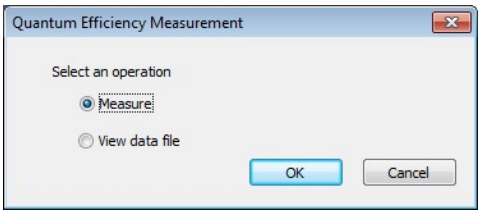
11 Quantum Efficiency Application

11.1 Mode Selection

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



Quantum Efficiency 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 or no integrating sphere has been registered, 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 efficiency measurement.
Measurement is performed according to the following steps.

Step	Window	Operation
Step 1	"Preparation window"	Set the parameters required to perform quantum efficiency measurement.
Step 2	"Preparation window"	Click [Start] on the main toolbar.

Step 3	"Starting blank measurement"	Check that an optional integrating sphere is installed in the sample chamber and that no sample is set.
Step 4	"Starting blank measurement"	Click [Measurement] to start blank spectrum measurement.
Step 5	"Setting sample information"	Set the unknown sample in the sample chamber and enter the sample information (such as sample name and sample ID).
Step 6	"Setting sample information"	Click [Measurement] to start measurement of the unknown sample.
Step 7	"Main window"	When performing an additional sample measurement, click [Start] on the main toolbar and perform steps 3 to 6 or steps 5 and 6.

■File check mode

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

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

Editing of some of the table information (including the area calculation range) is also possible.

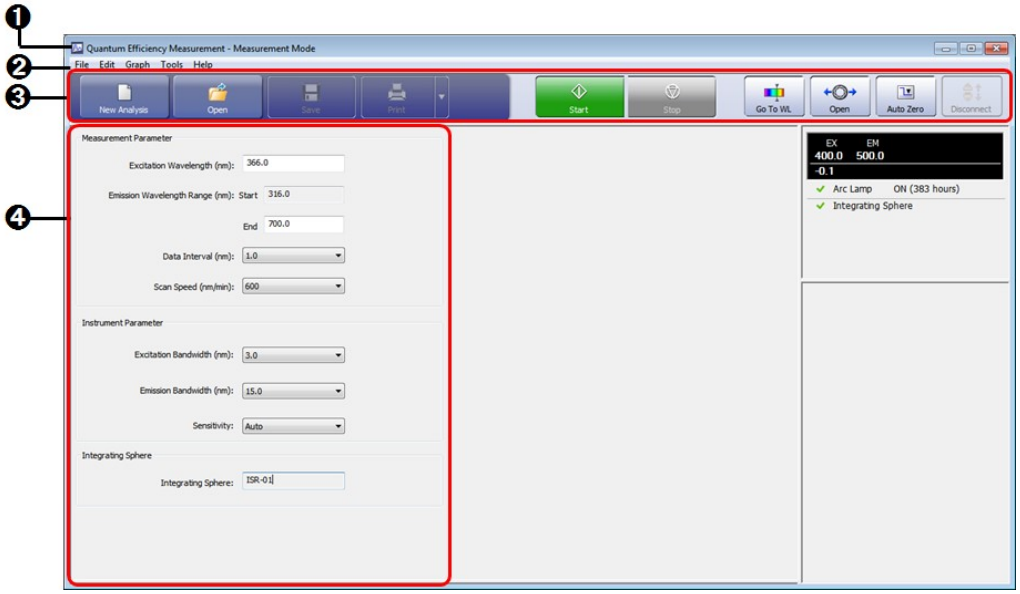
> **NOTE** Measurement of additional samples cannot be performed.

11.2 Window Layout

■Preparation window

Set the parameters required to perform quantum efficiency measurement.

This window is displayed first when starting in measurement mode.

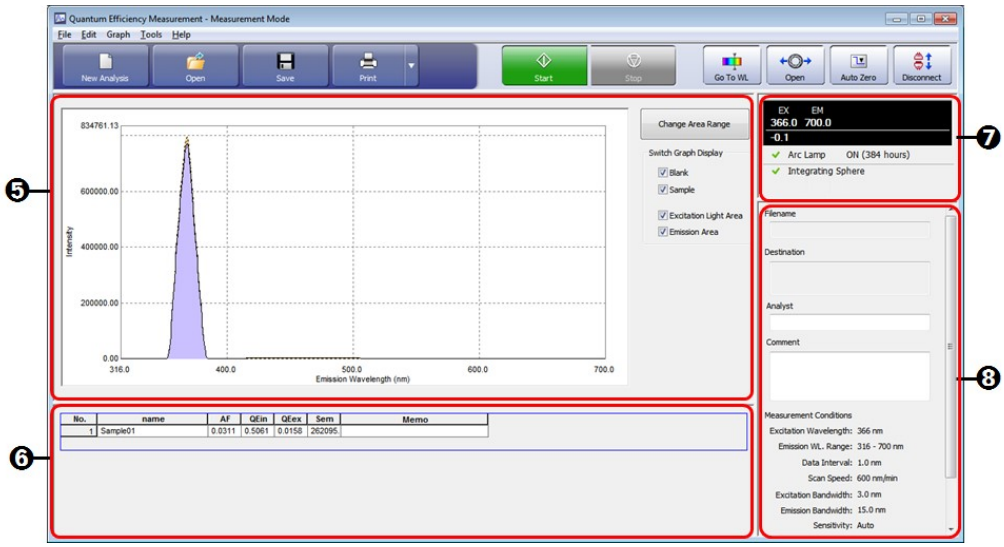


Window Layout of the Preparation Window (Measurement Mode)

■Main window

Check and edit data and perform sample measurement.

> **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 efficiency 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	Parameter view	Set or display the measurement parameters, instrument parameters, and integrating sphere when performing a new analysis.
❺	Graph view	Plots the fluorescence spectrum undergoing analysis or the fluorescence spectrum loaded from existing measurement result data. Clicking displays a blue frame and deselects the analysis result view.
❻	Analysis result view	Displays the results of the analysis in progress or the results loaded from existing measurement result data in the sample table. Clicking displays a blue frame and deselects the graph view.
❼	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"
❽	File information view	Displays information on the saved or loaded file. The analyst and comment information can be edited.

11.3 Menu Bar

11.3.1 [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). The loaded data (all measured values and calculation results) is closed and the window is displayed 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 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 whether printing was selected in the analysis result view or graph view. • When selected in the analysis result view (sample table in blue frame): Summary report • When selected in the graph view (graph in blue frame): Detailed report Reference "Printing layout"
[Exit]	Exit the quantum efficiency measurement application and close the window.

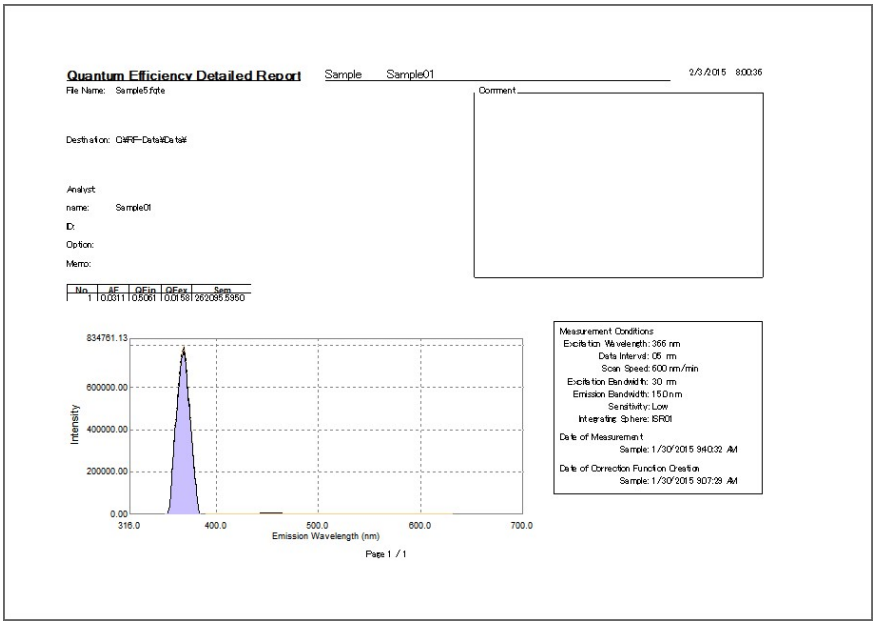
■Printing layout

In file check mode, the printing layout changes depending on the view in which printing was selected (analysis result view or 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.

Graph view

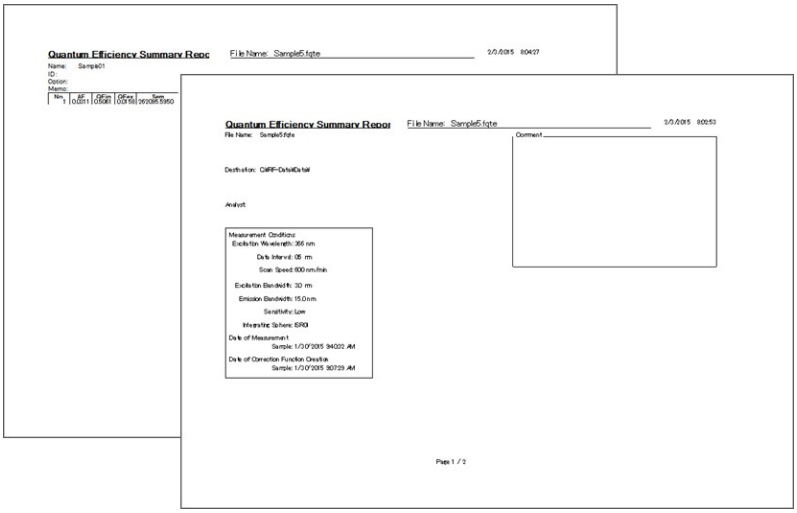
In this view, analysis results (fluorescence spectrum, measurement conditions, file information, and standard table information) of the sample selected (highlighted) in the sample table are printed.



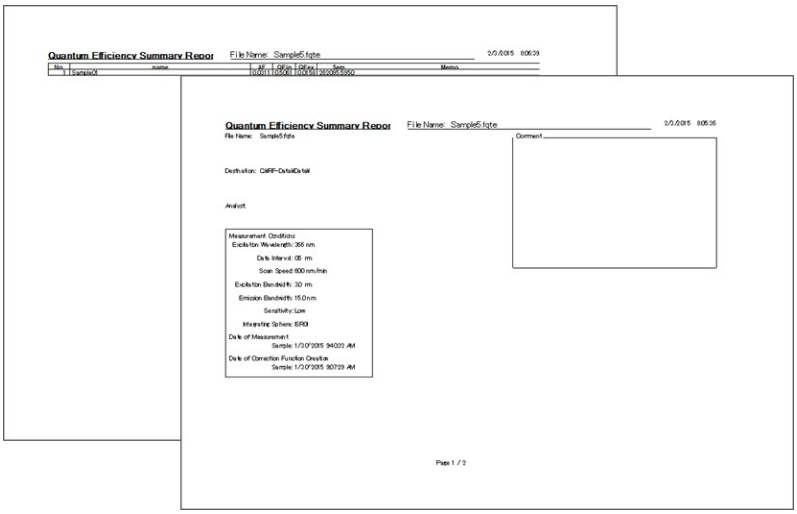
Example of a Printed Detailed Report

Analysis result view

In this view, all analysis results (measurement conditions, file information, and sample table information) 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])

11.3.2 [Edit] Menu

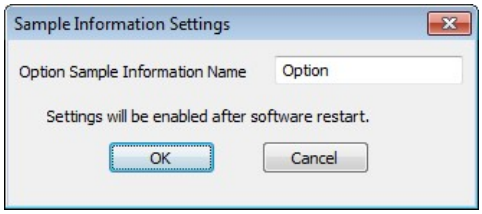
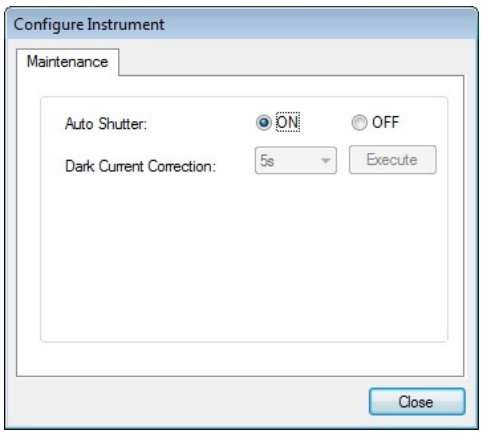
Command	Description
[Cut]	Move the selected content to the clipboard.
[Copy]	Copy the selected content to the clipboard.
[Paste]	Paste the item on the clipboard to the selected position.
[Select All]	Select all selectable items.

11.3.3 [Graph] Menu

Command	Description

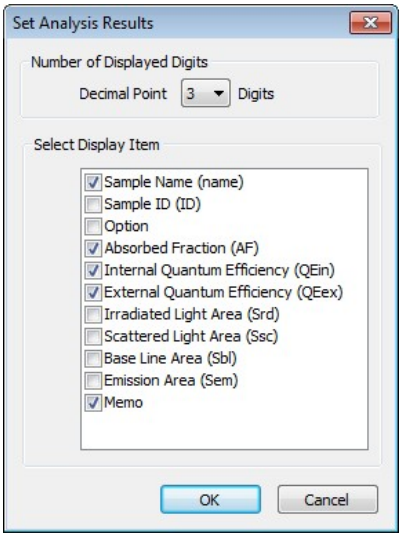
[Cursor]		Select the cursor type to display on the graph.
	None	A normal cursor is displayed (default).
	[Crosshairs]	Displays a cursor with an intersecting vertical and horizontal line. The intersecting point is moved in the graph view using the mouse and the coordinates are displayed on both scales.
	[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 Excitation Light Area]		Display or hide the excitation light area for each sample.
[Display Emission Area]		Display or hide the emission light area for each sample.

11.3.4 [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" NOTE The arc lamp cannot be turned ON or OFF in quantum efficiency measurement.  [Configure Instrument] Window
[Set Analysis Results]	Set the number of digits to display for absorbance, internal quantum efficiency, external quantum efficiency, irradiation area, sample scattered light area, and emission light area in the analysis result view and whether to show or hide columns in the sample table grid. Reference "[Set Analysis Results] window"

[Recorrect]	Perform recorrection on the raw data of the sample in the analysis results 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"

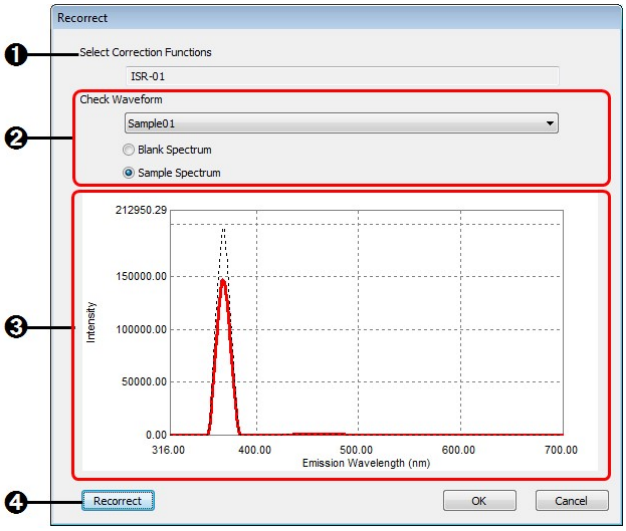
■[Set Analysis Results] window



[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 (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.
	[Absorbed Fraction (AF)]	Display the absorbance ((Srd - Ssc) / Srd).
	[Internal Quantum Efficiency (QEin)]	Display the internal quantum efficiency ((Sem - Sbl) / (Srd - Ssc)).
	[External Quantum Efficiency (QEex)]	Display the external quantum efficiency ((Sem - Sbl) / Srd).
	[Irradiated Light Area (Srd)]	Display the irradiation area (area within the excitation wavelength range of the blank spectrum).
	[Scattered Light Area (Ssc)]	Display the scattered light area (area within the excitation wavelength range of the sample spectrum).
	[Base line Area (Sbl)]	Display the baseline area (area within the emission wavelength range of the blank spectrum).
	[Emission Area (Sem)]	Display the emission light area (area within the emission wavelength range of the sample spectrum).
	[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**

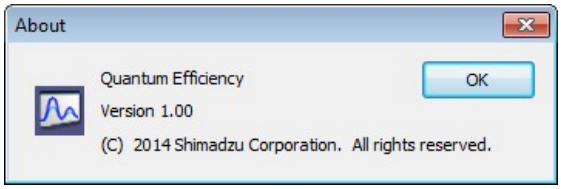


[Recorrect] Window

No.	Item	Description
❶	[Select Correction 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.
❷	[Check Waveform]	Select the sample for waveform checking.
	[Blank Spectrum]	Plot the blank spectrum waveform of the sample selected for [Check Waveform] in the graph plotting area.
	[Sample Spectrum]	Plot the sample spectrum waveform of the sample selected for [Check Waveform] in the graph plotting area.
❸	Graph plotting area	If ❹[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 ❹[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.
❹	[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.

11.3.5 [Help] Menu

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



[About] Window

11.4 Main Toolbar

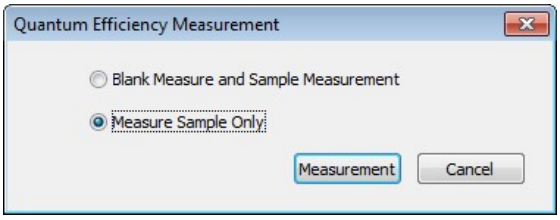
Main Toolbar (Function Operation Area)

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]	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"
[Print Preview]	Display a preview of printer output.
[Start]	Display the [Quantum Efficiency Measurement] window and start measurement. Reference "11.4.1 Selecting Quantum Efficiency Measurement"
[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.).
[Disconnect]	Disconnect from the instrument. Hint Connection to the instrument only occurs when selecting [Measure] at startup or selecting [New Analysis].

- 11.4.1 Selecting Quantum Efficiency Measurement

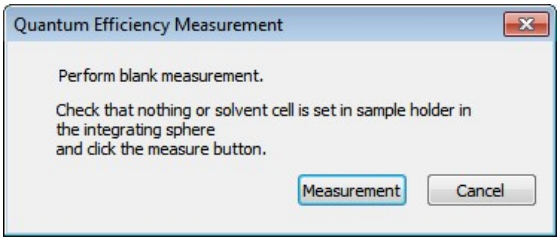
11.4.1 Selecting Quantum Efficiency Measurement



Quantum Efficiency Measurement Selection Window

Item	Description
[Blank Measure and Sample Measurement]	Select this option to perform both blank measurement and sample measurement.
[Measure Sample Only]	Select this option to only perform sample measurement.
[OK]	• When [Blank Measure and Sample Measurement] is selected, clicking [Start] on the main toolbar will display a confirmation message asking to make sure that the sample compartment is empty. Reference "Starting blank measurement" • When [Measure Sample Only] is selected, the sample information settings window is displayed. Reference "Setting sample information"
[Cancel]	Cancel any settings made and close the [Quantum Efficiency Measurement] window.

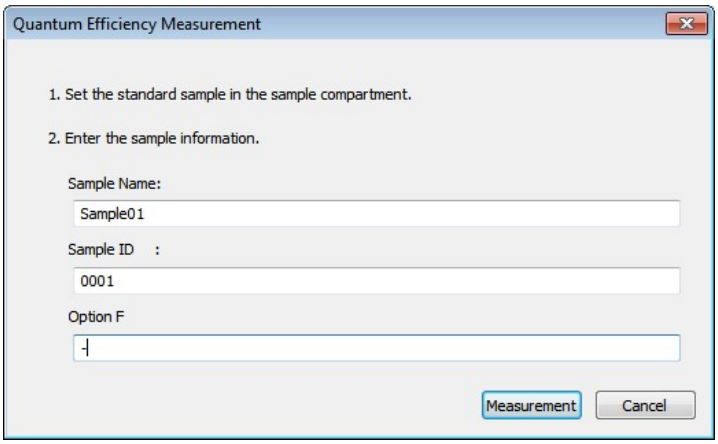
■Starting blank measurement



Starting Blank Measurement

Item	Description
[Measurement]	Start blank measurement.
[Cancel]	Cancel blank measurement and close the window.

■Setting sample information

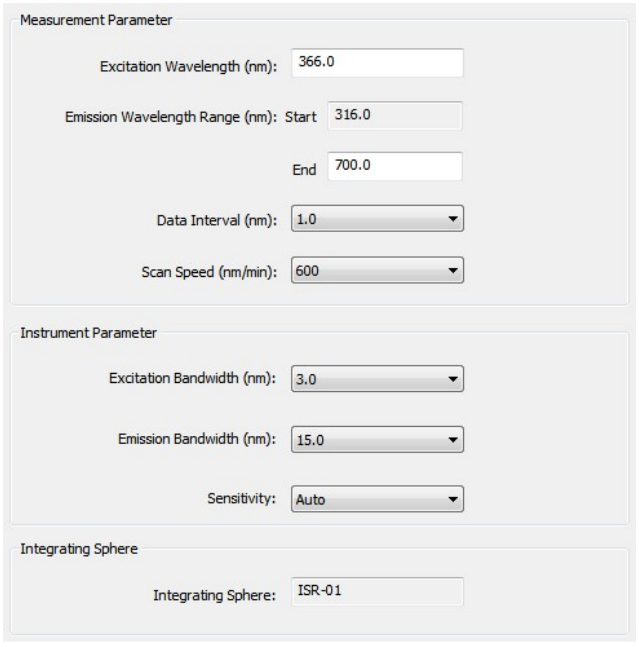


Setting Sample Information

Item	Description
[Sample Name]	Enter the sample name.
[Sample ID]	Enter the sample ID.
[Option]	Enter option information.
[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 any settings made and close the window.

11.5 Parameter View

Set the parameters required to perform quantum efficiency measurement.



Parameter View

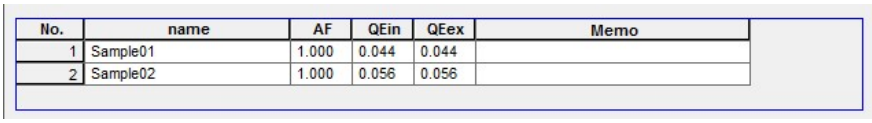
Item		Description
[Measurement Parameter]		
	[Excitation Wavelength (nm)]	Enter the excitation wavelength value. Settable range: 250.0 to 800.0 (to one decimal place)
	[Emission Wavelength Range (nm)]	• [Start]: Displays the emission wavelength at which measurement starts The displayed value changes according to the value of [Excitation Wavelength (nm)]. • [End]: Enter the end wavelength for emission wavelength measurement. Settable range: (start wavelength + 100) 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]		
	[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) Hint This section is only displayed when integrating spheres are registered.

11.6 Analysis Result View

Analysis results are displayed in the sample table.

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"



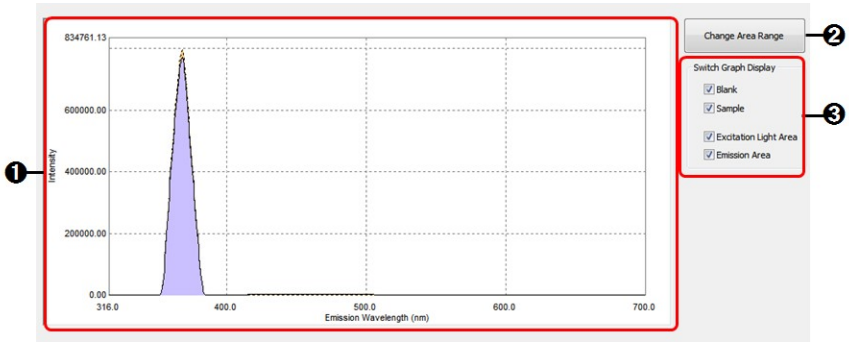
No.	name	AF	QEin	QEex	Memo
1	Sample01	1.000	0.044	0.044	
2	Sample02	1.000	0.056	0.056	

Analysis Result View

Item	Description
[No.]	Displays numbers in the order of measurement.
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.

11.7 Graph View

The blank spectrum and sample spectrum of the sample selected from the sample table in the analysis result view are displayed overlaid.

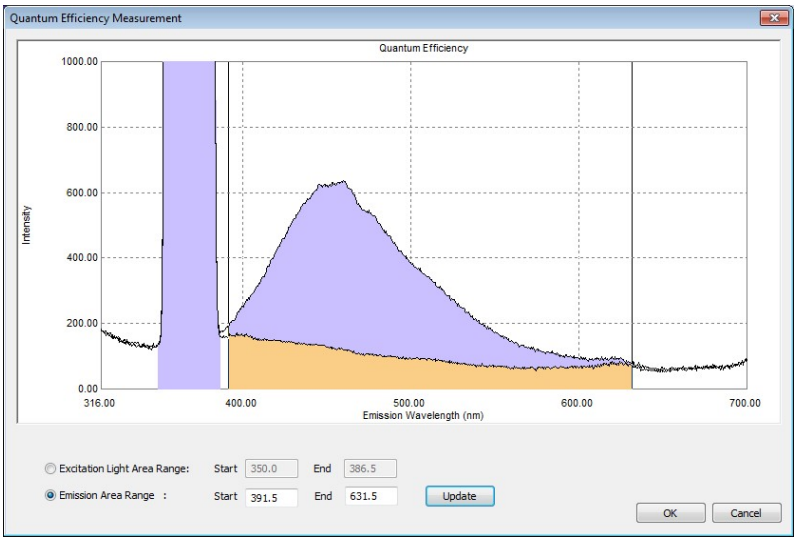


Graph View

No.	Item		Description
❶	Graph plotting area		Graphs are plotted according to the ❸[Switch Graph Display] checkboxes.
❷	[Change Area Range]		Display the window for changing the area range. This window allows the excitation light area range and emission light area range to be changed. Reference "11.7.1 Changing the Area Range"
❸	[Switch Graph Display]		
		[Blank]	Select this checkbox to display the blank spectrum in the graph plotting area.
		[Sample]	Select this checkbox to display the sample spectrum in the graph plotting area.
		[Excitation Light Area]	Check this checkbox to fill the excitation light area with light blue. Hint • This setting is linked to [Display Excitation Light Area] on the [Graph] menu. • This is only available when both the [Blank] and [Sample] checkboxes are selected.
		[Emission Area]	Check this checkbox to fill the emission light area with pink. Hint • This setting is linked to [Display Emission Area] on the [Graph] menu. • This is only available when both the [Blank] and [Sample] checkboxes are selected.

- 11.7.1 Changing the Area Range

11.7.1 Changing the Area Range

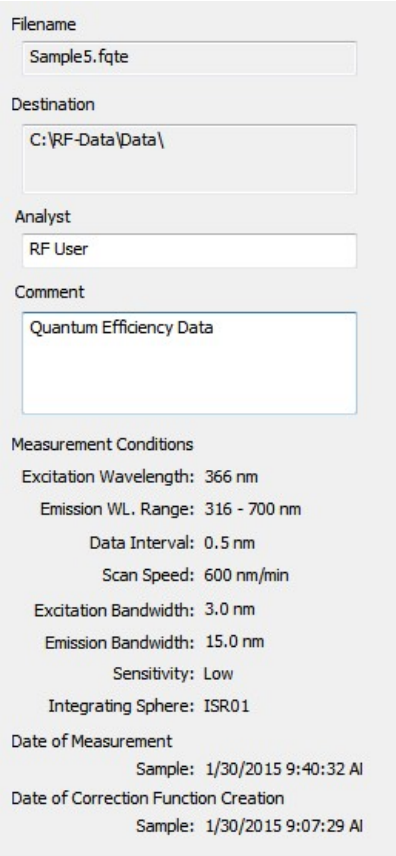


Changing the Area Range

Item		Description
Graph plotting area		The excitation light spectrum of the blank state and the spectrum of scattered light from the sample and fluorescence are plotted overlaid in this area.
[Excitation Light Area Range]		Set the range (wavelength range) of the excitation light area of the blank and sample spectra to use in calculation.
	[Start]	Set the start wavelength. Hint This value can also be set by dragging the vertical line for the start wavelength on the graph.
	[End]	Set the end wavelength. Hint This value can also be set by dragging the vertical line for the end wavelength on the graph.
[Emission Area Range]		Set the range (wavelength range) of the emission light area of the blank and sample spectra to use in calculation.
	[Start]	Set the start wavelength. Hint This value can also be set by dragging the vertical line for the start wavelength on the graph.
	[End]	Set the end wavelength. Hint This value can also be set by dragging the vertical line for the end wavelength on the graph.
[Update]		Click to plot the graph using the range entered for [Start] and [End]. If the set values are outside the allowable range, the values of [Start] and [End] are revised automatically and then the graph is plotted.
[OK]		Confirm the settings made and close the window.
[Cancel]		Cancel the settings made and close the window.

11.8 File Information View

This view displays information including the filename, save destination, and measurement conditions of fluorescence quantum efficiency measurement result data files.



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 date and time of sample measurement.
[Date of Correction Function Creation]	Displays the creation date and time of the correction function used when capturing the sample data.

11.9 Calculation Algorithm of the Quantum Efficiency 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 efficiency" in the quantum efficiency calculation program.

There exists a relationship between the number of photons emitted as fluorescent light and the area of the fluorescence spectrum. The quantum efficiency calculation program calculates the absorbance, internal quantum efficiency, and external quantum efficiency of a sample from the excitation light peak area and fluorescence area of the blank spectrum, measured without the sample in the sample compartment, and the sample spectrum, measured with the sample set in the sample compartment.

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) Determining the area calculation ranges

In order to determine the areas of peaks, an area calculation range must be specified for each peak.

For each peak, wavenumber transformation is performed on the two wavelengths (start wavelength and end wavelength for area calculation) specified by the user and the area calculation range is determined for the waveform resulting from wavenumber transformation.

In practice, the two data points immediately above and below the positions specified by the user are interpolated with a straight line to determine the intensity at the user-specified positions, and these locations are designated as the end points of the region used in area calculation.

Step 3) Area calculation

The excitation light peak area $S_b E_x$ and fluorescence peak area $S_b E_m$ of the blank spectrum and the excitation light peak area $S_s E_x$ and fluorescence peak area $S_s E_m$ of the sample spectrum are calculated using the trapezoidal rule.

Step 4) Quantum efficiency calculation

The absorbance, internal quantum efficiency, and external quantum efficiency are calculated according to the following formula.

$$\text{Absorptivity} = \frac{\text{Amount of absorbed light}}{\text{Amount of irradiated light}} = \frac{S_s E_x - S_b E_x}{S_b E_x}$$

$$\text{Internal quantum efficiency} = \frac{\text{Amount of radiated light}}{\text{Amount of absorbed light}} = \frac{S_s E_m - S_b E_m}{S_b E_x - S_s E_x}$$

$$\text{External quantum yield} = \frac{\text{Amount of radiated light}}{\text{Amount of irradiated light}} = \frac{S_s E_m - S_b E_m}{S_b E_x}$$

$S_b E_x$: Excitation light peak area of the blank spectrum

$S_b E_m$: Fluorescence peak area of the blank spectrum

$S_s E_x$: Excitation light peak area of the sample spectrum

$S_s E_m$: Fluorescence peak area of the sample spectrum