

ECLASSICAL 3200L UHPLC

USER MANUAL



Operation Manual for EClassical D3251L Fluorescence detector

V1.0.2

Statement

The manual is intended to help users to understand, use and maintain EClassical D3251L Fluorescence detector. Elite Analytical Instruments Co., Ltd. does not assume there responsibility caused by business or special purpose use of the manual.

The information in this document is subject to change without notice and should not be construed as a commitment by Elite Analytical Instruments Co., Ltd..

This manual is believed to be complete and accurate at the time of publication. Elite Analytical Instruments Co., Ltd. assumes no responsibility for any errors that may appear in this document.

In no event shall Elite Analytical Instruments Co., Ltd.be liable for incidental or consequential damages in connection with, or arising form, the use of this manual.

The Copyright of the manual is owned by Elite Analytical Instruments Co., Ltd..

This book or parts thereof may not be reproduced in any form without the written permission of Elite Analytical Instruments Co., Ltd..

Please read the document carefully before using EClassical D3251L Fluorescence detector.

Foreword

Thank you for purchasing the EClassical D3251L fluorescence detector.

The Elite EClassical D3251L fluorescence detector is combined with a liquid chromatography system to detect the composition of a sample using fluorescence. Do not use samples that may be infected with bacteria or viruses.

This instrument is a product made by people with basic knowledge of chemical analysis. If the wrong analytical instrument is used, the use of drugs/samples will not only not get the correct analysis results, but also lead to safety problems, so personnel with basic knowledge of chemical analysis should not use it.

Before you want to use it, be sure to read the instruction manual in detail, understand its content, and use the instrument correctly. The liquid chromatography system consists of a pump that sends the mobile phase, a sample importing part that injects the sample (automatic sampler, manual sampler), a column that separates the sample, a column temperature chamber that keeps the column at a certain temperature, and a detector that converts the separated sample into a signal for detection. Before use, please read the instructions of the various instruments that make up the liquid chromatography system and be sure to use this product correctly.

Matters needing attention

1. Warranty on the product

1) Product guarantee

Regarding the Elite D3251L fluorescence detector and its accessories, only when the instrument is used in accordance with the contents of this user manual, the company makes the following warranties based on the specifications specified in the user manual.

No warranty, expressed or implied, is given as to quality, performance, value or fitness for a particular purpose of use.

Without the approval procedures prescribed by the Company, the sales shop, special shop or its staff in oral or written communication of information is not consistent with the performance of the product, there is no guarantee.

2) Scope of warranty

Repair the instrument free of charge when it breaks down due to defects in the company's manufacturing.

However, repairs occur when parts are replaced or exchanged for equivalent equipment. There is also the possibility that the same type of product may not be available when the new generation of computers and printers used in the instrument is frequently introduced in the market. Instruments and consumables that have been abandoned or resold without contact with the Company, and parts of the limited warranty period that fail, are outside the scope of the warranty.

Data loss or application software damage due to instrument failure is outside the scope of the guarantee.

3) Warranty period

Within a year from the time the instrument is set up.

Warranty limits and items outside the scope of the warranty

In the following cases, they cannot be subject to free repair even during the warranty period.

(1) In the event of failure due to use in a place that does not conform to the Company's specified setting (refer to Appendix 1 setting).

(2) When the power supply (voltage, frequency) specified by the company is not used, and the fault occurs due to the abnormal power supply.

(3) Corrosion or deterioration of piping due to impure substances mixed in gas, air and cooling water provided by customers themselves.

(4) Corrosion of electrical circuits or deterioration of optical components due to strongly corrosive gases contained in the air.

(5) Failure due to the use of hardware, software or auxiliary supplies not provided by the company.

(6) Do not follow the use of this instruction manual, and do not contact the company, repair and cause failure.

(7) When a company or individual other than the company has carried out a repair.

(8) After the setting, the company has not allowed to move or shift the setting, resulting in failure.

(9) When the company does not permit the decomposition, modification or relocation.

(10) Due to fire, earthquake, geomantic disaster, thunder, commotion, riot, crime, terrorist activities, war, radioactive pollution, pollution caused by harmful substances and other force majeure accidents caused by failure.

(11) When a fault occurs due to a computer virus.

(12) Instruments and consumables that have been abandoned or resold without contact with the Company, and parts of the limited warranty period break down.

(13) When there is a failure due to a lifetime part beyond the replacement date.

4) Limitation of liability

The liability of the Company is limited to the liability for compensation of persons and other property caused by the defects of the products. We shall not be liable for any direct, indirect, special, incidental or inevitable damage caused by the use of the product.

5) The use of industrial ownership by third parties

The Company shall not be liable for any dissatisfaction with products made using instruments provided by the Company or its affiliates or patents held by third parties regarding methods of use of instruments.

2. About setup/relocation and post-guest service

1) Set/move

(1) Please do not set the instrument on delivery without permission.

In order to use the analytical instruments safely and with high precision, the setting up of the instruments at the time of delivery is carried out by the Company or by technicians who have been educated in the Company's setting and whose qualifications are recognized by the Company.

(2) When setting up, customers are expected to refer to the user manual and make various preparations in accordance with the setting conditions of the instrument (refer to Appendix 1 setting).

(3) After the delivery of the instrument, if it is necessary to move the instrument, in order to avoid disputes caused by the move, it is necessary to consult the dealer who purchased the instrument or the company.

2) After-customer service

(1) For post-customer service, please consult your local distributor or our company.

(2) For the service after the warranty period, the company has prepared a maintenance contract, inspection service contract, etc., please consult the local dealer or the company for details.

(3) Service parts/consumables are available for the instrument during its lifetime. Available parts or equipment can also be provided after the durability period (up to 10 years), but this does not guarantee the extension of the durability of the instrument. In addition, due to the cessation of production of the parts or equipment to be supplied, it is possible to provide some substitutes.

(4) Due to the shutdown of the body, it may not be possible to supply body components other than repair parts/consumables. If the instrument fails and cannot be repaired, please stop using it and hope that the customer can discuss the replacement of the instrument.

3. About customer facing seminars/training

Conduct customer-oriented seminars or training for the safe and accurate use of analytical instruments at our facilities or by visiting customers directly. Please consult your local sales department (paid service) for instruction procedures.

1) Durable period

When regular maintenance/inspection, replacement and repair of life parts are carried out in accordance with the provisions of this instruction manual, the durability of the instrument is 7 years after the beginning of use (set up) (when the instrument is used under standard conditions of use (8h/ day and 20 days/month)).

If you want to use the instrument after the service life, please ask the service company to conduct a safety check (it is a paid service). If the instrument is used continuously without safety checks, it may cause safety problems due to the failure of the instrument.

And accept the safety inspection at the same time, also expect customers to discuss the replacement of the instrument.

4. About abandonment

This instrument does not currently contain substances that should be distinguished from general waste materials and discarded. However, it may be changed by future laws or regulations. When abandoning the instrument, please confirm the relevant laws before proceeding, or please consult the company.

When disposing of the used computer or taking it to the recycle bin, please delete all data remaining on the hard drive under the customer's responsibility.

When you discard rechargeable batteries on your computer, you should entrust a professional recycling company, or tape the positive and negative electrodes to keep them insulated and hand them over to a recycling center.

Other points of attention

Use of drugs/samples

(1) When using this instrument for analytical services, the drug or sample used by the customer must be used, kept and handled according to the prescribed standards and under the customer's responsibility.

(2) Regarding the use/storage/disposal of test/standard solution/precision management samples, please follow the instructions of the respective distributors.

(3) Do not use samples that may be infected with bacteria or viruses.

About radio disturbances

This instrument complies with EN 61326-1(2013) Class A specification. If it is set near other electrical/electronic instruments, it may have adverse effects on each other. In particular, try not to set in the TV, radio and other electrical equipment next to. In addition, please do not bring electrical equipment * into the room where this instrument is installed

* A device that emits radio waves, such as a cell phone, a radio transceiver, or a radio phone, as if it were a specific power-saving device.

Safety precautions

Before using the Elite D3251L fluorescence detector, please read the following safety instructions carefully to fully understand their contents.

The instructions here are important matters related to safe operation, and must be followed. In this manual, according to the level of danger and damage, safety signs are divided into the following four categories:



This is a security alert marker. Used to call attention to a potential hazard to a person. To avoid possible harm or death, be sure to follow all safety information described after this sign.



[Warning]

Failure to properly follow the instructions and precautions indicated by this sign may result in serious injury or damage to health and property. The property damage includes the environment around and the instrument.



[Caution]

Failure to properly follow the instructions and precautions indicated by this sign may result in slight injury or damage to health and property. Slight injury means no hospitalization is needed to the wounded. Slight property damage means the instrument can be recovery through simple maintenance.



[Note]

The sign is used wherever information is given to ensure optimal performance of the instrument.

Precautions or notices concerning non-human safety are indicated by the following slogan.

Notice: This means that if not avoided, there is no harm to people, but may lead to property damage/major damage to the instrument/data damage/environmental pollution of dangerous conditions.

Note: In order to use the instrument correctly, avoid minor damage to the instrument, and give full play to its function and performance.

● Be sure to follow the warning label affixed to the instrument, the precautions and instructions in all instructions that come with the instrument. Failure to comply may result in death or serious injury and damage to equipment by electric shock or fire.

● The maintenance to be carried out by the customer is limited to the items listed in this manual. Please do not perform repairs that are not documented in the user manual. For example:

- Please do not modify the instrument or use other parts than those specified.
- Do not use the instrument without removing the safety device.
- Please do not use the accessory parts of this instrument on other instruments.
- Please do not set up or move the instrument after delivery.

● When using chemical substances such as test drugs, please check the properties of the substance and information about the use (MSDS, etc.), and use it exactly.

● If there is a problem on the instrument or an unknown point on the user manual, please contact our customer service staff.

● Although the precautions and instructions in the instrument or user manual have been thoroughly discussed, unexpected events may occur. When operating, not only follow these matters, but also hope that customers often pay attention to safety.

Common considerations regarding safety

The following represents the precautions described in this user manual.



[Warning] Accidents due to continued use under abnormal conditions

- Under abnormal conditions, continuous use of the instrument may result in electric shock/fire. When abnormal sound, odor, smoke and other abnormal phenomena occur during use, please cut off the power supply immediately and unplug the power cord. Then, after implementing safety countermeasures suitable for the situation, contact the dealer who purchased the instrument or the customer service department of the company.

Warning Precautions in the Manual

The following summarizes the warning marks described in this instruction manual.



[Warning]



Ignition of Flammable Chemicals

- This instrument is not explosion-proof. In unattended operation, do not use organic solvents having an ignition point below 70 °C.
- Beware of ignition hazard when using flammable chemicals such as organic solvents.
 - (a) Do not bring a heat or flame source near the instrument.
 - (b) Well-ventilate the laboratory room where the instrument is used.
 - (c) Always check the following conditions. If an abnormality is found, stop operation immediately.
 - Leakage of solvent or waste solution.
 - Leakage of solvent inside the instrument.
- When using flammable chemicals, be careful about possible ignition due to static electricity. To prevent the build-up of static electricity, use a conductive container for waste solution employ and provide proper grounding connection to it.



Explosion of Vapor from Flammable Chemicals

- If a flammable chemical such as organic solvent leaks from the flow path of the instrument and its vapor concentration reaches the explosion limit, it could cause spontaneous combustion with dangerously explosive results. When using a flammable and readily volatile chemical, be sure to check for leakage from the instrument flow path and ventilate the laboratory room adequately.



Electric Shock due to Contact with Inside of Instrument.

- **There is a possibility of death or serious injury due to electric shock from xenon lamp power supply (30kV). When removing the cover of the light source in order to replace the xenon lamp, the power supply must be cut**

off first;

- **Electric shock may result when replacing the fuse.**
- **Before replacing the fuse, be sure to cut off the power supply and unplug the power cord**



Electric Shock due to Improper Grounding

- This instrument is designed in conformity with the specifications of Class I in Annex H of the IEC 61010-1 (International Electrotechnical Commission Standards) – Issue 1.

To prevent an electric shock hazard, provide a proper grounding connection.

- (a) Be sure to use the grounded 3P power cable, which is supplied as a standard accessory for the instrument.

The use of a different type of power cable may result in an electric shock hazard. Connect the 3P power cable to a grounded 3P power outlet.

- (b) If a grounded 3P power outlet is not available, use a grounded 3P table tap or a 3P-2P adapter. In this case, be sure to provide proper grounding connection.

For grounding connection, use a screw having a diameter of M4 or higher and a turn count of 3 or more in threading, and a wire having a thickness of 1.25 mm.



Injury due to Xenon Lamp Explosion.

[In use]

- When the cumulative turn-on time of the xenon lamp exceeds the guaranteed useful lifetime, the electrodes evaporate and the scattered matter sticks to the bulb wall, so its blackening progresses and heat dissipation is hindered. As a result, the internal bulb temperature (pressure) will rise abnormally to cause a danger of burst, etc.

Replace the xenon lamp with a new one before its cumulative turn-on time exceeds the guaranteed useful lifetime.

Model	Part Name	Guaranteed life	Limit life
5J0-1890	150 W xenon lamp	500 hours	2500 hours

[At replacement]

- Before removing the xenon lamp for replacement, turn off the xenon lamp (turn off power to the instrument) and then wait for at least one hour until the xenon lamp becomes sufficiently cool to reduce its internal pressure the normal safety level.
- If a strong shock or impact is applied to the xenon lamp or if the surface of its quartz glass part is scratched, it may explode and scatter glass pieces, resulting in personal injury. Be sure to wear proper protective gear such as safety goggles, safety mask, thick long sleeves, and gloves when handling the xenon lamp.

[At mounting]

- When loosening or tightening the retaining nut for the xenon lamp, be careful not to apply excessive force to its glass bulb part. Never hold and turn the glass bulb part for loosening/tightening.
- Do not touch the quartz glass part of the xenon lamp with bare hands. If the quartz glass part of the xenon lamp is contaminated with dust or fingerprints, wipe it using a gauze sheet or absorbent cotton cloth slightly moistened with high-quality alcohol. If the xenon lamp is turned on with dust or fingerprints left on the surface of the quartz glass part, it may cause contamination burn-in to decrease the mechanical strength of the glass part, resulting in explosion of the xenon lamp.
- Be sure to mount the xenon lamp in the specified direction.

If the mounting direction (polarity) is wrong, the cathode will be consumed significantly to disable turn-on of the lamp.

Mount the lamp so that the '+' (anode) mark on it will be positioned at the support metal of the lamp holder.

If the lamp with its cathode consumed excessively is used continuously, pressure inside the lamp bulb may become too high, causing possible explosion. To prevent this, replace the lamp with a new one immediately if its cathode has been consumed substantially.
- If the nut on the lamp base and wiring part is loose, the contact resistance between them will increase due to poor contacting. This could generate a large amount of heat to make the lamp extremely hot, resulting in possible explosion. To prevent this, be sure to tighten the nut securely.

[At disposal]

- The xenon lamp is filled with high-pressure gas (approx. 1 MPa at room temperature, approx. 4 MPa under operating condition), and this high-pressure gas still remains in the xenon lamp after it is demounted for replacement. For disposal of the used xenon lamp, wrap it with a thick cloth

(e.g., triple-folded cotton cloth) completely and crush its glass part with a hammer or the like. Then, discard the xenon lamp as a dangerous waste item properly. If the xenon lamp is discarded without being crushed, it could explode due to possible impact at the time of disposal, scattering glass pieces to cause personal injury.



Inflammation or Injury due to Toxic, Corrosive or Stimulative Solvent.

When using a toxic, corrosive or stimulative solvent, be careful not to incur a physical inflammation or injury. For details of the properties of each solvent and how to handle it, refer to the relevant Safety Data Sheets (SDS). Be sure to handle each solvent properly.

- (a) Wear proper protective clothes (e.g., safety goggles) so that a solvent will not come into direct with the skin.
- (b) Ventilate the laboratory room adequately to prevent accidental inhalation of harmful solvent vapor.

Warning Precautions in the Manual (Continued)

The following summarizes the warning marks described in this instruction manual.



[Note]



Touching Hot Part Could Result in Burns.

The light source lamp and its cover become hot during operation and remain hot for a while even after power-off.

They can severely burn you if touched.

Before replacement of the lamp, turn power off and wait for about two hours until it cools down sufficiently.



Direct Gazing at Illuminating Xenon Lamp Could Cause Eye Damage.

The xenon lamp radiates intense ultraviolet light when it is on.

If you look at the illuminating xenon lamp during coarse adjustment of its position, your eyes could be damaged. Do not look at the xenon lamp directly when it is lit. Be sure to wear tinted safety glasses to prevent possible eye damage.



Prevention of Instrument Tip over

Personal injury could be incurred if the instrument tips over. When installing the instrument, provide a proper means for preventing the instrument from tipping over accidentally.



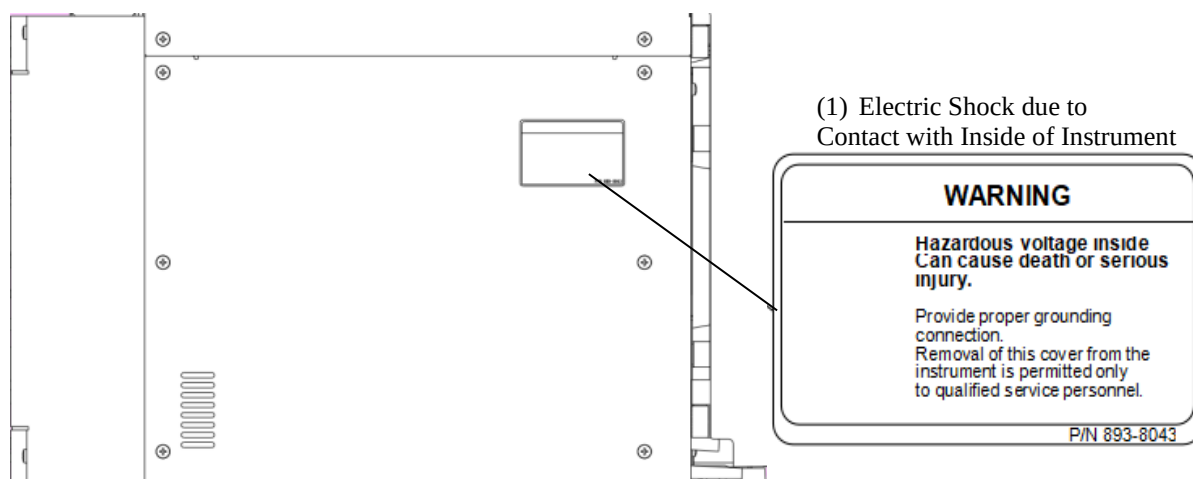
Fatigue due to Long-Hour Operation.

If you keep working with the display monitor and keyboard for long hours, your eyes and body will be fatigued to jeopardize your health. When working with the display monitor for a long time, take a break for 10 to 15 minutes per hour for health of your eyes and body.

Warning and Caution Labels on Instrument

The warning labels shown below are attached on the Elite D3251L fluorescence detector.

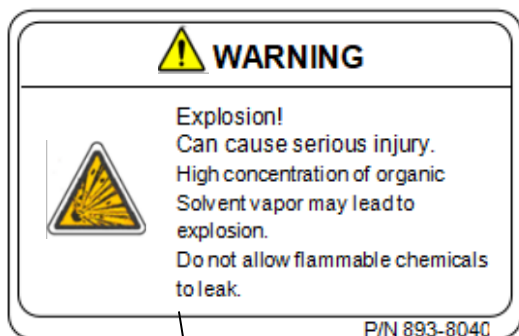
Read the warning labels carefully, and check the instructions on them to attain a clear understanding with reference to actual parts. Periodically check the appearances of these warning labels to see if they are clean to allow easy reading over a safe distance. If any one of the warning labels becomes illegible due to deterioration, contact your local Hitachi High-Tech Science Corporation sales representative or service office of Hitachi High-Tech Science Corporation sales representative for replacement with a new one.



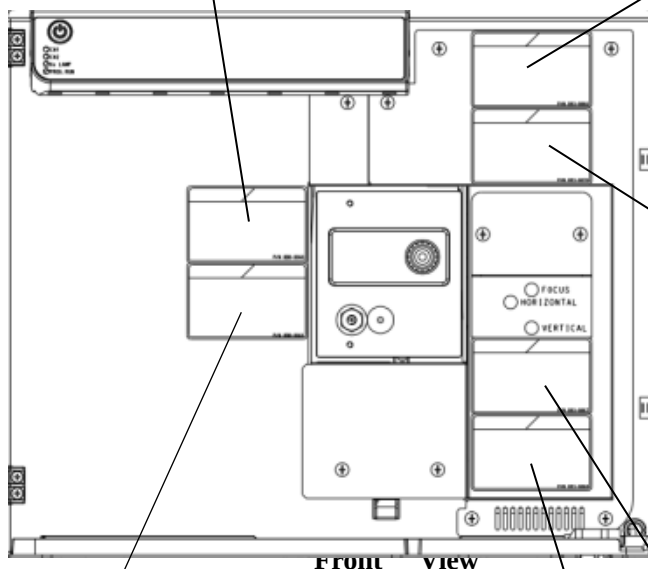
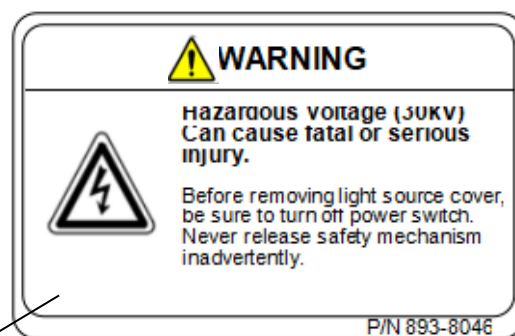
Left side panel

Warning and Caution Labels on Instrument (Continued)

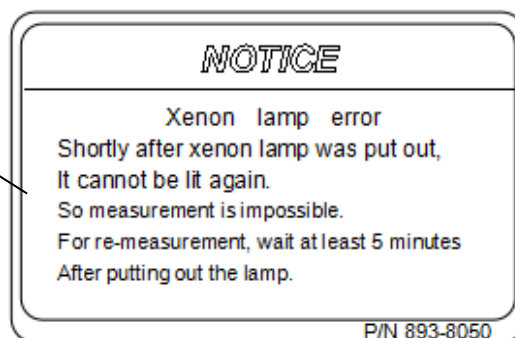
- (2) Explosion of Vapor from Flammable Chemicals



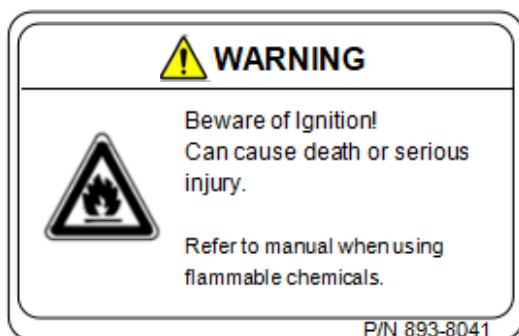
- (3) Electric Shock due to Contact with Light Source Power Supply



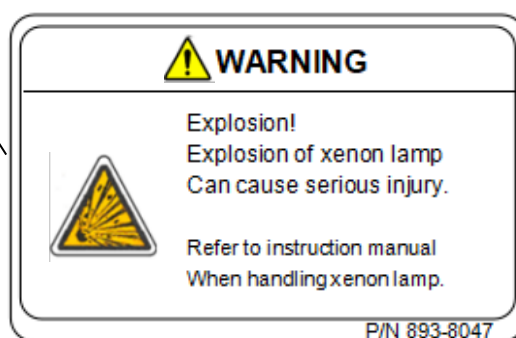
- (4) Before Turning on Xenon Lamp



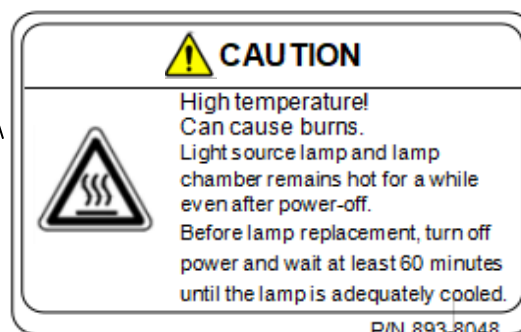
- (5) Ignition of Flammable Chemicals



- (6) Explosion of Xenon Lamp



- (7) Burns due to Contact with Hot Part



Precautions other than personal safety

Precautions on Use of Solvents

- Fluororesin, PEEK, quartz and SUS316 materials are used for the wetted parts of the instrument. Never use solvents that would corrode these materials.
- This instrument has internal parts made of materials that could be corroded by strongly acidic solutions, strongly alkaline solutions or organic solvents. Take care not to spill these corrosive solutions or solvents into the inside of the instrument. If a corrosive solution or solvent spills inside, wipe it off immediately.
- For use of the liquid leak sensor, follow the instructions given below.
 - a. Turn on the leak sensor function when feeding a solvent.
 - b. The leak sensor function may remain inactive in cases where the amount of leakage is too small. Furthermore, leakage of a highly volatile solvent may not be detected. Be sure to check for liquid leakage regularly to prevent a possible serious accident regardless of whether the leak sensor is activated or not.
 - c. In routine inspection of the instrument, visually check for liquid leakage before attempting measurements.
 - d. In periodic inspection of the instrument, check that the leak sensor works normally.
- Referring to the instruction manual accompanying each module unit of the liquid chromatograph system, set up the automatic liquid feed stop function (such as the pressure limiter function of the pump unit) to prevent a possible trouble.

Disposal of Waste Solution

- Be sure to collect waste solution and treat it for proper disposal in accordance with the relevant laws and regulations regarding water pollution control and sewage treatment.
Improper treatment of waste solution may result in environmental pollution and could also lead to a penalty.

Accuracy and Precision of Measured Values

- Carry out periodic inspection and check whether the system is operating normally. If necessary, conduct measurement on a control sample.

Possible Carryover

- When examining the results of measurements, take account of the possibility of occurrence of a carryover. Otherwise, a correct judgment may not be formed. Since fluororesin and quartz materials are used for the wetted parts of piping, particular attention should be paid when analyzing samples that are likely to be adsorbed by these materials

Before Turning on Xenon Lamp Again

- After the xenon lamp is turned off, it remains very hot for a while. Under this condition, it is not allowed to turn on the xenon lamp again for measurement. Before turning on the xenon lamp again, wait for at least five minutes.

CONTENTS

CHAPTER 1 OUTLINE	1
1.1 OUTLINE	1
1.2 TECHNICAL SPECIFICATIONS AND PERFORMANCE SPECIFICATIONS	1
1.3 PHYSICAL SPECIFICATIONS.....	3
CHAPTER 2 FUNCTIONS	1
2.1 THE NAME AND FUNCTION OF EACH PART	1
2.2 NAME AND FUNCTION OF EACH PART OF THE CONTROL KEY BOARD (OPTIONAL)	3
2.3 DESCRIPTION OF FLUOROMETRY	5
2.3.1 Principle of fluorometry.....	5
2.3.2 Advantages of fluorometry	6
2.3.3 Notes on fluorescence analysis.....	8
2.3.4 Principle of operation	12
2.3.5 Features of light source.....	12
CHAPTER 3 INSTALLATION AND TRANSPORTATION	1
3.1 UNPACKING INSPECTION	1
3.1.1 Unpack	1
3.1.2 Packing list.....	2
3.2 INSTALLATION REQUIREMENTS.....	2
3.2.1 Site requirements	2
3.2.2 Power Cables	3
3.2.3 Computer Requirements	4
3.3 ASSEMBLY	5
3.3.1 Removing Transport Fixing screws	5
3.4 CIRCUIT AND COMMUNICATION CONNECTION	6
3.5 VERIFICATION.....	7
3.6 TRANSPORTATION	7
CHAPTER 4 INSTRUMENT OPERATION.....	1
4.1 TURN ON AND TURN OFF	1
4.2 INSTRUMENT PREHEATING	2
4.3 KROMSTATION CHROMATOGRAPHIC DATA WORKSTATION.....	2
4.3.1 Configuration.....	3
4.3.2 Method Settings.....	5
4.4 D3251L MAINTENANCE SOFTWARE	7
4.4.1 Connecting Devices.....	8
4.4.2 Status.....	8
4.4.3 Wavelength Setting	9
4.4.4 Em Bandwidth setting.....	10
4.4.5 Lights and controls.....	11
4.4.6 Meta Parameter Setting	11
4.4.7 Displaying Maintenance Information.....	12
4.4.8 PMT Voltage Settings	14
4.4.9 Spectral scanning.....	15
4.4.10 Water Raman measurement	17
4.5 BEFORE PERFORMING ANALYSIS	21
4.5.1 Mobile phase selection and use of attention points.....	23
4.5.2 Characteristics of organic solvents	24
4.5.3 Cautions on static electricity	28
4.6 PREPARATIONS	28
4.6.1 Degassing and removing dust from mobile phase	28

4.6.2 Degassing mobile phase.....	29
4.6.3 Removing dust from mobile phase.....	31
4.6.4 Removing dust from sample.....	31
CHAPTER 5 CONTROL KEYBOARD OPERATION (OPTIONAL)	1
5.1 BASIC OPERATIONS.....	1
5.1.1 Powering on Power Supplies	2
5.1.2 Parameter Input Method.....	3
5.1.3 Automatically Return to zero.....	6
5.1.4 Setting of measurement wavelength	6
5.1.5 End Operation	7
5.1.6 Cleaning after use.....	7
5.2 UTILITY SETTINGS	8
5.2.1 Setting of OFFSET	8
5.2.2 Setting the TIME CONSTANT.....	9
5.2.3 Setting whether to USE the time program (USE PROG).....	10
5.2.4 Setting of the applied voltage of photomultiplier (PMT VOLT).....	11
5.2.5 Other settings.....	11
5.3 GLP ASSOCIATION FUNCTION.....	15
5.3.1 Xenon (Xe) lamp log (LOGBOOK) display.....	15
5.3.2 Setting xenon (Xe) lamp log (LOGBOOK)	16
5.3.3 Display of mercury (Hg) lamp LOGBOOK.....	18
5.3.4 Reset the Mercury (Hg) LOGBOOK.....	19
5.3.5 Setting the KEY LOCK.....	20
5.3.6 Wavelength Detection Accuracy with mercury lamp (WL CHECK)	21
5.3.7 Xe lamp energy check.....	22
5.4 OPERATING INSTRUCTIONS.....	24
5.4.1 Setting of time program	24
5.4.2 Start Time Program.....	29
5.4.3 Stopping the time Program.....	29
5.4.4 Time Program Operations.....	29
5.4.5 Spectrum measurement.....	32
5.4.6 Setting of recorder and spectrum output	36
5.4.7 Labeling Operations.....	39
5.5 SET UP SETTINGS	40
5.5.1 Setting the Communication Channel (CH).....	40
5.5.2 Setting LCD CONTRAST	41
5.5.3 Setting Xenon lamp exchange standard time.....	42
5.6 USE THE KEY PAD FOR INSTRUMENT MAINTENANCE/INSPECTION	44
5.6.1 Checking Basic Operations.....	44
5.6.2 Confirming Performance Specifications	44
5.6.3 Wavelength accuracy check and calibration	57
CHAPTER 6 MAINTENANCE AND INSPECTION	1
6.1 PERIODIC CHECK.....	1
6.2 CHECK/CLEAN THE LIQUID TRAY	2
6.3 CHECK AND CLEAN THE FLOW TANK.....	4
6.4 CHECK AND CLEAN THE FLOW TANK.....	5
6.4.1 Cleaning/cleaning of circulation pool.....	6
6.4.2 Decomposition and cleaning of flow pool.....	8
6.4.3 Leakage of the flow pool.....	10
6.4.4 Cautions on storage of the flow cell.....	12
CHAPTER 7 TROUBLESHOOTING AND DIAGNOSIS	1
7.1 FAULT MEASURES	1
7.2 ERROR MESSAGE.....	2

7.2.1 Workstation.....	2
7.2.2 Key Panel (Optional Module).....	4
CHAPTER 8 REPLACEMENT PARTS/CONSUMABLES.....	1
8.1 REPLACE PARTS AND CONSUMABLES	1
8.2 Xe LAMP REPLACEMENT.....	2
8.3 REPLACING A MERCURY (HG) LAMP	12
8.4 REPLACING THE FUSE.....	14
8.5 OPTIONAL COMPONENTS	15
APPENDIX	I
SAFETY INFORMATION.....	I
COMMON SOLVENT INFORMATION	II

Chapter 1 OUTLINE

1.1 Outline

The EClassical D3251L fluorescence detector (abbreviated D3251L detector) is one of the EClassical 3200L series products introduced by Elite

The D3251L detector is used as the test unit of this series of HPLC, which can be easily used with various liquid chromatography infusion pumps, automatic sampler, column thermostat and other units, or can be used as a test tool alone.

The D3251L detector is equipped with both xenon and mercury lamps. Enables universal fluorescence detection and automatic wavelength calibration.

1.2 Technical Specifications and Performance

Specifications

The technical parameters and performance specifications of the D3251L detector are as follows

Table 1-1 Technical specifications and performance specifications of the D3251L detector

NO.	Performance parameter	Performance index
1	Optical system	spectrophotometry Monitor the intensity of the emitted beam
2	Illuminant	150 W xenon lamp Mercury lamp
3	Set range of excitation light wavelength	200 to 850 nm/Zero order
4	Fluorescence wavelength setting range	250 to 900 nm/Zero order (Photomultiplier must be changed at emission wavelength greater than 731 nm)

NO.	Performance parameter	Performance index
5	Excitation side spectral bandwidth	15 nm
6	Spectral bandwidth on the fluorescence side	15 nm、30 nm (variable) In this instrument, the spectral bandwidth is defined by the half-width value. Therefore, in order to completely separate the spectrum, a wavelength interval of more than 30 nm is required.
7	Wavelength setting mode	Use communication (e-Line) or keyboard (optional) to set
8	Wavelength accuracy	± 3 nm
9	Wavelength reproducibility	$\leq \pm 0.5$ nm
10	Recorder output (optional)	10 mV Full scale. Full scale 1 to 1000 mFU (settable in steps of 1mFU).
11	Processor output (optional)	1 V Full scale, digital by e-Line. -40 (-40 mV) to 1000(1000 mV)
12	Time constant	0.05、0.1、0.5、1.0、2.0、4.0、8.0 s
13	Sensitivity	(a) With emission-side spectral bandwidth set at 15 nm S/N ratio for Raman peak of water--900 or more (Baseline method) S/N ratio for Raman peak of water--675 or more (Tangent method) (b) With emission-side spectral bandwidth set at 30 nm S/N ratio for Raman peak of water--4000 or more (BG method) Wavelength: 350 nm, Time constant: 2.0 s
14	Automatic zero range	0 to 1000 mFU
15	Offset range	0 to 1000 mFU (settable in steps of 1 mFU)
16	Spectral memory	Memorized contents--Excitation and fluorescence spectra Number of spectra memorized--4 Measuring wavelength range--Changeable in measurement condition setting
17	Marker (Option)	Place a marker (0.6 mV) on the recording
18	Instrumental conditions setup	Following conditions are set and saved in the memory. - Full scale range - Spectrum measurement range - Photo multiplier applied voltage - Offset value
19	Time program	Number of programs--9 Settable time--Up to 600 minutes in increments of 0.1 minute Number of steps storable--100 steps for a total of 9 files Programmable parameters--Measurement wavelength (Ex and Em) Baseline processing PMT gain

NO.	Performance parameter	Performance index
20	GLP support function	Lamp energy check Wavelength accuracy check Lamp lighting time and replacement record
21	Communication function	e-Line communication
22	External output terminal (option)	• Processor: 1 V Full scale • Recorder: 10 mV Full scale
23	External input/output contact terminals	Time Program Start Error Input/Output Busy Output (Above contacts are incorporated in e-Line connector, and effective with analog connection.) Auto Zero Input Lamp Off Input
24	Flow cell	Standard
25	Flow cell capacity	Standard 12 L (irradiated capacity)
26	Flow cell withstand pressure	1.0 MPa
27	Operating temperature range	4 ~ 35 °C (non-condensation)
28	Operating humidity range	25 ~ 85 %RH

1.3 Physical Specifications

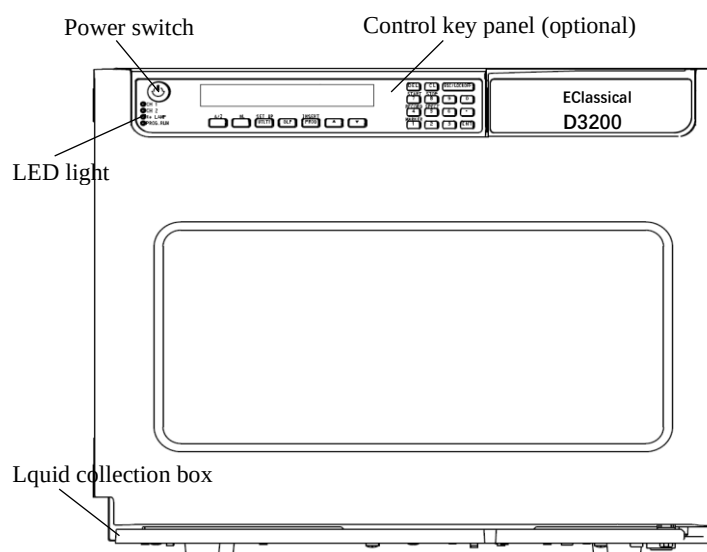
The physical specifications of the D3251L detector are as follows.

Table 1-2 Physical specifications of the D3251L detector

Serial numb	Performance parameter	Performance index
1	Weight	25kg
2	Dimensions (L × W × H)	470 mm × 340 mm × 300mm
3	Power requirement	100 ~ 240 VAC、50/60 Hz
4	Rated power	250 W

Chapter 2 FUNCTIONS

2.1 The name and function of each part



- Power switch: Turns power ON/OFF.
- Drip tray: A tray for liquid leakage.
- Control key plate: Keyboard required for independent operation of the instrument (optional).
- LED: Four LED indicators are provided for indicating the following states.

Table 2-1 LED status display

Name	LED status
CH1 (blue, orange)	Blue: CH1 operation mode.
	Orange: An error has occurred in CH1 operation mode.
CH2 (blue, orange)	Blue: Time program is in execution.
	Blue: CH2 operation mode.
Xe LAMP (blue)	Orange: An error has occurred in CH2 operation mode.
	Blue: Xe lamp is lit.
PROG. RUN (blue)	Blue: Time program is in execution.

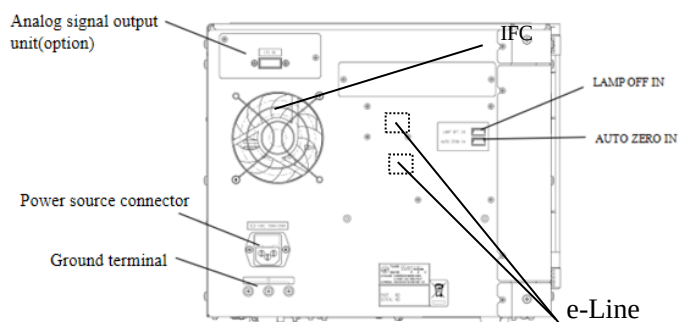


Figure 2-2 Rear Panel

- Analog signal output unit (Option): Provides PROCESSOR, RECORDER, MAKER IN terminal.
- Ground terminal: The terminals for grounding connection (three terminals).
- IFC substrate: DC IN, ACC, e-Line, System DAD, PC interface available.
- Power source connector: Connects the power supply cable.

Table 2-2 Input/output terminals

No.	Indication	Function	Remarks
1	e-Line	1. Used to transmit the acquired signal (or the acquired spectral data) to other units on the e-line. 2. Used for input/output of each contact signal. ① START IN ② ERROR IN ③ ERROR OUT ④ BUSY OUT	1. The fluorescence intensity data is output. 2. ① The program is initiated. ② An error condition in another instrument is checked. ③ An error condition on this instrument is indicated. ④ The operational status of the instrument is indicated.
2	LAMP OFF IN	The lamp is turned off by means of a contact signal input.	
3	AUTO ZERO IN	The auto zero function is activated by means of contact signal input.	
4	PROCESSOR (option)	Analog output for data processor (integrator) 1. The fluorescence intensity value at each point of time 2. Stored spectral data	The fluorescence intensity value data is output.
5	RECORDER (option)	Analog output for recorder 1. The fluorescence intensity value at each point of time 2. Stored spectral data	The fluorescence intensity value data is output.
6	MARKER IN (option)	The marker function is activated by means of contact signal input.	The marker function is started when the short-circuit signal of more than one second is input.

2.2 Name and function of each part of the control key board (optional)

A control key board will be used when a chromato data station won't be used for an instrument controlling. The control key board will be invalid when the chromato data station is connected.

Description is given for each key on the optional UI pad control panel.

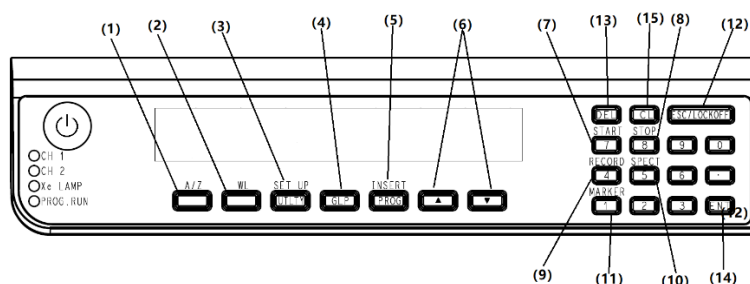


Figure 2-3 Controlling the key pad

Note: Press the **ESC** key to return to the initial state when you made incorrect operation.

Table 2-3 Key Functions

No.	Indication	Function	Remarks
1	A/Z  (Automatic zero setting)	Sets display and output to zero at each measuring wavelength.	
2	WL  (Wave length)	Used to set each measuring wavelength.	
3	SET UP  (Utility, Set up)	(1) Sets offset value, time constant, etc (UTILITY). (2) Sets digital communication channel via the e-Line, LCD contrast.	(2) Keep pressing it with  key simultaneously to change to the setup screen.
4	GLP  (GLP)	Selects GLP information. (1) Checks for lamp energy and wavelength accuracy. Displays and resets lamp logbook. (2) Sets the key lock.	(2) Press the ESC key to release the key lock
5	INSERT  (Set program)	Selects and sets the time program.	

6	△ ▽ (Arrow symbol)	(1) The monitor screen △key: Accesses the previous screen. ▽key: Accesses the next screen. (2) Editing program △key: Displays the previous screen. Indicates the last line if it is the first line. ▽key: Displays the next screen. Indicates the first line if it is the last line.	
7	START 7 (start)	(1) Starts the time program. (2) Starts analog output of the stored spectrum.	(2) Valid only when connect to the analog signal output unit.
8	STOP 8 (stop)	(1) Stops the time program. (2) Stops analog output of the stored spectrum	
9	RECORD 4 (recorder)	Used to specify the recorder full-scale range, recorder output speed, and output data.	
10	SPECT 5 (spectrum)	Obtains spectrum over a specified wavelength range and stores the spectrum in memory.	
11	MARKER 1 (mark)	Places a marker on the recording.	
12	ESC/ LOCKOFF (Exit, key lock)	(1) Used to return from data input mode to the monitor screen. (2) Used to interrupt the recording of the spectrum. (3) Used to release the key lock when keys are locked.	
13	DEL (delete)	Deletes the step which the cursor is pointed while editing a time program. - Deletes the step which the cursor is pointed in the input part of TIME. - Deletes the item when the cursor points a part other than the input part of TIME. - When the ESC key is pressed while “-” is displayed on the screen, the screen returns to the monitor screen, and cancels the DEL key processing. “-” is displayed after pressing the DEL key, and the processing will be determined by pressing the ENT key.	
14	ENT (press enter)	Accepts entered information.	
15	CL (clear)	(1) Used to remove the present data input on the display before the ENT key is pressed (eg. to erase incorrect data). (2) Also used to reset system when an error condition is indicated.	

2.3 Description of Fluorometry

2.3.1 Principle of fluorometry

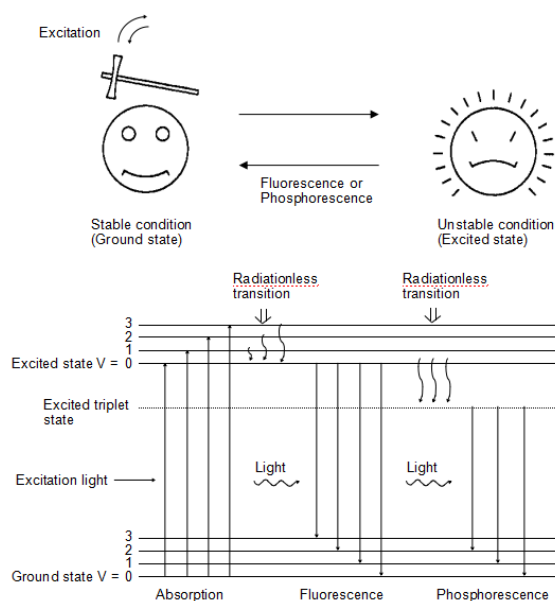


Fig. 2-4 Typical Organic Molecular Energy Level Diagram

Figure 2-4 shows the energy level transitions of an organic molecule for the various processes involved in absorption, fluorescence and phosphorescence.

When light strikes an organic molecule in the ground state, it absorbs radiation of specific wavelengths and several excited states are populated. A part of the excitation (absorbed) energy is lost in vibrational relaxation, i.e. radiation less transition to the lowest vibrational level in the excited state.

The molecule can return to the ground state by;

- (1) Emitting radiation (Fluorescence)
- (2) Undergoing a radiationless transition to populate the triplet state. The triplet state can emit radiation (Phosphorescence). Generally, phosphorescence persists for 10^{-4} seconds or longer due to the selection rule imposed on the triplet-to-singlet transition. In contrast, luorescence takes place over a period of 10^{-8} to 10^{-9} seconds.
- (3) Going through a radiationless transition to return directly to the

ground state.

Since a part of the radiation absorbed by the substance is lost as vibrational energy; the energy emitted by the excited state is less than that absorbed by the compound (i.e. the fluorescence wavelength is longer than the excitation wavelength, ***Stokes' Law**).

The ratio of the number of photons emitted during fluorescence to the number of photons absorbed is called the quantum efficiency of fluorescence (Fluorescence Yield). If two compounds absorb the same number of photons, the fluorescence intensity of the compound with the larger fluorescence quantum yield will be greater than that from a compound with a lower fluorescence quantum yield. Also, the intensity of the fluorescence emitted by a compound is proportional to the number of photons absorbed by it.

Therefore, when a dilute sample is used, the intensity of fluorescence is expressed by:

$$F = KI_0cl\epsilon\phi$$

F : Fluorescence intensity

K : Instrumental constant

I_0 : Intensity of exciting radiation

c : Concentration of the compound of interest

l : Optical path length of cell

ϵ : Absorptivity of substance

ϕ : Quantum efficiency of substance

2.3.2 Advantages of fluorometry

For verifying the advantage of fluorometry, the limitation of absorbance measurement in its application to low-concentration samples is explained first.

In this discussion, we will consider the detection of a sample that has a transmittance of 99% (relative to the blank). If we assume that the inaccuracy of the % transmittance measurement is 0.1%, the reliability of the observed data is as follows:

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

In this example, the uncertainty in the concentration measurement is ±20%. In contrast, in fluorometry, a difference in the signal is directly proportional to the concentration of sample.

An analysis of the error in the measurement is as follows:

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

As is evident from this example, the error percentage by fluorometry is not dependent on sample concentration theoretically, so this method is very advantageous for low sample concentrations.

In fact, when the concentration is extremely low, there will be a relative increase in the error factor, but in general, compared with the absorption method, low concentrations of 3 digits or more can be measured.

The above description can be illustrated in Figure 2-5. In absorptiometry, the signal I_s is used to represent the difference between the incident light amount I_0 and the transmitted light amount I_t , and the point where I_s is the same as the noise level is the detection limit. In contrast, in the fluorescence method, because the fluorescence amount I_F itself is a signal, so as long as a little fluorescence is emitted, this increase can also be detected. Moreover, because the fluorescence wavelength is different from the excitation light (incident light) wavelength, it is not easily affected by the excitation light and can be measured with high sensitivity.

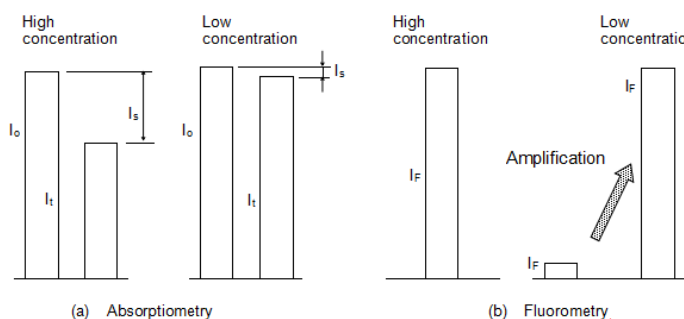


Fig. 2-5 Comparison of Absorptiometry and Fluorometry

In addition to high sensitivity, there is also the advantage of a large amount of information available. In addition to the excitation light, which is equivalent to the absorption spectrum, there is also the fluorescence spectrum.

If the two wavelengths are selected appropriately, or the excitation wavelength is exactly selected, and the fluorescence spectrum (or its inverse operation) is recorded, the quantitative and qualitative analysis of the sample composed of complex components can be carried out.

Figure 2-6 shows the measurement of a sample composed of multiple components. In the absorption method, what can be measured is limited

to the absorption spectrum, so there are more than two components mixed or the absorption wavelength is almost the same, and the components cannot be separated out for measurement. In contrast, in the fluorescence method, even if the absorption wavelength is the same, as long as the fluorescence wavelength is different, the components can be separated and measured by selecting the fluorescence wavelength appropriately.

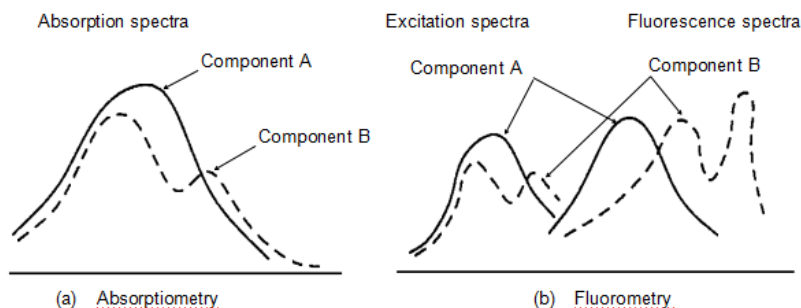


Fig. 2-6 Spectroscopic Measurement of a Sample Containing Two Components

Table 2-4 Comparison of Analytical Information Attainable with Absorptiometry and Fluorometry

Absorptiometry	Fluorometry
Absorption spectra only (corresponding to excitation spectra)	• Excitation spectra • Fluorescence spectra

2.3.3 Notes on fluorescence analysis.

(1) Raman Scattering

When fluorescence is measured, two additional peaks may appear in the spectrum. The Rayleigh peak appears at the excitation wavelength and is due to scattered light, while the Raman peak appears at longer wavelength than the excitation. The position of the Raman band is dependent on the excitation wavelength, while the position of the fluorescence is independent of the excitation wavelength though its peak height changes. Both Rayleigh and Raman scatterings occur due to a solvent. Take care not to mistake them for fluorescence peaks.

Table 2-2 presents the position of the Raman peak for a variety of excitation wavelengths. In liquid chromatography, the Raman scattering appears as a background rise.

This means that baseline noise increases to disturb high-sensitivity measurement.

Table 2-5 Raman Spectral Peak Position at Each Excitation Wavelength

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

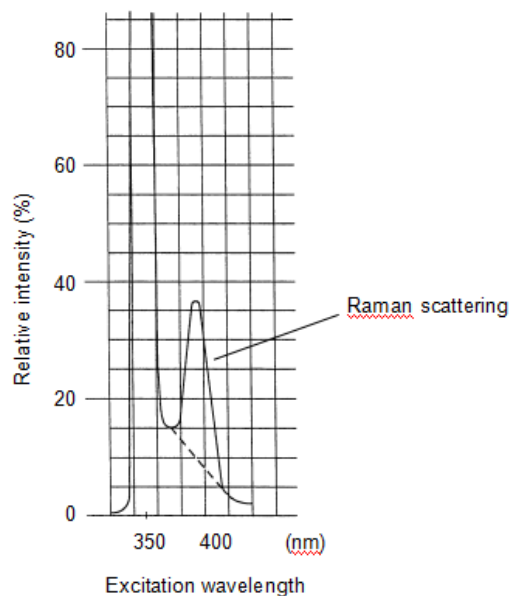


Fig. 2-7 Raman Spectrum of Water

- (2) Notes on Handling Samples that Contain a High Concentration of the Compound of Interest.

When the concentration of the compound of interest is relatively high, various error factors will arise.

The greatest factor is that the excitation beam is substantially absorbed near the entrance slit of a cell and it cannot reach the cell center adequately.

An extreme situation is shown in Fig. 2-8. Fluorescence is emitted near the entrance slit of a cell, but it will not be taken into the emission monochromator.

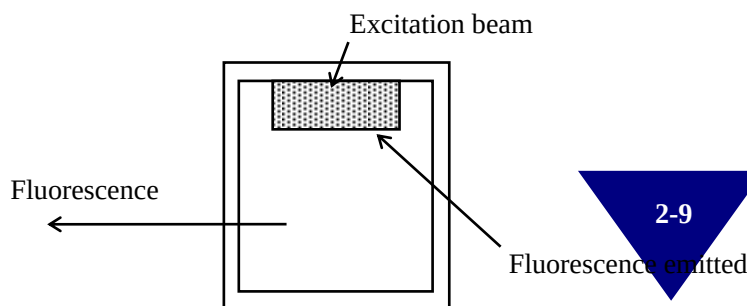


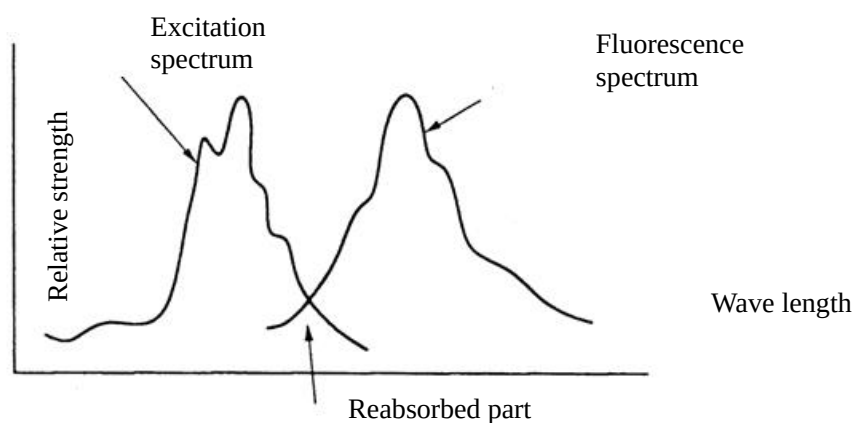
Fig. 2-8 Sample Having Extremely High Concentration

If emission occurs only in the entrance area for excitation beam, such a sample needs to be diluted with an appropriate factor before measurement.

The second factor is called “concentration quenching” where activity is disturbed by interaction of molecules.

Another potential error that may occur when fluorescence detection is used is the re-absorption of fluorescence (self-absorption of fluorescence). An example of this phenomenon is shown in Figure 2-9. Re-absorption of fluorescence occurs when the tail of the shorter-wavelength side of fluorescence spectrum overlaps the tail of the long-wavelength side of the excitation spectrum. When this phenomenon occurs, the fluorescence spectrum appears to be somewhat shifted toward the longer-wavelength.

This phenomenon will very rarely cause a substantial error in quantitation when fluorescence detection is used, and should not be a cause for concern.

**Fig. 2-9 Re-absorption of Fluorescence**

Anyway, if a high concentration may have caused a measurement error, dilution with an appropriate factor is necessary.

(3) Second-order and Third-order Radiation

The Raman peak is described as a cautionary item when the excitation and emission wavelengths are comparatively close to each other. Conversely, when these wavelengths are comparatively distant from each other, the analyst should be aware of the second and third-order radiation phenomenon.

The second-order radiation occurs at a double wavelength of the excitation beam and the third-order radiation arises at a triple wavelength.

For instance, if the excitation wavelength is 240 nm, the second-order and third-order excitation occur at 480 and 720 nm, respectively. To eliminate second and third order radiation, it is simply necessary to place a short-wavelength cutoff filter in the path of fluorescing radiation.

When the second and/or third-order radiation poses a particular problem, it is recommended to use the optional filter set accessory. In measurement with a chromatograph, the second and third-order radiation appears as a background rise. Therefore, baseline noise will increase to disturb high-sensitivity measurement.

(4) An Example of a Fluorescence Spectrum

Figure 2-10 provides an example of a fluorescence spectrum.

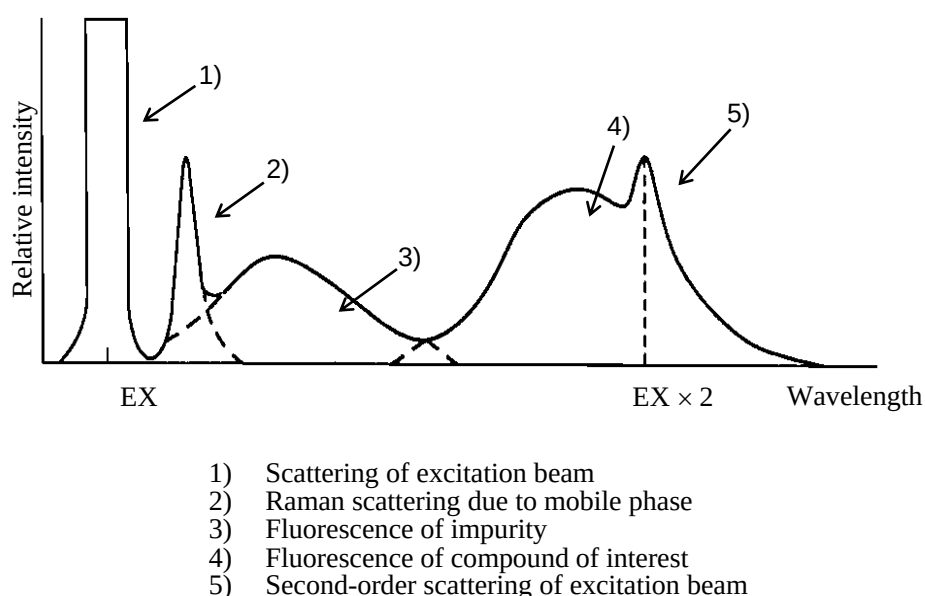


Fig. 2-10 Measurement Example of Fluorescence Spectrum

In measurement of a fluorescence spectrum, many peaks appear besides that of sample fluorescence. It is necessary to find the correct sample peak according to the explanation given above.

(5) Temperature Dependency of Fluorescence Intensity

In most cases, the fluorescence intensity decreases by 1 to 2% as the temperature of sample increases by 1 °C.

It should be noted, however, that in some biochemical samples, the fluorescence intensity decreases by approx. 10% as the temperature of sample increases by 1 °C.

2.3.4 Principle of operation

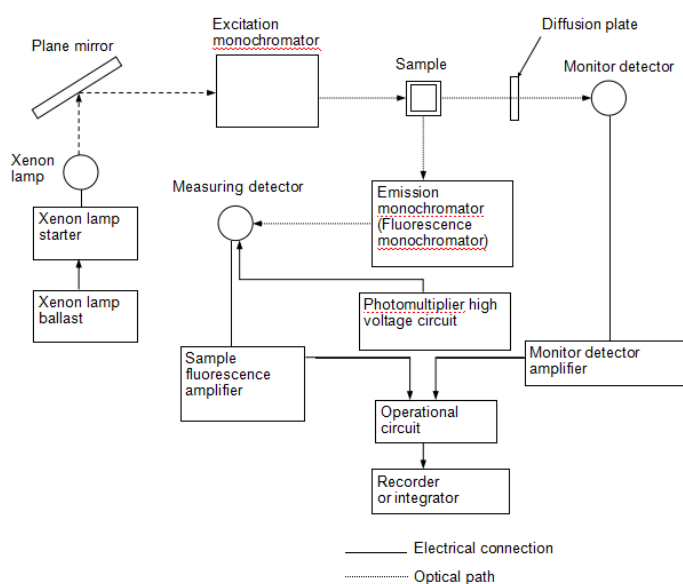


Fig. 2-11 Functional Block Diagram

The beam from the xenon lamp strikes the excitation monochromator that disperses the radiation. Only radiation of the specified wavelength is allowed to pass through the exit slit. After the exit slit, the excitation radiation strikes the sample.

The fluorescence (emitted radiation) enters the emission monochromator, where it is dispersed by the monochromator.

The selected radiation strikes the measuring detector.

On the other hand, the excitation beam which passed through the sample enters the monitor detector via the diffusion plate.

2.3.5 Features of light source

The light source thermally dissociates ozone via the heat generated by the xenon lamp itself. This allows the use of a quartz-bulb xenon lamp that produces intense radiation even in the short wavelength region.

As a safety measure against failure or stoppage of the cooling fan, a thermal sensor works to extinguish the xenon lamp in about 15 minutes if the fan stops during analysis at a room temperature of 27 °C, or in about 20 minutes if the fan was stopped from the beginning, in order to prevent failure or damage of the instrument.

Chapter 3 INSTALLATION AND TRANSPORTATION

3.1 Unpacking inspection

The D3251L detector is packaged with corrugated paper and plastic liner. After receiving the instrument, please check whether the outer package of the instrument is complete and intact. If there are signs of damage to the outer package, please contact Elite or your local distributor in time.



[Warning]

If the instrument you receive shows signs of damage, please do not attempt to install the instrument, you can ask Elite to inspect and assess the condition of the instrument.

3.1.1 Unpack

Place the box containing the D3251L detector face up on a level floor. Use a wallpaper knife to cut off the tape at the top, open the instrument package, and ensure that the instrument is not connected to any cables or pipelines when taking out the instrument. Hold the bottom of the instrument with both hands when handling it. For safety reasons, it is recommended that two persons carry the device together to avoid personal injury. Remove the foam material successively and open the protective film of the instrument.

The instrument weighs about 25 kilograms. It is necessary to firmly grasp the left and right sides of the instrument and move the instrument carefully so as not to accidentally fall and get injured.

3.1.2 Packing list

Table 2-1 Packing list

NO.	Name	Unit	Quantity
1	D3251L Fluorescence detector	machine	1
2	D3251L Fluorescence detector starter kit	suit	1

3.2 Installation Requirements

3.2.1 Site requirements

● Environmental Requirements

The detector must work under the environmental conditions described in Table 2-2. It is recommended that the ambient temperature change be less than 2°C /h. If the ambient temperature fluctuates greatly, the collected signal may be affected.

Table 2-2 Environment requirements

NO.	Type	Demand
1	Service temperature	The range is 4 to 35°C, and the temperature change during the measurement is small. And no dew on the instrument
2	Service humidity	25~85 %RH
3	Environment (gas)	(a) Well-ventilated areas. (b) where there are few gases that strongly corrode metals, such as acids and alkalis. (c) where there is less gas to dissolve organic solvents (especially gasoline, diluent). Avoiding direct sunlight will disturb the optical performance and discolor the instrument. Please avoid the window. Do not set it in a place with direct wind. Do not exert strong vibrations or shocks that can be felt by the body. Do not approach gas stove, electric heater, oven and other heating objects.
4	other	Do not approach instruments that generate strong electric and magnetic fields (welding machines, high-frequency electric furnaces, transformers on columns, etc.). Do not set it in a place with too much garbage or dust. Will disturb the optical properties. Do not drastically change the supply voltage. Will be a source of noise. The power supply of the motor (agitator, vibrator, etc.) without noise prevention instruments cannot be connected to the same power line with the power supply of the photometer, and the on/off operation cannot be repeated frequently.

**[Caution]**

- ◆ Do not use the detector under fluctuating temperature conditions;
- ◆ If the ambient temperature is too low, raise the room temperature slowly to avoid condensation caused by rapid temperature rise.

- ***Laboratory instrument placement requirements***

The D3251L detector can be placed on a normal desktop or bench. If the complete EClassical 3200L system is to be placed on the workbench, ensure that the workbench can support the weight of all the components, and allow an additional space of about 10cm on the left side, about 5cm on the right side, and about 15cm in the rear for air circulation and power cord connection.

**[Caution]**

- ◆ Keep the detector away from ventilation devices or areas with significant temperature changes. Do not place the detector near the air conditioning outlet, window, microwave oven, etc.
- ◆ Avoid placing the instrument in an area with excessive corrosive gases or dust. This will cause degradation of instrument performance and shortened service life.
- ◆ Ensure instrument power supply (electrical coupler or power outlet) when placing detectors in the laboratory.

**[Warning]**

The whole set of instruments should be placed in a horizontal position, otherwise there is a risk of falling!

3.2.2 Power Cables

- Power supply voltage: AC100 ~ 240 V relative to the rated voltage, the change should be within $\pm 10\%$

- Frequency: 50 or 60 Hz, frequency variation within ± 0.5 Hz
- Power socket: The power supply can be obtained near the system setting point of the instrument.
- Please use T5.0A fuse.



[Warning]

- ◆ Please use the special power cord provided by Elit, such as the use of other brands or unknown power cord may cause damage to electronic components or personal injury!
- ◆ If the instrument is connected to the power grid above the appropriate range, it may cause electrical shock or damage to the instrument!
- ◆ For personal safety and instrument protection, an external fuse is installed at the rear of the instrument.

3.2.3 Computer Requirements

Hardware requirements

- Minimum hardware requirements: Intel Core 2 CPU, 2 GB memory, 1 GB hard disk (PDA module requires more than 8 GB memory).
- Minimum display resolution: 1024×800, 64K color (16-bit true color).
- Computer accessories requirements: at least one USB port for the Hardware Key, at least one network interface (LAN) for device communication, and one USB port for software installation.
- Network management requirements: It is recommended that the computer used to connect the HPLC is not networked. If it is necessary to connect to the Internet, please complete the connection under the guidance of Elite engineers.

Software requirement

- Kromstation Chromatographic data workstation must run Windows 10 or later (such as Windows 10 or Windows 11).
- Verify that the operating system used to run the Kromstation

chromatography data workstation is genuine.

- Ensure that the firewall of the operating system is disabled.
- sets the option for putting your computer to Sleep in your operating system to Never.
- Set the properties of the network adapter and make sure that allow the computer to turn off this device to save power in the Power Management option for the network adapter is not selected.
- Anti-virus software is not recommended for computers connected to the EClassical 3200L high performance Liquid chromatograph, and you must ensure that the mobile storage device used for data copying is free of any computer viruses.

3.3 Assembly

3.3.1 Removing Transport Fixing screws

There are three transport screws on the underside of the main instrument. Remove the transport screws with a phillips screwdriver.

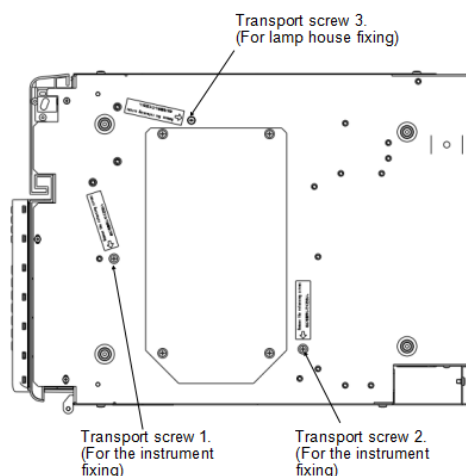


Fig. 3-1 Taking out transportation screws

3.4 Circuit and communication connection

EClassical D3251L fluorescence detector is connected to PC through USB interface to realize software control.

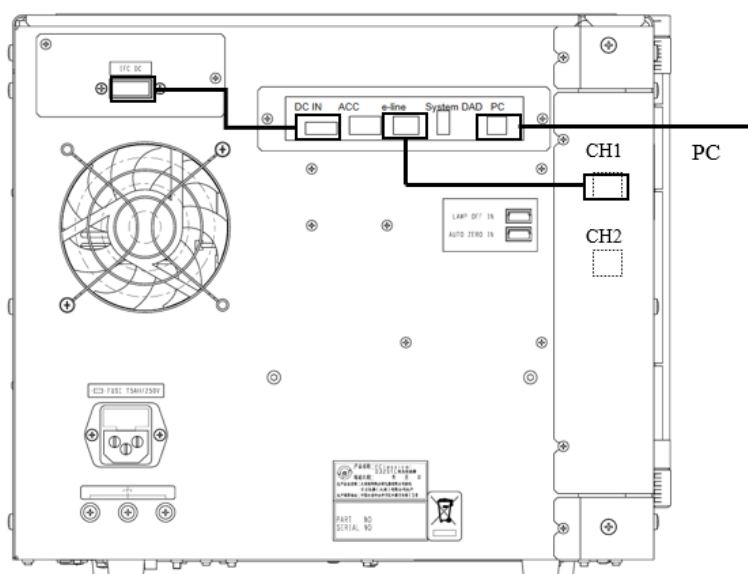


Fig. 3-2 D3251L circuit connections

D3251L detector please follow these steps to connect the circuit:

- 1) If the IFC board is optional, connect the e-Line cable, communication cable and white USB cable to the corresponding interface as shown in Figure 3-2.
- 2) If the analog board is optional, the analog board is directly connected to AD.
- 3) Plug the power cord into the power input outlet.



[Note]

The e-Line cable connecting the D3251L detector to the IFC substrate can only be connected to the CH1. If the interface is connected to the CH2. Interface, the detector and workstation cannot communicate.

3.5 Verification

Under normal circumstances, the instrument is verified after the factory, its performance meets the factory requirements, the user generally does not need to verify. If there is any doubt about the performance of the newly installed instrument, the user can verify the HPLC as follows.

- 1) Take a 150mm C18 column.
- 2) After the default parameters of the detector in the chromatographic data workstation were fully balanced, 7 needles of 1×10^{-7} g/mL naphthalene methanol standard solution were continuously analyzed at a flow rate of 1.0mL/min.
- 3) The chromatogram obtained, the calculation of qualitative and quantitative repeatability, meet the factory indicators that the whole liquid chromatography system meets the test requirements.

3.6 Transportation

D3251L detector is a precision instrument, for long-distance transportation, please pay attention to gently handle, violent vibration, drop may cause damage to the internal parts of the instrument. The use of random original packaging provided by Elite can effectively buffer the physical vibration of electronic parts and mechanical parts, so that the instrument is best protected.

When the instrument needs to be transported or returned to the factory for repair, please follow the following steps to pack:

- 1) Power off all instruments.
- 2) Unplug the detector's power cord and network cable and put it into a special sealed bag.
- 3) Remove the connection pipe between the detector and other components of the chromatograph.
- 4) Carefully remove the detector from the stack configuration, place it on a wide platform, and put it into a special sealed bag.
- 5) Put the original packaging foam into the detector frame and fix it.
- 6) Put the fixed detector into the original packing box carefully, and put other accessories in the shipping list.

- 7) Seal the mouth of the packing box with tape. To prevent liquid from entering, it is recommended to cover the sealed packing box with a layer of plastic wrap.
- 8) The packaged instrument will be transported.



[Warning]

When packing, be sure to check the packing box, if the original packing box has been damaged, do not use, should consult the local agent or contact with the customer service staff of Ilit company to solve!

Chapter 4 INSTRUMENT OPERATION

4.1 Turn on and turn off

Boot Up: Turn on the main power switch on the front panel of the detector, and the detector enters the startup self-test state for about 0.5 min. The indicators on the front panel are blue, indicating that the self-test passes and the self-test ends. If there is an alarm state, the instrument will appear "drip" sound, and the front panel indicator light will turn red, representing a fault. You need to rectify the fault cause. You can try to clear the problem by using the "Clear fault" function given in the software.

Shutdown: Click the main power switch on the rear panel of the detector, the instrument status indicator will be extinguished, and the cooling fan will stop running. The device is shut down.



[Warning]

Even if the power switch on the rear panel of the detector is off, the inside of the instrument is not charged. Without turning off the power switch, directly unplugging the power cord of the back panel of the instrument can also be powered off, but the process is an illegal operation.



[Note]

The wavelength, output range and other parameter values are the values set before the last shutdown. When you run the D3251L detector for the first time, all parameters are factory set defaults.

4.2 Instrument Preheating

The purpose of detector preheating is to make the circuit system and the light source reach a stable state. Circuit board electrical performance and light source to achieve a stable state, the general preheating time takes about half an hour.

4.3 Kromstation Chromatographic data workstation

The D3251L detector can be controlled using the Kromstation workstation. The parameters of the detector will be set up and analyzed using the Kromstation chromatographic data workstation. If the sampling spectrum function is required, please use the maintenance software of Elite D3251L. This section describes the functions and usage of the Kromstation workstation. Figure 4-1 shows the structure of the Kromstation chromatography data workstation.

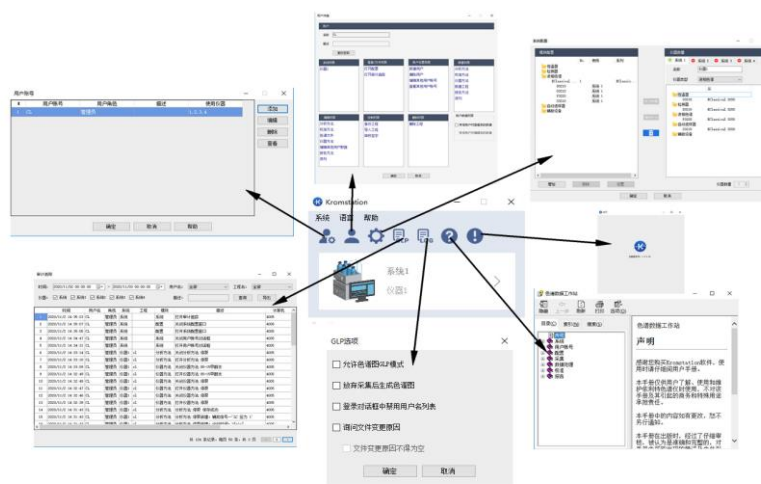
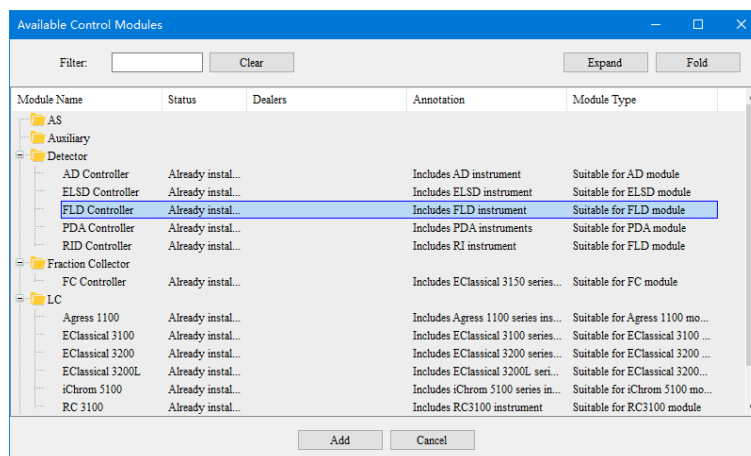


Fig.4-1 Workstation structure

4.3.1 Configuration

Double-click the workstation icon to enter the Kromstation workstation, click "Add" button, the following icon appears, double-click the "FLD" module; Enter the D3251L detector verification interface as follows:



Here you need to select the xenon lamp specifications according to the type of xenon lamp, the standard is 500h.



[Note]

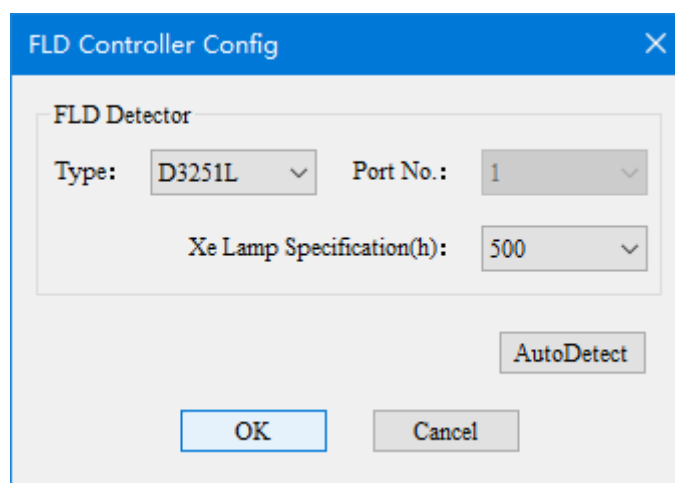
Please confirm the xenon lamp model and enter the following values

Model	Part name	Guaranteed life span	Useful lifetime	Value
5J0-1890	150 W xenon lamp(H)	500h	2500h	Below 2500

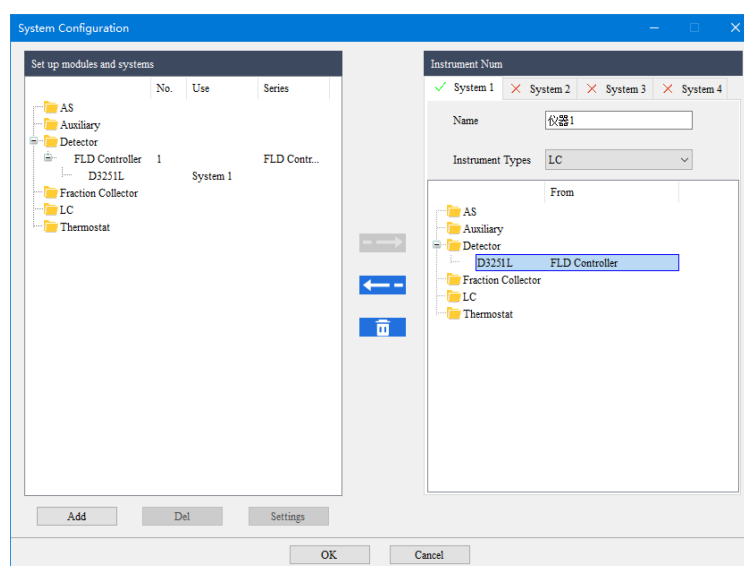
Initial value: 500 (Xenon lamp models at the time of shipment are 5J0-1890).

After the verification is successful, the following screen is displayed:

Select the "FLD" module below and click  Add the D3251L detector to the right



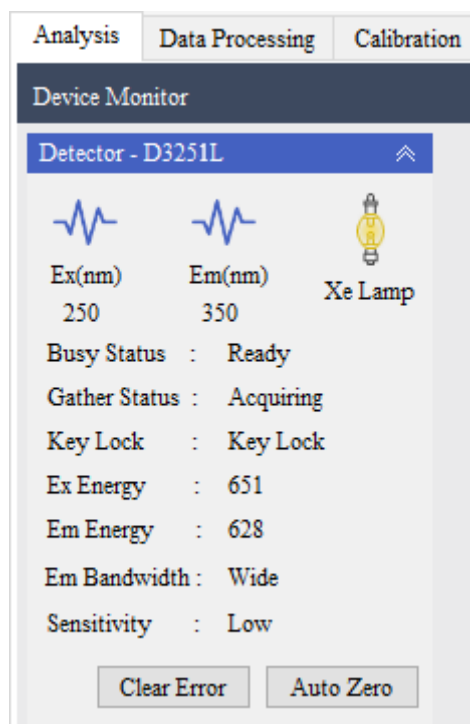
After joining successfully, the following figure is shown:



Click the "OK" button to enter the workstation.

The device monitor displays the following information about the D3251L module:

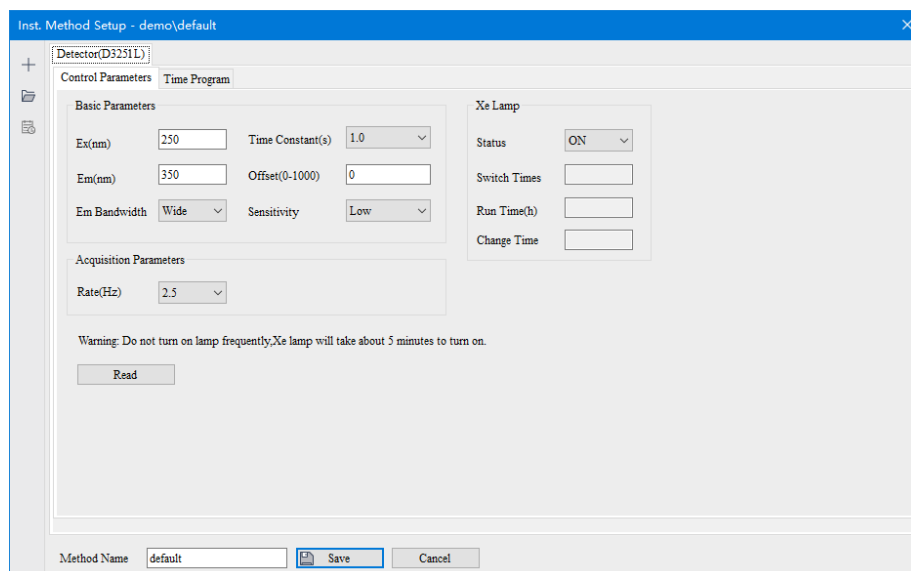
If the xenon lamp module is gray, it is considered that the xenon lamp is not turned on.



4.3.2 Method Settings

● Control parameter Settings

D3251L Fluorescence detector parameter control is shown in the figure below:



- Excitation wavelength Ex (nm): input range 200 ~ 850 nm;
- Emission wavelength Em (nm): input range 250 ~ 900 nm;
- Fluorescence side bandwidth: wide (30 nm), standard (15 nm) optional;

- d. Time constant (s): 0.05, 0.1, 0.5, 1.0, 2.0, 4.0, 8.0 Optional. The default value is 1.0
- e. Offset (0-1000): To expand the negative absorbance scale, specify the increase range (offset) of the 0 FLU position, which can be entered from 0 to 1000 mFU;
- f. Sensitivity: Five sensitivity levels, corresponding to five PMT voltage values, respectively, super high, high, medium, low, ultra-low five gears are optional;
- g. Sampling frequency (Hz): 0.3125, 0.625, 1.25, 2.5, 5, 10, 20 Optional. The default value is 2.5.
- h. Xenon lamp:

Open state: xenon lamp switch can be set;

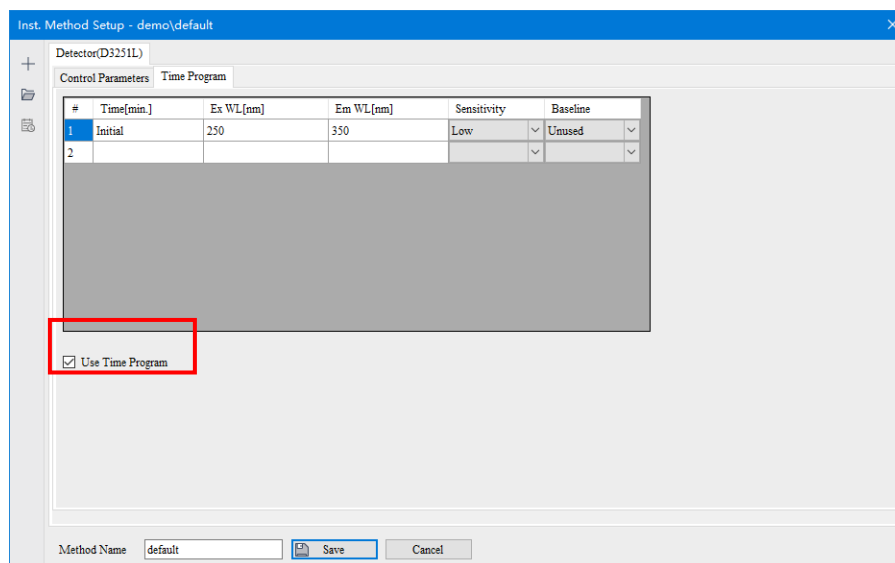
Read the instrument parameters: you can read the instrument [number of starts], [running time (h)], [replacement time].

After setting the above parameters, you can save and send the method to the next computer.

● Time program Settings

D3251L The fluorescence detector can set the excitation wavelength, emission wavelength, sensitivity and baseline state for different time periods, as follows:

In addition to the above configurable parameters, other parameters are the same as the parameter Settings in [Control parameters]. In addition, when using [time program], it is necessary to check [Collection according to time wavelength program], otherwise the time program setting is invalid.



Time: instrument parameters at this point in time;

Ex wavelength (nm): excitation wavelength size;

Em wavelength (nm): size of the emission wavelength;

Sensitivity: Five sensitivity levels, corresponding to five voltage values of PMT, respectively, super high, high, medium, low, ultra-low five gears are optional;

Baseline: [not used] - does not change the baseline status; [Automatic return to zero]- The baseline automatically returns to zero at this time;

[Hold]- The baseline remains the same as before;



[Note]

wavelength set in the Control parameter setting must be consistent with the wavelength set at 0.0 min.

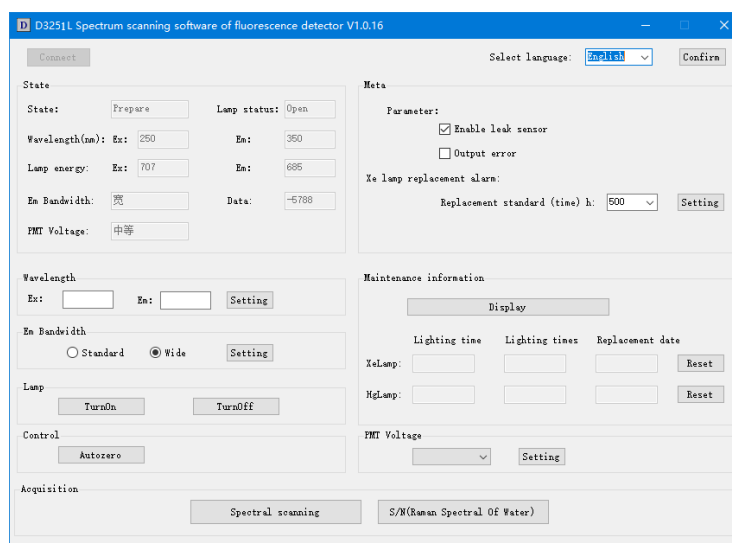
For example, Ex is set to 500 in the current setting, and Ex is set to 500 in the setting of the measured wavelength

At 250, even if the time program starts, Ex moves from 250 to 500, a time loss will occur.

4.4 D3251L Maintenance software

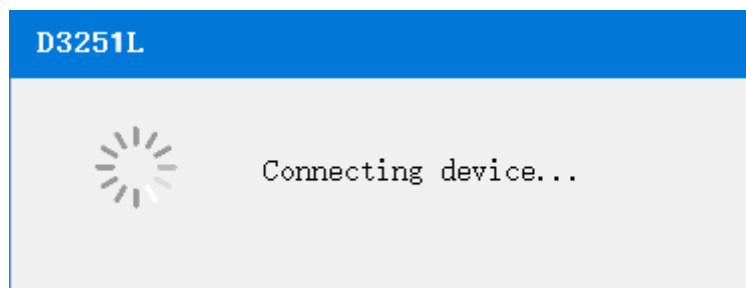
D3251L maintenance software main functions: check instrument status, wavelength setting, bandwidth setting, lamp control, automatic zero, equal energy check, Meta parameter setting, display maintenance information, PMT voltage setting, spectral scanning and water Raman measurement.

The main interface of D3251L software is shown as follows:



4.4.1 Connecting Devices

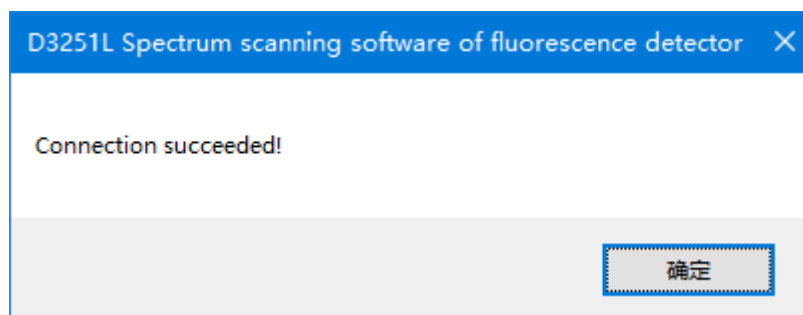
(1) Click the "Connect Device" button, and the workstation will display as follows:



[Note]

This process takes about 15s, please be patient

(2) After the connection is successful, it will display "Device connection success!" The dialog box is as follows:



[Note]

The device connection failed! Dialog box to check whether the communication cable is connected.

4.4.2 Status

Real-time detection information will be displayed in the [Status] column, including:

[Status]: Display detection is running, preparing and spectral scanning and fault information;

[Lamp status]: Displays whether the current xenon lamp status of the instrument is on or off;

[Wavelength (nm)]:

[Ex]: Display the excitation wavelength of the current detector;

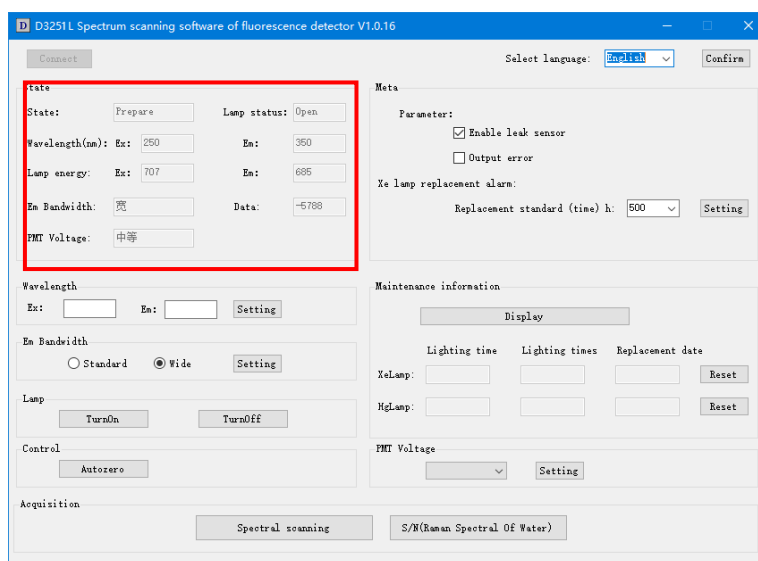
[Em]: Display the emission wavelength of the current detector;

[Lamp energy]:

[Ex]: Displays the excitation side energy value of the current detector;

[Em]: Display the fluorescence side energy value of the current detector;

[PMT voltage]: the reality of the current PMT voltage gear, ultra-low, low, medium, high and ultra-high five grades;



4.4.3 Wavelength Setting

The excitation wavelength and emission wavelength will be set in the column [wavelength] :

In the following dialog box, enter the excitation wavelength and the emission wavelength.



[Note]

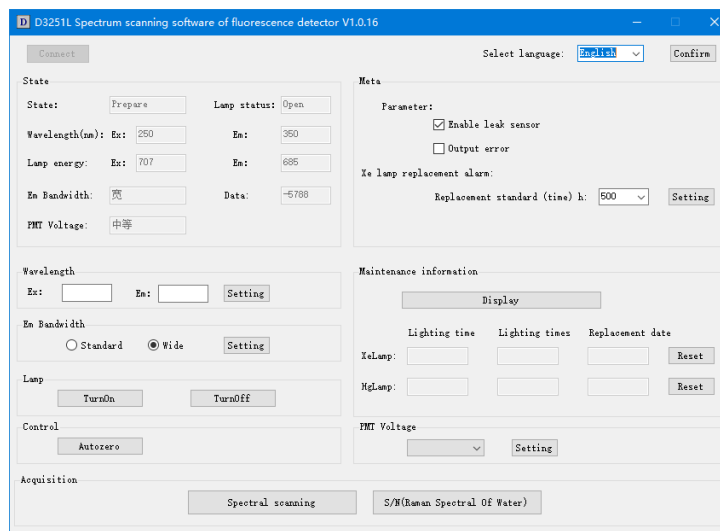
Excitation wavelength Ex (nm) : input range 200~850 nm; Emission wavelength Em (nm) : input range 250~900 nm;



[Note]

In order to completely separate the spectrum, it is recommended

that the excitation and emission wavelengths be set at intervals greater than 30 nm.



4.4.4 Em Bandwidth setting

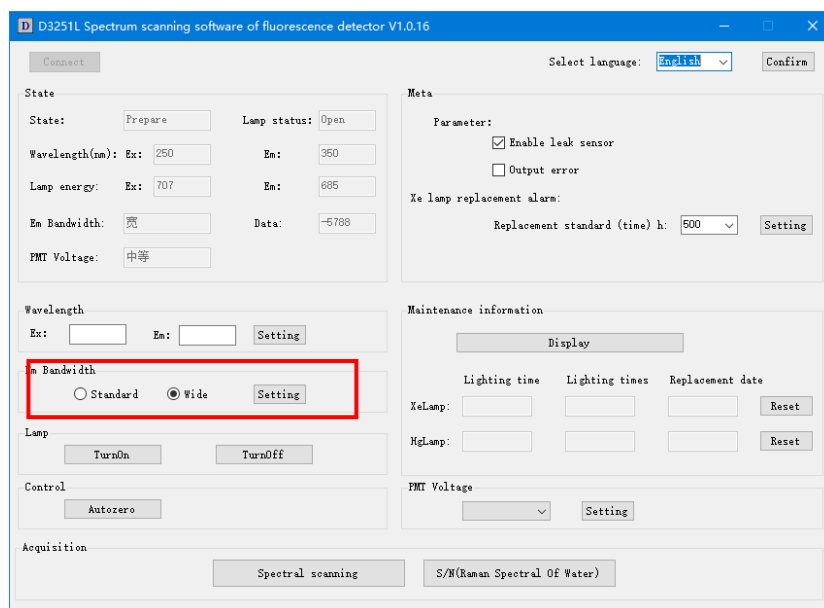
In the [Em bandwidth] setting column, the fluorescence side bandwidth is set:

In the following dialog box, select bandwidth

[Standard]: The fluorescence bandwidth is 15 nm;

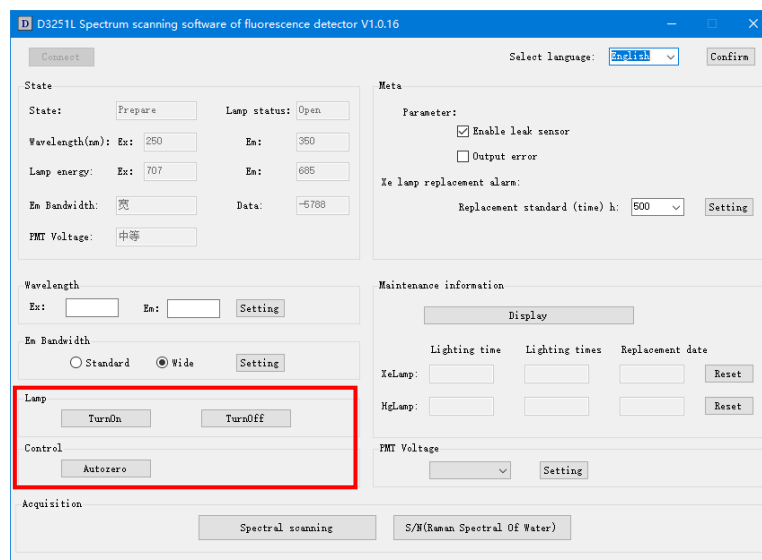
[Width] : The fluorescence bandwidth is 30 nm.

Click [Settings]]to run the machine according to the selected bandwidth.



4.4.5 Lights and controls

In this section, the lamp switch control and [automatic zero] control, and the [lamp energy] check operation will be performed.



- **Light**

As shown in the figure above, the switch of the instrument xenon lamp can be controlled separately in this column. After the operation of the method is completed, the xenon lamp can be turned off first, and the temperature of the xenon lamp can be turned off after the temperature of the xenon lamp is lowered.

- **Control**

As shown in the figure above, in this bar, you can perform both [auto zero] and [lamp energy] checks:

[Automatic zeroing] : manually zero the detector baseline;

[Lamp energy] : Check excitation and fluorescence measurement energy, see 4.4.2.

4.4.6 Meta Parameter Setting

In this section, the night leak alarm and xenon lamp life Settings will be performed.

[Practical parameters]:

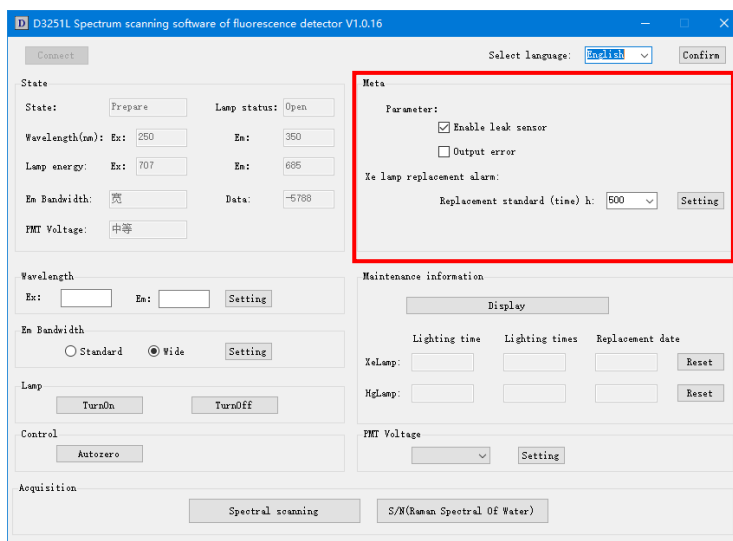
[Enable night leakage alarm]: the default option. If this option is checked, the alarm information will pop up when the D3251L detector is night leakage alarm, and the xenon lamp will immediately turn off the lamp protection.

The default is this option;

[Output error]: When this check is checked, the D3251L detector will pop up an alarm message when the night alarm is reported, but the xenon lamp will not turn off the light;

[Xe lamp replacement alarm]:

[Replacement standard (time) h]: 500 and 2500 optional, select 500, when the cumulative life of the xenon lamp reaches 500 h, the xenon lamp life prompt will pop up; Also select 2500, when the cumulative life of the xenon lamp reaches 2500 hours, the xenon lamp life message will be displayed.



* Please confirm the xenon lamp model and enter the following values.

Model	Part name	Guaranteed life span	Useful lifetime	Value
5J0-1890	150 W xenon lamp(H)	500h	2500h	Below 2500

* Initial value: 500 (Xenon lamp model 5J0-1890 at the time of shipment).

4.4.7 Displaying Maintenance Information

This topic describes how to display, maintain, and reset the Xe and Hg indicators. The interface is as follows:

Maintenance information

Display

	Lighting time	Lighting times	Replacement date	
XeLamp:				Reset
HgLamp:				Reset

[Display maintenance information]: After clicking this button, the related information of Xe lamp and Hg lamp will be displayed, as follows:

Maintenance information

Display

	Lighting time	Lighting times	Replacement date	
XeLamp:	0	2	2000/1/1	Reset
HgLamp:	0	0	2000/1/1	Reset

[Light up time] : displays the cumulative light up time of Xe and Hg;

[Lighting times] : displays the cumulative lighting times of Xe and Hg;

[Replacement date]: Display the replacement time of Xe and Hg;

[Reset] : This button is used to reset the replacement time of the lamp after replacing Xe or Hg, and then the "lighting time" and "lighting times" of the lamp will be counted from 0 again.

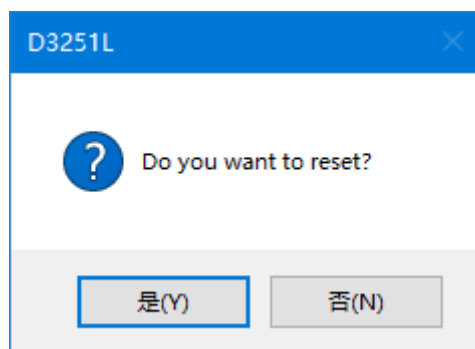
Click this button to display the following dialog box (take replacing the Xe lamp as an example)

Reset

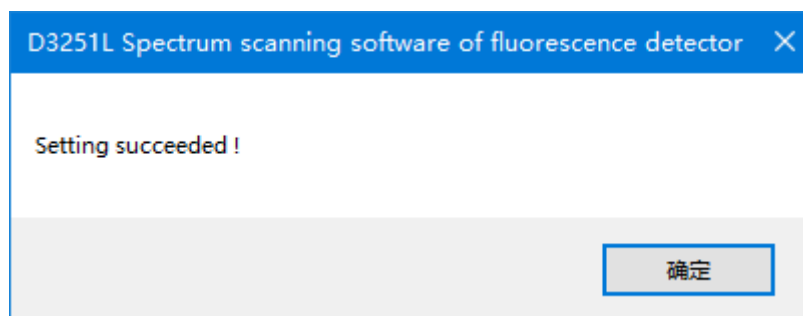
	Lighting time	Lighting times	Replacement date	
XeLamp:	0	2	2000年 1月 1日	

OK Cancel

Enter the replacement date →[OK], the following dialog box will pop up

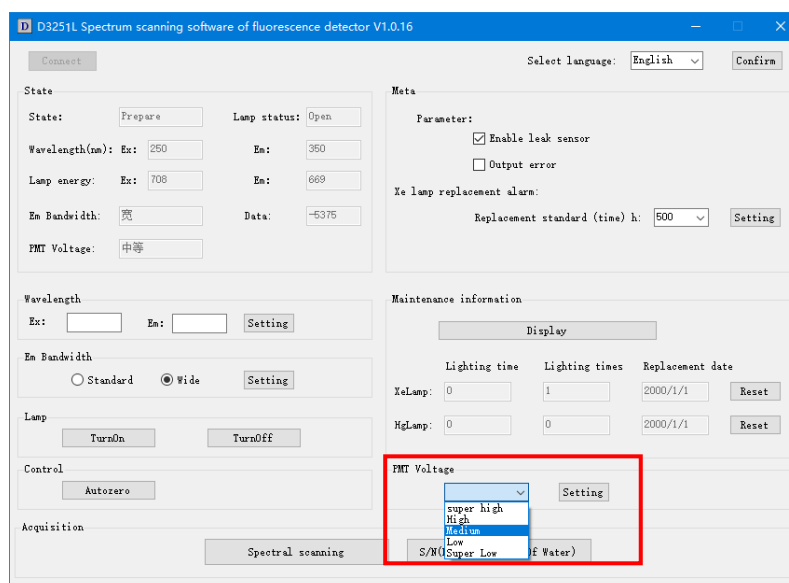


Confirm whether to replace or not, click [Yes] to reset the lamp time. After the setting is successful, the following dialog box will pop up:



4.4.8 PMT Voltage Settings

This section describes how to set the PMT voltage. The interface is as follows:

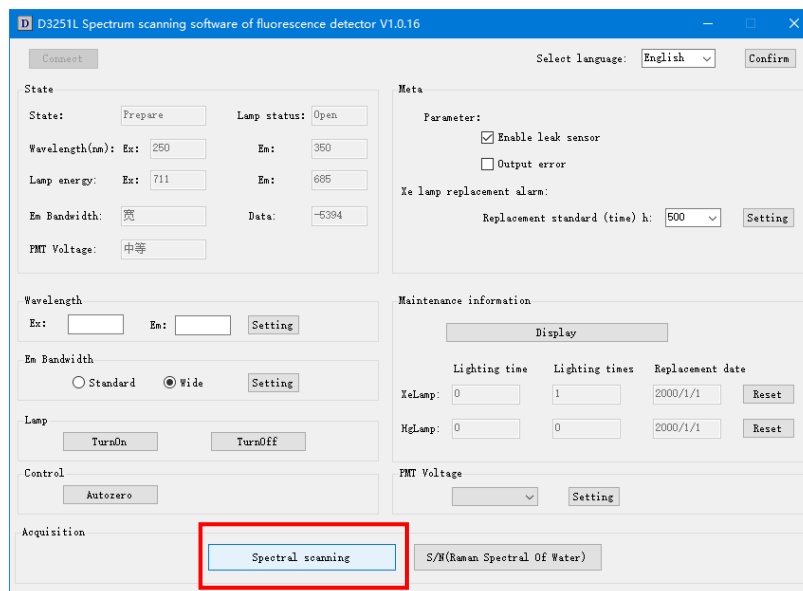


[PMT voltage]: There are five gears, which are ultra-low, low, medium, high and ultra-high, and the user chooses different PMT voltage gears according to the experimental requirements, that is, each gear has different magnification of the signal. The default value

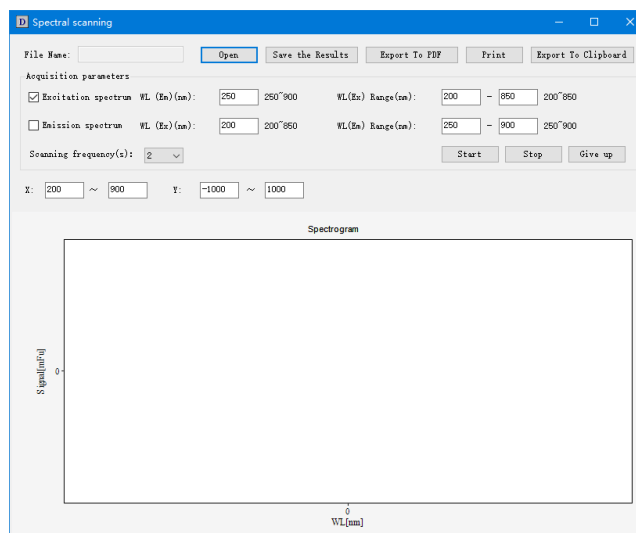
is medium.

4.4.9 Spectral scanning

This section describes the spectrum scanning function. Click the [Spectrum scanning] button:



Enter the spectrum scanning interface as follows:



In this interface, you can complete the spectrum scanning function.

(1) [Collection parameters]: Used to set spectrum scanning parameters.

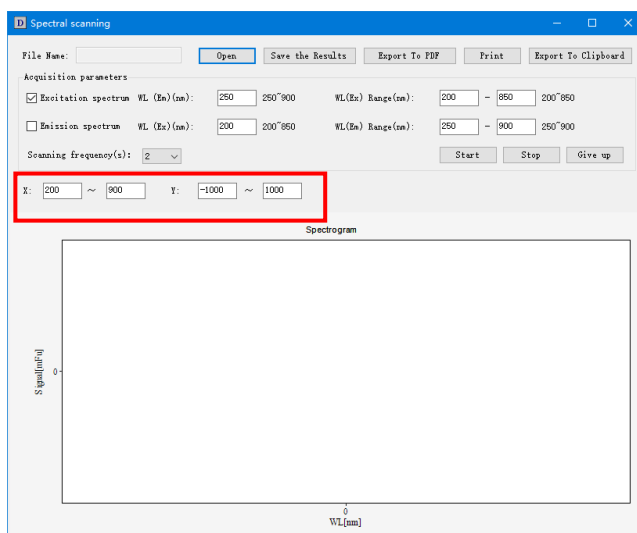
①[Excitation spectral scanning]: When this option is selected, the excitation spectrum scanning function will be performed, requiring the input:

- a. [Emission wavelength]: Input a fixed emission wavelength value, input range 250 nm ~ 900 nm;

- b. [Excitation wavelength range (nm)]: Enter the initial excitation wavelength and the termination excitation wavelength to be scanned, and the input range is 200 nm ~ 850 nm
- ② [emission spectrum scanning]: When this option is selected, the emission spectrum scanning function will be performed, and you need to enter:
 - a. [excitation wavelength]: Input a fixed excitation wavelength value, input range of 200 nm ~ 850 nm;
 - b. [Emission wavelength range (nm)]: Input the start emission wavelength and end emission wavelength to be scanned, and the input range is 250 nm ~ 900 nm
- ③[Scanning frequency (s)]: The larger the number, the slower the scanning speed. The default scanning speed is 2 s, and the optional speed is 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 s:
- ④ [Start] : Click this button to perform spectral scanning according to the parameters set above;
- ⑤[Stop] : Stop the current spectral scanning;
- ⑥[Abandon] : abandon the current spectral scan; (2) [Open]

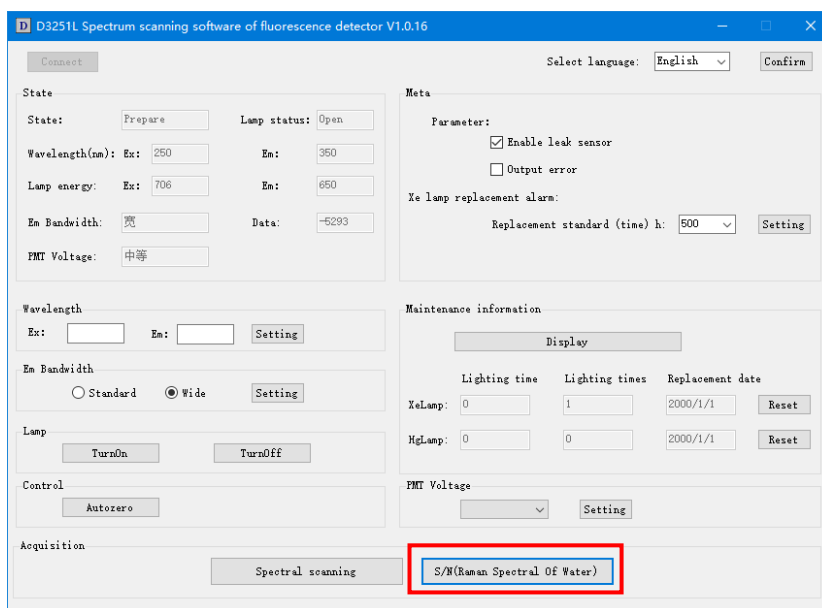
You can open any spectral scan file;

- (3) [Save]: Save the current spectral scanning results to any folder;
- (4) [Print to PDF]: You can print the currently open spectrogram to the PDF file;
- (5) [Print]: Print the current open spectrum;
- (6) [Export to clipboard]: Export the current spectral map to the clipboard.
- (7) [Coordinate range setting]: you can set the coordinate range size displayed by the spectrogram to facilitate users to view and display the spectrogram.

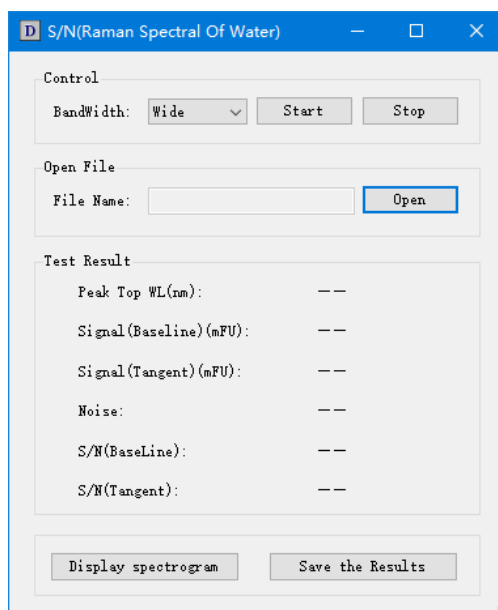


4.4.10 Water Raman measurement

In this section, the water Raman spectrum scanning function is introduced. Click on [S/N Determination (Water Raman spectroscopy)] as follows:



Open [S/N determination (Water Raman spectroscopy)] as follows:

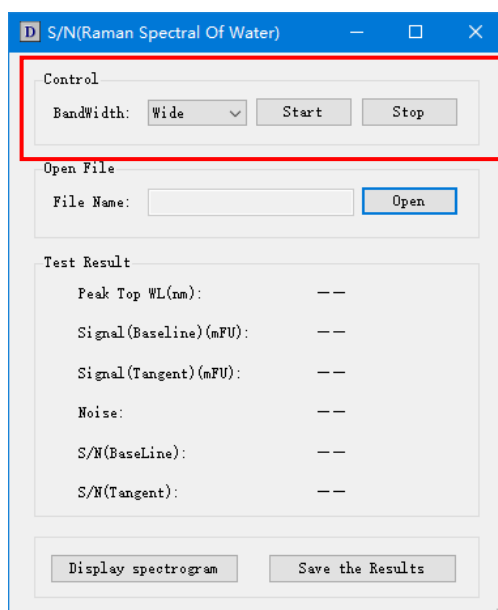


(1) Select the spectral bandwidth to be scanned:

[Standard] : 15 nm bandwidth;

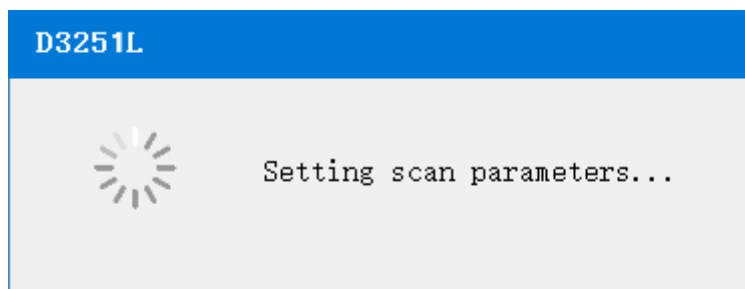
[Width]: 30 nm bandwidth.

- a. Fluorescence side spectral bandwidth: 15 nm
S/N ratio (baseline specification: S/N ratio ≥ 900)
S/N ratio (Tangent method specification: S/N ratio ≥ 675)
- b. Fluorescence side spectral bandwidth: 30 nm
S/N ratio (BG method, specification: S/N ratio ≥ 4000)



(2)[Start scanning]: The Raman spectrum scanning function is performed according to the set bandwidth. At this time, the parameters of the D3251L fluorescence detector were set as in 5.6.2.3, and the scanning frequency was 1.5s.

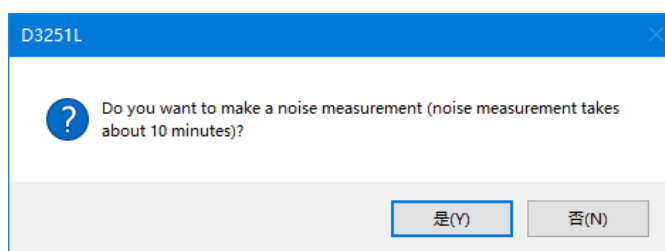
- ① Then the D3251L fluorescence detector begins to perform Raman spectral scanning.



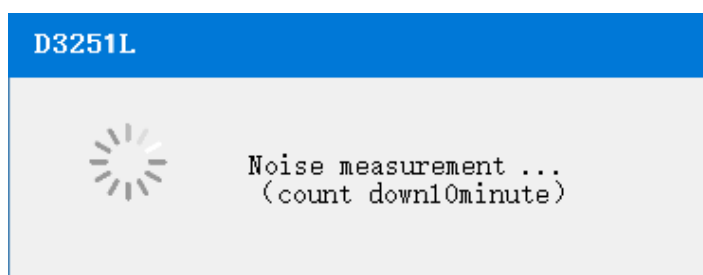
[Note]

Raman spectrum scanning process takes about 150s, please wait patiently.

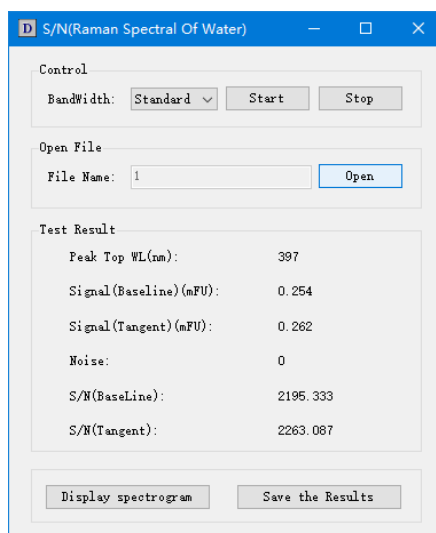
- ② The following dialog box is displayed after the spectrum scanning is complete



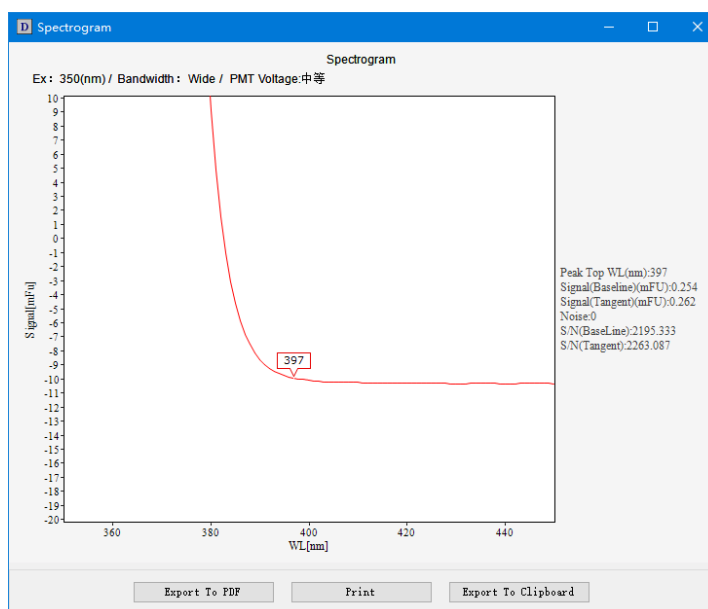
- a. Click [Yes]: The following operations will be performed to start the noise measurement. It takes about 10 minutes.



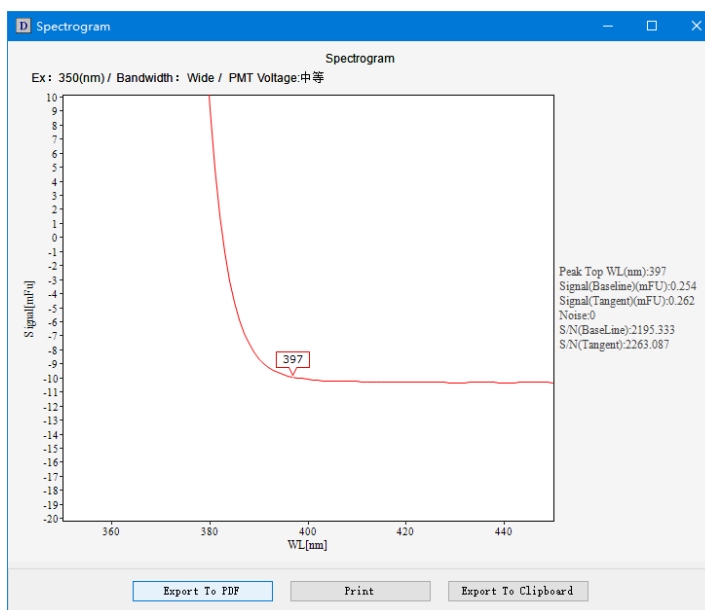
After the noise test is finished, the test result will automatically pop up;



Click [Show Spectrogram], the Raman spectrogram will be displayed as follows:



b. Click [No] : Directly display Raman spectra, no noise test, will only display spectra, no Raman results, as follows:



[Note]

At this time, there is no Raman value because there is no noise test.

- ③ After the spectral scan is finished, the results can be saved, printed, printed to PDF, exported to the clipboard and opened the spectrogram and result functions.

4.5 Before Performing Analysis

This chapter describes the basic items to be careful about before starting analysis.



[Warning]

Combustion of combustible drugs:

- This instrument is not explosion-proof. In unattended operation, do not use organic solvents having an ignition point below 70 °C.
- When using combustible medicines such as organic solvents, it may cause combustion.
 - (a) Please do not place fire near the instrument.
 - (b) Adequate ventilation in the room.
 - (c) Always check the following two points, if there is an

anomaly, please stop using immediately.

- There is no leakage of solvents or waste fluids used.
- Inside the instrument, there is no solvent leakage.
- When using combustible medicines, there is a risk of burning due to static electricity.

In order to avoid combustion due to static electricity, A grounded conductive substance is used to prevent the accumulation of static electricity on the waste liquid container.



[Warning]

Explosion of flammable pharmaceutical vapors:

Flammable substances such as organic solvents leak from the liquid path of the instrument, and the concentration of the vapor reaches the explosion limit value, which may cause an explosion.

When using combustible and easily vaporized drugs such as organic solvents, check that there is no leakage in the liquid channel of the instrument, and fully carry out indoor ventilation.



[Warning]

Body inflammation or injury caused by the toxicity, corrosiveness and irritation of solvents:

The use of toxic, corrosive, irritating solvents may cause inflammation or injury to the body. Information on the properties and use of the solvent (MSDS (Material Safety Data Sheet)) must be confirmed for exact use.

- (a) Please wear protective tools (protective glasses), etc., to prevent the solvent from sticking to the skin.
- (b) Please carry out adequate indoor ventilation to avoid inhalation.

4.5.1 Mobile phase selection and use of attention points

(1) Please pay attention to the restrictions on the characteristics and temperature of the mobile phase used. (See Table 4-2 for organic solvent characteristics.)

(2) When performing high sensitivity analysis in the UV detector, please use a solvent with particularly low UV absorption characteristics. And be sure to use solvents labeled for liquid chromatography. The commonly used super tests, such as methanol, ethanol, and acetonitrile, have very different absorption characteristics at short wavelengths, which vary by production batch and manufacturer. This point should be noted when performing high sensitivity analysis.

(3) degassing of the mobile phase must be carried out before use.

(a) Organic solvents generally contain more air and may result in loss of column efficiency due to changes in the baseline of bubbles, the incorrect operation of check valves or column efficiency. Therefore, degassing devices are recommended. Moreover, when mixing solvents (gradients, etc.), degassing devices are needed. Please refer to 4.6.2 "Degassing of mobile phase" for degassing before use.

(b) Degassing only prior to use may also occur due to changes in the baseline of bubbles when analysis is performed in the short wavelength domain with UV detectors, or in high sensitivity analysis with differential refractometric detectors. In this case, use a degassing device that can degasses the mobile phase in real time.

(4) About mobile phase

When the mobile phase of liquid chromatography is used, the material of the liquid part of the piping system such as the infusion pump and the separation column is generally stainless steel (SUS316), so the solvents and tests that may corrode stainless steel are sometimes limited to a certain extent. In particular, solvents and solutions containing halogen ions such as HCl, KCl, NaCl and NH₄Cl are representative test drugs. The restricted drugs are listed in Table 4-1 for your reference.



[Note] When restricted samples have to be used for component separation, component reaction, etc., or mobile phases that precipitate salts are used, be sure to fully flush the entire flow system with distilled water after the analysis. If not rinsed, it may result in wear of the plunger. In order to prevent salt from precipitating into the plunger and the plunger sealing ring, water is injected into the plunger cleaning assembly at the back of the pump head and cleaned after the analysis is completed. Connect the attached cylinder to the cleaning mechanism and fill with water. If a plunger cleaning pump (optional) is connected, drive the plunger cleaning pump to clean the plunger.

Table 4-1 Reagents that are restricted as mobile phases

Use is Possible	Use is Possible if 50% or Lower	Use is Possible if 10% or Lower	Reagents Unsuitable for SUS316
Phosphoric acid Sodium phosphate	Acetic acid Ammonium citrate Ammonium nitrate Citric acid Butyric acid Sodium nitrate	Disodium phosphate Ammonium formate Ammonium perchlorate Ammonium phosphate Boric acid Formic acid (up to pH3) Hydrochloric acid (up to pH3) Potassium nitrate Sodium bicarbonate Sodium carbonate	Ammonium chloride Potassium chloride Sodium chloride Trifluoroacetic acid Note 1: Max. concentration of above reagents should be 5-6%. Note 2: When the above reagents have been used necessarily, wash all the flow paths with distilled water completely without fail.

Do not use reagents that corrode SUS316 though they are not shown in the table above. If viscosity of solvent is high, pumping is impossible.

4.5.2 Characteristics of organic solvents

Table 4-2 lists the characteristics of the organic solvent used as a mobile phase in an industrial chromatograph.



[Warning]

Combustion of combustible drugs:

- This instrument is not explosion-proof. In unattended operation, do not use organic solvents with combustion points less than 70 ° C.

- There is a risk of combustion when combustible medicines such as organic solvents are used.

- (a) Keep gas sources away from the instrument.

- (b) Adequate ventilation of the premises.

- (c) Always check the following two points, when abnormal phenomenon occurs, please stop using immediately.

- The solvent or waste liquid used must not leak.

- There should be no solvent leakage inside the instrument.

- When using combustible medicines, there is a risk of combustion caused by static electricity.

To prevent combustion caused by static electricity, use a grounded conductive substance to prevent static electricity from accumulating in the liquid waste container.

Body inflammation or injury caused by the toxicity, corrosiveness and irritation of solvents:

The use of toxic, corrosive, irritating solvents may cause inflammation or injury to the body. Information on the properties and use of the solvent (MSDS (Material Safety Data Sheet)) must be confirmed for exact use.

- (a) Please wear protective tools (protective glasses, etc.) to prevent the solvent from sticking to the skin.

- (b) Please carry out adequate indoor ventilation to avoid attraction.



[Warning]

Explosion of flammable pharmaceutical vapors:

Flammable substances such as organic solvents leak from the flow path system of the instrument, and the steam may cause an explosion if it exceeds the explosion limit.

When using combustible and easily vaporized drugs such as organic solvents, be sure to check the flow system of the instrument for leakage, and fully carry out ventilation in the room.

**[Warning]**

Inflammation and personal injury due to the toxicity, corrosion and irritation of solvents:

The use of toxic, corrosive, irritating solvents may cause inflammation and injury to the body. Be sure to confirm the properties of the solvent and the information about the use (MSDS (Material Safety Data Sheet)) before using the solvent exactly.

(a) Wear proper protective tools (protective glasses, etc.) to prevent the solvent from sticking to the skin.

(b) Ventilation should be carried out adequately to avoid attraction.

Table 4-2 Characteristics of organic solvents

	Polarity E ² (Al ₂ O ₃)	Viscosity (cP20 °C)	Refractive Index	UV Cut off (nm)	Flash Point (°C)	Ignition Point			Vapor Density (Air: 1)	Boiling Point (°C)	Specific Gravity (Water: 1)
						°C	Lower Limit	Upper Limit			
Fluoroalkanes	-0.25		1.25								
n-Pentane	0.00	0.23	1.358	210	< -40	308.9	1.5	7.8	2.5	36.1	0.6
Hexane	0.00		1.375	210	-21.7	233.9	1.2	7.5	3.0	68.9	0.7
Isooctane	0.01		1.404	210							
Petroleum ether	0.01	0.3		210							
n-Decane	0.04	0.92	1.412		46.1	207.8	0.8	5.4	4.9	173.9	0.7
Cyclohexane	0.04	1.00	1.427	210	-20	260	1.3	8	2.9	81.7	0.8
Cyclopentane	0.05	0.47	1.406	210							
Diisobutylene	0.06		1.411	210							
i-Pentene	0.08		1.371		-17.8	272.8	1.5	8.7	2.4	30	0.7
Carbon disulfide	0.15	0.37	1.626	380	-30	100	1.3	44	2.6	46.1	1.3
Carbon tetrachloride	0.18	0.97	1.466	265							
Amyl chloride	0.26	0.43	1.413	225	12.8	343.3	1.6	8.6	3.7	106.1	0.9
Butyl chloride	0.26		1.436	220	-9.4	460	1.8	10.1	3.2		0.9
		0.81 [†]			o-17.2	463.9	1.0	6.0		144.4	
Xylene	0.26	0.61 [†]	~ 1.50	290	m-25	527.8	1.1	7.0	3.7	138.9	0.9
		0.64 [†]			p-25	528.9	1.1	7.0		138.3	
i-Propyl ether	0.28		1.368	220	-27.8	443.3	1.4	21	3.5	68.9	0.7
i-Propyl chloride	0.29	0.37	1.378	225	-32.2	593.3	2.8	10.7	2.7	35	0.9
Toluene	0.29	0.33	1.496	285	4.4	536.1	1.4	6.7	3.1	110.6	0.9
n-Propyl chloride	0.30	0.59	1.389	225	< -17.8		2.6	11.1	2.7	46.1	0.9
Chlorobenzene	0.30	0.35	1.525		32.2	637.8	1.3	7.1	3.9	132.2	1.1
Benzene	0.32	0.80	1.501	280	-11.1	562.2	1.4	7.1	2.8	80	0.9
Ethyl bromide	0.37	0.65	1.424			511.1	6.7	11.3	3.8	37.8	1.4
Ethyl ether	0.38		1.353	220	-45	180	1.9	48	2.6	35	0.7
Ethyl sulfide	0.38	0.23	1.442	290							
Chloroform	0.40	0.45	1.443	245							
Methylene chloride	0.42	0.57	1.424	245	-50	518.9	3.8	15.4	2.2	38.5	0.9
Methyl i-butyl ketone	0.43	0.44	1.394	330							
Tetrahydrofuran	0.45		1.408	220	-14.4	321.1	2	11.8	2.5	66.1	0.9
Ethylene dichloride	0.49		1.445	230	13.3	412.3	6.2	16	3.4	83.9	1.3
Methyl ethyl ketone	0.51	0.79	1.381	330	-6.1	515.6	1.8	10	2.5	80	0.8
i-Nitropropane	0.53		1.400	380	48.9	420.6	2.6		3.1	131.1	1.0
Acetone	0.56		1.359	220	-17.8	537.8	2.6	12.8	2.0	56.7	0.8
Dioxane	0.56	0.32	1.422	260	12.2	180	2.0	22	3.0	101.1	1.0
Ethyl acetate	0.58	1.54	1.370	260	4.4	460	1.8	8	3.5	90	0.9

Methyl acetate	0.60	0.45	1.362	210	-10	501.7	3.1	16	2.6	60	0.9
Amyl alcohol	0.61	0.37	1.410		32.8	300	1.2	10.0	3.0	137.8	0.8
Dimethyl sulfoxide	0.62	4.1									
Aniline	0.62	2.24	1.586		70	617.2	1.3		3.2	184.4	1.0
Dimethyl amine	0.63	4.4	1.387	275	< -17.8	312.2	1.8	10.1	2.5	56.7	0.7
Nitromethane	0.64	0.38	1.394	380	35	418.3	7.3		2.1	101.1	1.1
Acetonitrile	0.65	0.67	1.344	210	5.6				1.4	81.7	0.8
Pyridine	0.71	0.37	1.510	305	20		1.8	12.4	2.7	115	1.0
Butyl cellosolve	0.74	0.94		220							
i-Propanol n-Propanol	0.82		1.38	210	11.7	398.9	2.0	12	2.1	82.8	0.8
Ethanol	0.88	2.3	1.361	210	12.8	422.8	4.3	19	1.6	78.3	0.8
Methanol	0.95	1.20	1.329	210	11.1	463.9	7.3	36	1.1	63.9	0.8
Ethylene glycol	1.11	0.60	1.427	210	111.1	412.8	3.2			197.2	1.1
Acetic acid	Large	19.9	1.372								
Water	Large	1.26	1.333								
Salts & buffer	Larger										



[Note]

Points to note when using organic solvents as mobile phases:

(1) When using organic solvents, please pay attention to the ultraviolet wavelength that can be cut off.

* Ultraviolet cut-off wavelength means that light below this wavelength is not allowed to pass through.

For example, since the cut-off wavelength of chloroform is 245 nm, it should not be used at wavelengths with a wavelength value shorter than that (for example, 210 nm).

(2) The boiling point of the mobile phase

Solvents with low boiling points should not be used as mobile phases. Please use a solvent with a boiling point above 55 °C.

in the suction of the pump and may not be able to perform the infusion.

(3) When using UV detectors for high sensitivity analysis, please use solvents with minimal UV absorption characteristics. Special tests such as methanol and acetonitrile, which are commonly used, have great absorption characteristics at short wavelength, so please use liquid chromatography solvents.

4.5.3 Cautions on static electricity



[Warning]

Static electricity may lead to the combustion of combustible medicines:

When using combustible drugs, there is a risk of combustion caused by static electricity.

To prevent combustion caused by static electricity, use a grounded conductive substance to prevent static electricity from accumulating in the liquid waste container.

Because flammable organic solvents are often used in high performance liquid chromatography, great attention must be paid to temperature.

In liquid chromatographs, thin tubes are used, so static electricity is generated.

When the charged liquid accumulates in the insulated liquid waste container and generates discharge, if there is combustible material, it will burn.

4.6 Preparations



[Note]

When using ultrasonic cleaners, please follow the instruction manual of each instrument.

4.6.1 Degassing and removing dust from mobile phase

- (1) Be sure to degas before using the mobile phase.

If the oxygen, nitrogen and other gases contained in the mobile phase are used under high pressure, it may lead to the deterioration of the mobile phase sample, the loss of column efficiency of the bubble, the noise of the detector of the bubble, the change of pump pressure, and the reduction of the precision of the gradient mixing ratio.

- (2) The degassing method of mobile phase includes the degassing method using the degassing device and the manual degassing method. The degassing method will be described in item 4.6.2.

- (3) Be sure to use a clean mobile phase without dust.

If the pump into the "dust", may lead to the pump does not perform infusion, pressure changes and other failures.

4.6.2 Degassing mobile phase

There are various ways of degassing.

Perform degassing suitable for the purpose of use.

- (1) Example of Degassing by Ultrasonic Waves (when mobile phase is organic solvent)

Example: Methanol 100%

Mixture of methanol 80% and acetonitrile 20%

- (a) Please set the stirred * mobile phase container in the ultrasonic cleaner.

* When using a mixed mobile phase, be sure to use a stirring rotor to stir completely, so as not to cause poor reproducibility and baseline deviation.



[Note]

Be sure to degass the organic solvent mobile phase with an ultrasonic cleaner filled with water.

- (b) Please switch on the power of the ultrasonic cleaner.
- (c) When degassing is carried out, small bubbles will appear at first, and gradually become large bubbles, until no bubbles can be stopped. Degassing takes about 5-10 minutes.
- (d) Wait until there is no bubble, please cut off the power of the ultrasonic cleaner.
- (e) Remove the mobile phase from the ultrasonic cleaner, wipe it dry, and install it in the liquid chromatograph.

- (2) Examples of degassing by decompression method (when the mobile phase is an aqueous solution)

Example: Methanol 20% + water 80%, 0.2% citric acid solution need to prepare the appliance

- ① Ultrasonic cleaner

(The tank must be filled with water.)

- ② Suitable for mobile phase container mouth with rubber plug suction

- (a) Please set the stirred * mobile phase container in the

ultrasonic cleaner.

* When using a mixed mobile phase, be sure to use a stirring rotor to stir completely, so as not to cause poor reproducibility and baseline deviation.



[Note]

Be sure to degas the mobile phase of the aqueous solution by using ultrasonic water injection and vacuum suction. Each degassing time should remain the same.

- (b) Insert the rubber tube of the aspirator into the opening of the test bottle.
- (c) Please switch on the power of the ultrasonic cleaner.
- (d) Decompress by opening the tap plug connected to the pipette.
- (e) When degassing is carried out, small bubbles will appear at first, and gradually become large bubbles, until no bubbles can be stopped. Degassing takes about 5 minutes (see note).
- (f) When no air bubbles emerge, remove the pipette rubber tube attached to the test bottle.
- (g) Turn off the tap plug.
- (h) Cut off the power to the ultrasonic cleaner.
- (i) Remove the mobile phase from the ultrasonic cleaner, wipe it dry, and install it in the liquid chromatograph.



[Note]

The emergence of bubbles varies according to the composition of the mobile phase. When mixing the mobile phase with an organic solvent (e. g. , methanol 20%+ water 80%), the composition ratio of the mobile phase changes due to the degassing time (due to the different boiling points of the mobile phase in the reduced pressure state), so the degassing time should remain the same each time.

4.6.3 Removing dust from mobile phase

There are several methods for removing dust from mobile phase. Please choose a method suitable for the purpose of use to dust.

(1) Examples of mobile phase filtration by thin film filter

Filtration with a 0.45 μ m thin film filter allows for both dust removal and degassing.



[Note]

Remove dust from the mobile phase with a 0.45 μ m film filter (do not filter with filter paper as dust may also stick to the filter paper).

4.6.4 Removing dust from sample

(1) The sample containing fine dust must be filtered.

(2) Be sure to remove the doping in the sample.

(3) Collect the sample into the syringe, insert the 0.45 μ m needle type film filter, and filter it to remove the dust in the sample.



[Note]

There are several filtration methods for samples. Select a method suitable for sample size and filter.



[Note]

There are several ways to remove dopants from a sample. Use a method suitable for the purpose of analysis to remove the doping from the sample.

Chapter 5 Control keyboard

operation (optional)



[Note]

Fatigue caused by long-term use: When watching the display while operating, keeping the same position and staring at the display for a long time will make the eyes and body very tired. When using for a long time, in order to protect your health, take 10 to 15 minute rest every 1 hour to relax your eyes and body.

5.1 Basic Operations

Initially, turn on the power and display the 1Monitor screen before proceeding. In order to make the xenon lamp stable, after turning on the power, please leave it for more than one hour. If the placement time is short, the measurement accuracy will be reduced. The value displayed

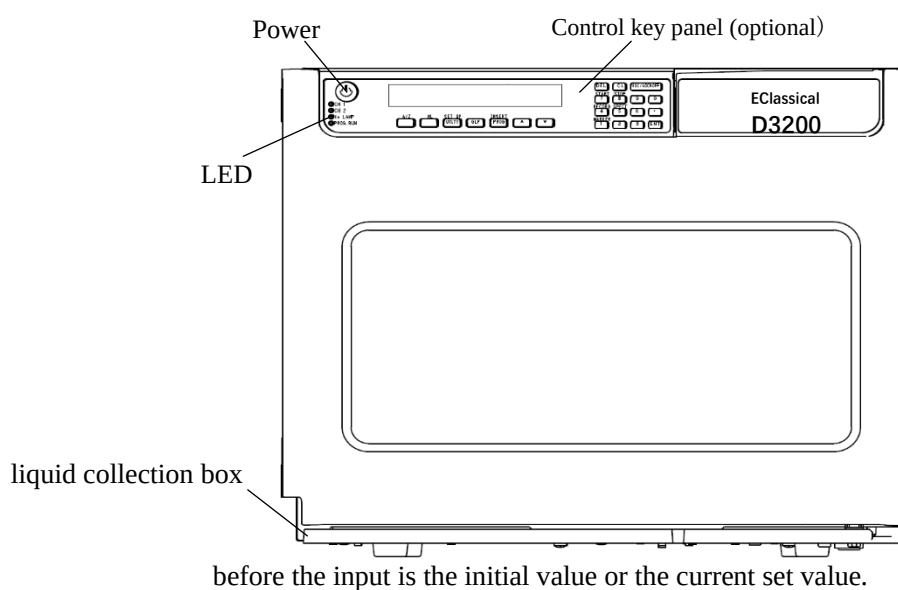


Fig 5-1 Appearance view

5.1.1 Powering on Power Supplies



[Note]

If the Monitor screen is still not displayed on the LCD of the control keyboard or an error message is displayed more than three minutes after the power is turned on, refer to Section 7.1 "Troubleshooting" or Section 7.2 "Error Message" in Chapter 7.

(1) Turn on the power switch.

(2) After a few seconds, the LCD will display the initial screen. At the same time, a self-diagnostic (initial treatment) test will be performed.

* Both the LED for the current channel and the LED for the Xe LAMP will turn green.

Initial interface:

WL DRIVE & LAMP INITIALIZING

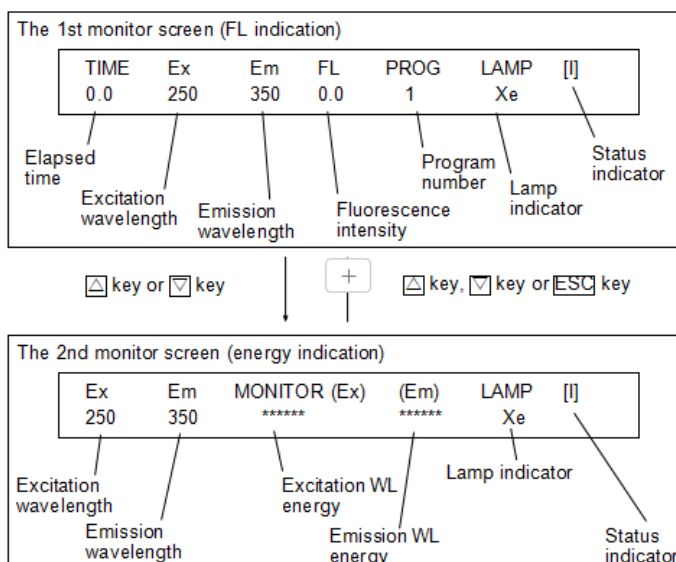
During the initialization process, the wavelength shift (check the moving reference point), the lights are turned on and the automatic zero is set.

(3) When the initial processing normally ends, the 1Monitor screen will be displayed.

Press the $\triangle$ or $\triangle$ key to display the 2Monitor screen. To return to the No

1Monitor screen, press $\triangle$ key or ∇ key or [ESC] key

* When an exception occurs during initialization, an error message is displayed (see section 7.2).



- ① The indicated values are the default values or currently set values.
 - ② The indicated program number is the currently selected one. However, when the time program is not used, “-” is indicated.
 - ③ In lamp indication, Xe stands for the lit status of the xenon lamp and OFF stands for the extinguished status.
 - ④ Fluorescence intensity (FL) is indicated within a range from -1000.0 to 1000.0. If the intensity is outside this range, “*****” is indicated.
 - ⑤ Status indicators should be read as follows:
 - [I]: Initial status.
 - [R]: Time program running status
 - [B]: Busy status
 - [L]: Key-locked status
- (4) Turn off the power switch of the detector.

5.1.2 Parameter Input Method

- (1) Input is performed by the numeric key and the [ENT]]key
 - (a) Single numeric input

Parameter name: PROGRAM NO. (1-9)

Input range: (1-9)

Input field: 1

(b) Plural numeric input

Parameter name: LAMP CHANGE (MM, DD, YYYY)

Input item: (MM, DD, YYYY)

Input range: <1-12>

Input field: -

(c) Numerical value selection

Parameter name: TIME CONSTANT (0.05s=1, 0.1s=2, 0.5s=3, 1.0s=4, 2.0s=5, 4.0s=6, 8.0s=7)

Select item: (0.05s=1, 0.1s=2, 0.5s=3, 1.0s=4, 2.0s=5, 4.0s=6, 8.0s=7)

Input field: 4

(d) Program setup screen

Input item			
TIME	Ex Em	BAS E	PM T
			1

Input field: 1

Steps for entering values

- ① The cursor is displayed at the beginning of the input field. And in the input field, the value that has been set the previous time is displayed.
- ② Can enter numeric keys and decimal points (.) (The number of bytes entered should be within the range of bytes that can be entered).

- ③ Use the [ENT] key to determine the entered value and move to the next project.

If an incorrect value is entered, the incorrect value will still be displayed in the input field, and the cursor will be set in the first position of the input field, waiting for the value to be entered again.

Use the [ESC]key to return to the original screen (Monitor screen).

If you press the [ESC]key in the middle of the input value, the input parameter is not updated (the value is still the previous setting).



[Note]

When the power supply is turned on, defaults will be indicated in the item selection number.

(2) Deleting the Present Data Entry

(a) If you made an error in entering data, but have NOT pressed the ENT key, press the CL key, then re-edit the value.

(b) If you made an error in entering data and HAVE pressed the ENT key, press the ESC key for return to the 1st monitor screen then re-edit the value.

(c) Press the DEL key to delete the item in the input part while editing the program.

When the ESC key is pressed while setting plural parameters, the item, which the ESC key has pressed, will be updated.

(3) For canceling the setup conditions, press the ESC key for return to the 1st monitor screen followed by re-setup.

(4) For accessing the next window without changing or entering values, press the ENT key, or s key.

For accessing the previous or next window, press r key, or s key.

(5) If the time program is not in use, spectral output can be performed at any time.

If spectral output is started during data acquisition, the data is automatically directed to the integrator.

- (6) The UI pad operation is disabled when connecting with the chromatography data station

5. 1. 3 Automatically Return to zero

If the data baseline deviates from the zero point of the recorder, or if the zero point adjustment is necessary, the output value is set to 0. Press

- ① Press [A/Z] key.
- ② The FL indication on the 1st monitor screen is reset to zero and output signal becomes zero.

<The 1Monitor screen>

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0	1	Xe	

* Automatic zero adjustment range: 0 to 1000

When out of range, an error message is displayed (see Section 7.2)

5. 1. 4 Setting of measurement wavelength

The predicted excitation light wavelength (Ex) and fluorescence wavelength (Em) were set.

- ① Press [WL] key.
- ② The wavelength setting screen will be displayed. Set the excitation light wavelength (Ex) (cursor is displayed on Ex). W

<Wavelength setting screen> (Ex display)

Ex	Em (200-850.0) nm
250	350

Press the number key ([200]~[850]) → press the [ENT] key.

* Setting range: 200 ~ 850 nm, the minimum unit is 1 nm.

* Initial value: 250

* If you do not want to change the excitation wavelength, press the [ESC] key or the Δ key. It will return to the 1Monitor screen.

* When pressed, the excitation wavelength is not changed, and the

fluorescence wavelength is directly moved to the setting. The cursor will then move over Em as well.

③ Set fluorescence wavelength (Em)

<Wavelength setting screen>(Em display)

Ex	Em (250-900.0) nm
250	<u>3</u> 50

Press the number key ([250]~[900]) → press the [ENT] key.

- * Setting range: 250 ~ 900 nm, the minimum unit is 1 nm.
- * Initial value: 350
- * If you do not want to change the fluorescence wavelength, press the [ESC] key, or the ▽ key. It will return to the 1Monitor screen.
- * When pressing △, the fluorescence wavelength is not changed, and the excitation wavelength is directly moved to the setting.

④End the measurement wavelength setting and return to the 1Monitor screen.

Ex and Em will display the values set.

<The 1Monitor screen>

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0	1	Xe	

5. 1. 5 End Operation

The operation can be finished by cutting off the power supply of the instrument.

Even if the power is cut off, the set value of the measurement condition is still saved, and when the power is switched on, the set condition that was saved before the power is cut off can be used to measure.

5. 1. 6 Cleaning after use

When a buffer (e.g., salt solution) is used, the flow tank must be cleaned with distilled water after the measurement. If neglected, the circulation

pool will be blocked and may cause damage.

5.2 UTLTY Settings

Use the UTILITY key to set the following instrument conditions.

- OFFSET
- TIME CONSTANT
- USE PROG (whether to use time program or not)
- PMT VOLT (photomultiplier voltage)
- OTHERS

5.2.1 Setting of OFFSET

The way the value in the measurement is added to the signal value that sets the input value and then output is called offset

- ① Press the  key.
- ② The following display will be presented.

<Utility initial screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
_1 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select OFFSET.

Press numeric key  and then the  key.

- ③ The following display will be presented.

<Offset setting screen>

OFFSET (0 - 1000)
_0

Set an offset value.

Press numeric key ( to , usually input ) and then the  key.

* An offset value is settable within a range from 0 to 1000 in increments of 1 step.

* Default value: 0

In the general data processing instrument, since the input range of the negative side is about -10 mV, if the baseline value is less than or equal to this value, it will not be able to integrate the chromatographic peak (Figure 5-2 (a)). At this time, the offset function is used to change the baseline changed to the negative side into the signal value within the input range of the data processing instrument (Figure 5-2 (b)).

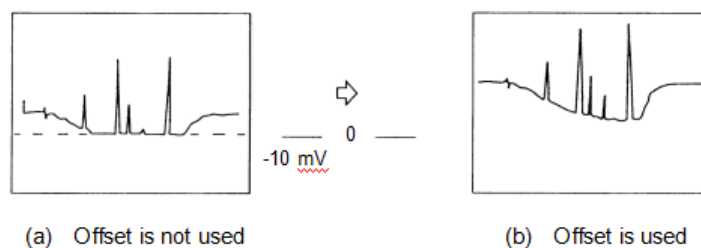


Fig. 5-2 Example of offset

- ④ Offset setting is now completed and the 1st monitor screen returns.

Though no value is indicated for FL, “+*” will be displayed when selecting a value other than 0.

<1st monitor screen>

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0+*	1	Xe	

5.2.2 Setting the TIME CONSTANT

Specify electrical response time.

- SET UP
UTLTY
- ① Press the UTLTY key.
 - ② The Utility Settings screen will display.

<Utility setting screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
_2 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select TIME CONSTANT.

Press numeric key 2 and then the ENT key.

- ③ The following display will be presented.

<Time constant setting screen>

TIME CONSTANT (0.05s=1, 0.1s=2, 0.5s=3,
4 1.0s=4, 2.0s=5, 4.0s=6, 8.0s=7)

Set a time constant value.

Press numeric key (1 to 7), usually input 4 and then the ENT key.

* Default value: 4

④ Constant setting is now completed and the 1st monitor screen returns.

5.2.3 Setting whether to USE the time program (USE PROG)

It is selectable whether or not to use automatic change of measurement parameters in response to measurement time (time program).

- ① Press the SET UP
UTLTY key.
- ② The following display will be presented.

<Utility initial screen>

UTILITY (OFFSET=1, TIME CONSTANT=2, _3 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select USE PROG.

Press numeric key 3 and then the ENT key.

- ③ The following display will be presented.

<Time program setting screen>

USE TIME PROGRAM (YES=1, NO=0) 1

Select use (YES=1) or non-use (NO=0) of a time program.

Press a numeric key (1 or 0) and then the ENT key.

- * 1: Time program starts by start input.
- * 0: Time program does not start despite start input.
- * Default value: 1

④ Selection or avoidance of a time program is now completed and the 1st monitor screen returns.

When use of a time program is avoided, “-” is indicated for PROG.

<1st monitor screen>

TIM E	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0	-	Xe	

5.2.4 Setting of the applied voltage of photomultiplier (PMT VOLT)

Set photomultiplier voltage for fluorescence detection.

- ① Press the SET UP
UTLTY key.
- ② The following display will be presented.

<Utility initial screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
4 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select PMT VOLT.

Press numeric key 4 and then the ENT key.

- ③ The following display will be presented.

<PMT voltage setting screen>

PMT VOLT (SUPER HIGH=1, HIGH=2, MID=3
3 LOW=4, SUPER LOW=5)

Set a photomultiplier voltage.

Press numeric key (1 to 5, usually input 3) and then the ENT key.

* Default value: 3

- ④ Photomultiplier voltage setting is now completed and the 1st monitor screen returns.

5.2.5 Other settings

Here, set the following items.

- Em BANDWIDTH
- ERROR OUT
- LEAK SENSOR
- LAMP

(1) Em BANDWIDTH setting

A bandwidth value of the emission monochromator is settable. Standard is 15 nm and wide setting is 30 nm

- ① Press the SET UP
UTLTY key.
- ② The following display will be presented.

<Utility initial screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
5 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select OTHERS.

Press numeric key 5 and then the ENT key.

- ③ The following display will be presented.

<Others setting screen>

OTHERS (Em BANDWIDTH=1, ERROR OUT=2,
1 L EAK SENSOR=3, LAMP=4)

Select Em BANDWIDTH.

Press numeric key 1 and then the ENT key.

- ④ The following display will be presented.

<Em bandwidth selecting screen>

Em BANDWIDTH (STANDARD=1, WIDE=2)
1

Set an Em bandwidth value.

Press numeric key (1 or 2, usually input 1) and then the ENT key.

* Default value: 1

- ⑤ Em bandwidth setting is now completed and the 1st monitor screen returns.

(2) ERROR OUT setting

It is selectable whether to enable or disable the error signal output.

When enabled, the following errors can be indicated: Xenon lamp error, ADC error, DAC error, Data buffer overflow (CDS system) and Overheat (Refer to Section 7.2)

SET UP

- ① Press the UTLTY key.

- ② The following display will be presented.

<Utility initial screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
5 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select OTHERS.

Press numeric key 5 and then the ENT key.

- ③ The following display will be presented.

<Others setting screen>

OTHERS (Em BANDWIDTH=1, ERROR OUT=2,
2 L EAK SENSOR=3, LAMP=4)

Select ERROR OUT.

Press numeric key 2 and then the ENT key.

- ④ The following display will be presented.

<Error out signal selecting screen>

ERROR OUT SIGNAL (DISABLE=0, ENABLE=1)
1

Invalid (0) or valid (1) status of error out signal output is settable.

Press numeric key (0 or 1) and then the ENT key.

* Default value: 1

- ⑤ Error out setting is now completed and the 1st monitor screen returns.

(3) LEAK SENSOR setting

Whether to use the liquid leak sensor or not is settable.

SET UP

- ① Press the UTLTY key.

- ② The following display will be presented.

<Utility initial screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
5 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select OTHERS.

Press numeric key 5 and then the ENT key.

- ③ The following display will be presented.

<Others setting screen>

OTHERS (Em BANDWIDTH=1, ERROR OUT=2,
3 L EAK SENSOR=3, LAMP=4)

Select LEAK SENSOR.

Press numeric key 3 and then the ENT key.

- ④ The following display will be presented.

<Leak sensor selecting screen>

LEAK SENSOR (OFF=0, ON=1)
1

Select use (ON=1) or non-use (OFF=0) of the leak sensor.

Press a numeric key or , usually input) and then the key.

- * Default value: 1
- * If the leakage sensor (1) is set, an error message will be displayed when detecting leakage (see section 7.2).
- * When the power is turned on again, it is automatically set to the use state of the leakage sensor (1).

⑤ Selection or avoidance of the leak sensor is now completed and the 1st monitor screen returns.

(4) LAMP setting

Select xenon lamp on or off.

- ① Press the key.
- ② The following display will be presented.

<Utility initial screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select LAMP.

Press numeric key and then the key.

- ③ The following display will be presented.

<Others setting screen>

OTHERS (Em BANDWIDTH=1, ERROR OUT=2,
 L EAK SENSOR=3, LAMP=4)

Select LEAK SENSOR.

Press numeric key and then the key.

- ④ The following display will be presented.

<Xe lamp selecting screen>

Xe LAMP ON/OFF (OFF=0, ON=1)

Select ON (1) or OFF (0) status of the xenon lamp.

Press numeric key or , usually input) and then the key.

- * Default value: 1
- ⑤ Xenon lamp setting is now completed and the 1st monitor screen

returns.

When the xenon lamp is lit, Xe is displayed for LAMP. And when the lamp is extinguished, OFF is displayed for LAMP.

<1st monitor screen>

TIME	Ex	Em	FL	PROG	LAMP	[I]
0.0	250	350	0.0	1	Xe	

* The Xe lamp will light up when this unit is powered on.



[Note]

After the light is turned off, the xenon lamp cannot be turned on again because it is in a high temperature state, so it cannot be measured.

After the xenon lamp is turned off, wait for more than 5 minutes.

5.3 GLP Association Function

In this function, set the following items.

- Xenon (Xe) lamp LOGBOOK display and reset
- Mercury (Hg) lamp LOGBOOK display and reset
- KEYLOCK Settings
- Detection of wavelength accuracy with mercury lamp (WL CHECK)
- Xenon lamp for energy CHECK.



[Note]

The log is reset by entering the value of the year and the [ENT] key.

Re-enter the year even if the value displayed in the year entry is the same as the current year.

5.3.1 Xenon (Xe) lamp log (LOGBOOK) display

It can display the cumulative lighting time (standard life), lighting times and replacement date of the xenon lamp so far.

- ① Press the GLP key.

- ② The following display will be presented.

<GLP initial screen>

GLP (LOGBOOK=1, KEY LOCK=2,
1 WL CHECK=3, Xe CHECK=4)

Select LOGBOOK.

Press numeric key **1** and then the **ENT** key.

- ③ The following display will be presented.

<Lamp selecting screen>

LAMP SELECT (Xe=1, Hg=2)
1

Select Xe.

Press numeric key **1** and then **ENT** key.

- ④ The following display will be presented.

<Logbook selecting screen>

LOGBOOK (REPORT=1, RESET=2)
1

Select REPORT.

Press numeric key **1** and then the **ENT** key.

- ⑤ On the logbook report screen, the total on period of the Xe lamp and its standard lifetime (in parentheses under Xe LAMP), the number of switching-on times and the date of the last lamp change will be indicated.

<Log display screen>

Xe LAMP	SWITCHING	LAST CHANGED DATE
123h (500)	23 times	1/ 1/2022

Press the **ENT** or **ESC** key.

- ⑥ Logbook display is now completed and the 1st monitor screen returns.

5.3.2 Setting xenon (Xe) lamp log (LOGBOOK)

Total on period of the Xe lamp, the number of switching-on times and the date of the last lamp change are reset.

- ① Press the **GLP** key.

- ② The following display will be presented.

<GLP initial screen>

GLP (LOGBOOK=1, KEY LOCK=2,
1 WL CHECK=3, Xe CHECK=4)

Select LOGBOOK.

Press numeric key 1 and then the [ENT] key.

- ③ The following display will be presented.

<Lamp selecting screen>

LAMP SELECT (Xe=1, Hg=2)
1

Select Xe.

Press numeric key 1 and then the [ENT] key.

- ④ The following display will be presented.

<Logbook selecting screen>

LOGBOOK (REPORT=1, RESET=2)
2

Select RESET.

Press numeric key 2 and then the [ENT] key.

Next, advance to the Xe lamp change date setting screen for setting the date of xenon lamp change.

- ⑤ Set the xenon lamp replacement date.

- Ⓐ Enter the month in MM (the cursor will appear on the MM bar).

Setting screen of lamp replacement date (show month)

Press the number key ([1]~[12]) → press the [ENT] key.

After that, the cursor will move to the DD field.

- Ⓑ Enter the date in DD

<Setting screen of lamp replacement date> (display date)

Xe LAMP CHANGE (MM DD YYYY)
<1-31> 1 1 2022

Press the number key ([1]~[31]) → press the [ENT] key.

After that, the cursor will move to the YYYY field.

- Ⓒ Enter the year in YYYY

<Setting screen of lamp replacement date> (show year)

Xe LAMP CHANGE (MM DD YYYY)
<2000~21000> 1 1 2022

Press the number key ([2000]~[2100]) → press the [ENT] key.

* Be sure to re-enter the year even if the preset year is displayed.

⑥ End the log setting and return to the 1Monitor screen.

* When the replacement date of the xenon lamp is set,
the cumulative lighting time and lighting times of
the xenon lamp are also reset.

5.3.3 Display of mercury (Hg) lamp LOGBOOK

Total on period of the Hg lamp, the number of switching-on times and the date of the last lamp change can be indicated.

① Press the GLP key.

② The following display will be presented.

<GLP initial screen>

GLP (LOGBOOK=1, KEY LOCK=2,
1 WL CHECK=3, Xe CHECK=4)

Select LOGBOOK.

Press numeric key 1 and then the ENT key.

③ The following display will be presented.

<Lamp selecting screen>

LAMP SELECT (Xe=1, Hg=2)
2

Select Hg.

Press numeric key 2 and then the ENT key.

④ The following display will be presented.

<Logbook selecting screen>

LOGBOOK (REPORT=1, RESET=2)
1

Select REPORT.

Press numeric key 1 and then ENT key.

⑤ On the logbook report screen, the total ON period of the Hg lamp, the number of switching-on times and the date of the last lamp change will

be indicated.

<Logbook report screen>

Hg LAMP	SWITCHING	LAST CHANGED DATE
12h	56 times	1/ 1/2022

Press the **ENT** or **ESC** key.

- ⑥ Logbook display is now completed and the 1st monitor screen returns.

5.3.4 Reset the Mercury (Hg) LOGBOOK

Total on period of the Hg lamp, the number of switching-on times and the date of the last lamp change are reset.

- ① Press the **GLP** key.
 ② The following display will be presented.

<GLP initial screen>

GLP (LOGBOOK=1, KEY LOCK=2, 1 WL CHECK=3, Xe CHECK=4)
--

Select LOGBOOK.

Press numeric key **1** and then the **ENT** key.

- ③ The following display will be presented.

<Lamp selecting screen>

LAMP SELECT (Xe=1, Hg=2) 2

Select Hg.

Press numeric key **2** and then **ENT** key.

- ④ The following display will be presented.

<Logbook selecting screen>

LOGBOOK (REPORT=1, RESET=2) 2

Select RESET.

Press numeric key **2** and then **ENT** key.

Next, advance to the Hg lamp change date setting screen for setting the date of mercury lamp change.

- ⑤ Set the date of mercury lamp change in the following procedure.
 ① Enter the month in MM (the cursor will appear on the MM bar).

<Setting screen of lamp replacement date >(show month).

Hg LAMP CHANGE	(MM DD YYYY)
<1-12>	<u>1</u> 1 2022

Press the number key ([1]~[12]) → press the [ENT] key.

After that, the cursor will move to the DD field.

⑤ Enter the date in DD

<Setting screen of lamp replacement date> (display date)

Hg LAMP CHANGE	(MM DD YYYY)
<1-31>	1 <u>1</u> 2022

Press the number key ([1]~[31]) → press the [ENT] key.

After that, the cursor will move to the YYYY field.

⑥ Enter the year in YYYY

<Setting screen of lamp replacement date >(show year)

Hg LAMP CHANGE	(MM DD YYYY)
<2000~21000>	1 1 <u>2</u> 022

Press the number key ([2000]~[2100]) → press the [ENT] key.

* Be sure to re-enter the year even if the preset year is displayed.

⑥ End the log setting and return to the 1Monitor screen.

* When the replacement date of the mercury lamp is set, the cumulative lighting time and lighting times of the mercury lamp are also reset.

5.3.5 Setting the KEY LOCK

Lock the keyboard to prevent incorrect operation.

① Press the GLP key.

② The following display will be presented.

<GLP initial screen>

GLP (LOGBOOK=1, KEY LOCK=2,
<u>2</u> WL CHECK=3, Xe CHECK=4)

Select KEY LOCK.

Press numeric key 2 and then the ENT key.

- ③ The following display will be presented.

<Key lock selecting screen>

KEY LOCK (YES=1, NO=0)

1

Set selection 1 or avoidance 0 of KEY LOCK.

Press numeric key (1 or 0, usually input 1) and then the ENT key.

* Default value: 0

* Even with the keys locked, the monitor screen can be changed with
△ and ▽ keys.

Key lock can be released by pressing ESC/ LOCKOFF key. It is also released when power is turned on again or when an error occurs, Set selection 1 or avoidance 0 of KEY LOCK.

- ④ Key lock setting is now completed and the 1st monitor screen returns.

5.3.6 Wavelength Detection Accuracy with mercury lamp (WL CHECK)

The bright line on 254 nm of mercury lamp is used to detect whether the wavelength is normal.

- ① Press the GLP key.
- ② The following display will be presented.

<GLP initial screen>

GLP (LOGBOOK=1, KEY LOCK=2,

3 WL CHECK=3, Xe CHECK=4)

Select KEY LOCK.

Press numeric key 3 and then the ENT key

- ③ The detection wavelength accuracy screen will be displayed, and the detection wavelength is normal.

<Detection wavelength accuracy screen > (Detection wavelength accuracy)

CHECKING WL ACCURACY

* If the mercury lamp is abnormal, the following error message

will be displayed. At this point, press [CL] Key to return to the 1Monitor screen.

<Display error message>

Hg LAMP ERROR PRESS "CL" KEY TO CLEAR MESSAGE
--

- ④ Automatic display of excitation wavelength (Ex) detection results.

<Display Ex test results>

Ex SIDE ACCURACY (254 nm) GOOD -1 nm

Press the [ENT] key.

* When the deviation is within the ± 3 nm range, "GOOD" will be displayed, and beyond ± 3 nm

Range, "FAIL" is displayed.

* Press the [ESC] key before pressing the [ENT] key, or when the original state lasts for 5 minutes,

Automatically returns to the 1Monitor screen.

- ⑤ The fluorescence wavelength (Em) test results will be displayed.

<The Em detection results>

Em SIDE ACCURACY (254 nm) GOOD -1 nm

Press the [ENT] or [ESC] key.

* When the deviation is within the ± 3 nm range, "GOOD" will be displayed, and beyond ± 3 nm Range, "FAIL" is displayed.

* Before pressing the [ENT] key, press the [ESC] key, or when the original state lasts for 5 minutes,

Automatically returns to the 1Monitor screen.

- ⑥ End the log setting and return to the 1Monitor screen.

5.3.7 Xe lamp energy check

Energy level of the xenon lamp can be checked using the xenon lamp beam at 254 nm.

- ① Press the GLP key.
- ② The following display will be presented.

<GLP initial screen>

GLP (LOGBOOK=1, KEY LOCK=2,
4 WL CHECK=3, Xe CHECK=4)

Select Xe CHECK.

Press the number key ([4]) → press the [ENT] key.

- * If the Xe lamp is turned off, the system will return
to the 1st monitor screen without performing an energy check.

- ③ The xenon lamp energy detection screen will be displayed and the
energy detection will be performed

<Xenon lamp energy detection screen> (energy detection in progress)

CHECKING LAMP ENERGY

- ④ Automatic display of xenon lamp energy detection results.

<Displays xenon lamp energy test results>

Xe ENERGY
1200

Press [ENT] or press [ESC].

- * If 5 minutes or more elapses without pressing
ENT or ESC key, the system will return automatically to the 1st
monitor screen.

- ⑤ When the Xe lamp energy check is finished, the system returns to the
1st monitor screen.



[Note]

When the temperature of the Xe lamp is high, the sealed gas pressure will fluctuate and the lamp may be difficult to ignite. Before restarting the instrument or re-lighting the lamp, wait at least five minutes for the lamp to cool down after turning it off.

5.4 Operating Instructions

5.4.1 Setting of time program

A time program is used for automatic change of the chromatograph measurement conditions with time. The time program takes priority over other settings.

The following items can be set.

- Measurement time (TIME)
- Excitation wavelength (Ex)
- Emission wavelength (Em)
- Baseline processing (BASE)
- Photomultiplier voltage (PMT)

* The number of time program files that can be set is 9, and the number of steps is 9 files combined to set a total of 100steps.

The set time program will be saved even if the power is cut off.

* Use the [CL] key to correct the entered values. Use the [DEL] key to delete the saved values. Use the $\triangleleft$ and $\triangleright$ keys to move to the previous or next Step. After the setting of each Step, press [ESC] key to return to the 1Monitor screen, and the time program is also set.



[Note]

When the TIME program is set, the time of step 1 must be 0.0. It cannot be changed or deleted. In addition, on the last Step of the time program, be sure to set the measurement end time. Thus, the time program will be executed until the time entered in the final Step.



[Note]

Between each Step, the measurement time should be set with an interval of more than 0.3 minutes.



[Note]

5.1.4 The wavelength set in item "Measurement Wavelength Setting" must be consistent with the wavelength set at 0.0 min. For example, if Ex is set to 500 in the current setting and Ex is set to 250 in the measurement wavelength setting, a time loss will occur even if Ex is moved from 250 to 500 when the time program is started.

- ① Press the PROG key.
- ② The time program No. setting screen appears.

<Time program No. setting screen>

SET PROGRAM NO. (1-9)

1

Create, change, or select the number of a saved program.

Press the number key ([1]~[9]) → press the [ENT] key.

* Initial value: 1

* Press the [ESC] key if you do not want to change the Settings. It will return to the 1Monitor screen.

- ③ The mode Settings screen will be displayed. Displays 0 all the time.

<Mode setting screen>

EDIT MODE (MODIFY=0, NEW=1)

1

Select whether you want to use (0) or delete (1) the selected time program in the above item

Conditions within the number.

Press the numeric key ([0] or [1]) → press the [ENT] key.

* 0: Use the conditions in the selected time program number in the above item, and can be advanced

Line time program additions and changes.

1: Delete the conditions in the selected time program number in the above item, and can reset the conditions of the time program.

Secondly, in order to set the time program, migrate to the Step setting screen and perform the first step 1Step Settings (the cursor will be displayed on the TIME bar).

④ Set step 1 of the time program (start condition)

① Set TIME。

<Step setting screen (TIME indicated)>

TIME	Ex	Em	BASE	PMT
0.0			1	1

Press the number key ([0.0]) → press the [ENT] key.

* The value entered in the TIME field must be 0.0.

* Initial value: 0.0

* The program number in the Settings will be displayed on the lower right side of the screen.

The cursor will then move to the Ex field.

② Set Ex.

<Step setting screen (Ex indicated)>

TIME	Ex	Em	BASE	PMT
0.0	<u>2</u> 50		1	1

Press the number key ([200]~[850]) → press the [ENT] key.

* Setting range: 200 ~ 850 nm

However, it needs to be set to the same wavelength value as Ex in the setting of the measured wavelength.

* Initial value: blank

The cursor will then move to the Em bar.

③ Set Em。

<Step setting screen (Em indicated)>

TIME	Ex	Em	BASE	PMT
0.0	250	<u>3</u> 50	1	1

Press the number key ([250]~[900]) → press the [ENT] key.

* Setting range: 250 ~ 900 nm

However, it needs to be set to the same wavelength value as Em in the setting of the measured wavelength.

* Initial value: blank

The cursor will then move to the BASE bar.

④ Set BASE。

<Step setting screen (BASE indicated)>

TIME	Ex	Em	BASE	PMT
_ 0.0	250	350	<u>1</u>	1

Press the numeric key ([1] or [2] : General input [1]) → press the [ENT] key.

* 1: Run AUTO ZERO.

The data baseline serves as the zero point of the logger.

2: Execute the HOLD command

Leave the baseline unchanged. The Hold function is disabled by automatically zeroing.

* Initial value: 1

Ⓒ Set PMT.

<Step setting screen (PMT indicated)>

TIME	Ex	Em	BASE	PMT
_ 0.0	250	350	1	<u>3</u>

Press the number key ([1]~[5]) → press the [ENT] key.

Ⓓ ends the setting of Step 1 and returns to the TIME setting of the STEP setting screen.

* The number on the right of the time program setting screen is the time program number in the setting.

* If you want to change the condition, do the Settings after 2Step, rather than changing the bar

The last Step is set.

Ⓔ The setting after the 2Step of the time program

Ⓐ sets the TIME for the pre-change.

<Step setting screen (display TIME)>

TIME	Ex	Em	BASE	PMT
_ 10.0			1	1

Press the number key ([0]~[600]) → press the [ENT] key.

* Setting range: 0 ~ 600 points, the minimum setting range is 0.1 points.

After that, the cursor moves to Ex.

Ⓑ Set pre-changed Ex.

<Step setup screen (show Ex)>

TIME	Ex	Em	BASE	PMT
10.0	<u>400</u>		1	1

Press the number key ([200]~[850]) → press the [ENT] key.

After that, the cursor moves over Em.

③ sets the pre-changed Em.

<Step setup screen (show Em)>

TIME	Ex	Em	BASE	PMT
10.0	400	<u>5</u> 00	1	1

Press the number key ([250]~[900]) → press the [ENT] key.

After that, the cursor moves to BASE.

④ sets the pre-changed BASE.

<Step setup screen (BASE display)>

TIME	Ex	Em	BASE	PMT
10.0	400	500	<u>2</u>	1

Press the number key ([1] or [2]) → press the [ENT] key.

* In order to align the baseline set in the previous Step with the baseline set in the current Step, you need to enter 2.

⑤ Defaults sets the pre-changed PMT.

<Step setup screen (display PMT)>

TIME	Ex	Em	BASE	PMT
10.0	400	500	2	<u>4</u>
				1

Press the number key ([1]~[5]) → press the [ENT] key.

① ends the setting of Step 2 and returns to the TIME setting of the STEP setting screen.

* Repeat the above if there are still conditions you want to change.

If there are no conditions you want to change, set the last Step.

⑥ Setting of the last Step

① <Step Setting screen (display TIME)>

TIME	Ex	Em	BASE	PMT
<u>2</u> 0.0			1	<u>4</u>
				1

Press Value key → Press [ENT] key → Press [ESC] key.

* Setting range: 0 ~ 600 points, the minimum setting unit is 0.1 points.

* You do not have to change the Settings for items other than TIME.

② End time program Settings, and return to the 1Monitor screen.

⑦ Add Step

If you want to add a Step while entering a Step, press the [INSERT] key.

The Step setting screen will be displayed, please use the same method as above to append.

5.4.2 Start Time Program

Press the [START] key to start the time program.

The status of the 1Monitor screen is displayed as R (executing the TIME program), and the elapsed time is displayed in the time bar. And, PROG.RUN's LED will turn green.

<The 1Monitor screen>

TIME	Ex	Em	FL	PROG	LAMP	[R]
***	250	350	0.0	1	Xe	

5.4.3 Stopping the time Program

Press the STOP key, then the time program under execution stops and the 1st monitor screen returns.

5.4.4 Time Program Operations

① Time program setting

Described next is the procedure for newly creating a time program shown in the figure.

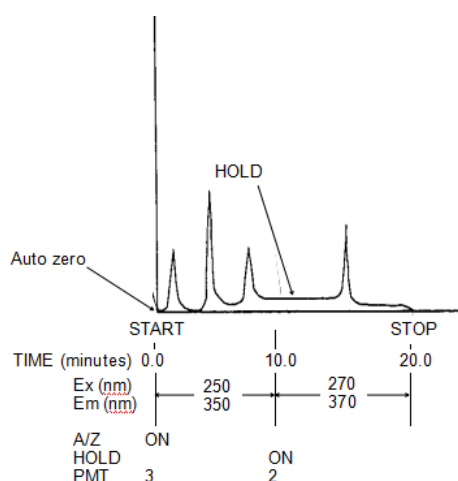


Fig. 5-3 Time program and measured wavelength

In this example, assume that a new time program is set in program number 1. This time program, ① within 10 minutes after the start, the voltage applied by the photomultiplier tube is changed from MID to LOW, the excitation wavelength is changed from 250 nm to 270 nm, and the optical wavelength is adjusted from 350 nm to 370 nm and ② the measurement is finished within 20 minutes.

Press **PROG** key.



SET PROGE NO. (1 - 9)
<u>1</u>

Press **1** key and then the **ENT** key.



EDIT MODE (MODIFY=0, NEW=1)
<u>0</u>

Press **1** key and then the **ENT** key.



TIME	Ex	Em	BASE	PMT
<u>0.0</u>			1	1

Press **0** key and then the **ENT** key.

Press **2** **5** **0** keys and then the **ENT** key.

Press **3** **5** **0** keys and then the **ENT** key.

Press **1** key and then the **ENT** key.

Press **3** key and then the **ENT** key.



TIME	Ex	Em	BASE	PMT
<u>0.0</u>	250	350	1	3 1

Press **1** **0** keys and then the **ENT** key.

Press **2** **7** **0** keys and then the **ENT** key.

Press **3** **7** **0** keys and then the **ENT** key.

Press **2** key and then the **ENT** key.

Press **2** key and then the **ENT** key.



TIME	Ex	Em	BASE	PMT
<u>10.0</u>	270	370	2	2 1

Press **2** **0** keys and then the **ENT** key.



TIME	Ex	Em	BASE	PMT
<u>20.0</u>				1

Press the **ESC** key.

Time program setting is now finished.



TIME	Ex	Em	FL	PROG	LAMP	[I]
<u>0.0</u>	250	350	0.0	1	Xe	

Press the **START** key.

The time program starts and measurement is initiated.

② Check of time program

Take the following procedure for checking the program that was set in the above.

Press the **PROG** key.



SET PROGE NO. (1 - 9)
<u>1</u>

Press **1** key and then the **ENT** key.



EDIT MODE (MODIFY=0, NEW=1)
<u>0</u>

Press **0** key and then the **ENT** key.

Step at startup is indicated.



TIME	Ex	Em	BASE	PMT
<u>0.0</u>	250	350	1	3 1

Press **▽** key to access the next step.



TIME	Ex	Em	BASE	PMT
<u>10.0</u>	270	370	2	2 1

Press **▽** key to access the next step.



TIME	Ex	Em	BASE	PMT
<u>20.0</u>				1

Press the **ESC** key, and the 1st monitor screen returns.



TIME	Ex	Em	FL	PROG	LAMP	[I]
<u>0.0</u>	250	350	0.0	1	Xe	

5.4.5 Spectrum measurement

Set the wavelength scan range (maximum and minimum values) for excitation (Ex) and emission (Em), then measure and record the spectral data for each.

* Carry out recording of spectral data with sample contained in the cell (sample not flowing, but stationary in cell).



[Note]

The spectral bandwidth of the excitation side and the fluorescence side of the instrument is 15 nm (about 30 nm when the peak base width is wide). The difference between the excitation wavelength and the fluorescence wavelength is less than 30 nm, which cannot be correctly measured. Use the instrument when the difference between the excitation wavelength and the fluorescence wavelength is greater than 30 nm.



[Note]

The maximum wavelength scanning range of excitation light and fluorescence (end wavelength to start wavelength) is 400 nm. For spectrum acquisition above this range (400 nm), take two separate measurements.



[Note]

The spectral data that has been memorized is not saved after the power is turned off.

(1) Spectral measurement and memory of excitation light (Ex) scanning range

- ① Press [SPECT] key.
- ② The scan mode setting screen will be displayed.

<Scan mode setting screen>

SPECTRUM MODE (FL (Ex) =1, FL (Em) =2)

1

Select FL (Ex).

Press Number key [1] → Press [ENT] key.

For the next scan range setting, enter the scan range setting screen and enter Start

Wavelength (cursor displayed on WL1)

- ③ Enter the starting wavelength (minimum) in WL1 of the scanning range.

<Scan range setting screen (display WL1)>

SCANNING WL RANG (WL1 WL2) nm FL (Ex)

<200-800>

200-600

Press the number key ([200]~[800]) → press the [ENT] key.

* Setting range: 200 ~ 800 nm

* Initial value: 200

* If you do not want to change the Settings, press the ▽ key to move to the Settings bar of WL2

* After that, the cursor moves to WL2

- ④ Enter the end wavelength (maximum value) in the WL2 field.

<Scan range setting screen (display WL2)>

SCANNING WL RANG (WL1 WL2) nm FL (Ex)

<250-850>

200-600

Press the number key ([250]~[850]) → press the [ENT] key.

* Input value: WL1+50 ~ WL1+400, and less than 850

* Setting range: 250 ~ 850 nm

* Initial value: 600

*Pressing key prior to the ENT key will return to WL1.

- ⑤ A memorize spectrum setting screen will now appear.

<Light Pu memory file setting screen>

MEMORIZE SPECTRUM NO. (1 ~ 4)

1

Set the memory file number for the spectral data.

Press the number key ([1]~[4]) → press the [ENT] key.

* Initial value: blank

* Upon entering a memorize spectrum No., the previous spectral

data will be deleted and the new data will be memorized.

- ⑥ The scan monitor screen appears, and spectral acquisition within the set range is carried out.

<Scan Monitor screen (collecting spectrum)>

SCANNING SPECTRUM NO.:1	MODE:FL (Ex)
Ex:***nm	Em:350nm

* Pressing the ESC key during spectral acquisition will cancel the process. Spectral data will not be memorized.

* The memorize spectrum No. set in the preceding step will be indicated at SCANNING SPECTRUM NO.

* Ex is effective within the scan range set above.

- ⑦ When the spectral data memorization is completed, the system returns to the 1st monitor screen automatically.

(2) Measurement and Recording of Spectrum for Emission(Em) Scan Range:

- ① Press [SPECT] key.
② The scan mode setting screen will be displayed

<Scan mode setting screen>

SPECTRUM MODE (FL (Ex) =1, FL (Em) =2)

2

Select FL (Em).

Press Number key [2]→ Press [ENT] key.

Now proceed to the scan range setting screen for setting a scan range, and enter a start wavelength (a cursor will appear at WL1).

- ③ Enter the starting wavelength (minimum value) in WL1 of the scanning range.

<Scan range setting screen (display WL1)>

SCANNING WL RANG (WL1 WL2) nm FL (Ex)

<250-850>

250-700

Press the number key ([250]~[850]) → press the [ENT] key.

* Setting range: 250 ~ 850 nm

* Initial value: 250

* If you do not want to change the setting, press the ▽ key to

proceed to WL2 setting.

The cursor now moves to WL2.

④ Enter an end wavelength (maximum value) at WL2.

<Scan range setting screen (display WL2)>

SCANNING WL RANG (WL1 WL2) nm FL (Ex)

<300-900>

250-600

Press the number key ([300]~[900]) → press the [ENT] key.

* Input value: WL1+50 ~ WL1+400, and less than 900

* Setting range: 300 ~ 900 nm

* Initial value: 600

* Pressing the $\triangle$ key before pressing the [ENT] key will return you to the WL1 Settings.

⑤ The setting screen of the optical memory file will be displayed.

<Light Pu memory file setting screen>

MEMORIZE SPECTRUM NO. (1 ~ 4)

1

Set the memory file number for the spectral data.

Press the number key ([1]~[4]) → press the [ENT] key.

* Initial value: blank

* Upon entering a memorize spectrum No., the previous spectral data will be deleted and the new data will be memorized.

And the new spectral data will be memorized.

⑥ The scanning Monitor screen will be displayed, and the spectrum acquisition within the set range will be carried out.

<Scan Monitor screen (collecting spectrum)>

SCANNING SPECTRUM NO.:1 MODE:FL (Ex)

Ex:250nmEm:***nm

* Pressing the ESC key during spectral acquisition will cancel the process. Spectral data will not be memorized.

* The memorize spectrum No. set in the preceding step will be indicated at SCANNING SPECTRUM NO.

* Em is effective within the scan range set above.

⑦ When the spectral data memorization is completed, the system returns to the 1st monitor screen automatically.

5.4.6 Setting of recorder and spectrum output

The following items are settable as factors used in recorder and/or spectrum output.

- Full scale range of recorder
- Output speed of recorder
- Output spectrum data



[Note]

With an emission spectrum, scattered excitation light, Raman scattering due to solvent, emission due to solvent or impurities in it, and second (or third) order emission may be superimposed on the emission of the analyte element. Similarly, an excitation spectrum may be affected by scattered light at the emission wavelength, by Raman scattering due to solvent, by emission due to solvent or impurities contained in it, and by second (or third) order scattered light of the excitation beam. These factors must be taken into consideration when selecting the optimum excitation and emission wavelengths for spectrum acquisition. Refer to section 2.3.3 for details.



[Note]

The excitation and emission spectra output are not corrected for the wavelength-dependent sensitivity of the photomultiplier. The obtained spectra therefore differ from the true excitation and emission spectra of the analyte element. This difference is especially noticeable at wavelengths below 300 nm.

- (1) Set the measuring range of the recorder
 - ① Press the [RECORD] key.
 - ② The recorder setting screen will be displayed.

RECORD (RCD RANGE=1, RCD SPEED=2,

1 SPECT RCD=3)

Select RCD RANGE.

Press the number key ([1]) → press the [ENT] key.

③ The recorder range setting screen will be displayed.

<Recorder range setting screen>

RCD RANGE (1-1000)

1000

Set the recorder range.

Press the number key ([1]~[1000]) → press the [ENT] key.

* Initial value: 1000

④ End the setting of the recorder range and return to the 1Monitor screen.

(2) Set the output speed of the recorder

① Press the [RECORD] key.

② The recorder setting screen will be displayed.

<Recorder setting screen>

RECORD (RCD RANGE=1, RCD SPEED=2,

2 SPECT RCD=3)

Select RCD SPEED.

Press Number key ([2]) → press [ENT] key.

③ The recorder range setting screen will be displayed. Recorder range setting screen

<Recorder speed setting screen>

RCD SPEED (40 nm/min=1, 60 nm/min)

2

Set recorder speed.

Press the number key ([1] or [2]) → press the [ENT] key.

* Initial value: 2

④ End the setting of the recorder range and return to the 1Monitor screen.

(3) Set the output spectral data

① Press the [RECORD] key.

③ The recorder setting screen will be displayed.

<Recorder setting screen>

RECORD (RCD RANGE=1, RCD SPEED=2,

3 SPECT RCD=3)

Select SPECT RCD.

Press Number key ([3]) → press [ENT] key.

③ The optical number screen for setting output data will be displayed.

<Set the optical number screen of the output data>

RECORD SPECTRUM NO. (1-4)

1

Set the optical number you want to output.

Press the number key ([1]~[4]) → press the [ENT] key.

* Initial value: 1

④ The setting screen of the background spectrum number will be displayed.

The background spectrum is used to correct the absorption of the solvent or flow cell.

To obtain the correct corrected spectrum, when modifying the solvent or wavelength scanning range, etc., measure the spectrum that becomes the background and memorize it.

<Setting screen for background spectrum number>

BACKGROUND SPECTRUM NO. (0 ~ 4)

0

Set output spectral correction whether or not.

Press the number key ([0]~[4]) → press the [ENT] key.

* 0: Output the selected SPECTRUM in RECORD SPECTRUM NO., without moving.

* 1 to 4: RECORD SPECTRUM NO will be output. Subtracts the specified background data from

After the spectral data

* Initial value: 0

* When the energy spectrum and spectrum type are different, the following error message will be displayed.

* Press [CL] to return to the 1Monitor screen.

<Display error>

SPECTRA ARE MISMATCHED PRESS "CL" KEY TO CLEAR MESSAGE
--

⑤ The waiting Monitor screen will be displayed.

<Wait for the Monitor screen to output>

RCD NO.	SPEED	WL-RANGE (Em)	WIDTH:STD
1	60	200-600 PRESS	"START"

* Press the [START] key.

* Before pressing the [START] key, when pressing the [ESC] key, the contact operation will be performed.

* RCD NO. The number of the output spectrum that has been set above will be displayed.按[START]键。

⑥ The output Monitor screen will be displayed and the spectrum output will be performed

<Monitor screen (Output spectrum)>

RCD NO.	Ex	Em	FL	SPEED	MODE
1	250	350	0.0	60	Em

* When the spectrum is being output, press the [STOP] key to cancel the output operation.

* RCD NO. The number of the output spectrum that has been set above will be displayed.

⑦ After the output of the spectrum is finished, it will automatically return to the 1Monitor screen.

5.4.7 Labeling Operations

A marker line is indicated only on the recorder. It is usable as an event marker to indicate, for example, the start of measurement or the point where set conditions were changed during measurement.

*Press the

MARKER
1

 key to indicate a marker line on the recorder data.。

5.5 SET UP Settings

Before the analysis operation, set the communication channel (CH), set the LCD CONTRAST.

- This setting does not have to be done every time. If you do not need to modify the original setting, please skip the setting.
- The value displayed before the input is the initial value or the current set value.

5.5.1 Setting the Communication Channel (CH)

Set the communication channel using E-LINE.

- ① Turn on the power switch with the  key held down.
- ② The Set up screen will be displayed.

<SETUP screen>

SETUP.	DETECT	THERMO	CONTRAST	S. NO.
	CH1	NO USE	4	02AB-111

Press the [ENT] key

- ③ The Set up screen will be displayed.

<SETUP Setting screen>

SETUP ITEMS (DETECTOR=1, CONTRAST =2,	
1	EXCHANGE STANDARD=3)

Select DETECTOR.

Press the number key ([1]) → press the [ENT] key.

- ④ The communication channel setting screen will be displayed.

<Communication channel setting screen>

DETECTOR (CH1 = 1, CH2 = 2)	
1	

Select a channel (CH).

Press numeric key ([1] or [2]) → press the ENT key.

- * Default value: 1
- * Two channels can be selected for two detectors.

- ⑤ Channel setting is now completed and the setup initial screen appears.

Under DETECTOR, the selected channel setting is indicated.

<SETUP screen>

SETUP.	DETECT	THERMO	CONTRAST	S. NO.
	CH1	NO USE	4	02AB-111

* When you press the ENT key here, the setup items selecting screen is presented to allow reentry.

Or you can advance to LCD contrast setting.

* The selected setting will be fetched upon turning on power supply at the next time.

⑥ Turn off the power switch.

5.5.2 Setting LCD CONTRAST

Set the brightness of the LCD screen.

- ① Turn on the power switch with the SET UP
UTLTY key held down
- ② The following display will be presented.

<Setup initial screen>

SETUP.	DETECT	THERMO	CONTRAST	S. NO.
	CH1	NO USE	4	02AB-111

Press the ENT key.

- ③ The following display will be presented.

<Setup items selecting screen>

SETUP ITEMS (DETECTOR=1, CONTRAST =2, 2 EXCHANGE STANDARD=3)

Select CONTRAST.

Press Number key ([2]) → press [ENT] key.

- ④ The communication channel setting screen will be displayed.

<Communication channel setting screen>

LCD CONTRAST (1-7) 3

Select LCD CONTRAST.

Press the number key ([1]~[7] : General input [3]) → press the [ENT] key.

* [1] : Dark → [7] : light

* Initial value: 4.

⑤ LCD contrast setting is now completed and the setup initial screen appears.

Under CONTRAST, the selected contrast value is indicated.

<SETUP screen>

SETUP.	DETECT	THERMO	CONTRAST	S. NO.
	CH1	NO USE	3	02AB-111

* When you press the ENT key here, the setup items selecting screen is presented to allow reentry.

* The selected setting will be fetched upon turning on power supply at the next time.

⑥ Turn OFF the power switch.

5.5.3 Setting Xenon lamp exchange standard time



[Warning]

Xenon lamp rupture:

When the cumulative lighting time of xenon lamp exceeds the use limit, the evaporation and flying objects attached to the electrode on the tube wall promote the blackening of the tube wall, the heat dissipation will be reduced, and the temperature (pressure) in the tube will be abnormally high, which may cause damage such as the rupture of the lamp.

Xenon lamps that exceed the use limit time, please replace them with new ones as soon as possible.

Set standard xenon lamp life.

When the xenon lamp is on and exceeds the standard life of the xenon lamp, an error message will be displayed on the screen, informing the user that the xenon lamp needs to be replaced.

- ① Turn on the power switch with the **SET UP** **UTLTY** key held down.
- ② The following display will be presented.

<Setup initial screen>

SETUP.	DETECT	THERMO	CONTRAST	S. NO.
	CH1	NO USE	4	02AB-111

Press the **ENT** key.

- ③ The following display will be presented.

<Setup items selecting screen>

SETUP ITEMS (DETECTOR=1, CONTRAST =2,
3 EXCHANGE STANDARD=3)

Select EXCHANGE STANDARD.

Press numeric key **3** and then **ENT** key.

- ④ The following display will be presented.

<Standard Xe lamp lifetime setting screen>

XE LAMP EXCHANGE STANDARD (1-2500) H
500

The standard lifetime of the Xe lamp is settable here.

Press numeric key (to input **1** to **2500**) and then the **ENT** key.

- * Check the model of your Xe lamp and
input the correct value listed below.

Model	Part name	Guaranteed life span	Useful lifetime	Value
5J0-1890	150 W Xe lamp(H)	500 hours	2500 hours	2500 or less

- * Default value: 500 hours (5J0-1890 Xe lamp is attached with shipment.)

- ⑤ The setting of a standard Xe lamp lifetime is now completed and the following display will be presented.

<Setup initial screen>

SETUP.	DETECT	THERMO	CONTRAST	S. NO.
	CH1	NO USE	3	02AB-111

- * When you press the **ENT** key here, the setup items selecting screen is presented to allow reentry.
- * The selected setting will be fetched upon turning on power supply at the next time.
- ⑥ Turn OFF the power switch.

5.6 Use the key pad for instrument maintenance/inspection

5.6.1 Checking Basic Operations

Perform the following operations during setting.

- (1) Please prepare the instrument according to the appendix.
- (2) Switch on the power supply of the instrument.
- (3) Confirm whether the measurement conditions can be set (time constant, output range, etc.).
- (4) Use the [WL] key to check the operation of the wavelength.
- (5) Use the [A/Z] key to check the operation of automatic zeroing.

5.6.2 Confirming Performance Specifications

Confirm the performance specifications during the configuration.



[Note]

After adjusting the position of the xenon lamp (see Item 8.2), perform a performance check. Proper performance checks cannot be performed without position adjustment.

5.6.2.1 Xe lamp energy check

Detect the energy of the xenon lamp (wavelength 254 nm) (see item 5.3.7).



[Note]

When the energy value of the Xe lamp has decreased by 50% or more in comparison with the value at the start of use, the useful life of the lamp is considered to have expired. Use the decrease in energy value plus baseline fluctuation (data variation or noise) in judging the lifetime of the lamp.

5.6.2.2 Wavelength accuracy check by using Hg lamp

Check the wavelength accuracy by using the bright line at 254 nm of the Hg lamp (see section 5.3.6). “GOOD” will appear on the display when the wavelength error is within 3 nm, or “FAIL” will appear when the error exceeds 3 nm



[Note]

Wavelength calibration is necessary when “FAIL” appears. See section 5.6.3 (2) for the calibration.

5.6.2.3 Checking the Sensitivity

The sensitivity of the fluorescence detector is measured by using the Raman spectrum of water to measure the S/N value. Before measuring, prepare the following instruments.

- pump
- Recorder or D-2500 chromato-data processor
- Distilled water

(1) Inject distilled water

Connect tubing as shown in Fig.5-4 supply distilled water at a flow rate of 1 mL/min for 3 minutes with the Primaide 1110 Pump in Manual mode, then set the flow rate to 0 and stop the supply of water. Remove the flow cell unit and check for any bubbles in the flow cell.



[Note] If the flow tank is placed in an empty state (including the setting), first inject methanol or acetonitrile into the flow tank, wet the inner wall of the flow tank, and then inject water. If water is injected without first injecting organic solvents, sensitivity may be reduced.

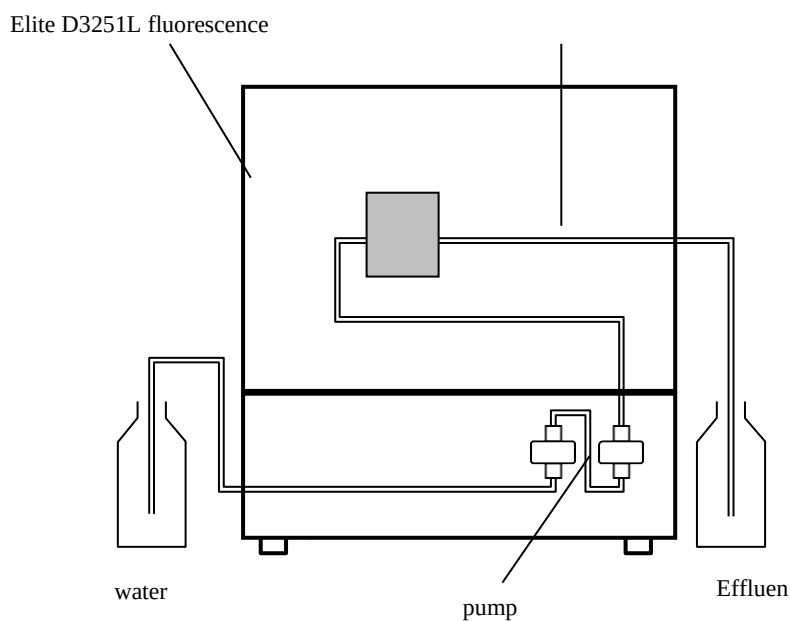


Fig. 5-4 Pipe configuration

(2) Measure the Raman spectrum of water

After the flow tank is installed, the Raman spectral data of the water is measured according to the following conditions and stored in the memory (refer to items 5.4.2, 5.4.3, 5.4.5, 5.4.6 and 5.5.2).

Follow these steps to measure.

- Excitation wavelength (Ex) : 350 nm
- Time constant (response time) : 2 seconds
- Photomultiplier printing voltage: MID
- Bandwidth of the fluorescence side spectrum: STANDARD(15 nm) or WIDE(30 nm)
- Measurement wavelength of fluorescence spectrum: 350 ~ 450 nm

- Spectrum number: any one from 1 to 4

(a) After the power is turned on, the 1Monitor screen will be displayed.

(b) Set the excitation light wavelength

- ① Press [WL] key, the wavelength setting screen will be displayed.

Here, set the excitation light

<Wavelength (Ex). Wavelength setting screen (show Ex)>

Ex	Em	(200-850.0)
350	350	

Press numeric keys **3****5****0** and then the **ENT** key.

- ② The fluorescence wavelength (Em) is not set, and the status quo is maintained.

<Wavelength setting screen (show Em)>

Ex	Em	(200-850.0)
350	<u>350</u>	

Press the **ENT** key

- ③The 1Monitor screen will display

(c) Set the time constant and the bandwidth of the photomultiplier's incising voltage and fluorescence side spectrum

An error message will be displayed informing the user that the xenon lamp needs to be replaced.

- ① Press the **SET UP**
UTLTY key, and the utility setting screen appears.

<Utility setting screen>

UTILITY (OFFSET = 1, TIME CONSTANT = 2,
<u>2</u> USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Press numeric key **2** and then the **ENT** key.

- ② The time constant setting screen appears.

<Time constant setting screen>

TIME CONSTANT (0.05s=1, 0.1s=2, 0.5s=3,
<u>3</u> . 1.0s=4, 2.0s=5, 4.0s=6, 8.0s=7)

Select EXCHANGE STANDARD.

Press numeric key **5** and then the **ENT** key

③ The 1st monitor screen appears.

④ Press **SET UP**
UTLTY key. And the utility setting screen reappears

<Utility setting screen>

UTILITY (OFFSET = 1, TIME CONSTANT = 2,
4 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Press numeric key **4** and then the **ENT** key

⑤ The pmt voltage setting screen appears.

<Pmt voltage setting screen>

PMT VOLTAGE (SUPER HIGH = 1, HIGH = 2, MID = 3,
3 . LOW = 4, SUPER LOW = 5)

Select EXCHANGE STANDARD.

Press numeric key **3** and then the **ENT** key.

⑥ The 1st monitor screen reappears.

⑦ Press **SET UP**
UTLTY key, and the utility setting screen reappears

<Utility setting screen>

UTILITY (OFFSET = 1, TIME CONSTANT = 2,
5 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Press numeric key **5** and then the **ENT** key.

⑧ The others setting screen appears.

<Others setting screen>

OTHERS (Em BANDWIDTH=1, ERROR OUT=2,
1 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Select Em BANDWIDTH.

Press numeric key **1** and then the **ENT** key.

⑨ The Em bandwidth setting screen appears.

<Em bandwidth setting screen>

Em BANDWIDTH (STANDARD=1, WIDE=2)

1

Set the bandwidth of the fluorescence side spectrum.

Press numeric key 1 or 2 and then the ENT key. ◦

* 1: STANDARD = emission spectrum bandwidth 15 nm

2: WIDE = emission spectrum bandwidth 30 nm

⑩ The 1st monitor screen returns.

(d) Setting of spectrum measurement:

① Press the SPECT key. The scan mode setting screen appears.

<Scan mode setting screen>

SPECTRUM MODE (FL (Ex) = 1, FL (Em) = 2)

2

Press numeric key 2 and then the ENT key.

② Set a scanning range.

Ⓐ Enter a starting wavelength (minimum value) for WL1.

<Scan range setting screen>

SCANNING WL RANGE (WL1WL2) nm FL (Em)

<250-850>

350 - 700

Press numeric keys 350 and the ENT key.

Ⓑ Enter an end wavelength (maximum value) for WL2.

<Scan range setting screen>

SCANNING WL RANGE (WL1WL2) nm FL (Em)

<400-700>

350 - 450

Press numeric keys 450 and the ENT key.

③ The memorize spectrum No. setting screen appears.

<Memorize spectrum No. setting screen>

MEMORIZE SPECTRUM NO. (1-4)

1

Press a numeric key (1 to 4) and the ENT key.

- ④ The scan monitor screen appears, and spectrum acquisition begins.

<Scan monitor screen (spectrum acquisition under way)>

SCANNING SPECTRUM NO.: 1 MODE: FL (Em)
Ex: 350 nm Em: *** nm

- ⑤ When the recording of spectral data finishes, the 1st monitor screen returns automatically.

(e) Setting of recorder full scale range, speed and memory No.:

- ① Press the **RECORD** key and the recorder setting screen appears.

<Recorder setting screen>

RECORD (RCD RANGE = 1, RCD SPEED = 2,
1 SPECT RCD = 3)

Press numeric key **1** and then the **ENT** key.

- ② The recorder full scale setting screen appears.

<Recorder full scale setting screen>

RCD RANGE (1 - 1000)
1000

Press numeric keys **1000** and then the **ENT** key.

- ③ The 1st monitor screen returns.

- ④ Press the **SPECT** key. The recorder setting screen reappears.

<Recorder setting screen>

RECORD (RCD RANGE = 1, RCD SPEED = 2,
2 SPECT RCD = 3)

Press numeric key **2** and then the **ENT** key.

- ⑤ The recorder speed setting screen will appear.

<Recorder speed setting screen>

RCD SPEED (40 mm / min = 1, 60 mm / min = 2)

2

Press numeric key 2 and then the ENT key.

⑥ The 1st monitor screen returns.

⑦ Press the SPECT key. The recorder setting screen reappears.

<Recorder setting screen>

RECORD (RCD RANGE = 1, RCD SPEED = 2,

3 SPECT RCD=3)

Press numeric key 3 and then the ENT key.

⑧ The output data spectrum No. setting screen appears.

<Output data spectrum No. setting screen>

RECORD SPECTRUM NO. (1-4)

1

Press a numeric key 1 to 4 and then the ENT key.

* Set the same number as was entered when making the setting for spectrum measurement.

⑨ The background spectrum No. setting screen appears.

<Background spectrum No. setting screen>

BACKGROUND SPECTRUM NO. (0 ~ 4)

0

Press numeric key 0 and then the ENT key.

⑩ The output ready monitor screen appears.

<Output ready monitor screen>

RCD NO.	SPEED	WL-RANGE (Em)	WIDTH: STD
1	60	350-450	PRESS "START"

Press the START key.

* A spectrum is recorded by starting the recorder or chromatograph data processor simultaneously with pressing the START key.

- ⑪ The spectrum output screen appears, and spectrum output begins.

<Spectrum output screen>

RCD NO.	Ex	Em	FL	SPEED	MODE
1	350	350	1000.0	60	Em

- ⑫ When the spectrum output is completed, the 1st monitor screen returns automatically.



[Note]

When connecting the detector to the data processing instrument with the IFC substrate (digital), use the [ALL START] key of the data processing instrument to start the output.

(3) Read Raman semaphore

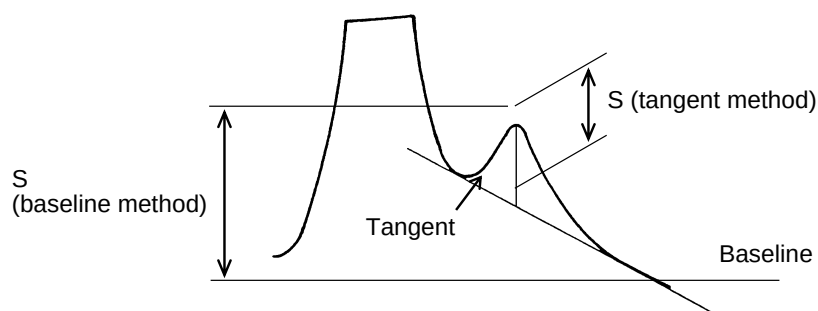


Fig.5-5 Reading the Raman semaphore

Read a voltage value equivalent to S.

When using the Kromstation workstation for data collection, the length of S on the chart (L mm) can be calculated.

$$S = \frac{L}{162} \times 2^x mV$$

L: Length of S on the chart (mm)

x: ATT value of the data processing instrument used for measurement



[Note]

Data acquisition using the Kromstation workstation can be automatically calculated with the included maintenance software. Refer to the maintenance software manual for more information.

(4) Measuring noise

The excitation wavelength is maintained at 350 nm, and the fluorescence wavelength is set close to the peak wavelength of 397 nm (other measurement conditions are the same as (2) above), and the amount of variation in fluorescence intensity is obtained.

The [WL] key is used to move the fluorescence wavelength (Em) and set the fluorescence wavelength so that the fluorescence intensity is the maximum value. Start the logger or Kromstation chromatographic data processing instrument and record the baseline.

* Drifts can also be measured after 15 minutes of measurement.



[Note]

When the spectral bandwidth of the fluorescence side is WIDE (30 nm), the peak wavelength of the fluorescence wavelength will shift to the wavelength side shorter than 397 nm. When the spectral bandwidth of the fluorescence side is WIDE (30 nm), the peak wavelength of the Raman light will move to the wavelength side of the scattered light due to the wider spectral bandwidth of the scattered light and the scattered light of the water. Refer to Section 5.4 for more information.

(5) Calculate noise

The noise value is calculated using the method shown in Figure 5-1 above.

(6) Calculate the Raman S/N ratio of water

It can be calculated using the S and N obtained in (3) and (5) above.

① Spectral bandwidth of fluorescence side: 15 nm

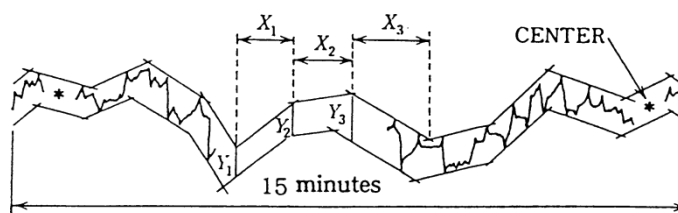
S/N ratio (baseline specification: S/N ratio ≥ 900)

S/N ratio (Tangent method specification: S/N ratio

≥ 675)

② Spectral bandwidth of fluorescence side: 30 nm

S/N ratio (Baseline specification: S/N ratio ≥ 4000)



$$\text{Noise value} = \sum_{R=1}^{R-n} Y_{R/n} \quad (X_n \text{ in } 0.5 \text{ bits})$$

Fig.5-6 Calculating noise (using JAIMAS 0005-2008 formula)



[Note]

Note that Raman spectra or noise levels are affected by distilled water or bubbles.

(7) Measurement method of Raman spectrum of water (when using time program)

(The Raman semaphore obtained in (2) above is smaller)

When the sensitivity (S/N ratio of the Raman peak of water) is low, please check the following items again.

(a) When the noise is loud

① Check the distilled water

* Please use distilled water that is not long after refining. Distilled water that has been stored in resin containers for a long time may be mixed with fluorescent substances.

② Position and life of xenon lamp

(b) when the separation of spectra is poor

① Check the distilled water

* Please use distilled water that is not long after refining. Distilled water that has been stored in resin containers for a long time may be mixed with fluorescent substances.

② Check bubbles

When water is trapped in an empty flow tank, it may not be

possible to remove small bubbles in the flow tank. Please fill with ethanol, etc., and then seal with water.

③ Change the measurement method of the spectrum
(when using the time program)

The Raman spectrum of water is measured using a time program. At this time, the wavelength shift is used to confirm the change of FL value in advance.

First, press the [WL] key to set the excitation wavelength (Ex) to 350 nm and the fluorescence wavelength (Em) to 450 nm. Next, press the [A/Z] key to set the FL value to 0.

After pressing the [WL] key, confirm the maximum fluorescence wavelength (Em) near 397 nm

The FL value is associated with this wavelength (Em), and the fluorescence wavelength (Em) is near 380 nm and the minimum FL value is associated with this wavelength. Moreover, the value where the minimum value of FL is less than half of the maximum value is the expected value.

The Raman spectrum of water uses a time program to create the following time program.

TIME	Ex	Em	BASE (A/Z = 1, HOLD = 2)
0.0	350	450	1
0.5		360	
0.6		370	
0.7		375	
0.8		380	
0.9		385	
1.0		390	
1.1		395	
1.2		400	
1.3		405	
1.4		410	
1.5		415	
1.6		430	
1.8		450	
2.0			



[Note]

In the above time program, for example, the minimum value of FL is 380 nm and the maximum value is 400 nm. Set the Em entry of the time program to contain wavelengths that represent the maximum and minimum values of FL. (For example, when the table

indicates that the wavelength of the maximum FL is 397 nm, the wavelength of 1.1 minutes is regarded as 397 nm).

Read the value equivalent to S from the spectrum obtained using the time program.

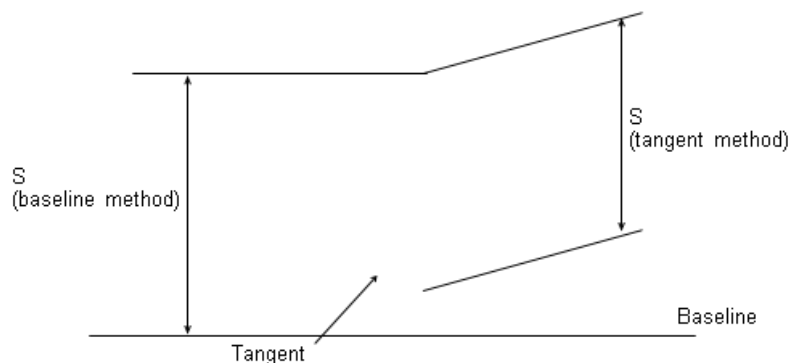


Fig. 5-7 Measuring method of Raman spectrum of water (when using time program)



[Note]

When using the IFC substrate for data acquisition, Raman spectroscopy can be measured directly through the relevant maintenance software.

(8) Measuring drift

The drift is measured with the Raman spectral data of the water and the baseline (the measurement conditions are the same as in (4) above). However, the measurement condition should be set to 15 minutes).

In simple terms, the output value at a fluorescence wavelength of 450 nm is calculated as level zero.

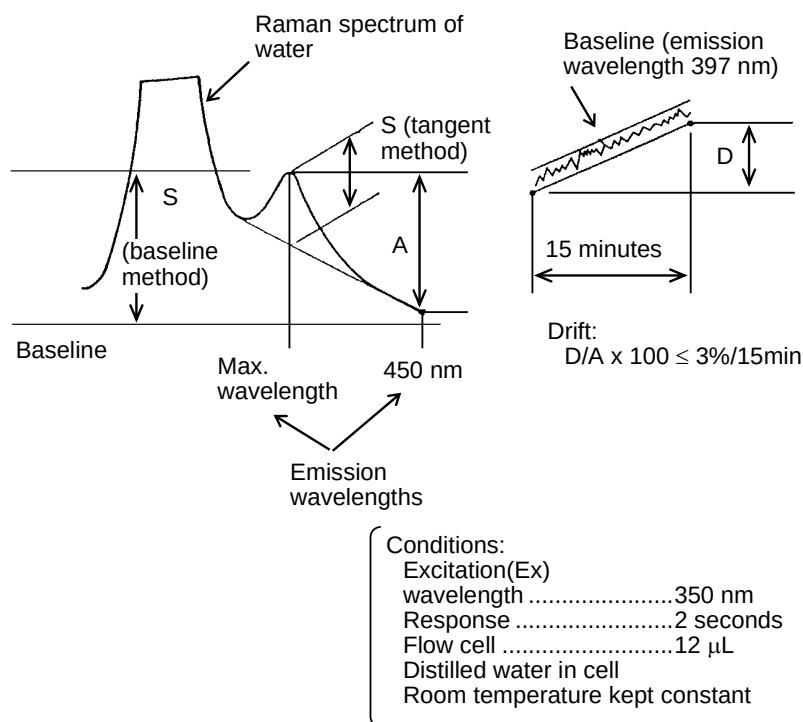


Fig.5-8 Drift Measurement



[Note]

Wait until the xenon lamp is stable, and then take measurements.
(after turning on the lights for an hour).

5.6.3 Wavelength accuracy check and calibration

(1) Check of Wavelength Accuracy

(a) Principle of measurement

Wavelength accuracy of the emission side monochromator is checked by using the bright line at 254 nm of the builtin Hg lamp.

As for the wavelength accuracy of the excitation side monochromator, the Em side is fixed at the wavelength position where the abovementioned bright line was detected, then the Ex wavelength where a Rayleigh scattering peak of 254 nm is produced is measured.

(b) Check of wavelength accuracy

Check the wavelength accuracy by using the bright line at 254 nm of the Hg lamp (see section 5.3.6). "GOOD" will be indicated if the wavelength error is within ± 3 nm, or "FAIL" will be indicated if the error exceeds ± 3 nm.



[Note] Please check the accuracy of the wavelength after adjusting the position of the xenon lamp (see item 8.2). If the position is not adjusted, the correct wavelength will not be confirmed.



[Note] If FAIL is displayed, the wavelength needs to be corrected. Please refer to 5.6.3 (2) for wavelength correction.

(2) Calibration of Wavelength Accuracy

If there is a wavelength deviation, then adjust the wavelength in the wavelength calibration mode (special mode).

(a) Calibration of excitation wavelength

- ① Press the SET UP
UTLTY key, and the utility setting screen appears.

<Utility setting screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
99 USE PROG=3, PMT VOLT=4, OTHERS=5)

Press numeric keys 99 and then the ENT key.

- ② The excitation wavelength calibration mode screen appears.

<Excitation wavelength calibration mode screen>

Ex WL ADJUST (1-20)
10 (ONLY FOR FACTORY USE) (-2)

The present offset value is indicated at Ex WL ADJUST. Indicated at the bottom right of the screen is the wavelength check result (deviation of wavelength; indication range of -10 to 10).

Wavelength calibration is conducted by entering a new offset value (value to which the deviation is added).

Press numeric keys (from 1 to 20) and then the ENT key.

* Input range for offset value: 1 to 20

* Calculation of new offset value:

$$\text{New offset value} = \text{present offset value} - \text{wavelength deviation}$$

$$\text{Example: } 12 = 10 - (-2)$$

③ The 1st monitor screen appears.

(b) Calibration of emission wavelength

① Press the **UTLTY** key, and the utility setting screen appears.

<Utility setting screen>

UTILITY (OFFSET=1, TIME CONSTANT=2,
98 USE PROG = 3, PMT VOLT = 4, OTHERS = 5)

Press numeric keys **9 8** and then the **ENT** key.

② The emission wavelength calibration mode screen appears.

<Emission wavelength calibration mode screen>

Ex WL ADJUST (1-20)
10 (ONLY FOR FACTORY USE) (-2)

The present offset value is indicated at Em WL ADJUST. Indicated at the bottom right of the screen is the wavelength check result (deviation of wavelength; indication range of -10 to 10).

Wavelength calibration is conducted by entering a new offset value (value to which the deviation is added).

Press numeric keys (from **1** to **2 0**) and then the **ENT** key.

* Input range for offset value: 1 to 20

* Calculation of new offset value:

$$\text{New offset value} = \text{present offset value} - \text{wavelength deviation}$$

$$\text{Example: } 12 = 10 - (-2)$$

③ The 1st monitor screen appears.

<Offset setting screen>

OFFSET (0 - 1000)
_0

Press numeric keys (from **1** to **1 0 0 0**): Generally enter [0] → press the **[ENT]** key

* Set range: 0 ~ 1000, the minimum unit is 1Step.

* Initial value: 0

Chapter 6 Maintenance and inspection

6.1 Periodic Check

No.	Check Item	Frequency of Check	Check Procedure
1	Drain tray	Daily	Check the drain tray, and wash it when needed.
2	Lighting of lamp	Daily	Check that “Xe” is indicated on LEDs.
3	Liquid leakage	Daily	Visually check that liquid is not leaking from the mobile phase bottle, tubing and waste bottle.
4	Fluorescence intensity check	Daily	Press A/Z key, and check if FL indication is zeroed.
5	Lifetime of lamp	Monthly	1) Check lamp for service life by energy check, or by cumulative lamp turn-on time. 2) Check if baseline drifts considerably (or if baseline fluctuates or noise becomes high).
6	Leak sensor	Monthly	1) Drip water onto the leak sensor and confirm the occurrence of an error. 2) After confirmation, wipe off water.
7	Wavelength accuracy	Yearly	Check wavelength by using bright line of Hg lamp at 254 nm.



[Note]

After adjusting the position of the xenon lamp, check the energy of the xenon lamp and the wavelength accuracy of the mercury lamp. If you do not adjust the position, you will not get the correct result.

6.2 Check/clean the liquid tray

The D3251L fluorescent detector is equipped with a leakage sensor for sensing leakage. However, due to the dirt on the sensor and other reasons, it may not be possible to correctly perceive the leakage. Please follow the steps below for regular cleaning and cleaning.

(1) Check the operation of the leakage sensor

Drop 0.5 mL of water onto the leakage sensor to check for leakage errors.

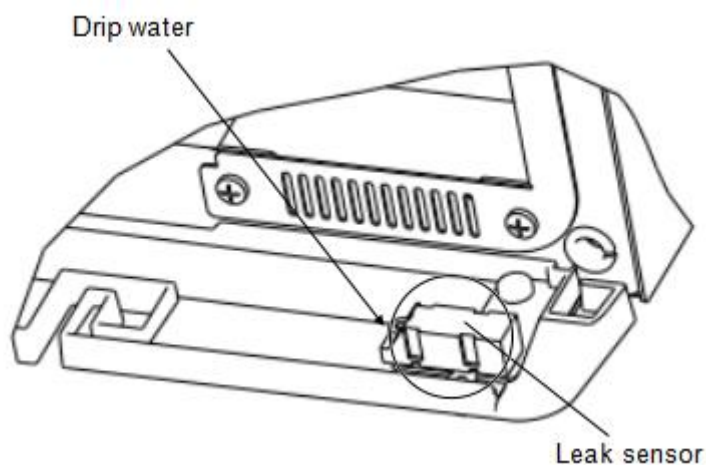


Fig. 6-1 Check the operating status of the leakage sensor



[Note]

When using a leakage sensor that senses leakage, please pay attention to the following points.

(a) When using solvents, activate the leakage sensing function before using.

(b) The leakage sensor of this instrument may not be able to detect the leakage of a small amount of liquid.

Moreover, it may not be perceived when using highly volatile solvents. Even if the leakage sensing function

is activated, it is necessary to verify the presence of leakage frequently to avoid major accidents.

(c) As a routine check, the presence of leakage is checked visually prior to measurement.

(d) As a regular check, confirm that the leakage sensor is functioning properly.

(2) Cleaning of leakage sensor

<preparative>

- Hex wrench (size: 2.5#)

(a) With the hex wrench equipped with the instrument, unscrew the fixing screw of the leakage sensor, and then remove the leakage sensor from the liquid dish.

(b) After wiping the water on the leakage sensor and the liquid tray, fix the leakage sensor to the liquid tray again.



[Note]

When cleaning the leakage sensor, be careful not to damage the tip of the leakage sensor. If damaged, the leakage sensor may not function properly, or an error may occur.

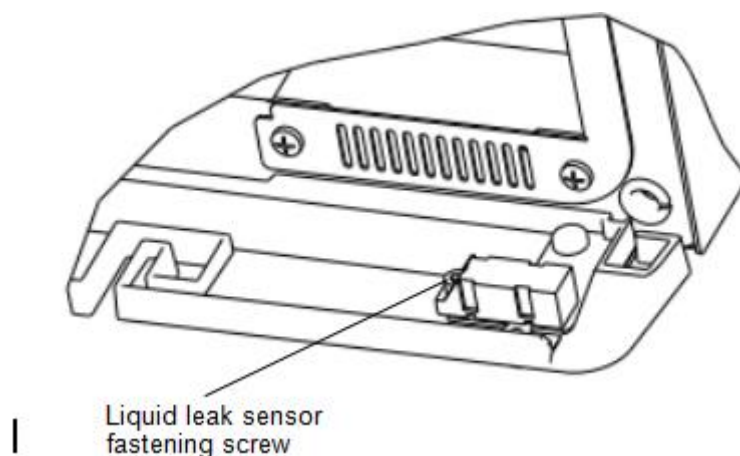


Fig.6-2 Screws securing the leakage sensor



[Note]

Install the leakage sensor in the correct position. When the installation position is not appropriate, the leakage sensor may not function properly, or an error may occur.

6.3 Check and clean the flow tank



[Warning]

Physical inflammation or injury caused by toxic, corrosive, irritating solvents: The use of toxic, corrosive, irritating solvents may cause inflammation or injury to the body. Information on the properties and use of the solvent (MSDS (Material Safety Data Sheet)) must be confirmed for exact use.

- (a) Please wear protective tools (protective glasses), etc., to prevent the solvent from sticking to the skin.
- (b) Please carry out adequate indoor ventilation to avoid attraction.

If the liquid tray is dirty, please follow the following steps to clean the liquid tray.

- (1) Unscrew the fixing screw and remove the leakage sensor from the liquid tray.



[Note]

Be sure to use the removed leakage sensor carefully to avoid damage to the leakage sensor. When damaged, it may not function properly.

- (2) Draw out the liquid tray from the instrument.
 - (3) Wipe the liquid tray with a soft cloth such as gauze and clean it. When there's stubborn dirt, Please rinse with running water.
 - (4) After completely drying the liquid tray, press it into the instrument.
 - (5) Wipe the dirt on the leakage sensor with soft cloth such as gauze.
- Fix the leakage sensor to the liquid tray with screws.

6.4 Check and clean the flow tank



[Warning]

The burning of combustible drugs

- This instrument is not explosion-proof. Do not use organic solvents with combustion points less than 70 ° C when no one is operating.
- Combustible medicines such as organic solvents may cause combustion.
 - (a) Do not place the source of fire near the instrument.
 - (b) Please perform adequate ventilation in the room.
 - (c) Always check the following two points, if abnormal, please stop using immediately.
 - There is no leakage of solvents or waste fluids used.
 - Inside the instrument, there is no solvent leakage.
- There is a risk of combustion due to static electricity when using combustible medicines.
- In order to avoid combustion due to static electricity, a grounded conductive substance is used to prevent the accumulation of static electricity on the liquid waste container.



[Warning]

Explosion of flammable vapor

- Flammable pharmaceutical products, such as organic solvents, leak from the liquid passage of the instrument at concentrations above the explosive limit, which may cause an explosion.
- When using combustible and easily vaporized drugs such as organic solvents, check that there is no leakage in the liquid channel of the instrument and carry out adequate ventilation in the room.

6.4.1 Cleaning/cleaning of circulation pool

If there is dirt in the flow tank, please clean it.

<preparative>

- syringe (glass, about 10 mL)
- cleaning solution

Flow cell unit (P/N 8DD-5350)

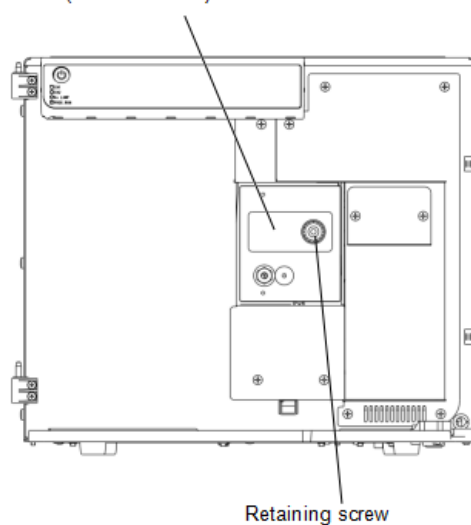
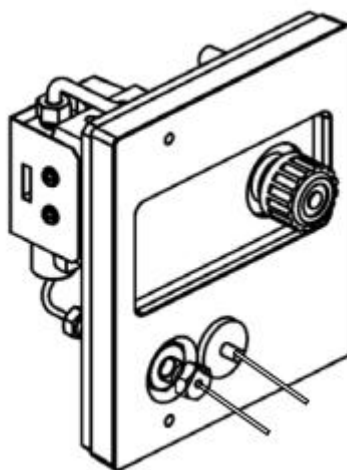
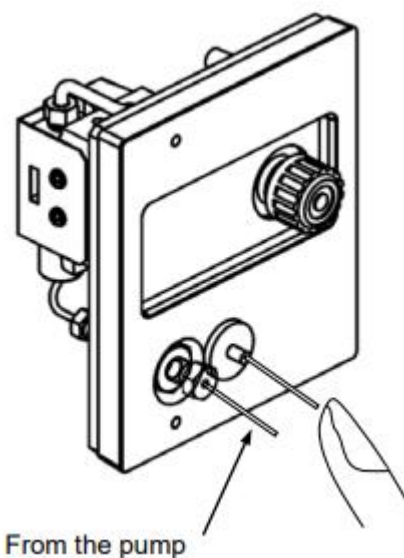


Fig. 6-3 Check the check points for connecting the pool

- (1) Loosen the fixing screws of the flow pool assembly (Figure 6-3).
- (2) Pull out the flow pool set along the horizontal direction.
- (3) Visually check whether there is foreign matter (crystallization, etc.) or dirt in the circulation tank.
- (4) Visually check whether there is leakage around the flow pool.
- (5) The internal organs of the pool need to be cleaned. Cleaning fluids vary depending on the mobile phase used. If the mobile phase is water, inject distilled water into the syringe first. If the mobile phase is organic solvent, inject ethanol (acetone) into the syringe, insert the syringe into the tip of the liquid tray, and clean while injecting (Figure 6-4).

**Fig6-4 Pool assembly**

- (6) In the same manner after changing to the mobile phase used.
- (7) When bubbles are mixed in the flow pool, use the pump to inject at a flow rate of 1.0 mL/min and a pressure below 1.0 MPa while blocking the outlet of the drain pipe. After applying pressure to the flow pool for 2 to 3 seconds, remove the finger blocking the drain pipe accordingly. Under normal circumstances, this is done 3 to 4 times, you can remove the bubble clean. If the bubbles are not completely removed after three or four attempts, repeat the preceding steps several times.

**Fig. 6-5 When air bubbles are mixed into the flow pool****[Note]**

If too much pressure is applied, the flow tank may be damaged.

When cleaning the flow tank, please set the upper pressure of the pump to 1.0 MPa before cleaning.

6.4.2 Decomposition and cleaning of flow pool

1) Decomposing the circulation pool

When the dirt in the circulation tank cannot be removed after cleaning, decompose and clean according to the following steps.

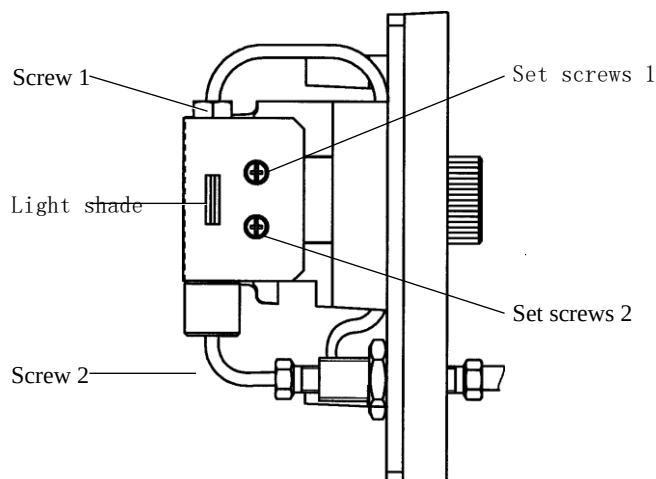


Fig. 6-6 Circulation pool set

- ① After loosening the fixing screw, remove the light shield.
- ② Take down the flow pool window.
- ③ Remove the pressure rod of the flow tank.
- ④ Loosen the screws.
- ⑤ Take out the circulation tank.

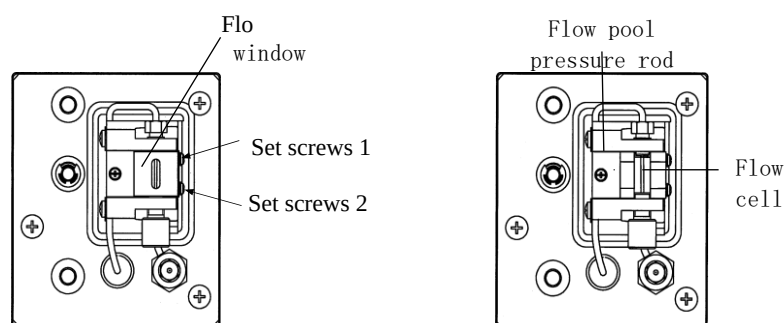


Fig.6-7 Flow pool decomposition

2) Clean the circulation tank

- ① Please flush the circulation tank with running water.
- ② When the dirt cannot be removed after rinsing with running water, please clean it with an ultrasonic cleaner. When performing ultrasonic cleaning, be sure to use a container made of paper or plastic to avoid damage to the circulation pool.

After wetting the gauze with acetone or ethanol, wipe the dirt on the surface of the circulation tank.



[Note]

When decomposing the flow pool, be careful not to damage the surface of the flow pool.



[Note]

When cleaning the flow tank, always use a container made of paper or plastic. If a glass container is used, the flow tank will be damaged and the original performance of the instrument may not be achieved.

3) Assembly of Flow Cell

- ① Assemble the flow cell into the cell holder in the orientation shown in Fig.6-8 and fix it with the cell clamp.
- ② Tighten the fixing screws 1 and 2 on the upper and lower sides.

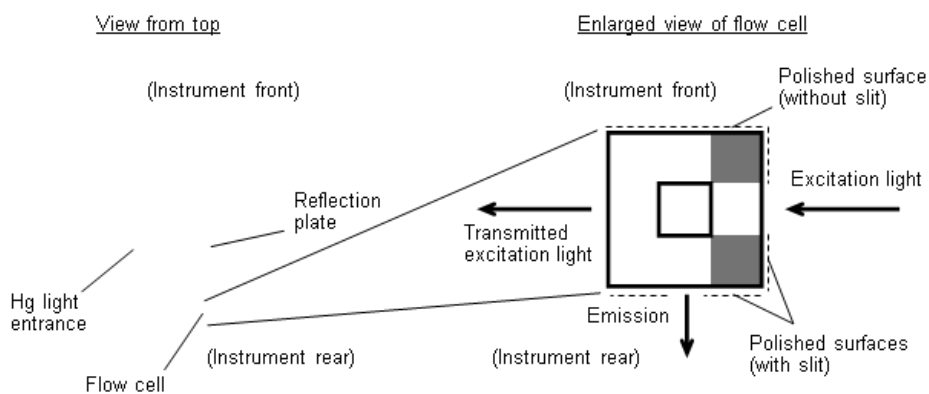


Fig.6-8 Assembly of a flow pool

- ③ Attach the cell window and shielding plate.
- ④ After the assembly is completed in accordance with the steps contrary to the decomposition, the solution is exiled to confirm whether there is leakage.



[Note]

When installing the flow pool, be careful not to damage the surface of the flow pool.



[Note]

When the flow pool is set to the pool base, please be careful not to mistake the installation direction of the gloss surface of the flow pool (see Figure 6-8). If the installation direction is wrong, the original performance of the instrument may not be obtained.



[Note]

After completely tightening screw 1 by hand, tighten screw 2 by hand for about 30° , or tighten screw 1 by using a wrench.

6.4.3 Leakage of the flow pool

It is important to check for any liquid leakage from the flow cell unit prior to analysis. If a leak is found, stop the pump immediately and remove the flow cell unit. Take the following procedure to remedy the leak.

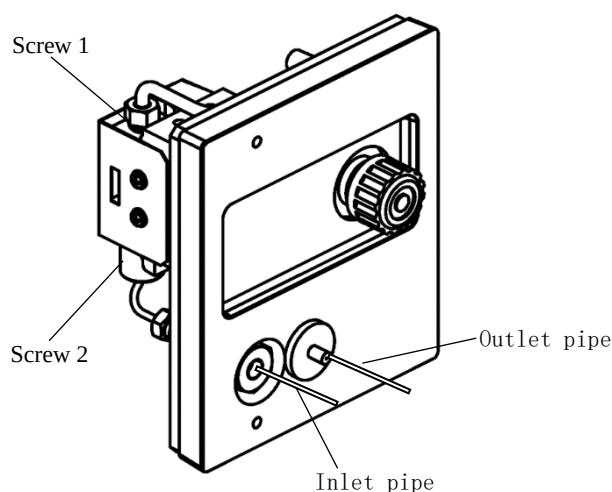


Fig. 6-9 Check whether the flow pool set is leaking

- 1) Use the pump to inject the fluid and confirm whether the solution is discharged from the outlet hose of the flow pool.
- 2) If no solution is produced, it indicates that the outlet hose has been blocked. At this time, clean and replace with a new outlet hose.
- 3) Although the normal discharge solution also occurs when leakage, the degree of screw tightening may be insufficient. At this point, use a wrench to tighten screw 1 slightly more (about 10° to 20°), or use your hand to tighten screw 2 slightly more (10° to 20° left).
- 4) Wipe the liquid around the circulation tank clean.
- 5) When the flow tank is taken off, infusion is performed with the pump to confirm whether the liquid is leaking.



[Note]

If you do not check whether the OUTLET hose is blocked, it is easy to judge that it is not

When the screw is tightened with a wrench, the flow tank may be damaged. Make sure your OUTLET hose is not blocked.



[Note]

When leakage occurs in the sample chamber, please wipe the liquid first and then clean it with water.

6.4.4 Cautions on storage of the flow cell

When the circulation tank is not used for more than two or three days in a row, please flush the inside of the distilled water, alcohol, etc., and then store it. If it lasts less than two or three days, it can be stored in the circulation tank filled with solvent such as ethanol and acetonitrile.

Chapter 7 Troubleshooting and diagnosis

7.1 Fault Measures

Symptom	Cause	Judgment/Check	Remedy
1. Self-diagnosis (initialization) is not performed when the POWER switch is turned on.	The power cord plug is not securely plugged in.	Visual check	Plug in the power cord securely.
	The fuse is blown.	Check the fuse for continuity.	Replace the fuse with a new one.
	The light source cover is not properly attached. (The cover activates a safety interlock switch.)	Visual check	Retighten the retaining screws of the light source cover.
2. The keyboard does not work.	The instrument is not ready for operation.	Check the monitor screen.	Press the ESC key. The READY status will be presented.
3. The  key does not work.	The display does not present the 1st monitor screen (FL indication).	Check the monitor screen.	Return to the 1st monitor screen (FL indication).
4. The time program does not start.	The instrument is not ready to operate.	Check the monitor screen.	Press the ESC key. The READY status will be presented.
5. The xenon lamp does not light.	The xenon lamp is still hot.	Turn on the lamp again.	For turning on the lamp again, wait at least 5 minutes after the Xe lamp went out.
	The Xe lamp has reached the end of its useful life.	Check the cumulative turn-on time of Xe lamp.	Replace the Xe lamp with a new one.
6. Excessive noise	The flow cell is contaminated.	Visual check	Clean the flow cell.
	The mobile phase is not sufficiently degassed.	Spike noise occurs.	Degas the mobile phase thoroughly.
	The mobile phase contains impurities.	Check the mobile phase.	Purify the mobile phase, or replace it with mobile phase of a higher quality.
	The gain level of the recorder is too high.	Check the gain level of the recorder.	Adjust the gain level of the recorder.
	The pump is faulty.	Check to ensure the operation of the pump is smooth.	Check the pump.
		Check for noise when the pump is stopped.	

	The xenon lamp has reached the end of its useful life.	Check the cumulative turn-on time of Xe lamp.	Replace the xenon lamp with a new one.
7.Excessive drift	The warm-up period is not sufficient.	-	Wait until the instrument becomes stable (at least 10 minutes).
	Mobile phase is leaking from a fitting.	Check each fitting.	Retighten the offending fitting.
	An impurity is eluting from the column.	Stop feeding liquid from the pump.	Wait until elution is completed, or replace the column with a new one.
	There is a significant change in the ambient temperature. Or low purity solvent is used.	Check solvent.	Control the temperature variation. Purify the solvent or replace it with one of a higher quality.
8.Poor S/N ratio	Flow cell contaminated	Visual check	Clean the flow cell
	Xenon lamp off-position	Check lamp energy level	Adjust lamp position
	Error in flow cell mounting	Visual check	Correctly mount flow cell
	Flow cell surface scratched	Visual check	Replace flow cell
9.Poor wavelength accuracy	Water is not injected in the flow cell.	$\frac{3}{4}$	Inject water into the flow cell.
	Bubbles have entered the flow cell.	Visual check	Remove bubbles
	Poor adjustment of xenon lamp position	Check lamp energy level.	Adjust Xe lamp position properly.
	Error in flow cell mounting	Visual check	Correctly mount the flow cell
	Monochromator off-position	Check wavelength accuracy.	Perform wavelength accuracy
10.Leak error occurs	Liquid has leaked.	Visual check	Wipe off the liquid which has leaked to the drain receptacle. Also wipe off the liquid stuck to the leak sensor
	Contaminant has stuck to the leak sensor.	Visual check	Wipe off the contaminant stuck to the leak sensor

7.2 Error Message

7.2.1 Workstation

Display error message	Error message displayed by the module	Cause
Self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: Wavelength origin error (Ex)	Ex SIDE OF WL DRIVE MECHANISM ERROR	During initialization (self-diagnosis), the wavelength origin on the Ex side is displayed when it is not successfully detected.
The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: ADC initialization error	ADC INITIALIZE ERROR	When initializing (self-diagnosing), the ADC does not have a normal initial Displayed during initialization.

The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: an ADC error	ADC ERROR	When CDS is connected, ADC data transfer stops.
The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: a DAC error	DAC ERROR	DAC(Signal Output option) Displays when data transfer stops.
Self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: wavelength origin error (Em)	Em SIDE OF WL DRIVE MECHANISM ERROR	During initialization (self-diagnosis), the wavelength origin on the Em side is not successfully detected.
The self-diagnosis of the D3251L detector Ch1/2 detected an error: ROM error	ROM ERROR	When the instrument is started (self-diagnosis), the program's SUM value is not correct (ROM exception).
The self-diagnosis of the D3251L detector Ch1/2 detected an error: a RAM error	RAM ERROR	When the instrument is started (self-diagnosis), the RAM is detected and displayed if there is an anomaly (poor RAM).
The D3251L detector Ch1/2 detects an anomaly in its self-diagnosis: a parameter error. The parameters of the D3251L detector Ch1/2 have been initialized.	PARAMETER ERROR	Displayed when the storage space of the saved method parameter is poor (damaged).
The D3251L detector Ch1/2 detects an anomaly in its self-diagnosis: the log message is incorrect. The maintenance log of D3251L detector Ch1/2 has been initialized.	LOG INFORMATION ERROR	Displays when the space for storing LOG information is poor or damaged.
Self-diagnosis of the D3251L detector Ch1/2 An exception was found in: EEPROM error. The instrument information for the D3251L detector Ch1/2 has been initialized.	EEPROM ERROR	Display when the storage space of saved instrument parameters is poor (damaged).
The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: a leakage error	LAMP OFF BY LEAKAGE	There was a leak in the solvent tray. Displays when the leakage sensor detects a leak.
The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: data buffer overflow	DATA BUFFER OVERFLOW	Unable to transfer data to IFC during data collection. Displays this information when the data buffer overflows.
The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: automatic zeroing was out of range	A/Z OVER RANGE	Although the automatic zero is implemented, it is displayed when the zero range is exceeded.
The D3251L detector Ch1/2 has detected an anomaly: an incorrect Hg lamp	Hg LAMP ERROR	When starting or after turning off the light, the Hg light should be lit but cannot be lit. When the light is to be turned off but cannot be turned off.
Turn off this light by turning off the light contact input.	LAMP OFF BY EXTERNAL INPUT	IN the LAMP OFF IN terminal, it is displayed when the lamp off signal is entered (deliberately off the lamp).
An error contact signal was received from the module.	LAMP OFF BY EXTERNAL ERROR	An error signal is received from an externally connected module and is displayed when the lights are forced to turn off.
The self-diagnosis of the D3251L detector Ch1/2 revealed an anomaly: overheating	LAMP OFF BY OVER HEAT	After the thermostatic fan has stopped, this message is displayed when the lamp is turned off to prevent overheating.
The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: the Xe lamp error	Xe LAMP ERROR	When starting up or after turning off the lights, the Xe light cannot be lit. When the light is to be turned off but cannot be turned off.
The self-diagnosis of the D3251L detector Ch1/2 detected an anomaly: wrong spectral type	SPECTRA ARE MISMATCHED	When the spectrum is collected, it is displayed when the spectrum type is inconsistent with that read from the module.
The Xe lamp of the Ch1/2 of the D3251L detector needs to be replaced.	Xe LAMP EXCEEDED EXCHANGE STANDARD TIME	When the Xe lamp is turned on, it is displayed if the use time of the Xe lamp exceeds the preset switching time.

7.2.2 Key Panel (Optional Module)

Message	Description	Remedy
ROM ERROR PRESS “CL” KEY TO CLEAR MESSAGE.	ROM is faulty.	1) Press CL key, and 1st monitor screen returns. 2) Contact your nearest service office of Hitachi High-Tech Science sales representative.
RAM ERROR PRESS “CL” KEY TO CLEAR MESSAGE.	RAM is faulty.	1) Press CL key, and 1st monitor screen returns. 2) Contact your nearest service office of Hitachi High-Tech Science sales representative.
PARAMETER ERROR PARAMETERS ARE SET TO DEFAULT VALUE.	Analytical parameters are inadequate.	1) Press CL key, and 1st monitor screen returns. 2) Turn on power again. 3) Contact your nearest service office of Hitachi High-Tech Science sales representative.
LOG INFORMATION ERROR LOG INFORMATION IS INITIALIZED.	Log information is inadequate.	1) Press CL key, and 1st monitor screen returns. 2) Turn on power again. 3) Contact your nearest service office of Hitachi High-Tech Science sales representative.
EEPROM ERROR INSTRUMENT INFO. IS INITIALIZED.	Instrument parameters are inadequate.	1) Press CL key, and 1st monitor screen returns. 2) Turn on power again. 3) Contact your nearest service office of Hitachi High-Tech Science sales representative.
Ex (Em) SIDE OF WL DRIVE MECHANISM ERROR	Failure in detecting Ex or Em wavelength origin during self-diagnosis test (initialization).	1) Cannot be released by key operation. 2) Turn on power again. 3) Contact your nearest service office of Hitachi High-Tech Science sales representative.
Ex (Em) SIDE ACCURACY (254 nm) NOT DETECTED	Wavelength error exceeds ± 10 nm during wavelength accuracy check.	1) Carry out troubleshooting (4.5). 2) Contact your nearest service office of Hitachi High-Tech Science sales representative.
LAMP OFF BY OVERHEAT! PRESS “CL” KEY TO CLEAR MESSAGE.	Abnormal temperature detected	1) Press CL key, and 1st monitor screen returns. 2) Contact your nearest service office of Hitachi High-Tech Science sales representative.
	Xe lamp turned off to prevent its overheating.	1) Press CL key, and 1st monitor screen returns. 2) Use UTLTY key to light up the lamp again.
LAMP OFF BY EXTERNAL INPUT PRESS “CL” KEY TO TURN ON.	Lamp has been turned off due to an external contact input. Busy status is assumed.	1) Release the key lock if it has been activated. 2) Press CL key and light up the lamp again.
LAMP OFF BY LEAKAGE PRESS “CL” KEY TO	Liquid leakage has occurred. Xe and Hg lamps have been turned off. Busy status is assumed.	1) Press CL key, and 1st monitor screen returns. 2) Use UTLTY key to light up the lamp

CLEAR MESSAGE.		again.
Xe LAMP ERROR PRESS "CL" KEY TO CLEAR MESSAGE.	Xe lamp fails to come on or go off. Temperature upper limiter of Xe lamp power source is activated.	1) Press  key, and 1st monitor screen returns. 2) Wait at least 5 minutes after turning off lamp for the lamp to cool down, then turn on power again or re-light the lamp. 3) Replace lamp with a new one.
Hg LAMP ERROR PRESS "CL" KEY TO CLEAR MESSAGE.	Hg lamp fails to come on or go off.	1) Press  key, and 1st monitor screen returns. 2) Replace the lamp with a new one.
XE LAMP EXCEEDED EXCHANGE STANDARD TIME PRESS "CL" KEY TO CLEAR MESSAGE.	The cumulative turn-on time of xenon lamp has exceeded its standard exchange time.	1) Press  key, and 1st monitor screen returns. 2) Replace the lamp with a new one.
A/Z OVER RANGE PRESS "CL" KEY TO CLEAR MESSAGE.	Allowable auto zero range has been exceeded.	1) Press  key, and 1st monitor screen returns. 2) Change the wavelength. 3) Replace the solvent with another one. 4) Clean the flow cell.
NO SPECTRA PRESS "CL" KEY TO CLEAR MESSAGE.	Spectrum of specified No. is not found.	1) Press  key, and 1st monitor screen returns. 2) Enter a number corresponding to an existing spectrum. 3) Perform spectrum acquisition.
SPECTRA ARE MISMATCHED PRESS "CL" KEY TO CLEAR MESSAGE.	Scan mode incorrect, Em bandpass incorrect, fixed wavelength incorrect, background WL range doesn't include entire spectrum WL range, background spectrum mode incorrect, or energy spectrum incorrect.	1) Press  key, and 1st monitor screen returns. 2) Select correct parameters.
DATA BUFFER OVERFLOW PRESS "CL" KEY TO CLEAR MESSAGE.	During data acquisition, data transfer to the interface board has been disabled, causing an overflow of the data buffer.	1) Press  key, and 1st monitor screen returns. 2) Check the connection with the interface board.
ADC INITIALIZE ERROR	An error has occurred during ADC initialization.	1) Cannot be released by key operation. 2) Turn on power again. 3) Contact your nearest service office of Hitachi High-Tech Science sales representative.
ADC ERROR	Data transfer from ADC has been interrupted.	1) Cannot be released by key operation. 2) Turn on power again. 3) Contact your nearest service office of Hitachi High-Tech Science sales representative.

DAC ERROR	Data transfer to DAC has been interrupted.	1) Cannot be released by key operation. 2) Turn on power again. 3) Contact your nearest service office of Hitachi High-Tech Science sales representative.
-----------	--	---

Chapter 8 Replacement

parts/Consumables

8.1 Replace parts and consumables

The main replacement parts and consumables used in this instrument are shown in 8-1. The life of most components (lamps, etc.) varies greatly with the use of the instrument.

Standard life is as follows.

- **160 hours/month (8 hours per day)**
- **1920 hours/year (240 days of operation per year)**

Since a product with an unrecorded standard life is not a consumable, replace it if it is lost or damaged.

Table 8-1 Replacement parts and consumables

Model	Part Name	Estimated Service Life	Remarks
5J0-1890	150 W Xenon lamp(H)	Guaranteed life: 500 hours Limit: 2500 hours Warranty period: 6 months after delivery to customer	
890-2662	Mercury lamp	Guaranteed life: 100 hours/2 years	
J821399	Time-lag fuse	5 A	
890-2932	Flow cell (12 mL)	9600 hours/5 years	
890-2684	Drain tube ID 0.33 ´ 0.62 T	9600 hours/5 years	Length: 2 m Outside diameter:1.57 mm
893-5360	Inlet tube ID 0.25 ´ 0.66 T	9600 hours/5 years	Length: 60 mm Outside diameter: 1.57 mm (inlet ferrule included)
8DD-5038	Ferrule front	9600 hours/5 years	Part for connecting column and inlet tube Material: PEEK
M790019	Screw S (SUS)	9600 hours/5 years	Example use: Connecting pipe
893-0827	Connecting pipe (SUS, coated)	9600 hours/5 years	Example use: Piping between column outlet and inlet tube For standard flow cell Length: 350 mm Inner diameter: 0.25 mm, Outer diameter: 1.57 mm, Material: SUS, PTFE (for coating)
L329109	Ferrule (SUS)	9600 hours/5 years	Example use: Connecting pipe
893-0825	Fitting	9600 hours/5 years	Example use:Column outlet

			connection For outer diameter:1.57 mm
--	--	--	--

8.2 Xe lamp replacement



[Warning]

Injured by the rupture of the xenon lamp!

After turning off the lamp (cut off the power supply), wait for more than 1 hour until the xenon lamp is sufficiently cool and the pressure of the xenon lamp is sufficiently reduced, and then start the replacement work.



[Warning]

Burn from the heat!

When replacing a xenon lamp, contact with a high temperature xenon lamp or the fixed frame of the xenon lamp may cause injury.

After cutting off the power supply, please place for more than 1 hour, until the xenon is fully cool, then replace the work.

Just after using the xenon lamp, immediately replace the xenon lamp, because the xenon lamp is in a high temperature state, it is necessary to cool the xenon lamp first.

Replace the xenon lamp when it has been sufficiently cooled.

<preparative>

- **150 W Xenon lamp (H)(Model 5J0-1890)**
- **Safety glasses**

1) Cooling of xenon lamp

Turn off the xenon lamp as described in 5.2.5. Although the xenon lamp has been turned off, the fan is still running and cooling will be carried out. In this state, please do not cut off the power supply, at least for 1 hour.

2) Replace the xenon lamp



[Warning]

Electrocution from touching the inside of the instrument!
There is a possibility of death or serious injury due to electric shock from xenon lamp power supply (30kV). When removing the cover of the light source in order to replace the xenon lamp, the power supply must be cut off first.

**[Warning]**

Injured by the rupture of the xenon lamp!

When the xenon lamp is strongly impacted, or the surface of the quartz glass is cracked, the quartz glass will be broken, and the flying glass pieces may cause injuries. When using xenon lamps, it is necessary to wear protective masks, thick cloth gowns, protective glasses, gloves and other protective tools.

- ① Turn OFF the power switch and unplug the power cord.
- ② Loosen Four retaining screws of the light source cover, and detach the cover (Figure 8-1).

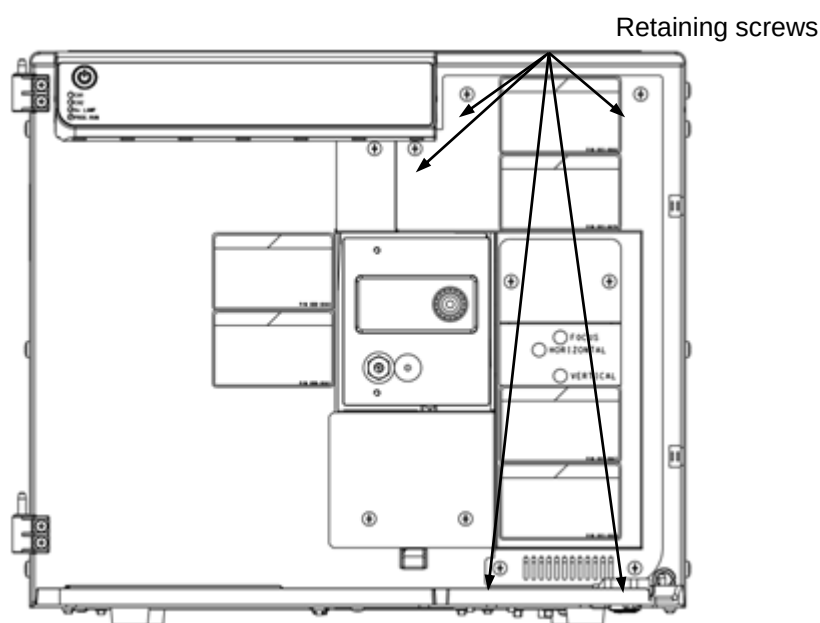


Fig.8-1 Removing the cover of the light source

- ③ Wear safety glasses.
- ④ Remove the socket connecting the lamp shade and the host, and loosen the lamp shade fixing screw.

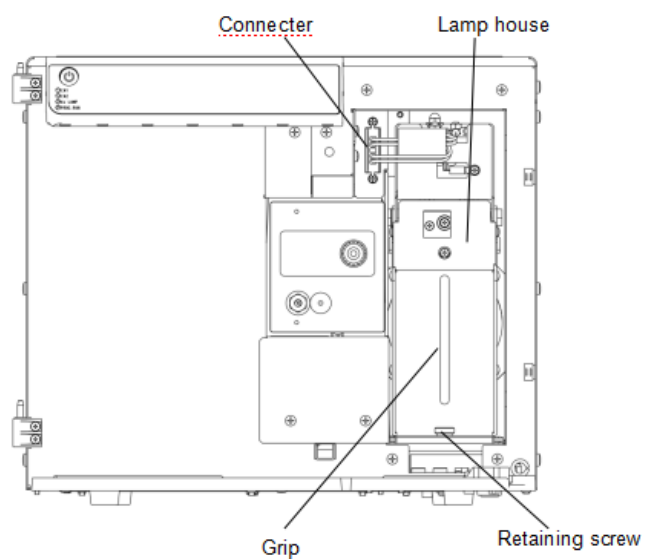


Fig.8-2 Removing the socket and fastening screws

- ⑤ Hold the lampshade handle, gently lift the handle side up, and pull the lampshade out of the main engine.

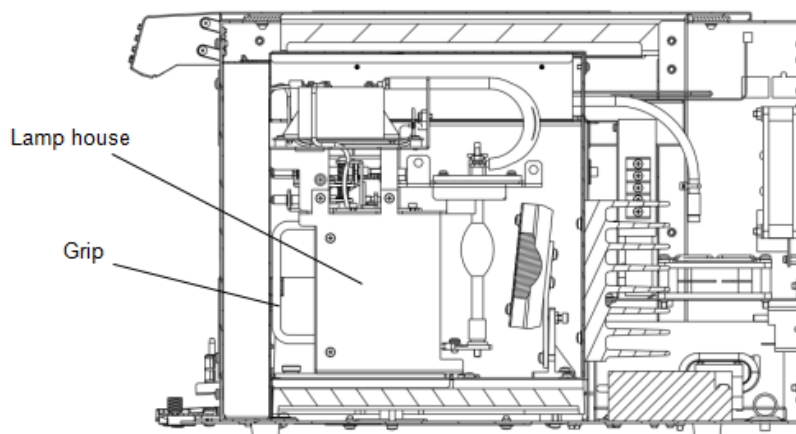


Fig. 8-3 Taking out the lampshade

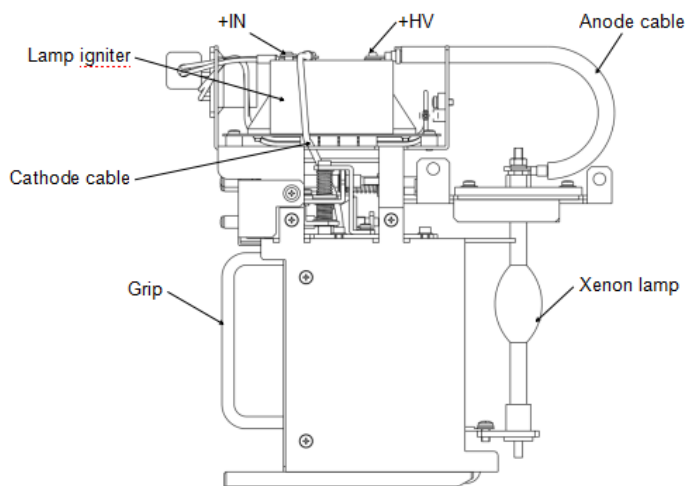


Fig. 8-4 Removed lampshade

- ⑥ Take out the used xenon lamp



[Warning]

When abandoned, the xenon lamp was broken and injured!

The xenon lamp that has been used will also have residual high pressure (room temperature: about 1Mpa, operation: about 4MPa) gas, so be sure to use a thick cloth (3 pieces of cotton stacked together) and other completely wrapped, from the above with a hammer to break the glass, and dangerous goods and waste gas. If it is not broken and discarded, it will be broken due to the impact and other factors during the abandonment, and the flying glass may lead to injury.



[Warning]

Injured by the rupture of the xenon lamp!

When loosening or tightening the retaining nut, do not grasp the glass part of the lamp.

If excessive pressure or impact is applied to the glass part of the lamp, the glass part may break. Hold the xenon lamp body when loosening or tightening the nut.

Loosen the anode nut of the lamp and remove the anode side of the lamp (+ mark side) from the support buckle secured to the ceramic plate (Figure 8-5).

At this time, please do not touch the lamp valve part. And for the convenience of turning on the lamp, the center of the lamp valve is tied

with a thin metal wire, please do not cut it off.

- ⑦ Loosen the cathode nut and remove the reed plate from the cathode side of the lamp (Figure 8-5).

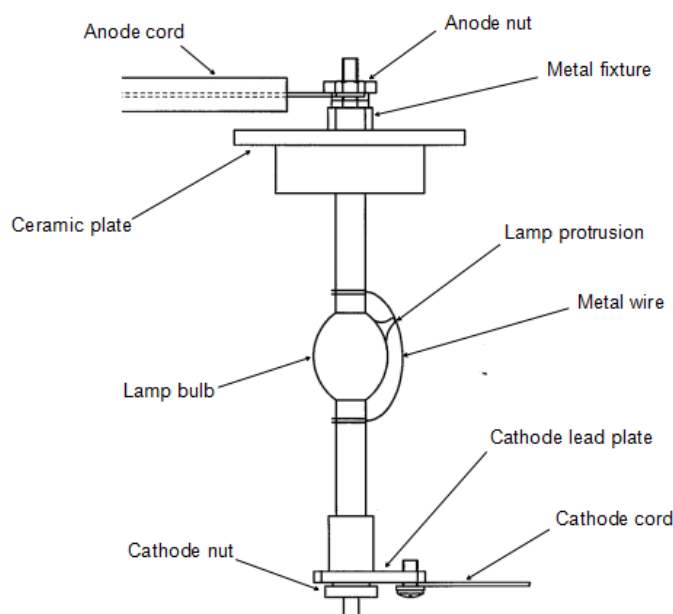


Fig. 8-5 Removing an indicator

- ⑧ Install new xenon lamps



[Warning]

Injured by the rupture of the xenon lamp!

Do not touch the glass part of the xenon lamp directly with your hand.

In case of hand dirt and other dirt, please stick a little high quality alcohol with gauze or absorbent cotton and wipe gently.

If the lamp is turned on in a state of hand dirt on the surface of the lamp (quartz glass), the dirt will burn and stick together, reducing the mechanical strength of the glass, which may lead to the rupture of the xenon lamp.



[Warning]

Mistaken xenon lamp installation direction (polarity) and rupture, will lead to injury!

When installing a xenon lamp, if the polarity is wrong, the cathode will be consumed, and the xenon lamp will not light

up.

At this time, the xenon lamp should be replaced as soon as possible. If you continue to use a xenon lamp with a depleted cathode, the pressure in the electron tube of the xenon lamp will increase abnormally, which may lead to the rupture of the xenon lamp.

Be sure to follow the mounting direction of the xenon lamp. Insert the "+" (anode) marking side of the xenon lamp into the metal part of the lamp holder with the "+" (anode) marking side.



[Warning]

Due to the careless installation of xenon lamp broke, resulting in injuries!

If the nut connecting the xenon lamp port to the wiring part is loosened, due to poor contact, the contact resistance will be increased, generating heat and becoming high temperature, which may lead to rupture. Please tighten the nut.

Install new xenon lamps.

Connect the hole of the spring plate to the cathode side screw of the lamp, and secure the cathode spring plate with the cathode nut (Figure 8-6). Be sure to install the lamp valve protrusion facing the front panel of the instrument (Figure 8-7).

At this time, please do not touch the lamp valve part. And in order to light the lamp easily, the center of the lamp valve is installed with a thin metal wire, please do not cut off this line. If the wire is not on the protrusion side in the center of the lamp valve, move the wire to the protrusion side.

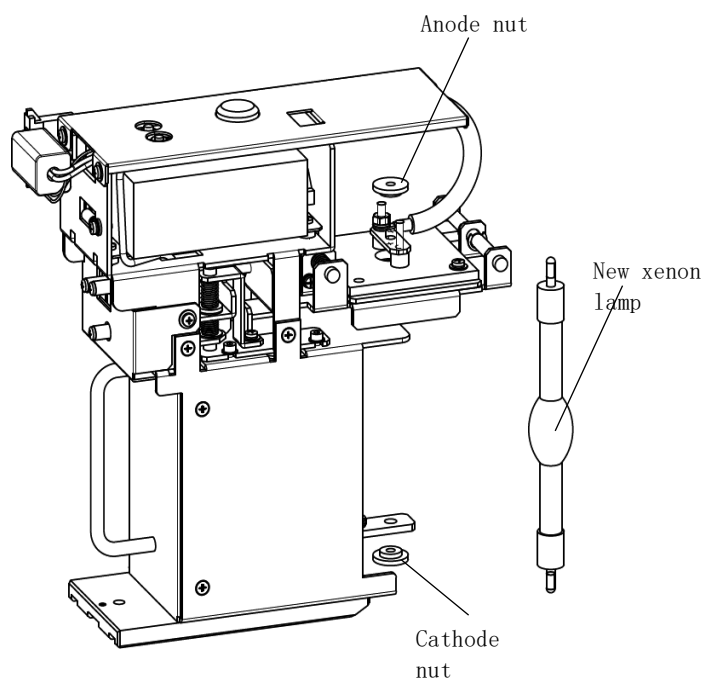


Fig. 8-6 Installing the xenon lamp

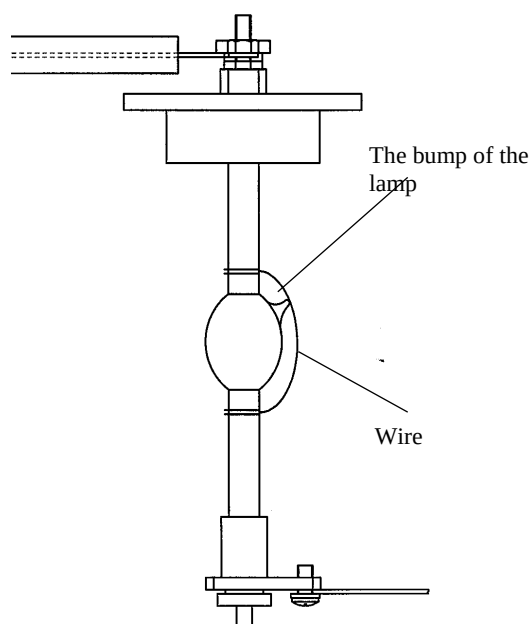


Fig. 8-7 Projection of the lamp valve

- ⑨ As shown in Fig. 8-6, put the anode terminal (marked “+”) of the lamp into the metal fixture on the ceramic plate. Then, using the anode nut, mount the lamp so that the protrusion on the bulb is positioned as shown in Fig. 8-7. Refer to Fig. 8-4 for a view of the lamp when assembled.



[Note]

When installing the xenon lamp, keep the bulb protrusions facing

the front panel side of the device (Figure 8-4). If the installation direction is not correct, the loss of light will be larger, and sometimes the original performance of the device cannot be obtained.

- ⑩ After mounting the Xe lamp in the lamp house, assemble it into the main body (Fig. 8-2).

Raise the lamp house and insert it into the main body, then lower it and press it downward into place.

The lamp house will not move if installed correctly. Finally, secure the lamp house with retaining screws and connect its connector with the main body.

- ⑪ When installation of the lamp house is finished, close the light source cover and turn ON the power (check that the lamp comes on).

Now proceed to positional adjustment of the Xe lamp.

3) *Please adjust the xenon lamp position*



[Note]

If you look directly at the xenon lamp in the light, you will hurt your eyes!

When making a rough adjustment of the lamp position, if you look directly at the excited light emitted by the xenon lamp, you will injure your eyes. Because the xenon lamp in the bright lamp emits strong ultraviolet light, please do not look directly at the xenon lamp, and be sure to wear protective glasses.

The position of the light emitting part of the xenon lamp varies according to the type of lamp, so the position of the lamp needs to be adjusted when it is initially used or after replacing the xenon lamp.

Use the 3 adjusting screws on the right side of the instrument to adjust the position of the lamp.

HORIZONTAL: Screws are used to adjust the horizontal position.

FOCUS: Screw for adjusting the position of the focus of the light source.

VERTICAL: Screw for adjusting the position in the vertical direction.

There are two methods of adjustment: coarse adjustment while looking at the image of the light source and micro adjustment while looking at the data, which need to be coarse adjustment first, and then micro adjustment.

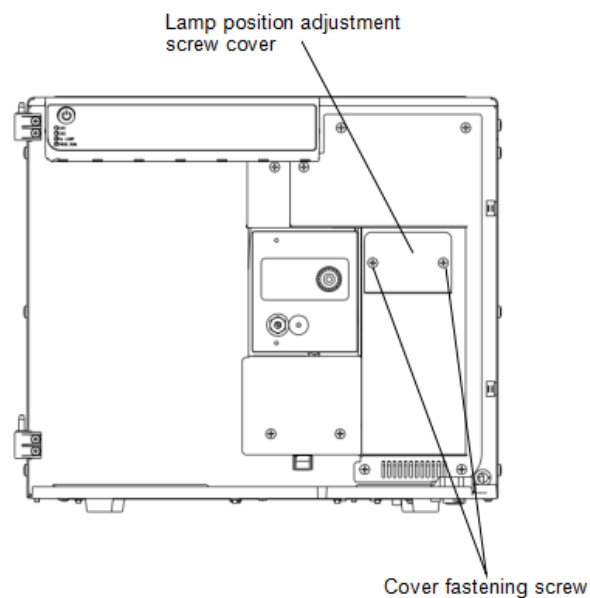


Fig. 8-8 Screw cover for indicator positioning

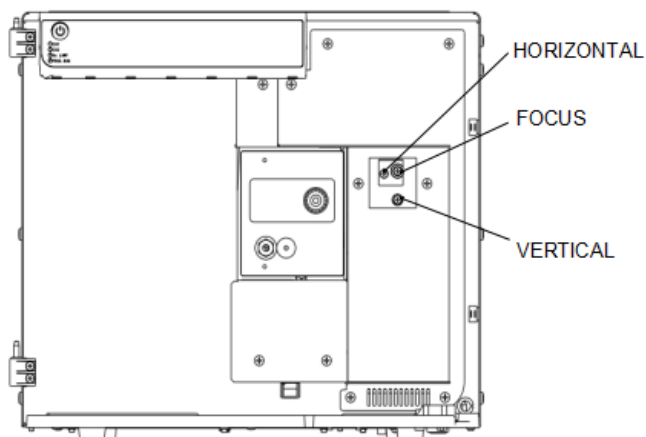


Fig. 8-9 Adjusting the indicator position

(a) Coarse regulation

The excitation light wavelength (Ex) is set to 550 nm and adjusted while observing the light image. (Refer to 5.1.4)

- ① Power on
- ② In this state, take out the flow pool (Figure 8-2)
- ③ Set the excitation wavelength at 550 nm and the emission wavelength at 350nm.
- ④ In this state, as shown in Figure 8-10, place a piece of paper at

the side of the excitation light outlet of the sample chamber (the center of the excitation light outlet), and observe the state of the excitation beam.

Repeat the VERTICAL, HORIZONTAL and FOCUS adjustments in this order little by little until the excitation beam (rainbow color) becomes brightest. The coarse adjustment of the light source position is thus completed. Please adjust VERTICAL first in installation because the lamp position is shifted downward for fixation of the transport screw.

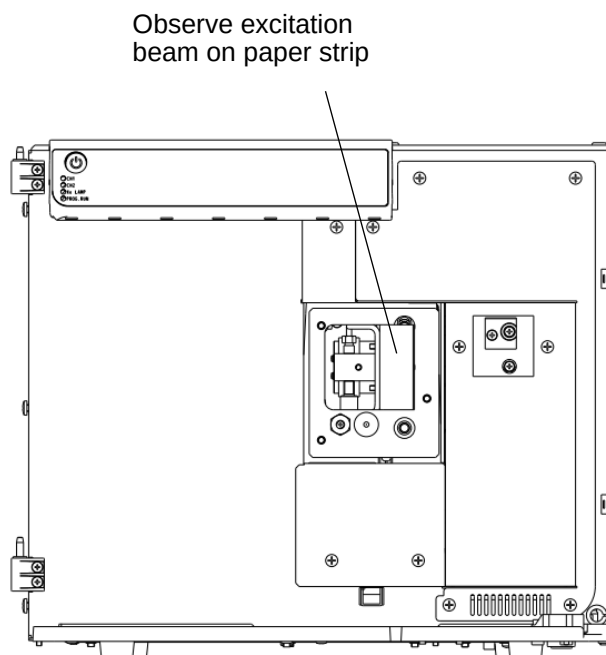


Fig. 8-10 Observation of the excited beam

(b) Fine-tuning

After the rough adjustment is completed, the excitation light wavelength (Ex) is set to 350 nm and fine-tuned while looking at the data.

- ① After completing the rough adjustment, please install the flow pool.
- ② Set the excitation wavelength at 350nm and the emission wavelength at 350nm;
- ③ In this state, look at the Ex energy value of the monitor, and turn it repeatedly little by little in the order of FOCUS, HORIZONTAL and VERTICAL until the display value becomes the maximum. When the display value becomes the maximum value, the adjustment of the lamp position can be finished.

4) Enter the replacement date of the lamp

After completing the above operations, enter the replacement date of the lamp. From this input, the cumulative light time becomes 0, and the number of lights becomes 1.

8.3 Replacing a mercury (Hg) Lamp

<preparative>

890-2662 mercury lamp

(1) Replace the mercury lamp

- ① Cut off the power supply and unplug the power cord.
- ② Loosen the four screws securing the light source cover, and remove the cover (Figure 8-11).

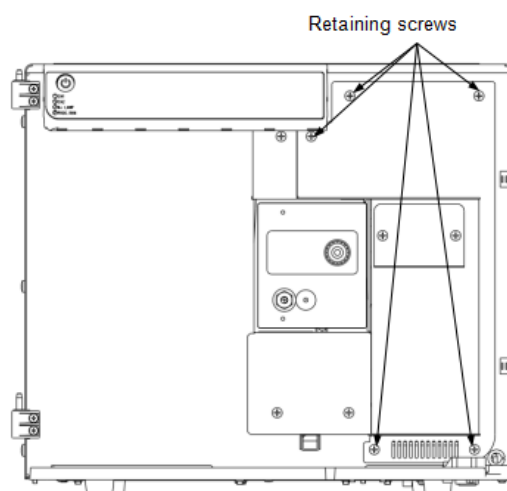


Fig.8-11 Removing the cover of the light source

- ③ Loosen the screws securing the mercury lamp cover (Figure 8-12).

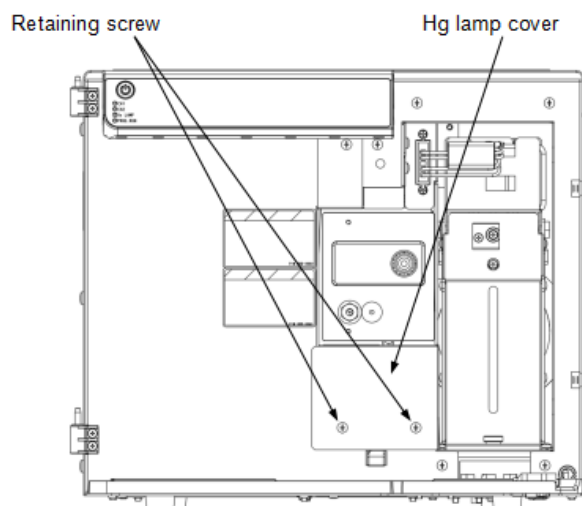


Fig. 8-12 Removing the mercury lamp cover

- ④ Remove the flow tank.
- ⑤ After removing the cover of the mercury lamp, you can see the mercury lamp fixed on the right side of the instrument (Figure 8-13).
- ⑥ Remove the socket connecting the mercury lamp to the main engine, loosen the two fixing screws, and take out the mercury lamp.

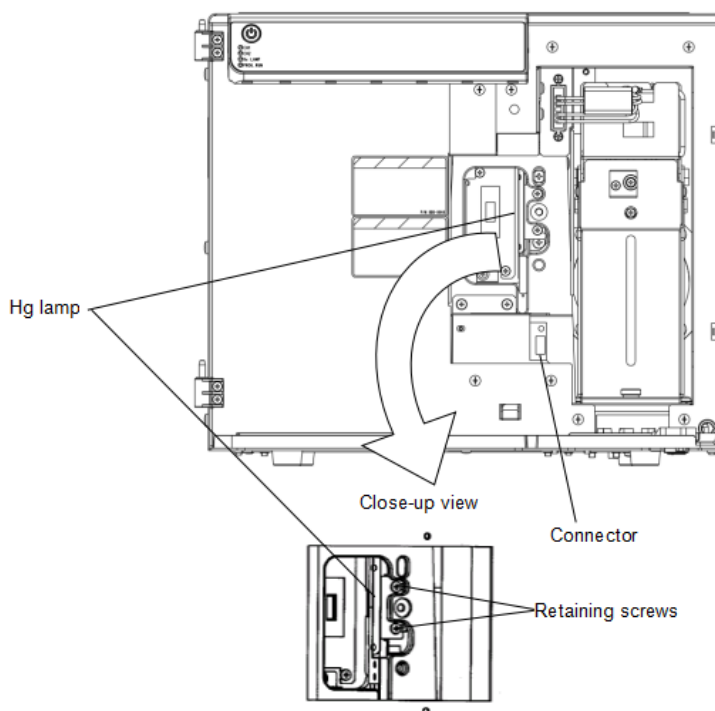


Fig.8-13 Removing the mercury lamp

- ⑦ Install new mercury lamps.

Use two fixing screws to secure the mercury lamp and connect the jack to the main engine.

- ⑧ Install the outer cover of the mercury lamp and the outer cover of the light source to the original position.

- (2) Enter the replacement date of the lamp

After completing the above operations, enter the replacement date of the lamp. From this input, the cumulative light time becomes 0, and the number of lights becomes 1.

8.4 Replacing the Fuse



[Warning]

Electrocution from touching the inside of the instrument!

Electric shock may result when replacing the fuse.

Before replacing the fuse, be sure to cut off the power supply and unplug the power cord.

<preparative>

- **New fuse (Model J821399 T5A (two pieces))**

< confirmation >

- **Please make sure that the power to the instrument has been cut off and that the power cord has been unplugged.**

- (1) Replace the fuse

The fuse holder can be removed by pressing the upper side handles.

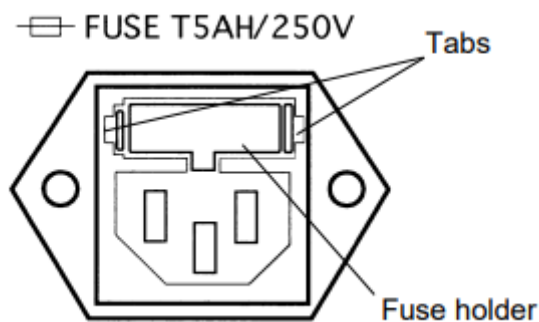


Fig.8-14 After replacing (two) fuses, push the fuse holder forward.

8.5 Optional Components

NO.	Describe	P/N
1	D3251L keypad	18020823
2	IFC base board	18020822
3	D3251L analog output board	18020820
4	D3251L analog output signal cable	17000176
5	Filters	16060134

Appendix

Safety information

● *General safety information*

The following general safety precautions must be followed at all stages of instrument operation, maintenance and repair. Failure to comply with the special warnings elsewhere in this manual will violate the safety standards for the design, manufacture and use of the instrument, and Elite will not be liable for any damage caused by the user's failure to comply with these requirements.

● *Safety standards*

This instrument is a Class I safety device (that is, it provides a protective grounding terminal) and is manufactured and tested according to national safety standards.

Safety symbol

Symbol	Description
	For equipment marked with this symbol, the user should refer to the instruction manual to avoid injury to the operator and damage to the instrument
[Warning]	Warn you of situations that may result in injury or death. Do not operate beyond the warning unless you have fully understood and met the required conditions.
[Caution]	Warning You may cause data loss or damage to the equipment, unless you have fully understood and met the required conditions, do not exceed caution.
[Note]	Warning you may cause the experimental data is not ideal or the instrument does not work properly, unless you have fully understood and met the required conditions, do not operate beyond the scope of attention.

Common solvent information

Volatile buffers and volatile mobile phase modifiers are commonly used

Name	pKa	pKb	pH Range	BP	MP
Acid					
Trifluoroacetic acid	0.3	13.70		72.4 °C	
Formic acid	3.75	10.25		100.7 °C	
Acetic acid	4.75	9.25		116.0 °C	
Carbonic acid	6.37	7.63		-	
alkali					
ammonia	9.25	4.75		-33.35 °C	
methylamine	10.81	3.19		16.6 °C	
ethylamine	10.66	3.34		-6.3 °C	
triethylamine	11.01	2.99		89.3 °C	
Buffer fluid					
Ammonium formate			3.0-5.0		120 °C
Pyridinium formate			3.0-5.0		
Ammonium acetate			3.8-5.8		111 °C
Pyridinium acetate			4.0-6.0		
Ammonium carbonate			8.0 (adjust)		
Ammonium carbonate			5.5-7.5and9.3-11.3		
Ion pair reagent					
Pentafluoropropionic acid	≈0.6			96-97 °C	
Heptafluorobutyric acid	≈0.6			120 °C	
Fluoropentonic acid	≈0.6			140 °C	
Pentafluooctanoic acid	≈0.6			189 °C	
Tridecyl fluoroheptanoic acid	≈0.6			175 °C	

ELITEHPLC

About Elite

Suzhou Elite Science & Technology Co., Ltd.
701#, Building 10, Liandong U Valey, No. 179 Zhujiawan Street,
Gusu District, Suzhou, Jiangsu, China.
Tel: +86-512-67997535
E-mail: info@eliteHPLC.com
Web: <http://www.elitehplc.com>
