INSTRUCTION MANUAL

FOR DW-F98

FLUOROSPECTROPHOTOMETER

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Artist of Science

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Preface

Thank you for purchasing F98 Fluorescence Spectrophotometer.

Please read this manual carefully before installation or first time using F98 Fluorescence spectrophotometer.

Fluorometry is a high sensitive, high selective and modern analysis method. Fluorometry provides excitation spectrum, emission spectrum, including luminous intensity, luminous life, fluorescence polarization and other information. It's linear range of working curve is wide enough to become an important analysis method in Measure analysis.

For proper use of F98 Fluorescence spectro-photometer, basic knowledge of optical instruments and molecular fluorometry is needed. Computer skills are needed as well.

For more detail of F98 specifications, please check manual chapter 1.4.

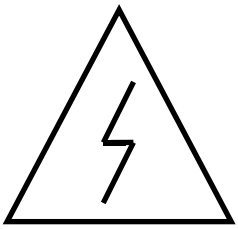
F98 Fluorescence spectrophtometer is a dual-monochromator spectrophtometer with fluorescence excitation wavelength scan, emission wavelength scan, 3D scan, Synchronous scan, time scan, quantitative analysis and other functions.The Fluorescence spectrophtometer should be connected to a computer.

This manual includes instrument instruction, software instruction and appendix.

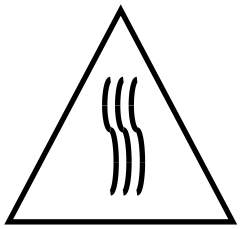
Chap. 6.3.5 is a value-added part. If you need information of this part, please contact us.

Safety Instructions

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



This sign indicates possible electric hazard. It shall be handled by a qualified person according to the corresponding procedure 。（On power switch and trigger）



This sign indicates heat on surface.

NOTICE

1. **The instrument is suitable for analysis in laboratory. If the instrument is needed outside the lab, please make the field work environment meets the environmental requirements of the laboratory.**
2. **Please use the original package when moving the instrument.**
3. **Please boot the instrument in sequence. First, turn on the Xenon lamp power supply. Then turn on the main power. When shutting down, turn off the main power first, then turn off the Xenon lamp power supply. There will be 30 min before Xenon lamp becomes steady. Please wait 60 sec to retrigger the Xenon lamp if the Xenon lamp power shut down.**
4. **If the Xenon lamp power is not triggered and making noise, shut down the Xenon lamp power immediately and retrigger after 60 sec. Due to the fact that Xenon lamp life is closely related to the switching times, please minimize unnecessary trigger.**
5. **When the instrument is on, the temperature of the vents on top left corner is high. Please keep the air circulating and away from the vents surface. DO NOT observed Xenon light directly with naked eye.**
6. **Please make sure the fans on the left side and top left corner operate normally. If the fans are not functioning, please turn off the instrument for repairs.**
7. **As to protect PMT, DO NOT let light into the sample cell when the gain is higher than 6. When using unknown sample in the test, set the gain from low to high gradually from 1 to 17.**
8. **Please check the Fluorescent zero and adjust zero after setting gain.**
9. **When an error occurred by wrong operation or other machine or instrument error, shut down the**

instrument immediately. When the software is not operating properly, Start TaskManager to end the"NeoLG.exe"process, then restart the software and the instrument.

10. **DO NOT loose the screws in the monochromator. Keep the environment clean.**
11. **Cut the power before opening the instrument. Pay attention to the high-voltage electrical components on the left rear of the instrument.**
12. **Cover the instrument with dust proof if the instrument is not used for a long time.**

Menu

PART I:

INSTRUMENT MANUAL

1. Apperance & Performance

1.1 Apperance

1.1.1 Body

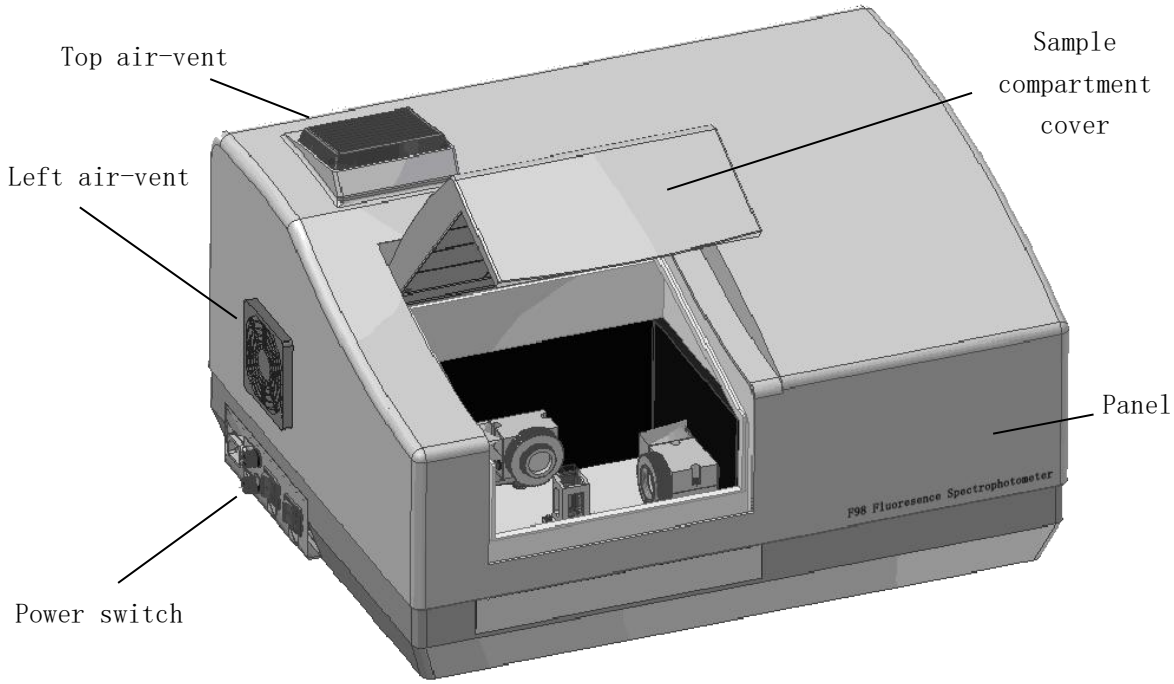


Fig. 1-1 Main body

Top air-vent: Air circulating for xenon lamp. Please don't block the ventilation opening.
Left Air-vent: Air circulating for xenon lamp. Please don't block the ventilation opening.
Power switch: Please turn to chap. 1.1.3 for more information.
Sample Compartment Cover: The cover can be opened up to less than 90 degrees.
Pilot Light: Power light.
Panel: Shows the instrument type

1.1.2Interface

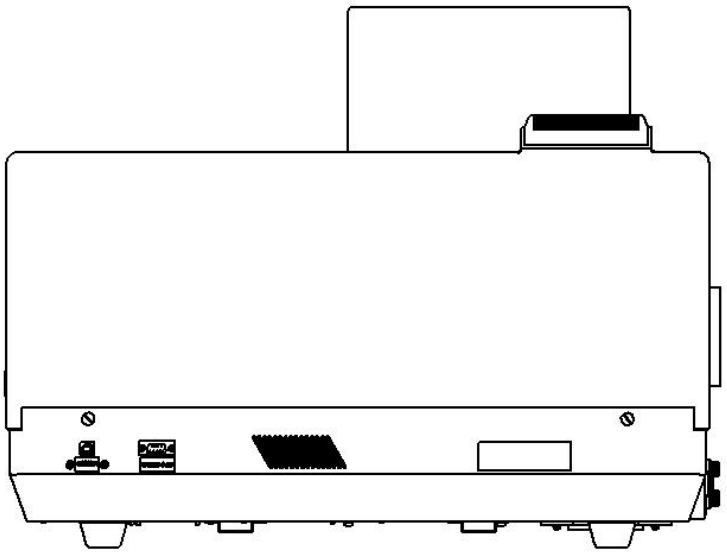


Fig.1-2 Back View

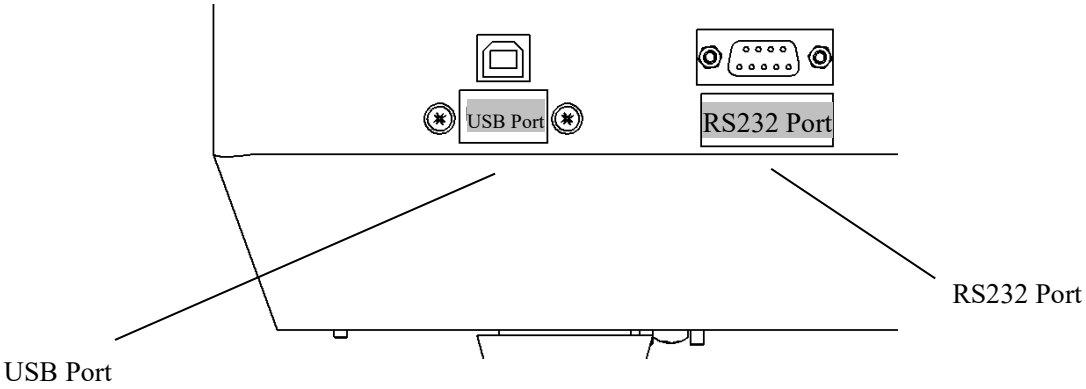


Fig.1-3 Interface

USB Port：Link to PC with a USB cable
RS232Serial Port：For debug only

1.1.3 Power Switch

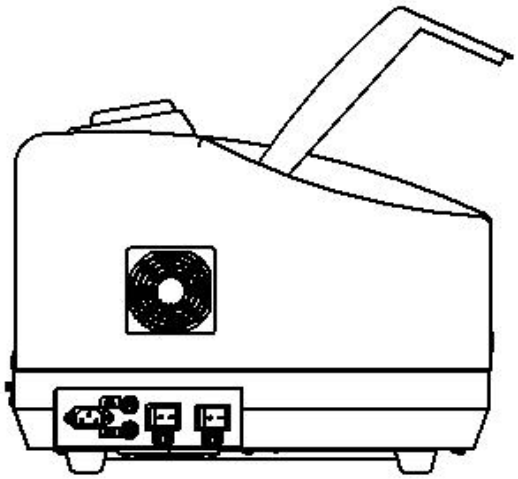


Fig.1-4 Side View

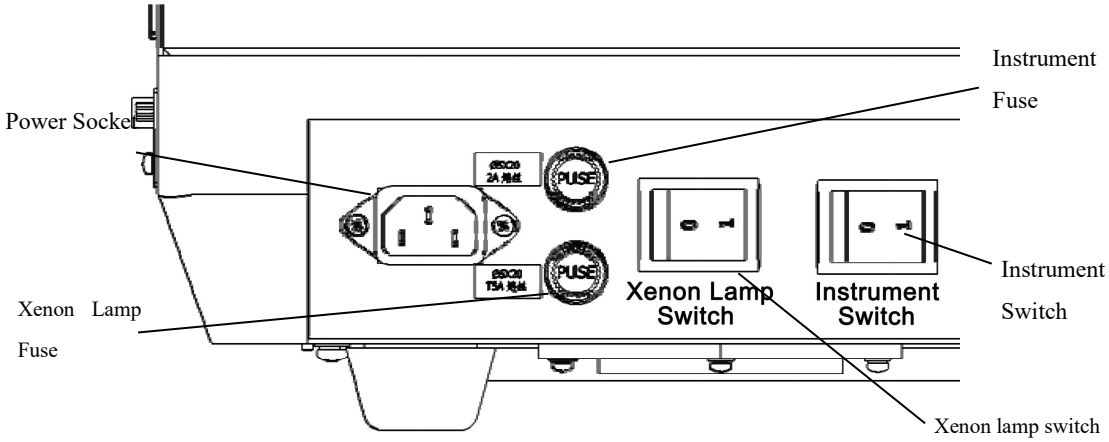


Fig.1-5 Power & Switches

Power Plug-in：For connecting power cable.
Xenon Light Fuse：For xenon light fuse.
Xenon Light Switch：Turn on/off the xenon light.
Instrument Fuse：For main power fuse.
Instrument Switch：Turn on/off the instrument.

1.1.4 Sample Compartment

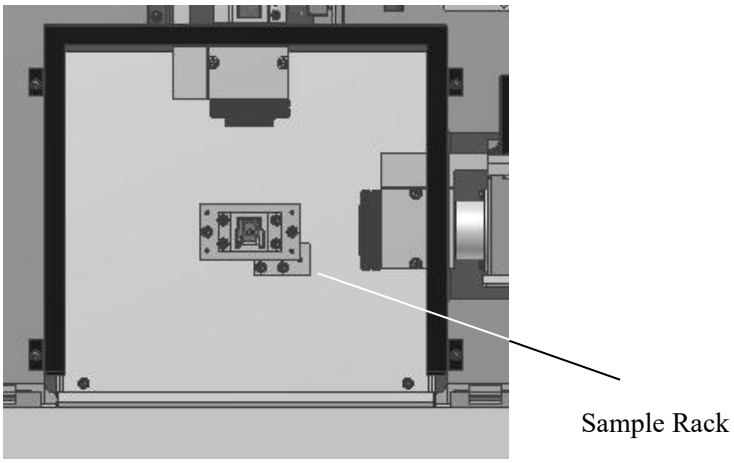


Fig.1-6 Sample Compartment

Sample Compartment：Sample hold inside.
Sample Hold：For fixing sample pool.

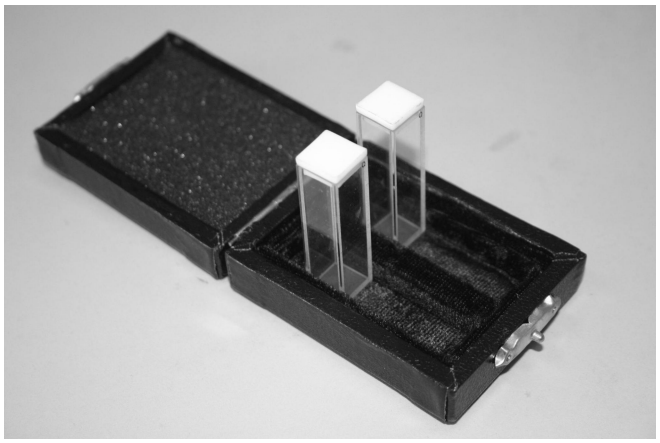


Fig.1-7 Quartz Fluorescence Sample Pool（10mm）

1.2 Principle

1.2.1 Signal processing and control systems

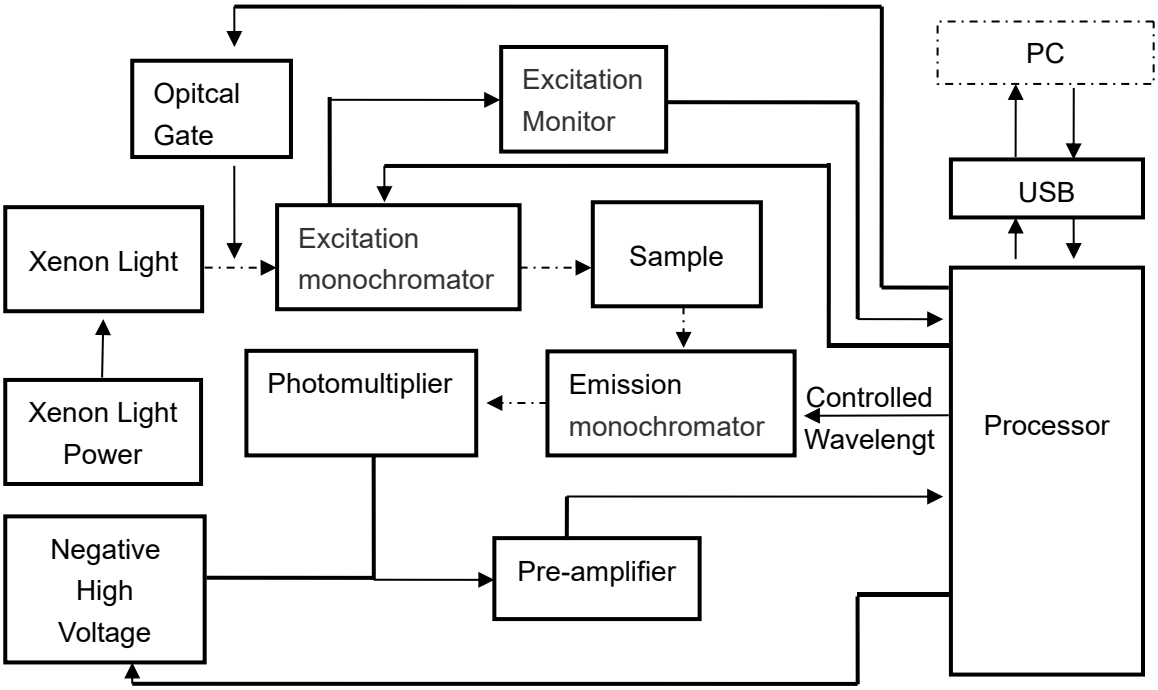


Fig.1-8 Signal Processing & Control System

1.2.2 Light Path

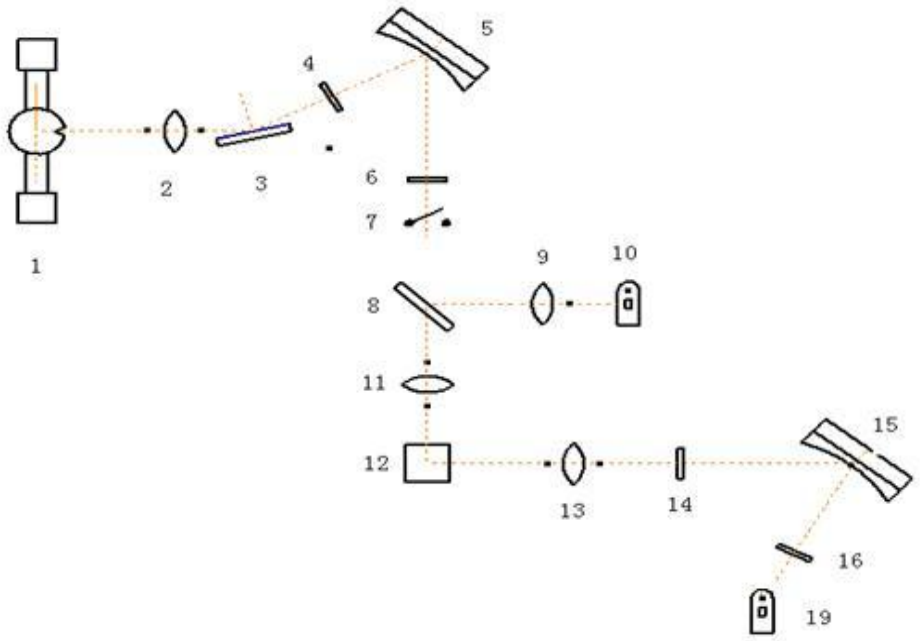


Fig.1-9 Light Path

1. Xenon lamp
2/9/11/13. Convex lens
3. Reflector
4/6/14/16. Slit
5/15. Grating
7. Optical Gate
8. Splitter
10. Photocell
12 Sample Cell
19. Photomultiplier

1.3 Functions

1.3.1 Modes For Measurement

1. **Wavelength scan**. Excitation wavelength scan provides spectrums of fluorescence intensity which changes along with excitation wavelength under fixed emission wavelength. Emission wavelength scan provides spectrums of fluorescence intensity which changes along with emission wavelength under fixed excitation wavelength.
2. **Time scan**. Time scan provides spectrums which changes along with time under fixed emission wavelength and excitation wavelength.
3. **Quantitative Analysis**. According to the fluorescent spectraphotometry, the fluorescence intensity(F) is proportional to the concentration ofthe test sample(C) under given conditions (the test group is dilute solution). Use fluorescent power F and known sample concentration C to get standard curve. Then measure the fluorescence of unknown sample to get the sample concentration.
4. **3D Scan**. 3D scan includes excitation wavelength, emission wavelength and fluorescence information.
5. **Synchronous scan**. The excitation side and the emission side scan at the same time.

1.3.2 Self Tests & Adjustments

1. **Self Tests & Adjustments**

   The instrument will initialize and self test while booting, including connection detection, database detection, AD module detection, signal gain detection, motion parts detection etc. Test result will be displayed on the screen and easy for users to find problems.

1.4 Performance

Tab.1-1 Performance of F98

Item	Content
Light Source	150W xenon lamp（Hamamatsu）
Excitation Wavelength	200nm~900nm
Emission Wavelength	200nm~900nm
Excitation Slit	1nm,2nm,5nm,10nm,20nm
Emission Slit	1nm,2nm,5nm,10nm,20nm
Wavelength Accuracy	±0.4nm
Wavelength Repeatability	≤0.2nm
Signal-to-Noise Ratio	Raman peak of water（P-P）：S/N＞350
Limit	≤5×10^{-11}g/ml（Sulfuric acid quinine solution）
Linearity	γ≥0.995
PeakRepeatability	≤1.5%
Stability(10min)	Zero Drift：±0.3
	Value Limit：±1.5%
Wavelength Scan Speed	Multi-speed Level, Maximum at 60000nm/min
Photometric Range	0.00-10000.00
Data Transportation	USB2.0
Power	200W
Power Source	AC 220V/50Hz； 110V/60Hz
Demension	600×460×380（mm）
Weight	Net Weight: 21kg Gross Weight：40kg

2. Booting & Shutting Down

2.1 Booting Status

2.1.1 Booting

Put the instrument on steady platform. Make sure the main power and light power switch are off (switch to 0). Plug in the power line.

There is a certain probability that the high voltage trigger of the Xenon light power will affect other electrical equipment around. We highly recommend you check the following status before turning on the Xenon light power:

1） Unplug the USB cable connecting PC and the instrument.

2） Make sure the grounding of power source is reliable.

3） If other instruments in the same platform were affected by the high trigger voltage before, please shut down those instruments and turn on after the xenon light is triggered.

4） Turn on the xenon light power, then turn on main power after the xenon light is triggered.

Connect PC and the instrument with the USB cable, then run the PC software.

ATTENTION：

①If the xenon light is not triggered properly and making high noise, shut down the xenon light power immediately. Please turn on the xenon light after a few seconds.(It happens only when the power is not stable or the xenon light is reaching its limit)

②As xenon lamp life and the switching times are closely related, Please reduce unnecessary trigger.

③Xenon light needs 30 minutes to stable.

④Don’t turn on Xenon light without turning on the main power after.

2.1.2 Fans Condition

Please make sure the fans on the left side and on the top left are functioning every time startup. If the fans are not working, shut it down and check.

2.1.3 Multi-instruments Booting

When using multiple instruments, please turn on all the xenon lights first, then turn on all the main power to reduce the influence of the high trigger voltage.

2.1.4 Initialization

Initialization status will be displayed on computer software as Fig.1-10.

2.2 Power Off

When connecting to PC, close the software first; then turn off the main power; at last turn off the xenon light power.

ATTENTION: To restart the Xenon light power, please wait for 60 seconds after power off.

3. Installation

3.1 Environment

The instrument is suitable for analysis in laboratory environment.For its work with computers, so need to meet the following workingcondition.

3.1.1 Laboratory Environment

Temperature 10～30 ℃, humidity under 85%. Avoid corrosive gas and the organic and inorganic gases which are absorptive within the range of ultraviolet.

3.1.2 Workbench

The workbench should be smooth and solid. Avoid vibration, dust, direct sunlight.

3.1.3 Power

AC 220V±22V，50Hz±1Hz or 110V±11V，60Hz±1Hz.

3.1.4 Environment Changes

If the instrument is needed in the field, please make sure the environment meets the requirements above. Please use the original package moving instrument. If there are special requirements please inform us when ordering.

3.2 Package

The instrument adopts carton packaging. Long-distance transport may require additional outside wooden box.

3.2.1 Check the Package

Before unpacking, make sure the packaging is intact. If the package is damaged, please contact with the transportationinsurance.

3.2.2 Unpack

Open the case and carefully take out the instrument (Please keep the package for

transportation). Make sure the instrument and all accessories are correct according to the package list. Please contact us if there is any mistake.

Table.3-1 Fluorescence spectrophotometer package list

Standard package	Instrument	1 pc
	Fluorescence spectrophotometer software	1 set
	Power Cable	1 pc
	USB Cable	1 pc
	Quartz fluorescence cell 10mm	1 pair
	Fuse（2A/5A）	2 pairs
	Instruction Manual	1 copy
	Applications Manual	1 copy
	Certification of products	1 copy
	Packing list	1 copy
	Warranty	1 copy
Optional spare parts	Quartz fluorescence cell 10mm	
	Glass fluorescence cell 10mm	
	Fuse（2A/5A）	
	USB cable	
	Power cable	
Optional accessories		
	Membrane kind sample accessories	
	Powder kind sample accessories	
	Microscale capillary sample accessories	
	Jacket sample pool accessories	
	200μL centrifuge tube accessories	

3.3 Installation

3.3.1 Cleaning

Remove the tape and clean the surface.

3.3.2 Check the Power Source

Make sure the instrument power supply voltage and area voltage are correct.

3.3.3 Plug In

Put the instrument on a stable work table about 10 cm away from wall. Plug in the power cable to the lab power.

3.4 Tests

3.4.1 Signal to Noise Ratio Test

1. Turn on the instrument and preheat.

Light source and electronic components require to reach heat balance after startup.Start operation after 30 minutes preheating.

2. Put in sample

Choose a clean quartz fluorescence cell. Fill the quartz fluorescence cell with sample (double distilled water). Then put it in the sample cell.

ATTENTION: Dirty quartz fluorescence cell will affect the accuracy of the test.

3. Run emission wavelength scan

Choose “Wavelength Scan” –“Emission wavelength scan”. Set the excitation wavelength at 350nm, the emission wavelength at 300nm to 500nm. Set slit to 10nm. Set scan speed at 60nm/min. Response: auto. Gain: high. Run wavelength scan, then check the peak around emission wavelength 397nm. This peak is signal S.

4. Run time scan.

Choose “Time scan”. Set the excitation wavelength at 350nm. Set the emission wavelength at 397nm. Scan time 120 seconds. Slit 10nm. Response time 2 seconds. When the signal is stable(Let doubly distilled water expose more than three minutes above conditions), run time scan.

5. Calculate

Note the peak signal S and the Peak-valley value N. S/N is the Raman signal to noise ratio.

Attention：

① The energy of Raman is weak, so the measurement is easy to be interfered. If the result looks bad, please test again.

②In order to protect the PMT tube, when the gain is high(above 6), please don’t put high energy light source into the sample cell.

③Setting the gain will affect the fluorescent zero. Please reset zero after changing gain.

3.4.2 Wavelength Test

- The instrument will automatically adjust wavelength. If there is any wavelength mistake, trained professionals are allowed to test wavelength with mercury-arc lamp or fluorescent lamp. Learn the test method through training.

  Mercury-arc lamp spectral figure and wavelength are as below.

Table.3-2Emission spectra of low pressure mercury lamp in UV and visible region

NO.	Wavelength/nm	NO.	Wavelength/nm
1	253.65	7	404.66
2	296.73	8	407.78
3	302.15	9	435.84
4	313.16	10	546.07
5	334.15	11	576.96
6	365.01	12	579.07

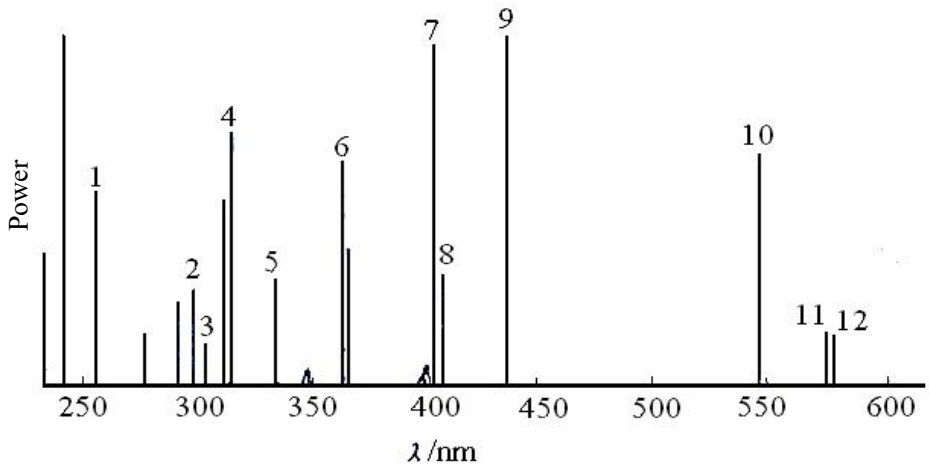


Fig.3-1Spectra of mercury-arc lamp

4. Maintenance

4.1 Routine maintenance

1. Always check whether it meets the requirements of the work environment in daily use.
2. Keep the vents functioning while the instrument is on.

ATTENTION: High temperature vents. Keep distance.

3. Keep the instrument clean. Add a dust cover when not in use.Use water to clean the instrument appearance.DO NOT use alcohol, ether, acetone and other organic solvents. Do not clean when the instrument is working.
4. Keep the quartz fluorescence cell clean.

4.2 Light Source Maintenance & Replacement

4.2.1 Maintenance

a) Keep the light source clean.
b) Strictly in accordance with the order of operations when turn on/off xenon light.

ATTENTION: When power on, turn on the xenon light power first, then the main power. When power off, turn off the main power first, then the xenon light power.

c) Xenon light will be hard to trigger when the power is not stable or the xenon light is reaching its limit.

ATTENTION:If the xenon light is not triggered properly and making high noise, shut down the xenon light power immediately. Please turn on the xenon light after a few seconds.

d) Avoid repeated triggering xenon lamp.

As xenon lamp life and the switching times are closely related, Please reduce unnecessary trigger.

e) Make sure that the instrument cooling fan is working properly. Make sure the surface of the instrument top vents maintain good ventilation.

4.2.2 Light Source Replacement

Professionals are allowed to replace the light source.Specific methods and calibration procedures will be introduced through training.

Part II：Software Manual

5. Software Installation

Before reading this section, please read Part I carefully. Windows XP system is recommended.

In order to run the software properly in Windows 7, please run the software in administrator account.

5.1 Requirements

5.1.1 Hardware Requirements

Hardware	Minimum requirements
CPU	Intel P4 2.0GHz or same level CPU
Memories	512M
Hard disk	No less than 200M disk space
USB	USB2.0
CD drive	CD-ROM
Monitor	resolution 1024*768 16-bit color

Table.5-1 Hardware requirements

5.1.2 System Requirements

Windows XP or higher version is recommended. Please turn off the screen saver and power management program while F98 Fluorescence Spectrophotometer data processing software is running.

5.2 Install F98 Software

Insert the USB flash disk. Run “Setup.exe” to start installation.

6. How to Use the Software

6.1 Before Use

6.1.1 Connect to PC

The instrument connects to the computer via USB cable, the computer will automatically install the driver the first time connected, and please run software after the driver is successfully installed.

6.1.2 Link Procedure

1. Turn on the instrument

First turn on the xenon lamp, then turn on main power when the xenon light is lit. The instrument will enter online mode.

2. Link via USB cable

Connect the instrument to PC with USB cable.

3. Initialization

Run F98 software. The software will start initializing and self tests.

4. Work modes

Fig.6-1 Tool bar

Click the first button on the tool bar in fig.6-1 to create a new measurement. Choose a work mode in “Wavelength Scan”, “Time Scan”, “Quantitative Analysis”,“3D Scan” and “Synchronous Scan”.

5. Power Off

Close the software, and then shut down main power and xenon light power.

If you shut down main power first, there will be a communicate error on the software. Use task manager to close the NeoLG.exe.

6.2 Functions

6.2.1 Measurement Modes

There are 5 measurement modes:

a) Wavelength Scan:

（1） Shows the excitation spectra of the sample.
（2） Shows the emission spectra of the sample.
（3） Phosphorescence wavelength scan is available.
（4） Supports automatic repeat scan.
（5） Supports data printout.

b) Time Scan:

（1） Shows the sample fluorescence spectra change with time.
（2） Phosphor Kinetics scan is available.
（3） Supports automatic repeat scan.
（4） Supportsspectrum data processing.
（5） Supports data printout.

c) Quantitative:

（1） Supports single-wavelength, dual wavelength and three-wavelength quantitative analysis.
（2） Supports 1 to 3 times curve fitting.
（3） Data decimal can be changed.
（4） Programmable optical gate control.
（5） Supports data printout.

d) 3D Scan:

（1） Shows 3D spectrum of excitation wavelength, emission wavelengthand fluorescence data.
（2） Supports 3D view of the spectrum.
（3） Supports cross-sectional view of the spectrum.
（4） Supports 3D contour map of the spectrum.
（5） Supports data printout.

e) Synchronous Scan:

（1） Shows synchronous fluorescence spectroscopy spectrum.
（2） Supports automatic repeat scan.
（3） Supports data printout.

6. 2. 2 **Interface**

6.2.2.1 Modules

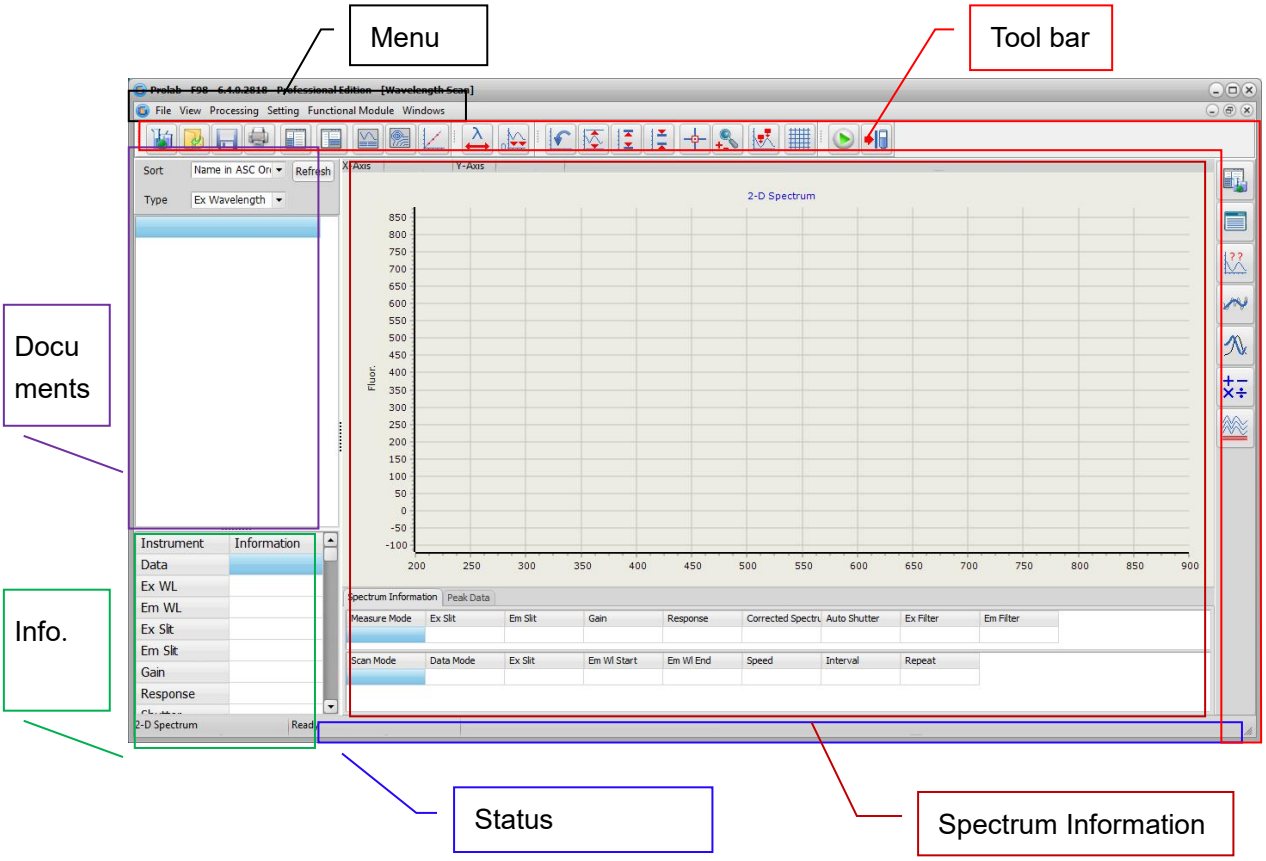


Fig.6-2 Interface modules

6.2.2.2 Modules & Functions

a) Menu & Tools

Fig.6-3 Menu& Upper Tool bar

Fig.6-4 Right Tool bar

1) Provides instrument controls and settings.
2) Tool bar are shortcuts for common features.

b) Document Browser

Document Brower shows files saved in Wavelength Scan, Time Scan and Quantitative Analysis mode. Double click to open a file.

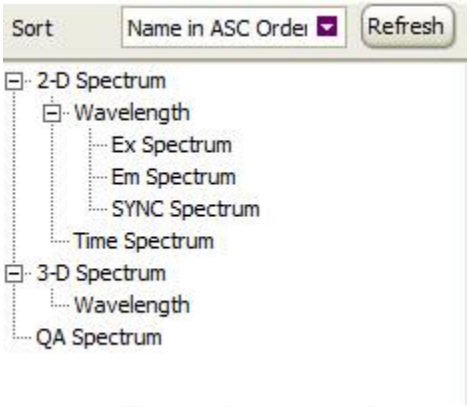


Fig.6-5 Document Browser

1) To reset the file saving path, click menu “Settings”->“Instrument Settings”. Set a new saving path in the pop out window as Fig.6-6.

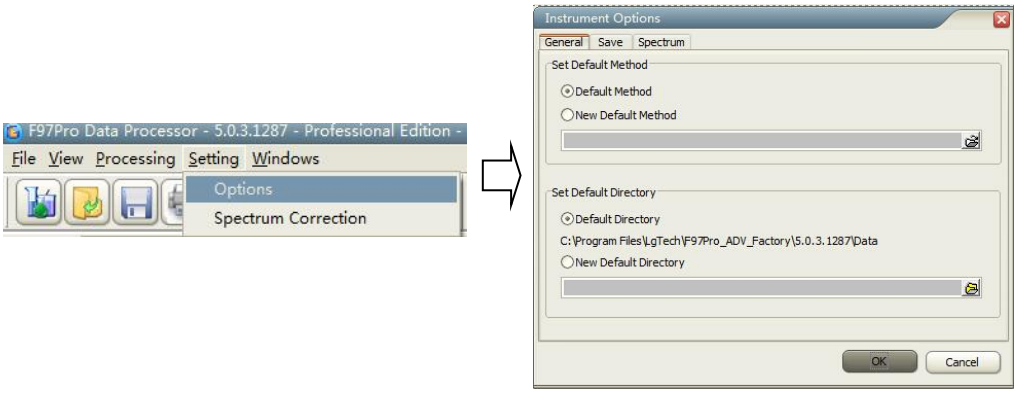


Fig.6-6 Set file path

2) Double click a spectrum file will show the spectrum and refresh.
3) Right click a file to open, rename or delete.

4) Way of sorted files can be changed.

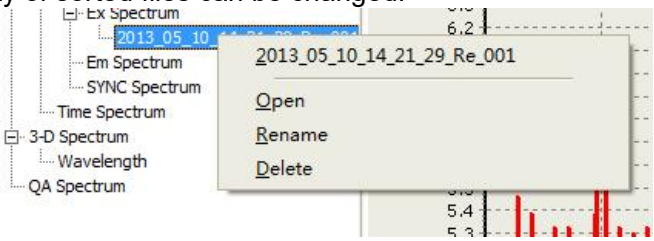


Fig.6-7 File menu

c) Information Window

Information window shows current data and info of the instrument.Including current fluorescence value, excitation wavelength, emission wavelength, excitation and emission slit, gain, the response time and optical gate condition. Double click to modify instrument parameters.

Instrument	Information
Fluor.	
Ex WL	
Em WL	
Ex Slit	
Em Slit	
Gain	
Response	
Shutter	

Fig.6-8 Information Window

Any changes made here will not affect the parameters in Method Settings. Information window is usually for status check.

d) Spectrum Window

1) Shows spectrum information.

2) Use mouse to zoom in and out. Press the left mouse button, drag the mouse from top left to bottom right to draw a square, then release the button. Spectrum in that square will be zoomed in. Drag the mouse the opposite way to zoom out.

3) Click “Peaks” to show the peaks in the spectrum.

e) Status Window

Status window shows the current status of the instrument.

6.2.2.3 Tool Bar

Icon	Function
	New Measurement
	Open Spectrum
	Save Spectrum
	Print Spectrum
	Show/Hide Status
	Show/Hide Spectrum Information
	Activate 2D Window
	Activate 3D Window
	Activate Quantitative Analysis Window
	Back to original coordinate
	Auto coordinate
	Y-axis enlarge 2 times
	Y-axis reduce 2 times
	Get/Cancel Axis Data
	Zoom In/Out
	Show/Hide Peaks
	Show/Hide Grid

	Start/Stop
	Open/Close Optical Gate
	Set Wavelength
	Run/Cancel Zero Adj.
	Spectrum Properties
	Print Data
	Peak Threshold Setting
	Spectrum Smoothing
	Spectrum derivation
	Spectrums Calculation
	Spectrums Comparison

Table.6-1 Tool Bar Icons

6. 3 **Software Operation**

6. 3. 1 **Wavelength Scan**

Wavelength Scan Flow chart

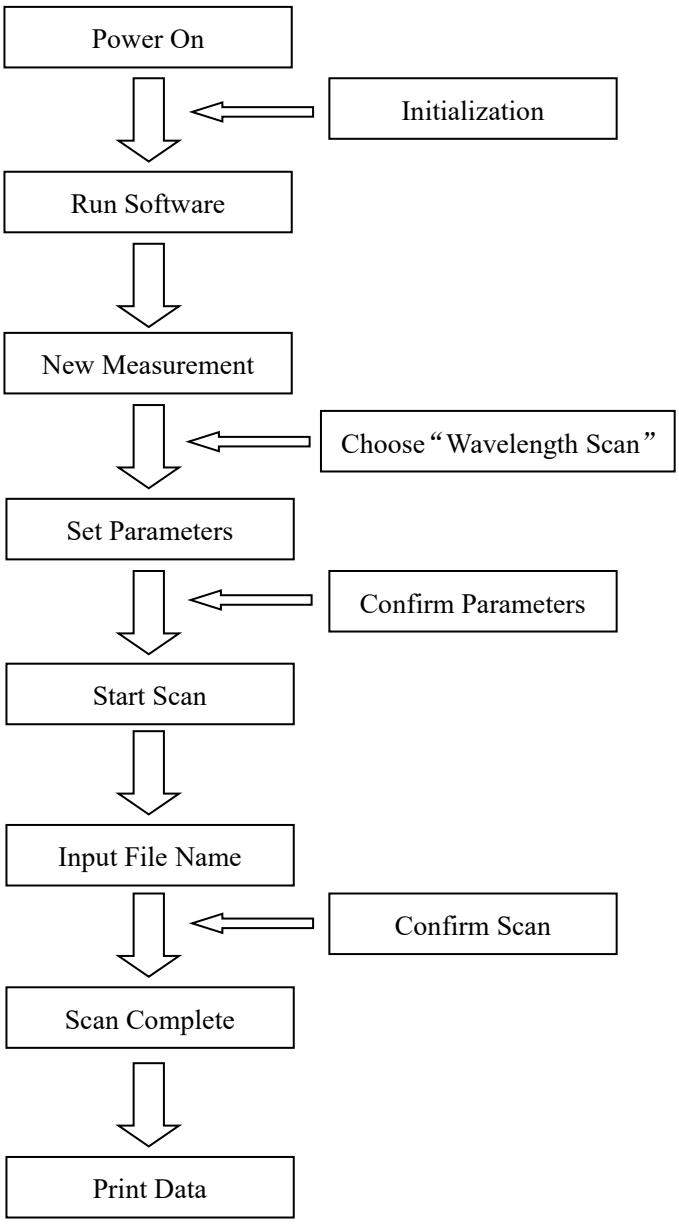


Fig.6-2 Wavelength Scan Flow chart

6.3.1.1 New Measurement

Create a new measurement.

Select “Files”->“Create Method” or click  to enter Create Method Window.

1. Measurement Summary:

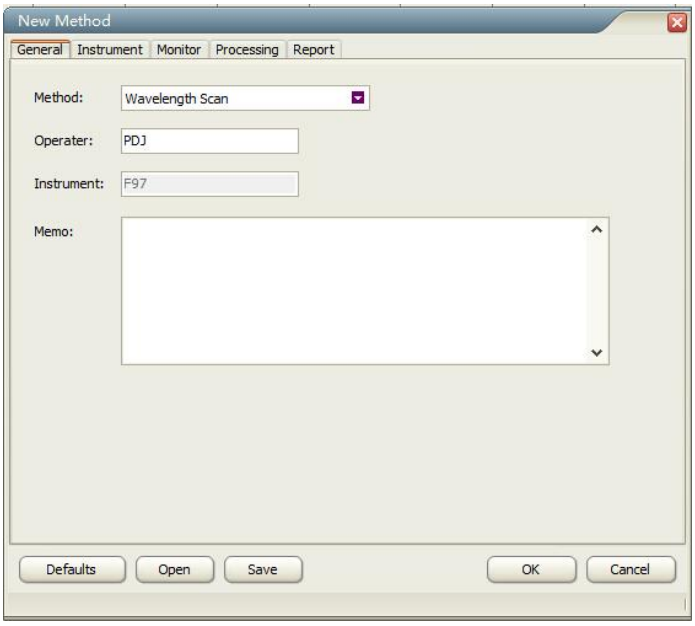


Fig.6-3 Measurement Summary

1) Measure Mode: Choose “Wavelength Scan”.
2) Operator: Input operator’s name.
3) Instrument: The model of the connected instrument is indicated.
4) Comment: Enter a description or notes on measuring conditions.

2. Instrument Tab:

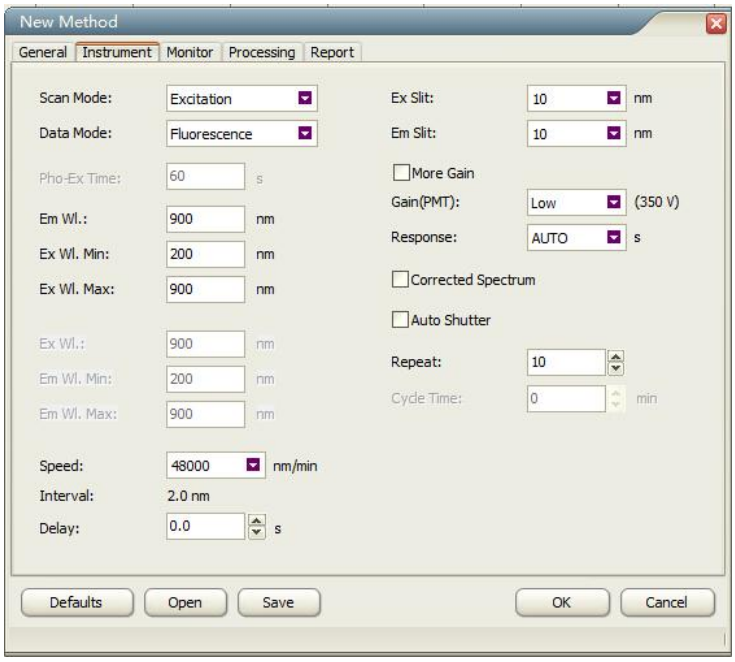


Fig.6-4 Scan Settings

（1） Scan Mode: Excitation & Emission.

1) In excitation mode, X axis is excitation wavelength, Y axis is fluorescence value. The instrument will do an excitation wavelength scan under a fixed emission wavelength.

2) In emission mode, X axis is emission wavelength, Y axis is fluorescence value. The instrument will do an emission wavelength scan under a fixed excitation wavelength.

（2） Data Mode: Fluorescence and phosphorescence mode available.*

1) The instrument will do Fluorescence scan in Fluorescence mode.

2) The instrument will do phosphorescence scan in phosphorescence mode.A phosphorescence excitation time input window will be activate before the scan.*

（3） Phosphorescence excitation time: Set the excitation time in phosphorescence mode.*

（4） Emission wavelength, excitation start wavelength, excitation end wavelength are available in excitation mode.

Emission Wavelength: Input emission wavelength(200nm-900nm).

Excitation Start Wavelength:Input excitation start wavelength (200nm-900nm).

Excitation End Wavelength:Input excitation end wavelength (200nm-900nm).

（5） Excitation wavelength, emission start wavelength, emission end wavelength are available in excitation mode.

Excitation Wavelength: Input excitation wavelength(200nm-900nm).

Emission Start Wavelength:Input emission start wavelength (200nm-900nm).

Emission End Wavelength:Input emission end wavelength (200nm-900nm).

（6） Scan Speed: Choose scan speed. The faster the noise get higher.

（7） Scan Interval: Shows data sampling interval according to the scan speed.

（8） Delay: After pressing the Measure button, measurement isstarted following the delay time set here. It is used fortemperature stabilization,

etc.In repeat measurement,it is the time until the start of the first measurement.

（9） Excitation Slit: Set excitation slit.

（10） Emission Slit: Set emissionslit.

（11） Gain(PMT): Set gain level by changing the PMT negative high voltage.

（12） More Gain: Enlarge the gain range.

There will be a negative high voltage value besides each gain.This value is for reference only.There will be some deviation from the actual value.

（13） Response： Set the signal's response time.Usually automatically set.

（14） Spectral Correction： The instrument will use the last correction result to adjust the wavelength parameter to correct sample spectrum when the Spectral Correction is selected.(Please turn to Appendix II for more detail)

（15） Shutter: To control the excitation time or condition of the sample.

1) When Shutter is selected, the instrument will open optical gate only when it's scanning to excite sample.When the scan stops, the optical gate will automatically close.This function is for samples which are not stable when excite by light.

2) When Shutter is NOT selected,the optical gate will be open.Sample will always be excite.

3) Shutter will be on in phosphorescence mode.*

（16） Replicates: Set the number of repeat measurements.The instrument will only scan once when it's 1.

（17） Cycle time:Set a repetition interval.

3. Monitor：

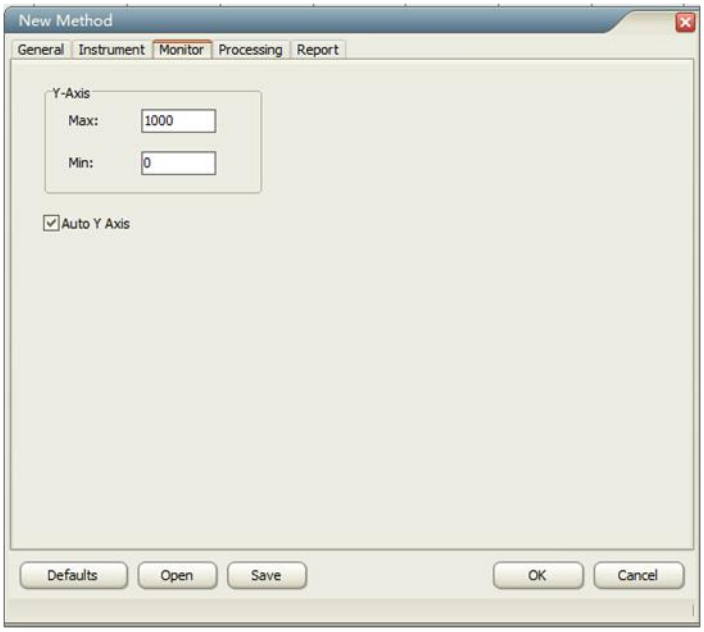


Fig.6-12 Monitor Tab

1) Y Axis: Enter the max and min point of Y axis.The max point should be larger.

2) Auto Adjust Y Axis: Y axis will automatically set by spectrum data.

4. Processing：

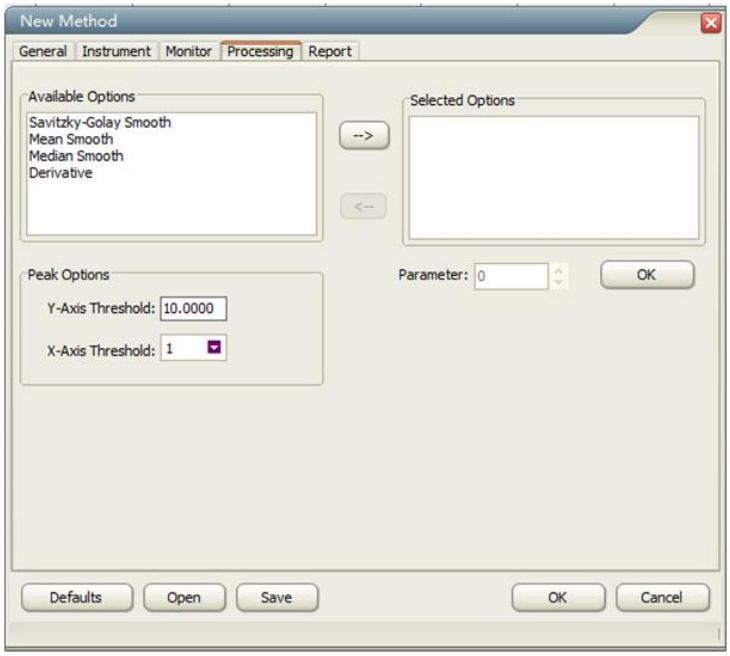


Fig.6-13 Spectrum Processing

1） 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.

2） 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.

3） Parameters: Click the “+” in Methods Chosen box to modify the parameters. Click “OK” to confirm.

4） Peak Finding: Automatically find peaks by giving threshold when the scan is complete.

5. Report：

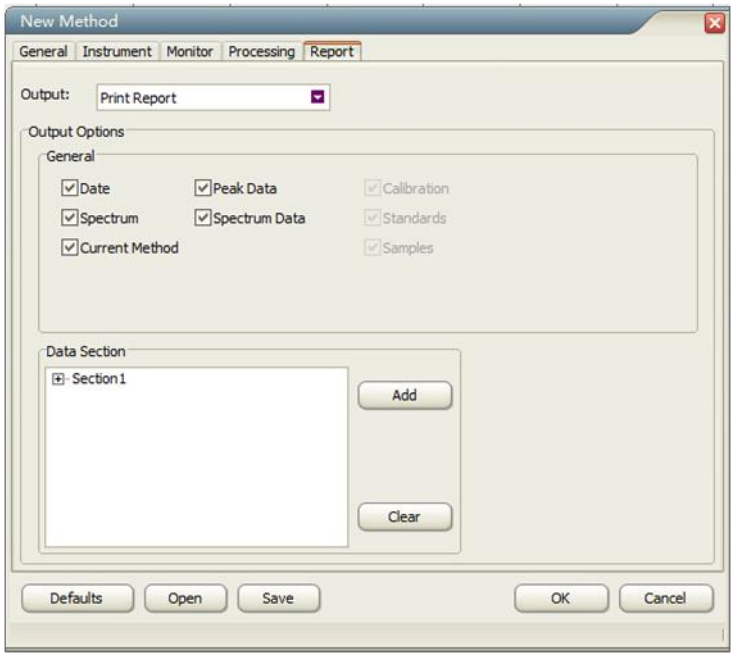


Fig.6-14 Data Printout

1、 Output: Print Report or Save as Microsoft（R）Excel file.

2、 Output options: Choose the printout data. Check the content in “Properties” button on the left after the scan.

3、 Add Data: When “Spectrum Data” is checked, you can choose data section to printout. Set the start wavelength, end wavelength and interval in the pop out window, then click OK. Click the “+”to see the data section.

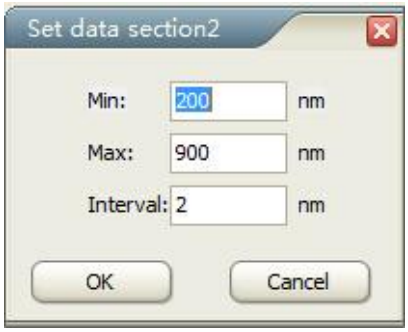


Fig.6-15 Set data section

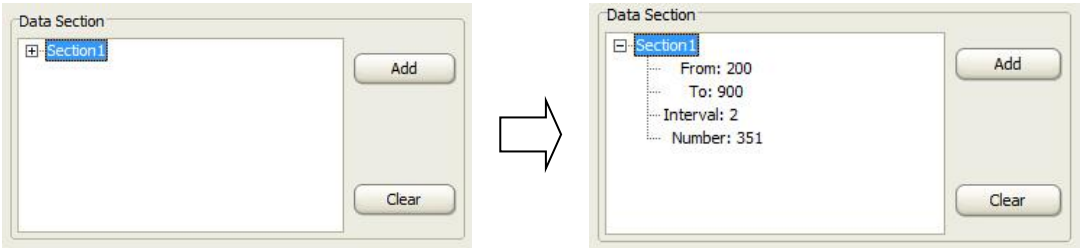


Fig.6-16 Data section

4、 Clear Data: Clear current data section.

5、 Click “Defaults” to reset the settings to default.

6、 Click “Open” to open a saved method. It’s a *.FMTD file.

7、 Click “Save” to save current settings.

6.3.1.2 Run Wavelength Scan

Wavelength scan procedure: Standby->Ready->Start Wavelength Scan ->Move to Excitation(Emission) Wavelength -> Standby.

1. **File Name**

Click  button to start a measurement.Input a file name or use system time as file name.

2. **Stop Scan**

Click  to stop the scan.

6.3.1.3 Spectrum

The instrument will do wavelength scan with all the parameters. Spectrum will be displayed in the spectrum window. The spectrum file will be automatically saved in the file browser window.

Click  to see details of the spectrum.

Icons	Function
	Reset Original Coordinate.
	Auto Adjust Coordinate.
	Enlarge Y Axis 2 Times
	Reduce Y Axis 2 Times
	Get/Cancel Axis Data
	Zoom In / Out
	Show/Hide Peaks

	Show/Hide Grid
	Peak Finding Details
	Start/Stop
	Open/Close Optical Gate
	Set Wavelength
	Run/Cancel Zero Adj.
	Spectrum Smoothing
	Spectrum derivation
	Spectrums Calculation
	Spectrums Comparison

Table.6-2 Functions for Spectrums

6.3.1.4 Printout

Icon	Functions
	Spectrum Properties
	Print Data

Table.6-3 Functions for Printout

6.3.2 Time Scan

Time Scan Flow Chart

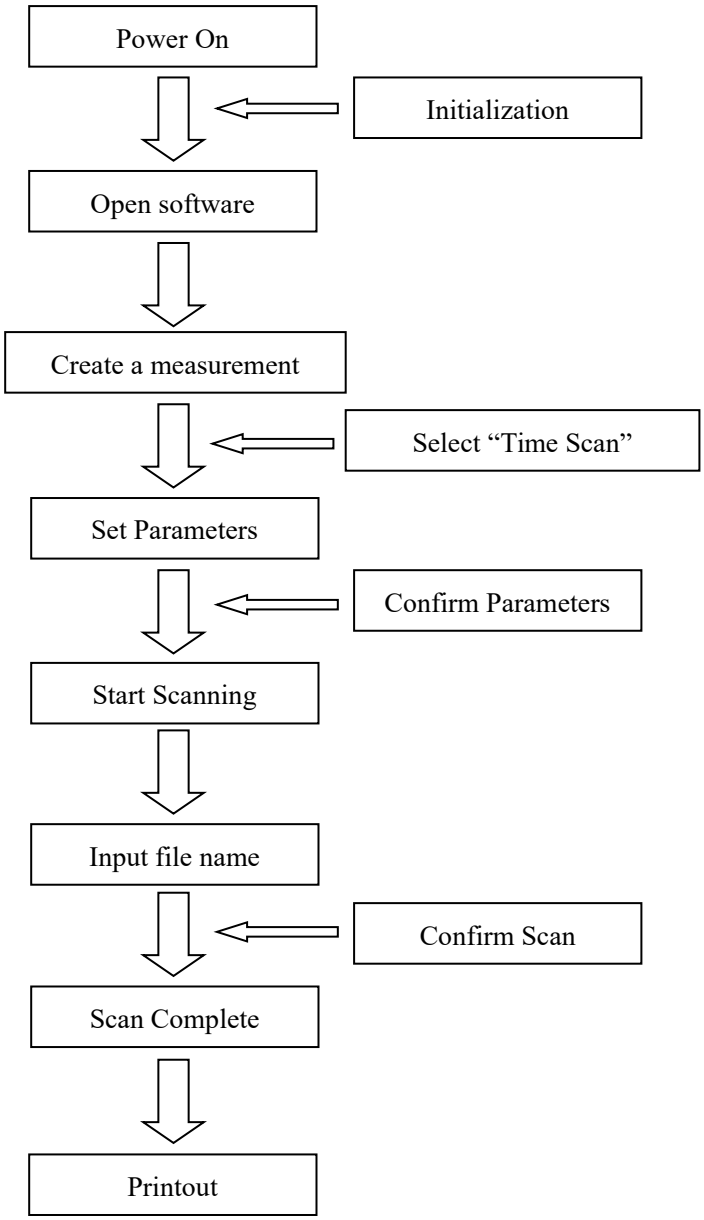


Fig.6-18 Time Scan Flow Chart

6.3.2.1 Create a Measurement

Click “Files”->“Create Measurement” or click  to create a new measurement.

1. General：

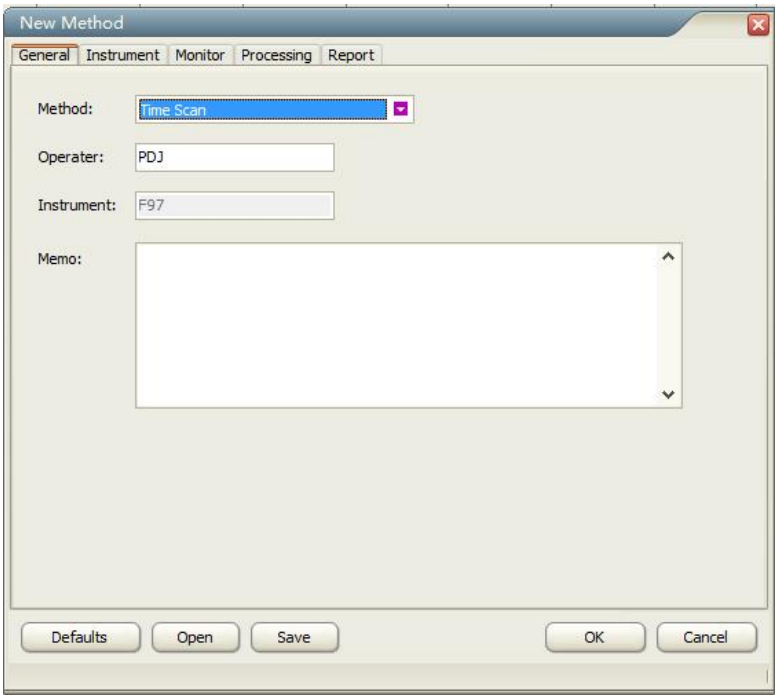


Fig.6-19 General

1) Measurement: Choose “Time Scan”.
2) Operator: Input operator’s name.
3) Instrument: The model of the connected instrument is indicated.
4) Comment: Enter a description or notes on measuring conditions.

2. Instrument:

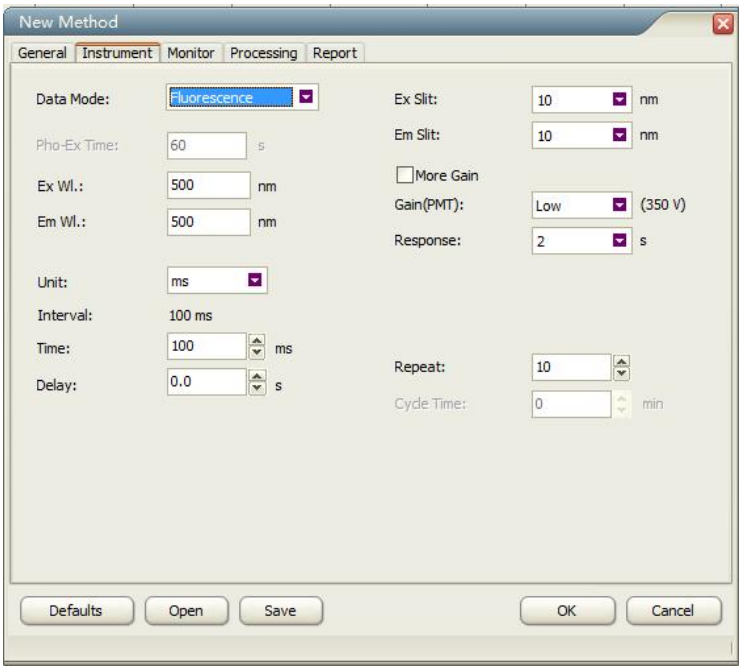


Fig.6-20 Scan Settings

1) Scan Mode: Fluorescence and phosphorescence mode available*
   A. The instrument will do Fluorescence scan in Fluorescence mode.
   B. The instrument will do phosphorescence scan in phosphorescence mode.A phosphorescence excitation time input window will be activate before the scan.*
2) Phosphorescence excitation time: Set the excitation time in phosphorescence mode.*
3) Emission Wavelength: Input emission wavelength (200nm-900nm).
4) Excitation Wavelength: Input excitation wavelength (200nm-900nm).
5) Scan Interval: This is a fixed value as 0.1s(100ms).
6) Scan time: Set thescan time.
7) Delay: After pressing the Measure button, measurement isstarted following the delay time set here. It is used fortemperature stabilization, etc. In repeat measurement,it is the time until the start of the first measurement.
8) Excitation Slit: Set excitation slit.
9) Emission Slit: Set emissionslit.
10) Gain(PMT): Set gain level by changing the PMT negative high voltage.
11) More Gain: Enlarge the gain range. There will be a negative high voltage value besides each gain.This value is for reference only.There will be some deviation from the actual value.
12) Response: Set the signal's response time from "0.1","0.5","1","2","4".The shorter time,the more noise.
13) Replicates: Set the number of repeat measurements.The instrument will only scan once when it's 1.
14) Cycle time:It's available when Replicates is more than 1.Set a repetition interval.

3. Monitor：

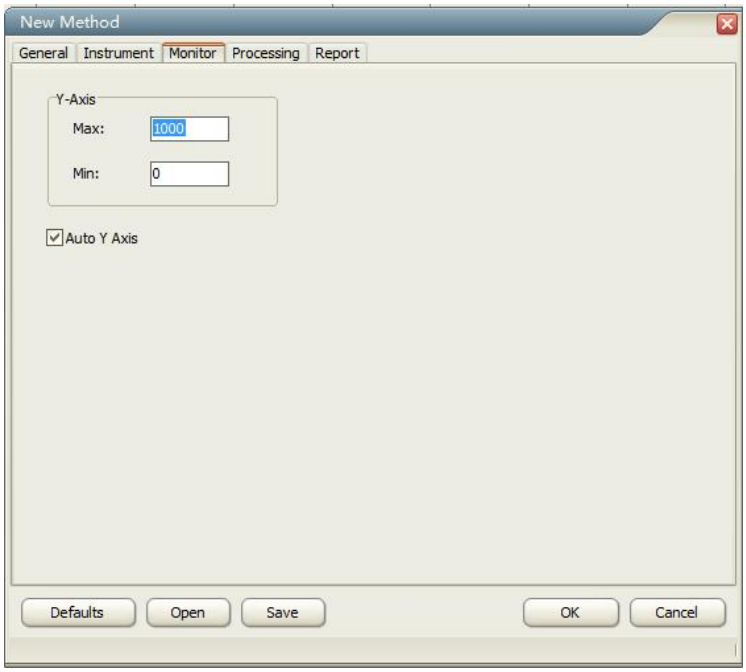


Fig.6-21 Data Display

1. Y Axis: Input the min point and max point of Y axis.
2. Auto Adjust Y Axis: Y axis will automatically set by spectrum data.

4. Processing：

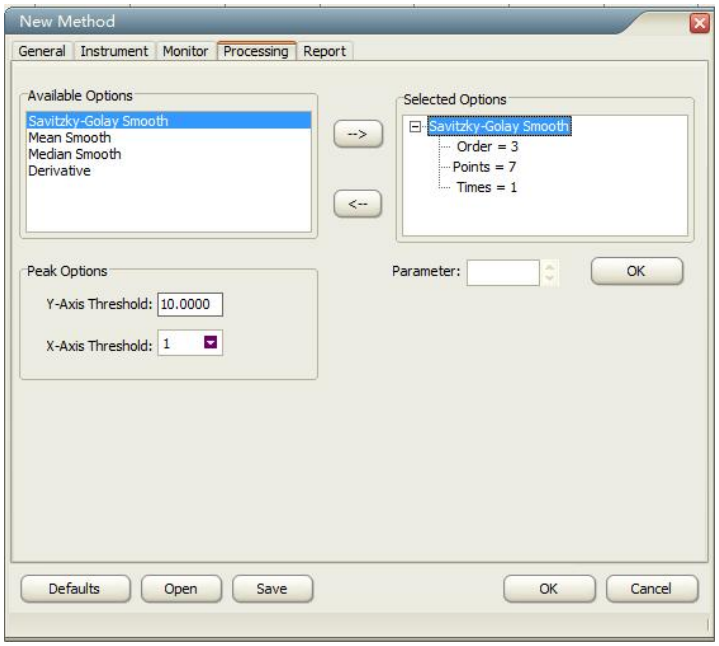


Fig.6-22 Spectrum Processing

1） Available Options: 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 available options and the selected options box. Then, the selected method appears in the available options box.

2） Selected Options: The processing sequence is displayed. To delete a processing method, first select the method, and then click the leftward-pointing arrow key between the available options and the selected options box. Then, the selected method disappears from the selected options box.

3） Parameters: Click the “+” in Methods Chosen box to modify the parameters. Click “OK” to confirm.

4） Peak Finding: Automatically find peaks by giving threshold when the scan is complete.

5. Report：

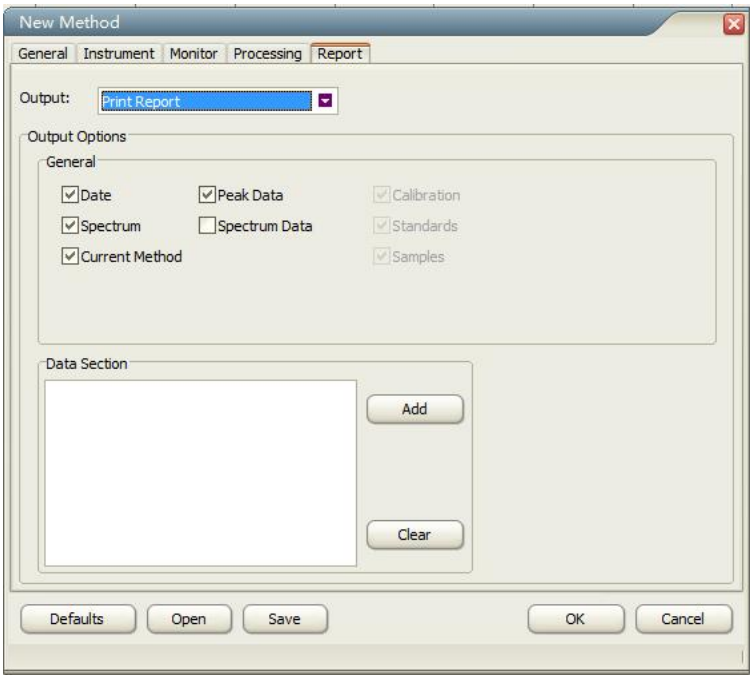


Fig.6-23 Data Printout

1、 Output: Print Data or Save as Microsoft（R）Excel file.

2、 Output options: Choose the printout data.Check the content in “Properties” button on the left after the scan.

3、 Add Data: When “Spectrum Data” is checked,you can choose data section to printout.Set the start wavelength, end wavelength and interval in the pop out window,then click OK.Click the “+”to see the data section.

4、 Clear Data: Clear current data section.

5、 Click “Defaults”to reset the settings to default.

6、 Click “Open”to open a saved method. It’s a *.FMTD file.

7、 Click “Save”to save current settings.

6.3.2.2 Run Time Scan

Time scan procedure: Standby->Ready->Start Time Scan -> Standby.

A. Save File:

Click  to start scan.Input file name in the pop out window or use system time as file name.

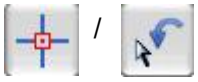
B. Stop Scan:

Click  to stop the scan.

6.3.2.3 Spectrum Processing

The instrument will do Time Scan with all the parameters. Spectrum will be displayed in the spectrum window. The spectrum file will be automatically saved in the file browser window.

Click  to see details of the spectrum.

Icon	Functions
	Reset Original Coordinate.
	Auto Adjust Coordinate.
	Enlarge Y Axis 2 Times
	Reduce Y Axis 2 Times
	Get/Cancel Axis Data
	Zoom In / Out
	Show/Hide Peaks
	Show/Hide Grid
	Peak Finding Details

	Start/Stop
	Open/Close Optical Gate
	Set Wavelength
	Run/Cancel Zero Adj.
	Spectrum Smoothing
	Spectrum derivation
	Spectrums Calculation
	Spectrums Comparison

Table.6-4 Functions for Spectrum

6.3.2.4 Printout Data

Icon	Functions
	Spectrum Properties
	Print Data

Table.6-5 Functions for Printout

6.3.3 Quantitative Analysis

Quantitative Analysis flow chart

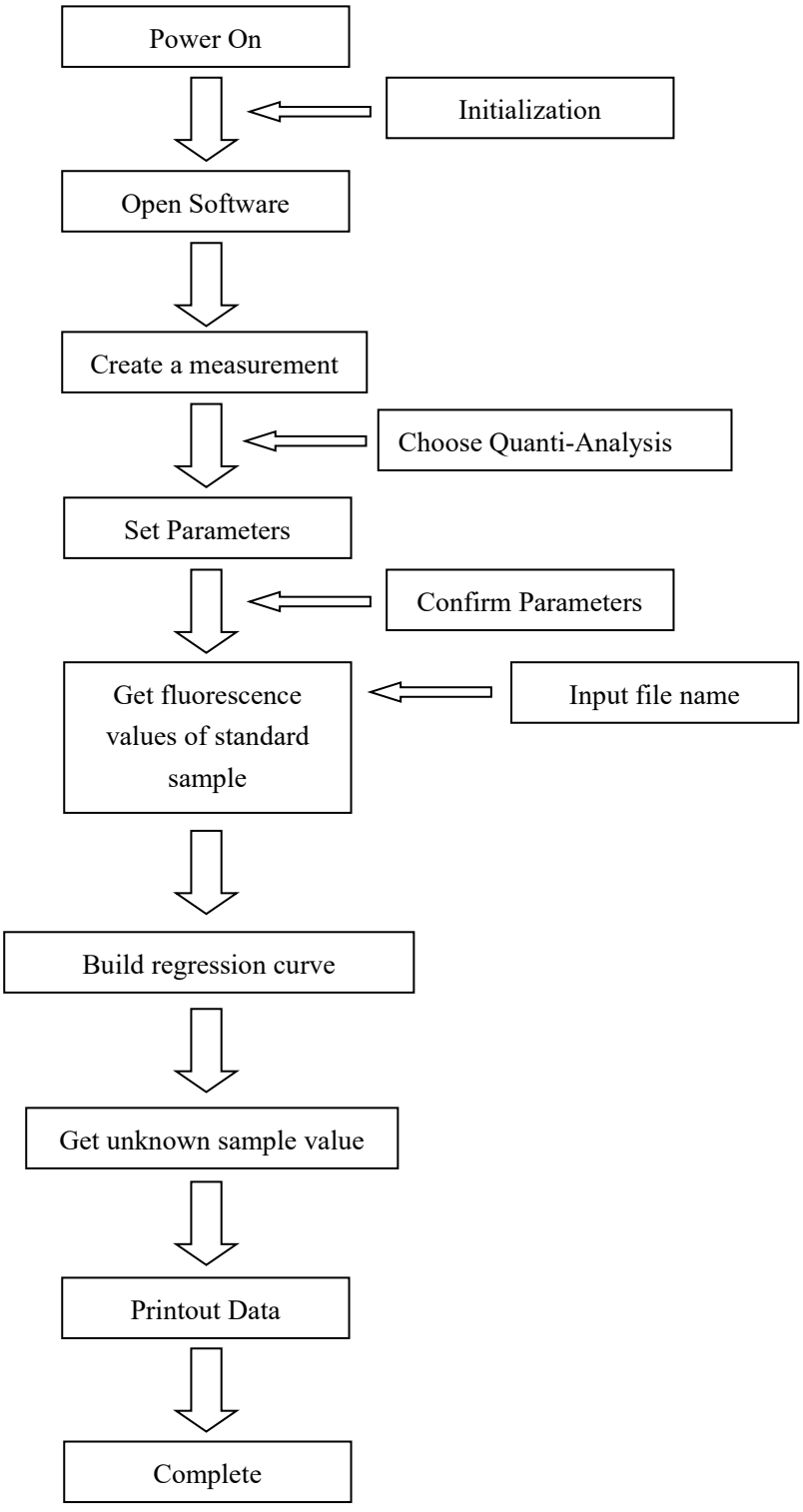


Fig.6-24 Quantitative-Analysis Flow Chart

6.3.3.1 Create a Measurement

Click “Files”->“Create Measurement” or click  to create a new measurement.

1. General:

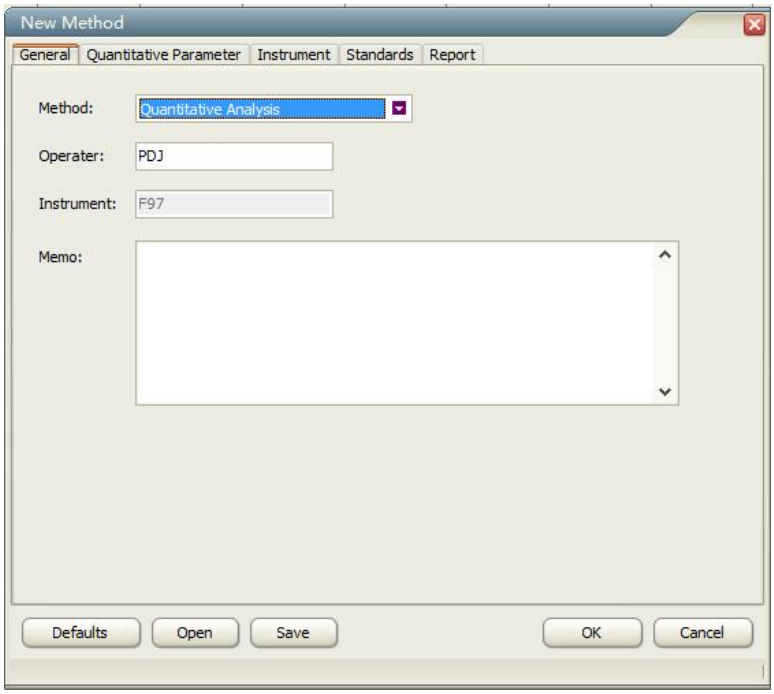


Fig.6-25 General

1) Measurement:Choose “Time Scan”.
2) Operator: Input operator’s name.
3) Instrument: The model of the connected instrument is indicated.
4) Comment: Enter a description or notes on measuring conditions.

2. Quantitative analysis

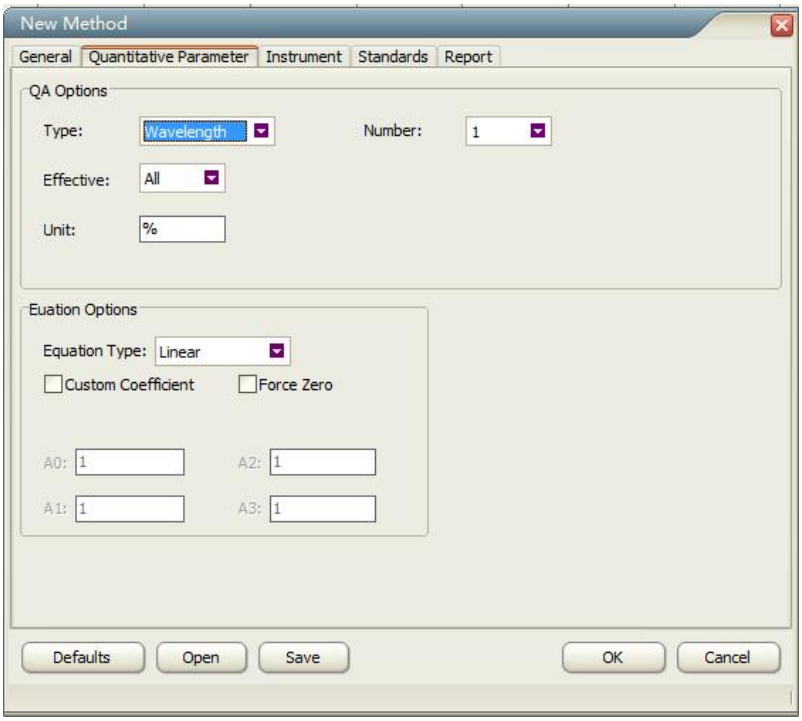


Fig.6-26 Quantitative analysis

1、 QA Options:

1) Type: Select the method of creating a calibration curve. Wavelength only.

2) Number of wavelengths: the number of wavelengths used in Quantitative analysis. Available from 1 to 3.(Details in appendix 4)

3) Significant Figures: Set Significant figures of the calculated value. Available from 2 to 6.

4) Conc. Unit: Set Conc. Unit.

2、 Equation Parameters: Set equation type or curve fitting equation parameters

1) Equation Type: Choose from “1st order”, “2nd order” and “3rd order”.

2) Custom Parameters: Choose to input your own parameters A0,A1,A2,A3.

The equation will be $Conc = A0 + A1 * X^1 + A2 * X^2 + A3$.(Details in Appendix 5)

3) 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.

3. Instrument

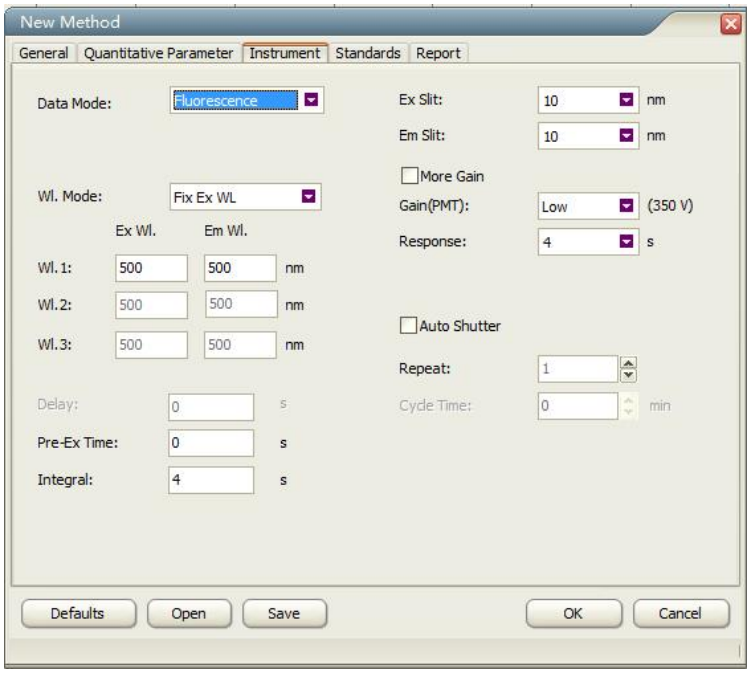


Fig.6-27 Instrument

1、 Data Mode: Fluorescence mode.

2、 Wavelength Mode:“Fixed Excitation Wavelength” and “Fixed Emission Wavelength”. The Wavelength 1, 2, 3 are related to the number of wavelengths in tab “Quantitative Parameters”.

1) When the number of wavelengths is 1, it would be the same whether in “Fixed Excitation Wavelength” or “Fixed Emission Wavelength” mode.

2) When the number of wavelengths is 2, Wavelengths 1 & 2 are available. ① If you choose “Fixed Excitation Wavelength”, then the Excitation Wavelength is fixed. When the instrument is doing measurement, it will go to emission wavelength 1,then go to emission wavelength 2. ② If you choose “Fixed Emission Wavelength”, then the Emission Wavelength is fixed. When the instrument is doing measurement, it will go to excitation wavelength 1, and then go to excitation wavelength 2.

3) When the number of wavelengths is 3, Wavelength 1, 2 & 3 are available.① If you choose “Fixed Excitation Wavelength”, then the Excitation Wavelength is fixed. When the instrument is doing measurement, it will go from emission wavelength 1 to emission wavelength 3. ② If you choose “Fixed Emission Wavelength”, then the Emission Wavelength is fixed. When the instrument is doing measurement, it will go from excitation wavelength 1 to excitation wavelength 3.

⚠ **ATTENTION:** When the number of wavelengths is 3, the value of wavelength 1, 2, 3 should be increasing or decreasing.

3、 Wavelength 1: Input Excitation Wavelength 1 and Emission Wavelength 1(200-900nm).

4、 Wavelength 2: Input Excitation Wavelength 2 and Emission Wavelength

2(200-900nm).

5、 Wavelength 3: Input Excitation Wavelength 3 and Emission Wavelength 3(200-900nm).

6、 Pre-Excitation Time: The instrument allows light to illuminate the sample pre-excitation time before measurement. During this time the instrument will not do fluorescence measurement. The time of this part is to stabilize the excitation light and the sample.

7、 Integration time: A function for obtaining data averaged over the specified time for the purpose of acquiring stabilized data.

8、 Excitation slit: Set excitation slit.(F97 is fixed to 10nm)

9、 Emission slit: Set emission slit.(F97 is fixed to 10nm)

10、 Gain(PMT): Set gain level by changing the PMT negative high voltage.

11、 More Gain: Enlarge the gain range. There will be a negative high voltage value besides each gain. This value is for reference only. There will be some deviation from the actual value.

12、 Response： Set the signal's response time from "0.1","0.5","1","2","4".The shorter the more noise.

13、 Shutter: To control the excitation time or condition of the sample.

1) When Shutter is selected, the instrument will only open the shutter when measuring samples. The shutter will automatically close when the measurement is complete. This function is for samples which are not stable when excited by light.

2) When Shutter is NOT selected, the shutter will be open. Sample will always be excited.

4. Standards：

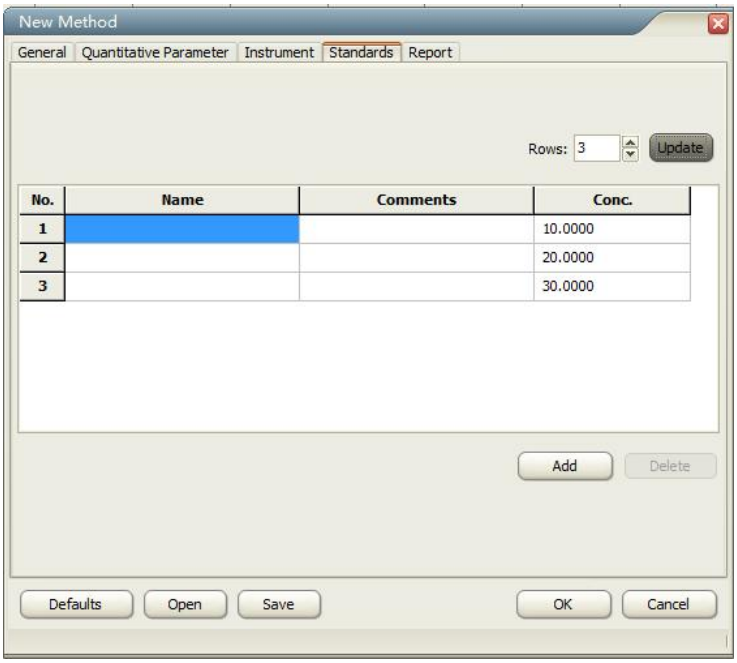


Fig.6-28 Standards

1. Sample table: Sample table gives a list of standards for sample measurement or calibration curve preparation. This table contains the items listed below.
2. Lines: Number of Standard Samples.
   1) “Update”:By clicking this button, sample numbers are set by the entered number of samples. The displayed sample names, comments, etc. are all cleared.
   2) “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.
   3) “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. Report：

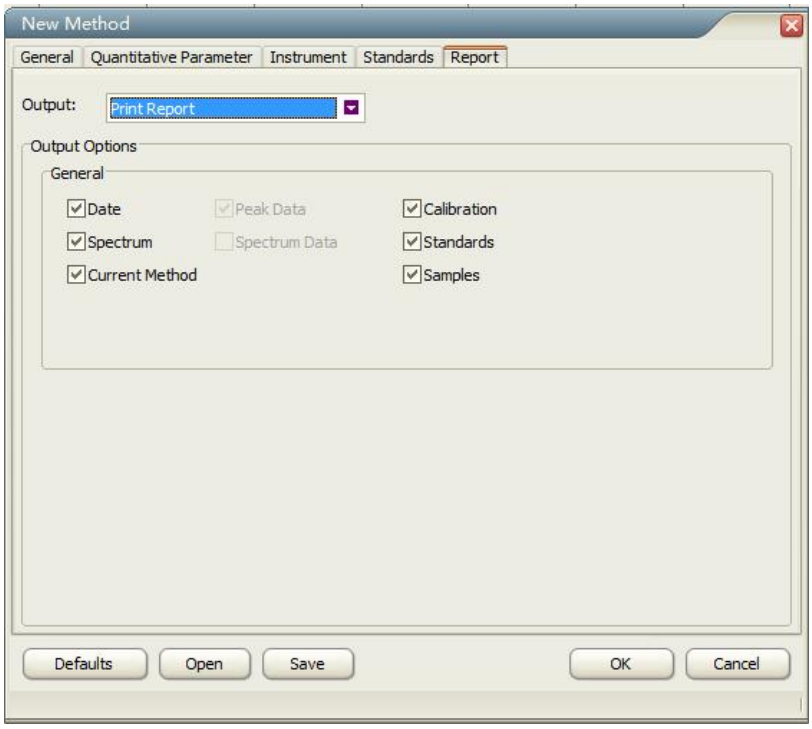


Fig.6-29 Report tab

1. Output: Print Data or Save as Microsoft（R）Excel file.
2. Output options: Choose the printout data. Check the content in “Properties” button on the left after the scan.
3. Clear Data: Clear current data section.
4. Click “Defaults” to reset the settings to default.
5. Click “Open” to open a saved method. It’s a *.FMTD file.
6. Click “Save” to save current settings.

6.3.3.2 Quantitative-Analysis Interface

Click ”OK” in the last step to enter Quantitative-Analysis interface.

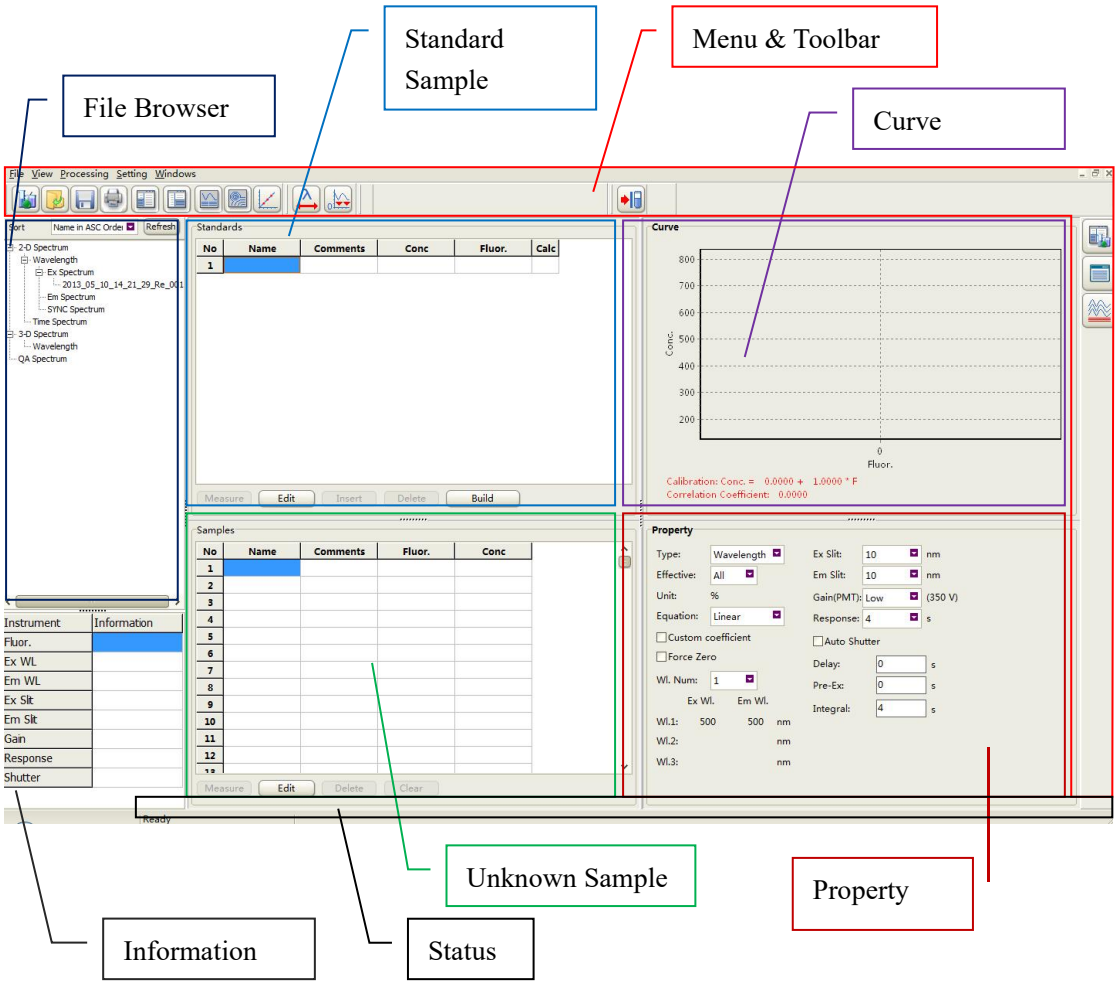


Fig.6-30 Quantitative-Analysis Interface

1. Menu, Toolbar, File Browser, Information and Status are the same as Chap.6.2.2.2.
2. Property: Check all the parameters of Quantitative-Analysis.

ATTENTION: The parameters cannot be modified in the window. Please create a new measurement to modify the parameters.

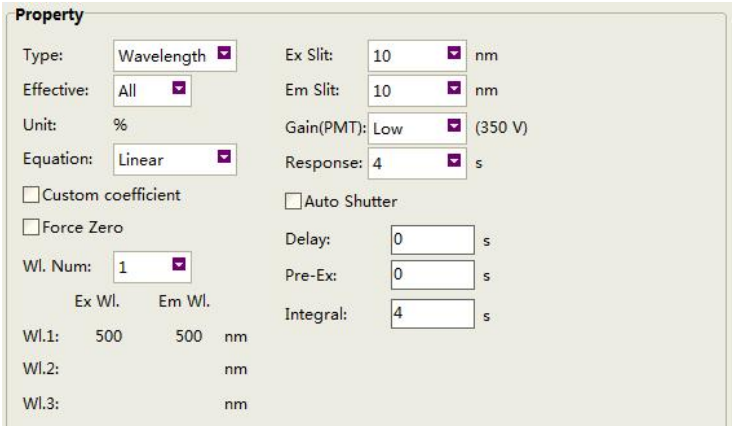


Fig.6-31 Property Window

6.3.3.3 Build Curve

1. In the Standardswindow, you can modify sample name, description and concentration; add or delete sample; check the fluorescence value of samples.

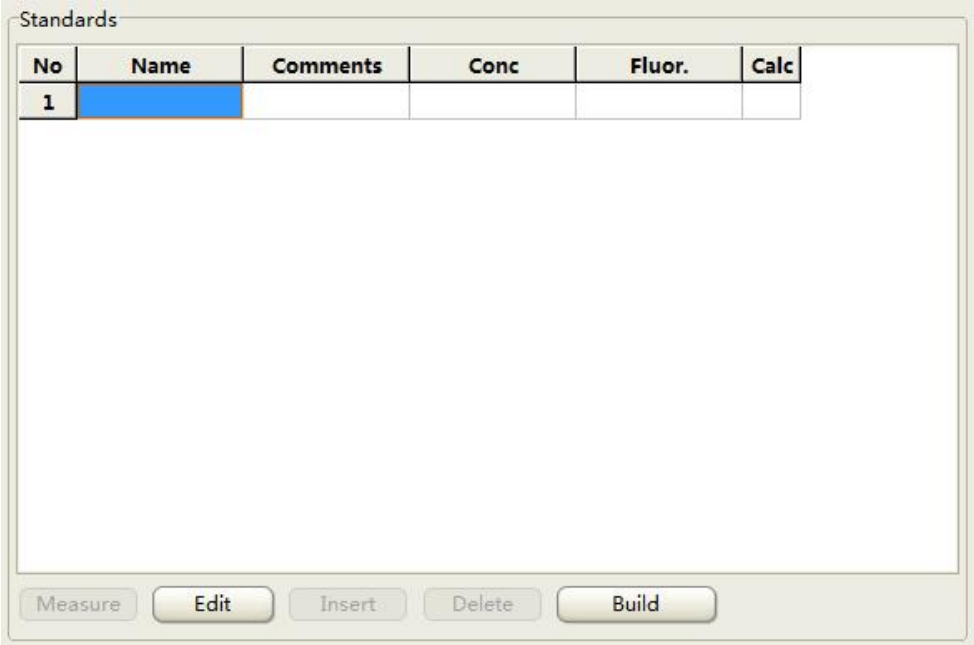


Fig.6-32 Standards Window

1） Modify sample name, description and concentration: Click “Edit”, then double click in the table to modify the content you want. Then click “OK” to confirm.

2） Measure a fluorescence value: Click to select a sample in the table, then click “Start” button. The instrument will start measurement. The Status Window will goes as “Standby”->“Remaining time **sec”->“Standby”.

3） Add Sample: Click “Edit”, then click “Insert”. There will be another line in the standards window. Click “OK” to finish.

4） Delete Sample: Click “Edit” and click a line you want to delete, then click “Delete”. Click “OK” to finish.

5） Choose the sample data needed in curve calculation: Click “Edit”, then click the check mark in “Calculate” row if you want to use this data for calculation.

ATTENTION: Pay attention to the Zero adjustment. Click to do Zero adjustment, click again to reset the Zero point.

2. Click “Build Equation” to build the curve of standard sample when finishing measurement as Fig.6-33. The abscissa is fluorescence value, the ordinate is the concentration value. The equation is under the figure.(Check the mathematical algorithms of the regression curve in Appendix(V).

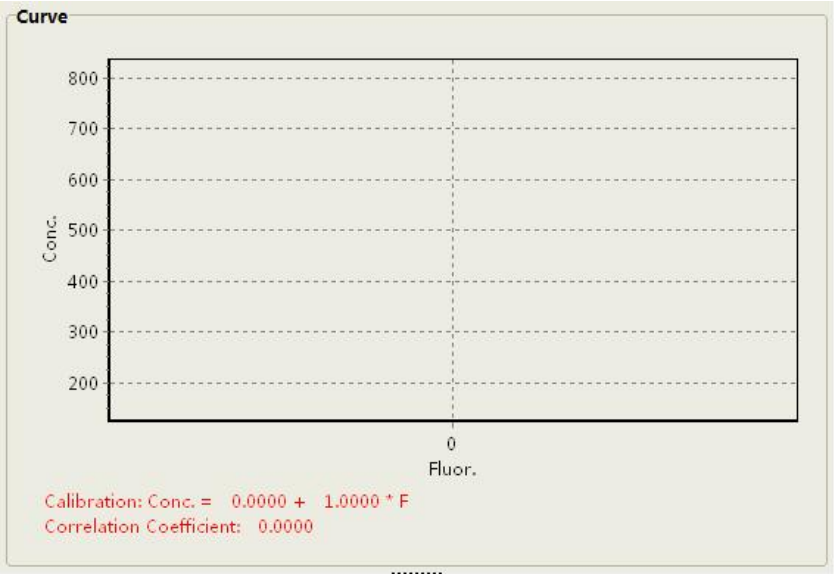


Fig.6-33 Regression Curve

6.3.3.4 Measuring unknown sample

When the regression curve is created, you can start measuring unknown sample. Operate the test sample in samples window as Fig.6-34. There are functions in the sample window: Measure, Modify, Delete and Clear.

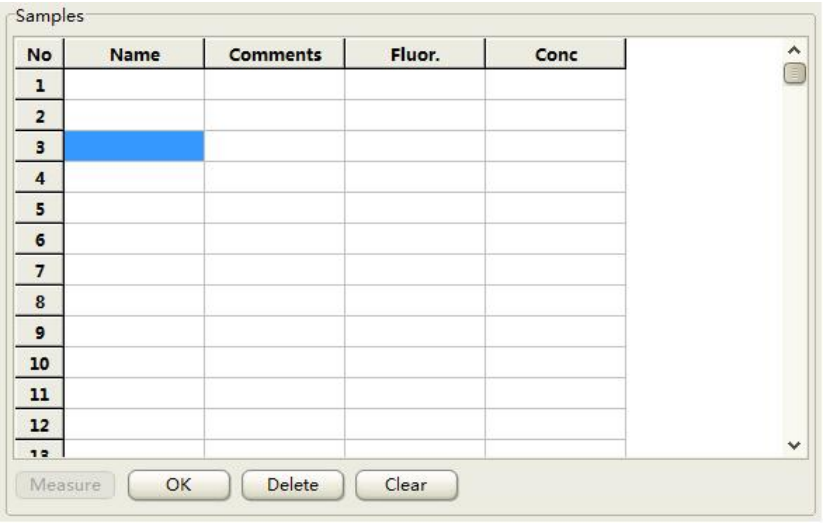


Fig.6-34 Sample Window

1、 Change sample name & note: Click “Edit” button, then double click the frame you want to modify. Click “OK” to confirm the modification and back to test sample window.

2、 Measure sample fluorescence value & concentration value: Click a sample fluorescence value or concentration value frame, then click “Measure” button to measure the sample. The fluorescence value and concentration value of the sample will be in “Fluorescence” and “Concentration” column. When measuring sample, the Status window will show current status as “Standby”→“Seconds Counting: ** sec”→“Standby”.

3、 Delete sample: Click “Edit” button, then click the line you want to delete and click “delete” button to delete the sample. Click “OK” to confirm the modification and back to test sample window.

4、 Clear sample list: Click “Edit” button, then click “Clear” button. Click “OK” to confirm the modification and back to test sample window.

6.3.3.5 Printout Data

Icon	Function
	View spectrum information & choose printout content and format.
	Printout Data.

Tab.6-6 Toolbar icon functions

6.3.4 3D Scan

3D fluorescence spectrum has a characteristic of the "fingerprint". We can get all kinds of information on the sample map through the analysis of three-dimensional fluorescence spectrum, Including the excitation wavelength of Rayleigh scattering and secondary scattering, Raman shift samples, the optimal excitation wavelength, the best fluorescence peak wavelength.

3D Scan Procedure

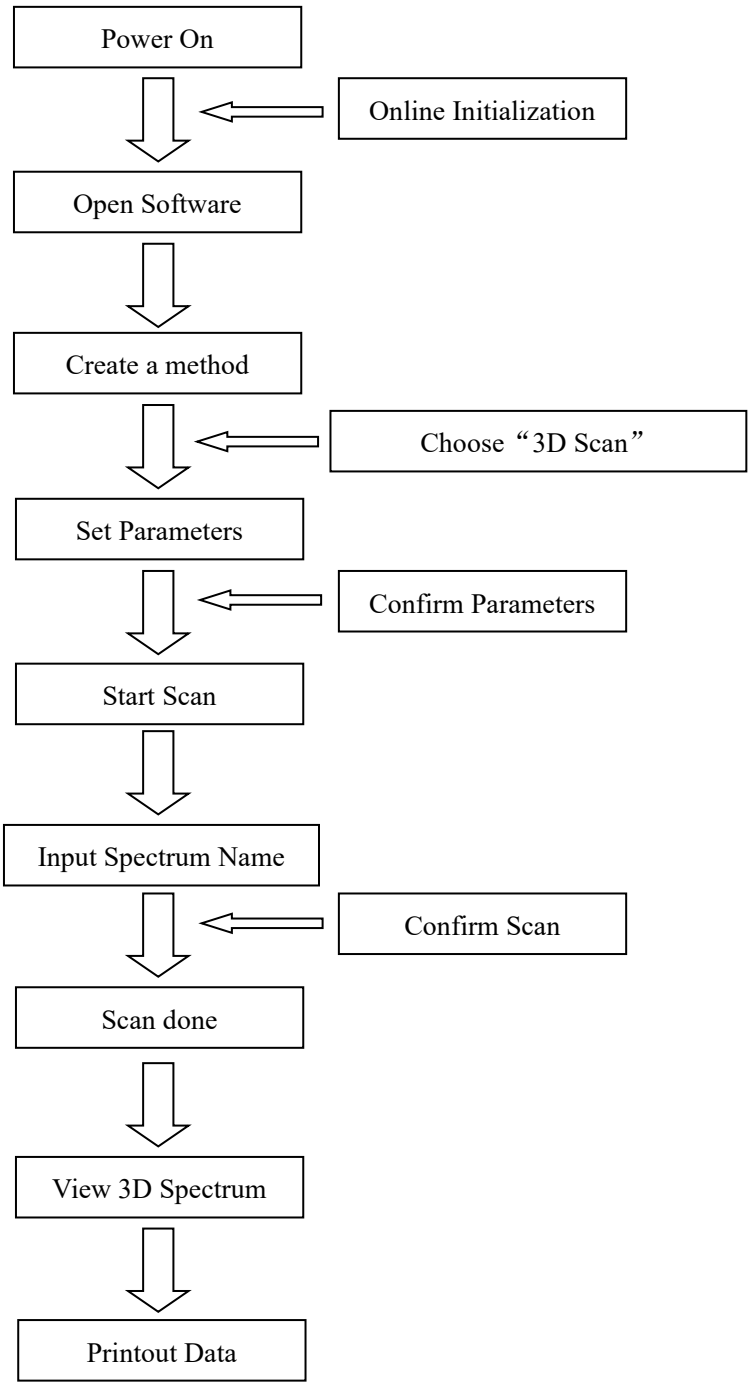


Fig.6-35 3D Scan Procedure

6.3.4.1 Create a method

Click “File”→“New Method” or click  to enter the window below in Fig.6-36 to create a method.

1. **General：**

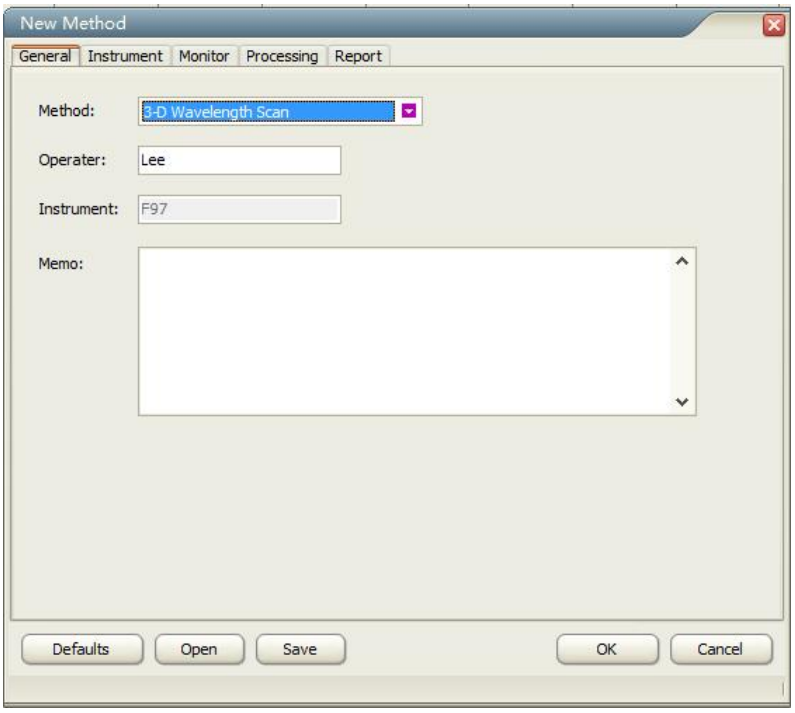


Fig.6-36 General tab

1) Measure Mode:Choose “3D Scan”.
2) Operator: Input operator’s name.
3) Instrument: The model of the connected instrument is indicated.
4) Comment: Enter a description or notes on measuring conditions.

2. Instrument:

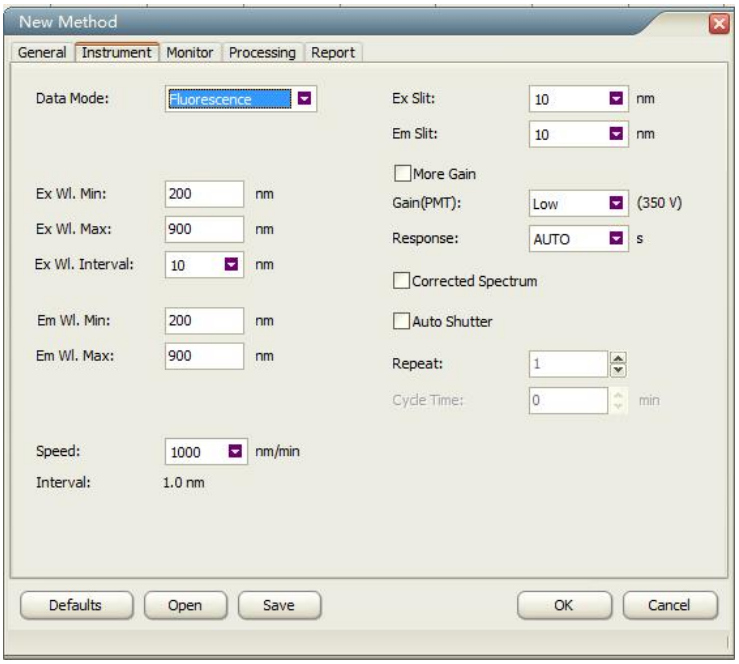


Fig.6-37 Instrument tab

1） Scan Mode: Fluorescence mode.

2） Excitation Start Wavelength: Input excitation wavelength(200nm-900nm).

3） Excitation End Wavelength: Input excitation wavelength(200nm-900nm).

4） Excitation 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.

5） Emission Start Wavelength: Input emission wavelength (200nm-900nm).

6） Emission End Wavelength: Input emission wavelength (200nm-900nm). End wavelength should be longer than start wavelength.

7） Scan Speed: Set scan speed. The faster the speed is, the shorter the scan takes, and with more noise.

8） Scan Interval: set the interval of the data point in the spectra according to the scan speed.

9） Excitation Slit: Set a slit width for the excitation side.(F97 is fixed in 10nm)

10） Emission Slit: Set a slit width for the emission side.(F97 is fixed in 10nm)

11） Gain (PMT): Set the voltage level of the photomultiplier tube to change the gain.

12） More Gain: More Gain levels to choose.

13） 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. When this setting is at ON, the instrument will use the last correct result to correct the spectra (Turn to Appendix for detail). The suitable wavelength range of Rhodamine B solution for spectral calibration is 250nm-600nm.

14） Response Time: Set a response time. Set auto usually.

15） Shutter Control: 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.

16） Replicates: Set the repeat times of scans.

17） Cycle time: Set the waiting time between two scans.

3. Monitor：

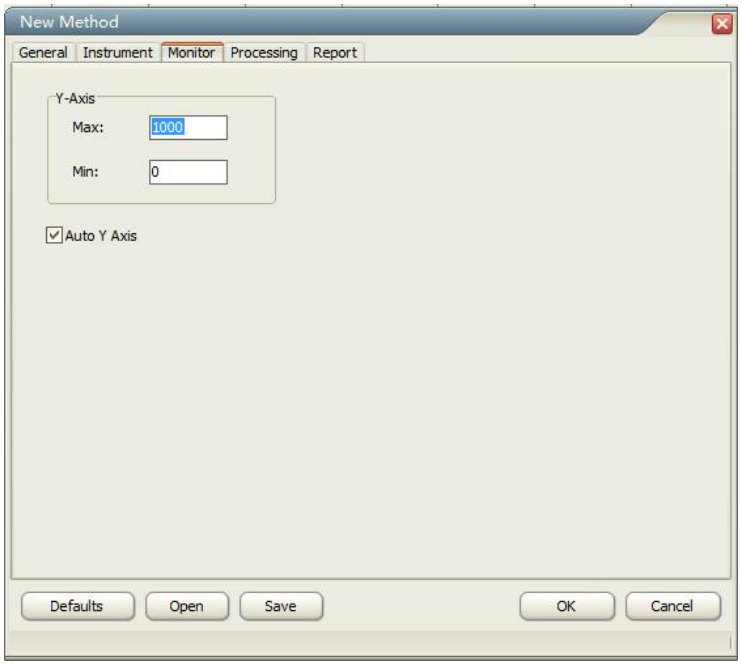


Fig.6-38 Monitor Tab

1） Y Axis: Input start point and end point of the Y Axis.

2） Auto set Y Axis: Y axis will automatically set according to Y axis data.

4. Processing：

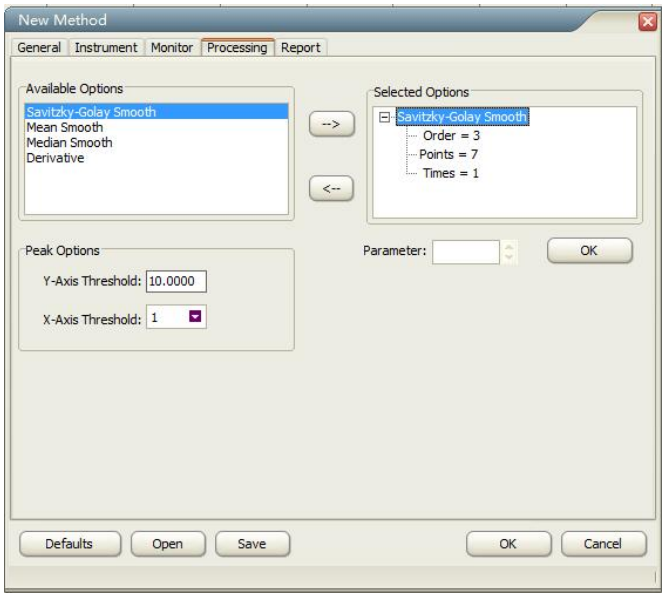


Fig.6-39 Processing Tab

1. Available Methods: There are 4 methods of data processing."Polynomial

smooth", "smooth mean", "median Smooth" and "derivative" are available.

1) Select a method in “available” window, then press --> to move the method to “selected” window.

2) Select a method in “selected” window, then press <-- to move the method to “available” window.

2. Selected: the selected methods are in this window. When the scan is done, the software will use selected methods to process scan data.
3. Modify Parameters: Click the “+” in front of a method in Selected window to show the parameters. Click to modify, then press OK to confirm.
4. Peak Finding: Set the threshold and sensitivity to find peak after the scan.

5. Report：

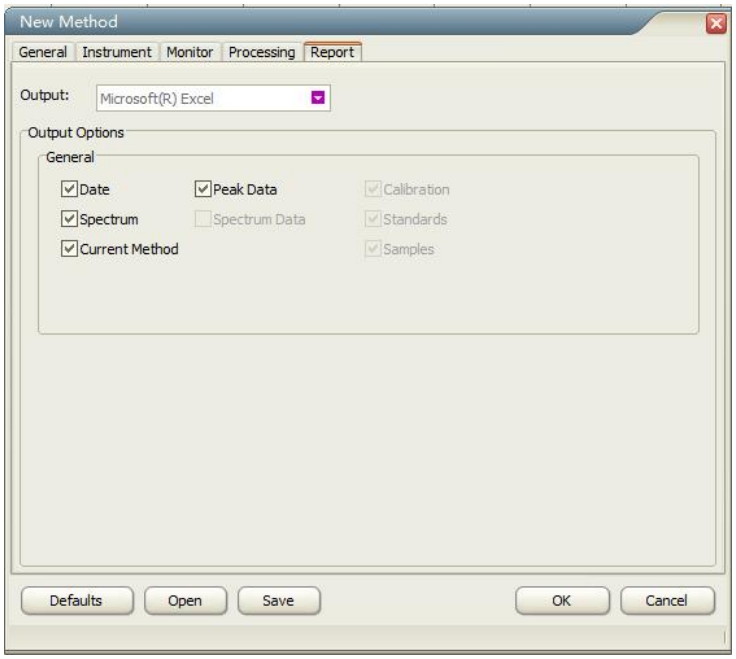


Fig.6-40 Report Tab

1、 Output: Transfer data into Microsoft Excel format.

2、 Output Options: Put a check mark to select the output data.

6.3.4.2 Run Wavelength Scan

The status will be: “Standby” → “Ready to scan” → “Moving excitation wavelength” → “Moving emission wavelength” → “Start wavelength scan” → “Move emission wavelength” → “Standby”.

A. File Name

Click button , there will be a popout window to input a file name. Press OK to start 3D scan. Keep it blank if you want to use the current system time as file name.

B. Scan Stop

Click to stop the scan.

6.3.4.3 3D Spectrum Processing

A. Spectrum Display

When the scan is running, spectrum data will be displayed in the spectrum window as 2D figure. The spectrum will be displayed in the spectrum window as 3D figure after scan.The spectrum file will be automatically saved.

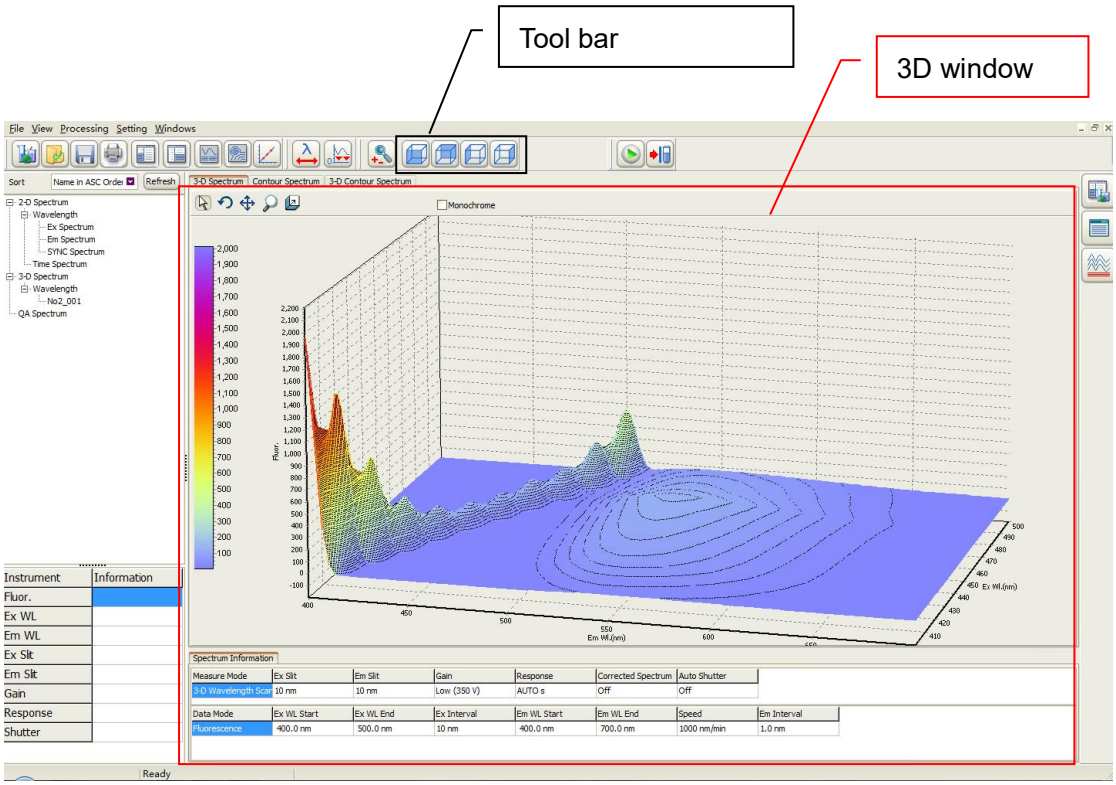


Fig.6-41 3D Scan Interface

B. 3D Scan Toolbar

Icon	Function
	Click to show the front view
	Click to show the rear view
	Click to show the left view
	Click to show the right view

Tab.6-7 3D Functions

C. Spectrum Window

1. 3D Spectrum Tab: 3-D Scan data can be displayed in 3D view.The spectrum is a composition of emission wavelength axis, the excitation wavelength axis fluorescence value axis, contour and color notification.

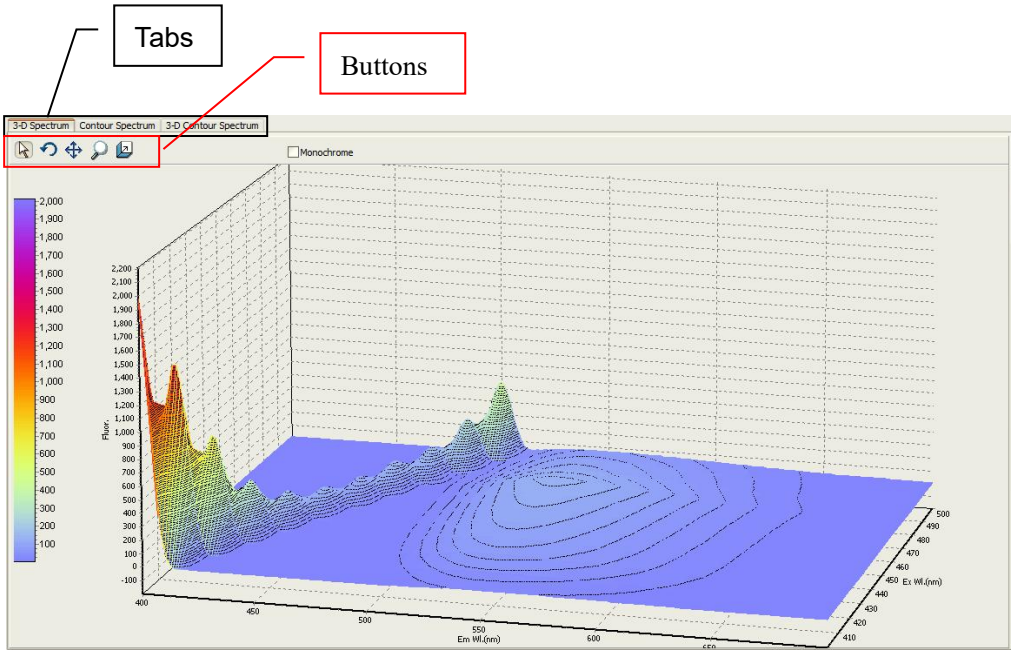


Fig.6-42 3D Spectrum Tab

Click these tabs  to show 3D view, Contour view and 3D contour view. Click buttons in tab 6-8 for more functions.

Icon	Function
	Click  to zoom and move the spectrum. Left key operations are the same as 6.2.2.2. Hold right key to move the spectrum.
	Click  to rotate the spectrum. Hold left key to rotate with mouse moving. Hold right key to move the spectrum.
	Click  to move the spectrum.
	Click  to zoom the spectrum. Hold left key and move the mouse down to zoom out. Hold left key and move the mouse up to zoom in. Hold right key to move the spectrum.
	Click  to change the depth. Hold left key and move the mouse to adjust the depth of the spectrum. Hold right key to move the spectrum.

Tab.6-8 Spectrum Controls

2. Contour Tab: Shows the contour line in 2D view as Fig.6-43.

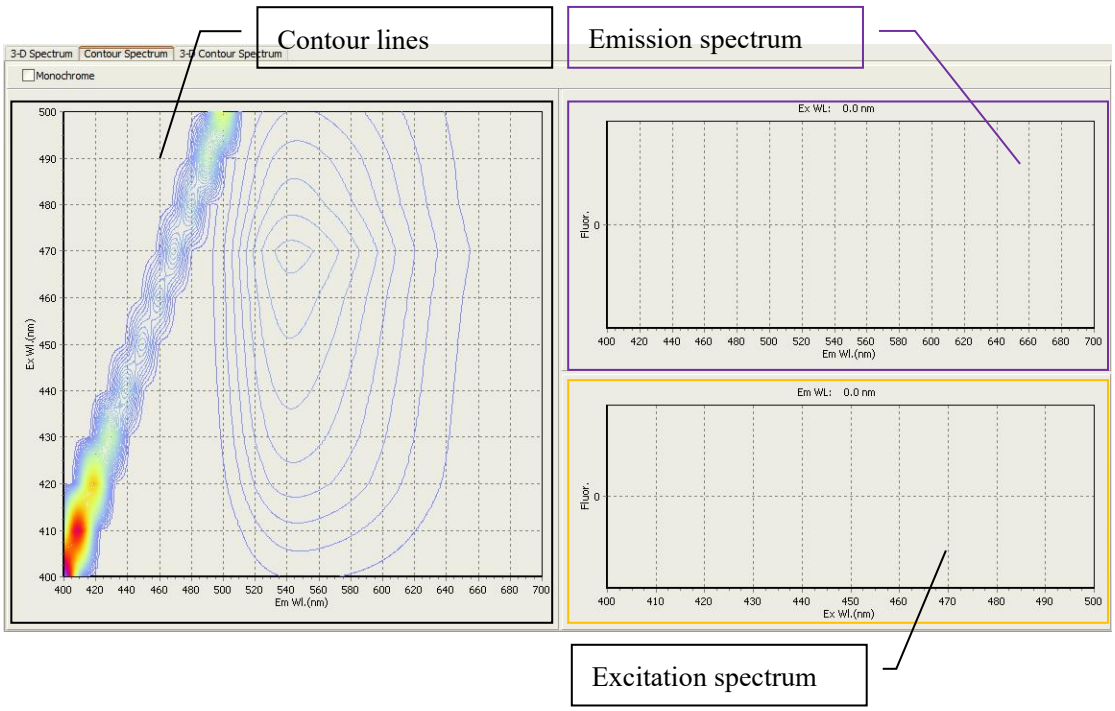


Fig.6-43 Contour Tab

Contour tab includes contour window, emission window and excitation window.

（1） Contour lines: X axis is emission wavelength. Y axis is excitation wavelength. 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 side window.

（2） Excitation Spectrum: An excitation spectrum at the cursor position in contour lines window is displayed. Wavelength and photometric value can be read in the window.

（3） An emission spectrum at the cursor position in contour lines window is displayed. Wavelength and photometric value can be read in the window.

3. 3D Contour Tab: Shows all the 2D spectrums in 3D view as Fig.6-44.

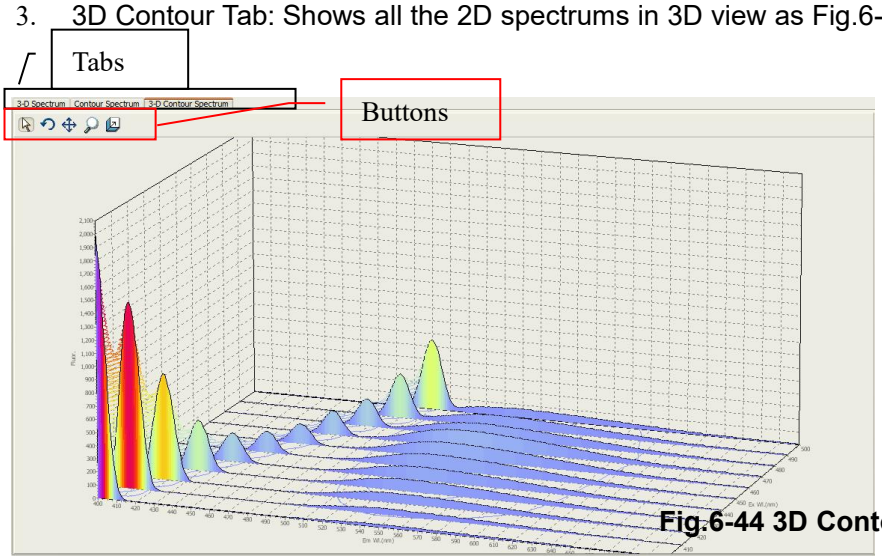


Fig.6-44 3D Contour Tab

6.3.4.4 Print out

Icons	Functions
	Spectrum Properties
	Print Data

Table 6-9 Toolbar

6.3.5 Synchronous Scan

Synchronous Scan flow chart

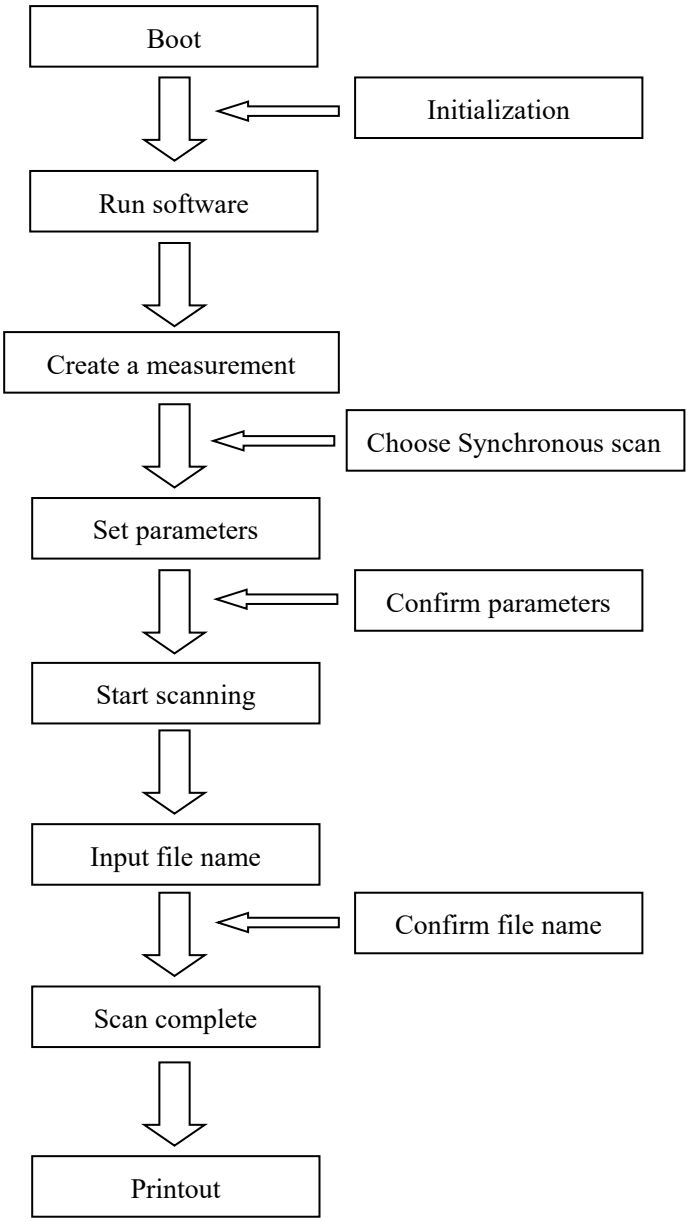


Fig.6-45 Synchronous Scan flow chart

6.3.5.1 Create a measurement

Click “File”→“Create measurement” or click icon  to create a measurement.

A. General：

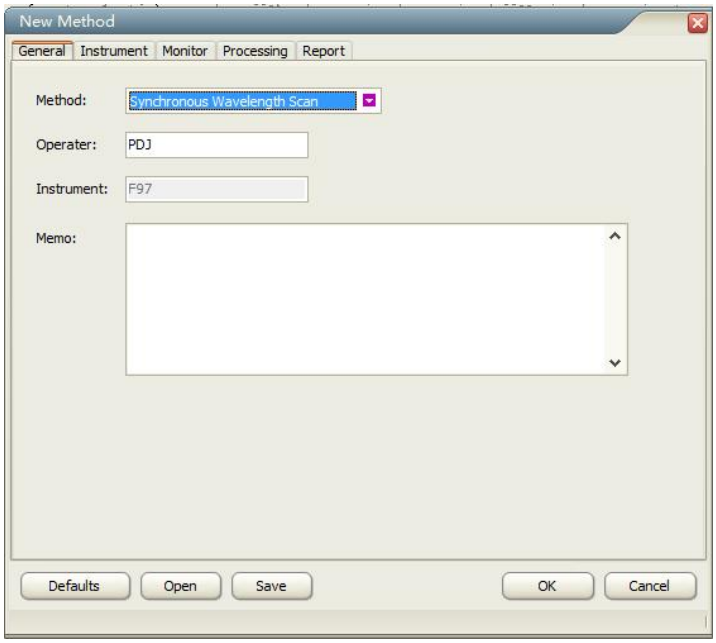


Fig.6-46 General

1. Measurement: Choose “Synchronous scan”
2. Operator: Input operator name.
3. Instrument: The model of the connected instrument.
4. Comments: Enter a description or notes on measuring conditions.

B. Instrument:

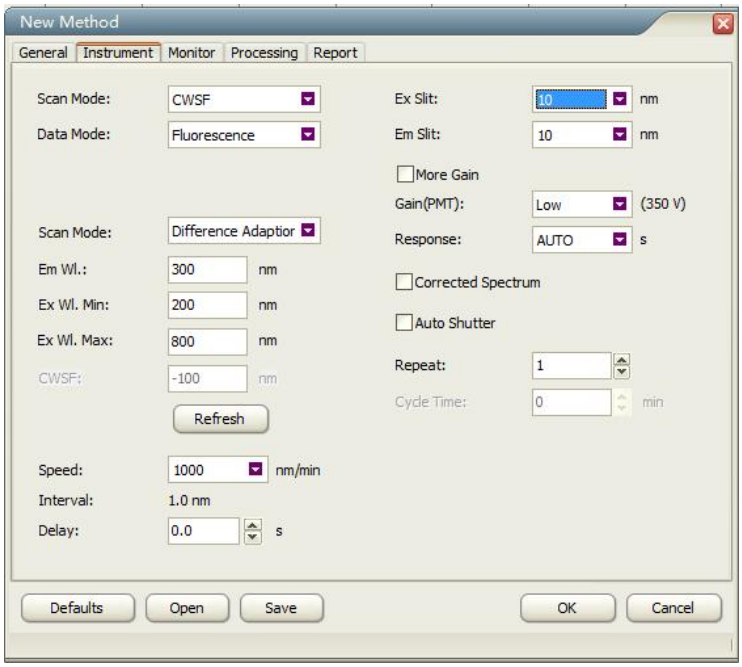


Fig.6-47 Instrument Tab

1. Scan mode: Constant Wavelength Synchronous Fluorescence (CWSF) & Constant Energy Synchronous Fluorescence (CESF). Constant Wavelength Synchronous Fluorescence: Excitation and emission sides scan at the same time in a fixed wavelength difference. Constant Energy Synchronous Fluorescence: Excitation and emission sides scan at the same time in a fixed energy difference.
2. Data mode: Fluorescence mode
3. Scan mode: If the scan mode is “WL Adaption”, “CWSF” is available. If the scan mode is “Difference Adaption”, “Emission wavelength” is not available. Click “Refresh” to see the emission wavelength.

   1) If the scanning style is “WL Adaption”, emission wavelength is not available. Edit excitation start wavelength or CWSF then click “Refresh”, emission wavelength will be available.

   2) If the scanning style is “Difference Adaption”, CWSF is not available. Edit emission wavelength and excitation start wavelength then click “Refresh”, CWSF will be available.
4. This is the start wavelength for wavelength scan on the emission side.(200 to 900 nm)
5. This is the start wavelength for wavelength scan on the excitation side.(200 to 900 nm)
6. This is the end wavelength for wavelength scan on the excitation side. (200 to 900 nm)
7. Constant Wavelength Difference & Constant Energy Difference: Constant wavelength difference is the difference between excitation start wavelength and emission start wavelength. Constant energy difference is the difference between energy of excitation start wavelength and emission start wavelength.

8. Scan Speed: Set a wavelength scan speed.
9. Scan Interval: Shows data sampling interval according to the scan speed.
10. Delay: After pressing the Measure button, measurement is started following the delay time set here. It is used for temperature stabilization, etc.
11. Excitation Slit： Select a slit width for the excitation side.（F97 is fixed to 10nm）
12. Emission Slit：Select a slit width for the emission side.（F97 is fixed to 10nm）
13. PMT Voltage: A function for controlling the voltage of the photomultiplier tube. It will change the gain.
14. More Gain: More gain level available. This negative high value is for reference only, and the actual negative high voltage value will have a bias.
15. Response: Response time of wavelength scan. Select Auto usually.
16. 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. Rhodamine B solution suitable for spectral calibration wavelength range is 250 to 600nm.

4. Shutter control: 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.

1) 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.

2) If it's not chosen, the shutter is open constantly.

17. Replicates: Set the number of repeat measurements.
18. Cycle time: Set a repetition interval.

C. Monitor：

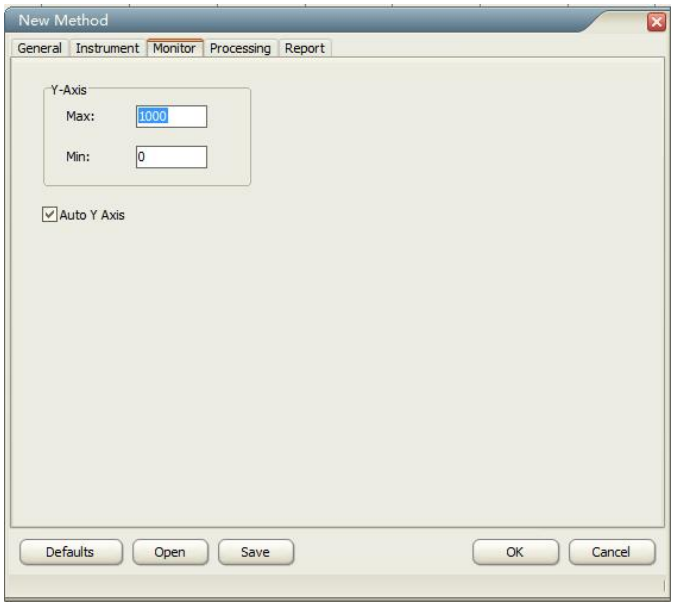


Fig.6-48 Monitor Tab

1. Y Axis: Enter the start point and end point of Y axis.
2. Auto Y Axis: Automatically adjust Y axis when a check mark on.

D. Processing：

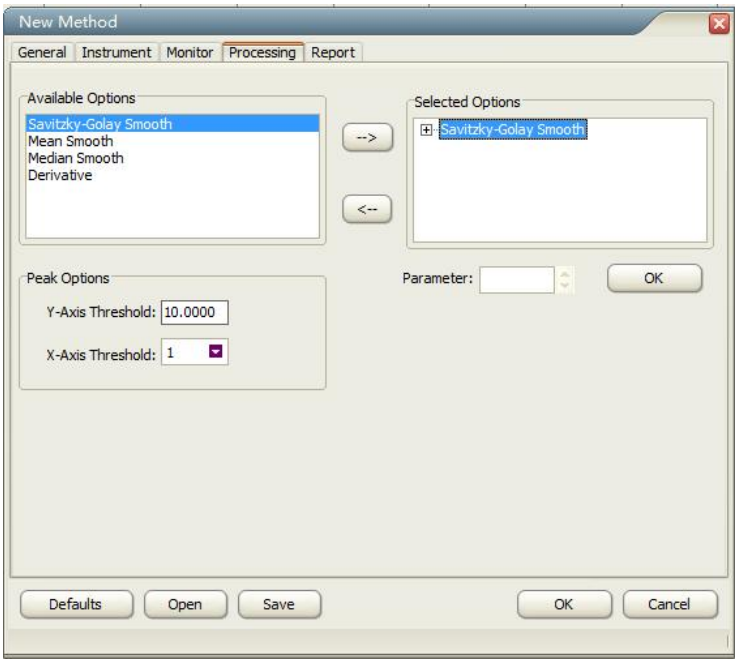


Fig.6-49 Processing Tab

1、 Available Options: Used for obtaining an average spectrum in repeat measurement. A list of data processing (Savitsky-Golay, Mean smooth, Median smooth, Derivative) is shown.

1) Select a data processing item, and click the rightward-pointing arrow key --> between the Available Options box and Selected Options box. Then, the selected method appears in the Selected Options box.

2) The processing sequence is displayed. To delete a processing method, first select the method, and then click the leftward-pointing arrow key <-- between the Available Options box and Selected Options box. Then, the selected method disappears from the Selected Options box.

2、 Change Parameters： If you want to carry out any smoothing in Selected Options box, click the + in front of the relevant item and the smoothing parameters can be changed.

3、 Peak Finding: Select a peak detection method indicated in the data processing window. Set a detection limit for the photometric value axis of peak and valley.

E. Report:

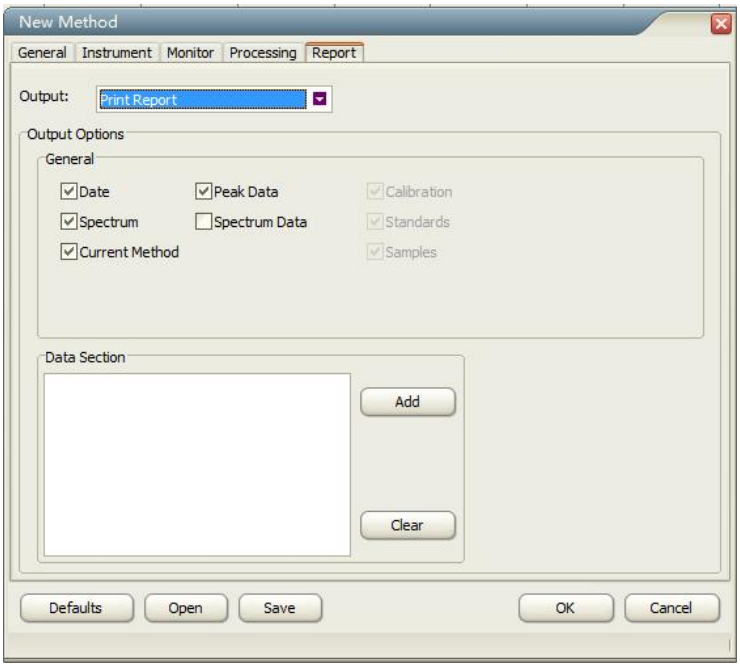


Fig.6-50 Report Tab

1、 Output: Print Report and Use Microsoft® Excel are available.

2、 Output options: Check these items to add into printout report. Click  to check out the chosen items.

3、 Add Data: When “Spectrum Data” is checked, you can choose data section to printout. Set the start wavelength, end wavelength and interval in the pop out window, then click OK. Click the “+”to see the data section.

4、 Clear Data: Clear current data section.

5、 Click “Defaults” to reset the settings to default.

6、 Click “Open” to open a saved method. It’s a *.FMTD file.

7、 Click “Save” to save current settings.

6.3.5.2 Run Synchronous Scan

There is a changing process running synchronous scan: “Standby”→“Ready”→“Start wavelength scan”→“Moving excitation and emission wavelength”→“Standby”

一、 **File Name**

Click  to enter a file name. Click OK to start the scan. Keep it blank to use the current system time as file name.

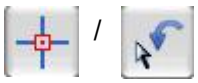
二、 **Stop Scan**

Click  to stop the scan.

6.3.5.3 Spectrum Processing

Spectrum will be displayed in the spectrum window. The spectrum file will be automatically saved in the file browser window.

Click  to see details of the spectrum.

Icon	Functions
	Reset Original Coordinate.
	Auto Adjust Coordinate.
	Enlarge Y Axis 2 Times
	Reduce Y Axis 2 Times
	Get/Cancel Axis Data
	Zoom In / Out
	Show/Hide Peaks
	Show/Hide Grid
	Peak Finding Details

	Start/Stop
	Open/Close Optical Gate
	Set Wavelength
	Run/Cancel Zero Adj.
	Spectrum Smoothing
	Spectrum derivation
	Spectrums Calculation
	Spectrums Comparison

Tab.6-10 Toolbar

6.3.5.4 Printout Data

Icon	Functions
	Spectrum Properties
	Print Data

Tab.6-11 Toolbar

6.3.6 MORE CONVENIENT OPERATING METHODS

6.3.6.1 Arithmetic Operation between Spectra

Arithmetic Operation Procedures

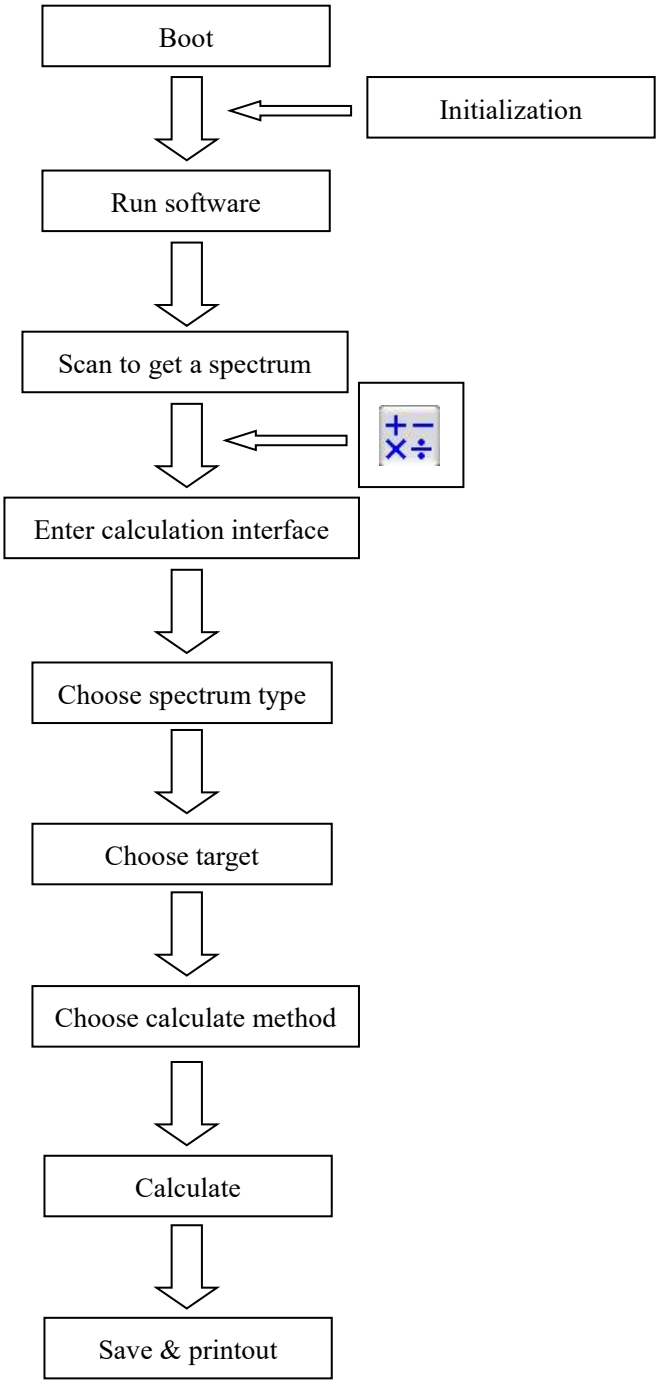


Fig.6-51 Flowchart

Arithmetic Operation includes addition, subtraction, multiplication and division among the same type of spectrums. Click  on the right to enter the spectrum compare window as Fig.6-52.

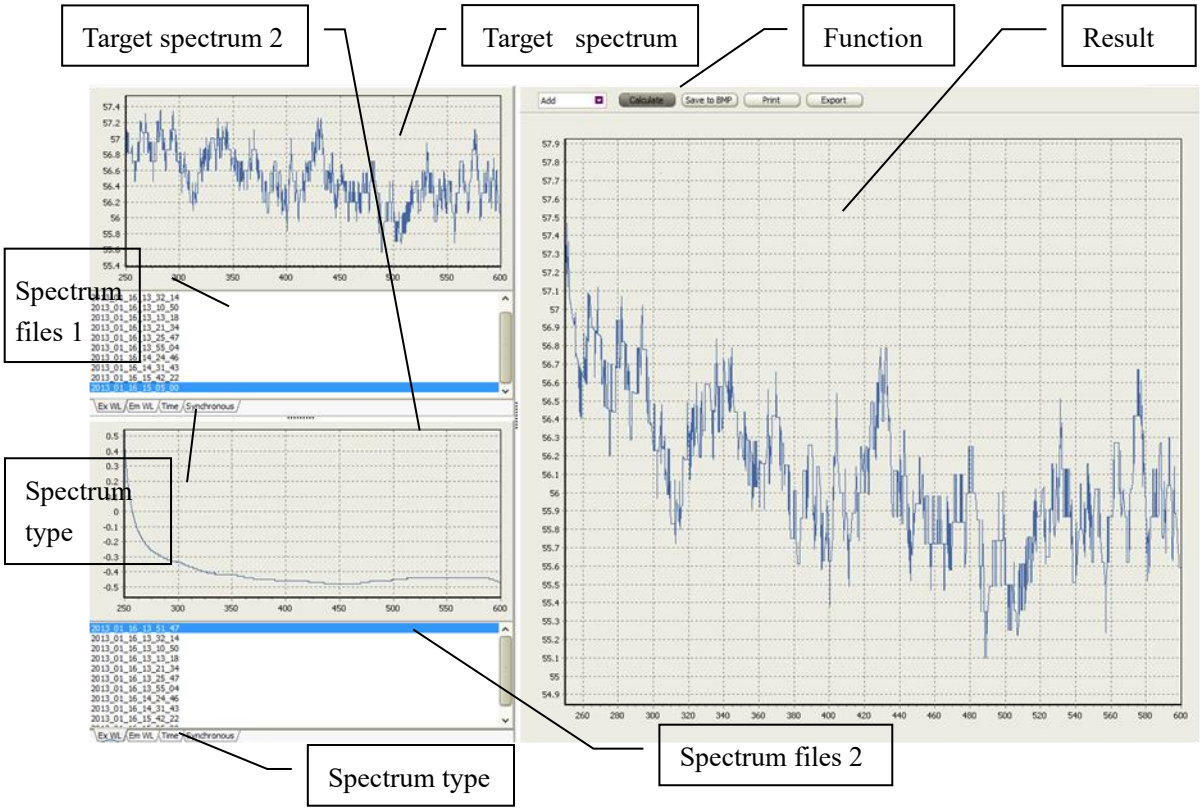


Fig.6-52 Arithmetic Operation

Arithmetic Operation Interface:

1. Functions: Calculations and data processing as Tab.6-12

Buttons	Function
Add	Click to choose Add, Minus, Plus and Division
Calculate	Click to calculate
Save to BMP	Click to save the spectrum
Print	Click to printout

Tab.6-12 Arithmetic Functions

2. Target spectrum 1 & Spectrum files section 1: Double click the spectrum file in section 1, then the spectrum will be displayed in spectrum 1.
3. Target spectrum 2 & Spectrum files section 2: Double click the spectrum file in section 2, then the spectrum will be displayed in spectrum 2.
4. Spectrum type: Click tag to change spectrum.
5. Result: Shows the result spectrum.

6.3.6.2 Overlaid Display of Spectra

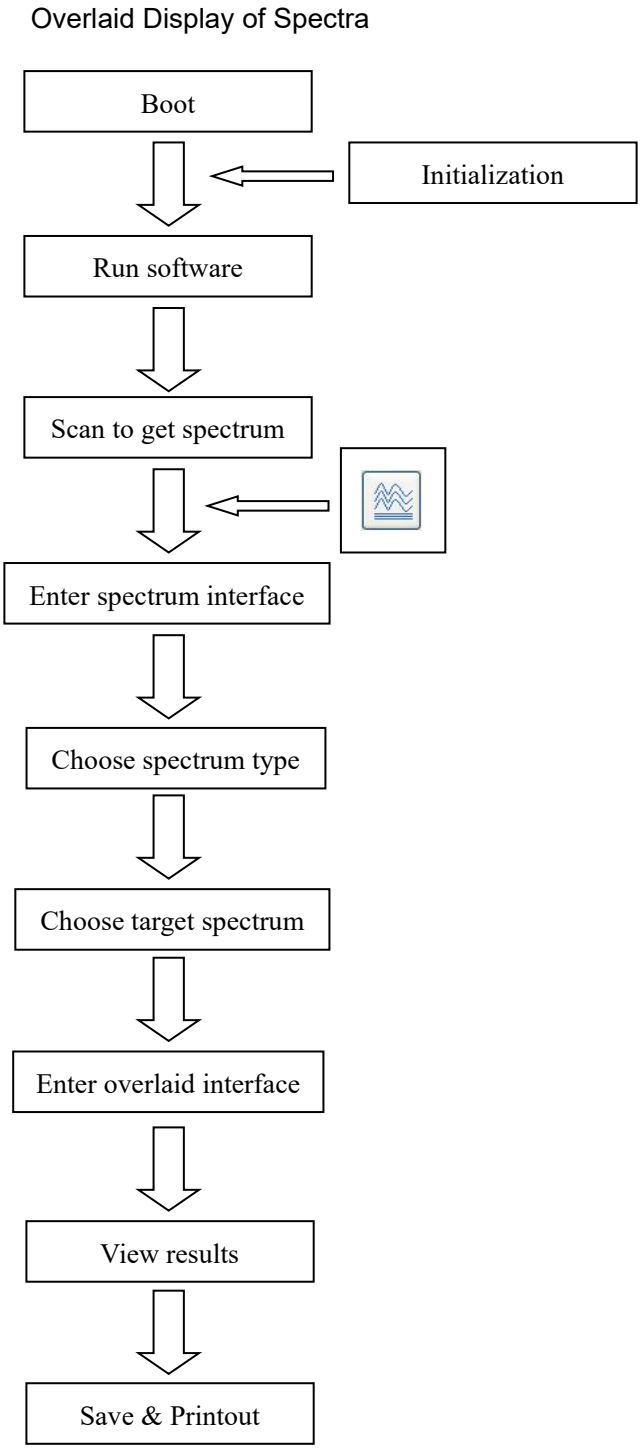


Fig.6-53 Flowchart

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

Click  to enter the overlaid interface as in Fig.6-54.

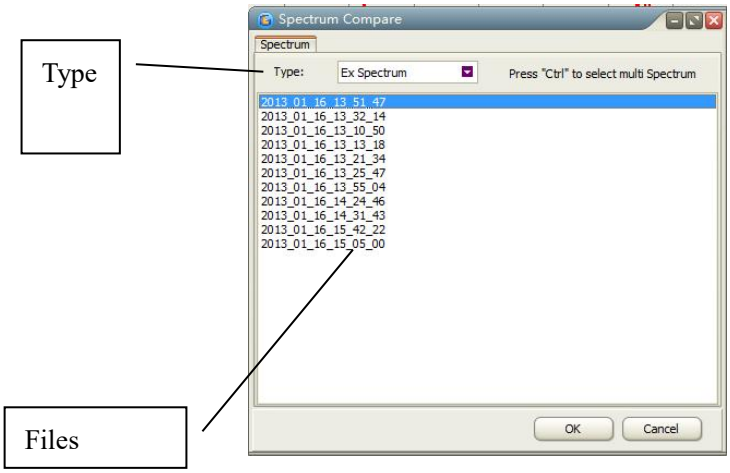


Fig.6-54 Overlaid Display Interface

A. Interface:

1. Type: Choose the spectrum type. Excitation spectrum, emission spectrum, time scan spectrum and synchronization scan spectrum are available.
2. Files: Shows the spectrum files of the type above.Hold “Ctrl” to select multiple spectrums.

CHOOSE NO MORE THAN 9 SPECTRUMS.

B. Click “OK” to enter interface as Fig.6-55.

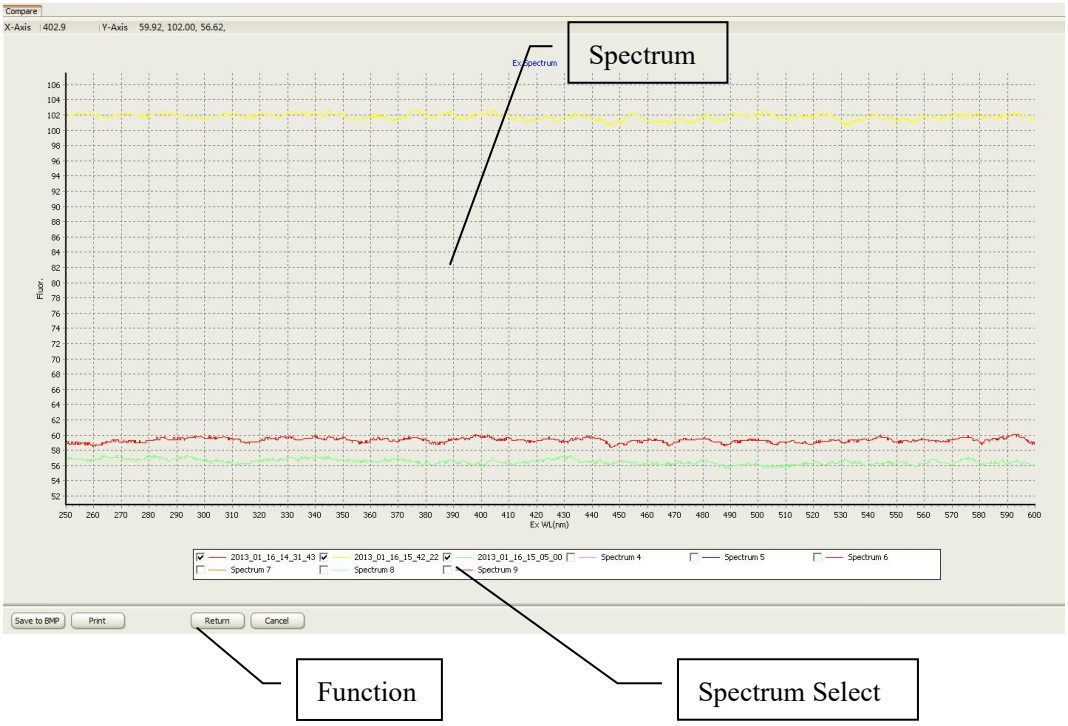


Fig.6-55 Overlaid Viewer

1. The selected spectrums are in the overlaid viewer in different color.
2. Spectrum Select: Choose the spectrums to compare.
3. Functions: To save, print the spectrum. Click “Return” to go back Fig.6-55. Click “Cancel” to exit overlaid.

6.3.6.3 Detection of Spectral Peaks

Detection of Spectral Peaks Procedure

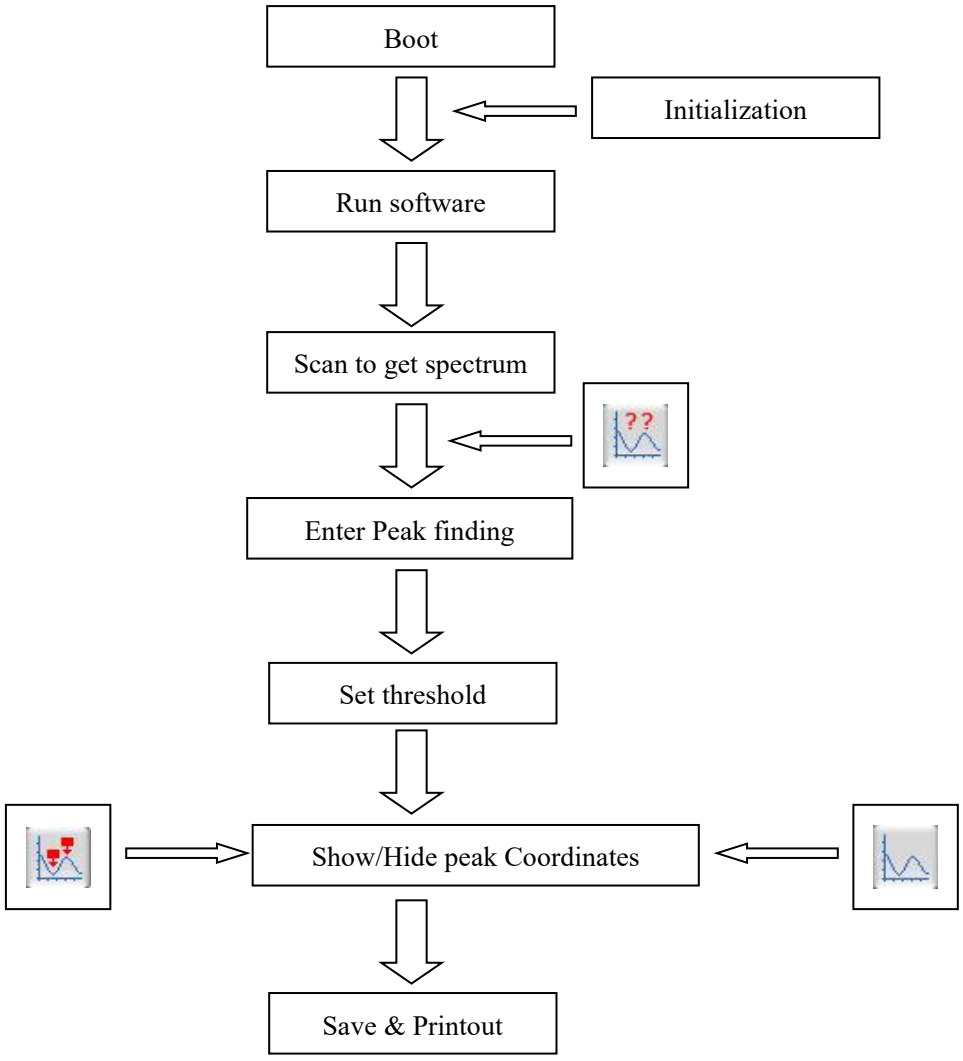


Fig.6-56 Flowchart

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. 6-57 appears. Set threshold of Y axis and X axis. If the peak is high, the Y axis threshold could be larger; if the peak is wide, the X axis threshold could be larger.

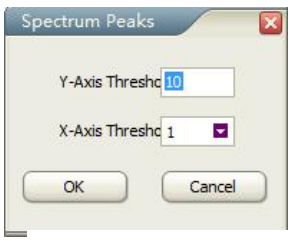
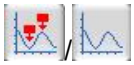


Fig.6-57 Peaks

Click  to show/hide information of the peak coordinates.

6.3.6.4 Smoothing of Spectrum

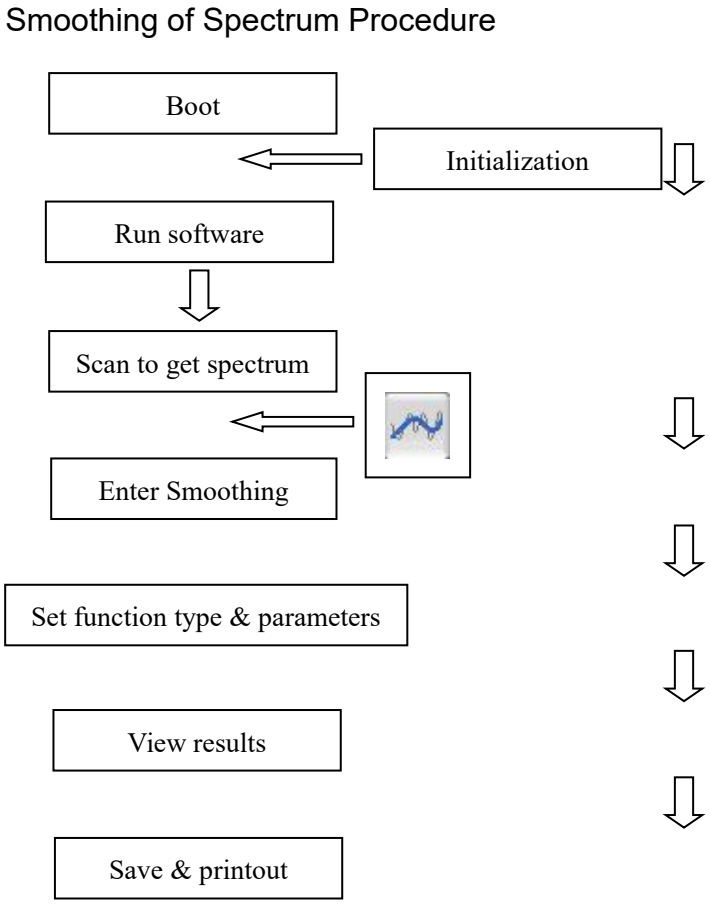


Fig.6-58 Flowchart

Smoothing is to reduce the noise of spectrum. Click  to set smoothing parameters in Fig.6-59. Select function type, Smoothing order, Number of points and Number of times then click “OK” to see the effect.

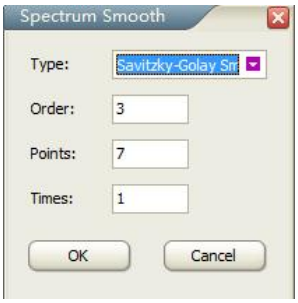


Fig.6-59 Smoothing parameters

Type	Smoothing order	Number of points	Number of times
Savitsky-Golay	The highest power of the polynomial	Set the number of points to be used in calculation.(odd number)	Set the number of smoothing operations.
Mean	----------	Set the number of points to be used in calculation.	Set the number of smoothing operations.
Median	----------	Set the number of points to be used in calculation.	Set the number of smoothing operations.

Tab.6-13 Smoothing Parameters

6.3.6.5 Derivative Operation on Spectrum

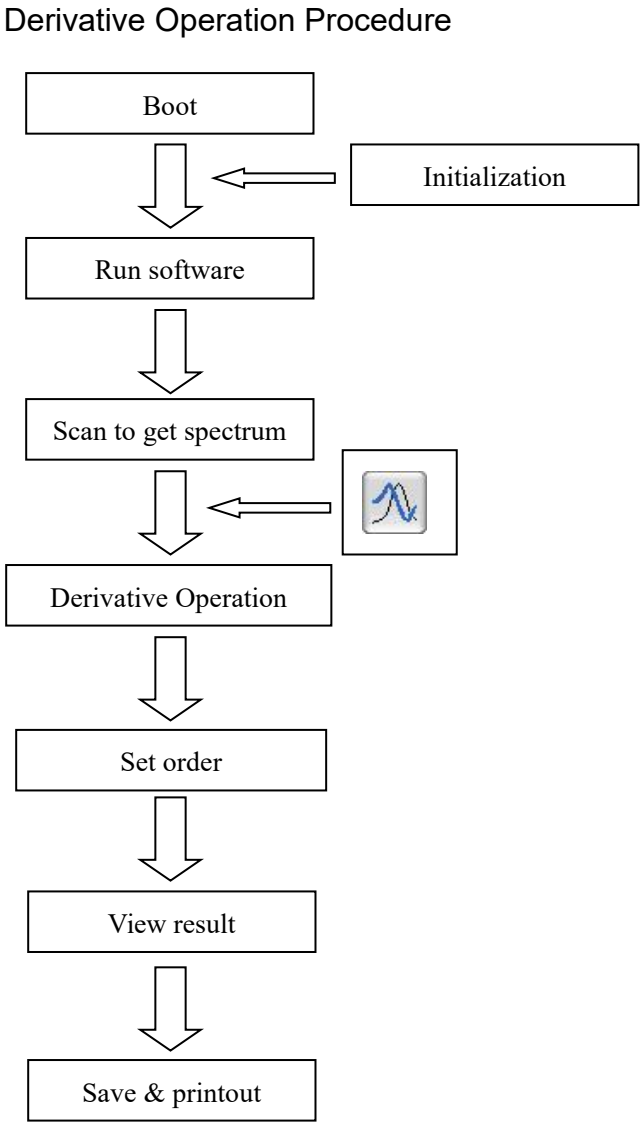


Fig.6-60 Flowchart

Derivative Operation on Spectrum is to enhance the resolution of peaks. Derivation can distinguish various disturbances affecting the shape of the spectrum peaks. Usually combining the smoothing operation.

Click  to open Derivative parameters window. Set Derivative order and click “OK” to see the result.

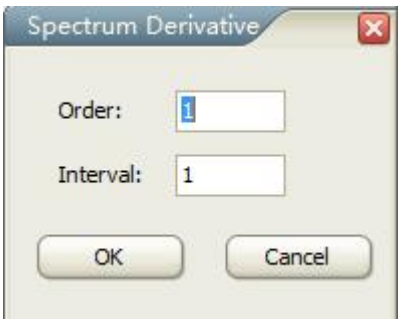


Fig.6-61 Derivative parameters

6.3.6.6 Instrument Settings

This is to change the save path, file name and spectrum type. Click “Settings” →“Options” 

to open settings window in Fig.6-62.

A. General tab:

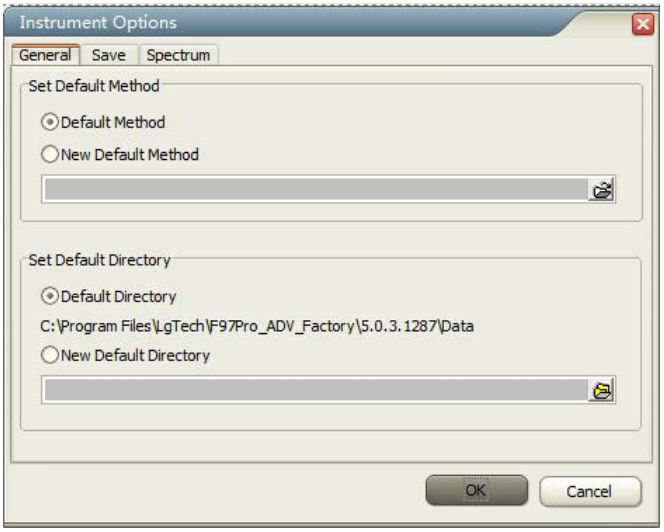


Fig.6-62 General tab

1. Default Method: Instrument use default parameters.
2. Specified method: Instrument uses specified parameters.
3. Default Directory: Instrument uses default path.
4. New Default Directory: Instrument uses specified path.

B. Storage Tab：

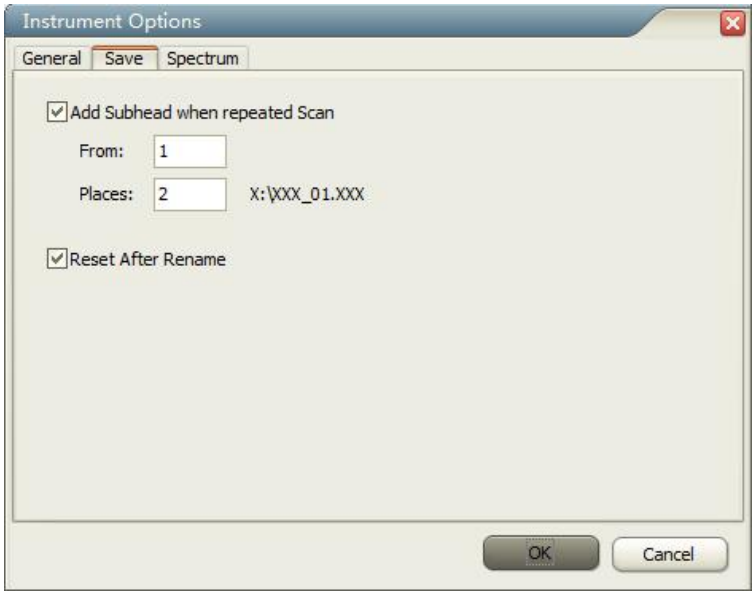


Fig.6-63 Save Tab

1. Add Subhead when repeated scan: Automatically add a number suffix to file names.

2. From: Set the start number.
3. Places: Set number digits.
4. Reset after rename: Auto reset number when using another name.

C. Spectrum Tab：

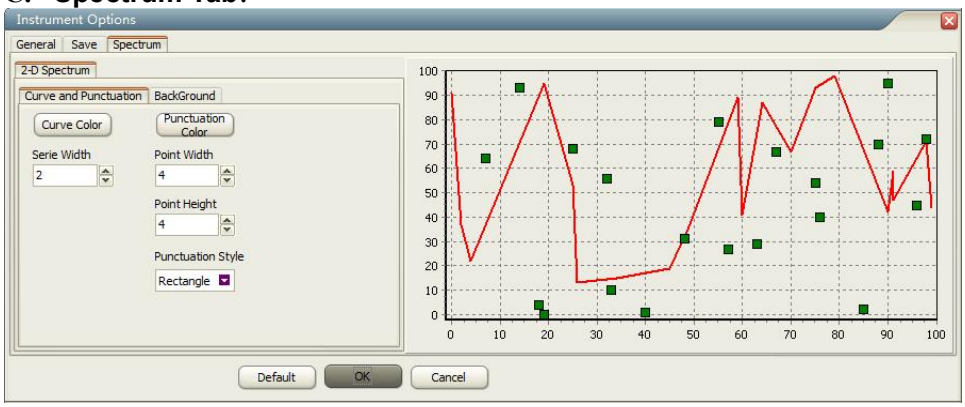


Fig.6-64Spectrum Tab (Curve & Coordinates)

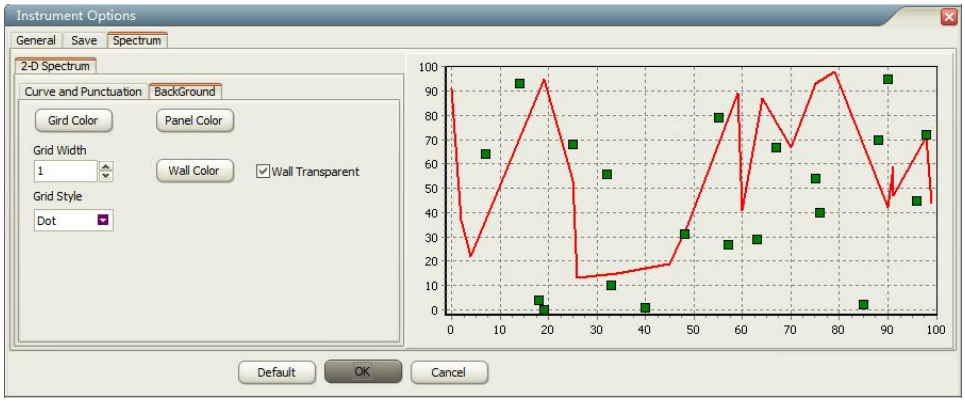


Fig.6-65 Spectrum Tab (Background Setup)

1. Curve & Coordinates Tab: Sets color and width of curves, and color, width, height and shape of dots.
   1) Curve color: Sets curve color.
   2) Serie width: Sets curve width.
   3) Punctuation color: Sets dot color.
   4) Point width: Sets dot width.
   5) Point height: Sets dot height.
   6) Punctuation style: Sets dot shape as rectangle, circle, triangle, down triangle, cross, diagcross, star and diamond.
2. Background Setup: Sets the background color, table color, table style and curve width in “Spectrum information” window.
   1) Grid color: Sets the table color in “Spectrum information” window.
   2) Grid width: Sets the curve width in “Spectrum information” window.
   3) Grid style: Sets table style as solid, dash, dot, dash dot, dash dot dot and clear in “Spectrum” tab.
   4) Panel color: Sets the background color in “Spectrum information” window.
   5) Wall color: Sets the coordinate board color.
   6) Wall Transparent: Check it to make the board transparent.
3. Click “Default” to restore the parameters to default.

6.3.6.7 Rename & Delete Spectrum Files

Right click on a file in file browser. You can delete or rename in the pop-out menu in Fig.6-66.

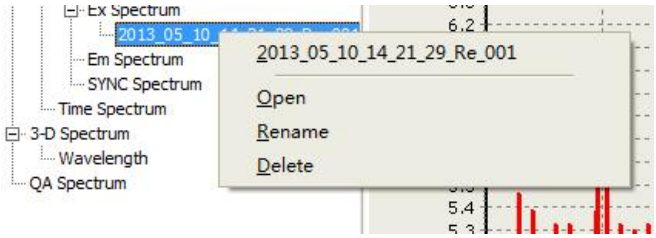


Fig.6-66 Edit File

MAKE SURE YOU WANT TO DELETE THE FILE BEFORE YOU DELETE IT.

6.3.6.7 Reorder Files

Sort by menu: There are 4 ways to sort files: “Name in ASC order”, “Name in DESC order”, “Time in ASC order” and “Time in DESC order” in Fig.6-67. Click “Refresh” to see new order.

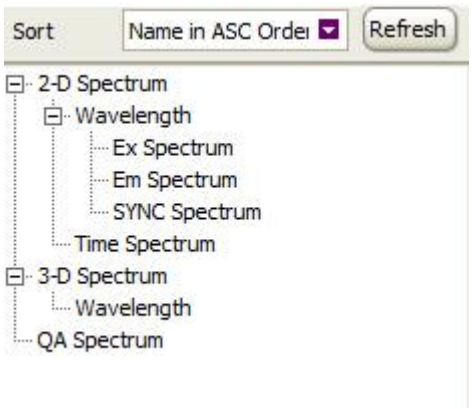


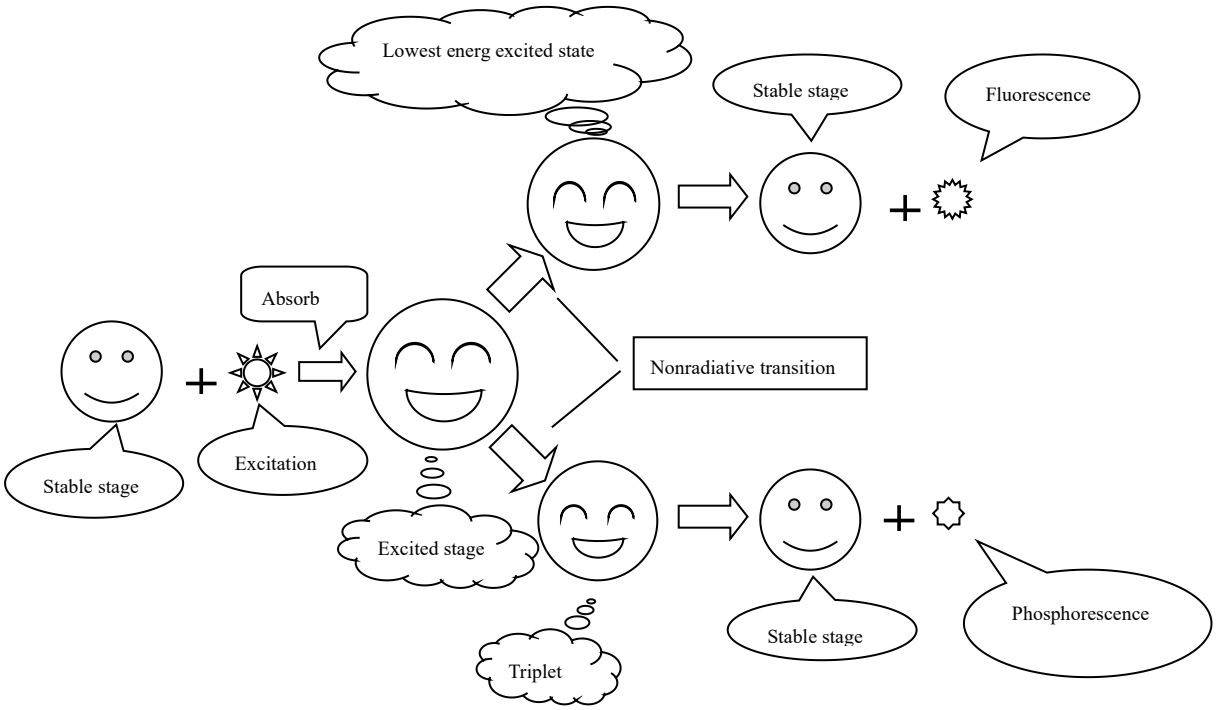
Fig.6-67 Orders

PART III：APPENDIX

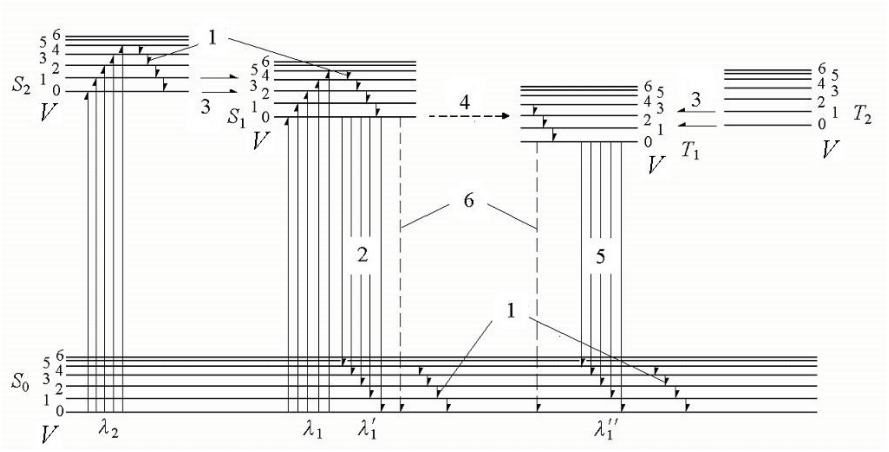
Appendix I: Fluorescence & Phosphorescence

F1.1 Theory

Schematic diagram of luminous



F1-1 Schematic diagram of luminous



F1-2 Typical Organic Molecular Energy Level

1-Vibrational relaxation；2-Fluorescence emission；3-Internal conversion；4-Intersystem crossing；5-Phosphorescence emission；6-External conversion；

S-Singlet；*T*-Triplet；*V*-Vibrational level

Figure F1-2 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).

Molecule in the excited state is very unstable, it can return to the ground state by a variety of ways like radiative transitions and non-radiative transitions, etc.

（1）**Vibrational energy relaxation.** Vibrational energy relaxation is a process in which the population distribution of molecules in quantum states of high energy level caused by an external perturbation returns to the Maxwell–Boltzmann distribution.

In solution, the process proceeds with intra- and intermolecular energy transfer. The excess energy of the excited vibrational mode is transferred to the kinetic modes in the same molecule or to the surrounding molecules. Through this process, the initially excited vibrational mode moves to a vibrational state of a lower energy. The relaxation is called the longitudinal relaxation, and the time constant of the relaxation is called the longitudinal relaxation time, or T1.

（2）**Internal conversion.** Internal conversion is a radioactive decay process where an excited nucleus interacts electromagnetically with an electron in one of the lower atomic orbitals, causing the electron to be emitted (ejected) from the atom.Thus, in an internal conversion process, a high-energy electron is emitted from the radioactive atom, but not from a nucleon in the nucleus. Instead, the electron is ejected as a result of an interaction between the entire nucleus and an outside electron that interacts with it. For this reason, the high-speed electrons from internal conversion are not beta particles, since the latter come from beta decay, where they are newly created in the process. Since no beta decay takes place during internal conversion, the element atomic number does not change, and thus (as is the case with gamma decay) no transmutation of one element to another is seen. However, since an electron is lost, an otherwise neutral atom becomes ionized. Also, no neutrino is emitted during internal conversion.

（3）**Fluorescence emission**. When the non-radiative excited state of molecules cross to singlet through the vibration relaxation, internal conversion and intersystem crossing, and then from singlet back to the ground state by emitting radiation (photons), the emitted photon is phosphorescence, a process known as "fluorescence emission ".

（4）**Intersystem crossing**. Intersystem crossing is a radiationless process involving a transition between two electronic states with different spin multiplicity.

When an electron in a molecule with a singlet ground state is excited (*via*absorption of radiation) to a higher energy level, either an excited singlet state or an excited triplet state will form. A singlet state is a molecular electronic state such that all electron spins are paired. That is, the spin of the excited electron is still paired with the ground state electron (a pair of electrons in the same energy level must have opposite spins, per the Pauli exclusion principle). In a triplet state the excited electron is no longer paired with the ground state electron; that is, they are parallel (same spin). Since excitation to a triplet state involves an additional "forbidden" spin transition, it is less probable that a triplet state will form when the molecule absorbs radiation.

When a singlet state nonradiatively passes to a triplet state, or conversely a triplet transitions to a singlet, that process is known as intersystem crossing. In essence, the spin of the excited electron is reversed. The probability of this process occurring is more favorable when the vibrational levels of the two excited states overlap, since little or no energy must be gained or lost in the transition. As the spin/orbital interactions in such molecules are substantial and a change in spin is thus more favourable, intersystem crossing is most common in heavy-atom molecules (e.g. those containing iodine or bromine). This process is called "spin-orbit coupling". Simply-stated, it involves coupling of the electron spin with the orbital angular momentum of non-circular orbits. In addition, the presence of paramagnetic species in solution enhances intersystem crossing.

The radiative decay from an excited triplet state back to a singlet state is known as phosphorescence. Since a transition in spin multiplicity is observed, phosphorescence is another manifestation of intersystem crossing. The time scale of intersystem crossing is on the order of 10^{-8} to 10^{-3} s, one of the slowest forms of relaxation.

（5）**Phosphorescent emitter.** When the non-radiative excited state of molecules cross to triplet through the vibration relaxation, internal conversion and intersystem crossing, and then from triplet-excited back to the ground state by emitting radiation (photons), the emitted photon is phosphorescence, a process known as "phosphorescent emitter".

（6）**External conversion.** Excited molecules go back to the ground state by interaction with the solvent or other solutes.

As there is energy loss before the fluorescent or phosphorescent emission, the fluorescence and phosphorescence emission wavelengths are greater than the excitation wavelengths.

F1.2 Fluorescence Analysis

During fluorescence emission, the ratio of the number of photons emitted and the number of photons that absorb the excitation light is called the quantum yield of fluorescence.

Quantum yield of fluorescence：$\Phi=\frac{I_F}{I_a}$ （Formula F1-1）

I_F is fluorescence emitted photons; I_a is the number of photons absorbed by molecules. The closer Φ is reaching 1, the stronger the fluorescence of the compound will be. If Φ equals 0 or close to 0, there is no fluorescence. The value of Φ should be between 0 to 1. The value depends on the chemical structure of the material molecules and the environment (such as temperature, pH, solvent, etc.) and other factors.

Fluorescence is emitted by the absorption of light from the material, so the fluorescence intensity of the solution F and the extent of the absorption of light and the quantum fluorescence efficiency of the material:

$$F \propto (I_0\text{-}I_t) \rightarrow F = K_0\Phi (I_0\text{-}I_t) \quad \text{（Fomular F1-2）}$$

As $\frac{I_t}{I_0}=10^{-\varepsilon bc} \Longrightarrow I_t=I_0*10^{-\varepsilon bc}$, According to Lambert's law:

$$F = K_0\Phi (I_0 - I_0 10^{-\varepsilon bc}) = K_0\Phi I_0 (1-10^{-\varepsilon bc}) = K_0\Phi I_0 (1-e^{-2.303\varepsilon bc})$$

（Formula F1-3）

Φ is the quantum efficiency of the fluorescent substance, which is a constant. K_0 is a constant of the instrument.

Expand $e^{-2.303\varepsilon bc}$ into formula F1-3:

$$F = K_0\Phi I_0[2.303\varepsilon bc - \frac{(2.303\varepsilon bc)^2}{2!} + \frac{(2.303\varepsilon bc)^3}{3!} + \cdots] \quad \text{(Formula F1-4)}$$

When $2.303\varepsilon bc \leq 0.05$, the items after the first item in the brackets are negligible:

$$F = K_0\Phi I_0 2.303\varepsilon bc \quad \text{（Formula F1-5）}$$

Where,
F: Intensity of fluorescence
K_0: Instrumental constant
I_0: Intensity of exciting radiation
b: The optical path of the cuvette
c: Concentration of substance
ε : Absorptivity of substance
Φ : Quantum efficiency of substance

Therefore, for a dilute solution of a fluorescent substance and constant K_0, I_0, b:

$$F=K\cdot c \quad \text{（Formula F1-6）}$$

This means if $2.303\varepsilon bc \leq 0.05$, the fluorescence intensity of the solution is approximately linear with the concentration of the fluorescent substance.

Appendix II：Spectral correction

F2.1 Theory

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.

Needed:

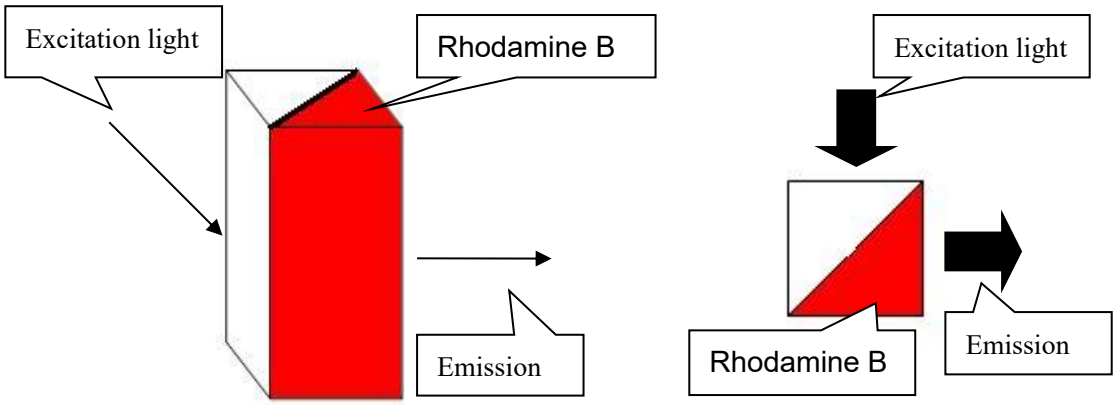
1. Fluorescence spectrophotometer
2. Triangle quartz cell
3. 3.0g/L—6.0g/L Rhodamine B
4. A scatterer

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.

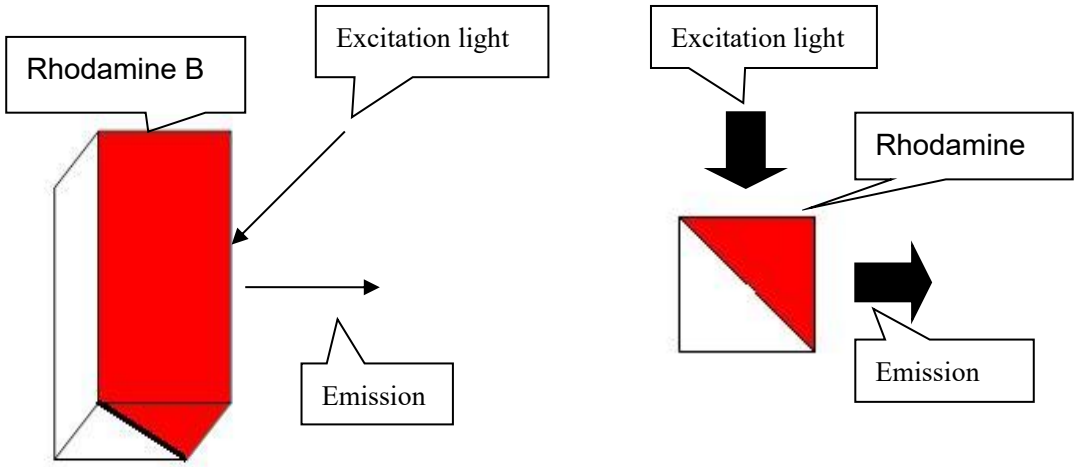
F2.2 Correction of the Exitation Monochromator

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.

The triangular cell filled with Rhodamine B should in principle be stored at a dark place.



1 Direction 1



F2-2 Direction 2

Pour Rhodamine B into a triangular cell in the procedure illustrated in F2-1 or F2-2.Directions in F2-1 is recommended. Reason 1: Reducing over absorption high concentration sample; 2: Reduce excitation light scattering interference.

Click “Settings” →“Spectrum Correction” 

to set calibration parameters.

1. Emission wavelength: 640nm
2. Excitation start wavelength: 250nm
3. Excitation end wavelength: 600nm
4. Scan speed: According to the actual situation
5. Delay: Time before scan.
6. EX Slit:: According to the actual situation.
7. EM Slit:: According to the actual situation.
8. PMT: According to the power.
9. Response: Auto.

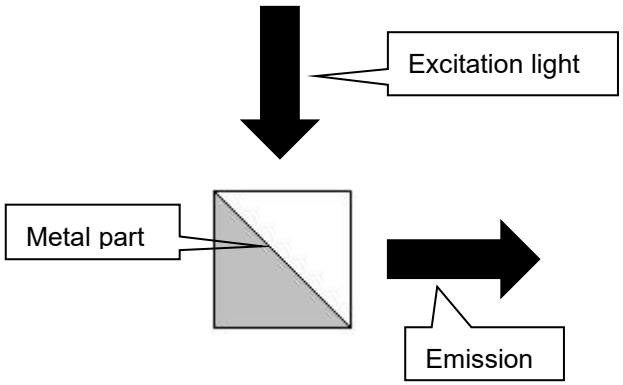
Click “Start Calibration” to start. Correct data will be automatically saved.

F2.3 Correction of the Emittion Monochromator

This is the function to obtain the instrumental response on the emission side such as wavelength characteristics of the emissionmonochromator 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.

Pour Rhodamine B into a triangular cell in the procedure illustrated in Fig. F2-3. The triangular cell filled with Rhodamine B should in principle be stored at a dark

place.



F2-3 Direction

Click “Emission Calibration” tab to set parameters.

1. Excitation wavelength: 640nm
2. Emission start wavelength: 250nm(same as excitation)
3. Emission end wavelength: 600nm(same as excitation)
4. Scan speed: According to the actual situation.
5. Delay: Time before scan.
6. EX Slit: F97:10nm; F97Pro & F97XP: According to the actual situation.
7. EM Slit: F97:10nm; F97Pro & F97XP: According to the actual situation.
8. PMT: According to the power.
9. Response: Auto.

Click “Start Calibration” to start scan. At the end of scan there will be a confirm window, click “OK” to confirm all the corrections are done. Correct data will be automatically saved.

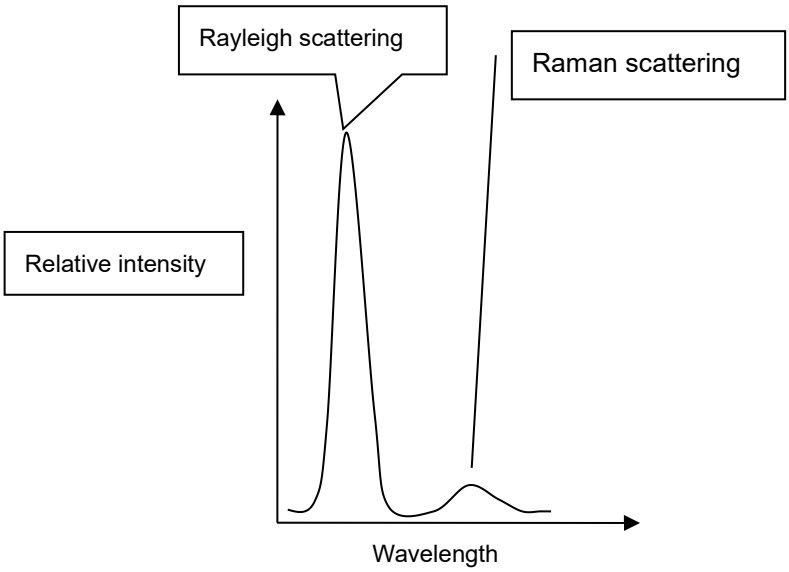
Note: If you want to recalibrate the EM side, you should start from EX side.

Appendix III： Raman Scattering of Water & Detection Limit of Quinine Sulfate

F3.1 Raman Scattering of Water

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.



F3-1 Water Raman Scattering

F3.2 Water Raman Scattering S/N Ratio

Max peak value is S_{max}. Minimum peak value is S_{min}:

Signal：S= S=$\frac{S_{max}+S_{min}}{2}$（Formula F3-1）

Noise：N= $S_{max} - S_{min}$（Formula F3-2）

S/N：$\frac{S}{N} = \frac{S_{max}+S_{min}}{2(S_{max}-S_{min})}$（Formula F3-3）

F3.3 Detection Limit of Quinine Sulfate

Set the excitation wavelength at 350nm and the emission wavelength at 450nm. Alternately measure 1×10^{-9} quinine sulfate and 0.05mol/L sulfuric acid blank solution fluorescence values.

$F_i = F_{i1} - F_{i0}$（Formula F3-4）

F_{i1}: Fluorescence of standard quinine sulfate solution

F_{i0}: Fluorescent of Standard blank solution

Average fluorescent:

$$\bar{F} = \frac{1}{11}\sum_{i=1}^{11} F_i \text{（Formula F3-5）}$$

The detection limit is twice the standard deviation of the reading material concentration：

$$DL = \frac{C}{\bar{F}} \times 2 \times s \text{（g/ml）（Formula F3-6）}$$

DL: Detection limit

C: Concentration of quinine sulfate

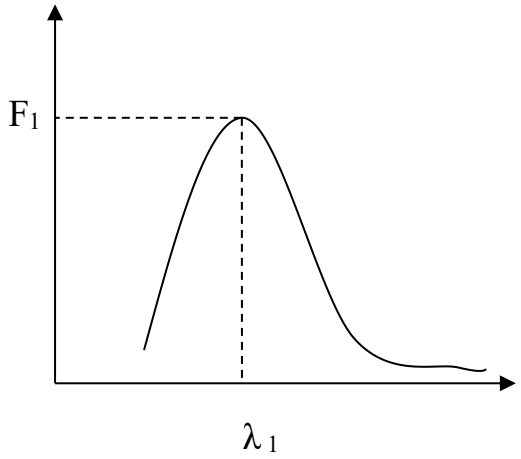
$\bar{F}$: Average fluorescent

S: The standard deviation

$$s = \sqrt{\frac{\sum_{i=1}^{11}(F_i-\bar{F})^2}{11-1}} \text{（Formula F3-7）}$$

Appendix IV：Quantitative analysis

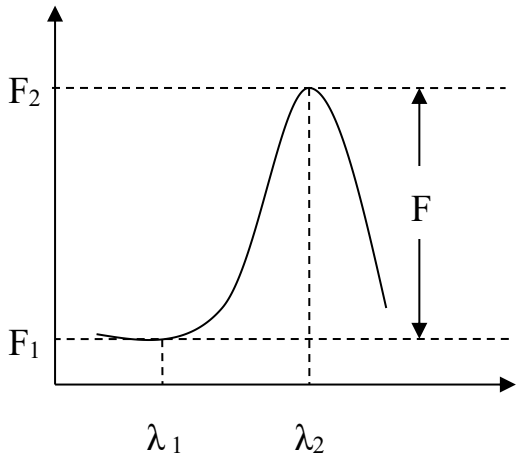
F4.1 Single Wavelength



F4-1 Single wavelength

Fluorescence F_1 is the value on the curve at λ_1.

F4.2 Double Wavelengths

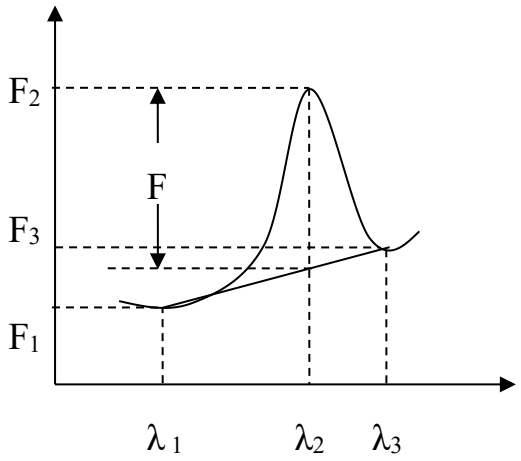


F4-2 Double wavelengths

In F4-2, F_1 and F_2 are the Fluorescence at λ_1 and λ_2.

$$F=F_2 - F_1$$

F4.3Triple Wavelengths



F4-3 Triple wavelengths

In F4-3, F_1 , F_2 and F_3 are the Fluorescence at λ_1 , λ_2 and λ_3.

$$F = F_2 - \frac{(\lambda_1 - \lambda_2) \times F_3 + (\lambda_2 - \lambda_3) \times F_1}{\lambda_1 - \lambda_3}$$

Appendix V： DETAILS ON QUANTITATIVE

F97 series provide 3 calibration types: Linear working curve, Quadratic working curve and Cubic working curve. All of them are not forced through the 0 coordinates.

F5.1 Linear Working Curve (1st order)

The calculation formula is as follow:

$$C = A_1 \times F + A_0$$ （Formula F5-1）

Where,

C : Concentration of each sample (input value)

F : Data on each sample (measured value)

A_1 and A_0 are calculated by the least squares method

Suppose there are ndata points(F_n， C_n)，then

$$A_1 = \frac{\sum_{i=1}^{n} F_i C_i - \frac{1}{n}\sum_{i=1}^{n} F_i \cdot \sum_{i=1}^{n} C_i}{\sum_{i=1}^{n} F_i^2 - \frac{1}{n}(\sum_{i=1}^{n} F_i)^2}$$ （Formula F5-2）

$$A_0 = \frac{\sum_{i=1}^{n} C_i}{n} - A_1 \times \frac{\sum_{i=1}^{n} F_i}{n}$$ （Formula F5-3）

F5.2 Quadratic Working Curve (2nd order)

The calculation formula is as follows：

$$C = A_2 \times F^2 + A_1 \times F + A_0$$ （Formula F5-4）

Where, C: Concentration of each sample (input value)

F: Data on each sample (measured value)

A_2 , A_1 and A_0 are calculated by the least squares method

Suppose there are n data points(F_n， C_n)，then：

$$A_2 = \frac{S(F^2C)S(FF) - S(FC)S(FF^2)}{S(FF)S(F^2F^2) - [S(FF^2)]^2}$$ （Formula F5-5）

$$A_1 = \frac{S(FC)S(F^2F^2) - S(F^2C)S(FF^2)}{S(FF)S(F^2F^2) - [S(FF^2)]^2}$$ （Formula F5-5）

$$A_0 = \frac{\sum_{i=1}^{n} C_i}{n} - A_1\frac{\sum_{i=1}^{n} C_i}{n} - A_2\frac{\sum_{i=1}^{n} F_i^2}{n}$$ （Formula F5-7）

$$S(FF) = \sum_{i=1}^{n} F_i^2 - \frac{(\sum_{i=1}^{n} F_i)^2}{n}$$ （Formula F5-8）

$$S(FC) = \sum_{i=1}^{n} F_iC_i - \frac{\sum_{i=1}^{n} F_i \cdot \sum_{i=1}^{n} C_i}{n}$$ （Formula F5-9）

$$S(FF^2) = \sum_{i-1}^{n} F_i^3 - \frac{\sum_{i=1}^{n} F_i \cdot \sum_{i=1}^{n} F_i^2}{n}$$ （Formula F5-10）

$$S(F^2C) = \sum_{i=1}^{n} F_i^2C_i - \frac{\sum_{i=1}^{n} F_i^2 \cdot \sum_{i=1}^{n} C_i}{n}$$ （Formula F5-11）

$$S(F^2F^2) = \sum_{i=1}^{n} F_i^4 - \frac{(\sum_{i=1}^{n} F_i^2)^2}{n}$$ （Formula F5-12）

F5.3 The correlation coefficient

The correlation coefficient R represents how the regression curve fitting.

Suppose there are n data points:

$$R = \frac{\sum_{i=1}^{n} C_iF_i - \frac{\sum_{i=1}^{n} C_i \cdot \sum_{i=1}^{n} F_i}{n}}{\sqrt{\left(\sum_{i=1}^{n} C_i^2 - \frac{(\sum_{i=1}^{n} C_i)^2}{n}\right)\left(\sum_{i=1}^{n} F_i^2 - \frac{(\sum_{i=1}^{n} F_i)^2}{n}\right)}}$$ （Formula F5-13）

Appendix VI：Synchronous Scan

The difference between Synchronous scan and other usual scan is the excitation side and emission side scan at the same time. The fluorescent value and wavelength spectrum is synchronous fluorescent spectrum.

F6.1 Constant Wavelength Difference

Constant wavelength difference is to keep a fixed wavelength difference between excitation wavelength and emission wavelength in the scanning process(Δλ= λex- λem = constant). The choice of Δλ is very important. This will directly affect the synchronous fluorescence spectra shape, slit and signal strength. Usually use Stokes shift as Δλ.

Constant wavelength difference method can be used for measuring multi-component PAHs or a variety of amino acid mix (such as mixing phenylalanine, tyrosine and tryptophan). PAHs properties are very similar, in spite of strong fluorescence, but a variety of excitation and emission spectra overlap is often severe for the classical fluorescence analysis. Constant wavelength difference method is more sensitive and less interference.

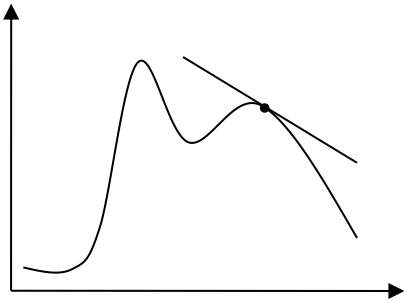
F6.2 Constant Energy Difference

Constant energy difference method shows significant effect in improving sensitivity and overcoming Raman scattering. Constant energy difference method is to keep Δσ as constant. $\Delta\sigma=(\frac{1}{\lambda_{ex}}-\frac{1}{\lambda_{em}})\times 10^{-2}$.

Constant energy difference is based on quantum characteristic energy vibration transitions of phosphor. If fixed energy difference $\Delta E=\Delta\sigma\times 10^{9}\times h$ (h is Planck's constant) equal to the difference between a vibrational energy, when the excitation energy and emission energy exactly matches a specific absorption and emission transition conditions, the synchronous spectrum will be at maximum. Constant energy difference method is good for the identification and determination of PAHs.

Appendix VII： Derivative Operation on Spectrum

The derivative of a function of a real variable measures the sensitivity to change of a quantity (a function or dependent variable) which is determined by another quantity (the independent variable).



F7-1 Derivative of the function

There are many ways of derivative operation on spectrum. Since the x-axis(time axis or the wavelength axis, etc.) of the original spectral data are equally spaced, then

First order derivative：

$$\frac{dy}{dx} = \frac{y_{i+1} - y_i}{\Delta x}$$ （Formula F7-1）

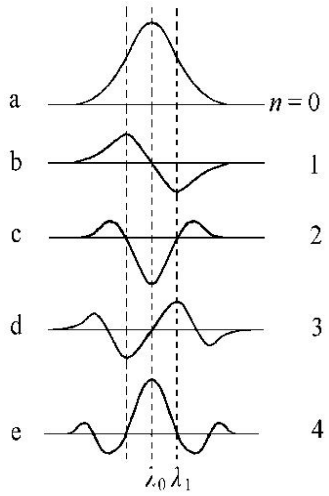
Second order derivative：

$$\frac{d^2y}{dx^2} = \frac{y_{i+1} - 2y_i + y_{i-1}}{\Delta x^2}$$ （Formula F7-2）

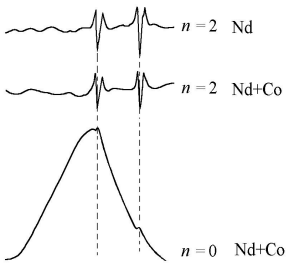
Where：y: fluorescence

x: wavelength, time, etc.

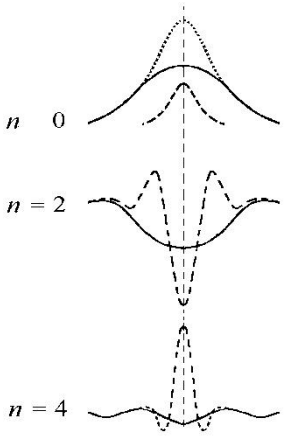
Derivative spectra not only can eliminate baseline drift or flat background interference, but also can provide a higher resolution than the original spectrum.



F7-2



F7-3



F7-4

In F7-2,there is a clear alternation of peaks. In F7-3, the acromion is higher after derivative. Second order derivative spectrum is clearer. In F7-4, the original spectrum two curves are seriously overlapping, but in n=2/4 the peaks are clearer.

Higher order derivative can eliminate the low order background curves. The spectrum shape is complicated after derivative, but it raises the resolution and sensitivity.

Appendix VIII： Smoothing

The basic idea of smoothing is to map a smooth point,thendepicte a number of points around the smooth point to be "fit" or "average" or "sort" in order to obtain the best estimate of the value of the smooth point to eliminate random noise. With modern analytical instruments increasing speed and automation, multiple accumulate and smoothing technology has become a common method of noise reduction.

F97 series provide 3smoothing methods: Savitsky-Golay, Mean and Median.

F8.1Savitzky–Golay

A **Savitzky–Golay filter** is a digital filter that can be applied to a set of digital data points for the purpose of smoothing the data, that is, to increase the signal-to-noise ratio without greatly distorting the signal. This is achieved, in a process known as convolution, by fitting successive sub-sets of adjacent data points with a low-degree polynomial by the method of linear least squares . When the data points are equally spaced an analytical solution to the least-squares equations can be found, in the form of a single set of "convolution coefficients" that can be applied to all data sub-sets, to give estimates of the smoothed signal, (or derivatives of the smoothed signal) at the central point of each sub-set.

F8.2 Mean

M**ean smoothing** is a calculation to analyze data points by creating a series of averages of different subsets of the full data set.

F8.3 Median

Median smoothing is to sort the selected data (the number of data points is odd), then take the intermediate value as the smoothed value.

Appendix IX： Phosphorescence

F9.1 Theory

Phosphorescence is a specific type of photoluminescence related to fluorescence. Unlike fluorescence, a phosphorescent material does not immediately re-emit the radiation it absorbs. The slower time scales of the re-emission are associated with "forbidden" energy state transitions in quantum mechanics. As these transitions occur very slowly in certain materials, absorbed radiation may be re-emitted at a lower intensity for up to several hours after the original excitation.

Most photoluminescent events, in which a chemical substrate absorbs and then re-emits a photon of light, are fast, on the order of 10 nanoseconds. Light is absorbed and emitted at these fast time scales in cases where the energy of the photons involved matches the available energy states and allowed transitions of the substrate. In the special case of phosphorescence, the absorbed photon energy undergoes an unusual intersystem crossing into an energy state of higher *spin multiplicity* (*see term symbol*), usually a triplet state. As a result, the energy can become trapped in the triplet state with only classically "forbidden" transitions available to return to the lower energy state. These transitions, although "forbidden", will still occur in quantum mechanics but are kinetically unfavored and thus progress at significantly slower time scales. Most phosphorescent compounds are still relatively fast emitters, with triplet lifetimes on the order of milliseconds.

Since phosphorescence usually last longer than fluorescence, it is possible to use F97 series for phosphorescent analysis. There is a shutter to do chopper operation. When the sample is excited excitation light, use the shutter to chop the light path, the sample is no longer irradiated by excitation light.The instrument can get phosphorescence because phosphorescence usually last longer than fluorescence.

F9.2 Phosphorescence Wavelength Scan

Phosphorescence wavelength scan is to scan the phosphorescence of samples, in order to gain information about the wavelength of phosphorescence. There are two scan modes: excitation scan and emission scan.

Phosphorescence Wavelength Scan Notes：

1. Phosphorescence Life. This is closely related to the chop time of the instrument. If the phosphorescence life is shorter than the chop time, the instrument can not get the phosphorescence signal. The max scan speed is important. The instrument can not get the phosphorescence signal if the phosphorescence died before the scan completed. We should make sure that the phosphorescence life is longer than chop time and scan speed.
2. Set the “Phosphorescence excitation time”. It is the excitation time of samples. Set the excitation time according to different samples.
3. Set the “Delay” time. This is the delay time before the scan. If the delay time is

too short, the excitation may not be chopped, then the phosphorescence may contain interference of excitation light. We recommend to set the delay time no less than 18ms.

F9.3 Phosphorescence Time Scan

Phosphorescence wavelength scan is to do a time scan of the phosphorescence of samples to get information of phosphorescence life or phosphorescence decay rate. Delay time should be no less than 18ms.

F9.4 Applications

Phosphorescence analysis is mainly used for the determination of organic compounds, such as the analysis of polycyclic aromatic hydrocarbons and petroleum products, the analysis of pesticides, alkaloids and plant growth hormones, drugs and clinical analysis. Phosphorescence analysis techniques have been applied to the study of biological active substances testing and cell biology, biochemistry etc.

Appendix X：Chemiluminescence

Chemiluminescence is the emission of light, as the result of a chemical reaction. There may also be limited emission of heat. Given reactants A and B, with an excited intermediate [◊],

[A] + [B] → [◊] → [Products] + light

The decay of this excited state[◊] to a lower energy level causes light emission. In theory, one photon of light should be given off for each molecule of reactant. This is equivalent to Avogadro's number of photons per mole of reactant. In actual practice, non-enzymatic reactions seldom exceed 1% Q_C, quantum efficiency.

In a chemical reaction, reactants collide to form a transition state, the enthalpic maximum in a reaction coordinate diagram, which proceeds to the product. Normally, reactants form products of lesser chemical energy. The difference in energy between reactants and products, represented as ΔH_{rxn}, is turned into heat, physically realized as excitations in the vibrational state of the normal modes of the product. Since vibrational energy is generally much greater than the thermal agitation, it rapidly disperses in the solvent through molecular rotation. This is how exothermic reactions make their solutions hotter. In a chemiluminescent reaction, the direct product of a reaction is an excited electronic state, which then decays into an electronic ground state

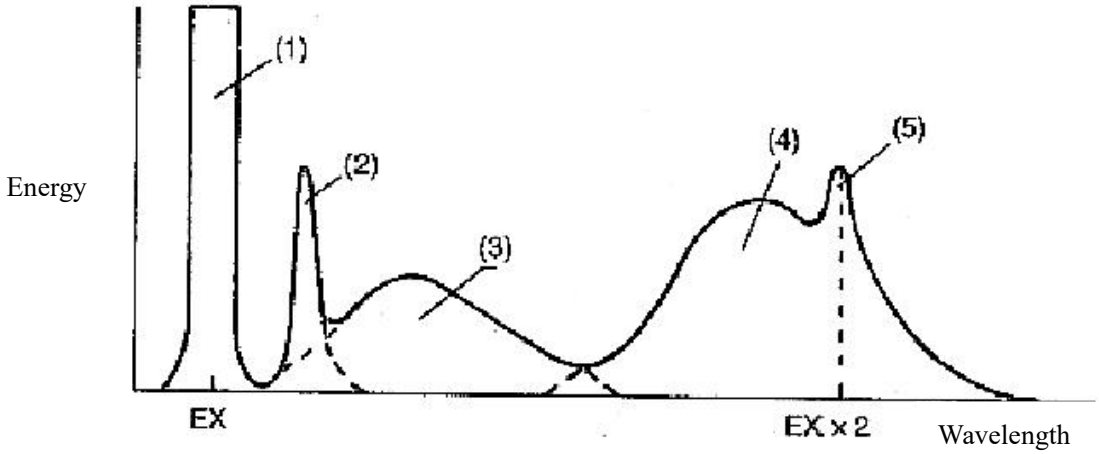
through either fluorescence or phosphorescence, depending partly on the spin state of the electronic excited state formed.

Chemiluminescence differs from fluorescence in that the electronic excited state is derived from the product of a chemical reaction rather than the more typical way of creating electronic excited states, namely absorption. It is the antithesis of a photochemical reaction, in which light is used to drive an endothermic chemical reaction. Here, light is generated from a chemically exothermic reaction.

Appendix XI：Multiple Excitation Scattering

Due to the grating monochromator factors, during the wavelength scan (including 2D& 3D), the photomultiplier tube will receive multiple scattered light from excitation light. Scattering occurs on the wavelengths which is 2 or 3 times of excitation wavelength.

Excitation light spectrum In F11-1.



F11-1 Excitation light spectrum

（1）Excitation scattering（2）Raman scattering；（3）Interfering peaks；（4）Fluorescence peaks；（5）2nd excitation scattering

Put a light cut filter in the excitation light will eliminate this effects.

Appendix XII： Accessories

Type	Name	Description
Optional Spare Parts & Accessories	Quartz fluorescence cell10mm	10mm 4-side-clear quartz cell
	Glass fluorescence cell10mm	10mm 4-side-clear glass cell
	Fuse（2A/5A）	Fuse
	USB cable	USB cable
	Power cable	Power cable
	Cut-off filters	Eliminate the scattering of excitation
	Multi-function base	Base of other holder
	Membrane sample holder	Combine with Multi-function base for membrane sample
	Powder sample holder	Combine with Multi-function base for powder sample
	10mm cell holder adapter	Combine with Multi-function base for 10mm cuvette
	Peltier temperature control holder	Set temperature within 15-45℃
	Auto Polarization Filter	Get polarized light both excitation side and emission side
	Fluorescence Quantum Yield accessory	Measuring the absolute quantum yield