Spectrofluorophotometer Manual

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1 Overview

Thank you for your purchase a Shimadzu spectrofluorophotometer system. The following instruments are supported:

- RF-5301PC
- RF-1501 (requires a program card)

For convenience and enhanced operability the system is designed to run on an MS Windows platform providing an intuitive, graphical user interface. As a result the operator can feel comfortable in a wide variety of applications ranging from routine analysis to state-of-the-art research work.

The spectrofluorophotometer unit is combined with the **panorama Fluorescence** Spectroscopy software package.

For trouble-free and efficient use of the spectrofluorophotometers be sure to study this manual thoroughly. You are advised to read the following chapters subsequently.

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1.4 Preparing the instrument for measurements

The following test procedures are applied to test the instrument and the software to produce reliable results:

Signal/Noise Test

Wavelength Accuracy Test

1.5 Measurements

Any measurements will be carried out from a central measurement window, where instrument parameters and follow-up actions are configured:

Measurement Window

Before a measurement can be started, the instrument must be initialized:

Instrument Initialization

The following measurement procedures are available:

2D Emission Measurement

3D Emission Increment Measurement

3D Emission Measurement

2D Excitation Measurement

3D Excitation Increment Measurement

3D Excitation Measurement

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2 What is Fluorescence?

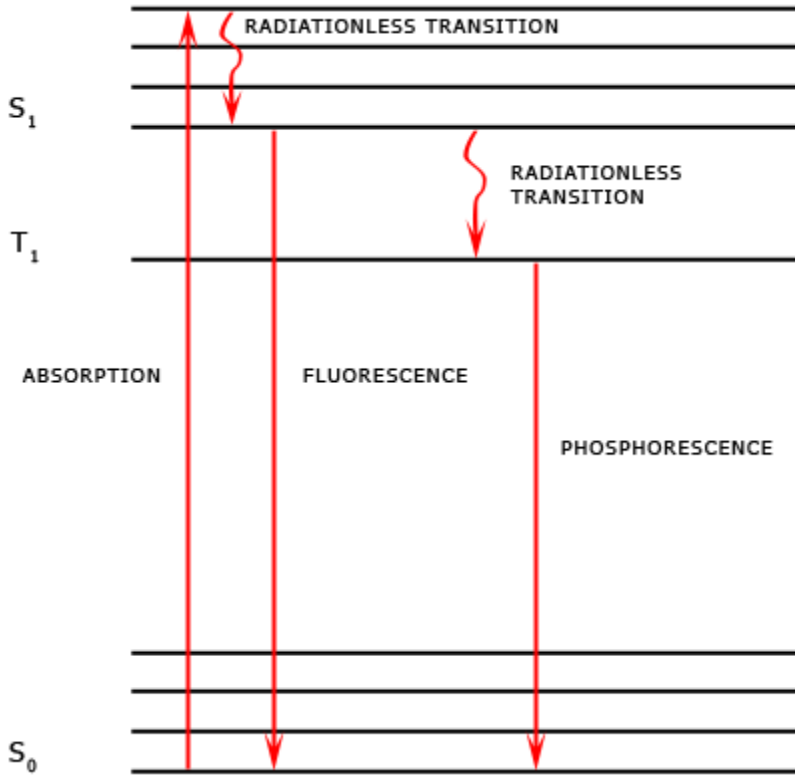
The phenomenon of certain kinds of substance emitting light on absorbing various energies, without involving heat generation, is called luminescence. That kind of luminescence that is emitted on exposure to ultraviolet or visible rays is called photo luminescence.

Fluorescence and phosphorescence, representative of photo luminescence, possess hues different from the reflected or transparent color a substance, emitting longer wavelengths of light than radiated light. Familiar examples are the green color observed in a red ink in the daylight (eosin aqueous solution) and a pale color in shirts.

2.1 Principles of Fluorescence

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

When a molecule in the base state S_0 is exposed to light, the kinetic energy of the electrons in the molecule is altered, moving the molecule into the excited state S_1 with higher energy level as shown in the following figure:



The excited state, however, soon changes back to the base state as the molecule is deactivated by radiating the energy in the form of heat or light. The molecule then transits, without radiation, to an excited state having a slightly lower energy level than the excited state S_1. The light the molecule emits as it returns further to the base state S_0 is called fluorescence.

Since part of the energy of the light absorbed has been lost as vibration or heat energy, the light covers a longer wavelength than the light to which the molecule has originally been exposed (Stokes law).

The light the molecule emits as it transits, without radiation, to the triplet state T_1 from the excited state S_1, the returns to the base state S0 is called phosphorescence. Phosphorescence has a life longer than 10^{-4} seconds because of the need for spin transformation.

2.2 Three Basic Laws of Fluorescence

- **Law 1**
  In order a substance to emit fluorescence, light absorption must take place.
- **Law 2**
  Generally, fluorescence has a longer wavelength than excitation light.
- **Law 3**
  The quantum yield of fluorescence (*Q*)is determined by the frequency of non-radiation of the absorption energy to heat or others.

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

where

n_e: Frequency of light emission
n_f: Frequency of non-radiation transition

Law 1 dictates that the task of testing an unknown sample begin with measurement of its absorption spectrum with a relatively higher concentration. If there is no absorption at all, then it is considered that fluorescence is not emitted even if the sample is excited at the wavelength. Conversely, fluorescence is emitted most intensely if the sample is excited with the absorption peak wavelength.

Law 2 indicates that, since part of the energy is reduced, shifting the wavelength of the fluorescence to longer side. Hence, the task of measuring the fluorescence spectrum can be reduced to a matter of scanning only the longer wavelength side of the excitation light.

Law 3 uses the quantum yield of fluorescence (*Q*) to indicate what proportion of energy absorbed is 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:

Compound	**Solution**	**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
Napthalene	Ethanol	0.12
Indole	Water	0.45
Chlorophyll a	Ether	0.32
Chlorophyll b	Ether	0.12

2.3 Advantages of Fluorometric Analysis

2.3.1 High selectivity

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

2.3.2 High sensitivity

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

2.4 Important Notes on Fluorometric Analysis

2.4.1 Effect of Sample Temperature

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

2.4.2 Photochemical reaction of samples

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

2.4.3 Fluorescence from impurities

Peaks caused by fluorescent components other than the component of interest during fluorescence spectrum measurement are called fluorescence from impurities. Fluorescence from impurities are associated with

1. Scattered light and its secondary light
2. RAMAN scattered light of the solvent
3. Fluorescence from the solvent or cell.

(1.) and (2.) are discussed in the following. For (3.), commercially available grades of reagents often detect fluorescence caused by the presence of impurities solvent.

Remember that high-sensitivity testing in the ultraviolet region is particularly susceptible to the effects of solvent fluorescence. Fluorescence-free solvents are available for fluorescence analysis use. To remove concern over the possible effects of solvent fluorescence, rather use these commercial solvents or refine your solvent by yourself.

General quartz cells will weak fluorescence when they are excited at around 260 nm because of impurities (aluminum) inherent in the cells. Use of a fluorescence-free cell that contains artificial quartz (P/N200-34591-03) is recommended for exciting traces of samples at around 260 nm.

2.4.4 Effects of scattered light

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

Depending on the characteristics of the grating monochromator, scattered light may also appear in the wavelength regions two and three times the excitation light wavelength as second order and third order light, respectively. With an excitation light wavelength of 220 nm, for example, second order light appears at 440 nm and third order light appears at 660 nm. A short wavelength cutoff is inserted at the emission side to remove such light.

Further, if the excitation light wavelength is set to visible, light having half the wavelength is also emitted from the excitation monochromator. With an excitation light wavelength of 450 nm, for example, light of 225 nm is also emitted. A short wavelength cutoff filter is inserted at the excitation side to remove such light. Use the filter set available as a special accessory if second order or third order light is of concern.

RAMAN scattering appears when the solvent has RAMAN activity. It appears on the longer wavelength side of excitation light as like fluorescence peak. RAMAN scattering is distinguishable because it remains essentially unchanged in intensity with changes of the sample concentration and also because changes of excitation light wavelength vary the position of the peaks caused by RAMAN scattering but not the peak position of fluorescence. The table below summarizes the relation-ships between the excitation light wavelength and RAMAN peak.

	Solvent and RAMAN peak wavelengths [nm]				
Excitation light wavelength [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

2.4.5 High-concentration samples

Too high sample concentrations can be a cause of various errors. Because spectrofluorophotometers are designed to detect fluorescence emitted from the center of the cell, the excitation light would be absorbed in the vicinity of the inlet of the cell if the sample concentration is too high. The failure of the excitation light to fully reach the center of the cell causes a loss of apparent fluorescence intensity.

Further, that portion of fluorescence emitted from the center of the cell having a shorter wavelength is re-absorbed by the sample in the cell, making the spectrum look as if it were shifted towards the longer wavelength side. Generally, samples with absorbances up to 0.02 (in a 10 mm cell) in the ultraviolet region are said to be free from these phenomena.

2.4.6 Effects of cell contamination

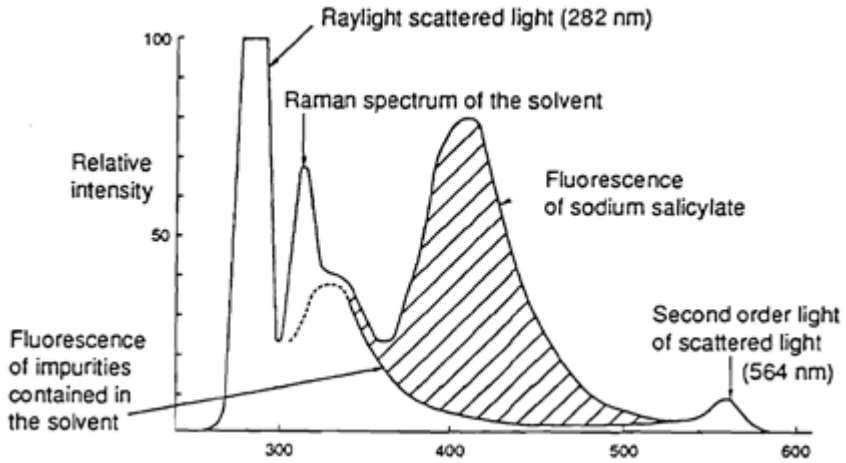
In fluorescence analysis, the slightest smear in the cell could affect measurement accuracy. Especially, if the cell is left with a sample being loaded inside. the solvent could be evaporated and adhere to the cell wall as persistent smear. In testing an extremely dilute sample, dirt on the external,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

2.4.7 Effect of dissolved oxygen

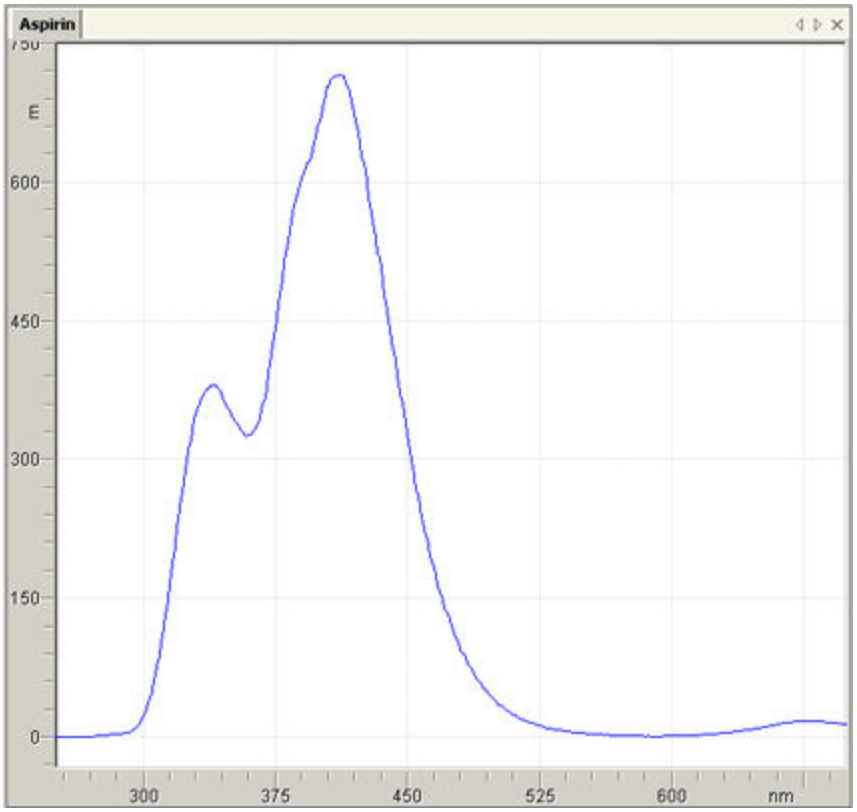
Generally, oxygen dissolved in a solvent exerts a marked fluorescence extinction effect on certain samples (quenching). If quenching by dissolved oxygen is not negligible, solvent degassing is required. Solvent degassing is conveniently accomplished by blowing a nitrogen gas into the solvent or decompressing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

2.5 Example Scan of Fluorescence Spectra

The figure below shows a fluorescence spectrum of an aqueous solution of sodium salicylate as an example. Generally, the fluorescence spectrum of a dilute solution not only provides a record of the fluorescence of the sample but involves a complication of various emission spectra, involving the scattered light (Rayleigh scattering) that is observed as a result of excitation light being scattered by dust or molecules in the solution, order light of the scattered light as shown in figure:

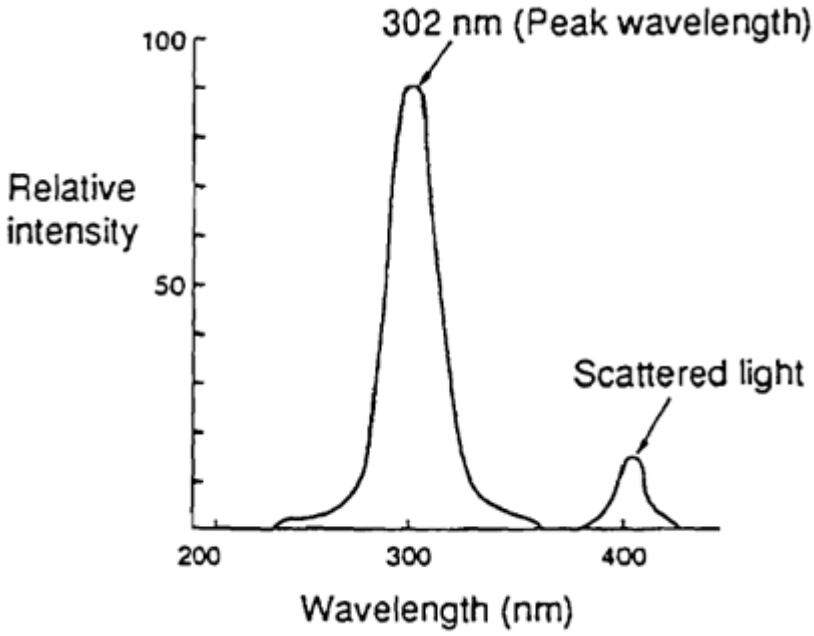


A measured emission spectrum of diluted Aspirin tablet looks like this:

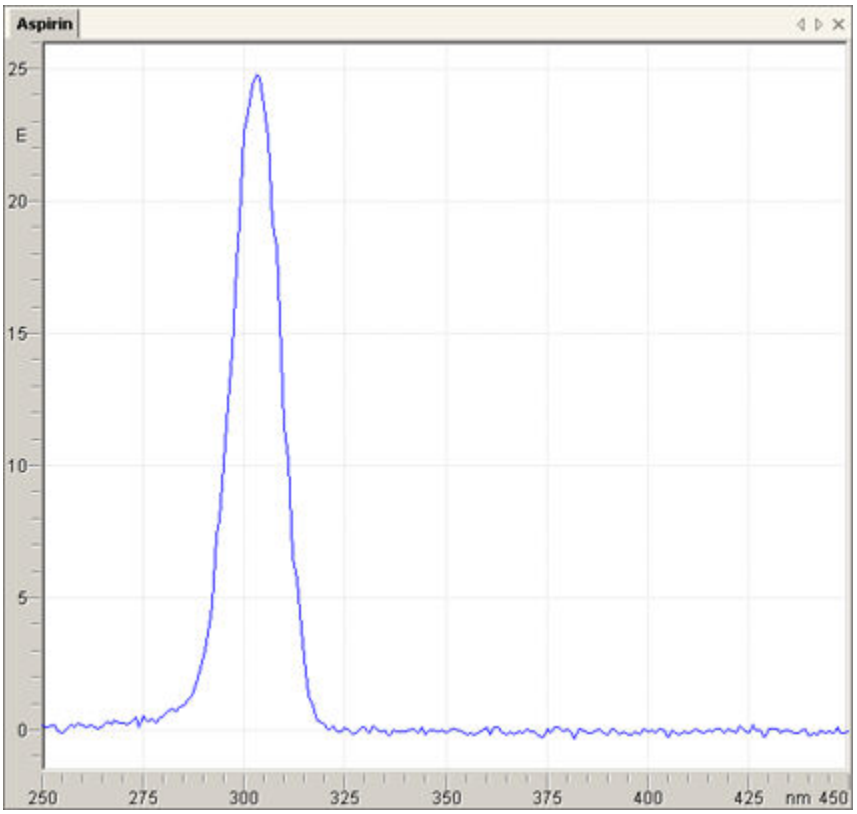


2.6 Example Scan of Excitation Spectra

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



A measured excitation spectrum of diluted Aspirin tablet looks like this:



2.7 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 shown in the following:

$$\partial b(\lambda^*) = \frac{P}{4\pi n^2} E_{\lambda} F_{\lambda}(\lambda^{*'}) K_{\lambda} \partial\lambda'$$

where

db (λ^*): Intensity of the fluorescence observed at wavelength

n: Refractive index

p: Reflection coefficient

E_{λ}: Intensity of the excitation light at wavelength

$F_{\lambda}(\lambda')$: True fluorescence intensity at wavelength in the spectrum emitted by the excitation light at wavelength

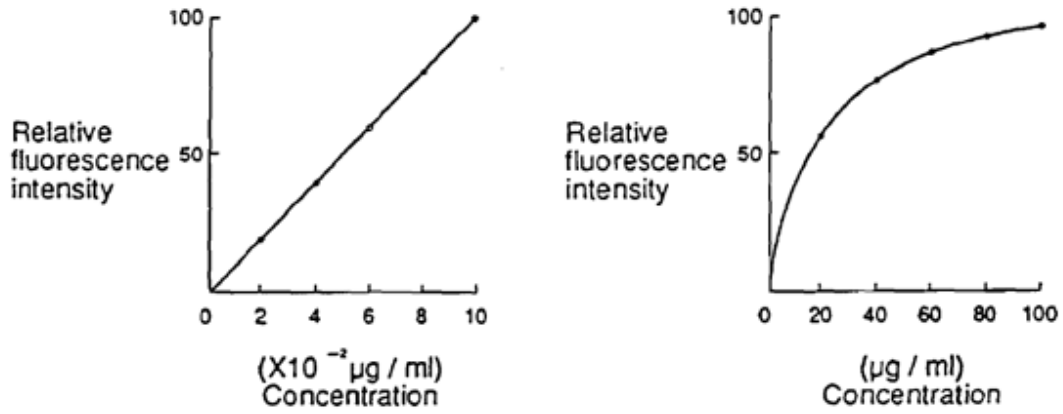
K_{λ}: Absorbance at wavelength

dλ' : Bandwidth of wavelength

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

$B(\lambda') = K\,C$

Hence, the calibration curve that is proportional to the concentration *C* is straight. As the concentration of the sample rises, however, the calibration curve would be curved as explained in "High-concentration samples" section. The following figure shows a calibration curve observed with a diaminostilbene aqueous solution:



2.8 References

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

3 Installation of the spectrofluorophotometer

3.1 Inspection of Parts

After unpacking the instrument check that all the following parts are included.

3.1.1 RF-5301PC Instrument

Type	Part Number	Quantity
Optical unit	P/N 206-81601-**	1
Power cable (100/120 VAC)	P/N 071-60814-01	1
I/F Cable	P/N 206-83579-91	1
Phillips-head screwdriver	P/N 200-94612	1
Sensitivity adjustment flat-blade screwdriver	P/N 200-94613	1
Fuse 5A (100/120 V)	P/N 072-01663-14	2
Xenon lamp 150 W	P/N 200-81500-01	1

3.1.2 RF-1501 Instrument

Type	Part Number	Quantity
Optical unit	P/N 206-62901	1
Power cable (100/120 VAC) (200-240 VAC)	 P/N 206-67 197 P/N 200-81009-02	1
I/F Cable	P/N 206-83579-91	1
Phillips-head screwdriver	P/N 200-94612	1
Sensitivity adjustment flat-blade screwdriver	P/N 200-94613	2
Fuse 5A(100-120VAC) 3A(200-240VAC)	P/N 072-01661-23 P/N 072-01661-20	1
Xenon lamp 150 W	P/N 072-01661-201	1

3.1.3 Necessary Optional Parts for RF-1501:

Type	Part Number	Quantity
RS-232C cable	P/N 200-86408	1
Communication Program Card	P/N 489-04196-24	1

3.1.4 panorama fluorescence software package

If you ordered only the software package, check the equipment items according to the next table:

Type	Part Number	Quantity
panorama fluorescence Software	P/N 980-00212	1

3.2 Safety Precautions

This chapter lits precautions that are particularly important for ensuring safe operation of the equipment. Be sure to observe these precautions when operating the instrument. It is dangerous not to comply with the following points:

1. Do not use the unit for any purpose other than the above-mentioned types of analysis.
2. Follow the procedures described in the user manual.
3. Observe all warnings and cautions.
4. Do not disassemble or modify the unit without the express approval of an authorized Shimadzu representative. Failing to do may lead to dangerous situations or damage of the unit.
5. Do not use methods which are not indicated in the instrument manual. Failing to do may lead to dangerous situations or damage of the unit.
6. For internal repair of the product contact your Shimadzu representative.

The precautions are classified into the following three types based on their importance:

Danger: Can lead to death or serious injury.

Warning: Can lead to injury.

Caution: Can cause damage, malfunction or fire to the equipment or loss of data.

Danger:

The positive electrode of the Xenon lamp is charged to a voltage as high as 30000 V when the lamp is turned ON. The lamp is energized to about 90 V and it reaches a temperature of 100 °C or more after it has been lit. Make sure that the cover of the lamp housing is installed in position before operating the instrument!

Before removing the cover of the lamp housing be sure to disconnect the power cable from the outlet!

Remember that even after the power switch is set to the OFF position, the lamp unit remains electrically charged and can cause electric shock. Disconnect the power cable from the power outlet, set the power switch to the OFF position and leave the instrument for five minutes or longer. Only then remove the cover of the lamp housing!

Danger:

The Xenon lamp contains high pressure gas. Impact, vibration or pressure on it may cause it to burst. Be extremely careful with handling it!

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

Caution: **While installing the lamp do not touch the bulb with bar fingers. Finger oil remaining on the bulb is be baked onto the bulb when the lamp is lit. That can cause the lamp to burst! Cleaning of the lamp is possible with ethyl alcohol or the special cleaning agent supplied with the lamp.**

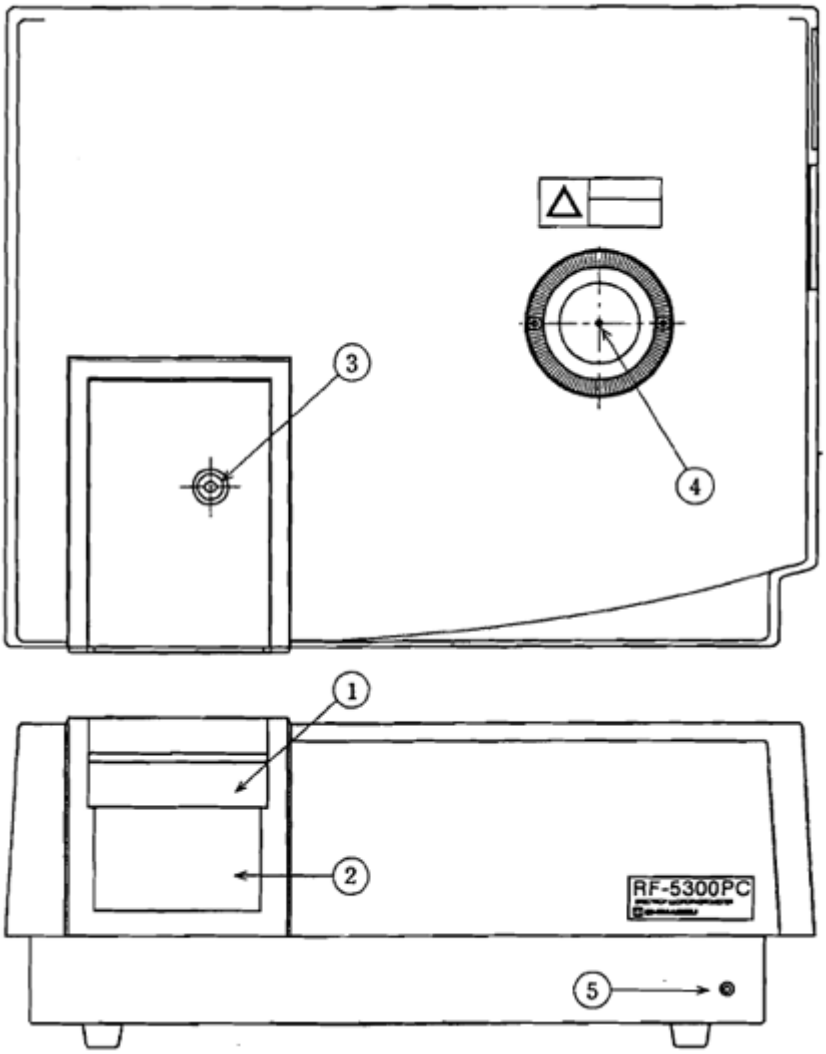
Caution: **When relocating or shipping the instrument be sure to remove the Xenon lamp! Store the removed lamp in the special case supplied with the lamp.**

Caution: **Replace the lamp immediately when ist total service life has exceeded 500 hours (guaranteed service life). Never use a lamp more than 1000 hours! A lamp used more than 1000 hours is liable to burst. A damage of the lamp unit is possible.**

3.3 Part Names and Functions

3.3.1 RF-5301PC System

Top View and Front View



(1) Sample compartment. Holds a sample being analyzed.

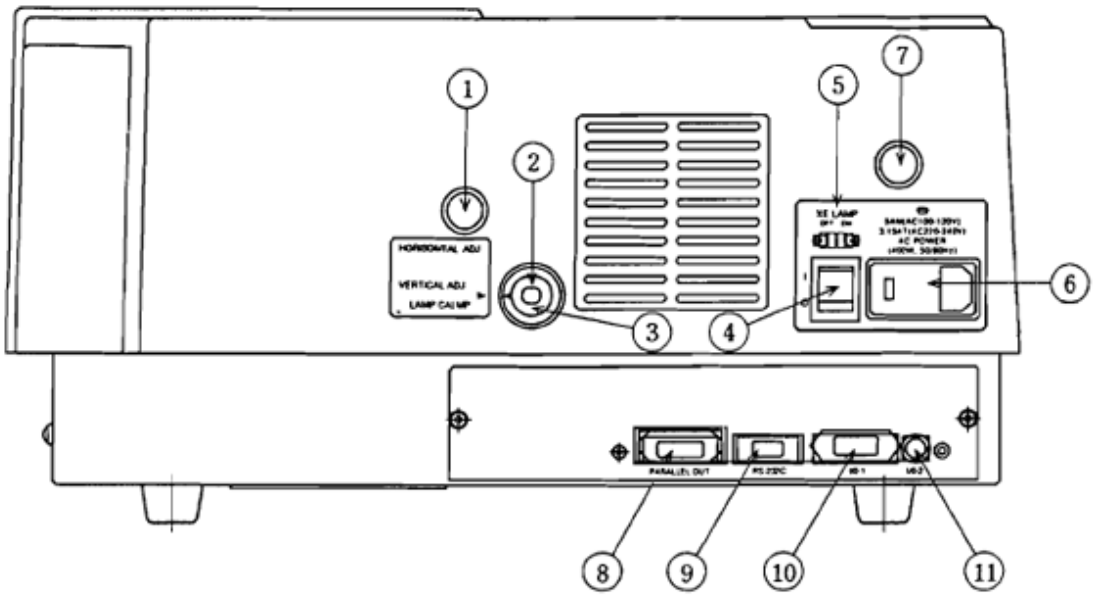
(2) Front panel Xenon lamp. Can be removed when installing an accessory, etc.

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

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

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

Right-side View



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

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

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

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

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

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

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

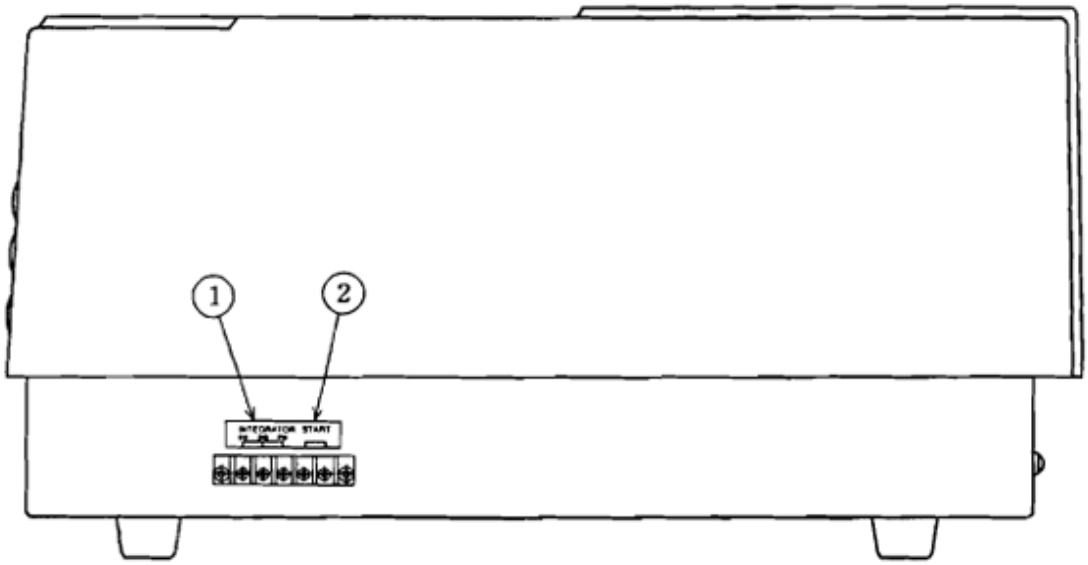
(8) Parallel I/F connector. Not used.

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

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

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

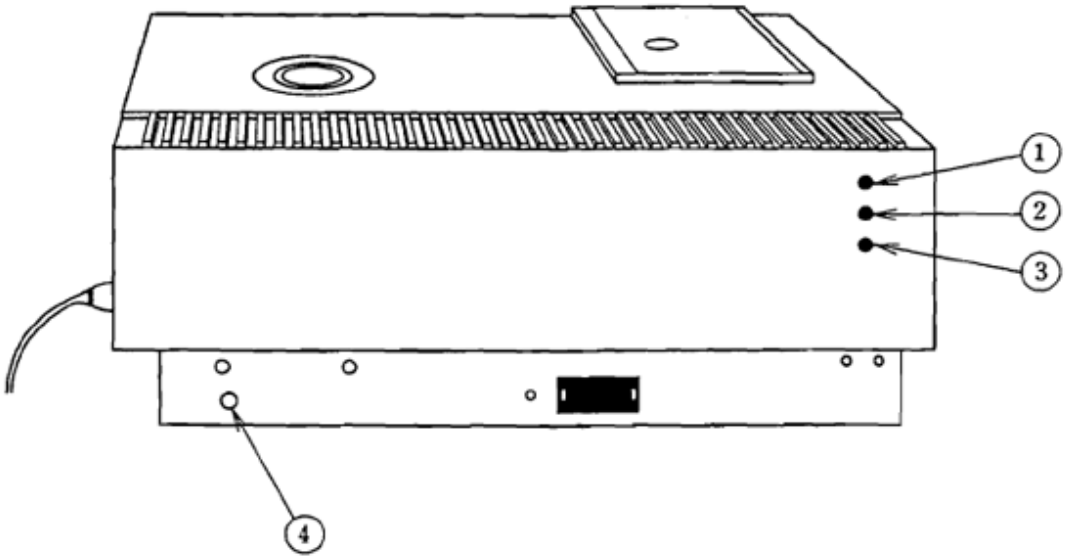
Left-side View



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

(2) Contact IN terminals. Analysis operations can be started by jumpering these terminals.

Rear View



(1) Sensitivity adjustment trimmer VR0. Used to adjust the gain.

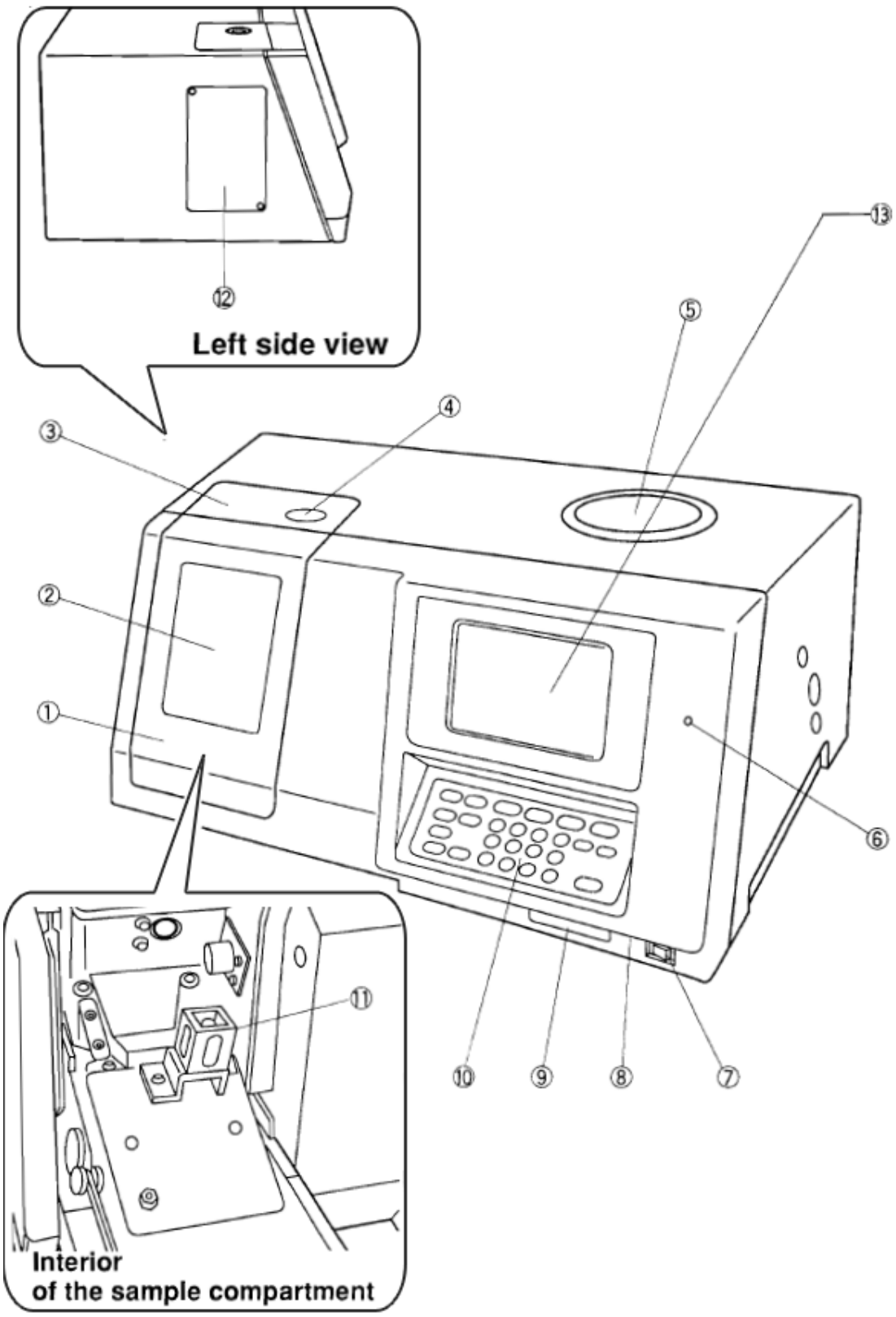
(2) Sensitivity adjustment trimmer VR1. Intended for adjustment of detection sensitivity when negative high-voltage is OFF. This trimmer has been factory-set to a suitable position. Under normal circumstances this trimmer should not be touched!

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

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

3.3.2 RF-1501 System

Front View



(1) Sample compartment

Set the sample to be measured in the sample compartment. Opening the sample compartment cover will expose the cell holder forward.

(2) Front panel

Detach the front panel to install accessories or the like.

(3) Top panel

Detach the top panel to install accessories or the like.

(4) Reagent injection hole cover

Detach the reagent injection hole cover to inject a reagent or the like into the sample compartment when a cell has been set in it.

(5) Light-source compartment cover

Detach the light-source compartment cover to mount or unmount the xenon lamp.

(6) Light-source illumination indicator lamp

This indicator lights when the xenon lamp is ON.

(7) Power switch

Use to turn the power to the unit on and off.

(8) Contrast control

Use to adjust the contrast of the LCD.

(9) IC card slot

Insert optional IC cards (data pack and program pack) into this slot.

(10) Operator panel

Use to operate the unit. (Refer to the description of chapter 4 OPERATING INSTRUCTIONS 4.1 Operator Panel Discription)

(11) Standard cell holder

Cell holder for 10 mm cells.

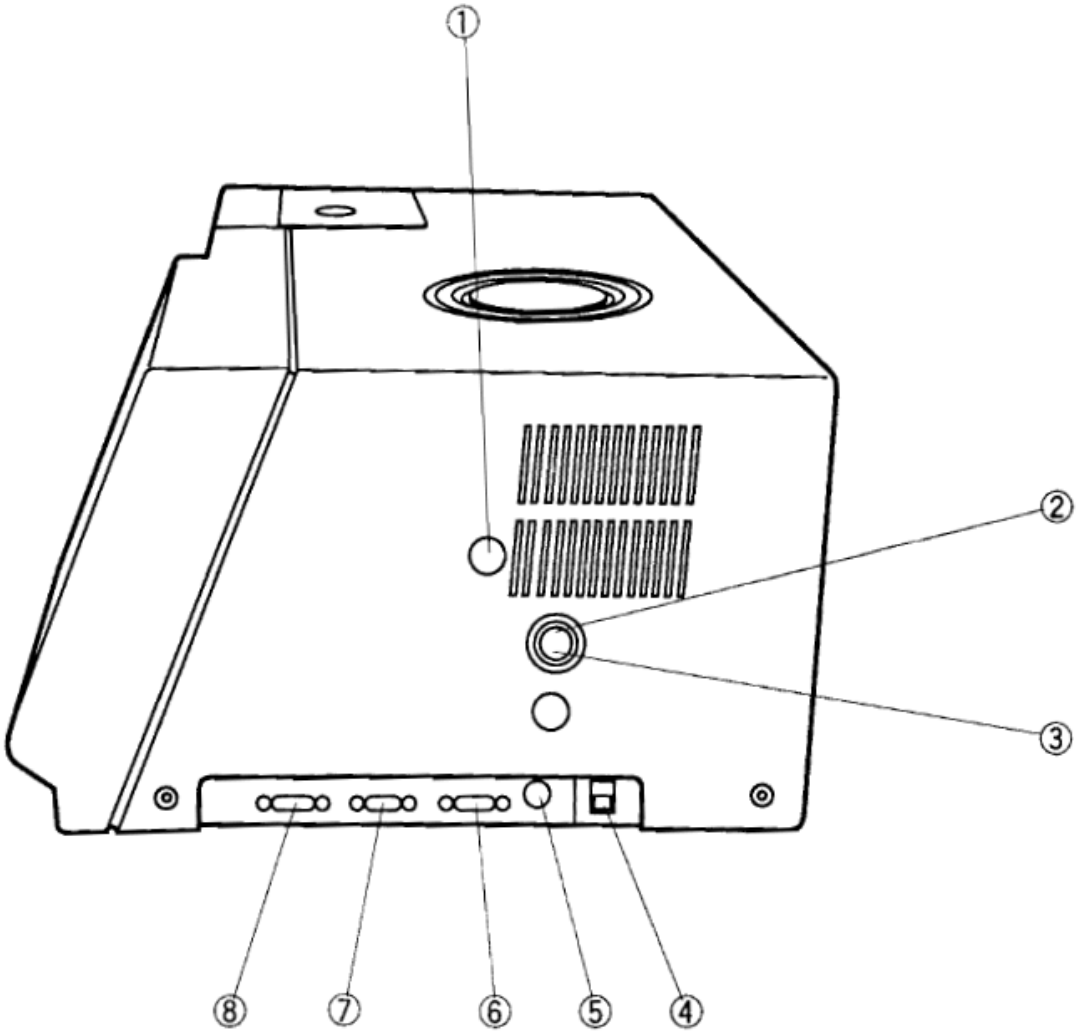
(12) Side panel

Detach the side panel to install accessories or the like.

(13) LCD (Liquid Crystal Display)

Display of the current mode, parameters, wavelengths and fluorescence intensity. It also displays messages and warnings or remedies of error. The selection modes are displayed in frames on the bottom. Those correspond to the function keys F1 to F4. By pressing function key to be selected, the operating mode is implemented. The selected mode is shown in the frame at upper left side of the screen.

Right Side View



(1) Light source horizontal dlrection adjusting screw

Use to adjust the horizontal direction of the light source.

(2) Lamp mounting screw

Use to mount or demount the xenon lamp.

(3) Light source vertical direction adjusting screw

Use to adjust the vertical direction of the light source.

(4) Lamp switch

Leave the lamp switch normally at the ON position.

(5) I/0-2 connector

Attach the sipper to this connector.

(6) I/0-1 connector

Attach the auto-sample changer to this connector.

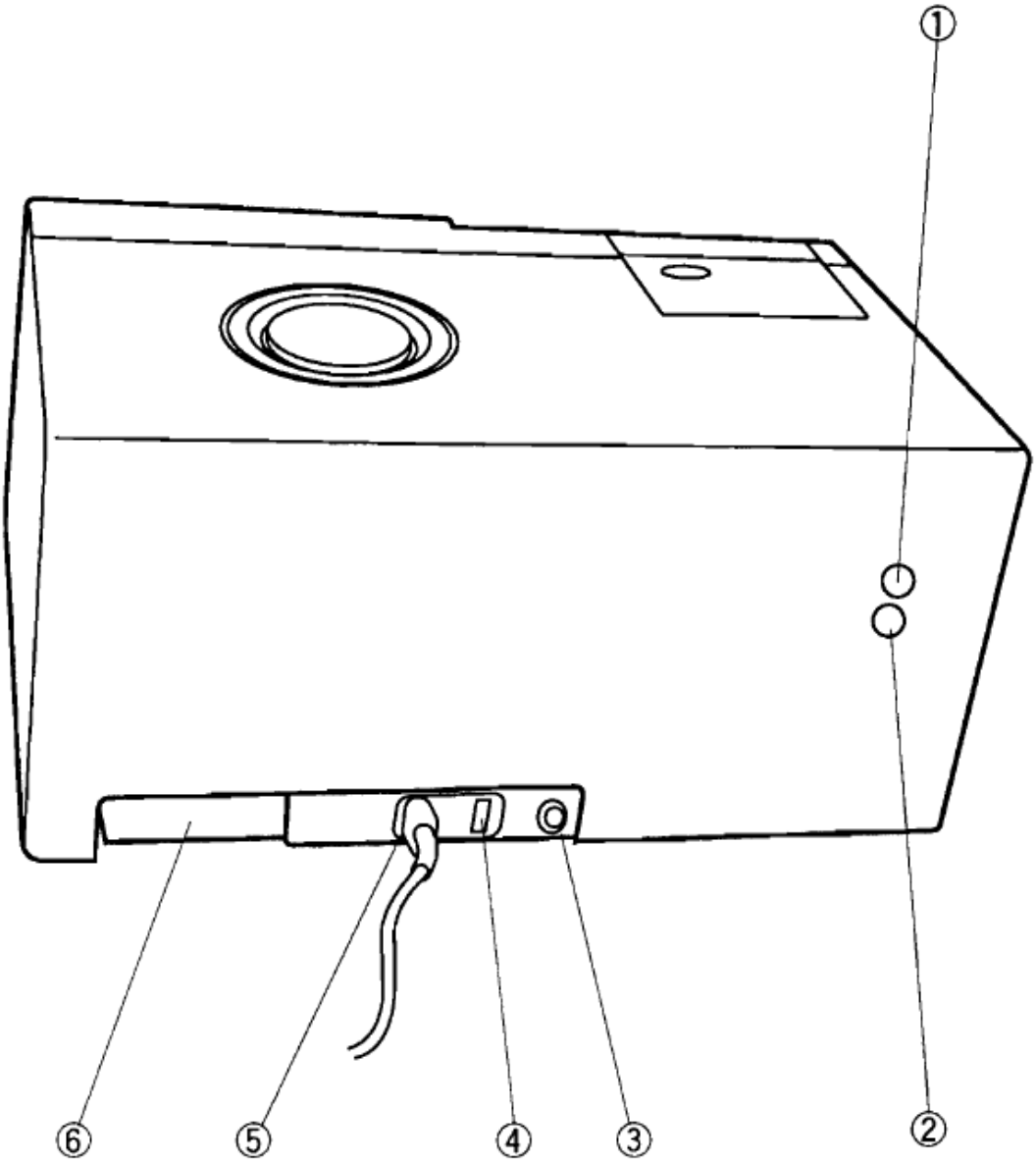
(7) RS-232C connector

Attach a computer In support of an RS-232C interface to the RF-1501.

(8) Printer connector

Attach a printer to the RF-1501.

Rear View



(1) Sensitivity control VR1

Use to adjust fluorescence intensity when adjusting the light source position.

(2) Sensitivity control VR2

The sensitivity control VR2 is used to set detector sensitivity. **It Is factory-adjusted and must not be touched.**

(3) Grounding terminal

Connect a grounding wire to this terminal.

(4) Voltage setting selector

The voltage setting selector offers a choice of four input voltages (100, 120, 220, and 240 VAC).

(5) Power cable Inlet

Plug the accessory power cable into this inlet to power the unit from an AC outlet.

(6) Expansion board slot

Set an optional interface board or output board into this slot. It is normally covered.

3.4 Hardware Specification

3.4.1 RF-5301PC System

Item	Description
Light source	150 W Xenon lamp
Lamp housing	Lamp housing with ozone self-decomposition
Emission/Excitation monochromator	Blazed holographic concave diffraction grating (F/2.5 for emission and excitation side)
Density of diffraction grating	1300 lines/mm
Wavelength scan range	220 - 900 nm and zero-order light (EX and EM)
Measuring wavelength range	220 - 750 nm and zero-order light (EX and EM)
Slit width	1.5/3/5/10/15/20 nm (excitation side) 1.5/3/5/10/15/20 nm (emission side)
Wavelength accuracy	±1.5 nm
Wavelength scan speed	SURVEY, SUPER, VERY FAST, FAST, MEDIUM, SLOW, VERY SLOW
Light source compensation system	Monochromatic light monitoring diode feedback system
Detector	Photomultiplier tube for photometry and monitor sides; Photometry: R3788-02, Monitor: R212-14
Sample compartment	Single non-constant temperature cell holder
Sensitivity selection	High/low selectable (difference approx. 50:1)
Response	Automatic cumulative smoothing depending upon wavelength; scan speed and manual response are selectable.

S/N ratio	150 or greater at the water Raman peak of distilled water, spectral bandwidth 5 nm for both EX and EM, EX 350 nm
Power supply	100/120/220/240 VAC, 50/60 Hz, 400 VA
Operating temperature range	15 - 35 °C
Operating humidity range	45 - 80 % (70 % or less at room temperature 30 °C or higher)
Dimensions	Photometer: 670(W) × 530(D) × 270(H) mm
Weight	Photometer: 43 kg

3.4.2 RF-1501 System

Item	Description
Light source	150 W Xenon lamp
Light source chamber	Ozone self-extinction lamp house
Monochromators	Ion-blazed holographic concave grating for both excitation and emission monochromator F12.4
Number of grating ruled lines	900 lines/mm
Wavelength scan range	220 - 900 nm and zero-order light (EX and EM)
Measuring wavelength range	220 - 750 nm and zero-order light (EX and EM)
Bandwidth	10 and 20 nm for both excitation and emission
Wavelength accuracy	± 5 nm
Wavelength scan speed	Selectable from among SUPER (about 3700 nm/min), FAST, MEDIUM, and SLOW
Slewing speed	Approx. 30.000 nm/min

Light source compensation system	Dynode feedback method by monitored excitation light.
Detector	Photomultipliers for both fluorescence measurement and monitoring
Sample compartment	Single non-constant temperature cell holder
Sensitivity selection	Two selectable settings, HIGH and LOW (sensitivity difference: about 50 times)
Response	0-98% 0.02, 0.03, 0.1, 0.25, 0.5, 2 and 8 sec
S/N ratio	The S/N ratio at the Raman peak of distilled water is 300 or higher under the measurement conditions: Excitation wavelength : 350 nm Emission wavelength : Peak wavelength of the Raman line Bandwidths : 10 nm for both excitation and emission Response : 2 sec
Display	Backlit LCD (320 x 200 dots)
I/O	One RS-232C port One printer interface (Centronics compatible) port Sipper interface Autosampler interface
Measurement functions	**Wavelength scanning** • Automatic search for optimal combination of excitation and emission wavelengths • Excitation and emission spectrum measurement • Spectrum enlarging and reduction • Differential spectra • Spectrum data printout • Saving of spectra (two spectra stored in the unit's internal memory and 9 stored per RAM card) **Quantitative measurement** • Calibration curve creation (up to 10 standard sample, first order approximation) • Repetitive measurement (up to 5 times) • Calibration curve storage and modification

	• Saving of quantitative measurement data files (two files stored in the unit and 9 stored per RAM card)
Storage of measurement files	Three files stored in the unit's internal memory and 60 per RAM card
Input Power	100 V,120 V, 220 V, 240 V AC, 50160 Hz
Power Consumption	400 VA
Operating temperature range	10 - 35 °C
Operating humidity range	45 - 80 % (70 % or less at room temperature 30 °C or higher)
Dimensions	500 (W) x 400 (D) x 255 (H) mm
Weight	23 kg

3.5 Installation of the RF-5301PC or RF-1501

3.5.1 Location

To provide optimal performance of the instrument and to ensure a long trouble-free operating life, be sure to install the instrument in a location that satisfies the requirements listed below.

Take care of your instrument location!

Note that Shimadzu Corporation shall not be responsible for any damages or costs arising from deteriorated performance or mechanical damage arising from the use of the instrument in location that fails to satisfy the following requirements even during the guarantee period.

- Room temperature within the range of 15 °C to 35 °C.
- A position not exposed to direct sunlight.
- A position not subject to strong vibration or any continuous (even weak) vibration.
- A position free from strong magnetic or electromagnetic fields.
- Relative humidity within the range 45 % to 70 %. (If the room temperature is 30 °C or higher the relative humidity must be more than 70 %.)
- A location free from exposure to corrosive gas or any organic or inorganic gas that has an absorption band in the UV region.
- A location substantially free from dirt or dust.
- Place the spectrofluorophotometer on a table or surface which can withstand the load of the spectrofluorophotometer (weight of RF 5301PC: 43 kg; weight of RF-1501: 23 kg).

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

3.5.2 Connection to Personal Computer

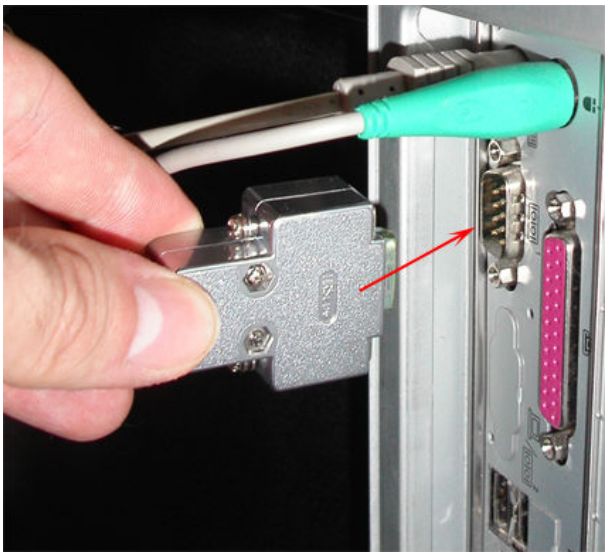
Using the RF-1501 with panorama Fluorescence.

When using the RF-1501 with the software, please make sure to have the **Program Pack** (see "IC Card Options for the RF-1501" below) installed properly. Otherwise the software cannot control the instrument!

The RF-5301PC or RF-1501 unit must be connected to the personal computer with the attached I/F cable.

1. Connect the male end of the cable to the connector (serial) on the right-side face of the RF-5301PC unit
2. Adjust the two screws to secure the connection.

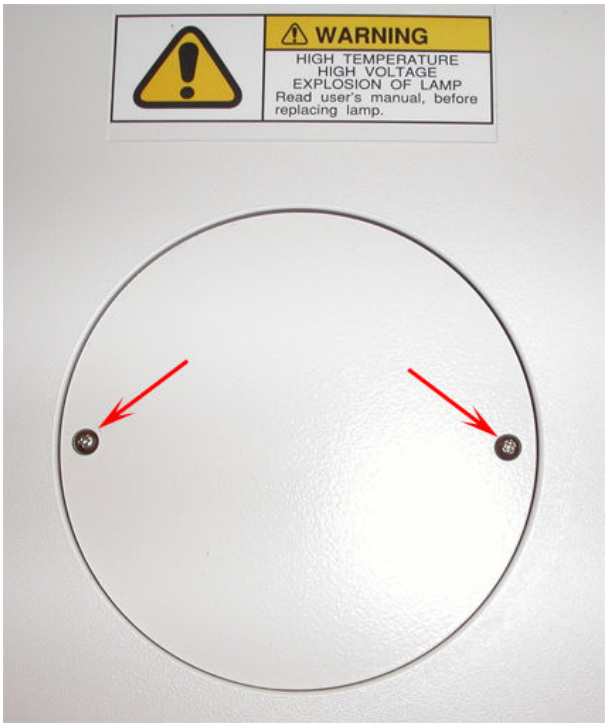
3. Connect the female end of the cable to the serial port
4. Tighten the two screws to secure the connection. expose the inside of the lamp housing.



3.5.3 Installation of the Xenon lamp

Before installing the Xenon lamp make sure that the power switch is in the OFF position and disconnect the power cable from the power outlet to prevent accidental electrical shock.

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

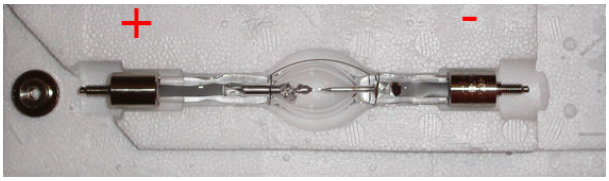


2. Loosen the fixing screws on the slide plate.

3. Shift the sliding plate to expose the inside of the lamp housing.

4. Inset the screw driver for fixing the lamp (standard accessory) into the opening provided for that purpose along the guide tube. Loosen the lamp fixing screw by turning it counterclockwise.

5. Remove the Xenon lamp from its shipping case and remove the knurled screws on the positive and negative sides.



6. Line up the lamp so that the black or red mark on the cement part of the negative side of the lamp is oriented in the direction of the lamp fixing screw. Then insert the lamp into the housing.

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

7. Insert the screw driver into the opening again and turn the lamp fixing screw clockwise to secure the lamp in position.

8. Close the sliding plate and secure it with the fixing screws.

Caution: **If the positive electrode side is subjected to a pull-force by the power cord while the lamp is lit, the lamp may be damaged. When securing the terminal of the positive electrode with the knurled screw make sure that the power cord at the positive side is a little slack.**

9. Connect the power cord terminal of the positive electrode with the positive end of the lamp.
10. Secure the terminal with the knurled screw.
11. Tighten the knurled screw by hand! Using a tool such as a wrench may damage the lamp.

12. Install the cover of the lamp housing. The installation of the Xenon lamp is complete.

3.5.4 IC Cards (Options for the RF-1501)

The following kinds of IC cards are available to add additional functions to the RF-1501 unit.

Caution: **Please pay attention to the following precautions in handling IC cards:**

- Do not give rough shocks to the cards.
- Do not fold or bend the cards.
- Do not expose the cards to high temperature or direct sunlight.
- Protect the cards against intense magnetic fields or electrostatic effects. The stored programs might be destroyed.
- The useful life of the data pack battery is about 7 years.

Program Pack (Gray-labeled IC card)

The program pack adds additional functions to the RF-1501. It supports two modes of measurements in standard:

- **Spectrum mode**
  in which the excitation or emission spectrum of a sample is measured by wavelength scanning.
- **Quantitative measurement mode**
  in which the concentration of an unknown sample is measured by creating a calibration curve from a standard sample with a known concentration.

Data Pack (Yellowish green-labeled IC card)

A single data pack provides two memory functions as described below. Battery backup protects the stored data from erasure when the data pack is removed from the slot in the RF-1501.

- **Parameter memory function**
  The RF-1501 can store three sets of measurement conditions by itself. An additional 60 Sets of measurement conditions can be stored per data pack.

- **Data memory function**
  The RF-1501 provides two files to store spectrum, quantitative measurement and other data. An additional 9 files can be stored per data pack.

Caution:	**Note on removing data packs**
⚠	If you remove the data pack while it is being accessed for reading or writing, damage to the stored data could result. Since reading from or writing to the data pack is completed in a short time, perform any key operation, then wait for about 3 seconds before removing the data pack.

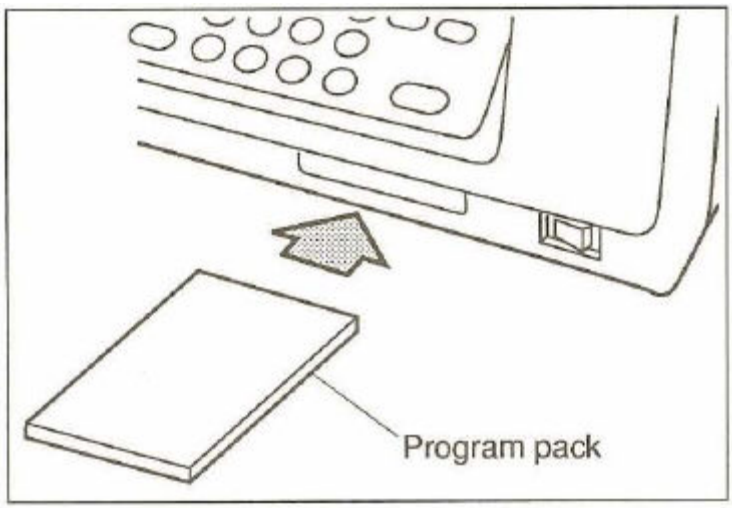
Installing the IC Card

Please follow the steps below to install the IC card correctly to your RF-1501 unit:

1. Insert the IC card firmly into the slot at the front, lower right of the RF-1501.

   You can insert or remove the **program pack** regardless of whether the power is on or off.

   Never remove the **data pack** while the instrument reads from or writes to the data pack!



3.5.5 Installation of external Accessories

The following external accessories are available with the RF-5301PC or RF-1501 instrument.

- **Standard cell holder (default)**
- **Sipper**
  Please refer to the section "Working with the Sipper Accessory" for details.
- **Auto-Sampler**
  Please refer to the section "Working with the Auto-Sampler Accessory" for details.
- **Constant temperature cell holder** (currently not supported by the **panorama fluorescence** software!)

Install the external accessory according to the steps described in the particular accessory sub-section.

As long as you run the instrument with external accessories together with the **panorama fluorescence** software, there is no need for a special setup of the instrument. The software will take over full control over all accessories and parameter settings.

In case you like to run the RF-1501 unit as a stand-alone instrument, the external accessories need to be installed separately via the on-screen display. Please refer to your RF-1501 operation instructions manual, chapter 4.7 for details.

3.5.6 Powering ON

After installing the Xenon lamp and optional IC cards or accessories power ON the instrument. Before setting the power switch to the ON position, make sure of the following aspects.

1. **Grounding**
   The power cable for the instrument is a three-wire type including a ground wire. Connect the cable to a three-wire type power outlet, and provide reliable grounding. If the power outlets available on-site are two-wire type, make sure to provide grounding using the grounding terminal on the power cable, ground the instrument directly.
2. **Connecting the power cable**
   First, be sure that the power switch is in the OFF position. Then insert the attached power cable into the power inlet located on the right-side face of the instrument. Make sure that the setting of the AC voltage selector conforms to the voltage of the site power.
3. **Xenon lamp ON/OFF switch**
   Set the Xenon lamp ON/OFF switch to the ON position.

If all items are satisfied please continue as follows:

1. Set the power switch to the **ON** position. The Xenon lamp will turn ON automatically.

   Xenon lamp ignition failure!

   If the Xenon lamp fails to light or a continuing ”crackling” sound can be heard, power OFF the instrument. For troubleshooting take a look at chapter "RF-5301PC Troubleshooting" or contact Shimadzu.

2. After powering ON the instrument power ON the computer and start MS-Windows.
3. Start the **panorama** **fluorescence** software package.

3.6 Lamp Adjustment

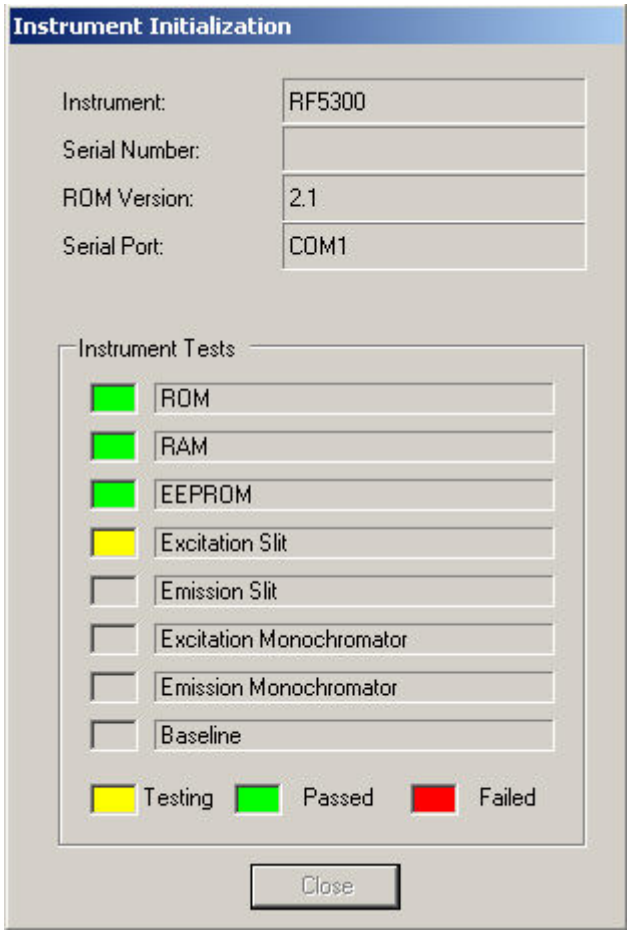
The lamp position must be adjusted according to the RAMAN spectrum obtained with distilled water. This adjustment must be preformed when installing the Xenon lamp for the first time and when replacing a lamp. Make sure that the instrument has been powered ON for at least 30 minutes before performing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

3.6.1 Coarse-adjustment of the lamp position

For coarse-adjustment of the Xenon lamp, please follow the instructions below:

1. Start the **panorama** **Fluorescence** software.

2. From the **RF-5301/RF-1501 menu**, select the **Go to Wavelength** command.
   The instrument will be initialized automatically on first time after powering ON showing the following dialog:

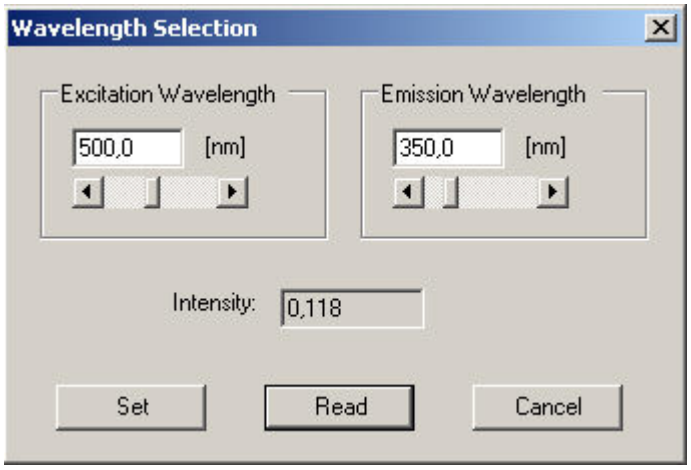


> **Instrument Initialization is not mandatory!**
>
> Instrument initialization is only done, if the spectrofluorophotometer is used the first time after powering ON or if connection was lost for any reason. If this dialog is omitted, the instrument has already been initialized successfully.

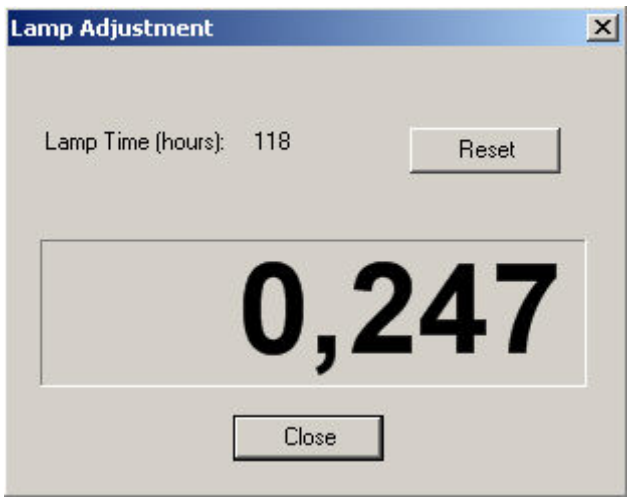
3. After completion of the initialization process, **click** the ***Close*** **button**.

> **What happens on Instrument Initialization Failure?**
>
> Please refer to the Troubleshooting section or contact Shimadzu support for help.

4. In the Go to Wavelength dialog, **set** the following **parameters**:
   - Excitation Wavelength = **500 nm**.
   - Emission Wavelength = **350 nm**.



5. **Click** the ***Read*** **button** to update the actual intensity value (optional).
6. **Click** the ***Set*** **button** to apply actual values.
7. From the **RF-5301/RF-1501 menu**, select the **Lamp Adjustment** command.
   The instrument shutter is opened and the following dialog is shown:

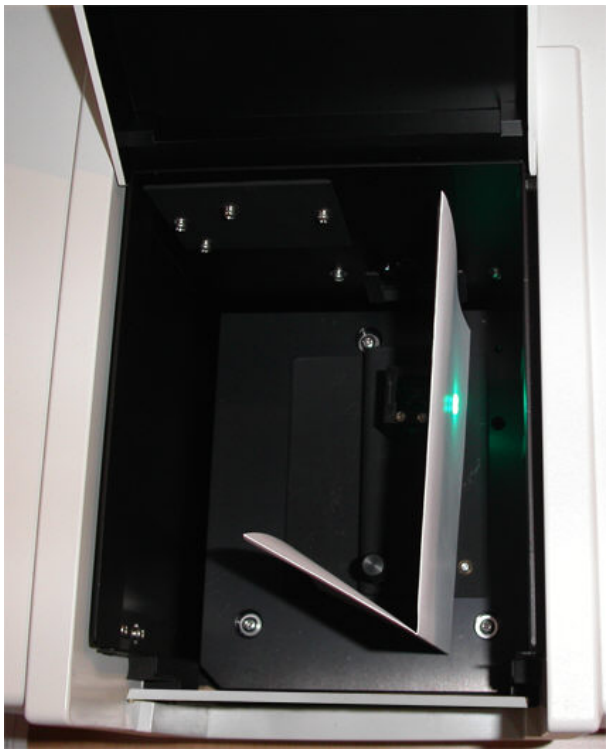


The intensity value is updated continuously.

> **The lamp time reset function is not available for the RF-1501 Instrument!**
>
> The current lamp time is only displayed for the RF-1501 instrument.

8. **Open** the lid of the sample compartment.

9. **Place** a sheet of **white paper** into the cell holder.
   The excitation monochromator should be emitting a green light beam.



10. Adjust the **screw** for **horizontal** positioning of the lamp so that the beam spot on the white paper is of maximum brightness.

Caution: ⚠	**Be sure to adjust the horizontal position of the lamp first. Adjusting the vertical position first may cause the light beam to deviate and make it impossible to correct the lamp position.**

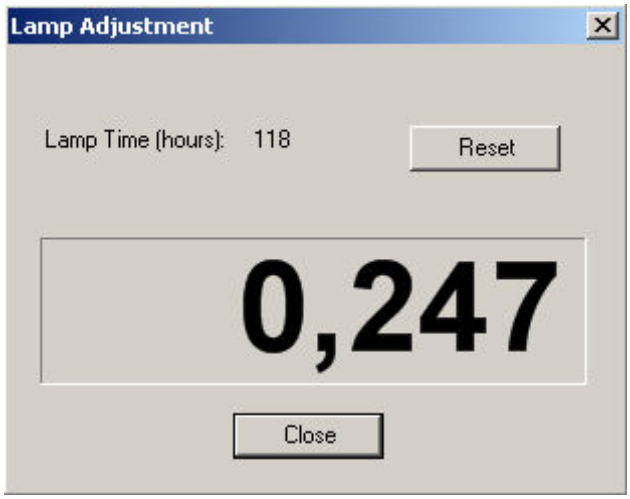
11. Similarly to the horizontal position adjust the **screw** for **vertical** positioning of the lamp.

12. **Remove** the **white paper**.
13. **Load** a **cell** containing **distilled water** into the cell holder.
14. **Close** the compartment lid.

3.6.2 Fine-adjustment of the lamp position

After coarse-adjustment of the lamp position a fine-adjustment is necessary. Follow the steps below:

1. **Activate** the **Lamp Adjustment** dialog.
   The display shows an energy value.



2. Adjust the **screw** for **horizontal** positioning of the lamp until the energy value indicates a maximum.
3. Adjust the **screw** for **vertical** positioning of the lamp until the energy value indicates a maximum.
4. If a new lamp was installed, **click** the **Reset button** to reset the lamp timer (optional).
5. **Click** the ***Close*** **button** to finish the procedure.

4 Performance Tests

4.1 Signal/Noise Test

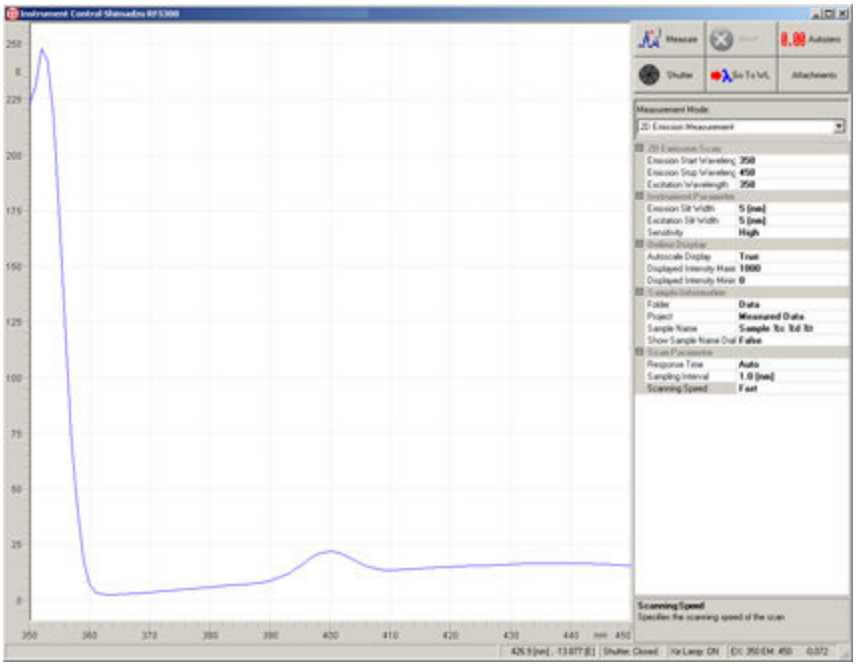
After installation of the Xenon lamp and adjustment of the lamp position the performance of the instrument should be verified to check that it is operating normally.

4.1.1 Prerequisites

A 2D Emission measurement is required in advance to check experimental conditions with the sample. Please follow the instructions below:

1. **Start** the **panorama fluorescence** software and make sure, the instrument has warmed up for at least 30 minutes.
2. From the **RF-5301/RF-1501 menu**, select the **2D Emission Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings.
3. In the measurement dialog, **set** the following parameters:
    - Emission start wavelength: **350 nm**
    - Emission stop wavelength: **450 nm**
    - Excitation wavelength: **350 nm**
    - Scanning speed: **fast**
    - Emission slit width:
      RF-5301 PC: **5 nm**
      RF-1501: **10 nm**
    - Excitation slit width:
      RF-5301 PC: **5 nm**
      RF-1501: **10 nm**
    - Sensitivity: **high**
    - Sampling interval: **1.0 nm**
    - Response time: **Auto**
4. **Open** the lid of the **sample compartment**.
5. **Load** a cell filled with **distilled water** into the cell holder.
6. **Close** the lid of the **sample compartment**.
7. **Click** the ***Autozero*** **button**.

8. **Click** the ***Measure*** **button** to start scanning.
The resulting spectrum is shown in the following figure:



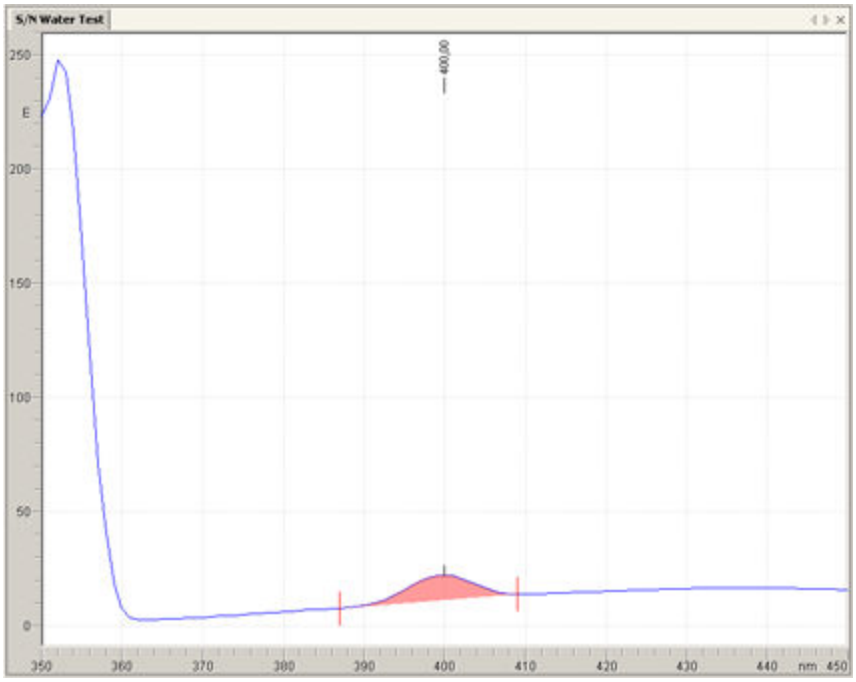
9. **Click** the **button** on top right of the measurement dialog to close it.
The measured 2D emission spectrum is now shown in the main workspace.

10. From the **Mathematics menu**, select the **Find Peaks** command.

11. In the **Mathematics tab** on bottom right, adjust the parameters and **click** the ***Calculate*** **button**.
The peaks should be detected automatically and a peak table will be created.

> **Too less or too many peaks are found!**
>
> Please refer to the Find Peaks command section for details on how to setup parameters for peak finding. Refer to the Find Peaks section in the chapter Mathematics for an introduction.

The resulting spectrum looks similar to the figure below:



(Determined wavelength of water band: 400.00 nm)

The obtained peak wavelength should be approximately 397 nm.

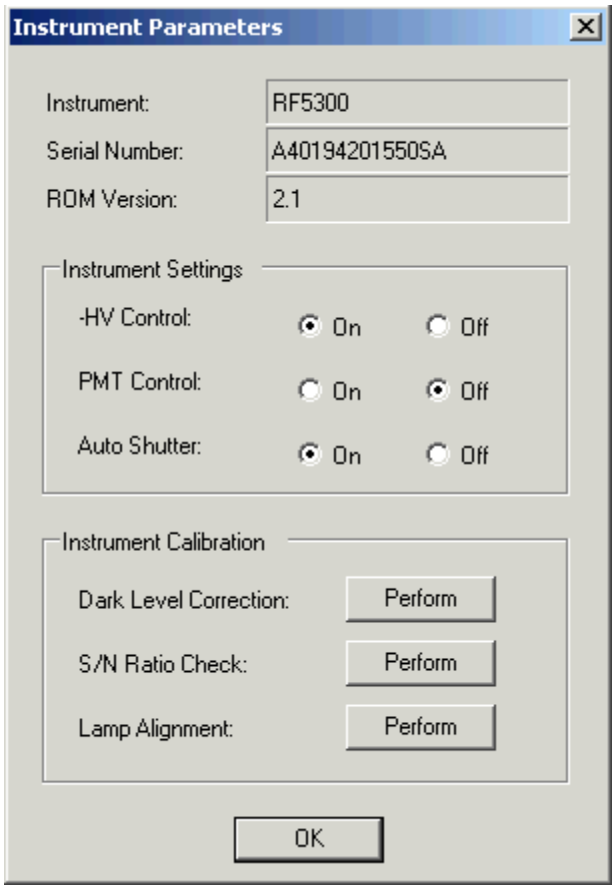
12. From the **File menu**, select the **Print** command to print a report for the spectrum and corresponding peak values.
    For details on printing and configuring report templates, please refer to the chapter "Printing".

4.1.2 Signal to Noise Test Procedure

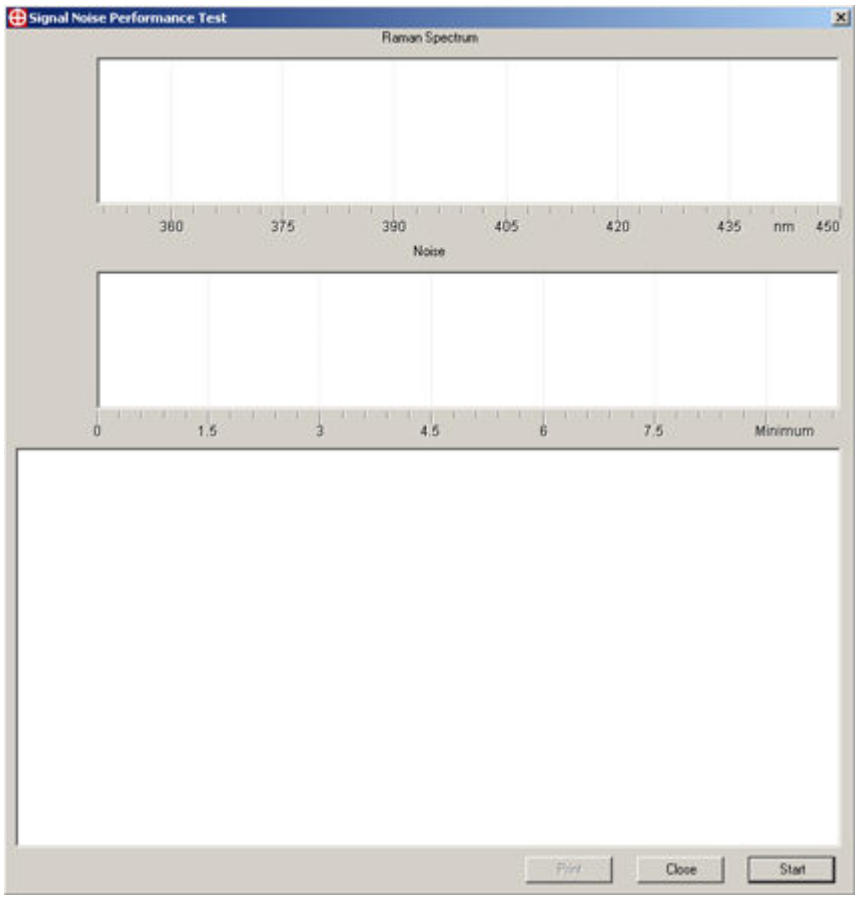
The automatic signal to noise test procedure will perform subsequent measurements among a time interval of 10 minutes. Emission spectra will be recorded automatically and the signal to noise ratio is calculated from the water peak. Finally a report is generated, which can be printed.

To perform the signal to noise test, please follow the instructions below:

1. From the **RF-5301 menu**, select the **Instrument Parameters** command.
   The instrument parameters dialog is opened:



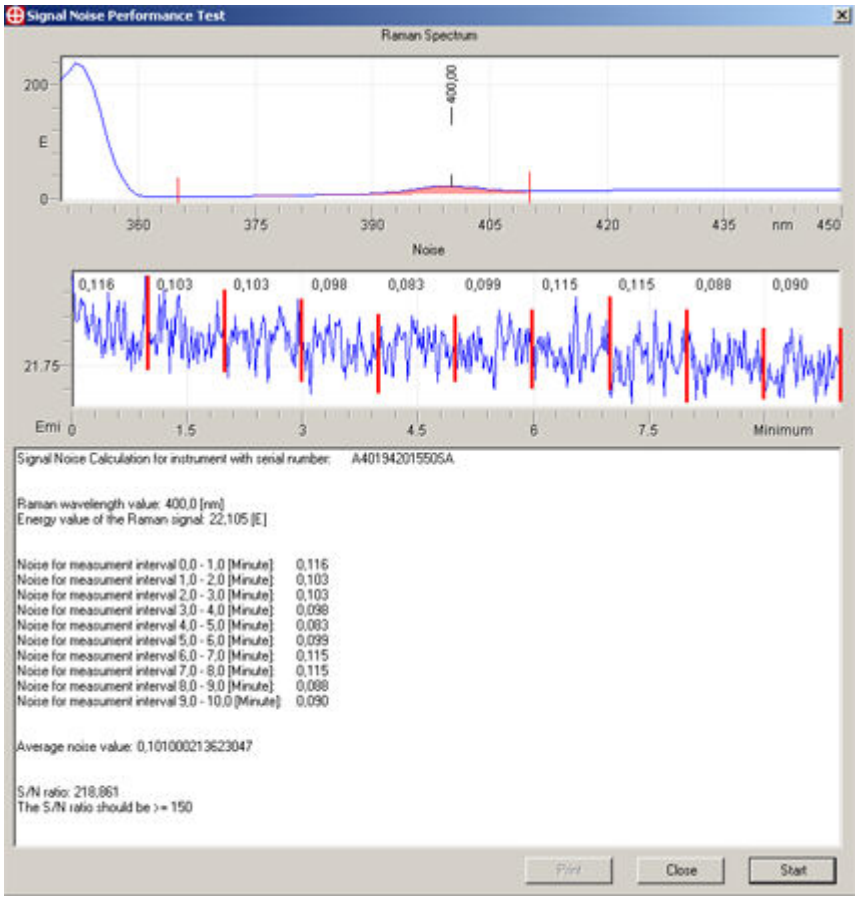
2. In the dialog, **click** the ***Perform*** **button** next to the **S/N Ratio Check** label.
   The Signal Noise Performance Test dialog is opened:



3. In the dialog, **click** the ***Start*** **button**.
   The user is prompted to insert a cell filled with distilled water into the cell holder:

4. **Open** the lid of the **sample compartment**.
5. **Load** a cell filled with **distilled water** into the cell holder.
6. **Close** the lid of the **sample compartment**.
7. **Click** the ***OK*** **button** to start measurement.

8. After the automatic test procedure is finished, the report is completed:



(Determined S/N ratio: 218.861)

The signal to noise value should be ≥ 150 for the RF-5301PC instrument.

4.2 Signal to noise report layout

This report shows the results of the RF-5301 signal to noise test.

Notwithstanding the basic print layout properties and general object properties, some individual properties can be adjusted for 2D data objects, that need to be printed.

4.2.1 Noise spectrum print layout properties

The same settings are applied as described for the Spectral image in the basic report layout section.

4.3 Wavelength Accuracy Test

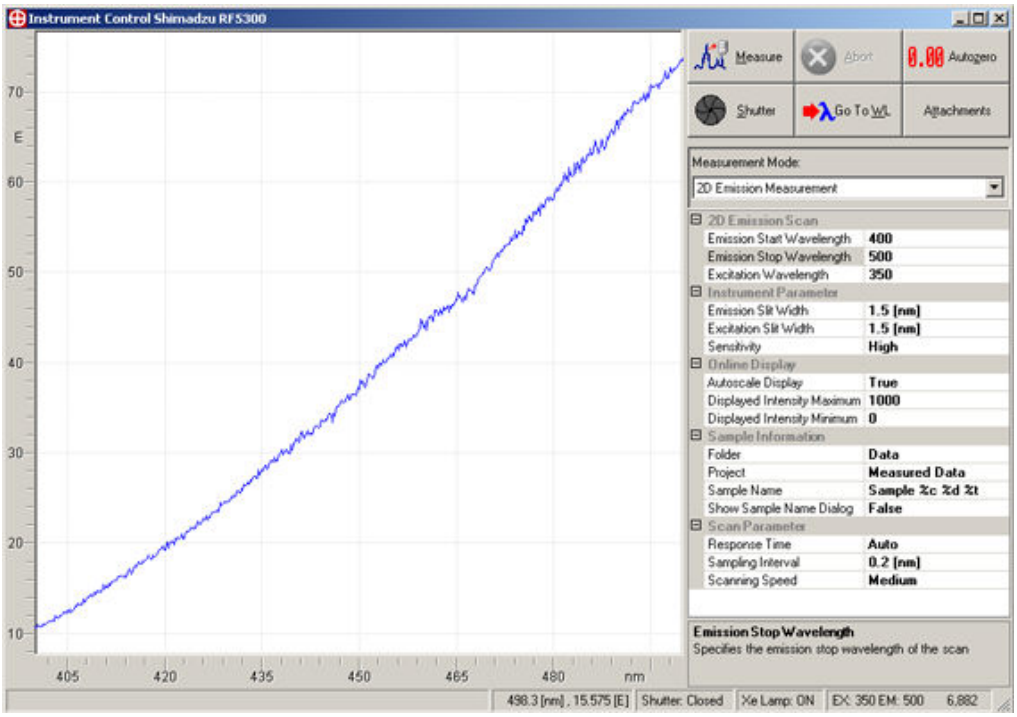
Based on the mercury emission line (435.8 nm) of the light from a fluorescent lamp, the wavelength accuracy can be verified for the monochromators.

4.3.1 Verifying wavelength accuracy for emission monochromator

To verify the emission monochromator a 2D emission measurement must be carried out.

1. **Start** the **panorama** **Fluorescence** software and make sure, the instrument has warmed up for at least 30 minutes.

2. From the **RF-5301/RF-1501 menu**, select the **2D Emission Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings:



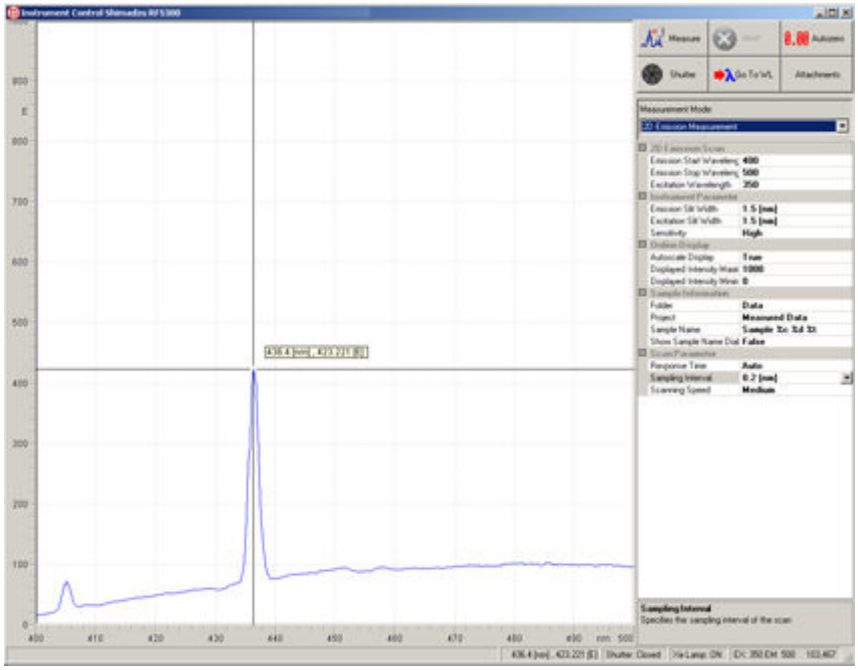
3. In the measurement dialog, **set** the following parameters:

   - Emission start wavelength: **400 nm**
   - Emission stop wavelength: **500 nm**
   - Excitation wavelength: **350 nm**
   - Scanning speed: **medium**
   - Emission slit width:
     RF-5301 PC: **1.5 nm**
     RF-1501: **10 nm**
   - Excitation slit width:
     RF-5301 PC: **1.5 nm**
     RF-1501: **10 nm**
   - Sensitivity: **high**
   - Sampling interval:
     RF-5301 PC: **0.2 nm**
     RF-1501: **1 nm**
   - Response time: **Auto**

3. **Open** up the lid of the **sample compartment** to expose the emission monochromator to light from a fluorescent lamp.
   Remove the cell from the sample compartment.

4. **Click** the ***Measure*** **button** to start scanning.

5. After scanning has stopped, check that the **resultant peak** in the spectrum is within the range of **435.8 ± 1.5 nm**.

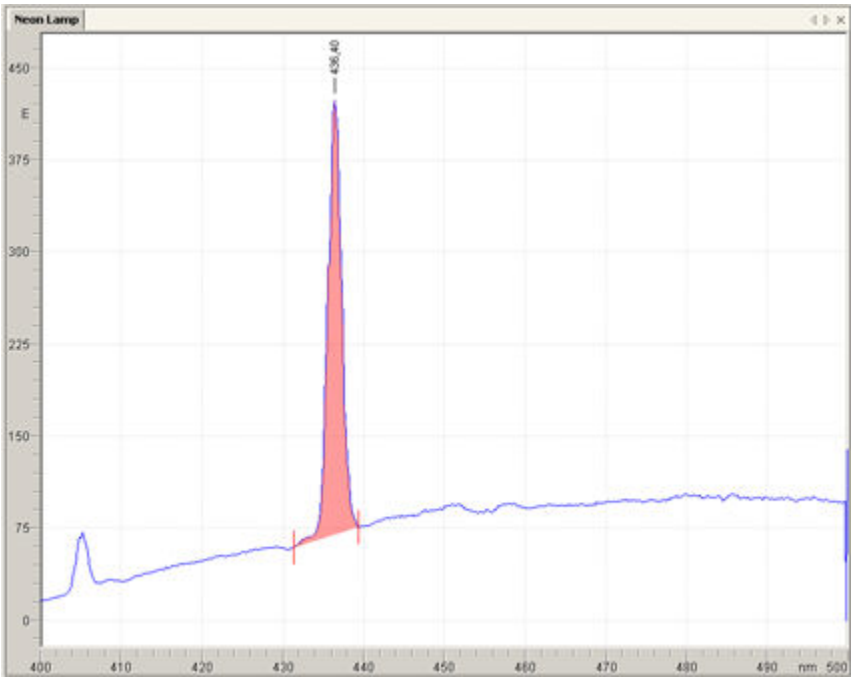


(Determined wavelength: 436.4 nm)

6. **Click** the **button** on top right of the measurement dialog to close it.
   The measured 2D Emission spectrum is shown in the main workspace now.
7. From the **Mathematics menu**, select the **Find Peaks** command.
8. In the **Mathematics tab** on bottom right, adjust the parameters and **click** the ***Calculate*** **button**.
   The peak should be detected automatically and a peak table will be created.

> **Too less or too many peaks are found!**
>
> Please refer to the Find Peaks command section for details on how to setup parameters for peak finding. Refer to the Find Peaks section in the chapter Mathematics for an introduction.

The resulting spectrum looks similar to the figure below:



(Emission spectrum of a Neon Lamp)

9. From the **File menu**, select the **Print** command to print a report for the spectrum and corresponding peak values.
   For details on printing and configuring report templates, please refer to the chapter "Printing".

> **Check the resultant peak position in the spectrum!**
>
> If the emission monochromator fails to satisfy the range mentioned above, contact Shimadzu or its nearest representative. You will find contact data in the "Contact Shimadzu" chapter.

4.3.2 Verifying wavelength accuracy for excitation monochromator

To verify the excitation monochromator a 2D Excitation measurement must be carried out.

1. From the **RF-5301 menu**, select the **2D Excitation Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings (see figures above).
2. In the measurement dialog, **set** the following parameters:
   - Excitation start wavelength: **400 nm**
   - Excitation stop wavelength: **500 nm**
   - Scanning speed: **medium**
   - Emission slit width: **3.0 nm**
   - Excitation slit width: **1.5 nm**
   - Sensitivity: **high**
   - Sampling interval: **0.2 nm**
   - Response time: **Auto**

3. **Set** the **Emission wavelength** parameter exactly to the **peak maximum value** determined from the Emission wavelength accuracy test above. The value should be within the limits of 435.8 ± 1.5 nm.

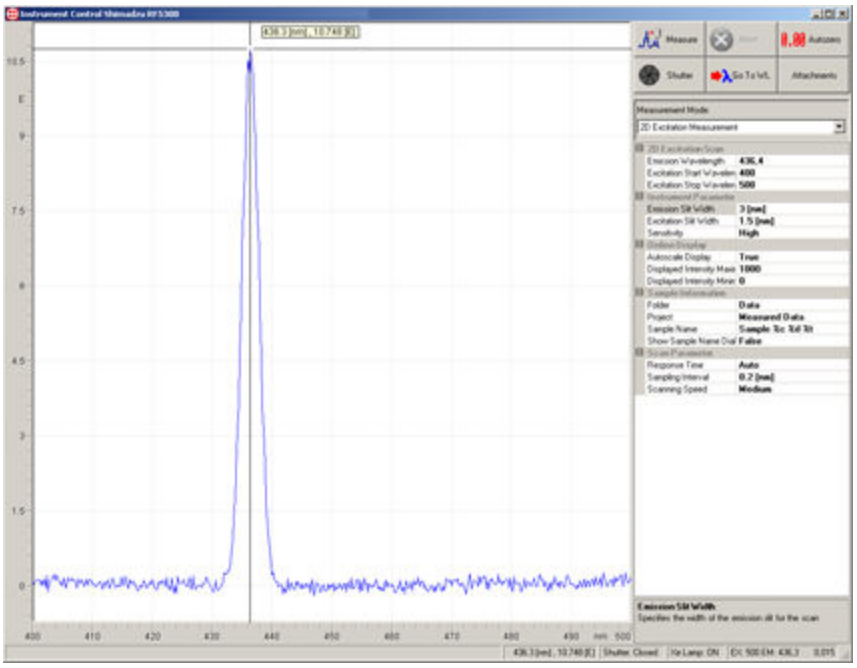
4. **Open** the lid of the **sample compartment**.

5. **Load** a cell filled with **distilled water** into the cell holder.

6. **Close** the lid of the **sample compartment**.

7. **Click** the ***Measure*** **button** to start scanning.

8. After scanning has stopped, check that the **resultant peak** in the spectrum is within the range of **435.8 ± 1.5 nm**.



(Determined wavelength: 436.3 nm)

9. To produce a **printed report**, please follow the instructions from step 7 on in chapter "Verifying wavelength accuracy for emission monochromator".

The wavelength of the resultant peak is around the emission wavelength that was recorded in chapter "Verifying wavelength accuracy for emission monochromator".

However, this is an easier method for obtaining the wavelength accuracy of the instrument. To obtain the exact wavelength accuracy you need a Mercury lamp. Please contact Shimadzu or its nearest representative for further information. You will find contact data in the "Contact Shimadzu" chapter.

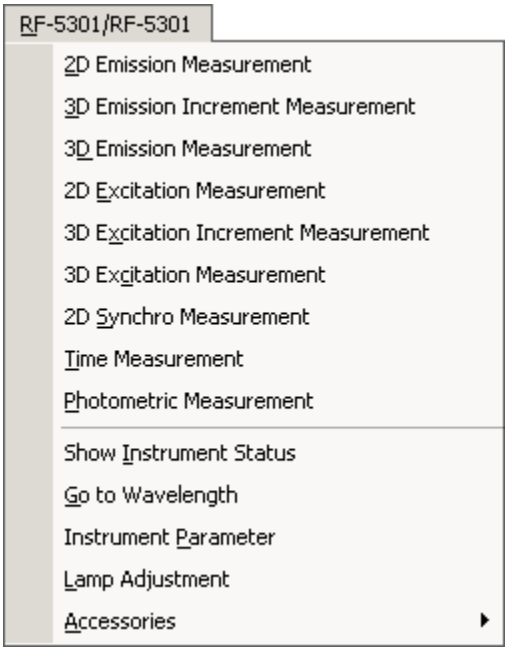
5 Menus

5.1 RF-5301/RF-1501 menu

The RF-5301/RF-1501 menu holds various commands to control and drive a Shimadzu RF-5301PC or a RF-1501 spectrofluorophotometer. It provides a collection of measurement commands being short hands to pre-defined instrument configurations. Some instrument maintenance commands are also available.

> **Why is the menu missing sometimes?**
>
> The RF-5301/RF-1501 menu is only available when running a **panorama fluorescence** software.

5.1.1 RF-5301/RF-1501 menu contents



The **RF-5301/RF-1501** menu provides the following commands:

Commands are dynamically updated according to the connected instrument type.

- 2D Emission Measurement
- 3D Emission Increment Measurement
- 3D Emission Measurement
- 2D Excitation Measurement
- 3D Excitation Increment Measurement
- 3D Excitation Measurement
- 2D Synchro Measurement

Why is the menu entry missing?

The 2D Synchro Measurement is only available for the RF-5301PC system! It is not supported with RF-1501 systems.

- Time Measurement
- Photometric Measurement
- Show Instrument Status
- Go to Wavelength
- Instrument Parameter
- Lamp Adjustment
- Accessories
    - Sipper

6 Measurements

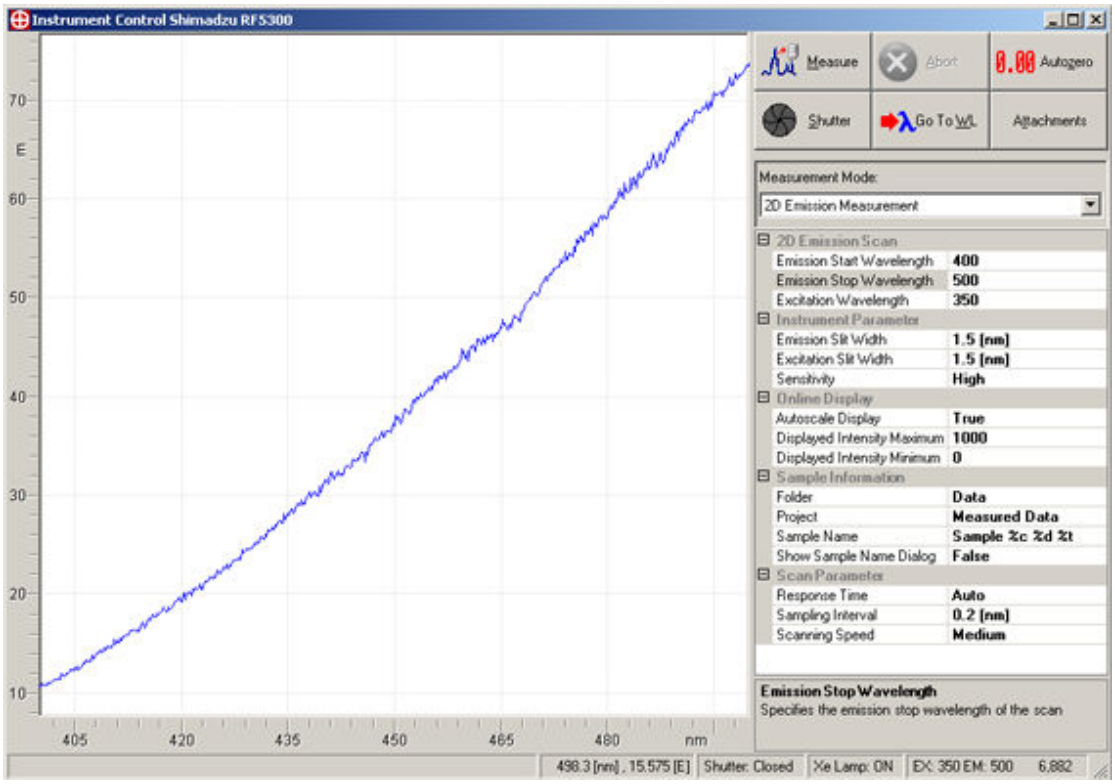
6.1 Measurement Window

The measurement window is the main dialog where all measurement parameters for the RF-5301PC and the RF-1501 instrument can be adjusted. The dialog always shows the last used settings and spectra. From this dialog, measurements can be started and stopped.

> **During measurement no other operations can be performed!**
>
> The measurement window is always shown on top of the application, because during measurement no other actions can be performed in the software.

The contents of the measurement window are described in more detail in the following:

To **show** the **measurement window**, select one of the measurement procedures from the RF-5301/RF-1501 menu. It looks like this:



6.1.1 Online Measurement Monitoring

In order to follow the measurement progress, on the left side of the dialog either a 2D spectrum view or a 2D and 3D spectrum view are displayed. The data views show the current measured spectra and will be updated subsequently during measurement.

6.1.2 Measurement Control Buttons

On top right of the dialog, several buttons are available to control the instrument and measurements. The following buttons are available:

Measure Button / Sip and Measure Button

To **start a measurement** with actual parameter settings, click this button. For details on measurement parameter settings, please see below.

In case a sipper accessory is connected to the instrument and activated,the button text shows **Sip and Measure**. For details on how to work with the sipper accessory, please refer to the section "Working with the sipper accessory".

Abort Button

To **abort** the current **measurement**, click this button.

> **Why is abort delayed sometimes?**
>
> Depending on the measurement, the response time of the instrument delays abort of a measurement sometimes.

Autozero Button

While working with the instrument adjustment of the photomultiplier must be re-calibrated from time to time to obtain a constant baseline for spectra.

Click this button to **reset the baseline**.

Shutter Button

The shutter protects the photomultiplier from permanent exposure to light in order to prevent damage. Usually, the shutter is controlled by the measurement procedures automatically. For maintenance purposes it might be useful to control the shutter manually.

Click this button to **open** or **close** the shutter manually. The image inside the button shows the actual shutter condition.

> **Automatic shutter function can be switched off!**
>
> The Auto-shutter function can be permanently switched off in the Instrument Parameter dialog. Please refer to this section for details.

Go to WL Button

Please refer to the Go to Wavelength section for details.

Close Button

Closes the measurement window and returns to the main software.

Reset Button

This button is only available with the photometric measurement. By default, results of photometric measurements will be appended to the current report on the left pane of the measurement window.

Click ***Reset*** to **clear** the current report and recent measurements and restart with a new report.

> **Recent data is stored automatically!**
>
> When performing a reset, all previous measurement results are stored in a report within the main software automatically.

6.1.3 Measurement parameters

Some general parameters are available in all measurement procedures. They are described in more detail in the following:

Instrument Parameters

The following parameters are available:

- Emission Slit Width
  This parameter specifies the slit width of the slit in front of the emission monochromator in [nm]. Allowed settings are
  RF-5301 PC: **1.5 nm**, **3 nm**, **5 nm**, **10 nm**, **15 nm** and **20 nm**.
  RF-1501: **10 nm** and **20 nm**.

- Excitation Slit Width
  This parameter specifies the slit width of the slit in front of the emission monochromator in [nm]. Allowed settings are
  RF-5301 PC: **1.5 nm**, **3 nm**, **5 nm**, **10 nm**, **15 nm** and **20 nm**.
  RF-1501: **10 nm** and **20 nm**.

- Sensitivity
  This parameter controls the receiver gain. It can be adjusted to **High** or **Low** sensitivity. The high setting is about 50 times more sensitive than the low setting.

Online Display

The following parameters are available:

- **Autoscale Display**
  This parameter controls the display of the 2D and 3D online monitors on the left of the dialog. On **Yes**, spectra will be automatically scaled after completion of measurement.

- **Displayed Intensity Maximum**
  This parameter specifies the upper limit of the intensity axis.

- **Displayed Intensity Minimum**
  This parameter specifies the lower limit of the intensity axis.

Sample Information

The following parameters are available:

- **Folder**
  This parameter specifies the destination folder in the **panorama** project, where measured data are stored. If the folder does not exist, it will be created automatically.

- **Project**
  This parameter specifies the destination **panorama** project, where measured data are stored. If the project does not exist, it will be created automatically. For details on how to work with projects please refer to the Projects and Project Explorer section of the **panorama** help manual.

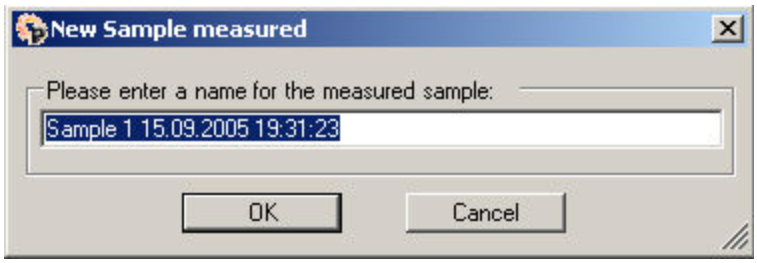
- **Sample Name**
  In the text field the name of the measured spectrum must be entered. In order to allow automatic naming, some wildcards can be used as described in the following:

  - %c
    This wildcard specifies a counter starting with 0. It will be increased automatically.

  - %d
    This wildcard specifies the actual date. The date format of the regional settings of the operating system is applied.

  - %t
    This wildcard specifies the actual time. The time format of the regional settings of the operating system is applied.

- **Show Sample Name Dialog**
  After completion of a measurement the user can be forced to enter a spectrum name. A default name is proposed automatically. The following sample name dialog is shown:



Scan Parameters

The following parameters are available:

- **Response Time**
  This corresponds to the response speed of the RF-5301 unit relative to the variation in the fluorescence intensity on a particular sample. The lower the setting (in seconds) the more swiftly the instrument can follow variations in the fluorescence intensity with time, although the resulting noise level will be greater. In contrast, the higher the setting, the less rapidly the instrument can follow variations, but the lower the noise. The allowed settings are
  RF-5301 PC: **0.02, 0.03, 0.1, 0.25, 0.5, 2.0, 4.0, 8.0** and **Auto.**
  RF-1501: **0.02, 0.03, 0.1, 0.25, 0.5, 2.0** and **8.0.**

- **Sampling Interval**
  Sets up the wavelength intervals for scanning. The allowed settings are
  RF-5301 PC: **0.2 nm**, **1.0 nm** and **2.0 nm**.
  RF-1501: **1.0 nm** and **2.0 nm**.

  > **Limitation of settings!**
  >
  > However, that the scanning interval of 2.0 nm is automatically selected only when the scan speed is set to "Survey" and cannot be used for other speed settings. Also, the setting of 0.2 nm cannot be selected for speed setting "Survey", "Super" and "Very Fast".

- **Scanning Speed**
  This parameter specifies the scanning speed for data acquisition. The seven settings are
  RF-5301 PC: **Survey, Super, Very Fast, Fast, Medium, Slow** and **Very Slow.**
  RF-1501: **Super, Fast, Medium** and **Slow.**

Sipper Parameters

For a general introduction into functionality of the sipper unit and how to use it with the instrument, please refer to the section "Working with the Sipper Accessory". The following parameters are available, which control the sipper accessory functions:

- **Use Sipper**
  Specifies, whether the sipper accessory is enabled and used during measurement or not. Parameters will be expanded or collapsed in the measurement window according to the settings.
  The parameter can be also adjusted from the Accessories sub menu in the RF-5301/RF-1501 menu.

  - **Yes**
    Make sure the sipper accessory is connected to the instrument before you change the flag status to Yes.

    - **No**
      The sipper accessory is not used.

- **Pump Speed**
  This parameter controls the pump speed of the built-in sipper pump. The following pump speed levels are applicable:

    - **Slow**
    - **Medium**
    - **Fast**

- **Sipping Time**
  Specifies the pumping duration in seconds.
- **Dwell Time**
  Specifies the rest period after pumping and before performing the measurement. This interval is applied to let the sample rest in the cuvette before measurement.
- **Purging Time**
  Specifies the purging duration after completion of the measurement in seconds. The sample is removed from the cuvette by pumping the solvent through the cuvette.
- **Number of Rinses**
  Specifies the number of purge intervals required to clean the cuvette.

Sipper Sampling Parameters (Auto-Sampler Control)

For a general introduction into functionality of the auto-sampler unit, please refer to the "Working with the Auto-Sampler Accessory" section. The following parameters are available to setup the auto-sampler unit:

- **Use Auto-Sampler**

    - **Yes**
      The auto-sampler is enabled
    - **No**
      The auto-sampler is disabled

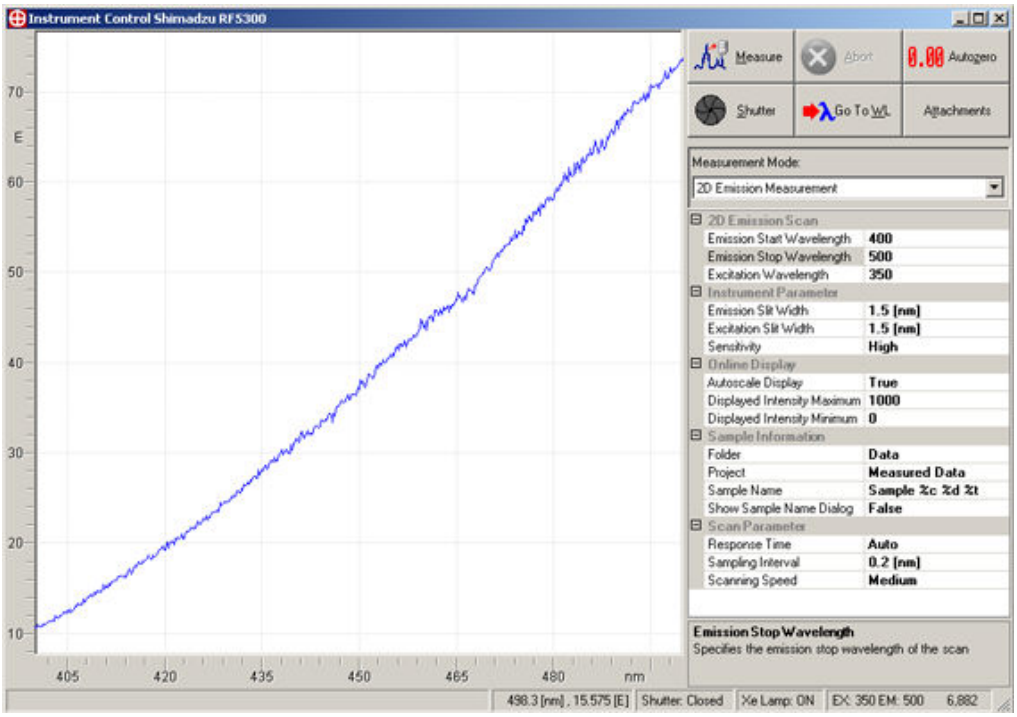
- **Number of Samples**
  Denotes the number of samples to be measured and provided in the auto-sampler.

6.2 2D Emission Measurement

The 2D emission measurement is performed to obtain a selective fluorescence spectrum of a chemical substance by excitation of a particular wavelength. Even within compound mixtures individual substances can be analyzed without prior separation.

A 2D emission measurement is carried out as described in the following:

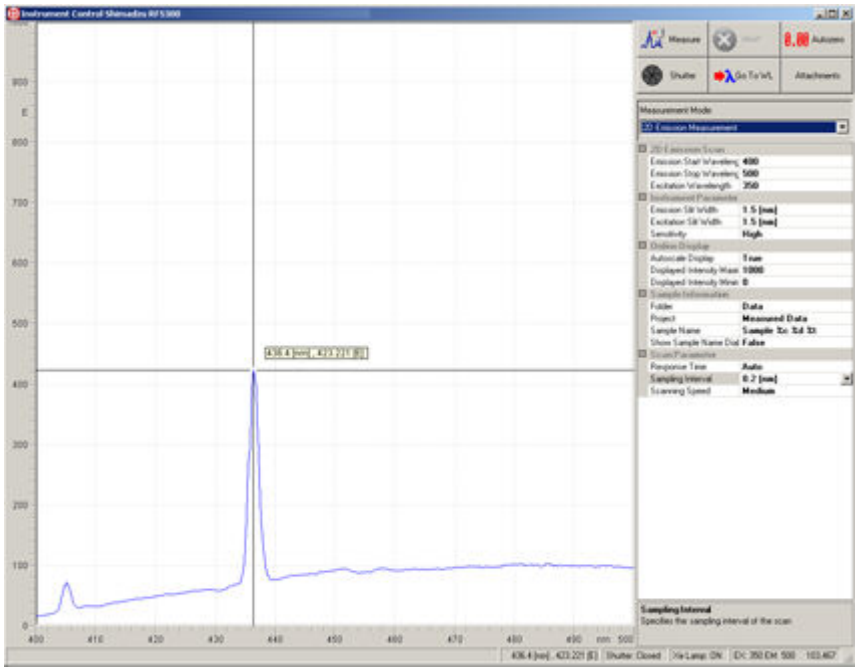
1. From the **RF-5301/RF-1501 menu**, select the **2D Emission Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings:



> **If the measurement window is already open...**
>
> ... the measurement type can be directly changed in the Measurement Mode drop down box.

2. **Set** the **Excitation Wavelength** to an appropriate value in [nm].
3. **Set** the **Emission Start Wavelength** to an appropriate start value of the detection range in [nm].
4. **Set** the **Emission Stop Wavelength** to an appropriate end value of the detection range in [nm].
5. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
6. **Open** up the lid of the **sample compartment**
7. **Load** a cell filled with a **sample** into the cell holder.
8. **Close the lid of the sample compartment.**
9. **Click** the ***Autozero*** **button**.

10. **Click** the ***Measure*** **button** to start scanning.
    During measurement the user can see evolving the spectrum. A sample spectrum of a neon lamp is shown below:



11. **Click** the **button** on top right of the measurement dialog to close it.
    The measured spectrum is now shown in the main workspace and a new spectrum node has been added to the destination project.

6.3 2D Excitation Measurement

The 2D excitation measurement is performed to observe emission signals at a discrete emission wavelength as a response to excitation. This way the fluorescence with a defined energy transition can be studied in different molecules.

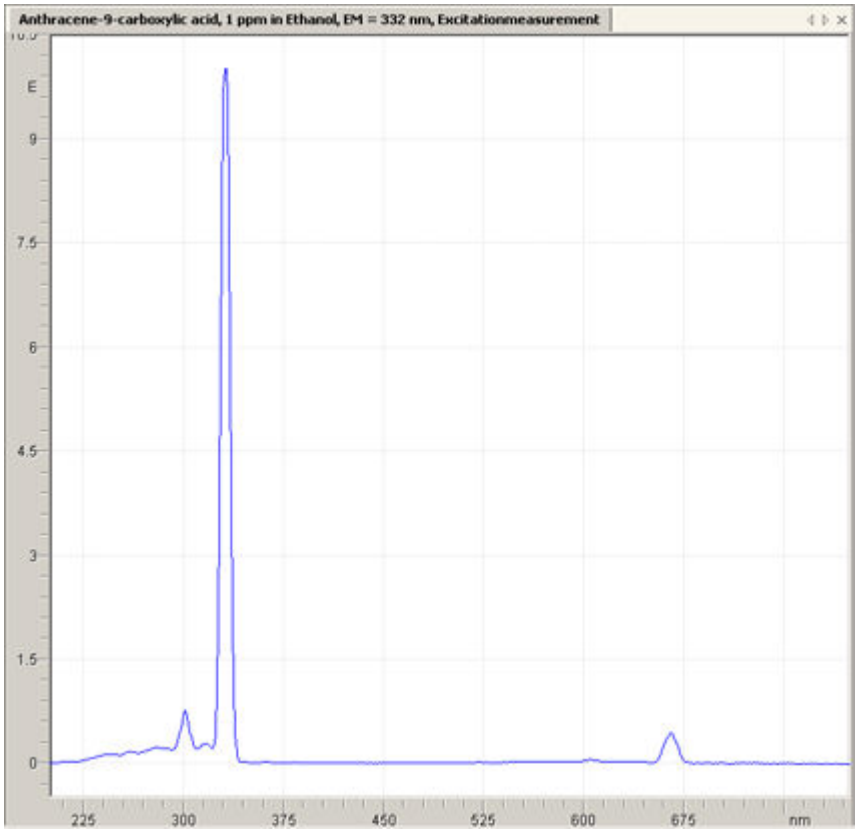
A 2D excitation measurement is carried out as described in the following:

1. From the **RF-5301/RF-1501 menu**, select the **2D Excitation Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings.

> **If the measurement window is already open...**
>
> ... the measurement type can be directly changed in the Measurement Mode drop down box.

2. **Set** the **Emission Wavelength** to an appropriate value in [nm].
3. **Set** the **Excitation Start Wavelength** to an appropriate start value of the detection range in [nm].
4. **Set** the **Excitation Stop Wavelength** to an appropriate end value of the detection range in [nm].
5. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
6. **Open** up the lid of the **sample compartment**

3. **Load** a cell filled with a **sample** into the cell holder.
4. Close the lid of the sample compartment.
5. Click the Autozero button.
6. Click the ***Measure*** **button** to start scanning.
   During measurement the user can see evolving the spectrum. A sample excitation spectrum of anthracene-9-carboxylic acid is shown below:



7. Click the **button** on top right of the measurement dialog to close it.
   The measured spectrum is now shown in the main workspace and a new spectrum node has been added to the destination project.

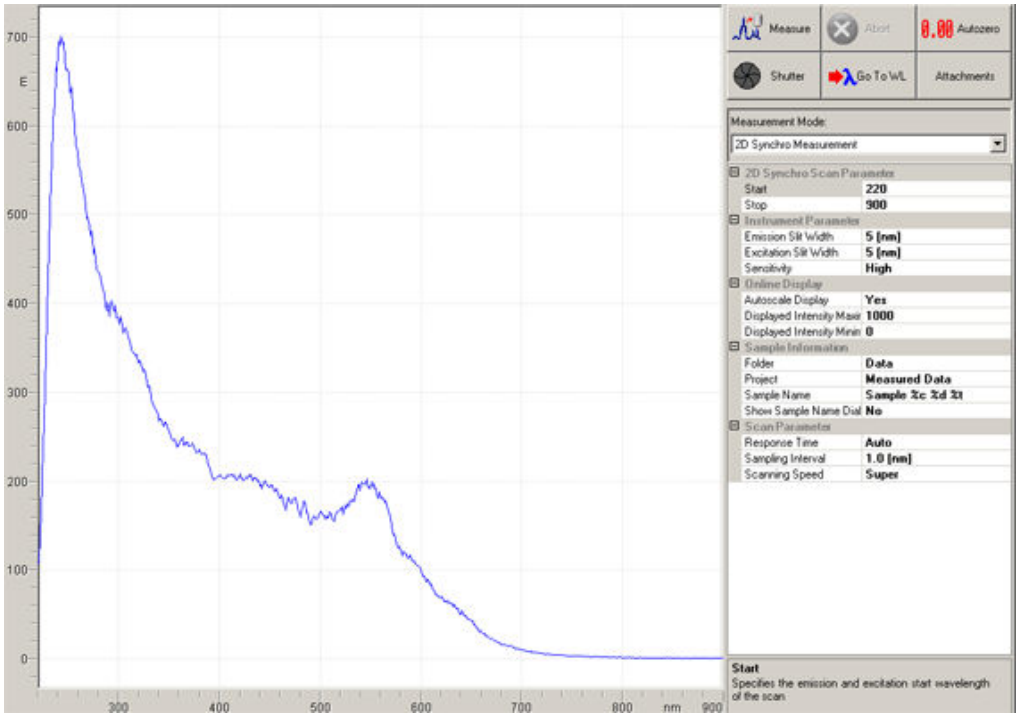
6.4 2D Synchro Measurement

The synchro measurement excites a sample at a particular wavelength and measures corresponding emission at the same wavelength as a direct response.

> **The 2D synchro measurement is not supported by the RF-1501 instrument!**

A 2D excitation measurement is carried out as described in the following:

1. From the **RF-5301/RF1501 menu**, select the **2D Excitation Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings:



> **If the measurement window is already open...**
>
> ... the measurement type can be directly changed in the Measurement Mode drop down box.

2. **Set** the **Start Wavelength** to an appropriate start value of the detection range in [nm].
3. **Set** the **Stop Wavelength** to an appropriate end value of the detection range in [nm].
4. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
5. **Open** up the lid of the **sample compartment**
6. **Load** a cell filled with a **sample** into the cell holder.
7. **Close** the lid of the **sample compartment**.
8. **Click** the ***Autozero*** **button**.
9. **Click** the ***Measure*** **button** to start scanning.
10. During measurement the user can see evolving the spectrum.
11. **Click** the **button** on top right of the measurement dialog to close it.
    The measured spectrum is now shown in the main workspace and a new spectrum node has been added to the destination project.

6.5 3D Emission Increment Measurement

The 3D emission increment measurement is performed to identify and separate fluorescent wavelengths of a sample caused by particular excitation. Significant emission wavelengths can be determined by scanning along the emission dimension.

This measurement is an automated sequence of 2D emission measurements collected in a 3D data object. The emission wavelength is increased subsequently by a user defined amount during measurements.

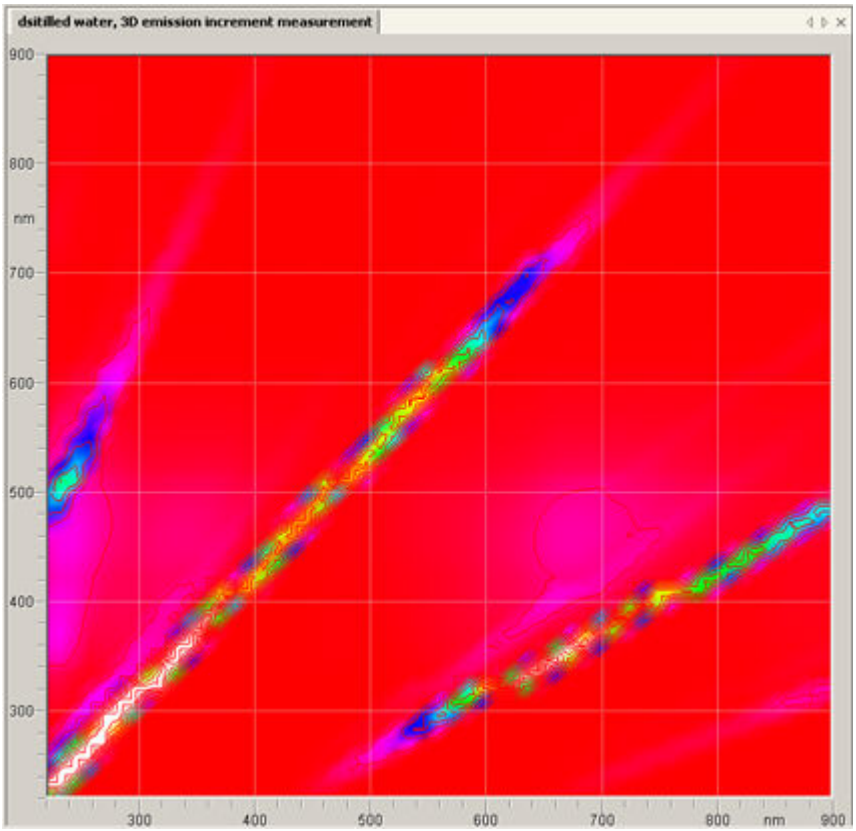
A 3D emission increment measurement is carried out as described in the following:

1. From the **RF-5301/RF-1501 menu**, select the **3D Emission Increment Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings.

   > **If the measurement window is already open...**
   >
   > ... the measurement type can be directly changed in the Measurement Mode drop down box.

2. **Set** the **Excitation Wavelength** to an appropriate value in [nm].
3. **Set** the **Emission Start Wavelength** to an appropriate start value of the detection range in [nm].
4. **Set** the **Emission Stop Wavelength** to an appropriate end value of the detection range in [nm].
5. **Set** the **Wavelength Increment** to an appropriate value in [nm].
6. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
7. **Open** up the lid of the **sample compartment**
8. **Load** a cell filled with a **liquid sample** into the cell holder.
9. **Close** the lid of the **sample compartment**.
10. **Click** the ***Autozero*** **button**.

11. **Click** the ***Measure*** **button** to start scanning.
    During measurement the user can see the 2D spectrum of the current cycle and the 3D spectrum evolving. As an example, the top view of a 3D emission increment measurement of distilled water is shown below:



> **The 3D spectrum can be displayed in several ways!**
>
> For 3D data several display modes are available. They can be selected from the 3D View menu:
>
> - 3D View
> - Top View
> - 2D Overlay

12. **Click** the **button** on top right of the measurement dialog to close it.
    The measured spectrum is now shown in the main workspace and a new spectrum node has been added to the destination project.

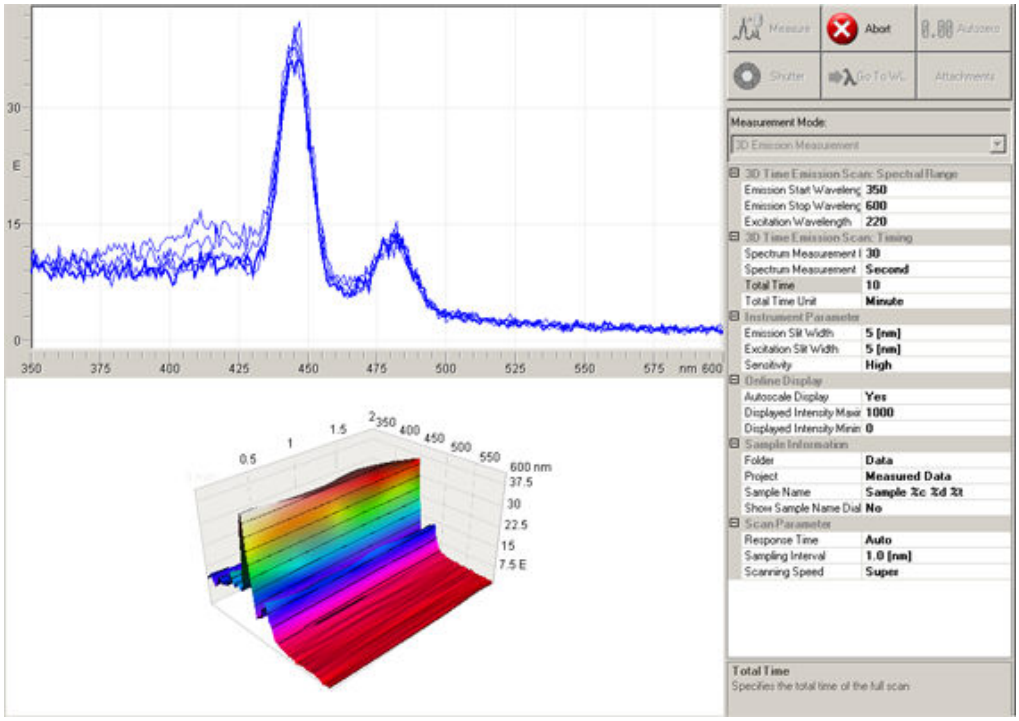
6.6 3D Emission Measurement

The 3D emission measurement is performed to collect several 2D emission spectra in fixed time intervals. This is useful to study reactions and see evolving of reactant signals.

All recorded 2D emission spectra will be collected in a 3D data object. This measurement is similar to the time measurement.

A 3D emission measurement is carried out as described in the following:

1. From the **RF-5301/RF1501 menu**, select the **3D Emission Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings:



> **If the measurement window is already open...**
>
> ... the measurement type can be directly changed in the Measurement Mode drop down box.

2. **Set** the **Excitation Wavelength** to an appropriate value in [nm].
3. **Set** the **Emission Start Wavelength** to an appropriate start value of the detection range in [nm].
4. **Set** the **Emission Stop Wavelength** to an appropriate end value of the detection range in [nm].
5. **Set** the **Timing Conditions** for the measurement. Changing one of the following parameters will cause automatic recalculation of dependent parameters:
   - Acquisition Rate
   - Total number of Acquisitions
   - Total time.
6. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
7. **Open** up the lid of the **sample compartment**
8. **Load** a cell filled with a **sample** into the cell holder.
9. **Close** the lid of the **sample compartment**.
10. **Click** the ***Autozero*** **button**.
11. **Click** the ***Measure*** **button** to start scanning.
    During measurement the user can see evolving the 2D spectra and the 3D spectrum.

> **The 3D spectrum can be displayed in several ways!**
>
> For 3D data several display modes are available. They can be selected from the 3D View menu:
>
> - 3D View
> - Top View
> - 2D Overlay

12. **Click** the  **button** on top right of the measurement dialog to close it.
    The measured spectrum is now shown in the main workspace and a new spectrum node has been added to the destination project.

6.7 3D Excitation Increment Measurement

The 3D excitation increment measurement is performed to identify fluorescent wavelengths of a sample. Particular excitation will return corresponding response signals in the emission dimension of the 3D excitation spectrum. Significant wavelengths can be determined and used in further analyses of the sample.

This measurement is an automated sequence of 2D excitation measurements collected in a 3D data object. The excitation wavelength is increased by a user defined amount during measurements.

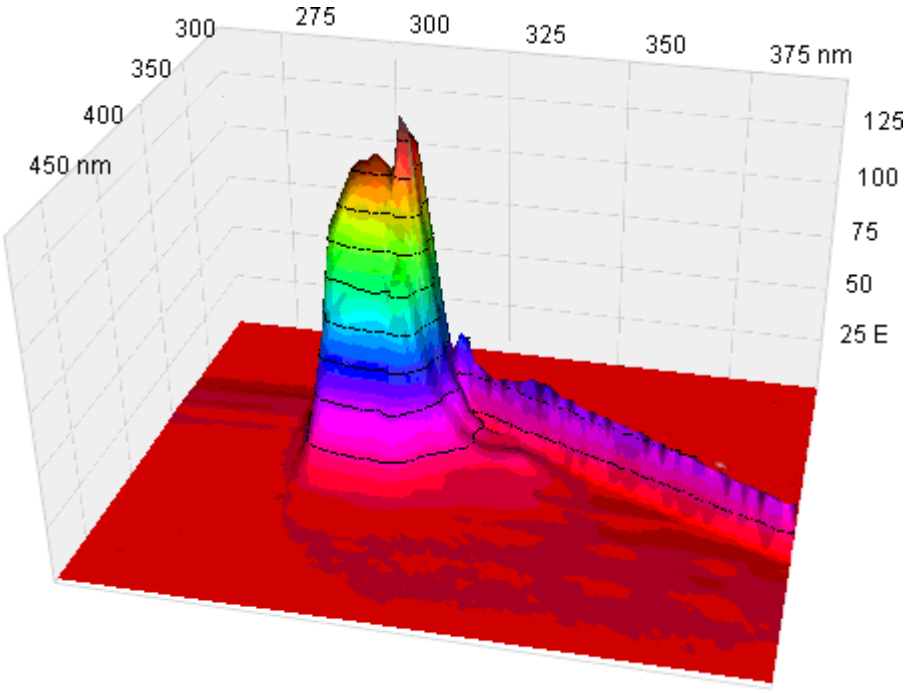
A 3D excitation increment measurement is carried out as described in the following:

1. From the **RF-5301/RF-1501 menu**, select the **3D Excitation Increment Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings.

   > **If the measurement window is already open...**
   >
   > ... the measurement type can be directly changed in the Measurement Mode drop down box.

2. **Set** the **Emission Wavelength** to an appropriate value in [nm].
3. **Set** the **Excitation Start Wavelength** to an appropriate start value of the detection range in [nm].
4. **Set** the **Excitation Stop Wavelength** to an appropriate end value of the detection range in [nm].
5. **Set** the **Wavelength Increment** to an appropriate value in [nm].
6. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
3. **Open** up the lid of the **sample compartment**
4. **Load** a cell filled with a **liquid sample** into the cell holder.
5. **Close** the lid of the **sample compartment**.
6. **Click** the ***Autozero*** **button**.

7. **Click** the ***Measure*** **button** to start scanning.
   During measurement the user can see the 2D spectrum of the current cycle and the 3D spectrum evolving. As an example, the 3D excitation increment measurement of a mixture of anthracene and naphthaline is shown below:



> **The 3D spectrum can be displayed in several ways!**
>
> For 3D data several display modes are available. They can be selected from the 3D View menu:
>
> - 3D View
> - Top View
> - 2D Overlay

8. **Click** the  **button** on top right of the measurement dialog to close it.
   The measured spectrum is now shown in the main workspace and a new spectrum node has been added to the destination project.

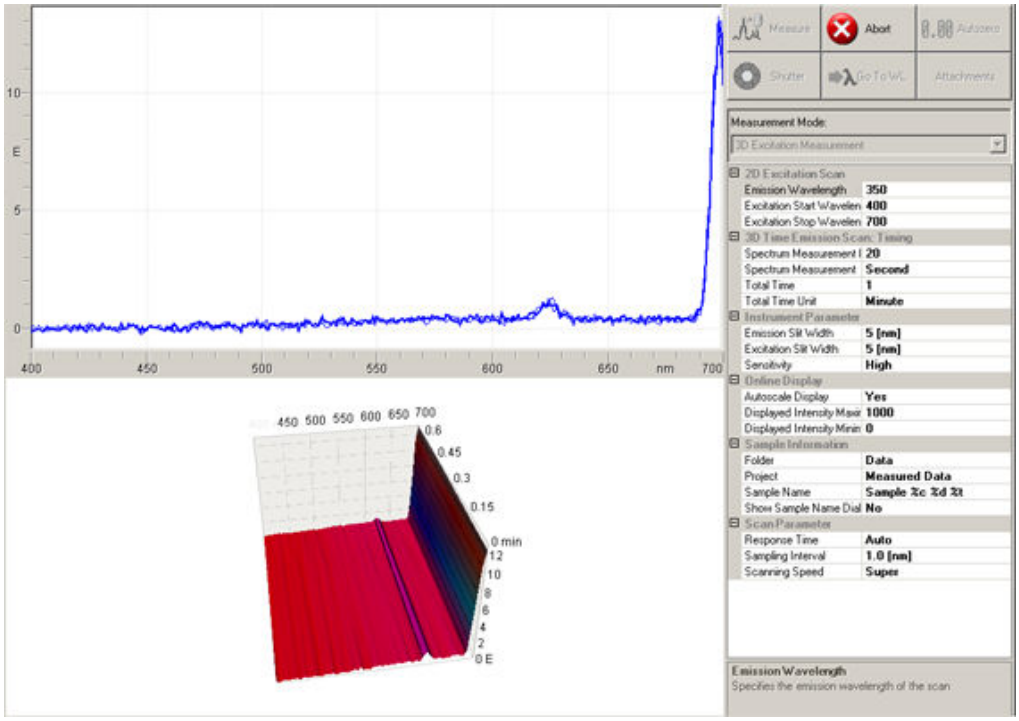
6.8 3D Excitation Measurement

The 3D excitation measurement is performed to collect several 2D excitation spectra in fixed time intervals. This is useful to study reactions and see evolving of reactant signals.

All recorded 2D excitation spectra will be collected in a 3D data object. This measurement is similar to the time measurement.

A 3D excitation measurement is carried out as described in the following:

1. From the **RF-5301/RF-1501 menu**, select the **3D Excitation Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings:



> **If the measurement window is already open...**
>
> ... the measurement type can be directly changed in the Measurement Mode drop down box.

2. **Set** the **Emission Wavelength** to an appropriate value in [nm].
3. **Set** the **Excitation Start Wavelength** to an appropriate start value of the detection range in [nm].
4. **Set** the **Excitation Stop Wavelength** to an appropriate end value of the detection range in [nm].
5. **Set** the **Timing Conditions** for the measurement. Changing one of the following parameters will cause automatic recalculation of dependent parameters:
   - Acquisition Rate
   - Total number of Acquisitions
   - Total time.
6. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
7. **Open** up the lid of the **sample compartment**
8. **Load** a cell filled with a **sample** into the cell holder.
9. **Close** the lid of the **sample compartment**.
10. **Click** the ***Autozero*** **button**.
11. **Click** the ***Measure*** **button** to start scanning.
    During measurement the user can see evolving the 2D spectra and the 3D spectrum.

The 3D spectrum can be displayed in several ways!

For 3D data several display modes are available. They can be selected from the 3D View menu:

- 3D View
- Top View
- 2D Overlay

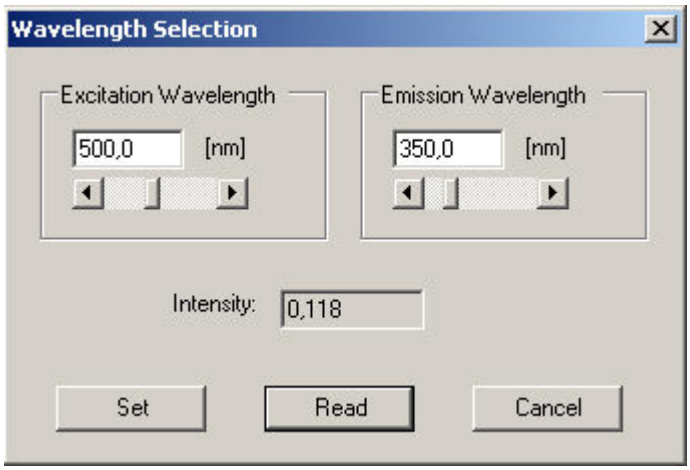
12. Click the **button** on top right of the measurement dialog to close it.
The measured spectrum is now shown in the main workspace and a new spectrum node has been added to the destination project.

6.9 Go to Wavelength

The Go to Wavelength function is used to adjust a particular excitation and emission wavelength directly. By adjusting the pair of parameters, the response signal can be directly detected. This procedure is useful for single value detection or instrument maintenance.

To set particular excitation and emission wavelengths, please follow the instructions below:

1. From the **RF-5301/RF-1501 menu**, select the **Go to Wavelength** command. The Go to Wavelength dialog is opened as shown in the following:



Go to Wavelength button is also available in measurement window!

The go to wavelength function is also accessible from the Measurement Window. Please refer to this section for details.

2. **Set** the **Excitation Wavelength** to an appropriate value in [nm] or move the slider to adjust.
3. **Set** the **Emission Wavelength** to an appropriate value in [nm] or move the slider to adjust.
4. **Click** the ***Read*** **button** to update the actual intensity value (optional).
5. **Click** the ***Set*** **button** to apply actual values and close the dialog.

Setting a wavelength...

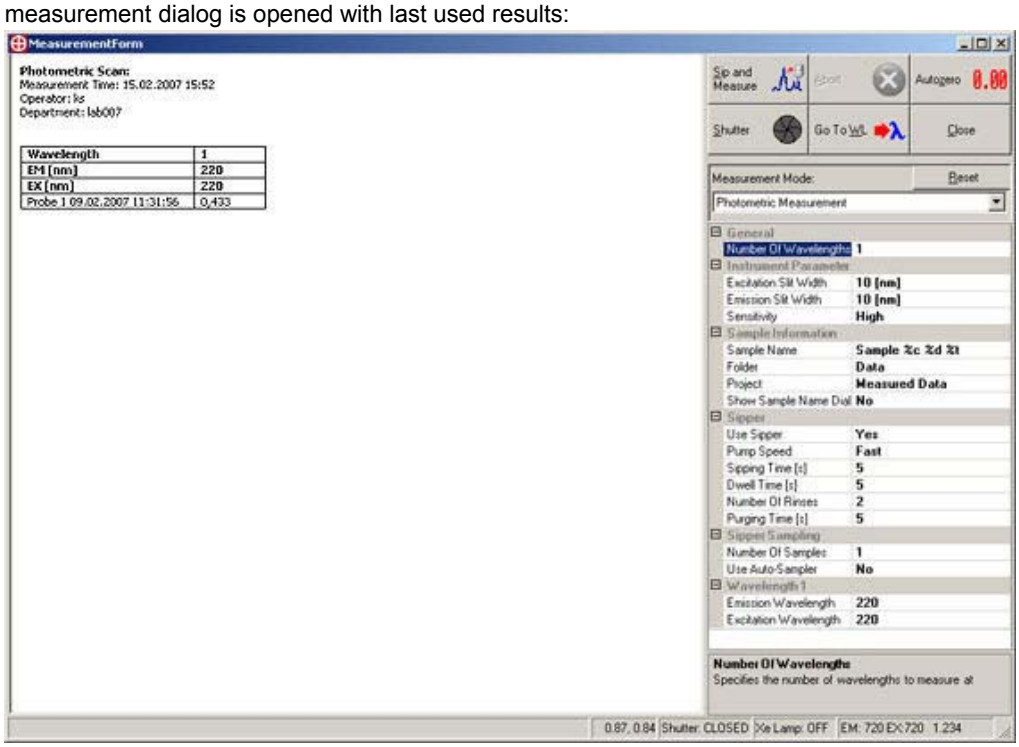
... will be applied instantly! The instrument will move to the selected excitation and emission wavelength and will keep this settings until new positions are applied by any other measurement procedure.

6. **Click** the ***Cancel*** **button** to discard changes and close the dialog.

6.10 Photometric Measurement

The photometric experiment allows single point measurements of up to 10 different excitation/emission pairs. results are displayed in a comprehensive report and trend plot. Follow the steps below to setup a photometric measurement:

1. From the **RF-5301/RF-1501 menu**, select the **Photometric Measurement** command. The measurement dialog is opened with last used results:



2. **Click** the ***Reset*** **Button** to clear current report contents.
   If you like to append the current measurements to the most recent ones, skip this step
3. **Set** the **Show Trend Plot** flag, if you like to perform multiple measurements of the same wavelengths (see below).
4. **Set** the **Number of Wavelengths** to the total number of wavelengths being measured simultaneously .
   Up to 10 different wavelengths can be measured at a time.
5. For each wavelength, a new "Wavelength n" category is appended to the properties with the following parameters:
   - **Set** the **Excitation Wavelength** to an appropriate value of the detection range in [nm].
   - **Set** the **Emission Wavelength** to an appropriate value of the detection range in [nm].
6. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
7. **Open** up the lid of the **sample compartment**.
8. **Load** a cell filled with a **sample** into the cell holder.
9. **Close** the lid of the **sample compartment**.
10. **Click** the ***Autozero*** **button**.
11. **Click** the ***Measure*** **button** to start scanning.

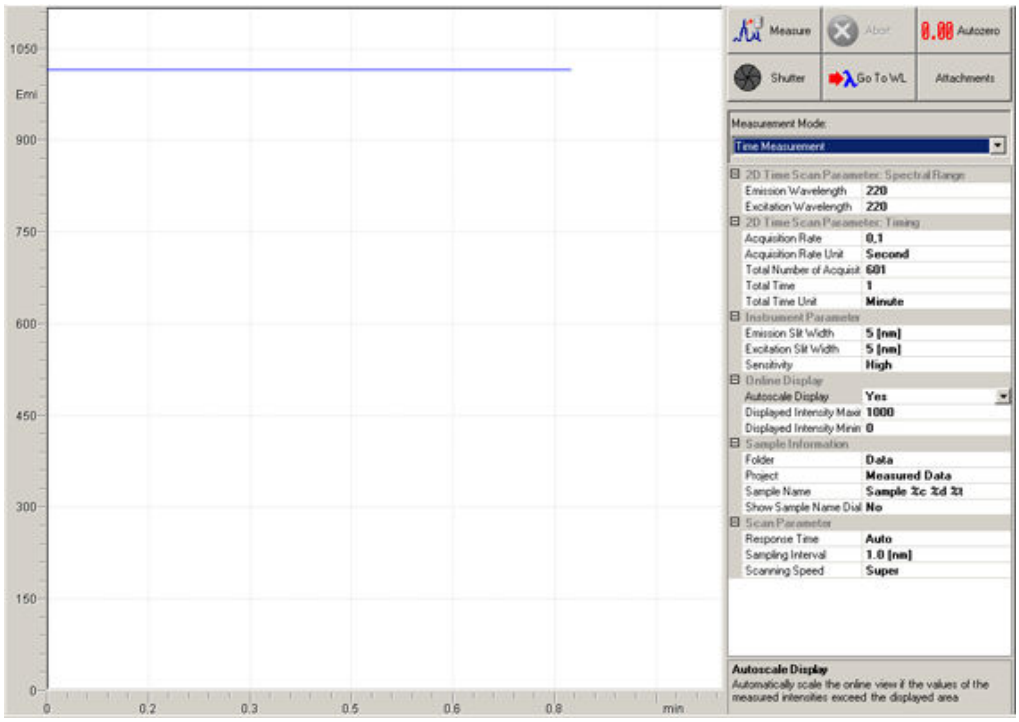
After completion of a measurement, the trend plot and report are updated accordingly.

6.11 Time Measurement

The time measurement is useful for studies of chemical reactions or life time of excited states. A substance is excited at a particular wavelength and its response is detected at the emission wavelength.

A time measurement is carried out as described in the following:

1. From the **RF-5301/RF-1501 menu**, select the **Time Measurement** command. The measurement dialog is opened showing the last measured spectrum and last used parameter settings.



2. **Set** the **Excitation Wavelength** to the appropriate value in [nm].
3. **Set** the **Emission Wavelength** for detection of the response signal to the appropriate value in [nm].
4. **Set** the **timing conditions** for the measurement. Changing one of the following parameters will cause automatic recalculation of dependent parameters:
    - Acquisition Rate
    - Total number of Acquisitions
    - Total time.
5. **Set** the **other parameters** in the dialog optionally. For details on general parameter settings, please refer to the Measurement Window section.
6. **Open** up the lid of the **sample compartment**
7. **Load** a cell filled with a **liquid sample** into the cell holder.
8. **Close** the lid of the **sample compartment**.
9. **Click** the ***Measure*** **button** to start scanning.
   During measurement the user can see the emission evolving.

7 Hardware and Maintenance

7.1 Maintenance

7.1.1 Cautions for transferring the instrument

When transferring or shipping the RF-5301PC/RF-1501 instrument be sure to remove the Xenon lamp. Store the removed lamp in a special case. The Xenon lamp contains high pressure gas. Impact, vibration or pressure on it may cause it to burst, posing a serious danger. Be extremely careful when handling it. If the lamp is touched with naked hand, clean the surface before lighting it. Cleaning is possible with ethyl alcohol or the special cleaning agent included with the lamp. Finger oil remaining on the bulb can be baked onto the bulb when the lamp is lit, possibly causing the lamp to burst.

7.1.2 Service life of the Xenon Lamp

The Xenon lamp is a consumable part. When the lamp is used for a long time, the emission point moves around or flickers. If such a problem occurs the noise level will become greater and an accurate data acquisition will be impossible. The time during which the lamp remains operative before the first occurrence of flickering is called the flicker life.

The guaranteed flicker life of a 150 W Xenon lamp is 500 hours. When the lamp time used reaches 500 hours, replace the lamp immediately.

Caution: ⚠	**Never use a lamp in excess of 1000 hours. A lamp used for more than 1000 hours may burst, possibly damaging the lamp unit.**

7.1.3 Safe disposal of the Xenon lamp

Special care must be taken to dispose the used Xenon lamp. The lamp will pose a potential danger in ordinary waste disposable processes, because it contains high pressure gas. Before disposing it, wrap it up carefully in thick cloth (to prevent glass fragments from flying) and crash the bulb portion with a hammer, etc. Be careful not to injure yourself with glass fragments.

7.1.4 Replacing fuse

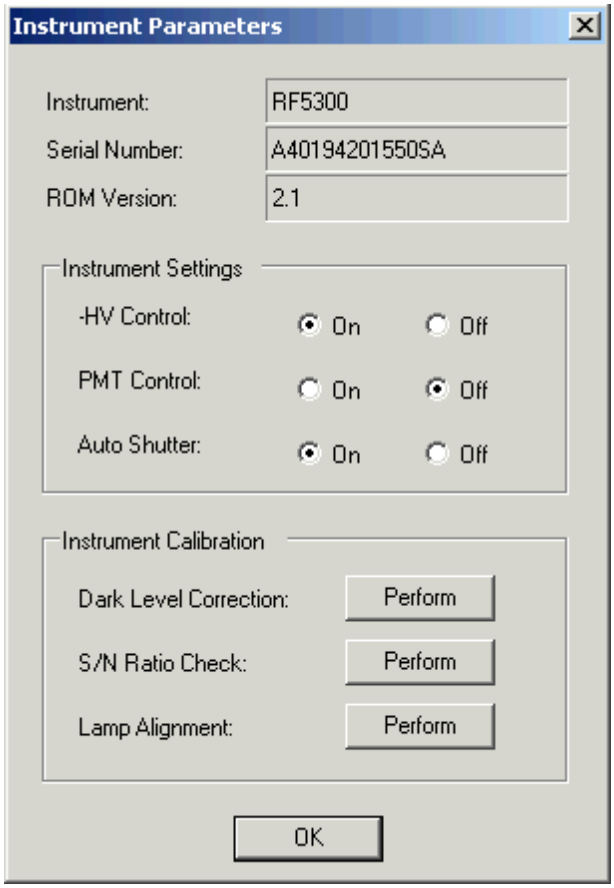
When a fuse is blown replace it. The fuses are right beside the ON/OFF power switch of the instrument RF-5301PC/RF-1501.

7.2 Instrument Parameter

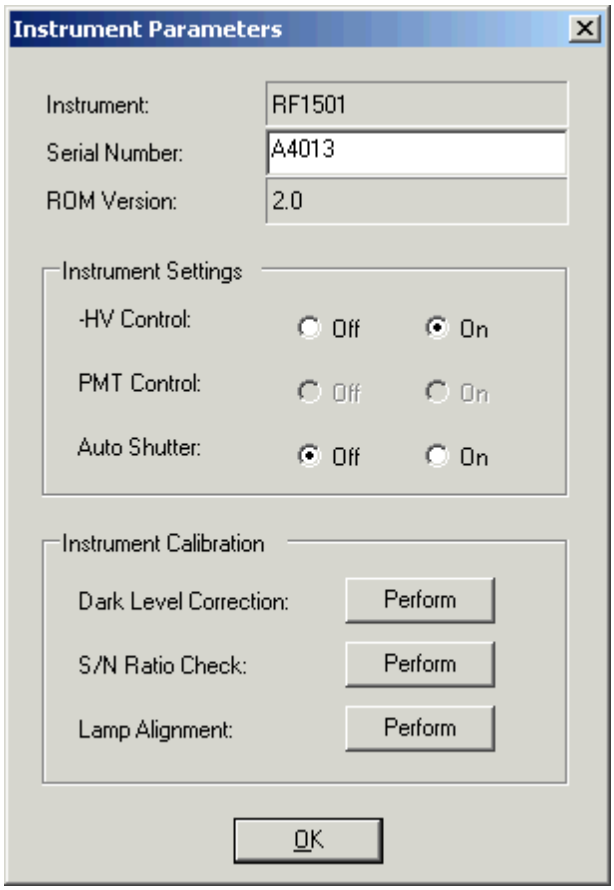
Some general instrument settings can be adjusted here. These parameters are most of all used for maintenance purposes and should not be changed.

To open the instrument parameter dialog, please follow the instructions below:

1. From the **RF-5301/RF-1501 menu**, select the **Instrument Parameter** command.
   For the **RF-5301PC system** the instrument parameter dialog is opened as follows:



For the **RF-1501 system** the dialog looks like this:



2. Enter the **serial number** of the **RF-1501** instrument, which is located on a label on the right side of the instrument. (RF-1501 System only!)

Caution: ⚠	**Entering the serial number is only required for the RF-1501 instrument!** **With the RF-5301PC system, the serial number is available automatically.**

3. **Configure** the parameters in the **Instrument Settings** section.

Caution: ⚠	**These options should only be used by service persons or experienced users. Wrong settings might cause permanent damage to any instrument components!** **With the RF-1501 system, the PMT control is not available!**

4. **Perform** any instrument calibration from the **Instrument Calibration** section.
5. **Click** the ***Close*** **button** to finish.

7.2.1 Instrument Settings

HV Control

This parameter controls the high voltage gain (HV) of the Xenon lamp which is regulated automatically by default. Regulation is done with regard to the sensitivity of the photomultiplier. For instrument maintenance, automatic regulation can be switched of. E.g. for measurement of specific instrument backgrounds or stray light measurements, which can be used for background correction of spectra.

- **On (default)**
  Automatic regulation of Lamp voltage is applied.
- **Off**
  No regulation of Lamp voltage is applied.

PMT Control

This parameter controls the sensitivity of the photomultiplier tube (PMT) with regard to the amount of light exposure. By default the sensitivity of the PMT will be controlled automatically. For service and maintenance purposes automatic regulation can be deactivated.

- **On (default)**
  PMT sensitivity is regulated automatically.
- **Off**
  The PMT sensitivity is not regulated.

Auto Shutter

This parameter controls the shutter which protects the photomultiplier from permanent irradiation with light. By default, the auto shutter is on to avoid damages to the photomultiplier. For some dark or background measurements carried out by service persons the shutter must be controlled manually.

- **On (default)**
  The shutter opens and closes automatically during measurements.
- **Off**
  The shutter must be opened and closed manually before starting measurements.

7.2.2 Instrument Calibration

This section provides the following operations:

- **Dark Level Correction**
  The dark level correction measures the background noise of the instrument. The characteristic noise level will be used to adjust the photomultiplier.
- **S/N Ratio Check**
  Please review the Signal to Noise Test section in the chapter "Performance Tests" for details.
- **Lamp Alignment**
  Please review the Lamp Adjustment section in the chapter "Hardware and Maintenance" for details.

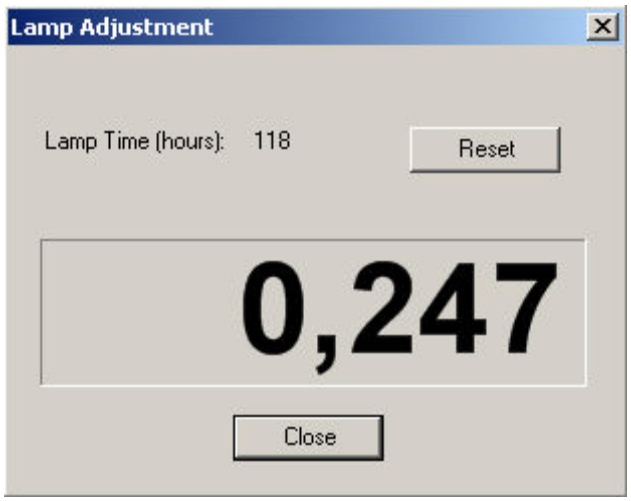
Click the ***Perform*** **button** next to the desired operation to start it.

7.3 Checking Lamp Time

The spectrofluorophotometer keeps a record of the total amount of time that the Xenon lamp has been lit. The time is displayed in units of hours in the Lamp Adjustment dialog.

After installing the lamp for the first time or after replacing it, zero the lamp time as followed:

1. From the **RF-5301/RF-1501 menu**, select the **Lamp Adjustment** command.
   The instrument shutter is opened and the following dialog is shown:



   The actually measured intensity value is updated continuously.
   In the upper part of the dialog the amount of time the lamp has been lid is shown.
2. To zero the lamp time **click** the ***Reset*** **button**.

> **The lamp time reset function is not available for the RF-1501 Instrument!**
>
> The current lamp time is only displayed for the RF-1501 instrument.

3. **Click** the ***Close*** **button** to finish.

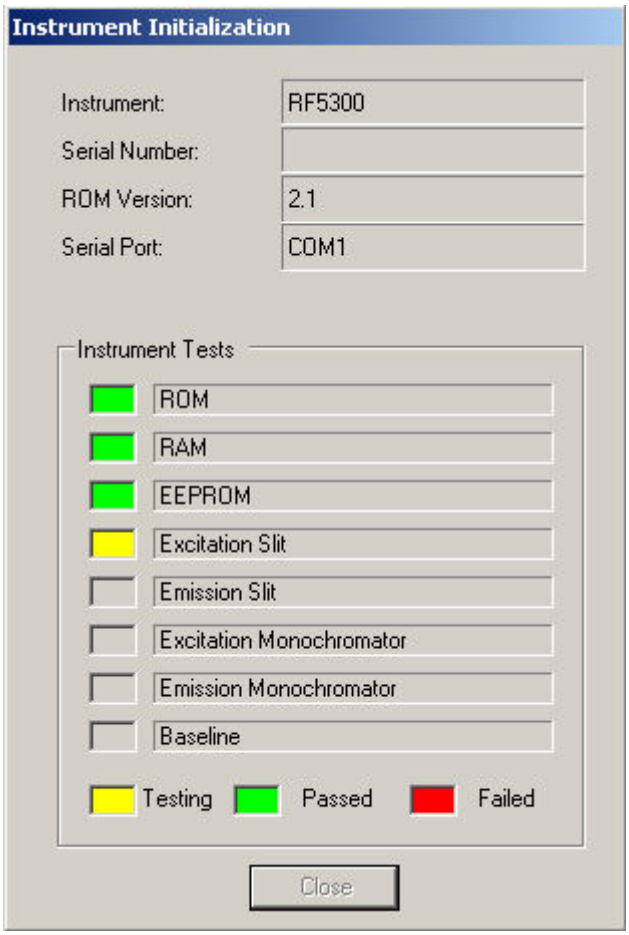
7.4 Show Instrument Status

The instrument status shows the actual status of the spectrofluorophotometer or is used for initialization. Initialization is required to ensure correct communication between the instrument and the **panorama fluorescence** software. The initialization procedure includes various internal self test procedures, which must be passed to ensure reliable measurement results.

When is the instrument initialized?

Initialization is only required, when the instrument is used the first time after powering ON and after a certain standby period. In this case, initialization is done automatically before the next measurement is started.

The steps of the initialization process are displayed on screen:



7.4.1 General instrument properties

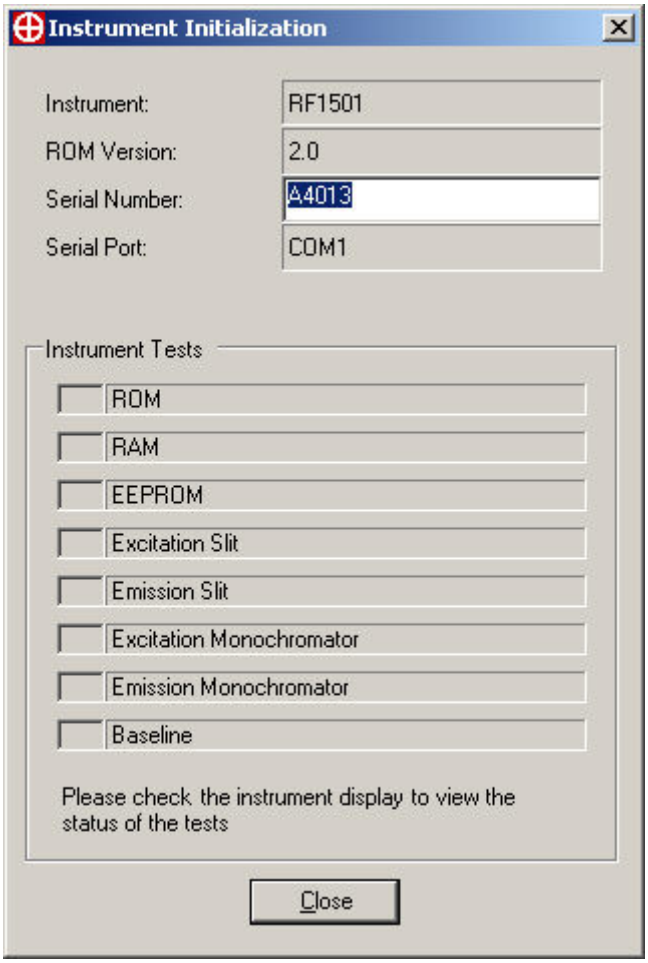
In the upper part of the dialog, some general instrument information are displayed. During initialization process all COM ports will be scanned for attached instruments. After successful auto-detection of the RF-5301 spectrofluorophotometer the following data is read from the instrument:

- Instrument name
- Serial number

RF-1501 Instrument Serial Number!

For the RF-1501 system the serial number needs to be entered manually. It is imprinted on a label on the right side of the device.

With RF-5301PC instruments, the serial number is stored on the internal EPROM and will be displayed automatically.



If no valid serial number is entered, you will be prompted by the following message when you try to close the dialog:

- ROM version
- Serial port

7.4.2 RF-5301 Instrument self test

Various internal self tests are performed to check the basic instrument functions. The test status is shown as colored square in front of the particular test item.

All tests need to be passed before working with the instrument will be possible.

> **What shall I do, if any self test fails?**
>
> If any internal self test fails, this might be due to any damage on one of the system components. Please contact Shimadzu for assistance.

After a successful initialization the instrument is ready for measurement.

In case of an unsuccessful initialization an error message appears as shown below:

For details on remedial actions please follow the instructions in the error message or refer to the RF-5301 troubleshooting section of this manual.

Click the ***Close*** **button** to finish.

7.5 Troubleshooting

7.5.1 Communication problems

The following items should be checked:

Items to be checked	**Remedial action**
Cable connection	Check the cable connections between the instrument and the computer. Next, try again to establish a communication.
COM port setting	Study the documentation for your computer to check that the COM port in question has been addressed correctly.

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

7.5.2 Before suspecting malfunction

The following items should be checked:

Problem	**Cause**	**Remedial action**
Instrument is not powered although the power switch is in the ON position.	Power cable is not securely connected.	Securely insert both end of the cable into the inlet on the instrument and the outlet at the site.
	Power fuse is blown.	Install a new fuse.

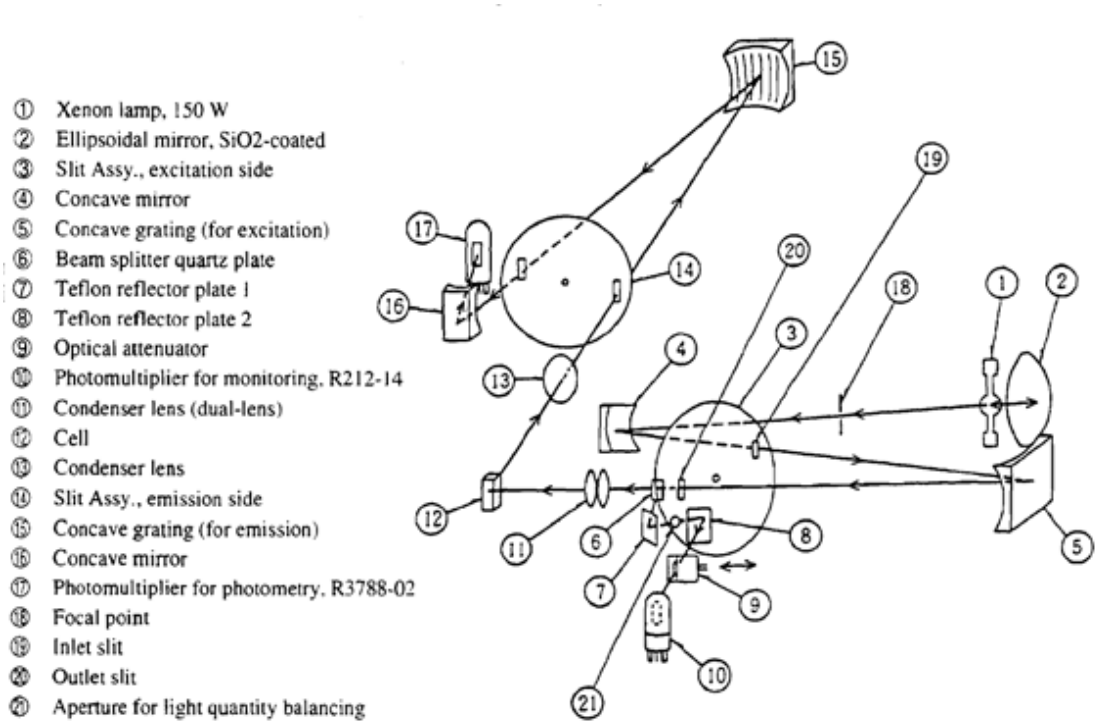
	Other causes.	Contact Shimadzu or its nearest representative.
Xenon lamp does not light.	Lamp ON/OFF switch is in OFF position.	Move the switch in ON position.
	Wiring is disconnected.	Before opening the cover of the lamp housing be sure to disconnect the power cable from the outlet!
	Lamp is still hot.	Allow the lamp to cool down for about 10 minutes.
	Other causes.	Contact Shimadzu or its nearest representative.
Signal does not come out.	Lamp is unlit.	See problem above.
	Xenon lamp is misaligned.	Correct the lamp position.
	The Shutter or the slit on the emission side is closed.	Open the shutter or the slit.
	Wrong acquisition parameters.	Correct the parameters.
	Other causes.	Contact Shimadzu or its nearest representative.
S/N ratio does not satisfy the guaranteed value.	Xenon lamp is not stably lit.	After powering ON the instrument wait 30 minutes until the Xenon lamp is stably lit.
	Xenon lamp is aged.	Use a new Xenon lamp.
	Distilled water is contaminated.	Use only clean distilled water.
	Other causes.	Contact Shimadzu or its nearest representative.

7.6 Instrument Construction

7.6.1 The optical system of RF-5301PC

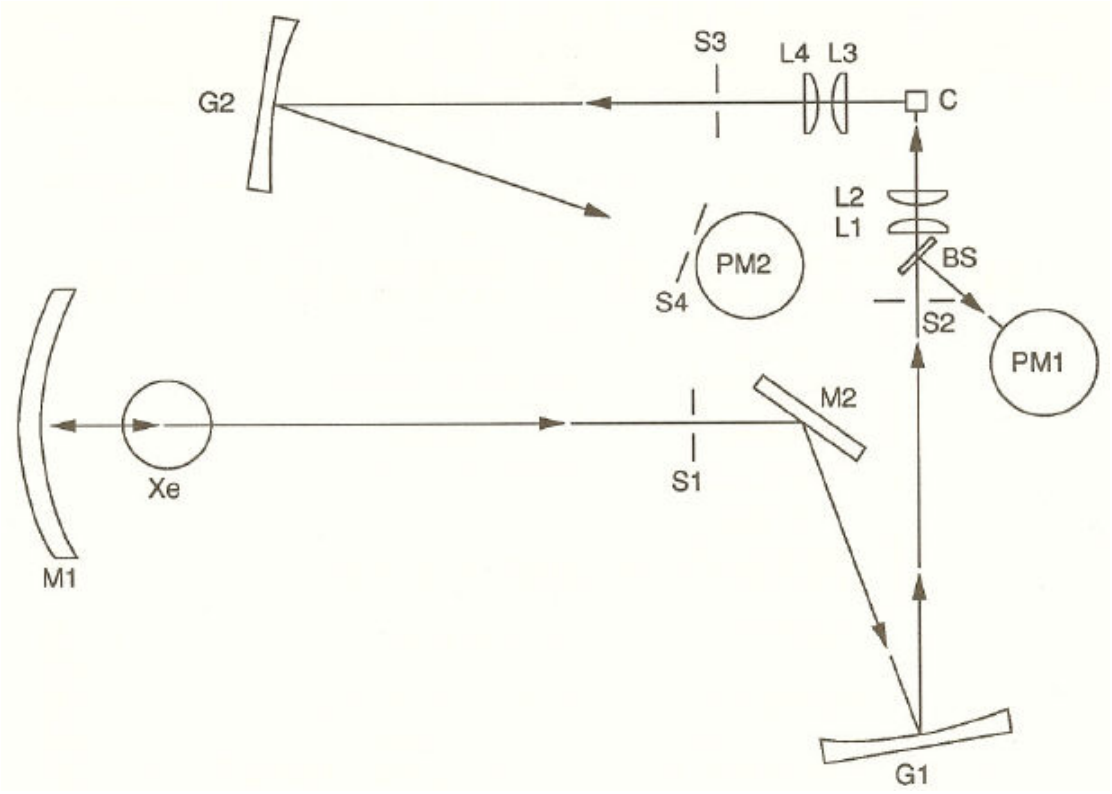
The optical system of the RF-5301PC instrument is illustrated in the figure below. A 150 W Xenon lamp (1) serves as the light source. The lamp housing contains generated ozone. The ozone is decomposed by means of the heat produced by the lamp. The bright spot of the Xenon lamp is expanded and collected by the ellipsoidal mirror (2). Then, the beam is collected again onto the entrance slit of the excitation-side slit assembly (3) by the concave mirror (4). A part of beam scattered by the concave grating (5) passes through the exit slit to irradiate the sample cell thought the condenser lens (11). To achieve light source compensation a part of the excitation light is reflected by the beam splitter quartz plate (6). Next it is directed to the teflon reactor plate 1 (7). The diffusely reflected light from the reflector plate 1 (7) then passes through the aperture for light balancing (21) and illuminates the teflon reflector plate 2 (8). Reflected by the reflector plate 2 (8) the diffuse light is attenuated to a specific ratio by the optical attenuator (9). Then it reaches the photomultiplier for monitoring (10).

The fluorescence occuring from the cell passes the condenser lens (13) and then it is introduced into the emission monochromator which comprises the slit assembly (14) and the concave grating (15). Then the isolated light is introduced through the concave mirror (16) into the photomultiplier for photometry (17). The resultant electrical signal is conducted to the preamplifier.



7.6.2 The optical system of RF-1501

The Optical system of the RF-1501 is explained below with reference to an Optical system diagram (see Figure below).



Light emitted from the xenon lamp is concentrated in the entrance slit S1 at the excitation monochromator by the light-concentrating ellipsoidal mirror M1. Light passing the slit S1 is reflected by the plane mirror M2 and enters the excitation grating G1 before it is dispersed to enter the exit slit S2.

Light emitted from S2 (excitation light) is concentrated in the sample cells through the lenses L1 and L2. Part of the excitation light is dispersed by the beam splitter 82 and enters the monitor photomultiplier PM1 for light source compensation.

Fluorescence passing the slit S3 is dispersed by the emission grating and, passing through the exit slit S4, enters the fluorescence photomultiplier PM2 for fluorescence intensity measurement.

8 Contacting

8.1 Contact Shimadzu

Shimadzu Deutschland GmbH is located in Germany:

Address:	**Shimadzu Deutschland GmbH** Albert-Hahn-Str. 6-10 D-47269 Duisburg Telephone: Telefax: e-mail:
Telephone:	+49 (0)203-7687-0
Fax:	+49 (0)203-766625
World Wide Web:	www.shimadzu.de

Further headquarters are located around the world. Please find the nearest headquarter in the internet at

http://eu.shimadzu.de/company/international

9 Appendix

9.1 Working with the Auto-Sampler Accessory

The auto-sampler unit is an optional add-on accessory for measurement automation. It provides an option to prepare a set of samples being measured subsequently in a predefined sequence. For details on how to install, setup and use the auto-sampler, please refer to the AUTO-SAMPLER OPERATION MANUAL.

> **The auto-sampler can only be used together with the sipper!**
>
> For technical reasons, the auto-sampler can only be used in conjunction with the sipper accessory. It allows to provide the samples one after the other to the instrument fully automated.

9.1.1 Enabling and disabling the Auto-Sampler

The sipper needs to be enabled before the auto-sampler can be enabled or disabled, because it can only be used in combination with the sipper unit. For details on how to enable the sipper, please refer to the section "Working with the Sipper Accessory".

In the Measurement Window, set the **Use Auto-Sampler** flag

- **Yes**
  The auto-sampler is enabled
- **No**
  The auto-sampler is disabled

9.1.2 Measurements with Auto-Sampler and Sipper

Performing measurements with the auto-sampler, please follow the steps below to setup experiments:

1. Prepare a number of samples in suitable reservoirs for the auto-sampler.
2. Put the samples in correct order into the auto-sampler.
3. Setup the auto-sampler itself to support the desired number of samples.
   Please refer to the AUTO-SAMPLER OPERATION MANUAL for details.
4. Now setup the measurement in **panorama Fluorescence** by selection of the desired measurement in the Measurement Window.
5. In the **Sipper Sampling** category of the measurement properties on bottom right of the Measurement Window set the **Use Auto-Sampler** flag to **Yes**.
6. Set the **Number of Samples** value to the number of prepared samples available in the auto-sampler.
7. Setup the **sipper parameters** as described in the section "Working with the Sipper Accessory".
8. Click the ***Sip and Measure*** **button** to start the measurement sequence.
   Alternatively, press the ***Start*** **button** on the auto-sampler.

All samples will be processed and measured one after the other automatically. On any error the system will stop and an error message is displayed.

Choose the automatic sample naming to avoid intermediate breaks and messages!

In the measurement Window setup the following parameters correctly to avoid interruptions:

1. In the **Sample Information** category set the flag **Show Sampler Name Dialog** to **No.**
2. Setup **Sample Name** parameter.

For details, please refer to the section Sample Information in the chapter Measurement Window.

9.2 Working with the Sipper Accessory

The sipper accessory is an optional accessory to the sample compartment for subsequent measurement of various liquids and dissolved samples. It has a built-in cuvette chamber, which is filled and emptied with a pump via a pipe system. Liquid samples can be easily measured without extra sample preparation. The liquid is directly pumped from your sample reservoir into the cell for measurement.

9.2.1 Installing the Sipper

The following steps are required to prepare the instrument for measurements with the sipper accessory:

1. **Power OFF** the instrument.
2. **Open** the sample **compartment lid** and **demount** the standard **sample holder**.
3. **Mount** the **sipper accessory** into the sample compartment.
4. **Connect** the sipper cable to the **I/O-2 connector** (Please refer to section "Part Names and Functions" to locate it).
5. **Power ON** the instrument again.

9.2.2 Enabling and disabling the Sipper Unit

Before the sipper can be used in **panorama Fluorescence**, it needs to be enabled in the software. There are two ways to do this as described in the following:

1. From the RF-5301/RF-1501 menu, expand the **Accessories** sub-menu.
2. Check or uncheck the **Sipper** option
   - **Checked**
     The sipper is enabled
   - **Unchecked**
     The sipper is disabled

Alternatively, the sipper can be enabled or disabled directly in the Measurement Window. The sipper unit has a few parameters that can be adjusted in the software as described in the Measurement Window section.

9.2.3 Performing Measurements with the Sipper

The work flow for routine measurement using the sipper accessory is described in the following:

1. Prepare the sample and provide the liquid in a reservoir.
2. Setup the desired measurement and corresponding parameters.
   For details on available measurements, please refer to the particular "Measurements" section.
   The Measurement Window is shown after selection of the measurement.

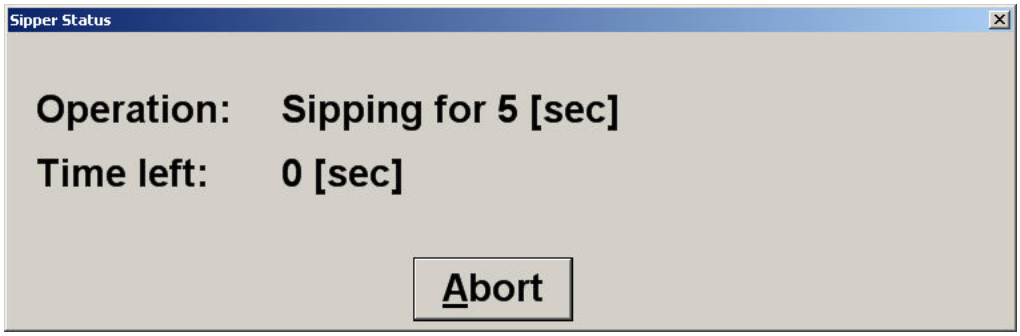
3. In the Measurement Window, setup the Sipper parameters.
   For detailed parameter explanations, please refer to the "Sipper Parameters" section.
   Example:
   - Pump Speed = Fast
   - Sipping time = 4.0 seconds
   - Dwell time = 1.0 seconds
   - Purge time = 4.0 seconds
   - Number of Rinses = 1
4. **Click** the ***Sip and Measure*** **button** to start the measurement.

> **Starting a measurement with the external sipper button.**
>
> The whole front panel of the sipper accessory is a button. If you press the front panel, the measurement will be started with current settings.

5. Put the tube to the ground of the reservoir to avoid bubbles being pumped into the cuvette chamber and confirm the message box with ***OK***.

The measurement progress and steps are shown in the status dialog:

1. The sample is pumped into the cell.
   The following status dialog is shown:



> **Aborting measurements.**
>
> Whenever you like to abort the current measurement, click the ***Abort*** **button** in the status dialog.
> Abort takes some time to complete already started operations. This may take up to 30 seconds.
>
> The sample remains in the cuvette chamber on abort. It will not be removed automatically!

2. Pumping stops and the instrument is idle for the specified dwell time in order to let the sample rest for a while.

3. The measurement is carried out.
   If not aborted, the sample is removed from the cell by purging and rinsing after completion of the measurement.

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