

INSTRUCTION MANUAL
HITACHI FLUORESCENCE SPECTROPHOTOMETER
FL SOLUTIONS PROGRAM
(OPERATION MANUAL)

Hitachi High-Technologies Corporation

PREFACE

Thank you very much for purchasing the Hitachi High-Technologies Corporation fluorescence spectrophotometer.

This instrument is intended for use by persons having a basic knowledge of chemical analysis. Remember that improper use of analytical instruments, chemicals or samples would result not only in wrong analytical data but also in consequences adverse to safety.

Carefully read this instruction manual before attempting operation. For proper use of the fluorescence spectrophotometer, please acquaint yourself with it.

ABOUT THIS MANUAL

The Hitachi High-Technologies Corporation fluorescence spectrophotometer is accompanied by the following two instruction manuals:

- F-2500/F-4500 Fluorescence Spectrophotometer Instruction Manual - Maintenance
- Hitachi High-Technologies Corporation Fluorescence Spectrophotometer Instruction Manual - FL Solutions Program - Operation

The cautionary instructions concerning safety are contained in the F-2500/F-4500 Fluorescence Spectrophotometer Instruction Manual - Maintenance. Be sure to read them before attempting operation.

This instruction manual describes the operational procedures for the FL Solutions program formulated for the Hitachi High-Technologies Corporation fluorescence spectrophotometer. Be sure to read through this instruction manual before attempting operation.

IMPORTANT

Precautions on Electromagnetic Wave Interference

1. Possible Electromagnetic Wave Interference Caused by The Instrument

When the instrument is used in a residential area or an adjacent area thereto, it may cause interference to radio and television reception.

To prevent this, use the specified system connection cables in strict accordance with the instruction manual.

The instrument is designed to minimize possible electromagnetic wave interference caused by it if the specified cables are connected properly.

However, there is no guarantee that electromagnetic wave interference will not be caused by the instrument.

If the instrument does cause interference to radio or television reception, which can be determined by turning the instrument off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Increase separation between the instrument and the radio/TV receiver.
- Connect the instrument to an outlet on a circuit different from that to which the radio/TV receiver is connected.

2. Possible Electromagnetic Wave Interference Affecting the Instrument

If the instrument is used near an intense electromagnetic source, interference noise may be given to the instrument to incur an adverse effect on its performance or functionality.

To prevent this, use the specified system connection cables in strict accordance with the instruction manual.

The instrument is designed to minimize possible electromagnetic wave interference affecting it if the specified cables are connected properly.

However, there is no guarantee that electromagnetic wave interference will not occur in the instrument.

If the instrument does incur electromagnetic wave interference, which can be determined by turning on and off possible sources of electromagnetic interference nearby, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient the instrument.
- Increase separation between the instrument and possible sources of electromagnetic interference.
- Increase separation between the power cable of the instrument and possible sources of electromagnetic interference.
- Connect the instrument to an outlet on a circuit different from that to which possible sources of electromagnetic interference are connected.
- Check that any other device connected with the instrument is not affected by electromagnetic interference.

WARRANTY ON PRODUCT

The Hitachi fluorescence spectrophotometer is warranted to be free from defects in material or workmanship under normal use within the product specifications indicated in this manual and under conditions given below. This warranty is void if the instrument is not used in accordance with the instruction manual.

(1) Scope of Warranty

- (a) Any parts which prove to be defective in design or workmanship during the warranty period will be repaired, adjusted or replaced without charge.
- (b) A substitute part may be used for repair, or replacement with an equivalent product may be made instead of repair.
- (c) Such system components as a personal computer and printer to be updated frequently for improvement may not be available in original versions at the time of replacement.

(2) Warranty Period

One year from the date of initial installation
(Excluding consumables such as a xenon lamp)

(3) Availability of Technical Support Service

Technical support service for the instrument is available within regular working hours on workdays predetermined by us.

(4) Limitations and Exclusions on Warranty

Note that the following cases are excluded from the scope of this warranty.

- (a) Failure due to operation at a place not meeting the installation requirements specified by us.
- (b) Failure due to power supply voltage/frequency other than specified by us or due to abnormality in power supply.
- (c) Corrosion or deterioration of the tubing due to impurities contained in reagent, gas, air or cooling water supplied by the user.
- (d) Corrosion of the electric circuits or deterioration of the optical elements due to highly corrosive atmospheric gas.

- (e) Failure due to use of hardware, software or spare parts other than specified by us.
- (f) Failure due to improper handling or maintenance by the user.
- (g) Failure due to maintenance or repair by a service agent not approved or authorized by us.
- (h) After disposal of the instrument, or after its resale without our approval.
- (i) Failure due to relocation or transport after initial installation.
- (j) Failure due to disassembly, modification or relocation not approved by us.
- (k) Consumables, and failure of parts that have reached the end of specified useful life.
- (l) Failure of parts excluded from the warranty in the instruction manual or other documents.
- (m) Failure due to acts of God, including fire, earthquake, storm, flood, lightning, social disturbance, riot, crime, insurrection, war (declared or undeclared), radioactive pollution, contamination with harmful substances, etc.
- (n) Failure of the hardware, or damage to the system software, application software, data or hard disk due to computer virus infection.
- (o) Failure of the personal computer connected with the instrument, or damage to the system software, application software, data or hard disk due to power interruption or momentary power voltage drop caused by lightning or the like.
- (p) Failure of the personal computer connected with the instrument, or damage to the system software, application software, data or hard disk due to disconnection of main power to the personal computer without taking the specified normal shutdown procedure.

DISCLAIMER OF WARRANTY

- (1) WE MAKE NO WARRANTIES, EITHER EXPRESS OR IMPLIED, EXCEPT AS PROVIDED HEREIN, INCLUDING WITHOUT LIMITATION THEREOF, WARRANTIES AS TO MARKETABILITY, MERCHANTABILITY, FOR A PARTICULAR PURPOSE OR USE, OR AGAINST INFRINGEMENT OF ANY PATENT. IN NO EVENT SHALL WE BE LIABLE FOR ANY DIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES OF ANY NATURE, OR LOSSES OR EXPENSES RESULTING FROM ANY DEFECTIVE PRODUCT OR THE USE OF ANY PRODUCT.
- (2) NO ORAL OR WRITTEN INFORMATION OR ADVICE GIVEN BY US, OUR DEALERS, DISTRIBUTORS, AGENTS OR EMPLOYEES SHALL CREATE A WARRANTY OR IN ANY WAY INCREASE THE SCOPE OF THIS WARRANTY.

Installation, Relocation and After-sale Technical Service

Installation of the instrument shall be carried out by or under supervision of our qualified service personnel or our authorized service agent.

Before installation of the instrument, the customer shall make preparations for satisfying the installation requirements in accordance with this manual.

When relocation of the instrument becomes necessary after initial installation (delivery), please notify our local sales representative or service office nearest you.

Technical Seminars and Training for Customers

For the customers to acquire in-depth understanding of the analytical instruments, technical seminars and customer training courses are available at our or your site. For further information, contact our local sales representative.

(The technical seminars and customer training courses are available at charge.)

OTHER PRECAUTIONS

Handling of Chemicals and Samples

- (1) The user is responsible for following relevant legal standards and regulations in the handling, storage and discarding of chemicals and samples used in analytical operations of the instrument.
- (2) Reagents, standard solutions and accuracy-control samples shall be handled, stored and discarded as instructed by the respective suppliers.

Notice on This Instruction Manual

- (1) The information contained in this manual is subject to change without notice for product improvement.
- (2) This manual is copyrighted by Hitachi High-Technologies Corporation with all rights reserved.
- (3) No part of this manual may be reproduced or transmitted in any form or by any means without our express written permission.

Trademark Acknowledgments

Microsoft, Windows, Microsoft Excel, Microsoft Word, and Windows 98 are registered trademarks of Microsoft Corporation USA.

All other brand names and product names used in this manual are trademarks, registered trademarks, or trade names of their respective holders.



SAFETY SUMMARY

PRECAUTIONS ON SAFETY

Before using the Hitachi fluorescence spectrophotometer, be sure to read the following safety instructions carefully.



General Safety Guidelines

- Follow all the operating procedures provided in this manual.
- Installation and maintenance of this instrument shall be carried out by service personnel qualified therefore.
- Be sure to observe the warnings indicated on the product and in the instruction manual. Failure to do so could result in personal injury or damage to the product.
- The hazard warnings which appear on the warning labels on the product or in the manual have one of the following alert headings consisting of an alert symbol and signal word DANGER, WARNING, or CAUTION.



DANGER : Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury.
(This warning does not apply to this product.)



WARNING : Indicates a potentially hazardous situation which, if not avoided, can result in death or serious injury.



CAUTION : Indicates a hazardous situation which, if not avoided, will or can result in minor or moderate injury, or serious damage to the product.



: The alert symbol shown at left precedes every signal word for hazard warnings, and appears in safety-related descriptions in the manual.

"NOTE" and "NOTICE" are heading words which do not concern personal safety directly.



SAFETY SUMMARY



General Safety Guidelines (Continued)

NOTICE: Used to indicate an instruction for preventing damage to the product.

NOTE : Used to indicate an instruction for ensuring correct use of the product and accurate analysis therewith.

- Do not modify the instrument, replace parts that are not user-serviceable, use non-specified parts, nor remove safety devices, as it could be hazardous.
- Installation at delivery, maintenance and relocation should be referred to service personnel qualified by us.
- Do not perform any operation or action other than described in this manual. When in doubt, please contact our local sales representative or service office nearest you.
- When using a chemical for analytical operation, be sure to provide proper ventilation in the laboratory room as per local requirements. Inadequate ventilation could endanger your health.
- Keep in mind that the hazard warnings in this manual or on the product cannot cover every possible case, as it is impossible to predict and evaluate all circumstances beforehand.
Be alert and use your common sense.



SAFETY SUMMARY

Safety Notices on Fluorescence Spectrophotometer

Electricity

- Make sure that the fluorescence spectrophotometer is supplied with power of 100V AC, 1 kVA or more (50 Hz or 60 Hz). If the power voltage fluctuates or noise is applied to the power line, the spectrophotometer may be adversely affected, resulting in any trouble in it.
- Be sure to provide grounding connection together with power line connection. The grounding resistance should be 100 ohms or less (conforming to Class-D grounding installation of electric facility technical standards). Check that the grounding connection is proper. If the grounding connection is improper, the spectrophotometer may become vulnerable to external noise or stray voltage may be produced in it, resulting in an electric shock hazard.
- The high voltage circuit is used inside the spectrophotometer. To prevent electric shock, do not open covers during operation unless otherwise necessary.

Source of Heat/Flame

Do not smoke or hold a flame near the fluorescence spectrophotometer.

Data Backup

Data stored on the hard disk may become unusable due to a system failure, wrong operation, computer virus infection, etc. To ensure data integrity in case of accidental damage to the hard disk, periodically make backup copies of hard disk files onto floppy disks.

To prevent an erroneous operation on the hard disk, always reserve approx. 100 MB of free space as a working area for application software.



SAFETY SUMMARY

Protection against Computer Viruses

If any program/data is damaged suddenly or an unexpected operation/screen is encountered, the personal computer is suspected of being infected by a computer virus.

Computer viruses are malicious programs that sneak into personal computers to cause misbehavior or damage to data. And, a program designed to offer protection against computer viruses is called a vaccine program.

Possible causes of virus infection are:

- Downloading a virus-laden program through communication.
- Using a floppy disk or other storage medium infected by a virus.

Note also that once any personal computer is infected by a virus, it may spread to other computers via communication or storage medium.

Never use a program or storage medium that is suspected of containing a virus.

If there is a possibility of virus infection, check for a virus using a vaccine program. Note, however, that some kinds of vaccine programs cannot eradicate particular viruses. **So, be sure to make a backup of hard disk files beforehand.**

The user is requested to prepare a vaccine program and carry out virus removal on his or her own responsibility.

Power Interruption

On occurrence of momentary power voltage drop due to power interruption or lightning, the personal computer may become faulty or the system software, application software or data may be damaged. For protection against momentary power voltage drop, it is advisable to use an AC uninterruptible power supply unit (stated according to the Japanese Electronic Industry Development Association guidelines for protection against momentary power voltage drop in personal computers).



SAFETY SUMMARY

Turning On/Off Personal Computer

Do not turn off power to the personal computer while the hard disk or floppy disk drive is active. If power to the personal computer is turned off while the hard disk or floppy disk is being accessed, the personal computer may become faulty or data/software stored in it may be damaged.

Before turning off power to the personal computer, be sure to quit the fluorescence spectrophotometer control/data processing program (FL Solutions program) first and then take the shut-down procedure using the system software.

FOREWORD

- This instruction manual is intended for the users of the Hitachi fluorescence spectrophotometer. It describes the installation, maintenance, operation and measurement procedures.
- The fluorescence spectrophotometer is designed for the purpose of fluorescence analysis. Never use this instrument for any other purposes.
- The fluorescence spectrophotometer is intended for use by persons having a basic knowledge of chemical analysis. Other persons should use them in the presence of a person familiar with chemical analysis. Remember that improper use of analytical instruments, chemicals or samples would result not only in wrong analytical data but also in consequences adverse to safety.
- Before attempting installation/maintenance of the fluorescence spectrophotometer, read the instruction manual carefully and fully understand the cautionary instructions for the sake of safety.
- Keep this manual handy for easy reference when necessary.
- The information contained in this manual is subject to change without prior notice.

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1. FUNCTIONS AND SCREEN COMPOSITION

1.1 Functions

In this program, the following measurement modes are available:

- Wavelength scan
- Time scan
- Photometry
- 3-D Scan

(‘3-D Scan program’ (option) required for Model F-2500)

Wavelength Scan

- (1) Photometric value data can be printed at the specified wavelength intervals or at up to 12 specified wavelengths.
- (2) The function keys provide functions unique to the wavelength scan mode.
Measurement: [F4], Stop: [F6]
- (3) Wavelength scan measurement can be repeated max. 99 times. And, a repetition period is settable.

Time Scan

- (1) Kinetics, or rate calculation will be performed.
- (2) Photometric value data can be printed at the specified time intervals or at up to 12 specified time points.

Photometry

- (1) Quantification method is selectable among specified wavelength, peak area, peak height, peak ratio and derivative methods. Up to 20 standards can be measured. A calibration curve is prepared by drawing polygonal lines through each point of standard concentrations or a regression calibration curve is prepared by least square fit. It is also possible to measure up to 20 standards and prepare a calibration curve based on an average value of standard concentrations.
- (2) A calibration curve can be redrawn by re-measuring a specific standard. In a calibration curve graph on the screen window, a value at each point can be read out using the cursor.

1.1 Functions

- (3) A calibration curve can be printed.
A calibration curve can be drawn by inputting its factor value.
- (4) Measurement data and a calibration curve are displayed on the same screen window. It is therefore possible to carry out measurement while observing the calibration curve.
- (5) According to the coefficient of determination calculated automatically, judgment can be made on a degree of matching between standard values and a calibration curve. Two/three-wavelength calculation is permitted in preparation of a calibration curve.
- (6) Statistical calculation is possible.
Mean value of measured data and standard deviation (SD) can be calculated.
- (7) Upper/lower limit judgment will be performed.
- (8) Spectrum display under photometry
In the data measured by a quantification method; peak height, peak area, peak ratio or derivative, a spectrum due to wavelength scan is displayable.

3-D Scan

- (1) Spectral calculation can be performed using the measured data.
- (2) A peak table can be displayed.
- (3) A bird's-eye view/synchronous spectrum can be displayed and printed.
- (4) Printing conditions are selectable.
In use of the DDE function, the wavelength interval and start/end wavelengths of data list can be selected.

Secondary Processing of Data

Measured data can be saved, and a variety of processings can be carried out on the saved data.

- (1) Automatic reading of data (spectrum tracing)
By placing the cursor on the currently displayed spectral graph, a photometric value at the cursor position can be read out.
- (2) Peak detection
Peaks and valleys of the currently displayed spectrum are detected automatically.
- (3) Change of scale
A measured spectrum can be enlarged or reduced by changing the ordinate and abscissa scales.
- (4) Smoothing
A measured spectrum can be smoothed for elimination of noise components.
- (5) Derivative
Spectral derivative is obtainable. It is permitted to specify an order of derivative, that of smoothing and a count value of data.
- (6) Spectrum calculation
Fundamental arithmetic operations can be made between spectra or a spectrum can be multiplied with a factor.
- (7) Area
The area value of the specified peak will be calculated.
The average area value is also calculable among peaks.
- (8) File conversion
Measurement data can be converted into an ASCII text file (*.txt) or JCAMP-DX file (*.dx). It is also possible to save a spectrum and calibration curve graph in a Windows meta file (*.wmf).
- (9) Display/print font setting
The font, style and size of characters to be displayed and printed can be changed as desired.

1.1 Functions

- (10) Data acquisition
Photometric value data on the data processing window and monitor window can be acquired. Acquisition is allowed within 100 data.
- (11) Printing items collectively specifiable
Printing items on overlaid spectra can be specified collectively.
- (12) Specification of printing items
Items in a peak table can be selected and printed.
- (13) Portrait or Landscape printing settable
Printing can be made Portrait or Landscape.
- (14) Collective spectrum printing
Printing can be carried out just by specifying a file.
- (15) Selection of spectrum to be displayed
The spectrum to be displayed for overlaying is selectable.
- (16) Automatic saving mode ON/OFF settable
ON/OFF can be set in the sample name/comment input window.
Automatic saving is effective in all the modes that use a sample table.
- (17) Data in each mode can be transferred to Microsoft® Excel.
(This is valid when Microsoft® Excel 97 has been installed in PC.)
- (18) After copying spectral data into commercial software such as Microsoft® Word, its scale, line type, color, etc. are changeable on the software.
- (19) The report generator (separately available, P/N 250-0455) can be used.

1.2 Installing the FL Solutions Program

For using the FL Solutions program, it must be installed using the attached system disks.

Installation of the FL Solutions program is required when using it at the first time (not required later for each operation). In the following installation session, it is assumed that:

- The floppy disk drive is assigned as drive A.
- The destination of installation is assigned as drive C.

- (1) Using the mouse, click the Start button of the Windows 98 and select **Settings - Control Panel**. The Control Panel window will then appear as shown in Fig. 1-1.

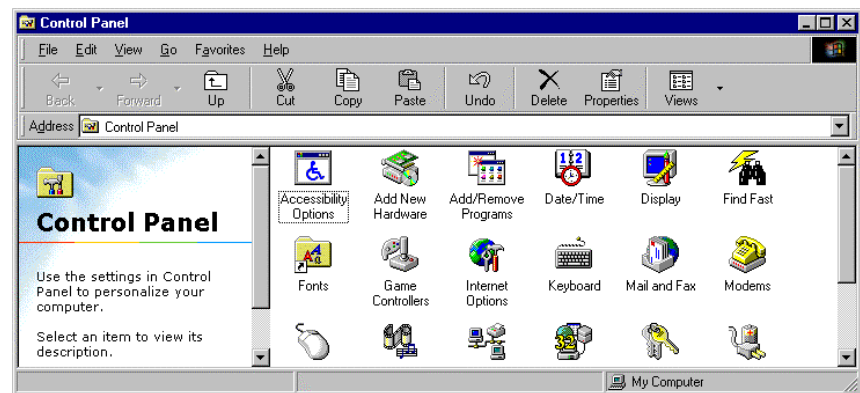


Fig. 1-1

- (2) In the Control Panel window, double-click the Add/Remove Programs icon. The Add/Remove Programs dialog box will then appear as shown in Fig. 1-2. In this dialog box, click **Install**.

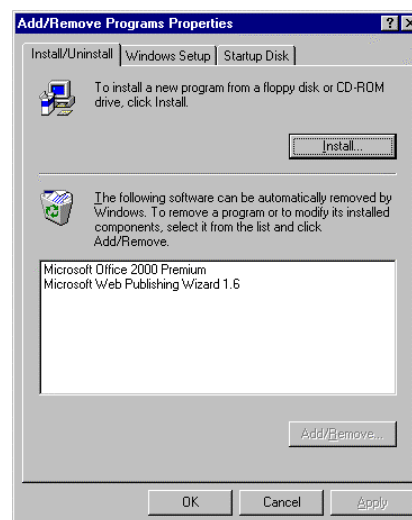


Fig. 1-2

1.2 Installing the FL Solutions Program

- (3) Then, when the dialog box shown in Fig. 1-3 appears, push in the first one of the attached system disks and click **Next**.

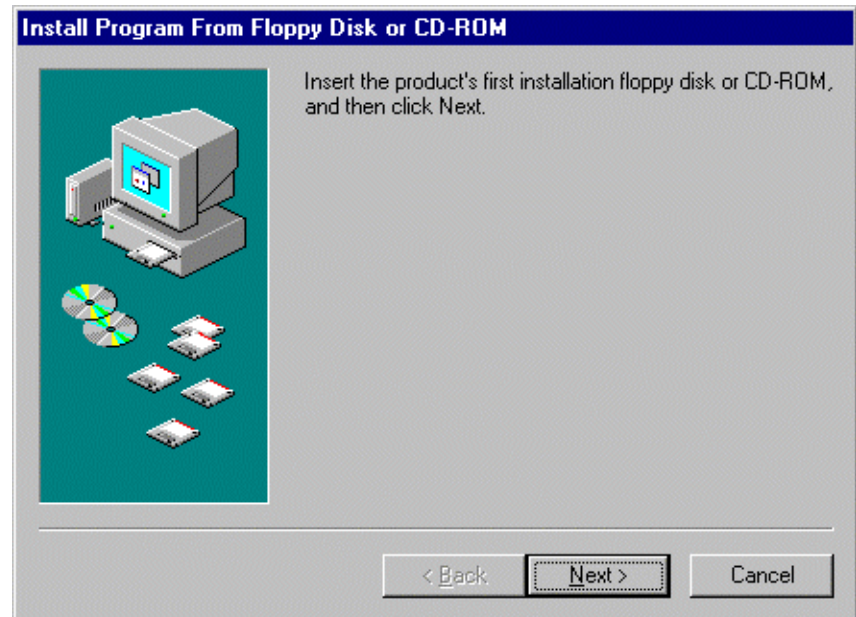


Fig. 1-3

- (4) Then, when the dialog shown in Fig. 1-4 appears, click **Finish**.

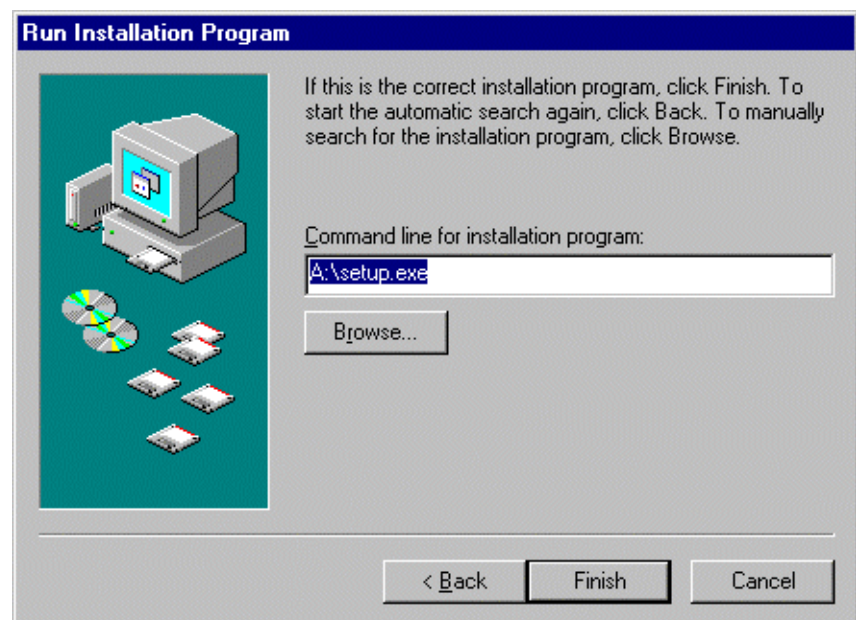


Fig. 1-4

Installation process will then begin. Take steps according to the instruction messages.

- (5) On completion of the installation process, restart the personal computer.
- (6) For startup of the FL Solutions program, refer to 1.3.

NOTES: 1. Monitor resolution must be set at 800 × 600 at minimum. Otherwise, each window of the FL Solutions program may not be displayed fully.

2. When the large font size of display is selected, words may not be indicated normally on each window. In this case, font size should be changed to the small one. For change, select **Control Panel - Display Properties - Settings - Font Size**.

1.2 Installing the FL Solutions Program

Reference:

Re-installation of FL Solutions Program

For re-installing this program, remove it first and then install it in the following procedure.

Select **Start - Settings - Control Panel**. In the Control Panel window, double-click the Add/Remove Programs icon, and the dialog box shown in Fig. 1-5 will then appear.

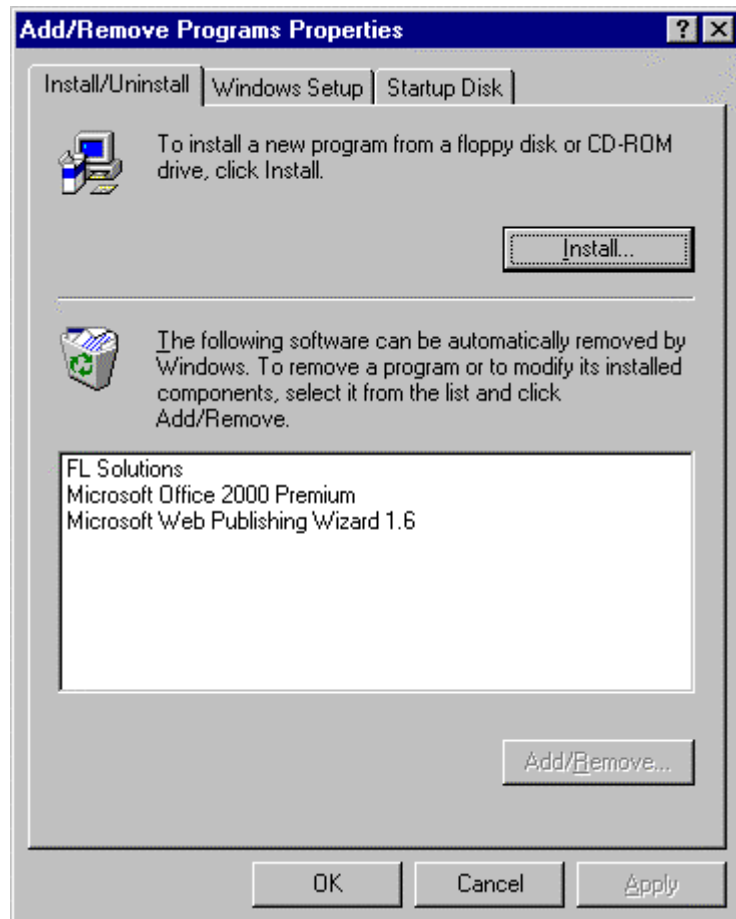


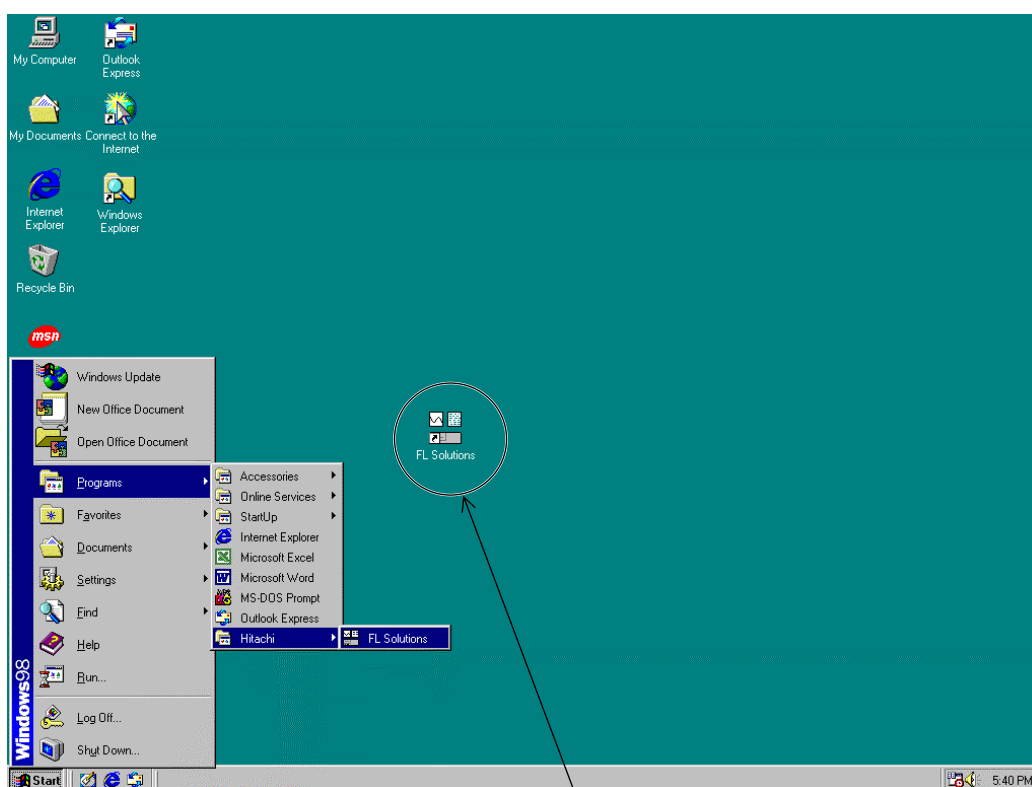
Fig. 1-5

In this dialog box, select "FL Solutions" and click the **Add/Remove** button. When it is removed, click **OK**. Thereafter, install the FL Solutions program again.

1.3 Startup of FL Solutions Program

To start up the FL Solutions program, take the following procedure.

- (1) Connect the communication cable (standard accessory) between the fluorescence spectrophotometer and the personal computer. Then, turn on power to the spectrophotometer and the computer in this order.
- (2) The Windows 98 starts.
- (3) Click the Start button. Point to Programs and Hitachi Applications and select FL Solutions. (See Fig. 1-6.) Or double-click the FL Solutions program icon.
If the FL Solutions program is set as a startup application in the Windows 98, the program automatically starts when turning on power supply of the personal computer. This setting can be selected at installation of the program.



Icon of FL Solutions program

Fig. 1-6

1.3 Startup of FL Solutions Program

- (4) The fluorescence spectrophotometer connected to the communication COM port of computer is now recognized and the window shown in Fig. 1-7 is displayed. Then, the spectrophotometer is initialized.

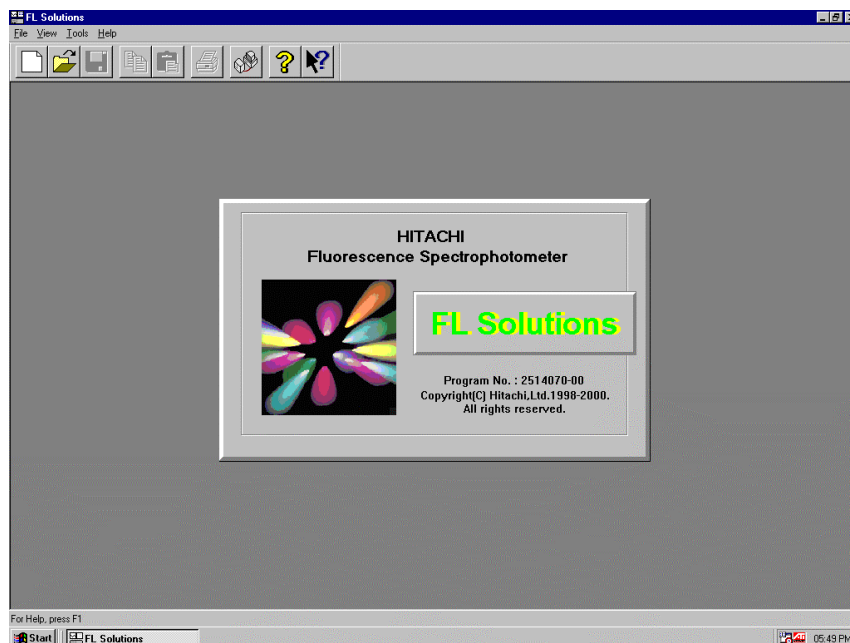


Fig. 1-7

- (5) A gauge of initialization is displayed as shown in Fig. 1-8, so its progress can be visually checked.

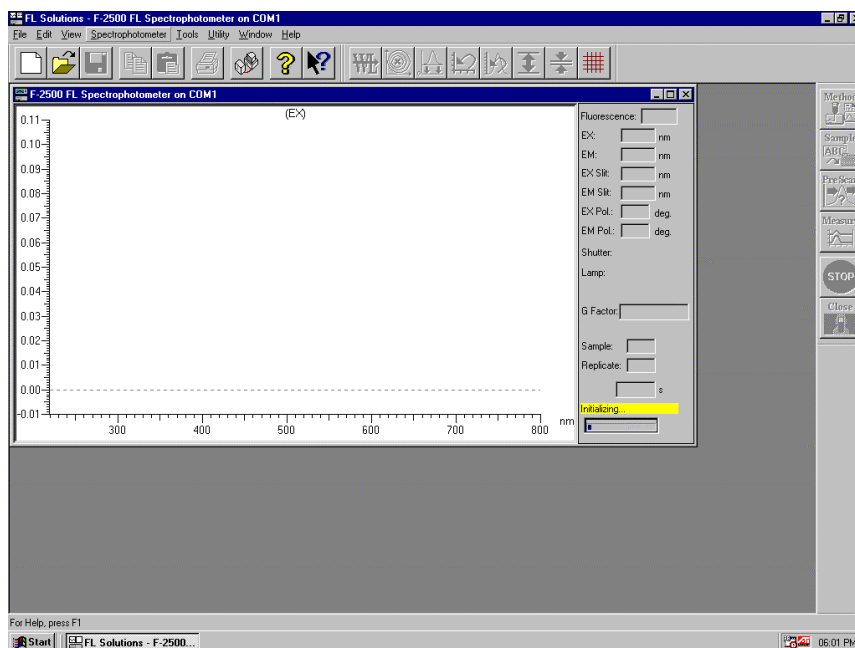


Fig. 1-8

NOTE: If the spectrophotometer is not connected with the COM port or its power is not turned on, the monitor window ("F-2500 Fluorescence Spectrophotometer on COM1" indicated on its title bar) is not presented. In this status, functions for other than measurement are usable. For measurement, first confirm connection of the cable, and then turn on power supply of the spectrophotometer. Next, select the Instruments (I) command from the Tools (T) menu. When selecting and connecting the spectrophotometer, the window shown in Fig. 1-8 is presented.

In execution of initialization, self-diagnosis is performed for the following items:

- ROM
- RAM
- Wavelength drive system
- Xenon lamp turn-on
- EEPROM
- Slit mechanism
- Chopper
- Backup battery voltage (only with Model F-4500)
- Contents of wavelength and slit calibration values

If an abnormality is found in the self-diagnosis, the relevant error message will be displayed. In this case, a corrective measure should be taken, referring to 4.7 of the maintenance edition.

If necessary, notify our local repair service office.

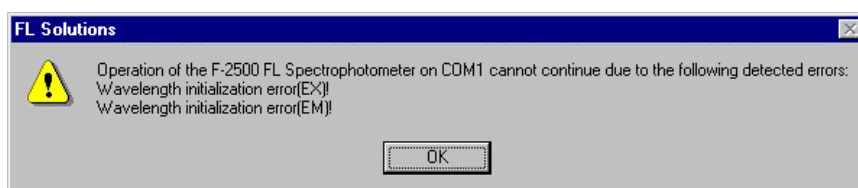


Fig. 1-9

1.3 Startup of FL Solutions Program

- (6) On completion of initialization, the window shown in Fig. 1-10 is displayed.

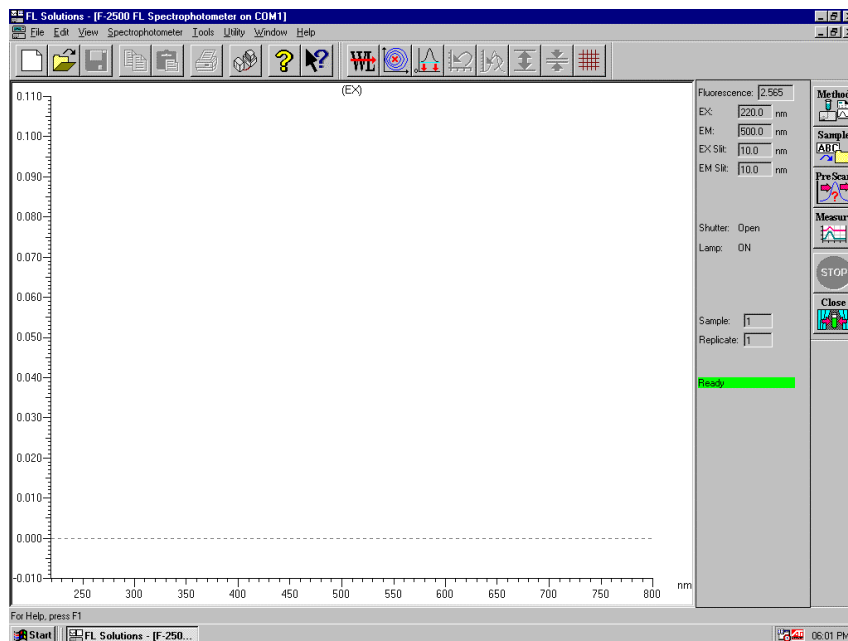


Fig. 1-10

1.4 Screen Composition

1.4.1 Basics of Window Operation This manual contains multiple methods for executing a function. For implementing each method, explanation is given using the naming described below.

(1) Menu bar and Command

Menu bar is a bar on which menus such as “File (F)” and “Edit (E)” are arranged. Commands are contained in each menu.

In the case shown in Fig. 1-11, an expression of “Scan (S) command of Spectrophotometer (S) menu” is used.

A menu or command is selectable by 2 methods.

- Move the mouse pointer to a menu and click on it.
- Key in the alphabetic letter after a menu name with the Alt key held down. Then, key in the alphabetic letter after a command name.

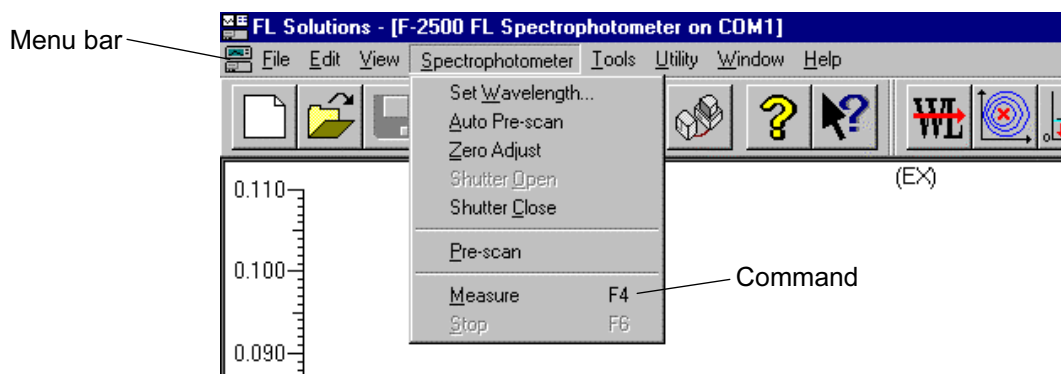






Fig. 1-11 Menu Bar and Command

1.4 Screen Composition

(2) Toolbar and Tool button

Toolbar is a row of tool buttons such as  and tool buttons provide shortcuts for common menu commands. In using , it is expressed as “ button of Measurement toolbar” or “ (analytical condition) button.”

For details of each button, refer to 1.4.4.

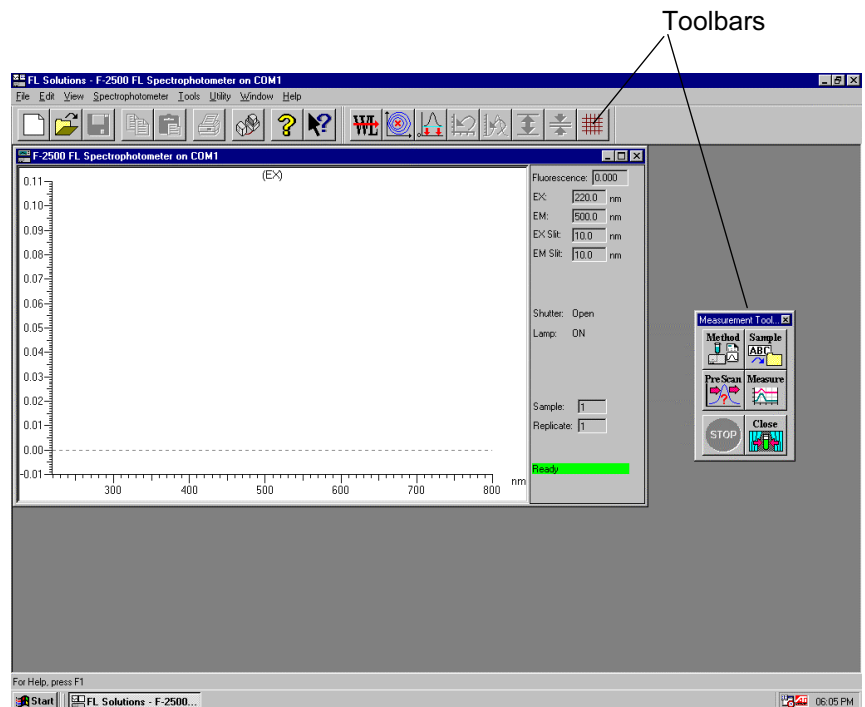



Fig. 1-12 Toolbar

(3) Dialog box

When you click the  button of Measurement toolbar, a small window as shown in Fig. 1-13 is presented. This window is called a “dialog box.”

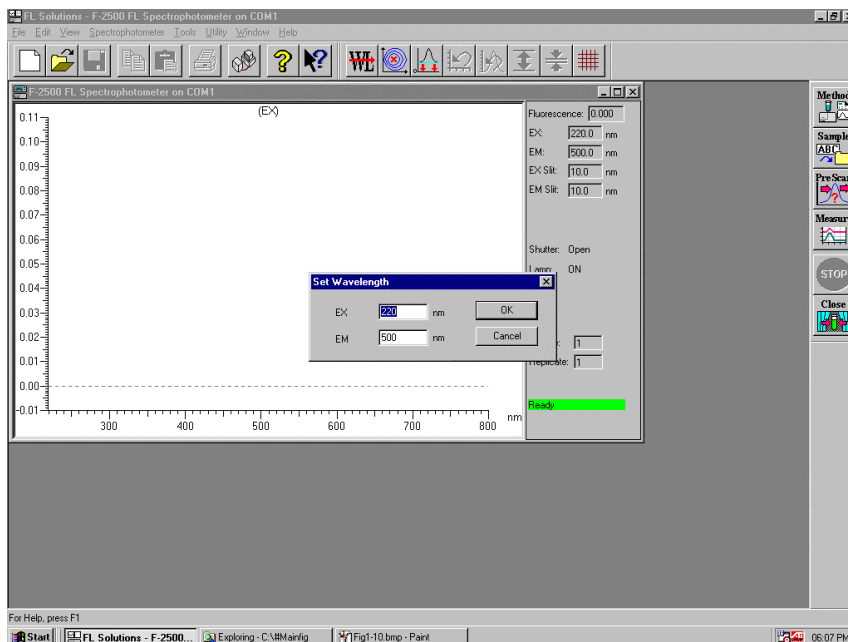


Fig. 1-13 Dialog Box

(4) Tab

Tabs are used in the Analysis Method window or Property window. Click a tab for changing display. Each tab contains setting items. The display shown in Fig. 1-14 is expressed as “Instrument tab.”

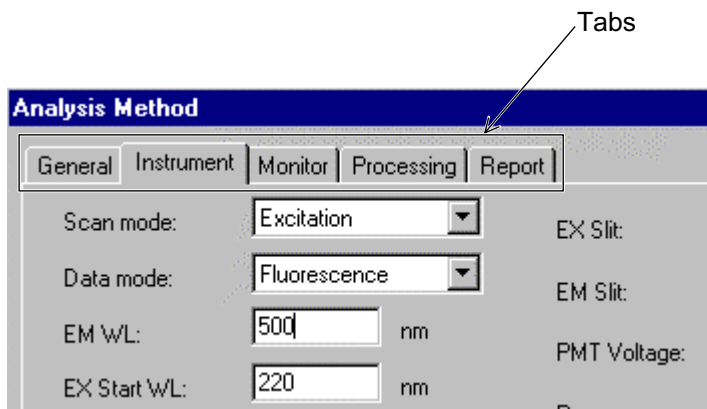




Fig. 1-14

1.4 Screen Composition

(5) Spin button

This button is used for inputting a numeral. Click  or  for setting a numeral. However, a value below decimal point cannot be set. Such a value must be input with the keyboard.

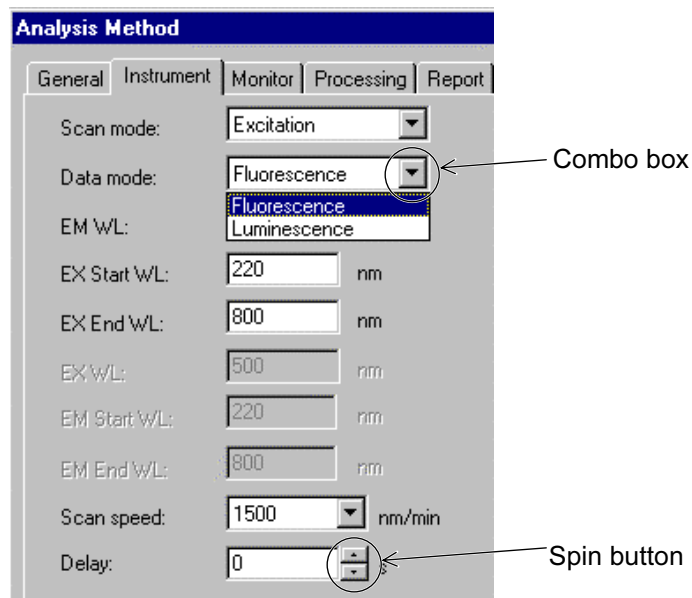



Fig. 1-15 Spin Button and Combo Box

(6) Combo box

Selection is allowed among the predetermined conditions. Click  for selecting the desired condition.

(7) Check box

For turning on a check box, put a check mark in it by clicking.

The check box is then set in the  status.

For turning off the check box, click it again to remove the check mark.

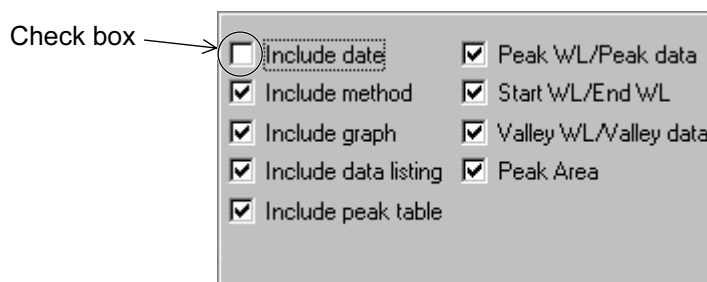


Fig. 1-16 Check Box

1.4.2 Kinds of Windows The FL Solutions program have two kinds of windows; monitor window and data processing window.
Set the monitor window active for measurement, or the data processing window for data processing.
The toolbar differs between these windows.

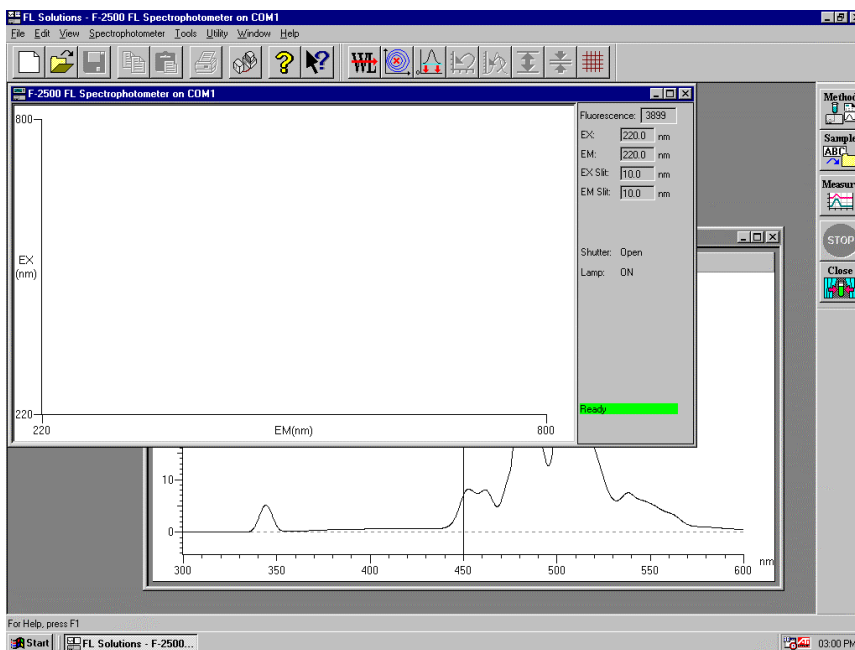


Fig. 1-17 Monitor Window

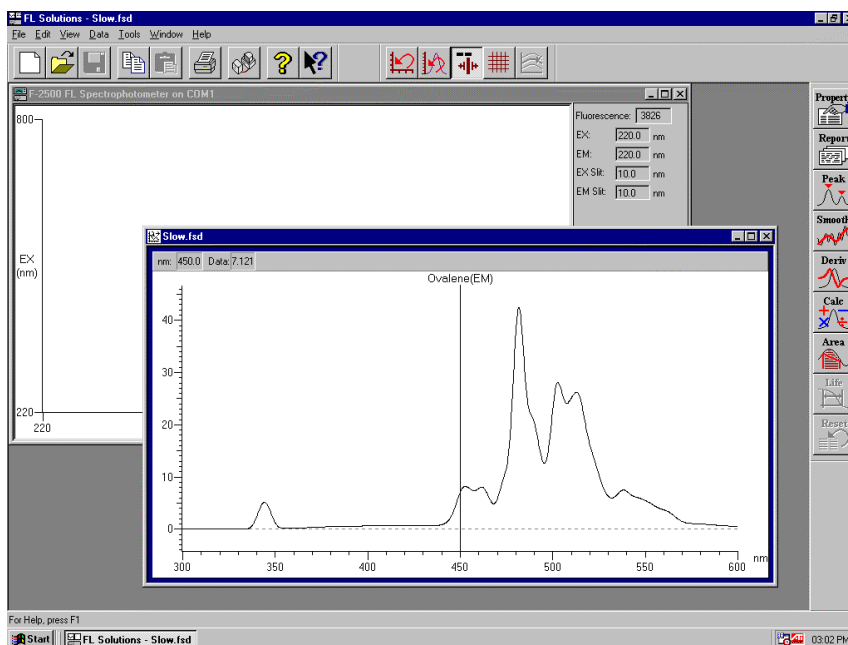


Fig. 1-18 Data Processing Window

1.4 Screen Composition

1.4.3 Data Processing Window

The items displayable in each measurement mode are shown below.

(1) Wavelength Scan

A spectrum alone can be displayed or both spectrum and peak table can be displayed as shown in Fig. 1-19.

Display of the peak table can be turned ON/OFF from the View menu.

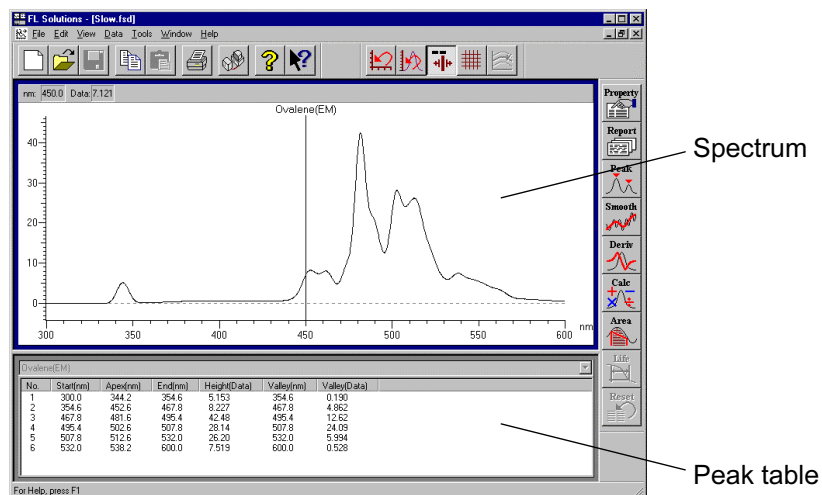


Fig. 1-19 Wavelength Scan

(2) Time Scan

A spectrum alone can be displayed or both spectrum and kinetics result table can be displayed as shown in Fig. 1-20.

Display of the kinetics result table or peak table can be turned ON/OFF from the View menu.

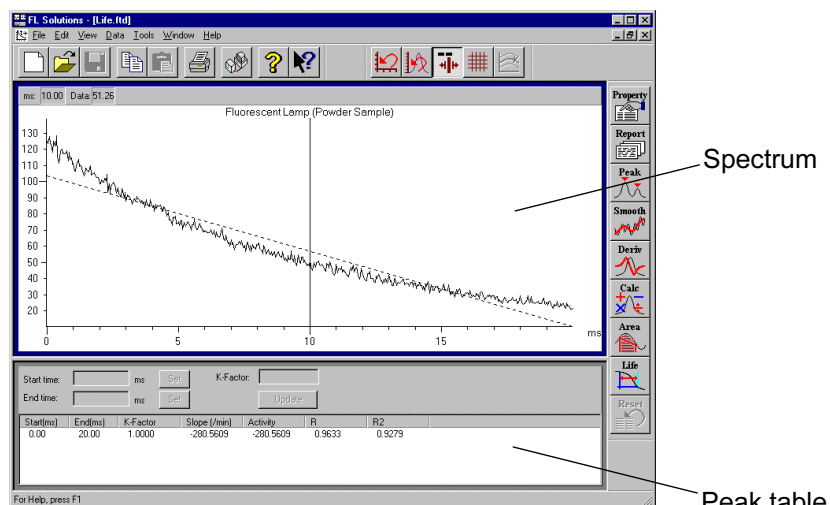


Fig. 1-20 Time Scan (kinetics)

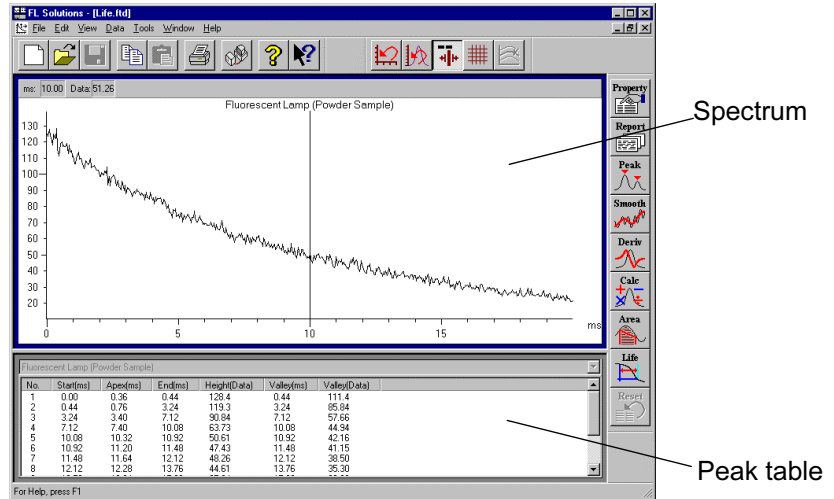


Fig. 1-21 Time Scan (peak table)

(3) Photometry

The items shown in Fig. 1-22 are selectively displayed depending on measurement method.

- On measurement starting with calibration curve

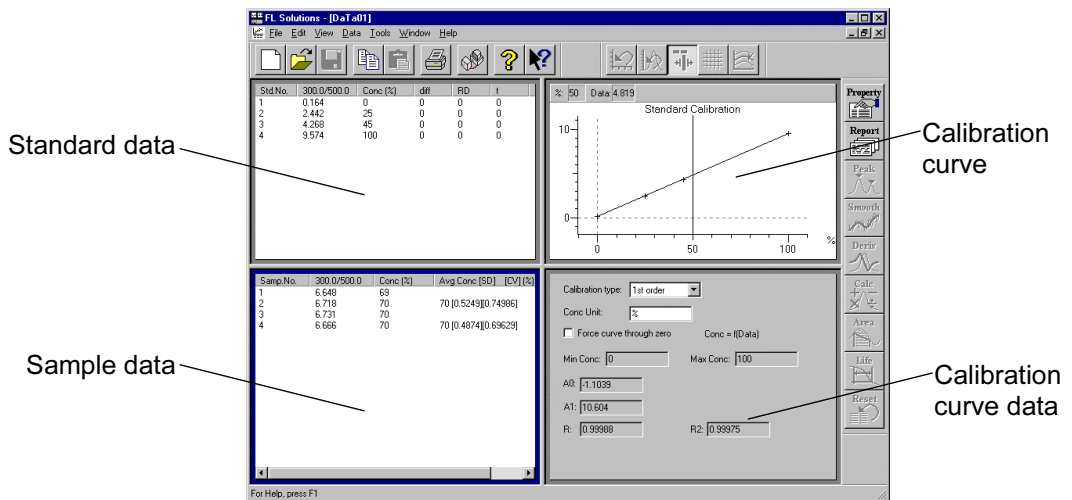


Fig. 1-22

1.4 Screen Composition

- On generation of calibration curve through factor input

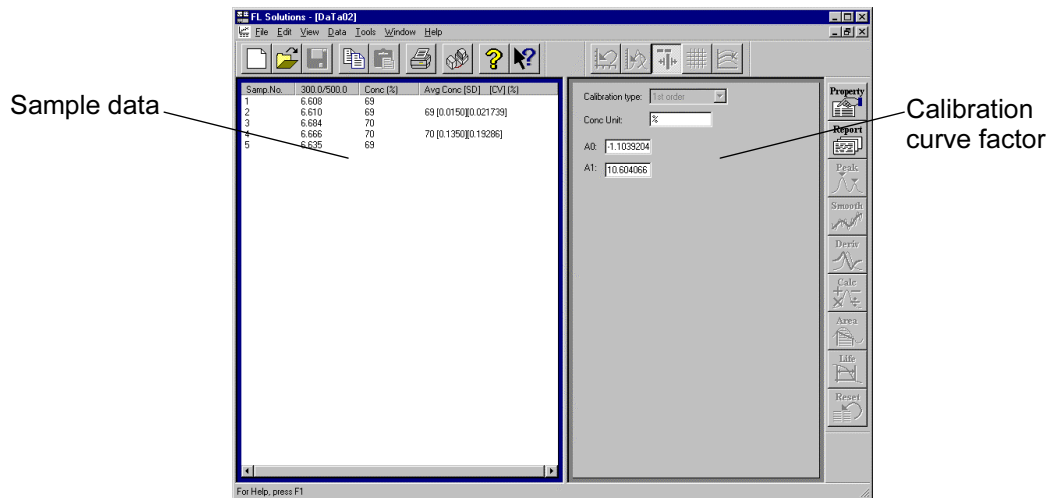


Fig. 1-23

- On display of photometric value at each wavelength in multi-wavelength mode

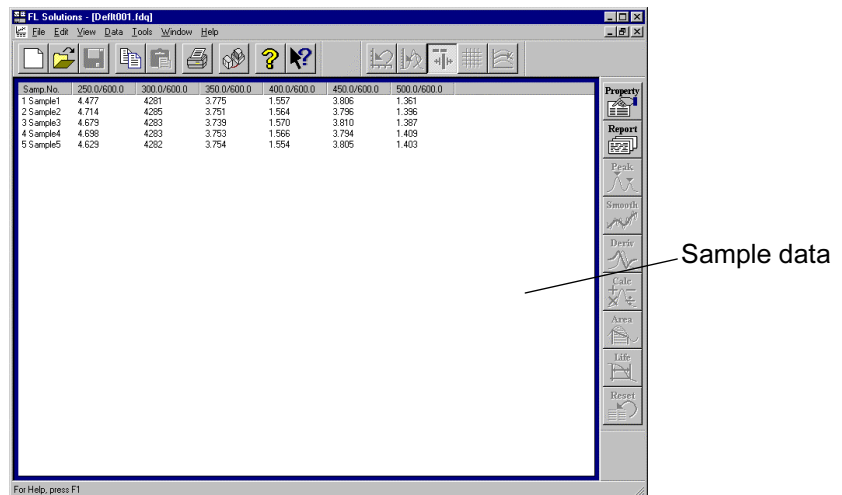


Fig. 1-24

1.4.4 Explanation of Tool Buttons

The FL Solutions program provides five kinds of toolbars as shown below. You can select a command by pulling down each menu from the menu bar. But for quick and easy operation, it is advisable to use these tool buttons.

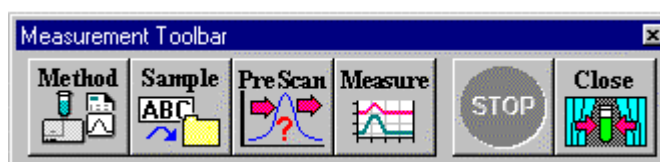
- Standard Toolbar
Has buttons related to printing and file handling.



- Instrument Toolbar (displayed in monitor window)
Has buttons related to spectrophotometer control such as wavelength drive and baseline measurement.



- Measurement Toolbar (displayed in monitor window)
Has buttons related to measurement such as analysis method setting and sample name input.

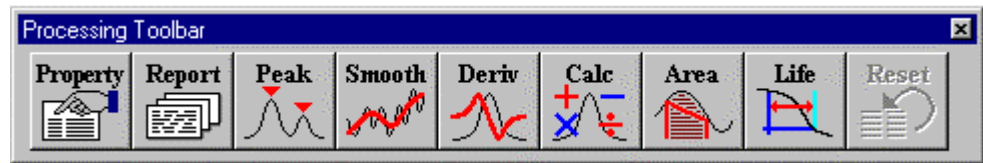


- Spectrum Toolbar (displayed in data processing window)
Has buttons related to spectral display such as auto scaling, ruled line indication and spectrum tracing.



1.4 Screen Composition

- Processing Toolbar (displayed in data processing window)
Has buttons for smoothing, differentiation, etc.



These five toolbars are independent and each of them can be located at a desired position as shown in Fig. 1-25 (monitor window).

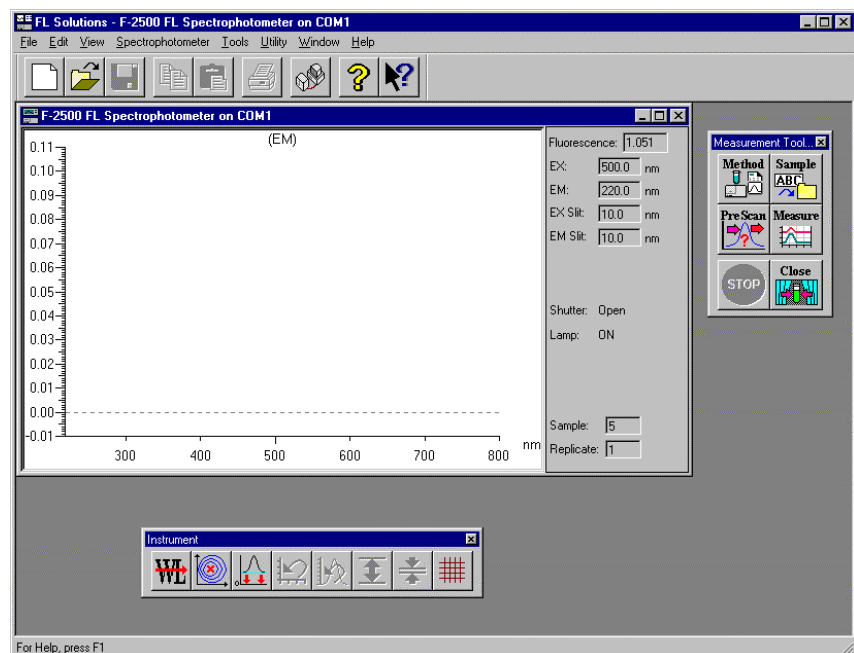


Fig. 1-25 An Example of Toolbar Arrangement

The following tables present the functional descriptions of the buttons on each toolbar.

The function keys on the keyboard can also be utilized. For the functional assignment to each function key, refer to 1.5.

Table 1-1 Standard Toolbar










Button	Function
	Creates a new analysis method/sample table.
	Opens data files.
	Saves the current file.
	Stores a selected character string or graphic item into the clipboard.
	Pastes the contents of the clipboard.
	Opens the print window.
	Indicates the present status of the spectrophotometer connected.
	Indicates the information (version number, etc.) on the FL Solutions program.
	Calls up the help window.

Table 1-2 Measurement Toolbar









Button	Function
	Opens the Analysis Method window.
	Displays the sample name/comment name entry window.
	Displays the sample table for sample name/comment name input.
	For manual input of standard sample values.
	Starts measurement.
	Stops measurement.
	Closes the shutter.
	Opens the shutter.

Table 1-3 Spectrum Toolbar












Button	Function
	Restores the scale to its original condition.
	Indicates ruled lines.
	Indicates a bird's-eye view.
	For display from the rear.
	For display from the right side.
	Selects a spectrum to be displayed.
	Indicates the trace cursor.
	For display in a contour map.
	For display from the front.
	For display from the left.
	Automatically sets the scale.

Table 1-4 Instrument Toolbar




















Button	Function
	Performs wavelength drive.
	Carries out automatic pre-scanning.
	Conducts zero-point adjustment.
	Enlarges the vertical-axis double.
	Reduces the vertical-axis to half.
	Restores the scale to its original condition.
	Adjusts the vertical-axis display automatically so that you can view the entire spectral graph.
	Indicates ruled lines.

Table 1-5 Processing Toolbar

Button	Function
	Opens the Spectrum Properties window.
	Outputs a report of data file.
	Performs peak detection.
	Performs differentiation.
	Performs peak detection in 3-dimensional measured data.
	Performs area calculation.
	Performs fundamental arithmetic calculations between spectra.
	Calculates the life of phosphorescence.
	Performs smoothing.
	Resets data.
	Performs fundamental arithmetic calculations of 3-dimensional spectra.

1.5 Functions of Function Keys

1.5 Functions of Function Keys

The function keys on the keyboard provides the following functions.

Each function can be directly executed.

Key No.	Wavelength Scan	Time Scan	Photometry	3-D Scan
[F4]	Measurement	Measurement	Sample measurement	Measurement
[F5]			Blank measurement	
[F6]	Stop	Stop	Stop	Stop
[F7]			Interrupting measurement	
[F8]			Remeasurement	
[F9]			End	

1.6 Explanation of Monitor Window

The monitor window contains the following information.

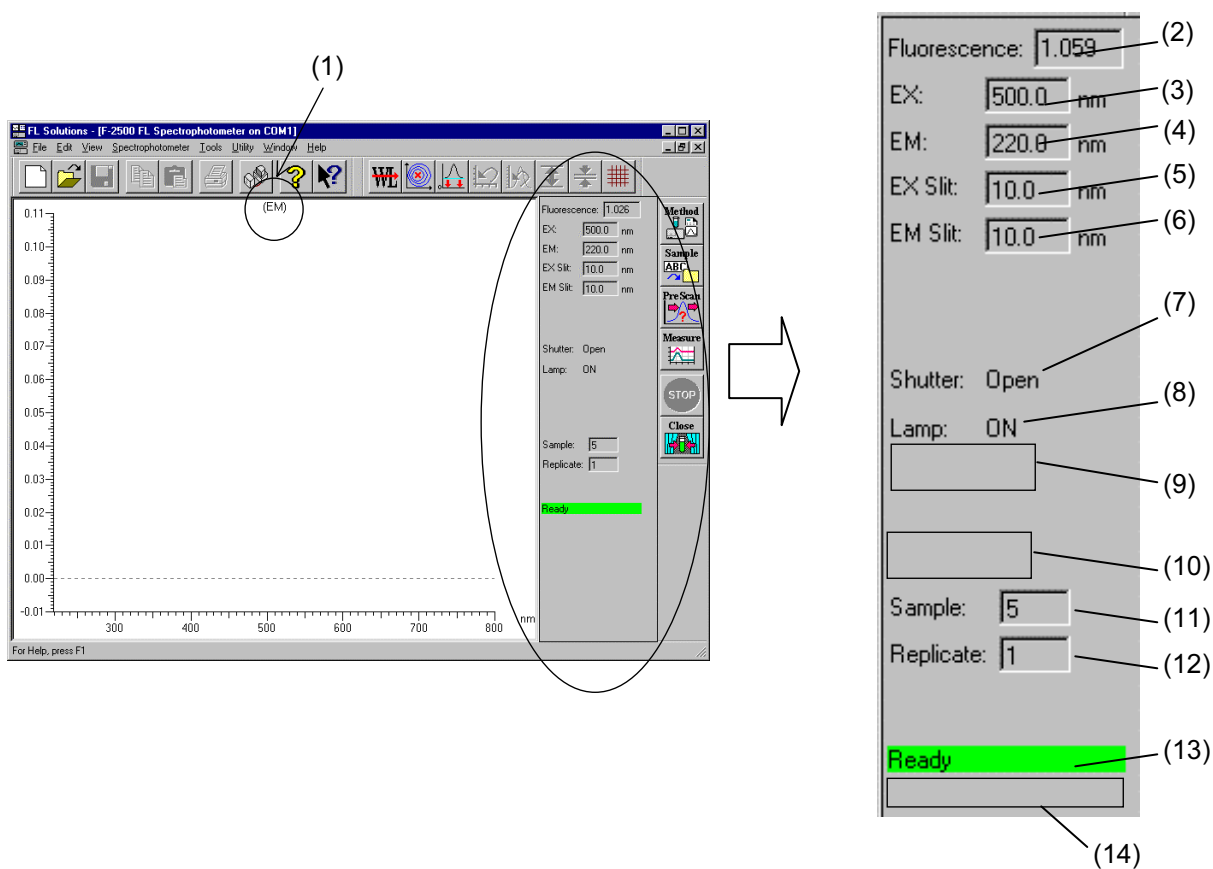


Fig. 1-26 Monitor Window

(1) Sample name indication

A sample name is indicated. For its input, use the sample table or sample name/comment input window.

(2) Current photometric value indication

The current photometric value is indicated.

(3) EX

The current excitation wavelength is indicated.

(4) EM

The current emission wavelength is indicated.

1.6 Explanation of Monitor Window

(5) EX slit

The current excitation slit width is indicated.

(6) EM slit

The current emission slit width is indicated.

(7) Shutter

Shutter opening or closing is indicated.

(8) Lamp

ON/OFF state of the light source lamp is indicated.

(9) Shutter control

The active state of shutter control is indicated.

(10) Corrected spectra

Setting of spectrum correction is indicated.

(11) Sample


The number assigned to the sample under measurement is indicated.

(12) Replicate count

A count in repeated measurement is indicated.

(13) Message indication

An information message such as "Ready," "Scanning" or "Parameter" is presented.

When the message "Ready" is indicated, it is allowed to press  (measurement) button.

(14) Error message indication

An error message such as "Xe Lamp Error!" is presented.


If any error message appears, take a proper countermeasure referring to 4.7 of the maintenance edition.

Table 1-6 Possible Error Messages

Message	Indication
ROM Status Error!	A ROM error has occurred.
RAM Status Error!	A RAM error has occurred.
Xe Lamp Error!	The Xe lamp is not lit.
Momentary Power Failure!	Power supply of the spectrophotometer was cut off. Restart the spectrophotometer for initialization.
Shutter Initialize Error	Shutter position cannot be detected.
Zero Adjustment Error	The spectrophotometer is abnormal.
Auto Prescan Error	Peak cannot be detected.
Common Prescan Error	Peak cannot be detected.
EX Wavelength Initialize Error	The initial position of the wavelength drive screw cannot be detected.
EM Wavelength Initialize Error	The initial position of the wavelength drive screw cannot be detected.
Battery Voltage Low	Line voltage has dropped.
EEPROM Error	An EEPROM error has occurred.
H16 Program Error	A program error of H16 has occurred.
Chopper Error	Chopper position cannot be detected.

1.7 Input of Spectrophotometer Serial No.

1.7 Input of Spectrophotometer Serial No.

Select the Instruments (I) command from the Tools (T) menu or click the  button. The dialog box shown in Fig. 1-27 opens.

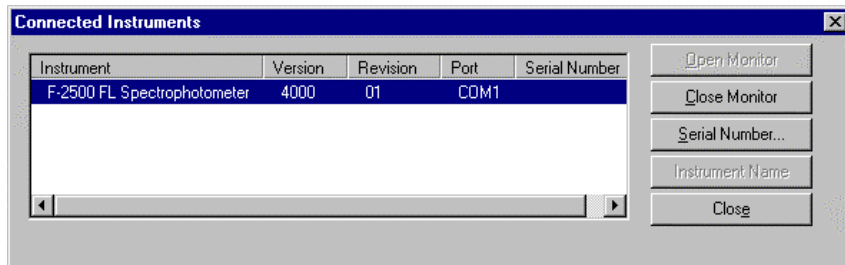


Fig. 1-27

Click the Instrument area and then **Serial Number**. The dialog box shown in Fig. 1-28 will open. In this box, input the serial No. of your spectrophotometer.

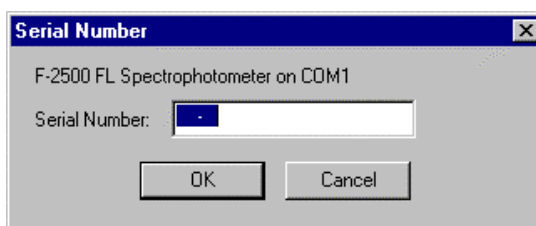


Fig. 1-28

After input, click **OK**.

The serial No. input here is saved as an analytical condition item into the data file and can be printed. In connection with the F-4500 series (F-4500 or F-4510), the relevant spectrophotometer needs to be selected. Click **Instrument Name**. In the dialog box shown below, select the model of your spectrophotometer.

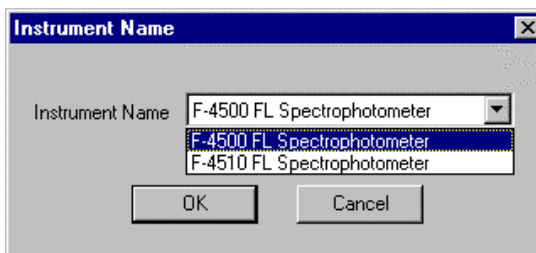


Fig. 1-29 Instrument Name

1.8 Window Setting

The information to be displayed upon starting the FL Solutions program or opening the data processing window can be selected and unnecessary one can be excluded from display.

Select Options (O) command from the Tools (T) menu.

In the Options dialog box, the following settings can be made.

After your setting, click **OK**.

The set functions are validated when starting the monitor window again or opening the data processing window.

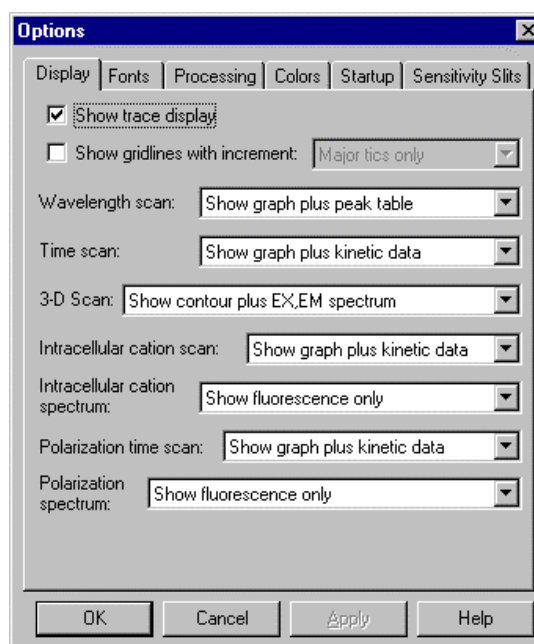


Fig. 1-30 Options Dialog Box

This box has the following tabs.

- Display tab
- Fonts tab
- Processing tab
- Colors tab
- Startup tab
- Sensitivity Slits tab

1.8 Window Setting

(1) Display Tab

Items to be displayed when opening a saved data file or data immediately after measurement can be set here. Set the number of data columns to be displayed. The items other than the number of display columns to be set here can also be set by a tool button or View menu after measurement.

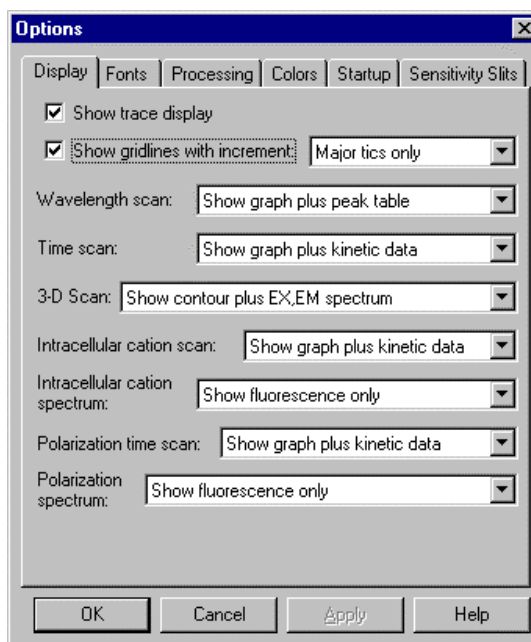


Fig. 1-31 Display Tab

- (a) Trace
Put a check mark in this box, and spectrum can be traced when the data processing window is open.
- (b) Ruled line
The contents of monitor and data processing windows can be displayed with ruled lines.
 - 1) Wide
 - 2) Intermediate
 - 3) Narrow
- (c) Wavelength scan
Items to be displayed when opening the data processing window in the wavelength scan mode can be set.
 - 1) Show graph only
 - 2) Show graph plus peak table

- (d) Time scan
Items to be displayed when opening the data processing window in the time scan mode can be set.
 - 1) Show graph only
 - 2) Show graph plus kinetics data
 - 3) Show graph plus peak table

- (e) 3-dimensional (effective for Model F-2500 when the '3-D Scan program' (option) is installed)
Items to be displayed when opening the data processing window in the time scan mode can be set.
 - 1) Show contour plus EXEM spectrum
 - 2) Show contour, EXEM spectrum plus peak table

- (f) Intracellular calcium (effective when the 'intracellular calcium measurement program' (option) is installed)
Items to be displayed when opening the data processing window in the intracellular calcium mode can be set.
 - 1) Show graph only
 - 2) Show graph plus kinetics data
 - 3) Show graph plus average data
 - 4) Show graph plus markers

- (g) Intracellular calcium spectrum (effective when the 'intracellular calcium measurement program' (option) is installed)
Items to be displayed when opening the data processing window in the intracellular calcium mode can be set.
 - 1) Show fluorescence only
 - 2) Show fluorescence plus ratio
 - 3) Show fluorescence plus conc.
 - 4) Show fluorescence, ratio plus conc.

- (h) Fluorescence polarization (effective when the 'fluorescence polarization measurement program' (option) is installed)
Items to be displayed when opening the data processing window in the fluorescence polarization mode can be set.
 - 1) Show graph only
 - 2) Show graph plus kinetic data
 - 3) Show graph plus markers

1.8 Window Setting

- (i) Fluorescence polarization spectrum (effective when the 'fluorescence polarization measurement program' (option) is installed)

Items to be displayed when opening the data processing window in the fluorescence polarization mode can be set.

- 1) Show fluorescence only
- 2) Show fluorescence plus polarization
- 3) Show fluorescence plus anisotropy
- 4) Show fluorescence, polarization plus anisotropy

(2) Fonts Tab

Display font, character size, etc. can be set.

Printer font, character size, etc. are also settable.

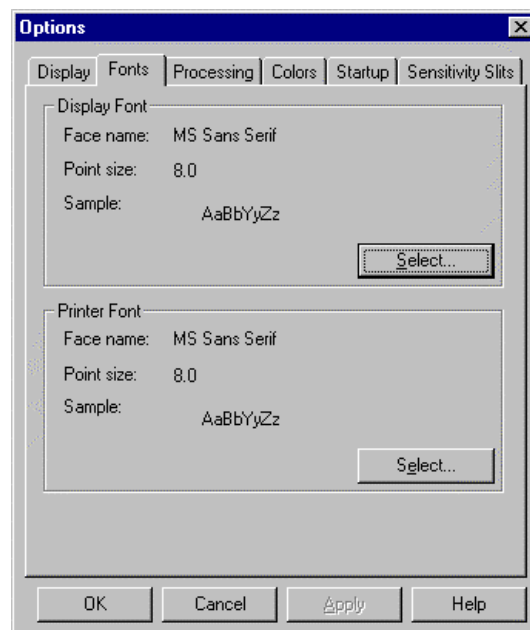


Fig. 1-32 Fonts Tab

(a) Display Font

Font, character size, etc. in the spectrum area under display can be set.

(b) Printer Font

Printer font is settable.

Clicking **Select (S)** presents the following dialog box.

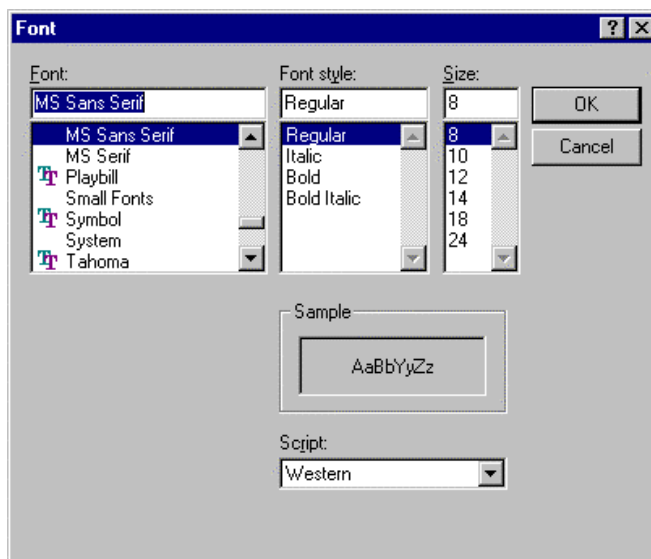


Fig. 1-33 Font Specification

Set Font, Font style and Size.

(3) Processing Tab

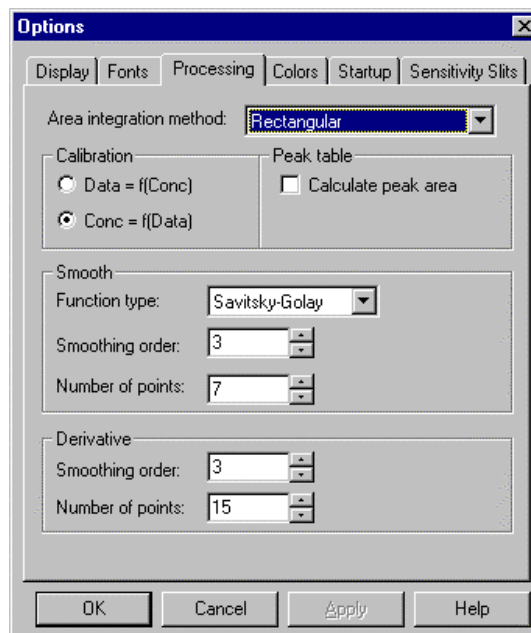


Fig. 1-34 Processing Tab

1.8 Window Setting

- (a) Area integration method
 - 1) Rectangular
 - 2) Trapezoid
 - 3) Romberg
 - (b) Calibration

A calibration curve formula to be used for the Photometry mode can be set.

 - 1) $Data = f(Conc)$
 - 2) $Conc = f(Data)$
 - (c) Peak table

Inclusion of area calculation item in the peak table can be selected or avoided.
 - (d) Smooth

The initial values of data processing function parameters can be set. However, this setting will not be reflected on the parameters of Analysis Method window.
 - (e) Derivative

The parameters of smoothing in an analysis method or to be carried out after measurement can be set.
- (4) Colors Tab

The color of the spectrum to be overlaid is settable here. Color 1 (bottom) is the color of the first spectrum.

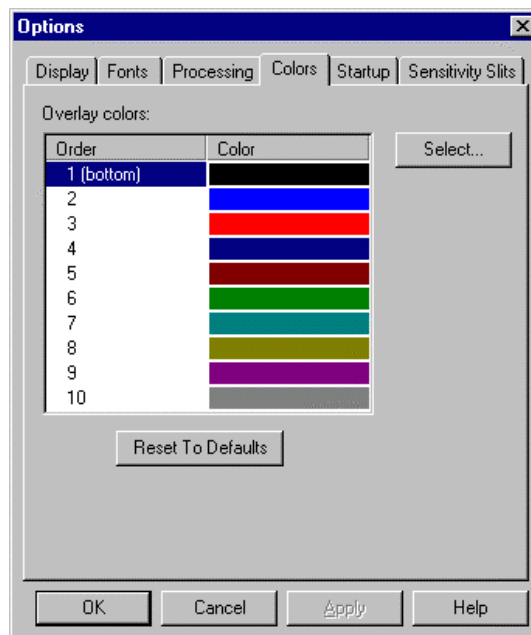


Fig. 1-35 Colors Tab

Click **Select**. The dialog box shown in Fig. 1-36 is then presented. A basic color or custom color is selectable. If there is no basic color you want, click **Define Custom Colors (D)**.



Fig. 1-36 Color Setting

The dialog box shown in Fig. 1-37 will appear. Select hue, saturation, luminance, etc. and click **Add to Custom Colors (A)**. Your color is then added to the custom colors. So select it and click **OK**.

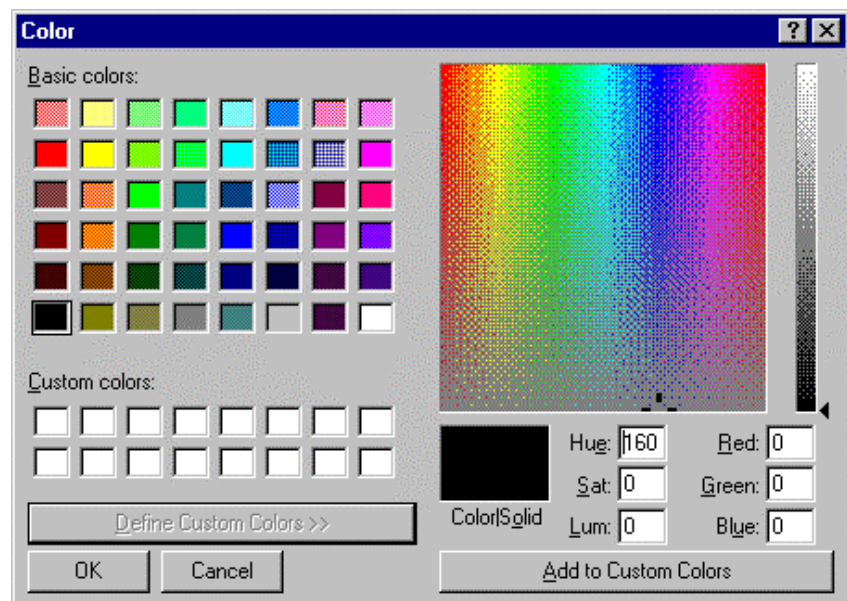


Fig. 1-37 Addition of Color

(4) Startup Tab

Analysis method to be taken upon startup of the FL Solutions program is settable here.

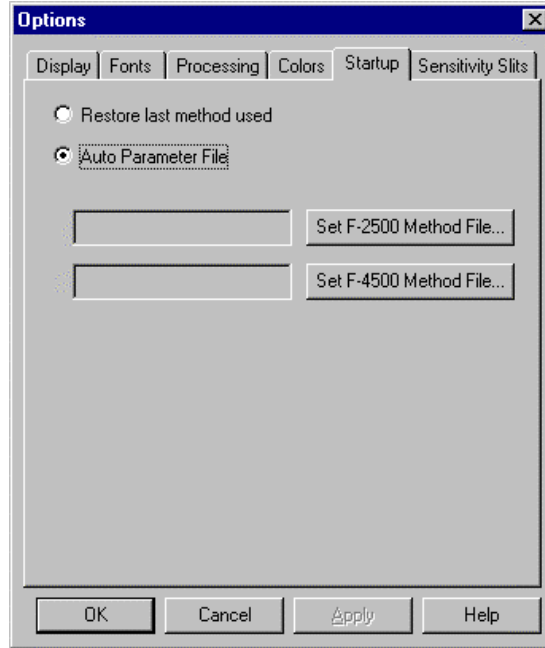


Fig. 1-38 Startup

- (a) Restore last method used
The method used last will be brought in.
- (b) Auto Parameter File
The instrument will start with the fixed method brought in. Click the model name of your instrument.

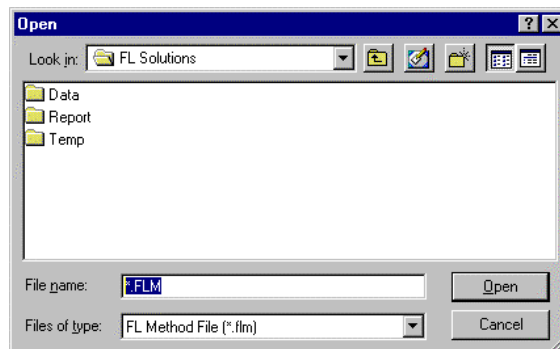


Fig. 1-39

Specify a file and click **Open (O)**.

1.9 Saving a Data File

Data can or cannot be saved depending on setting. So perform setting with reference to the following table. Setting is any of patterns ① to ⑥ is recommended.

Setting		“Use sample table” ON/OFF		Sample Name/Comment Input (“Use sample table” OFF)			
		—		ON		OFF	
Automatic saving		—		ON		OFF	
Analysis Method - Monitor tab		ON	OFF	ON	OFF	ON	OFF
“Open data processing window after data acquisition”							
Result	Display	○	×	○	×	○	×
	Automatic saving	○	○	○	○	—	—
	Manual saving	—	—	—	—	Possible	Impossible
Pattern		①	②	③	④	⑤	⑥

Read ○ and × symbols in the Result - Display row as follows.

○: Icons or data processing window is open immediately after measurement.

×: The data processing window is not open immediately after measurement. In patterns ② and ④, data is saved. So the relevant data file can be opened later.

1.10 Entry of File Name for Saving

1.10 Entry of File Name for Saving

File name needs to be set before measurement. Select the Sample Table (S) command in the Edit menu (E), and the dialog box shown in Fig. 1-40 will open. Input a sample name and comment. Then, bring the cursor to the file name column and click the button at (1).

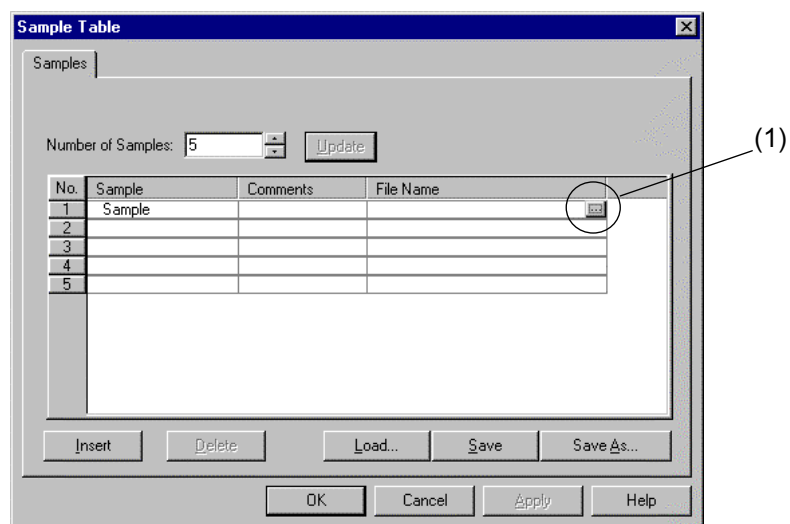


Fig. 1-40

The dialog box shown in Fig. 1-41 will appear. Enter your choice for “Save In” and specify “File Name.”

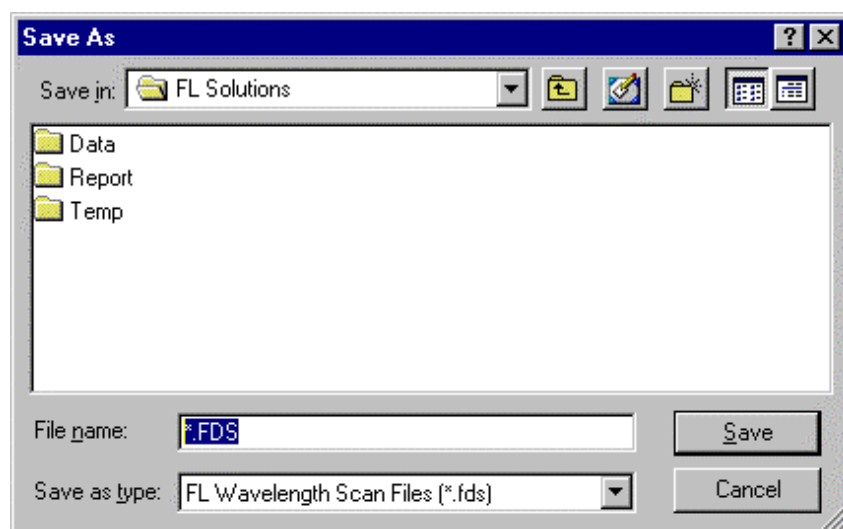


Fig. 1-41

After input, click **Save**.

If the file name field remains blank, a file name such as default001.uds is automatically given.

Once a measurement has been performed, you may perform the next ones with no change in setting (using the same sample table). In this case, file names "Sample 001.uds", "Sample 002.uds" and so on will be given. However, when there is no file name in a loaded analysis method file, file names will be given using the analysis method name as follows; Method .uds, Method 001.uds and Method 002.uds.

Enterable number of characters

Sample name : 20 characters

Comment : 255 characters

NOTE: If the specified storage medium (such as floppy disk or MO) does not have an adequate free area for new storage, the relevant file will be saved in the FL Solutions folder. This also occurs when the specified folder does not have an adequate free area.

1.11 Photometer Menu Commands

1.11 Photometer Menu Commands

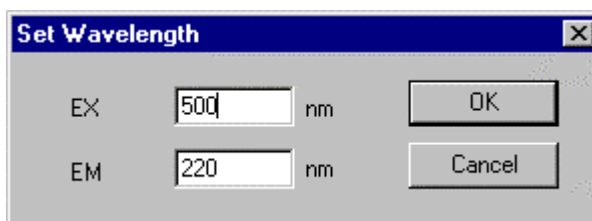
The following commands are available in the Spectrophotometer menu.

- Set Wavelength
- Zero Adjust
- Auto Pre-scan
- Shutter Open/Close

(1) Set Wavelength

This command sets the excitation/emission wavelength to a specified wavelength.

Click the Set Wavelength command or button, and the dialog box shown below will come up.



(2) Auto Pre-scan

This command automatically searches for an optimum excitation/emission wavelength for the sample under measurement. Other peaks than the fluorescence peak (such as peaks of excitation scattered light and Raman spectrum) are ignored. On completion of automatic pre-scanning, the optimum excitation/emission wavelength is indicated on the monitor window (in the current wavelength indication field detailed in 1.6).

(3) Zero Adjust

This command automatically closes the shutter and measures a zero point in detection. When the zero point is changed, e.g., after the photomultiplier voltage is changed, automatic zero-point adjustment is carried out.

(4) Shutter Open/Close

This command is available for opening/closing the excitation beam shutter. The current state is indicated on the right side of the monitor window.

1.12 Utility Menu Commands

The Utility menu contains the following commands:

- Instrumental Response
- Analog Output Gain
- EX Power Monitor
- Sens. Check
- Lamp Usage
- Lamp Off (only with Model F-2500)

(1) Instrumental Response

When you use this function. You need the Spectral correction accessories kit (P/N 251-0126).

(2) Analog Output Gain

Use this command for setting a data value corresponding to 1 V of analog output. For details, refer to 8.4.

(3) EX Power Monitor

Use this command for checking output of the monitor detector. When the EX Power Monitor is turned on (clicked), monitor value data is displayed regardless of measurement condition.

(4) Sens. Check

Use this command for checking sensitivity. You can check sensitivity/drift with this command. For details, refer to the maintenance edition.

(5) Lamp Usage

Use this command for checking/resetting the usage period of the xenon lamp. A cumulative turn-on time of the xenon lamp is indicated. After lamp replacement, reset this indication. For details, refer to the maintenance edition.

(6) Lamp Off (only with Model F-2500)

Use this command for turning off the xenon lamp. For turning on the xenon lamp again, restarting the spectrophotometer is necessary.

1.13 Termination of FL Solutions Program

1.13 Termination of FL Solutions Program

After completion of measurement, shut down the instrument in the following procedure.

- (1) From the File (F) menu, select the Exit (X) command.

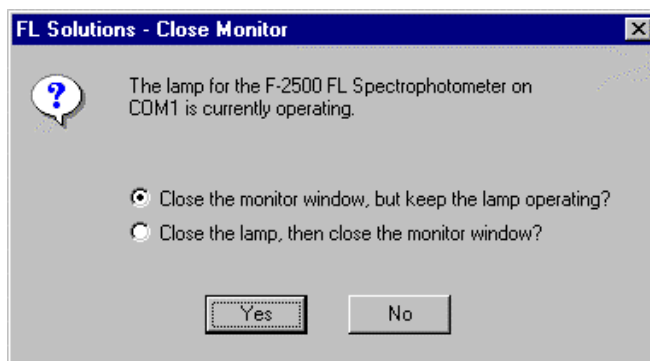


Fig. 1-42

Select **Yes**, and the FL Solutions program will then be terminated.

- (2) Turn off the power switch of the spectrophotometer main unit.
- (3) Click the Start button of the Windows 98 and select the Shut Down (U) item. In the Shut Down Windows dialog box, select "Shut down" and click **OK**.
- (4) Turn off power to the personal computer and the CRT display monitor.
(At step (3), the personal computer may be turned off in some settings.)
- (5) Turn off power to the printer.

2. WAVELENGTH SCAN

2.1 Flow of Operation

This section explains the condition setting and operating method for wavelength scan.

Measurement is made in the following procedure.

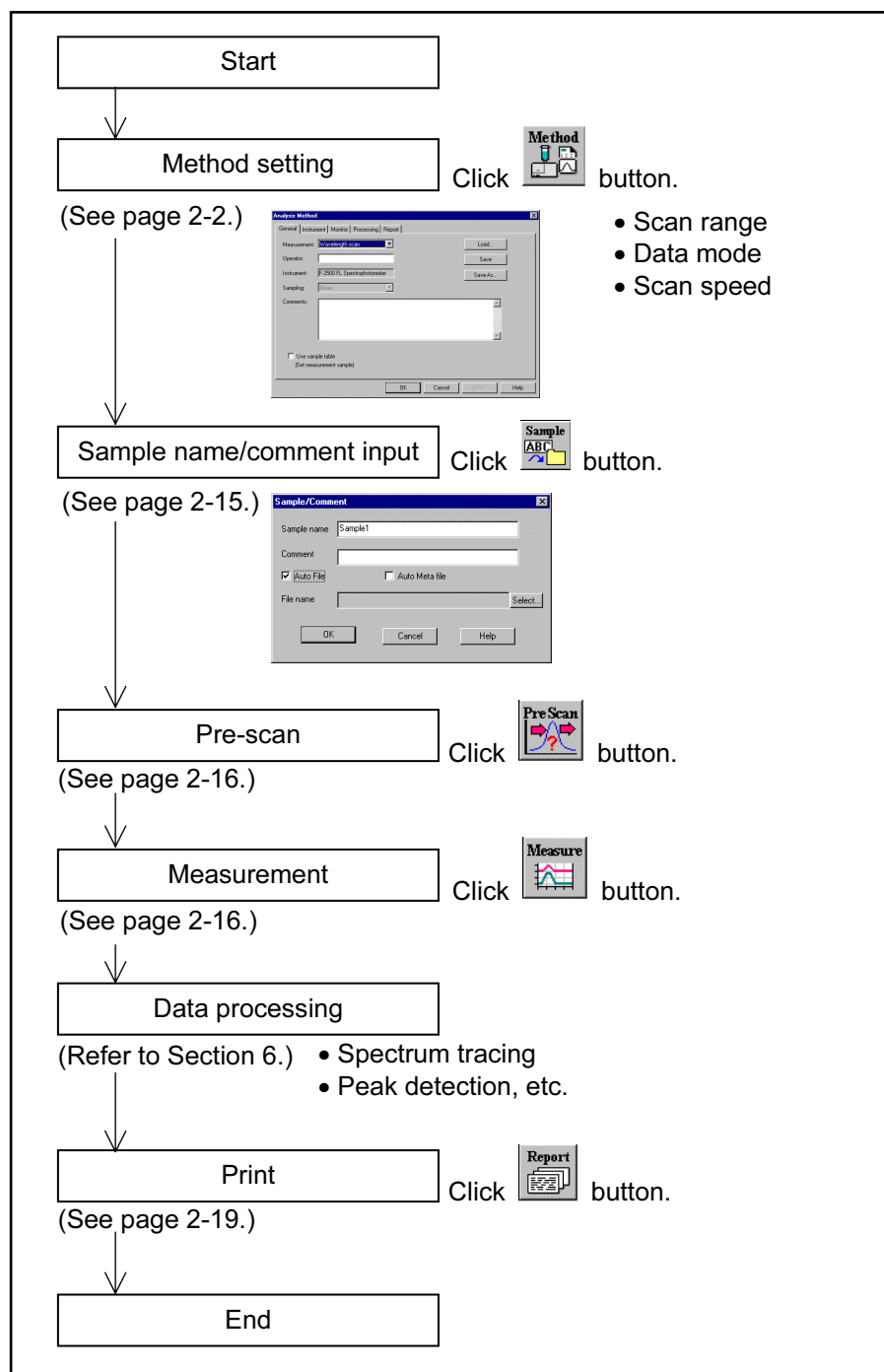



Fig. 2-1 Operational Flow (wavelength scan)

2.2 Creating an Analysis Method

2.2 Creating an Analysis Method

Set the analytical conditions (method) prior to measurement. From the Edit menu, select the Method command or click the  button. The dialog box shown in Fig. 2-2 will appear.

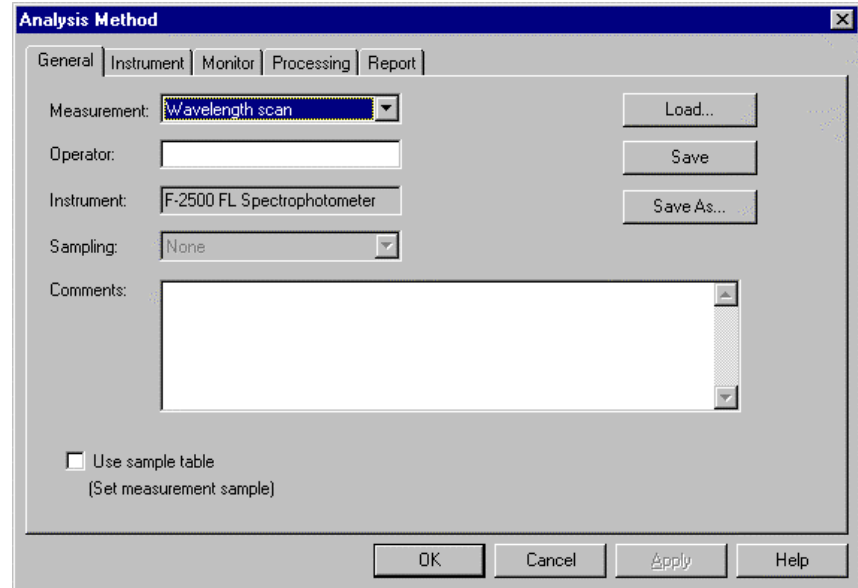


Fig. 2-2 Analysis Method

The following tabs are provided on the Analysis Method window for the wavelength scan mode. These tabs are explained on the subsequent pages.

- General
- Instrument
- Monitor
- Processing
- Report

(1) General

The parameters shown in Fig. 2-3 are provided under the General tab.

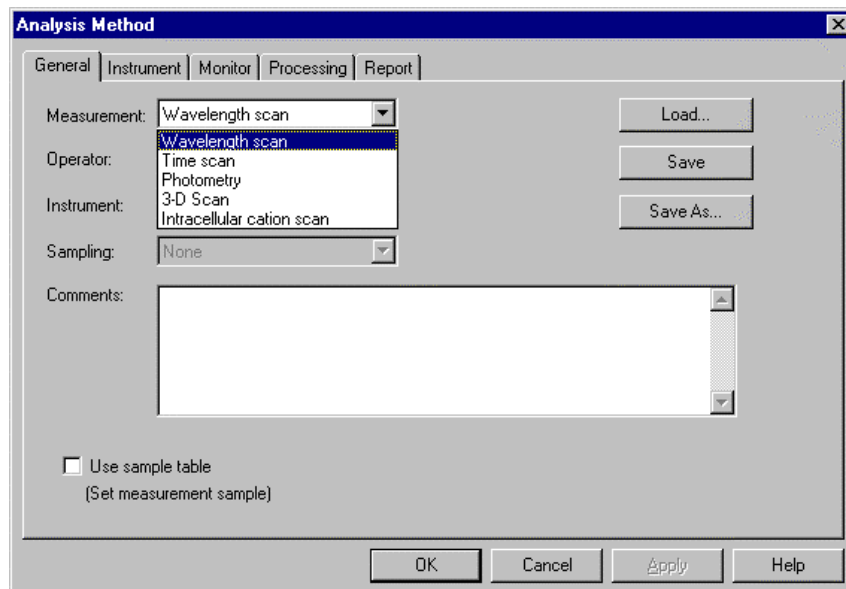


Fig. 2-3 General Tab

(a) Measurement (mode)

Select a measurement mode from among the four listed below.

- Wavelength scan
- Time scan
- Photometry
- 3-D Scan (displayed when the '3-D Scan program' (option) is installed in combination with Model F-2500)

Here, select "Wavelength scan." For Photometry, Time scan and 3-D Scan, refer to Sections 3, 4 and 5.

(b) Operator

Enter an operator name.

(c) Instrument

The model of the connected instrument is indicated.

(d) Use sample table (Set measurement sample)

Apply a check mark here when a sample table is used.

2.2 Creating an Analysis Method

- (e) Sampling
 - Standard
 - When sample is exchanged manually.
 - (f) Comments
 - Enter a description or notes on measuring conditions.
 - (g) Load
 - Click this button to read out saved analytical conditions.
 - The file opening window appears upon clicking, so select an analytical conditions file.
 - (h) Save
 - By clicking this button after setting the parameters for all five tabs, the analytical conditions are saved.
 - Clicking this button overwrites parameters on the existing ones.
 - (i) Save As
 - Click this button for renaming the set conditions for saving. The “Save As” window will appear, so enter a file name.
- (2) Instrument

When you click the Instrument tab, the window in Fig. 2-4 appears.

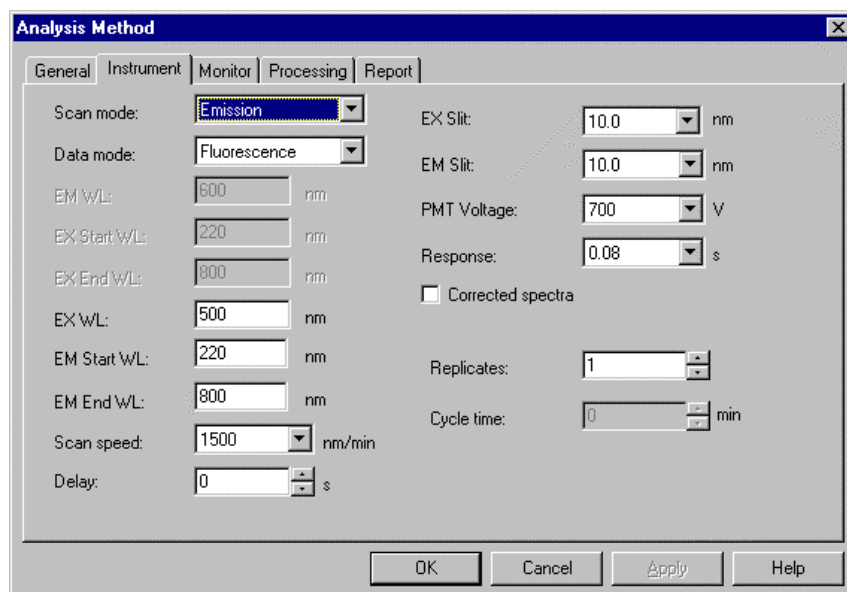


Fig. 2-4 Instrument Tab

(a) Scan mode

Select one of the following scan modes. In case of Synchronous, excitation wavelength appears on the abscissa of the graph.

- Excitation
- Emission
- Synchronous

(b) Data mode

Select one of the following data modes.

The Luminescence mode is for measuring luminescence in the biological/chemical field and only the sample side signal is acquired without performing ratio photometry. The Phosphorescence mode is for acquiring only phosphorescence after extinction of excitation beam while turning a chopper on the excitation side.

- Fluorescence
- Luminescence
- Phosphorescence (with Model F-4500)

(c) EM WL

This is the start wavelength for wavelength scan on the excitation side. Input the shorter wavelength side of measuring wavelength range.

Model	Input Range
F-4500	200 to 900 nm or 200 to 890 nm in Synchronous scan mode
F-2500	220 to 800 nm or 220 to 790 nm in Synchronous scan mode

(d) EX Start WL

This is the start wavelength for wavelength scan on the excitation side. Input the shorter wavelength side of measuring wavelength range.

Model	Input Range
F-4500	200 to 890 nm
F-2500	220 to 790 nm

2.2 Creating an Analysis Method

(e) EX End WL

This is the end wavelength for wavelength scan on the excitation side. Input the longer wavelength side of measuring wavelength range.

Model	Input Range
F-4500	210 to 900 nm
F-2500	230 to 800 nm

(f) EX WL

An excitation wavelength can be input. It is the end wavelength for wavelength scan on the excitation side. Input the longer wavelength side of measuring wavelength range.

Model	Input Range
F-4500	200 to 900 nm
F-2500	220 to 800 nm

(g) EM Start WL

This is the start wavelength for wavelength scan on the emission side. Input the shorter wavelength side of measuring wavelength range.

Model	Input Range
F-4500	200 to 890 nm
F-2500	220 to 790 nm

(h) EM End WL

This is the end wavelength for wavelength scan on the emission side. Input the longer wavelength side of measuring wavelength range.

Model	Input Range
F-4500	210 to 900 nm
F-2500	230 to 800 nm

(i) Scan speed

Set a wavelength scan speed. (Unit: nm/min)

F-4500	F-2500
30000	3000
12000	1500
2400	300
1200	60
300	15
60	
15	

For the Phosphorescence data mode, select one of the following values.

- 240 nm/min
- 60 nm/min
- 15 nm/min

(j) Delay

After pressing the Measure button, measurement is started following the delay time set here. It is used for temperature stabilization, etc. In repeat measurement, it is the time until the start of the first measurement.

The delay time is invalid for the second and subsequent measurements.

Input range: 0 to 9999 s

(k) EX Slit

Select a slit width for the excitation side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

2.2 Creating an Analysis Method

(l) EM Slit

Select a slit width for the emission side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

(m) PMT Voltage

A function for controlling the voltage of the photomultiplier detector (photomultiplier tube).
Select one of the following values. Changing from 400 V to 700 V increases the data value about two digits and changing from 700 V to 950 V increases it about 1 digit.

- 950 V (only with F-4500)
- 700 V
- 400 V

(n) Response

Select a response time from the following.

F-4500	F-2500
0.0004	0.04
0.01	0.08
0.05	0.4
0.1	2
0.5	8
2.0	Auto
8.0	
Auto	

By selecting Auto, a response time is set automatically according to scan speed. Select Auto usually.

- (o) **Corrected spectra**
A function for determining the spectrum inherent to a sample by correcting the photometer wavelength characteristic using the saved instrument parameters, following measurement with the instrument parameters for photometer control. If there are no instrument parameters for the wavelength range to be measured, only the wavelength range that allows correction is scanned. And if there is no wavelength area measurable, an error will occur.
For using this function, a Spectra Correction Parts Set (P/N 251-0126) is required. The Instrument Response command is provided in the Utility menu for operating this function.
When this setting is at ON, "Corrected spectra" appears on the monitor window. (See Fig. 1-26 in 1.6.)
- (p) **Shutter control (only with F-4500)**
The shutter can be automatically closed in other than measurement for suppressing sample deterioration due to the energy of excitation beam and opened when measurement starts. When you put a check mark at the head, the shutter will close and open at start of measurement. The shutter will close again when measuring wavelength begins returning to the start wavelength after measurement in the wavelength scan mode.
- (q) **Replicates**
Set the number of repeat measurements. Input range is 1 to 99. An average spectrum can also be determined in repeat measurement. Setting is available on the Processing tab.
- (r) **Cycle time**
Set a repetition interval. Input range is 0.0 to 180.0 min.

2.2 Creating an Analysis Method

(3) Monitor

When selecting the Monitor tab, the window in Fig. 2-5 appears.

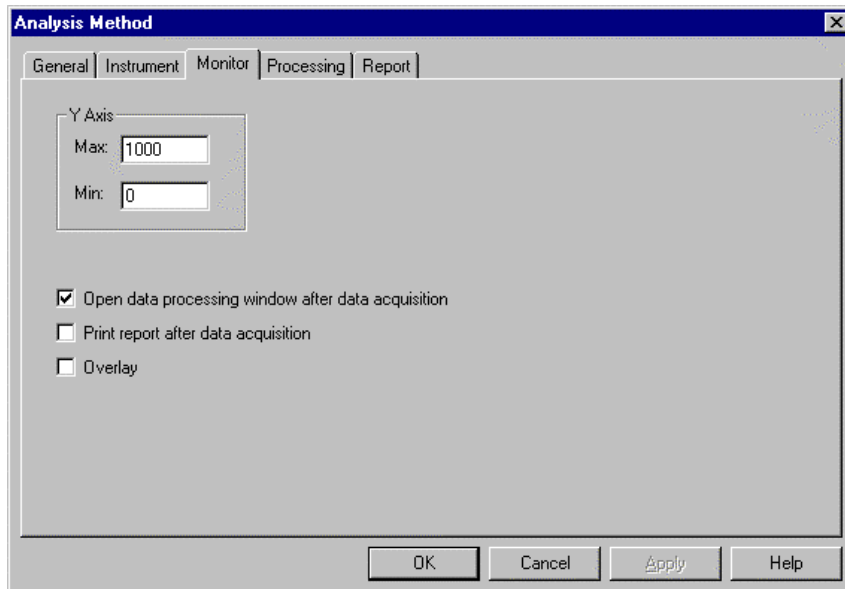


Fig. 2-5 Monitor Tab

Assign the following parameters.

- (a) Y-Axis Max (maximum)
Input a maximum value for Y-axis on the monitor window.
- (b) Y-Axis Min (minimum)
Input a minimum value for Y-axis on the monitor window.
- (c) Open data processing window after data acquisition
Select whether or not to conduct data processing after sample measurement. When selected (check mark is applied), an icon is displayed for the data processing window at the end of measurement. And by opening this icon, data processing such as peak detection is available.

NOTE: When the remaining memory capacity of PC in operation is very small (system resource is less than 20%), the data processing window will not open even if this item is turned ON. Either close the presently opened data processing window or restart the FL Solutions program.

(d) Print report after data acquisition

Select whether or not to print a report after sample measurement. When selected (check mark is applied), printing will be made automatically after measurement. The items set under the Report tab will be printed. When this item is not selected, data processing can be made by reading out a file via **File - Open**.

(e) Overlay

Spectra can be overlaid in the monitor window. This setting cannot be selected for repeat measurement.

(4) Processing

On selection of the Processing tab, the window in Fig. 2-6 will be displayed.

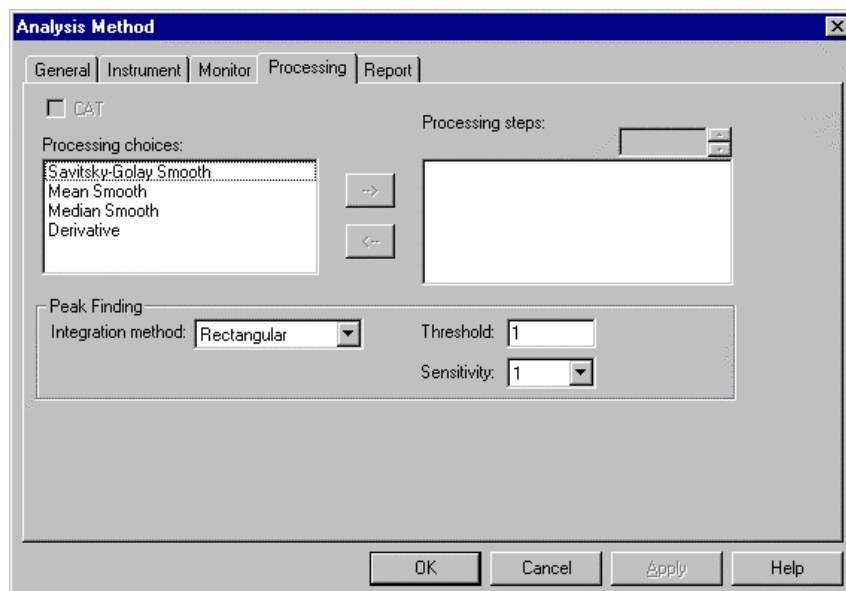


Fig. 2-6 Processing Tab

2.2 Creating an Analysis Method

- (a) CAT (Computing for Averaging Transient)
Used for obtaining an average spectrum in repeat measurement. This is especially effective for measuring samples with which the emission intensity varies with time.
- (b) Processing choices
A list of data processing (Savitsky-Golay, Mean smooth, Median smooth, Derivative) is shown. Select a data processing item, and click the rightward-pointing arrow key between the Processing choices and Processing steps display fields. Then, the selected method appears in the Processing steps field.
- (c) Processing steps
The processing sequence is displayed. To delete a processing method, first select the method, and then click the leftward-pointing arrow key between the Processing choices and Processing steps display fields. Then, the selected method disappears from the Processing steps field.
Also, in case you want to carry out any smoothing in Processing steps, double-click the relevant item and the smoothing parameters (order, data points, etc.) can be changed.
- (d) Peak Finding
Select a peak detection method indicated in the data processing window.
- (e) Threshold
Set a detection limit for the photometric value axis of peak and valley. Peaks or valleys below the setting will not be detected. Input range is 0.001 to 1000.
- (f) Sensitivity
Select the number of data points in the horizontal axis direction (abscissa). Select a sensitivity of "1" for detecting sharp peaks. Or select "8" for detecting broad peaks.
 - 1
 - 2
 - 4
 - 8

(5) Report

Select the Report tab, and the window in Fig. 2-7 will appear.

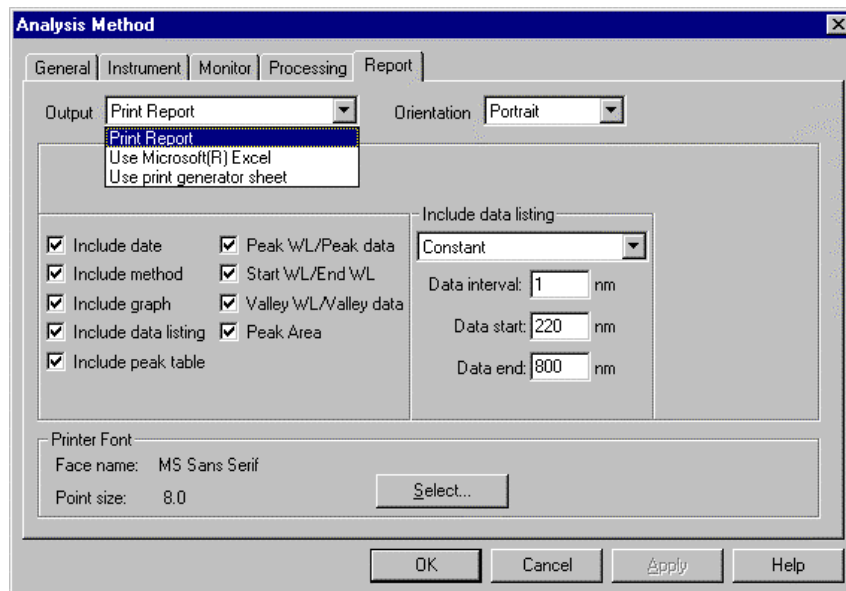


Fig. 2-7 Report Tab

This window is used to select an output method and items of measurement result.

(a) Output

1) Print Report

A report will be printed. For printing method, refer to 2.6.

2) Use Microsoft® Excel

Data will be transferred to Microsoft® Excel. For transfer method, refer to 5.13.

3) Use print generator sheet

Select this method for using the report generator (option). For usage, refer to the instruction manual of the report generator.

(b) Orientation

Selectable when Report is chosen for Print Method. Select a printing orientation. Either Portrait or Landscape orientation is selectable. When the Landscape orientation is selected, date and method can be printed.

2.2 Creating an Analysis Method

(c) Printable (transferable) items


- 1) Include date
- 2) Include method
- 3) Include graph
- 4) Include data listing
 - Constant
Data will be printed at fixed intervals between 2 wavelength points. Input Data interval, Start wavelength and End wavelength.
 - Select data
Data at the specified wavelength will be printed. Up to 12 wavelengths can be specified.
- 5) Include peak table
Select output items in the peak table.
 - Peak WL/Peak data
 - Start WL/End WL
 - Valley WL/Valley data
 - Peak Area

(d) Printer Font

Click this button for changing the font to be used in the report.

2.3 Defining Your Samples

When “Use Sample Table” has been set for the Analysis Method, the samples must be defined before measurement.

- (1) Open the monitor window and click the  button.
- (2) When the Sample Table window in Fig. 2-8 appears, fill in the blank section. For instance, if there are five samples, click the **Insert** button five times.

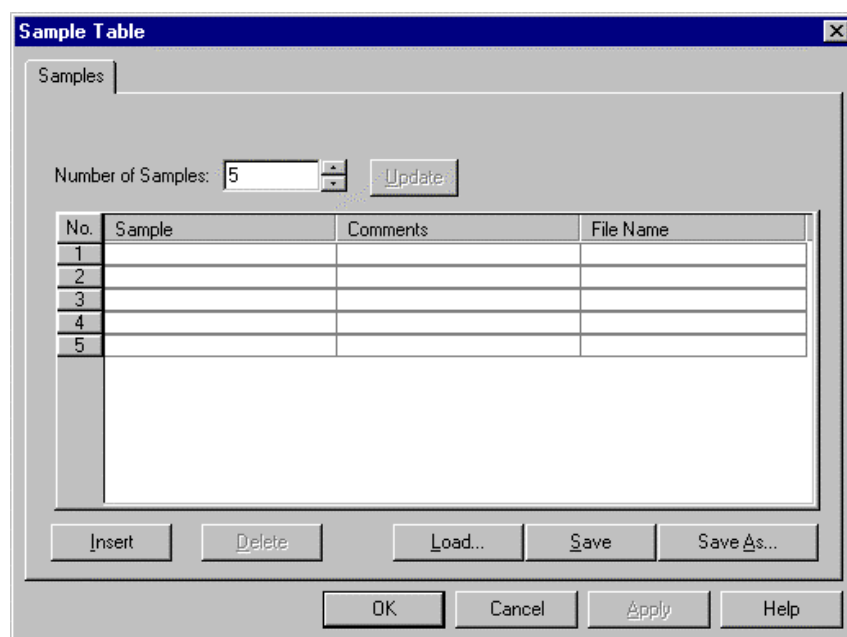


Fig. 2-8 Sample Table Window

- (3) Input the Sample, Comments and File Name for each sample. The sample names input here appear on the monitor window also. And the sample names input here are not changeable.


Maximum number of characters to be input

Sample : 20 characters

Comments : 255 characters

2.3 Defining Your Samples

- (4) When input is finished, check the entries and then click the **OK** button.

If a sample table is not used, then click the  button. The window in Fig. 2-9 will appear. Make entries for Sample and Comment. For automatic saving, put a check mark in the “Auto File” box and specify a file name for saving.

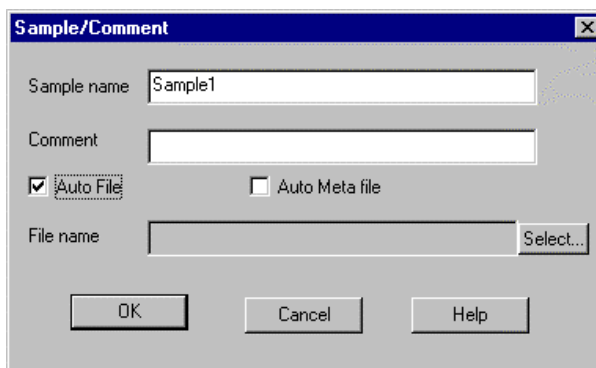



Fig. 2-9

2.4 Prescan

Select Pre-scan command from the Spectrophotometer menu or click the  (pre-scan) button.

This function executes high-speed scan under the set measuring conditions, detects the peak wavelength and moves the system to that wavelength. Also, the upper limit photometric value is automatically set so that the peak-wavelength value becomes approximately 70% of that upper limit value.

Scan ranges are as follows.

Excitation spectra : Start wavelength to emission wavelength - 20 nm or shorter wavelength side of end wavelength




Emission spectra : Excitation wavelength or longer wavelength side of start wavelength + 20 nm to end wavelength

Synchronous spectra: Start wavelength to end wavelength

After prescan, the upper limit photometric value is automatically set.

2.5 Conducting a Wavelength Scan

2.5 Conducting a Wavelength Scan

- (1) There are two methods of starting the scan.
 - (a) Select the Measure command from the Spectrophotometer menu.
 - (b) Click the  (measurement) button on the measurement toolbar.
For halting the measurement midway, click the  button on the toolbar.
- (2) Upon clicking the  button, the window shown in Fig. 2-10 appears when using a sample table. Now click **Yes**.

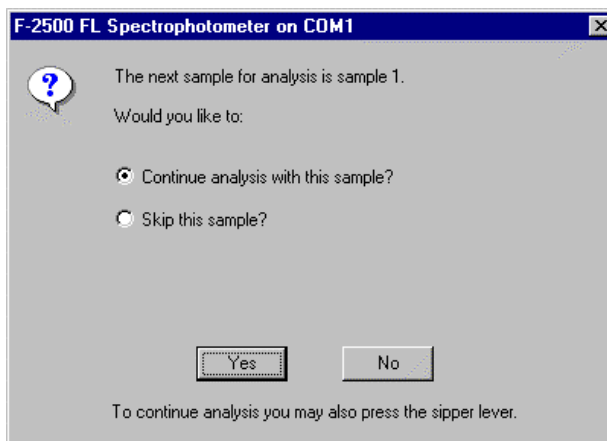


Fig. 2-10

- (3) The dialog box as in Fig. 2-11 will open. Set each sample and click **OK**.

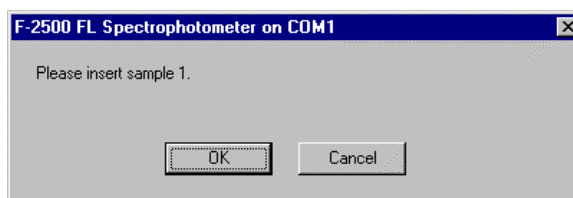


Fig. 2-11

- (4) When automatic saving is set, data will be saved after completion of measurement. Upon opening its file, a data processing window will appear as in Fig. 2-12. Unless automatic saving is set, select the Save As command from the File menu and save data in file.

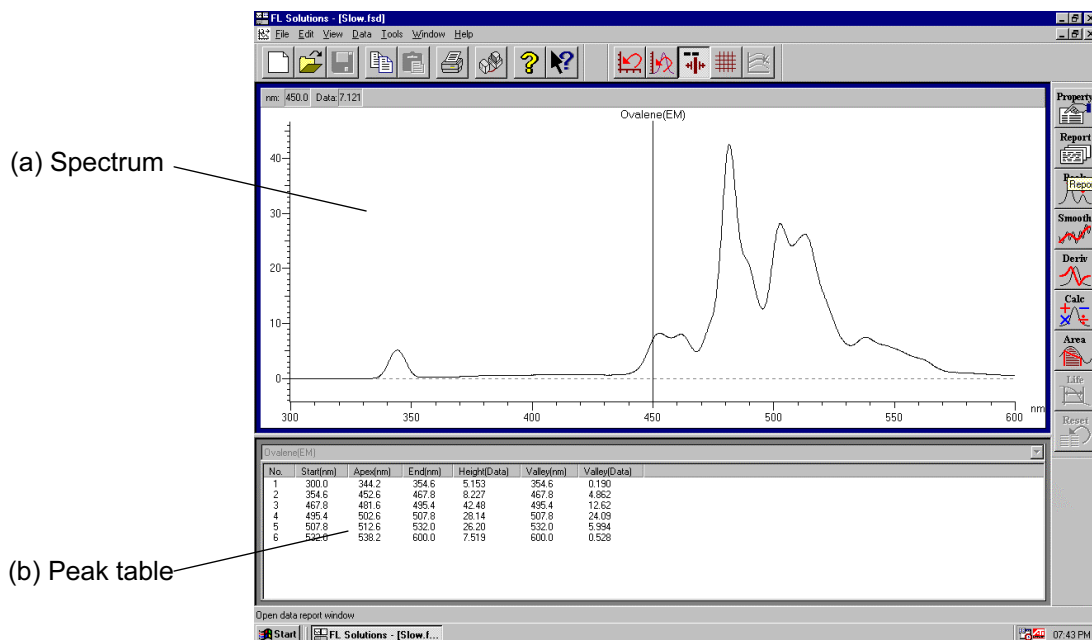



Fig. 2-12 Data Processing Window (wavelength scan)

- (a) Spectrum
The spectrum of a measured sample is traced.
- (b) Peak table
Peak wavelength, peak height and valley wavelength are displayed.

2.6 Data Printout

2.6 Data Printout

Printout can be provided even when the function for automatic printout after measurement is not used. The following two procedures are available.

- (1) Select the Print command from the File menu. Or click the  print button on the toolbar. The field active on the data processing window (e.g., (a) in Fig. 2-12) will be printed. What is displayed by clicking the Print Preview command in the File menu will be printed.

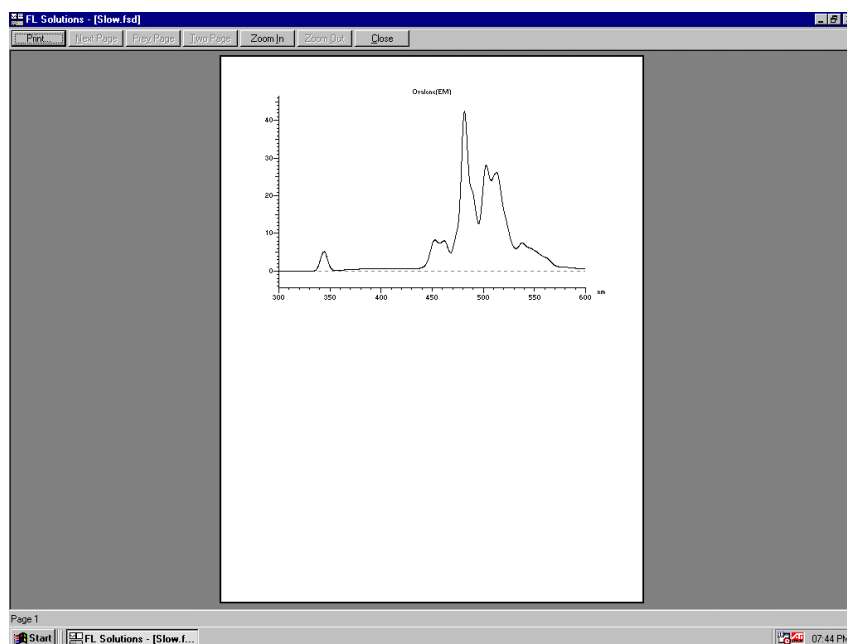



Fig. 2-13

- (2) Select the Report command in the Data menu. Or click the  button on the toolbar. The Print Preview window will appear, so click **Print**. Select the items to be printed at the Report tab under Properties.

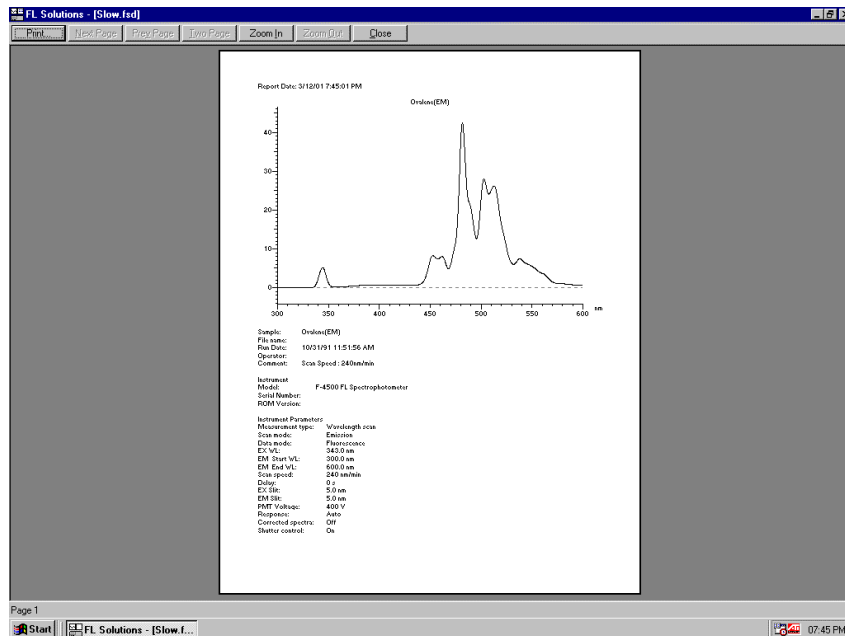


Fig. 2-14

NOTE: If characters overflow from the print frame in repeat measurement or in overlaying, then change the printing orientation from vertical to horizontal.

3. TIME SCAN

3.1 Flow of Operation

This section explains the parameter setting and operating method for time scan.

Measurement is performed in a flow shown below.

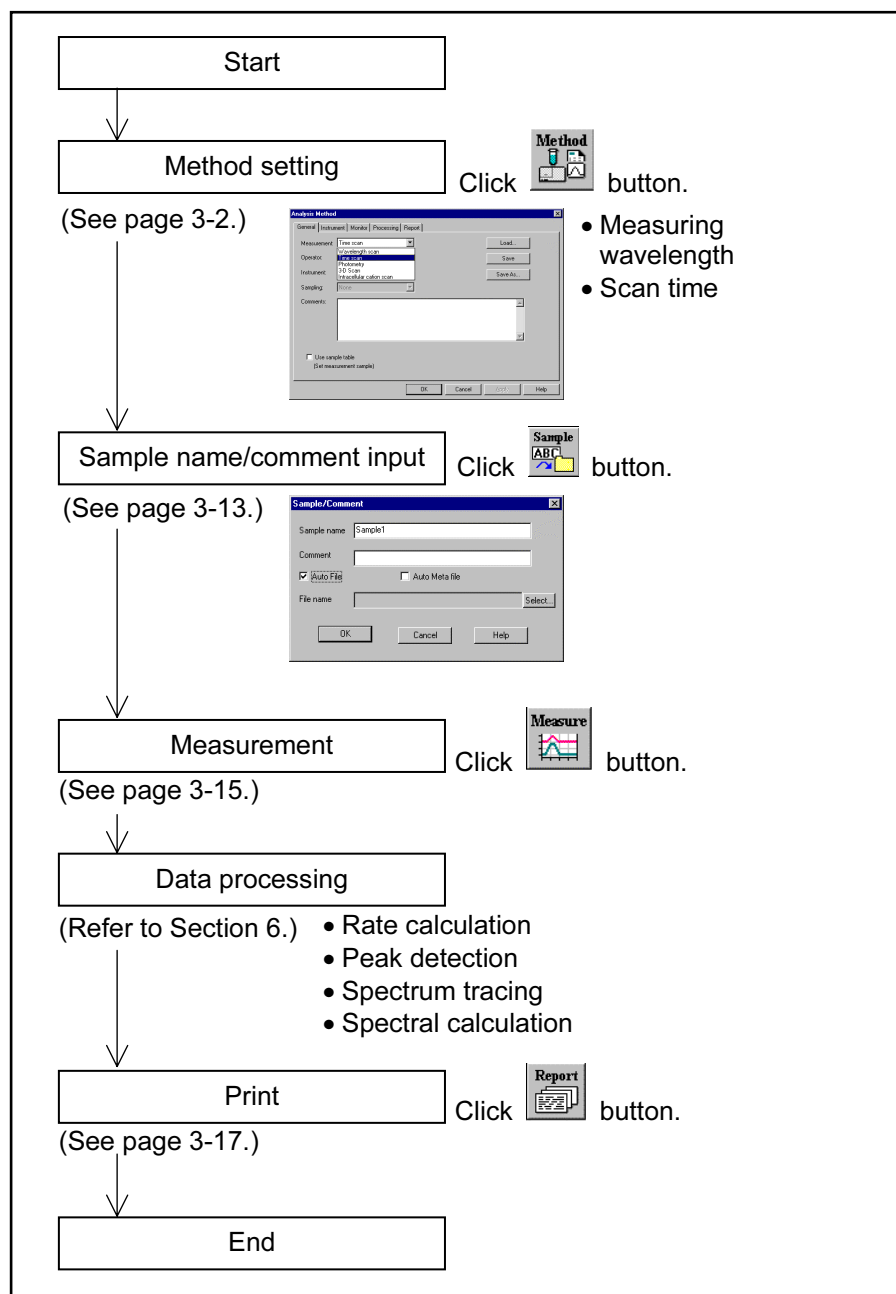



Fig. 3-1 Operational Flow (time scan)

3.2 Creating an Analysis Method

3.2 Creating an Analysis Method

Set the analytical conditions (method) prior to measurement. From the Edit menu, select the Method command or click the  button. The dialog box shown in Fig. 3-2 will appear.

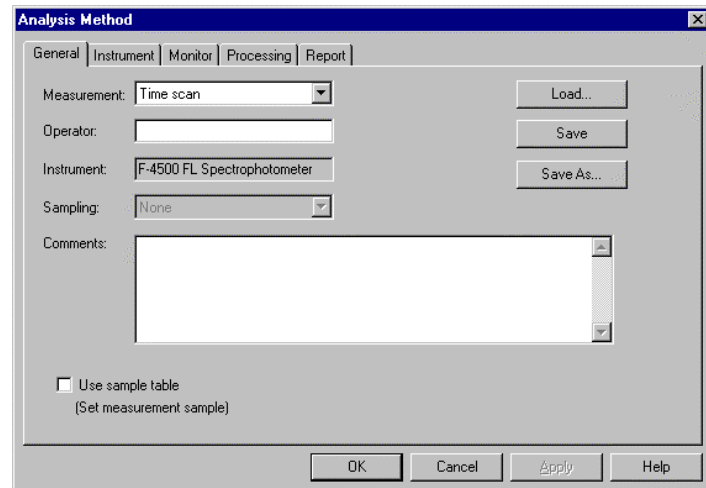


Fig. 3-2 Analysis Method

The following tabs are provided on the Analysis Method window for the time scan mode. These tabs are explained on the subsequent pages.

- General
- Instrument
- Monitor
- Processing
- Report

(1) General

The parameters shown in Fig. 3-3 are provided under the General tab.

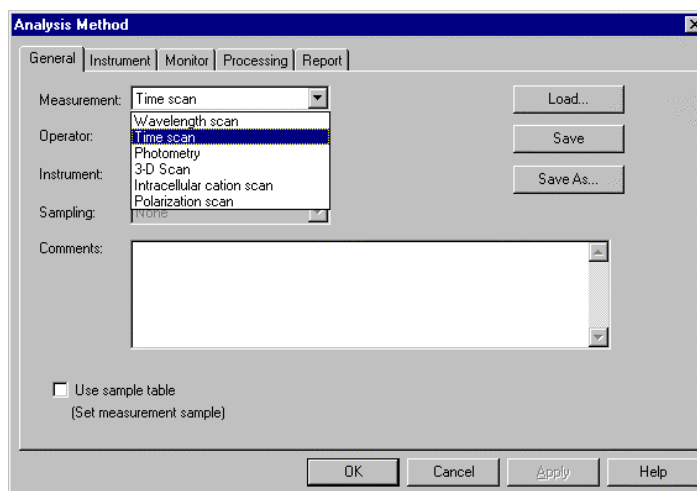


Fig. 3-3 General Tab

(a) Measurement (mode)

Select a measurement mode from among the four listed below.

- Wavelength scan
- Time scan
- Photometry
- 3-D Scan (displayed when the '3-D Scan program' (option) is installed in combination with Model F-2500)

Here, select "Time scan." For Wavelength scan, Photometry and 3-D Scan, refer to Sections 2, 4 and 5.

(b) Operator

Enter an operator name.

(c) Instrument

The model of the connected instrument is indicated.

(d) Use sample table (Set measurement sample)

Apply a check mark here when a sample table is used.

3.2 Creating an Analysis Method

- (e) Sampling
 - Standard
 - When sample is exchanged manually.
 - (f) Comments
 - Enter a description or notes on measuring conditions.
 - (g) Load
 - Click this button to read out saved analytical conditions.
 - The file opening window appears upon clicking, so select an analytical conditions file.
 - (h) Save
 - By clicking this button after setting the parameters for all five tabs, the analytical conditions are saved.
 - Clicking this button overwrites parameters on the existing ones.
 - (i) Save As
 - Click this button for renaming the set conditions for saving. The “Save As” window will appear, so enter a file name.
- (2) Instrument

This window appears when Time scan is specified for Measurement under the General tab on the Analysis Method window. When you click the Instrument tab, the window in Fig. 3-4 appears.

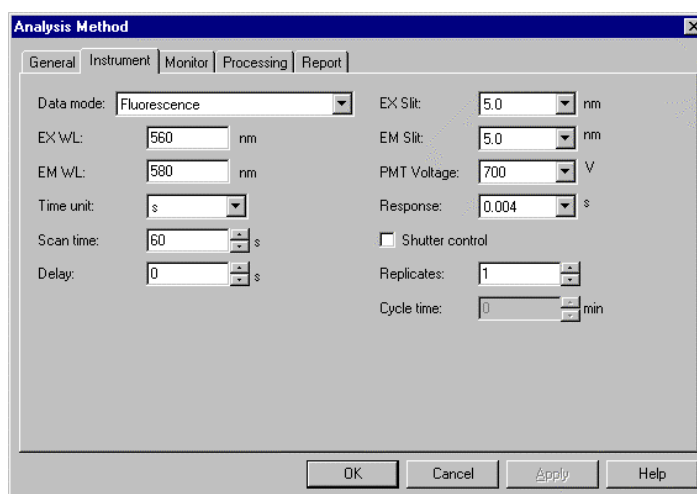


Fig. 3-4 Instrument Tab

(a) Data mode

Select one of the following data modes.

The Luminescence mode is for measuring luminescence in the biological/chemical field and only the sample side signal is acquired without performing ratio photometry.

If phosphorescence life is shorter than 20 ms, "Phosphorescence short life" should be selected.

In this case, unit time and measuring time are automatically set to ms and 20 ms.

- Fluorescence
- Luminescence
- Phosphorescence (only with Model F-4500)
- Phosphorescence Life Time (only with Model F-4500)
- Phosphorescence Life Time (Short) (only with Model F-4500)

(b) EX WL

Input an excitation wavelength.

Model	Input Range
F-4500	0, 200 to 900 nm
F-2500	0, 200 to 800 nm

(c) EM WL

Input an emission wavelength.

Model	Input Range
F-4500	0, 200 to 900 nm
F-2500	0, 200 to 800 nm

(d) Time unit (only with Model F-4500)

Set a time unit.

- s
- ms

3.2 Creating an Analysis Method

- (e) Scan time
Set a scan time.

Model	Input Range
F-4500	10 to 3600 s 1000 to 9999 ms
F-2500	10 to 9000 s

According to the set scan time, data sampling interval is set as listed below.

- Model F-4500

Measuring Time (s)	Sampling Interval (ms)
~ 3	1
~ 7	2
~ 18	5
~ 36	10
~ 72	20
~ 180	50
~ 360	100
~ 720	200
~ 1800	500
~ 3600	1000

In the Phosphorescence mode, the minimum sampling interval is 50 ms. It is fixed at 40 μ s in phosphorescence short-life measurement.

- Model F-2500

Measuring Time (s)	Sampling Interval (ms)
~ 120	100
~ 240	200
~ 600	500
~ 1200	1000
~ 2400	2000
~ 6000	5000
~ 9000	10000

(f) Delay

After pressing the Measure button, measurement is started following the delay time set here. It is used for temperature stabilization, etc. In repeat measurement, it is the time until the start of the first measurement.

The delay time is invalid for the second and subsequent measurements.

Input range: 0 to 9999 s

(g) EX Slit

Select a slit width for the excitation side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

(h) EM Slit

Select a slit width for the emission side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

(i) PMT Voltage

A function for controlling the voltage of the photomultiplier detector (photomultiplier tube).

Select one of the following values. Changing from 400 V to 700 V increases the data value about two digits and changing from 700 V to 950 V increases it about 1 digit.

- 950 V (only with F-4500)
- 700 V
- 400 V

3.2 Creating an Analysis Method

(j) Response

Select a response time from the following.

F-4500	F-2500
0.04	0.04
0.01	0.08
0.05	0.4
0.1	2
0.5	8
2.0	Auto
8.0	
Auto	

By selecting Auto, a response time is set automatically according to scan speed. Select Auto usually.

(k) Shutter control (only with F-4500)

The shutter can be automatically closed in other than measurement for minimizing sample deterioration due to the energy of excitation beam and opened when measurement starts. When you put a check mark at the head, the shutter will close and open at start of measurement. The shutter will close again when measuring wavelength begins returning to the start wavelength after measurement in the time scan mode.

(l) Replicates

Set the number of repeat measurements. Input range is 1 to 99.

(m) Cycle time

Set a repetition interval. Input range is 0.0 to 180.0 min.

(3) Monitor

When selecting the Monitor tab, the window in Fig. 3-5 appears.

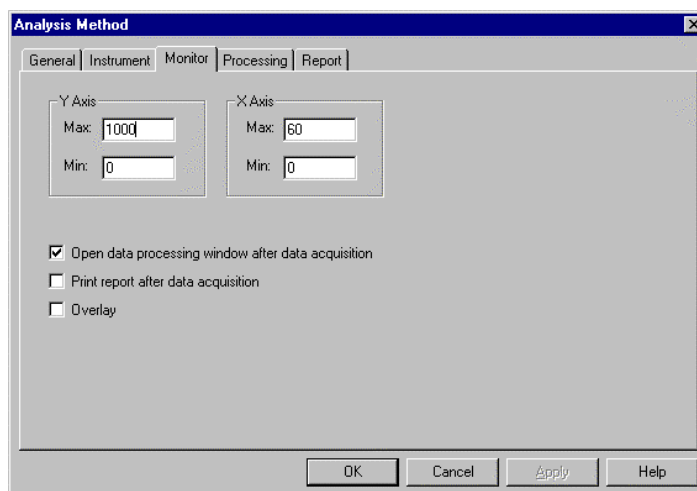


Fig. 3-5 Monitor Tab

Assign the following parameters.

- (a) Y-Axis Max (maximum)
Input a maximum value for Y-axis on the monitor window.
- (b) Y-Axis Min (minimum)
Input a minimum value for Y-axis on the monitor window.
- (c) X-Axis Max (maximum)
Input a maximum value for X-axis on the monitor window.
- (d) X-Axis Min (minimum)
Input a minimum value for X-axis on the monitor window.

NOTE: X-Axis Max and Min correspond to a display interval on the monitor window.

For example, when Max and Min are 60 and 30, display on the window is made within 30 to 60 s, 60 to 90 s, 90 to 120 s and so on.

3.2 Creating an Analysis Method

- (f) Open data processing window after data acquisition
Select whether or not to conduct data processing after sample measurement. When selected (check mark is applied), data is automatically saved after measurement.

NOTE: When the remaining memory capacity of PC in operation is very small (system resource is less than 20%), the data processing window will not open even if this item is turned ON. Either close the presently opened data processing window or restart the FL Solutions program.

- (g) Print report after data acquisition
Select whether or not to print a report after sample measurement. When selected (check mark is applied), printing will be made automatically after measurement. The items set under the Report tab will be printed.

- (h) Overlay
Spectra can be overlaid in the monitor window. This setting cannot be selected for repeat measurement.

(4) Processing

On selection of the Processing tab, the window in Fig. 3-6 will be displayed.

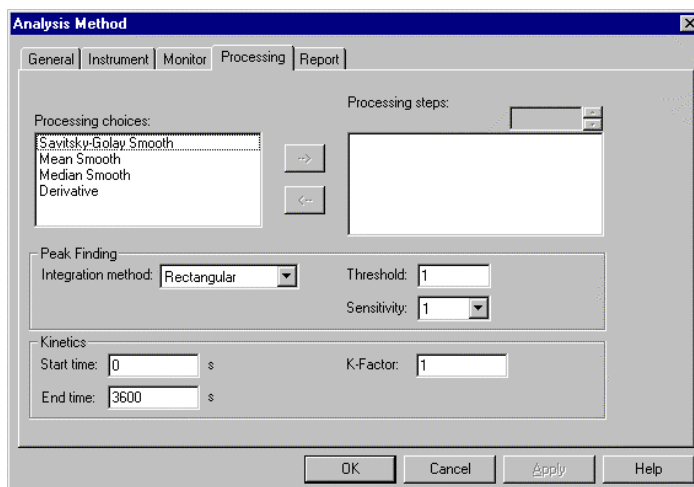


Fig. 3-6 Processing Tab

- (a) Processing choices
A list of data processing (Savitsky-Golay smooth, Mean smooth, Median smooth, Derivative) is shown. Select a data processing item, and click the rightward-pointing arrow key between the Processing choices and Processing steps display fields. Then, the selected method appears in the Processing steps field.

- (b) Processing steps
The processing sequence is displayed. To delete a processing method, first select the method, and then click the leftward-pointing arrow key between the Processing choices and Processing steps display fields. Then, the selected method disappears from the Processing steps field.
Also, in case you want to carry out any smoothing in Processing steps, double-click the relevant item and the smoothing parameters (order, data points, etc.) can be changed.

- (c) Peak Finding
Set threshold and sensitivity values for peak detection.

- (d) Kinetics
Input a start time, end time and K-factor for kinetics (rate calculation).

3.2 Creating an Analysis Method

(5) Report

Select the Report tab, and the window in Fig. 3-7 will appear.

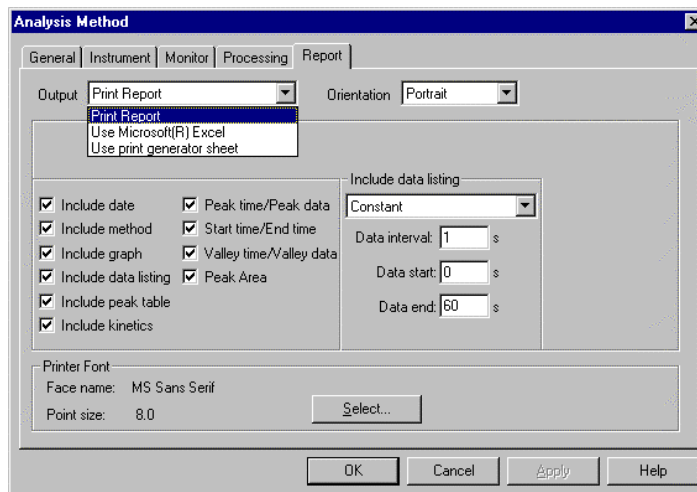


Fig. 3-7 Report Tab

This window is used to select an output method and items of measurement result.

(a) Output

1) Print Report

A report will be printed. For printing method, refer to 3.6.

2) Use Microsoft® Excel

Data will be transferred to Microsoft® Excel. For transfer method, refer to 6.13.

3) Use print generator sheet

Select this method for using the report generator (option). For usage, refer to the instruction manual of the report generator.

(b) Print format

Selectable when Report is chosen for Print Method. Select a printing orientation. Either Portrait or Landscape orientation is selectable. When the Landscape orientation is selected, date and method can be printed.

(c) Printable (transferable) items

1) Include data

The date and time of analysis can be printed.

Printing time will always be printed.

2) Include method

3) Include graph

4) Include data listing

- Constant

Data will be printed at fixed intervals between 2 wavelength points. Input Data interval, Start wavelength and End wavelength.

- Select data

Data at the specified wavelength will be printed.

Up to 12 wavelengths can be specified.

5) Include peak table

Select output items in the peak table.

- Peak time/Peak data

- Start time/End time

- Valley time/Valley data

- Peak Area

6) Include kinetics


(d) Printer Font

Click this button for changing the font to be used in the report.

3.3 Defining Your Samples

3.3 Defining Your Samples

When “Use Sample Table” has been set for the Analysis Method, the samples must be defined before measurement.

- (1) Open the monitor window and click the  button.
- (2) When the Sample Table window in Fig. 3-8 appears, fill in the blank section. For instance, if there are five samples, click the **Insert** button five times.

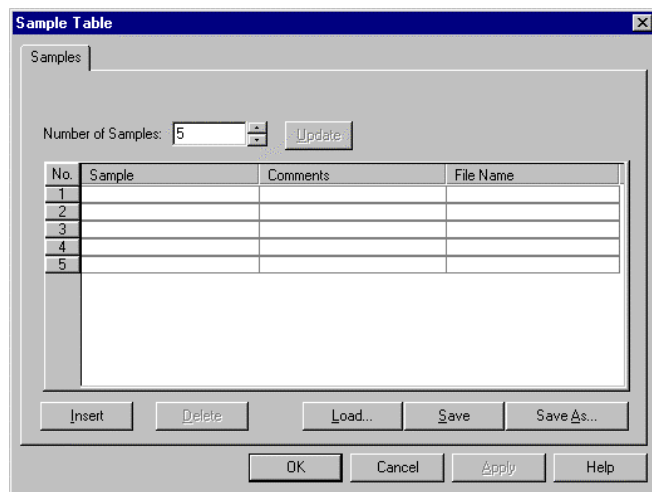


Fig. 3-8 Sample Table Window


- (3) Input the Sample, Comments and File Name for each sample. The sample names input here appear on the monitor window also.

Maximum number of characters to be input

Sample : 20 characters

Comments : 255 characters

- (4) When input is finished, check the entries and then click the **OK** button.

If a sample table is not used, then click the  button. The window in Fig. 3-9 will appear. Make entries for Sample and Comment. For automatic saving, put a check mark in the “Auto file saving after measurement” box and specify a file name for saving.

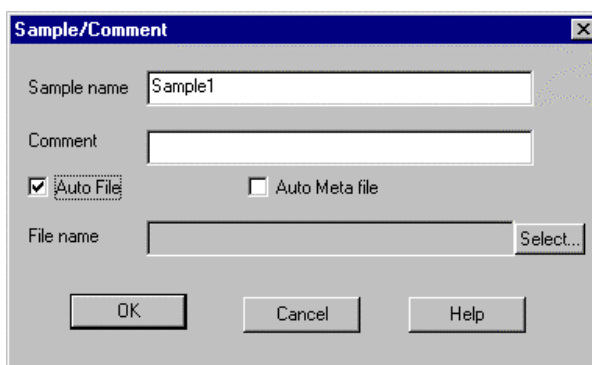





Fig. 3-9

3.4 Conducting a Time Scan

3.4 Conducting a Time Scan

- (1) There are two methods of starting the scan.
 - (a) Select the Measure command from the Spectrophotometer menu.
 - (b) Click the  (measurement) button on the measurement toolbar.

For halting the measurement midway, click the  button on the toolbar.

- (2) Upon clicking the  button, the window shown in Fig. 3-10 appears when using a sample table. Now click **Yes**.

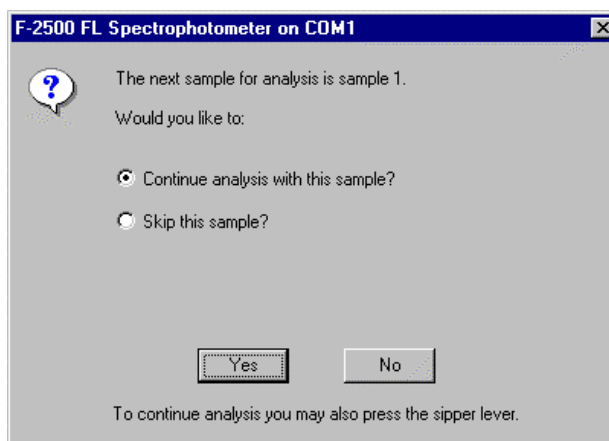


Fig. 3-10

- (3) The dialog box as in Fig. 3-11 will open. Set each sample and click **OK**.

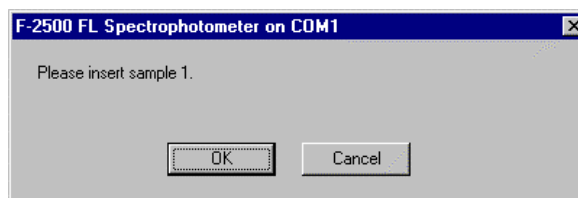


Fig. 3-11

- (4) When automatic saving is set, data will be saved after completion of measurement. Upon opening its file, a data processing window will appear as in Fig. 3-12. Although a peak table can be displayed, simultaneous display of rate calculation (kinetics) result and peak table cannot be selected. Fig. 3-12 shows a spectrum and the result of rate calculation. This is selectable from the View menu. Unless automatic saving is set, select the Save As command from the File menu and save data in file.

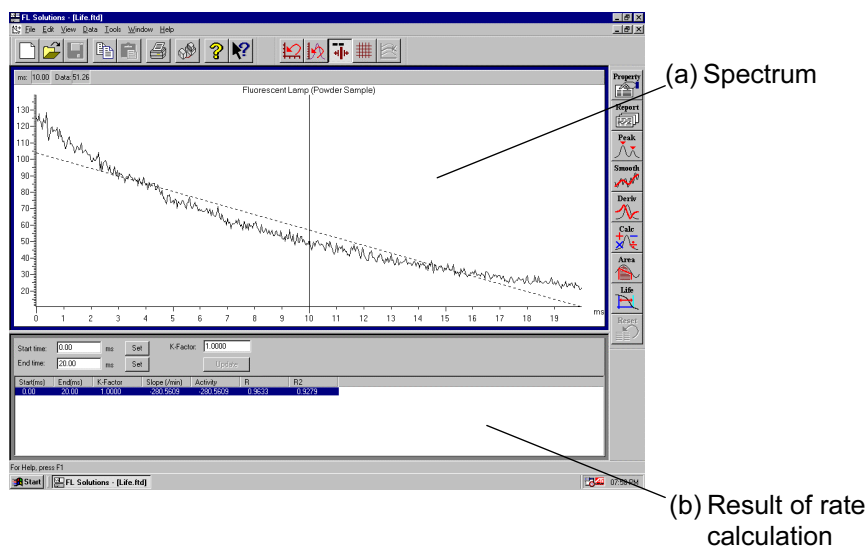



Fig. 3-12 Data Processing Window (time scan)

- (a) Spectrum
The spectrum of a measured sample is traced.
- (b) Result of rate calculation
The results of rate calculation (gradient, activity, R, R2) are displayed. Start time and end time are settable by numeric input or clicking **Set** with the line cursor aligned. After setting, click **Update**. For rate calculation, refer to APPENDIX B.
- (c) Peak table
Peak wavelength, valley wavelength, peak start/end wavelengths and peak area are displayed.

3.5 Data Printout

3.5 Data Printout

Printout can be provided even when the function for automatic printout after measurement is not used. The following two procedures are available.

- (1) Select the Print command from the File menu. Or click the  (print) button on the toolbar. The field active on the data processing window (profiled in blue when clicked, field (a) in Fig. 3-12) will be printed. The contents, that are displayed by selecting the Print Preview command from the File menu as shown in Fig. 3-13, will be printed.

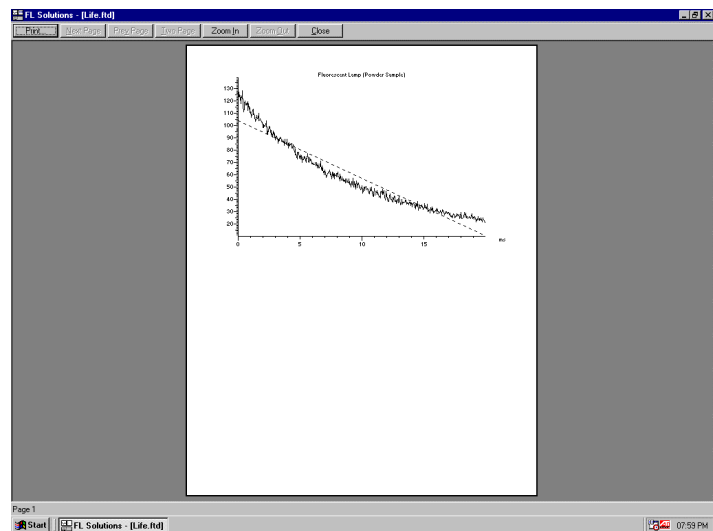



Fig. 3-13

- (2) Select the Report command in the Data menu. Or click the  (report) button on the toolbar. The Print Preview window will appear as shown in Fig. 3-14. Then, click **Print**. Select the items to be printed at the Report tab under Properties on the Edit menu.

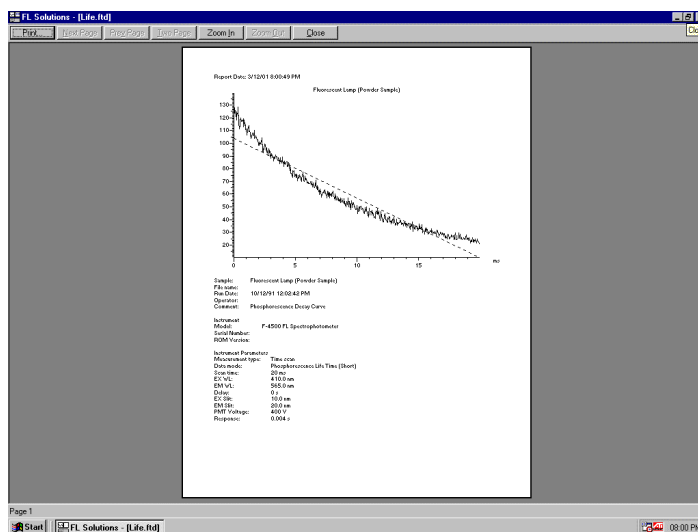



Fig. 3-14

NOTE: If characters overflow from the print frame in repeat measurement or in overlaying, then change the printing orientation from Portrait to Landscape.

3.6 Calculating the Phosphorescence Life

3.6 Calculating the Phosphorescence Life

This is the function for calculating phosphorescence life from the measurement result (phosphorescence attenuation curve). Replacing data value at T1 with I_0 , $I_{0/e}$ time (T2) is found, and T1, T2 and τ (phosphorescence life) = T2 - T1 are displayed. Select the phosphorescence life command from the data (D) menu or click the  button on the Processing toolbar. A window as in Fig. 3-15 will appear.

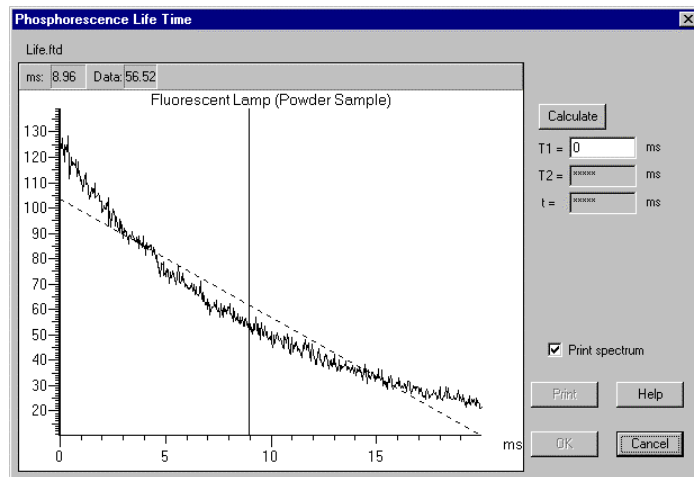


Fig. 3-15

Move the line cursor to the start point (T1) of calculation by the mouse or arrow keys [←] and [→] on the keyboard. Right-click the mouse at the start point. Or input a numeral in the T1 field and click **Calculate**. The result of calculation will be displayed.

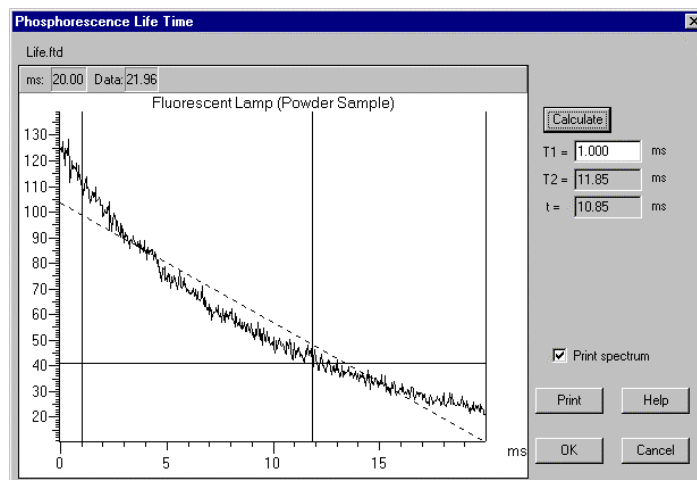


Fig. 3-16

For printout of the calculation result, click **Print**. For printout of the spectrum and calculation result, apply a check mark to “With spectrum record” and click **Print**.

For scale expansion, specify the area to be enlarged with the mouse.

Draw a rectangle with the mouse button held down as shown in Fig. 3-17.

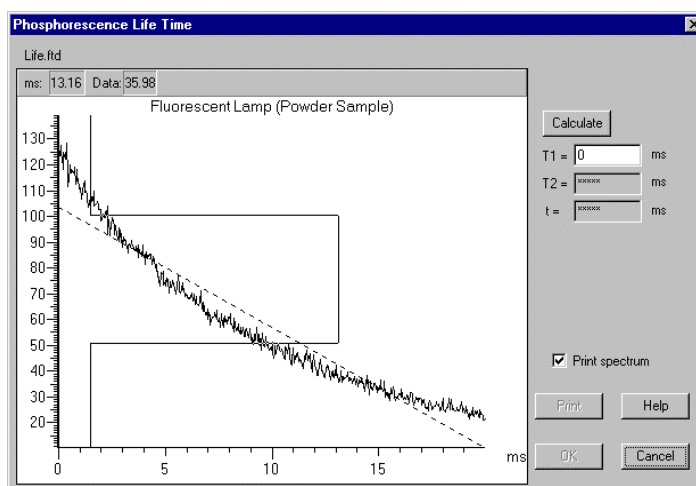


Fig. 3-17

When releasing the mouse button, the specified area will be enlarged as shown in Fig. 3-18.

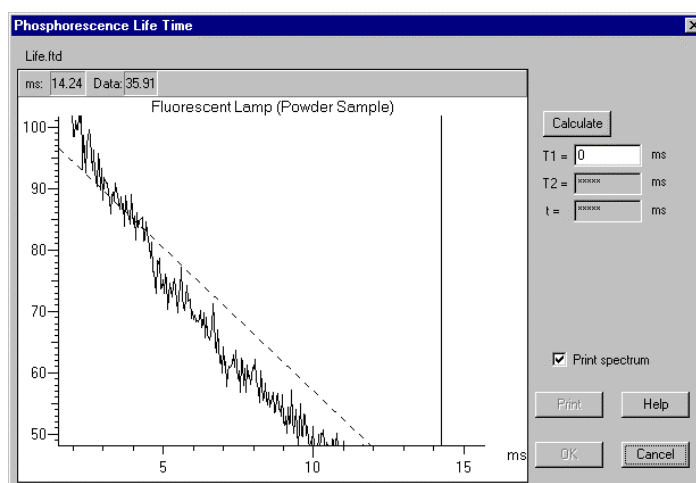


Fig. 3-18

Double-clicking on the spectrum selects auto scale.

4. PHOTOMETRY

4.1 Flow of Operation

This section explains the method of determining sample concentration with a calibration curve prepared. Following is the operational flow.

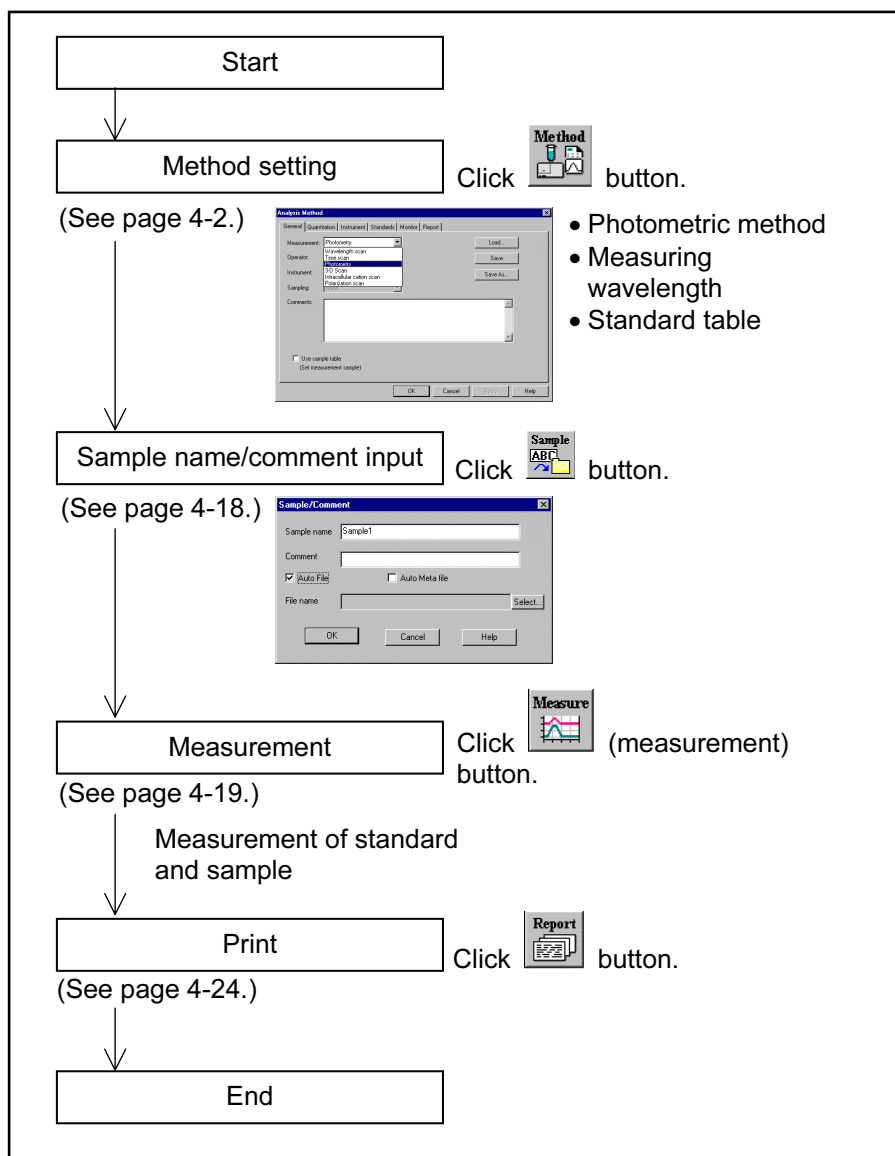


Fig. 4-1 Operational Flow (photometry)

4.2 Creating an Analysis Method

4.2 Creating an Analysis Method

Set the analytical conditions (method) prior to measurement. From the Edit menu, select the Method command or click the button. The dialog box shown in Fig. 4-2 will appear.

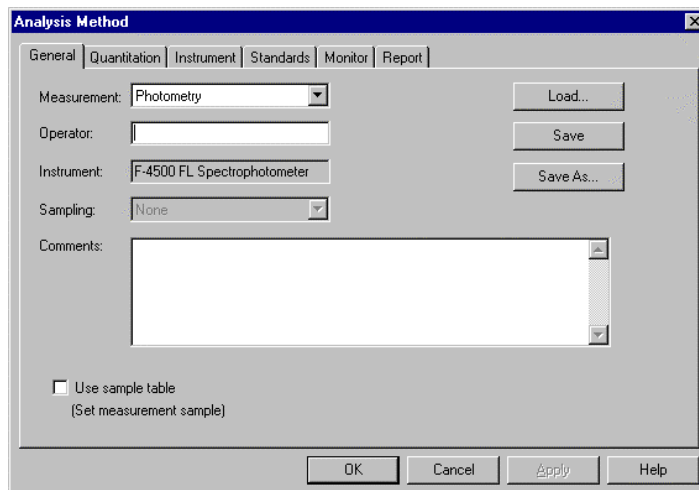


Fig. 4-2 Analysis Method

The following tabs are provided on the Analysis Method window for the photometry mode. These tabs are explained on the subsequent pages.

- General
- Quantitation
- Instrument
- Standards
- Monitor
- Report

(1) General

The parameters shown in Fig. 4-3 are provided under the General tab.

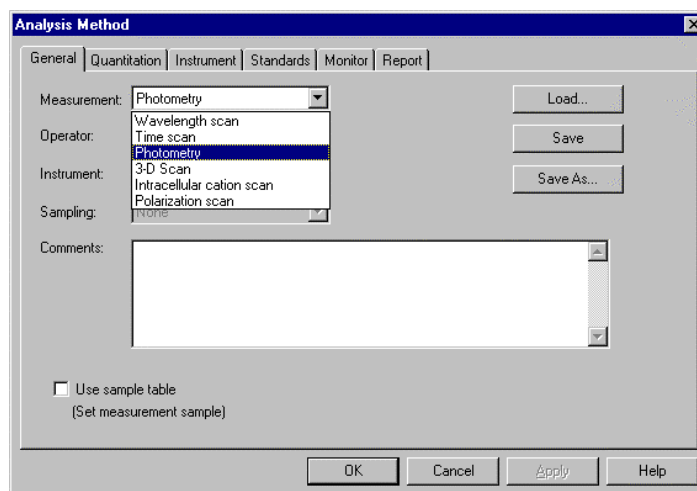


Fig. 4-3 General Tab

(a) Measurement (mode)

Select a measurement mode from among the four listed below.

- Wavelength scan
- Time scan
- Photometry
- 3-D Scan (displayed when the '3-D Scan program' (option) is installed in combination with Model F-2500)

Here, select "Photometry." For Wavelength scan, Time scan and 3-D Scan, refer to Sections 2, 3 and 5.

(b) Operator

Enter an operator name.

(c) Instrument

The model of the connected instrument is indicated.

(d) Use sample table (Set measurement sample)

Apply a check mark here when a sample table is used.

4.2 Creating an Analysis Method

- (e) Sampling
 - Standard
 - When sample is exchanged manually.
 - (f) Comments
 - Enter a description or notes on measuring conditions.
 - (g) Load
 - Click this button to read out saved analytical conditions.
 - The file opening window appears upon clicking, so select an analytical conditions file.
 - (h) Save
 - By clicking this button after setting the parameters for all six tabs, the analytical conditions are saved.
 - Clicking this button displays the “Save As” window in creation of a new analytical conditions file. So input a file name.
 - (i) Save As
 - Click this button for renaming the set conditions for saving.
- (2) Quantitation

Upon selecting the Quantitation tab, the window shown in Fig. 4-4 appears.

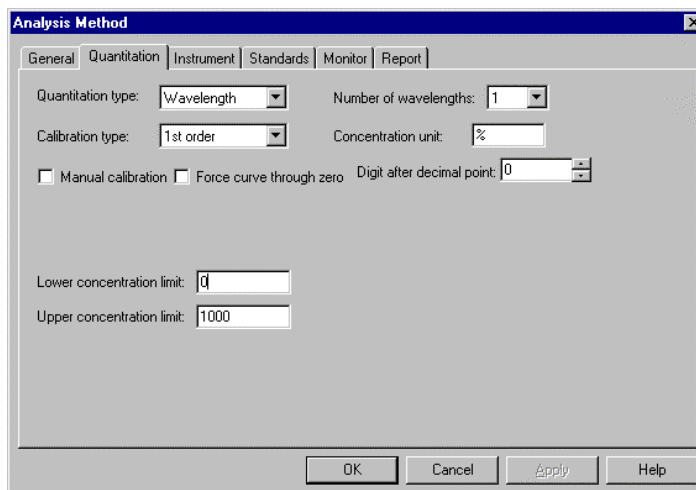


Fig. 4-4 Quantitation Tab

(a) Quantitation type

Select the method of creating a calibration curve.

Wavelength, Peak area, Peak height or Derivative method is selectable.

Spectra measured by the Peak area, Peak height and Derivative method can be checked.

1) Wavelength

Upon selecting "None" for calibration curve type in this quantitation method, up to 6 wavelengths can be set. When any other than "None" is selected for quantitation type, up to 3 wavelengths can be set.

(i) One wavelength calculation

This is the most generally used method.

As shown in Fig. 4-5 (a), one wavelength is specified, and the concentration is determined from the data I_1 at that time.

(ii) Two wavelength calculation

As shown in Fig. 4-5 (b), assuming I_1 and I_2 are the absorbance values at two different wavelengths, concentration is calculated through determination of I by the equation below.

$$I = I_2 - I_1$$

(iii) Three wavelength calculation

As shown in Fig. 4-5 (c), assuming I_1 , I_2 and I_3 are the data values at three different wavelengths, concentration is calculated through determination of I by the equation below. Photometry is made in the order of 1, 2, 3.

$$I = I_2 - \frac{1 - 2 \times I_3 + 2 - 3 \times I_1}{1 - 3}$$

But the following relation must hold:

$$1 < 2 < 3 \text{ or } 1 > 2 > 3$$

4.2 Creating an Analysis Method

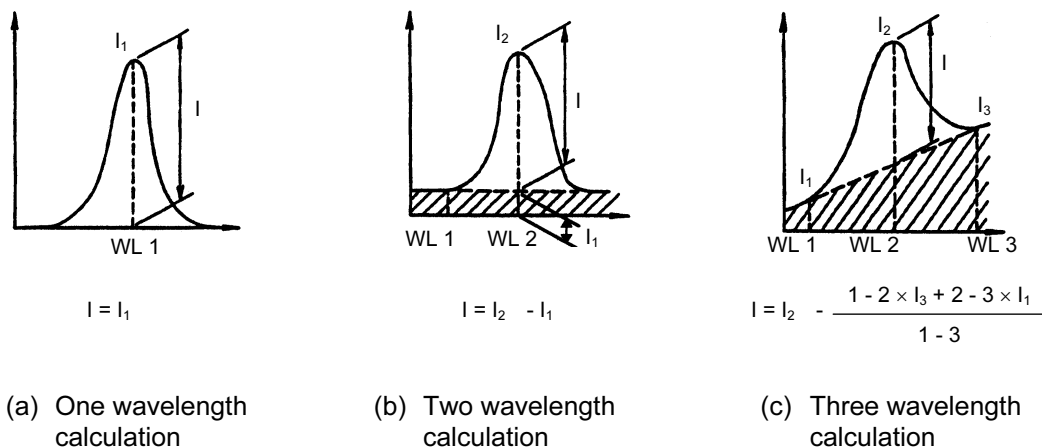


Fig. 4-5 Calculation Method

2) Peak area

Input the peak wavelength and its accuracy. Measurement is made in the wavelength range specified at the Instrument tab.

Peak wavelength is obtained and a calibration curve is prepared using the peak area of relevant peak. Select an integration method among Rectangular, Trapezoid and Romberg. Also set a threshold value and sensitivity for defining the peak.

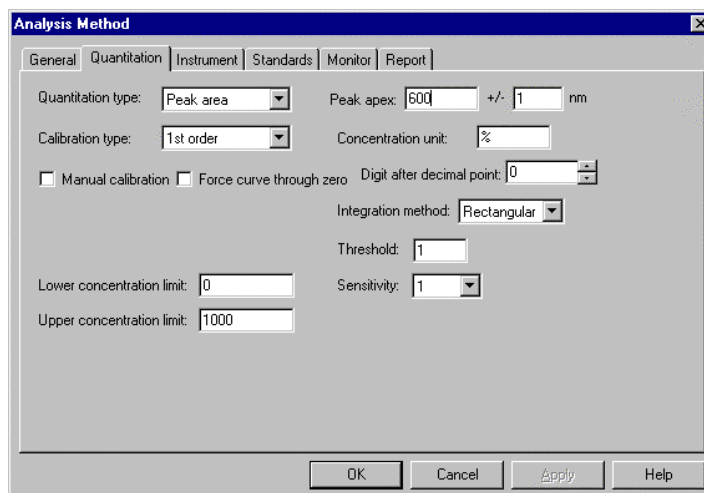


Fig. 4-6 Quantitation Tab (peak area)

3) Peak height

Input the peak wavelength and its accuracy. Measurement is made in the wavelength range specified at the Instrument tab. Peak is determined within the accuracy set here and calculation is made in the height of that peak.

The screenshot shows the 'Analysis Method' dialog box with the 'Quantitation' tab selected. The 'Quantitation type' is set to 'Peak height'. The 'Peak apex' is set to 600 nm with a +/- accuracy of 1 nm. The 'Calibration type' is '1st order' and the 'Concentration unit' is '%'. There are checkboxes for 'Manual calibration' and 'Force curve through zero', both of which are unchecked. The 'Digit after decimal point' is set to 0. The 'Lower concentration limit' is 0 and the 'Upper concentration limit' is 1000. Buttons for 'OK', 'Cancel', 'Apply', and 'Help' are at the bottom.

Fig. 4-7 Quantitation Tab (peak height)

4) Derivative

Input a peak wavelength (or wavelength to be used for calibration curve preparation). Measurement is made in the wavelength range specified at the Instrument tab and a calibration curve is created using the derivative value at the peak wavelength. Set the derivative order, smoothing order and data points as derivative parameters.

The screenshot shows the 'Analysis Method' dialog box with the 'Quantitation' tab selected. The 'Quantitation type' is set to 'Derivative'. The 'Peak apex' is set to 600 nm. The 'Calibration type' is '1st order' and the 'Concentration unit' is '%'. There are checkboxes for 'Manual calibration' and 'Force curve through zero', both of which are unchecked. The 'Digit after decimal point' is set to 0. The 'Derivative order' is set to 2, the 'Smoothing order' is 3, and the 'Number of points' is 15. The 'Lower concentration limit' is 0 and the 'Upper concentration limit' is 1000. Buttons for 'OK', 'Cancel', 'Apply', and 'Help' are at the bottom.

Fig. 4-8 Quantitation Tab (derivative)

4.2 Creating an Analysis Method

5) Ratio

Upon selecting Ratio, an input column for three factors (K0, K1, K2) and a setting column for calculation method (+, -, *) are displayed.

NOTE: By this quantitation method, a calibration curve cannot be created.

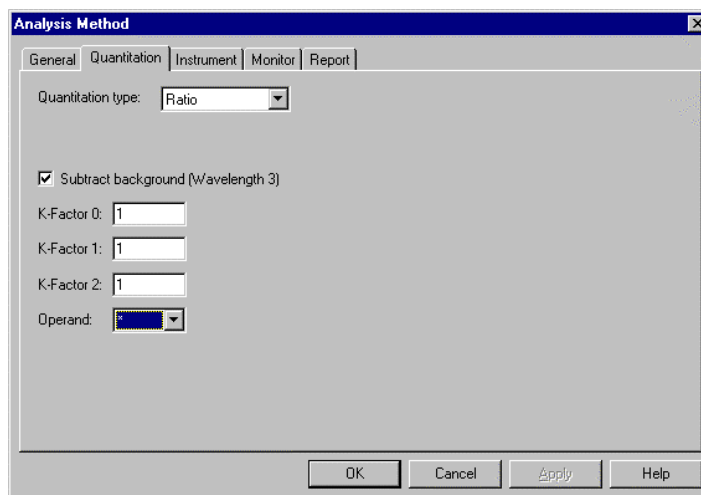


Fig. 4-9 Quantitation Tab (peak ratio)

Calculation is made by the following equation.

$$\text{RATIO} = \frac{K1 (A (WL1) - A (WL3))}{K2 (A (WL2) - A (WL3))} \#K0$$

(calculation method): +, -, *

WL1, 2, 3 : Specified wavelengths

WL3 : Background correcting wavelength

A () : Measured value at specified wavelength

(b) Calibration type

Set a calibration type. When selecting factor input (when standard calibration curve and its factors are already known), put a mark in the check box and enter values for A₀, A₁, A₂ and A₃.

- 1) None
Mode in which calibration curve is not used.
When this mode is selected with Wavelength set for Quantification type, multi-wavelength measurement at up to six wavelengths is possible. However, no spectrum can be displayed.
- 2) 1st order
This specifies the most generally used calibration curve. A linear calibration curve is to be created. The formula of calibration curve is as follows.

$$x = A_1y + A_0$$

Here, x is sample concentration and y is sample data.

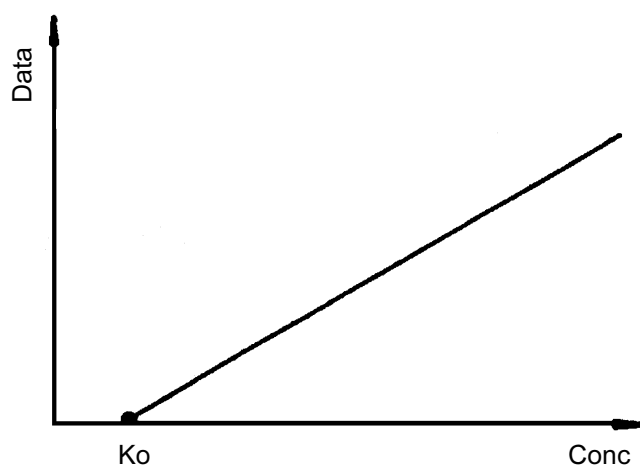


Fig. 4-10 1st Order

4.2 Creating an Analysis Method

3) 2nd order

A calibration curve is created using a quadratic curve. The formula of calibration curve is as follows.

$$x = A_2y^2 + A_1y + A_0$$

Here, x is sample concentration and y is sample data.

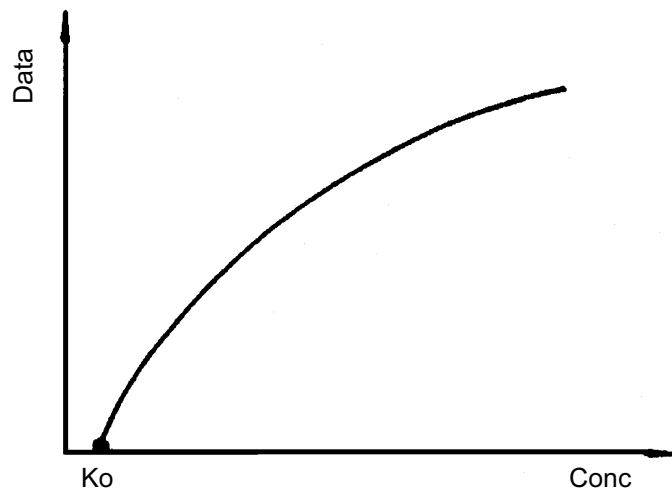


Fig. 4-11 2nd Order

4) 3rd order

A calibration curve is created using a cubic curve.
The formula of calibration curve is as follows.

$$x = A_3y^3 + A_2y^2 + A_1y + A_0$$

Here, x is sample concentration and y is sample data.

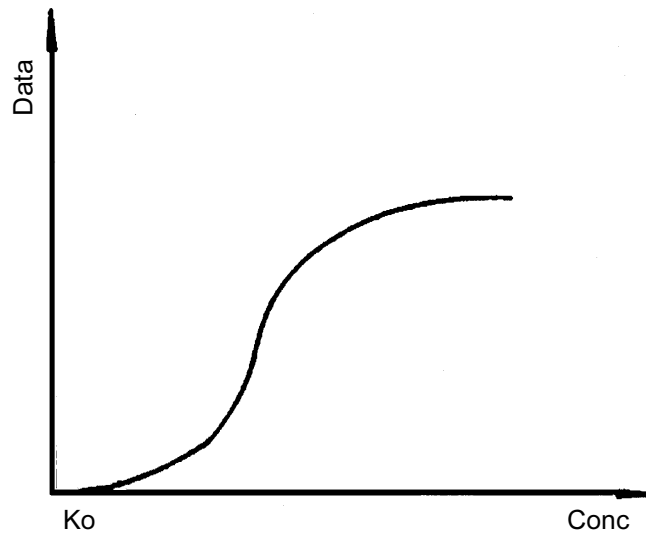


Fig. 4-12 3rd Order

4.2 Creating an Analysis Method

- 5) Segmented
A calibration curve created by connecting standard samples linearly according to the measured values of respective samples or input data.

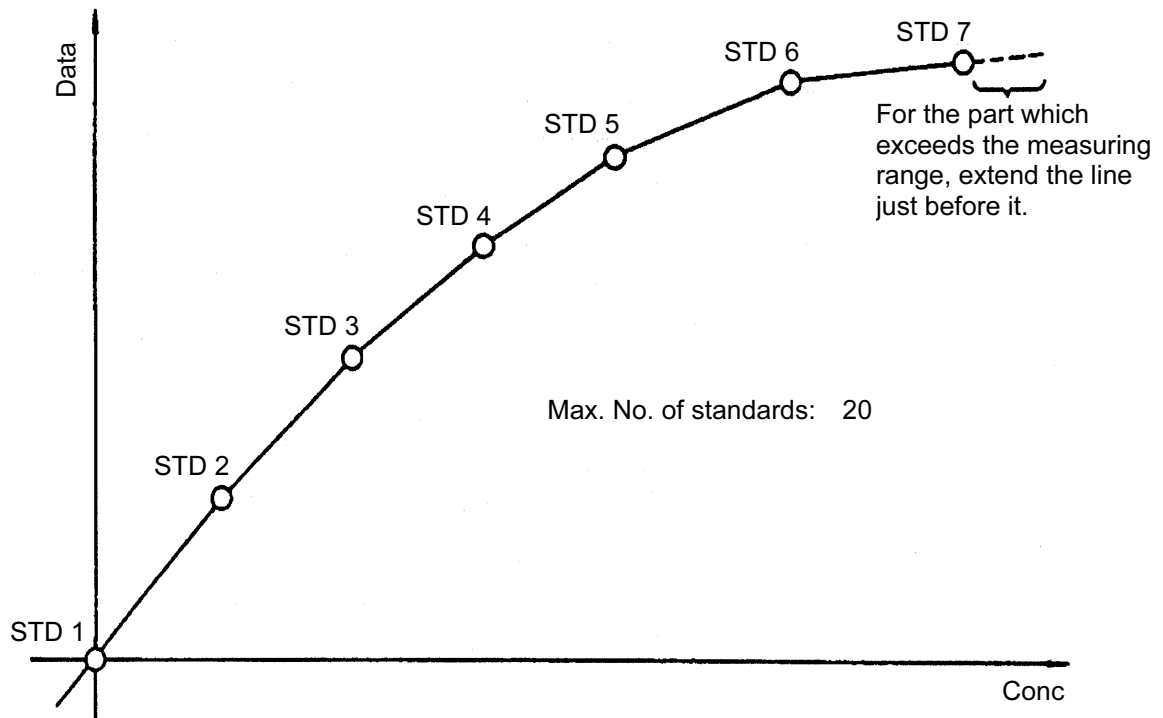
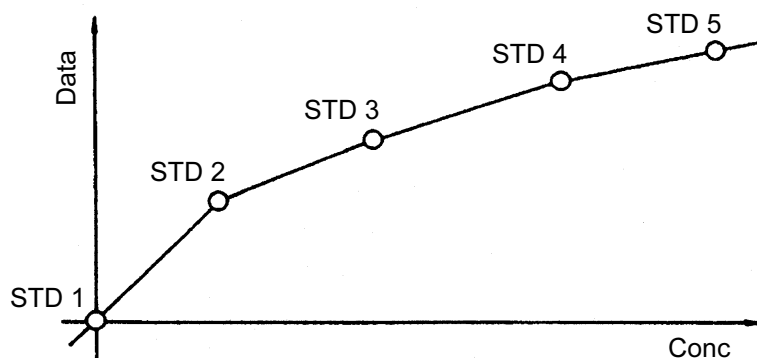
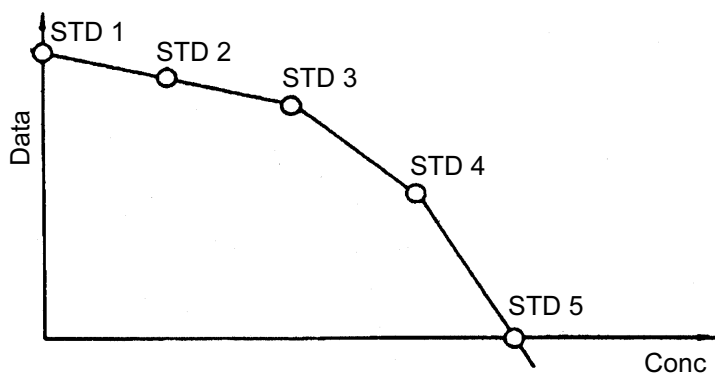


Fig. 4-13 Segmented

NOTE: A proper calibration curve can be created when the photometric value increases or decreases monotonously versus the concentration value. With a monotonic increase, the calibration curve is created with standard 1 (STD1) as origin. And with a monotonic decrease, the calibration curve is created with STD1 plotted on the photometric value axis and STD5 on the concentration axis. In these examples, the number of standards is set at 5.



Calibration curve with monotonous increase



Calibration curve with monotonous decrease

- (c) Number of wavelengths
When "Calibration type" is "None," up to six wavelengths are selectable, and with other calibration types, 1 to 3 wavelengths are selectable.
- (d) Concentration unit
Input a concentration (in mg/mL for example) from the keyboard.
- (e) Manual calibration
Put a check mark in this box for creating a calibration curve using factors. The number of factors (A_0 , A_1 , A_2 , A_3) varies with the calibration type selected.

4.2 Creating an Analysis Method

- (f) Force curve through zero
By putting a check mark in this box, a calibration curve is created so that its factor A_0 passes through "0" automatically.
- (g) Digit after decimal point
Set the number of digits after decimal point for the calculated concentration of the measured sample. The concentration value is rounded off to the set number of digits.
Input range: 0 to 3
- (h) Lower concentration limit/Upper concentration limit
This is used to set a normal value range for the concentration of the measured sample. If the concentration is higher than the upper limit set here, "H" will appear in the Avg Conc column, and if lower than the lower limit set here, "L" will appear in the Avg Conc column.

(3) Instrument

When you click the Instrument tab, the window in Fig. 4-14 appears.

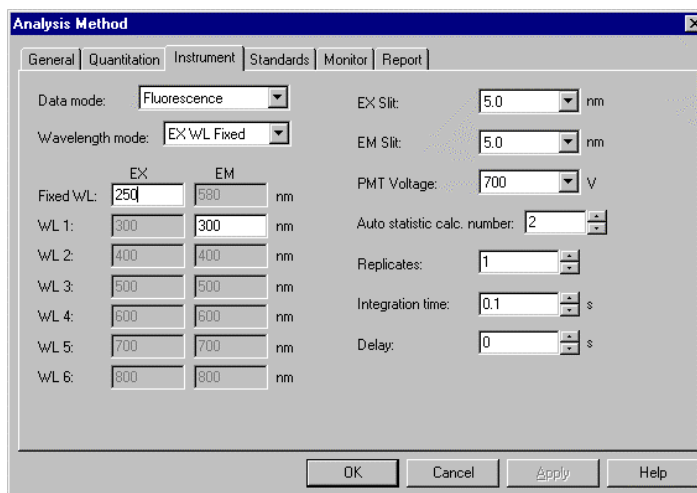


Fig. 4-14 Instrument Tab

- (a) Data mode
Select one of the following data modes.
The Luminescence mode is for measuring luminescence in the biological/chemical field and only the sample side signal is acquired without performing ratio photometry.
- Fluorescence
 - Luminescence
 - Phosphorescence
- (b) Wavelength mode
Select a wavelength for creating a calibration curve.
- EX WL Fixed
 - EM WL Fixed
 - Both WL Fixed
- (c) Wavelength (1 to 6)
Input the number of wavelengths specified at the Quantitation tab.

Model	Input Range
F-4500	0, 200 to 900 nm
F-2500	0, 220 to 800 nm

- (d) EX Slit
Select a slit width for the excitation side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

- (e) EM Slit
Select a slit width for the emission side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

4.2 Creating an Analysis Method

- (f) **PMT Voltage**
A function for controlling the voltage of the photomultiplier detector (photomultiplier tube).
Select one of the following values. Changing from 400 V to 700 V increases the data value about two digits and changing from 700 V to 950 V increases it about 1 digit.
- 950 V (only with F-4500)
 - 700 V
 - 400 V
- (g) **Auto statistic cal. number**
Calculation is made for mean value, SD (standard deviation) and CV (coefficient of variation). Set the number of samples for this purpose.
Input range: 2 to 4000
- (h) **Replicates**
Set the number of measurements.
Input range: 1 to 20
- (i) **Integration time**
A function for obtaining data averaged over the specified time for the purpose of acquiring stabilized data.
F-4500: 0.1 to 10.0 s
F-2500: 1, 2, 5, 10 s
- (j) **Delay**
After pressing the Measure button, measurement is started following the delay time set here. It is used for temperature stabilization, etc. In repeat measurement, it is the time until the start of the first measurement. The delay time is invalid for the second and subsequent measurements.
Input range: 0 to 9999 s

(4) Standards

Clicking the Standards tab opens the window shown in Fig. 4-16.

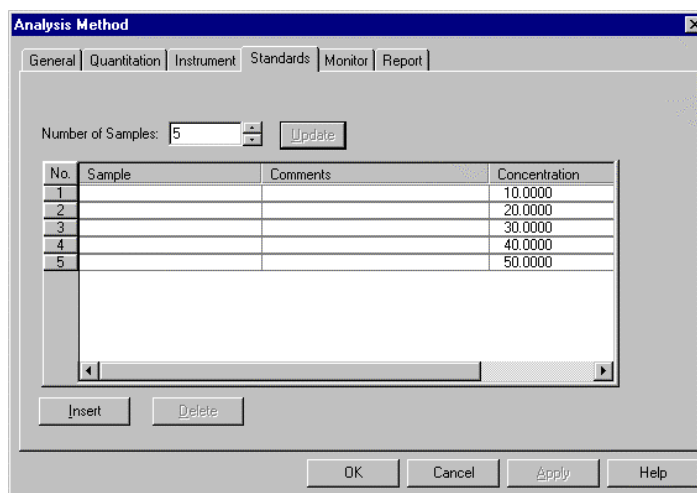


Fig. 4-16 Standards Tab

(a) Sample table

Sample table gives a list of standards for sample measurement or calibration curve preparation. This table contains the items listed below.

NOTE: The size of the sample table columns (cells) is changeable. Bring the cursor to a boundary line between left and right table headers, and it changes into the one shown below.



Now click the mouse button on the line and drag it to the left or right to obtain a suitable column size.

- 1) No.
This is the number given to each sample.
- 2) Sample
Enter a sample name in up to 20 characters.
- 3) Comments
Enter comments on each sample.
- 4) Concentration
Enter a sample concentration.

4.2 Creating an Analysis Method

- (b) Update
By clicking this button, sample numbers are set by the entered number of samples. The displayed sample names, comments, etc. are all cleared.
 - (c) Insert
When the initial screen is opened, the **Insert** button becomes active. Click this button to insert data at the end of the sample list.
 - (d) Delete
Click the column of the sample No. to be deleted, and it becomes active. Now click the **Delete** button and the item is deleted.
- (5) Monitor

When selecting the Monitor tab, the window in Fig. 4-17 appears.

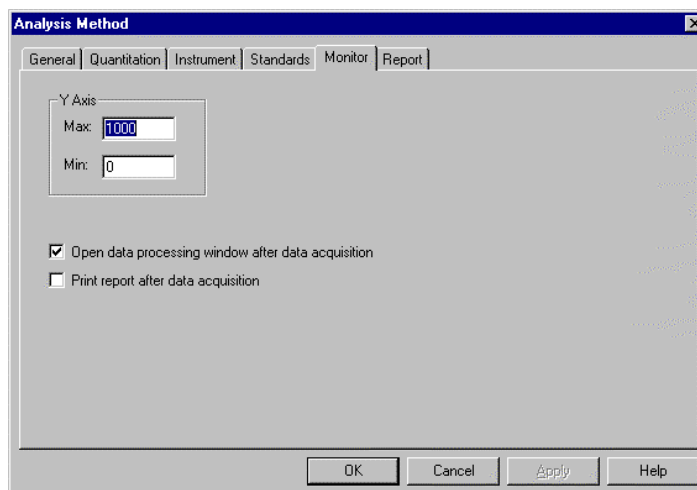


Fig. 4-17 Monitor Tab

Assign the following parameters.

- (a) Y-Axis Max (maximum)
Input a maximum value for Y-axis on the monitor window.
- (b) Y-Axis Min (minimum)
Input a minimum value for Y-axis on the monitor window.

- (c) Open data processing window after data acquisition
Select whether or not to conduct data processing after sample measurement. When selected (check mark is applied), the data is saved automatically after measurement and its file is displayed as an icon on the window. And by opening this icon, data processing such as peak detection is available. When this function is not selected, data processing can be achieved by reading out the file via **File-Open**.
- (d) Print report after data acquisition
Select whether or not to print a report after sample measurement. When selected (check mark is applied), printing will be made automatically after measurement. The items set under the Report tab will be printed.
- (6) Report

Select the Report tab, and the window in Fig. 4-18 will appear.

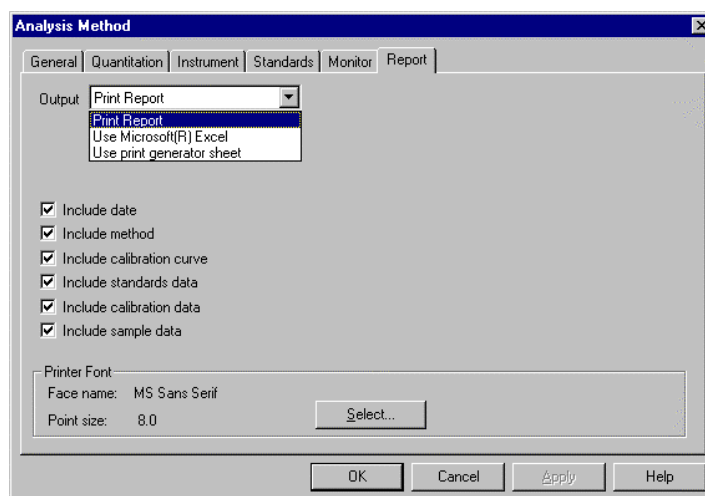


Fig. 4-18 Report Tab

On this window, select the items to be contained in an analysis report.

4.2 Creating an Analysis Method

(7) Output

(a) Print Report

A report will be printed. For printing method, refer to 4.5.

(b) Use Microsoft® Excel

Data will be transferred to Microsoft® Excel.
For transfer method, refer to 6.13.

(c) Use print generator

Select this method for using the report generator (option). For usage, refer to the instruction manual of the report generator.

<Printable (transferable) items>


- Include date
- Include method
- Include calibration curve
- Include standards data
- Include calibration data
- Include sample data

(8) Printer Font

Click this button for changing the font to be used in the report.

4.3 Defining Your Samples

When “Use Sample Table” has been set for the Analysis Method, the samples must be defined before measurement.

- (1) Open the monitor window and select the Sample table command in the Edit menu or click the  button.
- (2) When the Sample Table window in Fig. 4-19 appears, fill in the blank section. For instance, if there are five samples, click the **Insert** button five times. Or enter “5” for Number of Samples and click the **Update** button.

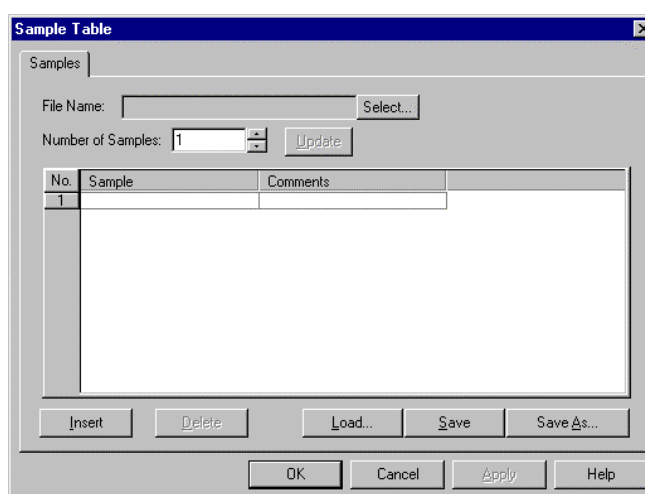


Fig. 4-19 Sample Table Window

- (3) Input the sample name of each sample and comments on it. Since sample names cannot be changed after measurement, input them here.
Maximum number of characters to be input
Sample : 20 characters
Comments : 255 characters
- (4) Input the name of the data file after measurement.
- (5) When input is finished, check the entries and then click the **OK** button.

4.4 Conducting Measurement

4.4 Conducting Measurement

First, create a working curve, and then proceed to sample measurement.

- (1) Set the analysis method, and the window in Fig. 4-20 will appear provided a sample table is not used.

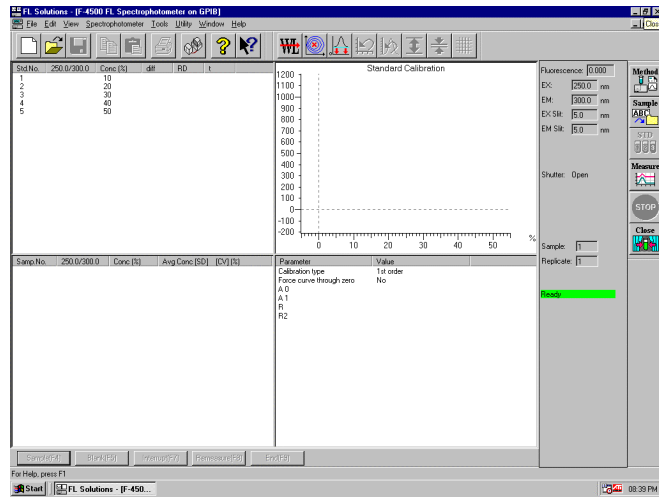


Fig. 4-20

When a sample table is used, the window in Fig. 4-21 will appear.

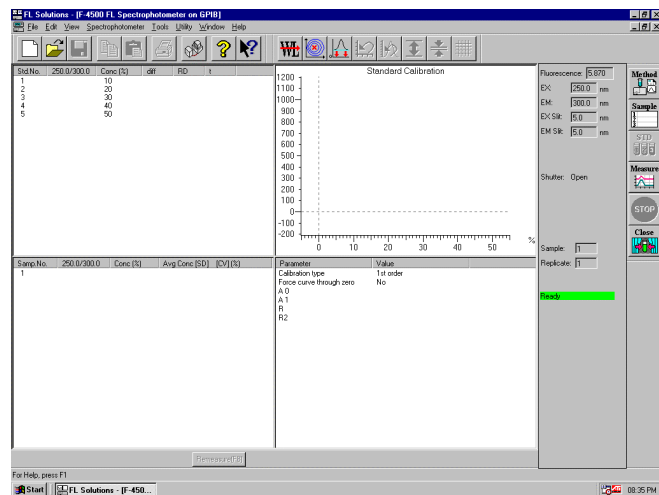


Fig. 4-21


- (2) There are two methods of starting measurement.
 - (a) Select the Measure command from the Spectrophotometer menu.
 - (b) Click the  (measurement) button on the measurement toolbar.
- (3) Standards will be measured. When finished, the window in Fig. 4-22 will appear. So click the **OK** button.



Fig. 4-22

- (4) Now measure the samples. If a sample table is not used, click the **Sample** button. To interrupt the measurement for other samples, click the **Interrupt** button. The window in Fig. 4-23 will appear, so enter a sample No.

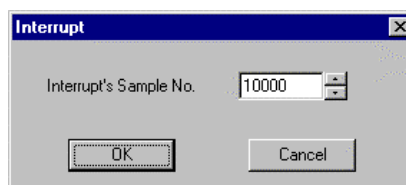



Fig. 4-23

Click the **OK** button and measurement starts. When a sample table is used, a message box is displayed, so conduct operation according to the messages.

- (5) When sample measurement is finished and a sample table is not used, click the **End** button. From the File menu, select the Save As command and save the data. When a sample table is used, the data will be saved automatically.

4.4 Conducting Measurement

- (6) For conducting remeasurement after completing a series of measurements, click the data area to be remeasured as shown in Fig. 4-24 and then click the **Remeasure** button. For saving data after remeasurement, click the End button or  button. The procedure is the same when a sample table is used. Both standards and samples can be remeasured.

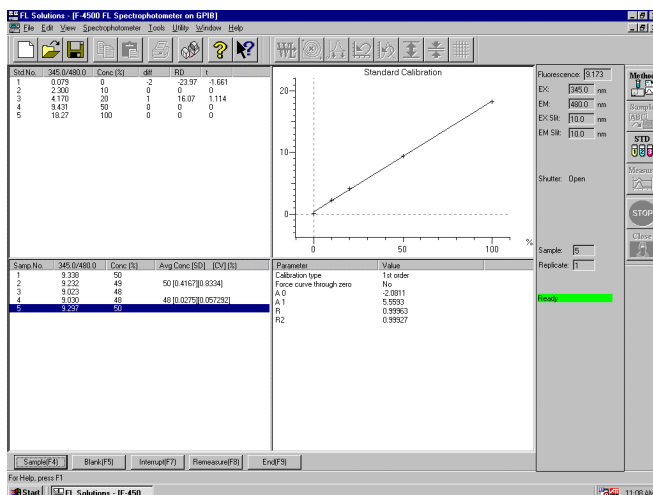


Fig. 4-24

- (7) For conducting a blank measurement, click the **Blank** button.
- (8) Data is saved on completion of measurement. When its file is opened, a data processing window shown in Fig. 4-25 appears.

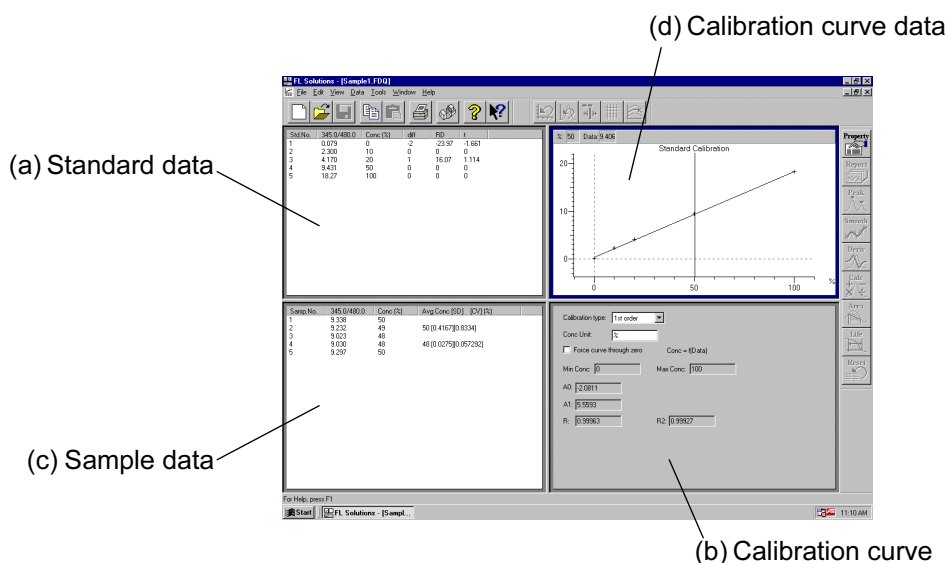


Fig. 4-25 Data Processing Window (photometry)

(a) Standard data

The measurement results of standards used for preparing a calibration curve are displayed.

Std.No.	345.0/480.0	Conc (%)	diff	RD	t
1	0.079	0	-2	-23.97	-1.661
2	2.300	10	0	0	0
3	4.170	20	1	16.07	1.114
4	9.431	50	0	0	0
5	18.27	100	0	0	0

Fig. 4-25-1 Standard Data

(b) Calibration curve

A calibration curve obtained from standard data is displayed. The curve can also be traced.

Concentration and photometric value are indicated at the top left of this field.

(c) Sample data

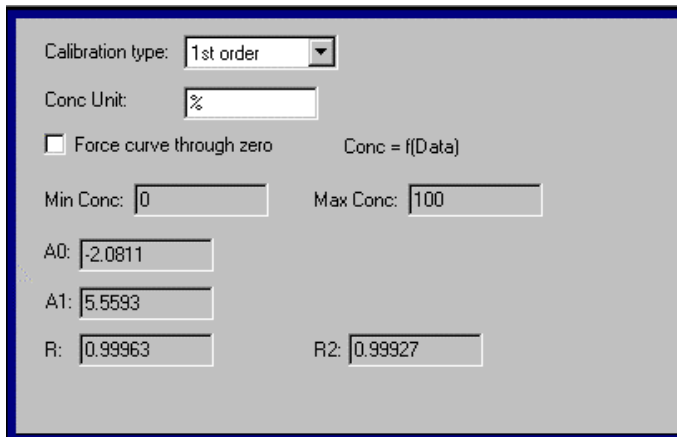
Sample data such as photometric value (Abs), concentration (Conc), mean value (Avg Conc), standard deviation (SD), coefficient of variation (CV) and upper/lower limit judgment results (HIGH/LOW) are displayed.

Samp.No.	345.0/480.0	Conc (%)	Avg Conc [SD] [CV] (%)
1	9.338	50	
2	9.232	49	50 [0.4167][0.8334]
3	9.023	48	
4	9.030	48	48 [0.0275][0.057292]
5	9.297	50	

Fig. 4-25-2 Sample Data

4.4 Conducting Measurement


- (d) Calibration curve data
Data such as determination coefficient and factors (A_0 , A_1 , A_2 , A_3) of calibration curve are displayed. And the calibration type can be changed.

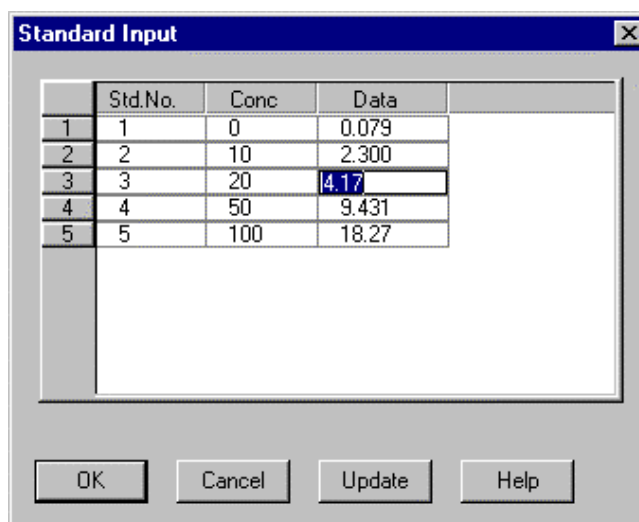


The screenshot shows a dialog box for calibration curve data. It includes a dropdown menu for 'Calibration type' set to '1st order', a text field for 'Conc Unit' set to '%', a checkbox for 'Force curve through zero' which is unchecked, and a formula 'Conc = f(Data)'. Below these are text fields for 'Min Conc' (0) and 'Max Conc' (100). At the bottom, there are text fields for coefficients: 'A0' (-2.0811), 'A1' (5.5593), 'R' (0.99963), and 'R2' (0.99927).

Fig. 4-25-3 Calibration Curve Data

4.4.1 Manual Input of Standard Data

The standard data can be input manually. This function is usable after measurement of standards. Either select **Standard** from the Edit menu of monitor window or click the  button. A window as in Fig. 4-26 will appear. So enter values in the Data column. After entry, click the **OK** or **Update** button. Then, the calibration curve is recalculated and displayed.



The screenshot shows a dialog box titled 'Standard Input' with a table for entering standard data. The table has columns for 'Std.No.', 'Conc', and 'Data'. The data is as follows:

	Std.No.	Conc	Data
1	1	0	0.079
2	2	10	2.300
3	3	20	4.17
4	4	50	9.431
5	5	100	18.27

At the bottom of the dialog box are buttons for 'OK', 'Cancel', 'Update', and 'Help'.

Fig. 4-26

4.4.2 Statistical Calculation

Statistical calculation is performed for the measured sample. The results are displayed in the "Avg Conc" column of the data processing window as shown in Fig. 4-25-2.

$$\text{MEAN} = \frac{\sum X_i}{N}$$

$$\text{CV} = \frac{\text{SD}}{\text{MEAN}} \times 100$$

$$\text{SD} = \sqrt{\frac{\sum X_i^2 - (\sum X_i)^2 / N}{N - 1}}$$

Here, N = number of standards

MEAN = mean value

SD = standard deviation


CV = coefficient of variation (%)

NOTE: "*****" is indicated for SD and CV values when they are nearly "0."

4.5 Printing Data

4.5 Printing Data

Printout can be provided even when the function for automatic printout after measurement is not used. The following two procedures are available.

- (1) Select the Print command from the File menu. Or click the  print button on the toolbar. The field active on the data processing window (e.g., (b) in Fig. 4-25) will be printed. What is displayed by clicking the Print Preview command in the File menu will be printed.

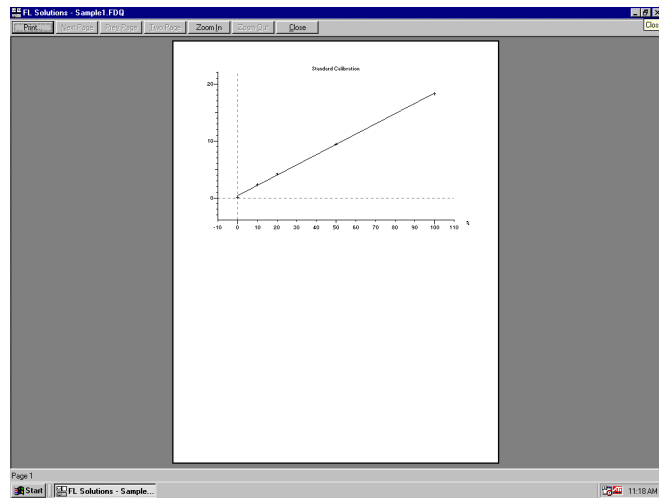



Fig. 4-27

- (2) Select the Report command in the Data menu. Or click the  button on the toolbar. The Print Preview window will appear. So click the **Print** button. Select the items to be printed at the Report tab with the Properties command chosen in the Edit menu. The measuring parameters will also be printed.

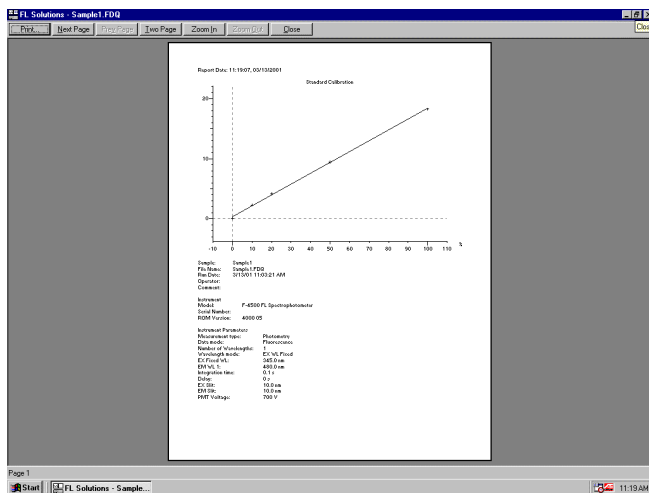


Fig. 4-28

4.6 Remeasuring Standard Data and Sample Data

4.6 Remeasuring Standard Data and Sample Data

Standard data and sample data can be remeasured at the midpoint of a series of measurements or after it.

For remeasurement, implement the following procedure.

- (1) Open the monitor window after completion of measurement.
- (2) Click the data to be remeasured.

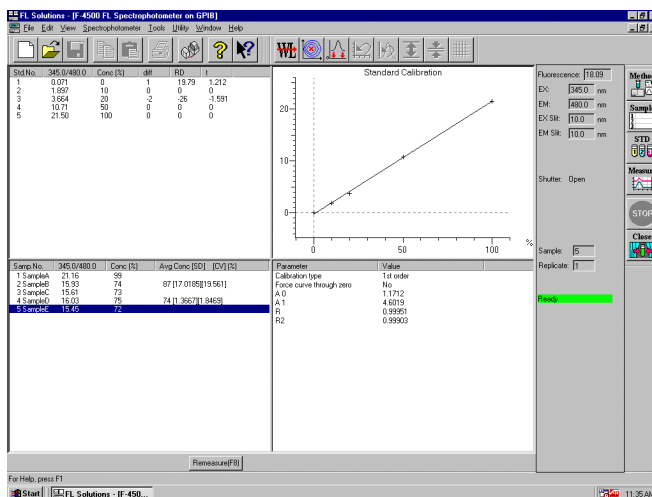


Fig. 4-37

- (3) Click **Remeasure** or select the Remeasure (E) command from the Spectrophotometer (S) menu. The dialog box in Fig. 4-38 will open.

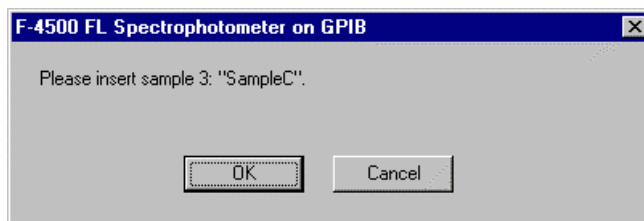


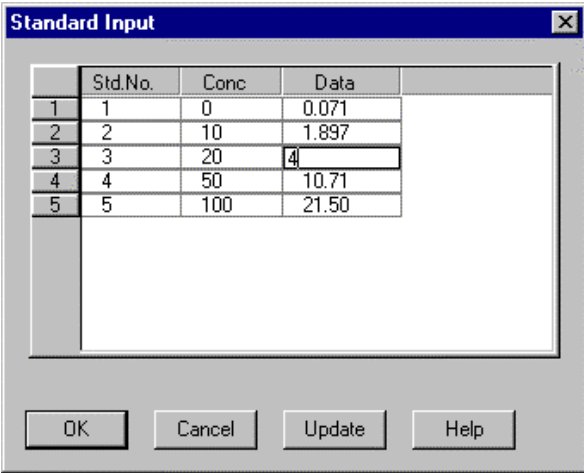


Fig. 4-38

- (4) Click **OK**, and measurement will start. When a standard is remeasured, the calibration curve will be recalculated and displayed.
- (5) Click the  button, and the data will be saved.

4.7 Manual Input of Standard Data

The standard data can be input manually. This function is usable after measurement of standards. Either select Standard (T) from the Edit menu of monitor window or click the  button. A window as in Fig. 4-39 will appear. So enter values in the Data column. After entry, click the **OK** or **Update** button. Then, the calibration curve is recalculated and displayed.



The image shows a dialog box titled "Standard Input" with a table containing five rows of data. The columns are labeled "Std.No.", "Conc", and "Data". The data values are: Row 1: 1, 0, 0.071; Row 2: 2, 10, 1.897; Row 3: 3, 20, 4; Row 4: 4, 50, 10.71; Row 5: 5, 100, 21.50. Below the table are four buttons: "OK", "Cancel", "Update", and "Help".

	Std.No.	Conc	Data
1	1	0	0.071
2	2	10	1.897
3	3	20	4
4	4	50	10.71
5	5	100	21.50

Fig. 4-39

4.8 Measurement Using Saved Calibration Curve

4.8 Measurement Using Saved Calibration Curve

Call a calibration curve in the following way.

- (1) Set measurement mode in Photometry.
- (2) From the File (F) menu, select the calibration curve load command. A window as shown below will appear. Select the file of the calibration curve to be used.

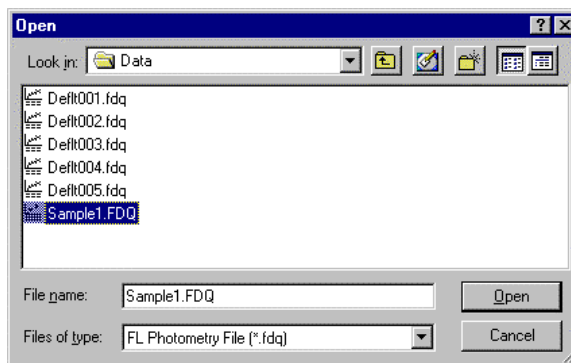


Fig. 4-40

- (3) When selecting the file, the window as shown in Fig. 4-41 will appear. The sample data section is blank.

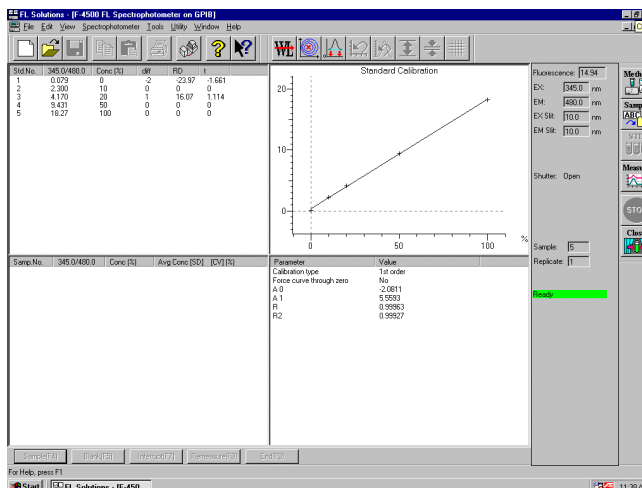




Fig. 4-41

- (4) Edit a sample table.
- (5) Set a blank sample to carry out auto zero.
From the Spectrophotometer (S) menu, select the Auto zero (Z) command. Or click the  button.
- (6) From the Spectrophotometer (S) menu, select Scan (S) command. Or click the  button on the toolbar.
- (7) Set and measure a sample.

5. 3-D SCAN

5.1 Flow of Operation

This section explains the parameter setting and operating method for 3-D Scan.

For using the 3-D Scan mode in the Model F-2500, the optional '3-D Scan program' needs to be installed.

Measurement is made in the following procedure.

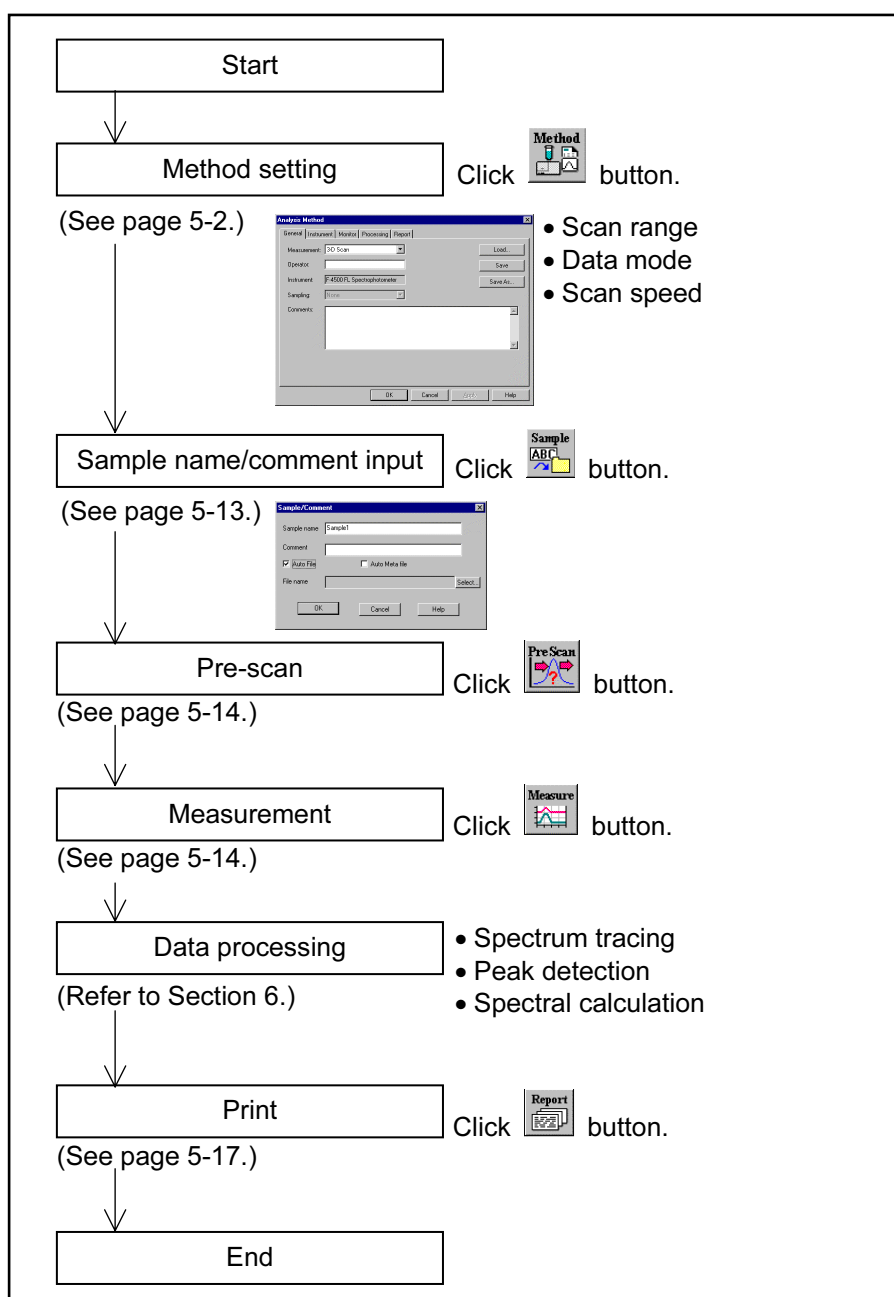



Fig. 5-1 Operational Flow (3-D Scan)

5.2 Creating an Analysis Method

5.2 Creating an Analysis Method

Set the analytical conditions (method) prior to measurement.

- (1) From the Edit menu, select the Method command or click the  button. The dialog box shown in Fig. 5-2 will appear.

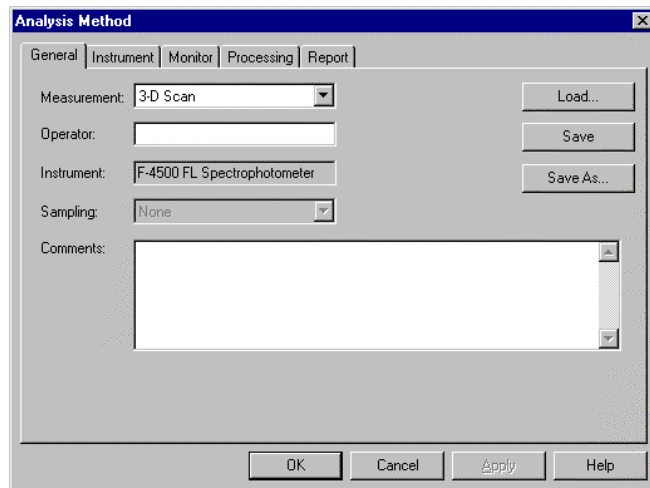


Fig. 5-2 Analysis Method

- (2) Set the analysis method on this window. For details of each tab, refer to 5.1.2 onward.

The following tabs are provided on the Analysis Method window for the 3-D Scan mode.

These tabs are explained on the subsequent pages.

- General
- Instrument
- Monitor
- Processing
- Report

(3) General

The parameters shown in Fig. 5-3 are provided under the General tab.

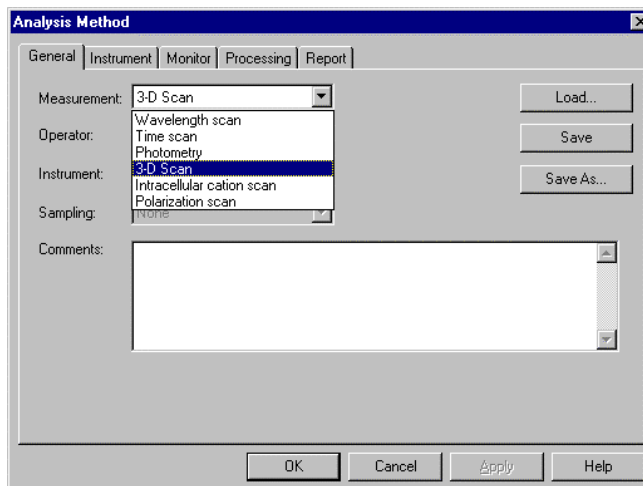


Fig. 5-3 General Tab

(a) Measurement (mode)

Select a measurement mode from among the four listed below.

- Wavelength scan
- Time scan
- Photometry
- 3-D Scan

Here, select “3-D Scan.” For Wavelength scan, time scan and Photometry, refer to Sections 2, 3 and 4.

(b) Operator

Enter an operator name.

(c) Instrument

The model of the connected instrument is indicated.

(d) Sampling

- Standard
When sample is exchanged manually.

(e) Comments

Enter a description or notes on measuring conditions.

5.2 Creating an Analysis Method

- (f) Load
Click this button to read out saved analytical conditions. The file opening window appears upon clicking, so select an analytical conditions file.
 - (g) Save
By clicking this button after setting the parameters for all five tabs, the analytical conditions are saved. Clicking this button overwrites parameters on the existing ones.
 - (h) Save As
Click this button for renaming the set conditions for saving. The "Save As" window will appear, so enter a file name.
- (4) Instrument

This window appears when 3-D Scan is selected for the Measurement under the General tab of the Analysis Method window. When you click the Instrument tab, the window in Fig. 5-4 appears.

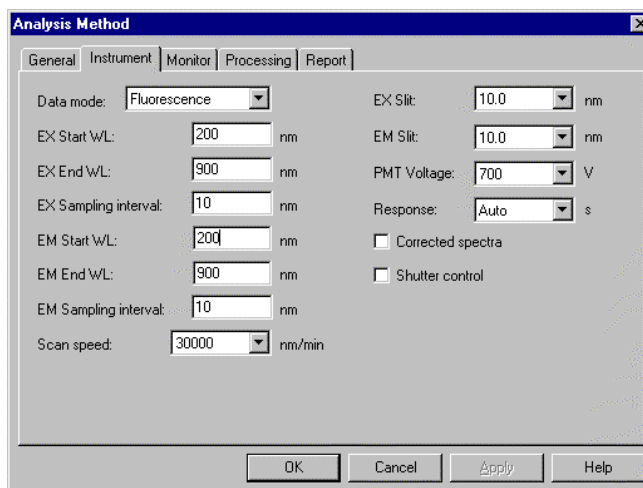


Fig. 5-4 Instrument Tab

(a) Data mode

Select either of the following data modes.

The Phosphorescence mode is for acquiring only phosphorescence after extinction of excitation beam while turning a chopper on the excitation side.

- Fluorescence
- Phosphorescence (with Model F-4500)

(b) EX Start WL

This is the start wavelength for wavelength scan on the excitation side. Input the shorter wavelength side of measuring wavelength range.

Model	Input Range
F-4500	0, 200 to 850 nm
F-2500	0, 200 to 750 nm

(c) EX End WL

This is the end wavelength for wavelength scan on the excitation side. Input the longer wavelength side of measuring wavelength range.

Model	Input Range
F-4500	0, 200 to 900 nm
F-2500	0, 200 to 800 nm

(d) EX Sampling interval

In the 3-dimensional measurement mode, emission spectrum measurement is repeated while shifting the excitation wavelength. Therefore, a shorter sampling interval on the excitation side will result in a longer measurement time.

Input range: 1 to 50 nm

(e) EM Start WL

This is the start wavelength for wavelength scan on the emission side. Input the shorter wavelength side of measuring wavelength range.

Model	Input Range
F-4500	0, 200 to 850 nm
F-2500	0, 200 to 750 nm

5.2 Creating an Analysis Method

- (f) EM End WL
This is the end wavelength for wavelength scan on the emission side. Input the longer wavelength side of measuring wavelength range.

Model	Input Range
F-4500	0, 200 to 900 nm
F-2500	0, 200 to 800 nm

- (g) EM Sampling interval
In the 3-D Scan mode, emission spectrum measurement is repeated while shifting the excitation wavelength. Therefore, a shorter sampling interval on the emission side will result in a longer measurement time.

Input range: 1 to 10 nm

- (h) Scan speed
Set a scan speed. (Unit: nm/min)

F-4500	F-2500
30000	3000
12000	1500
2400	300
1200	60
300	15
60	
15	

- (i) EX Slit
Select a slit width for the excitation side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

(j) EM Slit

Select a slit width for the emission side. Unit: nm

F-4500	F-2500
1.0	2.5
2.5	5.0
5.0	10.0
10.0	20.0
20.0	

(k) PMT Voltage

A function for controlling the voltage of the photomultiplier detector (photomultiplier tube).
Select one of the following values. Changing from 400 V to 700 V increases the data value about two digits.

- 950 V (only with F-4500)
- 700 V
- 400 V

(l) Response

Select a response time from the following.

F-4500	F-2500
0.04	0.04
0.01	0.08
0.05	0.4
0.1	2
0.5	8
2.0	Auto
8.0	
Auto	

By selecting Auto, a response time is set automatically according to scan speed. Select Auto usually.

5.2 Creating an Analysis Method

(m) Corrected spectra

A function for determining the spectrum inherent to a sample by correcting the photometer wavelength characteristic using the saved instrument parameters, following measurement with the instrument parameters for photometer control. If there are no instrument parameters for the wavelength range to be measured, only the wavelength range that allows correction is scanned. And if there is no wavelength area measurable, an error will occur.

For using this function, a Spectra Correction Parts Set (P/N 251-0126) is required. The Instrument Response command is provided in the Utility menu for operating this function. When this setting is at ON, "Corrected spectra" appears on the monitor window. (See Fig. 1-26 in 1.6.)

(n) Shutter control (only with F-4500)

The shutter can be automatically closed in other than measurement for suppressing sample deterioration due to the energy of excitation beam and opened when measurement starts. When you put a check mark at the head, the shutter will close and open at start of measurement. The shutter will close again when measuring wavelength begins returning to the start wavelength after measurement in the wavelength scan mode.

(5) Monitor

When selecting the Monitor tab, the window in Fig. 5-5 appears.

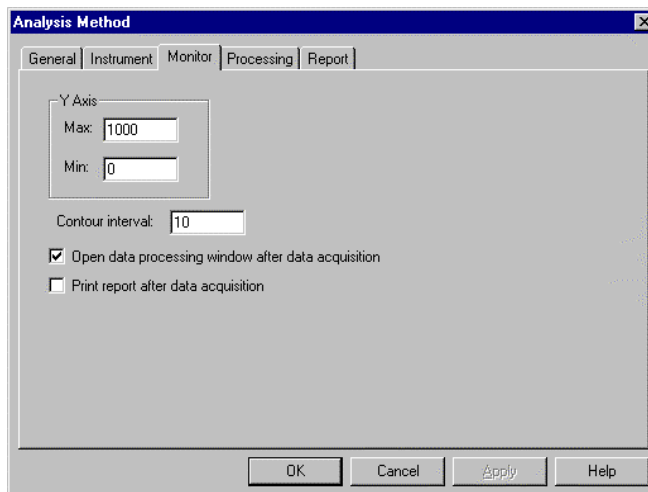


Fig. 5-5 Monitor Tab

Assign the following parameters.

- (a) Y-Axis Max (maximum)
Input a maximum value for Y-axis on the monitor window.
- (b) Y-Axis Min (minimum)
Input a minimum value for Y-axis on the monitor window.
- (c) Contour interval
Function for setting intervals of drawing contour lines.
Input range: 0.01 to 1000
- (d) Open data processing window after data acquisition
Select whether or not to conduct data processing after sample measurement. When selected (check mark is applied), an icon is displayed for the data processing window at the end of measurement. And by opening this icon, data processing such as peak detection is available.

5.2 Creating an Analysis Method

NOTE: When the remaining memory capacity of PC in operation is very small (system resource is less than 20%), the data processing window will not open even if this item is turned ON. Either close the presently opened data processing window or restart the FL Solutions program.

(e) Print report after data acquisition

Select whether or not to print a report after sample measurement. When selected (check mark is applied), printing will be made automatically after measurement. The items set under the Report tab will be printed. When this item is not selected, data processing can be made by reading out a file via **File-Open**.

(6) Processing

On selection of the Processing tab, the window in Fig. 5-6 will be displayed.

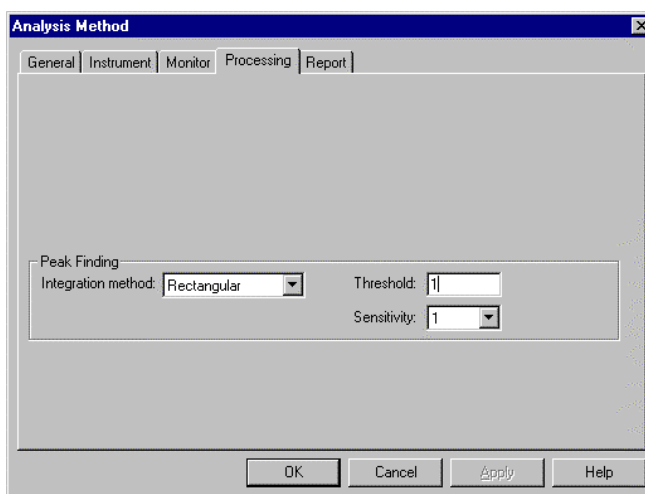


Fig. 5-6 Processing Tab

(a) Peak Finding

Select a peak detection method indicated in the data processing window.

(b) Threshold

Set a detection limit for the photometric value axis of peak and valley. Peaks or valleys below the setting will not be detected. Input range is 0.001 to 1000.

(c) Sensitivity

Select the number of data points in the horizontal axis direction (abscissa). Select a sensitivity of "1" for detecting sharp peaks. Or select "8" for detecting broad peaks.

- 1
- 2
- 4
- 8

(7) Report

Select the Report tab, and the window in Fig. 5-7 will appear.

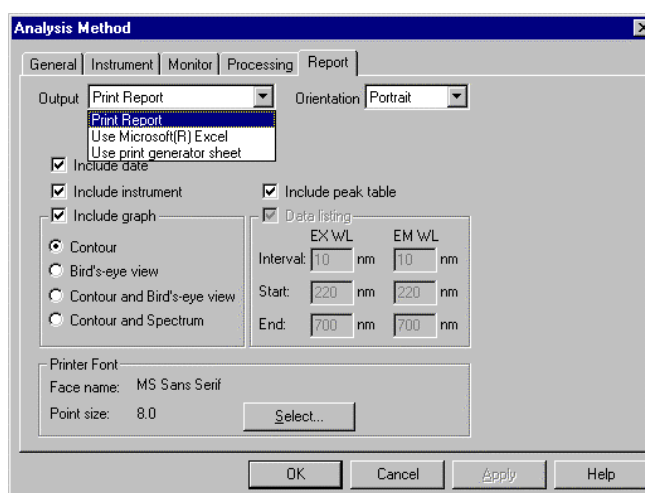


Fig. 5-7 Report Tab

(8) Output

(a) Print Report

A report will be printed. For printing method, refer to 5.7.

(b) Use Microsoft® Excel

Data will be transferred to Microsoft® Excel. For transfer method, refer to 6.13.

5.2 Creating an Analysis Method


- (c) Use print generator sheet
Select this method for using the report generator (option). For usage, refer to the instruction manual of the report generator.

- (9) Orientation
Selectable when Report is chosen for Print Method.
Select a printing orientation. Either Portrait or Landscape orientation is selectable. When the Landscape orientation is selected, date and method can be printed.
 - (a) Printable (transferable) items
 - 1) Include date
 - 2) Include instrument
 - 3) Include graph
 - Contour
 - Bird's-eye view
 - Contour and bird's-eye view
 - Contour and Spectrum
 - 4) Include peak table

 - (b) Printer Font
Click this button for changing the font to be used in the report.

5.3 Defining Your Samples

Samples must be defined before measurement.

Click the  button. The dialog box in Fig. 5-8 will appear.

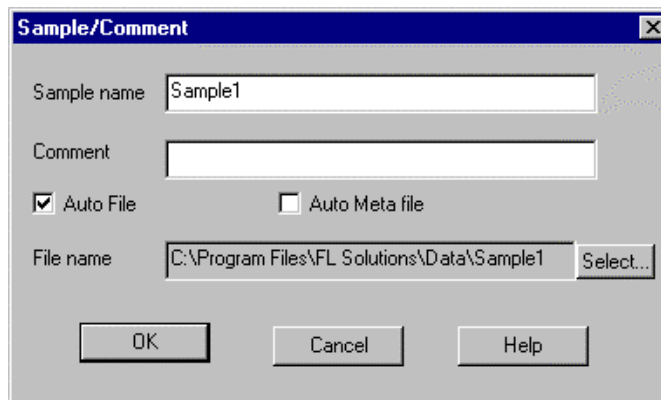




Fig. 5-8

Input for Sample and Comments. For automatic saving, put a check mark in the “Auto File” box and specify a file name. To specify a file name, click **Select**.

5.4 Conducting Measurement

5.4 Conducting Measurement

- (1) There are two methods of starting the measurement.
 - (a) Select the Measure command from the Spectrophotometer menu.
 - (b) Click the  (measurement) button on the measurement toolbar.
For halting the measurement midway, click the  button on the toolbar.
- (2) When automatic saving is set, data will be saved after completion of measurement. Unless automatic saving is set, select the Save As command from the File menu and save data in file. Upon opening its file, a data processing window will appear as in Fig. 5-9.

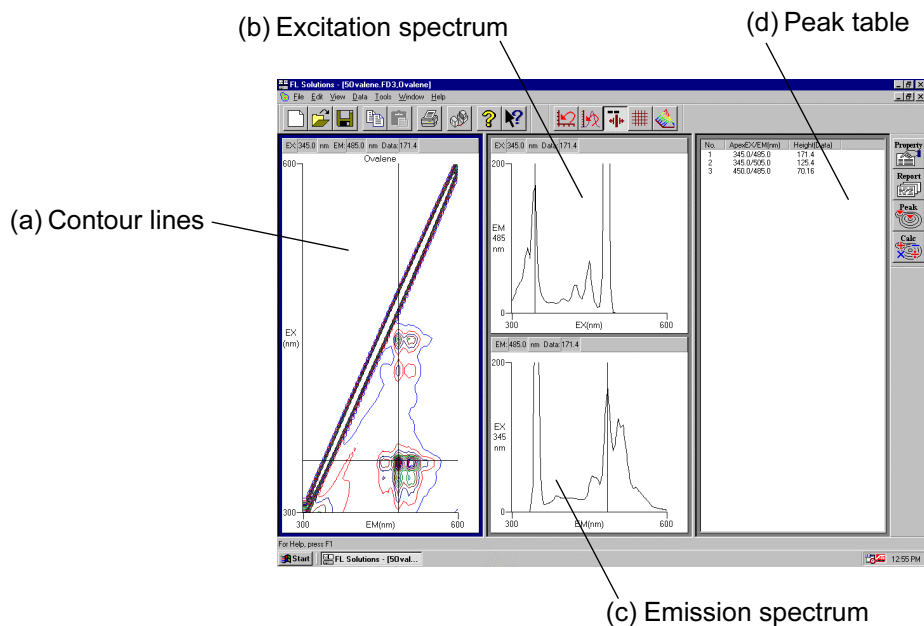




Fig. 5-9 Data Processing Window (3-D Scan)

- (a) Contour lines
From these contour lines, excitation and emission spectra can be read out.
As the cursor is moved, the cursor-specified excitation and emission spectra are displayed in (b) and (c).


(b) Excitation spectrum

An excitation spectrum at the cursor position in (a) is displayed. Wavelength and photometric value can be read by selecting the trace command from the View menu or clicking the  button.

(c) Emission spectrum


An emission spectrum at the cursor position in (a) is displayed. Wavelength and photometric value can be read by selecting the trace command from the View menu or clicking the  button.

(d) Peak table

Peak wavelength and peak height are indicated. For setting threshold and sensitivity values, click the  button.

5.5 Displaying a Bird's-Eye View

5.5 Displaying a Bird's-Eye View

3-D Scan data can be displayed in a bird's-eye view. Select the Bird's-eye view command from the View menu or click the  (bird's-eye view) button on the Spectrum toolbar.

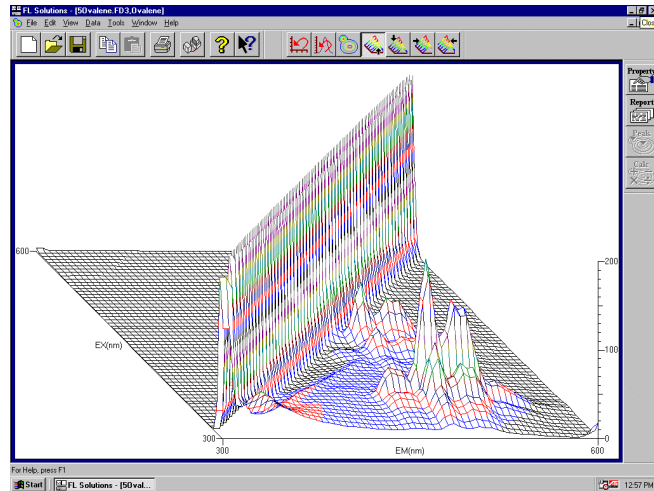








Fig. 5-10

Viewing direction is changeable by clicking  (from front),  (from rear),  (from left) or  (from right). Clicking  (contour line diagram) displays a contour line diagram.

5.6 Data Printout

Printout can be provided even when the function for automatic printout after measurement is not used. The following two procedures are available.

- (1) Select the Print command from the File menu. Or click the  print button on the toolbar. The field active on the data processing window (e.g., (a) in Fig. 5-9) will be printed. The contents of display upon clicking the Print Preview command in the File menu will be printed.

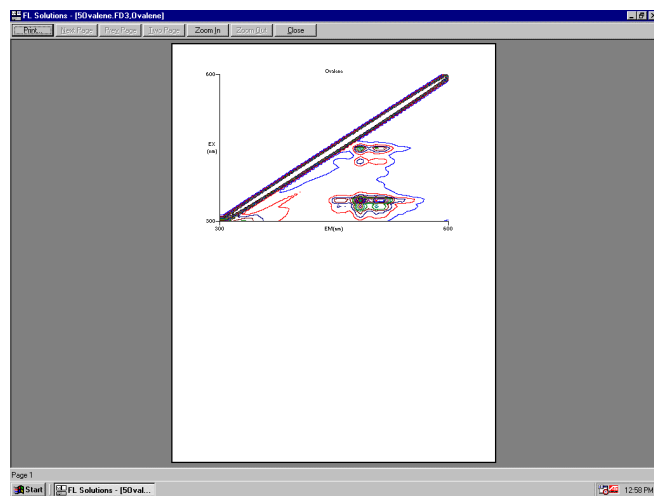



Fig. 5-11

5.6 Data Printout

- (2) Select the Report command in the Data menu. Or click the  button on the toolbar. The Print Preview window will appear, so click **Print**. Select the items to be printed at the Report tab under Properties.

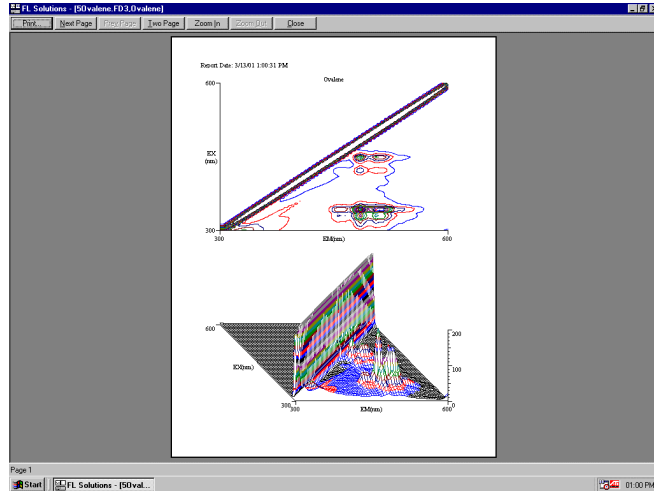



Fig. 5-12

6. DATA PROCESSING

This section explains the operation method for data processing after measurement.

6.1 Loading of Data

Load saved data as follows.

Either click the  (Open) button or select the Open command from the File menu. A window as in Fig. 6-1 will appear.

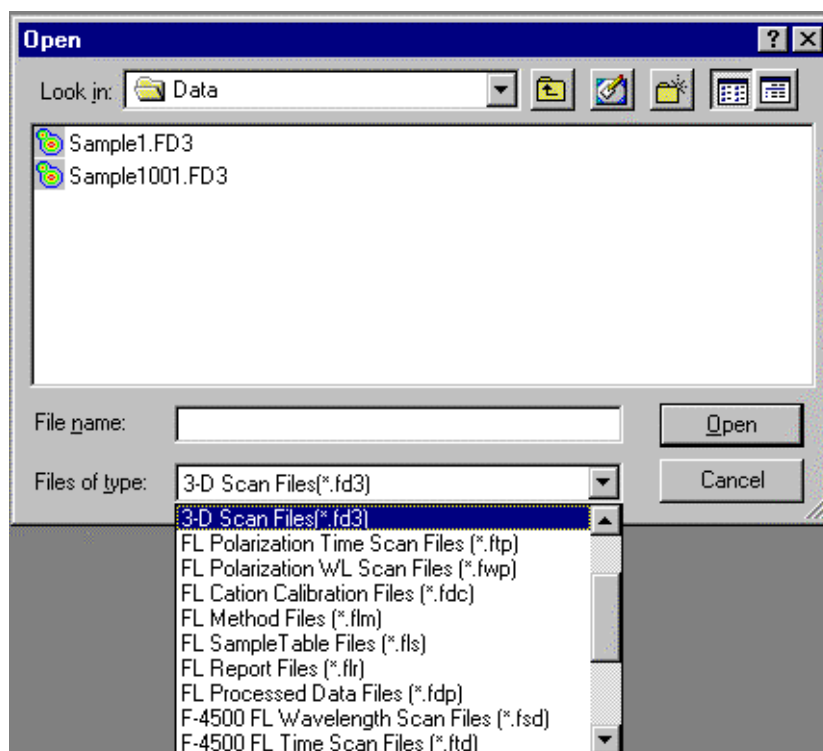


Fig. 6-1

Select the file type of data file in “Files of type” where file types are listed. Specify a desired file and click **Open**, and the data file will open.

The following types are included in “Files of type.”





- Wavelength scan files (*.fds)
- Time scan files (*.fdt)
- Photometry files (*.fdq)
- 3-D Scan files (*.fd3)
- Method files (*.flm)
- Sample table files (*.fls)
- Report files (*.flr)

6.1 Loading of Data

- Processed data files (*.fdp)
- F-4500 wavelength scan files (*.fds)
- F-4500 time scan files (*.fdt)
- F-4500 photometry files (*.fdq)
- F-4500 3-D Scan files (*.fcd)
- All files (*.*)

The window (Fig. 6-1) has four tool buttons. Table 6-1 lists the tool buttons and their functions.

Table 6-1 File-Open Window Buttons

Button	Function
	Displays a folder of one level higher.
	Creates a new folder.
	Lists the file names included in selected folder.
	Displays file details such as file name, size and type.

NOTE: When the remaining memory capacity of PC in operation is very small (system resource is less than 20%), the data processing window will not open. Either close the presently opened data processing window or restart the FL Solutions program.

6.2 Spectrum Tracing

This function is used to read the photometric value at a certain wavelength on the measured spectrum. First, bring the cursor onto the displayed spectrum, then right-click the mouse and a window as in Fig. 6-2 appears.

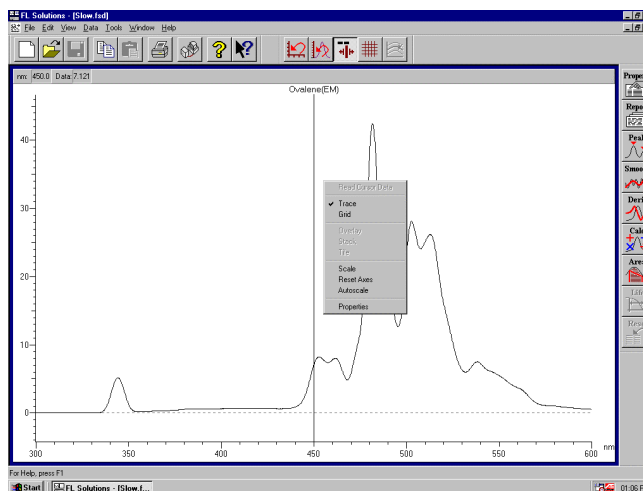



Fig. 6-2

Put a check mark before Trace or click the  button. A line cursor appears on the spectrum. The wavelength and photometric value at the cursor position are indicated at the location (a) in Fig. 6-3.

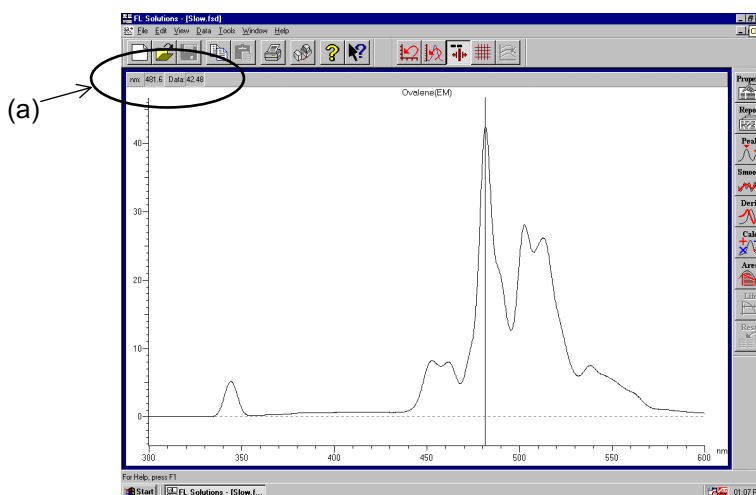


Fig. 6-3

6.2 Spectrum Tracing

The line cursor can be moved in the following two ways.

(1) Movement using mouse


Move the line cursor with the mouse to the position for readout.

(2) Movement using arrow keys on the keyboard

Click the [→] or [←] key and the line cursor moves in the corresponding direction.

6.3 Scale Change

The X and Y-axis scales of data can be changed.

Click the  (properties) button and select the Scale tab. Or right-click on the spectrum and select "Scale change."

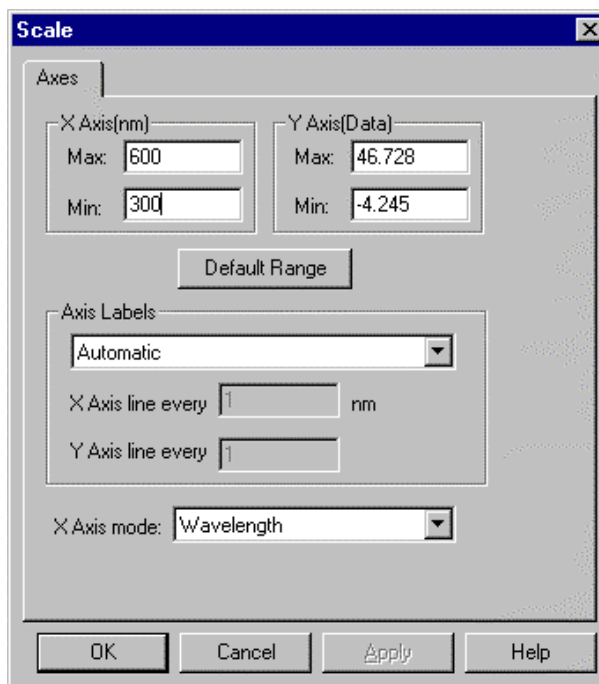



Fig. 6-4 Scale Tab


Enter values in the Maximum and Minimum columns for the X and Y axes. The initial values are those that have been saved. The above window contains the following setting items.

- (1) X Axis
Enter minimum and maximum values for the X axis.
- (2) Y Axis
Enter minimum and maximum values for the Y axis.
- (3) Default Range
System returns to initial values of X and Y axes.

Scale can be expanded with the mouse. Specify an area to be expanded. Holding the mouse button held down, draw a rectangle, and then release the button. Scale will be expanded. For returning to the original scale, click the Default Range button or the  button on the toolbar.

6.4 Change of Graph Axis

6.4 Change of Graph Axis

The horizontal axis of a graph is changeable on a spectrum. Click the  (properties) button and select the Scale tab.

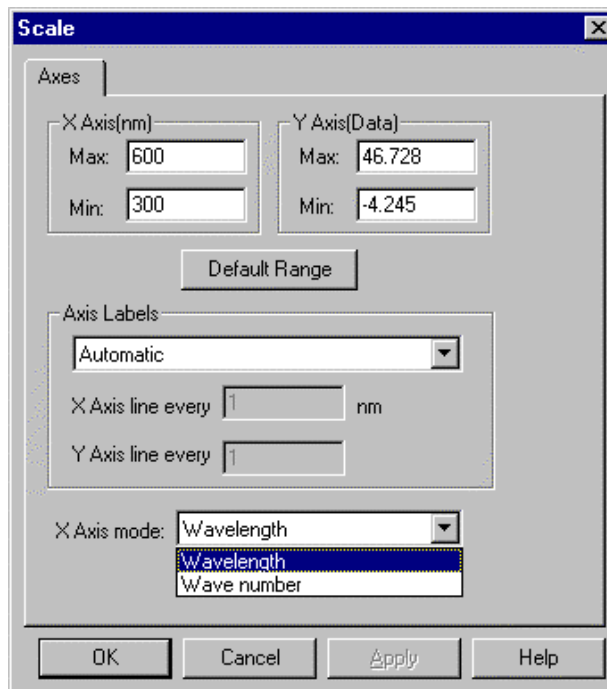


Fig. 6-5 Scale Tab

(1) X Axis mode

X-axis is indicated in either unit below.

(a) nm

Indicated in wavelength (nm).

(b) Kcm-1

Indicated in wavenumber (Kcm-1, K = 1000).

$x \cdot \lambda = 10000$ (x: Kcm-1, λ : nm)

6.5 Change of Spectral Line

The line and color of a spectrum can be changed. Select the Lines/Points tab of Spectrum Properties. Upon clicking this tab, the window below appears.

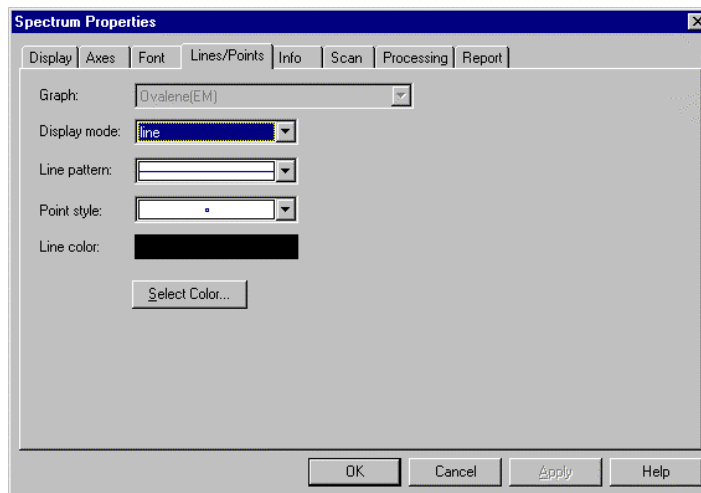


Fig. 6-6 Lines/Points Tab

The following setting items are contained in this window.

(1) Graph

Select a spectrum for setting lines/points.

(2) Display mode

Data can be indicated in the following modes.

- Lines
- Points
- Lines & Points

(3) Line pattern

Specify the kind of line such as dotted line or solid line.

(4) Point style

Select the point style to be used in graph printing or display.

6.5 Change of Spectral Line

(5) Line color

Use of the Color window enables you to change the color of spectral lines or points. There are 48 basic colors. By clicking the Define Custom Colors button, a smaller window is added on the right side of the Color window as shown in Fig. 6-7. And by clicking Define Custom Colors again, 16 colors are added. These colors are usable for the spectral lines and points. For defining the colors, entries can be made for Hue, Sat (saturation) and Lum (luminance), Red, Green and Blue. When the definition is finished, click the Add to Custom Colors button. Then, select the desired ones from the defined colors.

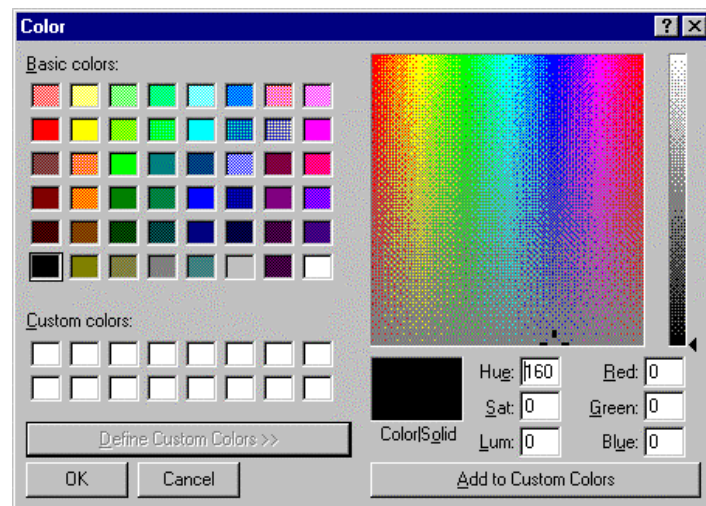



Fig. 6-7

6.6 Detection of Spectral Peaks

The spectral peaks and valleys are detectable automatically. Select the Find Peaks command from the Data menu or click  button, and a window as in Fig. 6-8 appears.

Set Integration method, Threshold and Sensitivity.

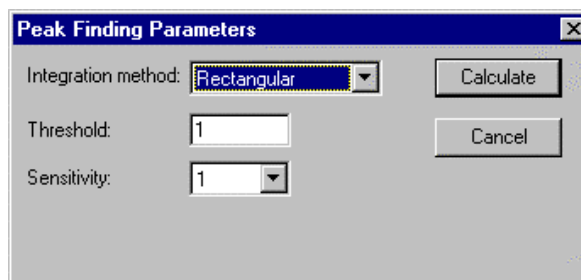


Fig. 6-8

(1) Integration method

The following three methods are available and any one may be selected for the peak wavelength. But the area calculation result indicated in the peak table will vary with the selected method.

- Rectangular
- Trapezoid
- Romberg

For details of these methods, refer to APPENDIX D.

(2) Threshold

Set a detection limit for the photometric value axis of peaks and valleys. Peaks and valleys below the set value will not be detected.

Input range: 0.001 to 1000


(3) Sensitivity

Select the number of data points in X axis direction. For detection of sharp peaks, select a sensitivity level of "1," and for broad peaks, select "8."

- 1
- 2
- 4
- 8

6.7 Indication of Peak Wavelength on Spectrum

6.7 Indication of Peak Wavelength on Spectrum

Select the Properties (O) command from the Edit (E) menu or click the  (properties) button on the Processing toolbar. The window in Fig. 6-9 will open.

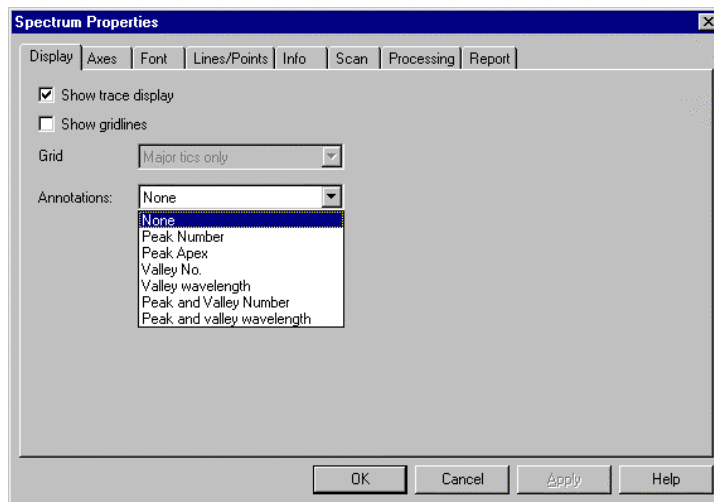


Fig. 6-9 Display Tab

In the Comments under this tab, the following settings can be made.

(1) None

Only a spectrum is displayed.

(2) Peak Number

Peak No. is indicated on a spectrum. This number is the one indicated in the peak table.

(3) Peak Apex

Peak wavelength is indicated on a spectrum. This wavelength is the one indicated in the peak table. It appears as shown in Fig. 5-9 and can be printed.

(4) Valley No.

Valley No. is indicated on a spectrum. This number is the one indicated in the peak table.

(5) Valley wavelength

Valley wavelength is indicated on a spectrum.
This wavelength is the one indicated in the peak table.
It can be printed.

(6) Peak and Valley Number

Peak and valley Nos. are indicated on a spectrum.
These numbers are the ones indicated in the peak table.

(7) Peak and valley wavelength

Peak and valley wavelengths are indicated on a spectrum.
These wavelengths are the ones indicated in the peak table.
They can be printed.

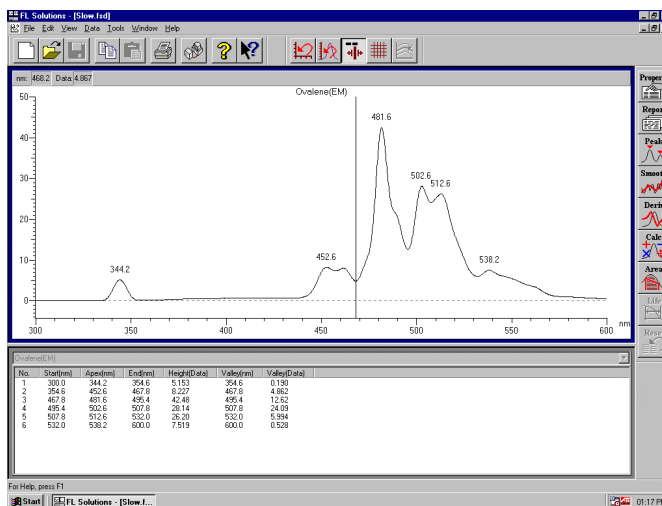



Fig. 6-10

6.8 Smoothing of Spectrum

6.8 Smoothing of Spectrum

Select the Smooth (S) command from the Data (D) menu or click the  button. The window in Fig. 6-11 will open.

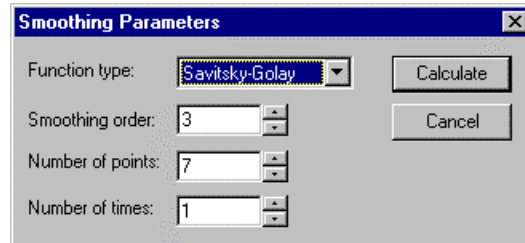


Fig. 6-11

Set the following parameters on this window.

(1) Function type

Select a smoothing method from the following three.

- Savitsky-Golay
- Mean
- Median

(2) Smoothing order

Set a smoothing order.

Input range: 2 to 4

(3) Number of points

Set the number of points to be used in calculation.

Input range: 5 to 101, Initial value: 7

(4) Number of times


Set the number of smoothing operations.

Input range: 1 to 100

For returning to the original status (before calculation), click



6.9 Derivative Operation on Spectrum

Select the Derivative (D) command from the Data (D) menu or click the  button. The window in Fig. 6-12 will open.

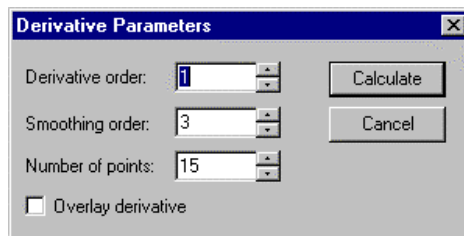


Fig. 6-12

(1) Derivative order

Set a derivative order.
Input range: 1 to 4

(2) Smoothing order

Set a smoothing order.
Input range: 2 to 4

(3) Number of points

Set the number of points to be used in calculation.
Input range: 5 to 101, Initial value: 15

(4) Overlay derivative

Spectra before and after derivative operation are overlaid for display. Note that the value on Y axis is not the one after derivative operation.

For returning to the original status (before calculation), click



6.10 Overlaid Display of Spectra

6.10 Overlaid Display of Spectra

This function is convenient for comparison of two or more spectra.

There are 3 methods selectable for this function.

- (1) Select the Overlay (O) command from the Data (D) menu.

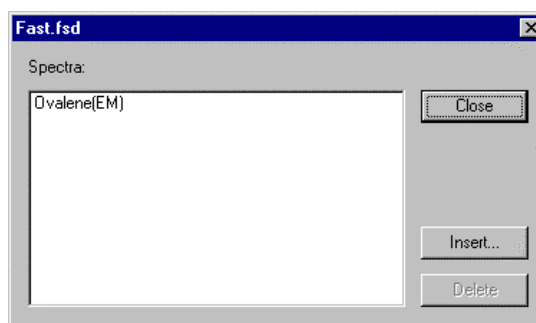


Fig. 6-13

Click the **Insert** button and a list of files that allow overlay is displayed. Select a file name and click the **Open** button. The selected sample name is added and displayed as in Fig. 6-14. Now click the **Close** button.

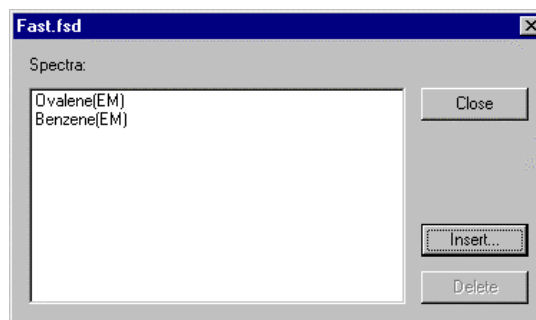


Fig. 6-14

- (2) Open a file to be overlaid to display its data.

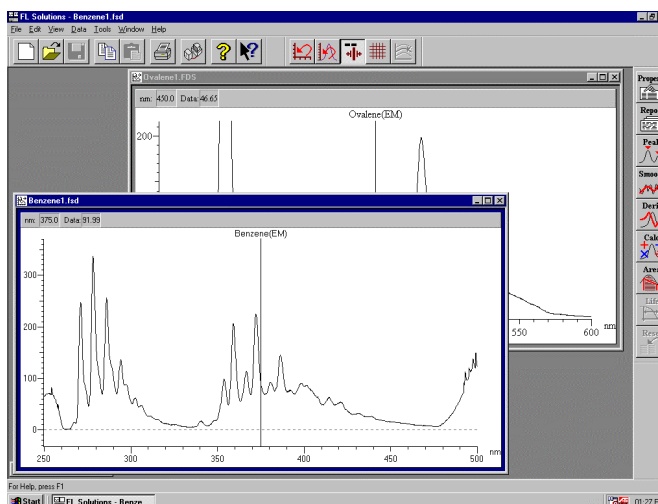


Fig. 6-15

Drag the blue profile of the displayed spectrum so that the mouse pointer comes onto the target spectrum. When the mouse pointer changes as shown in Fig. 6-16, drop the blue profile.



Fig. 6-16

- (3) Open a file as in (2) above. Click the spectrum to be overlaid and press **Ctrl+C** on the keyboard or select the Copy (c) command from the Edit (E) menu. Then click the target spectrum and paste the first spectrum on it by **Ctrl+V**. The spectra are now overlaid.

6.10 Overlaid Display of Spectra

(a) Deletion from Overlaid Display

Select the Overlay command from the Processing menu. In case you want to delete the spectrum having sample name "Filter 02," select the sample name and click **Delete**.

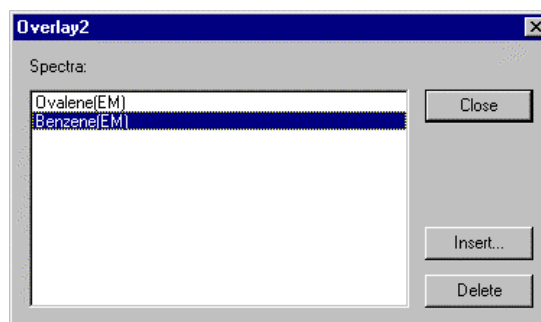



Fig. 6-17

(b) Display of Selected Spectrum Alone from Overlaid Spectra

Click  (Display spectrum) on the Spectrum toolbar.

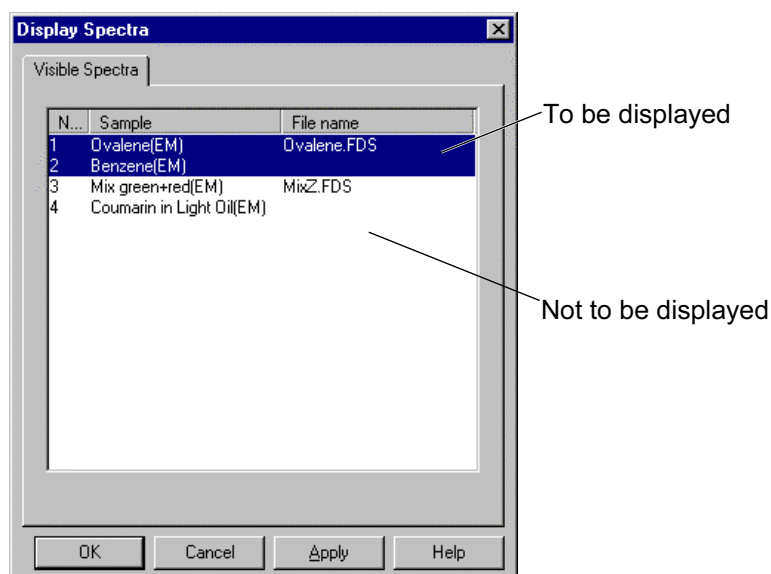


Fig. 6-18


Click the spectrum not to be displayed.

Click **OK**. In this case, spectra Nos. 1 and 2 will be displayed. Printout of this data covers not the spectra, but the sample names, analytical conditions, etc.

After file saving in the current state, 4 spectra will be displayed upon loading the file again.

6.11 Arithmetic Operation between Spectra

Shown here is an example of obtaining the difference between two overlaid spectra.

Display the spectrum for arithmetic operation. Now, click the Arithmetic (A) command on the Data (D) menu or click the  (spectral calculation) on the Processing toolbar. A window as in Fig. 6-19 will open.

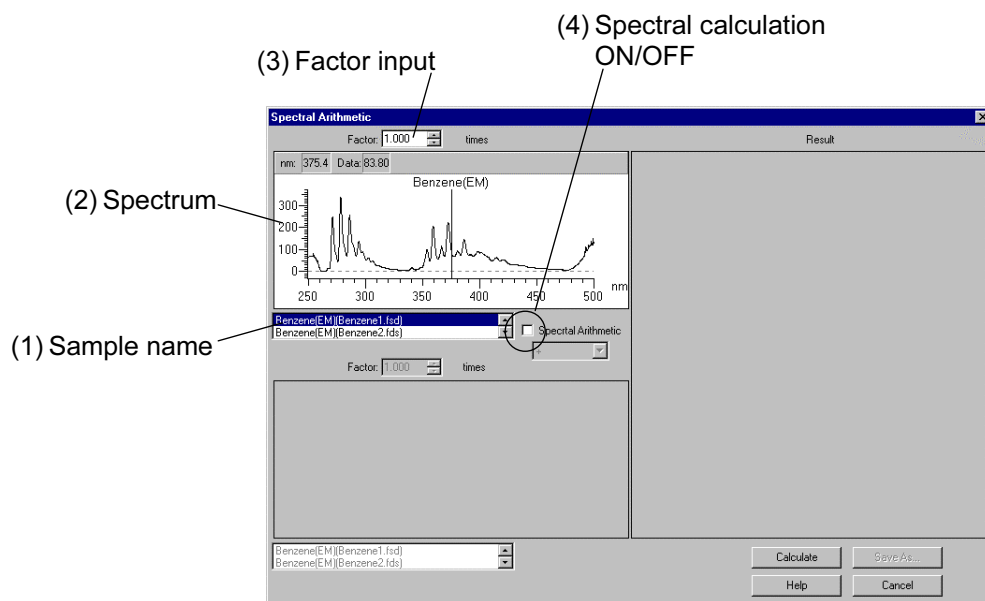


Fig. 6-19

- (1) The sample name of the file open in the window (file name in parentheses) is indicated. Select the file for arithmetic operation.
- (2) The spectrum selected in (1) is displayed here.
- (3) A factor for the spectrum selected in (1) is settable. For multiplying the spectrum by a factor, click **Calculate** here.
- (4) Apply a check mark here for arithmetic operation between two spectra. As a result, the window as in Fig. 6-20 appears.

6.11 Arithmetic Operation between Spectra

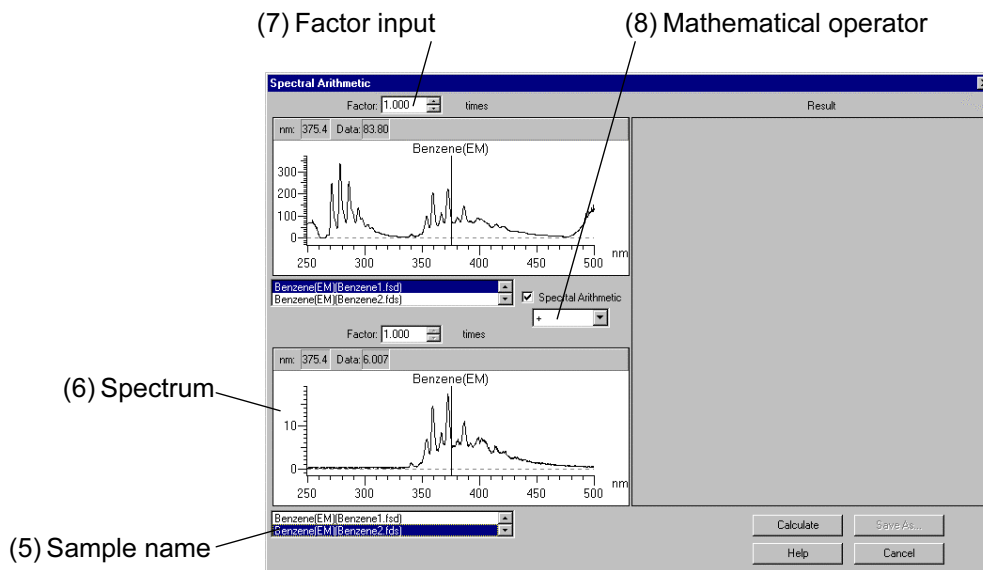


Fig. 6-20

- (5) Another sample name (file name in parentheses) is indicated. Select the file for arithmetic operation.
- (6) The spectrum selected in (5) is displayed.
- (7) Set the factor for the spectrum selected in (5).
- (8) Select a mathematical operator for the two spectra.
+: Addition, -: Subtraction, *: Multiplication, /: Division

After setting, click **Calculate**. The result of arithmetic operation is displayed as shown in Fig. 6-21.

Result of arithmetic operation

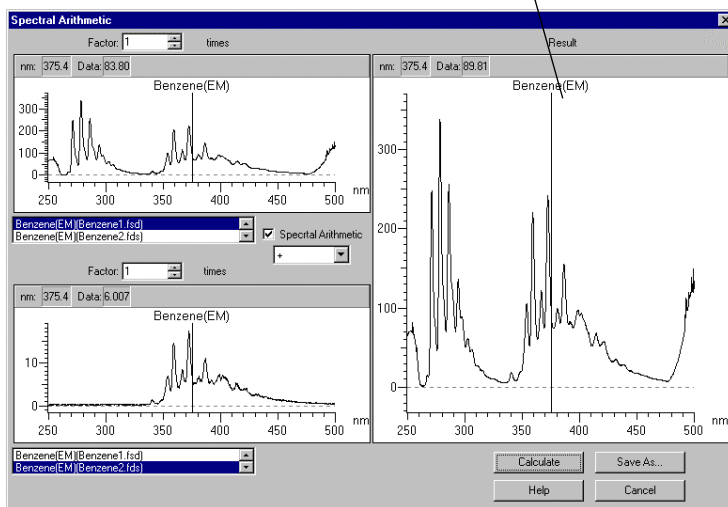



Fig. 6-21 After Arithmetic Operation

Click **Save As**, and the result of calculation can be saved in file.

6.12 Area Calculation

6.12 Area Calculation

An area value is obtainable by integration in the specified range of the presently displayed spectrum.

Select the Integration (I) command from the Data (D) menu or click the  (area calculation) button on the Processing toolbar. The window as in Fig. 6-22 will appear.

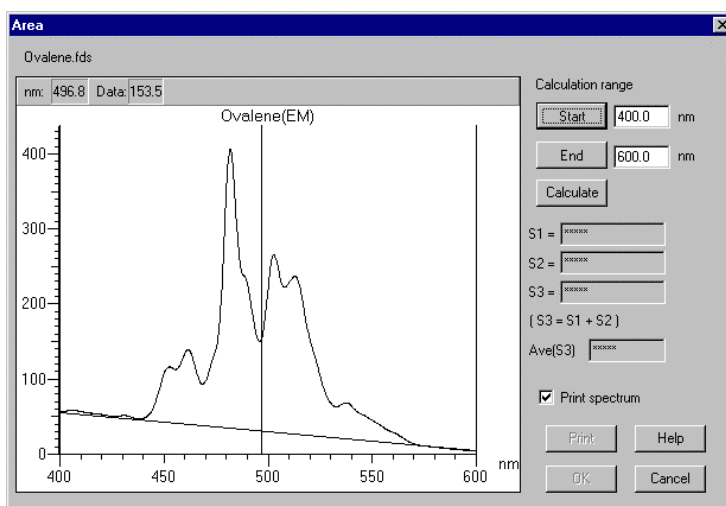



Fig. 6-22

Click **Start** and input the range for area calculation. Or move and right-click the cursor, and a value will be entered. In the same way, enter the end wavelength. After entry, click **Calculate**. The result will be displayed.

For printout, click **Print**. In order to print a spectrum and the result of calculation, apply a check mark to "With spectrum record" and click **Print**.

When clicking the **OK** button after calculation, its result will be saved. Operation will be reset by closing the data processing window or clicking the  button on the Processing toolbar. Clicking **Cancel** closes the window.

For scale expansion, specify the area to be expanded with the mouse. Draw a rectangle with the mouse button held down.

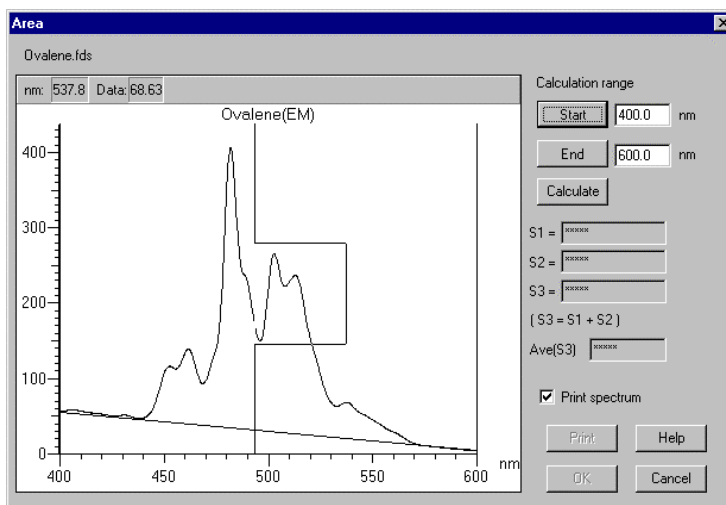


Fig. 6-23

Then, release the button, and the specified area will be expanded as shown in Fig. 6-24.

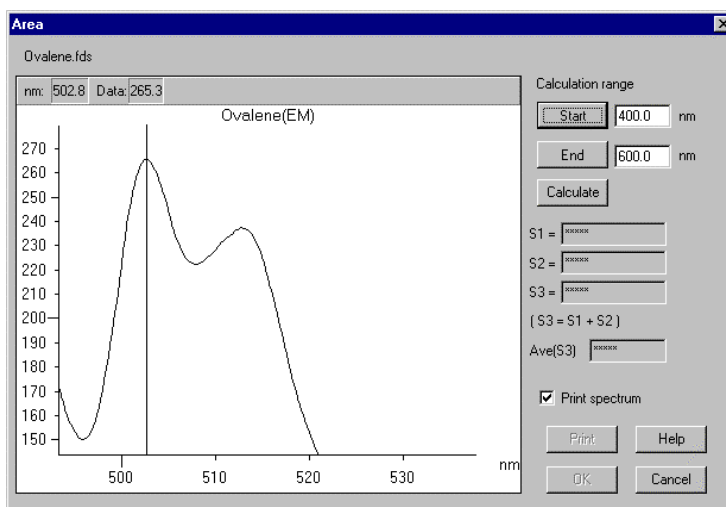


Fig. 6-24

Double clicking the right mouse button returns the scale to the original one.

6.12 Area Calculation

The principle of calculation is shown below.

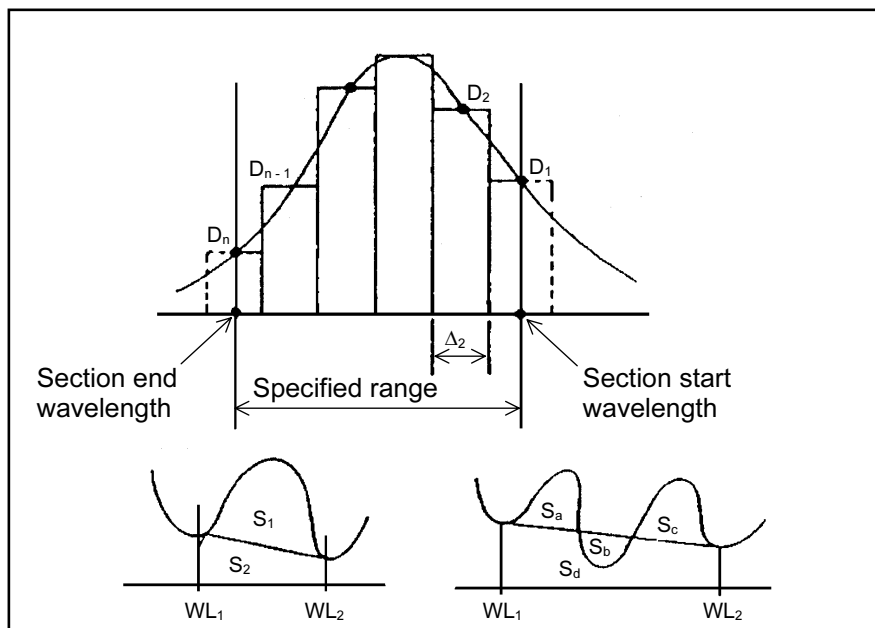


Fig.6-25

The elements (D_1 to D_n) of a spectrum are subjected to the following calculation for obtaining the area value.

$$S3 = \{D_2 + D_3 + \dots + D_{n-2} + D_{n-1} + D_1 + D_n\} / 2 * \Delta\lambda$$

$$S2 = (D_1 + D_n) * \{\Delta\lambda * (n-1)\}$$

$$(D_1 + D_n) * (\text{Section start wavelength (WL}_1) - \text{Section end wavelength (WL}_2))$$

$$S1 = S3 - S2$$

D_1 : Spectral data at section start wavelength

D_n : Spectral data at section end wavelength

$\Delta\lambda$: Sampling interval

In the time scan mode, change as follows.


Start wavelength (WL_1) → end time (T_2)

End wavelength (WL_2) → start time (T_1)

6.13 Transfer of Data to Microsoft(R) Excel

Measured data can be transferred to a worksheet of Microsoft(R) Excel.

Procedure is given below.

- (1) Click the  (properties) button and select the Report tab. A window as in Fig. 6-26 will open. In Print Method, select "Use Microsoft(R) Excel." Also select the data to be transferred (peak table and data list in case of wavelength scan). Then, click **OK**.

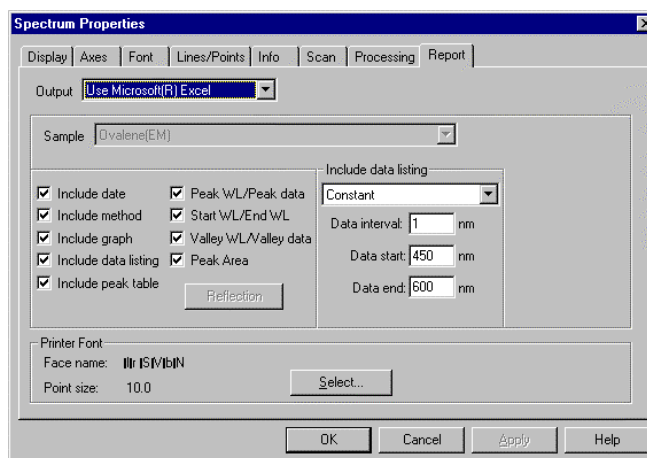



Fig. 6-26

- (2) Click the  (Report) button and the Microsoft(R) Excel will automatically start to read the data. The subsequent procedure concerns the Microsoft(R) Excel operation.

Report Date:	03/13/2001 14:41
Sample:	Ovalene(EM)
File name:	OVALENE.FDS
Run Date:	07/29/2000 15:34
Operator:	Administrator
Comment:	
Instrument:	F-4500 FL Spectrophotometer
Model:	5936-004
Serial Number:	4000 05
ROM Version:	
Instrument Parameters:	
Measurement type:	Wavelength scan
Scan mode:	Emission
Data mode:	Fluorescence
EX WL:	345.0 nm
EM Start WL:	400.0 nm
EM End WL:	600.0 nm
Scan speed:	60 nm/min
Delay:	0 s
EX Slit:	5.0 nm
EM Slit:	5.0 nm
PMT Voltage:	700 V
Response:	Auto
Connected spectra:	Off
Shutter control:	Off

Fig. 6-27

6.13 Transfer of Data to Microsoft(R) Excel

- (3) Data can be saved with extension code xls of Microsoft® Excel.
 Select **File - Save Copy As** and enter a file name.

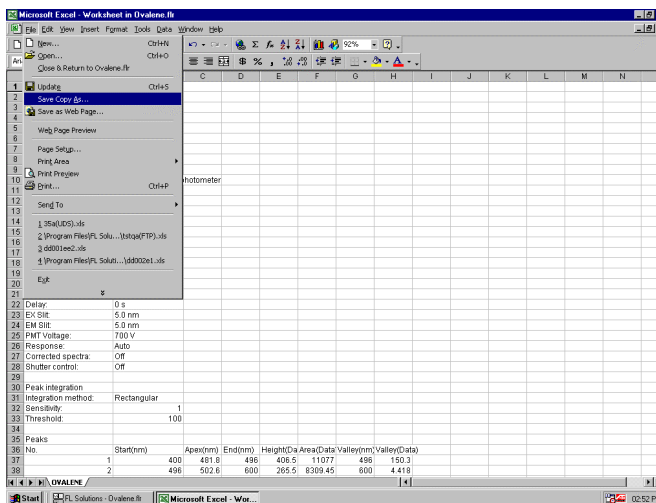


Fig. 6-28

- (4) Upon closing the Microsoft® Excel, the window in Fig. 6-29 appears. Now close this window. The original data processing window will appear again.

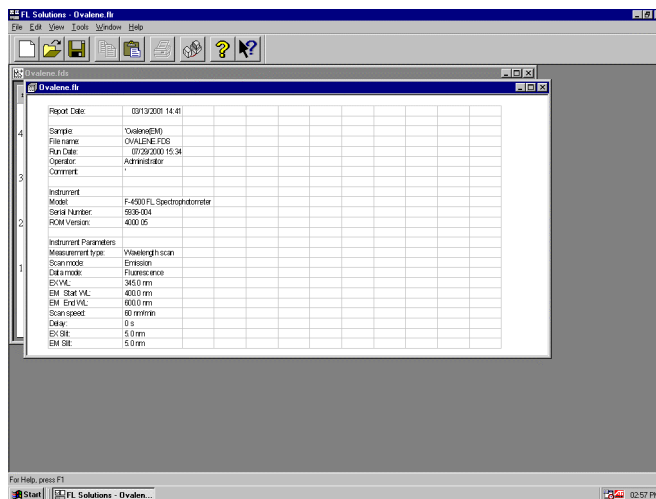


Fig. 6-29

6.14 Pasting of Spectra to Microsoft(R) Word

In report preparation, spectra can be pasted as they are. Make the spectral data active (click the relevant spectrum) and then select the Copy command from the Edit menu. Now open the Microsoft(R) Word and select the Paste command from the Edit menu. The data can be pasted to a Microsoft(R) Word text. Peak tables and/or kinetics data can also be pasted in the same way. By double-clicking the pasted spectrum shown in Fig. 6-30, functions such as scale change and calculation result display are applicable. The spectrum can be selected as a meta-file by the Paste Special (S) command with a form chosen. In the same way, peak tables and/or kinetics data can also be pasted.

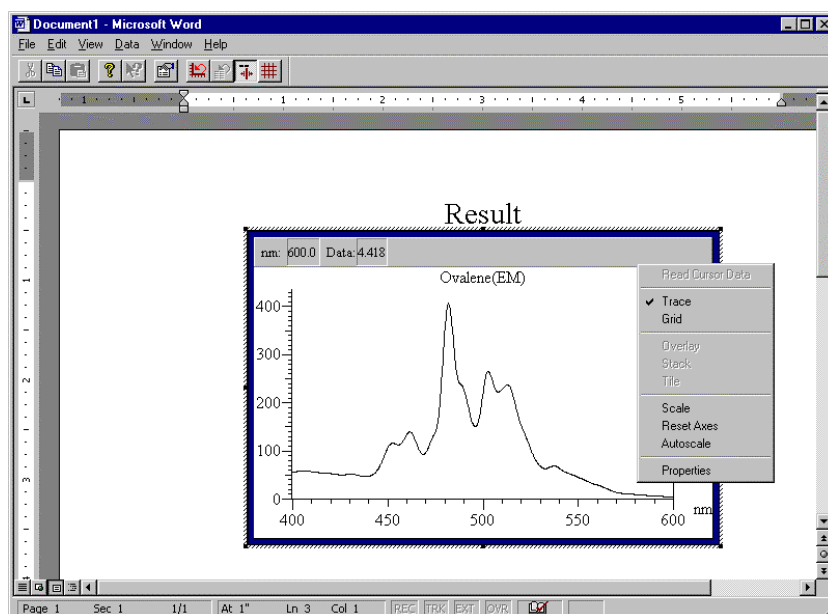


Fig. 6-30

When you double-click the pasted spectrum, you can edit it. Right-clicking on it permits editing such as scale change. A change made here will be brought into a Microsoft(R) Word text.

NOTE: The above editing in the Microsoft(R) Word can be made with a PC in which the Microsoft(R) Word and FL Solutions program are installed.

6.14 Pasting of Spectra to Microsoft(R) Word

For pasting a spectrum to the Microsoft(R) Excel, specify "Paste Special." The dialog box in Fig. 6-31 will appear.

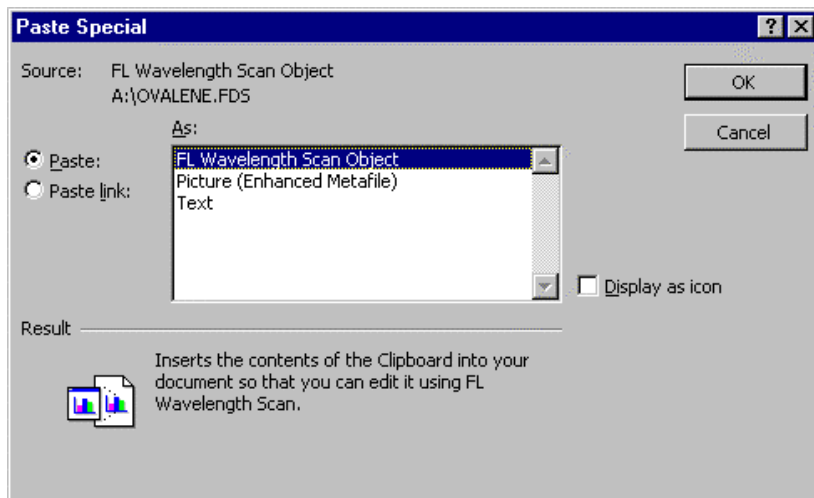


Fig. 6-31

Click **OK** will paste the spectrum.

6.15 Conversion of Data to ASCII Text File

A file conversion function is provided for processing of data with other spreadsheet software. Conversion can be made to the following three data files.

- JCAMP-DX file (*.dx)
- ASCII text file (*.txt)
- Graph meta file (*.wmf)

NOTES:

1. Date/time, analytical conditions and data list (raw data) will be converted into a JCAMP-DX file
2. The items selected at the Report tab of Properties will be converted into an ASCII text file.

- (1) Open the data processing window.
- (2) Click the Save As (A) command in the File (F) menu.
- (3) A window as in Fig. 6-32 will open.
Select "ASCII text files (*.txt)" at "Save as type."
And specify the folder for saving.

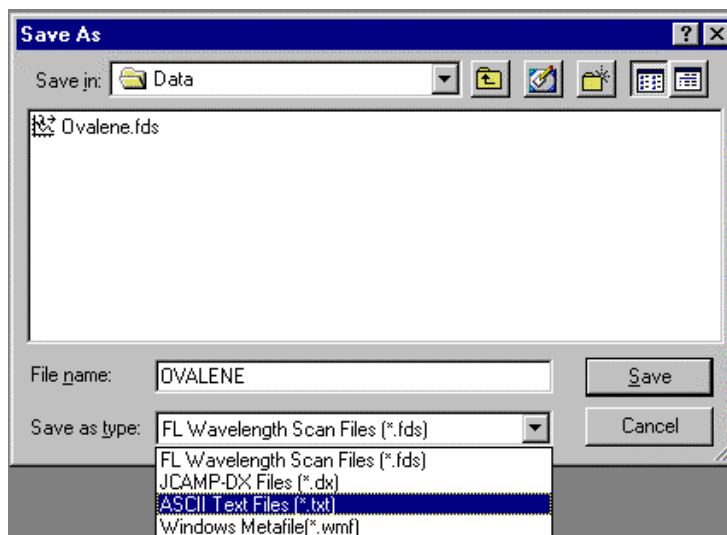


Fig. 6-32

6.15 Conversion of Data to ASCII Text File

(4) Enter a file name and click **Save**.

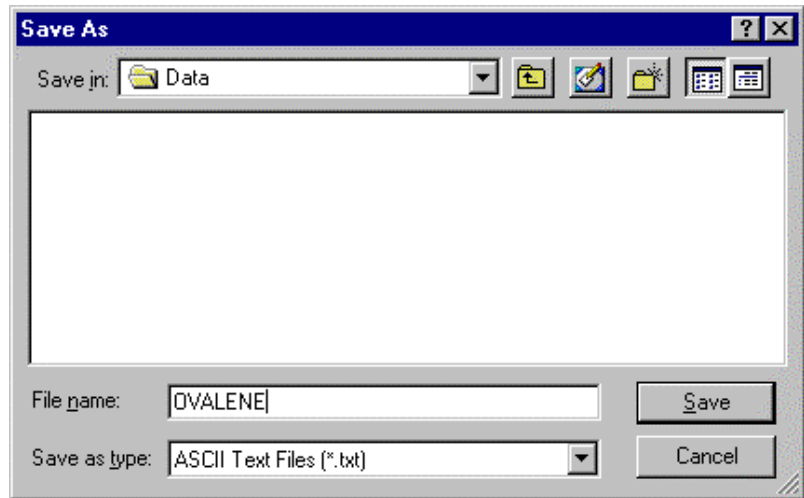



Fig. 6-33

File conversion is effective for a data file in any measurement mode. The selected data is separated by tab.

6.16 Renaming Your Sample

After measurement, the sample name of saved data can be changed.

Proceed s follows.

- (1) Open the data file for renaming.
- (2) Select the Properties (O) command from the Edit (E) menu or click the  button.
- (3) A window as in Fig. 6-34 will open. Click the Info tab.

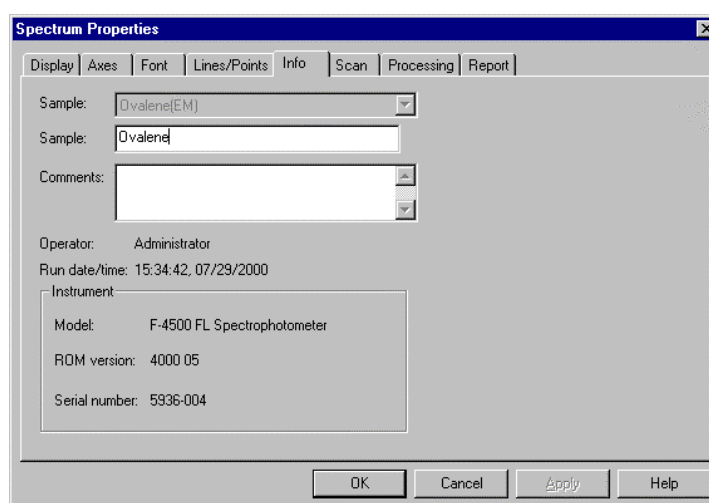


Fig. 6-34

- (4) Input a new sample name for “Sample name” and click **OK**.

NOTE: This function is effective in the wavelength scan, time scan and 3-D Scan modes.

For saving existing data in a different sample name, select the Save As (A) command from the File (F) menu.

6.17 Printing of Wavelength and Photometric Values under Display

6.17 Printing of Wavelength and Photometric Values under Display

This program provides a function for acquiring data such as presently displayed wavelength and photometric values, and traced value on the data processing window. These data can be printed.

Select the Data read (R) command from the Tools (T) menu. A window as in Fig. 6-35 will appear.

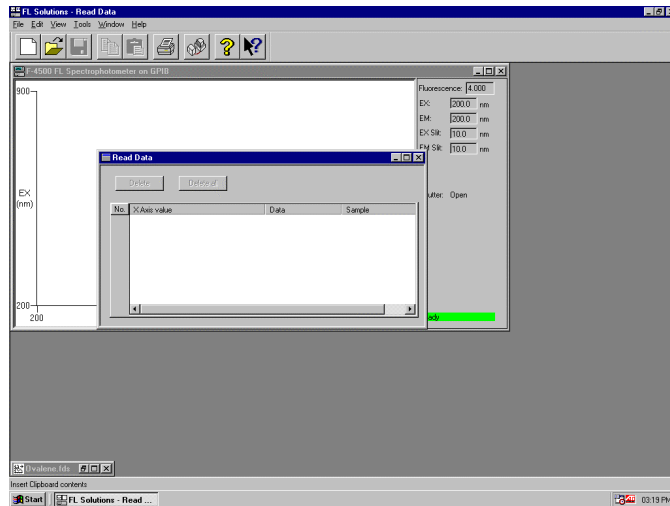


Fig. 6-35

For reading presently displayed wavelength and photometric values:

Move the mouse pointer onto the monitor window and right-click it there. A window as in Fig. 6-36 will be presented.

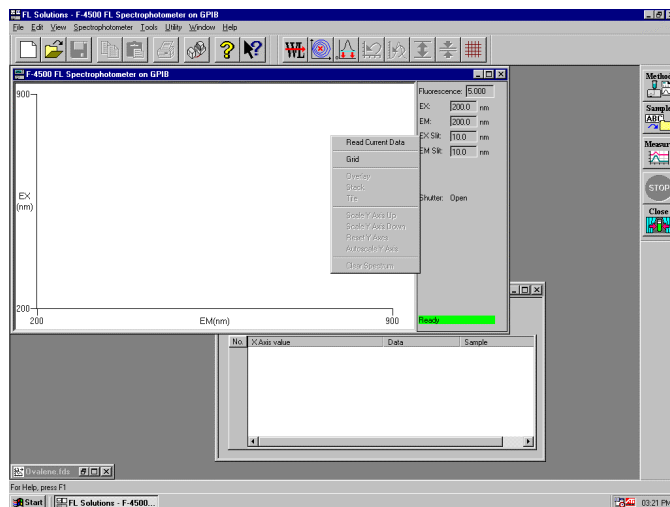


Fig. 6-36

Select "Present data read" and the photometric value on the monitor window will be read on the window shown in Fig. 6-35. Display becomes as shown in Fig. 6-37.

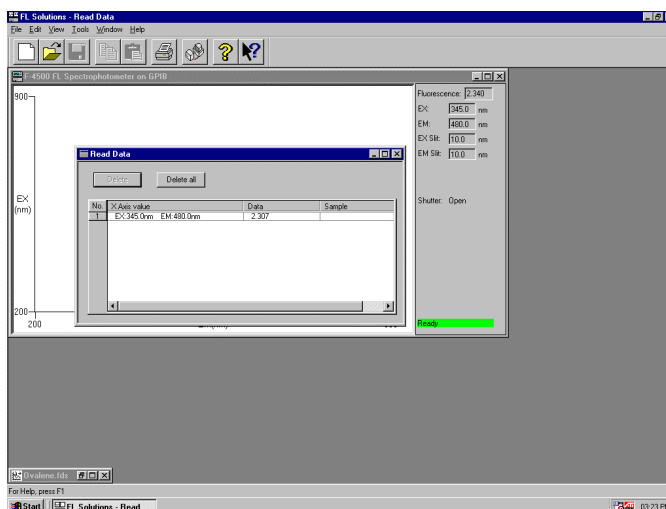


Fig. 6-37

For reading data from data processing window Display the window for data readout as instructed above and move the mouse pointer onto data processing window. Right-click the mouse there, and a window as in Fig. 6-38 will open.

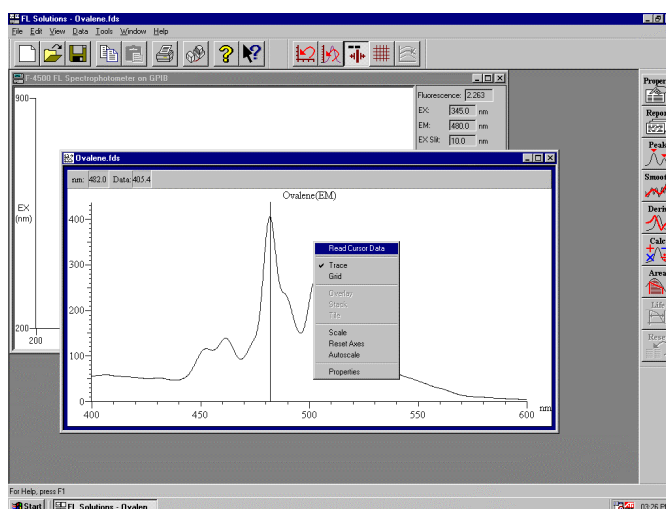


Fig. 6-38

6.17 Printing of Wavelength and Photometric Values under Display

When you select “Trace value read,” a window as in Fig. 6-39 will appear.

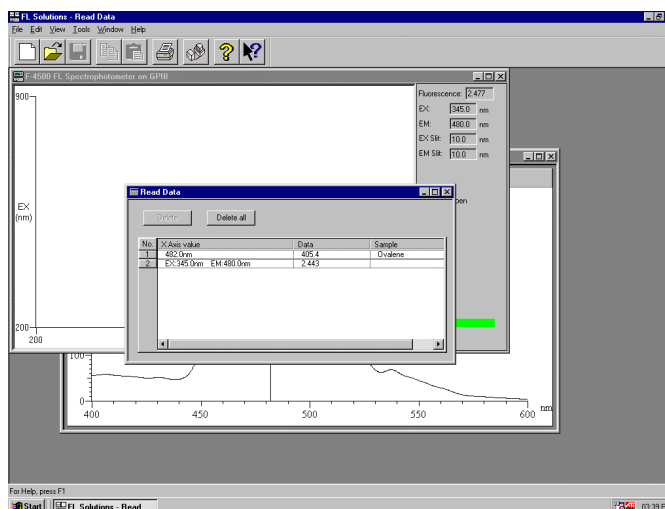



Fig. 6-39

In reading data from the data processing window, sample name information is displayed. Sample names can be edited. Click the sample name column, and input is allowed. The relevant data can be printed. From the File (F) menu, select the Print (P) command or click the  (Print) button. Data can be deleted by the following method. Click the data value and select **Delete**. For deletion of all data, select **Delete All**.

6.18 Reference to Spectrum Measured in Photometry Mode

When the quantification method is peak area, peak height or derivative in the photometry mode, wavelength scan will be carried out and according to its result, a calibration curve will be prepared. The spectrum measured in this case can be displayed and printed.

Open the data file. Right-click the numerical part of standard data or sample data and select "Spectrum reference." Or click a numeral and select the Spectrum reference (R) command from the Data (D) menu.

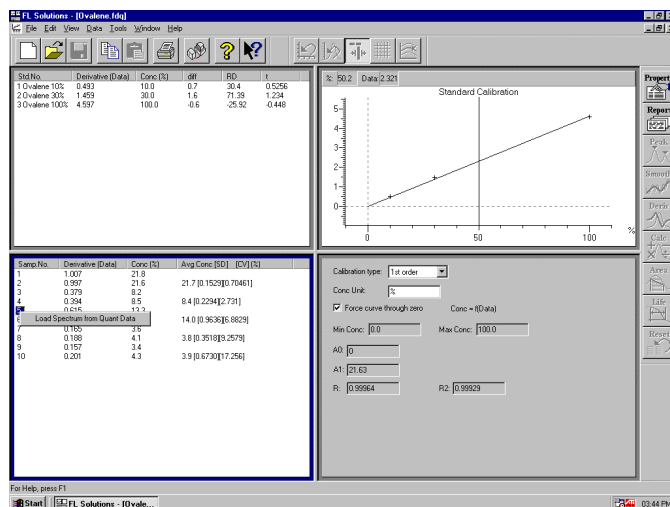



Fig. 6-40

6.18 Reference to Spectrum Measured in Photometry Mode

A spectrum appears as shown in Fig. 6-41. Spectrum tracing and scale change are possible. The spectrum can be printed by selecting the Print (P) command from the File (F) menu or click the  button.

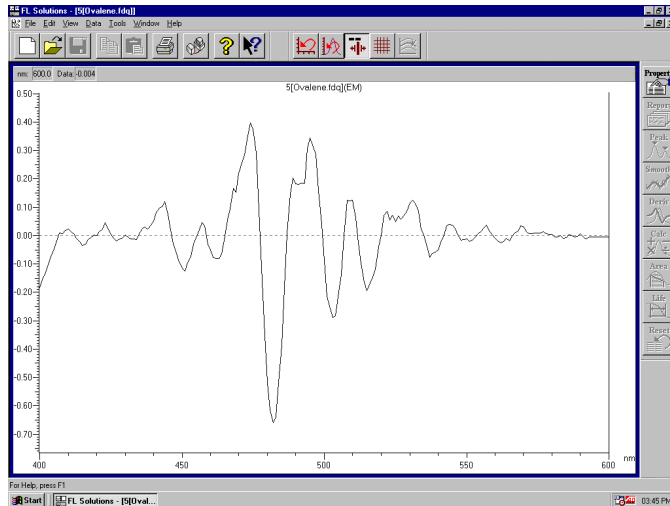


Fig. 6-41

7. MORE CONVENIENT OPERATING METHODS (APPLICATION PART)

7.1 For Always Starting Up with Same Analysis Method

The FL Solutions program can be started with the analysis method specified. Select **Tools - Options** as in Fig. 7-1.

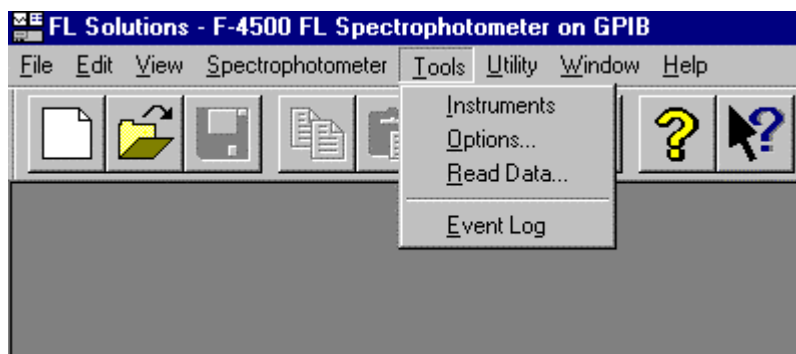


Fig. 7-1 Tools Menu

Now select the Startup tab as in Fig. 7-2.

Select "Use fixed method" and click **Set F-2500 Method** when the Model F-2500 is used or **Set F-4500 Method** when the Model F-4500 is used.

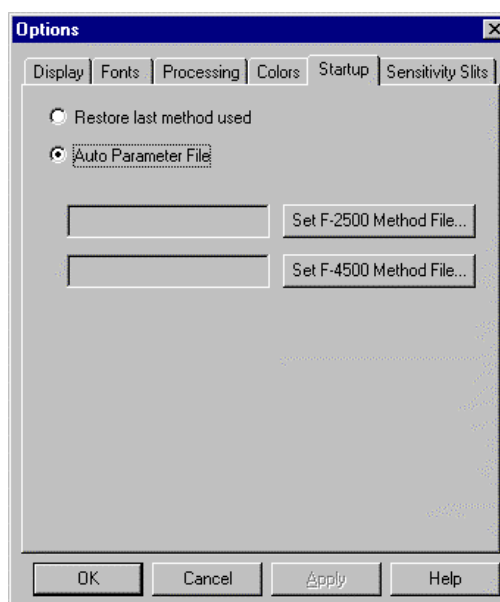


Fig. 7-2 Startup Tab

7.1 For Always Starting Up with Same Analysis Method

A window as in Fig. 7-3 will open. Set your method file here.

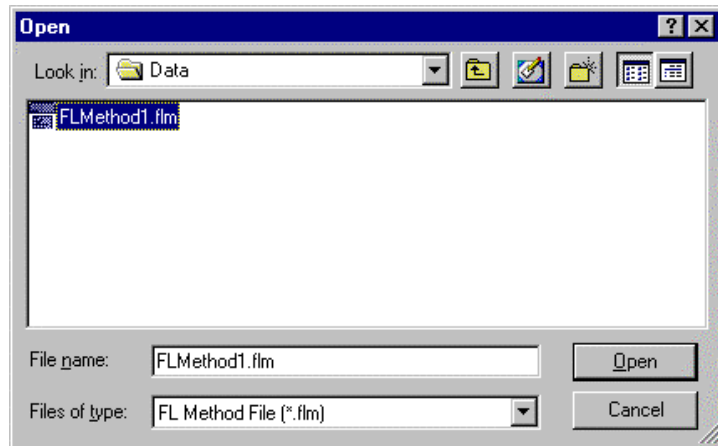



Fig. 7-3

This function is validated when the FL Solutions program is restarted.

7.2 Online/Offline Setting of Photometer

Connection with the photometer can be set offline. And this connection can be made again.

Select the Instrument command from the Tools menu or click the  button. The window in Fig. 7-4 appears. This window is for connection with the photometer.

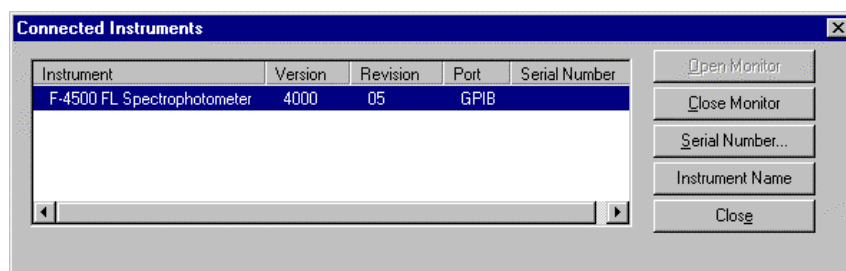


Fig. 7-4

Click the instrument name and then the **Cut** button. The offline status will be set. For online setting, click the **Connect** button. Initialization will be carried out and the monitor window will appear.

7.3 Check of Data File Information

7.3 Check of Data File Information

You can check the conditions under which specific data was measured.

For instance, when you want to know the analytical conditions or measurement date of a data file when opening it, specify and right-click the file and select Properties as shown in Fig. 7-5.

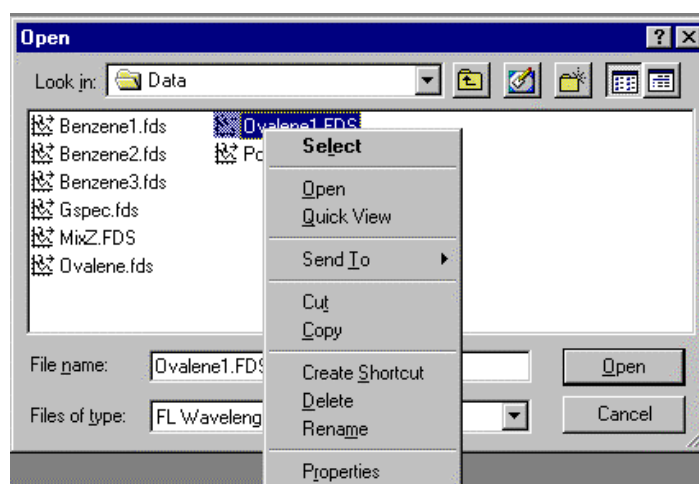


Fig. 7-5

The window in Fig. 7-6 will open. Information can be checked by clicking the Info and Scan tabs.

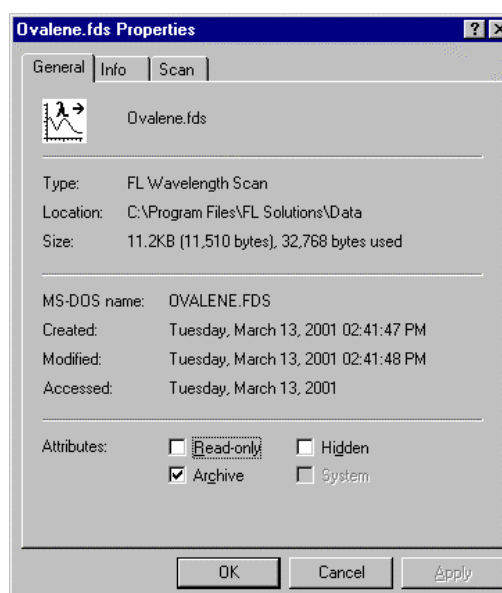


Fig. 7-6

On selection of Quick Viewer, a window appears as shown in Fig. 7-7. So a spectrum can be checked.

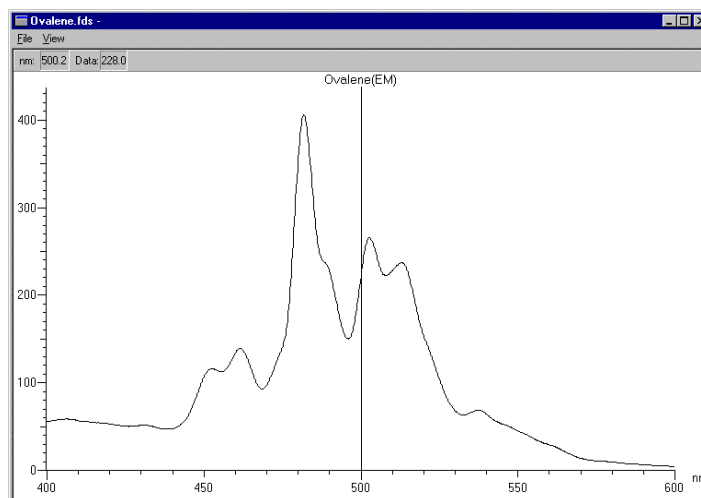



Fig. 7-7

Quick Viewer is usable when extension code is *.fds, *.fdt or *.fd3. The same operation as above can be performed by using Explorer.

The information on the open data such as analytical conditions can be checked.

Select the Properties (O) command from the Edit (E) menu or click the  button. The Info tab contains the date of measurement and the instrument used, and the Scan tab contains measuring conditions such as measuring wavelength, scan speed and slit width.

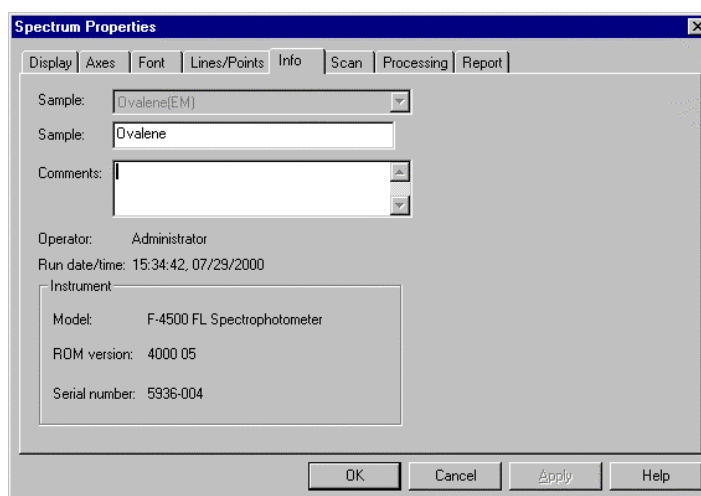


Fig. 7-8 Info Tab

7.3 Check of Data File Information

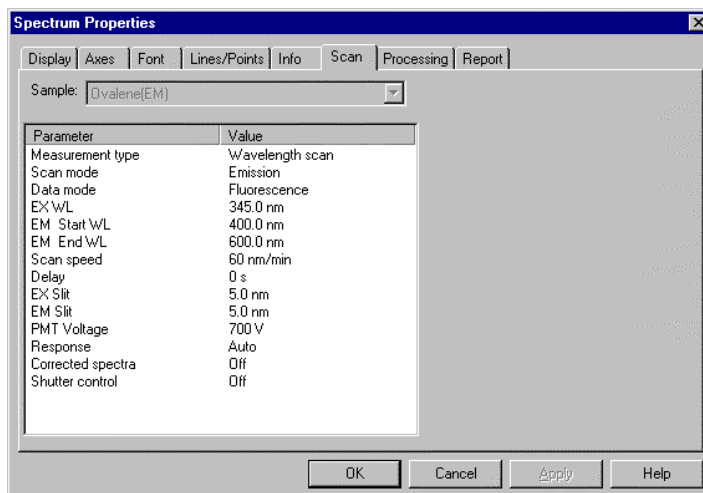



Fig. 7-9 Scan Tab

When multiple data are displayed, click the drop-down list box of sample names and select the sample name of the desired data.

7.4 Saving of Analysis Method

The set analysis method can be saved as a file. So instead of setting the method each time, you can just call out the file for setting. Select the Method command from the Edit menu on monitor window or click the  button.

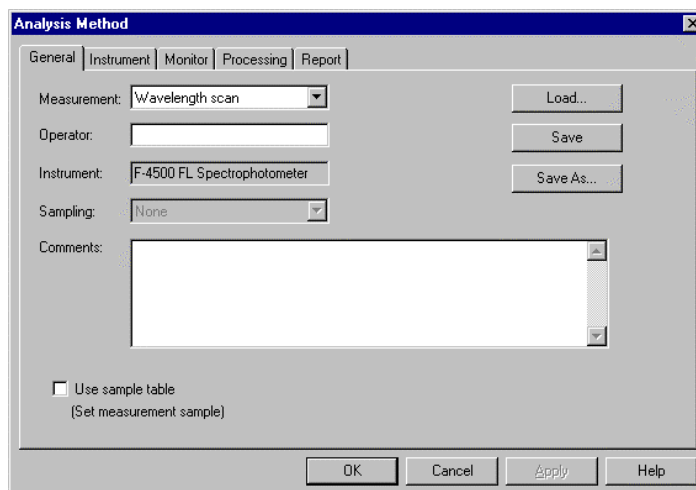


Fig. 7-10

When the setting is finished, click **Save As** and enter a file name. For calling out the saved file, click **Load** and select the file.

7.5 Even Log

7.5 Even Log

The date/time of lamp exchange and the occurrence of troubles can be recorded in a log. Select **Tools - Event Log**.

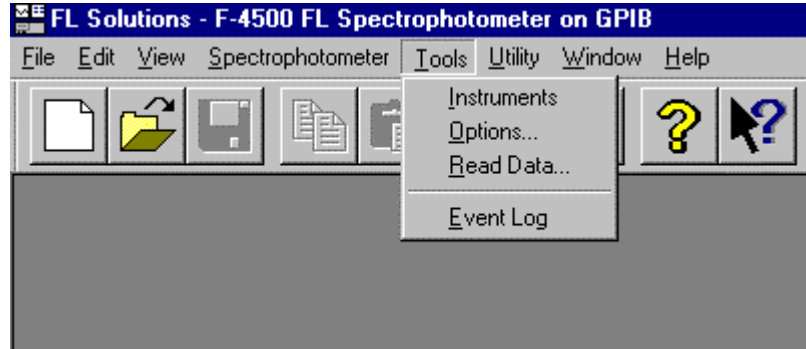


Fig. 7-11

The window in Fig. 7-12 shows the occurrence time of communication errors, etc., and user notes. For displaying only errors or only user notes, put a check mark at either item.

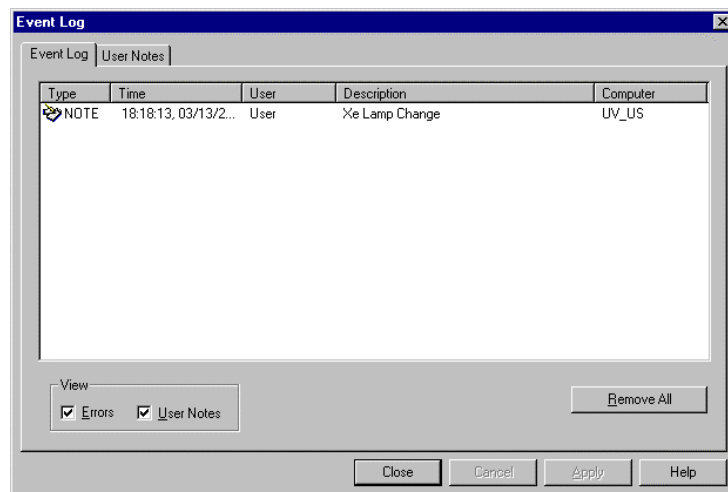


Fig. 7-12 Event Log Tab

For adding a note, select the User Notes tab, put an explanation in the Description field and click the **Add Note** button.
Maximum number of input characters: 127 characters

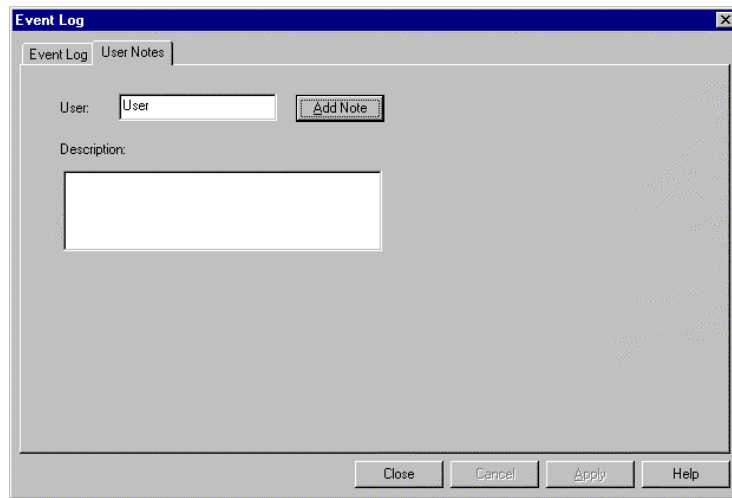


Fig. 7-13 User Notes Tab

7.6 Check of Program Version

7.6 Check of Program Version

The version of the program currently being used can be checked. Select the About FL Solutions (A) command from the Help menu.



Fig. 7-14

A window as in Fig. 7-15 will open. So check the program code.

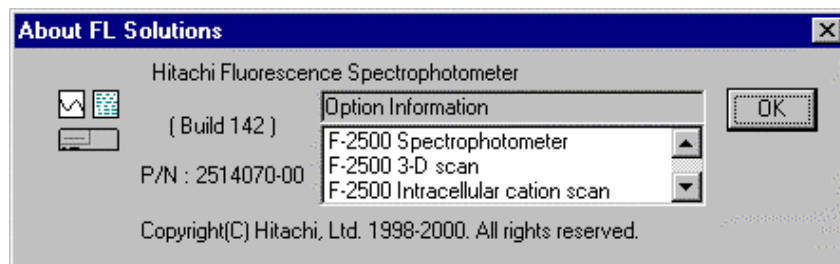


Fig. 7-15

7.7 Saving of Sample Table

Sample tables can be saved.

This is convenient for editing the sample table under the same name each time.

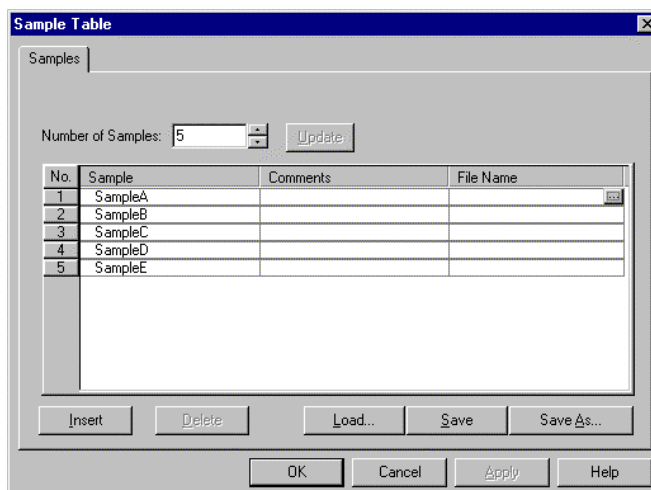


Fig. 7-16 Sample Table

After editing the sample table, click **Save As** and the window in Fig. 7-16 will appear. Enter a file name. For calling out the saved file (*.fls), click **Load** and select the file.

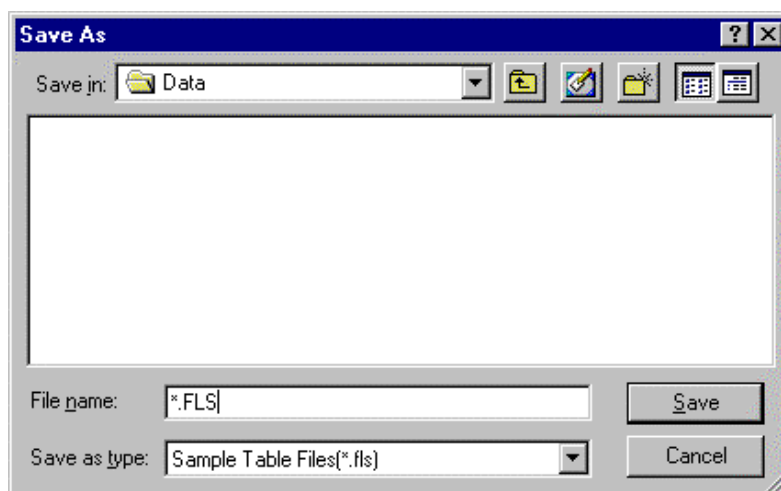


Fig. 7-17

7.8 Change of Window Display

7.8 Change of Window Display

When selecting the View (V) menu, display/non-display of toolbars and method of displaying multiple spectra can be selected.

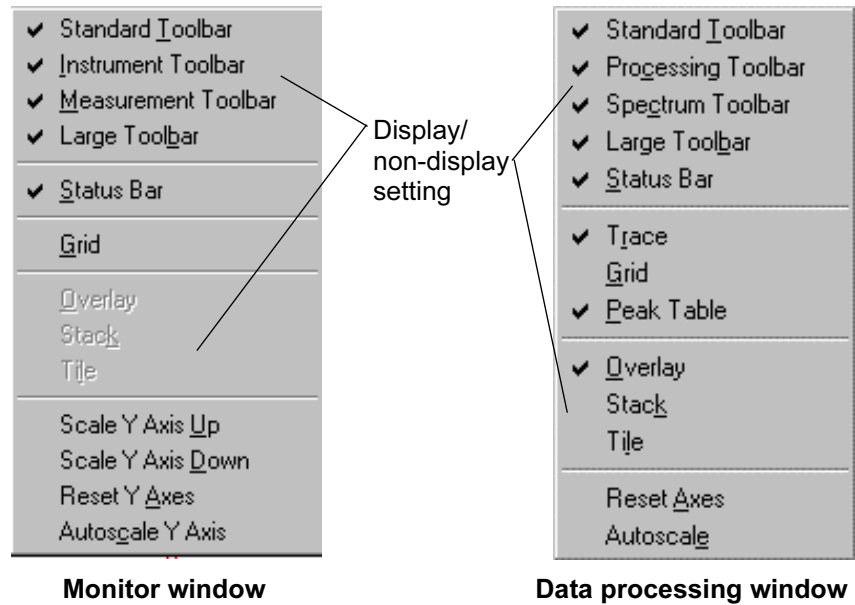


Fig. 7-20 View Menu Commands

Scale Y Axis Up : Y axis scale is doubled for display.

Scale Y Axis Down : Y axis scale is contracted by half for display.

Reset Y Axis, Reset Axes : The scale set under the Monitor tab is taken.

Autoscale Y Axis, Autoscale: Scale is adjusted to spectrum size for display.

The method of displaying multiple spectra is selectable.

- Overlay

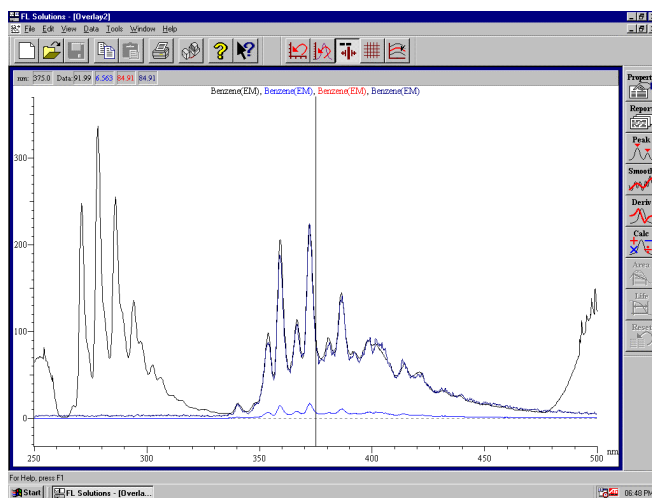


Fig. 7-21

- Stack

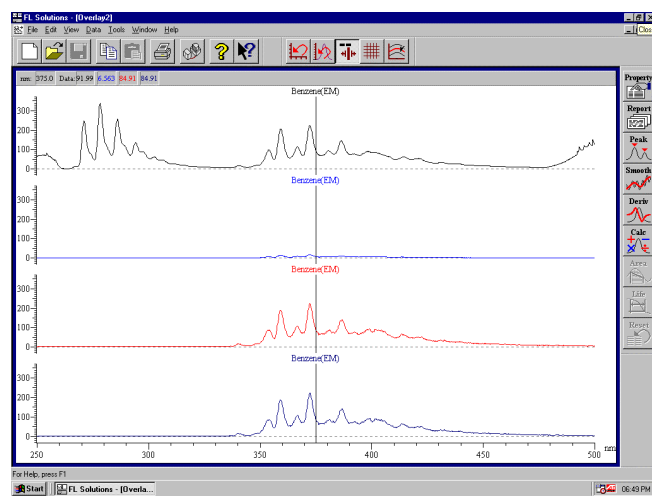


Fig. 7-22

7.8 Change of Window Display

- Tile

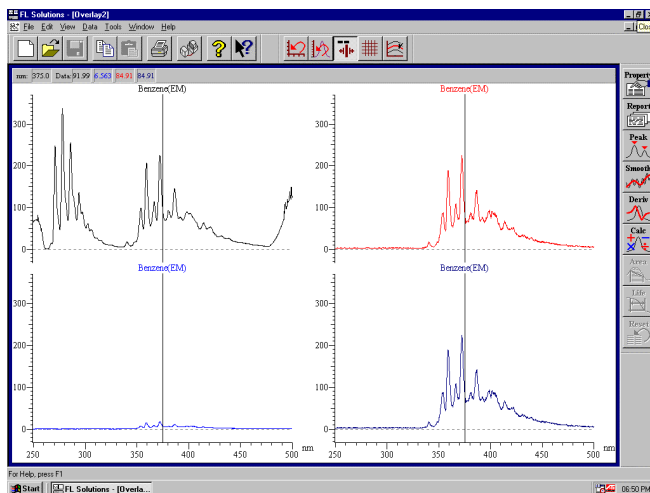



Fig. 7-23

NOTE: When six, seven or more spectra are displayed, there is a possibility of the spectra becoming too small, or not all the traced values can be displayed. In this case, use the Overlay (O) command of Data (D) menu to delete unnecessary spectra.

7.9 Check of Photometric Value at Specified Wavelength

This is a function for shifting to the specified wavelength. It is usable for checking the optical axis, etc.

Open the monitor window and select the Set Wavelength (W) command from the Spectrophotometer (S) menu or click the  (Set Wavelength) button.

A window as in Fig. 7-24 will open.

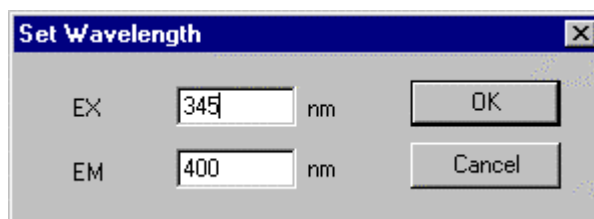


Fig. 7-24

Input target wavelength values and click **OK**.

Wavelength is now driven to the specified one and a photometric value is read on the monitor window. (Refer to (2) in 1.6.)

- **Wavelength drive to zero-order light**

For checking the optical axis, etc. in the zero-order light or moving the instrument, wavelength needs to be driven to the zero-order light. Open the window shown in Fig. 7-24 and set wavelength at 0. Then click **OK**, and wavelength will be adjusted to the zero-order light.

NOTE: For termination with wavelength kept at the zero-order light, select “Close the monitor window, but keep the lamp operating?” in shutting down the application (terminating the program). “Close the monitor window, but keep the lamp operating?” must also be selected for turning off the photometer power supply with the wavelength position kept unchanged.

7.10 Collective Printing of Files

7.10 Collective Printing of Files

Wavelength scan data files (*.uds) and time scan data files (*.udt) can be collectively printed.

From the File (F) menu, select the collective spectrum recording (L) command. A window as in Fig. 7-25 will appear.

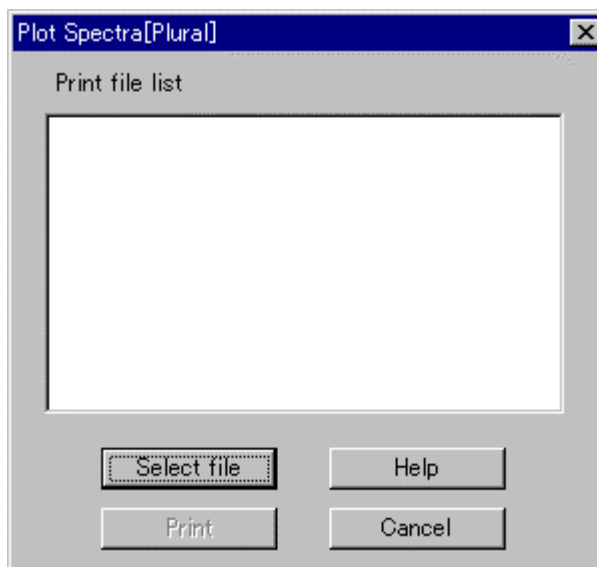


Fig. 7-25

Click **Select file**.

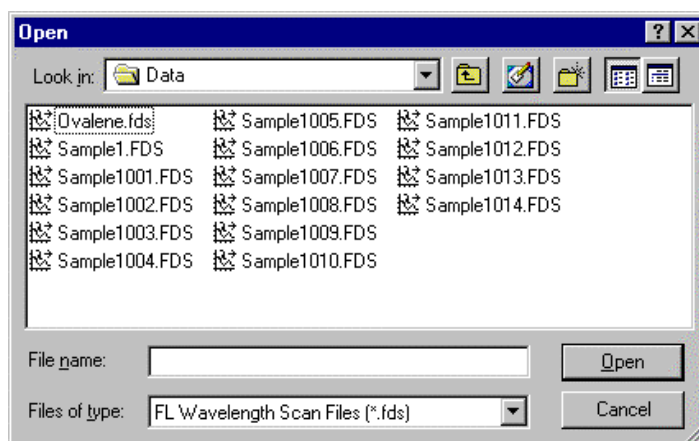


Fig. 7-26

Specify the folder of a file to be printed at “Look In” and specify the file. Click **Open (O)**.

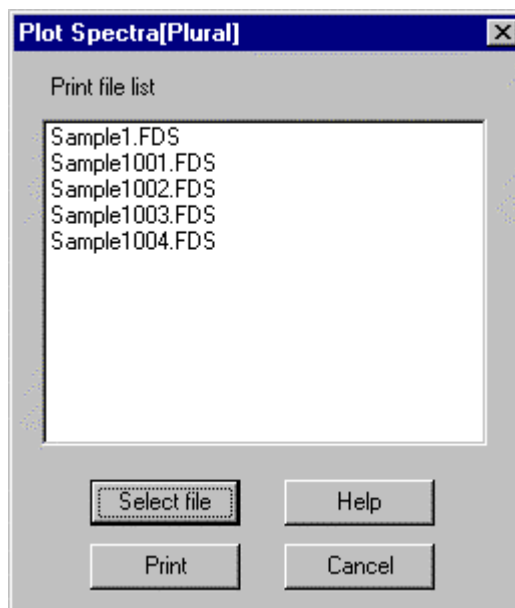


Fig. 7-27

After check, click **Print**. Printing starts. The items to be printed are the saved ones. A maximum of 100 files can be specified.

8. MEASUREMENT USING OPTIONAL ACCESSORIES

For the cable connection method for each accessory, refer to the instruction manual attached to the accessory. Explanation is given here on operations after setting of your analysis method, which is required as instructed in Sections 2 to 5.


8.1 Use of Sample Sipper

Hook up the sample sipper to the spectrophotometer. The parameters for the sipper are settable on its front panel. Explained here is the procedure for creating a calibration curve and measuring an unknown sample in the Photometry mode.

8.1.1 When Not Using a Sample Table

A message box appears in the standard measurement session, so operate in accordance with the messages. A message box does not appear in the sample measurement session. Measurement can be started just by pressing the sipping lever of the sample sipper.

- NOTES:**
1. When using the sample sipper, remeasurement, interrupt measurement and blank measurement cannot be made. But remeasurement of standards is possible.
 2. When repeat measurement is set, the standards will be measured by the set number of times. Samples will be measured only once.

Select the Measure command from the Spectrophotometer menu or click the  (measurement) button on the toolbar, and the window in Fig. 8-1 will open.

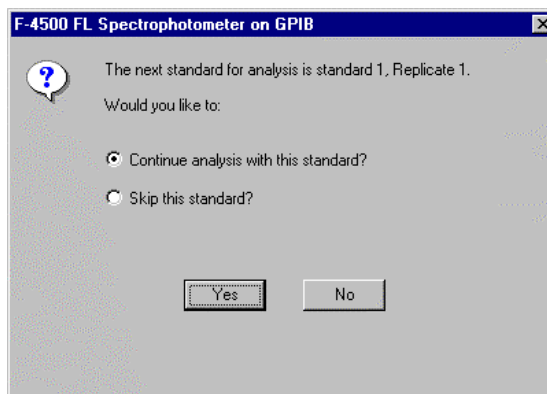



Fig. 8-1

8.1 Use of Sample Sipper

Press the sipping lever and measurement starts. A message box for the second measurement now appears.

For carrying out remeasurement or for skipping the second standard measurement, select the relevant item with the mouse (put a bullet in its radio button) and then click **Yes**.

When clicking **No**, the current measurement sequence will be terminated.

For restarting measurement, click the  button. Measurement then starts from the 1st standard.

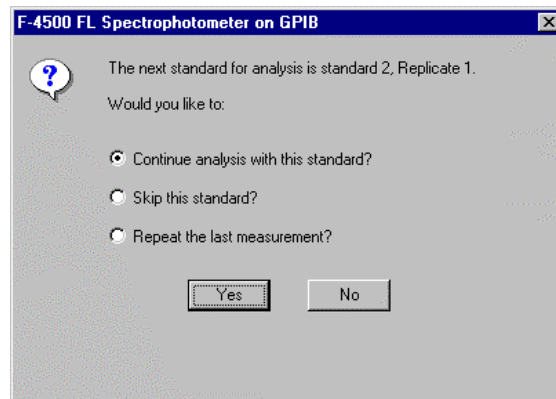


Fig. 8-2

On completion of standard measurement, the window in Fig. 8-3 will open.

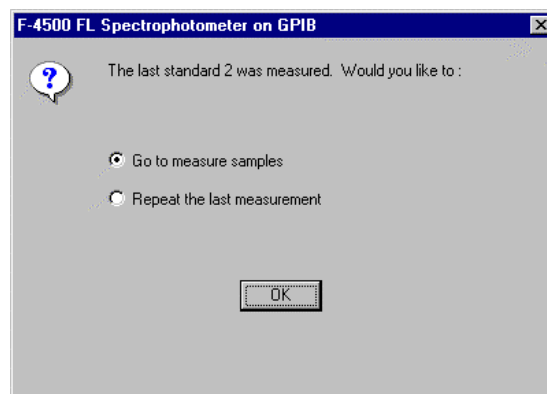


Fig. 8-3

By clicking **OK** with the mouse, a calibration curve is drawn and then samples can be measured. When the window in Fig. 8-4 appears, you should press the sipping lever. Measurement results appear at the area indicated by (1) in Fig. 8-5.

By clicking **Cancel**, the measurement is terminated, and the standards' data is not saved.

Now select "Repeat the last measurement" and click **OK**. Set the final standard in place and press the sipping lever. When repeat measurement has been set, measurement is started from the 1st one (Repl.No.1) for that standard.

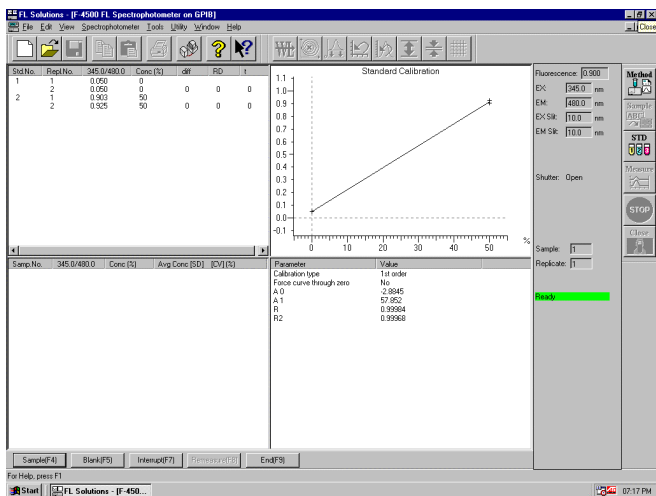


Fig. 8-4

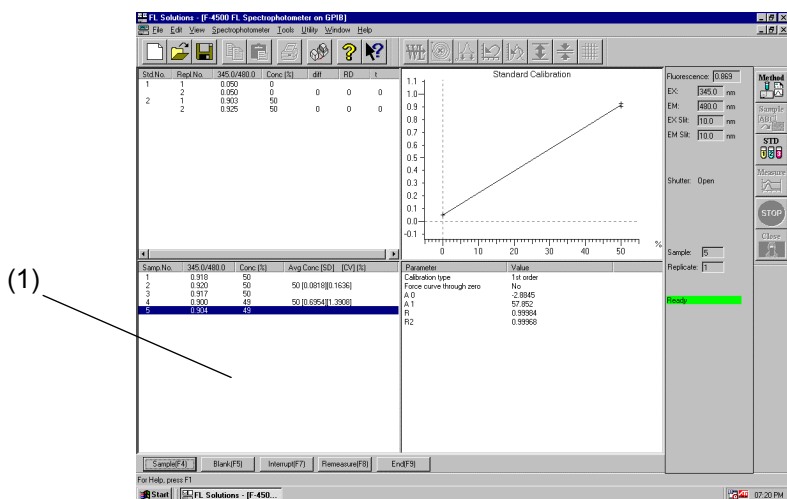


Fig. 8-5

When measurement is finished, click the **End** button, and the data will be saved.

8.1 Use of Sample Sipper

For remeasuring any of the standards after their measurements, click its data as shown in Fig. 8-6. Then click the **Remeasure** button.

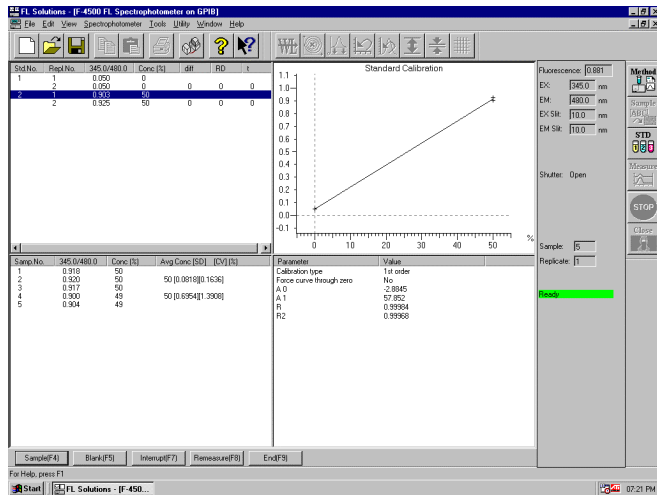


Fig. 8-6

A message box will open as in Fig. 8-7, so press the sipping lever.

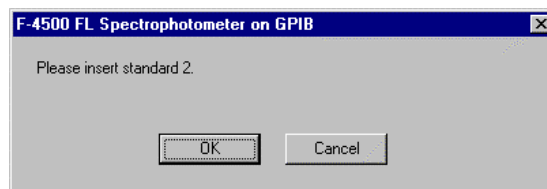




Fig. 8-7

When a standard is remeasured, the calibration curve is recalculated and sample data is also recalculated based on the curve and then displayed. To save the recalculated data, click the **End** button or the  (save) button on the toolbar.

8.1.2 When Using a Sample Table

A message box is displayed in both standard and sample measurement sessions. So operate according to the messages. When the message box appears, press the sipping lever of the sample sipper.

NOTE: When the sample sipper is used, blank measurement cannot be made.

Select the Measure command from the Spectrophotometer menu or click the  (measurement) button on the toolbar, and the window in Fig. 8-8 will open.

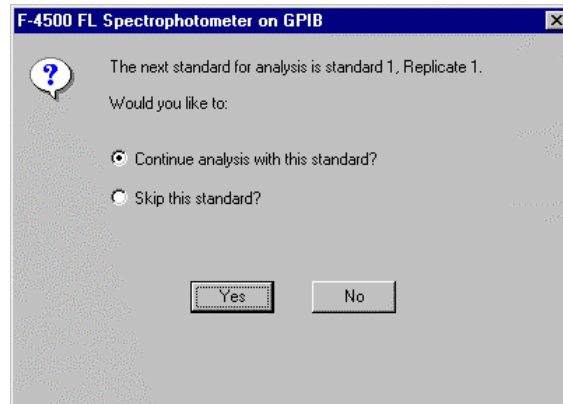



Fig. 8-8

Press the sipping lever and measurement starts.

For measurement of the 2nd and subsequent standards, a message box as in Fig. 8-9 will open. For remeasurement or for skipping measurement of the next standard, select the relevant item with the mouse (put a bullet in its radio button) and then click **Yes**. When clicking **No**, the current measurement sequence will be terminated.

For restarting measurement, click the  button. Measurement then starts from the 1st standard.

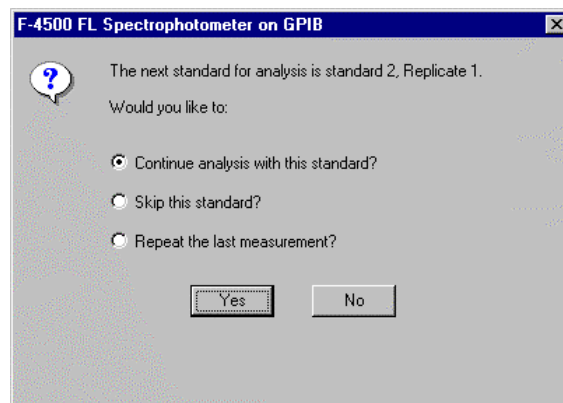


Fig. 8-9

8.1 Use of Sample Sipper

On completion of standard measurement, the window in Fig. 8-10 will open.

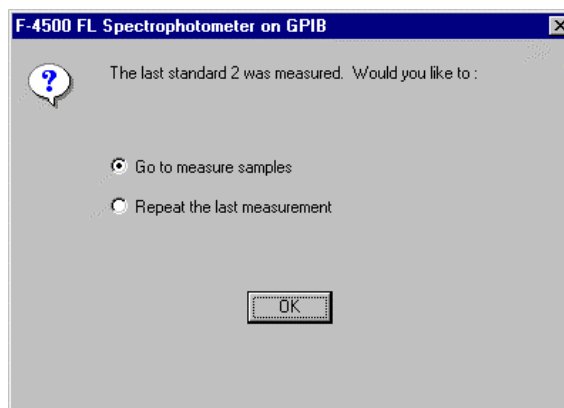


Fig. 8-10

By clicking **OK** with the mouse, a calibration curve is drawn and then samples can be measured.

By clicking **Cancel**, the measurement is terminated, and the standards' data is not saved.

For remeasurement of the final standard, select "Repeat the last measurement" and click **OK**. Set the final standard in place and press the sipping lever. When repeat measurement has been set, measurement is started from the 1st one (Repl.No.1) for that standard.

In the case of sample measurement also, a message box as in Fig. 8-11 will appear. Press the sipping lever, and measurement of the 1st sample starts.

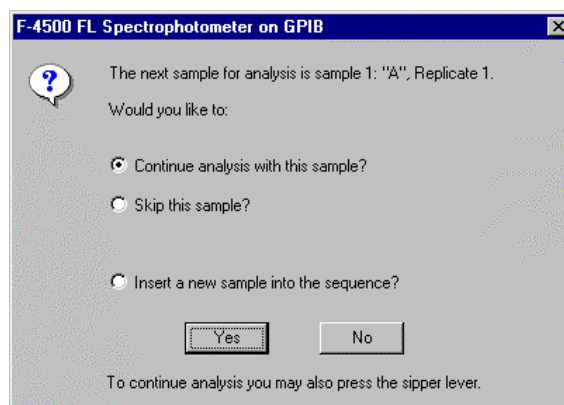


Fig. 8-11

For measurement of the 2nd sample, a message box as in Fig. 8-12 opens.

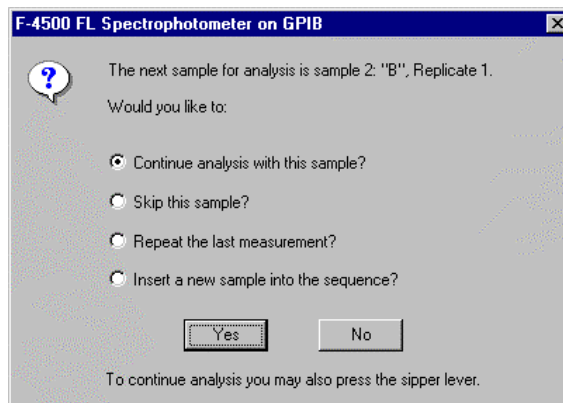


Fig. 8-12

Press the sipping lever for measuring the 2nd sample. For skipping this sample (and measuring the 3rd sample), select “Skip this sample?” and click the **Yes** button with the mouse. For remeasurement, select “Repeat the last measurement?” and click **Yes** button with the mouse. When repeat measurement has been set, measurement is started from the 1st one (Repl.No.1) for the 1st sample. In case of interrupt measurement, select “Insert a new sample into the sequence” and click the **Yes** button with the mouse. A message box as in Fig. 8-13 opens, so click the **OK** button.



Fig. 8-13

8.1 Use of Sample Sipper

For remeasurement after a series of measurements, click the data to be remeasured as in Fig. 8-14. Then click the **Remeasure** button.

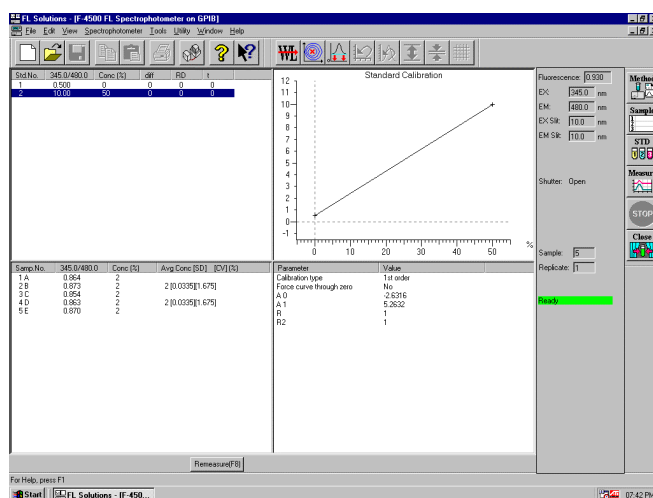



Fig. 8-14

A message box will open as in Fig. 8-15. Press the sipping lever, and measurement starts. When remeasuring a standard, the calibration curve is recalculated and sample data is also recalculated based on the curve and then displayed. To save the recalculated data, click the  (Save) button.

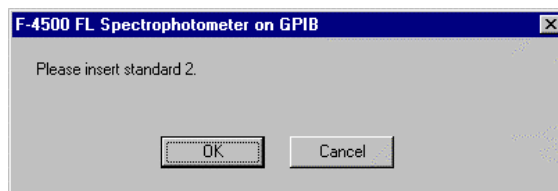



Fig. 8-15

8.1.3 In Wavelength Scan or Time Scan Mode

(1) When not Using Sample Table

Set your analysis method, and when the monitor window appears, press the sipping lever. Measurement starts.


(2) When Using Sample Table

Select the Measure command from the Spectrophotometer menu or click the  (measurement) button on the toolbar. A message box opens. Follow the messages given.

8.2 Use of AS-3000 Intelligent Autosampler

Set the rack parameters, injection volume and other items with the AS-3000 intelligent autosampler. For details, refer to the instruction manual attached to the AS-3000.

8.2.1 When Not Using a Sample Table


Select the Measure command from the Spectrophotometer menu or click the  (measurement) button on the toolbar. Then, press the **START** key on the AS-3000 intelligent autosampler. Measurement will start.

NOTE: In measurement without using a sample table, remeasurement, interrupt measurement and blank measurement cannot be done.

8.2.2 When Using a Sample Table

NOTE: In measurement when using a sample table, remeasurement, interrupt measurement and blank measurement cannot be done. But remeasurement of standards is possible. The standard to be remeasured should be set in the initial test tube position.

For measurement after completion of settings:

- (1) Select the Measure command from the Spectrophotometer menu in the FL Solutions program or click the  (measurement) button on the toolbar. The window in Fig. 8-16 opens.

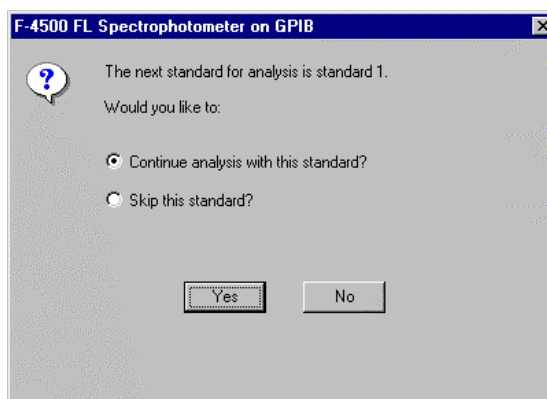


Fig. 8-16 For Start from Calibration Curve Creation

- (2) Press the **START** key on the AS-3000.
- (3) Measurement is started.

8.3 For Analog Output

8.3 For Analog Output

For connection, use the analog output terminal. Select the Analog Output Gain command from the Utility menu.

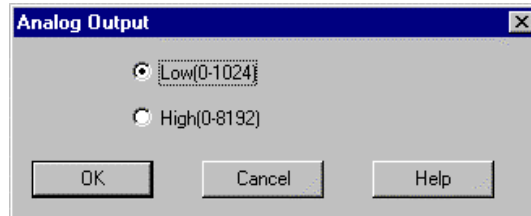


Fig. 8-17

This is a setting of data value corresponding to analog output of 1 V.

Analog output value (V) becomes as follows.

- Model F-4500
Low : $(\text{Data value} - \text{photometric value lower limit})/1024$
High: $(\text{Data value} - \text{photometric value lower limit})/8192$
- Model F-2500
Low : $(\text{Data value})/1024$
High: $(\text{Data value})/8192$

APPENDIX A DETAILS ON QUANTITATION

A.1 Foreword

In the Photometry mode of the F-2500 spectrophotometer, there are four calibration types.

- Linear working curve
- Quadratic working curve
- Cubic working curve
- Multiple segment working curve

These are explained in more detail below.

A.2 Linear Working Curve (1st order)

Regression line is determined via least squares method from a maximum of 20 data. The calculation formula is as follows :

$$x = A1 \cdot y + A0$$

$$A1 = \frac{\sum y_i x_i - \frac{1}{n} \sum y_i \cdot \sum x_i}{\sum y_i^2 - \frac{1}{n} (\sum y_i)^2}, A0 = \frac{\sum x_i}{n} - A1 \cdot \frac{\sum y_i}{n}$$

where, x : Concentration of each sample (input value)

y : Data on each sample (measured value)

n : Number of samples

A.3 Quadratic Working Curve (2nd order)

A quadratic curve is determined via least squares method from a maximum of 20 data. The calculation formula is as follows :

$$x = A2 \cdot y^2 + A1 \cdot y + A0$$

$$A2 = \frac{S(y^2x)S(yy) - S(yx)S(yy^2)}{S(yy)S(y^2y^2) - \{S(yy^2)\}^2} \quad S(yy) = \sum y_i^2 - \frac{(\sum y_i)^2}{n}$$

$$A1 = \frac{S(yx)S(y^2y^2) - S(y^2x)S(yy^2)}{S(yy)S(y^2y^2) - \{S(yy^2)\}^2} \quad S(yx) = \sum y_i x_i - \frac{\sum y_i \cdot \sum x_i}{n}$$

$$A0 = \frac{\sum x_i}{n} - A1 \frac{\sum y_i}{n} - A2 \frac{\sum y_i^2}{n} \quad S(yy^2) = \sum y_i^3 - \frac{\sum y_i \cdot \sum y_i^2}{n}$$

$$S(y^2x) = \sum y_i^2 x_i - \frac{\sum y_i^2 \cdot \sum x_i}{n}$$

$$S(y^2y^2) = \sum y_i^4 - \frac{(\sum y_i^2)^2}{n}$$

where, x : Concentration of each sample (input value)
y : Data on each sample (measured value)
n : Number of samples

A.4 Cubic Working Curve (3rd order)

A cubic curve is determined via least squares method from a maximum of 20 data. The calculation formula is as follows :

$$X = A3 \cdot Y^3 + A2 \cdot y^2 + A1 \cdot y + A0$$

where, x : Concentration of each sample (input value)
y : Data on each sample (measured value)
n : Number of samples

A.5 Multiple Segment Working Curve (Segmented)

The calibration curve is apt to bend when measuring turbid samples or the like. Use of a quantification program allows correcting the calibration curve by using up to 20 standards. Figure A-1 shows an example of curve correction. For the part which exceeds the measuring range of the standards, simply extend the line as it is.

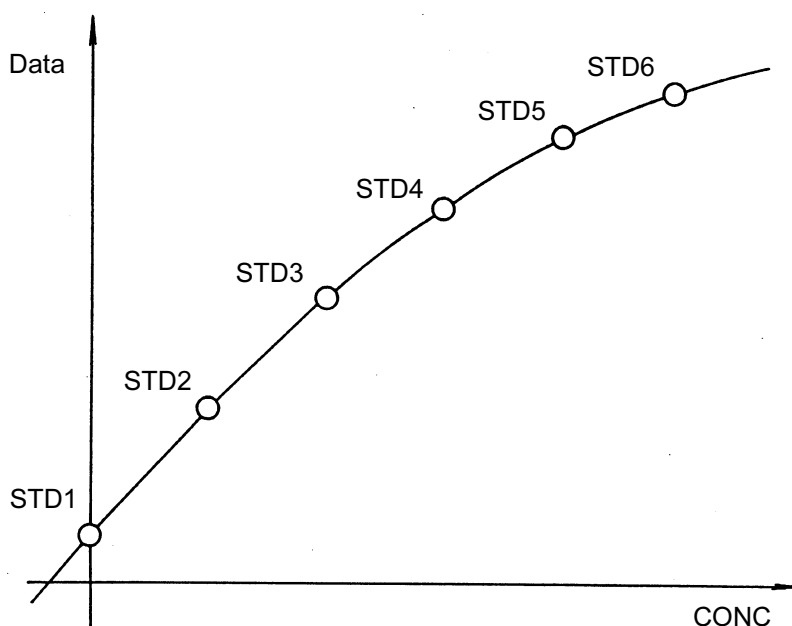


Fig. A-1 Correction of Curve Bending

<Notes on Preparation of Multiple Segment Working Curve>

- (1) A correct calibration curve can be created only when the measured value increases or decreases monotonically versus the concentration value. Especially when the inclination is negative-going, be sure to measure a blank having a data value larger than the other standards. A curve not showing a monotonic increase will appear as in Fig. A-2. And when the data value of the blank is small, regardless of a negative-going inclination, the curve will appear as in Fig. A-3.

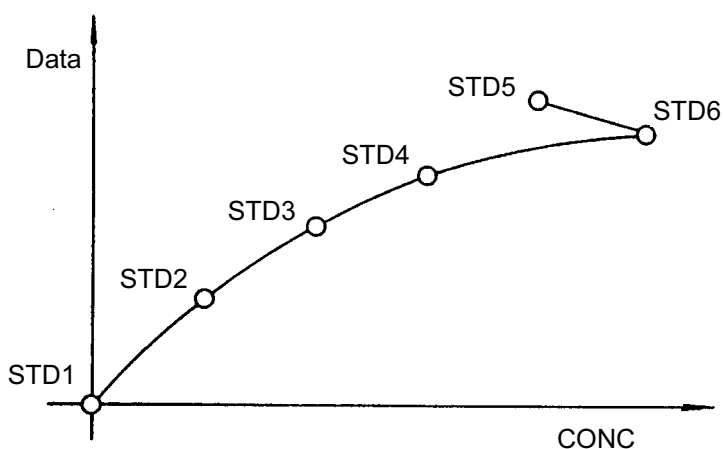


Fig. A-2 Example of Curve without Monotonic Increase (unsuitable curve)

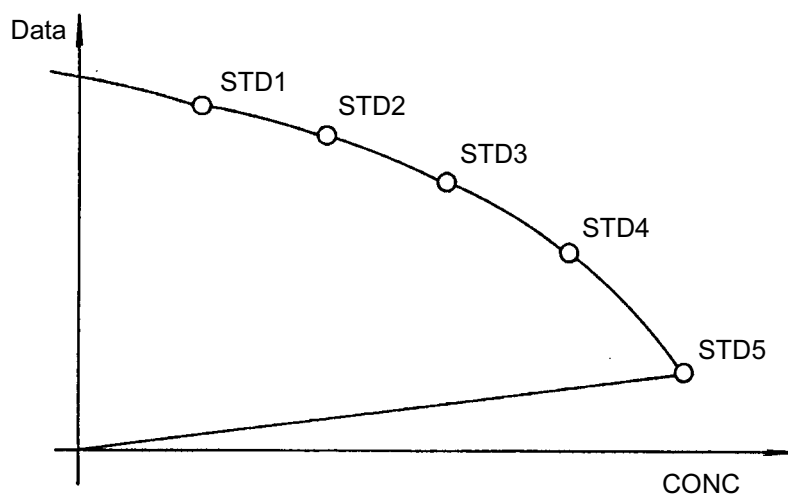


Fig. A-3 Example of Negative-going Curve where Measured Value of Blank is Small (unsuitable curve)

(2) Remeasurement of Standards

The standards can be remeasured after once preparing a calibration curve. The curve prepared in such case will appear as in Fig. A-4.

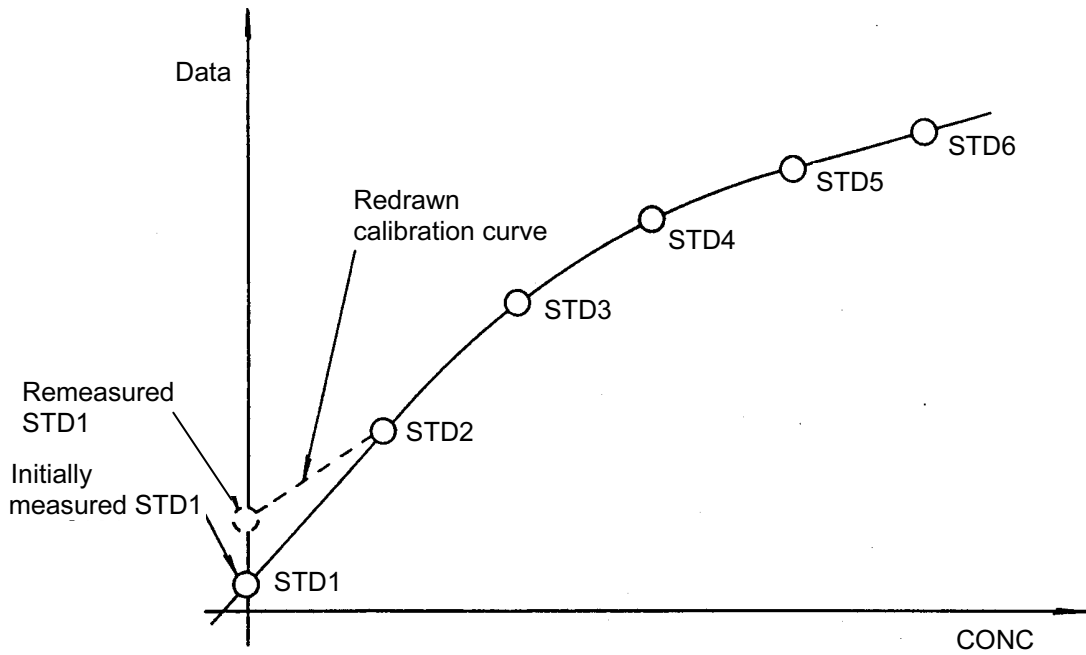


Fig. A-4 Calibration Curve when Standards are Remeasured

APPENDIX B DETAILS OF RATE ANALYSIS FUNCTION

B.1 Foreword

Rate analysis is used in the analysis of enzyme reactions. It is utilized for clinical and biochemical tests by reagent manufacturers, hospitals and so on. A computer is used to calculate the concentration from the variation in data per unit time, and the result is displayed and printed out.

B.2 Calculation Method

A timing chart for rate analysis is shown in Fig. B-1. Data is acquired when the initial delay time has elapsed after pressing the Measure button. A regression line is determined from this data via the least squares method, and the gradient and activity value are calculated. The calculation formula is as follows.

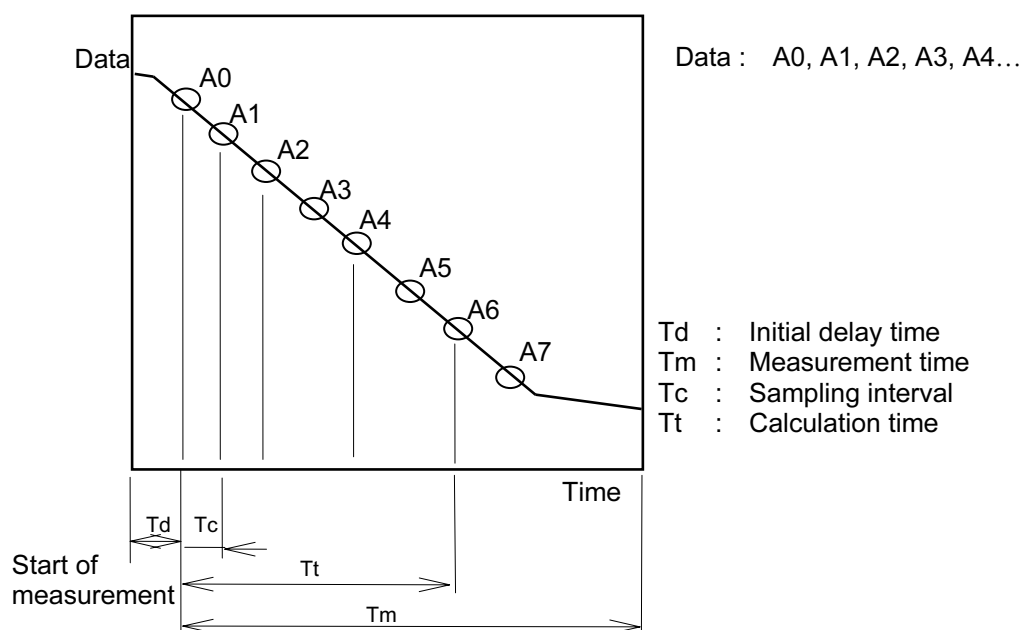


Fig. B-1

Prepare a regression line via least squares method from the measured data, and obtain a determination coefficient.

$$y = ax + b$$

where,

$$a = \frac{\sum x_i y_i - \frac{\sum x_i \sum y_i}{n}}{\sum x_i^2 - \frac{(\sum x_i)^2}{n}} \quad b = \sum y_i - \frac{a \cdot (\sum x_i)}{n}$$

x_i : Time (sec) of each data

y_i : Value of each data

n : Number of samples

The determination coefficient CD becomes as follows :

$$CD = \frac{(\sum x_i y_i - \frac{\sum x_i \sum y_i}{n})^2}{\left\{ \sum x_i^2 - \frac{(\sum x_i)^2}{n} \right\} \left\{ \sum y_i^2 - \frac{(\sum y_i)^2}{n} \right\}}$$

- Gradient (variation per minute)

$$D_i = \frac{a}{T_k} = 60a \text{ (/min)}$$

- Activity

$$C_i = k \cdot D_i$$

- R (determination coefficient)

$$R = \sqrt{CD} = \sqrt{\frac{(\sum x_i y_i - \frac{\sum x_i \sum y_i}{n})^2}{\left\{ \sum x_i^2 - \frac{(\sum x_i)^2}{n} \right\} \left\{ \sum y_i^2 - \frac{(\sum y_i)^2}{n} \right\}}}$$

- R2

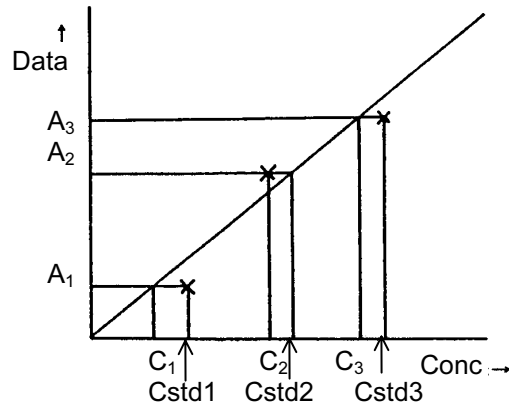
$$R^2 = (R)^2 = \frac{(\sum x_i y_i - \frac{\sum x_i \sum y_i}{n})^2}{\left\{ \sum x_i^2 - \frac{(\sum x_i)^2}{n} \right\} \left\{ \sum y_i^2 - \frac{(\sum y_i)^2}{n} \right\}}$$

NOTE : If the range for rate calculation does not coincide with the actual measured data range, then use only the measured data within that range for the calculation.

APPENDIX C DETERMINATION COEFFICIENT OF CALIBRATION CURVE

C.1 Calculation of Determination Coefficient

The determination coefficient and other factors are calculated via the following formula.



- An : Photometric or average value of standards
- Cn : Concentration on approximation curve versus A
- Cstdn : Concentration of standard (input value)
- N : Number of standards

DIFF : $DIFF_n = C_n - C_{stdn}$

RD : $RD_n = \frac{DIFF}{\bar{A}} \times 100$

$$\bar{A} = \frac{\sum A_n}{N}$$

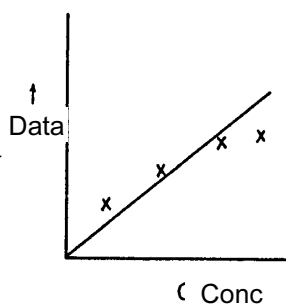
t : $t_n = \frac{DIFF_n}{\sqrt{\frac{\sum DIFF^2}{N-1}}}$

Determination coefficient : $R = \sqrt{\frac{\sum (C_n - \bar{C})^2 - \sum (C_n - C_{stdn})^2}{\sum (C_n - \bar{C})^2}}$,

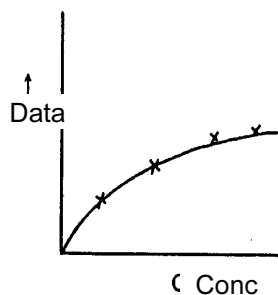
$$R_2 = (R)^2$$

C.2 Usage of Determination Coefficient

The determination coefficient indicates the goodness of fit of the measured standards and the prepared calibration curve. The closer this value is to "1", the better the fit of the measured values and calibration curve. If the value is far from "1", then the standards must be remeasured or the calibration curve mode must be changed. Examples of determination coefficients upon changing the calibration curve mode are given below.



x : standard measurement points
When calibration curve type is set to linear, determination coefficient < 1



When calibration curve type is set to quadratic, determination coefficient ≈ 1

With the above standard data, it can be seen that a quadratic curve provides a better result. A calibration curve, which was formerly judged through skill or experience, can thus be judged more easily through numerical means.

APPENDIX D INTEGRATION METHOD

D.1 Foreword

The integration methods used in the FL Solutions program are explained next.

D.2 Integration Methods

The following three methods are available in the FL Solutions program.

- Rectangular
- Trapezoid
- Romberg

D.2.1 Rectangular Method

The rectangular method is the simplest one among the above three. Since one sampling cycle is equivalent to the width of each sectional area, the total of all data points including peaks approximates the area to be obtained.

The object area shown in Fig. D-1 is the total of the rectangular sections obtained from linear approximation of the curve drawn via the data points. If a peak has few data points, the approximation will be a rough estimate.

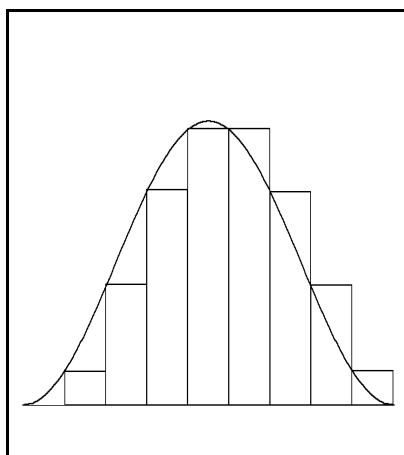


Fig. D-1

D.2.2 Trapezoid Method

This method is a further improvement on peak area calculation. The section of each sampling cycle is indicated by a rectangle on which a triangle is formed. The object area is the sum of all these areas.

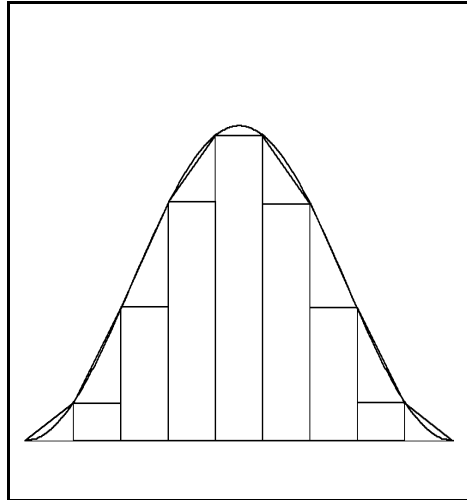
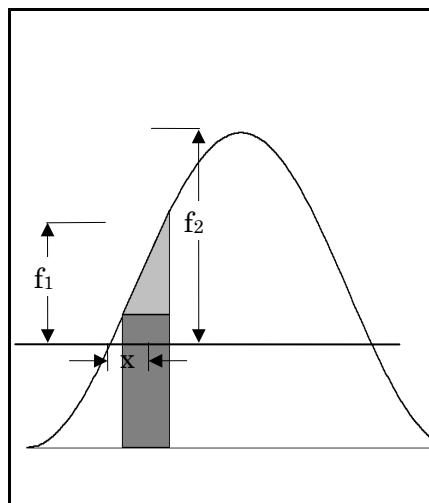


Fig. D-2

By taking just one rectangle, the area of the rectangular part I_r is expressed by the following formula.



$$I_r = f_1 x$$

where, x : Sampling interval
 f_1 : Height on left side of rectangle

Fig. D-3

A triangular part is added to this rectangle. Assuming the area of this component is I_T and the area of the triangular part is I_t , we obtain the following :

$$I_T = I_t + I_r = \frac{f_2 - f_1}{2} x + f_1 x = \left(\frac{f_1 + f_2}{2} \right) x$$

Considering this with respect to the whole area, we obtain :

$$I_T = \left(\frac{f_1}{2} + f_2 + \dots + f_{n-1} + \frac{f_n}{2} \right) x$$

D.2.3 Romberg Method

The Romberg method is the most accurate of the three methods discussed here. The trapezoid method provides different step sizes (sampling intervals) for area determination with high accuracy. In this method based on the sum of errors, two different step sizes for individual cases are available. However, unlike the conventional (classic) method of continuous approximation, an arbitrary decrease in step size is not allowed (as in case of an increase in the X-axis direction) for the purpose of accurate integration. This is because the data points that define the spectrum are handled as average data. Still, an increase in step size can be made using two or four factors if this is necessary for applying the Romberg method. The Romberg method will take the following form.

- (1) Integration is made via trapezoid method per data point.
- (2) Integration is made via trapezoid method per two data points.
- (3) By combining the above two results, the following formula is obtained.

$$I_R = I_n + \frac{I_n - I_{2n}}{3}$$

where, I_R = Integration by Romberg method

I_n = Trapezoid integration per data point

I_{2n} = Trapezoid integration per 2 data points

APPENDIX E DESCRIPTION OF FLUOROMETRY

E.1 Description of Fluorometry

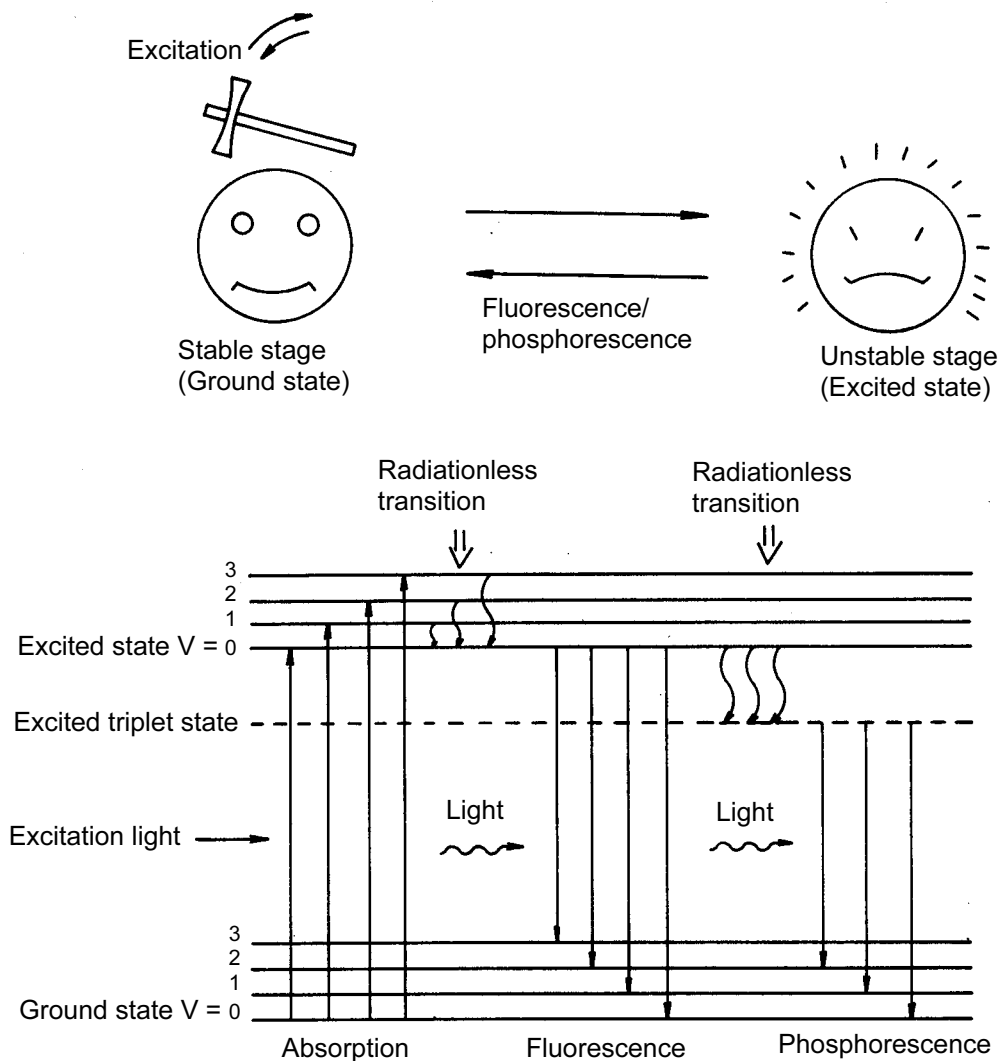


Fig. E-1 Typical Organic Molecular Energy Level

Figure E-1 illustrates the energy level transitions in an organic molecule in processes of light absorption and emission. When light strikes an organic molecule in the ground state, it absorbs radiation of certain specific wavelengths to jump to an excited state. A part of the excitation (absorbed) energy is lost on vibration relaxation, i.e., radiationless transition to the lowest vibrational level takes place in the excited state.

And, eventually the molecule returns to the ground state while emitting fluorescent radiation. Also, if radiationless transition to the triplet state takes place, then phosphorescence is emitted during triplet-to-singlet transition (from the excited triplet state to the ground singlet state). Generally phosphorescence persists for 10^{-4} sec or longer due to the selection rule imposed on the triplet-to-singlet transition. In contrast, fluorescence persists for a period of 10^{-8} to 10^{-9} sec in most cases.

As mentioned above, part of the radiation absorbed by the substance is lost as vibration energy, etc.; therefore, the fluorescence wavelength emitted from it is longer than the excitation wavelength (Stokes' law).

The ratio of the number of photons emitted during fluorescence to the number of photons absorbed is called the quantum efficiency of fluorescence. The larger the quantum efficiency a substance has, the more fluorescence it emits. Also, the intensity of fluorescence emitted from a substance is proportional to the quantity of light absorbed by it. When a dilute solution sample is measured, the intensity of fluorescence is expressed by

$$F = KI_0c l \epsilon \phi$$

where,

F : Intensity of fluorescence

K : Instrumental constant

I_0 : Intensity of exciting radiation

c : Concentration of substance

l : Cell path length (distance in the cell through which the radiation travels)

ϵ : Absorptivity of substance

ϕ : Quantum efficiency of substance

E.2 Advantages of Fluorometry

As contrasted with fluorometry, absorptiometry for a low-concentration sample is explained in the following :
 A sample having 99% transmittance to blank is taken as an example. In the absorbance measurement of such a substance, inaccuracies must always be taken into consideration. Here, the inaccuracy is assumed to be 0.1%.

Since it has an effect on both the blank and sample,

Percent transmittance of blank	100.0 ± 0.1%
Percent transmittance of sample	99.0 ± 0.1%
Difference (proportional to concentration of sample)	1.0 ± 0.2%

In this example, the uncertainty in concentration measurement is ±20%. While in the fluorometry, a difference from zero level corresponds to the concentration of sample.

Accordingly, measurement accuracy is as follows :

Output signal level at measurement of sample	100 ± 0.1
Value corresponding to blank	0 ± 0.1
Difference (proportional to concentration of sample)	100 ± 0.2

As can be seen from the above, the fluorometry is very advantageous for analyzing a low-concentration sample since its uncertainty is in most cases theoretically independent of the concentration of sample. Although actual practice may involve some error factors which increase as the concentration of sample becomes extremely low, the fluorometry is capable of measuring low concentration with an accuracy 100 times higher than in absorptiometry.

Figure E-2 is an explanatory illustration of the foregoing description. In absorptiometry, a difference between the quantity of incident radiation I_0 and the quantity of transmitted radiation is represented by signal I_s . A level at which the signal I_s becomes almost equal to a noise level is used as a detection limit.

In fluorometry, however, since the quantity of fluorescence I_f itself is represented as a signal, just a small amount of fluorescence can be amplified electrically for enabling detection. Still more, since a fluorescence wavelength of a substance is different from its excitation wavelength (incident light wavelength), the fluorescence wavelength is not readily affected by the exciting radiation, thereby contributing to ensuring high sensitivity.

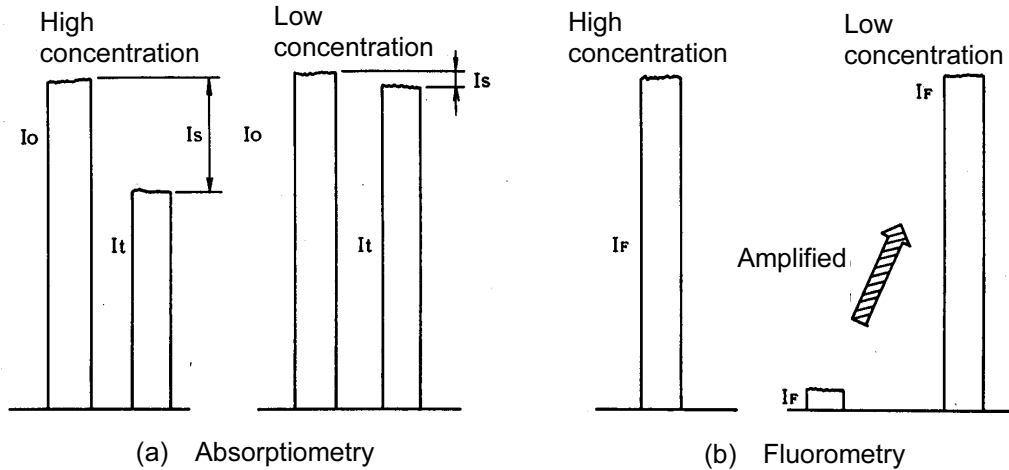


Fig. E-2 Comparison between Absorptiometry and Fluorometry

In addition to high sensitivity, fluorometry is advantageous in that more information is attainable. A fluorescence spectrum is also available besides an excitation spectrum which corresponds to an absorption spectrum in absorptiometry.

The two kinds of wavelengths can be selected as desired, and a fluorescence spectrum can be recorded using a properly selected excitation wavelength (or vice versa). Thus, quantitative and qualitative analyses can be made for a sample containing plural components.

Figure E-3 shows a simplified spectral graph of measurement of a sample containing multiple components. In absorptiometry, since only the absorption spectrum can be measured, two or more component wavelengths are presented. If the absorption wavelengths are similar to each other, each component cannot be separated in measurement. In fluorometry, even if the absorption wavelengths are similar, a difference in fluorescence makes it possible to select each fluorescence wavelength properly. Thus, each component can be separated in measurement.

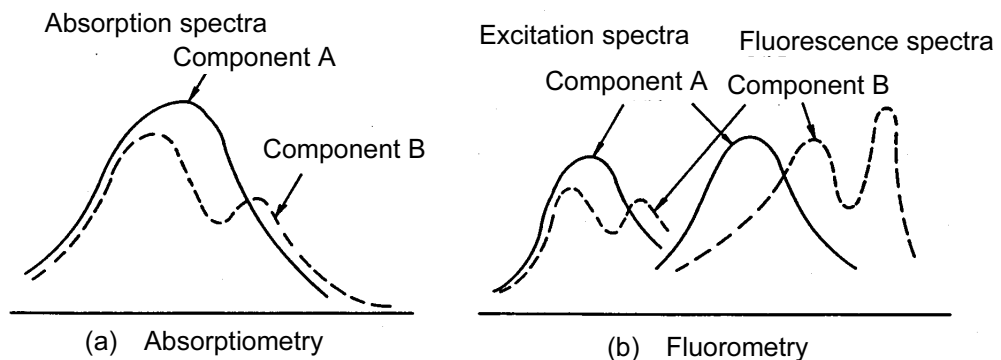


Fig. E-3 Measurement of Multi-Component Sample

Table E-1 compares information attainable in absorptiometry and that in fluorometry.

Table E-1 Comparison of Information Attainable in Absorptiometry and Fluorometry

Absorptiometry	Fluorometry
<ul style="list-style-type: none"> • Absorption spectrum only (Corresponding to excitation spectrum in fluorometry) 	<ul style="list-style-type: none"> • Excitation spectrum • Fluorescence spectrum

E.3 Notes of Fluorescence Analysis Measurement

E.3.1 Temperature Dependency of Fluorescence Intensity

For most kinds of samples, an increase of 1°C in the temperature of sample causes the intensity of fluorescence to decrease by 1 to 2%. It is also reported that for some kinds of biochemical samples, the intensity of fluorescence decreases as much as 10% as the temperature increases by 1°C. When analyzing a sample having a temperature-dependent property, it is advisable to use the constant-temperature cell holder (P/N 650-0150). Constant-temperature measurement can be carried out by circulating constant-temperature water through this cell holder.

E.3.2 Chemical Change in Sample due to Radiation

Some kinds of samples may be susceptible to a chemical change due to exciting radiation. In analysis of such a sample, keep the shutter closed to cut off an excitation beam until measurement is started, and then open the shutter immediately before measurement. If any chemical change due to exciting radiation is observed still, determine a signal level at the start time through extrapolation according to variation in signal level.

E.3.3 Raman Scattering

In fluorescence measurement, spectra having different natures from that of fluorescence may be observed. These are called Rayleigh scattering spectrum and Raman scattering spectra; the former appearing at the same wavelength position as the excitation spectrum, and the latter appearing at the longer-wavelength side near Rayleigh scattering. In a fluorescence spectrum, when the excitation wavelength is shifted, only the peak height is changed while the peak wavelength position remains intact. In a Raman scattering spectrum, when the excitation wavelength is shifted, the peak wavelength position is also changed accordingly. Both the Rayleigh scattering and Raman scattering are caused by a solvent which may be contained in the sample. When examining the spectral plot, be careful not to mistake these scattering effects of the fluorescence peak of interest. Table E-3 presents the Raman spectral peak position at each excitation wavelength for the purpose of reference.

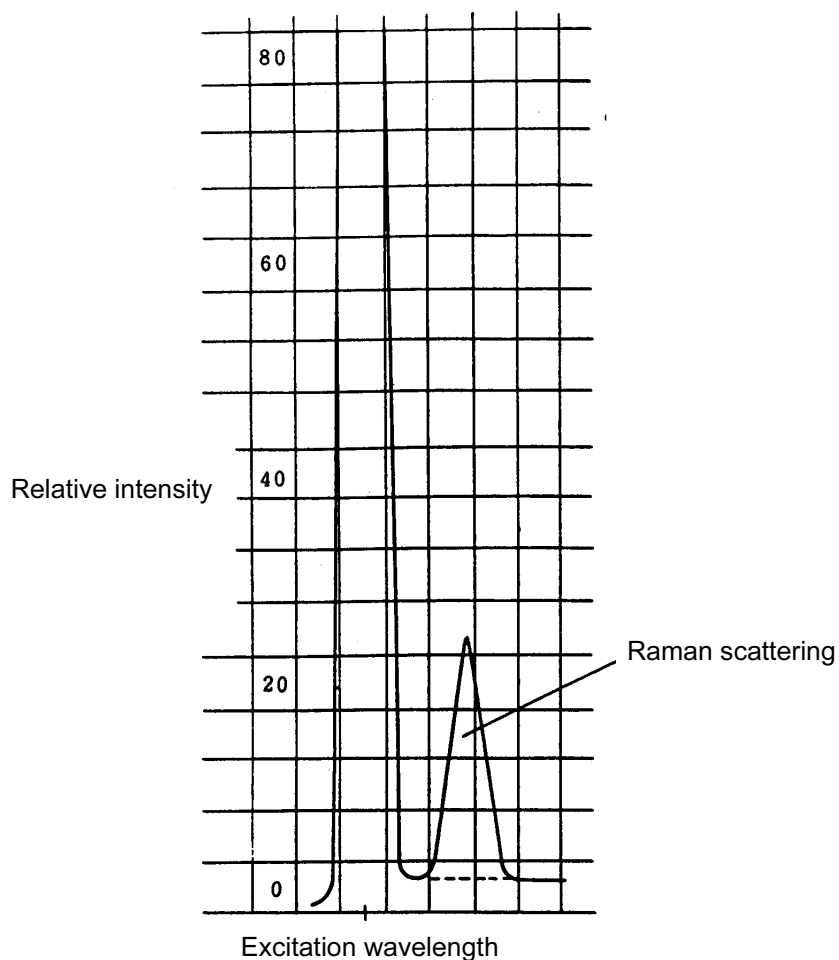


Fig. E-4 Raman Spectrum of Water

Table E-3 Raman Peak Positions at Respective Excitation Wavelengths

	(Excitation wavelength)	Water	Ethanol	Cyclohexane	Carbon Tetrachloride	Chloroform
Excitation wavelength and Raman peak position (nm)	248	271	267	267	—	—
	313	350	344	344	320	346
	365	416	405	408	375	410
	405	469	459	458	418	461
	436	511	500	499	450	502

E.3.4 Handling of High-Concentration Samples

In measurement of a high-concentration sample, a variety of error factors may be involved. The most significant error factor consists in that an excitation beam is absorbed at the entrance of a cell to prevent a sufficient level of excitation at the center of cell.

Figure E-5 illustrates an extreme case of this condition. Although fluorescence is emitted in the vicinity of the entrance for the excitation beam, it is not taken into the emission monochromator.

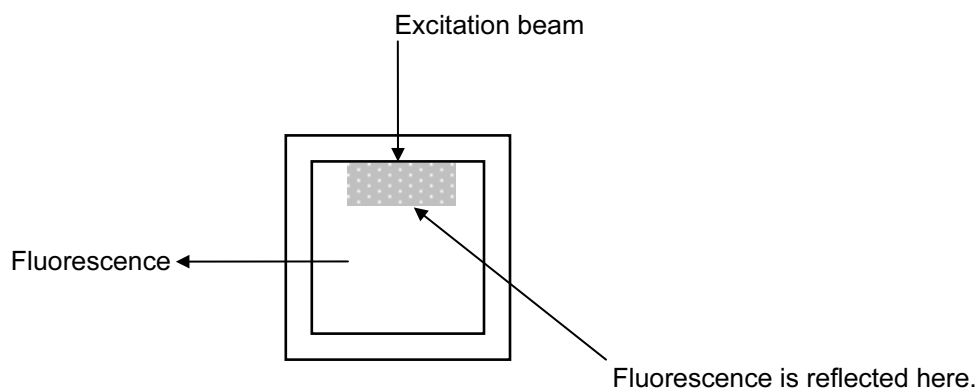


Fig. E-5 Sample Having an Extremely High Concentration

If only the incident point of excitation beam is bright, it is necessary to dilute the sample properly for measurement. The second significant error factor consists in extinction due to concentration. This condition is caused by preventing activation through interaction of molecules.

The third significant error factor consists in re-absorption of fluorescence. As shown in Figure E-6, this condition occurs due to overlapping between the short-wavelength side of fluorescence spectrum and the long-wavelength side of excitation spectrum. Therefore, it seems that the fluorescence spectrum has been shifted toward the long-wavelength side to some extent.

In measurement of an ordinary kind of sample, however, this condition will not impede quantitative determination significantly.

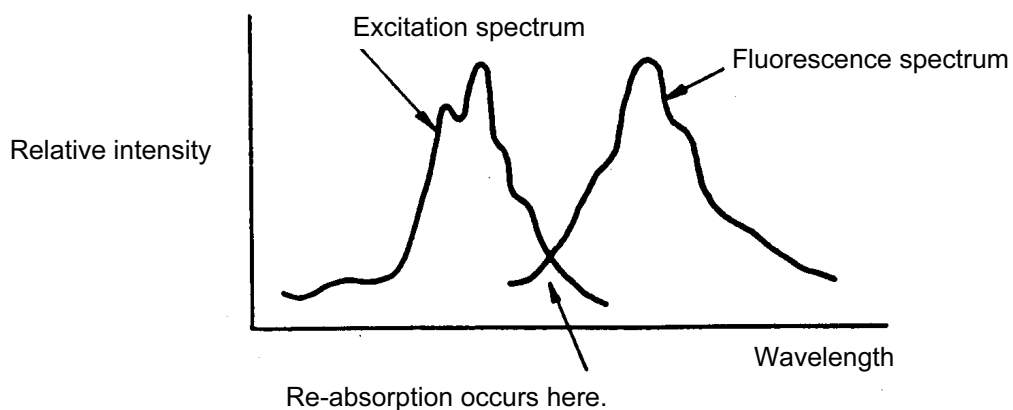


Fig. E-6 Explanatory Illustration of Re-absorption

In any case, if there is a possibility of a measurement error due to high concentration of a sample, dilute the sample properly or carry out surface fluorescence measurement using a solid sample holder.

E.3.5 Second-Order Scattered Radiation

Where the excitation and emission wavelengths are plotted near each other, care should be exercised not to mistake the Raman and Rayleigh scattering for the fluorescence spectrum as mentioned in E.3.3. Where the excitation and emission wavelengths are plotted apart from each other, care should be exercised not to mistake the second-order and third-order scattered radiations for the fluorescence spectrum.

The second-order scattered radiation appears at a wavelength two times longer than the excitation wavelength, and the third-order scattered radiation occurs at a wavelength three times longer.

For instance, if the excitation wavelength is 240 nm, the second-order and third-order radiations take place at 480 and 720 nm, respectively. For eliminating these scattered radiations, insert a short-wavelength cutoff filter in the path of fluorescing radiation (before the emission monochromator). It is advisable to use the filter set (P/N 650-0157) which is available as an optional accessory.

E.3.6 Contamination of Cell

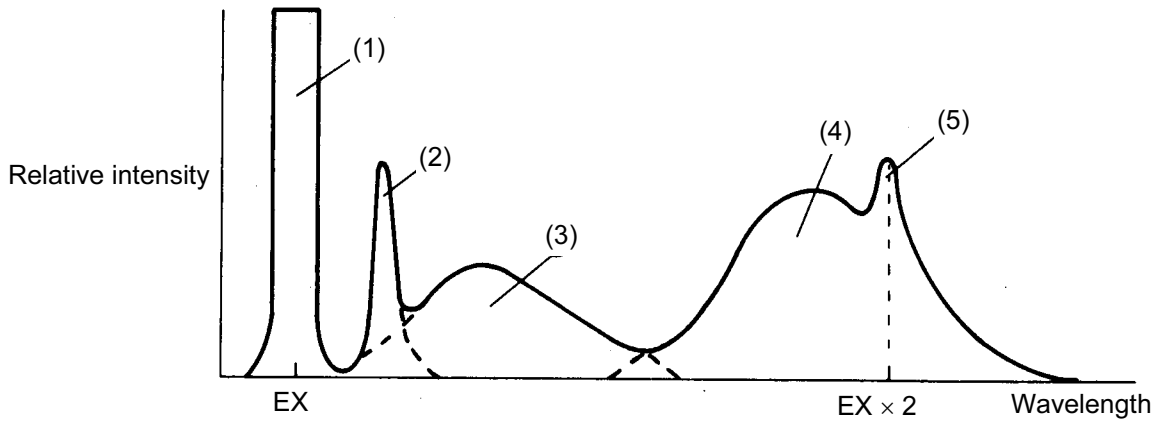
Since the fluorescence spectrophotometer provides high sensitivity, just a slight amount of contamination on a cell may have an adverse effect on results of measurement. To prevent this, treat the cell properly after its use.

Do not leave the cell containing say sample. In evaporation of a solvent, a residue of sample may adhere to the wall of the cell to cause contamination.

In measurement of a very dilute sample, contamination on the inner and outer walls of the cell may cause a problem. If a droplet of sample solution is put on the outer wall of the cell in sample injection into it, wipe off the cell with tissue paper and then set it on the cell holder.

E.3.7 Measurement Example of Fluorescence Spectrum

Figure E-7 shows a measurement example of fluorescence spectrum.



- (1) Scattering of exciting radiation
- (2) Raman spectrum of solvent
- (3) Fluorescence of impurities, solvent, etc.
- (4) Fluorescence of sample
- (5) Second-order spectrum of exciting radiation

Fig. E-7 Measurement Example of Fluorescence Spectrum

As shown in Figure E-7, other peaks than a fluorescence peak of sample appear in fluorescence spectral measurement. With reference to this example, it is necessary to identify a fluorescence peak of sample.

APPENDIX F MEASUREMENT OF INSTRUMENTAL RESPONSE (CORRECTED SPECTRA)

Spectrum correction is performed to enable measuring a true spectrum by eliminating instrumental response such as wavelength characteristics of the monochromator or detectors. The measurement of instrumental response is needed to perform spectrum correction. "Instrumental Response" is the function to measure and save the instrumental response.

F.1 Measurement of Instrumental Response on Excitation Side

This is the function to obtain the instrumental response on the excitation side such as wavelength characteristics of the excitation monochromator using Rhodamine B as a standard (quantum counter).

The instrumental response is automatically read with a single wavelength scan operation. A spectrum is correctable within a range of 200 to 600 nm.

(1) Handling of Rhodamine B

Pour Rhodamine B into a triangular cell in the procedure illustrated in Fig. F-1. The triangular cell filled with Rhodamine B should in principle be stored at a dark place.

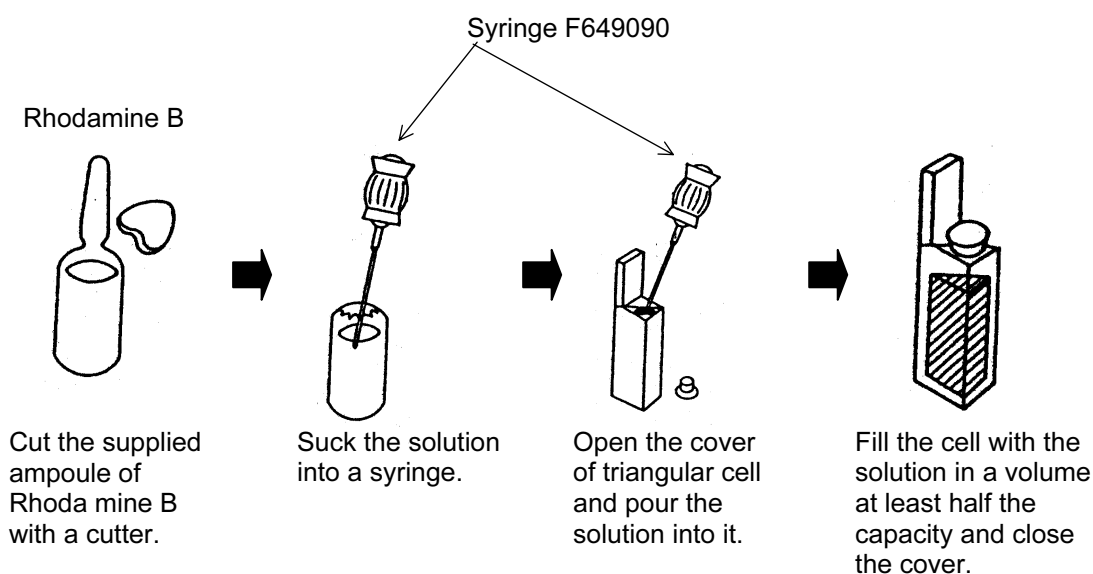


Fig. F-1 Handling of Rhodamine B

(2) Operating Procedures

- (a) Set PMT Voltage to 400 V and Data Mode to “Fluorescence”. Perform “Zero Adjust” to calibrate the zero point.
- (b) Set the triangular cell filled with Rhodamine B into the cell holder in the sample compartment. (See Fig. F-2).

The triangular cell must be set in the direction shown in Fig. F-3.

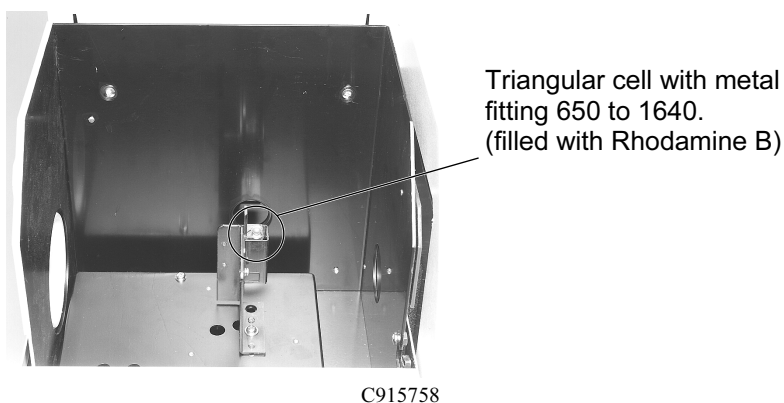


Fig. F-2 Setting of Rhodamine B

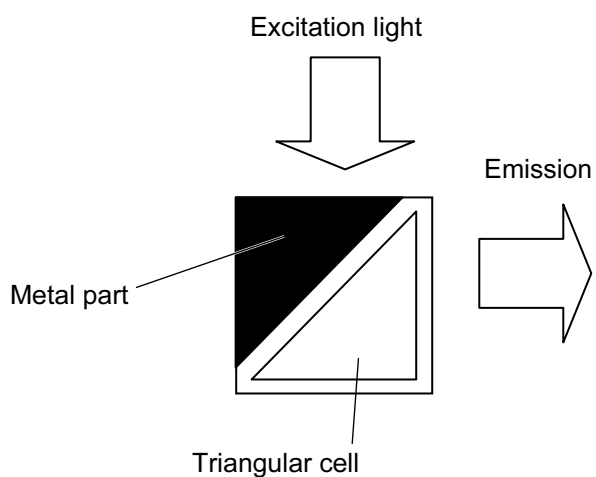


Fig. F-3 Setting Direction of Triangular Cell (Top view)

- (c) Select "EX" of "Instrumental Response: Utility".
EM wavelength is set at 640 nm and the instrument starts EX wavelength scan (from 198.6 nm to 601.4 nm).
At this stage, the instrument is automatically set in the following conditions.

Slit (EX/EM)5.0/20.0 nm
Scan speed.....60 nm/min
Scan mode.....Fluorescence
PMT Voltage400 V
Response.....Auto

- (b) Upon completing the measurement, all of measuring conditions are set to those set before.
Now, instrumental response on the excitation side has been measured and stored. Take out the triangular cell filled with Rhodamine B.

F.2 Measurement of Instrumental Response on Emission Side

This is the function to obtain the instrumental response on the emission side such as wavelength characteristics of the emission monochromator and detector (photomultiplier).

The instrumental response on the emission side is determined by measuring a combination of instrumental response on both the excitation and emission sides by synchronous wavelength scan, and dividing it by the instrumental response on the excitation side preliminarily measured. An emission spectrum is correctable within a range of 200 to 600 nm.

(1) Operating Procedures

- (a) Set PMT Voltage to 400 V and Data Mode to "Fluorescence".
Perform "Zero Adjust" to calibrate the zero point.
- (b) Set the diffuser into the cell holder in the sample compartment. (See Fig. F-4).

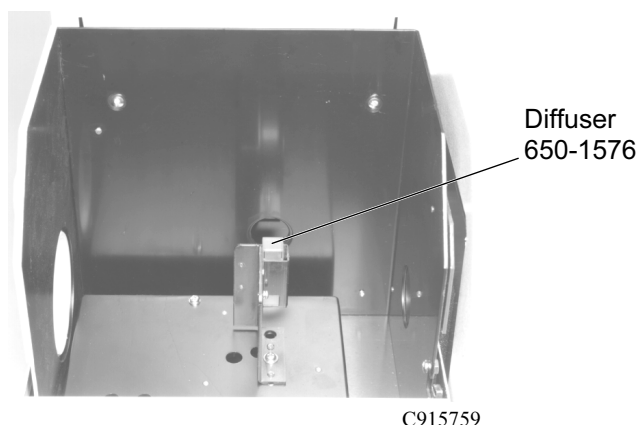


Fig. F-4 Setting of Diffuser

NOTE: Measurement of instrumental response on the excitation side must be completed before executing a measurement of instrumental response on the emission side.

- (c) Select “EM (200 to 600 nm)” of “Instrumental Response: Utility”.

EM wavelength is set at 198.6 nm and the instrument starts synchronous wavelength scan (from 198.6 nm to 601.4 nm).

At this stage, the instrument is automatically set in the following conditions.

Slit (EX/EM) 5.0/20.0 nm
Scan speed 60 nm/min
Scan mode Fluorescence
PMT Voltage 400 V
Response Auto

- (d) Upon completing the measurement, all of measuring conditions are set to those set before.

Now, instrumental response on the emission side has been measured and stored. Take out the diffuser.

F.3 Measurement of Instrumental Response on the Long Wavelength Range of Emission Side (only with Model F-4500)

When a corrected spectrum in the long wavelength area of 600 nm is required on the emission side, the beam of a lamp for which the wavelength characteristics are known is allowed to be irradiated to the emission optics, and the instrumental response in the long wavelength range on the emission side is read for the correction.

As a light source, a sub standard light source (P/N 250-0124 or 250-0125) is used. The correction range by it is 500 to 900 nm and, when combined with an instrumental response in the short wavelength range, a spectrum correction is available over the entire wavelength range (200 to 900 nm) on the emission side.

(1) Mounting and Positioning Sub Standard Light Source

Refer to the instruction manual for the installation of the sub standard light source (optional accessory P/N 250-0124 or 250-0125).

(2) Operating Procedures

(a) Close the cover of the sample compartment.

(b) Set PMT Voltage to 400 V.
Perform "Zero Adjust" to calibrate the zero point.

(c) Turn ON the power switch of the sub standard light source and wait for approx. 5 minutes until the lamp stabilizes.

(d) Select "EM (500 to 900 nm)" of "Instrumental Response: Utility".

The instrument starts EM wavelength scan (from 498.6 nm to 901.4 nm).

At this stage, the instrument is automatically set in the following conditions.

Slit (EM)..... 5.0 nm
Scan speed..... 60 nm/min
Scan Mode Luminescence
PMT Voltage 400 V
Response Auto

- (e) Upon completing the measurement, all of measuring conditions are set to those set before. Now, instrumental response on the emission side has been measured and stored. Remove the sub standard light source.

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