206-97851
Mar. 2015

Shimadzu Spectrofluorophotometer

RF-6000

Instruction Manual

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

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Introduction

Read this Instruction Manual thoroughly before using the product.

Thank you for purchasing this product.

This manual describes how to use this product.

Read this manual thoroughly before using the product and operate the product in accordance with the instructions in this manual.

The following instruction manuals are included with the product.

Document Name	Document No.	Description
Shimadzu Spectrofluorophotometer RF-6000 Instruction Manual (this manual)	206-97851	This manual explains installation, environment settings, and troubleshooting for the LabSolutions RF software.
LabSolutions RF Instruction Manual [Basic Operation Guide]	207-97926	This manual explains specifically how to perform basic operations using the LabSolutions RF software.

Keep this manual for future reference.

Important

- If the user or usage location changes, ensure that this manual is always kept together with the product.
- If this manual or a product warning label is lost or damaged, immediately contact your Shimadzu representative to request a replacement.
- To ensure safe operation, read "Safety Instructions" thoroughly before using the product.
- To ensure safe operation, contact your Shimadzu representative if product installation, adjustment, re-installation (after the product is moved), or repair is required.

Notice

- Information in this manual is subject to change without notice and does not represent a commitment on the part of the vendor.
- Any errors or omissions which may have occurred in this manual despite the utmost care taken in its production will be corrected as soon as possible, although not necessarily immediately after detection.
-
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Original version is approved in English.

Indications Used in This Manual

Warnings, cautions, and notes are indicated using the following conventions:

Indication	Meaning
⚠ 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.

The following symbols are used in this manual:

Indication	Meaning
Prohibition	Indicates an action that must not be performed.
Instruction	Indicates an action that must be performed.
Hint	Indicates information provided to improve product performance.
Reference	Indicates the location of related reference information.

Safety Instructions

To ensure safe product operation, read these important safety instructions carefully before use and follow all WARNING and CAUTION instructions given in this section.

■ Product Applications

⚠ WARNING

Only use this product for its intended purpose.

This product is a spectrofluorophotometer.
Using the product for any other purpose may result in accidents.

Safety regulations and standards

For notifications on installation and safety controls, follow the necessary procedures in compliance with the laws and regulations applicable in the country where the product is used.

■ Installation Site

⚠ WARNING

If using the product with dangerous samples that are inflammable or toxic, install equipment that can provide adequate indoor ventilation.

⚠ CAUTION

The weight of this product is 38 kg. A separate personal computer (hereafter abbreviated to "PC") is required to control the product. Install the product while taking into account the combined weight of other devices, such as the PC, monitor, and any options.

Prepare a table or bench that is level, stable, and strong enough to support the combined weight of all of these devices. The installation area that includes the product (width: 610 mm, depth: 565 mm), PC, 17" LCD monitor, and any options should have an approximate width of 1,100 mm and depth of 600 mm or more.
Failing to meet the above requirements may result in accidents where the table or bench collapses or units fall off.

Install the product away from walls at a distance of at least 50 mm on the left side and 100 mm on the right side.

The product has a cooling fan exhaust on the right side and a cooling air intake on the left side. Insufficient space on either side will prevent sufficient air cooling and may impair performance.
The power switch is located on the right side. Insufficient space can result in accidents due to difficulty in operating the power switch in an emergency.

⚠ CAUTION

Do NOT install this product in a location where there is corrosive gas, contaminants, or a lot of dust.

Such a location may impair the performance and shorten the service life of the product.

Install the product in a location that satisfies the following conditions in order to preserve performance.

- A location with temperatures within the range of 15 to 35 °C and minimal change in ambient temperature throughout the day
- A location where the product is not directly subject to cool air from air conditioning
- A location free from vibrations
- A location with humidity within the range of 30% to 80% and no condensation (however, when using the product with an ambient temperature of 30 °C or more, humidity must be 70% or less)
- An indoor location that corresponds to installation category II and pollution degree 2 standards and at an altitude of 2,000 m or less.

■ Installation

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

⚠ WARNING

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

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

Ground the instrument.

Grounding is necessary to prevent electric shock in the event of an equipment failure or short circuit, and important for ensuring stable operation.

⚠ WARNING

The power supply voltage is indicated on the power supply connector on the right side of the product. Only connect the product to a power supply of the voltage indicated. The power supply capacity required for the separate control PC and monitor must also be taken into account.

Otherwise, a fire or electric shock may 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.

Power Supply Voltage (indicated on the main unit)	Power Consumption	Frequency
100-240 V AC (100-240 V ∼)	300 VA	50/60 Hz

Do NOT place heavy objects on the power cord, and keep any hot items away. Do NOT modify the power cord in any way. Do NOT bend it excessively or pull on it.

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

Always use the power cord designated by Shimadzu.

⚠ CAUTION

Be aware of gaps around the instrument during installation.

If your fingers get caught, it may result in an injury.

Install the xenon arc lamp before turning ON the power switch.

The product is delivered without the xenon arc lamp installed. The product may be damaged if the power switch is turned ON before installing the xenon arc lamp.

■ Operation

⚠ WARNING

Do NOT measure explosive, ignitable, or flammable materials or inject them into the instrument.

They may ignite and cause a fire.

Do NOT use flammable sprays (hair sprays, insecticide sprays, etc.) near the product.

They may ignite and cause a fire.

Always wear protective gloves and eyewear when handling any toxic or biologically infectious samples.

We recommend using an air tight cell when handling any toxic, biologically infectious, or potentially flammable samples.

Do NOT spill any liquid on a PC or other electronic office equipment for use with the instrument.

Otherwise a fire or electric shock may result.

Do NOT place heavy objects on the instrument.

Do NOT place heavy objects such as a laptop PC on the instrument. Otherwise measurement results may be incorrect.

⚠ CAUTION

If any liquid such as water or organic solvent is spilt on the instrument, wipe it off immediately.

Spilt liquid can cause the electrical systems and functions of the instrument to break down.

If any sample is spilt, clean it up according to the handling and disposal methods described in the sample's SDS (safety datasheet).

If spilt sample is not cleaned up, vaporized sample will fill the sample chamber and may become a health risk. In addition, spilt sample may corrode the instrument internals and prevent the acquisition of correct measurement results.

Always create a backup to protect critical data from accidents.

The contents of the hard disk of the PC may be lost as a result of an unforeseen accident.

Ground the PC and any options with equal potential grounds.

Otherwise communications between units may be interrupted and equipment failures may occur.

NOTE Do NOT use cellular phones near this product. The use of cellular phones may cause abnormal data to be generated.

■ Inspection and Maintenance

⚠ WARNING

Prohibition

Never remove the main cover.

This may cause an injury or equipment failure.
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.

Instruction

Always turn OFF power to the product and unplug the power plug before performing inspections, maintenance, or part replacement.

Otherwise, electric shock or short-circuit accidents may occur.

Instruction

If the power plug gets dusty, remove the plug from the power outlet and wipe away the dust with a dry cloth.

Fire may break out if the power plug is used with dust on it.

Instruction

When replacing parts, use the parts described in "1.2 Package Contents" P.2 and "7 Maintenance Parts List" P.52.

If you use any other part, surrounding parts may be damaged, preventing normal use of the instrument.

⚠ CAUTION

Prohibition

Do NOT leave the product in a wet state or wipe the product with alcohol or thinner solvents.

Doing so may cause rust or discoloration.

NOTE Dispose of waste liquid properly and in accordance with the directions provided by your administrative department.

■ Repair, Disassembly and Modification

⚠ CAUTION

Prohibition

Do NOT modify or disassemble the product without permission.

This may result in accidents due to electric shock or short circuits. It may also result in an injury or equipment failure.

Instruction

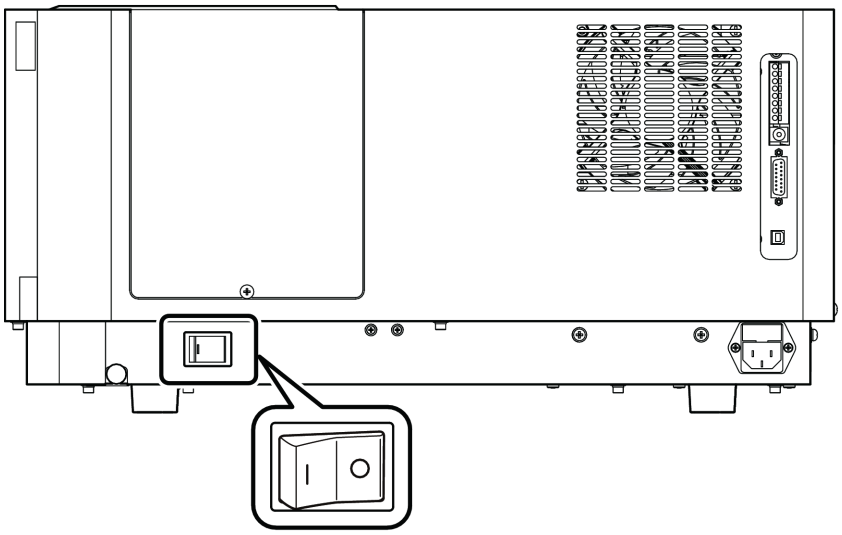
When repair is necessary, request your Shimadzu representative.

Failing to do so may result in an ignition, electric shock, or injury.

■ In an Emergency

If a problem is encountered, turn OFF the power switch according to the following procedure.

1 **Press the power switch, located on the lower section on the right side of the instrument, to the "O" position.**



How to Turn OFF the Power Switch in an Emergency

2 **Remove the power cord from lower section on the right side of the instrument.**

■ During a Power Outage

Take the following measures in the event of a power outage.

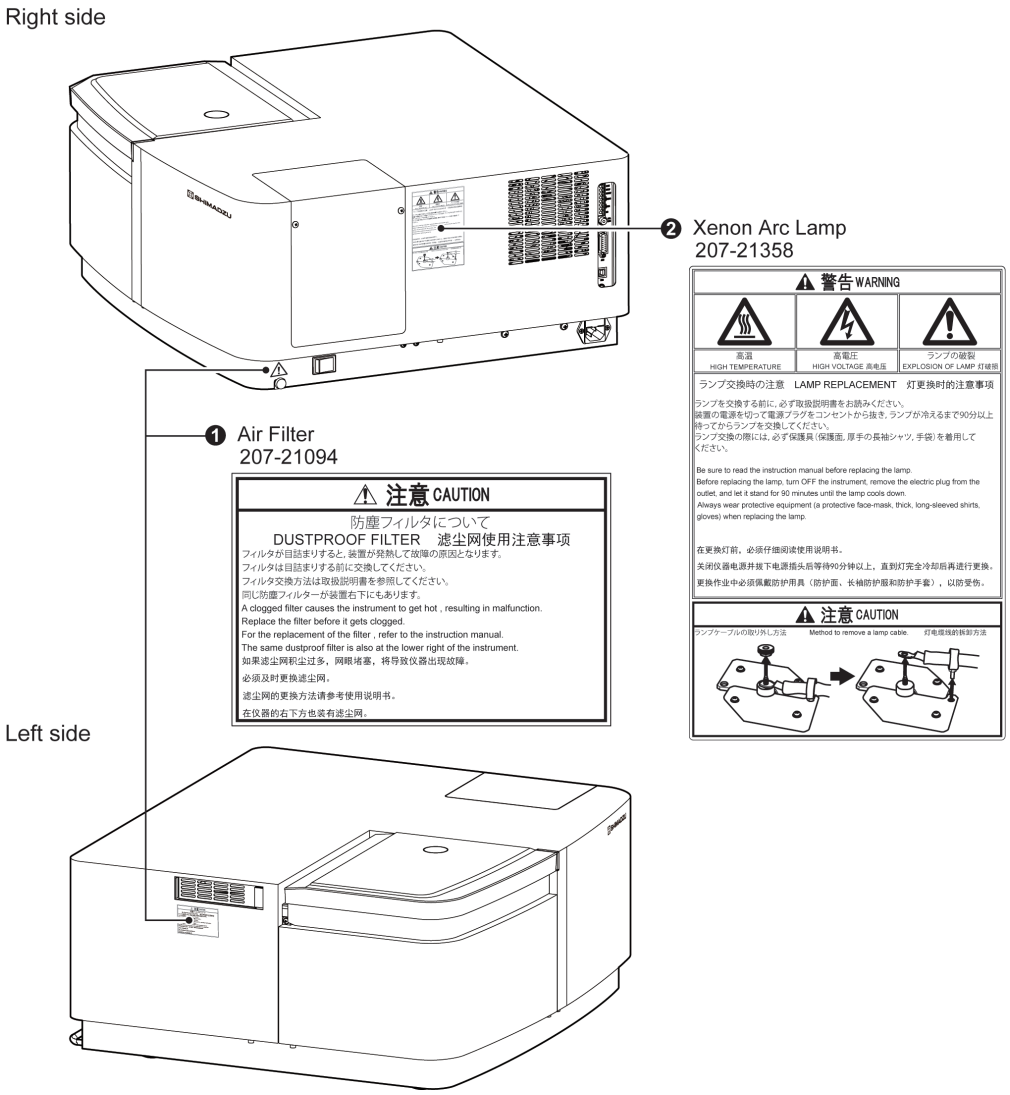
1 **Press the power switch, located on the lower section on the right side of the instrument, to the "O" position.**

2 **After power is restored, check the points described in the "Operation" section and then start the instrument normally.**

■ Warning Labels

In order to ensure safety, warning labels are attached in places requiring caution.

If a warning label is lost or damaged, obtain a new label through your Shimadzu representative and attach it in the correct position.



No.	Description
❶	(Part No.: 207-21358) Notes on the Air Filter (Dustproof filter) A clogged air filter will cause the instrument to heat up and may lead to instrument failure. Replace the air filter before it becomes clogged. An identical air filter (dust filter) is also installed on the lower right of the instrument. ▶▶ Reference "5.4 Air filter replacement" P.37
❷	(Part No.: 207-21094) Precautions for Replacing the Xenon Arc Lamp Turn OFF power to the instrument, remove the power plug from the outlet, and wait at least 90 minutes for the xenon arc lamp to cool down before replacing the lamp. When replacing the xenon arc lamp, always wear the following protective gear: a protective mask, thick long-sleeved shirt, and safety gloves. ▶▶ Reference "5.3.2 Replacing the Xenon Arc Lamp" P.32

Warranty

Shimadzu provides the following warranty for this product.

1. Period:

Please contact your Shimadzu representative for information about the period of this warranty.

2. Description:

If a product/part failure occurs for reasons attributable to Shimadzu during the warranty period, Shimadzu will repair or replace the product/part free of charge. However, in the case of products which are usually available on the market only for a short time, such as personal computers and their peripherals/parts, Shimadzu may not be able to provide identical replacement products.

3. Limitation of Liability:

(1) In no event will Shimadzu be liable for any lost revenue, profit or data, or for special, indirect, consequential, incidental or punitive damages, however caused regardless of the theory of liability, arising out of or related to the use of or inability to use the product, even if Shimadzu has been advised of the possibility of such damage.

(2) In no event will Shimadzu's liability to you, whether in contract, tort (including negligence), or otherwise, exceed the amount you paid for the product.

4. Exceptions:

Failures caused by the following are excluded from the warranty, even if they occur during the warranty period.

(1) Improper product handling

(2) Repairs or modifications performed by parties other than Shimadzu or Shimadzu designated companies

(3) Product use in combination with hardware or software other than that designated by Shimadzu

(4) Computer viruses leading to device failures and damage to data and software, including the product's basic software

(5) Power failures, including power outages and sudden voltage drops, leading to device failures and damage to data and software, including the product's basic software

(6) Turning OFF the product without following the proper shutdown procedure leading to device failures and damage to data and software, including the product's basic software

(7) Reasons unrelated to the product itself

(8) Product use in harsh environments, such as those subject to high temperatures or humidity levels, corrosive gases, or strong vibrations

(9) Fires, earthquakes, or any other act of nature, contamination by radioactive or hazardous substances, or any other force majeure event, including wars, riots, and crimes

(10) Product movement or transportation after installation

(11) Consumable items

Recording media such as floppy disks and CD-ROMs are considered consumable items.

* If there is a document such as a warranty provided with the product, or there is a separate contract agreed upon that includes warranty conditions, the provisions of those documents shall apply.

After-Sales Service and Availability of Replacement Parts

■ After-Sales Service

If any problem occurs with this product, perform an inspection and take appropriate corrective action as described in "6 Troubleshooting" P.41 of this manual.

If the problem persists, or the symptoms are not covered in the troubleshooting section, contact your Shimadzu representative.

■ Replacement Parts Availability

Replacement parts for this product will be available for a period of seven (7) years after the product is discontinued. Thereafter, such parts may cease to be available.

If Shimadzu receives notice of the discontinuation of units or parts, the necessary quantity for the above period is immediately calculated and secured. However, such units or parts may cease to be available within seven years after the discontinuation of the product, depending on the conditions of individual manufacturers and on changes in the quantity required.

Maintenance, Inspections, and Adjustment

In order to maintain the instrument's performance and obtain accurate measurement data, daily inspection and periodic inspection are necessary.

- For daily maintenance, inspection, and replacement parts, see "5 Maintenance and Inspection" P.27 of this manual.
- Periodic inspection should be requested to your Shimadzu representative.
- Replacement cycles described for periodic replacement parts are rough estimate. Replacement may be required earlier than the described replacement cycles depending on usage environment and frequency.

Disposal Precautions

Dispose of this product and any xenon arc lamps as industrial waste.

Dispose of this product yourself according to the waste disposal laws and regulations in your country or municipality or consign disposal to a suitable and authorized industrial waste disposal company.

■ Materials contained in the 150 W xenon arc lamp

The raw materials used in the xenon arc lamps of this product are listed below.

Lamp	Materials Used
Xenon arc lamp	• Metal (tungsten, brass, nickel-plated brass, stainless steel and nickel-plated zinc alloy) • Quartz glass

■ Disposal of xenon arc lamps

High-pressure xenon gas is enclosed in the xenon arc lamp. If it explodes, fragments of the lamp may scatter.

Dispose of the lamp according to the following procedure.

⚠ WARNING

Always wear the following protective gear: a protective mask, thick long-sleeved shirt, and safety gloves.

High-pressure gas is enclosed in the xenon arc lamp. If the lamp breaks, fragments of the glass may scatter and could cause injury.
Use a protective mask that covers your entire face with rigid plastic or similar material.

Keep the spent xenon arc lamp in the box in which it was packed at the time of delivery until you break it.

1 **Carefully wrap up the xenon arc lamp in a thick cloth to prevent fragments of glass scattering.**

2 **Strike the glass part of the xenon arc lamp, wrapped in the cloth, with a hammer to break it.**

3 **Dispose of the broken xenon arc lamp as industrial waste that is to be handled separately from general household waste.**

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1 Overview

The Shimadzu Spectrofluorophotometer is developed for high-performance and multi-function analysis. All functions are controlled from a PC using the LabSolutions RF software that provides excellent operability.

This instruction manual describes installation, operation, and maintenance of this instrument.

Reference For details on analysis operations using the LabSolutions RF software, refer to the "LabSolutions RF Instruction Manual (Basic Operation Guide)".

1.1 Features

■ Capable of fluorescence measurement at all wavelengths in the 200 to 900 nm range immediately after installation

The R928 model photomultiplier is installed as standard as the detector for fluorescence measurement.
This detector enables measurement across a wide range immediately after installation.

■ Excellent S/N performance

Excellent S/N ratio performance is achieved through improvements to the optical system and the design of electronics related to the detector.

■ Easy analysis using the LabSolutions RF software that provides excellent operability

Analysis is straightforward when using the LabSolutions RF software that features a user interface (UI) focused on operability. A 3D measurement function is included as standard and facilitates fluorescence measurement of unknown samples.

■ Equipped with a long-life xenon arc lamp

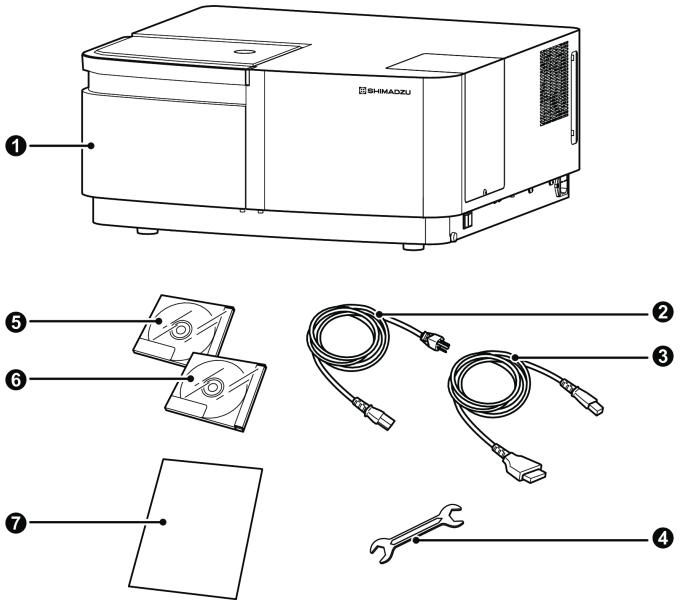
A long-life xenon arc lamp is installed on the RF-6000. This type of lamp reduces the frequency of replacement and shortens maintenance time. Even when the lamp needs replacing, the task of replacement is easy because position adjustment is not required after replacement.
The lamp illumination time is accurately recorded by the instrument and the LabSolutions RF software displays an alert when the time for replacement has been reached.

■ Incorporates a self-validation function

The LabSolutions RF software features a validation function for performance diagnostics. When using an optional Hg lamp unit, performance checks, which include wavelength accuracy, repeatability and resolution, can be easily performed.

1.2 Package Contents

This product consists of the following parts. Confirm that the package includes the correct number of each part.



Standard Contents

No.	Part Name	Part No.	Q'ty
❶	RF-6000 spectrofluorophotometer unit	S207-20400-58	1
❷	Power cord (120 V)	S071-60821-08	1
	Power cord (240 V)	S071-60825-51	1
❸	USB cable (2 m)	S088-52848-32	1
❹	Spanner 7×8	S086-03047-04	1
❺	LabSolutions RF software (CD)	S207-21705-92	1
❻	RF-6000 Instruction Manual (CD)	S207-21768-41	1
❼	LabSolutions RF Setup Procedure	S207-90012	1

1.3 Items Required for Operation

A PC that satisfies the specifications listed in the following table is required to use this instrument.

■ Recommended PC Specifications

Item	Description
OS	Windows 7 Professional (English) 32-bit version Windows 7 Professional (English) 64-bit version
CPU	Intel Core, Core2, or equivalent : Processor operation clock frequency 2 GHz min.
	Intel Pentium 4, Pentium D, or equivalent : Processor operation clock frequency 3 GHz min.
RAM	2 GB min.
Hard disk capacity	40 GB min.
Display	SXGA (1,280×1,024) or better resolution, with high color (65,536 colors) or better
Printer	Printer and drivers that are compatible with the installed version of Windows. (A functionally verified printer is recommended.)
Peripheral equipment	Installed with an optical drive that can read CD-ROM and DVD-ROM optical discs.

1.4 Optional Parts

The optional parts available for this instrument are listed below.

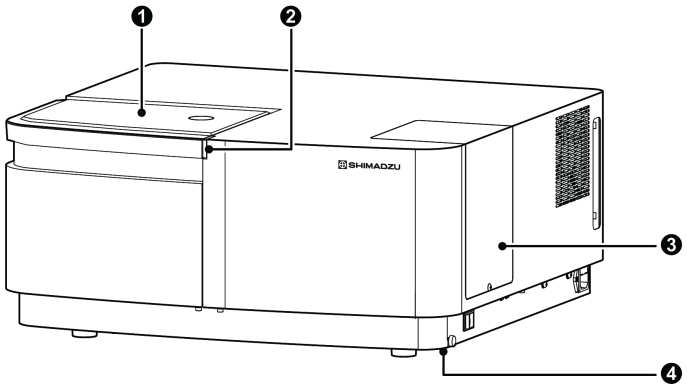
For optional parts that are not listed in the table below and details on optional parts, consult your Shimadzu representative.

Option Name	Part No.	Remarks
Hg lamp unit	S207-21700-41	Used for checking wavelength accuracy. This unit is used by the validation function of the LabSolutions RF software.
Integrating sphere unit	S207-21460-41	A Φ100 mm Spectralon integrating sphere unit for quantum efficiency measurement.
Sipper 6000	S207-21470-41	A peristaltic pump type sipper unit. This unit uses a 120 μL type flow cell. When combined with the ASC-5 autosampler, automatic measurement of 100 specimens can be performed.
Ultra-micro cell unit	S207-21455-41	An ultra-micro cell holder that enables measurement of minute sample quantities. An ultra-micro cell is also included. Sample quantities of approximately 50 μL can be measured.
Constant-temperature single cell holder (with stirrer)	S206-24930-91	A cell holder that maintains the specified temperature using a constant-temperature water circulator. The sample can be agitated using the provided stirrer. The available temperature range is 5 to 70 °C and the minimum required sample quantity is 2.5 mL.
Constant-temperature four-cell holder	S206-24940-91	A manual four-cell holder that maintains the specified temperature using a constant-temperature water circulator. The available temperature range is 5 to 80 °C.
High sensitivity cell holder	S204-26841-01	This cell holder increases fluorescence intensity by two to three times by utilizing the reflection effect of excitation light and fluorescent light with a reflective mirror.
Solid (powder) sample holder	S204-26836-01	In addition to solid and powder samples, a solution cell can be affixed to a special sample plate to perform fluorescence measurement. A light cutting filter for blocking scattered light is also included.
Flow cell (120 μL prism type)	S204-03285-04	A fused quartz flow cell that is used as a fluorescence monitor of eluate from a column chromatograph.
LC flow cell unit (12 μL cell)	S204-05566	This is used as a high sensitivity fluorescence monitor for a high performance liquid chromatograph.

Option Name	Part No.	Remarks
LC flow cell unit (120 μL cell)	S204-06249	This uses a two-sided reflective rectangular type flow cell to perform high sensitivity fluorescence monitoring measurement.
Peripheral device for polarization measurement (for ultraviolet and visible light)	S204-03290	A polarizer unit for measuring the degree of fluorescent polarization. The measurable wavelength range is 240 to 800 nm.
Micro cell unit	S204-27125	A micro cell unit with a minimum required sample quantity of 400 μL. A micro cell and special adapter are included.
Quartz cell polished on four sides (fused quartz)	S200-34441	This is a 10 mm rectangular type cell with four polished sides.
Non-fluorescent cell (special fused quartz)	S200-34594-03	Because fused quartz cells are slightly absorbent around 260 nm, there is weak fluorescence emission at around 400 nm. In particular, use the non-fluorescent cell when measuring low concentration samples at an excitation wavelength around 260 nm.
Filter set	S204-04691	A set of seven light cutting filters for blocking scattered light is also included.
8 ϕ test tube holder	S204-05853	A special holder for test tubes with an outside diameter of 8 ϕ. The minimum required sample quantity is 400 μL. This holder can be used with test tubes longer than 45 mm and shorter than 100 mm.
12 ϕ test tube holder	S204-03293	A special holder for test tubes with an outside diameter of 12 ϕ. This holder can be used with test tubes longer than 60 mm and shorter than 100 mm.
Bottom raising sample plate	S204-04811	By raising the bottom position of the cell, this special sample holder can reduce the minimum required sample quantity. The minimum required sample quantity when using this plate is 1.5 mL. Note that it cannot be used with the micro cell unit.
ASC-5 auto sample changer	S206-23810-91	When combined with the sipper unit, automatic measurement of multiple sample solutions can be performed. The maximum number of samples that can be set is 100.
Constant-temperature water circulator NTT-2200P	S208-97263	This is used to circulate constant-temperature water to the constant-temperature cell holder. The temperature control range is the ambient temperature +15 °C to 80 °C.

2 Part Names and Functions

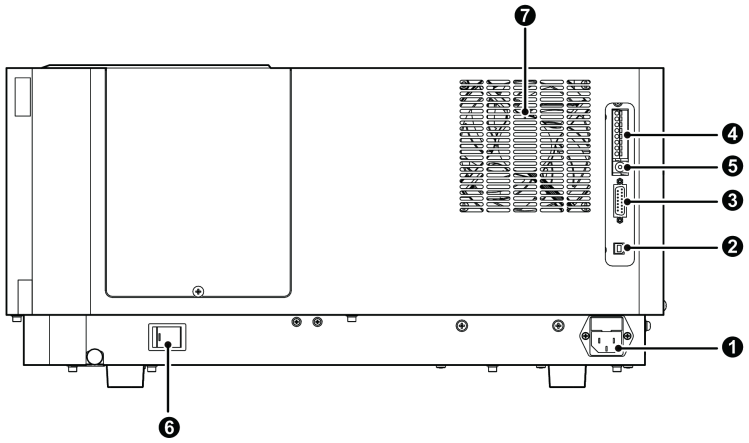
2.1 Front and Top



RF-6000 Front and Top

No.	Name	Description
❶	Sample chamber lid	Open and close this lid when setting the sample for measurement. Measurement cannot be started when the lid is open. If the lid is opened during measurement, measurement is aborted. Reference "2.4 Sample Chamber" P.9
❷	LED status indicator	Indicates the current status using different colors when power to the instrument is turned ON. • Normal state: Lit or blinking green. • Abnormal state: Lit or blinking red. • During measurement: Lit blue. Reference For details on status colors, see "6.3 Troubleshooting and Corrective Actions" P.43.
❸	Lamp housing lid	Open and close this lid when installing or replacing the xenon arc lamp. Reference "2.5 Lamp Housing" P.10 "5.3.2 Replacing the Xenon Arc Lamp" P.32
❹	Air intake	An air intake for cooling the lamp housing. It is fitted with an air filter to prevent the intake of dust. Reference "5.4 Air filter replacement" P.37

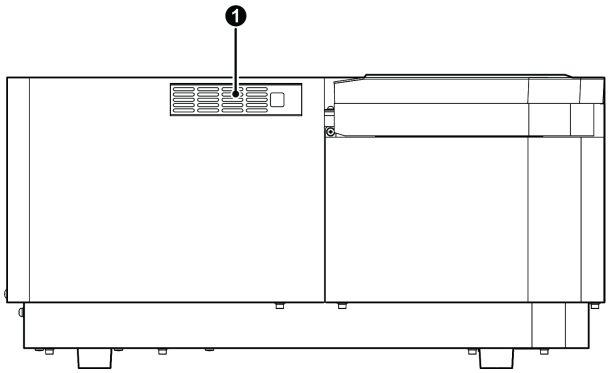
2.2 Right Side



RF-6000 Right Side

No.	Name	Description
❶	Power supply connector	Connect the provided power cord and supply power from an outlet. Reference "3.2 Power Supply Connection" P.13
❷	USB connector (to PC)	This connector is used to connect to the PC. Do not connect the PC and instrument until the USB driver has been installed on the PC. Reference "3.4.2 Connecting the USB Cable" P.19
❸	I/O connector	This is used to connect to the ASC-5 auto sample changer.
❹	External input/output terminals	Measurement can be controlled with external contact input.
❺	Analog output connector	This is used to connect to an external device, such as a recorder or chromatopac (integrator).
❻	Power switch	Turn power to the instrument ON and OFF. Power is ON when the switch is at the "I" position and OFF at the "O" position.
❼	Cooling fan	This fan cools the instrument interior.

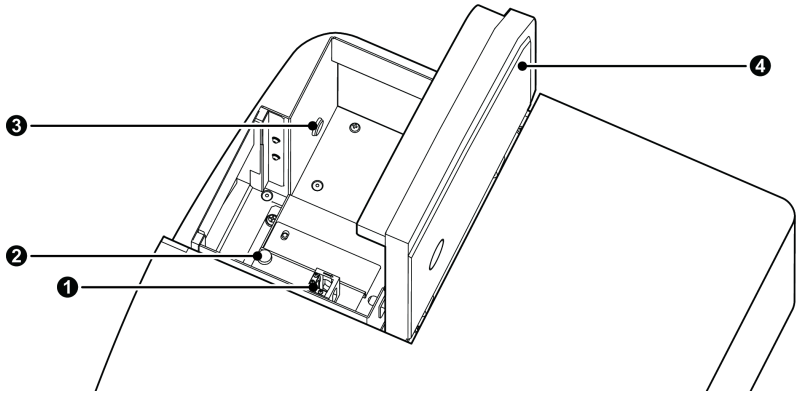
2.3 Left Side



RF-6000 Left Side

No.	Name	Description
❶	Air intake	An air intake for cooling the instrument interior. It is fitted with an air filter to prevent the intake of dust. Reference "5.4 Air filter replacement" P.37

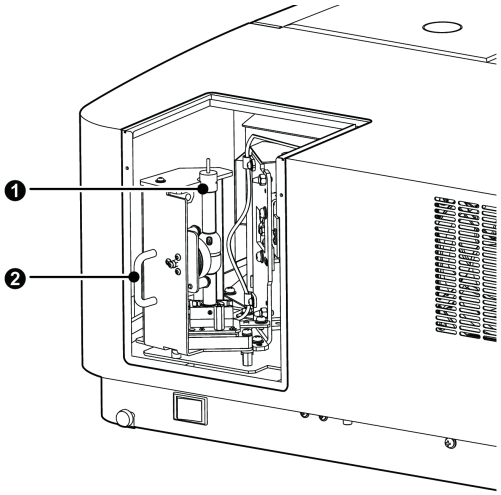
2.4 Sample Chamber



RF-6000 Sample Chamber

No.	Name	Description
❶	Cell holder	Set 10 mm rectangular type cells on this holder.
❷	Cell holder fixing screw	Options can be installed by removing the cell holder fixing screw.
❸	Sipper connector	This is used to connect to the optional sipper 6000. Connection of the sipper 6000 is performed by a Shimadzu service personnel.
❹	Sample chamber cover	Remove this cover when installing options into the sample chamber. NOTE The sample chamber cover is fitted with an open/close sensor. The detector protection function will activate if the sample chamber cover is open. Accurate measurement cannot be performed unless the cover is completely closed.

2.5 Lamp Housing



RF-6000 Lamp Housing

No.	Name	Description
❶	Xenon arc lamp	A 150 W xenon arc lamp. Reference For details on replacing the xenon arc lamp, see "5.3.2 Replacing the Xenon Arc Lamp" P.32.
❷	Lamp unit pullout handle	This handle is used to pull out the lamp unit when replacing the xenon arc lamp. Reference For details on replacing the xenon arc lamp, see "5.3.2 Replacing the Xenon Arc Lamp" P.32.

NOTE When the lamp housing cover is removed, the xenon arc lamp will not turn on for safety reasons.

3 Installation

3.1 Preparation for Installation

3.1.1 Suitable Locations and Preparation

To ensure safe operation, install the instrument in a suitable location that satisfies the following conditions.

⚠ WARNING

If using the product with dangerous samples that are inflammable or toxic, install equipment that can provide adequate indoor ventilation.

⚠ CAUTION

Do NOT install this product in a location where there is corrosive gas, contaminants, or a lot of dust.

Such a location may impair the performance and shorten the service life of the product.

Keep away from equipment that generates strong magnetic fields.

Failure to do so may prevent the instrument from working properly. If the power supply line is subject to noise, add a noise filter.

Install the product in a location that satisfies the following conditions in order to preserve performance.

- A location with temperatures within the range of 15 to 35 °C and minimal change in ambient temperature throughout the day
- A location where the product is not directly subject to cool air from air conditioning
- A location free from vibrations
- A location with humidity within the range of 30% to 80% and no condensation (however, when using the product with an ambient temperature of 30 °C or more, humidity must be 70% or less)
- An indoor location that corresponds to installation category II and pollution degree 2 standards and at an altitude of 2,000 m or less.

3.1.2 Installation Space

⚠ CAUTION

Instruction

The weight of this product is 38 kg. A separate PC is required to control this product. Install this product while taking into account the combined weight of other devices, such as the PC, monitor, and any options.

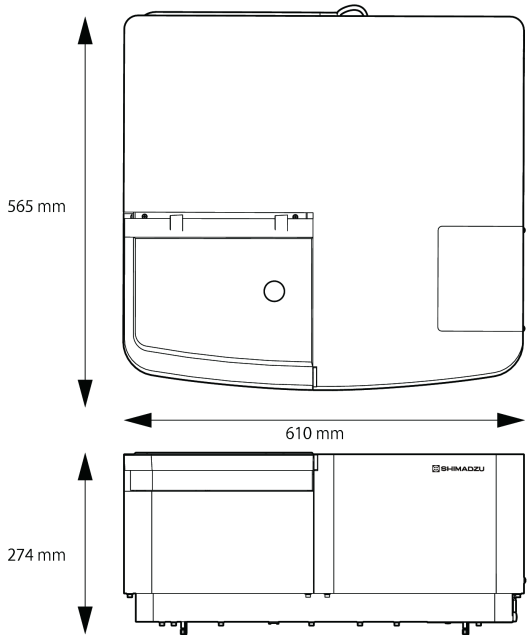
Prepare a table or bench that is level, stable, and strong enough to support the combined weight of all of these devices. The installation area that includes the product (width: 610 mm, depth: 565 mm), PC, 17" LCD monitor, and any options should have an approximate width of 1,100 mm and depth of 600 mm or more.
Failing to meet the above requirements may result in accidents where the table or bench collapses or units fall off.

Instruction

Install the product away from walls at a distance of at least 50 mm on the left side and 100 mm on the right side.

The product has a cooling fan exhaust on the right side and a cooling air intake on the left side. Insufficient space on either side will prevent sufficient air cooling and may impair performance.
The power switch is located on the right side. Insufficient space can result in accidents due to difficulty in operating the power switch in an emergency.

The dimensions of the instrument are shown below.



RF-6000 Spectrofluorophotometer Dimensions

3.2 Power Supply Connection

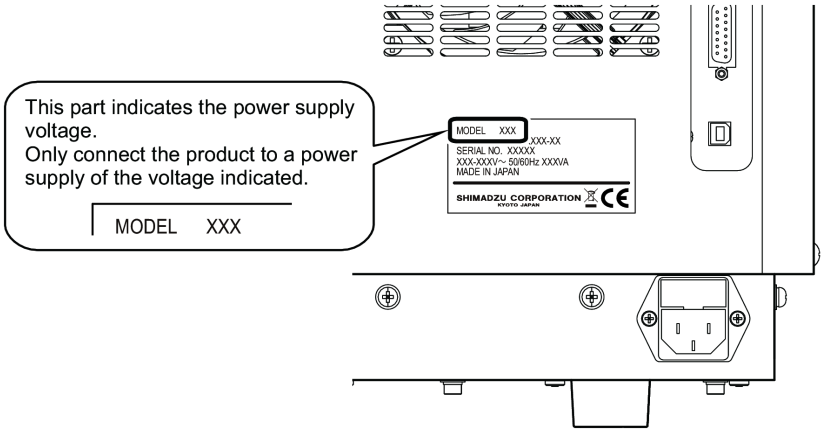
3.2.1 Checking the Power Supply Voltage

⚠ WARNING

Instruction

The power supply voltage is indicated on the power supply connector on the right side of the product. Only connect the product to a power supply of the voltage indicated. The power supply capacity required for the separate control PC and monitor must also be taken into account.

Otherwise, a fire or electric shock may 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.



Position of the Power Supply Voltage Indication

The following table shows the electrical voltage, power consumption, and frequency of the instrument.

Power Supply Voltage (indicated on the main unit)	Power Consumption	Frequency
100-240 V AC (100-240 V ∼)	300 VA	50/60 Hz

Verify that the power outlet to be used for connection has sufficient capacity.

If the capacity is insufficient, a power outage or voltage drop can occur, affecting not only this instrument, but other equipment connected to the same power supply.

The tolerance range for power supply voltage fluctuations is within ±10 %. If power supply voltage exceeds the tolerance range, use a voltage stabilizer.

3.2.2 Connecting to an Outlet

⚠ WARNING

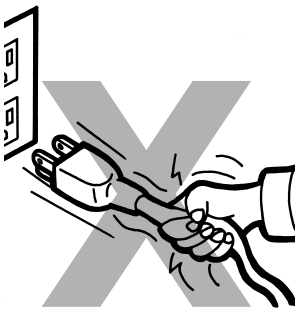
Instruction

Handle the power cord with care.

Observe the following precautions to avoid cord damage, fire, electric shock, or instrument failure.

- Do not put heavy objects on the power cord.
- Keep hot items away from the power cord.
- Do not modify the power cord.
- Do not bend the power cord excessively or pull on it.
- Always pull the plug to unplug the instrument, NOT the power cord.

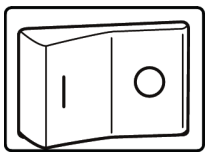
If the power cord is damaged, replace it immediately.



⚠ CAUTION

Instruction

Before connecting the power supply plug to the outlet, make sure that the instrument's power switch is OFF (pressed to the "O" position).



1 **Insert the female power supply plug into the power supply connector on the right side of the instrument.**

2 **Insert the male power supply plug into the outlet.**

3.2.3 Grounding

⚠ WARNING

Instruction

Ground the instrument.

Grounding is necessary to prevent electric shock in the event of an equipment failure or short circuit, and important for ensuring stable operation.

The power cord provided with the instrument has three wires that include a grounding wire. Always connect the power cord to a three-contact power outlet to ensure that the instrument is grounded.

3.3 Installing the Xenon Arc Lamp

⚠ WARNING

When handling the xenon arc lamp, always wear the following protective gear: a protective mask, thick long-sleeved shirt, and safety gloves

- The xenon arc lamp is filled with high-pressure gas. If the xenon arc lamp is subjected to a strong impact or the glass part is damaged, it may explode and scatter fragments.
- Use a protective mask that covers your entire face with rigid plastic or similar material.
- If you happen to touch the glass part with your bare hands, use a piece of gauze, or other suitable material, moistened with ethanol to wipe off any fingerprints. If the xenon arc lamp is lit with fingerprints on the glass part they will burn and may cause the lamp to explode.

Always turn power to the instrument OFF and unplug the power plug before replacing the xenon arc lamp.

An extremely dangerous high voltage of around 30 kV is applied to the positive (+) terminal of the xenon arc lamp at the start of ignition.

Make sure that the xenon arc lamp has cooled sufficiently before attempting to replace it.

The xenon arc lamp is extremely hot immediately after being turned OFF and touching it may result in burns.
The time required for the xenon arc lamp to cool is at least 90 minutes when turning OFF power to the instrument, or at least 30 minutes when manually turning OFF the lamp and leaving power to the instrument turned ON.
In the case when cooling the xenon arc lamp with the power to the instrument is left turned ON, always turn OFF power to the instrument and unplug the power plug before replacing the xenon arc lamp.

⚠ CAUTION

Replace the xenon arc lamp when the cumulative operating time of the lamp has exceeded 2,000 hours.

The xenon arc lamp may explode if it used passed its service life of 2,000 hours. It is dangerous if the xenon arc lamp explodes and units surrounding the lamp may be damaged.

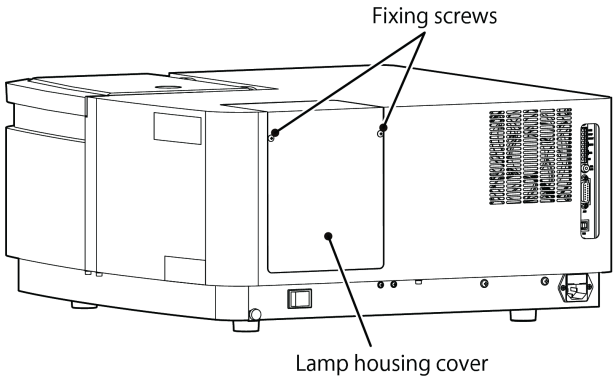
When replacing the xenon arc lamp, take care not to drop any screws.

Otherwise, instrument failure may result.

3.3.1 Installing the Xenon Arc Lamp

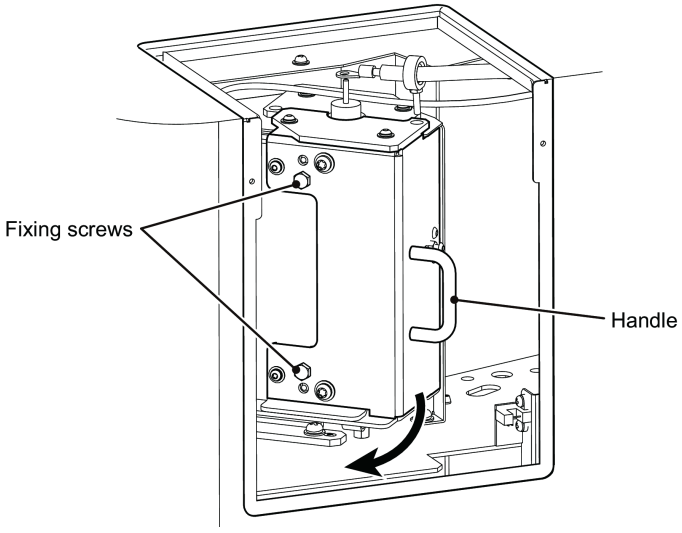
1 **Check that power to the RF-6000 is turned OFF and the male power plug has been unplugged from the outlet.**

2 **Loosen the two fixing screws on the lamp housing cover and remove the cover.**



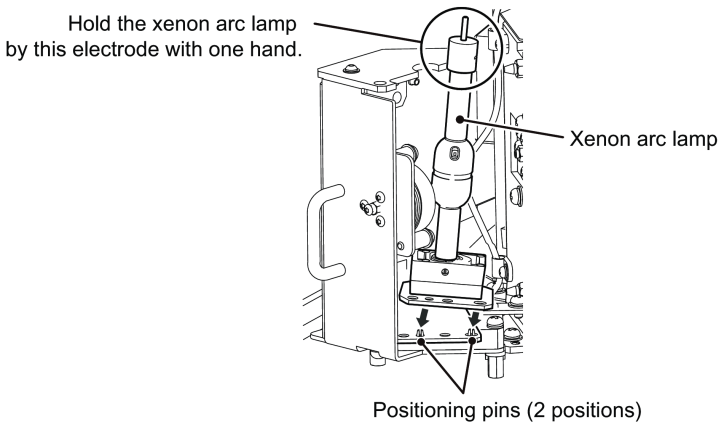
Removing the Lamp Housing Cover

3 **Completely loosen the two fixing screws on the lamp unit using the provided spanner, grip the lamp unit pullout handle, and pull the lamp unit out of the instrument.**



Pulling Out the Lamp Unit

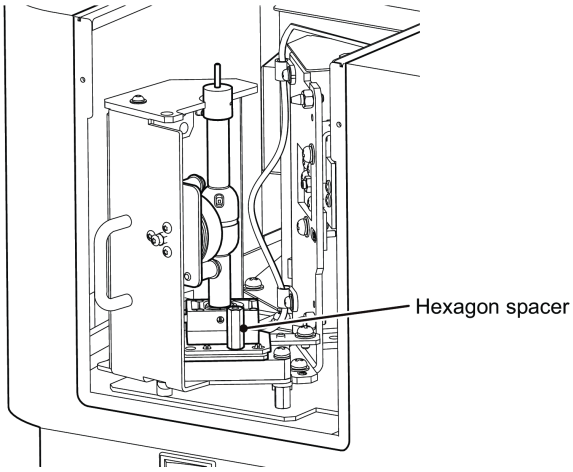
4 **Install the xenon arc lamp by aligning with the two positioning pins on the lamp unit.**



Installing the Lamp Unit

NOTE Hold the xenon arc lamp by the upper electrode with one hand.

5 **Tighten the single hexagon spacer in place with the provided spanner.**



Fixing the Lamp Unit in Place

6 **Grip the handle and return the lamp unit to its original position and then tighten the two fixing screws on the lamp unit using the provided spanner.**

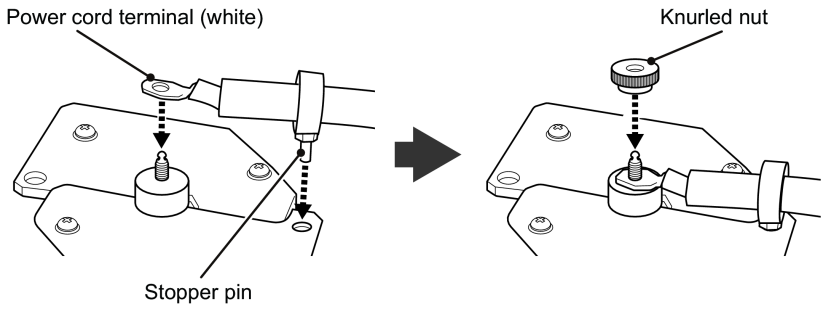
7 **Connect the power cord terminal (white) to the terminal of the xenon arc lamp while setting the stopper pin to the designated position and tighten the knurled nut at the top of the xenon arc lamp.**

⚠ CAUTION

Instruction

Always turn the knurled nut by hand.

Using a tool like a wrench may break the xenon arc lamp.



Connecting the Power Cord Terminal

8 **Attach and fix the lamp housing cover with the two fixing screws.**

3.4 Connecting to LabSolutions RF

The LabSolutions RF software must be set up and configured in order to control the RF-6000.

3.4.1 Installing LabSolutions RF

1 **Insert the installation CD of the LabSolutions RF software into the disc drive.**

2 **Install "LabSolutions RF" according to the onscreen instructions.**

3.4.2 Connecting the USB Cable

NOTE Installing the LabSolutions RF software also installs a USB driver.
After installation of the LabSolutions RF software is complete, connect the instrument and PC using the USB cable.

1 **Check that the task described in "3.4.1 Installing LabSolutions RF" is complete.**

2 **Check that power to the RF-6000 is turned OFF.**

3 **Insert the USB cable into the USB connector on the right side of the RF-6000.**

Reference "2.2 Right Side" P.7

4 **Insert the USB cable into a USB port on the PC.**

5 **Turn ON power to the RF-6000.**

Reference "3.5.1 Starting and Stopping" P.23

3.4.3 Instrument Registration in LabSolutions RF

Register information regarding the instrument to use to LabSolutions RF.

The information selected or entered here is recorded as file information in data files captured using LabSolutions RF.

1 **Turn ON power to the PC.**

A system check is performed and then Windows starts up.

2 **Double-click**  **(LabSolutions RF) on the desktop.**

Hint The software can also be started by clicking the [Start] button, navigating to [All Programs] - [Shimadzu] , and clicking [LabSolutions RF].

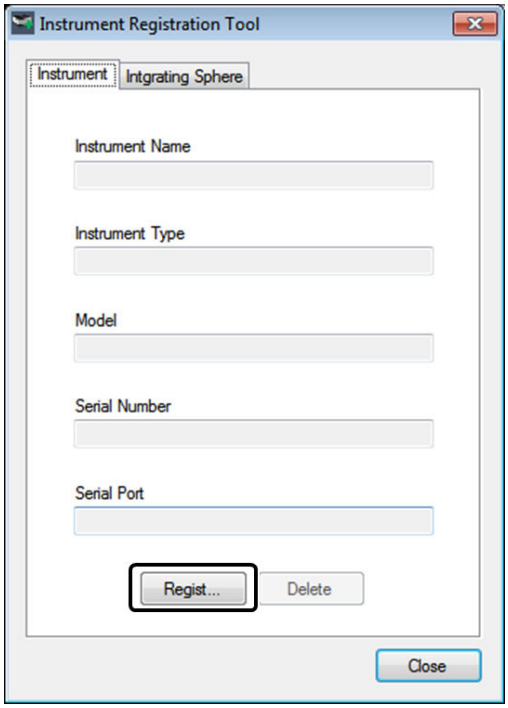
The LabSolutions RF launcher starts.

3 **Click [Register Device] on the [Manage] tab.**

LabSolutions RF Launcher

The [Instrument Registration Tool] window is displayed.

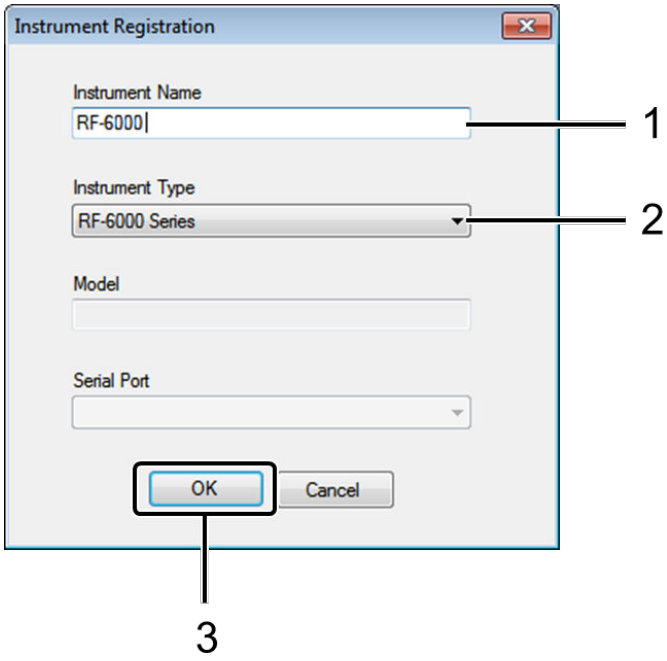
4 Click [Regist] on the [Instrument] tab.



[Instrument Registration Tool] Window

The [Instrument Registration] window is displayed.

5 Register information regarding the instrument to use to LabSolutions RF.



[Instrument Registration] Window

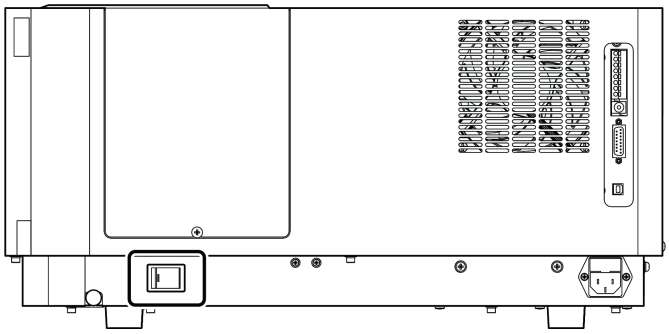
1 Enter "RF-6000" for [Instrument Name].

2 Select [RF-6000 Series] from the [Instrument Type] list.

3 Click [OK].

3.5 Starting, Stopping, and Initialization Operations

3.5.1 Starting and Stopping



RF-6000 Power Switch

■ Startup

1 **Turn ON power to the RF-6000.**

Turning ON power to the RF-6000 starts instrument initialization. The LED indicator blinks green during initialization and changes to solid green when initialization completes correctly.

Reference If the LED indicator displays a color other than green, see "6.1 LED Indicator Status List" P.41 and check the state of the instrument.

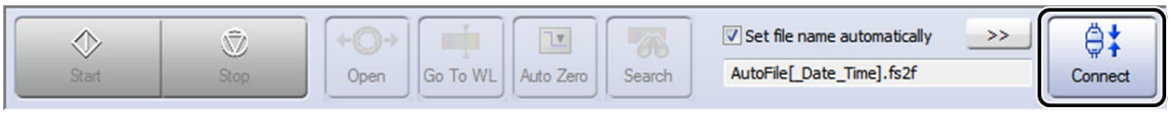
2 **Turn ON power to the PC.**

A system check is performed and then Windows starts up.

3 **Double-click**  **(LabSolutions RF) on the desktop to start the LabSolutions RF launcher.**

4 **Click to select the application to use for measurement from the LabSolutions RF launcher.**

5 **Click [Connect] on the measurement toolbar of the selected application.**



Connecting

6 **Click [OK].**

Measurement can now be performed.

⚠ CAUTION

Connect any options for use in measurement before turning ON power to the instrument.

Options that require a wired connection to the instrument, such as the sipper unit, must be connected to the instrument before turning the power ON. Connecting the wiring of options with the instrument power turned ON may damage the instrument.

■ Stop

1 **If measurement is underway, stop the measurement.**

2 **If LabSolutions RF and the RF-6000 are connected, click [Disconnect].**

Hint LabSolutions RF can also be closed by clicking [Exit] on the [File] menu.

3 **Turn OFF power to the RF-6000.**

3.5.2 Initialization Operation

Initialization operation starts automatically when power to the RF-6000 is turned ON.

When instrument initialization has completed correctly prior to connecting to LabSolutions RF, connecting to LabSolutions RF will change the instrument to the measurement standby state.

Also, the LED indicator on the front of the RF-6000 will light up green when initialization is completed correctly.

If a problem is detected during initialization, the LED indicator on the front of the RF-6000 will blink red. In this case, turn OFF power to the instrument and turn it back ON to perform initialization again.

If the problem persists, contact your Shimadzu representative.

3.6 Checking Performance After Installation

Once installation is complete, Shimadzu service personnel will perform a performance check for the following items.

- Wavelength accuracy
- S/N
- Stability of measured intensity values

4 Preparation

4.1 Precautions on Operation

■ Precautions Prior to Operation

When performing high-sensitivity analysis, allow adequate time for the instrument to stabilize and turn ON the xenon arc lamp in advance.

The xenon arc lamp takes about 1 hour to stabilize after being lit.

■ Precautions During Operation

Always keep all covers closed during measurement.

If a cover is opened or removed during measurement, the safety function activates automatically and stops measurement.

In this case, recover the instrument according to the displayed message.

Reference "6.4 Error Message List" P.47

■ Precautions on Air Conditioning

When performing high-sensitivity analysis, keep the ambient temperature constant.

Do not change the air conditioner setting during analysis.

■ Precautions After Operation

After analysis is complete, do not leave the sample in the sample chamber.

Highly volatile samples will cause contamination of the sample chamber. In addition, when using an option that employs a flow cell, such as the sipper unit, always flush the inside of the flow cell with distilled water to clean it after completing analysis. When planning not to use flow cells over a long period, in order to protect them, store them filled with methanol.

■ Performance Checks

An "S/N check" should be performed daily to maintain instrument performance.

5 Maintenance and Inspection

5.1 Notes on Maintenance and Inspection

This instrument requires maintenance and inspections to ensure safety during use.

It is possible to have periodic inspections performed by Shimadzu service personnel on a contractual basis.

For information regarding a maintenance and inspection contract, contact your Shimadzu representative.

⚠ WARNING

Never remove the main cover.

This may cause an injury or equipment failure.
The main cover does not need to be removed for routine maintenance, inspection, and adjustment. Have your Shimadzu representative perform any repairs that require removal of the main cover.

Always turn OFF power to the product and unplug the power plug before performing inspections, maintenance, or part replacement.

Otherwise, electric shock or short-circuit accidents may occur.

When replacing parts, use the parts described in "1.2 Package Contents" P.2 and "7 Maintenance Parts List" P.52.

If you use any other part, surrounding parts may be damaged, preventing normal use of the instrument.

5.1.1 Maintenance and Inspection Task List

The maintenance and inspection timings listed in this table are presented only as guidelines. These timings do not represent a guaranteed period of service.

These timings differ depending on the conditions of use.

Maintenance/Inspection Item	Daily	1 Year	2 Year	Remarks	See
Sample chamber inspection	✓	-	-	Wipe up any spilt sample.	P.28
Xenon arc lamp inspection/replacement	-	-	✓	Replace when the cumulative operating time is about 2,000 hours.	P.30
Air filter replacement	-	✓	-	Replace air filters when they become discolored or very dusty.	P.37
Performance checks	-	✓	-	Check that basic performance is maintained.	P.25

5.2 Sample chamber inspection

⚠ CAUTION

Instruction

If any liquid such as water or organic solvent is spilt on the instrument, wipe it off immediately.

Spilt liquid can cause the electrical systems and functions of the instrument to break down.

Instruction

If any sample is spilt, clean it up according to the handling and disposal methods described in the sample's SDS (safety datasheet).

If spilt sample is not cleaned up, vaporized sample will fill the sample chamber and may become a health risk. In addition, spilt sample may corrode the instrument internals and prevent the acquisition of correct measurement results.

When handling liquid samples, inspect the sample chamber before and after measurement for spilt sample solution.

If sample solution is spilt on the floor of the sample chamber, either wipe it up or check that the solution has completely passed through the floor.

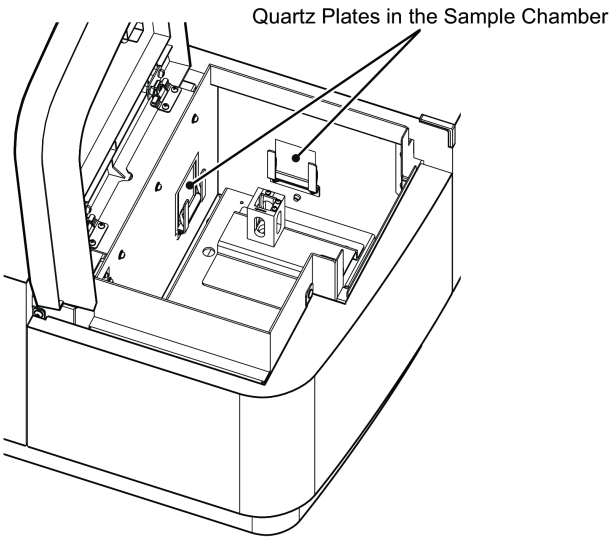
NOTE The floor of the sample chamber in this instrument has holes for solution to pass through. If sample solution is spilt on the sample chamber floor, immerse the floor in distilled water or diluted ethanol to wash off any sample solution.

5.2.1 Inspecting and Cleaning the Quartz Plates in the Sample Chamber

A quartz plate is fitted on both the excitation light entrance and emission light exit inside the sample chamber.

Check that the surface of each quartz plate in the sample chamber is neither significantly damaged nor dirty.

Gently wipe off any conspicuous stains with a dry cotton swab.



Quartz Plates in the Sample Chamber

NOTE

- A small amount of soiling or dust adhered to a quartz plate does not affect measurement. In particular, because the excitation light that enters through the quartz plate on the excitation light entrance is strong, even the smallest amount of soiling will be markedly observable. Before cleaning the quartz plates in the sample chamber, check the sensitivity (S/N ratio) using the validation function. If the validation function returns a pass result, cleaning or replacement is not required.
- If stains cannot be removed by the above method, the affected quartz plate must be replaced. Contact your Shimadzu representative to arrange replacement.

5.3 Xenon Arc Lamp Inspection

The instrument features a function that records and displays the cumulative operating time of the xenon arc lamp used as the light source.

Although the cumulative operating time is retained even when power to the instrument is turned OFF, a periodic inspection record of the displayed cumulative operating time of the xenon arc lamp should be kept because problems caused by unpredictable static electricity may reset the content of memory.

⚠ CAUTION

Replace the xenon arc lamp when the cumulative operating time of the lamp has exceeded 2,000 hours.

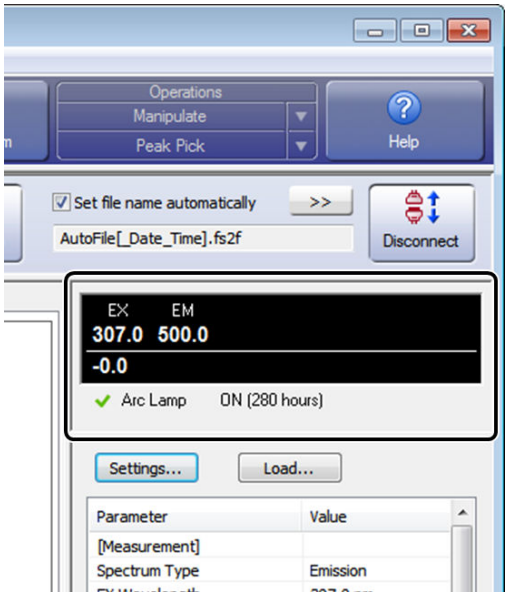
The xenon arc lamp may explode if it used passed its service life of 2,000 hours. It is dangerous if the xenon arc lamp explodes and units surrounding the lamp may be damaged.

5.3.1 Checking the Cumulative Operating Time of the Xenon Arc Lamp

1 **Connect LabSolutions RF to the RF-6000.**

Reference "Startup" P.23 in "3.5.1 Starting and Stopping"

2 **Check the cumulative operating time of the xenon arc lamp in the instrument status displayed on the upper right of the screen.**



Display Position of the Cumulative Operating Time of the Xenon Arc Lamp

NOTE If the cumulative operating time of the xenon arc lamp exceeds the service life time, the color of the mark on the instrument status changes to red. In this case, immediately replace the xenon arc lamp with a new one.

Reference "6.1 LED Indicator Status List" P.41

5.3.2 Replacing the Xenon Arc Lamp

⚠ WARNING

Instruction

When handling the xenon arc lamp, always wear the following protective gear: a protective mask, thick long-sleeved shirt, and safety gloves

- The xenon arc lamp is filled with high-pressure gas. If the xenon arc lamp is subjected to a strong impact or the glass part is damaged, it may explode and scatter fragments.
- Use a protective mask that covers your entire face with rigid plastic or similar material.
- If you happen to touch the glass part with your bare hands, use a piece of gauze, or other suitable material, moistened with ethanol to wipe off any fingerprints. If the xenon arc lamp is lit with fingerprints on the glass part they will burn and may cause the lamp to explode.

Instruction

Always turn power to the instrument OFF and unplug the power plug before replacing the xenon arc lamp.

An extremely dangerous high voltage of around 30 kV is applied to the positive (+) terminal of the xenon arc lamp at the start of ignition.

Instruction

Make sure that the xenon arc lamp has cooled sufficiently before attempting to replace it.

The xenon arc lamp is extremely hot immediately after being turned OFF and touching it may result in burns.
The time required for the xenon arc lamp to cool is at least 90 minutes when turning OFF power to the instrument, or at least 30 minutes when manually turning OFF the lamp and leaving power to the instrument turned ON.
In the case when cooling the xenon arc lamp with the power to the instrument is left turned ON, always turn OFF power to the instrument and unplug the power plug before replacing the xenon arc lamp.

⚠ CAUTION

Instruction

When replacing the xenon arc lamp, take care not to drop any screws.

Otherwise, instrument failure may result.

Part Used

Part Name	Part No.	Handling
Xenon arc lamp	S228-51511-95	Consumable part

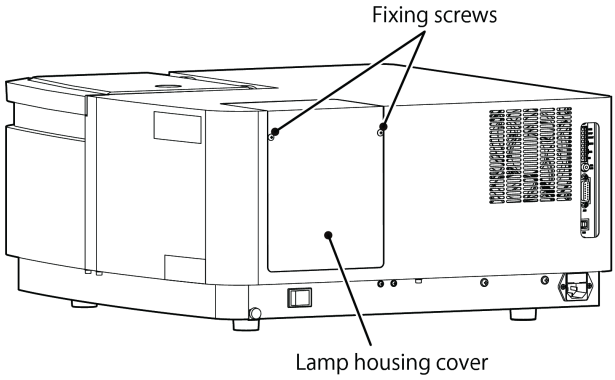
1 **Stop the RF-6000.**

Reference "Stop" P.24 in "3.5.1 Starting and Stopping"

2 **Disconnect the male power plug from the power outlet.**

3 **Wait until the xenon arc lamp has cooled down.**

4 **Loosen the two fixing screws on the lamp housing cover and remove the cover.**



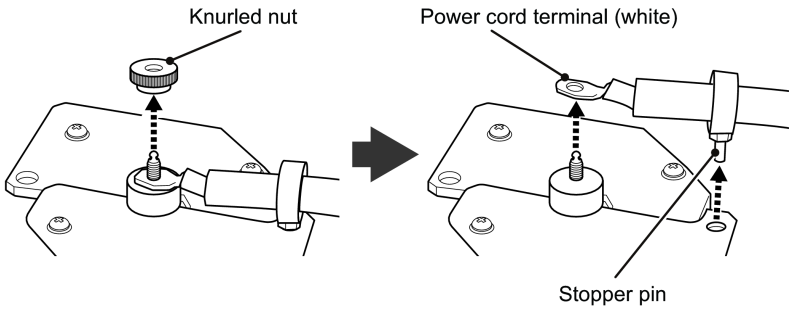
Removing the Lamp Housing Cover

5 **Remove the knurled nut at the top of the xenon arc lamp and then remove the power cord terminal (white) from the terminal of the xenon arc lamp while removing the stopper pin from the designated position.**

⚠ CAUTION

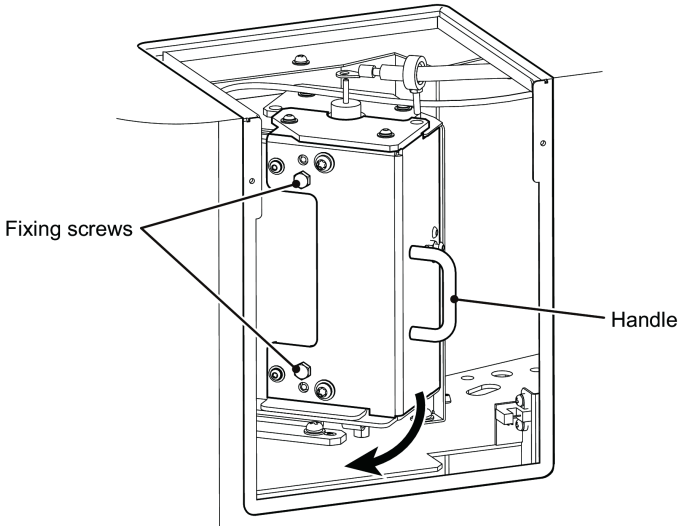
Instruction

Always turn the knurled nut by hand.
Using a tool like a wrench may break the xenon arc lamp.



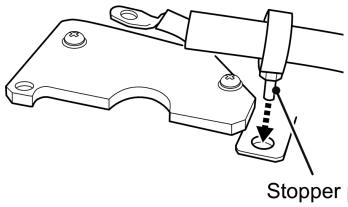
Removing the Power Cord Terminal

6 **Completely loosen the two fixing screws on the lamp unit using the provided spanner, grip the lamp unit pullout handle, and pull the lamp unit out of the instrument.**

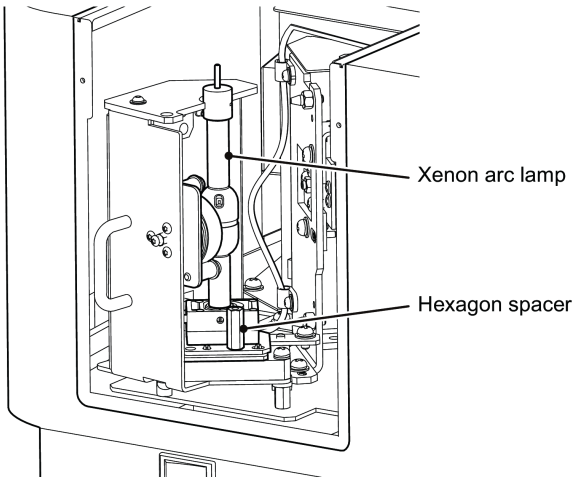


Pulling Out the Lamp Unit

Hint After pulling out the lamp unit, insert the power cord stopper pin removed in step 5 back into the same hole to facilitate the replacement work.

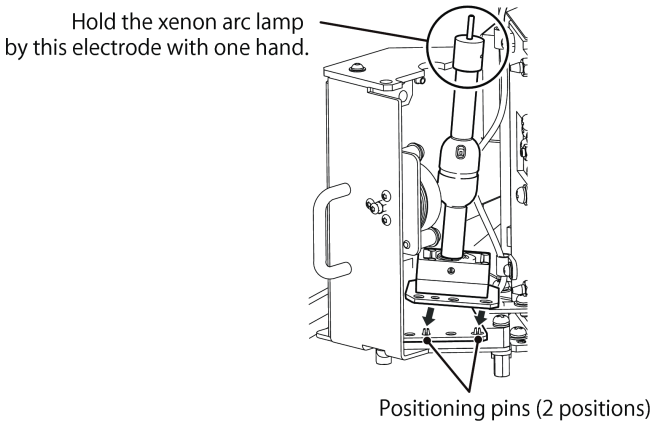


7 **Remove the single hexagon spacer using the provided spanner and then remove the xenon arc lamp.**



Removing the Lamp Unit

8 **Install the xenon arc lamp by aligning the two positioning pins of the lamp unit and then tighten the single hexagon spacer in place with the provided spanner.**



Installing the Lamp Unit

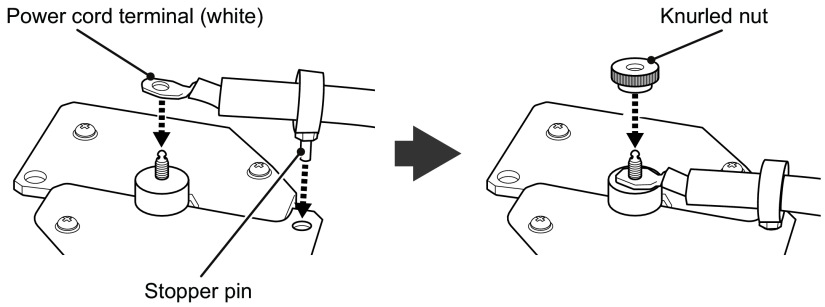
9 **Grip the handle and return the lamp unit to its original position and then tighten the two fixing screws on the lamp unit using the provided spanner.**

10 **Connect the power cord terminal (white) to the terminal of the xenon arc lamp while setting the stopper pin to the designated position and tighten the knurled nut at the top of the xenon arc lamp.**

⚠ CAUTION

Instruction

Always turn the knurled nut by hand.
Using a tool like a wrench may break the xenon arc lamp.



Connecting the Power Cord Terminal

11 **Attach and fix the lamp housing cover with the two fixing screws.**

5.3.3 Resetting the Lamp Cumulative Operating Time

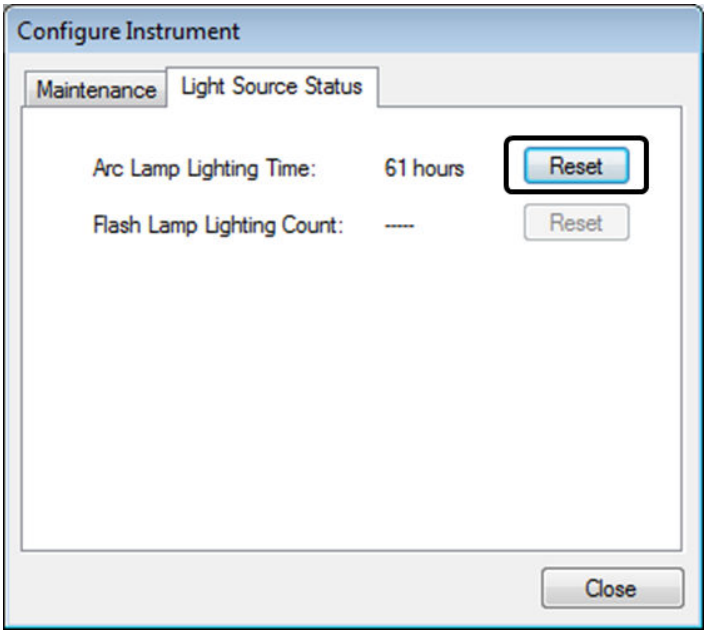
1 **Connect LabSolutions RF to the RF-6000.**

Reference "Startup" P.23 in "3.5.1 Starting and Stopping"

2 **Click [Configure Instrument] on the [Instrument] menu.**

The [Configure Instrument] window is displayed.

3 **Click the [Light Source Status] tab and then click [Reset] to the right of [Arc Lamp Lighting Time].**



[Configure Instrument] Window ([Light Source Status] Tab)

5.4 Air filter replacement

Air filters are installed on the bottom of the instrument at the front right and on the left side of the instrument.

If the air filters become clogged, the performance of the instrument will deteriorate and may cause instrument failure.

If an air filter has blackened or light is difficult to see when the air filter is held up to fluorescent lighting, replace the air filter with a new one.

⚠ WARNING

Instruction

Always turn OFF power to the instrument before replacing an air filter.

The cooling fan spins at all times when power to the instrument is turned ON. Removing the air filters when the cooling fans are spinning risks allowing large dust particles to enter into the instrument.

Part Used

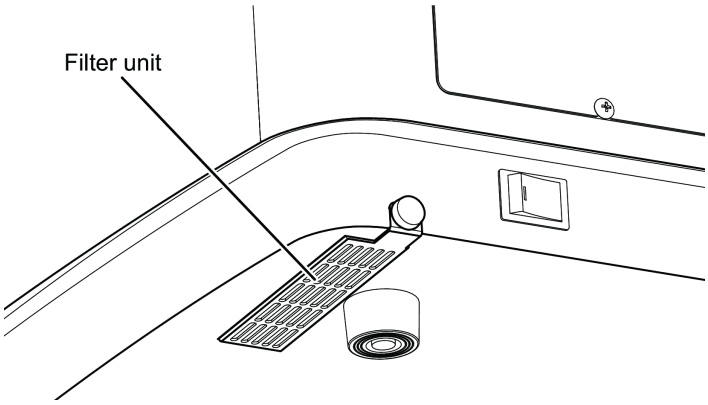
Part Name	Part No.	Handling
Air filter	S228-51147	Consumable part

5.4.1 Replacing the Air Filter on the Bottom at the Front Right

1 **Stop the RF-6000.**

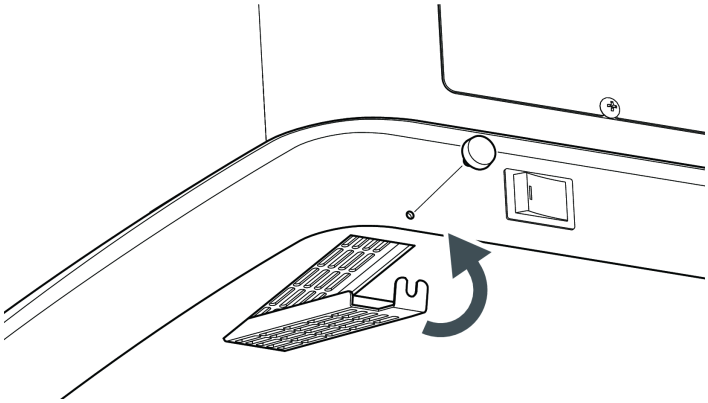
Reference "Stop" P.24 in "3.5.1 Starting and Stopping"

2 **Loosen the knurled screw for securing the filter unit on the bottom of the instrument at the front right and remove the filter unit.**



Filter Unit on the Bottom at the Front Right

3 **Replace the air filter and return the filter unit to its original position.**
Align the tab of the filter unit with the instrument cover and attach it.



Attaching the Filter Unit

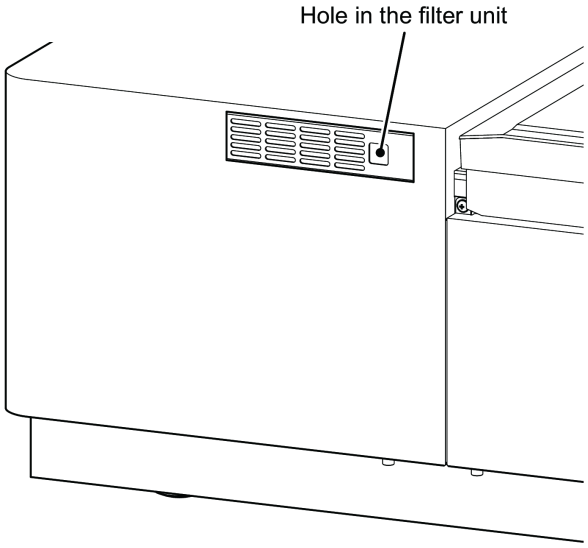
4 **Secure the filter unit in place by tightening the knurled screw.**

5.4.2 Replacing the Air Filter on the Left Side

1 **Stop the RF-6000.**

Reference "Stop" P.24 in "3.5.1 Starting and Stopping"

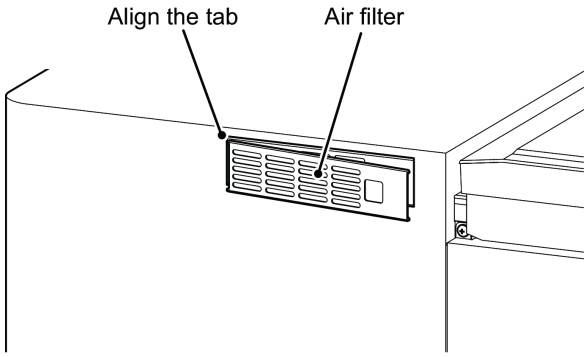
2 **Insert a finger into the hole in the filter unit and remove the filter unit from the instrument cover.**



Removing the Filter Unit

3 **Replace the air filter and return the filter unit to its original position.**

Align the tab of the filter unit with the instrument cover and attach it.



Attaching the Filter Unit

5.5 Cleaning the Exterior

If the instrument cover or sample chamber lid becomes dirty, wipe it clean with a soft dry cloth or tissue paper.

⚠ CAUTION

Prohibition

Do NOT wipe the sample chamber lid with organic solvent (alcohol, benzene, acetone, etc.)

Doing so may cause discoloration.

Prohibition

Do NOT leave the product in a wet state or wipe the product with alcohol or thinner solvents.

Doing so may cause rust or discoloration.

If the instrument cover is significantly dirty, clean the cover by the following procedure.

1 **Gently wipe the instrument cover with a cloth that has been dipped in a diluted neutral detergent and wrung out well.**

2 **Dip a piece of cloth into water, wring it out firmly, and wipe until no detergent remains on the instrument cover.**

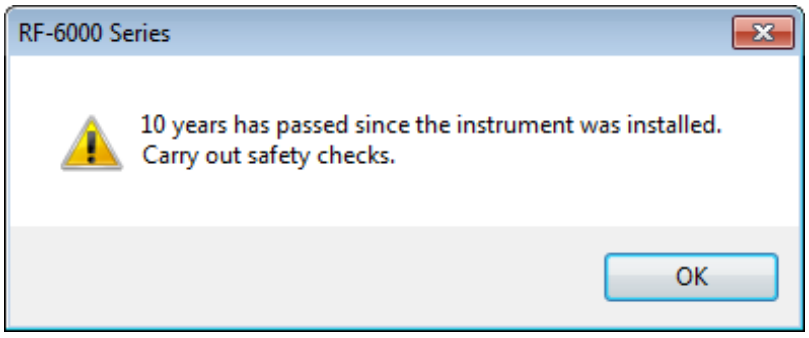
3 **Use a dry cloth to wipe any remaining moisture from the instrument cover.**

5.6 Safety Inspection Notice Window

This message window appears when the instrument has been used for 10 years.

Since this does not indicate a problem, simply close the message window to use the instrument. However, it is recommended that the instrument have a safety inspection as soon as possible.

Safety inspections are offered for a fee. Contact your Shimadzu representative.



Safety Inspection Notice Window

6 Troubleshooting

6.1 LED Indicator Status List

The instrument status is expressed according to LED indicator color and a buzzer tone. The following table lists the main operations and describes the corresponding display statuses.

Hint Buzzer tones comprise combinations of short tones ("bip": ・) and long tones ("beep": —).

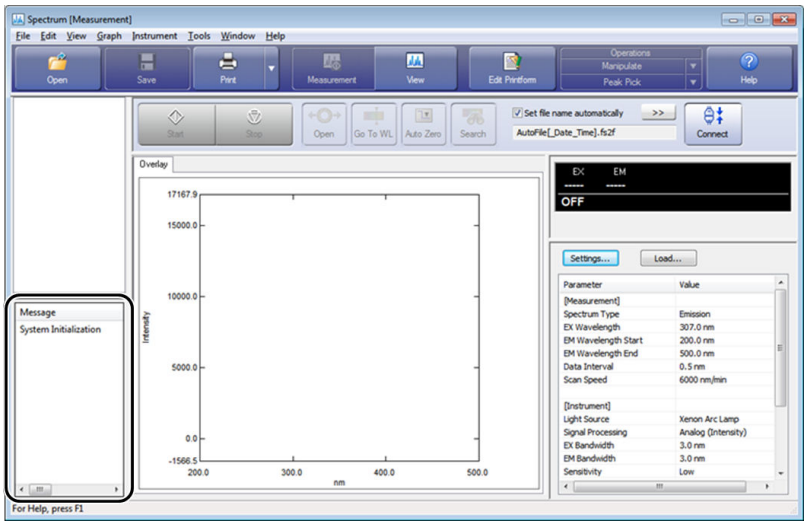
Operation	LED	Buzzer Tone	Status Description
Initialization in progress	Green (blinking)	None	The xenon arc lamp lit normally and initialization is being performed.
	Orange (blinking)		The xenon arc lamp was not ON when power to the instrument was turned OFF the last time the instrument was used so initialization is being performed with the xenon arc lamp unlit.
	Red (blinking)		Initialization is being performed even though the xenon arc lamp did not light normally.
Initialization complete	Green (lit)	None	Initialization completed correctly.
	Orange (lit)		Initialization completed correctly. However, the xenon arc lamp is not lit.
	Red (blinking)	— (once)	Initialization did not complete correctly. Connect the instrument to LabSolutions RF and check the error details.
Waiting for measurement	Green (lit)	None	Measurement can be performed in this state.
	Orange (lit)		The xenon arc lamp is set to OFF in the instrument conditions. The instrument is in the normal state.
	Red (blinking)	——— (continuous)	An error occurred in the instrument. Check the details of the error.
Measurement in progress	Blue (lit)	・ (once)	Measurement is being performed.

6.2 Initialization Items

Turning ON power to the spectrofluorophotometer automatically starts initialization in the following order.

No.	Initialization Items	Description
❶	ROM check	Checks the program ROM.
❷	RAM check	Checks the memory device (RAM).
❸	EEPROM check	Checks the instrument constants stored in EEPROM.
❹	Hardware configuration	Checks the connection status of attachments.
❺	Excitation side slit motor check	Detects the slit initialization position in the excitation monochromator.
❻	Excitation side grating motor check	Detects the wavelength origin position in the excitation monochromator.
❼	Shutter motor check	Detects the shutter initialization position.
❽	Emission side slit motor check	Detects the slit initialization position in the emission monochromator.
❾	Emission side grating motor check	Detects the wavelength origin position in the emission monochromator.

When a connection is established between the instrument and LabSolutions RF, the initialization results, light source usage, and connection of attachments are displayed in the log view in the lower left pane of the window.



If the LED indicator displays an abnormality (red) during initialization, check the problem item in the log view and then turn OFF power to the instrument.
Next, turn ON power to the instrument again to perform initialization.
If the problem persists, contact your Shimadzu representative.

> **NOTE** Measurement cannot be performed if even a single abnormality (red) occurs.

6.3 Troubleshooting and Corrective Actions

This section describes the probable causes of problems that can arise and the corrective actions for eliminating the causes.

For more detailed corrective action procedures, see the indicated page.

If the problem cannot be resolved even after performing corrective actions or the symptoms are not covered in the troubleshooting section, contact your Shimadzu representative.

6.3.1 Electrical System-Related

Problem	Probable Cause	Corrective Action	See
Power is not ON even after turning ON the power switch.	The power plug is unplugged.	Connect the power plug correctly.	P.14
	There is an object on the power cord or the power cord is bent.	Check the state of the power cord. If the power cord is damaged, replace it with the same type of cord.	—
	The wires inside the power cord are cut.	Replace the power cord with the same type of cord.	P.2
	The power supply does not meet the instrument specifications.	Use a power supply that meets the instrument specifications.	P.13
	The fuse is blown.	The fuse may have blown. Check the fuse and replace it if necessary. Contact your Shimadzu representative.	—
The LED status indicator is continuously lit red or yellow.	Initialization did not complete correctly.	Turn OFF power to the instrument and then turn it back ON again. If initialization does not complete correctly even after performing this corrective action several times, contact your Shimadzu representative.	P.42
	The corrective action corresponding to the error message has not been performed properly.	Check the error code number and error message and resolve the error by performing the indicated corrective action.	P.41
Cannot communicate with LabSolutions RF.	The provided USB cable is not being used.	Use the USB cable provided with the instrument.	P.2
	The USB cable is not connected correctly.	• Check that the USB cable is firmly connected to both the instrument and PC. • Connect the USB cable to a different USB port on the PC.	P.19

Problem	Probable Cause	Corrective Action	See
The xenon arc lamp will not light.	There is a problem with the wiring connection.	Unplug the power plug from the power outlet, open the lamp housing lid, and check the contact of the power cord connected to the positive (+) terminal of the xenon arc lamp.	P.16
	The xenon arc lamp is set to "OFF".	Check that the xenon arc lamp is set to "ON" in the instrument condition settings in LabSolutions RF.	—
	The lamp housing lid is open.	Check that the lamp housing lid is closed properly.	P.15
	The xenon arc lamp has exceeded its service life.	Replace the xenon arc lamp with a new one.	P.30
	The lamp housing is very hot.	• Check whether the LED indicator is blinking red. • Check whether the cooling fan has stopped.	P.41

6.3.2 Measurement Data-Related

Problem	Probable Cause	Corrective Action	See
The S/N ratio is bad. (low fluorescence intensity)	The cell is not filled with the required amount of sample.	When using a 10 mm rectangular cell holder, fill the cell with sample to about 15 mm from the cell floor.	—
	The wavelength is incorrect.	Use the validation function to check whether the Raman peak detection wavelength of water is significantly off.	P.25
	The slit is too narrow.	Widen the slit and perform measurement again.	—
	Incorrect sensitivity adjustment.	If the sensitivity (S/N ratio) check item of the validation function resulted in a fail, the detection sensitivity must be adjusted. Contact your Shimadzu representative.	P.25
	The sample concentration is too high.	Samples with an absorbance of 0.5 Abs or higher affect the absorption of excitation light and fluorescent light. Dilute the sample.	P.64
	The sample concentration is too low.	Increase the sample concentration and perform measurement again.	—
The S/N ratio is bad. (excessive noise)	The xenon arc lamp has exceeded its service life.	Replace the xenon arc lamp with a new one.	P.30
	Bubbles are present in the cell.	• Purge the air bubbles. • Replace the sample with one that does not contain air bubbles.	—
	External vibrations are affecting the instrument.	• Install the instrument on a sturdy table or bench. • Take measures to reduce external vibrations.	—
	The cell surface is dirty.	Clean or replace the cell.	P.61

Problem	Probable Cause	Corrective Action	See
The Raman peak of water is low.	The water used in measurement is contaminated.	Replace the water.	—
	The wavelength is incorrect by a significant amount.	Use the validation function to check whether the Raman peak detection wavelength of water is significantly off.	P.25
	The surfaces of the sample chamber quartz plates on the entrance and exit of the sample chamber are dirty.	Clean the surfaces of the sample chamber quartz plates.	P.29
	The cell surface is dirty.	Clean or replace the cell.	P.61
Significant amount of drift. (fluorescence intensity does not stabilize)	Measurement was started immediately after the xenon arc lamp was lit.	Approximately one hour is required for the instrument to stabilize after the xenon arc lamp is lit. Perform measurement after one hour has elapsed from lighting the xenon arc lamp.	P.26
	The ambient temperature is not stable.	• Stabilize the ambient temperature. • Install the instrument in a location where there is little temperature fluctuation.	P.26
	The instrument is affected by a strong draft, such as from air conditioning.	• Use an appropriate barrier to prevent directly exposing the instrument to drafts. • Change the installation location.	P.26
	The cumulative operating time of the xenon arc lamp is approaching its service life time limit.	When the xenon arc lamp is approaching its service life limit, replace it with an new one.	P.30

6.4 Error Message List

LabSolutions RF displays the following error messages according to the error status.

Hint The instrument emits a buzzer tone when an error occurs. Buzzer tones comprise combinations of short tones ("bip": ・) and long tones ("beep": —). Perform checks according to the described corrective action.

6.4.1 Error Messages in the Measurement Standby State

Error Message	LED	Buzzer Tone	Probable Cause	Corrective Action
Connection to instrument failed	—	—	The PC and instrument will not connect for some reason.	Check that the USB cable is connected correctly. In addition, turn power to the instrument OFF and then back ON, wait for initialization to complete, and then try connecting again. If the problem persists, contact your Shimadzu representative.
The total lighting time of the arc lamp is approaching the upper limit	—	・ (once)	The xenon arc lamp is approaching the end of its service life.	Prepare a new xenon arc lamp.
The total lighting time of the arc lamp has exceeded the upper limit	—	・・・ (3 times)	The xenon arc lamp has exceeded its service life.	The xenon arc lamp is at risk of exploding. Immediately replace the xenon arc lamp with a new one.
Arc lamp lighting failed Communication disconnected	Red (lit)	—・・ (3 times)	The xenon arc lamp did not light normally. The hardware may be faulty. Disconnect communication for safety reasons.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.
Arc lamp extinction failed Communication disconnected	Red (lit)	—・・ (3 times)	The xenon arc lamp could not be lit normally. The hardware may be faulty. Disconnect communication for safety reasons.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.

Error Message	LED	Buzzer Tone	Probable Cause	Corrective Action
Connection to the instrument has been terminated because the fan is detected to have stopped. If the arc lamp is on, it will be turned off automatically.	Red (lit)	— • • (3 times)	The cooling fan on the right side of the instrument was detected to have stopped. The fan may be faulty.	Perform initialization by turning OFF power to the instrument and turning it back ON. If the problem persists, contact your Shimadzu representative.
Connection to the instrument has been terminated because the lid of the light source chamber was opened. If the arc lamp is on, it will be turned off automatically. Close the lid of the light source chamber and then connect to the instrument again.	—	• • • (3 times)	Always close the lid on the lamp housing for safety reasons. If this message still appears even when the lamp housing lid is closed, the instrument may be faulty.	Turn OFF power to the instrument, close the lamp housing lid, and then turn ON the power again. Check whether the xenon arc lamp lights after performing initialization.
Connection to the instrument has been terminated because the light source protection function was turned on. If the arc lamp is on, it will be turned off automatically.	Red (lit)	— • • (3 times)	The xenon arc lamp was detected to have turned OFF even though no such instruction was given. The hardware may be faulty.	Turn OFF power to the instrument, close the lamp housing lid, and then turn ON the power again. Check whether the xenon arc lamp lights after performing initialization. If the problem persists, contact your Shimadzu representative.
Connection to the instrument has been terminated because an applied-voltage error was detected in the detector.	Red (lit)	— • • (3 times)	The negative high voltage actually applied to the photomultiplier detector did not match the value set for the negative high voltage to apply. The hardware may be faulty.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.
The dehumidification sensor error was detected. The cooling function will be turned off automatically.	—	• • • (3 times)	The humidity sensor inside the instrument could not be read correctly. The hardware may be faulty.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.

Error Message	LED	Buzzer Tone	Probable Cause	Corrective Action
Temperature sensor error detected. The cooling function will be turned off automatically.	—	• • • (3 times)	The temperature sensor inside the instrument could not be read correctly. The hardware may be faulty.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.

6.4.2 Error Messages During Measurement

Error Message	LED	Buzzer Tone	Probable Cause	Corrective Action
Measurement has been aborted and connection to the instrument has been terminated because the fan is detected to have stopped. If the arc lamp is on, it will be turned off automatically.	Red (lit)	— • • (3 times)	Measurement was stopped because the cooling fan on the right side of the instrument was detected to have stopped. The fan may be faulty.	Perform initialization by turning OFF power to the instrument and turning it back ON. If the problem persists, contact your Shimadzu representative.
Measurement has been aborted and connection to the instrument has been terminated because the lid of the light source chamber was opened. If the arc lamp is on, it will be turned off automatically.	—	• • • (3 times)	Always close the lid on the lamp housing for safety reasons. If this message still appears even when the lamp housing lid is closed, the instrument may be faulty.	Turn OFF power to the instrument, close the lamp housing lid, and then turn ON the power again. Check whether the xenon arc lamp lights after performing initialization.
Measurement has been aborted and connection to the instrument has been terminated because the light source protection function was turned on. If the arc lamp is on, it will be turned off automatically.	Red (lit)	— • • (3 times)	The xenon arc lamp was detected to have turned OFF even though no such instruction was given. The hardware may be faulty.	Turn OFF power to the instrument, close the lamp housing lid, and then turn ON the power again. Check whether the xenon arc lamp lights after performing initialization. If the problem persists, contact your Shimadzu representative.
Measurement has been aborted and connection to the instrument has been terminated because an applied-voltage error was detected in the detector.	Red (lit)	— • • (3 times)	The negative high voltage actually applied to the photomultiplier detector did not match the value set for the negative high voltage to apply. The hardware may be faulty.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.
Measurement has been aborted because a dehumidification sensor error was detected. The cooling function will be turned off automatically.	—	• • • (3 times)	The humidity sensor inside the instrument could not be read correctly. The hardware may be faulty.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.

Error Message	LED	Buzzer Tone	Probable Cause	Corrective Action
Measurement has been aborted because a temperature sensor error was detected. The cooling function will be turned off automatically.	—	• • • (3 times)	The temperature sensor inside the instrument could not be read correctly. The hardware may be faulty.	Turn OFF power to the instrument and then turn it back ON. If the problem persists, contact your Shimadzu representative.
Connection to the instrument has been terminated because the lid of the light source chamber was opened. The emission side slit will be closed automatically to protect the detector.	—	• (once)	The detector protection function activated because the sample chamber lid was opened during measurement.	Check that the sample chamber lid is closed before starting measurement.
Measurement data may be saturated.	—	• (once)	The data may have become saturated during measurement.	If saturated data becomes a problem, set a narrower slit width and perform measurement again.

7 Maintenance Parts List

7.1 Consumable part

Part Name	Part No.	Replacement Task	Remarks
Xenon arc lamp	S228-51511-95	User	150 W xenon arc lamp
Air filter	S228-51147	User	This is a filter for preventing dust being sucked into the instrument. An air filter is fitted on the bottom of the instrument at the front right and on the left side of the instrument.

7.2 Replacement Parts

Part Name	Part No.	Replacement Task	Remarks	Replacement Guideline
GR mirror adhesive, EX	S207-20451-45	Shimadzu service personnel	For excitation side grating	7 years
Ellipsoidal mirror ass'y	S228-51509-45	Shimadzu service personnel	Ass'y for condensing light from the xenon arc lamp	7 years
Back mirror, lamp	S207-20518-45	Shimadzu service personnel	Back mirror ass'y for condensing light from the xenon arc lamp	7 years
WINDOW PLATE PACKAGED,RF-6000	S206-25346-41	Shimadzu service personnel	For quartz plates on entrance and exit of sample chamber	7 years

8 Technical Information

8.1 Specifications

8.1.1 Hardware Specifications (RF-6000)

Item	Specifications
Lamp	150 W xenon arc lamp
Lamp Housing	Self-deozonating lamp house
Monochromators	Concave, blazed holographic grating, 1,300 grooves per mm, F/2.5 Excitation side: Off-plane optical system Blaze wavelength: 350 nm Emission side: In-plane optical system Blaze wavelength: 400 nm
Wavelength scale	200 to 900 nm and 0 order light
Measuring wavelength range	200 to 900 nm
Spectral bandwidth	Excitation side: 1.5, 3, 5, 10, 15, 20 nm Emission side: 1.0, 3, 5, 10, 15, 20 nm
Resolution	Emission: 1.0 nm
Wavelength accuracy	±1.0 nm
Wavelength repeatability	±0.2 nm
Wavelength scanning speed	Approx. 60,000 to 20 nm/min, 9-step selection
Wavelength slewing speed	Approx. 60,000 nm/min
S/N ratio	S/N ratio for Raman peak of distilled water: 350 min. (p-p), 1,000 min. (RMS) Excitation wavelength: 350 nm Spectral bandwidth: 5 nm for both excitation and emission Response: 2 seconds
Light source compensation method	Monochromatic light monitoring comparison operation method
Detectors	Monitoring side: Silicon photodiode Emission side: R928 photomultiplier
Sensitivity selection	2-step selection (High, Low, and Auto are available)
Dimensions	610W × 565D × 274H mm
Mass	38 kg
Operational temperature range	15 to 35 °C
Operational humidity range	30 to 80% (no condensation, below 70% for ambient temperatures higher than 30 °C).
Power supply	100 to 240 VAC (50/60 Hz)
Power Consumption	300 VA

8.1.2 Software Specifications (LabSolutions RF)

Item	Specifications
Compatible OS	Microsoft Windows 7 Professional (32-bit and 64-bit versions)
Interface	USB 2.0
Programs	Spectrum measurement, time course measurement, quantitation measurement, 3D measurement, quantum yield measurement, quantum efficiency measurement
Spectrum mode	Excitation spectrum measurement, fluorescence spectrum measurement, synchronous spectrum measurement
3D spectrum mode	Repeated measurement at specified time interval (3D excitation spectrum measurement, 3D fluorescence spectrum measurement, 3D synchronous spectrum measurement) Fluorescence spectrum measurement at specified excitation wavelength interval (3D spectrum measurement) Function for extracting excitation/fluorescence spectra from 3D spectrum data
Quantitation mode	Quantitation using the peak / maximum value / area etc. of single wavelengths, multiple wavelengths (including single, double, and triple wavelength methods), and specified wavelength ranges K-factor method, single-point calibration curve method, and multi-point calibration curve method (1st, 2nd, and 3rd order function-fitting, zero intercept can be specified) Photometric processing with user-defined functions (functions that use addition, subtraction, multiplication, and division can be embedded together with factors)
Photometric mode	Capturing the fluorescence intensity of single wavelengths and multiple wavelengths as well as peak / maximum value / area in specified wavelength ranges Photometric processing with user-defined functions (functions that use addition, subtraction, multiplication, and division can be embedded together with factors)
Time course mode	Time course recording using single wavelengths and the difference/ratio between two wavelengths Activity value calculation Event recording of reagent additions during measurement
Data processing functions (common to all modes)	Processing of waveform data (spectrum/time course) Data printing, point pick, peak pick, area calculation, constant calculation, data set calculation, 1st to 4th order differentiation, smoothing, common logarithm transformation, natural logarithm transformation, reciprocal transformation, exponentiation, square root, index transformation
Quantum yield measurement mode	Quantum yield calculation of unknown samples, list display and printing of multiple sample results, text conversion of spectrum data
Quantum efficiency measurement mode*1	Calculation of absorption factors, internal quantum efficiency, and external quantum efficiency as well as list display and printing of multiple sample results, text conversion of spectrum data

Item	Specifications
Printing functions	Creating Report Templates Printing using report templates
File functions	Automatic conversion to CSV file/text file (only manual text conversion in the quantitation and photometric applications)
Validation function	S/N ratio, resolution*2, wavelength accuracy*2, wavelength repeatability*2, noise level, drift
Instrument registration tool	Spectrofluorophotometer registration, integrating sphere unit registration
Spectrum correction function measurement tool	Creation of spectrum correction functions when an integrating sphere is installed
Hardware monitoring	Self-diagnosis function, instrument status monitoring function

*1 An integrating sphere unit (option) is required.

*2 A mercury lamp unit (option) is required.

8.2 Basics of Fluorometric Analysis

8.2.1 What Is Fluorescence?

The phenomenon of certain kinds of substance emitting light on absorbing various energies, without involving heat generation, is called luminescence.
The 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 of a substance, and emit light with longer wavelengths than irradiated light.
Familiar examples include the green color emitted from dye (eosin) contained in red ink under daylight and the pale blue color emitted from phosphorus contained in detergent adhered to the surface of dress shirts.

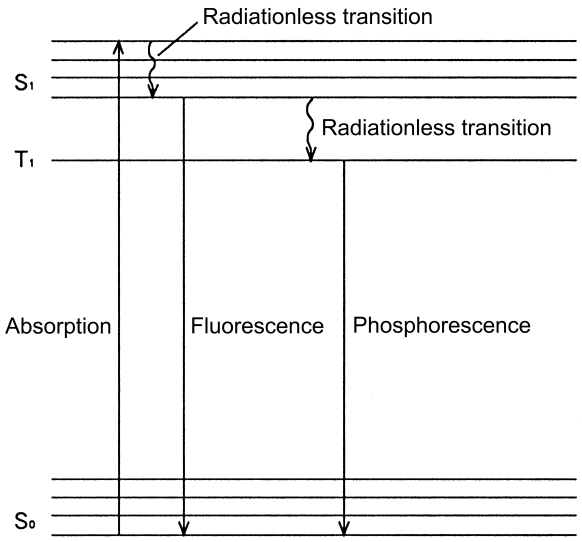
In addition to visible light, fluorescence is also emitted in the X-ray, radiation, and cathode-ray spectrums, and these are respectively referred to as "X-ray luminescence", "radioluminescence", and "cathodoluminescence". Note, however, that the light emitted by fireflies is the result of a chemical reaction in cells between oxygen and luciferin in the presence of luciferase, which is a process referred to as "chemiluminescence".

8.2.2 Principles of Fluorescence Process

This section explains the principles of fluorescence with reference to an organic compound as an example.

In real systems, factors including a larger number of energy levels, energy transfer from other molecules, and photochemical reactions must be taken into consideration.

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 the following figure.



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.

As indicated in the figure, 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, and then returns to the base state S_0 is called phosphorescence. In this case, phosphorescence has a longer life in general because of the need for spin transformation (by 10^{-4} to 10^{2} seconds).

8.2.3 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 the radiationless transition of the absorbed energy to heat etc.

$$Q = \frac{n_e}{n_e + n_f + \sum n_0}$$

n_e: Frequency of light emission n_f: Frequency of radiationless transition n_0: Other frequencies (chemical reaction etc.)

Law 1 indicates that, when measuring an unknown sample, an absorbance spectrum must be measured first with a comparatively high sample concentration. If absorption does not occur at all, it can be concluded that the sample does not emit fluorescence. 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 "Principles of Fluorescence Process", the residual radiation energy is reduced. 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 indicates whether the fluorescence intensity that a substance emits is high or low. The quantum yield of fluorescence (Q) indicates the proportion of energy that is absorbed and radiated as fluorescence. The higher the value of Q, the easier the substance produces fluorescence. The following table lists the quantum yields of typical fluorescent substances.

Compounds and Quantum Yield of Fluorescence

Compound	Solvent	Quantum Yield (Q)
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

8.2.4 Advantages of Fluorometric Analysis

■ 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.

■ High Sensitivity

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

8.2.5 Important Notes on Fluorometric Analysis

■ Effect of Sample Temperature

In many samples, each rise of 1 °C in sample temperature is said to produce a loss of 1 to 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.

■ 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 methods also include selecting the fastest scan speed available for spectrum measurement and selecting a narrow spectral bandwidth on the excitation side.

■ 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) scattered light and its second order light, (2) Raman scattered light of the solvent, and (3) fluorescence from the solvent or cell.
(1) and (2) are discussed in the following section. For (3), commercially available grades of reagents often detect fluorescence caused by the presence of impurities in solvent. Remember that high-sensitivity testing in the ultraviolet region is particularly susceptible to the effects of solvent fluorescence. Non-fluorescent solvents for fluorescence analysis are commercially available. You will either need to purchase such solvents or purify any applicable solvents yourself.

General quartz cells will produce weak fluorescence when they are excited at around 260 nm because of impurities (aluminum) inherent in the cells. In particular, when measuring samples with weak fluorescence by causing excitation in the ultraviolet region, we recommend using the optional non-fluorescent cell that is made from synthetic quartz.

■ 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.

Due to the characteristics of monochromators that utilize diffraction gratings, this scattered light also appears in the wavelength regions at twice and three times the size of the excitation wavelength and light at these wavelengths is referred to as second order light and third order light. For example, when the excitation wavelength is 220 nm, second order light appears at 440 nm and third order light at 660 nm on the fluorescence spectrum. In order to filter this light, measurement is performed with a short-wavelength cutting filter inserted into the filter holder on the emission side that blocks transmission at around 220 nm. If the effects of scattered light such as this become a problem, use an optional filter set that includes a number of filters.

Raman scattering appears when the solvent has Raman activity. Although a peak resulting from Raman scattering also appears at wavelengths longer than the excitation light, the peak intensity from Raman scattering does not significantly change even if the sample concentration changes. Also, when the excitation wavelength is shifted, the position of a peak resulting from Raman scattering changes but the peak position of fluorescence does not change. Therefore it is possible to discriminate between a fluorescence peak and peak resulting from Raman scattering.

The following table introduces the relationship between excitation wavelengths and peak wavelengths resulting from Raman scattering for each solvent.

Relationship Between Excitation Wavelengths and Raman Peak Wavelengths

Excitation Wavelengths (nm)	Solvents and Raman Peak Wavelengths (nm)				
	Water	Ethanol	Cyclohexane	Carbon Tetrachloride	Chloroform
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

■ High Concentration Samples

If the concentration of the sample for measurement is too high, a variety of error factors will occur. Conventional spectrofluorophotometers are designed to detect fluorescence emitted from the center of cells. This means that if the sample concentration is too high, excitation light may be absorbed around the cell entrance, preventing excitation light from completely reaching the cell center, and resulting in reduced fluorescence intensity. In addition, shorter-wavelength light in the fluorescence emitted from the cell center may be reabsorbed by sample in the cell causing the spectrum shape to apparently shift towards longer wavelengths.
These phenomena generally do not occur when sample absorbance is 0.05 Abs. (in a cell with an optical path length of 10 mm) or less.
If measurement of samples at high concentrations is unavoidable, measurement must be performed either using a triangular cell or by attaching a thin cell to the optional solid/powder sample holder.

■ Effects of Cell Contamination

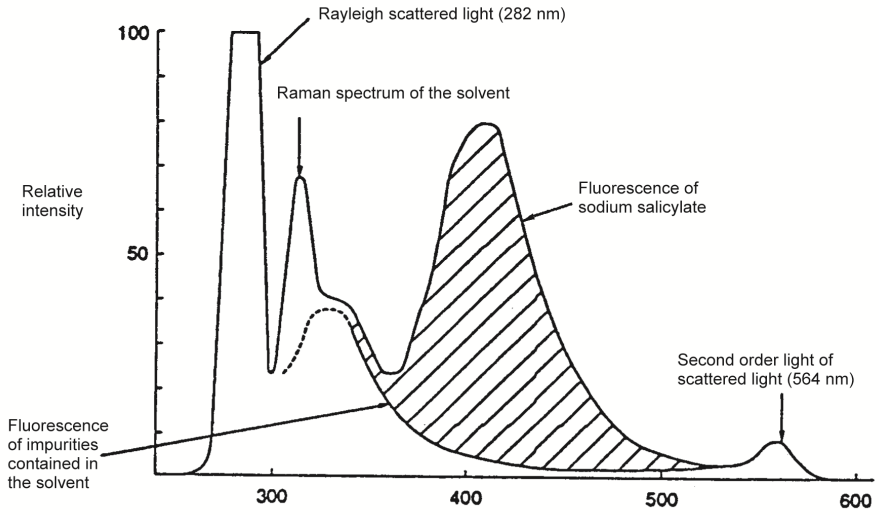
In fluorescence analysis, the slightest smear on the cell could affect measurement accuracy. In particular, if sample is left in the cell, it may adhere to the inner surfaces of the cell when the solvent evaporates and become stuck in the cell. When measuring highly diluted samples, the outer surfaces as well as the inner surfaces of the cell become problematic. If sample adheres to the outer surface of a cell, use a cloth to clean the cell before performing measurement.

■ Effect of Dissolved Oxygen

Dissolved oxygen in solvent generally has a fluorescence-quenching effect. Some samples show significant quenching. If the effect of quenching due to dissolved oxygen cannot be ignored, the solvent will require degassing. Degassing methods include injecting nitrogen gas into the solvent or lowering the pressure of the solvent using a vacuum pump.

8.2.6 Example Scan of Fluorescence Spectrum

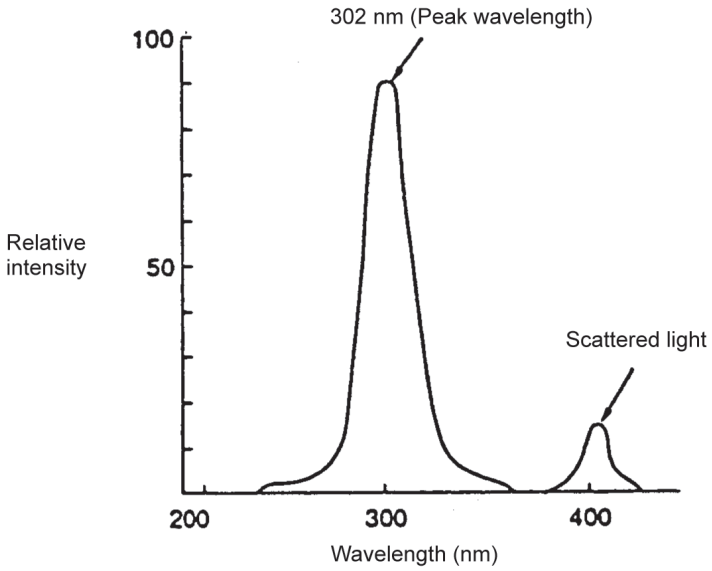
The following figure shows the fluorescence spectrum of salicylic soda aqueous solution measured with the excitation wavelength set to 282 nm.
Generally, the fluorescence spectra of diluted solutions contain a variety of overlapping emission spectra in addition to the fluorescence of the sample. The figure shows observed excitation light that was scattered (Rayleigh scattering) due to molecules and contaminants in the solvent, the corresponding second and third order light, Raman scattering light of the solvent, fluorescence of the solvent and impurities, and the fluorescence of salicylic soda.



Fluorescence Spectrum of Salicylic Soda Aqueous Solution

8.2.7 Example Scan of Fluorescence Spectrum

The following figure shows the excitation spectrum of salicylic soda aqueous solution measured with the fluorescence wavelength set to 405 nm.
The peak wavelength of 302 nm is the wavelength at which excitation will result in the highest excitation efficiency and strongest fluorescence.
The peak of 405 nm indicates the scattering of excitation light.
The excitation spectrum and absorbance spectrum are generally considered to match. For this reason, the optimal excitation wavelength required to obtain the highest fluorescence intensity can be generally reasoned by analogy for samples with a known maximum absorbance wavelength. Strictly speaking, however, the match is actually between the true, corrected excitation spectrum and the absorbance spectrum. The uncorrected, semblance excitation spectrum does not generally provide a complete match. Spectrum correction is explained in the next section.



Excitation Spectrum of Salicylic Soda Aqueous Solution

8.2.8 Determining the True Spectrum

The fluorescence spectrum and excitation spectrum recorded by the spectrofluorophotometer are the result of exciting the sample with monochromatic light output from the light source lamp and excitation monochromator, and measuring the fluorescence emitted by the sample using the emission monochromator and detector. This means that the fluorescence spectrum comprises both the spectral characteristics of the emission monochromator and the wavelength sensitivity characteristics of the detector. The excitation spectrum comprises both the spectral emission characteristics of the light source lamp and the spectral characteristics of the excitation monochromator. Therefore, a spectrum comprised of the overlapping spectral characteristics of monochromators and other components contained in the spectrofluorophotometer is referred to as a semblance spectrum.
Unless stated otherwise, spectra output from spectrofluorophotometers are generally semblance spectra. The following issues occur for such semblance spectra even when measuring the same sample.

- Spectra will vary between instruments from different manufacturers and between different models.
- Spectra will vary between individual instruments even from the same manufacturer.
- Spectra will vary over time even with the same instrument.

Therefore, when comparing data presented in research or at academic conferences, or comparing data measured using other instruments, comparisons must be performed using true spectra that exclude all of these spectral characteristics.

In order to remove this burden, the RF-6000 is registered with correction functions for various spectral characteristics at the time of installation to allow true spectra to be determined. This means that the instrument is designed to immediately obtain true spectra by performing automatic correction after measurement using the corresponding correction functions.

8.2.9 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

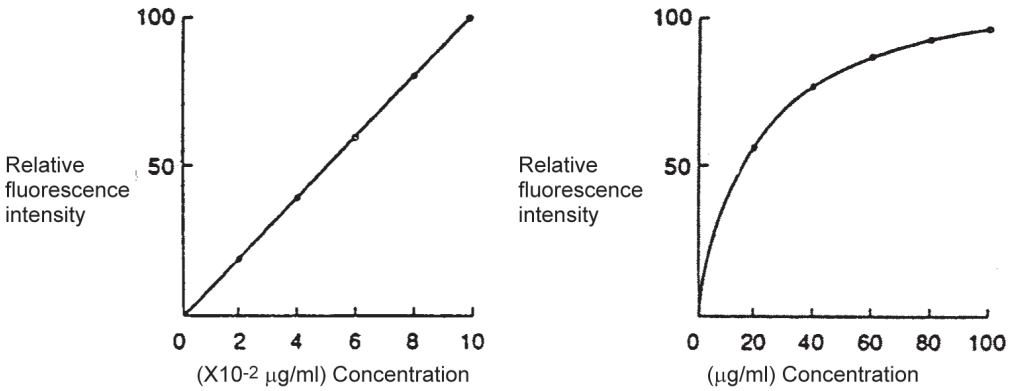
$$dB(\lambda') = \frac{\rho}{4\pi n^2} E\lambda F\lambda(\lambda') K\lambda d\lambda'$$

$dB(\lambda')$	:	Intensity of the fluorescence observed at wavelength
n	:	Refractive index
p	:	Reflection coefficient
$E\lambda$	:	Intensity of the excitation light at wavelength λ
$F\lambda(\lambda')$	:	True fluorescence intensity at wavelength λ' in the spectrum emitted by the excitation light at wavelength λ
$K\lambda$	:	Absorbance at wavelength λ
$d\lambda'$	:	Bandwidth of wavelength λ'

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

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

Since the absorbance is proportional to the concentration C, while the calibration curve should be a straight line, if the sample concentration is high, the calibration curve becomes curved due to the phenomena described in "High Concentration Samples" P.61. The following figure shows an example of calibration curves for diaminostilbene aqueous solution.

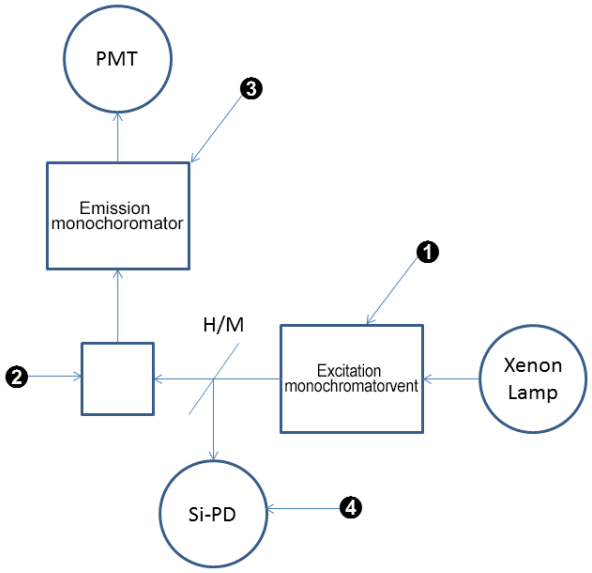


Working lines of a diaminostilbene aqueous solution

8.3 Operating Principle of the Instrument

8.3.1 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. This section provides an overview of the spectrofluorophotometer as depicted in the following figure and using the RF-6000 configuration as an example.

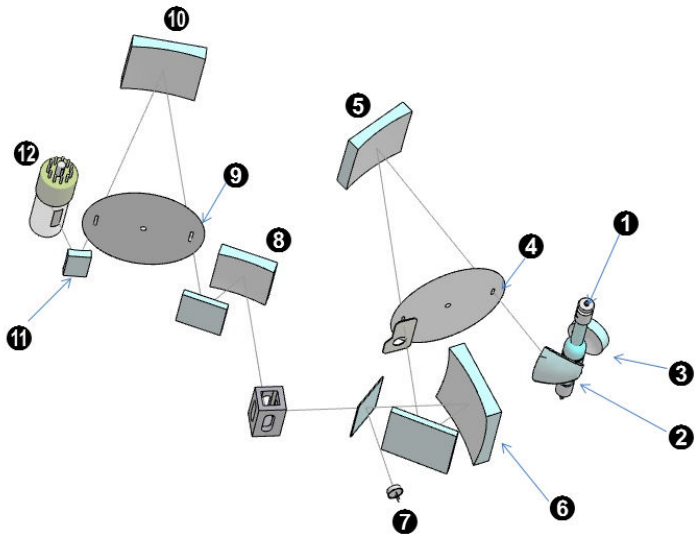


Constitution of RF-6000

❶ is the excitation monochromator. Excitation light is obtained by isolating a band of a particular wavelength from the light from the xenon lamp. 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.
❷ is the cell holder position. A cell that contains sample is set here for measurement.
❸ is the emission monochromator. Intensity of the fluorescence emitted from the sample is measured using a photomulitplier tube. 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.
❹ is a silicon photodiode detector used to monitor the intensity of excitation light irradiated onto the sample. The xenon arc lamps generally used by spectrofluorophotometers emit very high intensity light and have the advantage of providing radiation spectra that includes wavelengths in the ultraviolet and visible wavelength ranges. However, because there is a factor of instability in the emission of light, the fluorescence recorded by the emission monochromator at ❸ contains the fluctuations of the xenon arc lamp recorded as noise. In order to mitigate such a problem, measured values are always determined through a comparison operation performed between the measured fluorescence value recorded at ❸ and the measured monitor value recorded by the light intensity monitor.

8.3.2 Optical System of the Spectrofluorophotometer

The following figure shows the optical system of the RF-6000. A 150 W xenon arc lamp (❶) is used as the light source and the lamp housing employs a deozonating system that traps ozone and utilizes heat generated inside the housing. The bright spot of the xenon arc lamp is enlarged by the ellipsoidal mirror (❷) and back mirror (❸) and condensed by the entrance slit of the excitation side slit (❹). The light dispersed by the excitation side concave diffraction grating (❺) passes through the exit slit and is concentrated on the center of the cell holder for sample measurement by the spherical mirror (❻). The concave diffraction grating used by the monochromator is a high-efficiency, blazed holographic grating developed using Shimadzu's own technology. A diffraction grating of the same shape is also used on the emission side.
Between the spherical mirror (❻) and the cell holder, a quartz plate in the sample chamber is installed to guide the excitation light to the detector for monitoring light intensity (❼) for the purpose of performing light source compensation.
The fluorescence emitted from the cell is condensed by the spherical mirror (❽) into the entrance slit of the emission side slit (❾). The fluorescence is then dispersed by the emission side concave diffraction grating (❿) and concentrated by the concave mirror (⓫) onto the reception surface of the photomultiplier for fluorescence measurement (⓬).
The RF-6000 employs an off-plane optical system to provide an excitation monochromator with lower stray light. Neither the excitation monochromator nor emission monochromator use any lenses in the optical system. This allows for reduced chromatic aberrations and realizes an optical system that can effectively measure fluorescent light at all wavelengths.



Optical System of RF-6000

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