

Spectrofluorophotometer

# RF-6000



# Striving for the Ultimate in Accuracy and Easy Operability

The RF-6000 spectrofluorophotometer has been newly developed by combining Shimadzu's long-cultivated expertise and cutting-edge technologies.

Combined with LabSolutions™ RF software, designed for unrivaled measurement accuracy and easy operation, the RF-6000 offers the ultimate performance for a diverse range of customers' measurement needs.

Spectrofluorophotometer

# RF-6000



## Wide Variety of Spectral Techniques

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- Enhanced sensitivity and dynamic range enable fluorescence as well as bioluminescence, chemiluminescence, and electro-luminescence measurements.
- High-speed 3D scanning enables rapid acquisition of 3D spectra.
- Spectrum-Corrected Excitation\* and Emission spectra can be scanned.
- Fluorescence quantum yield and Fluorescence quantum efficiency measurements are available.

\*The correctable wavelength range is 250 to 850 nm.

## High Sensitivity, High Stability and High Speed

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- Highest level S/N Ratio in its class: 1,000 or more (RMS) /350 or more (P-P)
- High-speed scanning of 60,000 nm/min minimizes scan time.
- Extended range PMT offers scan wavelength range to 900nm.
- 2,000 hour long-life Xenon lamp.

## Excellent Usability

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- Easy-to-use LabSolutions RF software simplifies analysis.
- Validation routines included.
- Status bar in LabSolutions RF indicates lamp and accessory status.
- Large sample compartment for all analytical needs.



This product is certified as Shimadzu's Eco-products Plus. Energy savings: 45% reduction as compared to the previous model

# Wide Variety of Spectral Techniques

## Supports Applications in a Wide Variety of Fields

### Pharmaceuticals

Analysis of components in compounds  
Quality control of API or drug products

### Chemicals

Identification of artificial photosynthesis mechanisms  
Analysis of coumarin in light diesel oil

### Life Sciences

Spectral properties of fluorescence probes

### Foods

Quantitative analysis of additives  
Quality control of packaging

### Environmental

Evaluation of low-level contaminants in rivers and soils

### Electrical/Electronics

Spectral properties of fluorescent materials  
Measurement of quantum efficiency and quantum yield  
Analysis of LEDs, solar cells, and organic EL materials



## Electrical/Electronics

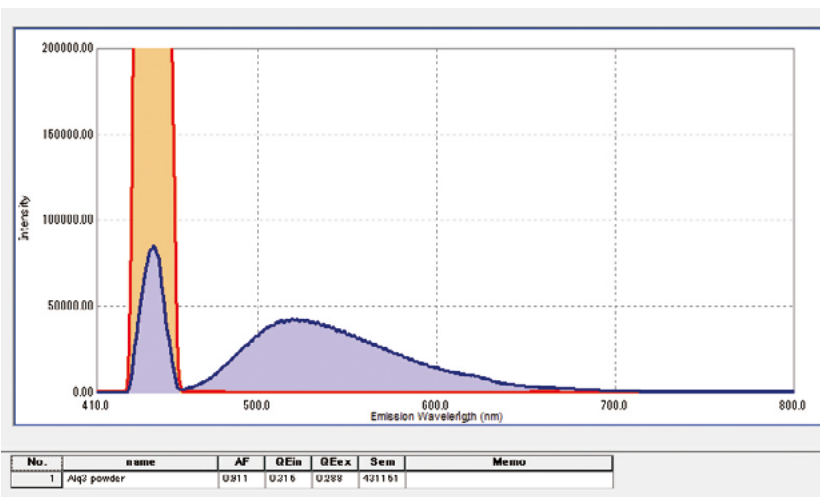
### Evaluating the Luminous Efficiency of Solid-State Semiconductor Materials

A 100 mm diameter Spectralon integrating sphere unit was used to measure the fluorescence quantum efficiency of the light-emitting layer of a solid-state semiconductor material (tris(8-hydroxyquinolino)aluminum) used in an organic EL device.

Using the quantum efficiency measurement application of LabSolutions RF software allows determining the fluorescence quantum efficiency easily using intuitive software commands.



The integrating sphere unit fits completely inside the sample compartment, eliminating the need for additional installation space.

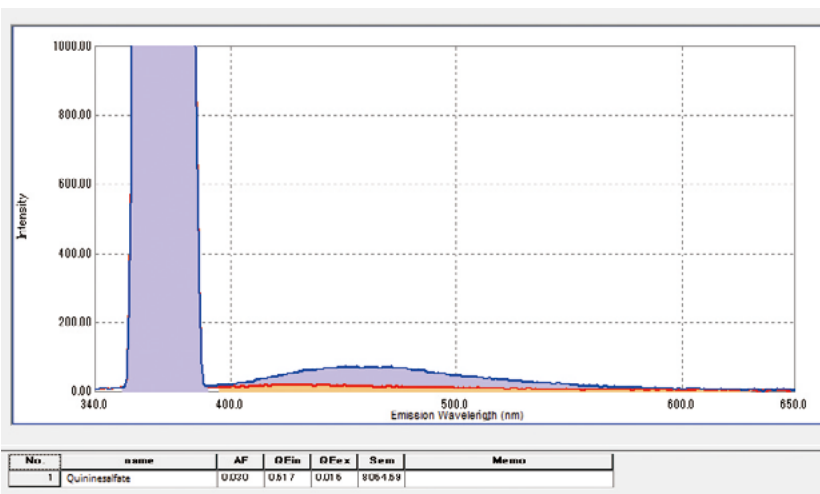


## Chemicals

### Measuring the Fluorescence Quantum Efficiency of Solution Samples

In addition to film and powder samples, the integrating sphere can secure a cuvette for measuring liquid samples.

The fluorescence quantum efficiency of a 1 N aqueous sulfate solution of quinine sulfate was measured (on an NIST SRM 936a substrate). Even tedious fluorescence quantum efficiency calculations can be performed readily using the LabSolutions RF quantum efficiency measurement function.

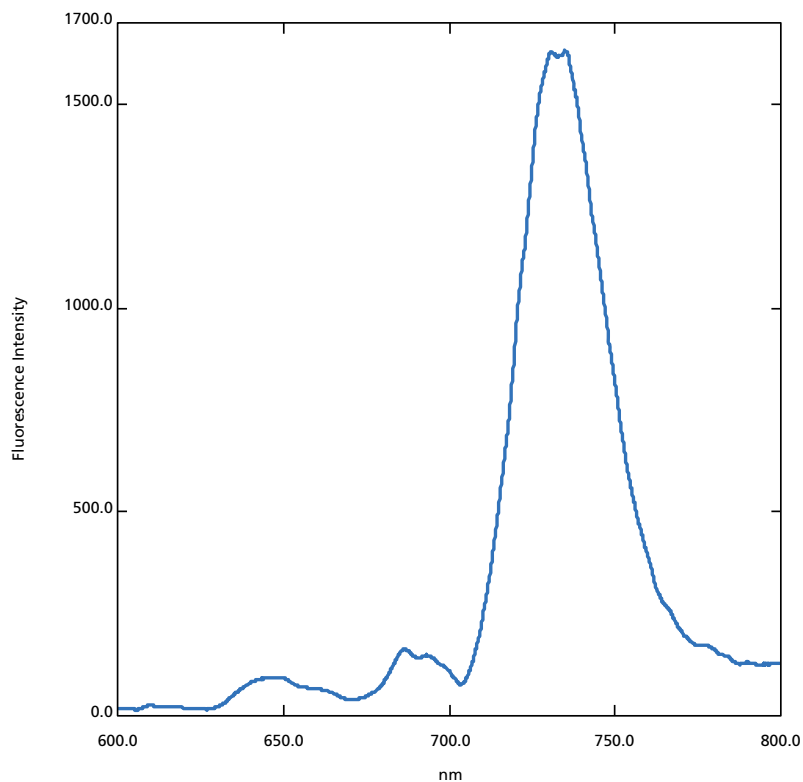


## Chemicals

### Long Wavelength Measurements

The RF-6000 can be used to research photosynthetic proteins for determining the mechanisms involved in artificial photosynthesis. In this case, a Thylakoid membrane solution was measured.\*1, 2

The fluorescence spectrum shows that the system can accurately measure the fluorescence peaks in the long wavelength region. A spectral correction function, allowing the accurate measurement of spectral shapes in real time, is also included standard.



Fluorescence Spectrum of Thylakoid Membrane Solution Cooled by Liquid Nitrogen

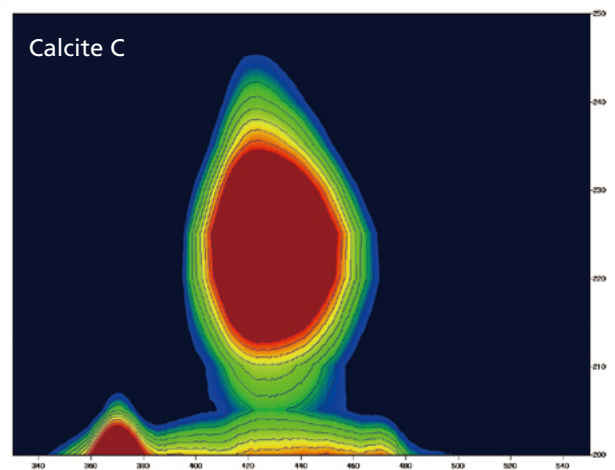
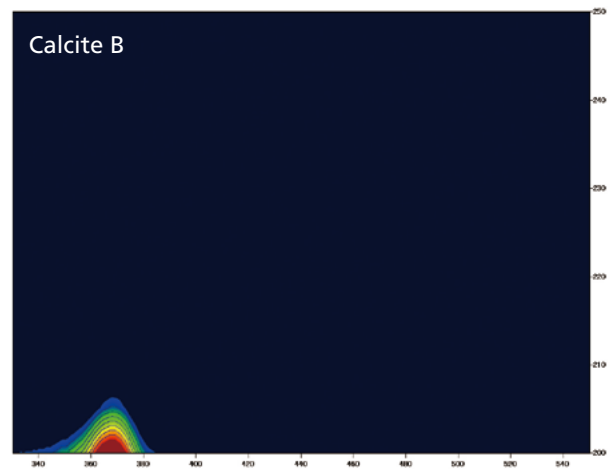
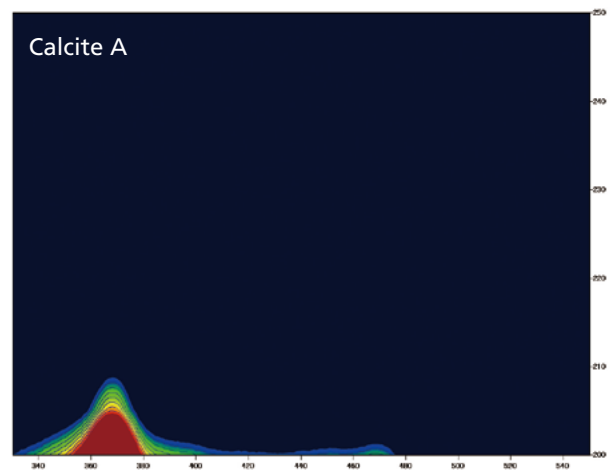
\*1: Measured with the help of professor Jian-Ren Shen of Okayama University.

\*2: Measured using a low-temperature measurement unit. Contact Shimadzu for further details about the low-temperature measurement unit.

# Chemicals

## Potential for Identifying the Source of Minerals

Calcite is a clear and colorless mineral consisting primarily of lime rock. Any impurities in the calcite may cause coloration. This example shows 3D fluorescence measurement (Excitation vs Emission) data for three types of calcite. Calcite A and B are clear and yellow whereas calcite C is clear and pink. 3D fluorescence patterns of calcite A and B were the same. In contrast, on the 3D fluorescence patterns of calcite C, there was a fluorescence peak at about 370 nm (EX; about 205 nm) and a strong fluorescence at about 430 nm (EX; about 225 nm). This fluorescence might be caused by metal-ions such as the manganese ion, which makes calcite pink. Because the fluorescence wavelengths caused by metal-ions are very sensitive against the size of the crystal field, they may readily show changes with mineral content.



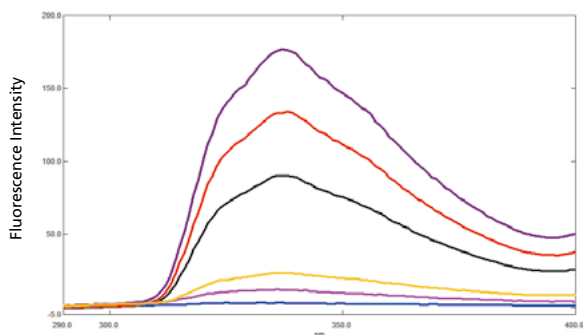
## Pharmaceuticals

### Measuring Duloxetine Hydrochloride (USP)

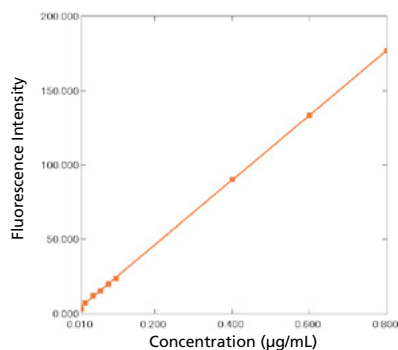
Duloxetine hydrochloride is a compound used as an antidepressant.

In this example, an RF-6000 was used to measure duloxetine hydrochloride.

The results indicated a lower limit of quantitation of 0.0007  $\mu\text{g/mL}$  and a lower limit of detection of 0.0002  $\mu\text{g/mL}$ , showing the ability of the RF-6000 to measure very low concentrations.



Fluorescence Spectra of Duloxetine Hydrochloride



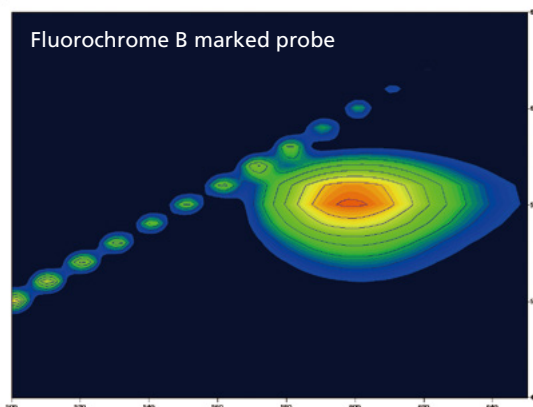
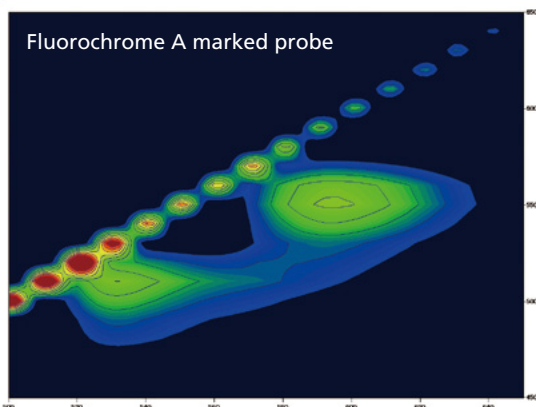
Calibration Curve

## Life Sciences

### Fluorescent Dyes for DNA Detection

Specified complementary DNA can be detected by using a DNA probe which is marked by fluorochrome. These probes become luminescent when connected to DNA.

The following shows the results of a 3D measurement of DNA marked by two different kinds of DNA probes. Unique fluorescent peaks according to specific DNA fluorescence probes can be quickly measured using the high-speed scanning function.





## Foods

### Classifying and Identifying Types of Milk

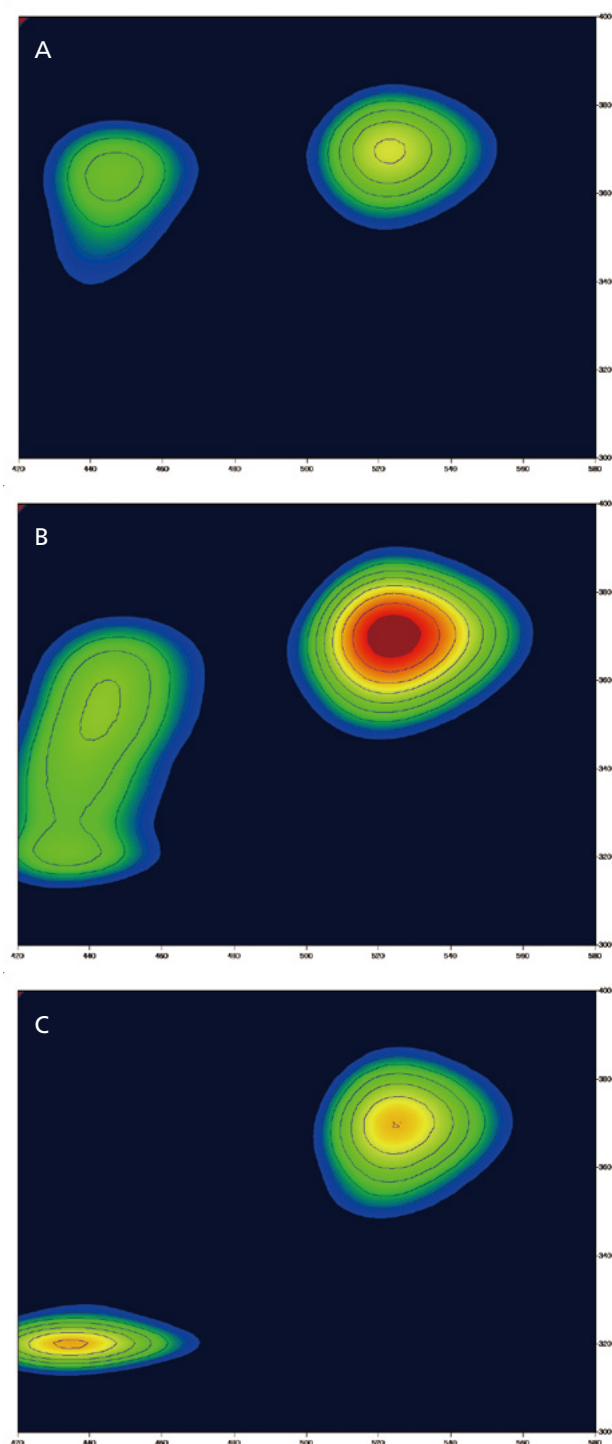
There are many types of milk products, such as raw or processed and those classified by fat content levels (low-fat, non-fat, and so on). These different types of milk products can have different 3D fluorescence spectra.

In this example, three different types of milk products (A, B, and C) were used to measure 3D fluorescence spectrum while varying the excitation wavelength.

Samples were diluted by five times with distilled water.

The results show that milk samples A and C have different fluorescence patterns. However, milk sample B has a fluorescence pattern that is found in both milk samples A and C.

Therefore, the 3D fluorescence spectrum can be used to discriminate between different types of milk products.



# Environmental

## Measuring Oil in Water - ASTM D5412

The American Society for Testing and Materials (ASTM) testing standard D 5412 specifies testing for polycyclic aromatic hydrocarbons contained in water as oil. In this example, the target substance was separated from a solution prepared with a mixture of five polycyclic aromatic hydrocarbons using synchronized scan\*. Fig. 1 is a fluorescence spectrum of the mixture of polycyclic aromatic hydrocarbons. Fig. 2 is a synchronized scan spectrum of Benzo[a]pyrene. Fig. 3 is a synchronized scan spectrum of the mixture. The fluorescence peak of Benzo[a]pyrene was not distinguished in the fluorescence spectrum of the mixture; however, it was clearly separated on the synchronized scan spectrum.

\* Synchronized scan

Synchronized scan mode simultaneously scans samples using both an excitation monochromator and a fluorescence monochromator that are offset by fixed wavelength intervals. Sharp fluorescence peaks can be detected for target substances even if multiple types of components are mixed together.

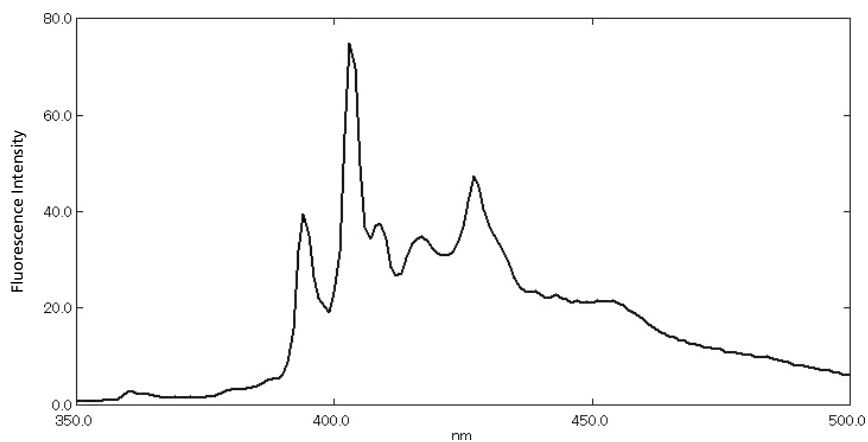


Fig. 1 Fluorescence Spectrum of the Mixture of Polycyclic Aromatic Hydrocarbons (Ex 300 nm)

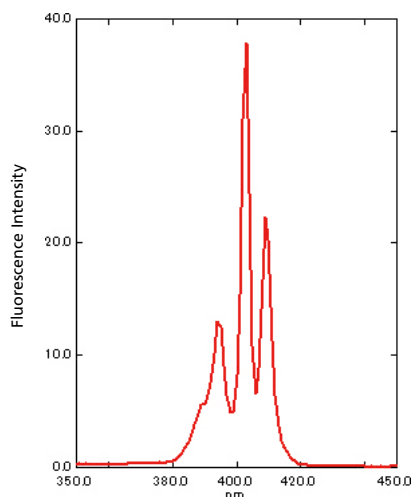


Fig. 2 Synchronized Scan Spectrum of Benzo[a]pyrene (Offset 6 nm)

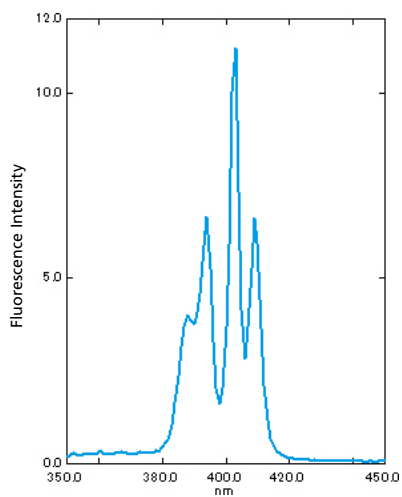


Fig. 3 Synchronized Scan Spectrum of the Mixture (Offset 6 nm)

# Environmental

## Trace Measurement of Chlorophyll

The chlorophyll content is commonly measured when inspecting the water quality of rivers and lakes. Since the concentration of chlorophyll is low in rivers and other such waters, high sensitivity is required for measurements. Chlorophyll emits fluorescent light when exposed to light, but the light exposure causes the fluorescence intensity to gradually diminish. Therefore, fluorescence measurements that apply only a slight amount of excitation light are required. If the same chlorophyll solution is measured repeatedly with a typical 5 nm bandwidth, the intensity varies as shown in Fig. 1. However, spectra with almost no variation can be obtained by narrowing a bandwidth, as shown in Fig. 2. These results show how low concentration chlorophyll solutions can be measured with good sensitivity.

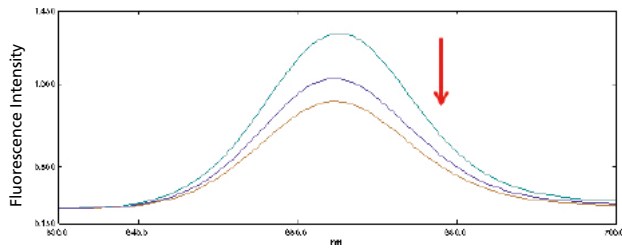


Fig. 1 Measurement with a Bandwidth of 5 nm

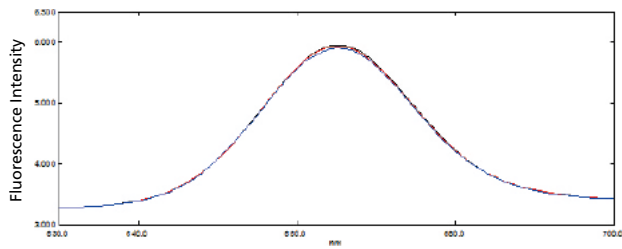


Fig. 2 Measurement with Very Low Excitation Intensity

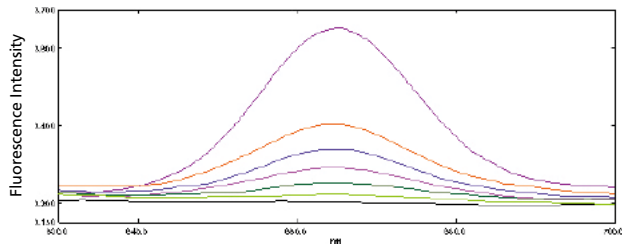


Fig. 3 Chlorophyll-a at Concentrations of 0.75, 1.5, 3.0, 4.5, 7.5, and 15 ppb

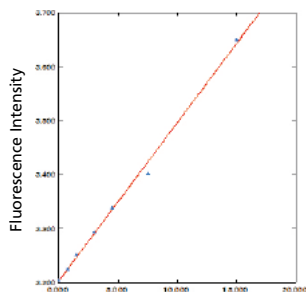


Fig. 4 Calibration Curve

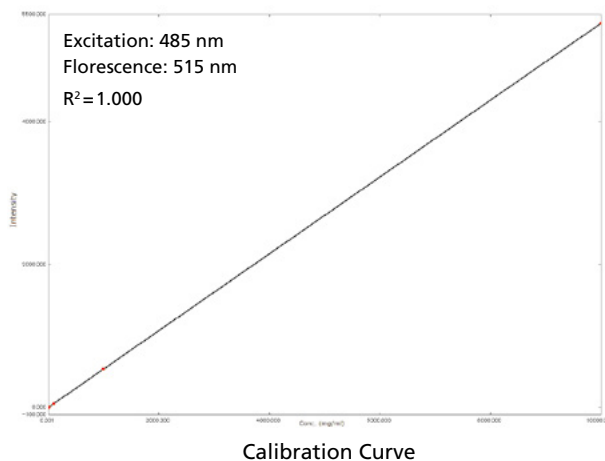
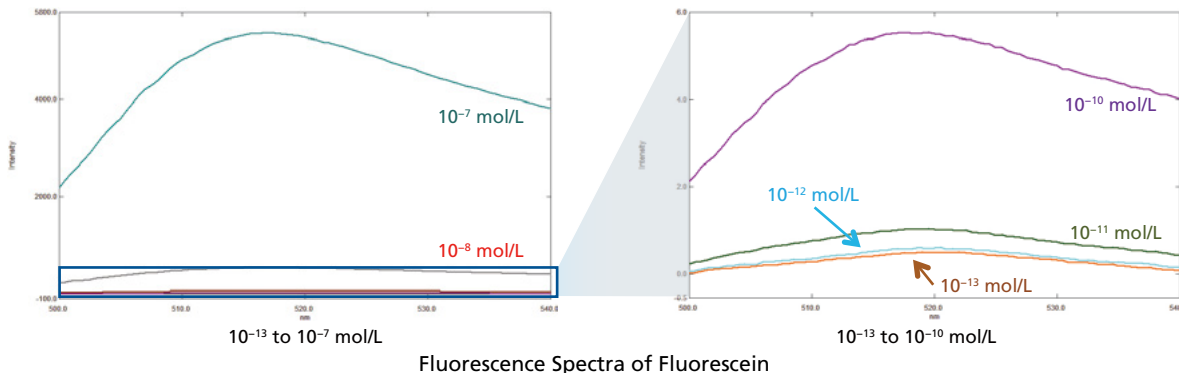
# High Sensitivity, High Stability and High Speed

## High-Sensitivity Measurements

- High-sensitivity measurements can be performed with S/N ratio of 1,000:1 (RMS) or 350:1 (P-P).
- Improved lower limit of quantitation. Measures concentrations up to  $1 \times 10^{-13}$  mol/L (fluorescein).

A redesigned optical system and signal processing system achieve the highest S/N ratio levels in its class. Even extremely dilute samples can be measured accurately.

The RF-6000 can measure fluorescence spectra from fluorescein concentrations as low as  $1 \times 10^{-13}$  mol/L. Furthermore, due to an auto-gain control function that ensures measurements are performed using optimal measurement parameters, the system can perform accurate and highly quantitative measurements over a wide six-order dynamic range, from  $10^{-13}$  to  $10^{-7}$  mol/L.



## High-Speed Measurements

Enables ultrafast scanning at speeds up to 60,000 nm/min. All wavelength regions can be measured in only one second. 3D fluorescence spectra can also be measured quickly.

## Stable Measurements

### ■ Includes a long-life Xenon lamp.

The Xenon lamp offers long service life and high stability. The 2000-hour service life significantly reduces running costs.

In addition, auto alignment technology allows customers to easily replace the lamp without tedious adjustment routines.



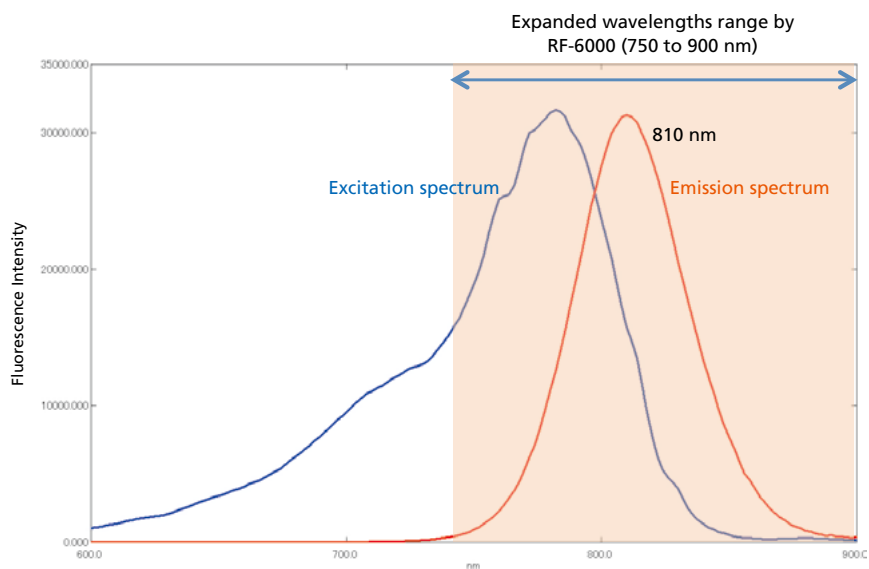
## Long Wavelength Measurements

### ■ The detector offers high sensitivity and a broad measurement wavelength range.

A low-noise photomultiplier is included standard. It offers high measurement sensitivity for long wavelengths.

Consequently, it can measure substances that exhibit fluorescence at longer wavelengths, such as chlorophyll and indocyanin green (ICG).

Below are Excitation and Emission spectra of indocyanin green, which is used for testing hepatic function and hepatic spare ability.



Excitation and Emission Spectra of Indocyanin Green

# Excellent Usability

## ■ LabSolutions RF software makes it easy for anyone to perform measurements.

LabSolutions RF software was developed to be easy for anyone to understand and operate.

From measurement to analysis, printing, and saving processes, windows are laid out in an easy-to-understand manner, which ensures that measurements can be performed easily.

All measurement programs feature the same main toolbar, menu, measurement toolbar, tree view, and log window configuration, so that each program can be operated in the same manner. This means the same operations can be used to operate all the specialized fluorescence analysis programs.

### Main Toolbar

The main toolbar includes buttons for all the main functions, such as file operations, printing, and data processing. The same main toolbar is used in all LabSolutions RF measurement programs.

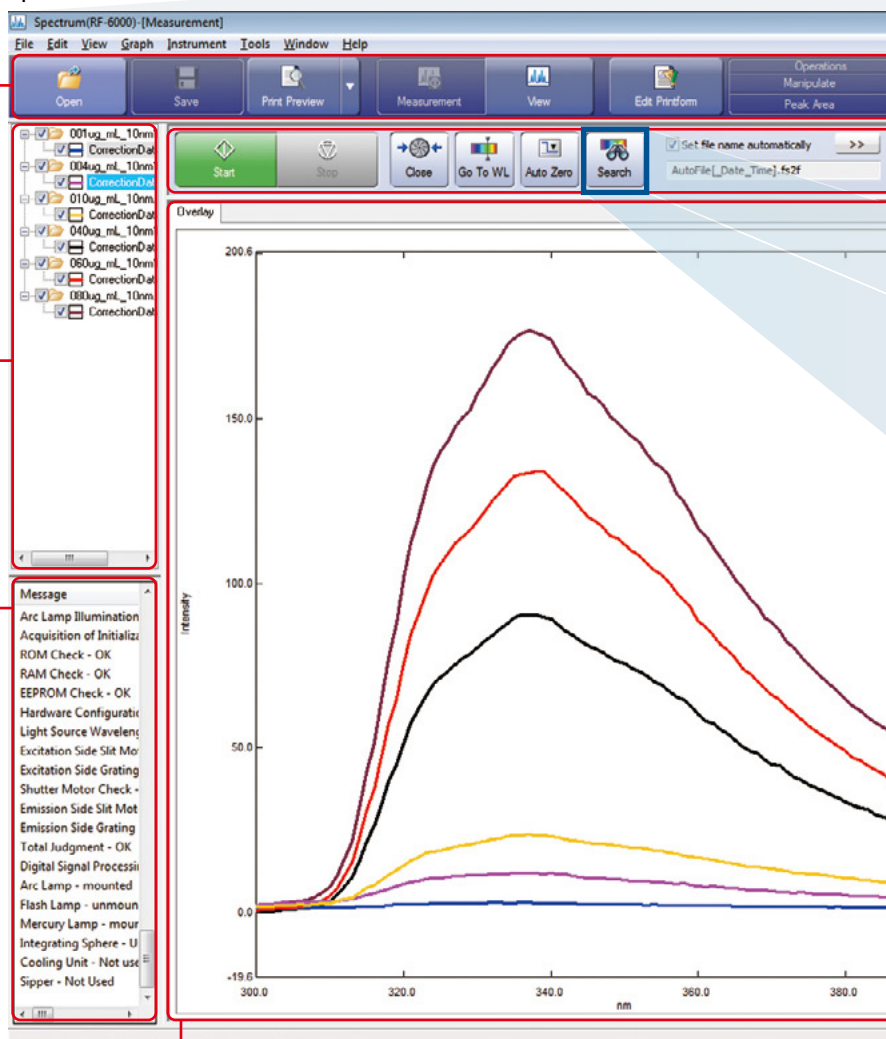
### Tree View

Lists all files currently displayed. It allows you to toggle between displaying, hiding, or closing data or converting data to text.

### Log View

This successively displays log and operational information to indicate the instrument status or measurement history information. The displayed log information is saved so post-operation review can be accomplished at any time.

### Spectrum Measurement Window



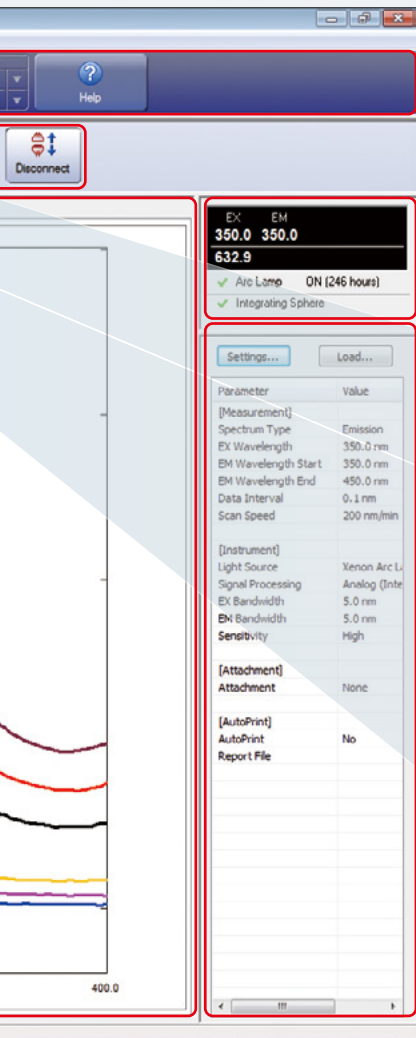
### Application Area

This area displays spectra, 3D data, data processing tables, quantitation tables, measurement parameters, and other information.

■ All functions can be launched from the LabSolutions RF launcher.

Relevant functions for each measurement action, such as spectral measurements and quantitative measurements, are organized conveniently on the LabSolutions RF launcher. That allows easy selection of the desired measurement function. In addition, frequently used Windows® applications can also be added to the LabSolutions RF launcher.

LabSolutions RF Launcher

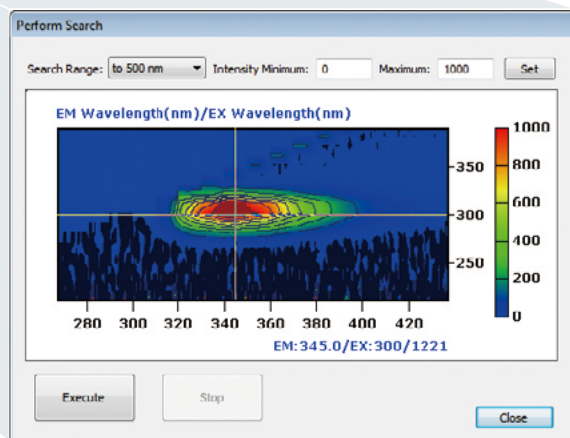


Instrument Status

This displays the total number of hours the xenon lamp has been illuminated, the recognition status of accessories, and the status of current actions. It also notifies the user about the status of errors and when it is time to replace the lamp.

Measurement Parameters

This area is used to set measurement parameters. For quantitative measurements, it also displays calibration curves.



Search

This allows searching for the optimal excitation wavelength or fluorescence wavelength.

\* Please contact Shimadzu representative to comply with ER/ES regulations including FDA 21 CFR Part 11 and PIC/S.

## Spectrum Correction

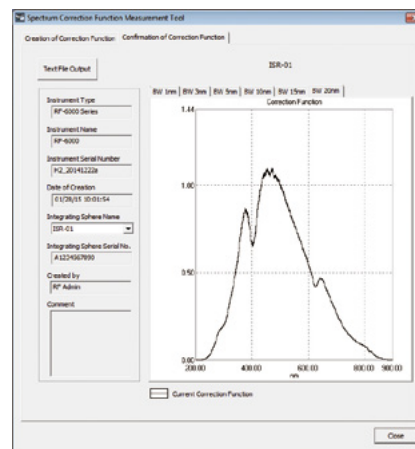
- Spectrum correction functions are included standard, which allows you to display spectrum corrected automatically!

A spectrum correction function for obtaining the true excitation and fluorescence spectrum determined by correcting the instrument function for instrument characteristics, such as the emission characteristics of the light source and spectrum characteristics of the optical system, is included standard. Because true spectrum can be obtained automatically, the spectra can be easily compared to spectrum measured using other instruments.

The spectrum correction functions preregistered in systems are determined using a calibrated standard light source and Shimadzu's proprietary correction techniques.

If an integrating sphere is installed as an accessory, a function for creating spectrum correction functions is included standard; this eliminates the need to install a special light source.

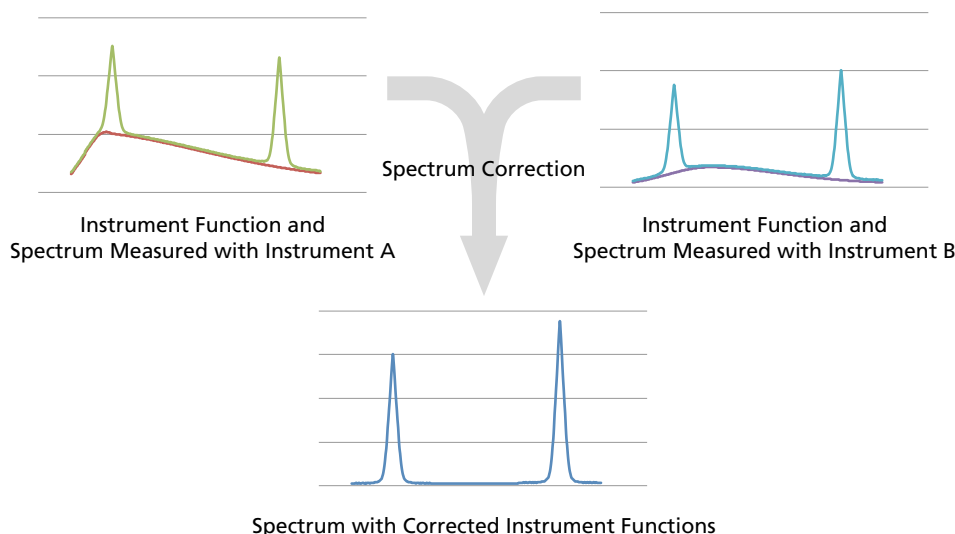
The correctable wavelength range (guaranteed range) is 250 to 850 nm, which is different from the measurable wavelength range (200 to 900 nm).



Window for Creating Spectrum Correction Functions

### ■ Overview of spectrum correction

A certain sample had two fluorescence peaks. When that sample was measured using instruments A and B, the fluorescence peak intensity was higher for the left peak with instrument A, whereas the right peak was higher for instrument B. In reality, the spectrum peak intensities and positions differed because instruments A and B each had the emission characteristics of light source and the spectrum characteristics of optical system. Correcting the spectrum by subtracting the difference in light source and optical system characteristics resulted in the same spectrum, which reveals that the two peaks are successively larger as the wavelength increases. By comparing the spectrum measured with different instruments the effects from differences in instrument characteristics cannot be ignored. Spectrum correction allows you to compare data measured using different instruments.





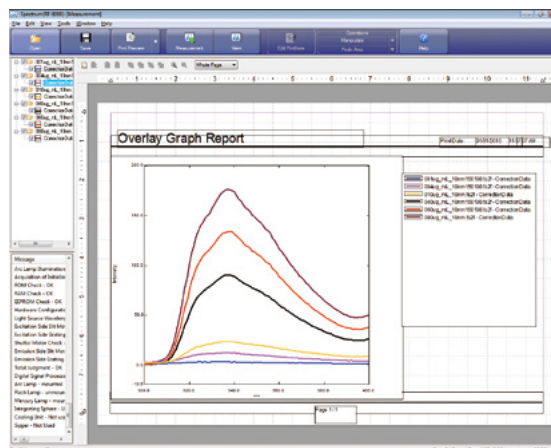


## Creating and Printing Reports

### ■ Report formats can be created easily.

Printed reports can be freely prepared by simply dragging the desired content to the desired layout.

This allows you reviewing the layout before printing, with content dragged into place, without using the print preview function.



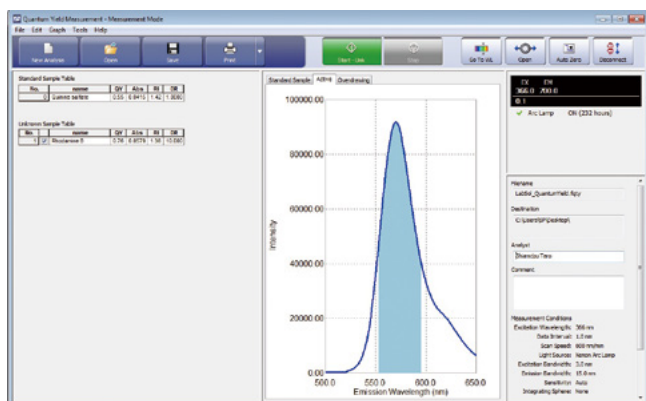
## Fluorescence Quantum Yield and Fluorescence Quantum Efficiency

### ■ Measuring fluorescence quantum yield and fluorescence quantum efficiency is simple.

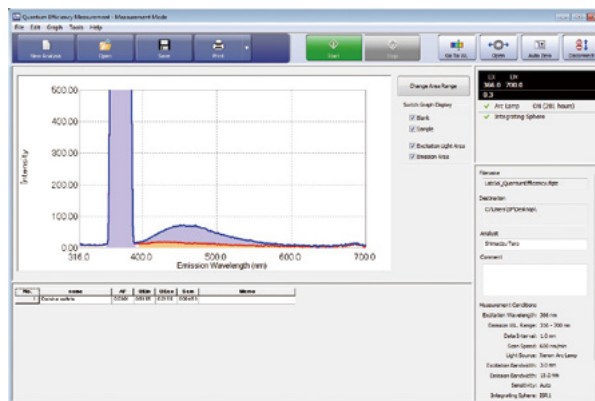
The fluorescence quantum yield can be calculated by comparing the fluorescence spectra of unknown samples with those of a standard sample with a known quantum yield.

The 100 mm diameter integrating sphere unit can also be used to calculate the fluorescence quantum efficiency.

The user-friendly window design allows anyone to easily measure the fluorescence quantum yield and fluorescence quantum efficiency using intuitive operations.



Fluorescence Quantum Yield Measurement Window  
(Fluorescence quantum yield measurement of  
rhodamine B using quinine sulfate)



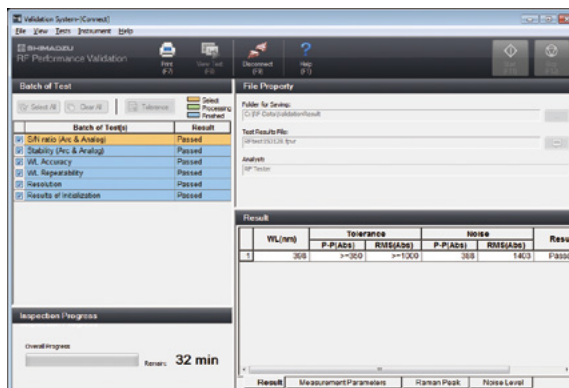
Fluorescence Quantum Efficiency Measurement Window  
(Fluorescence quantum efficiency measurement  
of quinine sulfate)

## Validation and Status Indication

### ■ Validation function allows you to diagnose performance.

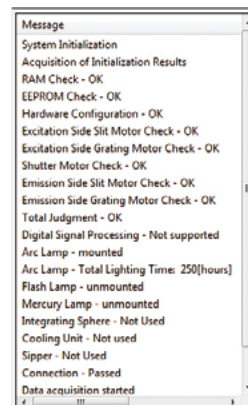
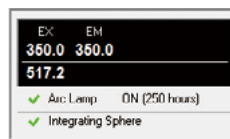
The system supports performance validation in accordance with procedures specified in JIS K 0120 General rules for fluorometric analysis.

Note: An optional mercury lamp is required for confirming wavelength accuracy, resolution, and wavelength repeatability.



### ■ Instrument status can be confirmed accurately.

The instrument status display in LabSolutions RF and LED indicators on the instrument provide notification when the lamp operating hours have exceeded the specified service life and indicate the recognition status of accessories as well as an abnormal instrument status.



Full color LED indicators on the front of the instrument indicate the current instrument status.

- Blue: Measurement in progress
- Green: Ready to measure
- Red: Instrument error or other problem



## Large Sample Compartment for All Analytical Needs

### ■ A larger sample compartment allows for mounting a 100 mm diameter integrating sphere unit.

The sample compartment size has been significantly increased, making it easier to place samples.



# Extensive Selection of Accessories

## Integrating Sphere Unit

(P/N 207-21460-41)

Used in combination with the dedicated application function for LabSolutions RF to determine the fluorescence quantum yield and fluorescence quantum efficiency.

Samples : Liquids, solids, or powders  
Internal diameter of integrating sphere : 100 mm  
Integrating sphere material : Spectralon  
Max. sample size : W12.5 × H45 × T12.5 mm  
Measurement wavelength range : 200 to 900 nm  
(Spectrum correction guarantee range: 250 to 850 nm)

Standard contents

- Integrating sphere attachment (main unit)
- Mesh for measurement of Spectral correction functions



## Ultra Micro Cell Holder Unit

(P/N 207-21455-41)

This ultra micro cell holder unit allows sample quantities below 100  $\mu\text{L}$  to be measured. It uses a commercial micro cell.

Standard contents

- Ultra micro cell holder

Available micro cell

- Hellma Cat. No. 105.250-QS-15 (Min. sample amount 100  $\mu\text{L}$ )
- Hellma Cat. No. 105.251-QS-15 (Min. sample amount 45  $\mu\text{L}$ )



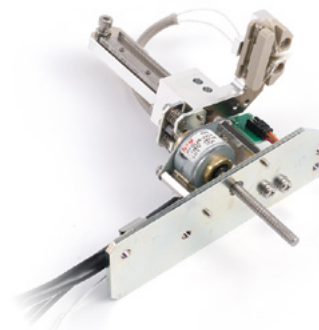
## Mercury Lamp Unit

(P/N 207-21700-41)

Used to inspect resolution, wavelength accuracy, and wavelength repeatability for validation.

Standard contents

- Mercury lamp (main unit)
- Scattering block



## Constant-Temperature Single Cell Holder with Stirrer

( P/N 206-24930-41 (100 V)  
206-24930-42 (115 V)  
206-24930-58 (220/240 V) )

Used to stir and measure temperature-controlled samples. This is ideal for measuring the fluorescence of suspended cells. Enables controlling the temperature of one cell by circulating temperature-controlled water. The operating temperature range is 5  $^{\circ}\text{C}$  to 70  $^{\circ}\text{C}$ . The stirrer rpm is variable. The minimum sample volume required is 2.5 mL.

For use with 100 V, 115 V, or 220/240 V power supplies

Note 1: This cannot be used if there is a large difference in specific weight between the sample and solvent.

Temperature-controlled water tubing connectors are compatible with 4 to 8 mm ID tubing.

Note 2: Requires a separate optional front cover (P/N 207-20490).



## Constant-Temperature Four Cell Holder

(P/N 206-24940-41)

Note: Temperature-controlled water tubing connectors are compatible with 4 to 8 mm ID tubing.

Enables simultaneously controlling the temperature of four cells by circulating temperature-controlled water. The operating temperature range is 5 °C to 80 °C (temperature of circulated water).

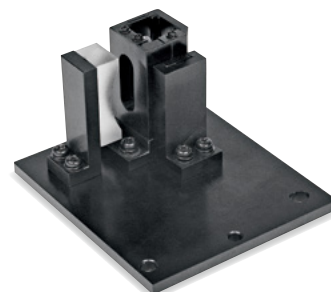
Note: Requires a separate optional front cover (P/N 207-20490).



## High-Sensitivity Cell Holder

(P/N 204-26841-42)

Using this attachment increases sensitivity by 2 to 3 times, which is especially useful for measuring dilute samples. It is designed to improve detection efficiency by using a mirror to reflect excitation light that passed through the cell back to the sample and by also reflecting the fluorescent light emitted in the opposite direction of the detector back to the fluorescence monochromator.



## Solid (Powder) Sample Holder

(P/N 204-26836-42)

In addition to solid and powder samples, it can hold a liquid solution cell to measure the reflected fluorescent light. The angle of the holder is designed to limit specular reflected excitation light from directly entering the fluorescence monochromator. Cutoff filters are included to block excitation light and scattered light (includes U-340, IHU-310, L-42, Y-50, O-56, and R-60). About 1 mL of sample is required for the powder holder.

Sample size range

- Between 5 mm and 140 mm wide
- Between 12 mm and 110 mm tall
- Max. 20 mm thick



## Polarized Light Measurement Attachment (for UV/VIS region: 240 to 700 nm)

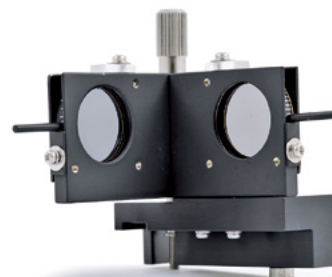
(P/N 204-03290-41)

## (for VIS region: 390 to 700 nm)

(P/N 204-03290-42)

Measuring fluorescence polarization provides information on the size, fluidity, and surrounding environment of molecules.

The wide wavelength range of this attachment, from 240 nm to 700 nm, allows measurement of the polarized light in UV and near infrared regions.



## Micro Cell Unit

(P/N 204-27125-41)

This allows measuring sample quantities of only 400  $\mu$ L. It is placed in the same cell holder as a regular 10 mm standard cell polished on four sides. However, it cannot be used with a sample elevation stage (P/N 204-04811).



## Cell Polished on Four Sides (Fused Quartz)

(P/N 200-34441)

## Non-Fluorescent Cell (Special Fused Quartz)

(P/N 200-34594-03)

Quartz cells with four sides polished absorb a small amount of light near 260 nm, which results in a slight fluorescence near 400 nm. Therefore, this non-fluorescent cell with special fused quartz is recommended for measuring low concentration samples with excitation near 260 nm.



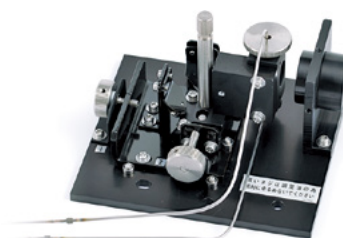
Non-Fluorescent Cell

## LC Flow Cell Unit (12 $\mu$ L Cell)

(P/N 204-05566-41)

With this attachment, the RF-6000 can be used as a highly sensitive spectral fluorescence monitor for high performance liquid chromatography. The excitation and fluorescence wavelengths are freely selectable, permitting selective detection. In addition, it allows you to record spectra with solvent delivery paused, which is very useful for identifying chromatography peaks. A rectangular quartz micro flow cell with minimized light scattering and a 12  $\mu$ L capacity is used.

Note: Requires a separate optional front cover (P/N 207-20490).



## LC Flow Cell Unit (120 $\mu$ L Cell)

(P/N 204-06249-41)

This LC flow cell unit is for analyzing catecholamine. A rectangular quartz micro flow cell with minimized light scattering, two reflective sides, and a 120  $\mu$ L capacity is used.

Note: Requires a separate optional front cover (P/N 207-20490).



## Filter Set

(P/N 204-04691)

Set of seven filters; includes IHU-310, U-340, L-42, Y-50, O-56, R-60, and B-390 filters

## 8 mm Diameter Test Tube Holder

(P/N 204-05853-41)

Holds 8 mm diameter test tubes. (The minimum sample volume required is 400  $\mu$ L.)  
Test tube size: 8 mm OD, between 45 mm and 100 mm long

## 12 mm Diameter Test Tube Holder

(P/N 204-03293-41)

Holds 12 mm diameter test tubes.  
Test tube size: 12 mm OD, between 60 mm and 100 mm long





## Sample Elevation Stage

(P/N 204-04811)

Minimizes dead volume by raising the cell position in order to reduce the sample volume required for measurement. (The minimum sample volume required is 1.5 mL.) However, it cannot be used with a micro cell unit (P/N 204-27125-41).



## Sipper Unit 6000

(P/N 207-21470-41)

Uses a stepping motor driven peristaltic pump for successively loading liquid samples for measurement. It can be operated in combination with the ASC-5 auto sample changer.

Flow cell : Quartz rectangular flow cell  
Cell capacity : 120  $\mu$ L (W4  $\times$  D3  $\times$  H10 mm)  
Aspiration rate : Three levels – fast, medium, or slow  
Min. sample volume required : 2 mL (less than 1 % carryover)  
Standard sample volume required : 3 mL

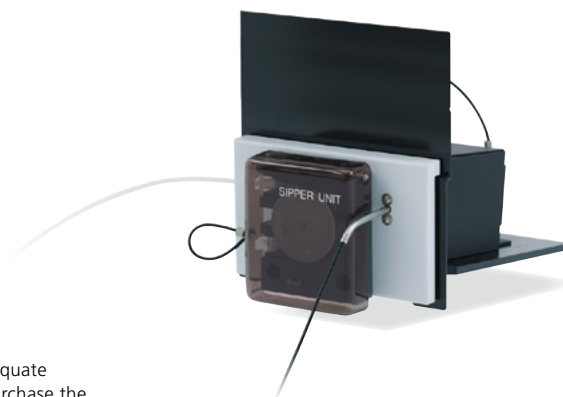
### Standard contents

- Sipper 6000 (main unit)
- Waste tank
- 2.5 m Tygon tube for pumps
- Spare fittings for peristaltic pumps
- ASC-5 connection cable

### Options and Consumables

- Solenoid valve unit (liquid contact surfaces made of fluoropolymer) (P/N 206-69824-41)
- SWA-2 sample waste unit (P/N 206-23820-58)  
The SWA-2 cannot be used for strong acids, strong alkalis, or ester solvents due to inadequate chemical resistance of the tube used in standard peristaltic pumps. To use the SWA-2, purchase the solenoid valve unit and sample waste unit indicated above.
- Tygon tube for pumps (P/N 200-54565-02)
- Spare fittings for peristaltic pumps (P/N 200-62050-24)

Note: Requires a separate optional front cover (P/N 207-20490).



## ASC-5 Auto Sample Changer

( P/N 206-23810-91 (100 V)  
206-23810-92 (115 V)  
206-23810-93 (220 V)  
206-23810-94 (240 V)

An automatic measurement system for multiple liquid samples can be configured by combining a sipper unit.

- Includes an accurate X–Y–Z 3-axis movement mechanism.
- Up to eight sets of parameters, such as rack size and number of test tubes, can be backed up by recording them in a file.
- Number of samples placeable: 1 to 100



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