5 Quantitation Application

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5.1 Window Layout

The quantitation application only has a "measurement mode". Measurement mode is used when performing measurement and offline tasks such as data analysis.

To edit quantitation report files or print using any created layout, click [Edit Printform] on the toolbar.

■Measurement mode

	Open	Save	Pirst		U leasurement	Edit Printform	Image: Weight of the second		
	Cont-01.fqqf Cont-01.fqqf Standard Table Sample Table		∲ Stat	Stop	Open L	Zero Search	Template: Open Save As. File Name: Qnt-01.fqqf		aconnect
		Ad	d Line Edit	Open Sav	ve As			EX EM 307.0 340.0	
I			Sample Name	Sample ID	Ex Co	nc EX307.0_EM34	0.0 Comments	0.0	
		1	Std-1	ID-01	1(.000 5855	.497	Arc Lamp	ON (2 hours)
Ī		2	Std-2	ID-02	20	.000 8604	.319		
I		3	Std-3	ID-03	40	.000 13766	.649	Sample Graph Paran	neters 4 >
I									
I								Settings	Load
	Message ^	Ad	d Line Edit						Indua A
I	Total Judgment - OK	-	Come la Norma	C	C	5×207.0 51240.0	Comments	Parameter [Tostrument]	value
I	Instrument Function A		Sample Name	Sample ID	Conc	EX307.0_EM340.0	Comments	EX Bandwidth	3.0 nm
I	Arc Lamp - Total Light		Unk-1	ID-091	21.681	89/4.3/2		EM Bandwidth	3.0 nm
	Mercury Lamp - mour	2	Unk-2	ID-U92	21.503	8927.622		Sensitivity	Low
	Mercury Lamp - Total	3	Unk-3	ID-U93	21.596	8952.041		Accumulation Time	10 ms
1	<u> </u>	4	Unk-4	ID-U94	21.785	9001.659		[Wavelengths]	
1	Sipper - Used 🛛	5	Unk-5	ID-U95	21.753	8993.399		Column Name 1	EX307.0_E
I	Connection - Passed							EX Wavelength	307.0 nm
н								EM Wavelength	240.0 mm

Window Layout of the Quantitation Application (Measurement Mode)

No.	Name	Function
0	Quantitation measurement toolbar	The buttons used for starting and stopping measurement and performing instrument control are located on this toolbar. Clicking ([Connect]) and establishing a connection with the instrument enables ([Start]) and other buttons.
0	Photometer status	The upper section displays the current wavelength and fluorescence intensity and the lower section displays the status of the spectrofluorophotometer.
		Displays the settings of the currently configured measurement parameters (settings

0	Parameter/graph view	such as parameters related to measurement and calibration curve equation), calibration curve graph, and sample graph. This view is used to configure, save, and load measurement parameters.
Ø	Standard table	This table is used to process data such as standard sample concentrations and fluorescence intensities in order to create calibration curves. Creating a standard table automatically creates a calibration curve. Calibration curves can be saved as files along with the data and information of measurement parameters and the standard table.
6	Sample table	This table is used to calculate sample concentration from measurement data (fluorescence intensity) using the K-factor method or a calibration curve created in advance, and display the result. If equations for the fluorescence intensity and concentration of each sample are created and registered in advance, the calculation results are displayed in the relevant table columns when measurement is performed.

■Edit print form



Window Layout of the Quantitation Application (Edit Print Form)

No.	Name	Function
0	Print form editing area	Edit properties such as position and size of printable objects placed on a report.
0	Properties view	Displays the properties of printable objects selected in the print form editing area. Properties such as link settings and text font can be changed.
0	Object list	Displays the printable objects that can be used in tree format. Either double-click on object names or drag object names into the print form editing area to add printable objects to a report file.

5.2 Menu Bar

- <u>5.2.1 [File] Menu</u>
- <u>5.2.2 [Edit] Menu</u>
- <u>5.2.3 [View] Menu</u>

- <u>5.2.4 [Graph] Menu</u>
- 5.2.5 [Instrument] Menu
 5.2.6 [Tools] Menu
- <u>5.2.7 [Window] Menu</u>
- 5.2.8 [Help] Menu

5.2.1 [File] Menu

Command		Description	
[New]		Close the currently open quantitation file and clear the measurement parameter settings.	
[Open]		Open a saved file.	
		Reference " <u>1.2 File Types</u> "	
	[Data]	Open a quantitation file (.fqqf) or calibration curve file (.fqcf).	
	[Parameters]	Open a measurement Parameters file (.fmqf).	
	[Template]	Open a quantitation template file (.fqtf).	
[Close]		Close the currently open quantitation file.	
[Sav	ze]	Save by overwriting the currently open quantitation file.	
[Save As]		Specify a filename and save a file such as a quantitation result or measurement parameter file.	
	[Data]	Save a quantitation file (.fqqf) or calibration curve file (.fqcf).	
	[Parameters]	Save the settings currently configured in the parameter view to a measurement parameter file (.fmqf).	
	[Template]	Save the currently configured measurement parameter, standard sample, and sample table information (excluding data) as a quantitation template file (.fqtf).	
[Text File Output]		Save the current standard sample, sample table data, and measurement parameter information to a text file (.txt) or CSV file (.csv). The format and conversion conditions for text file output are set via [User Setting] on the [Tools] menu.	
(Recent File)		The three most recently opened files are displayed.	
[Properties]		Display the [File Properties] window. This window is used to check data information and perform operations such as renaming data sets.	
		Reference <u>"[File Properties] window"</u>	
[Pri	nt Preview]	Display a preview of printer output.	
	4	Print the report file linked to the currently active table and view.	
[Prn	ntj	Reference <u>"[Ouick Print] tab"</u>	
[Exit]		Exit the quantitation application and close the window.	

■[File Properties] window

[File Summary] tab

e Summary His	tory	Patameters	
Software Name:	Lab	olutions RF	
Version:	1.0		
Filename:	C:\	F-Data \Data \QntData_001.fqqf	
	10/	6/2014 06:36:13 PM	
<u>Analyst:</u>	Use		
<u>C</u> omment:	sam	le comment	A.
			-
Instrument Name	RF-	000	
Instrument Type	RF-	000 Series	
<u>M</u> odel (S/N):			

[File Properties] Window ([File Summary] Tab) for Quantitation Files

Item	Description	
[Software Name]	Displays the name of the software that captured the data.	
[Version]	Displays the software version number.	
[Filename]	Displays the filename of the quantitation file.	
[Date/Time]	The date and time that the quantitation file was saved is displayed.	
[Analyst]	Displays the name of the analyst entered in the [Quantitation File Setting] window. The analyst name can be entered or changed on this tab.	
	Reference "[New Data Set] Window"	
[Comment]	Displays the comment entered in the [Quantitation File Setting] window. A comment can be entered or changed on this tab.	
	Reference "[New Data Set] Window"	
[Instrument Name]/ [Instrument Type]/ [Model (S/N)]	Displays information (instrument name, model, and serial number) regarding the instrument that captured the data.	
[OK]	Close the [File Properties] window and update the file with the entered information.	
[Cancel]	Cancel any entered information and close the [File Properties] window.	

[History] tab

Displays the data history of the quantitation file.

Description	Date/Time	User	-
Standard - Added EX240.0_EM365.0 Column	10/06/2014 03:56:55 PM		
Sample - Added EX240.0_EM365.0 Column	10/06/2014 03:56:55 PM		
Standard - Added Curve Parameters to EX24	10/06/2014 03:56:55 PM		=
Sample - Added Curve Parameters to EX240	10/06/2014 03:56:55 PM		
Modify Summary Analyst [] to [User]	10/06/2014 04:43:56 PM		4
Modify Summary Comments	10/06/2014 04:43:56 PM		
Standard - Added 1 Row	10/06/2014 05:09:57 PM		
Standard - Added 2 Row	10/06/2014 05:09:57 PM		
Standard - Added 3 Row	10/06/2014 05:09:57 PM		
Standard - Added 4 Row	10/06/2014 05:09:57 PM		
Standard - Added 5 Row	10/06/2014 05:09:57 PM		
Standard - Deleted 4 Row	10/06/2014 05:10:09 PM		
Standard - Deleted 5 Row	10/06/2014 05:10:09 PM		
Standard - Deleted 1 Row	10/06/2014 05:19:13 PM		
Standard - Deleted 2 Row	10/06/2014 05:19:13 PM		
Standard - Deleted 3 Row	10/06/2014 05:19:13 PM		-

[File Properties] Window - [History] Tab

[Parameters] tab

This tab displays the various parameters used in measurement.

File Properties			×		
File Summary History Patameters					
[Instrument]			_		
Light Source:	Xenon Arc Lamp				
Signal Processing:	Analog (Intensity)				
EX Band Width:	5.0 nm				
EM Band Width:	10.0 nm		=		
Sensitivity:	High				
Accumulation Time:	10 ms				
[Wavelengths]					
Column Name1	EX240.0 EM365.0				
EX Wavelength:	240.0 nm				
EM Wavelength:	365.0 nm				
[Calibration Curve]					
Column for Cal. Curve:	EX240.0 EM365.0				
Cal. Curve Type:	Multi Point				
Cal. Curve Unit:	mg/ml				
Selected Wavelength:	EX240.0_EM365.0		-		
<u></u>					
L					
		OK Can	icel		

[File Properties] Window - [Parameters] Tab

🛉 Hint

[Copy] and [Select All] can be selected from the right-click menu.

5.2.2 [Edit] Menu

Reference "2.2.1 [Edit] Menu"

5.2.3 [View] Menu

Reference "2.2.2 [View] Menu"

5.2.4 [Graph] Menu

Command		Description
[Display Sample Graph with]		Select the Y axis of the sample graph.
	[Intensity]	Display fluorescence intensity on the Y axis of the sample graph.
	[Concentrations]	Display concentration on the Y axis of the sample graph.
[Calibration Curve Statistics]		Select the statistics function to display on the calibration curve graph.
	[Equation]	 This is the calibration curve equation used when calculating concentration from an unknown sample. This equation can be used in two forms. Intensity = f (Conc) Conc = f (Intensit y)
		The calibration curve function can be selected according to the measurement parameters. When the coefficient of the calibration curve is changed, this function is also updated.
	[Square of Correlation Coefficient]	Display the value resulting from squaring the correlation coefficient, r, of the calculated calibration curve. This is used for first order calibration curves. Reference "Correlation coefficient"
	[Square of Multiple Correlation Coefficient]	Display the value resulting from squaring the multiple correlation coefficient, r, of the calculated calibration curve. Reference "Multiple correlation coefficient"
[Gra	ph Settings]	Display the graph settings window for [Standard Curve] or [Sample Graph].

■Correlation coefficient

This statistical value represents the degree to which the calculated calibration curve matches a linear curve. The range of the correlation coefficient is "-1.0" to "1.0". The closer this value is to "1.0" or "-1.0", it indicates that more linear calibration is being performed. The closer this value is to zero, it indicates that linear calibration is insufficient and the concentration calculated from the sample measurement values may be incorrect. The correlation coefficient is effective for first order calibration curves.

The correlation coefficient set of $\{(x_1, y_1), (x_2, y_2), \dots, (x_n, y_n)\}$ is calculated in the following manner using a weight of $\{w_1, w_2, \dots, w_n\}$.

$$r = \frac{S_{XY}}{\sqrt{S_{XX}}\sqrt{S_{YY}}}$$

where

$$Sxy = \sum w_i x_i y_i - \frac{\left(\sum w_i x_i\right)\left(\sum w_i y_i\right)}{\sum w_i}$$
$$Sxx = \sum w_i x_i^2 - \frac{\left(\sum w_i x_i\right)\left(\sum w_i x_i\right)}{\sum w_i}$$
$$Syy = \sum w_i y_i^2 - \frac{\left(\sum w_i y_i\right)\left(\sum w_i y_i\right)}{\sum w_i}$$

and

xi represents the concentration value, yi represents the measurement values, and wi is the user-entered weight factor.

■Multiple correlation coefficient

This value represents the degree to which the calculated calibration curve fits the standard data. The range of the correlation coefficient is "-1.0" to "1.0". The closer this value is to "1.0" or "-1.0", it indicates that satisfactory calibration is being performed. The closer this value is to zero, it indicates that calibration is insufficient and the concentration calculated from the sample measurement values may be incorrect. The multiple correlation coefficient set of $\{(y_1, f(x_1)), (y_2, f(x_2)), \dots, (y_n, f(x_n))\}$ is defined by the following equation.

$$r = \frac{Str}{\sqrt{Str}} \sqrt{Str}$$

where

$$\begin{split} & \mathbb{S}\mathcal{D} = \sum w_i f(x_i) y_i - \frac{\left(\sum w_i f(x_i)\right) \left(\sum w_i y_i\right)}{\sum w_i} \\ & \mathbb{S}\mathcal{D} = \sum w_i f(x_i)^2 - \frac{\left(\sum w_i f(x_i)\right) \left(\sum w_i f(x_i)\right)}{\sum w_i} \\ & \mathbb{S}\mathcal{D} = \sum w_i y_i^2 - \frac{\left(\sum w_i y_i\right) \left(\sum w_i y_i\right)}{\sum w_i} \end{split}$$

and

xi represents the concentration value, yi represents the measurement values, wi is the user-entered weight factor, and f (xi) is the calculated instrument reading obtained from the Standard Curve.

■[Customize Graph] window

[Line Colors] tab

Daţa:	Basic Color:
Annotation: Une Width: C	Created Color:

[Customize Graph] Window - [Line Colors] Tab for Calibration Curves

Item	Description
[Data]	For the graph settings of the calibration curve, the annotation, line type, and line color are displayed for [Standard Curve] and [Standard Data]. For the graph settings of the sample graph, the annotation, line type, and line color are displayed for [Sample Data]. The selected data set name is highlighted.
[Annotation]	Select the display style of the annotation used to display data points.
[Line]	Select the graph line from types such as solid line and dotted line.

[Width]	Set the width of the graph line.
[Basic Color]	Displays the colors that can be used as the graph line color. Selecting the target data set under [Data] and clicking a color in the pallet changes the graph line color.
[Created Color]	Displays the colors created using [Create Color].
[Create Color]	Display the [Create Color] window. This window is used to create colors absent from the basic color pallet.
[OK]	Confirm the settings made and close the [Customize Graph] window. The graph view is redrawn based on the settings made.
[Cancel]	Cancel any settings made and close the [Customize Graph] window.

[Limits] tab

ine Colors	Limits	Appearance	Settings	
X Axis				
Min:		10		
Max:		30		
Y Axis				
Min:		4.98	Min:	Hight
Max:		11.4	Max	1
			·	

Item	Description
[X Axis]	Set the upper and lower limit values of the X axis on the graph.
[Y Axis]	Set the upper and lower limit values of the Y axis on the graph.

[Appearance] tab

Reference "[Appearance] tab"

[Settings] tab

Reference <u>"[Settings] tab"</u>

5.2.5 [Instrument] Menu

Reference "2.2.3 [Instrument] Menu"

5.2.6 [Tools] Menu

Reference "2.2.4 [Tools] Menu"

■[User Settings] window (quantitation application)

The settings on the [Text File Formats], [Text Output], and [PDF Output] (Option) tabs are the same for all of the basic analysis applications.

Reference <u>"[User Setting] window (common)"</u>

[General] tab

Number of Displayed Decimal Places Standard Table and Calibration Cueve: Sample Table and Sample Graph: Rounding is used for only displayning, not for calculation. Rounded values are used for calculation.	General	Text File Formats	Quick Print	Text Output	1	
Standard Table and Calibration Cueve: Image: Complex of the second s	Numb	er of Displayed Dec	cimal Places		-	-
Sample Table and Sample Graph: 3 (*) Rounding is used for only displayning, not for calculation. Rounded values are used for calculation.	Stand	lard Table and Calib	oration Cueve:	3		
 Rounding is used for only displayning, not for calculation. Rounded values are used for calculation. 	Samp	le Table and Sampl	e Graph:	3	A V	
Rounded values are used for calculation.	Restaurant line line line line line line line line	ounding is used for	only displaynin	g, not for calc	culation.	
	R	ounded values are u	used for calcu	ation.		

[User Settings] Window - [General] Tab

Item		Description	
[Number of Displayed Decimal Places]		Configure settings such as the number of decimal places displayed for numerical values in tables and scale values on graphs.	
		NOTE Settings related to calculation cannot be changed after measurement parameters are created.	
	[Standard Table and Calibration Curve]	Select the number of decimal places for numerical values, such as data and concentration in the standard table, and for the scale of the calibration curve graph. Selection options: 1 to 6	
	[Sample Table and Sample Graph]	Select the number of decimal places for numerical values, such as data and concentration in the sample table, and for the scale of the sample graph. Selection options: 1 to 6	
	[Rounding is used for only displaying, not for calculation]	Select this setting when performing concentration calculation and operations with an equation using unmodified fluorescence intensity (internal data that contains undisplayed values) captured from the instrument. (Default setting)	
		NOTE When this setting is selected, the number of displayed digits can be changed even after measurement parameters are created.	
	[Rounded values are used for calculation]	Select this setting when performing concentration calculation and operations with an equation using fluorescence intensity rounded to the set number of display digits.	

[Quick Print] tab

eneral Te	ext File Formats Quick Print Text Output
<u>P</u> rint Item:	Standard Table Sample Table Calibration Curve Sample Graph Mesurement Parameters
<u>R</u> eport File C:\RF-Dat	a\Report\QuantitationStandardTable.frpt
	Reset

[User Settings] Window - [Quick Print] Tab

Item	Description	
	Displays the views and data tables that can be linked to report files.	
[Print Item]	The assumement Parameters] refers to the [Parameters] tab in the parameter view.	
[Report File]	Clicking an item in the [Print Item] list selects it and displays the name and save destination of the report file to which it is linked.	
[Browse]	Display the report file selection window.	
[Reset]	Return links to their initial state.	

5.2.7 [Window] Menu

Command	Description
[Edit Printform]	Change to the edit print form mode window.

5.2.8 [Help] Menu

Reference <u>"2.2.6 [Help] Menu"</u>

5.3 Parameter/Graph View

In addition to measurement parameters, the calibration curve, sample graph, and spectrum are displayed in the same view.

NOTE The calibration curve is only displayed in the quantitation application.

- 5.3.1 [Calibration Curve] Tab
- <u>5.3.2 [Sample Graph] Tab</u>
- <u>5.3.3 [Parameters] Tab</u>
- <u>5.3.4 [Spectrum] Tab</u>

5.3.1 [Calibration Curve] Tab

This tab displays a calibration curve graph, calibration curve equation, and statistical function of the calibration curve.

The calibration curve created based on the standard table data (that is not set as excluded) and according to the calibration curve conditions defined in the measurement parameters is drawn in the calibration curve graph area. The data points used in the calibration curve are displayed overlaid on the calibration curve graph. The units are concentration on the X axis and fluorescence intensity on the Y axis.



■Calibration curve right-click menu

Click the right mouse button on the calibration curve to display the following right-click menu.

Command		Description		
		Copy the graph on the [Calibration Curve] tab to the clipboard.		
[Co	ру]	Hint When the statistical function of the calibration curve is displayed, it is also copied.		
		Save a graph image in metafile format to the clipboard.		
	[Picture]	Even if the calibration curve function and statistics function are displayed on the graph, only the calibration curve graph is copied as a picture.		
		The graph can be pasted as an embedded graph object in the print form editing mode.		
	[For Report File]	Hint An embedded graph object is an object that is not linked to the graph view state.		
[Auto Scale]		Perform automatic scaling in both the X-axis and Y-axis directions. This setting displays the graph using the entire graph range in the X-axis direction and with a margin of 10% of the graph height in the Y-axis direction.		
ſCu	stomizel	Set the calibration curve line type, line color, line width, point shape, and whether points are displayed or not for each waveform.		
	1	Reference "[Customize Graph] window"		
[Vertical axis]		Select [Linear] or [Logarithmic] for the vertical axis of the calibration curve graph.		
[Horizontal axis]		Select [Linear] or [Logarithmic] for the horizontal axis of the calibration curve graph.		
[Print] [Properties]		Perform a quick print. Set the report file to use via [User Settings] on the [Tools] menu.		
		Reference "[User Settings] window (quantitation application)"		
		Display the calibration curve graph properties window. This window is used to check the calibration curve equation and the statistical function selected for display on the graph.		

Reference "[Standard Curve Properties] window"

■[Standard Curve Properties] window

Standard Curve Properties	X
Statistics Equation	
<u> </u>	
Calibration Equation	
Sguare of Multiple Correlation Coefficient : r(m)2	

Standard Curve Properties			X
Statistics Equation			
Order 1st			
K0: 2.34753	K1:	218.37121	
Zero Intercept: False			

[Standard Curve Properties] Window

Item	Description
[Graph]	Select this checkbox to display a calibration curve graph.
[Calibration Equation]	Select this checkbox to display the calculation function of the calibration curve below the graph.
[Square of Correlation Coefficient r2]	Select this checkbox to display the squared correlation coefficient r2 below the graph.
[Square of Multiple Correlation Coefficient r (m)2]	Select this checkbox to display the squared multiple correlation coefficient r(m)2 below the graph.
[Order]	Displays the order of the calibration curve equation.
Calibration curve coefficients (K0 to K3)	Displays the coefficients of the calibration curve equation.
[Zero Intercept]	Displays whether the calibration curve equation passes through the origin.

5.3.2 [Sample Graph] Tab

This tab plots the points of data (that is not set as excluded) from the sample table. The horizontal axis of the sample graph can be changed between [Concentration] and [Intensity] on the [Graph] menu.

Reference "5.2.4 [Graph] Menu"



■Right-click menu of the sample graph

Click the right mouse button on the sample graph to display the following right-click menu.

Command		Description				
[Coj	py]	Copy the graph on the [Sample Graph] tab to the clipboard.				
[Picture]		Save a graph image in metafile format to the clipboard.				
	[For Report File]	The graph can be pasted as an embedded graph object in report editing mode. Image: The graph can be pasted as an embedded graph object is an object that is not linked to the graph view state. Image: The graph can be pasted as an embedded graph object is an object that is not linked to the graph view state.				
[Auto Scale]		Perform automatic scaling in both the X-axis and Y-axis directions. This setting displays the graph using the entire graph range in the X-axis direction and with a margin of 10% of the graph height in the Y-axis direction.				
[Customize]		Set the sample graph line type, line color, line width, point shape, and whether points are displayed or not for each waveform.				
[Vertical axis]		Select [Linear] or [Logarithmic] for the vertical axis of the sample graph.				
[Print]		Perform a quick print. Set the report file to use via [User Setting] on the [Tools] menu.				
		Reference "[User Settings] window (quantitation application)"				

5.3.3 [Parameters] Tab

This tab is used to check or change the measurement parameters currently set on the instrument.

Reference <u>"Measurement mode"</u>

5.3.4 [Spectrum] Tab

This tab draws a spectrum in real time during range measurement.

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Reference "5.4 [Quantitation Measurement Parameters] Window"
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■Right-click menu of the spectrum graph

Click the right mouse button on the spectrum graph to display the following right-click menu.

Command		Description			
[Copy]		Copy the graph on the [Spectrum] tab to the clipboard.			
[Picture]		Save a graph	Save a graph image in metafile format to the clipboard.		
		The graph c	an be pasted as an embedded graph object in report editing mode.		
	[For Report Item]	🋉 Hint	An embedded graph object is an object that is not linked to the graph view state.		
[Auto Scale]		Perform auto [Limits] tab	omatic scaling of the graph. Automatic scaling is configured on the in the [Customize Graph] window.		
		Reference	"[Customize Graph] window"		
		🋉 Hint	Double-clicking on the graph will also perform automatic scaling.		
[Customize]		Configure so font.	ettings such as graph line type, line color, background color, and scale		
L	-	Reference	"[Customize Graph] window"		
[Ver	tical axis]	Select [Line	ar] or [Logarithmic] for the vertical axis of the graph.		

5.4 [Quantitation Measurement Parameters] Window

This window is used for configuring and saving measurement parameters. When nothing is displayed in the parameter display area, the required parameters are displayed in wizard format.

Wint The tabs are displayed in the order of [Instrument] tab → [Wavelengths] tab → [Calibration] tab → [Measurement (Standard)] tab → [Measurement (Sample)] tab and the [Quantitation Measurement Method] window is displayed last.

The [Quantitation Measurement Method] window is displayed from the second time onwards. Click the tab for configuration and set the parameters.

■[Wavelengths] tab

Measu	urement (Sample)	Equations	Pass/Fail	Instrument	Attachment
V	Vavelengths	Calibra	ation	Measurement	(Standard)
Type:	Point •]	<u>C</u> olumn Name:	EX350.0_EM400.0	
EX W	avelength (nm):	350	350		
EM W	avelength (nm):	400			
Entrie	8:				
	Columns	Тур	e	Ad	d
	EX350.0_EM400.0 EX350.0_EM450.0	Poir Poir	nt nt	Rem	ove

[Quantitation Measurement Method] Window - [Wavelengths] Tab

[Type] Select the measurement method. [Point] Perform measurement using the set excitation and fluorescence wavelengths. Perform scanning measurement of the specified range and use the single data obtained from the spectrum data with the method specified for [Range] as the measurement data. While the start wavelength and end wavelength must be specified, the other nargemeters are fixed.	
[Point] Perform measurement using the set excitation and fluorescence wavelengths. Perform scanning measurement of the specified range and use the single data obtained from the spectrum data with the method specified for [Range] as the measurement data. While the start wavelength and end wavelength must be specified, the other nargemeters are fixed.	
Perform scanning measurement of the specified range and use the single data obtained from the spectrum data with the method specified for [Range] as the measurement data. While the start wavelength and end wavelength must be specified, the other parameters are fixed	
 RF-5300 series: [Data Interval]: 0.2 nm, [Scan Speed]: Fast RF-6000 series: [Data Interval]: 0.1 nm, [Scan Speed]: 600 nm/min 	set
 The data at the last point in the measured range is not used in range measurement. The wavelength values (X-axis values) that correspond to the extracted data of peaks, valleys, maximum values, and minim values specified with [Range] are displayed in the comment from the relevant measurement row. 	um eld
[Column Name] Displays the column name in the table. While column names are automatically generated from wavelength values, they can be edited.	ý
[EX Wavelength (nm)] Enter the excitation wavelength. Effective range: 220.0 to 900.0 (RF-5300 series), 200.0 to 900.0 (RF-6000 series)	ries)
[EM Wavelength (nm)] Enter the fluorescence wavelength for measurement. If [Range (EM)] is select [Type], this item changes to range setting items ([Emission Start Wavelength and [End Wavelength (nm)]). Effective range: 220.0 to 900.0 (RF-5300 series), 200.0 to 900.0 (RF-6000 series)	ted for (nm)] ries)
[Range] Select the data to use. Selection options: Peaks, Valleys, Max, Min, Area (within the scan range)	
[Entries] Displays a list of point wavelengths and ranges for measurement. A maximum three entries can be displayed.	n of
[Add] Add the set wavelengths to the wavelength registration list.	

[Remove] Remove the specified wavelengths from the wavelength registration list.

■[Calibration] Tab

Measure	ment (Sample)	Equations	Pass/Fail	Instrument	Attachment
Wa	velengths	Calibrati	on	Measureme	nt (Standard)
Туре:	Multi Point	•		Column Name:	Result
<u>F</u> ormula:	Difference	•		<u>U</u> nit:	mg/ml
NL <u>1</u> : EX	(350.0_EM400.0 🔻	WL <u>2</u> : EX35	0.0_EM450.0	✓ \//L <u>3</u> :	Ŧ
F1*I	nt(WL1)-F2*Int(WL2)	F <u>1</u> : 1		F <u>2</u> : 1	
Parame	eters				
	Int = f (Conc)	() <u>C</u> onc = f	(Int)		
- 3	Int = K1*(Conc) + K0				
	Order of Curve:	1st	•		
1	Zero Interception				

[Quantitation Measurement Parameters] Window - [Calibration] Tab

Item	Description
	Select one of the following calibration curve methods.
	• [Multi Point]: This method determines concentration by drawing a calibration curve using multiple data points.
[Type]	• [Single Point]: This method determines concentration using a linear curve that joins the origin to the single point of a standard sample.
	 [K Factor]: This method determines concentration from calibration curve coefficients known in advance.
	Select one of the following quantitation methods.
	[Fixed Wavelength]: Wavelength at specified position
	• [Ratio]: Data difference of two wavelengths Int(WL1) / Int(WL2)
	• [Difference]: Data ratio of two wavelengths F1*Int(WL1) - F2*Int(WL2)
	• [3 Wavelength]: Three-wavelength quantitation calculation
	Hint The area (which is used to determine the concentration) calculated in three-wavelength quantitation is the difference between the fluorescence intensity of the middle wavelength (WL2) and the fluorescence intensity on the (base) line that joins the fluorescence intensities of the long wavelength (WL3) and short wavelength (WL1).
[Formula]	

		A(WL2) A(WL2) - A(WL2) - A(WL2) - (WL3 - WL1) + (WL2 - WL1) × A(WL3) (WL3 - WL1) WL1 WL2 WL3 (Emission)		
[WI	_1] to [WL3]	Set the measurement wavelengths to use for the calibration curve.		
[F1], [F2]		Enter the coefficients to use in the equation when [Difference] is selected for [Formula]. The default value for [F1] and [F2] is "1".		
[Col	lumn Name]	Enter the column title name when [Ratio], [Difference], or [3 Wavelength] is selected for [Formula] and the calculation result is to be displayed in the standard table.		
[Unit]		Enter the concentration unit of the standard sample.		
[Par	ameters]	Set the parameters to use according to the calibration curve method selected for [Type].		
	[Int]/[Conc]	Select intensity ([Int]) or concentration ([Conc]) for the equation to use in calibration curve calculation.		
	[Order of Curve]	Set the order of the calibration curve.		
	[Zero Interception]	Select this checkbox to perform calculation so that the calibration curve passes through the origin.		
	[STD Concentration]	Set the standard sample concentration when [Single Point] is selected for [Type]. Effective range: 0.0 to 1000.0		
		Set the coefficients of the calibration curve when [K-Factor] is selected for [Type].		
		• [K0:0]: Effective range: -10000 to 10000		
	[K0] to [K3]	• [K1:1]: Effective range: -10000 to 10000		
		• [K2:0]: Effective range: -10000 to 10000		
		• [K3:0]: Effective range: -10000 to 10000		

■[Measurement (Standard)] tab

Measurement (Sample)	Equations	Pass/Fail	Instrument	Attachment
Wavelengths	Calibrati	ion	Measurement	(Standard)
)ata Acguired By: O	ser Entry 💿	Instrument		
ample Repetitions: 1	E	rompt before rep	eat	
Settings of the decimal pla	ce to display			
Use Rounded Value for Calculation:	OFF	Folle carr	Follow [Tool] - [User Setting] to carry out this setting. The number of decimal places' digits display can be changed.	
Settings of the decimal place to display:	3	- num disp		
(for standard sample tabl	e and calibration	curve)		
(ioi stanuaru sample tabi				
(ioi stanuaid sample tau				
(ioi stanuaiu sample tau				
(ioi stanuaru sampie tau				

Parameter View - [Measurement (Standard)] Tab

Item	Description
	Set the method for capturing standard table data.
[Data Acquired By]	• [User Entry]: Directly enter fluorescence intensities into the table.
	• [Instrument]: Capture fluorescence intensities by performing measurement.
[Sample Repetitions]	Enter the number of times to repeat measurement on the same wavelength during measurement. Effective range: 1 to 100
[Prompt before repeat]	Select this checkbox to display a message each time one measurement finishes when performing repeated measurements. Clicking [OK] will resume measurement. This is used when repeating measurements while exchanging samples.

■[Measurement (Sample)] tab

Truvolonguia	Calibrati	on	Measurement	(Standard)
Measurement (Sample)	Equations	Pass/Fail	Instrument	Attachment
Data Acquired By	Iser Entry	Instrument		
Sample Repetitions: 1		rompt before rep	eat	
Settings of the decimal pla Use Rounded Value for Calculation:	ace to display	Follow [Tool] - [User Setting]		etting] to
Settings of the decimal place to display:	3	num disp	number of decimal places' digits display can be changed.	
(For sample table and sa	ample graph)			

Parameter View - [Measurement (Sample)] Tab

Item	Description		
	Set the method for capturing sample table data.		
[Data Acquired By]	• [User Entry]: Directly enter fluorescence intensities into the table.		
	• [Instrument]: Capture fluorescence intensities by performing measurement.		
[Sample Repetitions]	Enter the number of times to repeat measurement on the same wavelength during measurement. Effective range: 1 to 100		
[Prompt before repeat]	Select this checkbox to display a message each time one measurement finishes when performing repeated measurements. Clicking [OK] will resume measurement. This is used when repeating measurements while exchanging samples.		

■[Equations] tab

Wavele	engths	Calibrati	on	Measurement (Standard)
Measuremer	nt (Sample)	Equations	Pass/Fail	Instrument	Attachment
Type:	Custom	•			
Column <u>N</u> ame:	EQU_1	<u>U</u> nit:	mg/ml	Ea	ctors
Equation:	Conc*FACT	OR_1		(Clear
Build (doub	e click to add	an item to equatio	in)		
<u>C</u> olumns:	EX350.0_EI EX350.0_EI FACTOR_1 Result Conc	M400.0 M450.0	Operators:	* - / /	•
Entries:					
	Columns				<u>A</u> dd
	EQU_1			<u>B</u> e	emove

Parameter View - [Equations] Tab

Item		Description			
		Select the type of equation.			
[Type]		• Equations for [Ratio], [Difference], [Area], and [3 Wavelength] are incorporated. When using any of these settings, select the target data from the [Columns] list.			
		• Use [Custom] to freely configure an equation that includes any data and operators.			
[Column Name]		Enter the column title name for displaying the calculation result in the table.			
[Unit]		Enter the unit to use.			
[Equation]		Create a calculation equation. There are two methods for creating equations: directly entering data column names and operators, or selecting data and operators from the [Columns] and [Operators] lists. The latter method involves double-clicking on the items to use in the [Columns] and [Operators] lists.			
		Display the [Factor] window.			
[Factors]		Reference <u>"[Factor] window"</u>			
[Clear]		Clear the equation in the [Equation] field.			
[Build]		Displays lists of the data and operators that can be used in equations.			
[Columns]		Displays a list of the column names that can be used in equations. Double-clicking the column name of data to target in calculation inserts the column name at the cursor position in the [Equation] field.			

		Columns that can be used in equations are the fluorescence intensity column, factor column, calculation result column, and concentration column.
	[Operators]	Displays a list of the operators that can be used in equations. Double-clicking an operator to incorporate into an equation inserts the operator at the cursor position in the [Equation] field. If [Custom] is selected for [Type], create an equation by repeatedly selecting data and operators. The following operators can be used. + addition, - subtraction, × multiplication, / division, () parentheses
[Ent	ries]	Displays the column names registered in the equation. Select a column name in the list to reference the equation to which it is set.
	[Add]	Add the created equation to the list.
	[Remove]	Remove the specified wavelengths from the [Entries] list.

[Factor] window

actor			×
Column <u>N</u> ame: FACTOR_1	⊻alue:	1	
Registered Factor:			
Columns			Add
FACTOR_1			Delete
			Close

[Factor] Window

Item	Description
[Column Name]	Enter the title name of the column in the table for displaying the factor.
[Value]	Enter the factor value.
[Registered Factor]	Displays a list of currently set factor columns. Enter the [Column name] and the [Value], and click [Add] to add a factor to the list.
[Add]	Add the set factor to the list.
[Delete]	Remove the specified factor from the [Registered Factor] list.
[Close]	Close the [Factor] window.

∎[Pass/Fail] tab

Waveleng	ths	Calibrat	ion		Measurement	(Standard)
Measurement (Sample)	Equations	Pass	/Fail	Instrument	Attachment
Column <u>N</u> ame:	PF_1					
Passed Text:	Pass	Failed	Text: F	ail	E	actors
Equation:	EQU_1>=	1000				Clear
Build (double d	click to add	an item to equatio	in)			
<u>C</u> olumns:	EX350.0_ EX350.0_ FACTOR_ Result	EM400.0 EM450.0 .1	<u>O</u> pera	tors:	> < != <=	▲ ■ ▼
Entries:						Add
	Columns					Zuu
	PF_1				E	lemove
	L				50	

	Item	Description				
[Col	umn Name]	Enter the column title name for displaying the judgment result in the table.				
[Pas	sed Text]	Set the text to display in the table when the result of the set judgment equation is a pass. The default text is "Pass".				
[Fail	led Text]	Set the text to display in the table when the result of the set judgment equation is a fail. The default text is "Fail".				
[Equation]		Create a judgment equation. There are two methods for creating judgment equations: directly entering data column names and operators, or selecting data and operators from the [Columns] and [Operators] lists. The latter method involves double-clicking on the items to use in the [Columns] and [Operators] lists. E.g.: (EX350_EM400>10)&(WLEX350_EM500 <result) Meaning of above judgment equation When the value of the "EX350_EM400" column is greater than 10 and the value of the "WLEX350_EM400" column is less than the value of the "Result"</result) 				
		column, the text set for [Passed Text] is displayed in the [Column Name] field.				
[Fac	tors]	Display the [Factor] window.				
	_	PReference <u>"[Factor] window"</u>				
[Cle	ar]	Clear the judgment equation in the [Equation] field.				
[Bui	ld]	Displays lists of the data and operators that can be used in judgment equations.				
	[Columns]	Displays a list of the column names that can be used in pass/fail judgment. Double-clicking the column name of data to target in judgment inserts the column name at the cursor position in the [Equation] field. Columns that can be used in judgment equations are the fluorescence intensity column, factor column, calculation result column, and concentration column.				
	[Operators]	Displays a list of the operators that can be used in pass/fail judgment. Double-clicking an operator to incorporate into a judgment equation inserts the operator at the cursor position in the [Equation] field. The following operators can be used. = equal to, > greater than, < less than, != not equal to, >= greater than or equal to, <= less than or equal to, & AND operator, OR operator, () parentheses				
[Ent	ries]	Displays the column names registered in the judgment equation. Select a column name in the list to reference the judgment equation to which it is set.				
	[Add]	Add the created judgment equation to the list.				
	[Remove]	Remove the specified wavelengths from the [Entries] list.				

■[Instrument] tab

Reference "[Instrument] tab"

■[Attachment] tab

Reference "[Attachment] tab"

• <u>5.4.1 [Parameter Properties] Window</u>

5.4.1 [Parameter Properties] Window

Right-click on the parameter view and select [Properties] to display the parameter properties window. The selected items are displayed in the parameter view display area.

Parameter Properties	
Check items to be shown.	
Instrument	
Wavelength	
Calibration Curve	
Measurement(Standard)	
Measurement(Sample)	
Attachment	
V Formula	
Pass/ Fail	
OK Cance	2
Pass/ Fail OK Cance [Parameter Properties] Wi	ndow

5.5 Standard Table

NOTE Entries in the table other than factors such as comments and values are determined once the file is saved and closed. Therefore these entries can no longer be edited the next time the file is opened.

Add I	ine Edit	Open Save As.				
	Sample Name	Sample ID	Ex	Conc	EX240.0_EM365.0	Comments
1	SampleName1	SampleID1		10.0	5.6	1
2	SampleName2	SampleID2		20.0	8.0	
3	SampleName3	SampleID3		30.0	10.9	

Standard Table

Item	Description
[Add Line]	Add a row to the standard table.
	Select a row to click this button to display the [Edit Table] window. Sample name, sample ID, and option name can all be edited at once in the [Edit

		Table] window.			
Ed	ıtj	Reference <u>"5.5.1 [Edit Table] Window"</u>			
[Ca	libration Curve File]	Calibration curve files can be saved and loaded.			
	[Open]	Open a calibration curve file (.fqcf).			
	[Save As]	Save the standard table currently being created to a calibration curve file (.fqcf).			
Star	ıdard table	The following columns can be displayed. Show or hide columns by setting their display status in the [Standard Table Properties] window that is displayed by clicking [Properties] on the right-click menu. Standard Table Properties Columns Status Sample Name Show Detion Hide K Show Date Hide K Show Sho			
	[Sample Name]	Displays sample names. ^{*1}			
	[Sample ID]	Displays sample ID names			
	[Option]	Displays option names *1			
	[Type]	Displays option names. " Displays the measurement type. "Standard" is displayed for the standard table, "Unknown" for the sample table, and "Std-Repeat" for standard sample repetition data.			
	[Fy]	Selecting this checkbox when excluding points on the calibration curve that correspond to the sample deletes the data from the calibration curve.			
		Hint Clicking [Hide Excluded Lines] on the right-click menu hides excluded rows.			
	[Date]	Displays the date of data capture.			
	[Time]	Displays the time of data capture.			
	[Conc]	Displays concentrations of standard samples.			
	[EX**_EM**]	Displays the fluorescence intensity values of the set wavelengths. Image: Hint Values can be directly entered when [Data Acquired By] is set to [User Entry] on the [Measurement (Standard)] tab.			
	[Result]	Displays the value calculated from the measurement value (fluorescence intensity) when performing two- or three-wavelength quantitation.			
	[Wgt.Factor]	This changes the weight that data has on the calibration curve. For example, when the factor for all points is "1", the curve is calculated with all points having equal weight. However, when the factor for one point is set to "2", the curve is calculated as two points. In other words, the weight of this point is doubled. ^{*1}			
	[Comments]	Enter a comment. ^{*1}			

*1 This can be changed in the [Edit Table] window or using direct entry.

• <u>5.5.1 [Edit Table] Window</u>

5.5.1 [Edit Table] Window

Sample Name			
		🔽 Use seque	ntial number
Name:		First No.:	Step:
SampleName	+	1	1
Sample ID			
		🔽 Use seque	ntial number
Name:		First No.:	Step:
SampleID	+	1	1
Option			
		🔽 Use seque	ntial number
Name:		First No.:	Step:
Option	+	1	1

 Item
 Description

 [Name]
 Set the sample name, sample ID, and option name.

 [Use sequential number]
 Select this checkbox to display the start number and number of steps of the number that is sequentially suffixed onto [Name].

 [First No.]
 Set the start number.

 [Step]
 Set the number of steps.

Confirm the settings made and close the [Edit Table] window. Cancel any settings made and close the [Edit Table] window.

5.6 Sample Table

[OK]

[Cancel]

NOTE Entries in the table other than factors such as comments and values are determined once the file is saved and closed. Therefore these entries can no longer be edited the next time the file is opened.

	Sample Name	Conc	EX240.0_EM365.0	Comments		
1	Sample-1	16.4	7.2			
2	Sample-2	17.1	7.4			
3	Sample-3	21.3	8.5			
4	Sample-4	22.8	8.9			
5	Sample-5	27.3	10.1			

Sample Table

Item	Description
[Add Line]	Add a row to the sample table.

[Edit]		Select a row to click this button to display the [Edit Table] window. Sample name, sample ID, and option name can all be edited at once in the [Edit Table] window.
		Reference "5.5.1 [Edit Table] Window"
		The following columns can be displayed. Show or hide columns by setting their display status in the [Sample Table Properties] window that is displayed by clicking [Properties] on the right-click menu.
		Sample Table Properties
		Columns Status
Sample table		Sample Name Show Sample ID Hide Option Hide Type Hide Ex Show Date Hide
		[Sample Table Properties] Window
	[Sample Name]	Displays sample names. ^{*1}
	[Sample ID]	Displays sample ID names. ^{*1}
	[Option]	Displays option names. ^{*1}
	[Type]	Displays the measurement type. "Unknown" is displayed for normal data, "Unk-Repeat" for repetition data, and "Average" for averaged values.
	[Ex]	Select this checkbox to exclude the corresponding data point from the sample graph.
		Hint Clicking [Hide Excluded Lines] on the right-click menu hides excluded rows.
	[Date]	Displays the date of data capture.
	[Time]	Displays the time of data capture.
	[Conc]	Displays the concentration calculated from the calibration curve.
	[EX**_EM**]	Displays the fluorescence intensity values of the set wavelengths. Displays multiple wavelength pair values calculated using the quantitation method in separate columns.
		Hint values can be directly entered when [Data Acquired By] is set to [User Entry] on the [Measurement (Standard)] tab.
	[Result]	Displays the value calculated from the measurement value (fluorescence intensity) when performing two- or three-wavelength quantitation.
	[Comments]	Enter a comment. ^{*1}

*1 This can be changed in the [Edit Table] window or by direct entry.