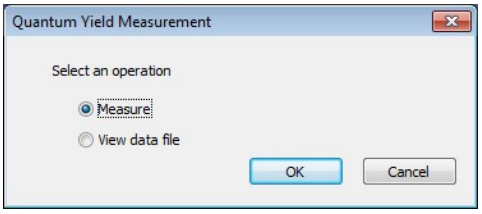
10 Quantum Yield Application

10.1 Mode Selection

Select the mode to start in the following window when starting quantum yield measurement from the LabSolutions RF launcher.



Quantum Yield Application Mode Selection

Command	Description
[Measure]	Select this mode when performing a new measurement. The preparation window starts in measurement mode and a connection with the instrument is established. Instrument power must be turned ON in advance and you must confirm that a connection can be established with the instrument (initialization of instrument settings is completed). NOTE When the RF-5300 series has been registered as the instrument to be used, this check box is disabled. Hint The RF-6000 series automatically performs initialization of settings when the power is turned ON, and initialization completes in about one minute when no problems are encountered. Reference "Measurement mode"
[View data file]	Select this mode to view an existing data file. The main window is displayed in file check mode. Reference "File check mode"

■Measurement mode

This mode is used to perform quantum yield measurement.
Measurement is performed according to the following steps.

Step	Window	Operation
		Select the method used to capture standard sample data and set the parameters required for quantum yield measurement.

Step 1	"Preparation window"	• When measuring a standard sample: Proceed to step 2. • When using standard sample data in an existing file: Proceed to step 5.
Step 2	"Preparation window"	Click [Start - Std] on the main toolbar.
Step 3	"Setting standard sample information"	Set the standard sample in the sample chamber and enter the sample information (such as sample name and quantum yield value) for the standard sample as well as the scan range.
Step 4	"Setting standard sample information"	Click [Measurement] to start measurement of the standard sample.
Step 5	"Setting unknown sample information"	Set the unknown sample in the sample chamber and enter the sample information (such as sample name and absorbance value) for the unknown sample as well as the scan range.
Step 6	"Setting unknown sample information"	Click [Measurement] to start measurement of the unknown sample.
Step 7	"Main window"	When performing an additional unknown sample measurement, click [Start - Unk] on the main toolbar and perform steps 5 and 6.

■File check mode

This mode is used to check the contents of saved quantum yield measurement data files. A connection cannot be established with the instrument in this mode.

In this mode, the main window appears displaying the standard table, unknown sample table, and spectrum data of each sample contained in the loaded file.

Editing of some of the table information of the open file is also possible.

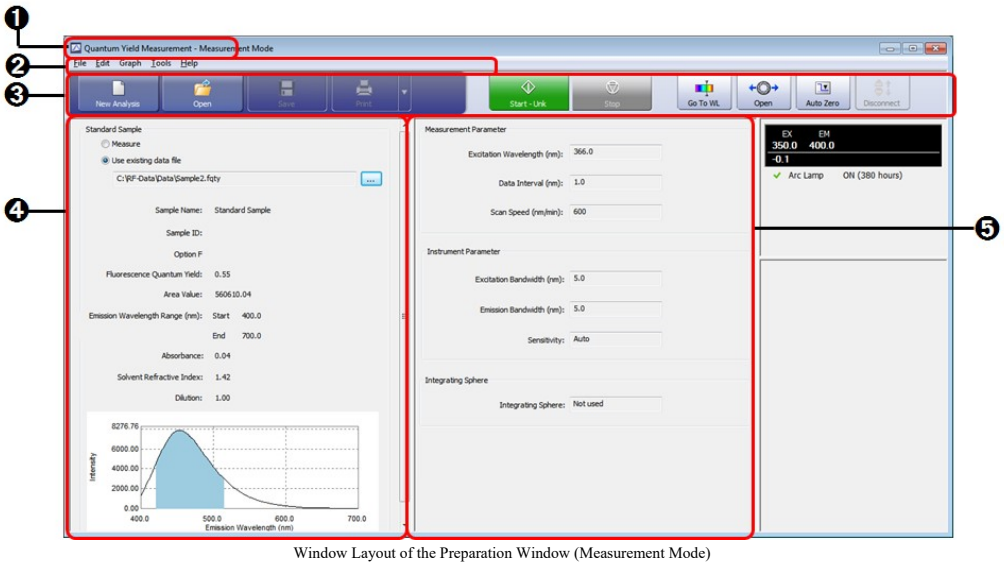
> **NOTE** Additional measurements of unknown samples cannot be performed.

10.2 Window Layout

■Preparation window

Select the method used to capture standard sample data and set the parameters required for quantum yield measurement.

This window is displayed first when starting in measurement mode.

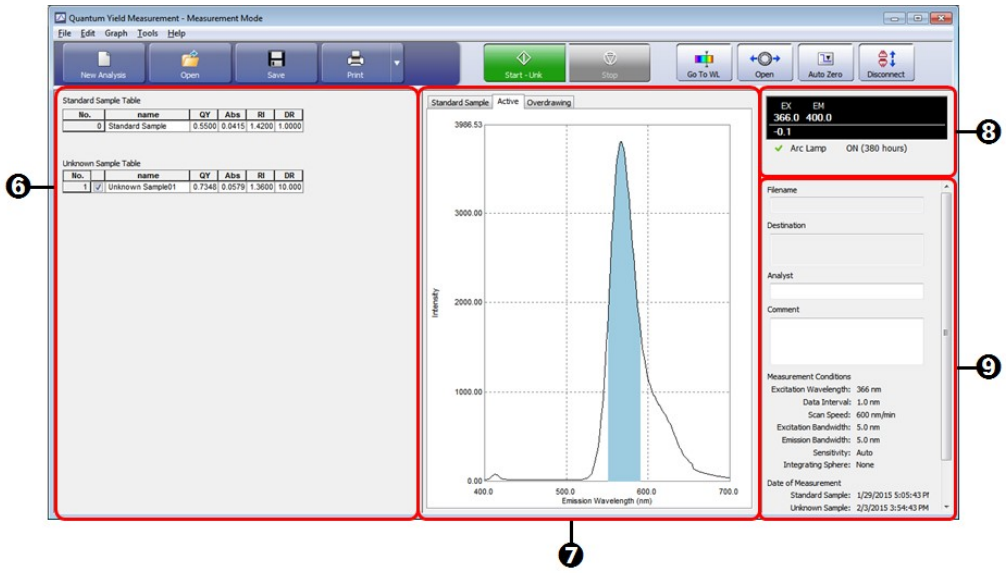


Window Layout of the Preparation Window (Measurement Mode)

■Main window

Check and edit data and perform measurement of unknown samples.

> **NOTE** Sample measurement is only possible when starting in measurement mode. Additional measurements cannot be performed with respect to existing files.



Window Layout of the Main Window (Measurement Mode)

No.	Name	Function
❶	Title bar	Displays the application name and window mode ([Measurement Mode] or [File Check Mode]).
❷	Menu bar	Displays the menus for quantum yield measurement.
❸	Main toolbar	Displays tool buttons for executing main functions, such as starting and stopping measurement, performing file operations, and printing. **NOTE** When starting in file check mode, the buttons for measurement and instrument control are disabled.
❹	Standard sample view	Select [Measure] to perform a new analysis of a standard sample or select [Use existing data file].
❺	Parameter view	Set the excitation wavelength of the standard sample as well as instrument parameters and measurement parameters common between standard and unknown samples when performing a new analysis.
❻	Analysis result view	Displays the results of the analysis in progress or the results loaded from existing measurement result data in the standard table and unknown sample table.
❼	Graph view	Plots the fluorescence spectrum undergoing analysis or the fluorescence spectrum loaded from existing measurement result data on the [Standard Sample] tab, [Active] tab, and [Overdrawing] tab.
❽	Istrument Status	The upper section displays the current wavelength and fluorescence intensity and the lower section displays the current status of the spectrofluorophotometer. **Reference** "2.7 Photometer Status"
❾	File information view	Displays information on the saved or loaded file. The analyst and comment information can be edited.

10.3 Menu Bar

10.3.1 [File] Menu

Command	Description
[New]	Close the loaded data (all measured values and calculation results) so that a new measurement can be started. If LabSolutions RF is disconnected from the instrument, a connection is established and the window is displayed in measurement mode.
[Open]	Open a data file (.fqty). Close the loaded data (all measured values and calculation results) and display the window in file check mode.
[Save]	Save by overwriting the currently open data file.
[Save As]	Save the currently open data file to a new file.
[Text File Output]	Output the spectrum data of the active sample (selected in the unknown sample table) to a text file.
[Print Preview]	Display a preview of printer output.
[Print]	Print a report file. The printing layout changes depending on the tab ([Standard Sample], [Active], or [Overdrawing]) displayed in the graph view. • [Standard Sample] or [Active] tab: Detailed report • [Overdrawing] tab: Summary report Reference "Printing layout"
[Exit]	Exit the quantum yield measurement application and close the window.

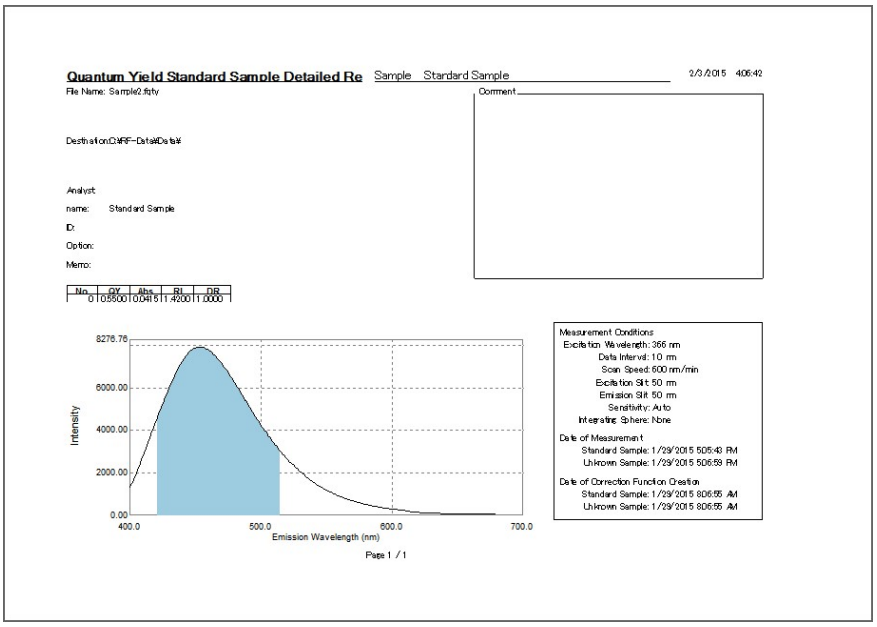
■Printing layout

The printing layout changes depending on the tab ([Standard Sample], [Active], or [Overdrawing] tab) displayed (selected) in the graph view.
The printing layout can be checked via [Print Preview] on the main toolbar or [Print Preview] on the [File] menu.

NOTE The printing layout cannot be edited.

[Standard Sample] tab

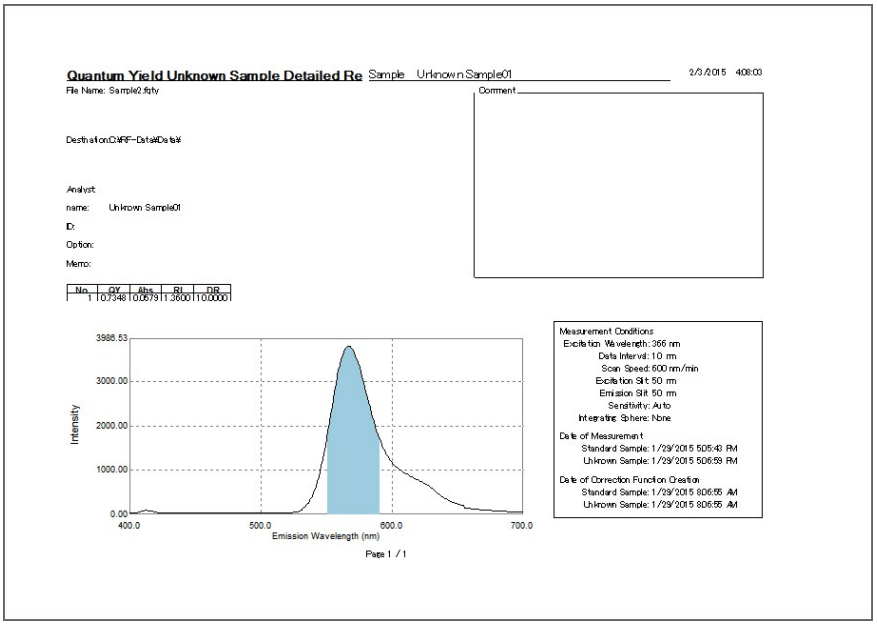
When this tab is selected, analysis results (fluorescence spectrum, measurement conditions, file information, and standard table information) of the standard sample are printed.



Example of a Printed Detailed Report (Standard Sample)

[Active] tab

When this tab is selected, analysis results (fluorescence spectrum, measurement conditions, file information, and unknown sample table information) of the sample selected (highlighted) in the unknown sample table are printed.

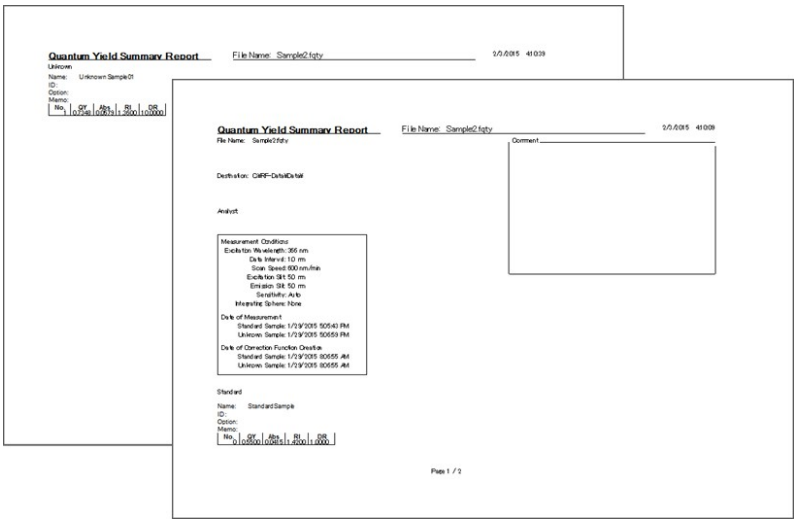


Example of a Printed Detailed Report (Unknown Sample)

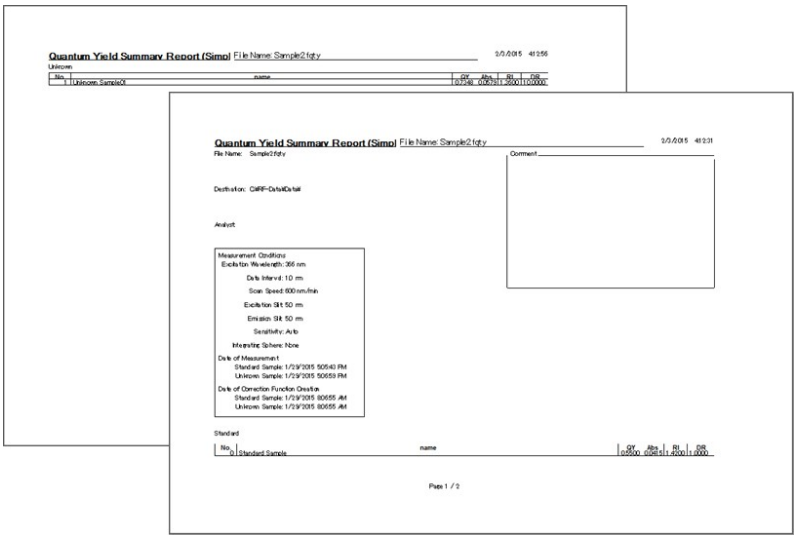
[Overdrawing] tab

When this tab is selected, all analysis results (measurement conditions, file information, standard table information, and unknown sample table information) for both the standard sample and unknown sample are printed.

The two provided printing layouts of [Print Table] and [Print Simple Table] can be selected via [Print Layout] on the [Tools] menu.



Example of a Printed Summary Report ([Print Table])



Example of a Printed Summary Report ([Print Simple Table])

10.3.2 [Edit] Menu

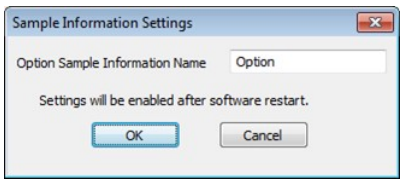
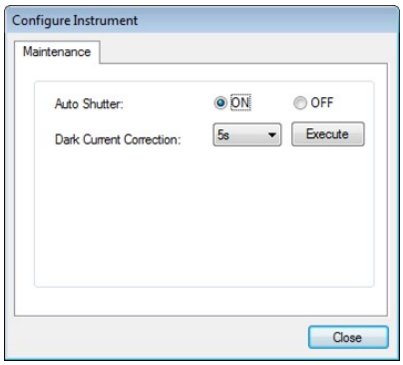
Command	Description
[Cut]	Move the selected content to the clipboard.
[Copy]	Copy the selected content to the clipboard.
[Paste]	Paste the item on the clipboard to the selected position.
[Select All]	Select all selectable items.

10.3.3 [Graph] Menu

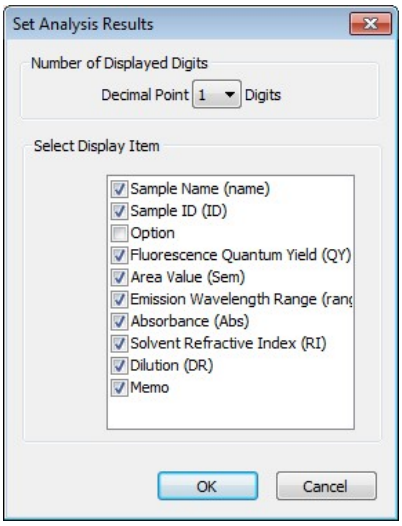
Command		Description
[Cursor]		Select the cursor type to display on the graph.
	None	A normal cursor is displayed (default).

[Crosshairs]	Displays a cursor with an intersecting vertical and horizontal line. The intersecting point is moved in the graph view using the mouse and the coordinates are displayed on both scales.
[Surfing]	Displays a cursor with an intersecting vertical and horizontal line. The intersecting point is moved across the spectrum graph using the mouse and the coordinates are displayed on both scales. This cannot be selected when the graph view is displaying the [Overdrawing] tab.
[Auto Scale]	Adjust the scale automatically based on the data.
[Graph Setting]	Set the display conditions of the graph in the [Graph User Setting] window. Reference "[Customize Graph] window"
[Display Area]	Display or hide the area calculation region on the graph.

10.3.4 [Tools] Menu

Command	Description
[Set Sample Information]	Set the option name of the sample information in the [Sample Information Settings] window.  [Sample Information Settings] Window
[Configure Instrument]	Configure settings related to the instrument in the [Configure Instrument] window. Reference "[Configure Instrument] window"  [Configure Instrument] Window
[Set Analysis Results]	Set the number of digits to display for fluorescence quantum yield, area value, absorbance, and solvent refractive index in the sample table and whether to show or hide columns in the standard sample grid and unknown sample grid. Reference "[Set Analysis Results] window"
[Recorrect]	Perform recorrection on the sample spectrum data in the standard table and unknown sample table using the current correction function. Reference "[Recorrect] window"
[Print Layout]	Select the printing layout of the summary report ([Print Table] or [Print Simple Table]). Reference "Printing layout"

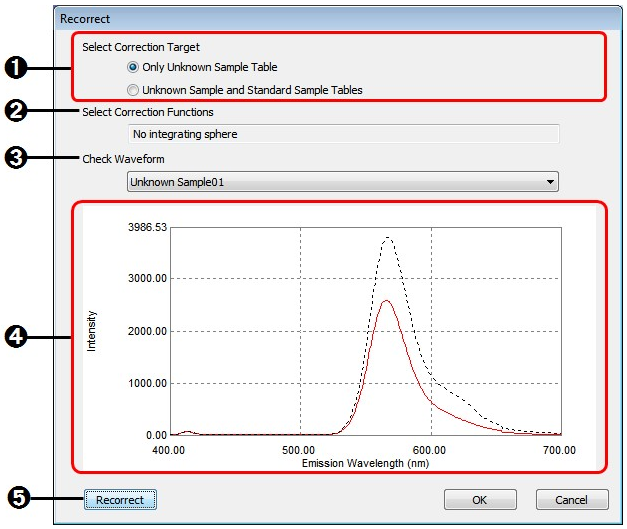
■[Set Analysis Results] window



[Set Analysis Results] Window

Item		Description
[Number of Displayed Digits]		Set the number of decimal places to a value from 0 to 4.
[Select Display Item]		Set the items to show or hide in the analysis results (standard table and unknown sample table).
	[Sample Name (name)]	Display sample names.
	[Sample ID (ID)]	Display sample IDs.
	[Option]	Display the names of options registered in the registry. Hint Set option names via [Set Sample Information] on the [Tools] menu. This setting applies to all applications.
	[Fluorescence Quantum Yield (QY)]	Display the quantum yield.
	[Area Value (Sem)]	Display the area value. Hint In measurement mode, the value calculated in data processing is displayed. In file check mode, the saved data value (with recalculation) is displayed.
	[Emission Wavelength Range (range)]	Display the emission wavelength range of "start wavelength - end wavelength".
	[Absorbance (Abs)]	Display the absorbance.
	[Solvent Refractive Index (RI)]	Display the refractive index of the solvent.
	[Dilution (DR)]	Display the dilution factor.
	[Memo]	Display a memo. (The default value is either empty or a saved data value.)
[OK]		Update the analysis results with the settings made and close the [Set Analysis Results] window.
[Cancel]		Cancel any settings made and close the [Set Analysis Results] window.

■[Recorrect] window

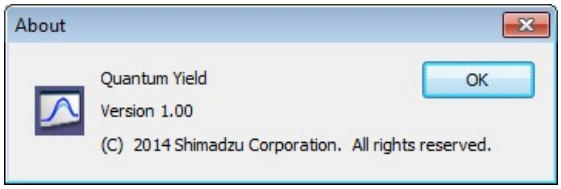


[Recorrect] Window

No.	Item		Description
❶	[Select Correction Target]		Select the target of processing.
		[Only Unknown Sample Table]	Perform recorrection on all spectra in the unknown sample table.
		[Unknown Sample and Standard Tables]	Perform recorrection on all spectra in the unknown sample table and standard table.
❷	[Select Correction Functions]		Select the correction function. The list displays the names of integration spheres for which measurement and saving of correction functions is complete in the order of registration.
❸	[Check Waveform]		Select the sample for waveform checking. The list items change according to the setting of ❶[Select Correction Target]. • When [Only Unknown Sample Table] is selected: Sample names in the unknown sample table are displayed. • When [Unknown Sample and Standard Tables] is selected: Sample names in the unknown sample table and standard table are displayed.
❹	Graph plotting area		If ❺[Recorrect] has not been clicked even once, the waveform of the selected sample after the current correction is plotted on the graph (black/dotted line). If ❺[Recorrect] is clicked, the waveform of the selected sample after the current correction (black/dotted line) and the waveform after recorrection (red/solid line) are plotted overlaid on the graph.
❺	[Recorrect]		Perform recorrection using the correction function selected by the user.
-	[OK]		Displays a confirmation dialog box. • Click [Yes] to overwrite the correction data in memory and close the [Reconnect] window. • Click [No] to return to the state before clicking [OK].
-	[Cancel]		Cancel any settings made and close the [Recorrect] window.

10.3.5 [Help] Menu

Command	Description
[Help]	Display the help top page.
	Display version information of the quantum yield measurement software.

[About]	 [About] Window

10.4 Main Toolbar

Main Toolbar (Function Operation Area)

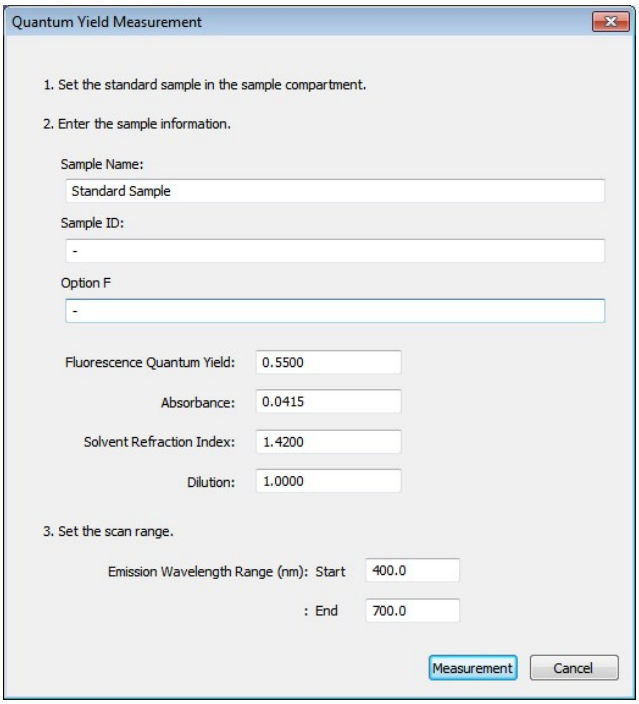
Main Toolbar (Instrument Control Area)

Item	Description
[New Analysis]	Close the loaded data (all measured values and calculation results) so that a new measurement can be started. If LabSolutions RF is disconnected from the instrument, a connection is established and the window is displayed in measurement mode.
[Open]	Open a data file (.fqty). Close the loaded data (all measured values and calculation results) and display the window in file check mode.
[Save]	Save by overwriting the currently open data file.
[Print] or [Print Preview]	It is possible to switch between [Print] and [Print Preview]. • [Print]: Print the printing layout linked to the tab ([Standard Sample], [Active], or [Overdrawing]) displayed in the graph view. • [Print Preview]: Display a preview of printer output. Reference "Printing layout"
[Start - Std]	Display the [Quantum Yield Measurement] window, enter parameters, and click [Measurement] to start standard sample measurement. This is displayed in measurement mode when the standard sample data to use is set to [Measure]. Reference "10.4.1 Setting Sample Information"
[Start - Unk]	Display the [Quantum Yield Measurement] window, enter parameters, and click [Measurement] to start unknown sample measurement. This is displayed when standard sample data already exists. Reference "10.4.1 Setting Sample Information"
[Stop]	Stop measurement. This is only available during measurement.
[Go To WL]	Display the [Wavelength Setting] window and move the excitation wavelength and emission wavelength. Reference "2.6.1 [Wavelength setting] Window"
[Open]/[Close] (shutter)	Open or close the shutter. The button shows [Open] when the shutter is closed and [Close] when the shutter is open.
[Auto Zero]	Set the fluorescence intensity to zero in the current state (wavelengths, instrument parameters, shutter open/close, etc.).
[Connect]/	Connect to or disconnect from the instrument.

[Disconnect]	NOTE Instrument connection and disconnection can only be performed in measurement mode.

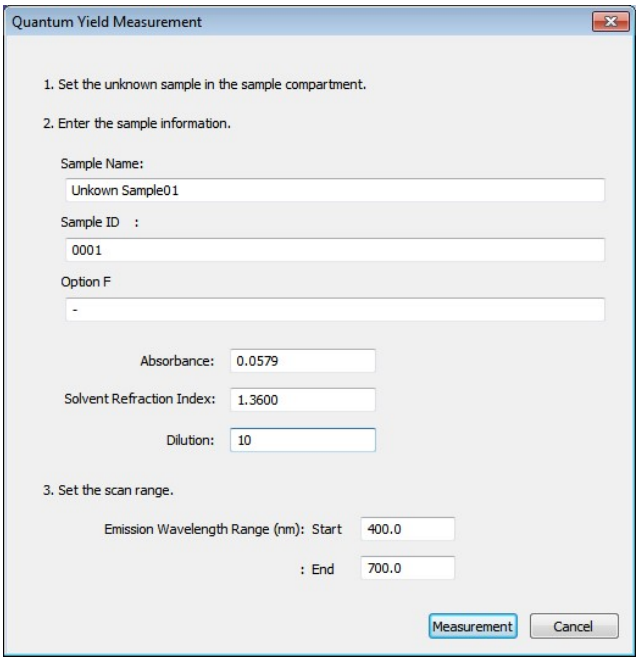
10.4.1 Setting Sample Information

■Setting standard sample information



[Quantum Yield Measurement] Window (Setting Standard Sample Information)

■Setting unknown sample information



[Quantum Yield Measurement] Window (Setting Unknown Sample Information)

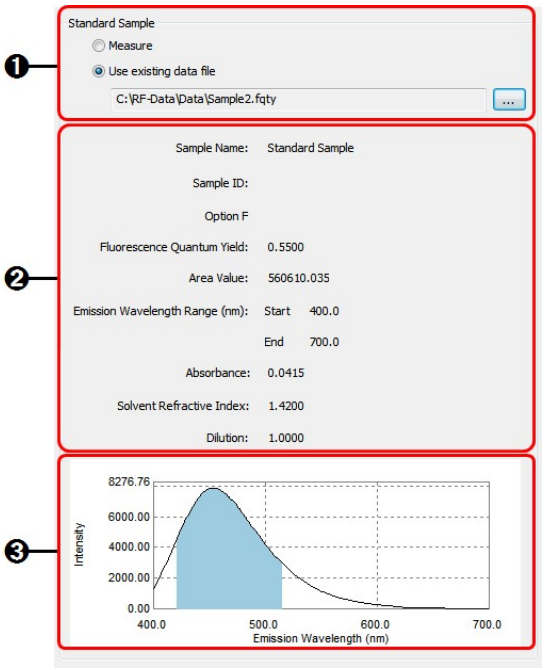
Item		Description
[Sample Name]		Enter the sample name.
[Sample ID]		Enter the sample ID.
[Option]		Enter option information.
[Fluorescence Quantum Yield]		Only displayed for standard samples. Enter the quantum yield. Settable range: 0.0001 to 10 (a value up to four decimal places can be entered)
[Absorbance]		Enter the absorbance for the excitation wavelength. Settable range: 0.0001 to 10 (a value up to four decimal places can be entered)
[Solvent Refraction Index]		Enter the refractive index of the solvent. Settable range: 0.0001 to 10 (a value up to four decimal places can be entered)
[Dilution]		Enter the dilution factor. Settable range: 0.0001 to 10000 (a value up to four decimal places can be entered)
[Emission Wavelength Range (nm)]		Set the wavelength range used to measure the fluorescence spectrum.
	[Start]	Enter the start wavelength for emission wavelength measurement. Settable range: 200.0 to 900.0 (to one decimal place) Hint The same wavelength entered for the excitation wavelength is set as the default.
	[End]	Enter the end wavelength for emission wavelength measurement. Settable range: 200.0 to 900.0 (to one decimal place)
[Measurement]		Check the entered information and start sample measurement. If there is a problem with any of the entered information, an error message is displayed.
[Cancel]		Cancel the settings made and close the window.

10.5 Standard Sample View

Select the method for capturing standard sample data to use in a new analysis.
This view is displayed in the following cases:

- When [Measure] is selected in the quantum yield mode selection window
- When [New] is selected on the [File] menu

- When [New Analysis] is selected on the main toolbar



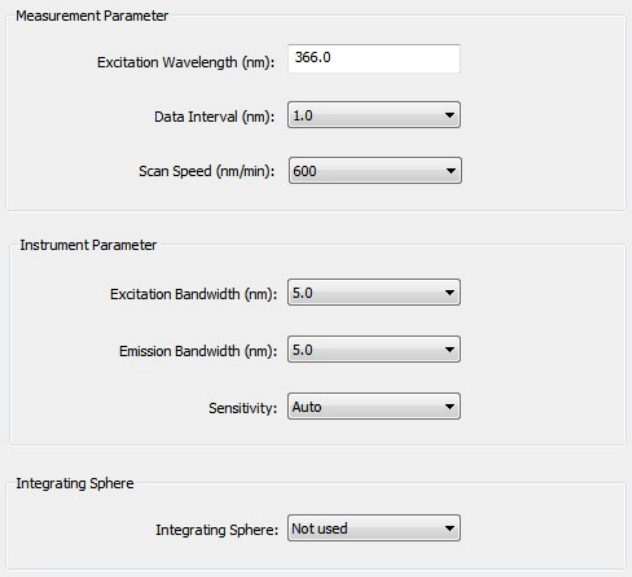
Standard Sample View

No.	Item		Description
❶	Standard sample selection		Select the method for capturing standard sample data.
		[Measure]	Measure a standard sample. • [Start - Std] is displayed on the main toolbar. • Default values are displayed in the analysis parameter view.
		[Use existing data file]	Click ... to open an existing quantum yield data file (.fqty). Standard sample information is loaded from the opened data file. [Start - Unk] is displayed on the main toolbar.
❷	Sample conditions		If [Use existing data file] is selected for ❶[Standard sample selection], the sample information (including analysis parameters) of the loaded standard sample data is displayed. • [Sample Name] • [Sample ID] • [Option] • [Fluorescence Quantum Yield] • [Area Value] • [Emission Wavelength Range (nm)] • [Absorbance] • [Solvent Refractive Index] • [Dilution]
❸	Spectrum		If [Use existing data file] is selected for ❶[Standard sample selection], the fluorescence spectrum of the loaded standard sample data is displayed on a graph. The region that underwent area calculation is displayed on the fluorescence spectrum graph.

10.6 Parameter View

Set the parameters required to perform quantum yield measurement.

If [Use existing data file] is selected in the standard sample view, the values loaded from the file are displayed.



Parameter View

Item		Description
[Measurement Parameter]		
	[Excitation Wavelength (nm)]	Enter the excitation wavelength value. Settable range: 200.0 to 900.0 (to one decimal place)
	[Data Interval (nm)]	Select the sampling interval. Selection options: 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10
	[Scan Speed (nm/min)]	Select the scan speed. The speeds that can be selected change depending on the lamp type and data interval.
[Instrument Parameter]		
	[Light Source]	Select the light source. • [Xenon Arc Lamp] • [Xenon Flash Lamp] **Hint** A light source that is not installed on the instrument is grayed out.
	[Excitation Bandwidth (nm)]	Set the spectral bandwidth of the excitation side monochromator. Selection options: 1.5, 3.0, 5.0, 10.0, 15.0, 20.0
	[Emission Bandwidth (nm)]	Set the spectral bandwidth of the emission side monochromator. Selection options: 1.0, 3.0, 5.0, 10.0, 15.0, 20.0
	[Sensitivity]	Select the sensitivity. Selection options: Auto, Low, High
[Integrating Sphere]		Select an integrating sphere. Selection options: Not used, registered integrating sphere name (in order of registration) **NOTE** Integrating spheres for which a dedicated spectrum correction function has not been created cannot be selected. Use the spectrum correction function measurement tool to create any required correction functions in advance. **Hint** This section is only displayed when integrating spheres are registered.

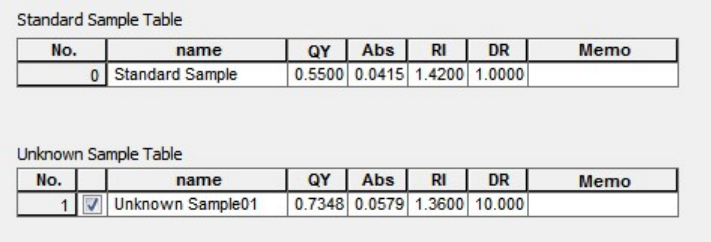
10.7 Analysis Result View

The analysis results are displayed in the standard table and unknown sample table.

After measurement, the spectrum of the sample selected in the unknown sample table is displayed on the [Active] tab of the graph view.

Setting the number of decimal places and displaying or hiding of columns is performed in the [Set Analysis Results] window.

Reference "[Set Analysis Results] window"



Analysis Result View (Standard Table, Unknown Sample Table)

Item	Description
[No.]	The standard table displays "No. 0" (one row only). The item whose checkbox is selected in the unknown sample table is displayed overlaid on the graph of the [Overdrawing] tab.
Display items	The display items set in the [Set Analysis Results] window are shown. Hint The [name], [ID], [Option], and [Memo] items can be edited at any time. (However, they cannot be edited when an existing data file is specified for the standard sample.)

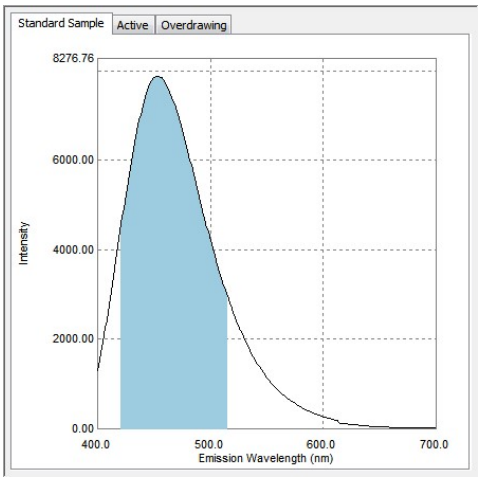
10.8 Graph View

The graph view comprises the three tabs of [Standard Sample], [Active], and [Overdrawing].

■[Standard Sample] tab

This tab displays a fluorescence spectrum graph of the standard sample and the area calculation range.

Hint Click [Display Area] on the [Graph] menu to display or hide the region on which area calculation was performed on the fluorescence spectrum graph.

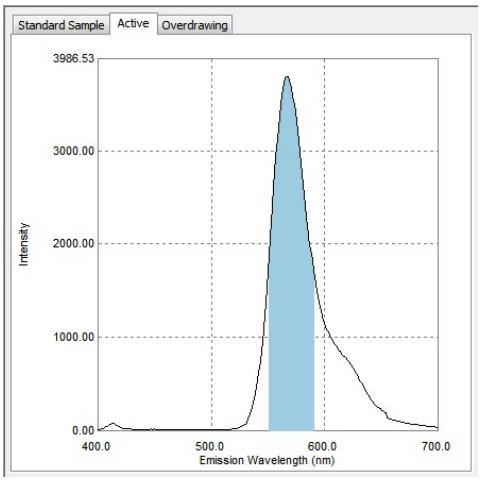


Fluorescence Spectrum Graph of a Standard Sample

■[Active] tab

This tab displays a fluorescence spectrum graph of the sample selected in the unknown sample table and the area calculation range

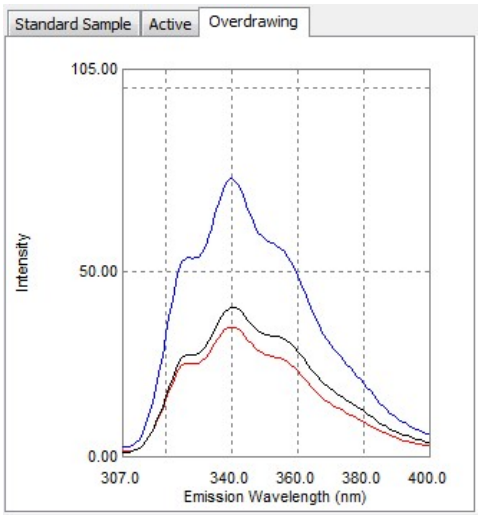
Hint Click [Display Area] on the [Graph] menu to display or hide the region on which area calculation was performed on the fluorescence spectrum graph.



Fluorescence Spectrum Graph of an Unknown Sample

■[Overdrawing] tab

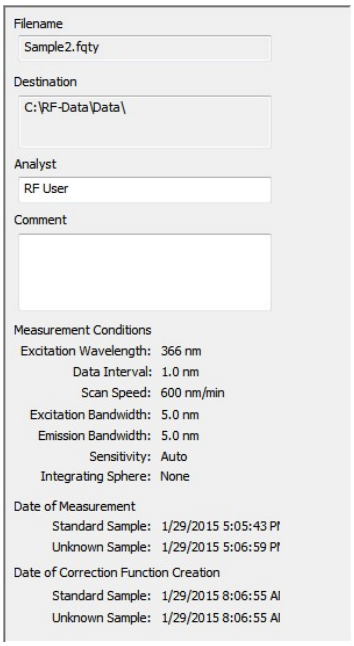
This tab displays overlaid fluorescence spectrum graphs of the samples whose checkbox is selected in the unknown sample table.



Overlaid Display

10.9 File Information View

This view displays information including the filename, save destination, and measurement conditions of data files for fluorescence quantum yield measurement results.



File Information View

Item	Description
[Filename]	Displays the filename of the displayed analysis results.
[Destination]	Displays the file path of the displayed analysis results.
[Analyst]	The analyst name saved in the data file can be edited.
[Comment]	The comment saved in the data file can be edited.
[Measurement Conditions]	Displays the measurement conditions saved in the data file.
[Date of Measurement]	Displays the measurement date and time of the standard sample and unknown samples. Hint The measurement date and time of the first unknown sample is displayed .
[Date of Correction Function Creation]	Displays the creation date and time of the correction function used when capturing data of the standard sample and unknown samples.

10.10 Calculation Algorithm of the Quantum Yield Calculation Program

The ratio between the number of photons emitted as fluorescent light from samples and the number of photons absorbed by samples is generally used as an index for evaluating the luminous efficiency of samples. This ratio is referred to as "quantum yield" in the quantum yield calculation program.

There exists a relationship between the number of photons emitted as fluorescent light and the area of the fluorescence spectrum. By comparing the fluorescence spectrum area of an unknown sample of an unknown quantum yield to that of a standard sample with a known quantum yield, the quantum yield calculation program can relatively calculate the quantum yield of the unknown sample.

The actual calculation procedure is described below.

Step 1) Wavenumber transformation of the fluorescence spectrum

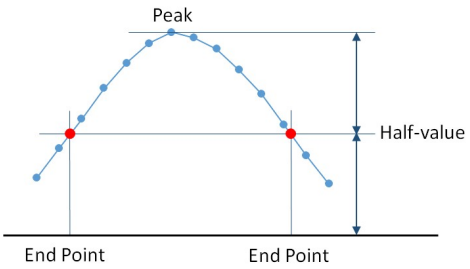
The horizontal axis of the fluorescence spectrum captured in measurement represents the wavelength λ and the vertical axis represents the energy intensity E. In this step, the waveform is transformed such that the horizontal axis becomes $1/\lambda$ and the vertical axis becomes $E \times \lambda$. For details on the reasoning behind this transformation, see "Note: Wavenumber transformation in area calculation".

Step 2) Peak detection

The peak position is searched for in the waveform resulting from wavenumber transformation.

Step 3) Half-value width calculation

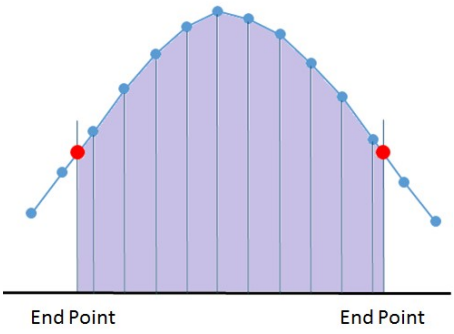
The intensity on the left and right of the central peak position is searched on the waveform resulting from wavenumber transformation to determine the position of the half peak intensity (half-value). In practice, on each side of the peak, the two data points immediately above and below the intensity half-value are interpolated with a straight line and the two points of intersection between this line and the peak (on the left and right of the peak at the actual half-value positions) are designated as the end points of the region used in area calculation.



Harf-value width

Step 4) Area calculation

The area between the two end points determined in step 3 is calculated using the trapezoidal rule.



Area calculation

Step 5) Quantum yield calculation

The quantum yield Φ is calculated according to the following formula.

$$\Phi = \Phi_s \times \frac{F \times A_s \times n^2 \times D}{F_s \times A \times n_s^2 \times D_s} \quad \text{----- (Formula 1)}$$

- Φ : Quantum yield of the unknown sample
- Φ_s : (Known) quantum yield of the standard sample
- F : Peak area of the unknown sample (calculated from the fluorescence spectrum)
- F_s : Peak area of the standard sample (calculated from the fluorescence spectrum)
- A : (Known) absorbance of the unknown sample at the excitation wavelength
- A_s : (Known) absorbance of the standard sample at the excitation wavelength
- n : (Known) refractive index of the unknown sample
- n_s : (Known) refractive index of the standard sample
- D : (Known) dilution factor of the unknown sample
- D_s : (Known) dilution factor of the standard sample

Note: Wavenumber transformation in area calculation

The energy $e(\lambda)$ of a single photon at wavelength λ is expressed using wavelength λ, blank constant h, and speed of light c in the following formula.

$$e(\lambda)=\frac{hc}{\lambda}\propto\frac{1}{\lambda} \quad \text{----- (Formula 2)}$$

If n represents the number of photons at wavelength λ, the following formula expresses the total light energy $E(\lambda)$ at wavelength λ.

$$E(\lambda)=n\times e(\lambda) \quad \text{----- (Formula 3)}$$

The following relationship can be derived from formula 2 and formula 3.

$$E(\lambda)\propto\frac{n}{\lambda} \quad \text{----- (Formula 4)}$$

Modifying formula 4 further obtains the following relationship.

$$n\propto E(\lambda)\times\lambda \quad \text{----- (Formula 5)}$$

The use of fluorescence spectrum area instead of handling the number of photons directly in the calculation of quantum yield and quantum efficiency (formula 1) is due to the assumption that the number of photons and area are proportional. Because the fluorescence spectrum captured in measurement comprises wavelengths on the horizontal axis and the energy intensity of light for each wavelength on the vertical axis, a value proportional to the number of photons cannot be obtained by simply determining the area of this waveform.

For this reason, formula 5 is applied to the vertical axis of the waveform captured in measurement to multiply the energy intensity $E(\lambda)$ by λ to transform vertical axis values into values proportional to the number of photons. Moreover, by reciprocating wavelengths λ on the horizontal axis, the distribution of photons with individual energy values is correctly expressed and this allows the number of photons to be correctly calculated using area calculation.