now the installation of the Xenon lamp is complete.

2.5 Powering ON

Upon completion of the installation of the Xenon lamp, power ON the instrument. Before setting the power switch to the ON position, make sure of the following.

NOTE

Check the following points before connecting the power.
* Power supply voltage and power supply capacity
120/220/230/240 V AC ± 10 % 450 VA 50 – 60 Hz
If the power supply voltage is unstable or power supply capacity is insufficient, the unit does not function properly. It is necessary to check the power supply of the entire unit before activating the power.
If fluctuation of the power supply voltage exceeds ± 10 %, use a voltage stabilizer.

Power source voltage varies according to the geographic region. A voltage selector is provided at the rear of the instrument (in the fuse holder compartment) to allow setting the voltage (100, 120, 220, 230 or 240 V) appropriate to the region.
The fuse to be used varies with the applied power voltage. The appropriate spare fuses are provided as standard with the instrument, but when new fuses are to be purchased, be sure that they correspond to the power voltage designated on the voltage selector.
To change the voltage, use a screwdriver to pry open the fuse holder cover. Remove the drum-shaped voltage selector. Re-insert the voltage selector, making sure that the appropriate voltage is displayed. Then re-mount the fuse holder cover.

WARNING

RISK OF ELECTRONIC SHOCK
Before changing a fuse or the inlet voltage, turn off the power switch and disconnect the power cable.

NOTE

REPLACING FUSE
When replacing the fuse, refer to Sec. 8.5 "Replacing Fuse".

NOTE

About the "Voltage selector 2"
Voltage selector 2 must be set according to the site power supply as follows:
Rated voltage: 100 – 120 V 100 V position
Rated voltage: 220 – 240 V 200 V position
* This selector has been factory-set to a suitable position. (See the right-side view in Chapter 3.)
* This selector is not provided for the 230 V model.

- **Grounding**
  The power cable for the RF-5301PC is a three-wire type including a ground wire. Connect the cable to a three-wire type power outlet, and provide reliable grounding. If the power outlets available on-site are two-wire type, be sure to use the grounding terminal of the power cable or the grounding terminal of the instrument.

CAUTION

RISK OF ELECTRONIC SHOCK
Be sure to ground the power cable to prevent any electric shock and to secure safe operation of the system.

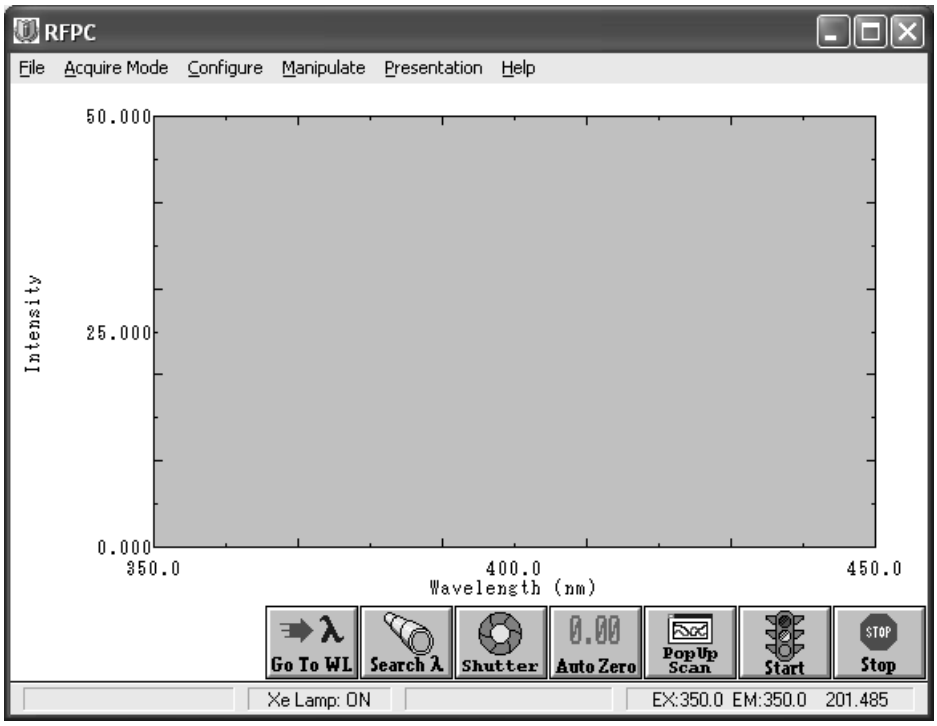
- **Connecting the power cable**
  First, be sure that the power switch is in the OFF position. Then, insert **the attached power cable** into the power inlet located on the right-side of the instrument. Make sure that the setting of the AC voltage selector conforms to the voltage of the site power.
- **XE LAMP ON/OFF switch**
  Set the XE LAMP ON/OFF switch (above the power switch) to the ON position. (See the right-side view in Chapter 3.)

Once all of the above items are satisfied, set the power switch to the ON position. The switch clicks and the Xenon lamp will automatically come on. Also, the power indicator lamp on the front face of the instrument will light up to indicate that the instrument is ON.

NOTE

If the Xenon lamp fails to light
If no click sound is heard or crackling sound continues by turning on the power and then the Xenon lamp doesn't light up, immediately turn off the power and refer to Sec. 9.3 "Before Suspecting Malfunction", to remedy the problem.

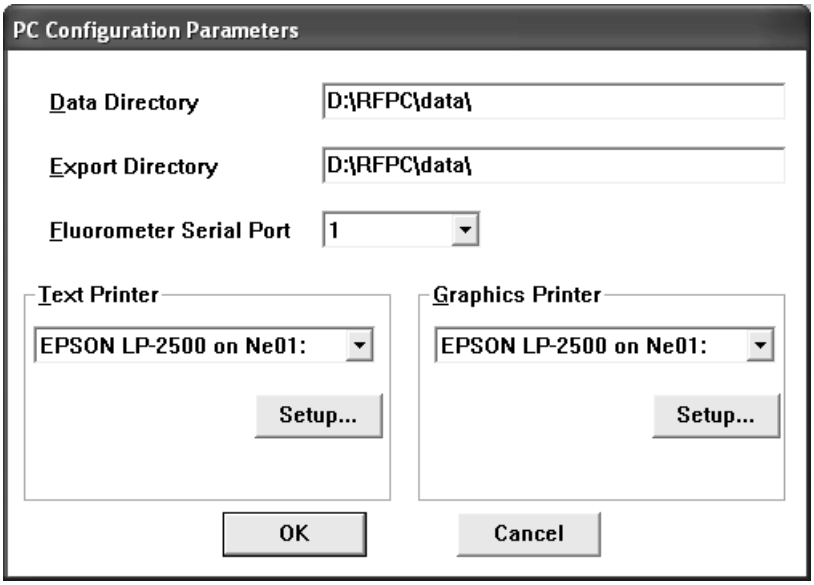
Next, power ON the computer, and start up Windows.
Once Windows is started, double-click the RFPC icon to launch the RFPC software. The message "OFF" should appear in the RFPC main window. This means that communication between the computer and the instrument has not yet been established.



Establish communication according to the procedure below.

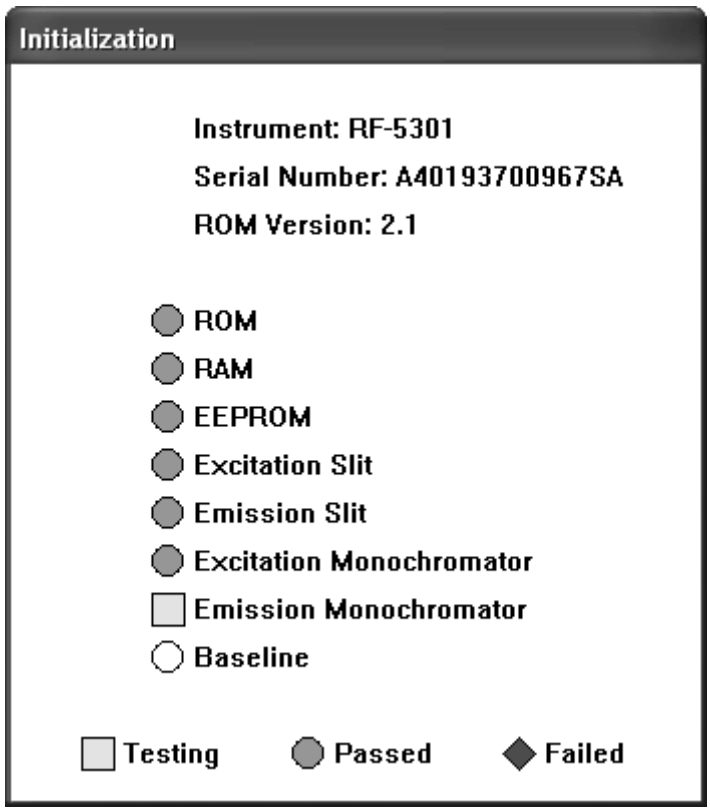
(1) Select [PC Configuration] from the "Configure" menu.

(2) The PC Configuration dialog box (see Sec. 7.4.3) will appear. Then, specify the COM port (for serial communication), data directory, and export directory to be used.



The diagram above indicates an example of the setup window. Be sure to enter the settings according to the user's actual system environment.

(3) Click the OK button.
Once communication is established, the screen shown below will appear, and the RF-5301PC software will initialize the instrument proper.

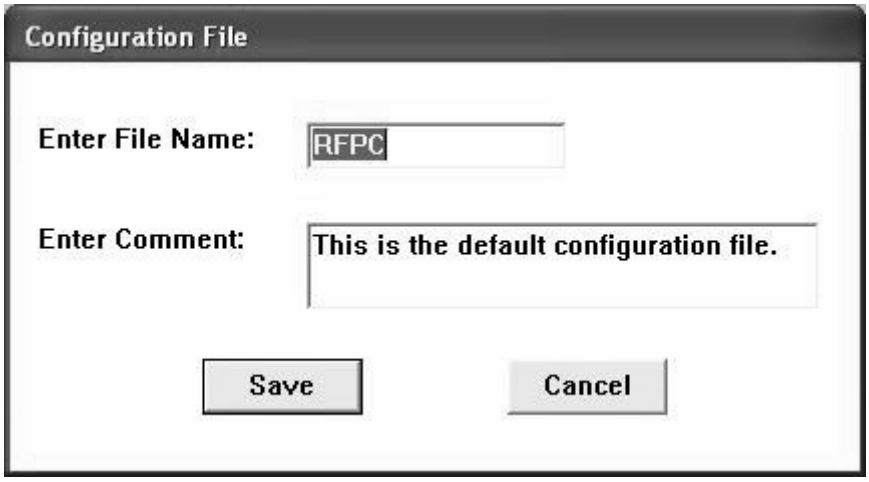


NOTE

When initialization fails to be completed successfully

When initialization fails to run normally and an error message appears, refer to Chapter 9 "Troubleshooting" to remedy the problem.

(4) Upon successful completion of initialization, select [Parameters] from the "Configure" menu, and save the communications parameters as the default configuration file (RFPC).

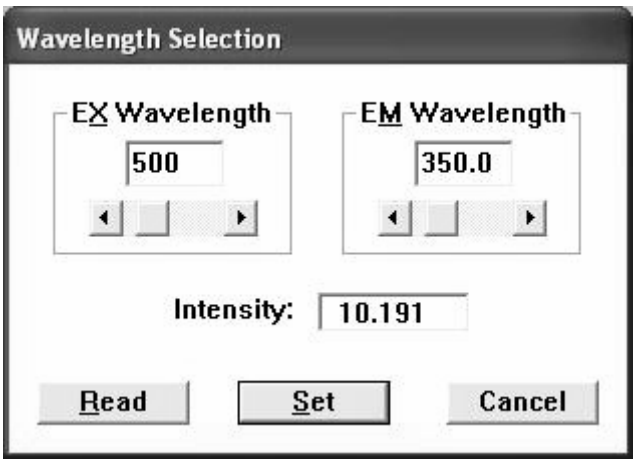


2.6 Adjusting the Lamp Position

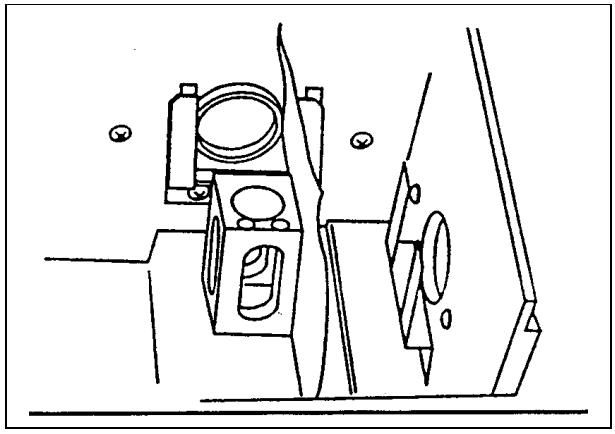
The lamp position must be adjusted according to the Raman spectrum obtained with distilled water. This adjustment must be performed when installing the Xenon lamp for the first time and when replacing a lamp.
Make sure that the instrument has been powered ON for at least 30 minutes before performing this adjustment.

2.6.1 Coarse-adjustment of Lamp Position

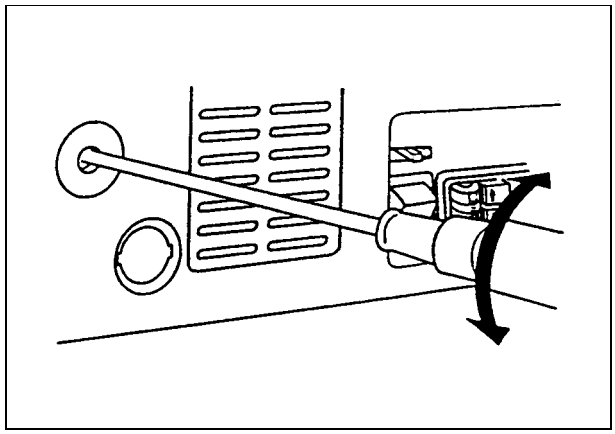
(1) Click the <Go to WL> button at the bottom of the screen, and select 500 nm (green light) as the excitation wavelength.



(2) Open the lid of the sample compartment. Place a sheet of white paper as illustrated. The excitation monochromator should be emitting a green light beam.



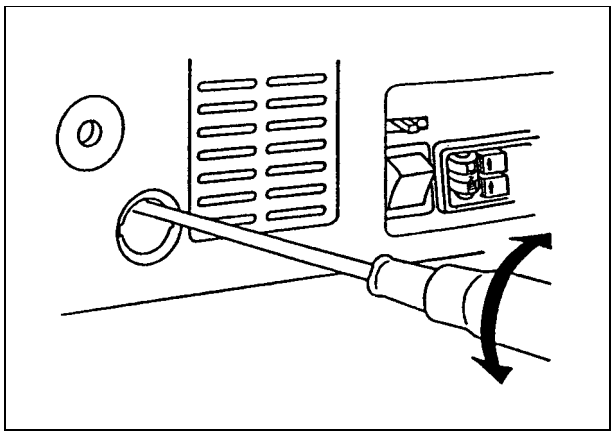
(3) Adjust the screw for horizontal positioning of the lamp so that the beam spot on the white paper is of maximum brightness.



NOTE

Be sure to adjust the horizontal position of the lamp first. Adjusting the vertical position first may cause the light beam to deviate and make it impossible to correctly position the lamp.

(4) Similarly, adjust the screw for vertical positioning of the lamp.

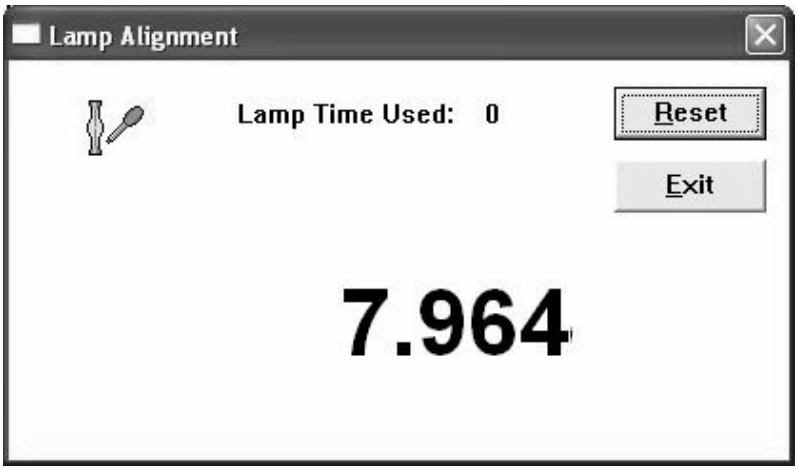


(5) Remove the white paper, and load a cell containing distilled water into a cell holder. Then, close the lid.

2.6.2 Fine-adjustment of Lamp Position

(1) Load a cell containing distilled water into the cell holder, and close the lid.

(2) Select "Instrument" from the Configure menu. The Instrument dialog box will appear. Click the button from the [Lamp Align] option in the dialog box. The Lamp Alignment dialog box will appear.



(3) Adjust the screw for horizontal positioning of the lamp until the intensity indication is at maximum.

(4) In the same manner, adjust the screw for vertical positioning of the lamp.

The lamp has now been correctly positioned. Next, reset the lamp total operating time (see the next section).

2.7 Resetting the Lamp Time Used

The RF-5301PC keeps a record of the total amount of time that the Xenon lamp has been lit. The time is displayed in units of hours.

NOTE
Be sure to always zero the lamp time used after installing the lamp for the first time and when replacing a lamp.

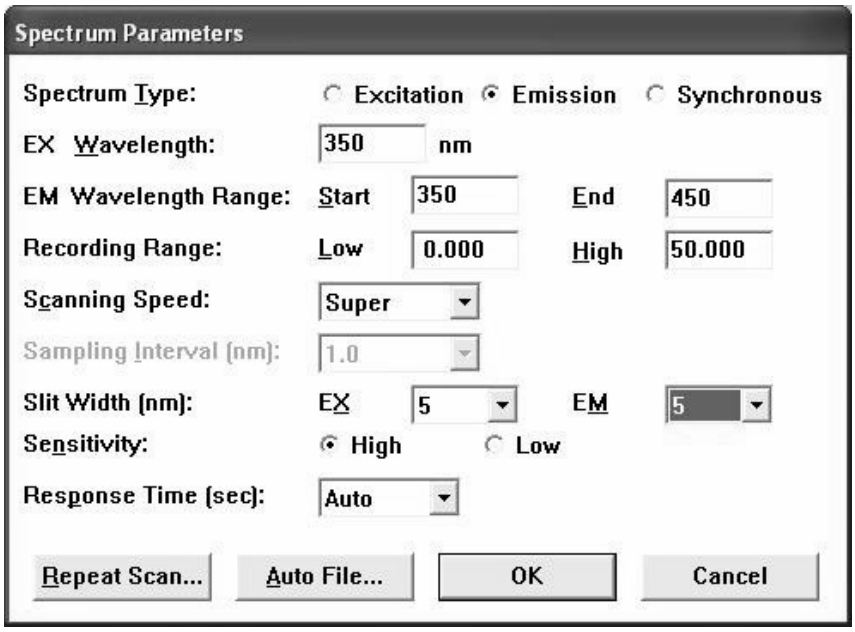
(1) Select "Instrument" from the Configure menu. The Instrument dialog box will appear. Click the <Perform> button of the [Lamp Align] option in the dialog box. The Lamp Align dialog box will appear. (This step is not necessary if the resetting procedure immediately follows the procedure described in the previous section.)

(2) Click the <Reset> button in the dialog box.

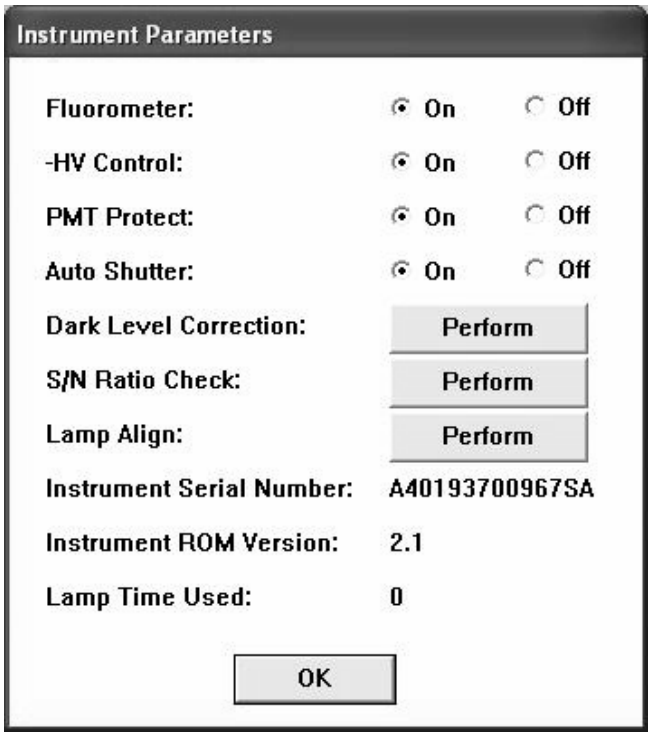
2.8 Adjusting VR0

The sensitivity of the instrument is calibrated according to the Raman peak obtained with distilled water.

(1) Select [Spectrum] from the "Acquire Mode" menu. Then select [Parameters] and set the parameters as follows.

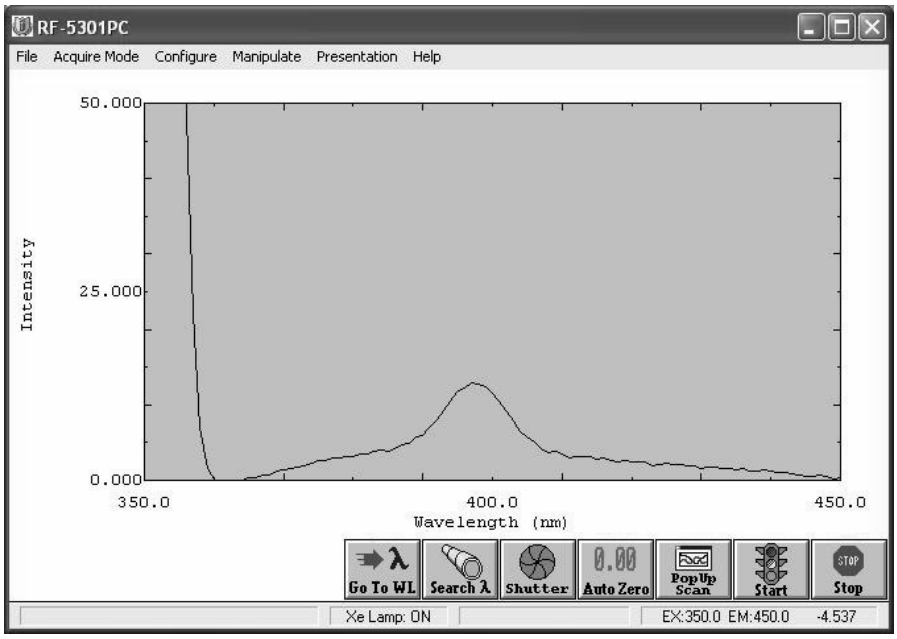


Select [Instrument] from the "Configure" menu and set the parameters as follows.



(2) Load a cell containing distilled water into the cell holder, and close the lid.

(3) Click the <Go to WL> button (at the bottom of the screen). Select 450 nm as the emission wavelength, and click the <Auto Zero> button. Now, click the <Start> button to start the measurement. The resultant spectral pattern will appear as shown below.



(4) Press the right button on the mouse. From the menu displayed, select [Cross Hair] and then the Display command from the submenu. A cross hair cursor for reading the data will appear. Using this cursor, obtain the peak wavelength (approx. 397 nm) and peak intensity value from the Raman spectrum.

(5) When the peak value falls within the range of 10 ± 2, no further adjustment (steps below) is needed. If, however, the peak intensity is outside this range, adjust the uppermost trimmer (VR0) of the three trimmers on the rear of the instrument.

NOTE
The trimmers other than VR0 (VR1 and VR2) have been factory-set. Do not adjust them.

(6) Repeat steps 3 and 4 above, to obtain the peak value.

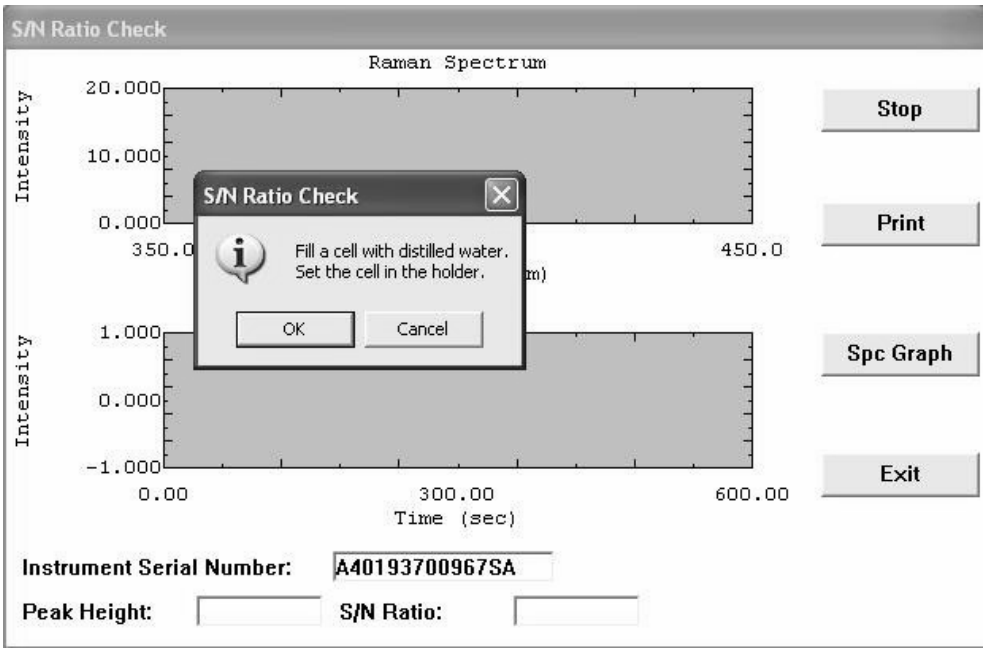
(7) Repeat steps 3 to 6 until the peak intensity falls within the specified range (10 ± 2).

2.9 Verifying Performance

After the instrument and the Xenon lamp have been properly installed, and the lamp position has been adjusted, the performance of the instrument should be verified to check that it is operating normally.

(1) Power on the instrument, and wait 30 minutes or longer until the lamp is stabilized.

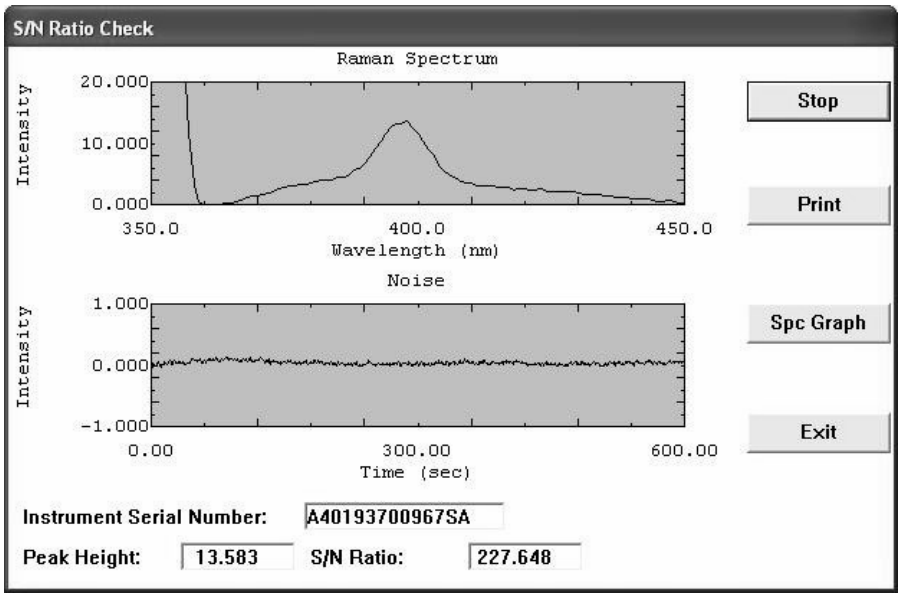
(2) Select [Instrument] from the Configure menu. The Instrument dialog box will appear. Click the <Perform> button of the S/N Ratio Test option in the dialog box. The S/N Ratio Test dialog box will appear.



(3) Load a cell containing distilled water into the cell holder, and close the lid.

(4) Click the <OK> button in the Information dialog box. The Raman peak for distilled water is measured, then a peak search is made, and the height of the Raman peak is indicated. Next, the Raman peak value is measured continuously over 10 minutes to take into account variation over time.

(5) Based on the results of these measurements of the Raman peak, the noise level is evaluated, and the S/N ratio is calculated. An S/N ratio of 150 or greater means that the instrument is operating normally.



(6) Peek height is normally within 10 ± 2. In another case, refer to Sec. 2.8 "Adjusting VR0" and adjust Peak height to normal level.

(7) If the S/N ratio is below 150, refer to Sec. 9.3 "Before Suspecting Malfunction" to check the main unit performance.

2.10 Electro Magnetic Compatibility

NOTE
Descriptions of this section only apply to the model for EU (European union) market: 206-55652-34

This instrument complies with European standard EN61326-1 as amended in 1998.
Emission : Industrial location
Immunity : Industrial location

2.10.1 Electro Magnetic Emission

This instrument can be used in industrial environments.
The test specifications are stated below.

(1) Radiated Emission (EN61326-1 : 1997 + A.1 : 1998 Class A)

(2) Conducted Emission (EN61326-1 : 1997 + A.1 : 1998 Class A)

NOTE
When an electro magnetic disturbance occurs to instruments being used close to this product, take an appropriate distance between the instruments to eliminate the disturbance.

2.10.2 Immunity to Electro Magnetic Interference

This instrument can be used in industrial environments.
The test specifications are stated below.

(1) Radiated Susceptibility (EN61000-4-3 : 1996)
Field strength : 10 V/m, Frequency range 80 – 1000 MHz, Modulation : 1 kHz AM80 %.

NOTE
In this condition, the datum of this instrument may be noisy at some frequencies. However, the instrument recovers automatically when the electromagnetic radiation has stopped.

(2) Power Magnetic Fields (EN61000-4-8 : 1993)
Magnetic field strength : 30 A/m

(3) Electrostatic Discharge (EN61000-4-2 : 1995)
Charge Voltage : 4 kV (Contact), 8 kV (Air Discharge)

(4) Fast Transient Bursts on AC Power Lines (EN61000-4-4 : 1995)
Test Voltage : 2 kV

(5) Surge Immunity of AC Power Lines (EN61000-4-5 : 1995)
Test Voltage : 1 kV line to line, 2 kV line to earth

(6) Radio Frequency Conducted Immunity of AC Power Lines (EN61000-4-6 : 1996)
Applied Voltage : 3 V, Frequency Range 150k – 80 MHz, Modulation : 1 kHz, AM80 %

(7) Immunity against Voltage Dips and Interruptions on AC Power Lines (EN61000-4-11)

NOTE

Compliance to the standard does not ensure that the instrument can work with any level of Electro Magnetic Interference stronger than the level tested.
Interference greater than the value specified in the condition above may cause malfunction of the instrument.
To avoid electro magnetic disturbance, following notices are recommended to be followed.

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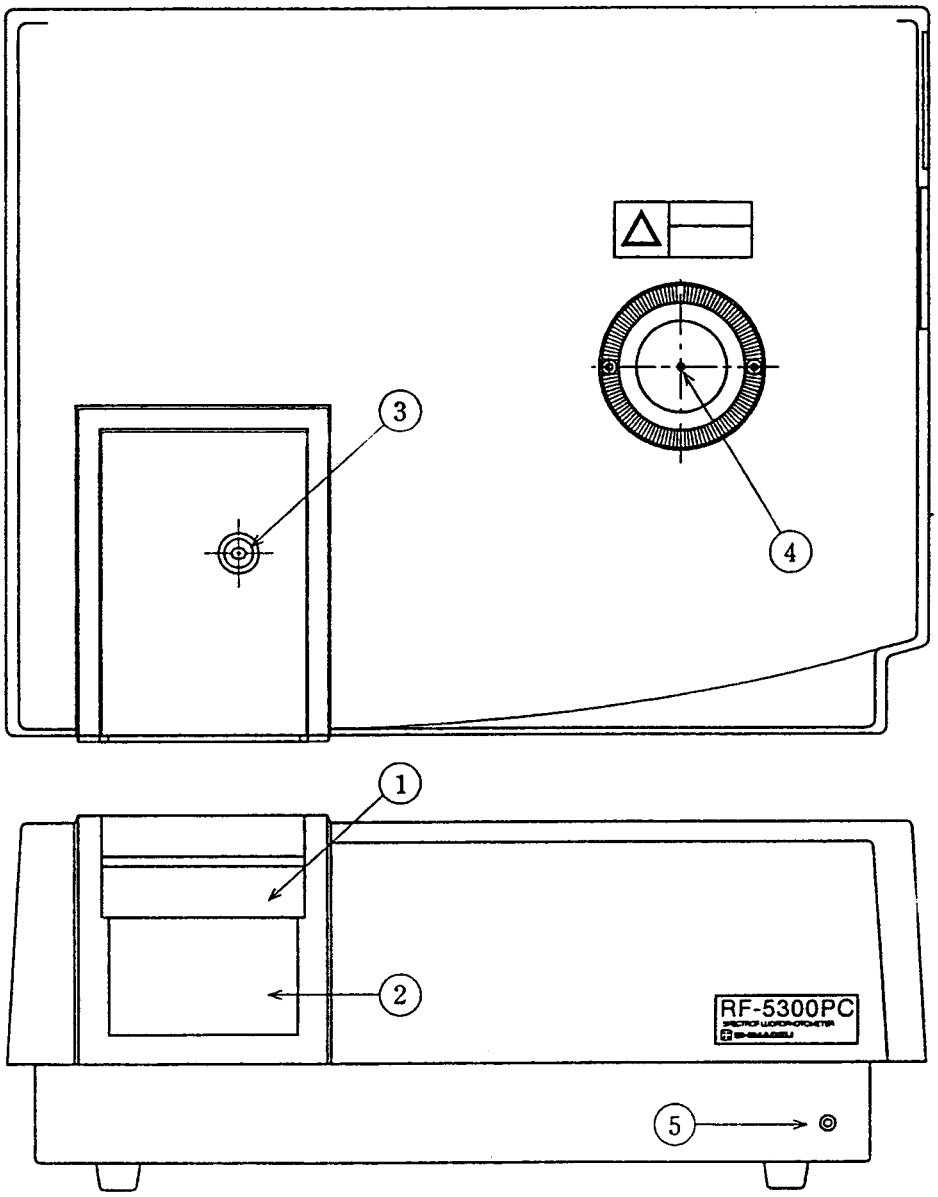
Chapter 3 Parts Names and Functions

CONTENTS

3.1 Front View



(1) Sample compartment
Holds a sample being analyzed.

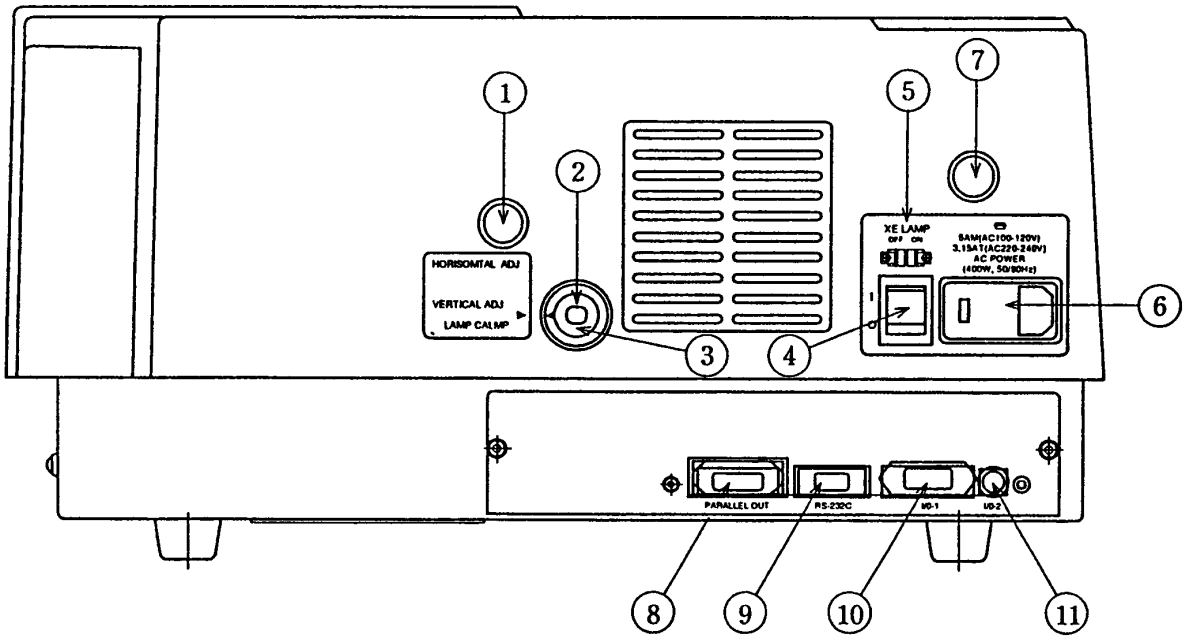
(2) Front panel
Can be removed when installing an accessory, etc.

(3) Cover of reagent injection hole
Can be removed to allow reagent to be injected into a cell loaded in the sample compartment

(4) Cover of lamp housing
Can be removed for installing or removing the Xenon lamp.

(5) Power indicator lamp
Lights up when the instrument is powered ON.

3.2 Right-side View



(1) Screw for horizontal positioning of lamp
For adjusting the horizontal position of the lamp.

(2) Screw for vertical positioning of lamp
For adjusting the vertical position of the lamp.

(3) Lamp fixing screw
Is tightened or loosened for installing or removing the Xenon lamp using the supplied screwdriver which is inserted through the guide tube.

(4) Power switch
Powers the instrument ON/OFF.

(5) Lamp ON/OFF switch
Usually, keep this switch in the ON position.

(6) Voltage selector 1
Must be set according to the site power supply (100/120/220/230/240 VAC).

(7) Voltage selector 2
Must be set according to the site power supply (100/200 VAC).

* This selector is not provided for the 230 V model.

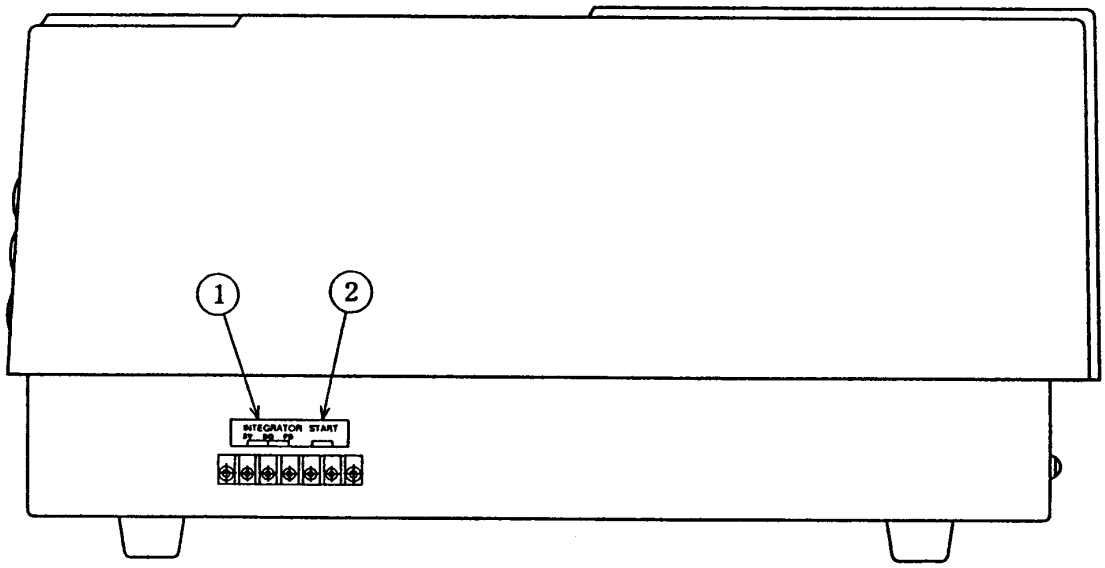
(8) Parallel I/F connector
Not used.

(9) RS-232C I/F connector
Used for communications with the computer.

(10) I/O-1 connector
For connecting the optional auto sample changer.

(11) I/O-2 connector
For connecting the optional sipper.

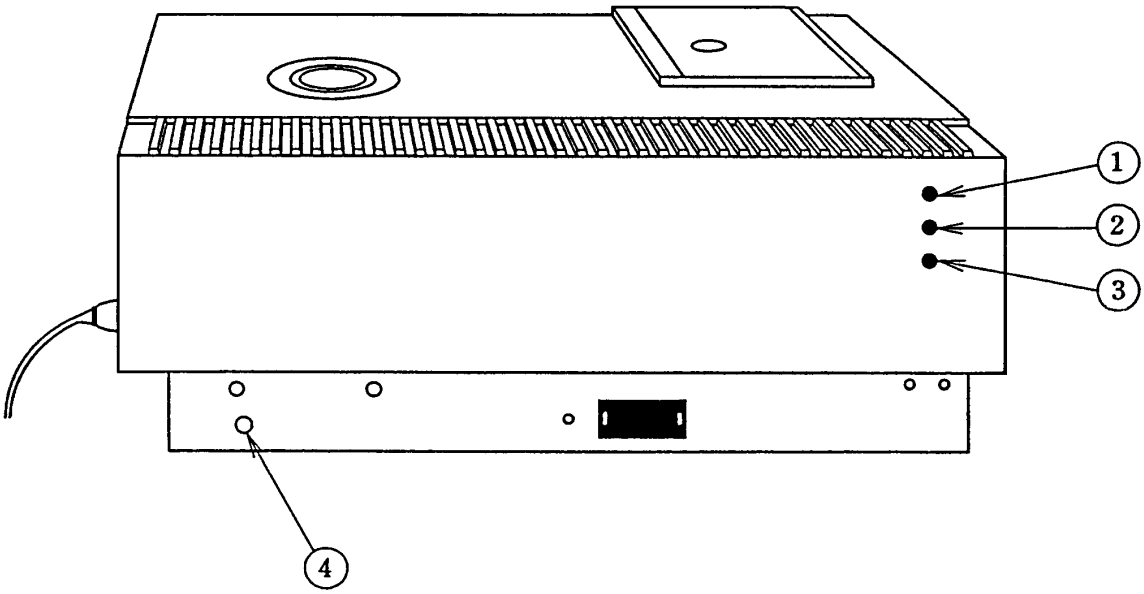
3.3 Left-side View



(1) Analog OUT terminals
Outputs voltage (1 V/FS) in proportion to the fluorescence intensity. The Chromatopack or other output devices can be connected here.

(2) Contact IN terminals
Analysis can be started by connecting between two terminals.

3.4 Rear View



(1) Sensitivity adjustment trimmer VR0
Used to adjust the gain. For details, see Sec. 2.8.

(2) Sensitivity adjustment trimmer VR1
Intended for adjustment of detection sensitivity when negative high-voltage control is OFF. This trimmer has been factory-set to a suitable position. Under normal circumstances, this trimmer should not be touched.

(3) Sensitivity adjustment trimmer VR2
Intended for adjustment of detection sensitivity when lamp compensation is ON. This trimmer has been factory-set to a suitable position. Under normal circumstances, this trimmer should not be touched.

(4) Grounding terminal
Can be connected to a grounding line. If the site power supply does not include grounding, be sure to connect this terminal to ground to protect the instrument.

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Chapter 4 Specifications

CONTENTS

4.1 Instrument Specifications

Item	Description
1. Light source	150 W Xenon lamp
2. Lamp housing	Lamp housing with ozone self-decomposition
3. Monochromator	Blazed holographic concave diffraction grating (F/2.5 for both excitation and emission sides)
3.1 Numbers of grating ruled lines	1300 lines/mm
3.2 Wavelength scan range	220 – 900 nm and zero-order light
3.3 Measurement wavelength range	220 – 750 nm and zero-order light
3.4 Slit width	1.5/3/5/10/15/20 nm (excitation and emission sides), and 6 nm (half-height, excitation side only)
3.5 Wavelength accuracy	± 1.5 nm
3.6 Wavelength scan speed	7 steps: Survey, Super, Fast, Very Fast, Medium, Slow, Very Slow Approx. 5,500 nm/min for Survey
3.7 Wavelength slewing speed	Approx. 20,000 nm/min
3.8 S/N ratio	150 or greater at Raman peak of distilled water
	Excitation wavelength: 350 nm, Emission wavelength: Raman peak
	Slit width: 5 nm both for excitation and emission, Response: 2.0 sec.
4. Light-source compensation system	Monochromatic Light monitoring dynode feedback system
5. Detector	Photomultiplier tube for both photometry and monitor sides
	Photometry: R3788-02, Monitor: R212-14
6. Sensitivity selection	High/low selectable (difference in sensitivity is approx. 50:1)
7. Response	Response at 0 – 98 % 0.02/0.03/0.1/0.25/0.5/2/4/8 sec.
8. Sample compartment	Single non-constant temperature cell holder
9. I/O	RS-232C I/F 1 (for communications with computer) Sipper I/F × 1 ASC I/F × 1 Analog Out I/F × 1
10. Power supply	100/120/220/230/240 VAC, 50/60 Hz, 450 VA
11. Operating temperature range	15 – 35 °C
12. Operating humidity range	45 – 80 % (70 % or less at room temperature 30 °C or higher)

4.2 Software Specifications

- **OS Requirements: WindowsXP SP2/Windows Vista Business**

Function	Description
1. Data acquisition mode	• Spectrum Analysis of excitation, emission and synchronous spectra • Time Course • Quantitative 1st-3rd-order working curves, repetitive analysis
2. File operations	• File saving Saves spectral, time course and quantitative measurement data. Saves parameter files. • File reading Reads spectral, time course and quantitative measurement data. Reads parameters files. • Format translation Converts measurement data into ASCII format. Converts measurement data into DIF format.
3. Data manipulation	• Arithmetic operation Scalar operation involving measurement data and constants • Data transformation 1st-4th order derivatives, smoothing, reciprocals, logarithms • File-to-file arithmetic operations Scalar operations involving two sets of measurement data • Data printing Printing of measurement data • Peak pick • Point pick Data printout for a specified wavelength and time span • Area calculation Calculates the area of a specified section. • Average Averages repetitive measurement data in quantitative analysis.

Function	Description
4. Presentation	• Channel status Sets up ON/OFF status of data presentation, etc. • Set graph limits Sets up new graph scale limits (this can be done using the mouse also). • Radar Sets up new graph limits automatically. • Plot Allows layout of measurement data and parameters and outputting them to the printer. • Display functions Allows control of display color, line type, grid, and fonts for measurement data.
5. User interface	• Help functions Provides on-line interactive help via a Help menu. • SpeedBox(R) Accelerates operation by assigning frequently used menu commands to graphical buttons which can be activated by simply clicking on them with the mouse.

Chapter 5 Getting Started

CONTENTS

5.1 Spectrum

This chapter describes basic procedures for the three respective data acquisition modes Spectrum, Quantitative, and Time Course modes through examples of actual analysis operations. For further details on each mode, refer to the next chapter onwards.

NOTE

- During data acquisition, do not open or operate any other application software.
- If another window application window overlaps the RFPC measurement window during data acquisition, the measurement window will be displayed in white until the next acquired data is plotted. This overlapping may affect the length of measurement time if it occurs repeatedly.

(1) First, prepare the RF-5301PC system for analysis. To do this, power ON the RF-5301PC unit and the computer.

After Windows has started up, double-click the RFPC icon to launch the instrument control software.

(2) Upon completion of the initialization of the optical bench, select [Spectrum] from the "Acquire Mode" menu to enter Spectrum mode. If Spectrum mode has been selected from another data acquisition mode, the Spectrum Parameters dialog box will automatically appear. In that case, set up the parameters as illustrated below. Or, if the current mode is already the Spectrum mode, select [Parameters] from the "Configure" menu to display the Spectrum Parameters dialog box.

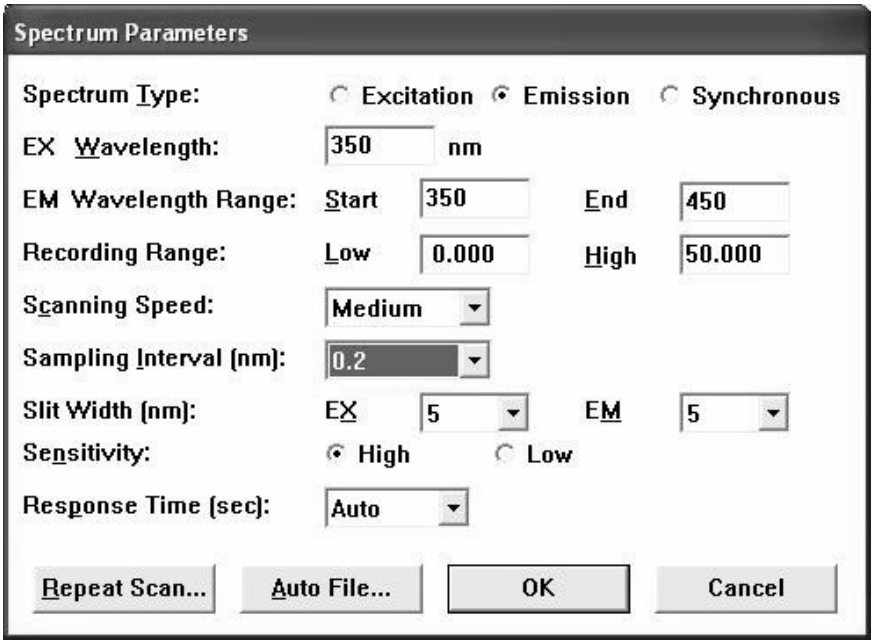


Fig. 5.1 Spectrum Parameters dialog box.

(3) After specifying all the parameters, click the <OK> button to enable the settings. During the parameter set-up procedure, the message "Set-up" is displayed in the status bar at the bottom right corner of the RF-5301PC window. When the wavelength and fluorescence intensity reappear on the screen, the settings are complete.

(4) Load a cell containing distilled water, and click the <START> button to start scanning. During scanning, the current spectral pattern is displayed on the screen, and the current wavelength and fluorescence intensity are indicated in the status bar.

(5) Upon completion of the scanning, the File Name dialog box will appear. Type the file name for the spectrum measured using up to eight alphanumeric characters. Do not enter an extension, since the default extension ".SPC" will be added to the file name automatically.
A comment may be entered for the file by moving the cursor to the Comment text box and typing. When all details have been entered, click the <Save> button to save the data in the channel. If the <Discard> button is clicked, the dialog box will close and all data entered will be lost.

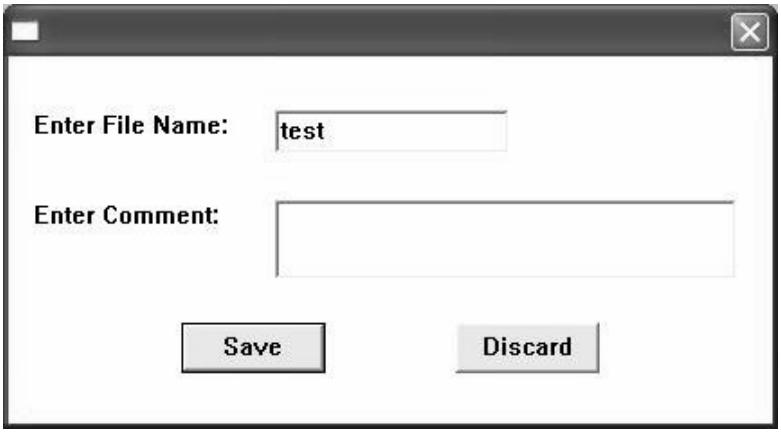


Fig. 5.2 Entering a file name

Channel
An area of computer memory allotted for particular data is called a "channel". In Spectrum mode, up to ten channels are available. Note that to save the data on a channel to the hard disk or a floppy disk, the Save command must be used.

(6) Clicking the mouse's right button will cause a menu to appear. Select [Radar] from the menu, and then [Both Axes] from the subsequent submenu. New graph limits will be set up automatically.

(7) To enlarge a specific area of a spectrum, point to the left top corner of that area with the mouse, and drag to define a rectangular box surrounding the intended area. Or, to set new graph limits select [Limits] from the same menu containing [Radar] (displayed by clicking the mouse's right button), and enter the upper and lower limits in the dialog box that appears.

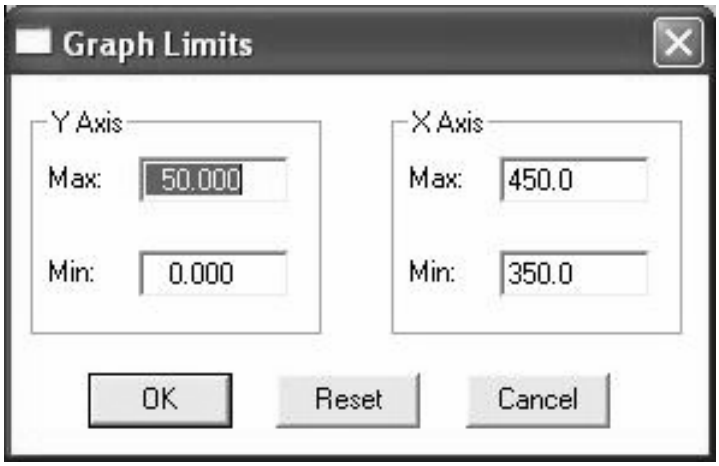


Fig. 5.3 Graph Limits dialog box

(8) To read the wavelength and fluorescence intensity at the position of the mouse pointer, click the mouse's right button. Then, select [Cross Hair] and then the [Display] command from the submenu.

(9) Move the pointer to the desired position.

(10) To erase the mouse pointer, select the Display command again.

(11) To save the obtained data, select the Save command from the File menu.

5.2 Quantitative

(1) Select [Quantitative] from the "Acquire Mode" menu to enter Quantitative data analysis mode. The Quantitative Parameters dialog box will automatically appear.
An example of an operation in Quantitative mode is described below, where a working curve is created and an unknown sample is analyzed quantitatively using quinine sulfate solution (0.1N H_2SO_4).
Prepare the following standard samples (quinine sulfate solution of different concentrations).
Blank, 12.5 ppb, 25 ppb, 50 ppb

(2) Specify the acquisition parameters as illustrated in Fig. 5.4.

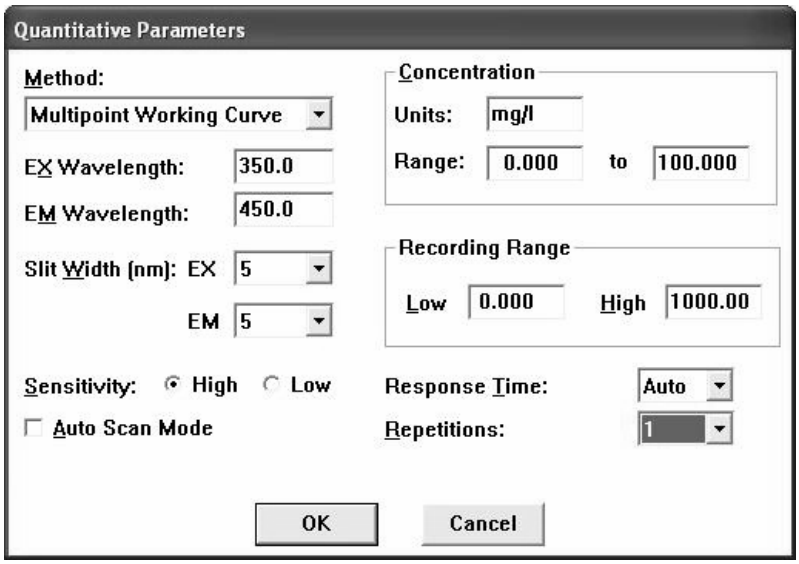


Fig. 5.4 Quantitative Parameters dialog box

When "Multipoint Working Curve" is selected under "Method," the Multipoint Working Curve dialog box will appear. Make the selection as illustrated below.

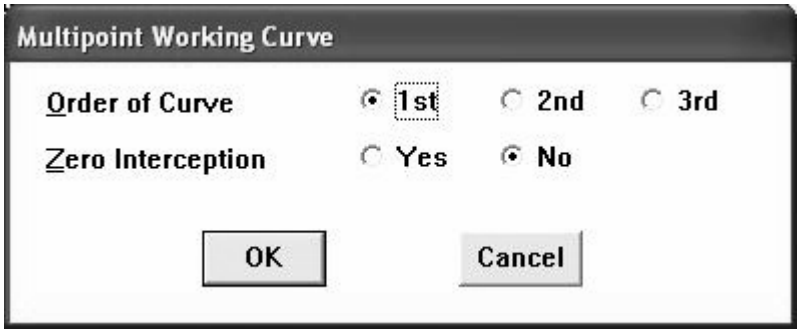


Fig. 5.5 Multipoint Working Curve dialog box

(3) Upon completion of the specification of all the parameters, click the <OK> button to enable the settings.

(4) Load a cell filled with blank sample into the cell holder and click the <Auto Zero> button to establish the zero point.

(5) Load the first standard sample into the cell holder and click the <Read> button. The Standard Sample Data Edit dialog box will appear. Enter the concentration value of the standard sample and click the <OK> button.

(6) Repeat step 5 above for the second standard sample onwards. Once data from a sufficient number of standard samples is acquired, the working curve will be created automatically and displayed on the screen. To automatically scale the graph, select [Radar] and then [Both Axes] from the menu appearing after clicking the mouse's right button. The screen will appear as illustrated in Fig. 5.6.
If an incorrect value was entered in the standard sample table, reverse-highlight that entry and double-click it or make corrections on the Standard Sample Data Edit dialog box which will appear when the Enter key is pressed.

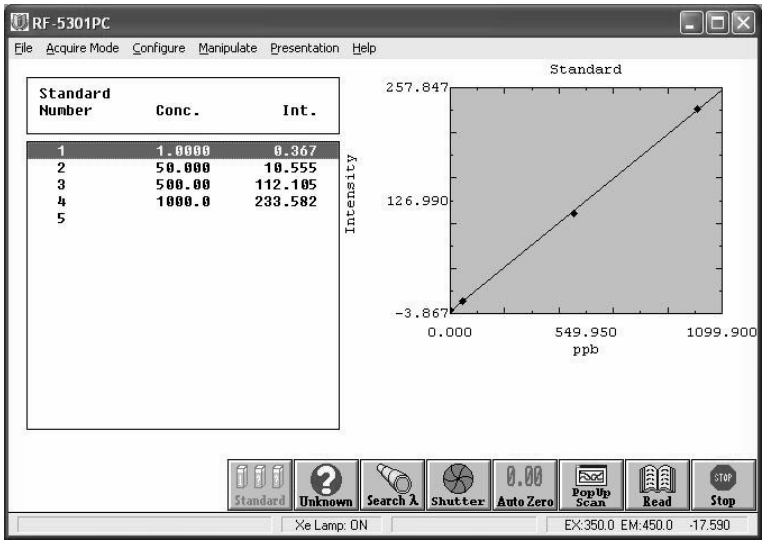


Fig. 5.6 Screen after standard data is entered

NOTE

The working curve may be created using the keyboard instead of using standard samples. In step 5 onwards, press the Enter key rather than clicking the <Read> button.
The Standard Sample Data Edit dialog box will appear on the screen. Then, enter the data for standard samples (those summarized in the following table).

	Concentration	Fluorescence intensity
Standard sample 1	0.0	0.0
Standard sample 2	12.5	12.5
Standard sample 3	25.0	25.0
Standard sample 4	50.0	50.0

(7) Upon creation of the working curve, it is now possible to determine the concentration of an unknown sample quantitatively. Click the <Unknown> button. The Unknown Acquisition screen will appear, and the Unknown button will appear gray.

(8) Load a cell filled with an unknown sample into the cell holder and click the <Read> button to start quantitative determination of the concentration.

(9) To save the obtained data, select [Save] from the File menu.

(10) Enter the file names to be assigned to the standard and unknown samples in the dialog box that will appear and then click the <OK> button.

5.3 Time Course

(1) Select [Time Course] from the "Acquire Mode" menu to enter Time Course data acquisition mode. The Time Course Parameters dialog box will automatically appear.

(2) Specify the acquisition parameters as illustrated in Fig. 5.7.

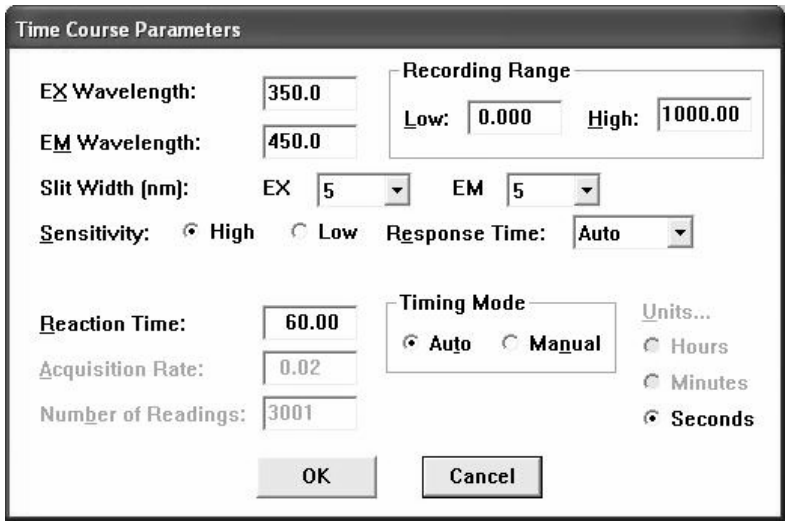


Fig. 5.7 Time Course Parameters dialog box

(3) Upon completion of the specification of all the parameters, click the <OK> button to enable the settings.

(4) Load a cell filled with a distilled water sample into the cell holder and click the <Auto Zero> button to establish the zero point. The following description is based on the assumption that the time course data is acquired on the basis of the Raman peak of distilled water.

(5) Click the Start button to run the data acquisition. Upon completion of the data acquisition, the File Name dialog box will appear. Type the file name for the obtained data using up to eight alphanumeric characters. Do not enter an extension, since the default ".TMC" will be added automatically to the file name.
A comment may be typed in the Comment text box by moving the cursor to the text box.
When all entries are complete, click the <Save> button to save the data in the channel. If the <Discard> button is clicked, all entered data will be lost.

(6) To save the data obtained, select [Save] from the File menu.

Chapter 6 Menus Summarized

CONTENTS

6.1 File Menu

This chapter summarizes the various menus used in the RF-5301PC control software (RFPC).
The RFPC software uses three data acquisition modes: Spectrum, Quantitative and Time Course.
In the Spectrum and Time Course data acquisition modes, up to ten scan data sets can be stored in temporary memory channels at any time. It is therefore necessary to save the data sets that will be needed later to a floppy disk or to the hard disk.
In Quantitative mode, two channels are available: the standard sample channel and an unknown sample channel. The standard sample channel can hold up to 20 data sets, and the unknown sample channel up to 400 data sets.
The selectable menu options differ depending on the data acquisition mode used. A non-selectable menu option appears in faint gray.
The File menu contains items relevant to channel management.
Each channel is capable of holding data temporarily, in the computer's (RAM) memory. On the other hand, a file contains data that has been saved on a floppy disk or the hard disk.

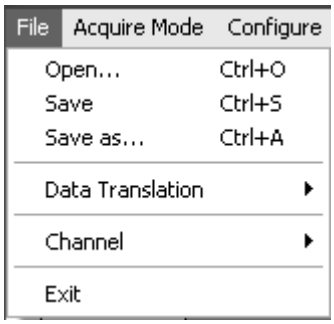


Fig. 6.1 File menu

[Open]
Loads a data file to a channel. It is also possible to check the parameters and channel status of the data file.

[Save]
Saves the data in a named channel. The channel name itself becomes the file name.

[Save as]
Use this command to specify the channel name and file name under which to save particular data. It is also possible to check the parameters in the channel.

[Data Translation]
Converts data in a channel into ASCII or DIF format.

[ASCII Export]
Converts a data file into ASCII format.

[DIF Export]
Converts a data file into DIF format.

[Channel]
Saves or erases a channel, or renames a channel.

[Save Channel]
Saves data in a channel.

[Erase Channel]
Erases data in a channel.

[Rename Channel]
Renames a file name and/or comment assigned to a channel. It is also used to name an unnamed channel.

[Exit]
Terminates the RFPC program.

6.2 Acquire Mode Menu

This menu is used to select a particular data acquisition mode from one of the following three modes.

[Spectrum]

This mode is used to acquire a spectrum.

[Quantitative]

This mode is used to analyze a sample quantitatively.

[Time Course]

This mode is used to acquire the fluorescence intensity at a specific wavelength over a period of time.

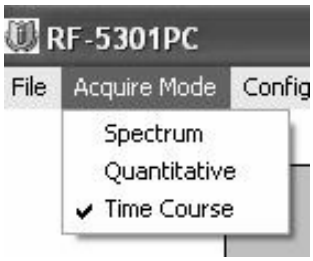


Fig. 6.2 Acquire Mode menu

6.3 Configure Menu

With this menu, the user can set up the parameters associated with data acquisition, communications with the computer, instrument, sipper, etc. It also includes menu options related to loading and saving of parameter configuration files. The Instrument menu is used for setting the instrument parameters, monitoring the Xenon lamp ON time, testing the S/N ratio, and positioning the lamp.

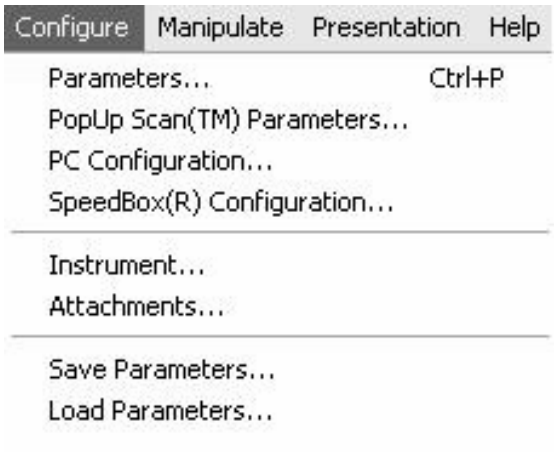


Fig. 6.3 Configure menu

[Parameters]
This command is used to set up data acquisition parameters.

[PopUp Scan(TM) Parameters]
Used for setting up the PopUp Scan(TM) parameters.

[PC Configuration]
Specifies the data and export directories used, and allows selection of the communications port and printer to be used.

[SpeedBox(R) Configuration]
Allows the user to customize the interface by altering the appearance, style and functions of the graphical buttons that constitute the SpeedBox(R).

[Instrument]
This menu item allows the user to switch communication between the computer and the instrument ON/OFF, and turn ON/OFF PMT (photomultiplier) protection and the auto shutter. Also, this menu is used to enable dark level correction, to monitor the total operating time of the Xenon lamp, and to check the ROM version of the RF-5301PC. The user can also test the S/N ratio to verify the performance of the instrument. Note that the [Lamp Align] option should be used to position the Xenon lamp correctly.

[Attachments]
This item allows setting of the parameters for the sipper.

[Save Parameters]
This command saves all the current parameters into a configuration file. Saving the parameters under a file name creates a directory containing "RFPC.EXE," on the hard disk into which all the current parameter configuration files are saved.

[Load Parameters]
This command is used to load a configuration file.

6.4 Manipulate Menu

This menu provides various arithmetic functions for channel data (scalar operations based on a constant, derivative operations, smoothing, logarithmic and reciprocal operations, channel-to-channel arithmetic operation, etc.) as well as data manipulation functions (data print, point pick, peak pick, peak area calculation).

Fig. 6.4 Manipulate Menu

[Arithmetic]

This command subjects the Spectrum or Quantitative or Time Course data in a particular channel to scalar operations (+, -,* , /) using a constant.

[Transforms]

This option subjects the Spectrum or Time Course data to various operations (smoothing, derivative, reciprocal and logarithmic transformation); only reciprocal and logarithmic transformations can be applied to the Quantitative data.

[File/Chnl Calc]

This command is used to apply vector operations (+, -,*, /) to Spectrum, Quantitative or Time Course data in more than one channel.

[Data Print]

The data in the designated channel is displayed in the window together with the associated parameters. The start and end points of the data as well as sampling interval can be selected. The activity value of an enzyme reaction is calculated based on the Time Course data. The data can be saved as a text file or output either to a printer or to the clipboard.

[Peak Pick] (Spectrum or Time Course modes only)

This function picks up the peaks in the specified channel and makes a list of the detected peaks. The peaks on the graph will be marked. Threshold adjustment, valley designation, replotting and printout in either tabular form or as graphic plots are possible. The peak table can be saved as a text file and/or copied to the clipboard.

[Point Pick] (Spectrum or Time Course modes only)

This is a tabulation function which allows X-axis designation of up to fifteen points to generate a list of the corresponding fluorescence intensity values. The X-axis values can be designated by directly typing them in or, by moving the pointer along the graph.

The X-axis values may be changed and the data table can be saved as a text file. It is also possible to mark the Point Pick positions on the graph. The table or graph may be output to a printer. The point list can be saved as a text file and/or copied to the clipboard.

[Peak Area] (Spectrum or Time Course modes only)

This option calculates the integral value (area) of up to four designated areas on a plotted graph. The ranges for integration can be designated by directly typing the start and end points or by moving the pointer on the graph. The baseline will be defined automatically, or may be zeroed.

The Peak Area table can be saved as a text file, and the table or graph may be output to a printer. The graph and/or table can be copied to the clipboard.

[Average] (Quantitative mode only)

In Quantitative mode, the average of the fluorescence intensity values for all sample data having the same ID number is determined.

[Working Curve] (Quantitative mode only)

In Quantitative mode, the parameters (order of the working curve, whether or not the curve must pass the zero point) for creating the working curve are specified.

6.5 Presentation Menu

This menu consists of choices for determining the presentation format of a data file on the screen or as a printout.

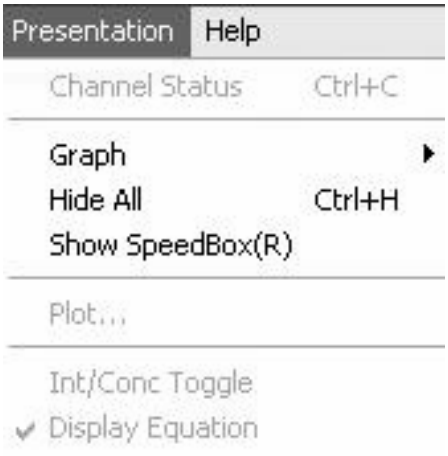


Fig. 6.5 Presentation menu

[Channel Status]

This command allows the information about all the active channels to be displayed on the screen, where the information includes the file name, the line type of the graph, and whether or not the channels are on the screen or have been saved to disk. The user can check the acquisition parameters of each channel on the graph or switch the presentation of particular channels ON/OFF. In Quantitative mode, the data will be displayed on the screen whether or not the data has been saved.

[Graph]

This command allows selection of the line color and line type of the graph and specification of the grids and fonts used in the graph. Also, it can change the limits of the graph presentation, provides access to the Auto Scale (Radar) function, and copies the currently displayed graph to the clipboard.

[Copy]

Copies the currently displayed graph to the clipboard.

[Display Grid]

Switches ON and OFF the grid display on the screen.

[Fonts]

Allows the user to specify the font formats as they will appear on the screen (type, size, style, color).

[Limits]

Allows changing of the upper and lower limits of X- and Y-axes to zoom in on an area for data display.

[Line Colors]

This option enables the user to assign unique colors, line type and annotations to each channel. The line color used may be either a default or customized color.

[Options]

This command allows the user to create custom grids and background colors for the display of graphs.

[Radar] (Auto Scale)

This function automatically sets the graph limits so that all the data in all displayed channels will be visible on the graph. This operation may be selected for either the X-axis or Y-axis or for both axes.

[Hide All]

This function temporarily clears and redisplays the graph for all displayed data on the screen.

[Show SpeedBox(R)]

This is an ON/OFF toggle switch for hiding and redisplaying the SpeedBox(R).

[Plot]

This menu item enables layout of a combination of graph overlays, parameters and text files for the four quadrants of a page to be output to a graphics printer. The resultant layout can be previewed on the screen before the actual printing operation.

[Int/Conc Toggle] (Quantitative mode only)

Toggles the Y-axis of the graph of an unknown sample between intensity and concentration units.

[Display Equation] (Quantitative mode only)

This is an ON/OFF toggle switch for hiding and redisplaying the equation of the working curve.

6.6 Help Menu

This menu consists of the Help choices that described how to run the RFPC software.

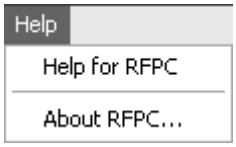


Fig. 6.6 Help Menu

[Help for RFPC]

This menu item offers online help information about how to use the various functions of the RF-5301PC.

[About RFPC]

This item provides details on the version of the RF-5301PC software.

6.7 PopUp Scan(TM) Menu

The PopUp Scan(TM) feature allows a sample to be scanned without having to change the current acquisition mode or parameters. The scan is displayed in a popup window which can be moved, resized, or closed. The window illustrated in Fig. 6.7 contains the following selections.

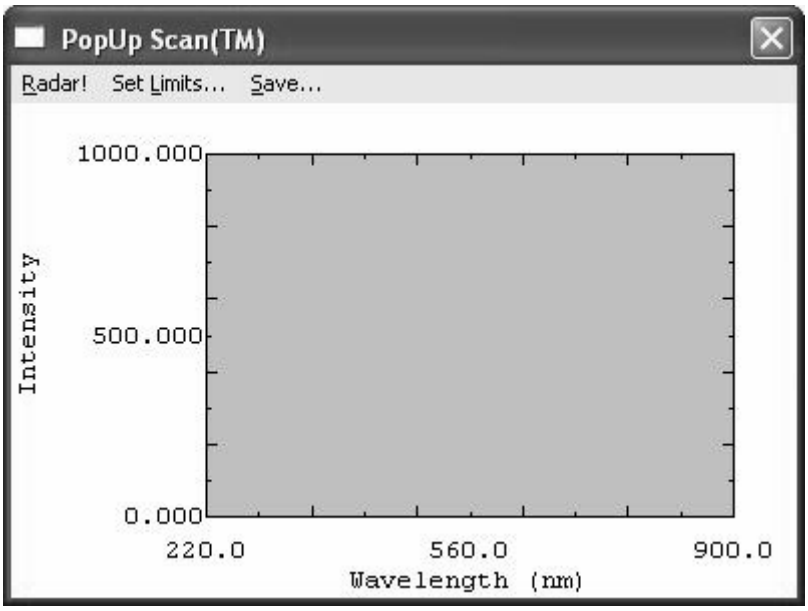


Fig. 6.7 PopUp Scan(TM) menu

[Radar]

The Radar function adjusts the graph limits on the X- and Y-axes so that all data will be visible on the graph. This option may be selected from the menu or directly from the keyboard (when the PopUp Scan window is active) by pressing ALT+R.

[Set Limits]

This option allows changing the X- and Y-axes limits of the graph in the PopUp window, or zooming in on an area without using the mouse.

When the Set Limits dialog box (see Sec. 7.6.2.4 "Limits") appears on the screen, enter upper and lower limit values for the X- and Y-axes and click the <OK> button to update the graph display. Select the <Cancel> button to exit the dialog box without changing the graph limits.

[Save]

This command allows a scan to be saved as a file that can later be loaded while in Spectrum to be manipulated like normally acquired data.

NOTE

Remember that closing the PopUp Scan(TM) window or starting another PopUp Scan(TM) will cause (without warning) the previous PopUp Scan(TM) to be discarded. Before doing this, therefore, be sure to save data if it may be needed later.

6.8 SpeedBox(R) Menu

The SpeedBox(R) is used to create graphical buttons to represent frequently used menu commands for quick convenient access. The SpeedBox(R) may be customized to the user's preferences via the SpeedBox(R) Configuration dialog box (see Sec. 7.4.4). When the software is quit, the last position of the SpeedBox(R) is remembered, and the SpeedBox(R) will be displayed in that same position next time the program is launched.

6.9 Right Button Menu

This menu appears when the right button of the mouse is pressed.
This menu is identical to the Graph submenu from the Presentation menu except that only the Right Button menu contains [Cross Hair] and only the Graph submenu contains the [Display Grid].

[Cross Hair]

The value of any point on the graph can be displayed on the screen by placing the cross hair cursor over it and pressing with the mouse.

[Display]

This command activates the cross hair cursor. Selecting it again causes the cursor to be erased.

[Lock]

This menu item will lock the cross hair cursor to the current position. Select it again to unlock the cursor.

Fig. 6.8 Right Button menu

For other menu items, refer to Sec. 7.6.2.

Chapter 7 How to Use the RF-5301PC

CONTENTS

7.1 File Menu

This chapter describes the commands contained in the menus listed across the top of the screen, from left to right. When a menu command appears in gray rather than in black, it cannot be selected until the appropriate conditions are met (i.e., until a file is loaded or saved, the fluorometer is turned ON, or the relevant data acquisition mode is selected).

7.1.1 Open

The Open command is used to load a file on disk into an empty channel.

(1) In Spectrum or Time Course data acquisition modes
Select the Open command from the File menu, or press CTRL+O from the main window. The Open dialog box, illustrated in Fig. 7.1 will appear on the screen.

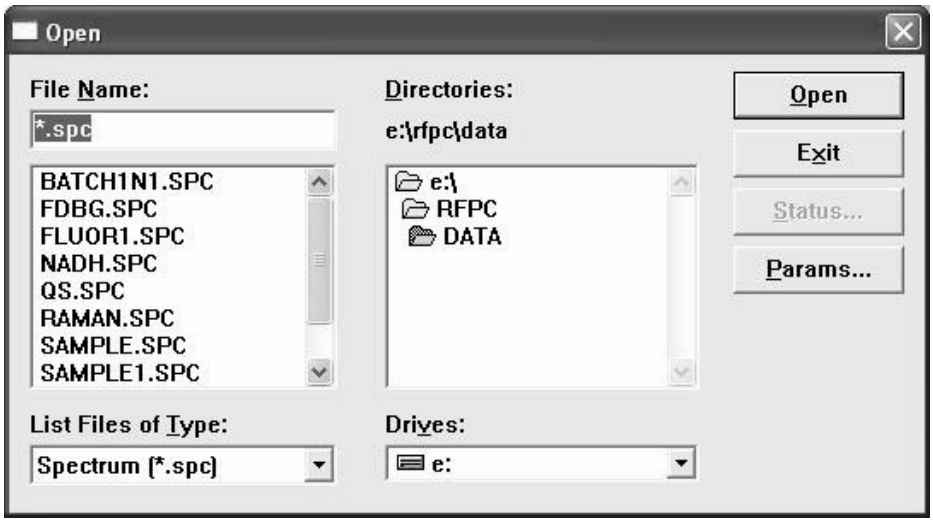


Fig. 7.1 Open dialog box

To load a file, reverse-highlight the intended file name in the list box, and select the Open button. The file will be loaded into an empty channel. Alternatively the file can be opened by double-clicking the file name. More than one file may be loaded as long as there is an empty channel (total of channels: 10) available. When a file is loaded, the file name and corresponding channel name will appear in the title bar.
Use the <Exit> button to return to the main window after (or without) loading a file. Alternatively, the ESC key may be pressed for the same purpose.
When the <Status> button is clicked with the mouse, or by typing ALT+S, the Channel Status dialog box will appear. This dialog box is identical to that which appears when the [Channel Status] command in the Presentation menu is selected (see Sec. 7.6.1 "Channel Status").

NOTE
When all channels are empty, the Status button appears gray.

When the <Parameter> button is selected, the data acquisition parameters for the reverse-highlighted (selected) file will appear.
The data will be presented based on the upper and lower limits of the current axes.
To change the upper and lower limits of the axes, use either the [Radar] (see Sec. 7.6.2.7) or [Limits] command (see Sec. 7.6.2.4) from either the Presentation or Right Button menu.

(2) In Quantitative data acquisition mode

Select [Open] from the File menu, or type CTRL+L using the keyboard. In Quantitative mode, there are two channels available standard and unknown. It is therefore necessary to specify a file type, either standard or unknown.

To load a file into an empty channel, reverse-highlight its file name in the list box, and select <Open>.

Alternatively, double-click the file name to open the file.

Use the <Exit> button to return to the main window after (or without) loading a file. Alternatively, the ESC key may be used for the same purpose.

When the <Status> button is clicked, the Channel Status dialog box will appear.

When the <Parameters> button is selected, the data acquisition parameters for the selected file will appear.

NOTE

In Quantitative mode, the data acquisition parameters and the equation for the working curve are stored together with the standard sample and unknown sample data. When a standard sample data file is loaded, the quantitative parameters are updated in accordance with the working curve. In this process, the concentrations of an unknown sample are recalculated based on the new working curve, and, at the same time, the concentration table is updated.

7.1.2 Save

Use this command to save the data on all the selected channels. The file names for saving data will remain same as the channel names. The file extensions corresponding to the file type will be added automatically. Note that an unnamed channel (a channel designated by "No Name" in the Channel Status dialog box instead of a file name) is not saved to disk even if it is selected. If there are any unnamed channels, the Save As dialog box will appear. Then, follow the procedure described below to save the data in that channel to disk.

7.1.3 Save As

Use this command to save data in an unnamed channel to disk. To activate the command, select it from the File menu with the mouse, or type CTRL+A. Once this command is selected, the Save As dialog box, illustrated in Fig. 7.2 will appear.

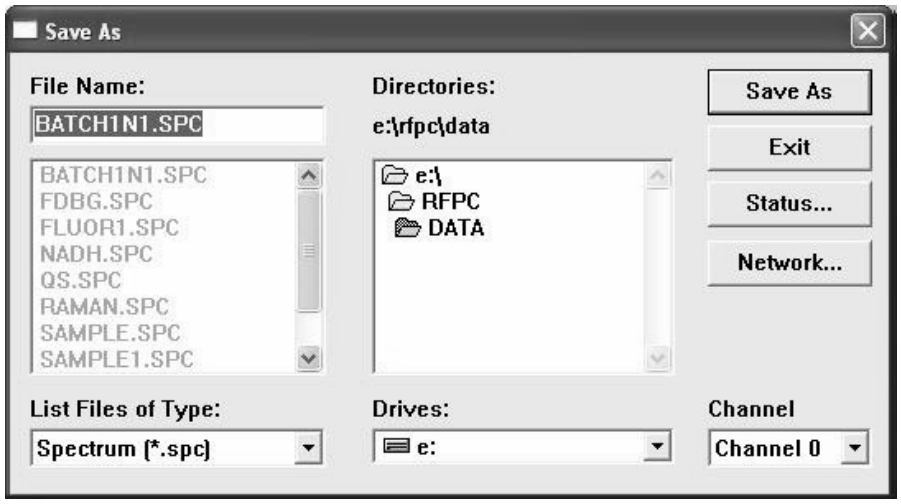


Fig. 7.2 Save As dialog box

Specify the channel, file name assigned to the data, drive name, and directory name, and then click the <Save As> button.
When the <Status> button is clicked, the Channel Status dialog box (see Sec. 7.6.1) will appear.
Use the <Exit> button to return to the main window.

7.1.4 Data Translation

This option converts the data in a channel into ASCII or DIF format. If the particular data is not on a channel, it must first be loaded into a channel using the [Open] command.

7.1.4.1 ASCII Export

This option saves the data in a channel into an ASCII (American Standard Code for Information Interchange) format file.

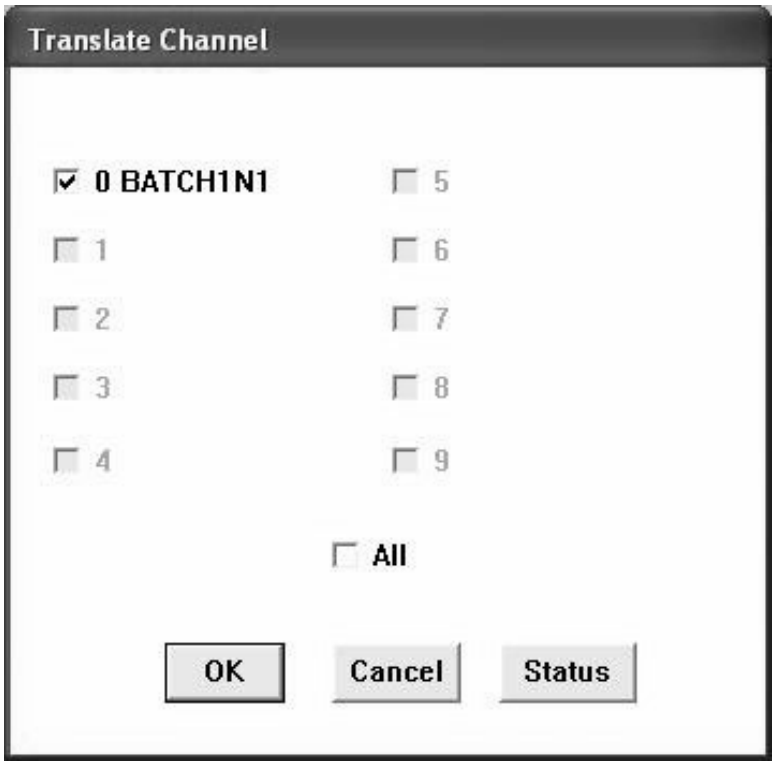


Fig. 7.3 Data Translation dialog box

Mark the check boxes of channels to be saved using the mouse or entering the number(s) corresponding to the underlined numbers. To cancel a marked check box, click that check box again. Select "All" to designate all the channels for data translation. After selecting all intended channels, click the <OK> button. The converted filed will be saved to the export directory on the specified disk drive, and will have a same file name (as the channel) except that is has file extension (.ASC).

When [ASCII Export] is selected in Quantitative (data acquisition) mode, a standard and/or unknown selection dialog box will appear. Mark the check boxes for the particular data and select the <OK> button.
The data in the channel remains unchanged. The descriptive information (comments) about the data in the file will not be carried over to the resultant ASCII file; only ordinate and abscissa information will remain. The general format of ASCII files is as follows:

X, Y <CR> <LF>
X, Y <CR> <LF>

wherein <CR> and <LF> mean carriage return and line feed codes respectively.
When the <Status> button is selected, the Channel Status dialog box (see Sec. 7.6.1) will appear.
To close this dialog box without executing data translation, click the <Cancel> button.

7.1.4.2 DIF Export

This command is identical to "ASCII Export" except that the resultant file format is DIF (Data Interchange Format) where the file has an extension ".DIF". The X-axis data are not translated for Spectrum and Time Course data. With an unknown sample, the X-axis data will represent the sample ID with a standard sample, the X-axis data will become concentration. The converted file will be saved into the export directory on disk.

7.1.5 Channel

This command is used for operations involving channels ("Save Channel," "Erase Channel," and "Rename Channel").

7.1.5.1 Save Channel

This command saves data present in a channel. When [Save Channel] is selected, the Channel Selection dialog box will appear. Mark the check boxes of the channels to be saved (in Quantitative mode, "Standard" and "Unknown"). Select "All" to save the data in all the channels. If the "Clear after Save" check box (in Quantitative mode) is marked, the data in the channel will be erased automatically after the data is saved. If a file is designated as "No Name," its data will not be saved to disk. To save the data in such a file, name it using the Rename Channel command (Sec. 7.1.5.3) or the Save As command from the File menu. Click the <OK> button to save the data.
When the <Status> button is selected, the Channel Status dialog box (see Sec. 7.6.1) will appear.
To close this dialog box without saving the data, click the <Cancel> button.

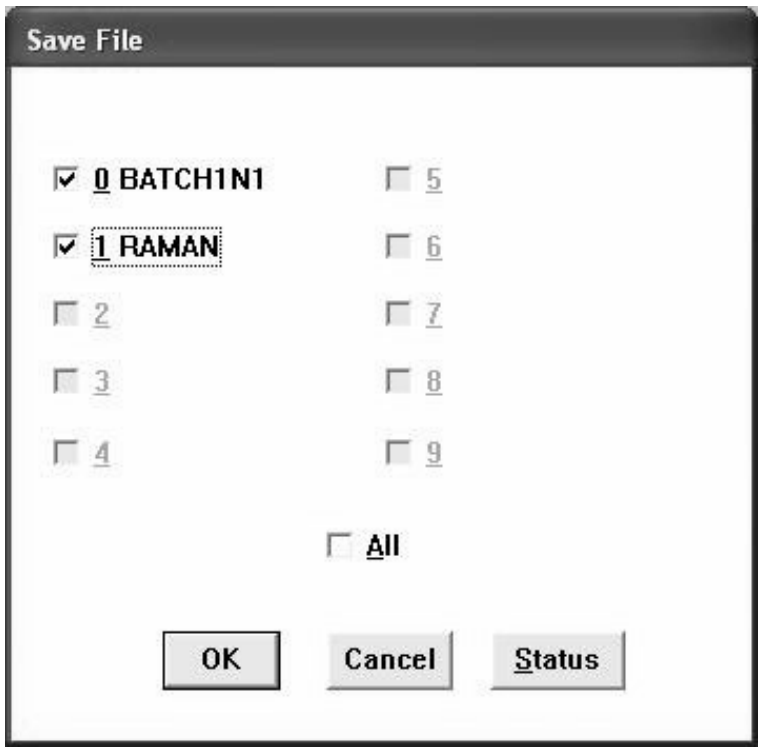


Fig. 7.4 Save File dialog box (Spectrum and Time Course modes)

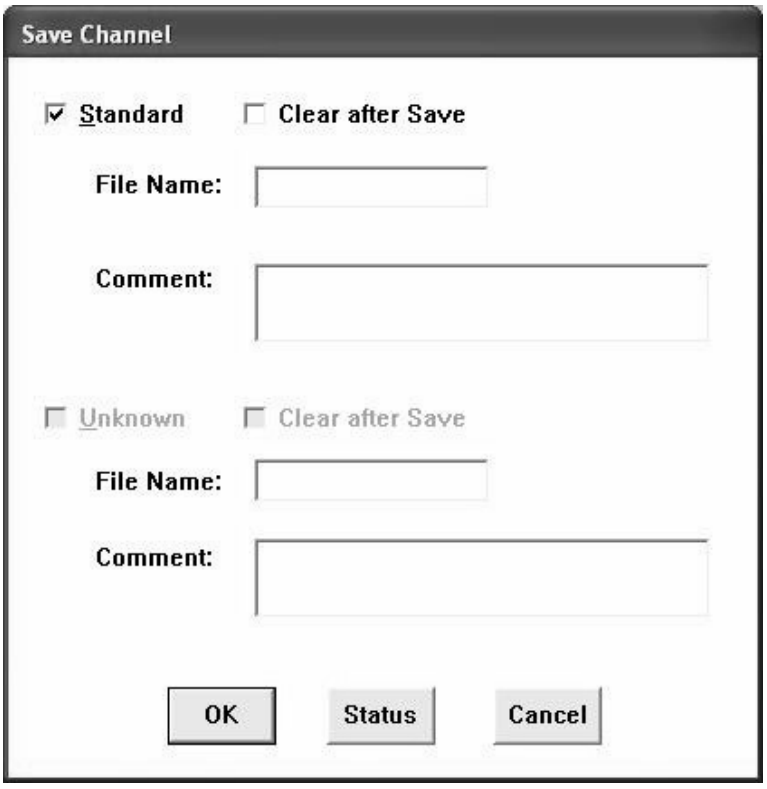


Fig. 7.5 Save Channel dialog box (Quantitative mode)

7.1.5.2 Erase Channel

This command deletes data from the computer's memory (channel(s)). Select the desired channel (in Quantitative mode, "Standard" or "Unknown"), and click the <OK> button. If any data in the channels has not been saved yet, the warning "Some data has not been saved! Do you wish to continue?" will be displayed. Select <Yes> to erase the data.
When the <Status> button is selected, whether or not the data has been saved can be checked (see Sec. 7.6.1).
To close this dialog box without erasing data, click the <Cancel> button.

7.1.5.3 Rename Channel

This option is used to change a file name and/or comment assigned to a channel. Note that the channels designated "No name" in the Channel Status dialog box cannot be saved using the Save and Channel Save commands. To save these channels, it is necessary to name these channels in advance.
When in Spectrum or Time Course data acquisition mode, and [Rename Channel] is selected, the Channel Selection dialog box will appear. Select a channel to be renamed, and then press the Enter key or click the <OK> button. The File Name/Comment Entry dialog box will appear, as illustrated in Fig. 7.6. The edit boxes in this dialog box show the current file name and comment. Type in the desired changes as required, and click the <OK> button. Click the <Cancel> button to close the dialog box without effecting any changes.

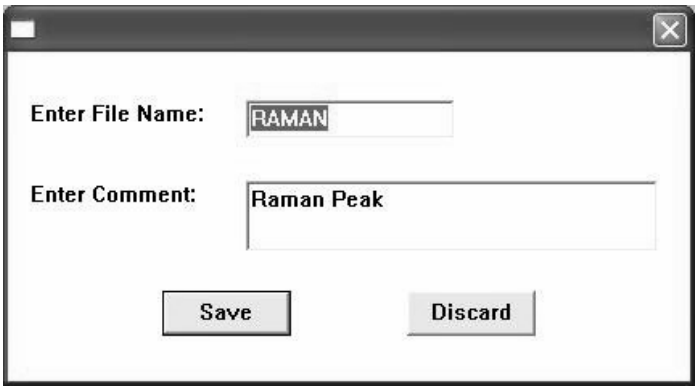


Fig. 7.6 File Name/Comment Entry dialog box

In Quantitative mode, the Rename Channel dialog box will appear, as shown in Fig. 7.7. Select "Standard" and/or "Unknown" by marking the check boxes, and enter or change the file name(s) and comment(s). Then, click the <Save> button or press the Enter key. Click the <Cancel> button to cancel the process and return to the main window.

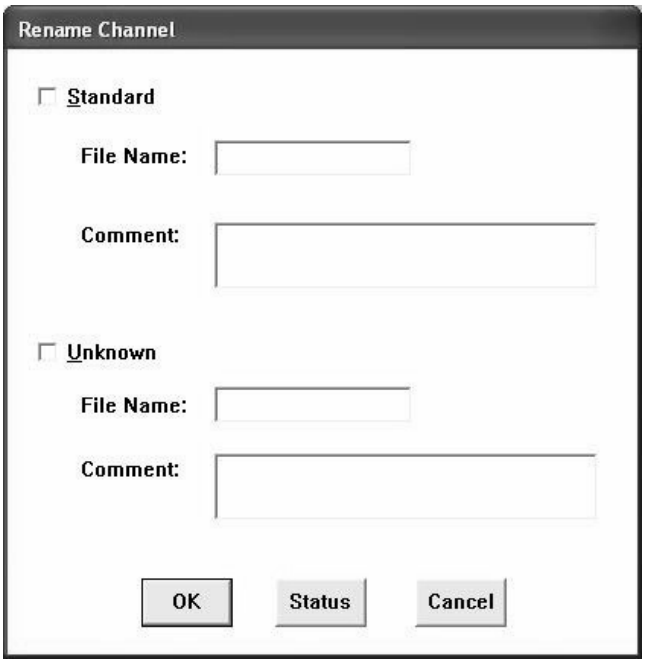


Fig. 7.7 Rename Channel dialog box (Quantitative mode)

NOTE

Naming channels will cause the data to be saved automatically as files when quitting the RF-5301PC program or when the current data acquisition mode is changed. Remember, therefore, that if any data in a file is altered without the file being renamed, the original contents in that file will be lost when the RF-5301PC program is shut down or when the current data acquisition mode is changed.

7.1.6 Exit

Select this menu command to exit the RFPC software. Before shutting down, data on "named" channels is automatically saved to disk. If any "unnamed" channels still remain, a warning message is issued.

7.2 Acquire Mode Menu

The RF-5301PC offers three different data acquisition modes, that is, Spectrum, Quantitative and Time Course. To select a new mode, use the "Acquire Mode" menu. When the current acquisition mode is switched, the data on all "named" channels is automatically saved to disk. If any "unnamed" channels still remain, a warning message is issued.

7.3 Buttons Described

Various graphical push buttons are located at the bottom of the RFPC screen. The buttons on the screen differ from mode to mode (Spectrum, Quantitative and Time Course). This section describes these buttons and their use in each acquisition mode.

7.3.1 Buttons in Spectrum mode

The Spectrum Mode screen contains seven buttons. Each button can be selected by clicking on it with the mouse or by pressing the corresponding function key (F2-F8) on the computer's keyboard. These buttons are mapped from left to right to the F2 through F8 function keys.

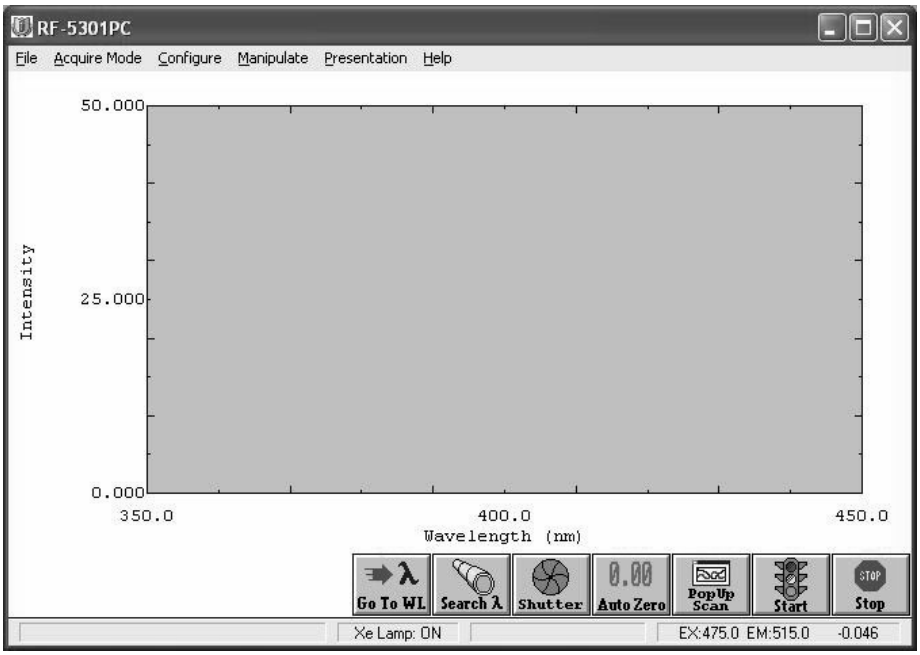


Fig. 7.8 Spectrum Mode screen

(1) Go To WL (Wavelength) button

This button allows setting of the excitation and emission wavelengths. Click this button with the mouse or press the F2 key. The Wavelength Selection dialog box (see Fig. 7.9) will appear, and then the user can enter the new EX and/or EM wavelength values or change them by directly typing numerals into the edit boxes or using the scroll bars below the respective edit boxes. The push buttons in the Wavelength Selection dialog box are described below.

[Read]

Selecting this button causes the optical bench to be adjusted to the entered wavelengths, and the fluorescence intensity value will appear in the dialog box. Note, however, that data acquisition parameter "Wavelength" will not be updated.

[Set]

Clicking this button causes the optical bench to be adjusted to the entered wavelengths, and the dialog box will close. The data acquisition parameter "Wavelength" will be updated.

[Cancel]

Selecting this button makes the original wavelength values effective again, and will close the dialog box.

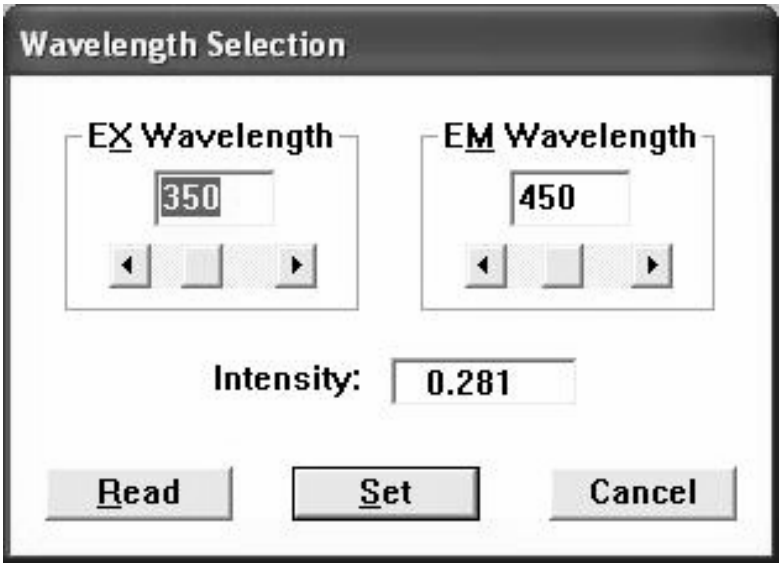


Fig. 7.9 Wavelength Selection dialog box

(2) Search button

Use this button to determine the optimal EX and EM wavelengths for a particular sample. When this button is clicked or the F3 key is pressed, the Search dialog box will appear.

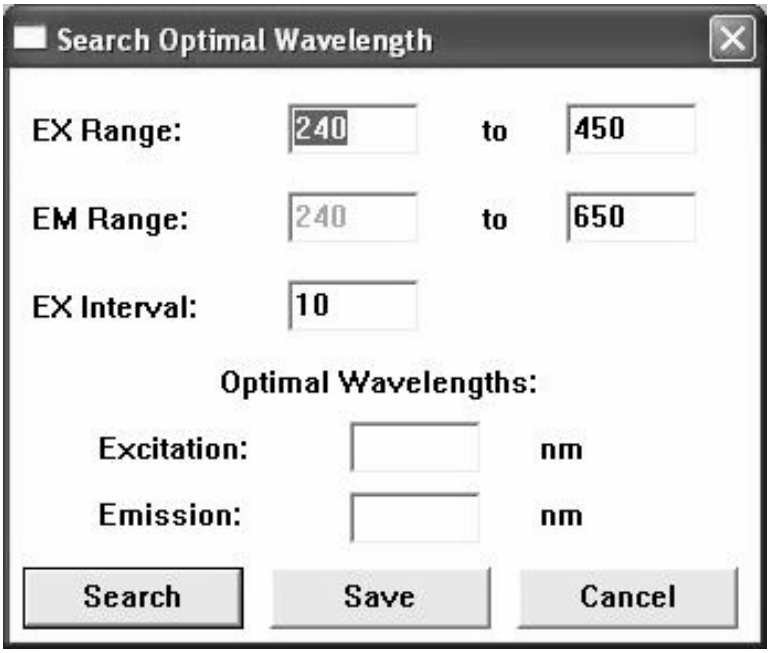


Fig. 7.10 Search dialog box

Enter values for the "EX search range", the "EM search range" and the "EX search interval" and click the <Search> button. The search process will begin.
Other than the peak resulting from the true fluorescence, peaks may also be obtained in a data acquisition run from scattered light, secondary light, or Raman scattering of the excitation light. These undesirable peaks will shift when the excitation wavelength varies, and can be discriminated from true fluorescence peaks. The search program at first analyzes the fluorescence spectrum at varying excitation wavelengths to detect the true fluorescence peak that gives the maximum fluorescence intensity. Then, it adjusts the emission monochromator to this peak wavelength, analyzes the excitation spectrum, and then determines the excitation wavelength that gives the maximum fluorescence intensity. Upon completion of the search process, the obtained wavelength set will appear in the Optimum Wavelength column. During the search process, the slit width is not altered. To change the slit width, use the Parameters command.
To optimize the wavelength, click the <Save> button.

(3) Shutter button
This button is used to open and close the shutter that blocks the excitation light. Click this button with the mouse or press the F4 key. The shutter will open and close. This button also visually indicates the position of the shutter by altering the graphic of the button according to the state of the shutter.

(4) Auto Zero button
This button is used to zero the fluorescence intensity. Click with the mouse or press the F5 key to execute Auto Zero. This function is used, for example, to zero the reading (fluorescence intensity) on solvent blanks.

(5) PopUp Scan(TM) button
Click with the mouse or press the F6 key to execute PopUp Scan(TM) ("survey scan"). The resultant spectrum is displayed in a pop-up window, allowing the spectrum of a sample to be viewed without having to change spectrum parameters.
The PopUp Scan(TM) uses a parameter set similar to that used for scanning in Spectrum mode.

(6) Start button
Click with the mouse or press the F7 key to start scanning. Upon completion of scanning, the File Name dialog box will appear. By entering a file name and clicking the <Save> button, the data will be saved to an empty channel. If a file name is not entered, the File Name list box will show "No Name". Note that if the Repeat Scan or Auto File feature is not in use, the data will not be stored to disk. Select the <Discard> button to delete the current data. When communication between the computer and the instrument is OFF, this button can be used to turn ON communication.

(7) Stop button
Click with the mouse or press the F8 key to terminate the scanning. Note, however, that the data acquired before the button is selected will be saved to a channel. This button also serves to interrupt communication between the computer and the instrument.

7.3.2 Buttons in Quantitative mode

The Quantitative Mode screen contains eight buttons. Each button can be selected by clicking on it with the mouse or pressing the function key (F1-F8) on the computer's keyboard.
These buttons are mapped from left to right to the F1 through F8 function keys.

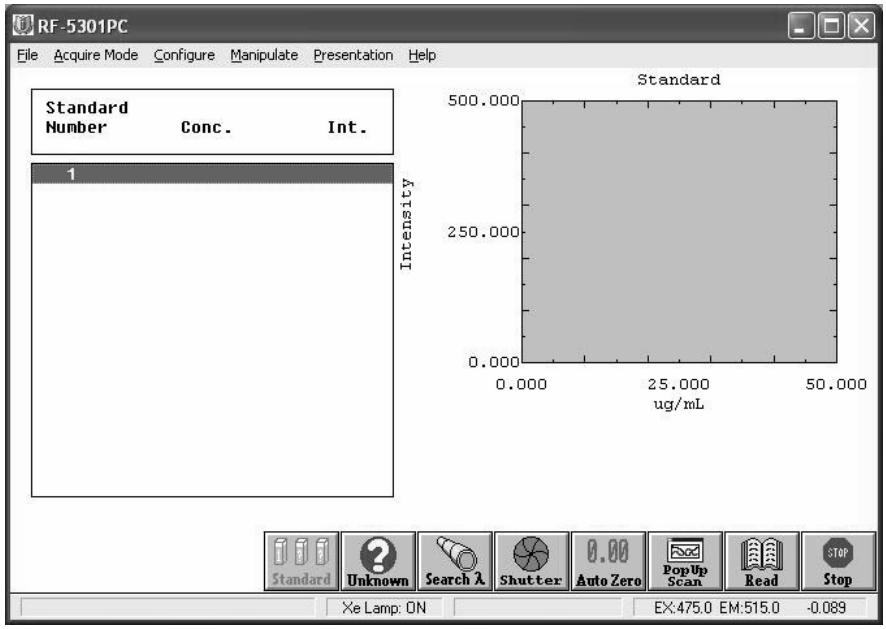


Fig. 7.11 Quantitative Mode screen

(1) Standard button (if not grayed)
Select with the mouse or press the F1 key to analyze a standard sample and generate the working curve.

(2) Unknown button (if not grayed)
Select with the mouse or press the F2 key to analyze an unknown sample quantitatively.

(3) Search button
Use this button to determine the optimal excitation and emission wavelengths for a particular sample. To do this, click with the mouse or press the F3 key. Also, see "Search button" in Sec. 7.3.1.

(4) Shutter button
This button is used to open and close the shutter that blocks the excitation light. Click this button with the mouse or press the F4 key. The shutter will open and close. This button also visually indicates the position of the shutter by altering the graphic of the button according to the state of the shutter.

(5) Auto Zero button
This button is used to zero the fluorescence intensity. Click with the mouse or press the F5 key to execute Auto Zero. This function is used, for example, to zero the reading (fluorescence intensity) on solvent blanks.

(6) PopUp Scan(TM) button
Click with the mouse or press the F6 key to execute PopUp Scan(TM) ("survey scan"). The resultant spectrum is displayed in a pop-up window, allowing a spectrum of a sample to be viewed without having to change the quantitative parameters.

(7) Read button
Select with the mouse or press the F7 key to read the current fluorescence intensity into the standard or unknown sample data table.
The concentration of a standard sample must be entered into the Edit Standard dialog box. Note that when communication between the computer and the instrument is OFF, this button can be used to turn ON communications.

(8) Stop button
Click with the mouse or press the F8 key to quit PopUp Scan(TM). This button also serves to interrupt communication between the computer and the instrument.

7.3.3 Buttons in Time Course mode

The Time Course Mode screen contains eight buttons. Each button can be selected by clicking on it with the mouse or pressing the function key (F1-F8) on the computer's keyboard.
These buttons are mapped from left to right to the F1 through F8 function keys.

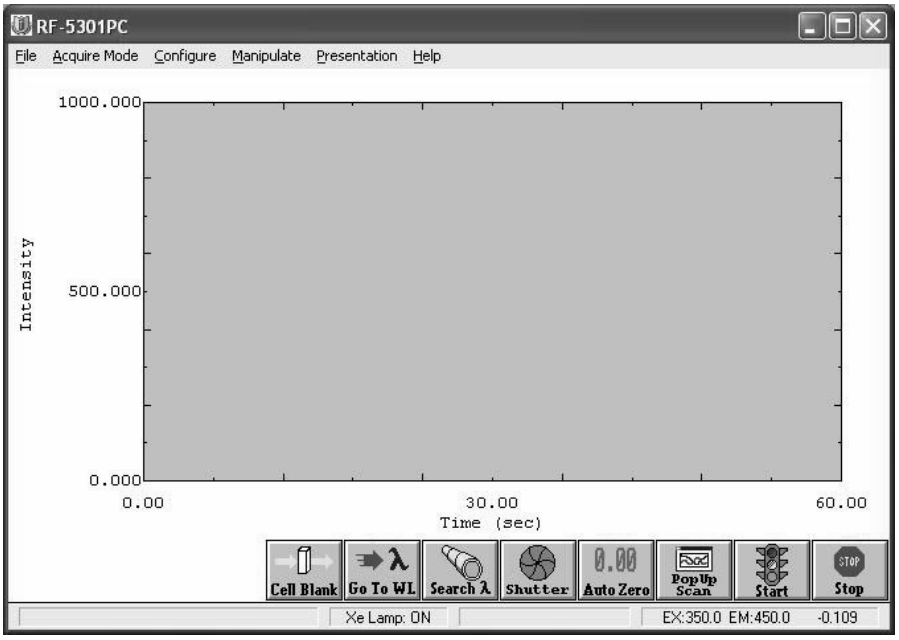


Fig. 7.12 Time Course Mode screen

(1) Cell Blank button
When this button is selected by clicking with the mouse or pressing the F1 key, the current fluorescence intensity reading will be stored in memory, and this reading will be subtracted from each of the time course data points. This cell blank reading will remain valid until a new acquisition mode is selected or the Auto Zero button is selected.

(2) Go To WL (Wavelength) button
This button allows setting of the excitation and emission wavelengths. Click this button with the mouse or press the F2 key. The Wavelength Selection dialog box (see Fig. 7.9) will appear, and then the user can enter the new EX and/or EM wavelength values or change them by directly typing into the text boxes or using the scroll bars below the respective text boxes. The push buttons in the Wavelength Selection dialog box are described below.

[Read]

Selecting this button causes the optical bench to be adjusted to the entered wavelengths, and the fluorescence intensity value will appear in the dialog box. Note, however, that the data acquisition parameter "Wavelength" will not be updated.

[Set]

Clicking this button causes the optical bench to be adjusted to the entered wavelengths, and the dialog box will close. The data acquisition parameter "Wavelength" will be updated.

[Cancel]

Selecting this button makes the original wavelength values remain effective, and will close the dialog box.

(3) Search button

Use this button to determine the optimal EX and EM wavelengths for a particular sample. To do so, click with the mouse or press the F3 key. Also, see "Search button" in Sec. 7.3.1.

(4) Shutter button

This button is used to open and close the shutter that blocks the excitation light. Click this button with the mouse or press the F4 key. The shutter will open and close. This button also visually indicates the position of the shutter by altering the graphic of the button according to the state of the shutter.

(5) Auto Zero button

This button is used to zero the fluorescence intensity. Click with the mouse or press the F5 key to execute Auto Zero. This function is used, for example, to zero the reading (fluorescence intensity) on solvent blanks.

(6) PopUp Scan(TM) button

Click with the mouse or press the F6 key to execute PopUp Scan(TM) ("survey scan"). The resultant spectrum is displayed in a pop-up window, allowing a spectrum of a sample to be viewed without having to change the Time Course parameters.

(7) Start button

Click with the mouse or press the F7 key to start the data acquisition. When the communication between the computer and the instrument is OFF, this button can be used to turn ON communication.

(8) Stop button

Click with the mouse or press the F8 key to quit data acquisition. Note, however, that the data acquired before this button is selected will be saved to a channel. This button also serves to interrupt communication between the computer and the instrument.

7.4 Configure Menu

The commands in this menu are associated with the setting of acquisition parameters, PopUp Scan(TM) parameters, communications with the instrument, SpeedBox(R) parameters, instrument parameters, and attachment (sipper) parameters. The menu also contains commands for opening and saving parameter files.

7.4.1 Parameters

When the Parameters command is selected, the Parameters dialog box corresponding to the currently effective acquisition mode (Spectrum, Quantitative or Time Course) will appear.
The Parameters dialog box will automatically appear whenever a new acquisition mode is selected from the Acquire Mode menu.

(1) Spectrum Parameters

A typical Spectrum Parameters dialog box will appear as illustrated in Fig. 7.13. The parameter items are described below.

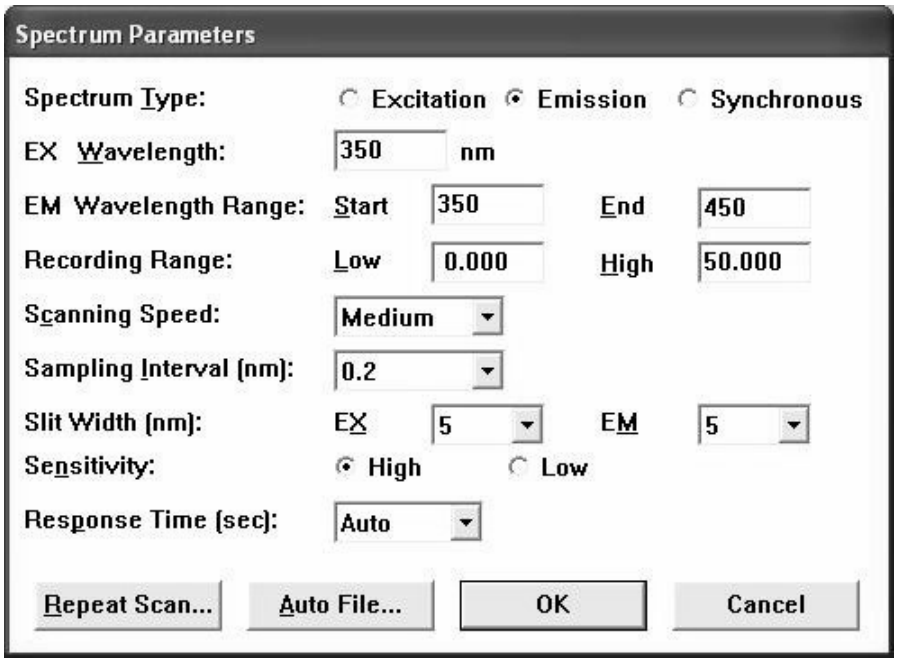


Fig. 7.13 Spectrum Parameters dialog box

[Spectrum Type]

Select the type of spectrum to be scanned from excitation, emission and synchronous.
If the excitation spectrum is selected, the data will be acquired with the excitation monochromator locked onto a fixed emission wavelength.
If the emission spectrum is selected, the data will be acquired with the emission monochromator locked onto a fixed excitation wavelength.
If the synchronous spectrum is selected, the data will be acquired while the emission and excitation monochromators are adjusted so that the difference between their wavelengths remains constant.

[EX/EM Wavelength]

When the excitation spectrum or emission spectrum is selected, use this text box to specify the fixed wavelength.

If the synchronous spectrum is selected, enter a value for the starting excitation wavelength. The allowable settings are 0, and 220 nm to 900 nm. The "0" means 0th order light. Note that the setting of 0th order light for synchronous spectra is impossible.

[EX/EM Wavelength Range]

Use these entries to specify the wavelength range for scanning. The allowable range is 220 nm to 900 nm.

If the synchronous spectrum is selected, the scan range of the emission wavelength will be specified. While in the synchronous spectrum mode, the emission and excitation monochromators are adjusted so that the difference between their wavelengths remains constant. Accordingly, the scan range for the emission monochromator will remain the same as that for the excitation monochromator. For example, when the excitation wavelength is set to 340 nm and the emission wavelength ranges from 350 nm to 450 nm, the resultant scan range for the excitation wavelength will be 340 nm to 440 nm.

The minimum scan range is 25 nm.

[Recording Range]

These entries correspond to the ordinate scale's upper and lower limits on the resultant graph. The allowable range is -100.0 to 1000.0.

[Scan Speed]

This entry specifies the scanning speed for data acquisition. The seven allowable settings are Survey, Super, Very Fast, Fast, Medium, Slow, and Very Slow.

[Sampling Interval]

Sets up the wavelength intervals for scanning. The allowable settings are 0.2 nm, 1.0 nm and 2.0 nm. Note, however, that the interval of 2.0 nm is automatically selected only when the scan speed is set to "Survey," and cannot be used for other speed settings. Also, the setting of 0.2 nm cannot be selected for speed settings "Survey," "Super" and "Very Fast".

[Slit Width (EX)]

This is the setting for the excitation monochromator. The allowable settings are 1.5 nm, 3 nm, 5 nm, 10 nm, 15 nm and 20 nm, and 6 HH. The setting of "6 HH" means the half-height slit used, for example, in analyzing a solid sample (slit width of 6 nm).

[Slit Width (EM)]

This is the setting for the emission monochromator. The allowable settings are 1.5 nm, 3 nm, 5 nm, 10 nm, 15 nm and 20 nm, and "Close". When the "Close" setting is selected, light is not directed to the emission monochromator. Note if the "PMT Protect" option within the "Instrument Parameters" menu (Sec. 7.4.5) is ON, this setting will be changed automatically to "Close" once the lid of the sample compartment is opened.

[Sensitivity]

Sets the desired sensitivity to "High" or "Low". The High setting is about 50 times as sensitive as the Low setting.

[Response Time]

This corresponds to the response speed of the RF-5301PC unit relative to the variation in the fluorescence intensity on a particular sample. The lower the setting (in seconds), the more swiftly the instrument can follow variations in the fluorescence intensity with time, although the resulting noise level will be greater. In contrast, the higher the setting, the less rapidly the instrument can follow variations, but the lower the noise level. The allowable settings are 0.02, 0.03, 0.1, 0.25, 0.5, 2.0, 4.0, 8.0 and Auto. The Auto setting allows the Response Time setting to be automatically optimized so that the peak position corresponds to the selected scanning speed.

[Repeat Scan]

This button causes the scan to be repeated automatically 2 to 100 times according to the selected time interval. When the <Repeat Scan> button is selected, the dialog box in Fig. 7.14 will appear.

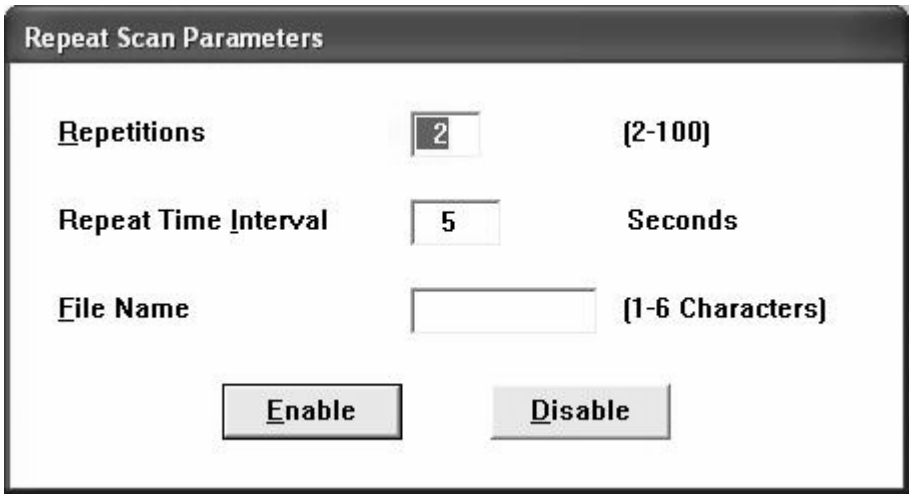


Fig. 7.14 Repeat Scan Parameters dialog box

Enter the number of repetitions to be performed ("Repetitions") and the time interval in units of seconds between the start of two consecutive scans ("Repeat Time Interval"). If the duration required for one cycle is longer than the time interval, the next scan starts as soon as the wavelength is reset to the starting wavelength.

If saving the obtained data to disk, enter a file name consisting of up to six alphanumeric characters.

If <Disable> is selected, the mode is not set to "Repeat Scan". This Disable button may also be used to exit from a previously set Repeat Scan mode.

If <Enable> is selected, the Scan Mode appears in the status bar on the main screen. This indication remains in position until <Disable> is selected in the Repeat Scan dialog box. The message "Repeat" is displayed in the status bar along with the designated file name and an additional two digits representing the number of the scan to be performed next. Thus, for the first scan, the status bar will display "Repeat-Test00" if "Test" was the file name entered. Upon completion of each scan, the data file will be saved to the data directory on disk with a ".SPC" extension. The buzzer will sound to report the completion of all the scans.

NOTE

(about "Auto File" and "Repeat Scan")

When the current scan mode selected is "Repeat Scan," attempting to select "Auto File" will cause an error message to appear. "Repeat Scan" must be cancelled first before "Auto File" can be selected. This also applies when attempting to select "Repeat Scan" if "Auto File" has already been selected.

The data from each scan is stored directly to disk rather than to a channel. The file must be loaded later as needed. If the file name entered already exists, it will be overwritten without any warning.

[Auto File]

Auto File allows a maximum of 100 data sets to be automatically saved in a specified file upon completion of each scan. Data will appear on the screen, but is not stored in a channel. When the Auto File button is selected, the dialog box illustrated in Fig. 7.15 will appear on the screen.

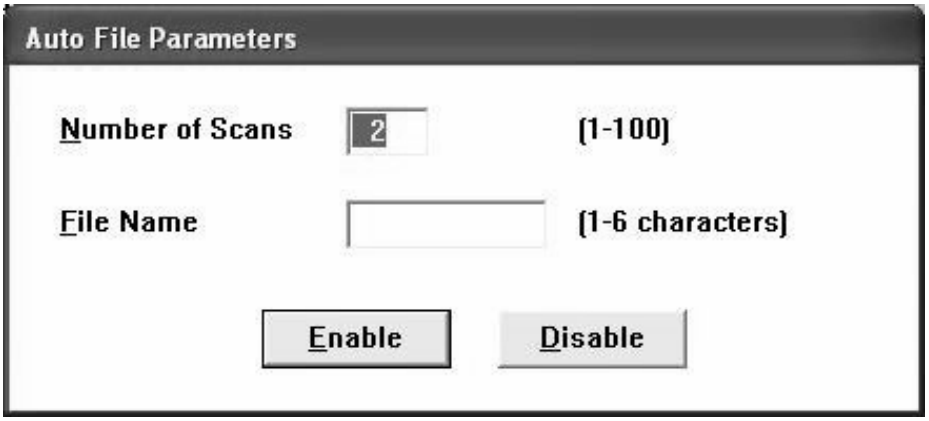


Fig. 7.15 Auto File Parameters dialog box

Enter a value for the "Number of Scans" being performed in Auto File mode, as well as the "File Name" for saving the resultant data.

Selecting the <Disable> button will cancel Auto File mode and clear the file name entered.

If <Enable> is selected, the scan mode is indicated in the status bar on the main screen. This indication remains in position until the specified number of scans is complete or the <Disable> is selected from the Auto File dialog box. Displayed in the status bar is "AF" (Auto File) and the designated file name together with an additional two digits representing the number of the scan to be performed next. Thus, for the first scan, the status bar will display "AF-Test00" if "Test" was the file name entered. If, after executing "Auto File", the file name from the previous set of Auto File runs is changed, or the number of Auto File runs is set to less than that of the current run (displayed in the Scan Mode window), a further Auto File run becomes impossible, and the next set of Auto File runs will begin at zero with the preset file name. To run scans in Auto File mode, select the <Start> button on the main screen. Upon completion of each scan, the data file will be saved to the data directory on disk with a ".SPC" extension.

(2) Quantitative Parameters

A typical Quantitative mode parameters dialog box will appear as illustrated in Fig. 7.16. The parameter items are described below.

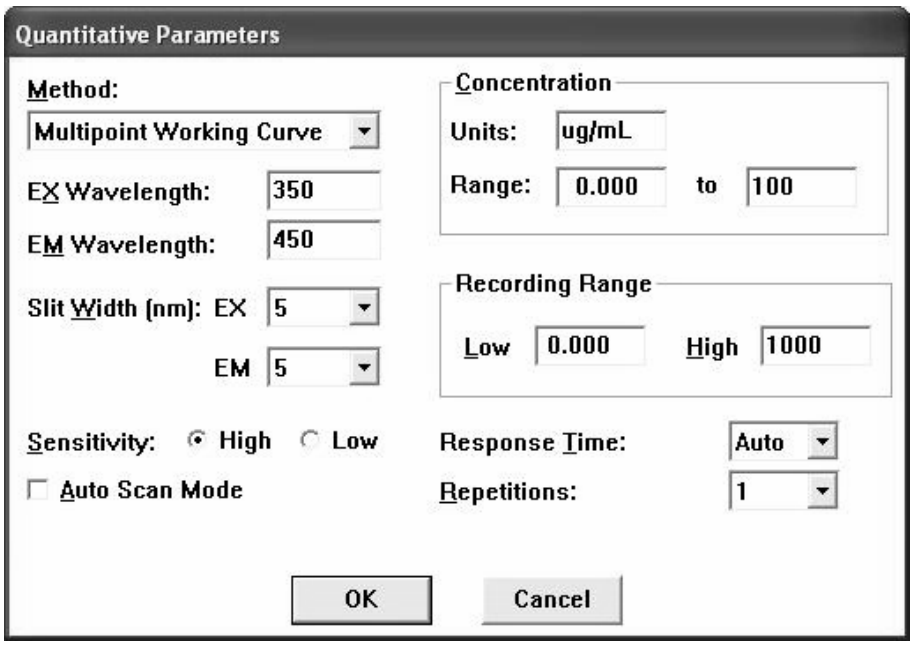


Fig. 7.16 Quantitative Parameters dialog box

[Method]

This parameter specifies the method for determining the concentration of an unknown sample. The selections include Raw Data Measurement, K-Factor, Single Point Working Curve, and Multi-Point Working Curve.

If no quantification is necessary, select "Raw Data Measurement".

Select "K-Factor" to enter the equation coefficients for the working curve. Enter the equation (1st order) into the dialog box that will appear.

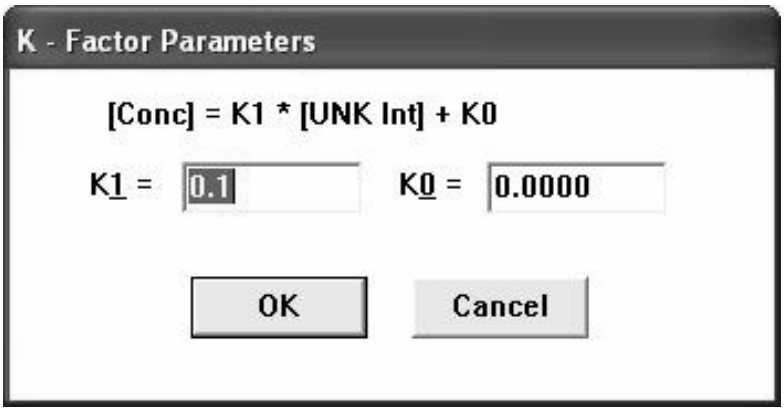


Fig. 7.17 K-Factor Parameters dialog box

Select "Single Point Working Curve" when using only one sample. A dialog box will appear for entering the concentration of a standard sample. Remember, however, that this method assumes that the blank (zero concentration) will have a relative fluorescence intensity of zero and that the working curve created will be a 1st order curve passing through the zero point.
Select "Multipoint Working Curve" when using two or more (up to 20) standard samples. The Multipoint Working Curve dialog box will appear. Specify the order of the curve (1st, 2nd, 3rd order) and whether or not the curve must pass through the zero point.

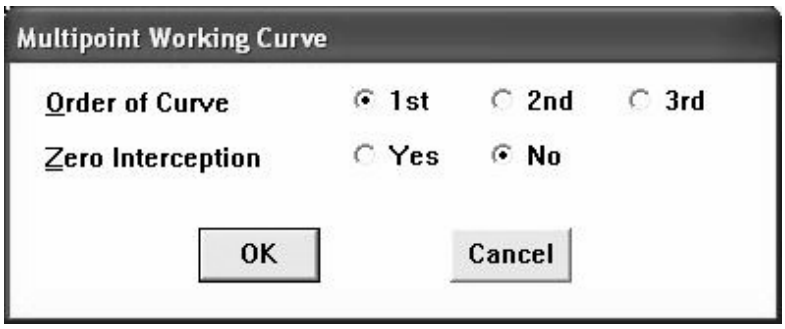


Fig. 7.18 Multipoint Working Curve dialog box

Cautions on using Multi Point Working Curve
The concentration values for an unknown sample will be displayed as 0.000 until a valid working curve is obtained. When information sufficient to determine the equation of the working curve has been acquired, the curve is displayed in the standard sample graph on the screen, and the concentration of the unknown samples will be calculated. The working curve is drawn over the concentration range (+10 %) of the standard samples. When using 2nd and 3rd order polynomials to calculate unknown sample concentrations, there may be multiple solutions (sample concentrations). In this case, the following algorithm is used to determine the best solution (concentration of the unknown sample):

(1) If a concentration value is less than or equal to the largest standard concentration and greater than or equal to the smallest standard concentration, the value is considered to be correct. If there are two or more concentration values for an unknown sample, the value nearest to the smallest standard concentration is taken as the correct concentration value.

(2) If none of the solutions satisfy the criteria in 1, the concentration value that is closest to the smallest standard concentration is considered to be correct.

[Concentration]

Use this selection to set the initial scale of the unknown concentration axis and specify the units of concentration and concentration range for display.

[Units]

Enter the units of concentration.

[Range]

Enter the concentration range for displaying.

[EX Wavelength]

Enter the excitation wavelength. The allowable settings are 0, and 220 nm to 900 nm.

[EM Wavelength]

Enter the emission wavelength. The allowable settings are 0, and 220 nm to 900 nm.

[Slit Width (EX)]

This is the setting for the excitation monochromator. The allowable settings are 1.5 nm, 3 nm, 5 nm, 10 nm, 15 nm and 20 nm, and 6 HH. The setting "6 HH" means a half-height slit is used, for example, to analyze a solid sample (slit width of 6 nm).

[Slit Width (EM)]

This is the setting for the emission monochromator. The allowable settings are 1.5 nm, 3 nm, 5 nm, 10 nm, 15 nm and 20 nm, and "Close". When the Close setting is selected, light is not directed to the emission monochromator. Note if the PMT Protect option in the Instrument Parameters dialog box (Sec. 7.4.5) is ON, this setting will be changed automatically to "Close" once the lid of the sample chamber is opened.

[Sensitivity]

Sets the desired sensitivity as "High" or "Low". The High setting is about 50 times as sensitive as the Low setting.

[Response Time]

This corresponds to the response speed on the RF-5301PC unit relative to the variation in the fluorescence intensity of a particular sample. The less the setting (in seconds), the more quickly the instrument can follow variations in the fluorescence intensity (over time) although the resulting noise level will be greater. In contrast, the higher the setting, the less quickly the instrument can follow variations, but the noise level is lower. The allowable settings are 0.02, 0.03, 0.1, 0.25, 0.5, 2.0, 4.0, 8.0 and Auto. In Auto setting Response Time is set unconditionally to 2.0.

[Auto Scan Mode]

Auto Scan Mode, if enabled, automatically starts PopUp Scan(TM) before quantitative acquisition of every sample, allowing the user to preview the spectrum of the sample. When Auto Scan Mode is effective, status information including the file name is displayed in the Scan Mode window.

[Repetitions]

This setting determines how many times a particular unknown sample is measured. For example, select 3 to measure an unknown sample three times under the same ID. By using "Average" from the Manipulate menu (see Sec. 7.5.8), an average fluorescence intensity can be calculated using samples of the same ID.

How to edit the Standard/Unknown Sample table

In Quantitative data acquisition mode, a graph will appear at the right side of the screen, and a standard or unknown sample table on the left side of the screen. The procedure for editing the standard or unknown sample table is described below.

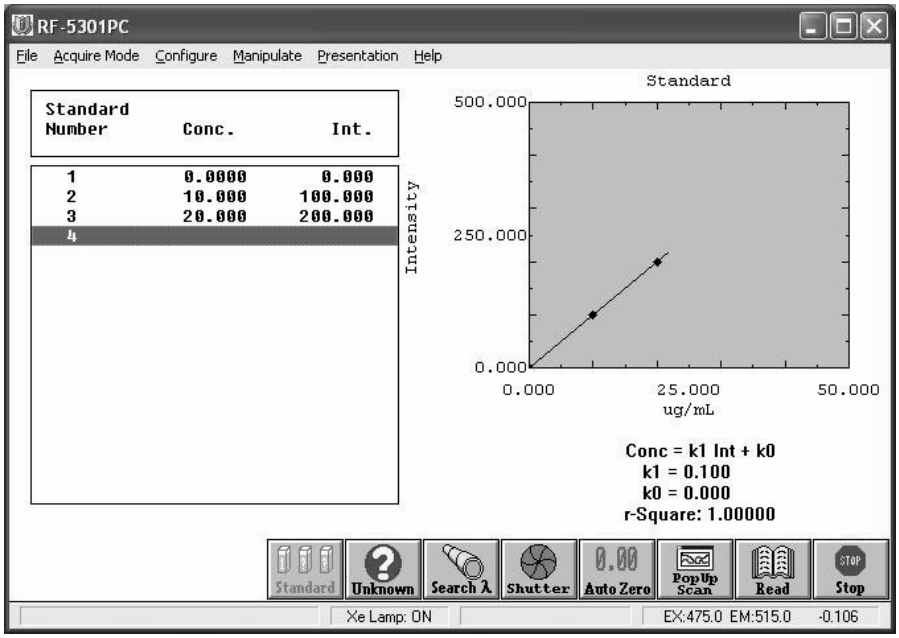


Fig. 7.19 Sample table

A reverse-highlighted item means that this item has been selected. To select an item, use the mouse or the keyboard cursor keys.

Press the Enter key or double-click with the mouse and the Data Edit dialog box will appear.

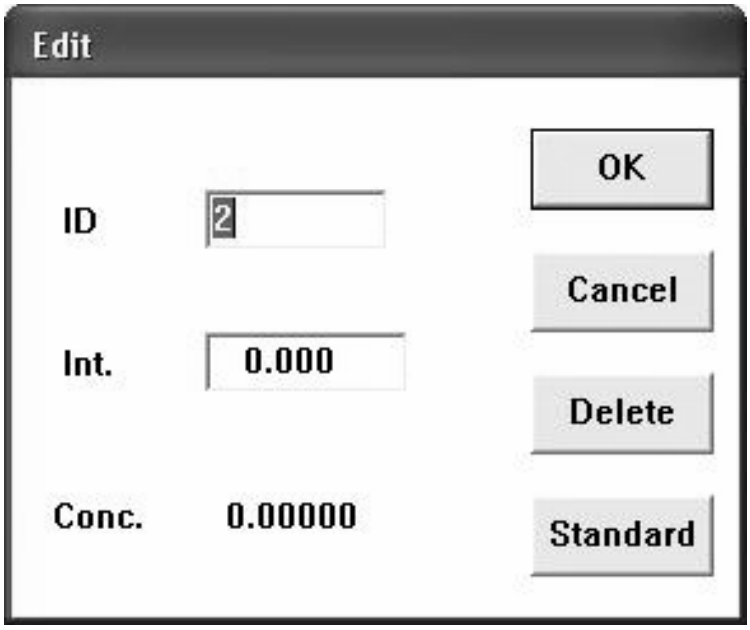


Fig. 7.20 Data Edit dialog box

The items within the Sample Data Edit dialog box are described below.
"ID" represents a sample ID (applicable only to unknown samples). By assigning the same ID to samples, the average fluorescence intensity for samples with the same ID can be determined later using the Average command (see Sec. 7.5.8).
"Concentration" (applicable only to a standard sample) means sample concentration. The concentration value for an unknown sample is automatically calculated.
"Intensity" stands for the fluorescence intensity of a sample. When the fluorescence intensity for an unknown sample is altered, the concentration value will automatically be recalculated.
Select the <Delete> button to delete a selected item.
Select the <Standard> button (applicable only to an unknown sample) to transfer a data item in the unknown sample table to a standard sample. Selecting this button will display a dialog box that prompts the user to enter the concentration value. Type in the concentration value, and click the <OK> button.
When all the entries have been correctly made, click the <OK> button. If the <Cancel> button is clicked, all entered data is cancelled, and the Data Edit dialog box will close.

(3) Time Course Parameters

In Time Course mode, if [Parameters] is selected, the Time Course Parameters dialog box will appear on the screen. The parameter items are described below.

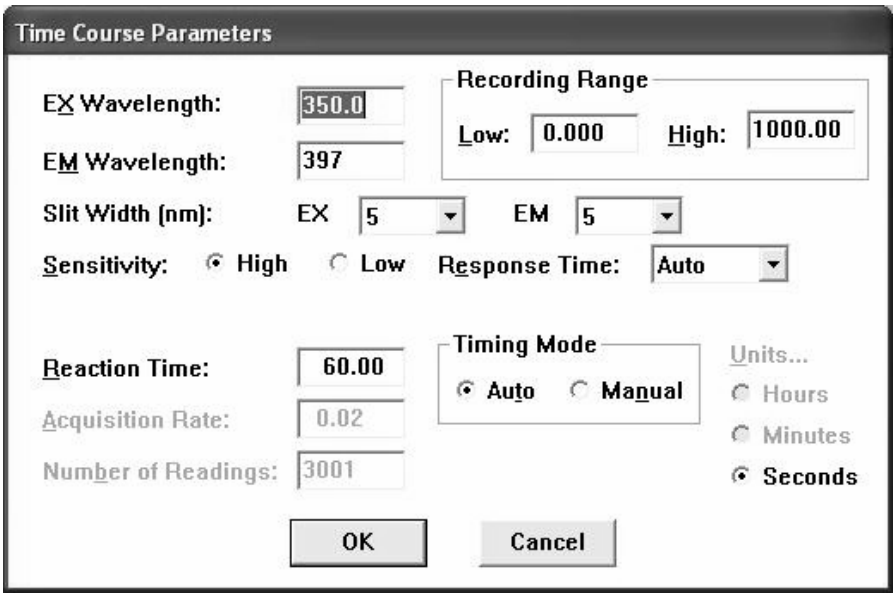


Fig. 7.21 Time Course Parameters dialog box

[EX Wavelength]

Enter the excitation wavelength for data acquisition. The allowable settings are 0, and 220 nm to 900 nm.

[EM Wavelength]

Enter the emission wavelength for data acquisition. The allowable settings are 0, and 220 nm to 900 nm.

[Recording Range]

These entries correspond to the ordinate scale's upper and lower limits in the resultant graph. The allowable range is -100.0 to 1000.0.

[Slit Width (EX)]

This is the setting for the excitation monochromator. The allowable settings are 1.5 nm, 3 nm, 5 nm, 10 nm, 15 nm and 20 nm, and 6 HH. The setting of "6 HH" means the half-height slit is used, for example, to analyze a solid sample (slit width of 6 nm).

[Slit Width (EM)]

This is the setting for the emission monochromator. The allowable settings are 1.5 nm, 3 nm, 5 nm, 10 nm, 15 nm and 20 nm, and "Close". When the Close setting is selected, light is not directed to the emission monochromator. Note, if the "PMT Protect" option from the Instrument Parameters dialog box (Sec. 7.4.5) is ON, this setting will be changed automatically to "Close" once the lid of the sample chamber is opened.

[Sensitivity]

Sensitivity can be set to either "High" or "Low". The High setting is about 50 times as sensitive as the Low setting.

[Response Time]

This corresponds to the response speed on the RF-5301PC unit relative to the variation in the fluorescence intensity of a particular sample. The less the setting (in seconds), the more quickly the instrument can follow variations in the fluorescence intensity although the resultant noise level will be greater. In contrast, the higher the setting, the less rapidly the instrument can follow the variation, but the noise level is lower. The allowable settings are 0.02, 0.03, 0.1, 0.25, 0.5, 2.0, 4.0, 8.0 and "Auto". The Auto setting allows the Response Time setting to be adjusted to approximately 1/4 that of the sampling interval setting.

[Timing Mode]

If "Auto" is selected, the user should enter a value for the total "Reaction Time". The "Units" should be specified to be in seconds. The values for "Acquisition Rate" and "Number of Readings" will be determined automatically. Channels whose units of time are other than seconds will be displayed after the time is converted into seconds.

If "Manual" is selected, the user should enter the time "Units", "Acquisition Rate" and "Number of Readings" (max. 12001 readings). The Reaction Time (Reaction Time = Acquisition Rate*Number of Readings) will be automatically calculated based on the values entered.

If an already loaded or newly loaded channel has different time units from the preset time units, the data is converted to the time units entered in this dialog box.

7.4.2 PopUp Scan(TM) Parameters

These parameters are for the PopUp Scan(TM) function, and are generally identical to those used in Spectrum mode, except that there are no parameters for Auto File and Repeat Scan. When PopUp Scan(TM) is executed, the current settings of acquisition parameters are temporarily stored, and the PopUp Scan(TM) parameters are sent to the instrument. Upon completion of the PopUp Scan(TM), the stored parameters will be restored.

7.4.3 PC Configuration

This option is for designating the default data and export directories (into which an ASCII-converted file, etc., are stored) and setting parameters for communications between the computer and the instrument and printers. When this option is selected, the dialog box illustrated in Fig. 7.22 will appear on the screen.

[Data Directory]

Enter the name of the directory into which data files are saved and from which data file are loaded, making sure that the directory name entered already exists. Be sure to strictly adhere to the MS-DOS protocol for assigning the directory (i.e. directory name must be preceded by a drive designation and end with "\," as in the case of C:\RFPC\DATA\).

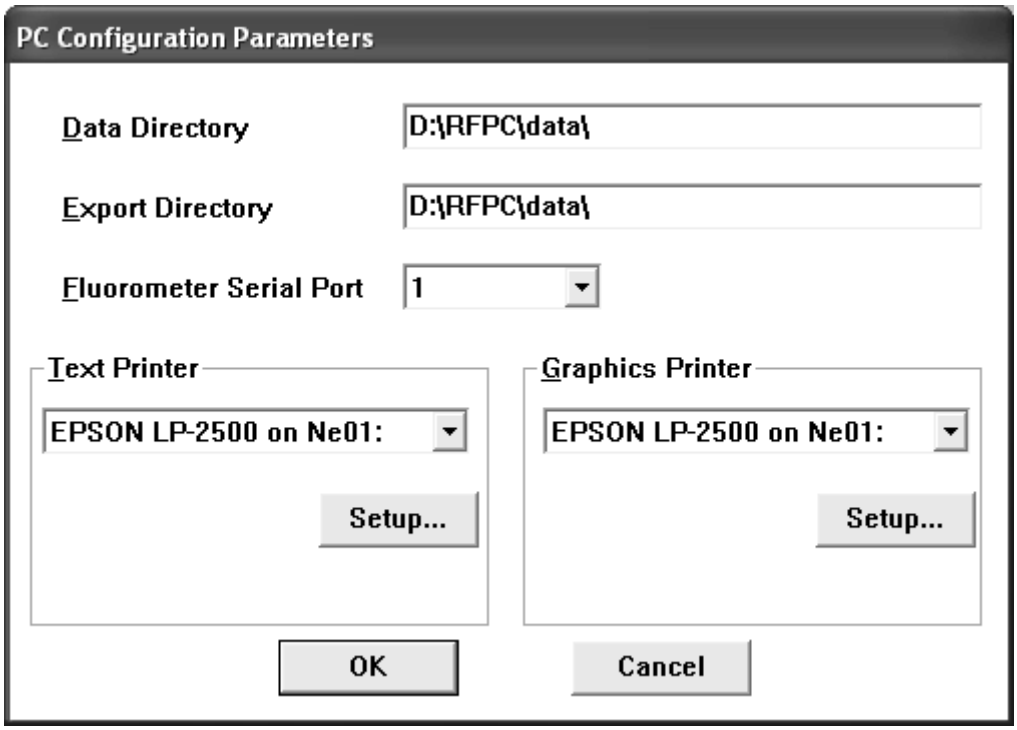


Fig. 7.22 PC Configuration Parameters dialog box

[Export Directory]

Type the name of the file into which to save export files (ASCII or DIF format), making sure that the directory name entered already exists. Be sure to strictly adhere to the MS-DOS protocol for assigning the directory (i.e. directory name must be preceded by a drive designation and end with "\," as in the case of C:\RFPC\EXPORT\).

NOTE

If you set the non-exist directory name to [Data Directory] or [Export Directory], you can not print data correctly with [Output] - [Graphics Plot] tool of [Peak Pick] or [Point Pick] mode.
Enter the name of the directory that has been designated at the installation of RFPC software to above two names of directory.

[Fluorometer Serial Port]

Select the serial port (1-4) on the computer to be assigned to the RF-5301PC (e.g. select 1 when connecting the instrument to COM1).

[Text/Graphics Printer]

Before the text and graphics printers can be selected through this dialog box, it is necessary to choose the printers in "Printer" from the Windows control panel. Select the printers to be used from the printer list within the list box. The selected Text Printer will be used for tabular output. The Graphics Printer will be used for graphics/table combinations. Select the Setup button (or use the control panel) to set up various printer features such as orientation and size of printer (or plotter) paper, and output resolution.

7.4.4 SpeedBox(R) Configuration

The SpeedBox(R) Configuration dialog box (Fig. 7.23) allows the user to select the appearance, functions and style of the buttons that constitute the SpeedBox(R). The commonly used commands can be assigned in advance to graphical buttons using the SpeedBox(R) Configuration dialog box, and then, any of these commands can be readily executed by simply clicking the corresponding button. To move the position of the SpeedBox(R) on the screen or to specify whether or not the SpeedBox(R) is displayed, select "Show SpeedBox(R)" from the "Presentation" menu.

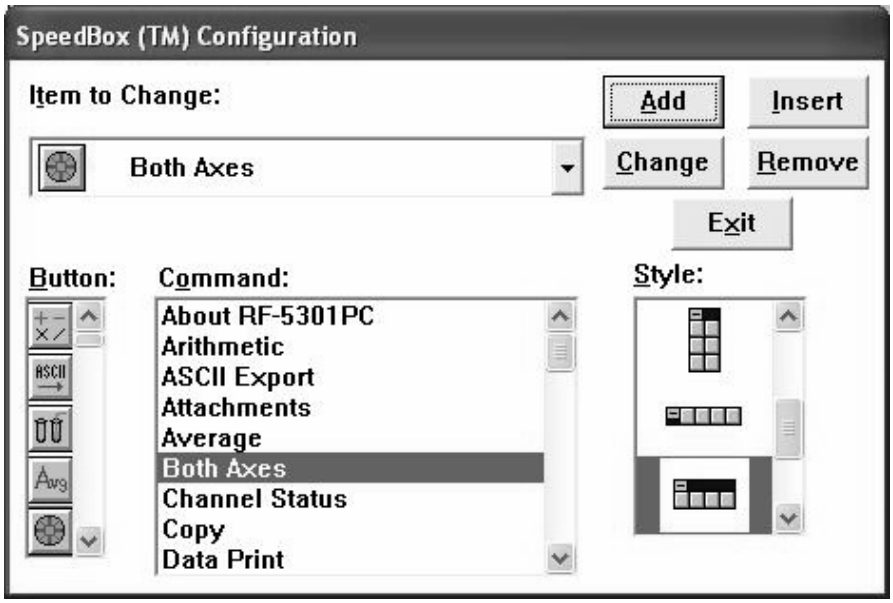


Fig. 7.23 SpeedBox(R) Configuration dialog box

[Item to Change]

This is a list box that includes all the buttons registered in the SpeedBox(R). The user can select one item from the list and alter or delete the item from the SpeedBox(R). When the list box is "closed," it indicates a button to be changed or deleted.

[Button]

This is a list of the graphical buttons. The user can select a button and an associated command and add or insert them into the SpeedBox(R).

[Command]

This is a list of the commands available in the program. When a command is selected together with a button, it is assigned to the button and can be added or inserted into the SpeedBox(R).

[Add]

This option should be used to add a new item to the SpeedBox(R). By selecting a Button together with its associated Command, and selecting <Add>, the new item can be added to the SpeedBox(R). Both a button and a command must be selected otherwise, no item will be added to the SpeedBox(R).

[Insert]

This option should be used to insert a new item into the SpeedBox(R). By selecting a Button together with an associated Command, and selecting <Insert>, the new item can be inserted after the currently selected item in the Item to Change list box. Both a button and a command must be selected otherwise, no item will be inserted in the SpeedBox(R).

[Change]

This button is used to change the Button or Command of a graphical button registered in the SpeedBox(R).

To change a registered item, follow the procedure below.

(1) Select the item to be changed from the Item to Change list box.

(2) Select both the Button and Command. It is possible to change either or both of the Button and Command. For example, when changing a Button only, select the Command corresponding to the item to be changed, and specify a new Button.

(3) Select the <Change> button. The selected item will be changed.

[Remove]

This option is used to remove an item from the SpeedBox(R). By selecting an item and pressing this button, the item will be removed from the SpeedBox(R). It is impossible to remove all the items.

[Exit]

Once all the changes are complete, press this button. The SpeedBox(R) configuration process will be complete. All the changes will be saved and the SpeedBox(R) will be updated accordingly. When the program is rebooted, the SpeedBox(R) will appear according to the stored settings.

[Style]

The "Style" list illustrates the graphical button layout of the SpeedBox(R). There are eight different styles available. The differences are in the orientation and number of rows of buttons (vertical or horizontal, and single or double row), and location of the title bar (top or side). To select a new style, click on a selection to highlight it.

7.4.5 Instrument

When [Instrument] is selected from the "Configure" menu, the Instrument Parameters dialog box will appear on the screen.

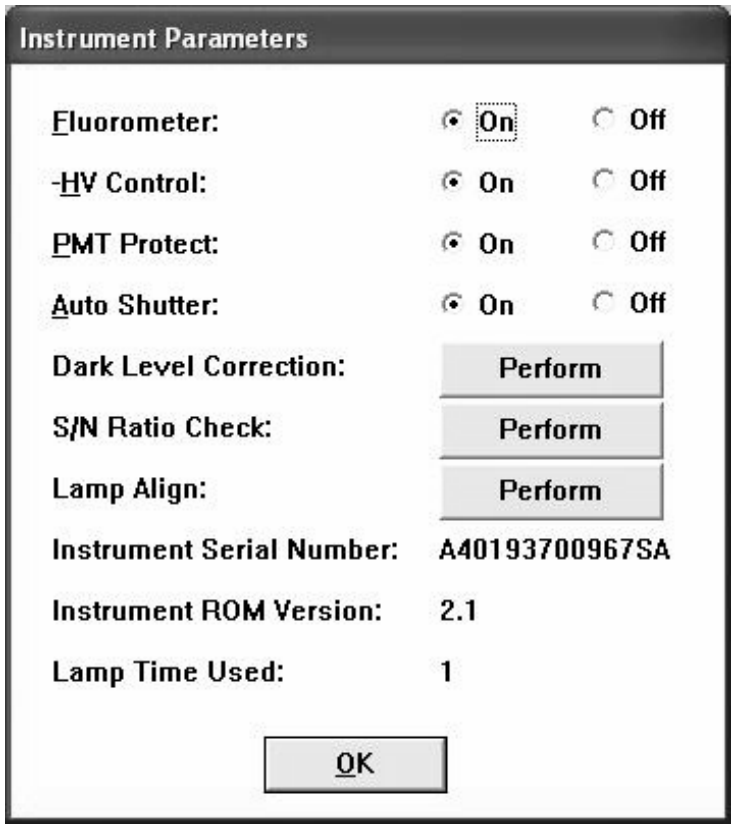


Fig. 7.24 Instrument Parameters dialog box

[Fluorometer]

This option turns ON/OFF the communications with the fluorometer.

[-HV Control]

If this choice is set to ON, the variation in fluorescence intensity owing to the variation in Xenon lamp power will automatically be compensated for. Usually, leave this setting ON. The compensation feature will automatically be turned OFF when the Xenon lamp is not lit.

[PMT Protect]

If this choice is set to ON, the slit of the emission monochromator will be closed automatically, when the lid of the sample compartment is opened to protect the photomultiplier tube against possible damage due to external light.

[Auto Shutter]

To protect a sample from decomposition or other chemical reactions, a sample should be free from irradiation by excitation light before actual analysis takes place. If this choice is set to ON, the shutter will automatically open only during an actual analysis run, to allow a sample to be irradiated with excitation light. If this choice is OFF, the user must manually open or close the shutter by selecting the shutter button at the bottom of the screen.

[Dark Level Correction]

The "dark current" refers to a signal current the photomultiplier outputs when it is not irradiated with light.

When the <Perform> button is selected, the shutter will be closed and "Auto Zero" will be executed, causing a zero point shift to correct for the dark current.

[S/N Ratio Test]

Use this feature to verify the performance of the instrument. Load a cell filled with distilled water into the cell holder and press the <Perform> button. Then, the S/N Ratio Test dialog box will appear on the screen.

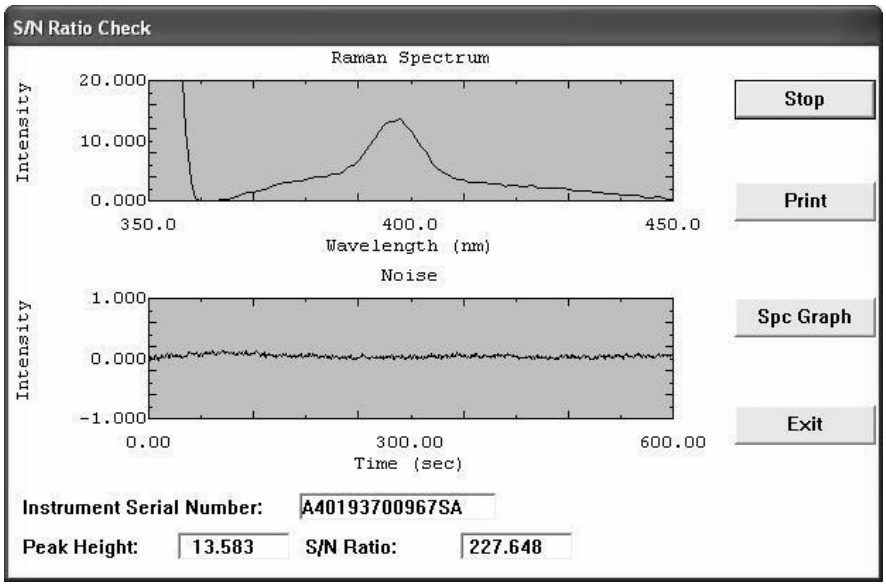


Fig. 7.25 S/N Ratio Test dialog box

Once the <OK> button is clicked, the Raman peak of the distilled water will be measured and the peak wavelength will be determined, and then the resultant fluorescence intensity at that wavelength will be displayed in the Peak Height box. The data acquisition parameters used are summarized next.

Spectrum Type:	Emission
EX Wavelength:	350 nm
EM Wavelength Range:	350 – 450 nm
Slit Width:	5 nm (for both excitation and emission)
Sampling Interval:	1 nm
Scan Speed:	Medium
Response Time:	Auto

Next, the wavelength for acquisition will be shifted to the Raman peak and will be "Auto Zero". Then, noise variation will be analyzed for ten minutes. Based on the obtained noise width, the S/N ratio will be calculated and displayed. The parameters for this operation are listed below.

EX Wavelength:	350 nm
EM Wavelength:	Wavelength at Raman peak
Slit Width:	5 nm (for both excitation and emission)
Response Time:	2.0 sec.
Sampling Interval:	1.0 sec.
Reaction Time:	10 min

To interrupt the data acquisition run, click the <Stop> button. In this event, the S/N ratio will not be determined.
Select the <Print> button to print out the test results.
When the right button of the mouse is clicked on the Raman spectrum graph or time course noise graph, the Right Button menu (Sec. 7.8) will appear on the screen, allowing the user to change the graph presentation style. Also, it is possible to display the Right Button menu by clicking on <SPC Graph (G)> or <TMC Graph (G)>.

After clicking the <SPC Graph (G)> button, the user will be able to modify a spectrum graph, after selecting the <TMC Graph (G)> button, a time course graph. The button indication is a toggle type, and clicking with the button of the mouse will alternate between displaying "SPC Graph (G)" "TMC Graph (G)".

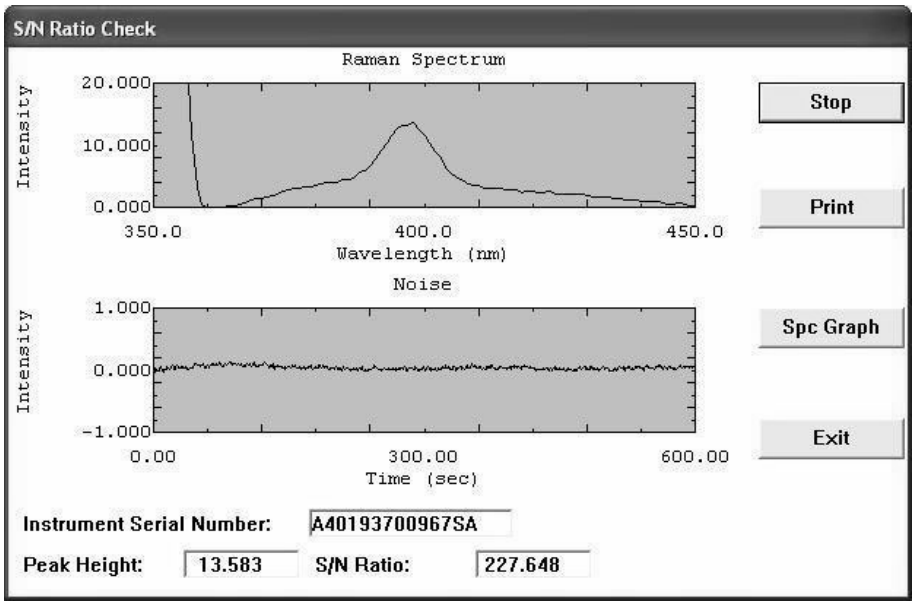


Fig. 7.26 SN Ratio Test dialog box (after acquisition)

[Lamp Align]

This menu should be used after replacing the Xenon lamp. First, load a cell filled with distilled water into the cell holder and click the <Perform> button. The Lamp Alignment dialog box will appear on the screen. Be sure to adjust the light source mirror so that the value in this window becomes a maximum (for details, see Sec. 2.6.2). Also, after replacing the lamp, use this dialog box to zero the "Lamp Time Used".

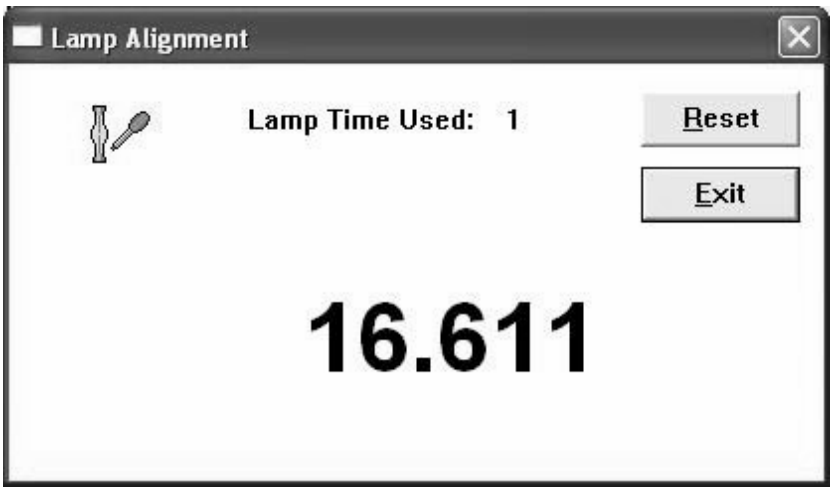


Fig. 7.27 Lamp Align dialog box

The Serial No. box indicates the serial No. of the user's instrument.
The ROM Version box shows the ROM version for the user's instrument.
The Lamp Time Used tells the user the total number of hours that the present Xenon lamp has been used. Be sure to replace the lamp if it has operated for 500 hours or longer (see Sec. 2.4 through Sec. 2.9).

7.4.6 Attachments

When the button on the sipper is pressed, the sipper automatically undergoes the sequence of sample sipping, data acquisition, sample purging, and rinsing.
This parameters menu is used for making settings of sipper-related parameters and executing sample sipping operations.

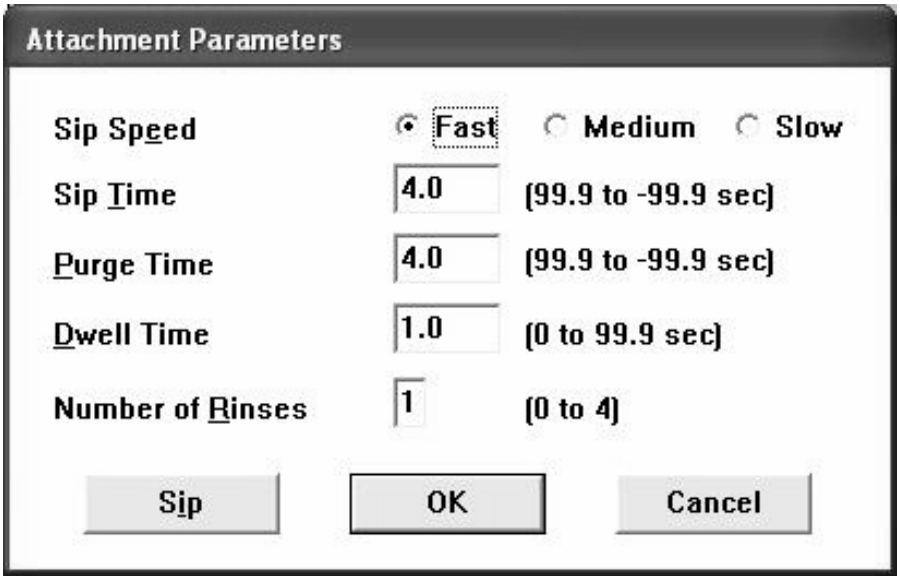


Fig. 7.28 Attachments Parameters dialog box

"Sip Speed" is used to specify the operating speed of the sipper. The allowable choices are Fast, Medium and Slow.
"Sip Time" specifies how long the sipper pump must run. The allowable entry range is -99.0 sec. to 99.0 sec. Enter a negative value to reverse pump rotation.
"Purge Time" is for selecting the duration of purging for a sample. The allowable entry range is -99.0 sec. to 99.0 sec. Enter a negative value to recover the sample.
"Dwell Time" should be specified from the range 0 sec. to 99.9 sec.
The Number of Rinses entry determines how many times (1 to 4 times) the sipper should be rinsed after purging the sipper of a sample.
A sample is drawn by the sipper as long as the <Sip> button is held down.

7.4.7 Save Parameters

This command saves all the current parameters (Spectrum, Quantitative and Time Course) into a configuration file. When this option is selected a dialog box, as illustrated in Fig. 7.29, will appear on the screen.

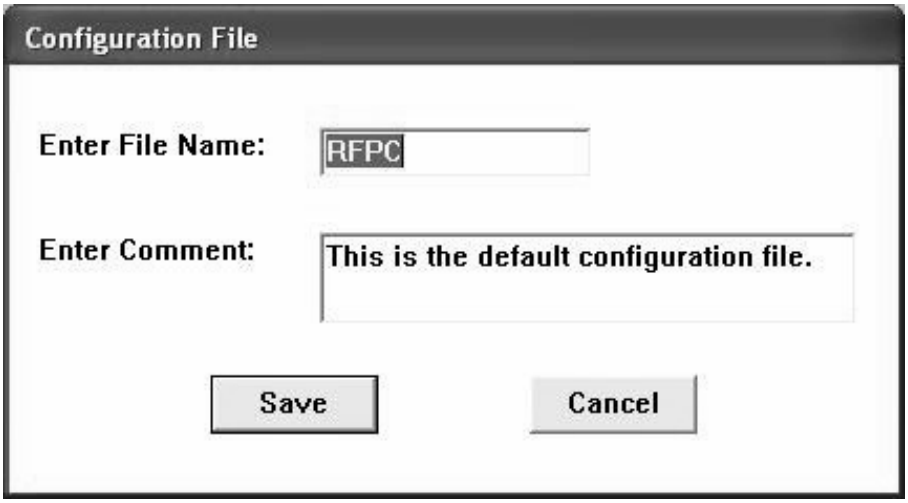


Fig. 7.29 Save Parameters dialog box

Type a file name (no extension) of up to eight characters. The user can also enter a comment into the comment text box.
Click the <Save> button to save the current parameters into the directory where the system program was installed. The file will have a ".CFG" extension. To change the program default configuration, select the file "RFPC.CFG".
"RFPC.CFG" will be loaded when the RF-5301PC software is launched, and new configuration is automatically set up. Note the current data acquisition mode remains same as that was effective when the program was terminated most recently.

7.4.8 Load Parameters

This option should be used to load a configuration file that has been saved using the Save Parameters command. When selected, a list box, as shown in Fig. 7.30 containing all the configuration files will appear on the screen.

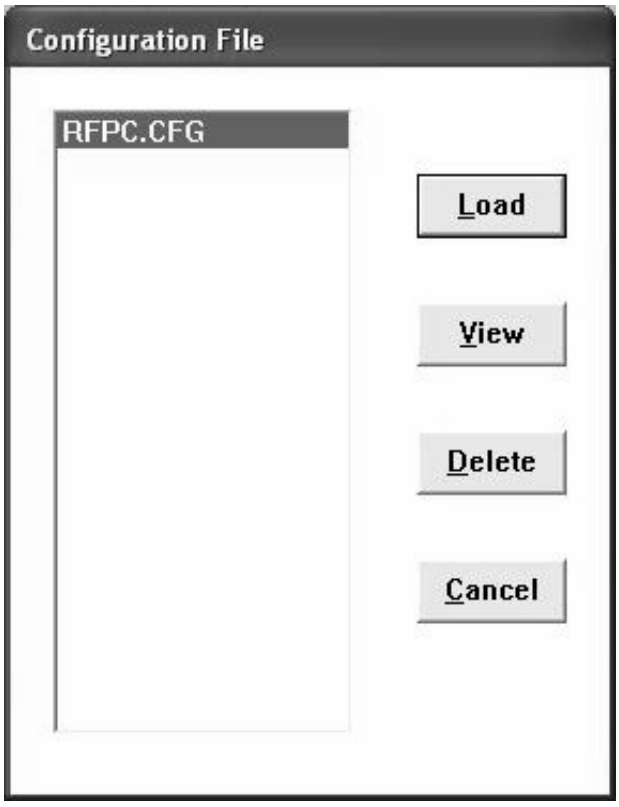


Fig. 7.30 Load Parameters dialog box

Move the cursor to an intended file name in the list box, and select the Load button. The current parameters will change to those contained in the selected file. The data acquisition mode will remain unchanged.
Selecting the <View> button will display a window that shows the file name, user's comment, and date and time of file creation.
When the <Delete> button is selected, a warning message will appear indicating that the user is about to delete the file which is highlighted. If <Yes> is selected in response to the inquiry "ARE YOU SURE?," the file will be deleted from the directory. Select <NO> to cancel the command. The "RFPC.CFG" is the default configuration file and cannot be deleted.

7.5 Manipulate Menu

The Manipulate menu contains commands which allow various manipulation schemes for acquired data. The "Arithmetic," "Transforms" and "File/Chnl Calc" options arithmetically process acquired data. To be able to execute any of these arithmetical operations in Spectrum or Time Course mode, the user must first specify the source channel, the intended operation, and the destination channel. The source is the channel number that stores the data to be subjected to the arithmetic operation. The destination is the channel number that stores the result of the operation. If the designated destination already contains data, the data in the channel will be overwritten (there will be a warning, and the user can either cancel or proceed). If an empty channel is selected, the user can enter the file name and comment as usual.
In Quantitative mode, the currently displayed data (standard or unknown) is used for the arithmetic operation. That is, the data in the source is identical to that of the destination, and the data in the source will be overwritten by the result of arithmetic operation.
Upon completion of the arithmetic operation, the result will be automatically plotted into a graph. However, the user must select the upper and lower limits of the graph so that the whole graph can appear within the screen (see Sec. 7.6.2.7 "Radar (Auto Scale)").

NOTE
The commands "Arithmetic," "Transforms," "File/Chnl Calc" and "Average" can replace the currently displayed data with the result of the operation (the data in Quantitative mode will be unconditionally overwritten). Therefore, before executing any of these commands, it will be necessary to save the data in the source channel to another file by using the Save As command.

7.5.1 Arithmetic

The arithmetic operations refer to scalar operations (+, -, *, /) that are based on the expression "S op C = D", wherein S is the source channel, C is a constant, D is the destination channel and "op" is an operator (in Quantitative mode, S=D, that is, the source is replaced with the result). These arithmetic operations are often used to obtain a relevant data presentation scheme (offset a spectrum, scale a derivative, etc.). When the Arithmetic command is selected in Spectrum or Time Course mode, the following dialog box will appear on the screen.

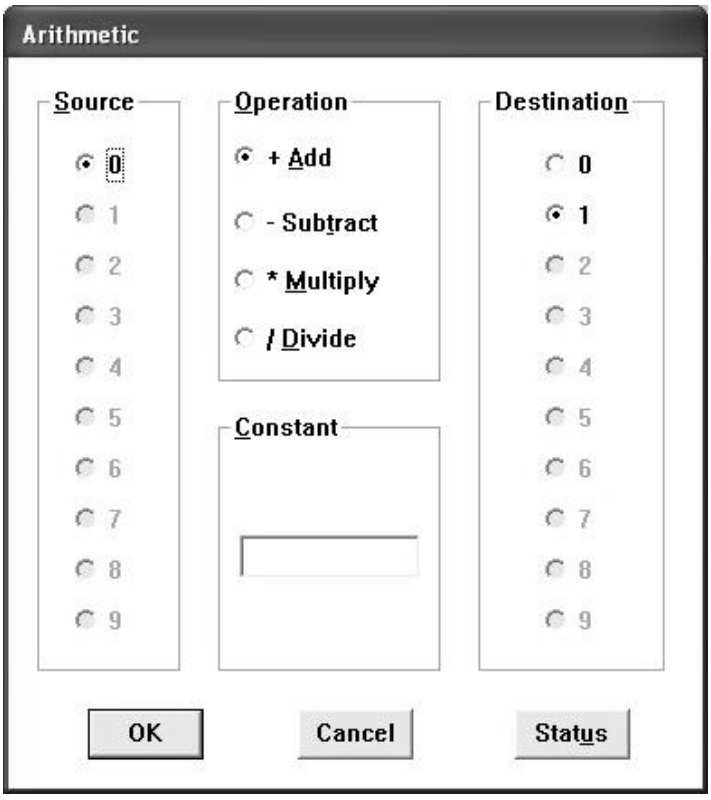


Fig. 7.31 Arithmetic dialog box (Spectrum and Time Course modes)

Select the intended operation, source and destination, and enter the constant and click the <OK> button. The calculation will be executed. Click the <Cancel> button to abort the operation.
Click the <Status> button to review the contents of the channels.
When the Arithmetic command is selected in Quantitative mode, the following dialog box will appear on the screen.

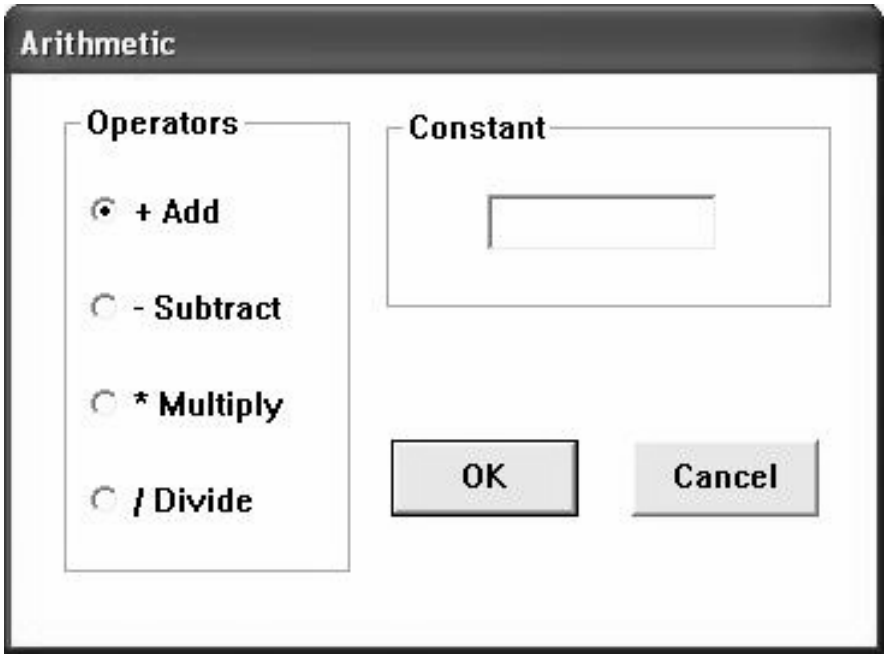


Fig. 7.32 Arithmetic dialog box (Quantitative mode)

NOTE

The data in the currently displayed data table (standard and unknown) will be replaced with the results of the operation. Therefore, before executing any of these operations, it will be necessary to save the data in the source channel as a file by using the Save As command. When operating with unknown data, the concentration values will be recalculated using the newly obtained fluorescence intensity values.

7.5.2 Transforms

The "Transforms" operations are function operations based on the expression "D = f(S)", wherein S is the source channel, D is the destination channel and "f" is a transform function. (In Quantitative mode, S = D, that is, the source data is replaced with the results of the transform.)
The transform functions available in Spectrum and Time Course modes are as follows:

Derivative	(0th through 4th orders, where 0 is smoothing)
Reciprocal	(1/Y)
Common logarithm	

The transform functions available in Quantitative mode are as follows:

Reciprocal	(1/Y)
Common logarithm	

When the Transforms option is selected in Spectrum or Time Course mode, the following dialog box will appear on the screen.

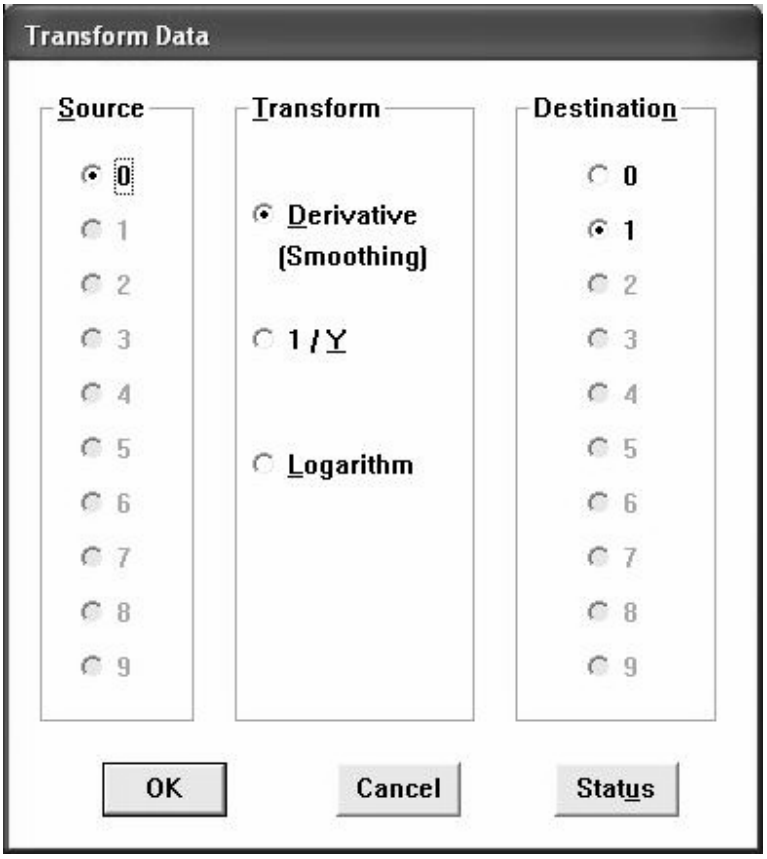


Fig. 7.33 Transforms dialog box (Spectrum and Time Course modes)

Select the source and destination, and specify the transform function and click the <OK> button. The calculation will be run. Click the <Cancel> button to abort the operation. Note that selecting <Status> allows the user to check the contents of the channel.

When the "Derivative" (smoothing) is selected, the Derivative dialog box (Fig. 7.34) will appear on the screen. Specify the order of the derivative and the size of the X value (dX) ("T" for Time Course mode, and "Lambda" for Spectrum mode).

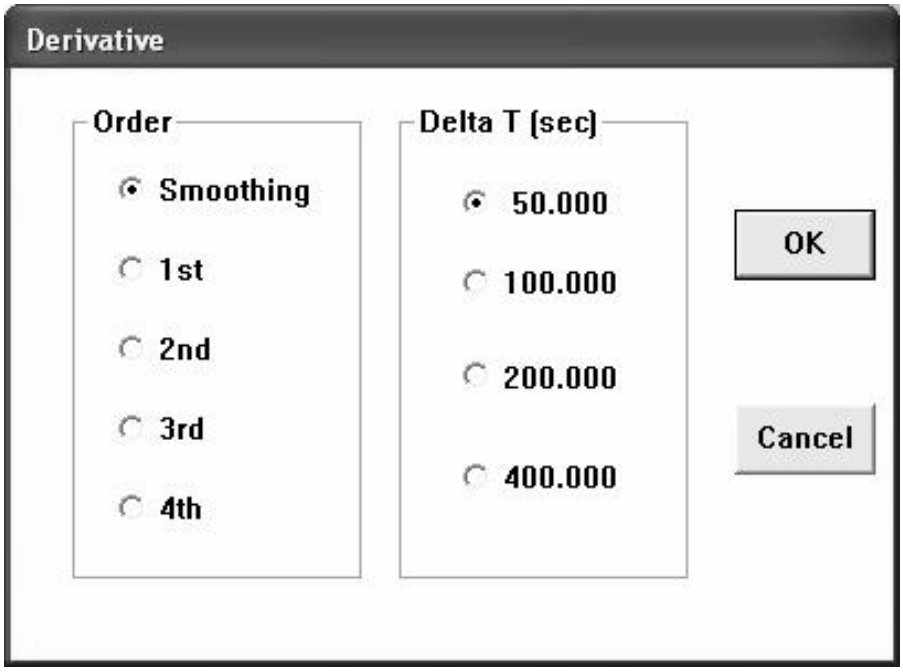


Fig. 7.34 Derivative dialog box

The smoothing and differentiation calculation is based on a convolution function using 17 data points. The size of the range (dX) subjected to the differentiation calculation is selectable in four steps. A large dX (delta X) will provide smaller noise at the cost of decreased spectrum resolution. Therefore, select values that will provide an optimum noise-resolution compromise.
The calculation of the derivative value requires the data points before and after the center wavelength. In other words, a differentiated or smoothed spectrum cannot be calculated at the two ends of the wavelength range. The original spectrum has units of intensity as its ordinate value. The derivative units are defined as follows:

Derivative	Spectrum	Time Course
1st derivative	Intensity/nm	Intensity/time
2nd derivative	Intensity/nm2	Intensity/time2
3rd derivative	Intensity/nm3	Intensity/time3
4th derivative	Intensity/nm4	Intensity/time4

When the Transform function is selected in Quantitative mode, the dialog box illustrated in Fig. 7.35 will appear on the screen.

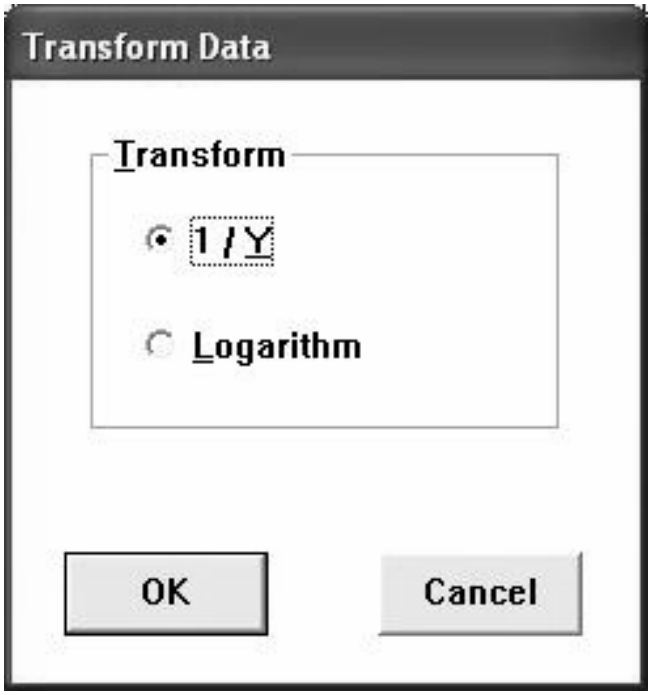


Fig. 7.35 Transforms dialog box (Quantitative mode)

The data in the currently displayed data table (standard and unknown) will be replaced with the result of the operation. Therefore, before executing any of these operations, it will be necessary to save the data in the source channel as a file by using the Save As command.
When operating with unknown data, the concentration values will be recalculated using the newly obtained fluorescence intensity values.
Select the desired transform function, and click the <OK> button to run the calculation. Click the <Cancel> button to abort the operation and exit the dialog box.

7.5.3 File/Chnl Calc

When the "File/Chnl Calc" command is selected in Spectrum or Time Course mode, the dialog box shown in Fig. 7.36 will appear on the screen.
The "File/Chnl Calculation" refers to vector operations (+, -,*, /) that are based on the expression "A op B = C," wherein A and B are each a source channel number, C represents the destination channel number (result) and "op" is an operator.
Note that B and C cannot be the same channel number. The range (wavelength or time) or pitch (sampling interval) of channels A and B need not be the same since any necessary interpolation will be performed using a cubic spline algorithm. The result (channel C) will have the same acquisition parameters as the original source channel (channel A).
After designating A, B and C, and the particular operation, click the <OK> button to run the calculation operation. Click the <Cancel> button to abort the operation and exit the dialog box. Click the <Status> button to review the contents of the channels.

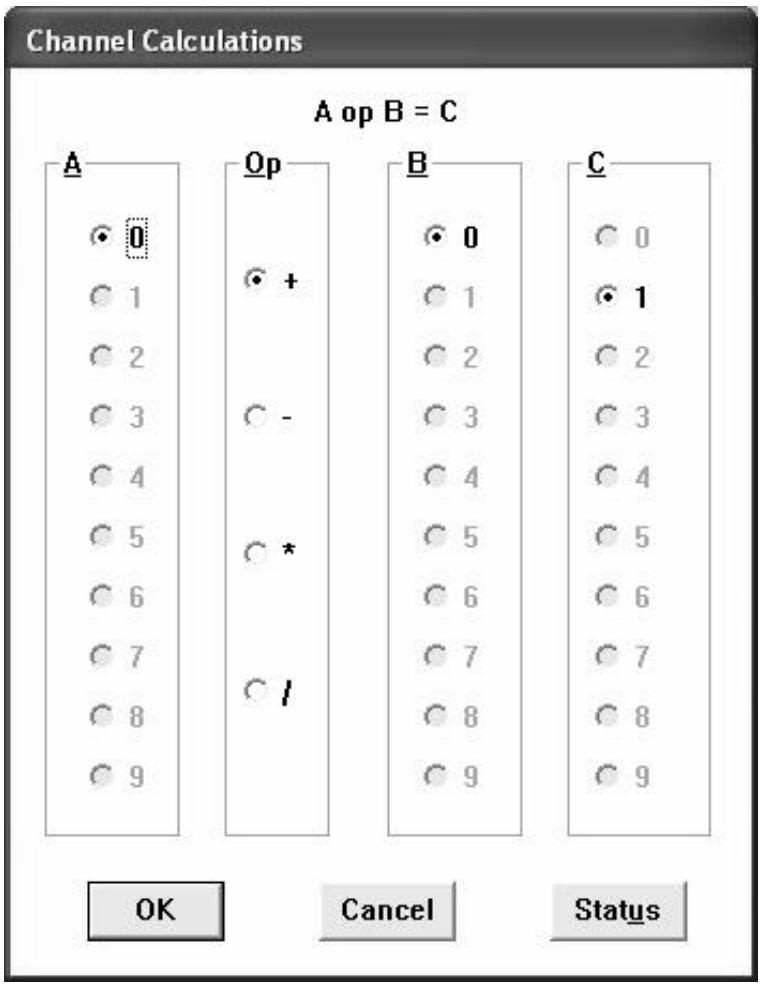


Fig. 7.36 File/Chnl Calc dialog box (Spectrum and Time Course modes)

When the File/Chnl Calc command is selected in Quantitative mode, the dialog box shown in Fig. 7.37 will appear on the screen.
The Arithmetic operation will involve both the currently displayed data table and the selected file. Select the file name and the operation type, and click the <OK> button to run the operation, or select the <Cancel> button to cancel the operation. The calculation will be performed with the table and the fluorescence intensity in the designated file (illustrated in the same line within the box). They will not change.

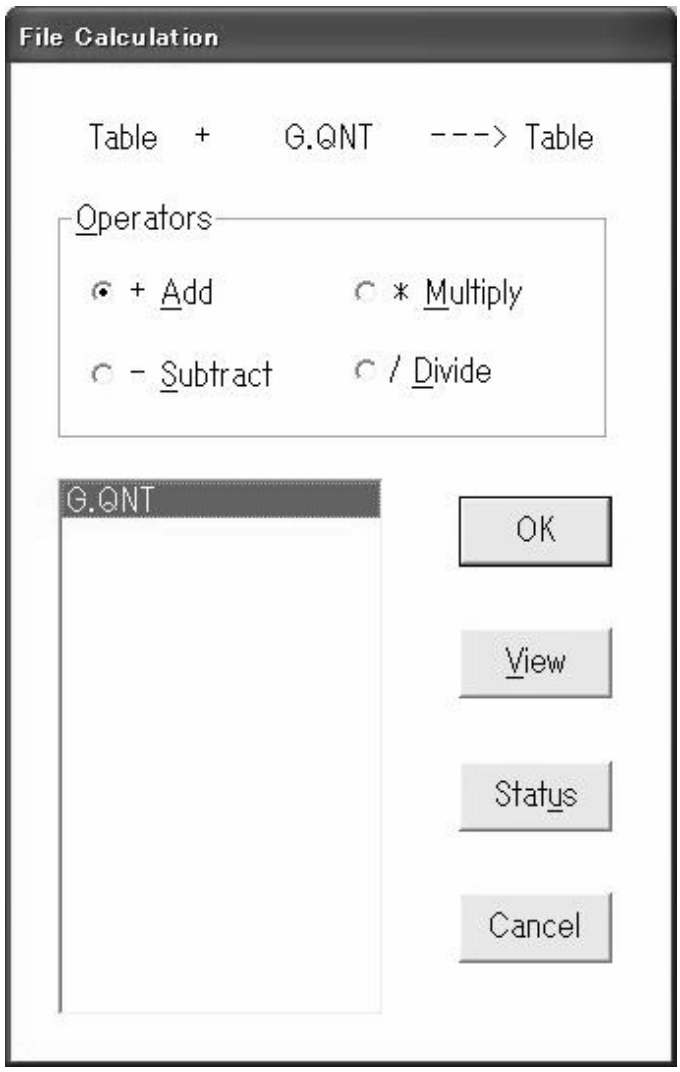


Fig. 7.37 File/Chnl Calc dialog box (Quantitative mode)

The data in the currently displayed data table (standard and unknown) will be replaced with the result of the operation. Therefore, before executing any of these operations, it will be necessary to save the data in a source channel as a file by using the Save As command. When operating with standard data, the equation for the working curve will be recalculated using the newly obtained data table. When operating with unknown data, the concentration values will be recalculated using the newly obtained fluorescence intensity values.

7.5.4 Data Print

The Data Print command will output the data on the selected channel to a window as values. When the Data Print command is selected, the channel selection dialog box will first appear. Select a channel whose data is to be displayed. The window illustrated in Fig. 7.38 will appear on the screen. This window is scrollable and movable and can be resized. The menu items in this window are described below. Note that more than one page of the Data Print window can be displayed for each channel.

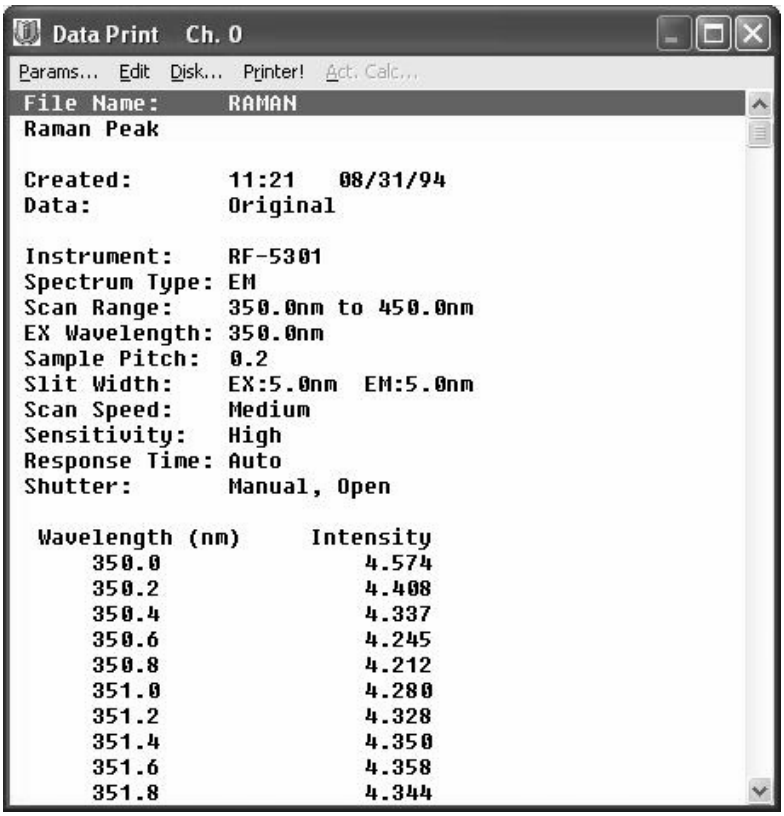


Fig. 7.38 Data Print window

When executing "Data Print" in quantitative mode, the standard and unknown data are printed out together with associated parameters. If two or more samples have the same ID, the average of these samples is also printed at the same time.
To close the Data Print window, select the control menu box at the top left corner of the window, and click the Close command, or double-click the control menu box itself.

[Params] (Spectrum and Time Course modes)

When the "Params" command is selected, the Data Print Parameters dialog box, shown in Fig. 7.39, will appear on the screen. Enter the "Start X Value" and "End X Value" as well as the "Skip Factor". If the <OK> button is clicked, the table will be updated with the new values. For time course data, the range entered in the Data Print Parameters dialog box will be used for the calculation of activity.

NOTE

The "Skip Factor" entered must be greater than the current Sampling Interval (see Sec. 7.4.1 "Parameters"). A value lower than the sampling interval setting will be ignored even if it is entered.

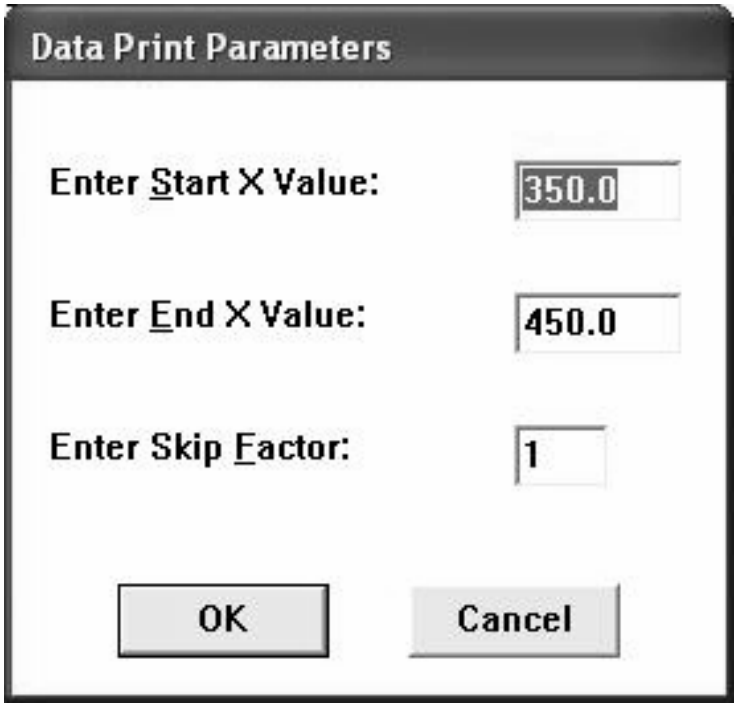


Fig. 7.39 Data Print Parameters dialog box

[Edit]

This command copies a designated part (Copy command) or all (Copy All command) of the window to the clipboard.

[Copy]

Specify the part of the window to be copied by dragging the mouse, and copy that area to the clipboard. The selected area will be reverse-highlighted.

[Copy All]

This option copies all the contents of the window to the clipboard.

[Disk]

When this command is selected, the following dialog box will appear on the screen.
Type in the intended file name and select the <OK> button. The data list currently in the window will be saved in the data directory with the designated file name. The file will have the extension ".TXT". The saved file can later be printed using the Plot option from the Presentation menu, or edited using a text editor.

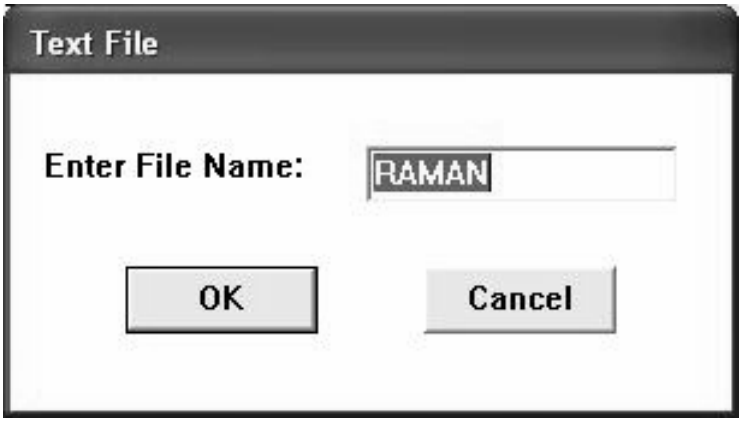


Fig. 7.40 Text File dialog box

[Printer]

To output the data list to the printer, select this menu item. A dialog will appear during a print run, allowing cancellation of the print job.

The units of time in Time Course mode are the same as those selected in the Time Course Parameters dialog box.

[Act. Calc.] (Time Course mode only)

To calculate the activity value, select this menu option. Define the range for the calculation using the Params menu. When the "Act. Calc." option is selected, the following dialog box will appear on the screen.

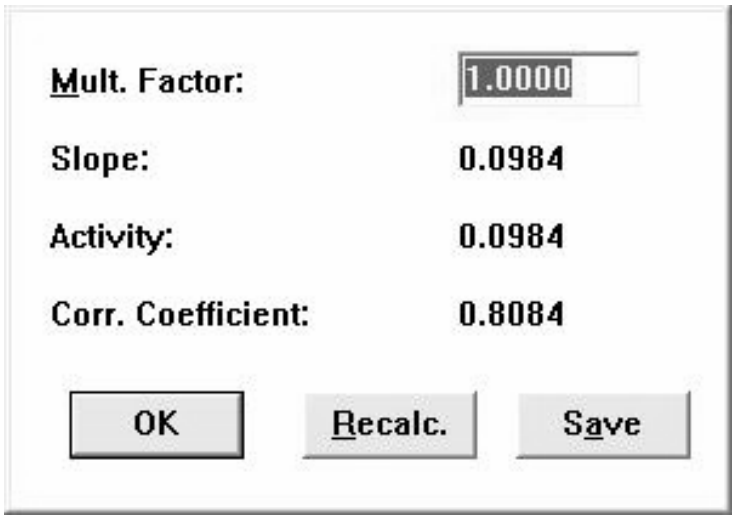


Fig. 7.41 Activity Calculation dialog box

"Mult. Factor" is a factor used to calculate the activity value. It is employed to correlate the obtained "slope" with the "activity" value of the actual enzyme reaction.
The Slope represents the gradient of the approximated straight line in the designated range.
The Activity is a normal activity and defined as [Activity = Slope * Mult. Factor].
The "Corr. Coefficient" means the correlation coefficient of the approximated straight line used in the Slope calculation.
If the "Mult. Factor" and/or the range for calculation were changed, select the <Recalc.> button to cause the "Activity" to be recalculated.
To save the result into the corresponding file, select <Save>. A dialog box will appear on the screen. Then, type in the file name and click the <OK> button. The file will have the extension ".TXT".

7.5.5 Peak Pick (Spectrum/Time Course mode)

Select this command to display the peak list for the selected channels in a window. When [Peak Pick] is selected, a dialog box will appear, allowing the user to select the intended channels. Select the channels for Peak Pick, and click the <OK> button.
The Peak Pick window provides, for every peak, its number (No.), an abscissa value (wavelength or time) and an ordinate value (fluorescence intensity). The peak will be indicated on the graph of the selected channel.

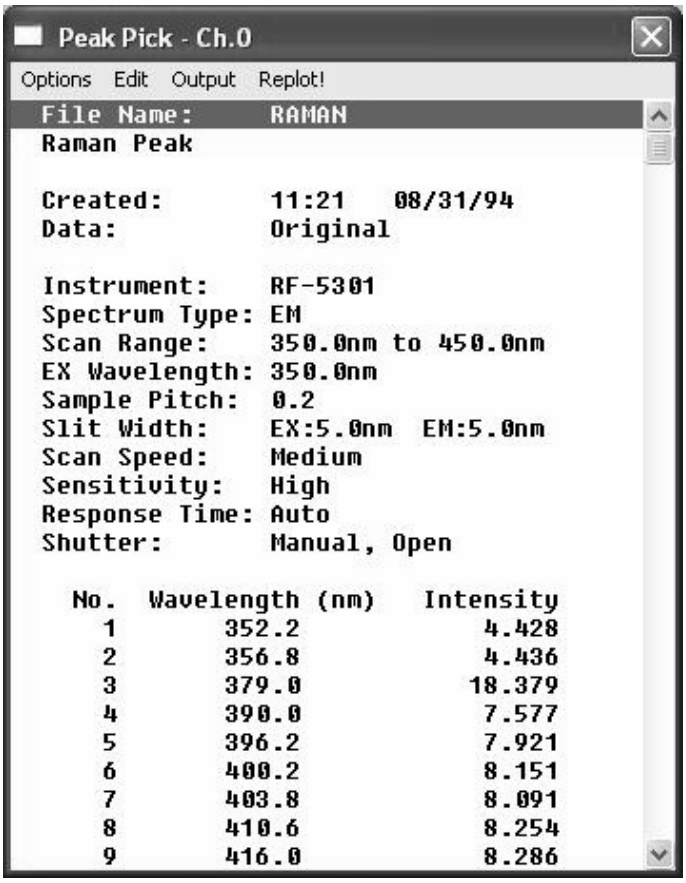


Fig. 7.42 Peak Pick window

Note that more than one page of the Peak Pick window can be displayed for each channel. This window is scrollable and movable, and can be sized to allow the user to view the data under the most convenient conditions.
To close the Peak Pick window, select the control menu box at the top left corner of the window, and click the Close command, or double-click the control menu box itself.
The menu items in the Peak Pick window are described below.

[Options]

[Find Valleys]

Selecting the "Find Valleys" option will cause a check mark to appear next to the [Find Valleys] option and the valley value list to appear in the window. Selecting the [Find Valleys] option again will cause the check mark to disappear and the peak list to reappear in the window.

[Change Threshold]

For the definition of the "threshold", see the description in "About Peak Pick Algorithm" below. Generally, the greater the threshold is, the less the number of peaks detected, the lower the threshold is, the more peaks are detected.

[Mark Graph]

When Peak Pick is executed, the graph will be replotted and each peak will be marked with a number. After the upper and lower limits are changed (Sec. 7.6.2.4 "Limits", and Sec. 7.6.2.7 "Radar (Auto Scale)") and the graph is replotted, all the marks (peaks, valleys and point pick marks) will be erased. Selecting the "Mark Graph" option will cause the peak (or valley) marks to reappear.

About Peak Pick Algorithm

A four-point successive comparison method is used to define a peak as at least four consecutive increases in value followed by at least four consecutive decreases in data. The top of the peak is the point before the decreases commence. In "Find Valley", the situation is reversed (four or more consecutive decreases, bottom, four consecutive increases).

The threshold is defined as the distance between a peak point and a line connecting the two adjacent valley points (or vice versa for valleys). If the threshold value of the peak is less than the threshold value entry (0.001 is the default), the peak is rejected. The threshold value can be adjusted to filter out unwanted peaks or noise.

The error resulting from this peak detection algorithm is estimated as 1/10th to 1/100th of the peak width at half the height of the peak. There may be possibility of slight error in detecting very sharp peaks. In such cases, it will be necessary to move the cursor to the intended point and directly read the value, or, use the Data Print function.

[Edit]

This command copies a designated part ("Copy" command) or all ("Copy All" command) of the window to the clipboard.

[Copy]

Define the part of the window to be copied with the mouse pointer, and copy that area to the clipboard. The selected area will be reverse-highlighted.

[Copy All]

This command copies all the contents of the window to the clipboard.

[Output]

The Output menu offers the following choices for outputting the Peak Pick data.

[Print Table]

When [Print Table] is selected, the Peak Pick data will be printed in a tabular form by the text printer.

[Save Table]

This option creates a tabular text file (with ".TXT" extension) and saves it in the data directory on disk. After selecting the Save Table command, enter a file name (without extension) in the "Save .TXT File" dialog box. The file that is created may be printed using the Plot command from the Presentation menu (see Sec. 7.6.5 and Sec. 7.6.6) or edited on a word processor or text editing software.

[Graphics Plot]

Selecting the Graphics Plot command will cause the data of a selected channel to be plotted graphically by the selected graphics printer. The plot will contain a list of the peaks as well as the acquisition parameters.

[Replot]

Select the Replot command to redraw the graph and clear all the marks (peaks, valleys and point pick marks).

7.5.6 Point Pick

The Point Pick option allows X-axis designation of up to 15 data points to be listed in a window as numerical values. These points are also marked on the graph.
Selecting this option will cause a channel selection dialog box to appear on the screen. Select the channel number for the Point Pick operation, and click the <OK> button. The following Point Pick dialog box will appear.

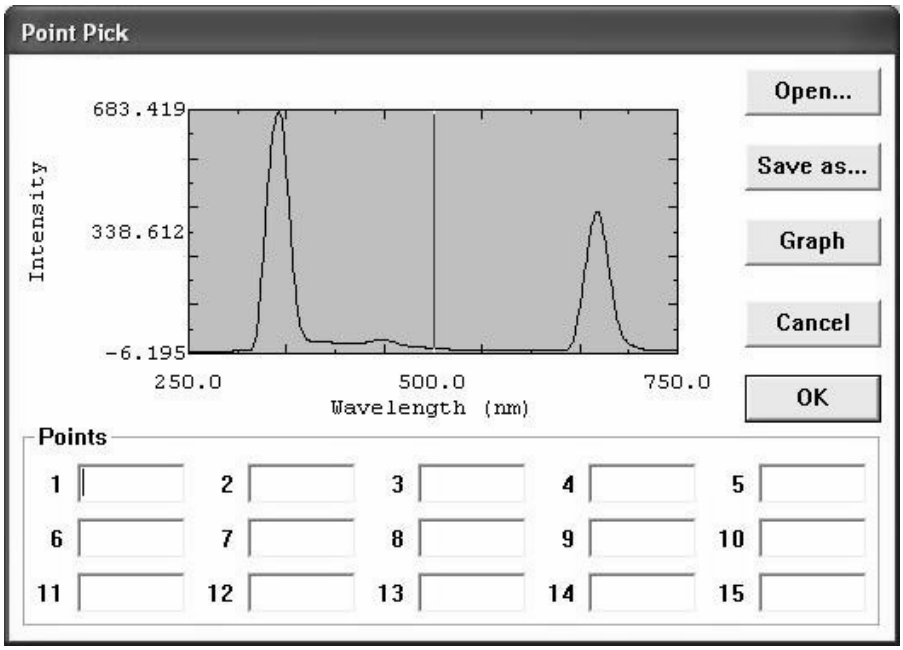


Fig. 7.43 Point Pick dialog box

The upper portion of this dialog box contains the graph of the selected channel. The lower portion of the dialog box provides text boxes for entering X-axis values.
To enter the X-axis values for the Point Pick operation, directly type the values into the text boxes or move the cursor around the graph. Moving the mouse cursor around the graph will cause the X-axis values at the cursor position to be entered to the text boxes.
The <Open> button will load the file that contains the list of the already saved X-axis values (with extension ".PTS" for Spectrum or ".PTT" for Time Course).
The <Save as> button will save the entered X-axis values. Type in the intended file name and save the values. The file will have the extension ".PTS" for Spectrum or ".PTT" for Time Course.
Selecting the <Graph> button will open a menu similar to the "Right Button" menu, allowing the user to change the graphic presentation parameters (see Sec. 7.8).
Clicking the <OK> button will cause the entered X-axis values to be stored and sorted in ascending order, and the Point Pick window illustrated in Fig. 7.44 will appear and show the Point Pick list. This window is scrollable and movable, and can be sized in the same manner as the Peak Pick window.

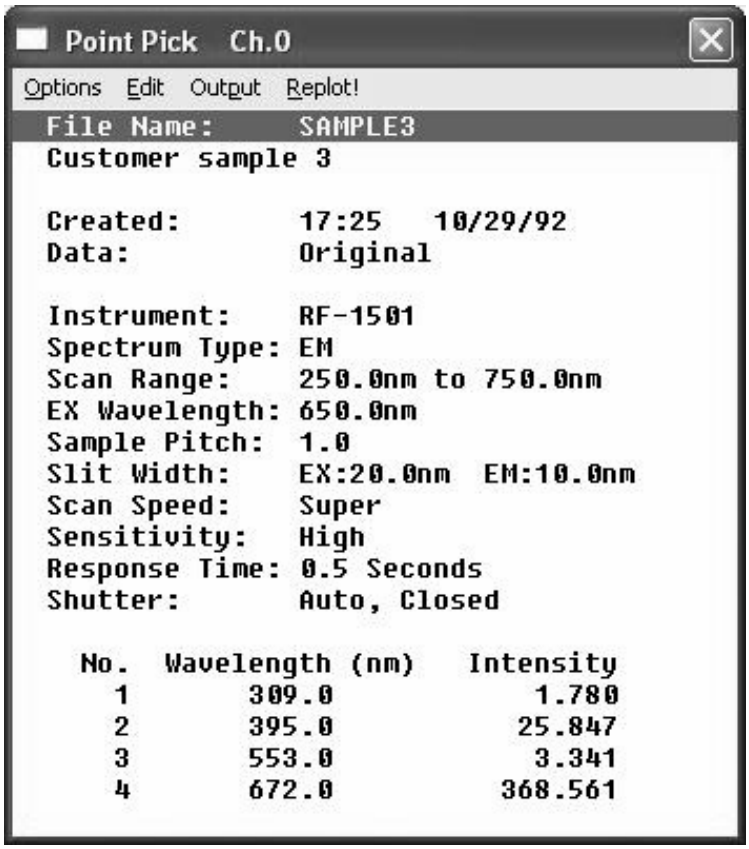


Fig. 7.44 Point Pick window

The menu items in the Point Pick window are described below.

[Options]

[Change Values]

Selecting the [Change Values] option will allow the user to reenter the X-axis values. When this menu item is selected, the Point Pick dialog box will reappear.

[Mark Graph]

When "Peak Pick" is executed, each peak will be marked with a number. After the upper and lower limits are changed and the graph is replotted, all the marks (peaks, valleys and point pick marks) will be erased. Selecting [Mark Graph] will cause the point marks to reappear.

[Edit]

This command copies a specified part ("Copy" command) or all ("Copy All" command) of the window to the clipboard.

[Copy]

Define the area of the window to be copied with the mouse pointer, and copy that area to the clipboard. The selected area will be reverse-highlighted.

[Copy All]

This option copies the whole window area to the clipboard.

[Output]

The Output menu offers the following choices for outputting the Peak Pick data.

[Print Table]

When [Print Table] is selected, the Peak Pick data will be printed in a tabular form by the text printer.

[Save Table]

This option creates a tabular text file (with ".TXT" extension) and saves it in the data directory on disk. After selecting the Save Table command, enter a file name (without extension) in the "Text File" dialog box. The file that is created may be printed using the Plot command from the Presentation menu (see Sec. 7.6.5 and Sec. 7.6.6) or edited on a word processor or using text editing software.

[Graphics Plot]

Selecting [Graphics Plot] will cause the Point Pick data of a selected channel to be plotted graphically by the graphics printer. The plot will contain a list of the points as well as the acquisition parameters.

[Replot]

Select the [Replot] command to redraw the graph and clear all the marks (peaks, valleys and point pick marks).

7.5.7 Peak Area

This command calculates the integral value for a designated area on a plotted graph. When [Peak Area] is selected from the Manipulate menu, the user will be prompted to select a particular channel. When the channel for the Peak Area operation is selected, the Peak Area dialog box will appear on the screen.

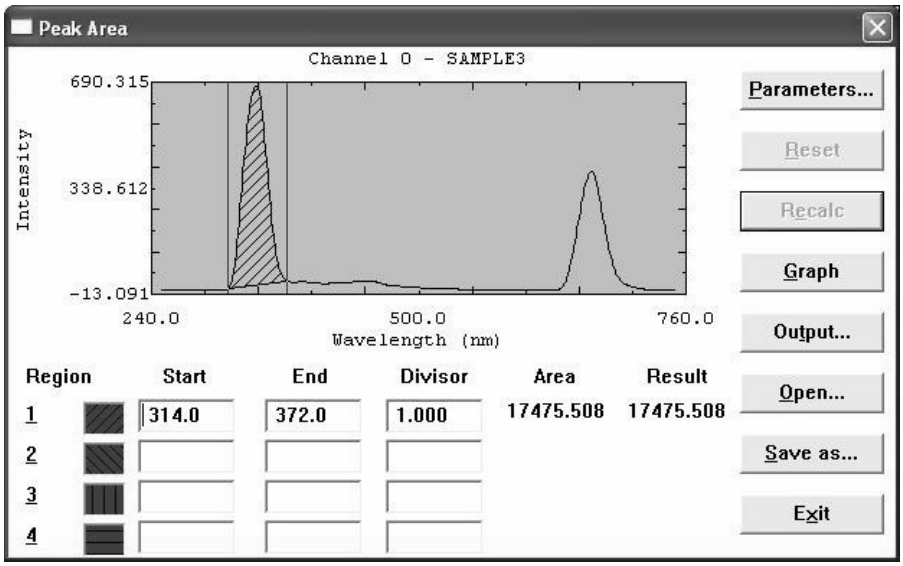


Fig. 7.45 Peak Area dialog box

The upper portion of this dialog box contains the graph of the selected channel. The lower portion of the dialog box provides text boxes for entering the "Start" and "End" X-axis values as well as the "Divisor" values.
To enter the Start and End X-axis values, directly type the values into the text boxes or use the two cursors on the graph.
This menu item allows the user to obtain integral values with up to four ranges within the graph. These ranges will be discriminated on the graph with different shades. After entering these values and pressing the <Recalc.> button or the Enter key, the baseline will be determined automatically and the peak area will be calculated. Note that it is possible to zero the baseline.
"Divisor" is used to calculate the result. When not specified by the user, it is automatically set at 1.0. The result is defined as "Result = Area / Divisor".

When <Reset> is selected, the range of integration will be a range that is defined by the acquisition parameters, and the "Divisor" will be set at 1.0.
The Parameter button allows setting of the parameters associated with the peak area calculation. "Factor" is a factor to be multiplied by the obtained peak area value and should be used when the obtained area value is too large or small. If "Zero the Baseline" is marked, the baseline will be zeroed.
When the <Recalc.> button is selected, a new area value is calculated. This button should be used after parameters are changed.
Selecting the <Graph> button will open a menu similar to the "Right Button" menu, allowing the user to change the graphic presentation parameters (see Sec. 7.8).
When the Output button is selected, the user can output the area data in various ways.

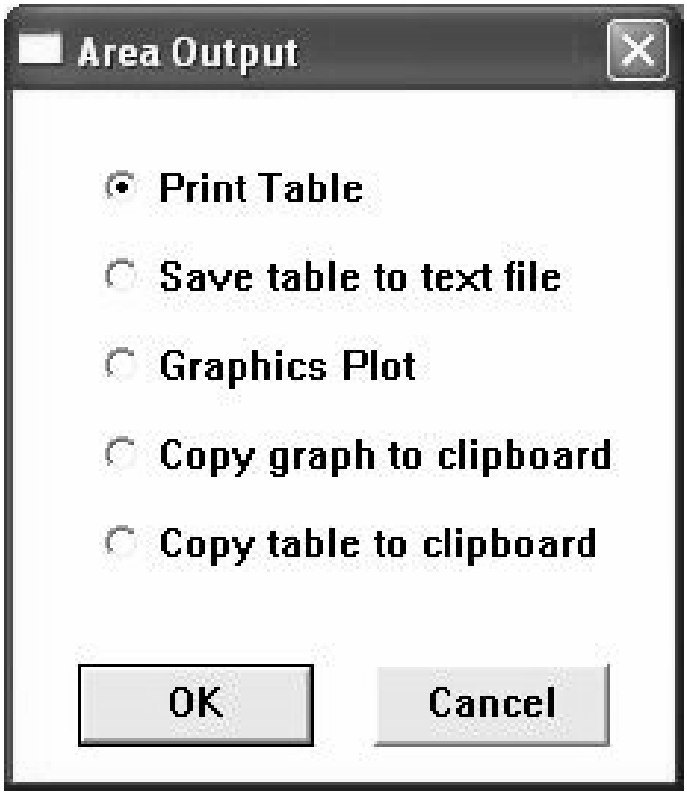


Fig. 7.46 Area Output dialog box

When the "Print Table" command is selected, the area data will be printed in tabular form.
When "Save table to text file" is selected, a tabular text file (with extension ".TXT") will be created and stored in the data directory. The file that is created may be printed using the Plot command from the Presentation menu (see Sec. 7.6.5 and Sec. 7.6.6) or edited on a word processor or using text editing software.
When "Graphics Plot" is selected, the graph of the specified channel will be plotted.
When "Copy graph to clipboard" or "Copy table to clipboard" is selected, the graph or tabular data will be copied to the clipboard.

The <Open> button will load the file (with extension ".PKA") that contains the list of the ranges that have been saved using the Save command.
The <Save> option will save the entered parameters. Type in a file name and save the parameters. The file will have the extension ".PKA".
Use the <Exit> button to close the Peak Area dialog box.

7.5.8 Average (Quantitative mode only)

When the Average command is selected from the Manipulate menu, the average value of the fluorescence intensity values for samples of the same ID will be calculated and the sample table will be overwritten based on the obtained average value. If the original data is needed, save it as a file with another name, and then execute "Average".

7.5.9 Working Curve (Quantitative mode only)

When the Working Curve command is selected from the Manipulate menu, the following dialog box will appear on the screen. The user can select the order (1st, 2nd or 3rd) of the working curve according to the multi-point working curve method and specify whether or not the curve must pass through the zero point. Note that the settings in this dialog box are valid only when the quantitative method is set to "Multipoint Working Curve" (refer to "Quantitative Parameters").

NOTE

After the parameters are changed and the <OK> button is clicked, the working curve will be replotted and the concentrations of unknown samples will be recalculated. At the same time, the unknown sample data table will be updated accordingly.

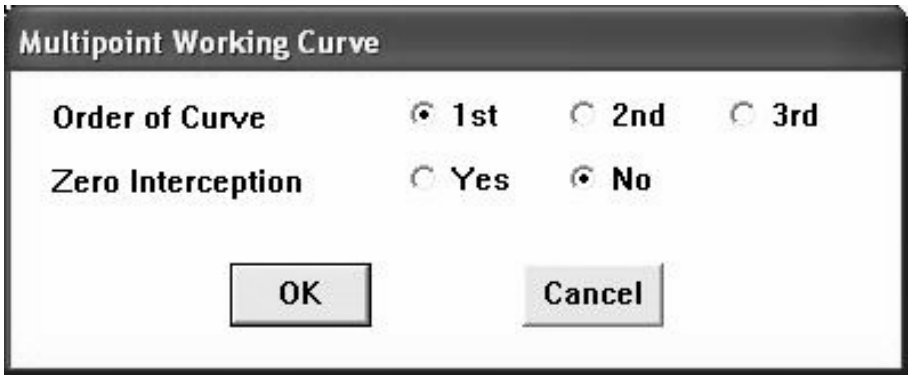


Fig. 7.47 Multipoint Working Curve Parameters dialog box

7.6 Presentation Menu

The Presentation menu items include "Graph", "Show SpeedBox(R)" and "Display Equation," allowing the user to control the presentation format of data files on the screen and define their printout format.

7.6.1 Channel Status

This command is used for monitoring the screen presentation of the active channels. The "Channel Status" window can be accessed via the Presentation menu or by pressing CTRL+C on the keyboard of the computer.
When the "Channel Status" option is selected in Spectrum or Time Course modes, the window in Fig. 7.48 will appear on the screen.

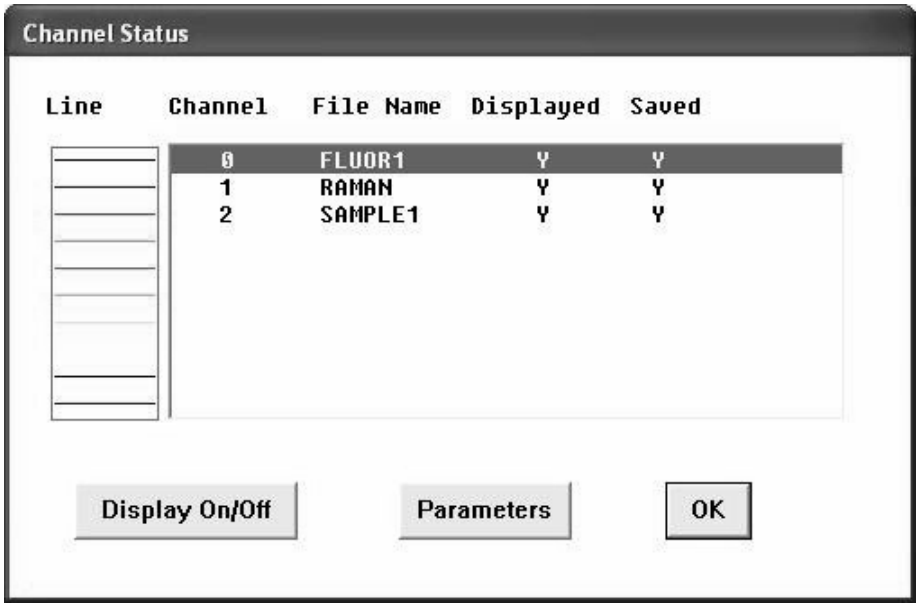


Fig. 7.48 Channel Status window (Spectrum and Time Course modes)

For each active channel, the window displays the following information.

(1) Line: Indicates the color and type of line.

(2) Channel: Means channel number (0 – 9).

(3) File Name: Displays a file name associated with channel number. "No Name" appears for an unnamed file.

(4) Displayed: Indicates whether or not ("Y" or "N") the channel is currently displayed on the graph.

(5) Saved: Indicates whether or not ("Y" or "N") the data has been saved to a disk.

The "Displayed" status of each channel can be switched ON or OFF by selecting the channel (with the up/down cursor keys or the mouse) and clicking the <Display On/Off> button at the bottom of the window or double-clicking on the intended channel.
The acquisition parameters of each channel can be viewed on the screen by selecting the channel (with the up/down cursor keys or the mouse) and clicking the <Parameters> button at the bottom of the window.
Select the <OK> button in the Channel Status window to return to the main screen. The graph will be updated based on any changes.
In Quantitative mode, the Channel Status window displays whether or not the data of standard and unknown samples has been saved to disk. The acquisition parameters of the selected channel can be displayed by selecting "Standard" or "Unknown" and then pressing the <Parameters> button.

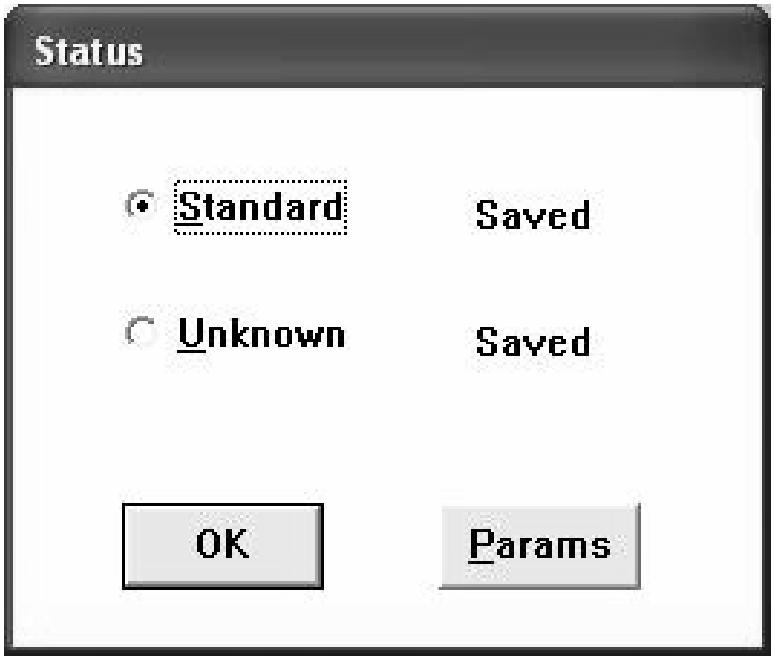


Fig. 7.49 Channel Status window (Quantitative mode)

7.6.2 Graph

The [Graph] option can be used to manipulate the display format of data. This menu option can also be selected by pressing the right button on the mouse. This [Graph] menu, however, does not contain the [Cross Hair] command included in the "Right Button" menu (Sec. 7.8).

7.6.2.1 Copy

When the [Copy] option is selected, the currently displayed graph (in Quantitative mode, a standard or unknown graph) is copied to the clipboard.
The graph copied into the clipboard can be pasted into another document.

7.6.2.2 Display Grid

The [Display Grid] option switches ON and OFF the grid display. To switch OFF the grid display, select [Display Grid] again.

7.6.2.3 Fonts

The [Fonts] option allows the user to specify the font for the coordinate values, axis labels and title of the graph to be displayed (in Quantitative mode, the standard or unknown graph).
When the [Fonts] option is selected, the font specification dialog box will appear on the screen. The user can specify the Font name, as well as the "Style", "Size", "Effects" and "Color" of the font to be used.
To enable the settings, click the <OK> button. Click the <Cancel> button to erase the settings and close the dialog box.

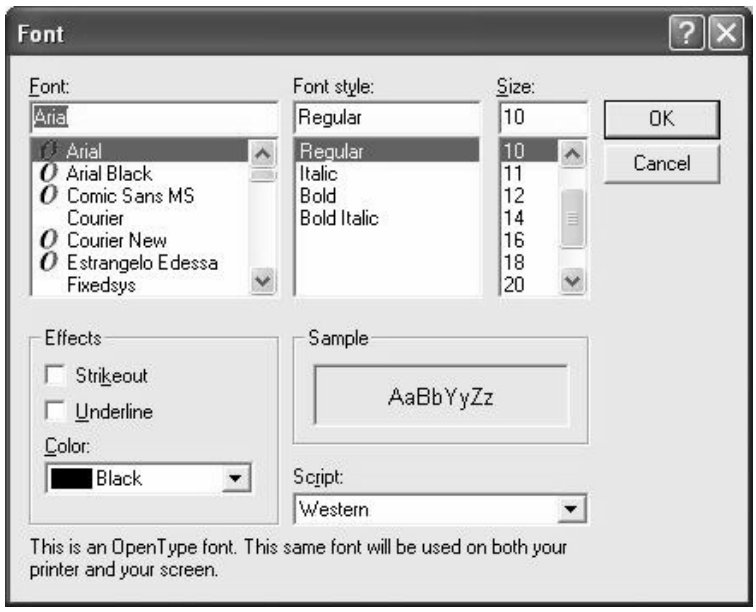


Fig. 7.50 Font Specification dialog box

7.6.2.4 Limits

This command allows the user to enter upper and lower limits for both the X- and Y-axes of the graph on the screen without using the mouse in order to expand or reduce the range of the graph display. When the [Limits] option is selected, the dialog box illustrated in Fig. 7.51 will appear.

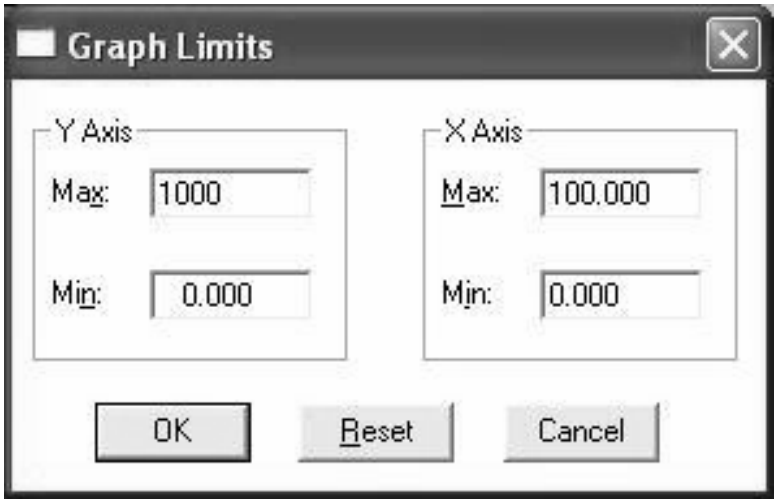


Fig. 7.51 Limits Specification dialog box

Enter the maximum and minimum values for the X- and Y-axes and click the <OK> button to update the graph axes. Selecting <Reset> will scale the graph to the acquisition parameters currently set from the Parameters dialog box in the Configure menu.
Click the <Cancel> button to exit the dialog box without changing the graph limits.

7.6.2.5 Line Colors

Select the [Line Colors] command to assign a custom color, line type or annotation to any of the ten channels based on the monitor or printer/plotter type and/or the user's preference. When the [Line Colors] command is selected, the dialog box illustrated in Fig. 7.52 will appear on the screen.

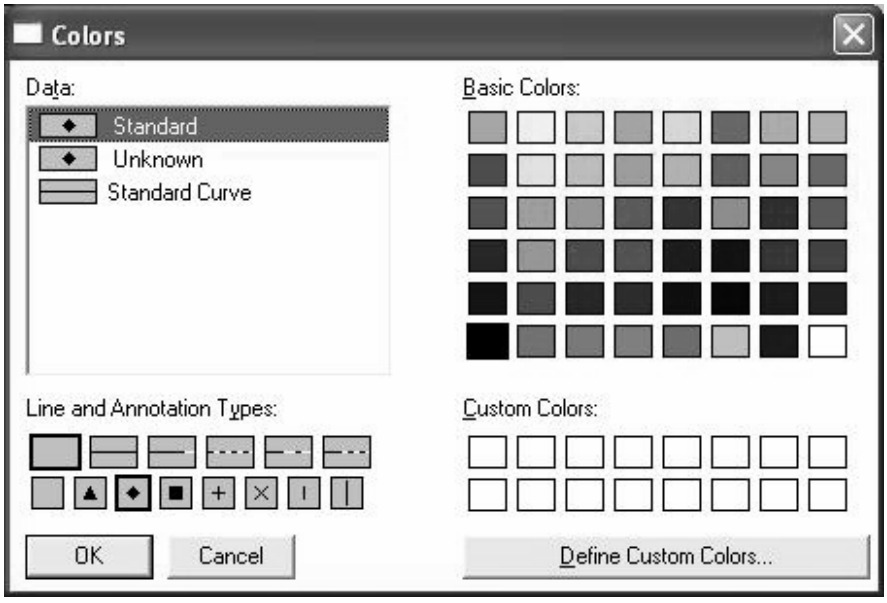


Fig. 7.52 Colors dialog box

To change the color settings, first select the intended channel number from the channel selection box. Next, select the line type, annotation type and color to be assigned to the selected channel from the "Line and Annotation Types" selection and the color selection boxes. If the computer is connected only to a monochromatic printer, each spectrum pattern can be identified by assigning a unique color. The user can select colors from a palette or can specify custom colors.
Upon completion of selection of the line type, annotation type and color for every intended channel, select the <OK> button to enable the settings. When necessary, save the new settings into a configuration file (with extension ".CFG") using the Save Parameters command. Click the <Cancel> button to exit the dialog box without changing the settings.

To customize a color, select any "Custom Colors" box and click the <Define Custom Color> button. The Custom Color Selector dialog box will appear on the screen. To define the intended color, directly type in the relevant values or designate the position in the color space with the mouse. Once all the color settings are satisfactory, click the <Add Color> button. Select <Close> to close the Custom Color Selector dialog box.

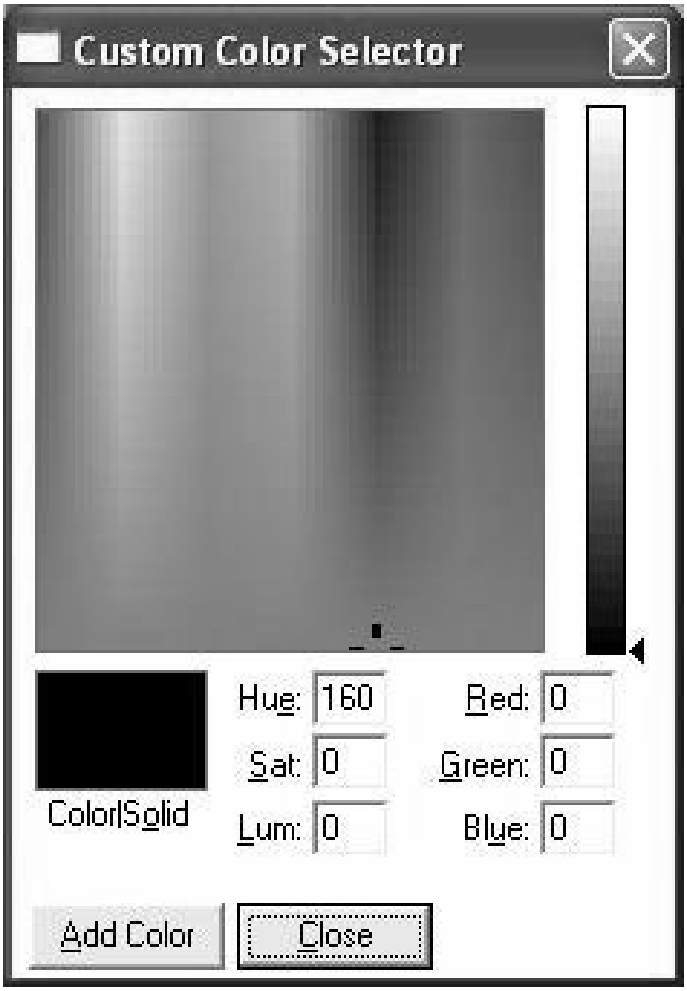


Fig. 7.53 Custom Color Selector dialog box

NOTE
The display color and line type selected for the channel 0 will also be used in the PopUp Scan(TM).

7.6.2.6 Options

Selecting the [Options] command allows the user to set up the grid type and background color of the graph to be displayed.

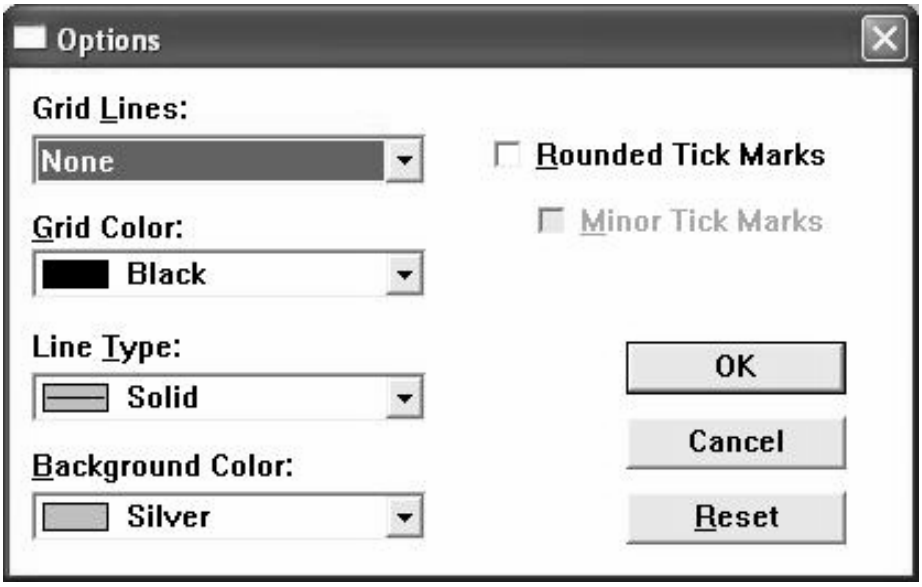


Fig. 7.54 Options dialog box

The items in the Options dialog box are described below.
The "Grid Lines" item allows the user to select the intended display style for the grid from "None", "Major" and "Major & Minor". "Major & Minor" can not be selected, if "Minor Tick Marks" is not checked.
The "Grid Color" choice defines the Color for the intended grid.
The "Line Type" specifies which grid line type is to be used.
When "Rounded Tick Marks" is marked, the tick marks will be arranged at uniform intervals along both the X- and Y-axes.
When "Minor Tick Marks" is marked, minor tick marks will be added to both the X- and Y-axes.
The "Background Color" choice allows the user to select the background color from the basic colors or "Custom Colors". To customize a color, double-click "Custom Colors" in the Colors dialog box and use the Custom Color Selector dialog box (Fig. 7.53).
Once all the settings are satisfactory, click the <OK> button to close this dialog box. The graph will be updated reflecting these new parameters.
Select the <Reset> button to erase the parameter settings. The original settings will be restored.
Click the <Cancel> button to exit the dialog box without changing the settings.

7.6.2.7 Radar (Auto Scale)

The [Radar] function adjusts the graph limits so that the data of all the displayed channels will be completely visible on the graph.
When the [X Axis] is selected, the Radar function will affect the X-axis.
When [Y Axis] is selected, the Radar function will affect the Y-axis.
When the [Both Axes] is selected, the Radar function will affect both the X- and Y-axes.

7.6.3 Hide All

Selecting [Hide All] from the Presentation menu will temporarily clear the graph of all displayed data. (Data will not be deleted.) A check mark will appear next to the [Hide All] option to indicate that it is selected. Selecting [Hide All] option when it is marked will cause all data that is currently loaded to be displayed on the graph, and will delete the check mark. This function is useful when the screen is crowded with previous data that cannot yet be stored or erased. The screen will be cleared for the next acquisition channel.

7.6.4 Show SpeedBox(R)

Selecting the [Show SpeedBox(R)] option from the Presentation menu will display or hide the SpeedBox(R). The SpeedBox(R) is an accelerator device that uses graphical buttons to represent commonly used menu commands. The SpeedBox(R) can be moved around the screen and situated at any convenient location. The SpeedBox(R) can be configured as necessary by selecting [SpeedBox(R) Configuration] option (Sec. 7.4.4) from the Configure menu.
Like ordinary windows, the SpeedBox(R) includes a control menu box. Clicking the control menu box with the mouse will cause the control menu to appear. The control menu is described below.
Selecting [Move] from the control menu allows the SpeedBox(R) to be moved around the screen via the keyboard. When this choice is selected, the cross hair cursor will appear. Then, move the cursor with the cursor keys, and when the SpeedBox(R) is situated at a suitable location, press the Enter key.
Selecting [Close] will hide the SpeedBox(R).
When [About] is selected, the version of the SpeedBox(R) is displayed.
The RF-5301PC software stores the configuration and location of the SpeedBox(R) when the program is shut down. When the software is launched the next time, the SpeedBox(R) will be displayed based on this stored information.

7.6.5 Plot (Spectrum/Time Course mode)

The Plot menu enables layout of a combination (maximum four items) of graph overlays, parameters and text files for the four quadrants of a page to be output to a graphics printer. Before the [Plot] menu can be selected, make sure that the graphics printer has been installed and is selected in the PC Configuration dialog box (Sec. 7.4.3).

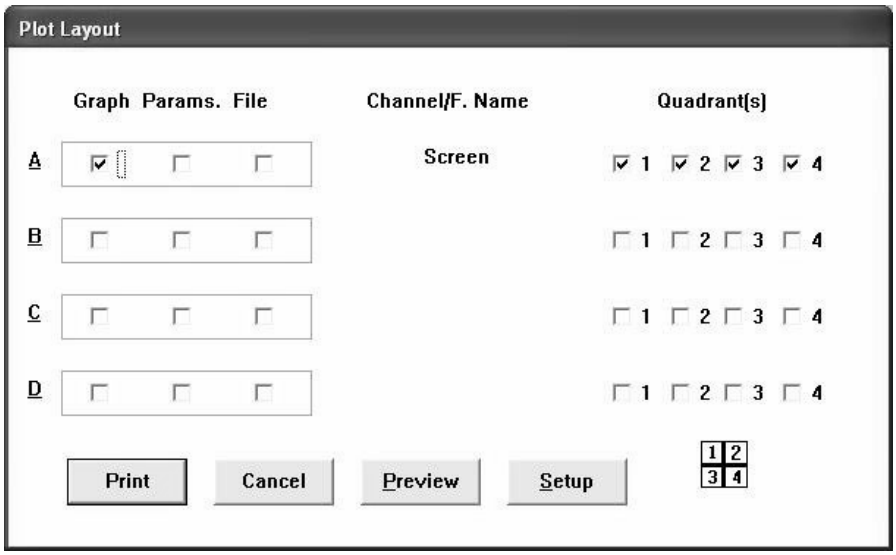


Fig. 7.55 Plot Layout dialog box (Spectrum and Time Course modes)

The Plot Layout dialog box is described below.

(1) Selection of items to be printed
Select any of "Graph", "Params" and "File" from each of the four items (Rows A through D) using the check boxes in the selection box adjacent to "A".

a. If "Graph" is selected, the "Plot Data" dialog box illustrated below will appear.

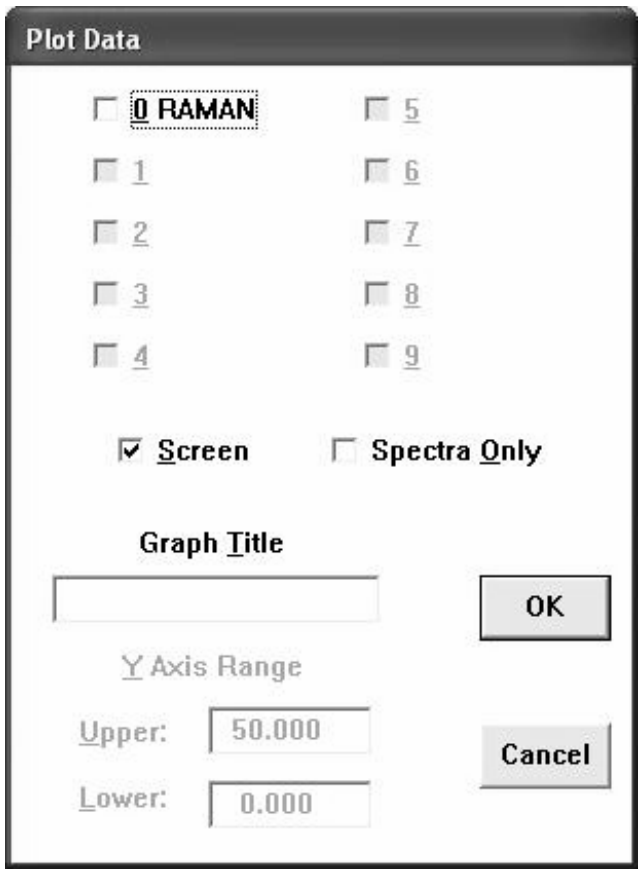


Fig. 7.56 Plot Data dialog box

If "Screen" is marked, the entire contents of the graph on the screen will be plotted as it appears on the screen. If "Screen", is not marked then select a channel and enter limits for the Y-axis. The "Spectra Only" option may be marked to overlay a graph on a page that already contains plotting.
A title may be added to this graph by entering characters in the "Graph Title" box. Once all the settings are satisfactory, click the <OK> button. Click the <Cancel> button to cancel the currently entered settings.

NOTE
The X-axis range is taken from the acquisition parameters of the lowest channel number selected, or from the screen selected.

b. To include the data acquisition parameters in the printout, select "Params", and choose the channel that contains the parameters to be printed.
c. Any of the text files may also be output to the printer. These include Data Print that is capable of being saved as text files, as well as the results of Peak Pick, Point Pick, area calculation and activity calculation, or any text file created using a word processing program (e.g. Windows Notepad). To do this, select "File" in the Plot Layout dialog box and specify the directory and file name in the text file list box that will appear (only one file name can be selected). Note that "paging" will not be performed for the print run.

(2) Definition of layout
Assign one quadrant of the page to each of the items (A through D) to be printed to determine the desired plot layout.
Press the <Preview> button to view the layout of the page on the screen as it will appear when printed out.
Select the <Print> button in the Plot Layout dialog box to start the print job on the graphics printer. If the <Print> button was selected, the Print Status dialog box will be displayed while the output is being written to disk (if using Print Manager). To abort the print job, click the <Cancel> button.

NOTE
In some cases, the use of the Print Manager may be undesirable (e.g. when the computer is incorporated into a network). Use of the Windows Print Manager can be disabled. For details, refer to the Windows User's Guide.

7.6.6 Plot (Quantitative mode)

The [Plot] menu enables layout of a combination (maximum four items) of graph overlays, tables, parameters and text files for the four quadrants of a page to be output to a graphics printer. Before the Plot menu can be selected, make sure that the graphics printer has been installed and is selected in the PC Configuration dialog box.

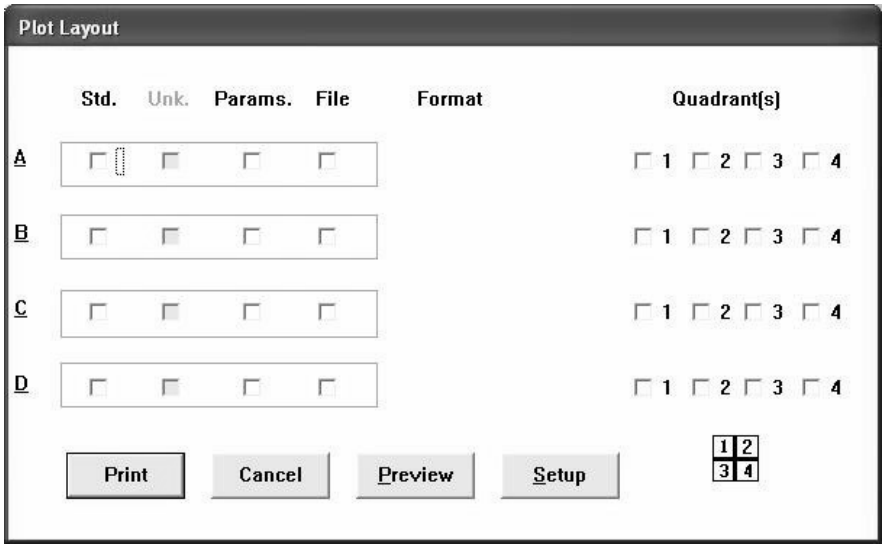


Fig. 7.57 Plot Layout dialog box

(1) Selection of items to be printed
Select any of "Std", "Unk", "Params". and "File" from each of the four items (Rows A through D) using the check boxes in the selection box adjacent to "A".

a. If "Std." or "Unk." is selected, the following Plot Data dialog box will appear.

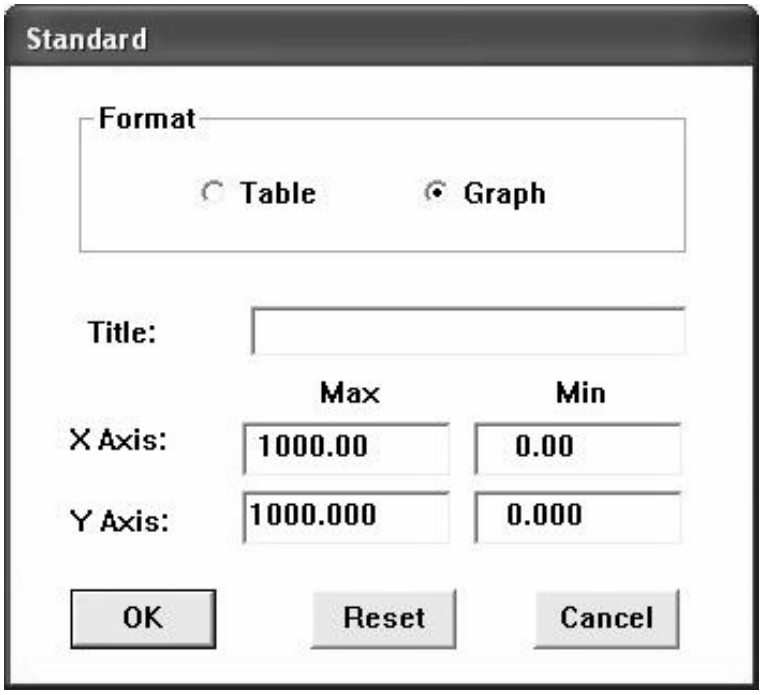


Fig. 7.58 Plot Data dialog box

To output a table, select "Table" from the "Format" selection box. To output a graph, select "Graph". A title may be added to the graph to be output by entering characters in the "Graph Title" box. After selecting "Graph", remember to enter upper and lower limits for the X- and Y-axes.
Once all the settings are satisfactory, click the <OK> button. Click the <Cancel> button to cancel the currently entered settings.

b. To include the data acquisition parameters in the printout, select "Params." and specify either "Standard Parameters" or "Unknown Parameters" in the dialog box that will appear.

c. Any of the text files may also be output to the printer. These include Data Print that is capable of being saved as text files, as well as the results of Peak Pick, Point Pick, area calculation and activity calculation, or any text file created on an editor or word processing program (e.g. Windows Notepad). To do this, select "File" in the Plot Layout dialog box and specify the directory and file name in the text file list box that will appear (only one file name can be selected).

(2) Definition of layout
Assign one quadrant of the page to each of the items (A through D) to be printed to determine the desired plot layout.
Press the <Preview> button to view the layout of the page on the screen as it will appear when printed out.

Select the <Print> button in the Plot Layout dialog box to start the print job on the graphics printer. If the <Print> button was selected, the Print Status dialog box will be displayed while the output is being written to disk (if using Print Manager). To abort the print job, click the <Cancel> button.

NOTE

In some cases, the use of the Print Manager may be undesirable (e.g. when the computer is connected to a network). Use of the Windows Print Manager can be disabled. For details, refer to the Windows User's Guide.

7.6.7 Int/Conc Toggle (Quantitative mode only)

Selecting [Int/Conc Toggle] from the Presentation menu will change the Y-axis of the unknown display between intensity and concentration units. This selection operates as a toggle, that is each time it is selected, the unknown graph Y-axis will change units.

7.6.8 Display Equation (Quantitative mode only)

Selecting [Display Equation] from the Presentation menu will display or hide the equation of the working curve. A check mark appearing adjacent to [Display Equation] means that this item is currently selected. To hide the working curve, select the [Display Equation] again.

7.7 Help Menu

Selecting "Help" from the Presentation menu will pull down the following Help menu items.

7.7.1 Help for RFPC

Selecting this menu item will load the RFPC Help file. The help system has a hierarchical structure. The user will be able to obtain the needed on-screen help information by moving through the help hierarchy from the top downward.

7.7.2 About RFPC

When selecting "About RFPC", the user will be able to find out the version of the software. To exit the box, click the <OK> button.

7.8 Right Button Menu

Pressing the right button on the mouse will display the [Graph] menu that is similar to the Presentation menu except that the "Right Button" menu contains the [Cross Hair] command not available on the Presentation menu.

[Cross Hair]

[Display]

This option will display the cross hair cursor for reading values on the graphs. When the cursor is moved to any position of the graph, the X and Y values at that position will be displayed below the respective axes. When this menu item is selected, a check mark will appear next to the menu item. To erase the cursor from the screen, select this menu item again. The check mark will disappear, too.

[Lock]

When this menu item is selected, a check mark will appear next to the menu item and the cursor will be locked at a particular position on the graph. To unlock the cursor select this menu item again. The check mark will disappear.

For other menu items, refer to Sec. 7.6.2.

7.9 Status Bar

At the bottom of the main screen is situated the status bar. The status bar shows the current wavelength setting, fluorescence intensity value, xenon lamp ON/OFF status, etc.

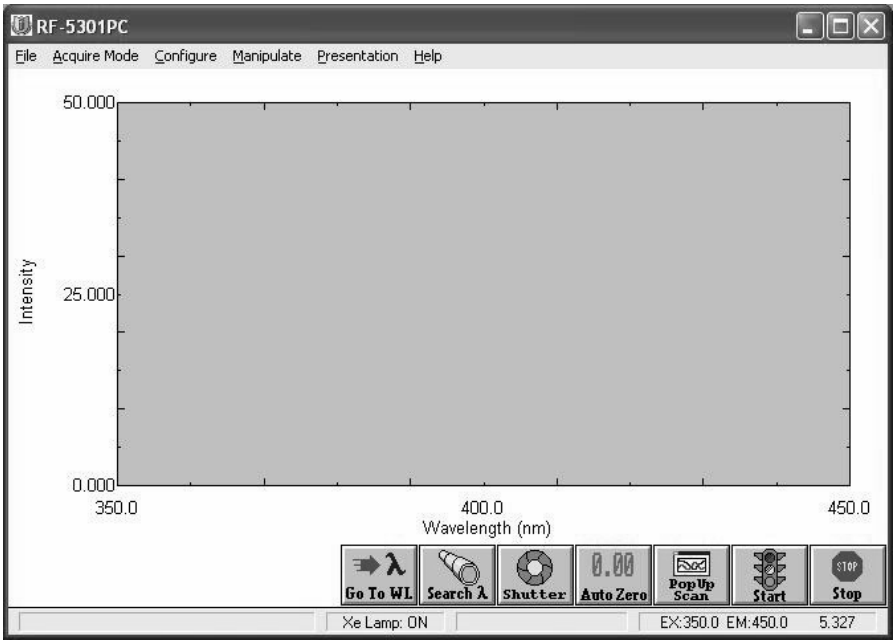


Fig. 7.59 Status Bar

The window items in the status bar, from right to left, are described below.

[Data Display Window]

Displays the current excitation and emission wavelength values and the current fluorescence intensity value. During scanning, the scan wavelength value and fluorescence intensity value will be displayed. During acquisition of time course data, the time elapsed and fluorescence intensity value will be displayed. If the communications with the instrument is OFF, this window indicates "OFF".

[Scan Mode Window]

In Repeat Scan or Auto File mode, the currently active mode and file name will be displayed. For details, see Sec. 7.4.1.

[Xe Lamp ON/OFF Window]

Indicates the current ON/OFF status of the Xenon lamp.

Chapter 8 Maintenance and Inspection

CONTENTS

8.1 Cautions for Transferring the Instrument

CAUTION

EXPLOSION OF LAMP

* When transferring or shipping the instrument, be sure to remove the Xenon lamp. Store the removed lamp in the special case. For information about reinstallation, adjustment and performance verification of the instrument after transferring or shipping, refer to Sec. 2.4 through Sec. 2.9.
* High pressure gas is charged in the Xenon lamp. If impact, vibration, or pressure is applied, the lamp may burst. Take most care in handling the lamp to avoid danger.
  Be sure to wear protective gears such as protective mask, gloves, and long sleeve shirts.
  For details of handling the Xenon lamp, refer to the caution during use indicated on the lamp inspection certificate.
* While installing the lamp, do not touch the bulb with bare fingers. If fingerprints remain on the bulb, remove them with gauze moistened with ethyl alcohol or the special cleaning agent included with the lamp. Finger oil remaining on the bulb can be baked onto the bulb when the lamp is lit, possibly causing the lamp to burst.

8.2 Service Life of the Xenon Lamp

The Xenon lamp is a consumable part. When the lamp approaches the end of its effective service life, the emission from the center of the lamp may fluctuate or the lamp itself may flicker. When such a problem occurs, the noise level will become greater, making accurate data acquisition impossible. The time during which the lamp remains operative before the first occurrence of flickering is called the "flicker" life.
The guaranteed "flicker" life of the Xenon lamp is 500 hours. When the lamp time used reaches 500 hours, replace the lamp immediately.
Once the lamp total time exceeds 500 hours, a warning message will be issued when the RF-5301PC software is launched.

CAUTION

EXPLOSION OF LAMP
Never use a lamp in excess of 1,000 hours. A lamp used for more than 1,000 hours may burst, possibly damaging the lamp unit.

8.3 Managing Lamp Time of Xenon Lamp

The RF-5301PC offers a monitoring function for the lamp total time used. To check the lamp total time on the screen, select [Instrument] from the Configure menu.

8.4 Safe Disposal of Xenon Lamp

CAUTION

EXPLOSION OF LAMP

It is very dangerous if the replaced Xenon lamp was disposed without giving any processing. Wrap the lamp completely with the thick cloth and break the bulb using a hammer to prevent scattering of pieces of glasses. On this processing, wear protective gears such as protective mask, gloves, and long sleeve shirts of bulky material. Be careful not to be hurt by piece of glass.

8.5 Replacing Fuse

When the fuse is blown, replace it according to the following procedure.

WARNING

RISK OF ELECTRONIC SHOCK
Before changing a fuse, turn off the power switch and disconnect the power cable.

CAUTION

REPLACEMENT OF FUSES
This unit uses the following fuse. Be sure to replace the fuse of same type and capacity.

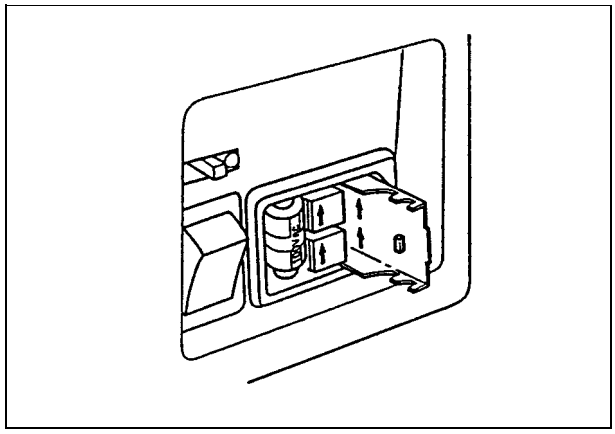
Rated voltage 100 – 120 V:	Part No. 072-01663-14 250 V 5 AT
Rated voltage 220 – 240 V:	Part No. 072-01663-12 250 V 3.15 AT

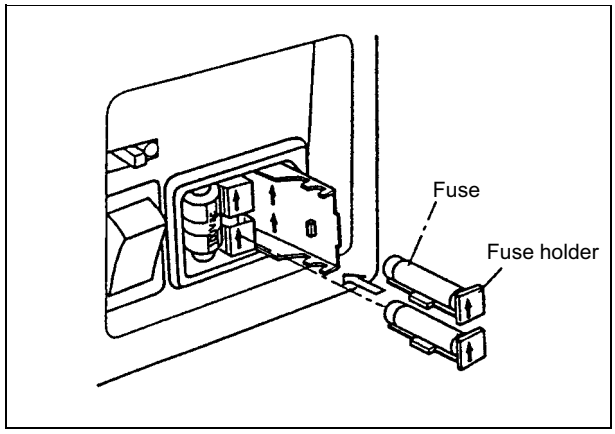
(1) Open up the fuse cover at the inlet on the right side of the instrument with a flat blade screwdriver.

(2) Draw out the fuse holders (2 pcs.)

(3) Replace a blown fuse with new one and insert the fuse holders to their original position. The arrow direction on the fuse holders must be identical to that on the fuse cover.

(4) Close the fuse cover.





8.6 Verifying Wavelength Accuracy

Based on the mercury emission line (435.8 nm) of the light from a fluorescent lamp, the wavelength accuracy can be verified for the monochromators.

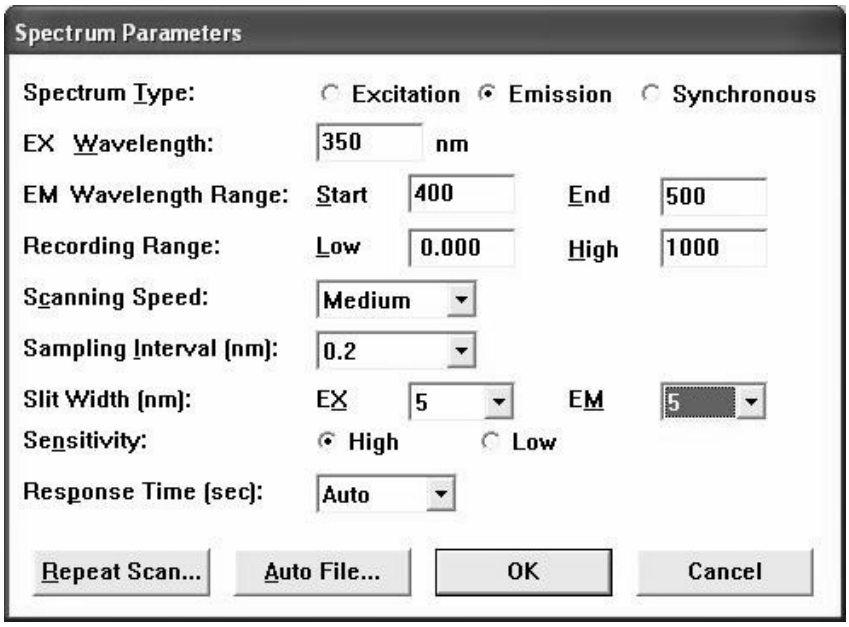
8.6.1 Verifying Wavelength Accuracy for Emission Monochromator

(1) Set up RF-5301PC to right below from a fluorescent light of room. Or prepare a desk lamp, set up it so as to light up sample compartment from just front.

(2) Set measurement mode to "Spectrum".

(3) Switch OFF "PMT Protect" and "-HV Control" options from the Instrument submenu under the Configure menu.

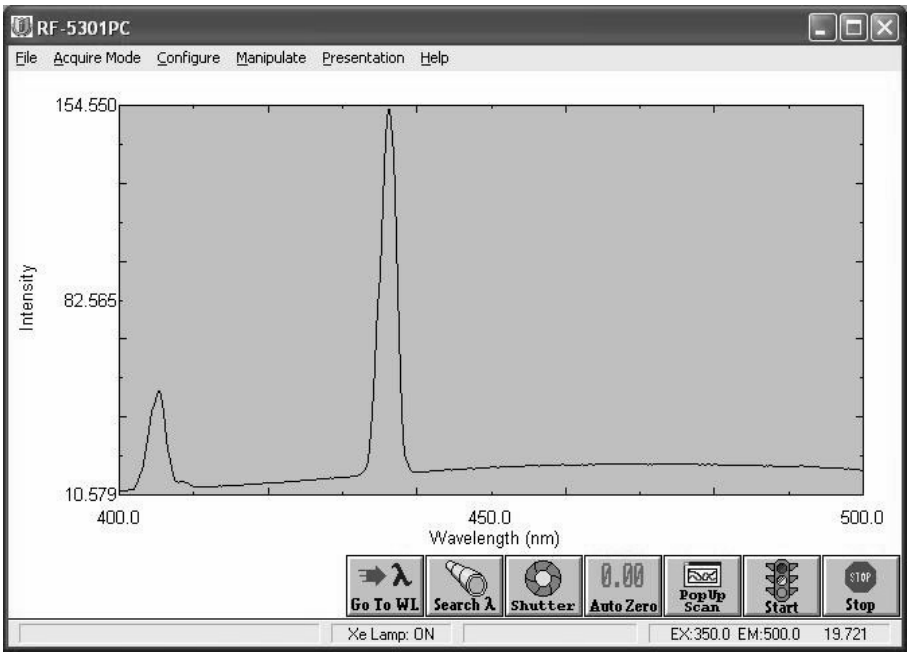
(4) Select [Parameters] from the Configure menu and set the parameters as follows.



(5) Remove the cell (or sample) from cell holder if include something samples in sample compartment.

(6) Open up the lid of the sample compartment to expose the emission monochromator to light from a fluorescent lamp of room. If using desk lamp, set up it so as to light up a back wall of sample compartment from RF-5301PC front side. And during measurement, a lid of sample compartment is state of being opened.

(7) Click the Start button to start scanning.
Upon completion of the scan, the following spectrum will be obtained. The spectral pattern may vary depending on the conditions of illumination from the fluorescent lamp.



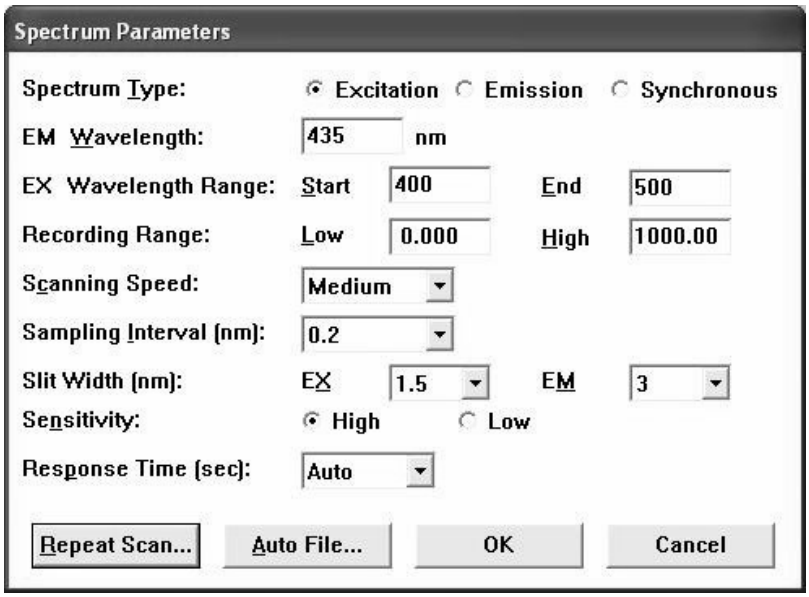
(8) Run [Peak Pick] (see Sec. 7.5.5) and check that the resultant peak in the Peak Pick window is within the range of 435.8 ± 1.5 nm In case that a peak intensity exceeds a full scale and can not read the peak wavelength correctly, adjust VR 1 trimmer (See Chapter 3) so as to be located peak within display. Make a note of the precise value of the peak wavelength (EM). Next, proceed to the verification of the wavelength accuracy of the excitation monochromator. If the emission monochromator fails to satisfy the range mentioned above, contact Shimadzu or its nearest representative.

8.6.2 Verifying Wavelength Accuracy for Excitation Monochromator

(1) Load a cell filled with distilled water into the cell holder, and close the lid of the sample compartment.

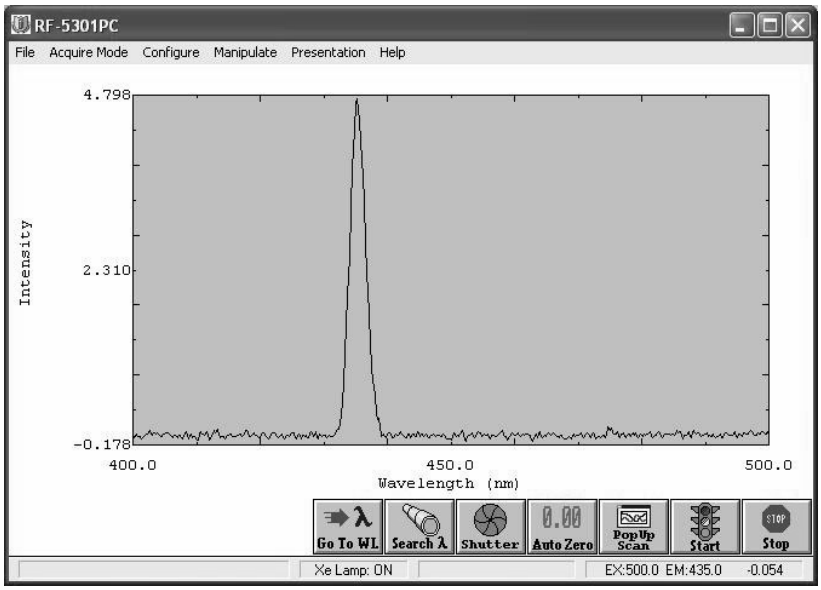
(2) Switch ON "PMT Protect" and "-HV Control" options from the Instrument submenu under the Configure menu.

(3) Select [Parameters] from the Configure menu and set the parameters as follows.



(4) Enter the emission wavelength value (EM) that was recorded in Sec. 8.6.1.

(5) Click the <Start> button to start scanning.
Upon completion of the scan, the following spectrum will be obtained.



(6) Run [Peak Pick] and verify the peak wavelength is around EM that was recorded in Sec. 8.6.1. However, this is an easier method for obtaining the wavelength accuracy of the instrument. Mercury lamp is necessary to obtain the exact wavelength accuracy. Please contact Shimadzu or its nearest representative for further information.

8.6.3 Testing S/N Ratio

Test the S/N ratio based on the Raman peak obtained with distilled water. For more information, see Sec. 2.9 "Verifying Performance".

8.7 Cleaning the Unit

The spectrofluorophotometer case and sample compartment should be kept clean. Cleaning should be done with a soft cloth slightly dampened with water or a solution of water and a mild detergent. Do not use an excessively damp cloth that liquid can drip into the spectrofluorophotometer.

CAUTION

Be very careful not to spill water, organic solvent, etc. on the instrument. Spilling liquid on the instrument can cause electric shock, fire, damage or malfunction of the instrument.

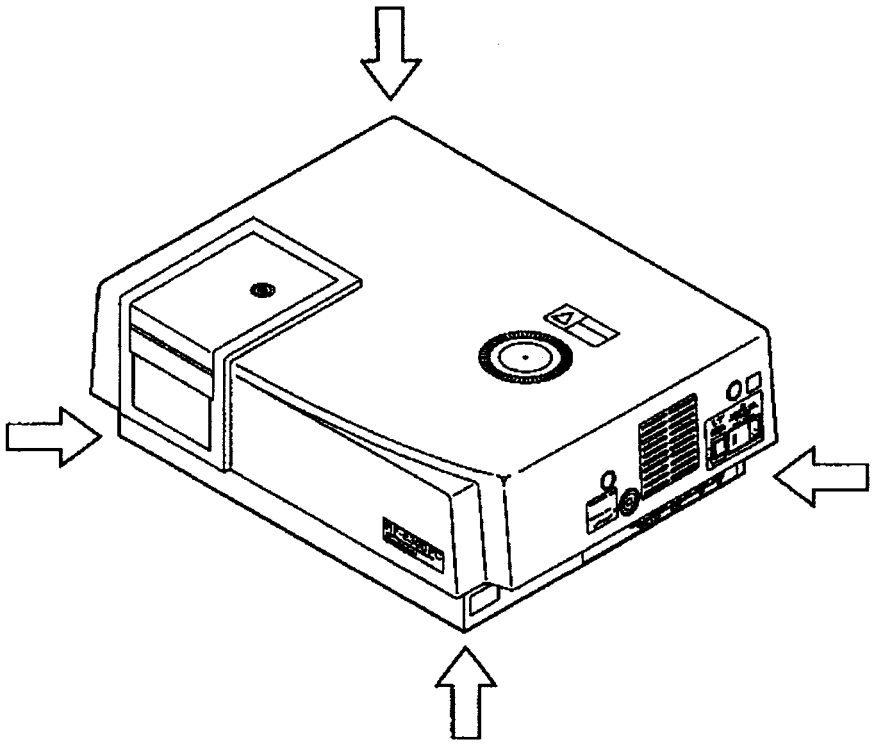
8.8 Service Parts List

1. Xenon lamp.....200-81500-01
2. Fuse, 5 AT (for 100, 120 V).....072-01663-14
3. Fuse, 3.15 AT (for 220, 230, 240 V).....072-01663-12

8.9 Precaution During Transportation of the Unit

CAUTION

This unit weighs about 43 kg. When moving this unit, it must be lifted by two persons firmly holding at the positions shown in the diagram.



Chapter 9
Troubleshooting

CONTENTS

9.1 Initialization Errors

During initialization after powering ON, the RF-5301PC executes a self-diagnosis (eight items listed below). Should any of these error items occur, take the relevant action specified below.
If an error occurs during the initialization process, the user can still establish communication between the computer and the instrument to make the instrument operative by canceling the error message on the screen and clicking the <Start> or <Read> button. REMEMBER, however, this feature was designed for use by Shimadzu service personnel only. Therefore, normal functioning of the instrument in such a situation is not guaranteed.

Error item	Remedial action
ROM RAM EEPROM Slit, excitation side Slit, emission side Excitation monochromator Emission monochromator	Contact Shimadzu or its nearest representative.
Zero setting	The lid of the sample compartment may be open. If the problem persists even after the lid is closed, contact Shimadzu or its nearest representative.

9.2 Error Messages

If the user attempts an improper software operation or if a problem occurs with the hardware, the RF-5301PC software will issue an error message. When an error occurs, correct it by following the instructions described below.

9.2.1 Communications Problems

If the communications between the computer and the instrument are not normal, take remedial action according to the table below. When there is a problem related to computer-instrument communications, the indication "OFF" will appear in the Data Display Window in the Status Bar.

Items to be Checked	Remedial Action
Cable connection	Securely insert the RS-232C cable into the RS-232C connectors on the computer and the instrument. Then, fasten the screws on the connectors and click the Start or Read button on the main screen to establish communication.
COM port setting	Study the documentation for your computer to check that the COM port (RS-232C port) in question has been addressed correctly. Select the PC Configuration command from the Configure menu to verify that the radio button corresponding to the COM port number connected to the cable has been checked (button appears as a block). If the COM port is not correctly addressed, select the correct number for that COM port. When satisfactory, close the PC Configuration dialog box and click the Start or Read button to establish communication. Once communications are established, run the Save Parameters command from the Configure menu to save the current configuration as a "RFPC.CFG" file.

If normal communications cannot be established in spite of the above remedial actions, contact Shimadzu or its nearest representative.

9.2.2 Software Operational Error Messages

The following tables summarize possible causes and remedial actions for software operational error messages.

Message	Possible cause and remedial action
Auto Zero Level Over!	Signal level was too high for auto zero operation. Select LOW sensitivity or set the slit narrower to lower the signal level. When analyzing a solid or power sample, the use of an appropriate color glass filter can be an effective remedial action. If the excitation wavelength is too near to the emission wavelength, separate them sufficiently. If the problem still persists despite the above action, even though the shutter is in the closed position, the instrument may be faulty. Contact Shimadzu or its nearest representative.
Bad Or Missing Configuration File	The configuration file (RFPC.CFG) is damaged or is not in the directory to which the RFPC software has been installed. Copy the RFPC.CFG file in the original set-up CD-ROM to the directory where the RFPC software is installed.
Cannot Establish Communications!!	The computer cannot communicate with the fluorometer unit. Check the cable connection. Also, select the PC Configuration menu to check that the COM port has been correctly addressed.
Communications Initialization Failure!	The designated COM port cannot be initialized. Reboot the computer. If the problem persists, the computer may have a hardware problem. Study the documentation of the computer to assess possible causes of the problem.
Communications Open Failure!	The designated COM port does not open. This error can occur if other software uses the same COM port or a Windows system error has occurred. Another possible cause is specification of a non-existing COM port in the PC Configuration dialog box.
Communications Time Out!	The computer cannot communicate with the fluorometer unit. Check the cable connection. Also, use the PC Configuration dialog box to check that the COM port has been correctly addressed.
General Communications Error!	Same as above.
Transmit or Receive Error!	Same as above.
Unknown Communication Error Status	Same as above.
DCB Initialization Failure!	Windows was unable to create a Device Control Block for the designated COM port. This is usually results from a damaged RFPC.CFG file.
Error Converting Channel Time Units! Delete Unnecessary Channels!	While in Time Course mode, when the time units are changed using Parameters from the Configure menu, all current channels are changed to those units accordingly. If there is not enough memory available, this message will appear. Delete any unnecessary channels to make space for any new data.

Message	Possible cause and remedial action
Error Creating Temporary File!	A temporary file will be written into the data directory for peak and point pick operations. If there is a file "TEMP PK.TXT" in the data directory, delete it. Also, refer to "File Creation Error".
File Creation Error!	The directory in which the file was to be created is non-existent, or a disk is full or damaged. If a floppy disk is used, check it has been inserted all the way into the floppy disk drive.
File Write Error!	The directory to which the file was to be written is non-existent, or the disk is full or damaged. If a floppy disk is used, check that it has been inserted all the way into the floppy disk drive.
Error Reading File!	The file on a disk is damaged or the disk drive itself may be faulty. Study the documentation of the computer and/or DOS.
Incorrect File Type!	The file specified is not valid for the current acquisition mode, and cannot be loaded.
Graphics Memory Error!	The amount of graphics memory is insufficient. Windows must be restarted.
Graphics Printer Error!	1) The printer driver has not been installed. Install the printer driver of the graphics printer to be used. 2) The printer is not online. 3) The printer does not recognize the connection with the computer. Power ON the printer and the computer, in this order. 4) The printer has not been connected correctly. Make sure that the correct interface port (LTP1, COM1, etc.) is selected to correspond to the hardware configuration.
Text Printer Error!	Same as above.
Graphics Printer Has NOT been Selected! See the PC Configuration command.	The intended graphics printer has not been selected. Select the printer using "PC Configuration". Save the settings using the Save Parameters command.
Text Printer Has NOT been Selected! See PC Configuration command.	The intended text printer has not been selected. Select the printer using "PC Configuration". Save the settings using the Save Parameters command.
Invalid Configuration File! Do You Want to Use the Default File?	The selected configuration (parameters) file is not for the RF-5301PC.
Command not Supported.	A control command not defined for controlling the fluorometer was used. This error may occur as the result of damaged "RFPC.CFG" file or faulty cable connection.
Memory Error!	There is insufficient available memory. Quit any other programs that are running to increase the available memory space.
No Enough Memory for Operation!	Available memory is insufficient. Quit any other programs that are running to increase the available memory space.

Message	Possible cause and remedial action
No More Empty Channels Available!	There are no empty channels for holding data. Delete any unneeded channels.
No Printers Installed!	A Windows-compatible printer has not been installed. Set up the printer using the Windows Control Panel.
No Replicate ID's found.	The Average command from the Manipulation menu cannot find any samples with common ID numbers, therefore no averaging operation can take place.
Reaction Time May not Exceed 100,000.	In Time Course mode, the total specified time may not exceed 100,000. Reduce either the sampling interval or the number of data points.
Table will not fit in area selected.	No "paging" is performed when printing data tables via the "Plot" command. Tables are too large to fit on regular page size. Change the paper orientation or select more quadrants. If the problem still remains unsolved, use "Data Print" from the Manipulation menu to change settings.
File will not be displayed entirely. Do you wish to continue?	When printing a test file using "Plot" from the Presentation menu, the file may be too large to fit within the defined plot area. If Continue is selected, the text will be trimmed and not fully printed.
Excitation Monochromator Slip	The excitation monochromator has detected a wavelength failure. Contact Shimadzu or its nearest representative.
Emission Monochromator Slip	The emission monochromator has detected wavelength failure. Contact Shimadzu or its nearest representative.
Lamp time is Invalid.	The management information on the total amount of time the lamp has been ON is faulty. Reset the lamp time to zero. If this problem still persists, contact Shimadzu or its nearest representative.
Can not set -HV mode ON (Because Lamp is off).	If the lamp remains unlit, the -HV control cannot be turned ON.

9.3 Before Suspecting Malfunction

If a fault should occur with the instrument, remedy it by referring to the table below. When a symptom in question is not found in the table, contact Shimadzu or its nearest representative.

Symptom	Possible Cause	Remedial Action
1) Instrument is not powered though the power switch is in the ON position.	Power cable is not securely connected.	Securely insert both ends of the cable into the inlet on the instrument and the outlet at the site.
	Power fuse is blown.	Install a new fuse.
	Other causes.	Contact Shimadzu or its nearest representative.
2) Xenon lamp does not light.	Lamp ON/OFF switch is in OFF position.	Move the switch (on the right side face of the instrument) to ON position.
	Wiring is disconnected.	Check the connection of the high-voltage lead on the positive (+) electrode side of the lamp. Before opening the cover of the lamp house, be sure to disconnect the power cable from the outlet. Inadvertently powering ON of the instrument with the cover open is extremely dangerous.
	Lamp is still hot.	Allow the lamp to cool down for about ten minutes.
	Other causes.	Contact Shimadzu or its nearest representative.
3) Signal does not come out.	Lamp is not lit.	See symptom 2 above.
	The Xenon lamp is misaligned.	Position the lamp correctly.
	Shutter is in the closed position, or the slit on the emission side is in "Close" status.	Open the shutter or slit.
	Incorrect acquisition parameters.	Correctly specify the wavelength, slit width, etc.
	Other causes.	Contact Shimadzu or its nearest representative.

Symptom	Possible Cause	Remedial Action
4) S/N ratio does not satisfy the guaranteed value.	Xenon lamp is not stably lit.	After powering ON the instrument, wait at least 30 minutes until the Xenon lamp is stably lit.
	Xenon lamp is aged.	Use a new Xenon lamp.
	Distilled water is contaminated.	Contaminated distilled water or a dirty cell will result in increased background noise owing to scattered light or impurity-induced fluorescence, decreasing the S/N ratio. Rinse the cell thoroughly. Use only clean distilled water.
	Other causes.	Contact Shimadzu or its nearest representative.

Chapter 10 Basics of Fluorometric Analysis

CONTENTS

10.1 What Is Fluorescence?

The phenomenon of certain kinds of substance emitting light on absorbing various energies, without involving heat generation, is called luminescence. That kind of luminescence that is emitted on exposure to ultraviolet or visible rays is called photo luminescence.
Fluorescence and phosphorescence, representative of photo luminescence, possess hues different from the reflected or transparent color a substance, emitting longer wavelengths of light than radiated light.
Familiar examples are the green color observed in a red ink in the daylight (eosin aqueous solution) and a pale color in shirts.

10.2 Principles of Fluorescence Process

This section explains the principles of fluorescence with reference to an organic compound as an example.
When a molecule in the base state S_0 is exposed to light, the kinetic energy of the electrons in the molecule is altered, moving the molecule into the excited state S_1 with a higher energy level as shown in Fig. 10.1.

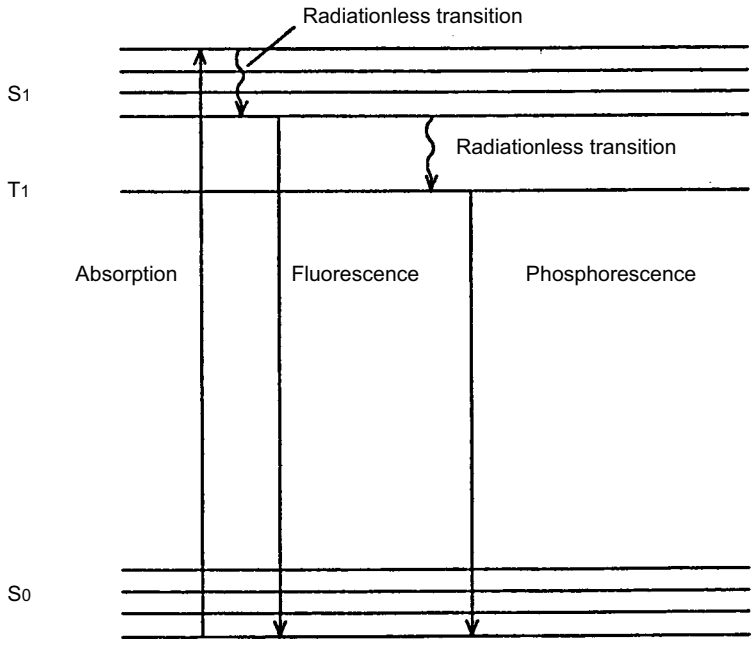


Fig. 10.1 Principles of fluorescence

The excited state, however, soon changes back to the base state as the molecule is deactivated by radiating the energy in the form of heat or light. The molecule then transits, without radiation, to an excited state having a slightly lower energy level than the excited state S_1. The light the molecule emits as it returns further to the base state S_0 is called fluorescence.
Since part of the energy of the light absorbed has been lost as vibration or heat energy, the light covers a longer wavelength than the light to which the molecule has originally been exposed (Stokes law).
The light the molecule emits as it transits, without radiation, to the triplet state T_1 from the excited state S_1, the returns to the base state S_0 is called phosphorescence. Phosphorescence has a life longer than 10^{-4} seconds because of the need for spin transformation.

10.3 Three Basic Laws of Fluorescence

Law 1
In order for a substance to emit fluorescence, light absorption must take place first.

Law 2
Generally, fluorescence has a longer wavelength than excitation light.

Law 3
The quantum yield of fluorescence (Q) is determined by the frequency of non-radiation of the absorption energy to heat or others.

$$Q = \frac{n_e}{n_e + n_f}$$

where
ne: Frequency of light emission
nf: Frequency of non-radiation transition

Law 1 dictates that the task of testing an unknown sample begin with measurement of its absorption spectrum with a relatively higher concentration. If there is no absorption at all, then it is considered that fluorescence is not emitted even if the sample is excited at the wavelength. Conversely, fluorescence is emitted most intensely if the sample is excited with the absorption peak wavelength.
Law 2 indicates that, since part of the energy of the light absorbed is lost as heat or vibration as explained in Sec. 10.2, the residual radiation energy is reduced, shifting the wavelength of the fluorescence to longer side. Hence, the task of measuring the fluorescence spectrum can be reduced to a matter of scanning only the longer wavelength side of the excitation light.
Law 3 uses the quantum yield of fluorescence (Q) to indicate what proportion of the energy absorbed is radiated as fluorescence. The higher the value of Q, the easier the substance produces fluorescence. Table 10.1 lists the quantum yields of typical fluorescent substances.

Table 10.1 Compounds and quantum yield of fluorescence

Compound	Solution	Quantum yield
Fluorescein/	0.1N-NaOH	0.92
Eosin/	0.1N-NaOH	0.19
Rhodamine B/	Ethanol	0.97
Riboflavin/	Aqueous solution, pH 7	0.26
Anthracene/	Ethanol	0.30
Naphthalene/	Ethanol	0.12
Indole/	Water	0.45
Chlorophyll a/	Ether	0.32
Chlorophyll b/	Ether	0.12

10.4 Advantages of Fluorometric Analysis

10.4.1 High Selectivity

Even if multiple substances are intermixed in a single sample, selective fluorescence measurement of a particular substance is made possible without having to remove the other substances if these other substances do not emit fluorescence.
Further, even though multiple fluorescence-emitting substances are intermixed in a sample, measurement can still distinguish them from each other by setting their wavelength in an appropriate manner if they vary in excitation or emission light wavelength.

10.4.2 High Sensitivity

Fluorescence analysis is 100 to 1,000 times more sensitive than absorptiometry, allowing measurement of ultra micro-quantity.

10.5 Important Notes on Fluorometric Analysis

10.5.1 Effect of Sample Temperature

In many samples, each rise of 1 °C in sample temperature is said to produce a loss of 1 – 2 % in fluorescence intensity, though this is dependent on the type of the sample. Certain biochemical samples reportedly produce a change of some 10 % in fluorescence intensity in response to a temperature change of 1 °C. Temperature-dependent samples need to be tested in the constant temperature cell holder.

10.5.2 Photochemical Reaction of Samples

Exposures to excitation light cause certain samples to produce a photochemical reaction, resulting in a change in fluorescence intensity. Testing of such samples should benefit from regulating the shutter to expose the sample to excitation light only for the duration of measurement. Other techniques available would be increase the spectrum scan speed to the extent possible or narrowing the bandwidth of the excitation light.

10.5.3 Fluorescence from Impurities

Peaks caused by fluorescent components other than the component of interest during fluorescence spectrum measurement are called fluorescence from impurities. Fluorescence from impurities are associated with (1) scatted light and its secondary light, (2) Raman scattered light of the solvent, and (3) fluorescence from the solvent or cell.
(1) and (2) are discussed in Sec. 10.5.4. For (3), commercially available grades of reagents often detect fluorescence caused by the presence of impurities solvent. Remember that high-sensitivity testing in the ultraviolet region is particularly susceptible to the effects of solvent fluorescence. Fluorescence-free solvents are available for fluorescence analysis use. To remove concern over the possible effects of solvent fluorescence, either use these commercial solvents or refine your solvent by yourself.
General quartz cells will produce weak fluorescence when they are excited at around 260 nm because of impurities (aluminum) inherent in the cells. Use of a fluorescence-free cell that contains artificial quartz (P/N200-34594-03) is recommended for exciting traces of samples at around 260 nm.

10.5.4 Effects of Scattered Light

In fluorescence testing, peaks caused by scattered light and Raman scattering may be observed in addition to the fluorescence components of primary interest. Scattered light is associated with the scattering of excitation light by solvent molecules (Rayleigh scattering) or by particulates or air bubbles, with the resultant scattered light entering the emission monochromator. Scattered light is manifest particularly in the testing of solid samples. These peaks are readily distinguished because they appear at the wavelength of the excitation light.

Depending on the characteristics of the grating monochromator, scattered light may also appear in the wavelength regions two and three times the excitation light wavelength as second order and third order light, respectively. With an excitation light wavelength of 220 nm, for example, second order light appears at 440 nm and third order light appears at 660 nm. A short wavelength cutoff filter is inserted at the emission side to remove such light.
Further, if the excitation light wavelength is set to visible, light having half the wavelength is also emitted from the excitation monochromator. With an excitation light wavelength of 450 nm, for example, light of 225 nm is also emitted. A short wavelength cutoff filter is inserted at the excitation side to remove such light. Use the filter set available as a special accessory if second order or third order light is of concern. Raman scattering appears when the solvent has Raman activity.
It appears on the longer wavelength side of excitation light as like fluorescence peak. Raman scattering is distinguishable because it remains essentially unchanged in intensity with changes of the sample concentration and also because changes of excitation light wavelength vary the position of the

peaks caused by Raman scattering but not the peak position of fluorescence. Table 10.2 summarizes the relationships between the excitation light wavelength and Raman peak.

Table 10.2 Relationships between the excitation wavelength and Raman peak

		Solvents and Raman peak wavelengths (nm)				
		Water	**Ethanol**	**Cyclohexane**	**Carbon tetrachloride**	**Chloroform**
Excitation light wavelength (nm)	248	271	267	267	-	-
	313	350	344	344	320	346
	365	416	409	408	375	410
	405	469	459	458	418	461
	436	511	500	499	450	502

10.5.5 High Concentration Samples

Too high sample concentrations can be a cause of various errors. Because spectrofluorophotpmeter are designed to detect fluorescence emitted from the center of the cell, the excitation light would be absorbed in the vicinity of the inlet of the cell if the sample concentration is too high. The failure of the excitation light to fully reach the center of the cell causes a loss of apparent fluorescence intensity. Further, that portion of fluorescence emitted from the center of the cell having a shorter wavelength is reabsorbed by the sample in the cell, making the spectrum look as if it were shifted towards the longer wavelength side.
Generally, samples with absorbance up to 0.02 (in a 10 mm cell) in the ultraviolet region are said to be free from these phenomena.

10.5.6 Effects of Cell Contamination

In fluorescence analysis, the slightest smear on the cell could affect measurement accuracy. Especially, if the cell is left with a sample being loaded inside, the solvent could be evaporated and adhere to the cell wall as persistent smear. In testing an extremely dilute sample, dirt on the external, as well as internal, walls of the cell would be of concern. If a sample solution should contact the external walls of the cell, wipe it off completely with tissue paper (not using a fluorescent dye) before mounting the cell in the cell holder.

10.5.7 Effect of Dissolved Oxygen

Generally, oxygen dissolved in a solvent exerts a marked fluorescence extinction effect on certain samples (quenching). If quenching by dissolved oxygen is not disregarded, solvent degassing is required.
Solvent degassing is conveniently accomplished by blowing a nitrogen gas into the solvent or decompressing the solvent with a vacuum pump.

10.6 Example Scan of Fluorescence Spectrum

Figure 10.2 shows a fluorescence spectrum of an aqueous solution of sodium salicylate as an example.
Generally, the fluorescence spectrum of a dilute solution not only provides a record of the fluorescence of the sample but involves a complication of various emission spectra, involving the scattered light (Rayleigh scattering) that is observed as a result of excitation light being scattered by dust or molecules in the solution, Raman scattered light of water, fluorescence of the solvent, impurities, and sodium salicylate, and second order light of the scattered light as shown in Fig. 10.2.

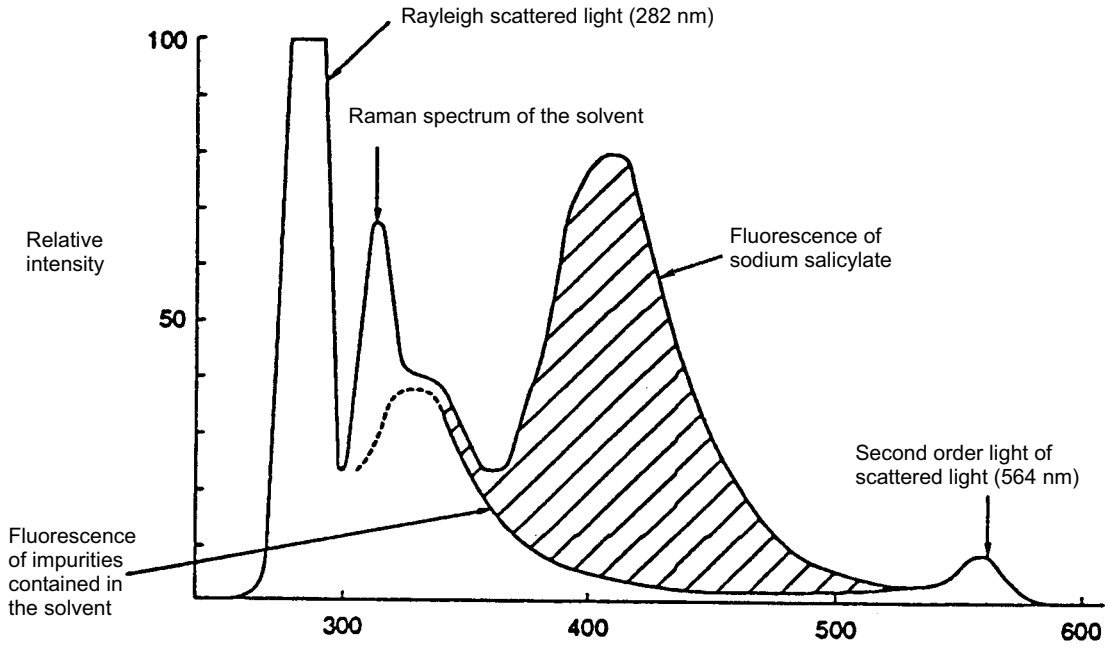


Fig. 10.2 Spectrum of a sodium salicylate aqueous solution

10.7 Example Scan of Excitation Spectrum

Figure 10.3 shows the excitation spectrum of a sodium salicylate aqueous solution. Excitation at the peak of 302 nm is found to maximize flurescence with the highest excitation efficiency. The peak of 405 nm is caused by a scattering of the excitation light.
Since the peak of the excitation spectrum and that of the absorption spectrum correspond essentially, the peak wavelength of the excitation spectrum of a particular sample can be estimated by analogy if its absorption maximum wavelength is known. Because, in precise terms, it is a corrected excitation spectrum that is analogous to the absorption spectrum, the apparent peak of the excitation spectrum does not completely match that of the absorption spectrum.

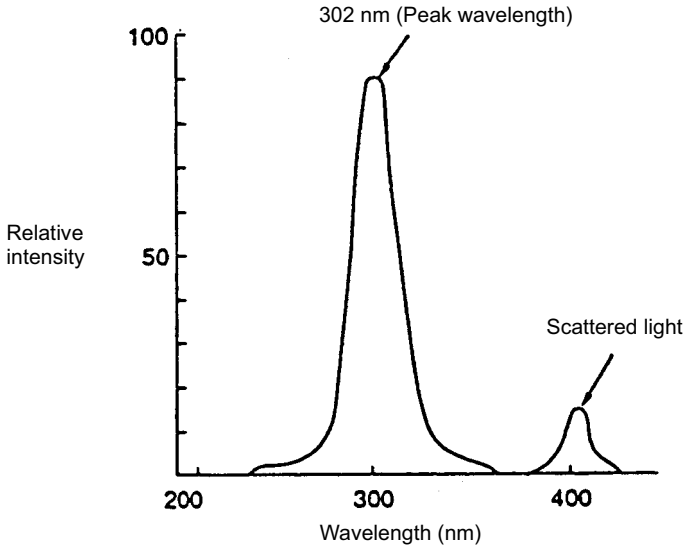


Fig. 10.3 Excitation spectrum of a sodium salicylate aqueous solution

10.8 Working Curve of Fluorescence

According to Foster, the relationship between the intensity and concentration of fluorescence emitted from a point in a cell can be stated in an expression as

$$db(\lambda') = \frac{p}{4\pi n^2} E\lambda F\lambda(\lambda'')K\lambda b\lambda'$$

where

db(λ'):		Intensity of the fluorescence observed at wavelength
n	:	Refractive index
p	:	Reflection coefficient
E	:	Intensity of the excitation light at wavelength
F(λ')	:	True fluorescence intensity at wavelength in the spectrum emitted by the excitation light at wavelength
Kλ	:	Absorbance at wavelength
dλ'	:	Bandwidth of wavelength

Since the absorbance is proportional to the concentration C, this equation can be transformed by integration to

$$B(\lambda') = KC$$

Hence, the calibration curve that is proportional to the concentration C is straight. As the concentration of the sample rises, however, the calibration curve would be curved as explained in (5), "High concentration samples". Fig. 10.4 shows calibration curve observed with a diaminostilbene aqueous solution.

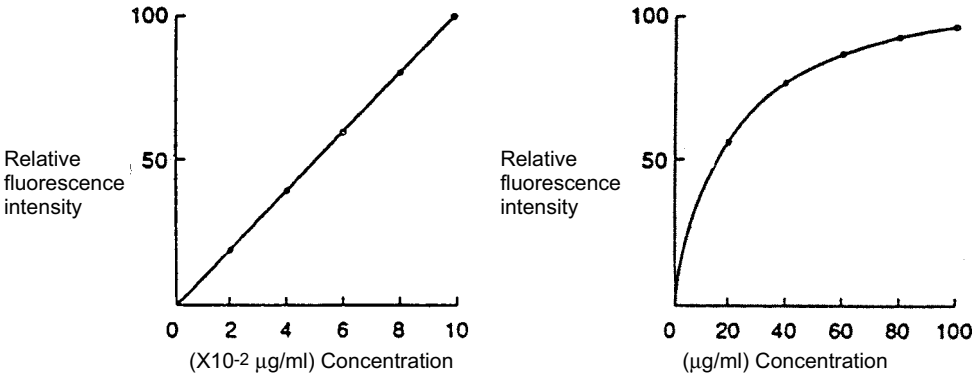


Fig. 10.4 Working lines of a dia minostilbene aqueous solution

(References)

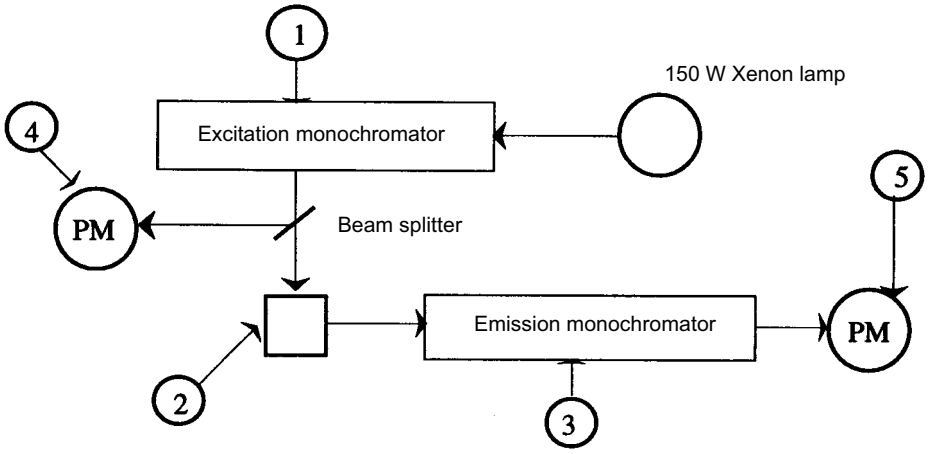
Applied Engineering Division, Shimadzu Corporation, Principles, Application, Application, and Equipment Structure of Fluorescence Analysis, Shimadzu Fluorescence Analysis Course, Shimadzu Corporation.

Chapter 11 Instrument Summarized

CONTENTS

11.1 About the Spectrofluorophotometer

The spectrofluorophotometer irradiates a sample with excitation light and measures the fluorescence emitted from the irradiated sample to perform a qualitative or quantitative analysis. A typical configuration of the spectrofluorophotometer is schematically described below (see Fig. 11.1) taking the RF-5301PC instrument as an example.



(1) Excitation monochromator
(2) Cell holder
(3) Emission monochromator
(4) Monitor side photomultiplier tube
(5) Fluorescence side photomultiplier tube

Fig. 11.1 Constitution of RF-5301PC

The excitation monochromator (1) isolates a band of a particular wavelength from the light from the Xenon lamp to obtain excitation light. Since brighter excitation light will contribute to higher sensitivity of the spectrofluorophotometer, the excitation monochromator incorporates a diffraction grating with a larger aperture to collect the largest possible amount of light.
The cell holder (2) holds a cell filled with sample.
The emission monochromator (3) selectively receives fluorescence emitted from the sample and its photomultiplier tube measures the intensity of the fluorescence. This monochromator has a diffraction grating whose size is the same as that of the excitation monochromator to collect the greatest possible amount of light.
The photomultiplier tube (4) is for monitoring. Generally, the Xenon lamps used on spectrofluorophotometers are characterized by very high emission intensity and an uninterrupted radiation spectrum. However, their tendency to unstable light emission will result in greater signal noise if no countermeasure is incorporated. In addition, the non-uniformity in the radiation spectrum of the Xenon lamp and in the spectral sensitivity characteristics of the photomultiplier tube (these criteria are generally called instrument functions) causes distortion in the spectrum. To overcome these factors, the photomultiplier tube (4) monitors a portion of excitation light and feeds the resultant signal back to the photomultiplier tube (5) for fluorescence scanning. (This scheme is called the light-source compensation system.)

11.2 Optical System of Spectrofluorophotometer

The optical system of the RF-5301PC instrument is illustrated in Fig. 11.2. A 150 W Xenon lamp (1) serves as the light source. The uniquely designed lamp housing contains generated ozone in it and decomposes the ozone by means of the heat produced by the lamp. The bright spot on the Xenon lamp is magnified and converged by the ellipsoidal mirror (2) and then further converged on the inlet slit of the slit Assy. (excitation side) (3) by the concave mirror (4). A portion of the light isolated by the concave grating (5) passes through the outlet slit, travels through the condenser lens (11) and illuminates the sample cell. (The concave grating in both the monochromators is a highly-efficient ion-blazed holographic grating, a product of Shimadzu's unique optics technology.) To achieve light-source compensation, a portion of the excitation light is reflected by the beam splitter quartz plate (6) and directed to the Teflon reflector plate 1 (7). The diffusely reflected light from the reflector plate 1 (7) then passes through the aperture for light quantity balancing (21) and illuminates the Teflon reflector plate 2 (8). Reflected by the reflector plate 2 (8), the diffuse light is attenuated to a specific ratio by the optical attenuator (9) and then reaches the photomultiplier for monitoring (10).
The fluorescence occurring on the cell is directed through the lens (13) to the emission monochromator that comprises the slit Assy. (14) and the concave grating (15). Then, the isolated light is introduced through the concave mirror (16) into the photomultiplier for photometry (17) and the resultant electrical signal is fed to the preamplifier.

① Xenon lamp, 150 W
② Ellipsoidal mirror, SiO2-coated
③ Slit Assy., excitation side
④ Concave mirror
⑤ Concave grating (for excitation)
⑥ Beam splitter quartz plate
⑦ Teflon reflector plate 1
⑧ Teflon reflector plate 2
⑨ Optical attenuator
⑩ Photomultiplier for monitoring, R212-14
⑪ Condenser lens (dual-lens)
⑫ Cell
⑬ Condenser lens
⑭ Slit Assy., emission side
⑮ Concave grating (for emission)
⑯ Concave mirror
⑰ Photomultiplier for photometry, R3788-02
⑱ Focal point
⑲ Inlet slit
⑳ Outlet slit
㉑ Aperture for light quantity balancing

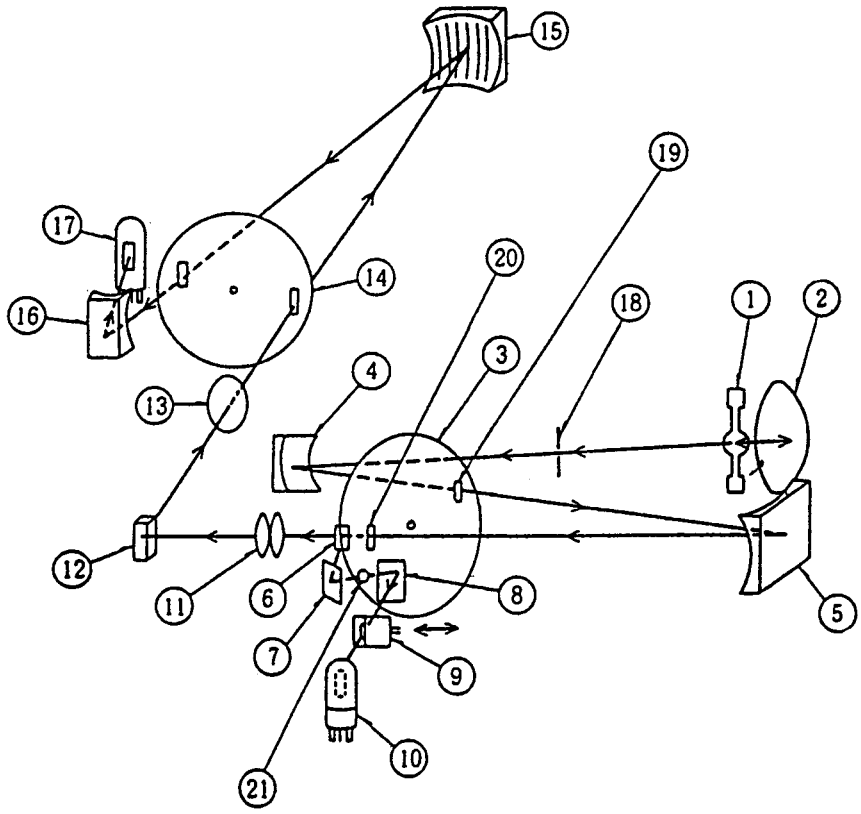


Fig. 11.2 Optical System of RF-5301PC

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Record of Revision

Date	Revision	Changed Page	Description
3/1995	A	Change of the check method of wavelength correctness.	Correction.
6/1995	B	PL/CE correspondence.	PL/CE
11/1996	C	Large reexamination of the contents.	Correction
4/1997	D	The addition of the Windows 95 installation method.	Addition
6/1999	E	Parameter change of a maintenance.	Correction
12/2000	F	CE2001 correspondence.	CE
11/2003	G	The evasion method of a printing error is added.	Addition
2/2004	H	A: Record of Revision	
1/2006	J	NOTE for WEEE mark was added.	
8/2006	K	Modification in layout Modification in phrases, pointed out by a native checker	
3/2008	L	1-2, 2-5, 2-6, 4-3, 4-4, 6-2, 6-9	Correction

Note)

A ...Added Page No.
D ...Deleted Page No.