

Operation Manual



BSFL-103

Fluorescence Spectrophotometer

Thank you for Choosing Biolab products. Please read the "Operating Instructions" and "Warranty" before operating this unit to assure proper operation.

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Preface

The method of fluorescence analysis (fluorometry) is a modern analytical method of high PMT level and high selectivity. It much can provide information, including an excitation spectrum, an emission spectrum, fluorescent intensity, fluorescence life, and fluorescence polarization. The linear range of working curve is wide and hence it has been an important analytical method in the field of trace analysis.

The BSFL-103 Fluorospectrophotometer is a fluorophotometer using filters with a monochromator. It has three functions, namely, wavelength scan, time scan for fluorescent intensity, and quantitative analysis. The instrument is capable of performing two modes of measurement: measuring with a single instrument only, and measuring with an on-line computer. The amounts of the functions and the convenience of measuring vary with the different modes of measurement.

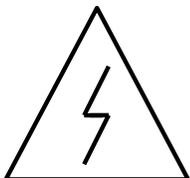
For proper use of the BSFL-103 Fluorospectrophotometer, the user of the instrument should have a basic knowledge of using optical instruments and a basic knowledge of molecular fluorescence spectroscopy. The user whose instrument is equipped with a computer also should have a corresponding knowledge and skills of operating the computer.

Before installation or the first use of this instrument, the user must read through this instruction manual carefully. According to the user's different equipment, the spectrophotometer main unit may be equipped with an external computer or a special printer. Our company guarantees that if the instrument is subject to damage or fails to work normally due to the imperfect manufacture of our product, the company will be responsible for the repair of the instrument (excluding quick-wear parts and consumables) without charge within the warranty period of 12 months from the delivery date under the condition that the user complies with the rules of transport, maintenance and operation. If the instrument is in need of repair, please send it and its accessories back to our company, along with the quality assurance card of our product.

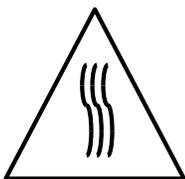
Alert Symbols of Safe Operation



Symbol indicates important information of operating the instrument. Please read carefully and operate according to the instructions following.



This symbol indicates a potentially hazardous electrical appliance through the operation, and indicates that a qualified professional man is needed to deal with such a hazard according to the corresponding procedures. (This label is attached on the power switch and trigger.)



This symbol indicates heating on the surface. Please perform cautiously according to the instructions following.

Points for Attention to Operation

1. The BSFL-103 Fluorospectrophotometer is suitably used in a laboratory room to perform analytical measurements. If the instrument needs to be used at a worksite, the working environment of the worksite should accord with the demands of the laboratory environment basically.
2. If the user needs to move the instrument to other working site for analytical measurements, please use original packing materials on moving.
3. Be sure to turn on/off the power of the instrument according to the operating procedure. When starting up the instrument, please turn on the power source of the xenon lamp first. Then turn on the power source of the spectrophotometer main unit after the xenon lamp is lit. When shutting down the instrument, please turn off the power source of the spectrophotometer first and then the power source of the xenon lamp. Otherwise, the instrument may not work normally. After the xenon lamp is lit, it is necessary to wait for 30 minutes over till the lamplight becomes stable. After the power source of the xenon lamp is turned off, please wait for 60 seconds, and then turn on the lamp if the lamp needs to be used again.
4. When the power source of the xenon lamp is turned on but the lamp fails to be lit and a squeaking sound of high frequency or a cracking sound of sparking is produced continuously, please turn off the power source of xenon lamp immediately and wait for several seconds, then turn on the lamp again. Because the life of the xenon lamp is closely related to the number of on-off of the lamp power source, the lamp should avoid being turned on/off repeatedly under a high voltage and the unnecessary number of on/off of the xenon lamp power source should be cut down as far as possible.
5. When the instrument is working, the surface temperature of the heat dissipation opening at the top of the left back side of the instrument (xenon lamp position) becomes very high. Be sure not to touch the surface of the heat dissipation opening with hands in order not to be burnt. The air around the heat dissipation opening should be kept circulating.
6. After each startup of the instrument, please first confirm that the heat dissipation fan at the side of the instrument runs normally in order to ensure normal work of the instrument. If the user finds anything wrong with the heat dissipation fan, the instrument should be closed down for an overhaul.
7. In order to protect the photomultiplier when PMT level is higher (>6), do not let high light

pass into the sample compartment. When a sample of unknown concentration is measured, PMT level should be set from low grade to high grade (1-17) gradually.

8. After PMT level is adjusted, the original set for zero-point of fluorescence should be checked and note that zero-point adjustment may be needed again.

9. When and only when a computer error is due to the operator¹'s false operation or other interferences, the power switch of the spectrophotometer main unit should be turned off immediately. When the software cannot be operated, please use task manager to end the "BSFL-103pc.exe" process, then restart the software and the instrument.

10. Within the monochromator the tightened screws must not be loosened. The optical devices and the working environment of the instrument should be kept clean.

11. When opening the cover to inspect the instrument, be sure to turn off the power source. Particularly after the upper cover of the instrument is removed, as there are electrical appliance parts with high voltage located at the left back of the instrument base, be sure not to contact these parts while the power source is still turned on.

01 Appearance and Performances of the Instrument

1. Appearance of the Complete Instrument and Main Components

1.138% The Complete InstrumentThe Complete Instrument

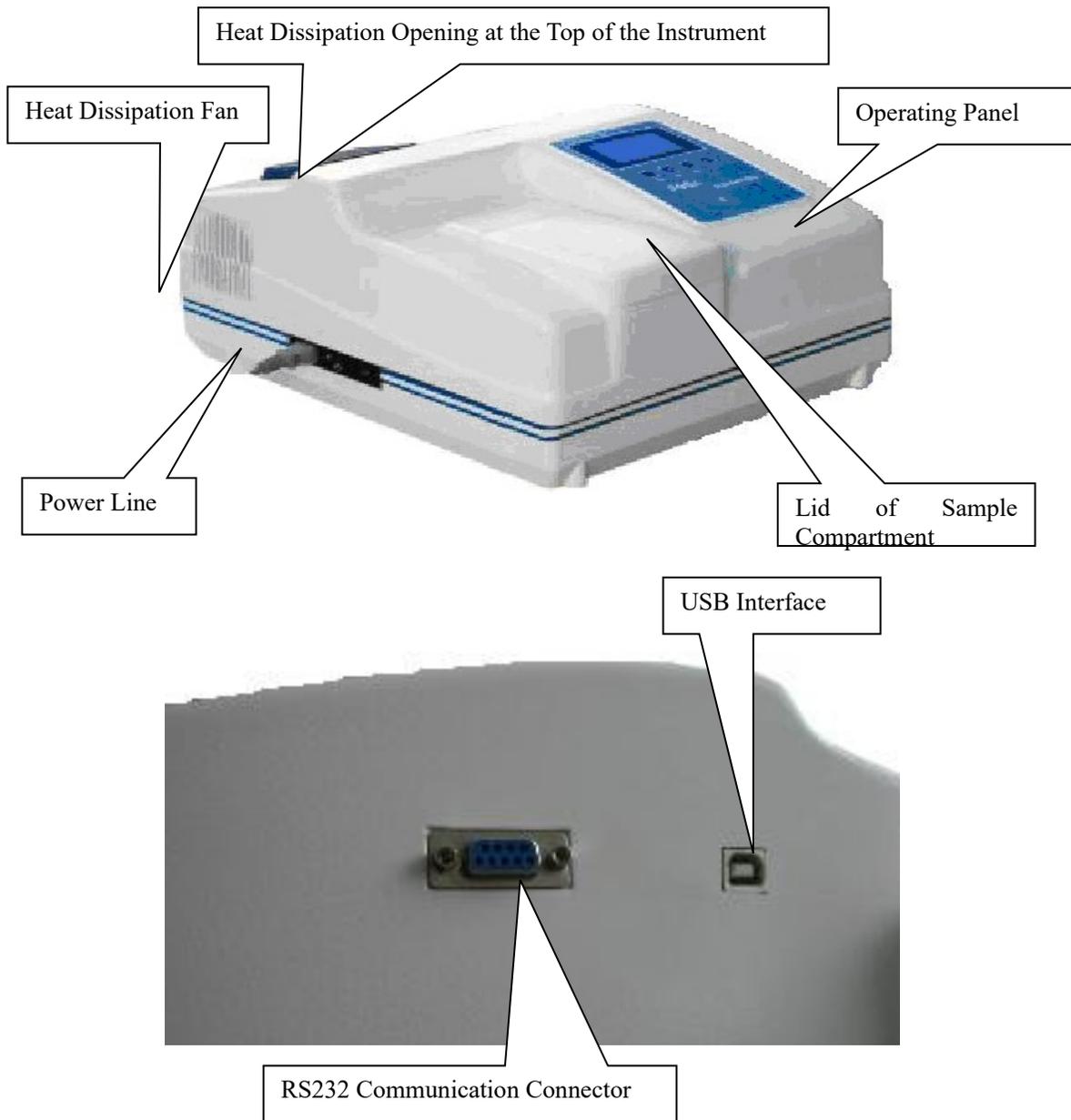


Fig. 1-1 BSFL-103 Fluorescence Spectrophotometer Main Unit

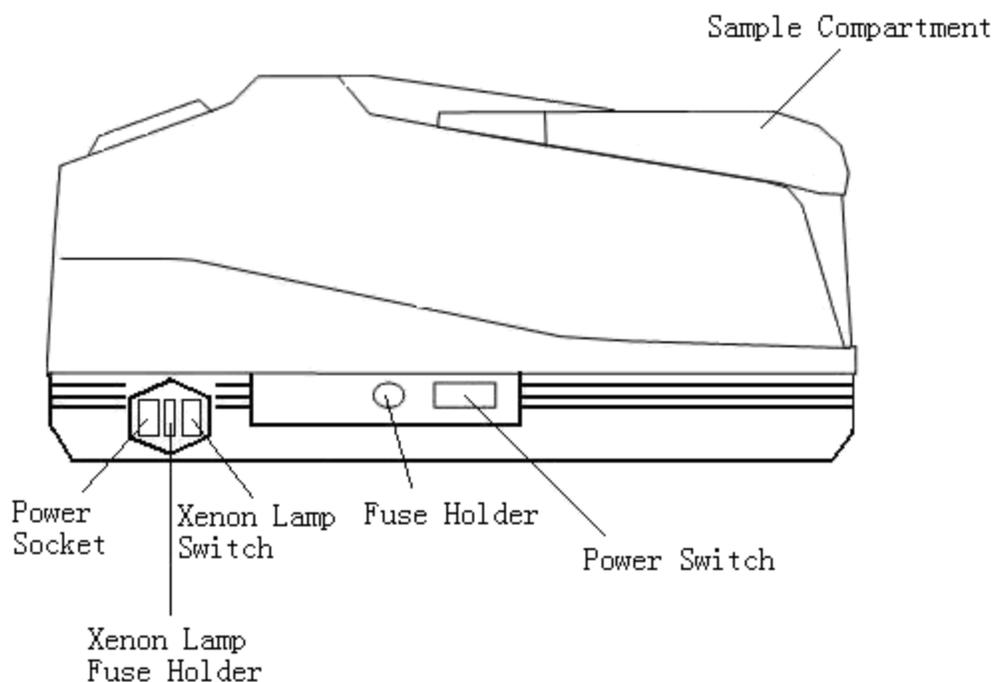


Fig. 1-2 The Left Side View of BSFL-103 Fluorescence Spectrophotometer Main Unit Sample Compartment (inside it a cell holder used to hold a sample cell), Power Switch (used to turn on/off the power source of the instrument), Fuse Holder (used to mount a fuse), Power Socket (used to interconnect a feed cable), Xenon Lamp Switch (used to turn on/off the Xenon lamp).

Main Components

1. Xenon Lamp

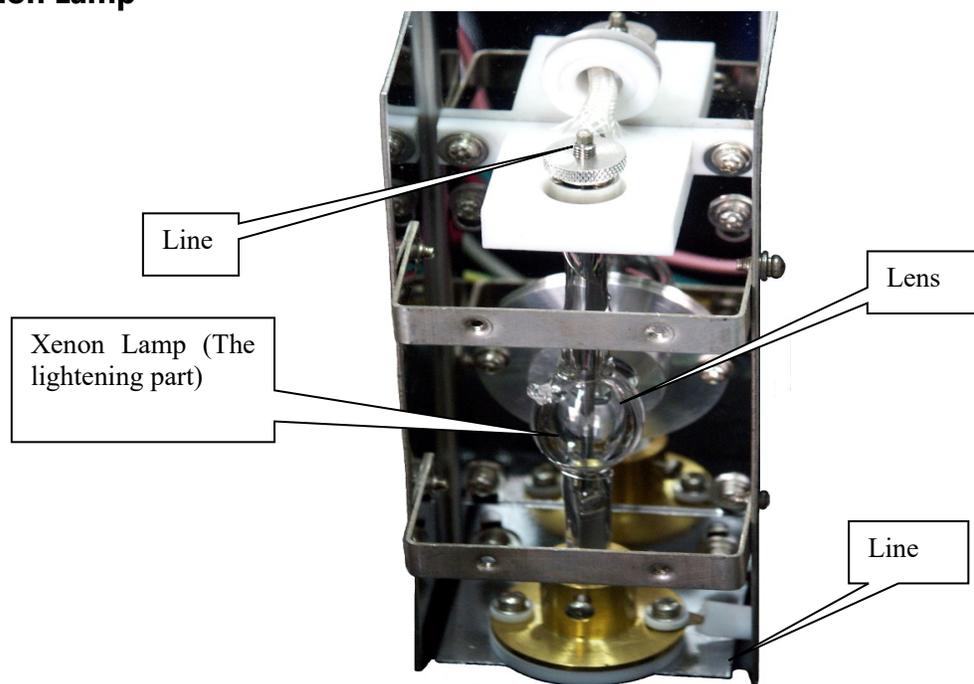


Fig. 1-3 Xenon Lamp and Lamp Holder

2. Sample Compartment

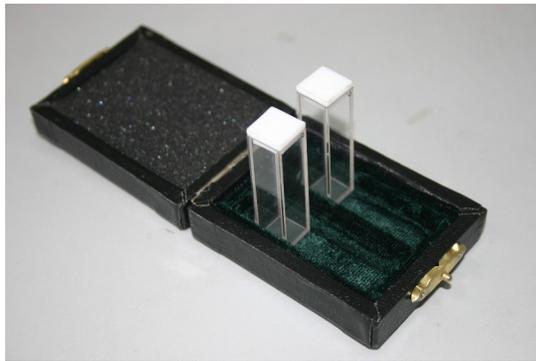
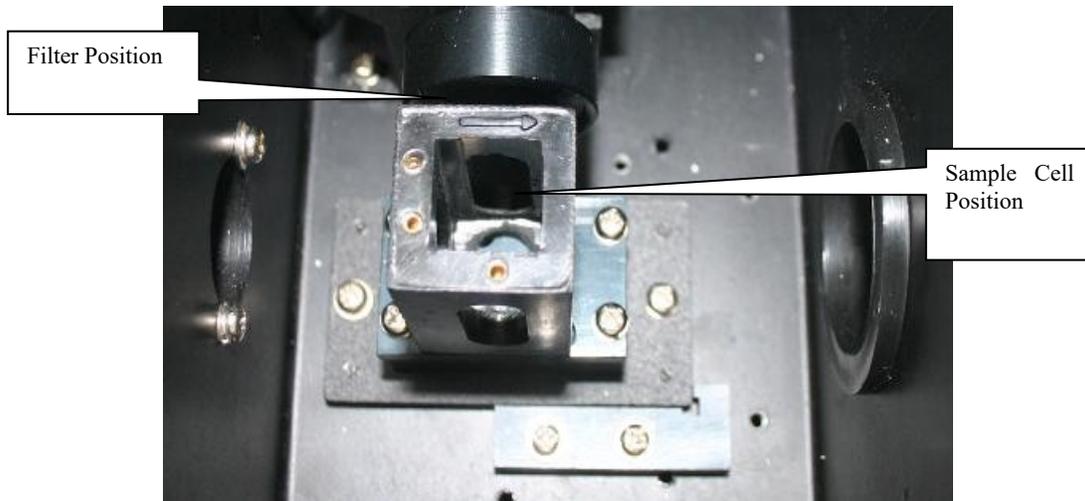


Fig. 1-4 Sample Compartment and Quartz Sample Cell (10 mm)

3. Filter



Fig. 1-5 Filter (center wavelength 365 nm)

1.2 Operating Panel of the Instrument



Fig. 1-6 Operating Panel of BSFL-103 Fluorescence Spectrophotometer Main Unit

1. Mode key, 2. Print key, 3. Print key, 4. Normalise key, 5. Display window.

1.3 Working Principles of the Instrument Signal Processing and Control System

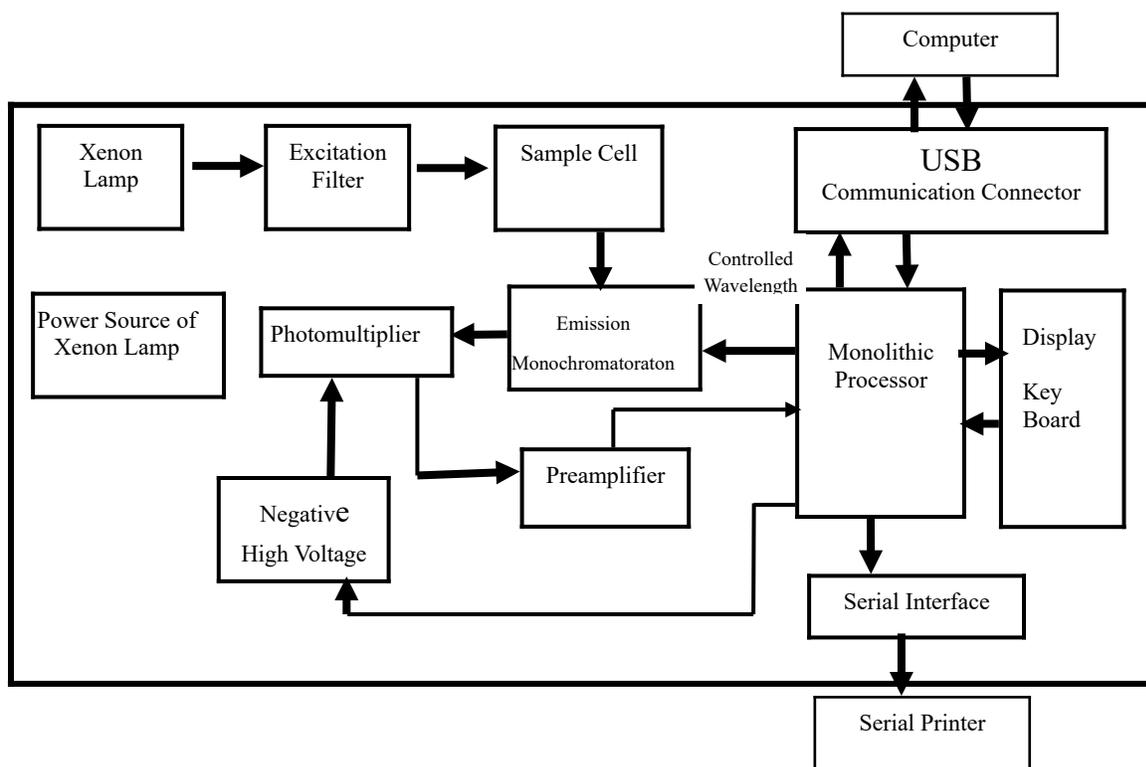


Fig. 1-7 Signal Processing and Control System

Optical System

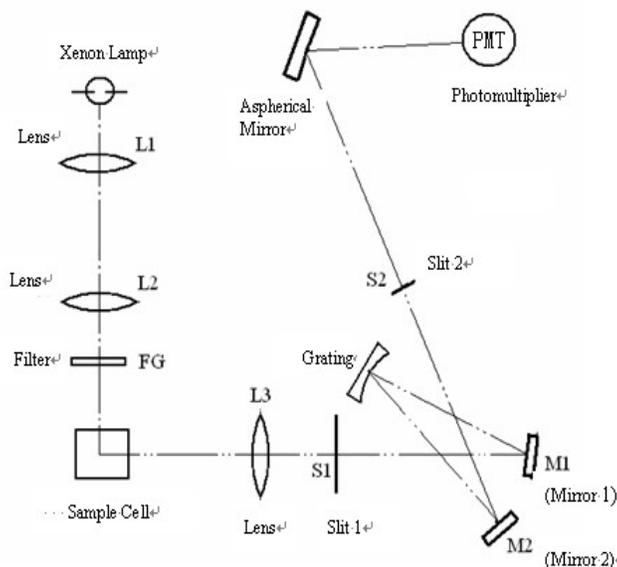


Fig. 1-8 Optical System Arrangement of BSFL-103 Fluorespectrophotometer

1.4 Measurement Functions of the Instrument

1.4.1 Working Modes of Measurement

1. Wavelength scan mode. At a fixed excitation wavelength a spectrum is obtained in which the fluorescent intensity of a sample varies with wavelength.

2. Time scan mode. At a fixed excitation wavelength, a spectrum is obtained in which the fluorescent intensity of a sample varies with time.

3. Quantitative analysis mode. According to the quantitative relationship of fluorophotometry $F=KC$, under a given experimental condition, fluorescent intensity (F) is directly proportional to the concentration (C) of a sample component to be determined. By measuring the fluorescent intensity of a standard solution, K is acquired by curve fitting. Then measure the fluorescent intensity of an unknown sample. From a quasi-linear equation calculate the concentration of the component to be determined in the unknown sample. A calibration can also be made by standard curve method or standard addition method. A concentration measurement can be made through direct reading from the instrument by single-point standard calibrating method.

1.4.2 Modes of Data Measurement

1. Measuring with a single instrument. By using the functions of the keys and the functional indicator lights on the instrument panel, read the measured data on the display window.

2. Measuring with an on-line computer. The instrument is connected with a computer through a communication connector. The instrument control, the parameters setting, and real-time measurement are all carried out by a computer software system. The software possesses powerful functions of spectrum processing, data processing and storage.

Note: For different modes of data measurement, the number of usable working modes and the convenience of use differ somewhat.

4.3 Self-checking and Self-correcting

1. Self-checking and self-correcting function of the instrument

The instrument will automatically initialize after startup, including self-checking and wavelength correcting. Self-checking contains on-line condition check, excitation source check, database module check, DAC module check, PMT module check and motor module check. The result of each check will be printed on the LCD or in the software window when connected to computer. Users can check out problems refer to table 1-2. Wavelength correcting program will guarantee the accuracy of wavelength.

1.5 Performance Index of the Instrument (Specifications)

Light source	150W Xenon lamp (consumable part)
Excitation filter	Filter character: $\pm 5\text{nm}$ labeled (An interference filter with center wavelength 365nm used as standard configuration. Users may separately purchase other $\phi 25\text{mm}$ interference filters with center wavelength within 200—850nm range.)
Emission monochromator	Emission monochromator wavelength range: (Em) 200—900nm Wavelength accuracy within $\pm 1\text{nm}$ Wavelength repetition $\leq 0.5\text{nm}$ Emission spectrum bandwidth: 10nm
S/N ratio	$S/N \geq 150$ (Using 1cm quartz sample cell, measure the signal noise ratio of Raman spectrum of water)
Check utmost	1×10^{-10} g/ml sulfuric acid quinine solution
Measure linearity	≥ 0.995
Peak value	$\leq 1.5\%$
Zero line drift	$\leq 0.3\%$ (Within 10 minutes)
Upper limit change of indicating value	$\pm 1.5\%$ (Within 10 minutes)
Power requirement	220V \pm 22V 110V/60HZ
Dimensions	442 \times 392 \times 250 (mm)
Weight	Net weight 11kg
	Gross weight 13kg

Table 1-1 Performance Index of the Instrument

02 Operation of the Instrument

2.1 Operation of the Instrument Panel

1 Display Window

The display window is a 128*64 pixels LCD, shows information including initialization status, fluorescence value, concentration value, emission wavelength value, PMT level, concentration factors, standard concentration, etc.

Symbol related:

1. "●" means the excitation light source is on.
2. "%" means the fluorescence value has been normalized.

2 Operation of the Function Keys

1. "**Mode**" Key : Select the operation mode. The switch circulation is as follow: Fluorescence Value Mode -> Wavelength Mode -> PMT Level Adjust -> Concentration Input Mode -> Factors Input Mode -> Concentration Mode -> Fluorescence Value Mode.

In Wavelength Mode and PMT Level Adjust, if parameter value set is different from current value, use "Mode" key to cancel current value and return the set value.

2. "**Print**" Key: The "Print" key can print the current value if a dedicated serial interface printer is installed in Fluorescence Value Mode and Concentration Mode. In other modes, the "Print" key confirm the changed parameters.

3. "**Adj 0**" Key: In Fluorescence Value Mode, The key can adjust the current value to zero. In Wavelength Mode, Concentration Input Mode and Factors Input Mode, the key can select every single number from left to right. While selected, the value can be changed. In PMT Level Adjust, The key can reduce PMT level.

4. "**Normalize**" Key: In Fluorescence Value Mode, The key will normalize current value to 100 and displays "%" symbol. Press again will cancel the normalization. In Wavelength Mode, Concentration Input Mode and Factors Input Mode, the key will add the selected number from 0-9 in circulation. In PMT Level Adjust, The key can add PMT level.

2.2 Normally Working Condition of Startup

2.2.1 Startup of the Instrument

First turn on the power source of the xenon lamp, and then turn on the power source of the BSFL-103 Fluorospectrophotometer main unit. The xenon lamp needs to stabilize for some time when it is lit. For precise measurement, the time should be 30 minutes over.

Note :

① If the xenon lamp fails to be lit and a squeaking sound of high frequency or a cracking sound of sparking is continuously produced, please turn off the power source of xenon lamp

immediately. Several seconds later, then turn on the xenon lamp again.(Such a case occurs only when the power source is not stable and capacity of the power source is not sufficient, or xenon lamp closes to the limit of service life).

② Because the life of the xenon lamp is closely related to the number of on-off of the lamp power source, the unnecessary number of turning on/off the xenon lamp should be cut down as far as possible and the xenon lamp should avoid being turning on/off repeatedly under a high voltage.

2.2.2 Confirmation of Heat Dissipation Fan Running

Every time after startup of the instrument, please first make sure that the heat dissipation fan at the side of the instrument runs normally in order to ensure that the instrument works normally. If finding anything wrong with the heat dissipation fan, should close down the instrument for an overhaul.

2.2.3 Initialization

The instrument will automatically initialize after startup. If the instrument is not connected to computer, the initialization status will be displayed on LCD. If connected to computer, the status will be displayed in software window, and the LCD will show "On-line".

Initialization includes self-checking and self-correcting. Information of every step is given in table 1-2.

	STEP	PASS	FAILURE
Self-checking	Online self-checking	Into on-line mode	Into single mode
	Database Module	Database initialized	Parameters error Memory chip error
	DAC Module	DAC Module initialized	DAC module chip error
	PMT Module	PMT Module initialized	Photomultiplier tube error PMT Module error
	Motor Module	Motor Module initialized	Motor error Motor driver board error
Self-correcting	Wavelength correcting	The value from bottom to top and to bottom again, shows a wave peak.	No obvious wave peak. Wavelength error Check if the sample cell is empty

Table 1-2 BSFL-103 Fluorospectrophotometer Initialization List

2.3 Measurement

After preheating for 30 minutes, the instrument can be used for measurement operation. The operating methods of the two measuring modes (measuring with a single instrument, measuring with an on-line computer) of the instrument are separately different.

When the BSFL-103 Fluorospectrophotometer main unit is connected with a computer, the selection of the different measuring modes and the operating methods are seen in detail in Section 7, Section 8 and Section 9 of Part Two.

This section is about the operation of quantitative analysis using a single instrument for measurement.

2.3.1 Pouring the Sample Solution into Sample Cell

Pour the sample solution to be measured into the sample cell up to about two thirds of height. Use filter paper to remove the solution left on the outside surface of the sample cell. Place it carefully into the sample cell holder.

Note: The sample cell is made of quartz material. When using, should take care to protect the window of light transmission. When taking the sample cell out or placing it into the holder with hand, should hold the sample cell diagonally with fingers.

2.3.2 Quantitative Analysis in Measurement Mode with a Single Instrument

Measuring mode with a single instrument is mainly used in the working mode for quantitative analysis. There are two methods, that is multipoint method of standard sample and single-point method of standard sample. The two operating methods are as follows.

1. Measuring procedures by Multipoint Method of Standard Sample

Prepare a series of standard solutions (including blank solution). After finishing the preparation of the solution of unknown sample, operate as the following sequence.

(1) Wavelength Adjustment. Press the "Mode" key to enter Wavelength Mode. "Current Wavelength: ***nm" shows current wavelength of emission monochromator. Use "Adj 0" key or the "Normalize" key to adjust target value. Press "Print" key after adjustment(See 2.1.2 for detail). The wavelength will be automatically set to target value.

(2) PMT Level Adjustment. Press the "Mode" key into Fluorescence Value Mode. Put the standard solution of maximal concentration into sample cell. Then press "Mode" to enter PMT level adjust. "Current PMT level: **" shows the current PMT level. The higher the level is, the more sensitive it will be. Use "Adj 0" key or the "Normalize" key to adjust the PMT level. Press "Print" key after adjustment. The PMT level will be automatically set to target value. Adjust the PMT level to make sure the fluorescence value of the maximal concentration standard solution is set between 200-400.

(3) Zero Adjustment. Place the blank solution in the light path. Press the "Mode" key into

Fluorescence Value Mode. Wait till the value is stable. Press "Adj 0" to adjust the fluorescence value to zero(± 0.1).

Attention: Zero adjustment is not allowed in the following measurement.

(4) Measurement for Standard Sample. Place a series of standard solutions (from dilute to concentrated) in the light path one by one. After the fluorescence value is stable, record the value. Fit a working curve equation according to "Data Processing with Microsoft Excel 2000 Software" provided by Appendix.

(5) Measurement for Unknown Sample. Place the solution of the unknown sample in the light path. After the fluorescence value is stable, record the value. Acquire the concentration of the unknown sample from the working curve equation.

2. Measuring Procedures by a Single-point Method of Standard Sample

Prepare one standard solution and one blank solution each. After finishing the preparation of unknown sample solution, operate as the following sequence.

Note: The concentration of standard sample should close to that of sample to be measured as far as possible.

(1) Wavelength Adjustment. Press the "Mode" key to enter Wavelength Mode. "Current Wavelength: ***nm" shows current wavelength of emission monochromator. Use "Adj 0" key or the "Normalize" key to adjust target value. Press "Print" key after adjustment(See 2.1.2 for detail). The wavelength will be automatically set to target value.

(2) PMT Level Adjustment. Press the "Mode" key into Fluorescence Value Mode. Put the standard solution of maximal concentration into sample cell. Then press "Mode" to enter PMT level adjust. "Current PMT Level: ***" shows the current level. The higher the level is, the more sensitive it will be. Use "Adj 0" key or the "Normalize" key to adjust the PMT level. Press "Print" key after adjustment. The PMT level will be automatically set to target value. Adjust the PMT level to make sure the fluorescence value of the maximal concentration standard solution is set between 200-400.

(3) Zero Adjustment. Place the blank solution in the light path. Press the "Mode" key into Fluorescence Value Mode. Wait till the value is stable. Press "Adj 0" to adjust the fluorescence value to zero(± 0.1).

Attention: Zero adjustment is not allowed in the following measurement.

(4) Direct-reading of Concentration. Press the "Mode" key to enter Concentration Input Mode. When the LCD shows "Input Concentration: ", use "Adj 0" key or the "Normalize" key to

adjust the value to be the concentration of standard solution. Then press the "Print" key to calculate the concentration factor and switch to Concentration Mode.

(5) Measurement for Unknown Sample. Place the unknown sample in the sample cell. The value on the display window is the concentration of the unknown sample in Concentration Mode. Press the "Print" key to print the current data if connected to a dedicated serial interface printer.

(6) Readout of Concentration Factor. Press the "Mode" key to switch Factor Input Mode. When the LCD shows "Input Factor: ", the value is the current concentration factor. Use "Adj 0" key or the "Normalize" key to adjust the value of concentration factor.

Note: When measuring the same sample, by setting the concentration factor, you may measure the sample directly. In this case, the PMT level should be in accordance with that of readout of concentration factor

2.3.3 Shutdown Instrument

Turn off the power source of the BSFL-103 Fluorospectrophotometer main unit first, and then the power source of the xenon lamp. (The order is reverse to that of startup.)

If in on-line mode, close the software first, then turn off the power source of the main unit, and turn off the Xenon lamp.

Note: After turning off the power source of the xenon lamp, please wait for 60 seconds at least. Then turn on the xenon lamp if you want to use it once again.

03 Installation of the Instrument

3.1 Installation Environment

The instrument is suitably used in a laboratory room to perform analytical measurements. Since it works in combination with a computer, the working conditions must meet the following demands.

3.1.1 Space Environment of Laboratory

Environment temperature range:10-30°C. Environment humidity is less than 85%.Corrosive gas and other organic or inorganic solvent gas that is absorptive within the range of violet radiation should not be contained in the atmosphere.

3.1.2 Working Table

The instrument should be placed on a sturdy and level table or the like, avoiding strong vibration, direct sun light, and dust.

3.1.3 Power Source

Alternating voltage must be within a range of 220V±22V, 110V±11V.

3.1.4 Environmental Alternation

If the instrument needs to be used at a worksite, the working environment should accord with the above demands for environment. If moving the instrument to other places, please use original packing materials. If users have any special demands, may place a special order for the instrument.

3.2 Unpacking for Checking

The BSFL-103 Fluorospectrophotometer main unit and the computer or the main unit and the printer are packed with a fiberboard box. For long-distance transport, users may make a request for a wooden box as additional outer packing.

3.2.1 Checking the Outer Packing

Before unpacking, check that the outer packing is intact. If finding the packing to be incomplete or finding marks of impact on the packing, please contact the transport insurance department.

3.2.2 Unpacking for Counting

Unpack at the position of adhesive seal tape. Take out the BSFL-103 Fluorospectrophotometer main unit carefully. (Please keep the outer packing so as to use it again when moving the instrument later. Check the main unit, the standard fittings, accessories purchased by choice and spare parts against the packing list. If anything is missing or damaged, please contact the local dealer or our company immediately.

Standard fittings	BSFL-103 Fluorospectrophotometer main unit	One piece
	365nm filter (installed in the main unit)	One piece
	Software package of data processing for F96 Pro Fluorospectrophotometer (set)	One set
	Power line	One piece
	USB wire	One piece
	Quartz sample cell 10mm	One pair
	Fuse (2A/5A)	two pieces
	Instruction Manual for BSFL-103 Fluorospectrophotometer	One copy
	Certification of products	One copy
	Packing list	One copy
Spare parts Purchased by choice	200—850nm Interference filter (φ25nm)	
	Quartz sample cell 10mm	
	Glass sample cell 10mm	

Accessories Purchased by choice	Fuse (2A/5A)	
	USB wire	
	Power line	
	Microcomputer main unit	
	Display device	
Accessories Purchased by choice	Dedicated serial interface printer	
	Membrane kind sample accessories	
	Powder kind sample accessories	
Accessories Purchased by choice	Microscale capillary sample accessories	

	Jacket sample pool accessories	
	200 μ L centrifuge tube accessories	
	Semi-auto sample introduction accessories (LG2.002.001)	

Table1-3 BSFL-103 Fluorospectrophotometer fitting list

Note: Accessories and spare parts purchased by choice are provided according to order contract.

3.3 Installation

3.3.1 Cleaning

Remove all adhesive tapes used for fixing during transport. Clean the surface of the instrument.

3.3.2 Check of Power Source Matching

Check that the power voltage to be supplied to the instrument should accord with that of the local.

3.3.3 Connecting the Power Cord

Place the BSFL-103 Fluorospectrophotometer main unit and the computer or the printer on a sturdy working table, and they should be 10cm over away from the wall. Connect the power cord accompanied with the instrument to the electric supply socket of the laboratory room.

3.4 Adjustment of Instrument

3.4.1 Signal Noise Ratio Test (Using Raman Spectral Peak of Water)

1. Testing Mode Using a Single Instrument

Make sure the sample cell is empty. First turn on the power switch of the xenon lamp. Check that the xenon lamp is lit. Then turn on the power switch of the instrument. After starting up, the instrument will automatically initialize. (The instrument possesses a self-checking function through a computer and a function of self-correcting wavelength). This process takes about several minutes.

(1) Preheating

After the instrument is started up, the xenon lamp and other electronic components need time to attain a thermal equilibrium. So measuring operation should be carried out after 30 minutes of starting up for preheating.

(2) Putting in Sample

Put the sample (redistilled water) into clean quartz sample cell. Hold the sample cell diagonally with fingers, and place it into the sample cell holder. See Fig.1-4.

Attention: If the quartz sample cell is not clean, it will affect the precision and reduce the S/N.

(3) Wavelength Adjustment

Adjust the value of current wavelength to 415 nm. More detail please turn to 2.3.2.

(4) PMT Level Adjustment

The purpose of PMT level adjustment is to let the displayed value of the sample to be measured be proper. Adjust the value of fluorescent intensity be between 100 and 200. Find the wavelength value (λ_{max}) of the maximum fluorescent intensity at about 415nm. For more information of adjustment please turn to 2.3.2.

Then observe for 2 minute at the wavelength λ_{max} . Record the values of maximum and minimum fluorescent intensity, respectively. The sum of the two values is divided by two to give an average value of fluorescent intensity at wavelength λ_{max} . The difference between the two values is the value of noise at wavelength λ_{max} .

After that adjust wavelength to 315nm. The displayed value of fluorescent intensity is the background value of Raman spectral peak of water. The difference between the above average value of fluorescent intensity at λ_{max} and the background value at 315nm is divided by the value of noise to give signal noise ratio (S/N) of the instrument.(The product index of the instrument $S/N \geq 150$).

Note:

- ① In order to protect the photomultiplier, when PMT level is higher ($\odot 6$), please not let high light pass into the sample compartment.
- ② When adjusting PMT level, readjustment of the automatic PMT level system of the instrument will influence the original set for zero-point of fluorescence. After adjusting PMT level, please check zero-point. If zero-point changes, it may be adjusted once again.

(5) Shutdown of Instrument

After finishing the test, shut down the instrument. Please turn off the power source of the xenon lamp first. And then turn off the power source of the BSFL-103 Fluorospectrophotometer main unit .

2. Testing Mode by using the Instrument in Combination with an On-line Computer

Finish the measurement for Raman Spectral Peak of water by using wavelength scan and time scan in the computer software. The theory is the same as Section 3.4.1 Part 1. The use of software please turn to Part Two.

3.4.2 Checking Wavelength of the Instrument

The instrument can automatically check the accuracy of indicated value of the instrument wavelength. If finding a mistake, may use a mercury-arc lamp to check (If there is no mercury-arc lamp, a fluorescent lamp can be used instead, but a professional man must be needed to do such work. The concrete method and the correcting procedures will be briefed through training.).

The wavelengths of spectral lines of a mercury-arc lamp are listed in the Table 1-1 and the spectrogram is seen in Fig 1-9.

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

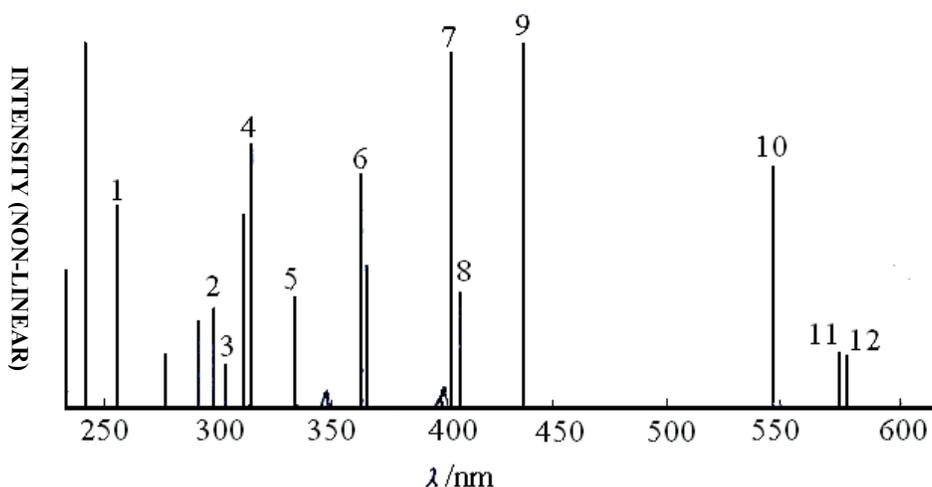


Fig. 1-9 spectrogram of mercury-arc lamp

04 Maintenance of the Instrument

4.1 Routine Maintenance

1. In routine operation, always check and make sure that the working conditions accord with the demands. If finding that the working conditions do not accord with the demands, should take measures without delay.

2. While the main unit of the instrument is working, keep good ventilation over the surface of the heat dissipation opening at the top of the instrument. It is unsuitable to stack other articles nearby.

Note: The temperature at the heat dissipation opening is very high. Be sure not to touch for fear of being burnt.

3. Keep the instrument clean. When the main unit of the instrument is not used, put a dust-proof cover over it. When cleaning the outside surface of the instrument, may wipe it with warm water. Do not use organic solvents such as alcohol, ether, and acetone. Please not clean while the instrument is still working.

4. The sample cell should be kept clean.

4.2 Maintenance and Replacement of Light Source Lamp

4.2.1 Maintenance of Light Source Lamp

1. A high-performance xenon lamp is used as the light source lamp of the instrument. It is mounted in a special lamp chamber as shown in Fig.1-3. The surface of the xenon lamp must be kept clean.

2. When turning on/off the xenon lamp, should strictly follow the operating sequence.

Note: When starting up the instrument, please turn on the power source of xenon lamp first. After the xenon lamp is lit, then turn on the power source of the main unit of the instrument. When shutting down the instrument, please turn off the power source of the main unit of the instrument first, and then turn off the power source of the xenon lamp.

3. When the voltage of power source is not stable and the capacity of power source is not sufficient, or the xenon lamp closes to the limit of service life, lighting xenon lamp is often difficult.

Note: If the xenon lamp fails to be lit and a squeaking sound of high frequency or a cracking sound of sparking is continuously produced, please turn off the power source of the xenon lamp immediately. Several seconds later, then turn on the xenon lamp again.

4. The xenon lamp should avoid being turned on/off repeatedly under a high voltage.

Note: Because the life of the xenon lamp is closely related to the number of on-off of the lamp power source, the unnecessary number of turning on/off the xenon lamp should be cut down as far as possible.

5. After starting up the instrument, make sure that the heat dissipation fan of the instrument runs normally, and be sure to keep the surface of heat dissipation opening at the top of the instrument well ventilated.

4.2.2 Replacement of Light Source Lamp

A precise combination light source that has been corrected in advance in the factory is used in the instrument. A professional man must be needed to replace a damage light source lamp. The concrete method and the correcting procedures will be briefed through training.

4.3.1 Maintenance of Filter

1. The surface of the filter should be kept clean.

Note: If having touched the surface of the filter, please wipe it clean with a piece of lens tissue and alcohol then dry it.

2. The filter stripped down must be cleaned then be wrapped up with lens tissue. After that it should be put into a container holding desiccant and be well preserved.

4.3.2 Replacement of Filter

1. Remove the cover of the sample compartment. Unscrew the knurled screw which is on the right side of the excitation part.

2. Open the cover board on the excitation part from the top left of the knurled screw.

3. Take out the excitation part.

4. Remove the front pressing ring of the excitation part anticlockwise.

5. After that hold the both sides of the filter edges with fingers and take it out. Then place a new one into the pressing ring. (There are no obverse and reverse sides for a filter.). Screw the pressing ring into the filter position in the front of the sample compartment clockwise and screw it tightly.

Note: When taking the filter out, please not touch the filter surface of light-transmission.

6. Make sure the cover board is open. Then put the excitation part on the frame.

7. Close the cover board. Tight the screws.

Part Two:

Operation Instruction for the Instrument Connected with a Computer

05 Environmental Requirements of Using Software and Installation

Before reading this part, please first read the "Operation Instruction for the Instrument" in Part Two and the operation manuals for the above versions of Windows 98.

5.1 Basic Configuration of Computer Hardware and Software

5.1.1 Hardware

CPU P2 Celeron is 600 or more. Computer memory is not less than 64 M. Computer hard disc should have enough space to run (about 200M). Two serial ports of the computer should run normally, and no interruption conflict occurs in the system.

Image resolution is more than 800×600 and color enhancement is more than 16-bit.

5.1.2 Software Requirements

The second edition of Windows 98 is used (The above versions of Windows98 can also be used.) in this part. While the computer is running, it is necessary to turn off the screen saver and to turn off power management in the BIOS program to ensure that the "Data processing software package of BSFL-103 Fluorospectrophotometer" (" Software " for shorter, the same below) can run normally.

5.2 Installation of "F96 Software"

Place CD-ROM into the drive. The computer will automatically install an application. In the absence of automatic installation, open the X: \ (double-click) SETUP.EXE documents (applications). When SETUP window appears, click "NEXT" button and type in the name and company name. Click "NEXT" button again and the Installation Path dialog box appears.

If there is no need of change, please click "NEXT" button directly. If hoping to amend path, click "Browse" button. Afterwards the Item Name dialog box will appear. The default name is "The Software Package of BSFL-103 Fluorospectrophotometer". You may click "NEXT" button directly or change the item name, then confirm the dialog boxes of all above steps.

If hoping to amend, click " Back" button, or click "NEXT" button directly. The computer begins to read CD-ROM for installation. After the installation has been completed, restart the computer to run the program.

Note: The used CD-ROM has its own serial number, and it must be connected with the instrument of the same serial number. It cannot be used with other instruments of different serial numbers in a crossing way. The software will not operate normally in such cross-use.

06 Operation of Software

6.1 Preparation before Use

6.1.1 Connection of BSFL-103 Main Unit with a Computer

The BSFL-103 main unit is connected through a USB port of a computer. The USB driver will be installed when the first time connected. Please run the software after the installation is finished.

6.1.2 Procedure for On-line Operation (Startup)

1. USB wire connection

Connect BSFL-103 main unit to a computer with USB wire.

2. Turn on BSFL-103

First turn on Xenon Lamp. Then turn on the power source of the main unit of the instrument after the Xenon Lamp is lit. The instrument will automatically check the connection, and enters on-line condition.

3. Initialization

Run "BSFL-103 Software". The software will check the connection and starts initialization. For more information please turn to 2.2.1.

On completion of initialization, the interface of wavelength scan mode shown in Fig. 2-1 is directly displayed.

Note: In the execution of initialization, please not place any sample into the sample compartment.

4. Relevant Working Modes

 Click "Working Mode" on the menu bar in Fig.2-1 to switch between the three modes: "Wavelength Scan", "Time Scan", and "Quantitative Analysis", as shown in Fig.2-2.

5. On-line Mode Power Off

 In on-line mode, close the software first, then turn off the power of the main unit.

**If the main unit is turned off first, that leads to a communication error in the software.
Run task manager to end pc.exe.**

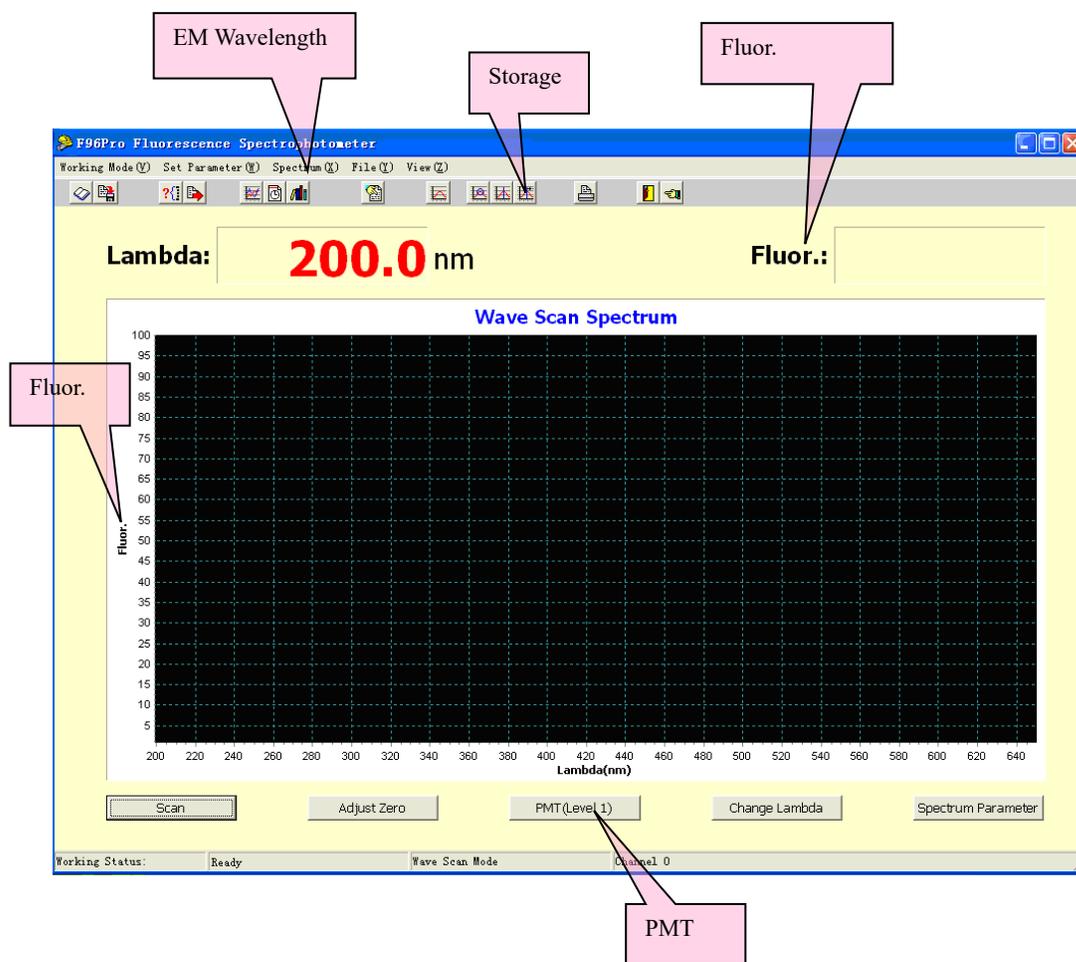


Fig. 2-1 Interface of Wavelength Scan

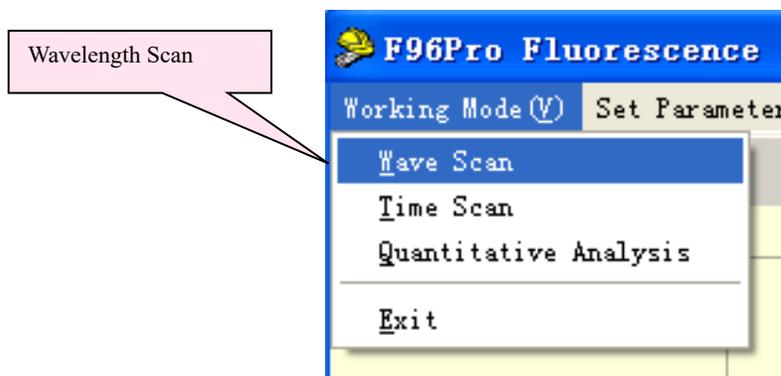


Fig. 2-2 Switchover of Working Mode

6.2 Three Interfaces of Working Mode

There are Three Interfaces of Working Mode, namely the interface of wavelength scan, the interface of time scan and the interface of quantitative analysis.

6.2.1 The Interface of Wavelength Scan

Wavelength scan means that in the conditions of selected excitation source (namely at excitation wavelength) people measure the fluorescent intensity which varies with fluorescent emission wavelength, that is, plot the fluorescence spectrum of a sample component.

Wavelength scan can be used as a selection of fluorescent emission wavelength measured in researches on the method of quantitative analysis for fluorescence and can also be used to study the emitting behavior of fluorescent molecules in solutions.

The interface of wavelength scan mode is displayed as shown in Fig. 2-1

6.2.2 The Interface of Time Scan

Time scan means that in the conditions of selected excitation source (namely at excitation wavelength) people observe how the fluorescent intensity of a sample component varies with time at a wavelength of fluorescent emission.

Time scan can be used as a method of kinetic analysis for measuring fluorescence, be used to develop researches on experimental conditions of quantitative analysis for measuring fluorescence and can also be used to measure drift and noise of the instrument.

The interface of time scan is displayed as shown in Fig. 2-3

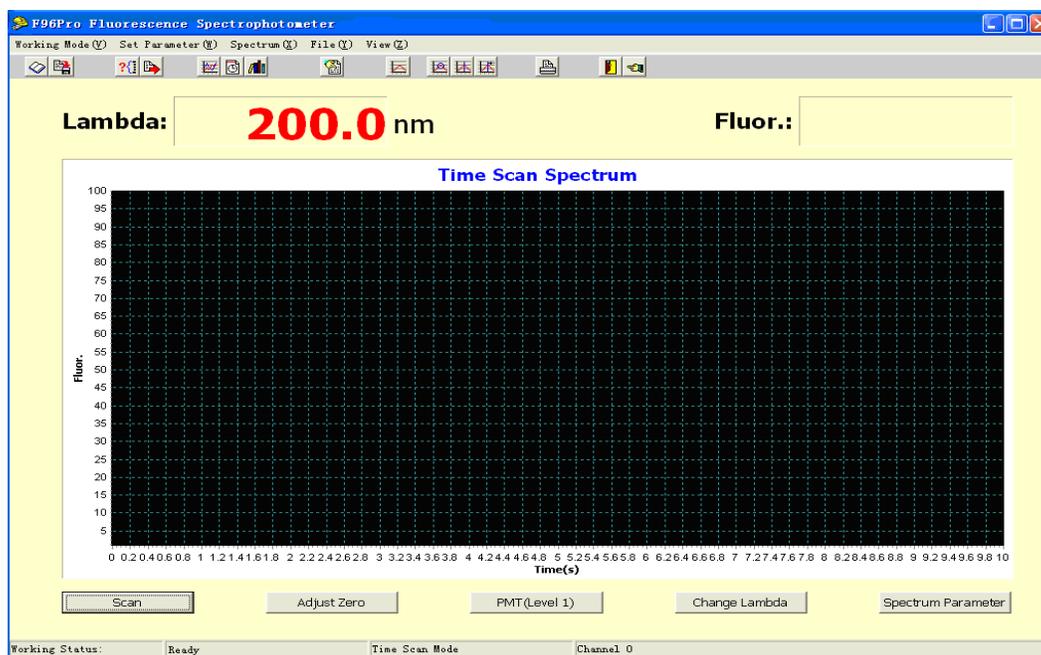


Fig. 2-3 Interface of Time Scan

Time scan differs from wavelength scan in that the unit of abscissa is time during scanning. In the functions such as "Set Parameter", "Spectrum", "File" and operation, the requirements for time scan are the same as those for wavelength scan.

6.2.3 The Interface of Quantitative Analysis

Quantitative analysis means that in the conditions of selected excitation source (namely at excitation wavelength) by measuring the fluorescent emission intensity (F) of the component (its unknown concentration C) to be determined, from the formula $F=KC$, the concentration of the component to be determined is acquired. K in the formula is a constant related to the test conditions and it can be acquired by measuring the fluorescent emission intensity of a standard solution of the component to be determined and then by calculating in the same test conditions. With the fluorescent emission intensity of many standard solutions it can also be acquired by linear fitting or graphical solution method. Quantitative analysis can be used to measure the content of the component of emitting fluorescence in the sample solution.

The interface of quantitative analysis is displayed as shown in Fig. 2-4.

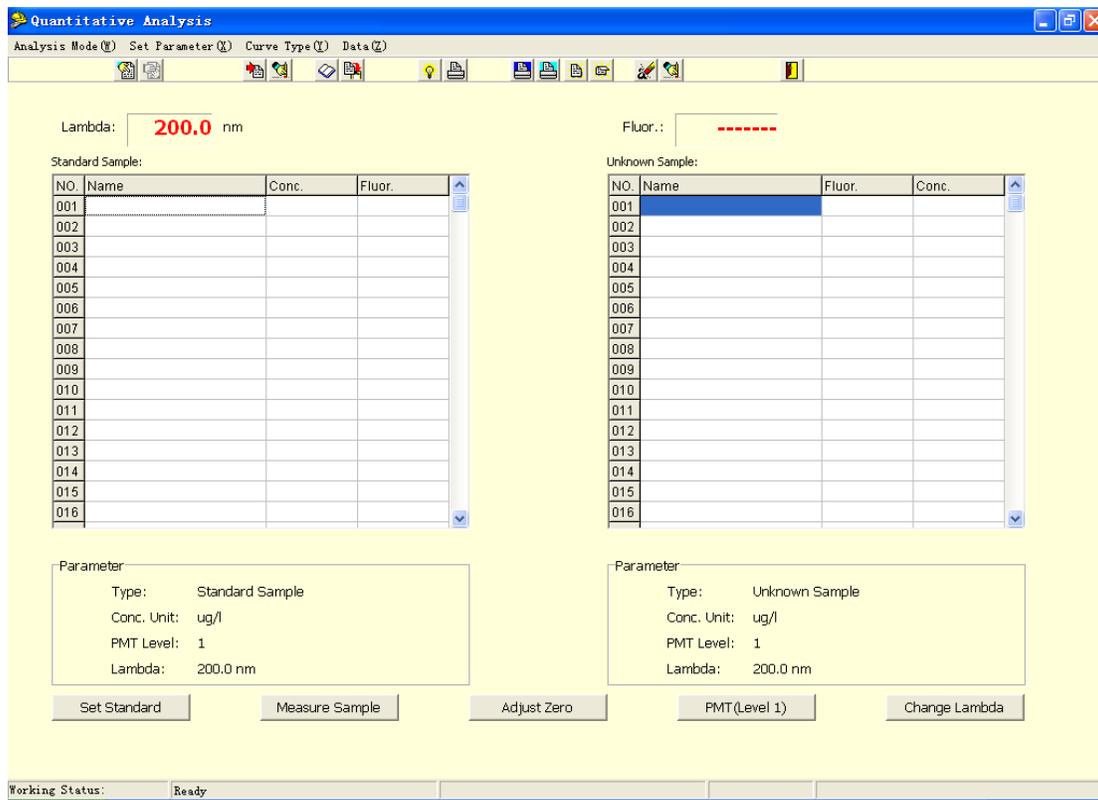


Fig. 2-4 Interface of Quantitative Analysis

07 Operation of Wavelength Scan Mode (Connected with a Computer)

7.1 The Interface of Wavelength Scan Mode

On completion of initialization for the instrument, the interface of wavelength shown in Fig.2-5 is immediately displayed. The first row at the top of screen is Menu Bar. In the second row are 15 "Shortcut Icons". On the screen are wavelength, display window of fluorescence, and coordinate graph with fluorescence value and wavelength. At the bottom of the screen there are 5 "Shortcuts".

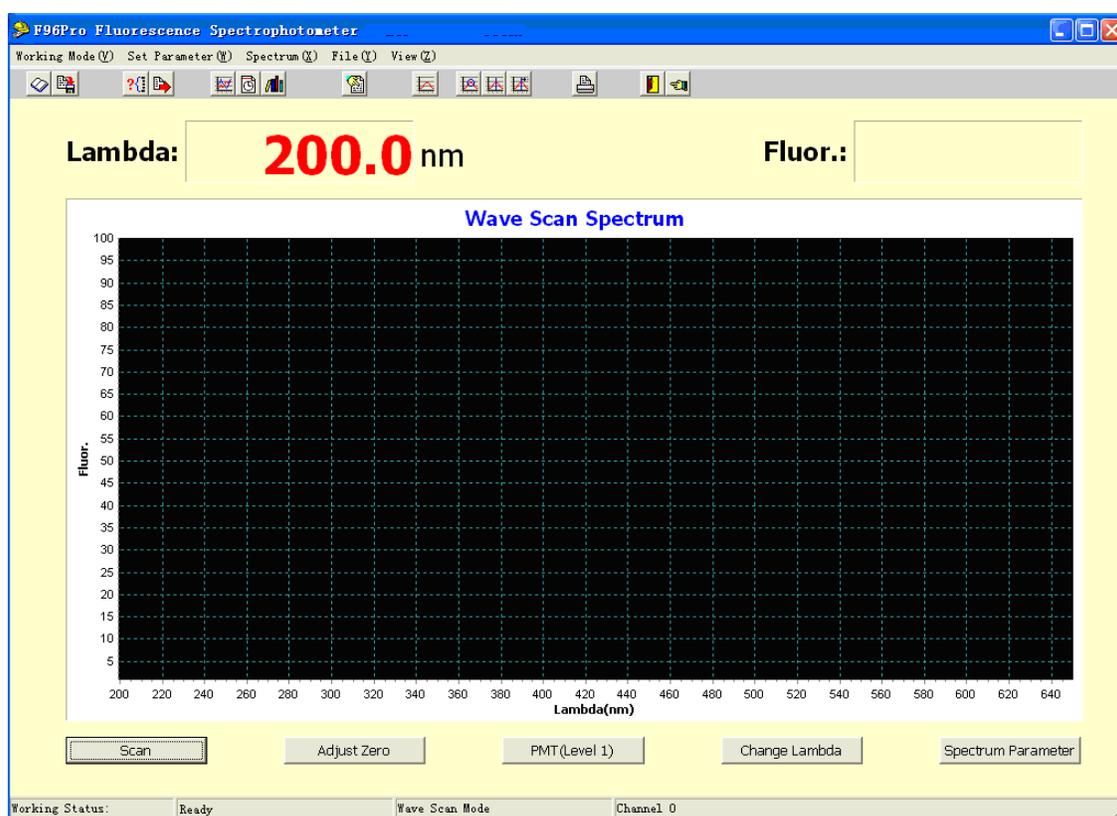


Fig. 2-5 Shortcuts in the Interface of Wavelength Scan of Fluorescence Spectrum

7.1.1 The Functions of Shortcuts at the Bottom of the Screen

"Scan" is the button for starting. After you click it, scanning a spectrum according to the parameters set.

“Adjust Zero” is the button for zero adjustment of the current value. It is used to deduct a background value.

“PMT(Level 1)” is the button for PMT level adjustment. Select a proper PMT level(1-17) in the pop-out window. Then click OK.

“Change Lambda” is the button for adjusting the current wavelength. The instrument main unit will automatically change the current wavelength to the wavelength set.

“Spectrum Parameter” is the button to show the parameters of current wavelength scan.

7.1.2 The Functions of Shortcut Icons

When the mouse pointer is moved onto a shortcut icon, the function of the shortcut icon will show. Icons are arranged in order from left to right. The functions are described bellow.



---“Load Spectrum” button means to open a previously saved spectrum.



---“Save Spectrum” button means to save the current spectrum.



---“Transmit Spectrum” button means to transmit a spectrum between the channels.



---“Transmit Parameter” button means to transmit the spectrum parameters in the current channel to other channels.



---“Wave Scan” button means to switch the current working mode to wavelength scanning mode.



---“Time Scan” button means to switch the current working mode to time scanning mode.



---“Quantitative Analysis” button means to switch the current working mode to quantitative analysis mode.



---“Parameter” button means to set scan parameters.



---“Restore Parameter” button means to restore the spectrum of current channel to its original status.



---“Smooth” button means to carry out operation of average value for the spectrum data, so that the spectrum becomes smoother.



---“P/V Detection” button means to detect the values of valley & peak of a spectrum.



---"Show P/V Data" button means to display the values of peak & valley in a spectrum after peak & valley detection.



---"Print" button means to print out a spectrum and data.



---"Exit System" button means to close F96 Software.



---"Locate Lambda to print" button means to select a wavelength you need for printing out.

7.1.3 Menu Bar at the Top of the Screen

"Working Mode": After clicking it with the mouse left button, from the pull-down menu select change of working mode or exiting from the program.

The other items on the menu bar are "Set Parameter", "Spectrum", "File" and "View" as shown in Fig.2-5. The operations and the setting methods refer to Section 7.2.

7.2 Wavelength Scanning Operation

Fluorescence spectrum can be acquired by wavelength scan, and the wavelength of fluorescent measurement used for quantitative analysis can be determined from the fluorescence spectrum.

7.2.1 Parameter Setting

On completion of initialization of the instrument, the interface of wavelength scan appears immediately. Before scanning operation, you need to set scanning parameters first.

After clicking "Set Parameter", the interface of "wavelength Scan Parameter" will appear as shown in Fig.2-6.

Wave Scan Parameter

Instrument: Replicate Scan | Abbreviatory Curve

EM Start: 200.0 nm EM End: 650.0 nm

Y Axis Min: 1 Y Axis Max: 100

Interval: 1 nm Replicates: 1

Scan Speed: 1000 nm/min PMT Level: 1

Multi-Curve: Overlap Draw Mode: Batch

Slit: 10.00 nm

Confirm Cancel

Working Status: Ready

Fig. 2-6 Interface of Wavelength Scan Parameter

1. **EM Start.** Set the start wavelength of scan.
2. **EM End.** Set the end wavelength of scan.
3. **Y Axis Min.** Set the minimum of Y axis in the scan spectrum.
4. **Y Axis Max.** Set the maximum of Y axis in the scan spectrum.
5. **Interval.** Set the data interval of wavelength scan.
6. **Replicates.** Set the number of scans.
7. **Scan Speed.** Set the scan speed. The value automatically changes with wavelength interval.
8. **PMT Level.** Set the PMT level of time scan.
9. **Multi-Curve.** Set the display mode of curves. "Overlap" shows different curves in the same window. "Overlay" will cover the last time scan curve, only showing the latest.
10. **Draw Mode.** Set the plot mode of wavelength scan. "Real Time" will show the current data of wavelength scan. "Batch" will show all data after the wavelength scan.
11. **Slit.** Its value is unchangeable and it has a bandwidth of 10nm.

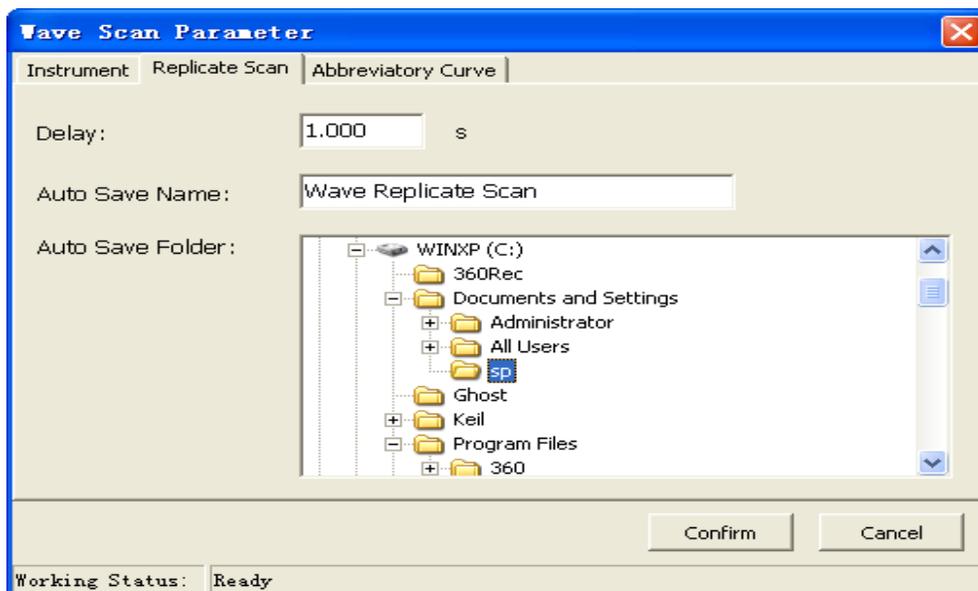


Fig. 2-7 Interface of Wavelength Scan Parameter – Replicate Scan Settings

12.Delay. Set the Delay between two scans.

13.Autosave Filename. Edit the filename of spectrum. The software will automatically add the current time of scan after the filename.

14.Autosave Position. Set the autosave position in your computer.

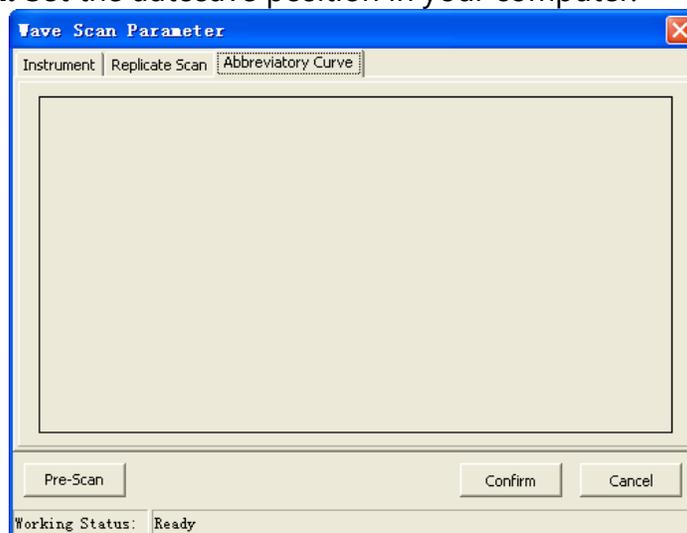


Fig. 2-8 Interface of Wavelength Scan Parameter – Abbreviatory Curve

15.Pre-Scan. It's a fast scan according to "Basic Settings" in "Wavelength Scan Parameter". After a Pre-Scan, a thumbnail is created and proper Y axis will be automatically set.

7.2.2 Spectrum Processing

Clicking the "Spectrum" button from Menu Bar in Fig.2-5 presents a pull-down sub-menu as shown in Fig.2-9.

1.Zoom. This means to reduce or enlarge a spectrum. After clicking this item you only need to input the scale data of a new spectrum.

For enlargement you may directly use the mouse to drag the spectrum on the screen. The method is to drag in the direction from the top left side of the spectrum to the bottom right side of the spectrum. If you want to return the enlarged spectrum to original one, you may drag in the opposite direction.

2.Differential Coefficient. This means to differentiate with respect to the spectrum. The 1st-4th order of derivative operation on the spectrum is performed. The set value of derivation interval must be twice the value of scan interval.

3.Restore. The spectrum may be restored to the original one.

4.Smooth. This means to perform calculation on the average value for each point of the spectrum to make the spectrum smoother.

5. P/ V Detection. It is used to detect the values of peaks and valleys of a spectrum.

(1) Click this item and "Input Sensitivity" dialog box will appear. Input a limited value of "Input Sensitivity" as a value of available peaks. All peaks above the limited value can be detected. For example, if the value of "Input Sensitivity" is 25, peak value 50 can be detected. If the value of "Input Sensitivity" is 60, peak value 50 cannot be detected.

(2) Then click "Show P/V Data" on the sub-menu, and the values of peaks & valleys will be displayed.

6.Arithmetic. Click "Arithmetic" in Fig.2-9, the interface of "Arithmetic" will open as shown in Fig.2-10. This calculation can be used as the operation between a single channel and constants, and can also be used as the arithmetic operation between channels.

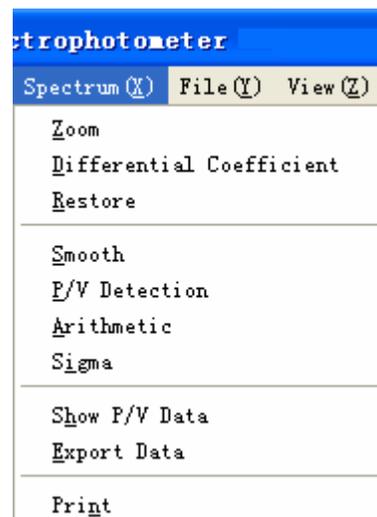


Fig. 2-9 Spectrum Menu

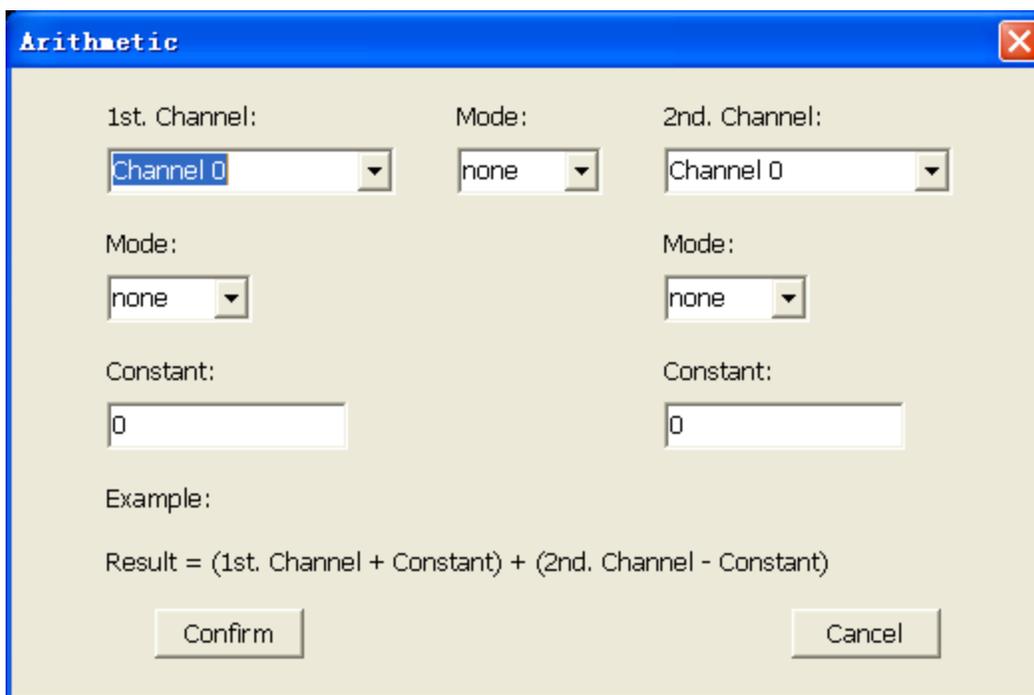


Fig. 2-10 Interface of Arithmetic Operation

7.Sigma. After clicking this item, input "Lambda Start" and "Lambda End", and then click the "Sigma" button. You will get the integral value of fluorescence within the range of set wavelength.

8. Show P/V Data. Shows the values of peaks & valleys.

9.Export Data. The output of the spectrum is in the form of data. There are two ways of data-out: discrete and continuous.

(1) Output of Discrete Data: Using the key ctrl or the key shift in combination with the mouse, from the list box select the wavelength values whose datum are required to examine.

(2) Output of Continuous Data: Input the range of wavelength whose datum are required to examine. Their values can be printed out.

9.Print. After it is clicked, "Input Spectrum Name" dialog box appears. Type in the spectrum name (Chinese name can be recognized.). Then click "OK".

Note: Before printing, please first confirm the condition of the printer installation.

7.2.1 Save Processing

Click "File" in Fig.2-5 and a pull-down sub-menu is shown in Fig.2-11.

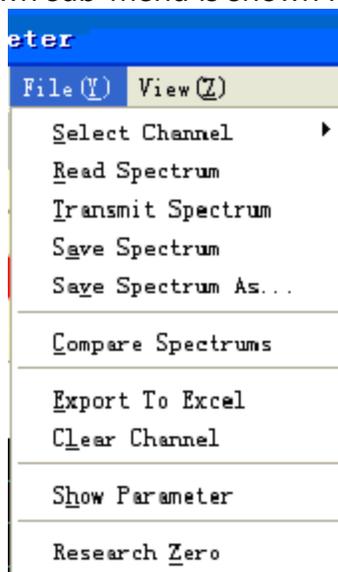


Fig. 2-11 Save Processing Sub-menu

- 1. Select Channel.** 10 channels (storage windows) altogether are provided for selection. Program can separately open 10 storage windows of wavelength scan or time scan at most for users' use.
 Note: 10 channels cannot run at the same time. Only the current channel can run. Quantitative Analysis, time scan and wavelength scan share the 10 channels.
- 2. Read Spectrum.** Show spectrum in the current channel. When the mouse pointer is moved onto the wavelength scan window and the right button is pressed down, the number of channel will be seen.
- 3. Transmit Spectrum.** Copy the spectrum of one channel into another channel.
- 4. Save Spectrum.** Save the selected spectrum and the file name onto the hard disc.
- 5. Save Spectrum As.** Save the spectrum onto the hard disc. After a dialog box appears, you can input another file name and click "Save".
- 6. Compare Spectrums.** This function compares different spectrums. For example, when comparing channel "0" with channel "1", first click channel "0", then press and hold the key Ctrl. At the same time click channel "1". At this time channel "0" and channel "1" are both selected. Then click "OK".
- 7. Export to Excel.** Export the spectrum data to excel file.
- 8. Clear Channel.** Clear a spectrum in the current channel.

9. Show Parameter. Display the parameters in the current channel.

10. Research Zero. This function will do wavelength correct of emission monochromator using grating zero order light.

7.2.4 Display Setting

Click "View" in Fig.2-5. A pull-down sub-menu is shown in Fig.2-12.

1. **Line Color.** Change the color of the current spectrum.
2. **Back Color.** Change the background color of the working window.
3. **Set Background.** Change the background color of scanning window.
4. **Set Netware.** Change the net type of the scanning window.
5. **Export Window as.** Export the current spectrum as BMP format. You can paste it to WORD documents for editing.

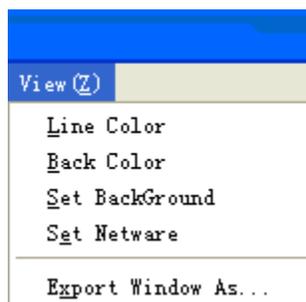


Fig. 2-12 View Sub-menu

08 Operation of Time Scanning Mode (When Connected with a Computer)

8.1 Interface and Operation of Time Scanning Mode

Time scan differs from wavelength scan in that the unit of X-axis is time during scanning. This working mode can be used to observe the situation in which the fluorescent intensity of a sample varies with time at a fixed emission wavelength.

8.1.1 Interface of Time Scanning Mode

Click "Working Mode" in the menu bar in Fig.2-1. Then click "Time Scan" in the pull-down sub-

menu and the interface of wavelength can be changed to the interface of time scan. See Fig.2-3.

The time scan interface is the same as that of wavelength scan interface except for the unit of X-axis. The functions of the shortcuts are also the same. For more details, please turn to Section 7.1.

8.1.2 Parameter Setting

Click "Parameter Settings" in Fig.2-3 to enter Time Scan Parameter Settings. As in Fig.2-13& 2-14.

Parameter	Value	Unit
Lambda	200	nm
Scan Time	10	s
Y Axis Min	1	
Y Axis Max	100	
Response	2	s
Replicates	1	
Interval	0.1	s
PMT	1	Level
Most Data	12000	
Multi-Curve	Overlap	

Fig.2-13 Time Scan Parameter Settings - Instrument

1. **Lambda.** Set the emission wavelength of the time scan.
2. **Scan Time.** Set the total scan time.
3. **Y Axis Min.** Set the minimum of Y axis in the scan spectrum.
4. **Y Axis Max.** Set the maximum of Y axis in the scan spectrum.
5. **Response.** Set the signal integral time of time scan.
6. **Replicates.** Set the number of scans.
7. **Interval.** Set the update interval of time scan. Update every 0.1 second.
8. **PMT.** Set the signal PMT level of time scan.
9. **Most Data.** Set the maximum sample size of time scan.
10. **Multi-Curve.** Set the display mode of curves. "Overlap" shows different curves in the same window. "Overlay" will cover the last time scan curve, only showing the latest.

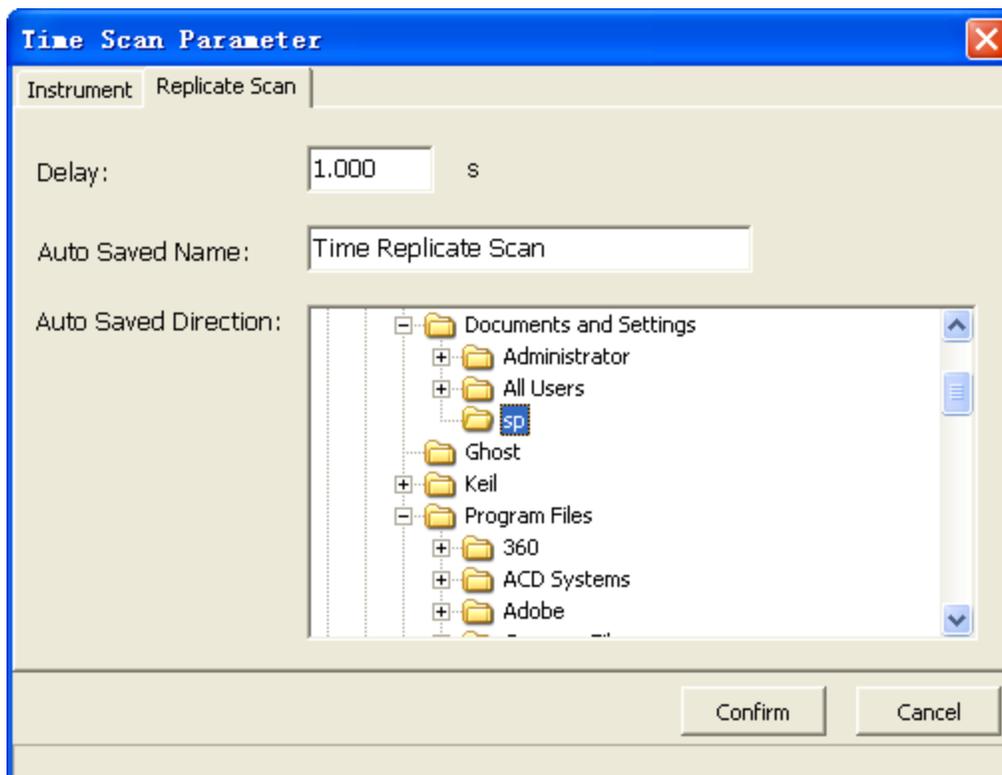


Fig.2-14 Time Scan Parameter Settings – Replicate Scan

11.Delay. Set the delay time between two scans.

12.Auto Saved Name. Edit the filename of spectrum. The software will automatically add the current time of scan after the filename.

13.Auto Saved Direction. Set the autosave position in your computer.

8.1.3 Spectrum Processing

The functions and the operation are the same as those of spectrum processing in wavelength scanning mode. For more details, refer to Section **7.2.2**.

8.1.4 Storage Processing

The functions and the operation are the same as those of spectrum processing in wavelength scanning mode. For more details, refer to Section **7.2.3**.

8.1.5 Display Setting

The functions and the operation are the same as those of spectrum processing in wavelength scanning mode. For more details, refer to Section **7.2.4**.

09 Operation of Quantitative Analysis Mode (When connected with a Computer)

9.1 Interface of Quantitative Analysis Mode

Click "Working Mode" on the upper left screen. The interface of quantitative analysis mode from the pull-down menu will appear as shown in Fig.2-15

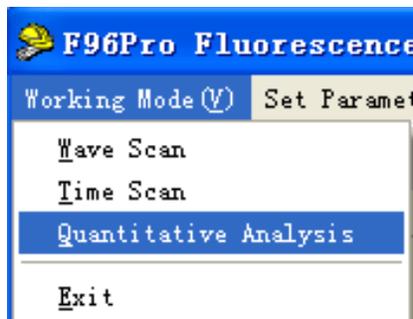


Fig. 2-15 Entering the interface of Quantitative Analysis

Above the interface of quantitative analysis on the top of the screen the first row is menu bar. The second row has 15 icons. The screen shows the Current Wavelength(Lambda), Fluorescence Value(Fluor.), and boxes of Standard Sample and Unknown Sample. On the bottom of the screen there are 5 buttons, as shown in Fig.2-16

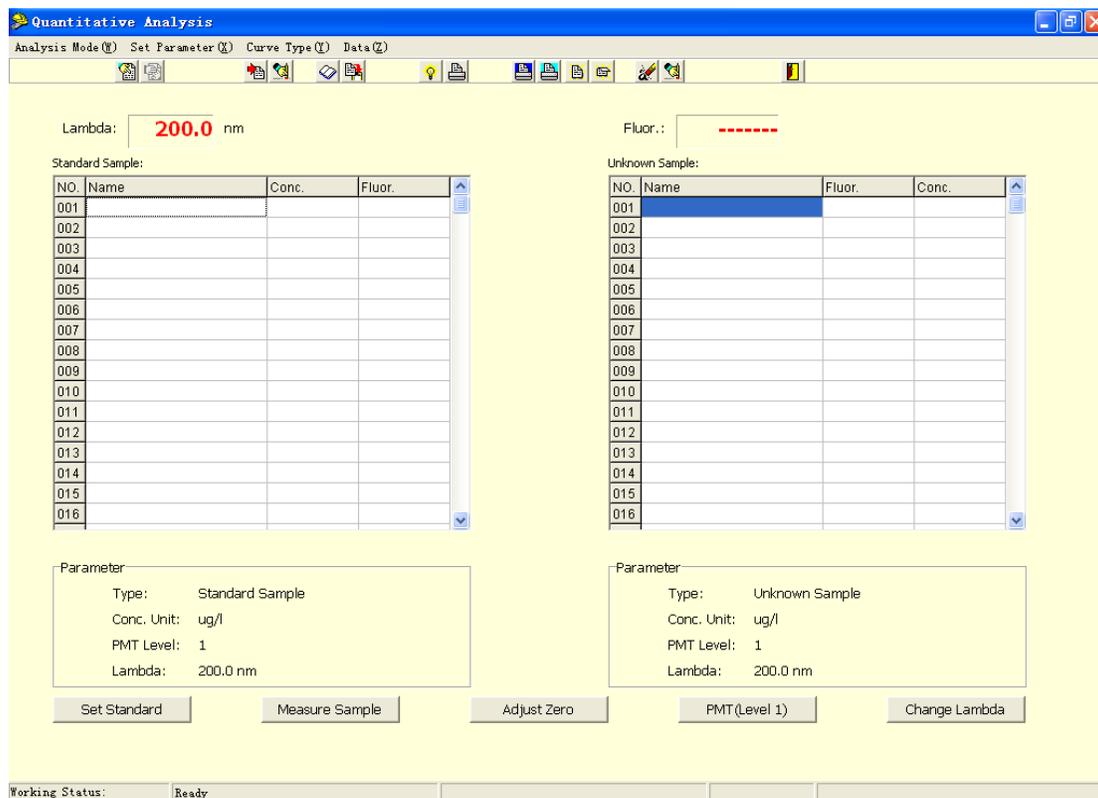


Fig. 2-16 Shortcuts in the Interface of Quantitative Analysis

9.1.1 The Functions of Short cuts on the Bottom of the Screen

The “Set Standard” button is used to set standard sample. After clicking it, a “Set Standard Sample” dialog will appear. For more details, see Fig. 2-20.

The “Measure Sample” button is used after the operation of sample measurement to display the measured fluorescence value of the sample and its corresponding concentration value in the “Display Box of Sample Measurement” when the shortcut is clicked.

The “Adjust Zero” button is used for zero-point adjustment of the current value and is used to deduct the background value.

The “PMT” button is used for PMT level adjustment. Select a proper PMT level(1-17) in the pop-out window. Then click OK.

The “Change Lambda” button is used to adjust the value of the current wavelength in the display window on the top of the screen. By clicking this shortcut the instrument main unit will change the current wavelength value to the set wavelength value automatically.

9.1.2 The Functions of Shortcuts of Icon

When the mouse pointer is moved onto a shortcut of icon, the functions of the shortcut of icon will show. Icons are arranged in order from left to right. The functions are described bellow.



---"Set QA. Parameter" button. This feature has not been activated yet.



---"Set Working Curve Parameter" button means to input coefficients directly to create an equation of a working curve. (The button can be used only under the condition of the method of coefficient input.)



---"Calculate Coefs." button means to create an equation of working curve by measuring standard samples.



---"Input Coefs" button means to input coefficients directly to create an equation of working curve



---"Load Spectrum" button. This feature has not been activated yet.



---"Save Spectrum" button. This feature has not been activated yet.



---"Build Curve" button means to build an equation of standard working curve.



---"Print Curve" button means to print out a working curve.



---"Print Std. Data" button means to print data of a standard sample.



---"Print Spl. Data" button means to print data of a sample.



---"Load QA. File" button means to read in a previously saved quantitation file.



---"Save QA. File" button means to save a current quantitation file.



---"Clear Std. Data" button means to clear all data of a standard sample.



---"Clear Spl. Data" button means to clear all data of a sample.



---"Exit QA" button means to exit from quantitative analysis.

9.1.3 Menu on the Top of the Screen

Menu on the top of the screen has columns such as "Analysis Mode", "Set Parameter", "Curve Type", "Spectrum", "Data".

1. Analysis Mode

This feature has not been activated yet.

2. Parameter Setting

Click "Set Parameter" to set the start number and concentration unit of sample.

3. Curve Types

By clicking "Curve Type", a pull-down sub-menu shown in Fig.2-17 appears. It has the following items (Mark "✓" is a default selection.)

(1) **Line-Linear Curve (1st order)**. The concentrations of a series of standard samples and their corresponding fluorescent intensity are fitted to form a linear equation.

(2) **Quadric-Quadric Curve (2nd order)**. The concentrations of a series of standard samples and their corresponding fluorescent intensity are fitted to form a quadric equation.

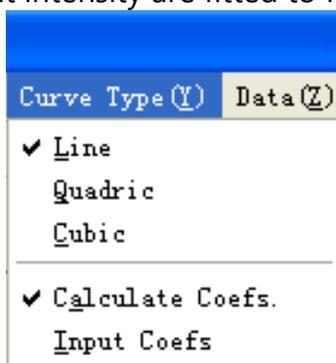


Fig. 2-17 Types of Curve Sub-menu

(3) **Cubic -Cubic Curve (3rd order)**. The concentrations of a series of standard samples and their corresponding fluorescent intensity are fitted to form a cubic equation.

(4) **Calculate Coefs.-** Method of Undetermined Coefficients. The method by which an equation of working curve is created through measuring standard samples is called Method of Undetermined Coefficients. For more details, refer to Section 9.2.4 ~9.2.6.

(5) **Input Coefs.-** Method of Coefficient Input. For a quantitative measurement of known working curve equation of standard samples. Click the "Coefficient Setting for Working Curve" icon and input coefficients "K0" and "K1" to create an equation of working curve. The method of measuring a sample by Method of Coefficient Input is described as below.

- ① Click "Input Coefs." in "Curve Type" menu.
- ② Click "Set Working Curve Parameter" icon. "Input Coefficient" dialog appears. After inputting coefficients "K0" and "K1" separately, click "OK" (In linear curve "K0" represents intercept and "K1" represents slope.). Thus an equation of the working curve is created and can be used for measuring a sample.

4. Data Processing

Click "Data" and pull down a sub-menu, which has the following items. See Fig.2-18.

- (1) **Build Curve.** Build a working curve.
 - (2) **Print Curve.** Print a working curve.
- Note: Please first confirm the setting condition of a printer in **Windows**.
- (3) **Save Curve As.** Save and rename the current curve.
 - (4) **Delete Single Spl. Data.** Delete the data of a single sample from the sample column.
 - (5) **Clear Std. Data .** Clear the data from the standard sample column.
 - (6) **Print Std. Data.** Print the data of standard samples.

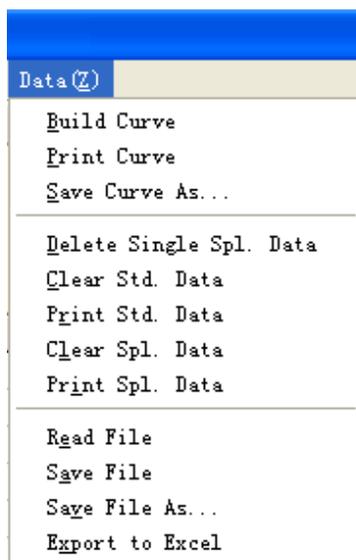


Fig. 2-18 Data Processing Sub-menu

- (7) **Clear Spl. Data.** Clear the data from the sample column.
- (8) **Print Spl. Data.** Print the data in the sample column.
- (9) **Read File.** Load the previously saved quantitation files from the hard disk.

Note: This function is often used to create a working curve by employing the previous data of standard solutions so as to make a direct measurement for samples of the same kind.

- ① After clicking "Build Curve" in "Data Processing" menu, you may see the working curve, the equation and the correlation coefficient immediately.
 - ② After closing the window, place the sample into the light path and click "Measure Spl.". The concentration of the sample will be displayed on the screen.
- (10) **Save File.** Save the data in the quantitation files onto the hard disk. After clicking this item,

a dialog box will appear. Enter a name of the file and click "Save".

(11) **Save File As.** Save the data in the quantitation files onto the hard disk. Rename the new file and click "Save".

(12) **Send to Excel.** The data in the quantitation files can be sent to Excel.

9.2 Operation of Quantitative Analysis

The interface of quantitative analysis is shown in Fig. 2-19. On the left is the display box of Standard Sample and on the right is the display box of Unknown Sample.

9.2.1 Setting of Fluorescence Wavelength for Measurement

Click "Set Standard" below. Input the value of fluorescence wavelength of the sample to be measured, then click "OK".

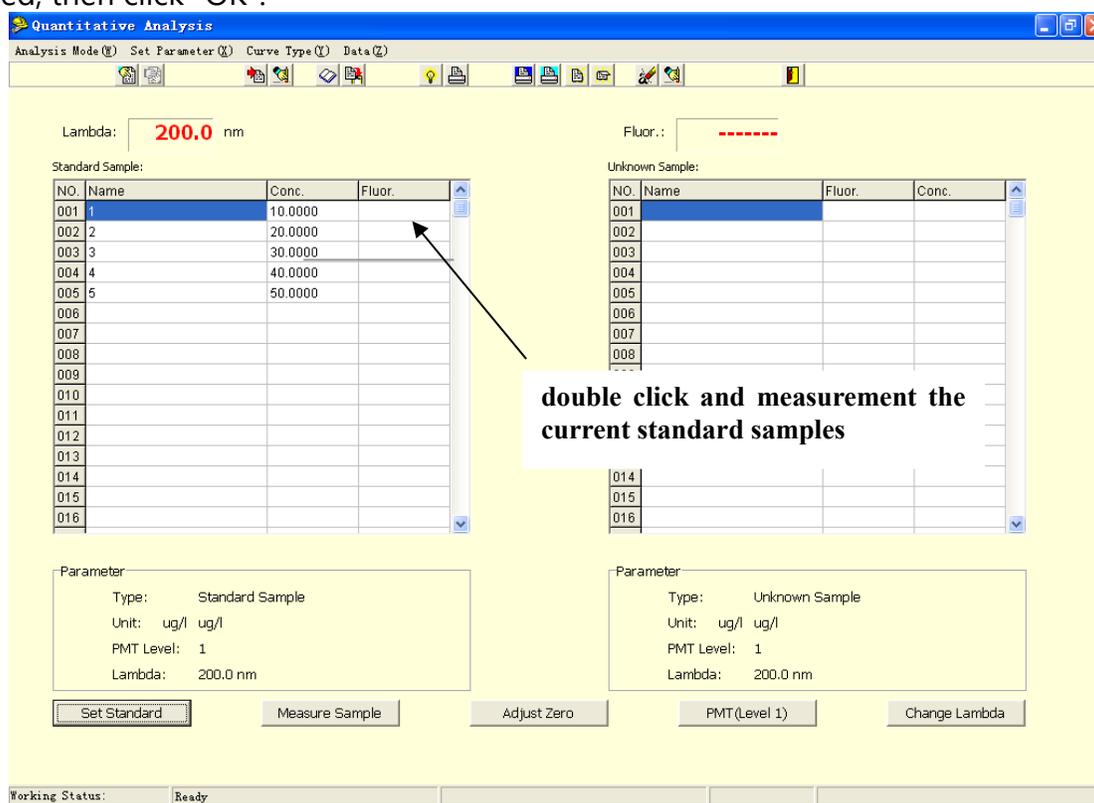


Fig. 2-19 Interface of Quantitative Analysis

9.2.2 PMT Setting

There are 17 grades (1-17). Default PMT level is 1. Generally adjust PMT level by using the highest concentration of the solution. Adjust the fluorescence value to make sure it is stable within the range of 300-400.

9.2.3 Zero-point Adjustment

Place the blank solution into the light path. Click the "Adjust Zero" button (See Fig.2-19). At this time the fluorescence value on the upper right of the interface becomes 0.0.

9.2.4 Standard Sample Setting

After clicking the "Set Standard." shortcut on the bottom left of the interface of quantitative analysis, a dialog box of "Set Standard Samples" shown in Fig.2-18 appears. Concentration unit can be set in "Unit" box. If there are 5 standard samples and their concentrations are 10 for No.1 sample, 20 for No.2 sample, 30 for No.3.....respectively. After inputting "1" in the "Name" and "10" in the "Concentration", click the "Add" button. The data input in will be displayed in the box below. Repeat the above operation for 5 times. Use the "Insert" and "Delete" buttons to edit your sample data. The data will be displayed in the "Standard Samples" box in the interface of quantitative analysis. See Fig.2-20.

Name	Conc.
1	10
2	20
3	30
4	40
5	50

Fig. 2-20 Dialog Box of Set Standard Samples

Note: ① The parameter setting of standard samples must follow the order of concentration from low to high. ② The names of standard samples (including sample names) can be set for Arabic numerals, English alphabet or Chinese characters.

9.2.5 Measurement of Standard Samples

In the "Standard Samples" in the interface of quantitative analysis, 5 rows of data of standard samples input in are listed respectively (See Fig.2-19). When measuring, put the No.1 standard sample into the sample compartment. Then double click the row of the No.1 standard sample. The display of "Under Measurement" will appear on the bottom of the interface. A few seconds later, fluorescence value (F) will be displayed. Repeat the above procedures until the fluorescence values of all standard samples are measured out.

9.2.6 Method of Undetermined Coefficients

Click "Curve Type" in the menu bar. Select the type of curve that you need in the pull-down sub-menu (The default setting is linear curve.).

Then click "Data" in the menu bar and select "Build Curve" in the sub-menu. A "Working Curve Window" will appear on the screen and a working curve of the standard sample will be plotted. Below the working curve there are an equation of the working curve and correlation coefficient.

The method as described above by which an equation of working curve is created through measurement of standard samples is called Method of Undetermined Coefficients.

If an equation of working curve is known, you may select "Input Coef.". As for the measuring of samples by the method of inputting coefficient, refer to Section **9.1.3**.

9.2.7 Measurement of Samples

After placing the sample into the sample compartment, click the "Measure Sample." shortcut on the bottom of the screen. The fluorescence value and the corresponding concentration value of the sample will be displayed in the display box of sample measurement.

9.2.8 Set Sample Name

Click one grid of "Sample Name" in the "Unknown Sample" box to edit the name of selected sample.

10 Appendix: Data Processing with Microsoft Excel 2000

Data Processing with Microsoft Excel 2000 Software

Experimental data processing of fluorophotometry by using Excel 2000 Software. Open Microsoft Excel 2000 software. The interface of the software will be displayed on the screen of the computer. Take the experimental data obtained by multipoint method of standard sample as an example.

1. Input Experimental Data

Input the recorded experimental data into Sheet1 of Excel.

No.	B0	B1	B2	B3	B4	B5
Concentration of Riboflavin Solution (g/mL)	0.000	0.020	0.040	0.060	0.080	0.100
Fluorescent Intensity	0.0	9.1	17.8	26.9	35.4	43.6

In the cell A1 input Concentration "c/ (g/mL)", in the cell B1 input "Fluorescent Intensity" and in the cells from A2 to A7 input the concentration data of riboflavin solutions. Input the corresponding data of fluorescent intensity in the cells from B2 to B7. Adjust the data format to let it be in the middle as shown in Fig.3-1 after inputting.

	A	B	C
1	c/(g/mL)	FL	
2	0	0	
3	0.02	9.1	
4	0.04	17.8	
5	0.06	26.9	
6	0.08	35.4	
7	0.1	43.6	
8			
9			
10			

Fig. 3-1 Screen Display after Inputting Fluorescent Intensity and Concentration

2. Enter the Chart Wizard System

(1) Preview Generated Graphics. Click the "Chart Wizard" button on the interface of Excel to go into "Chart Wizard 4-Procedure 1". From "Chart Types" on the "Standard Types" page, select "XY Scatter Chart". From the sub-chart types, select "Scatter Charts with Smooth Lines". Click the "Press down to View Examples" button. Preview the graphics to be generated and the setting of initial coordinates.

(2) Improve Generated Graphics. Click the "Next" button to go into "Chart Wizard 4-Procedure 2". Click "Series" page tab. Click the "Next" button to go into "Chart Wizard 4-Procedure 3". In the "Chart Title" field on the "Title" page, input "Standard Curve"; in the "Value (X) Axis" column input concentration "c/ (g/mL)" and in the "Value (Y) Axis" column input fluorescent intensity "F". Switch to the "Grid" page. Within the first three checkboxes select the check mark "✓". Then switch to the "Legend" page and delete the check mark "✓" for "Show Legend".

(3) Insert Graphics. Click the "Next" button to go into "Chart Wizard 4-Procedure 4". Select the location of inserting a graphics.

3. Data Processing by using Linear Regression

From the Excel menu select "Chart (C)" → "Add Trend Line (R)...". Select "Linear Regression". Switch to the "Options" page. Select "Show Formula" and "Display R-Square Value". Click the "OK" button.

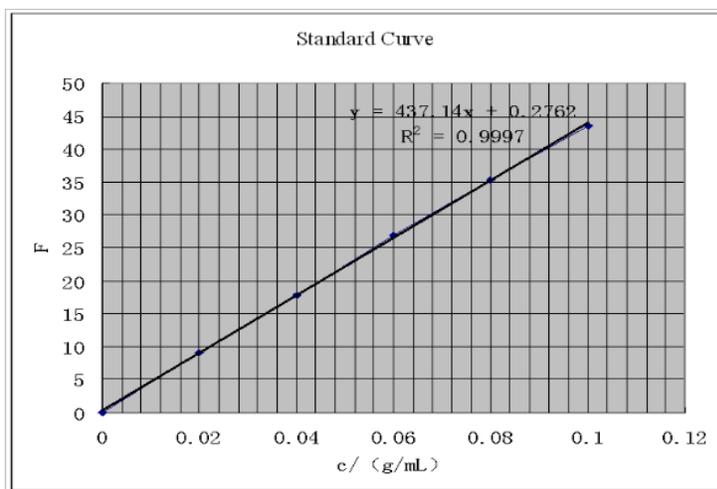


Fig. 3-2 The Standard Curve Plotted after Data Processing With Excel 2000 Software in Measurement of Riboflavin by fluorophotometry ($y=437.14x+0.2762$ the fitted equation, correlation coefficient $R^2=0.9997$)

4. Measurement of Unknown Sample

Under the same condition measure the fluorescent intensity of unknown sample solution. Comparing the fluorescence value with that in the above standard curve, or putting the value into the fitting equation, you may obtain the concentration of riboflavin in the unknown sample.



BIOLAB SCIENTIFIC LTD.

3660 Midland Avenue, Suite 300, Toronto, Ontario M1V 0B8 Canada

Email: contact@biolabscientific.com Tel: +1 707 533 1445

Website: www.biolabscientific.com