OLYMPUS®

User's Manual

FLUOVIEW FV3000

Confocal Laser Scanning Microscope

Notes

This user's manual is for the Olympus confocal laser scanning microscope.
To ensure the safety, obtain optimum performance and to familiarize yourself fully with the use of this system, we recommend that you study this manual thoroughly before operating this system, and always keep this manual at hand when operating this system. Retain this instruction manual in an easily accessible place near the work desk for future reference.
For products configuring this system, see page 3 in [Operation / Maintenance Manual].

- Research use only -

Optical Microscope and Accessory

AX8788

Introduction

This is a user's manual for confocal laser scanning microscope FV3000 in FLUOVIEW series.

Caution

This system is a Class 3B laser product.
The procedures for using this system are classified as follows:

- Operation
  "Operation" means the operations of observation functions of the microscope system described in FV3000 User's Manual provided by Olympus.
  The Class 3B laser beam is emitted only from the objective during the actual operation.

- Maintenance
  "Maintenance" means adjustments or procedures performed by users to maintain the proper operations of this system described in FV3000 User's Manual provided by Olympus.

- Service
  "Service" means the contents of any work, adjustment or repair performed by Olympus engineers or those who received the Olympus service training described in Service Manual provided by Olympus.
  The implementation of services may influence the system performances, and furthermore, there is a risk that the unintended laser equivalent to Class 3B may be emitted.

In order to maintain the full performance of this system and ensure your safety, be sure to read this user's manual, the instructions for the laser unit, and the light source unit before use.

For this system, in addition to manuals and instructions described above, there is also an on-screen manual ("Online Help") built in the software. For details of the standard image acquisition / analysis software for FV3000 FV31S-SW and the touch panel controller, see respective Online Help.

Registered Trademarks

Registered Trademarks

Microsoft and Microsoft Windows are registered trademarks of U.S. Microsoft Corporation.
Other company names or product names are either registered trademarks or trademarks of respective companies.

Caution

If this system is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the product may also be damaged. Always use the system according to this manual.

The following symbols are used in this manual.

⚠Caution : Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury.

Note : Indicates a potentially hazardous situation which, if not avoided, may result in damage to the equipment or other property.

TIP : Indicates commentary (for ease of operation and maintenance) .

Safety Manual

On this manual

This manual describes warnings and cautions for your safety use of this system. Before use, be sure to observe all the warnings and cautions.

 If this system is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the product may also be damaged. Always use this system according to this manual.

This system is applied with the requirements of standard IEC/EN61326-1 concerning electromagnetic compatibility.

- Emission Class A, applied to industrial environment requirements.
- Immunity Applied to industrial environment requirements.

Some interference may occur if this system is used in domestic location.

In accordance with European Directive on Waste Electrical and Electronic Equipment, this symbol indicates that the product must not be disposed of as unsorted municipal waste, but should be collected separately.
Refer to your local Olympus distributor in EU for return and/or collection systems available in your country.

Research Use Only

This system does not conform to FCC Part15 Class A.
This system is for biological research use, and it is categorized as a FCC Part15 Class A exempt device.

CALIFORNIA USA ONLY

The touch panel controller of this product uses a Lithium Battery which contains Perchlorate Material -special handling may apply, See www.dtsc.ca.gov/hazardouswaste/perchlorate.

For Korea only

but, exclude OBIS405-50LX, OBIS445-75LX, OBIS514-40LX, OBIS640-40LX, OBIS488-20LS, OBIS561-20LS, OBIS594-20LS, power supply for LD OBIS 6 Laser Remote and main laser combiner with 4 LDs FV31-MCOMB-P.

A급 기기 (업무용 방송통신기자재)

이 기기는 업무용 (A급) 전자파적합기기로서 판 매자 또는 사용자는 이 점을 주의하시기 바라 며, 가정외의 지역에서 사용하는 것을 목적으로 합니다.

Contents

1 Safety caution ⚠

1-1 Transportation and modification of this system

⚠CAUTION

- The assembling and setup of this system are exercised by Olympus engineers. If this system is moved or modified (disassembled or remodeled) by personnel other than Olympus engineers, the optical systems may not be adjusted properly. In case you need to move this system, contact Olympus. In an event that this system is moved by you and, if for any reason a problem related to laser safety occurred due to malfunction or breakage of this system, Olympus holds no responsibility.
- This system meets standards for product safety, laser safety and the like under circumstances that the system is setup by service personnel authorized by Olympus. In case any combination change or remodeling is made on this system once installed and/or any operating procedures different from those described in the instruction manual and/or safety manual are taken, it is excluded from the state of compliance with the system safety standards and/or the laser safety standards and the like. In this case, comply this system with the system safety standards and/or the laser safety standards, etc. once again under customer's responsibility.

1-2 Disposal of this system

⚠CAUTION

- The mercury burner used for the illumination unit of this system must be disposed of in accordance with the rules of industrial waste disposal. If you cannot dispose of it properly, contact Olympus for assistance.
- In order to replace the (lithium coin battery CR2032) built in the touch panel controller in this system, contact Olympus for assistance. When disposing of batteries, follow the local regulations.
- The GaAsP-PMT equipped with the high sensitivity spectral detector FV31-HSD contains arsenic and its compounds.
  When you dispose of this product, consign it to the authorized industrial waste disposer in accordance with the rules of industrial waste disposal. When you use this product in other countries and dispose of it there, follow the rules of industrial waste disposal there.

1-3 Handling precautions

⚠CAUTION

- Refer, also, to sections related to safety in the instruction manuals of peripheral units.
  However, if you use following units with this system, the instruction manuals provided with those units are invalid. See this manual for operation procedures of those units.
  - Research inverted microscope IX83
  - Light source U-HGLGPS
  - Z drift compensator IX3-ZDC2
  - Motorized DIC slider IX3-DICTA
- If this system seems to be damaged, discontinue use, shutdown Windows and exit the system according to the procedures described in [Operation / Maintenance Manual] on page 9 and contact Olympus for assistance. If this system is continuously used in such a state, it may cause injury to users, failure of this system or output of erroneous image data.
- Use each unit in accordance with specifications and environmental conditions described in this manual. Do not use each unit in any other method as it is dangerous.

1 Mechanical (heat) precaution

⚠CAUTION

- The system performance as well as the system safety cannot be guaranteed in case the system is disassembled, e.g. removing the cover or units with use of tools, or the connection cables are detached or attached. Never disassemble the parts which are not instructed to do so.
- Handle the movable parts, e.g. connectors or covers, etc. of connection parts with care, and avoid subjecting them to severe force or impact. Otherwise, it may cause a failure.
- This is not a waterproof system. Do not splash or spill water or other liquid on this system. If water or other liquid enters this system, discontinue use immediately and exit the system according to the procedures described in [Operation / Maintenance Manual] on page 9 and contact Olympus for assistance. If this system continues to be used in such a state, it may cause electric shock or other hazards and result in injuries of users, failures of this system, or output of erroneous image data.
- This system is a precision instrument. Handle it with care and avoid subjecting it to a sudden or severe impact.
- Cooling fans are provided at the rear panel of each power unit. Leave a space of 10 cm or more around the ventilation opening. Otherwise, it may cause a failure. (The temperature inside of the power units may rise to cause malfunctions or failure.)
- The fans to cool the laser units are provided at the side panel of the main laser combiner FV31-MCOMB and the sub laser combiner FV31-SCOMB. Leave a space of 10 cm or more around the ventilation opening. Otherwise, it may cause a failure. Do not set the main switch of the power supply for system FV31-PSU to O (OFF) while the laser unit is kept ON. Setting the main switch of the power supply for system FV31-PSU to O (OFF) stops the fan.
- Do not allow tools or metal fragments to get into the aperture of slits, etc. of each unit. Doing so could cause failure of this system or electric shock to the user.
- If any foreign substance, such as accessory tools or metal fragments enter this system through the vent hole or other openings, it may cause an electric shock and/or failure of this system. Discontinue use, exit the system according to the procedures described in [Operation / Maintenance Manual] on page 9 and contact Olympus for assistance.
- Do not put any substance on top of the product.
- Do not cover the product with a cloth, etc. during operation.
- When you store this system, put the dust cover over it to protect from dust.
- Under the dark environment, it may be hard to see this system clearly. Be careful not to come into contact with this system when you pass by.
- If the objective contacts the specimen, both of them may be damaged.
  For Z direction movement setting, the software limit can be set within the mechanical limit range of the IX83 2 port frame IX83P2ZF. Setting this software limit to the position where the objective comes closer to the specimen prevents the specimen from damage caused by the contact between objective and specimen.
  For details of the software limit, refer to Online Help of the standard image acquisition / analysis software for FV3000 FV31S-SW.
- Replacing consumables falls under the category of service work.
- Do not put your fingers or objects in the motorized area. You may be injured by pinching your fingers or the device may be damaged.

2 Electrical precaution

⚠CAUTION

- Product lifetime of illumination units is 8 years or 20,000 illumination hours, whichever occurs first, as a guideline. For details, see the preventive inspection sheet for illumination devices in [Operation / Maintenance Manual] on page 82.
- Always connect this system to ground correctly.
  Otherwise, our intended electric safety and performance of the system cannot be assured.
- In case that the fuse of an unit blows out, contact Olympus for replacement.
- This system is a Class A product used for industrial environment defined in EMC International Standards IEC61326-1. If you use this product in the residential environment, it may influence other equipment. Also, if you use the Class B product or the equipment intended to be used for residential environment together with this system, the malfunction may occur.
- Always use the power cord provided by Olympus. If the proper power cord is not used, the safety and performance of this system cannot be assured.
- The power cord and other connector cables are connected by Olympus technical personnel. Do not disconnect them.
- In case of emergency, unplug the power cord to stop supplying the power. For the power supply for system FV31-PSU, Power supply for LD OBIS 6 Laser Remote, control box IX3-CBH or light source U-HGLGPS, be sure to secure the space for reaching the power cord connector so that you can unplug the power cord immediately in case of emergency.
- When using the power strip, avoid it from getting dusty.
- Do not use the mercury burner exceeding its lifetime. For procedures to confirm the accumulated lighting time of the mercury burner, refer to [Operations/Maintenance] on page 35.
- The mercury burner seals high-pressure gas inside. If it continues to be used exceeding its lifetime significantly, the glass tube may eventually explode due to accumulated distortion.
- In case that the mercury burner explodes, follow the procedures below.

  Unplug the power cord from the outlet. Leave the place and ventilate for at least 30 minutes.
  After the mercury burner and the lamp house have cooled down, collect the remaining mercury with packing tape, paper or dropper, etc.
  Seal the collected mercury and tools used completely in a nonmetallic container, and ask the waste disposer to dispose of them.
  Should you have inhaled the mercury steam, consult the doctor immediately and follow his/her instructions.

3 Laser precaution

This system is specified as a laser product of the class as shown below, depending upon the laser to be combined.

[a] Laser combination

CLASS 3B (IEC60825-1:2007)

[a]

This product complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated June 24, 2007.

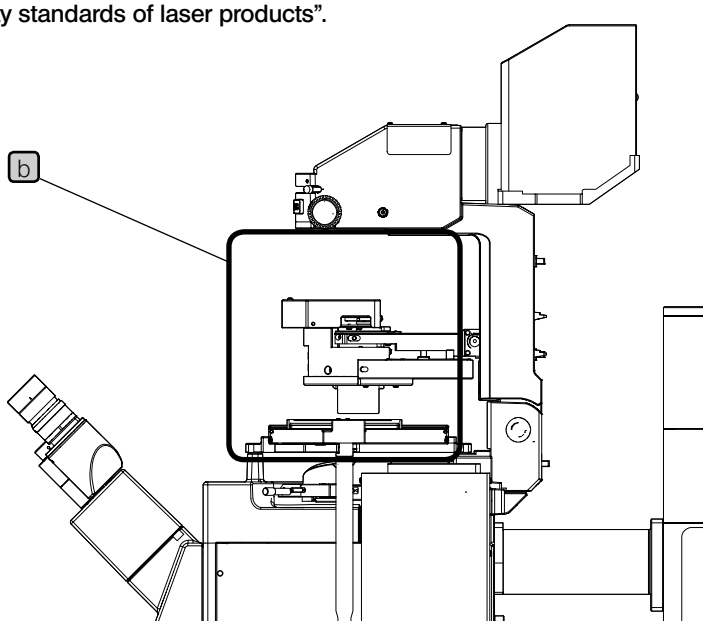
Laser products of Class 3B are products that can only be used under control of the laser safety officer.
Assign a laser safety officer and ensure safety in accordance with the instruction given by the assigned laser safety officer.
In "IEC60825-1, Safety standards of laser products", it requires safety preventive measures for users of laser products.
Before use, read IEC60825-1 of the latest edition carefully and then, use the products after taking appropriate measures.
Unless the safety measures indicated in IEC60825-1 are taken Olympus cannot ensure the safety of the product since it may cause exposure of dangerous laser emission.

⚠CAUTION **Use of controls or adjustments by procedures other than those specified herein may result in hazardous radiation exposure.**

TIP • If any help is required, contact Olympus for assistance.

• The wavelength and the power of the CW laser are described on the label. They are ensured only when the laser provided by Olympus is combined.

⚠CAUTION **NOHD (nominal ocular hazard distance) is defined as approximately 20 cm from the tip of the objective. Do not come close to the laser aperture [b] while the laser is emitted. For definition of NOHD, refer to "IEC60825-1, Safety standards of laser products".**



⚠CAUTION • Direct light, specular reflected light and diffused reflection light of laser are dangerous.

• The total laser radiation emitted from this system is as follows :

Maximum Permissible Exposure (for CW laser) : 1 W/m^2 (400 - 450 nm)
5.75 W/m^2 (488 nm)
10 W/m^2 (500 - 700 nm)

NOHD (Nominal Ocular Hazard Distance) : 20 cm in the vicinity of objective*
(When using UPLSAPO20X)

Exposure duration : 100 sec

* Hereafter, this area is called "Laser Hazard Area".

• Use the system at the correct click position. Since the revolving nosepiece or mirror units are made of plastic materials, they may be deteriorated if they are not used at the correct click position.

Laser class and danger level of this system

Class	Description of danger level
Class 1	The lasers of this class are always safe regardless of the optical systems where they are used.
Class 3B	The direct beam observation is dangerous.

⚠CAUTION • If the laser of Class 3B or upper enters your eye, blindness may occur.

• Make sure that the laser beam is not emitted before removing the objective or the nosepiece cap from the revolving nosepiece. If the objective, nosepiece cap and revolving nosepiece are removed, the laser beam in the parallel direction which is not diffused will be radiated, which causes the danger. If there is an empty hole in the revolving nosepiece, put a nosepiece cap to that hole.

• Do not put any substance having mirror surface close to the objective.

• Do not put flammable gas or liquid close to this system since it may cause fire.

• While the service work of this system is taking place, do not enter the room where this system is installed.

• Do not expose your hands or fingers to the laser beam emitted from the objective mount hole, tip of the objective or condenser lens. Otherwise it causes damage to your skin. In addition, never insert a mirror into the light path as the laser beam may come outside and it may enter your eye which is very dangerous.

• If the white LED of the emission indicator is turned ON, the laser may possibly be emitted from the tip of the objective.

• Place the specimen or the center plate horizontally on the stage. If they are tilted, the laser beam may reflect around the microscope system, which is extremely hazardous.

• Do not touch the specimen while the laser is emitted. The reflection angle of the laser may change, which is very dangerous.

• Do not bend or pull the optical fiber cable with an excessive force or step on it. If the optical fiber cable is damaged, the laser beam may leak outside it and cause an extremely hazardous situation. If such an event occurs, immediately turn OFF the laser power and contact Olympus for assistance.

Precautions to laser

⚠CAUTION

- The laser of this system is of Class 3B. If you look at a beam or scattered light directly, your eye may be damaged. Also, serious damage to your skin or fire hazard may occur.
- Do not expose your eyes or skin directly to a laser beam or scattered light. When using the laser, observe and exercise all safety measures described in this manual.
- When using this system, wear the protective eye glasses and make sure that no damage occurs to your eyes. However, even though you wear protective eye glasses, never look at a laser directly (front vision) . The protective eye glasses do not withstand the direct laser radiation.
- The laser of this system is of Class 3B. Do not stare at a laser or look at it with the optical instrument directly.

Eye damage

⚠CAUTION

- The laser emitted from this system focuses on retina of the eyeball.
- If the laser enters into your eyes, the retina may be damaged to result in blindness, etc.
- Use the protective eye glasses that match the class and wavelength of the laser of this system.
- The protective eye glasses to be used with this system cannot be used for other laser systems of different laser wavelength. Conversely, the protective eye glasses for other laser systems cannot be used with this system. It may cause damage to your eyes. Be sure to use the protective eye glasses suitable for this system.

Hazard of explosion/fire

⚠CAUTION

- Do not use any flammable gas that is ignited by laser or explosive gas. Also, do not use any other flammable substance or gas-producing substance.
- Do not put any combustibles over the objective. The fire hazard may occur by laser beam.

Burns

⚠CAUTION

- Burns may occur if the laser is mistakenly emitted to human body. Be sure to wear a protective suit. Use a protective suit of nonflammable cloth.

Reflected light

⚠CAUTION

Do not install substances that reflect, e.g. glass, metal or shinny plastic, etc. near the laser aperture. The reflected light may cause injuries such as burns and blindness and also may cause damage to this system.

Smoke produced by laser

⚠CAUTION

The smoke that may appear by applying the laser to substances may contain toxic substances. Remove the smoke produced by the laser with the equipment such as smoke-extraction measure so that the user should not breathe the smoke.

1-4 Necessary actions

OLYMPUS would like to take this opportunity to ask the customer to establish a system to ensure laser safety in line with the customer's facility. The items necessary for ensuring laser safety are listed below.

1 Appointing a laser safety officer

Assign a laser safety officer who is responsible for following actions to ensure the laser safety.

1 Duties of the laser safety officer

Secure the laser safety by combining following actions.

- Planning and implementing measures for preventing damages due to laser beam
- Setting and managing the laser controlled area
- Managing the key and so on which starts laser equipment
- Inspecting and maintaining laser equipment, and saving their records
- Inspecting and maintaining protection tools, and observing if it is being used properly
- Conducting the occupational health education and saving its records
- Other items necessary for preventing damages due to laser beam

2 Details of safety education and training

It is requested for the laser safety officer to conduct safety education and training for all of the persons who are to use laser equipment. Such training includes the followings :

- Characteristics of laser beam and its dangers and harmful effect
- Principles and structures of laser equipment
- Operating procedures of laser equipment
- Performance and handling procedures of safety equipment and protective tools
- Measures to be taken in an emergency and evacuation procedures

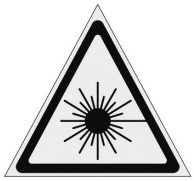
⚠CAUTION **The power supply for laser unit of Class 3B or Class 4 is controlled with the key. The laser safety officer should keep the key and exercise safety measures so that any unauthorized person cannot control the laser unit.**

2 Setting the laser controlled area (warning signs, off-limits and remote interlock switch)

The area where a laser beam of Class 3B or above is emitted is defined as the laser controlled area. The room where this system is installed is defined as the laser controlled area.

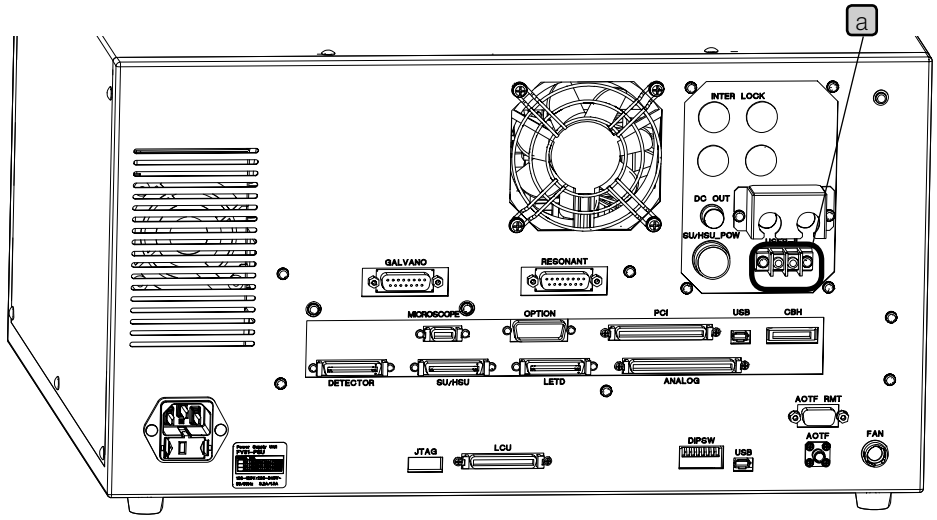
Place a warning sign near the laser controlled area that includes the following information :

- Name of the laser safety officer
- Descriptions of the dangers and harmful effects of laser radiation, and handling precautions
- A sign indicating the installation of laser equipment



⚠CAUTION

- **Be sure to use the remote interlock connector if you install the door interlock in the laser controlled area.**
- **The power supply for system FV31-PSU is equipped with the external interlock connector [a] (current rating : 30 mA, voltage rating : 24 V) . In order to connect the interlock switch to this connector, be sure to set the main switch of the power supply for system FV31-PSU to "O" (OFF) first and remove the short-circuit metal of the connector prior to connecting.**



Rear panel of the power supply for system FV31-PSU

TIP When you return the laser emission to be available after activation of a remote interlock, unlock the remote interlock. Then, turn OFF the start key on the front of the power supply for system FV31-PSU once and turn ON the start key again.

3 Preparing and wearing protective eye glasses and protective clothes

In Laser Hazard Area, it is essentially required to wear protective eye glasses and protective clothing. For this reason, when operating the microscope or placing the specimen, be sure to wear the protective eye glasses.

1 Using protective eye glasses

Use the protective eye glasses that match the class and wavelength of the laser of this system. Also, even though you wear protective eye glasses, never look at a laser beam directly.

Laser wavelength of this system

Wavelength nm	OD value
405, 445, 488, 514, 561, 594, 640	3 or more

2 Wearing protective clothing

If any work should be done in Laser Hazard Area, wear protective clothing made of flame-retardant material.

4 Ophthalmologic examination

The persons who use laser products of Class 4 or Class 3B are requested to have ophthalmologic examinations. Take the fundus examination in addition to the examination of the anterior eye part (corona and lens) . If the laser beam enters your eyes, take an ophthalmologic examination immediately.

1-5 Safety functions of this system

This system is provided with the safety measures corresponding to the specifications required for the laser products of Class 3B.

⚠CAUTION **Following safety functions are ensured in the state where Olympus delivered the microscope system. Never disassemble the microscope system containing the laser unit.**

1 Starting the unit using a key

The power supply for system FV31-PSU is equipped with a key to start the power supply.
When the unit is not in use, the key must be removed and must be kept and managed by the laser safety officer.

2 Radiation indicator

When the laser beam is emitted from the objective, the LED lamp of the emission indicator turns ON. When the LED is turned ON, use the utmost caution in operating this system.

TIP For details of the radiation indicator, see page 4 in [Operation / Maintenance Manual],

3 Beam blocking and beam attenuator

This product is provided with a built-in shutter to prevent the laser beam from entering the eye during visual observation.

4 Beam termination

This product is designed so that the laser beam is not inadvertently leaked out from the IX83 2 port frame or the laser combiner.

TIP
- The laser beam emitted from the object is terminated with the transmitted detector FV31-LETD.
- A cap is attached to the empty holes of the revolving nosepiece of the IX83 2 port frame IX83P2ZF to prevent the laser beam from coming out through the hole.

1-6 Appendix

1 Reference materials

TIP

- Food and Drug Administration Part 1040 “PERFORMANCE STANDARDS FOR LIGHT-EMITTING PRODUCTS”
- IEC60825-1 “Safety of laser products - Part 1 : Equipment classification and requirements”
- IEC/TR60825-14 “Safety of laser products - Part 14 : A user’s guide”

2 Warning labels

Safety symbols

The following symbols are attached to this system.
Study the meaning of the symbols, and always use this system in the safest possible manner.

Symbols	Meaning
	Indicates that the laser beam is applied. Take special care in handling.
	Indicates a non-specific general hazard. Follow the description given after this symbol or in the instruction manual.
	Indicates that the surface becomes hot, and should not be touched with bare hands. You may get burned.
	Indicates that care is required against your finger or hand being caught.
	Indicates high voltage (1kV or more) . Take special care to prevent electric shock.
I	Indicates that the seesaw type main switch is ON. (A seesaw type is a method to change the switch by pressing it to ON side or OFF side.)
O	Indicates that the seesaw type main switch is OFF.

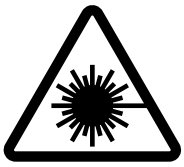
⚠CAUTION **When warning labels are dirty or peeled off, contact Olympus for replacement or inquiries.**

2-1 Warning labels for laser safety

For warning labels of the Z drift compensator IX3-ZDC2, refer to the Z drift compensator IX3-ZDC2 instruction manual.

1 Warning labels

a

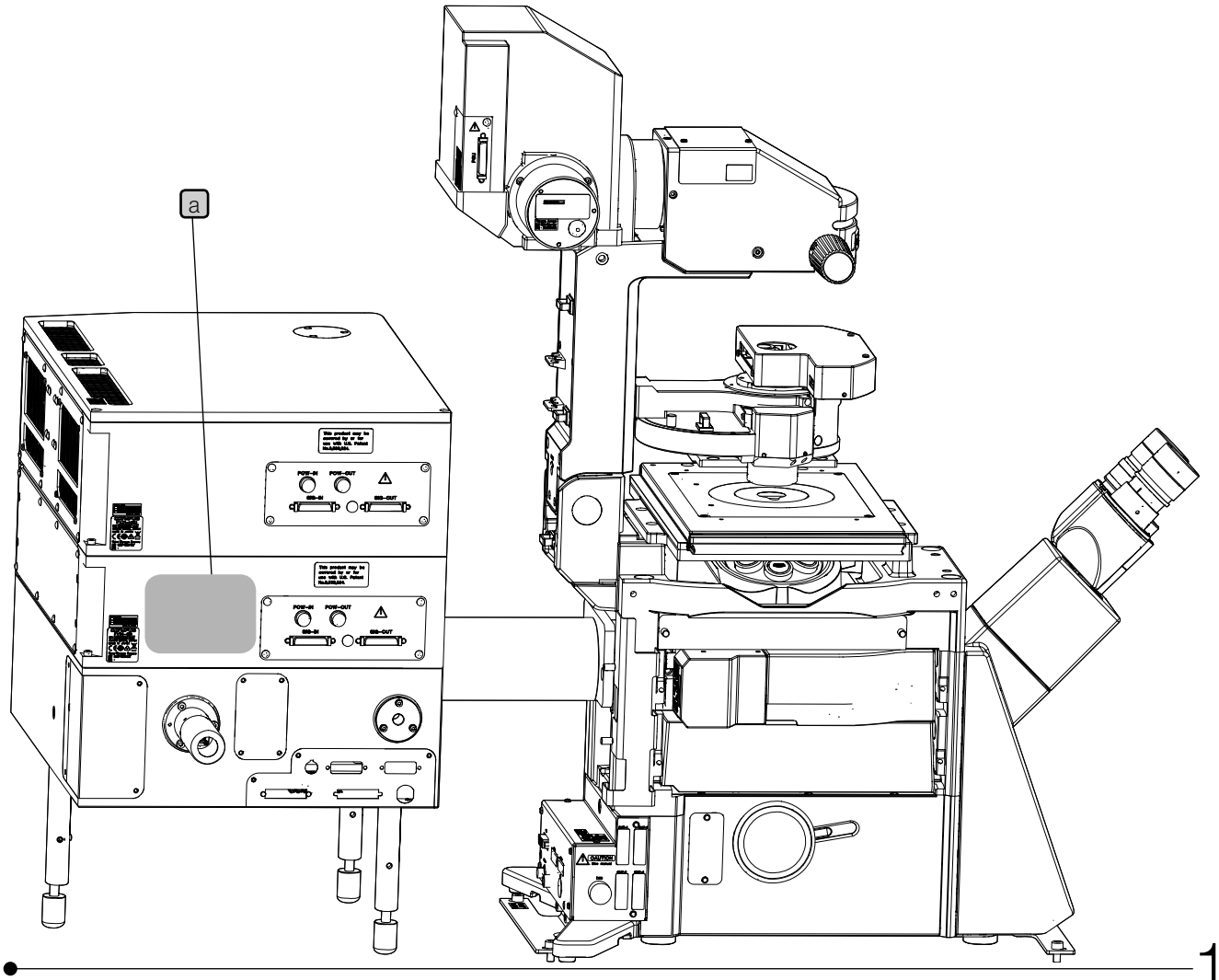


LASER RADIATION
AVOID EXPOSURE TO BEAM
CLASS 3B LASER PRODUCT

500mW MAX CW 400-700nm
IEC 60825-1:2007

Position to attach labels

· High sensitivity spectral detector FV31-HSD or spectral detector FV31-SD



2 Aperture label

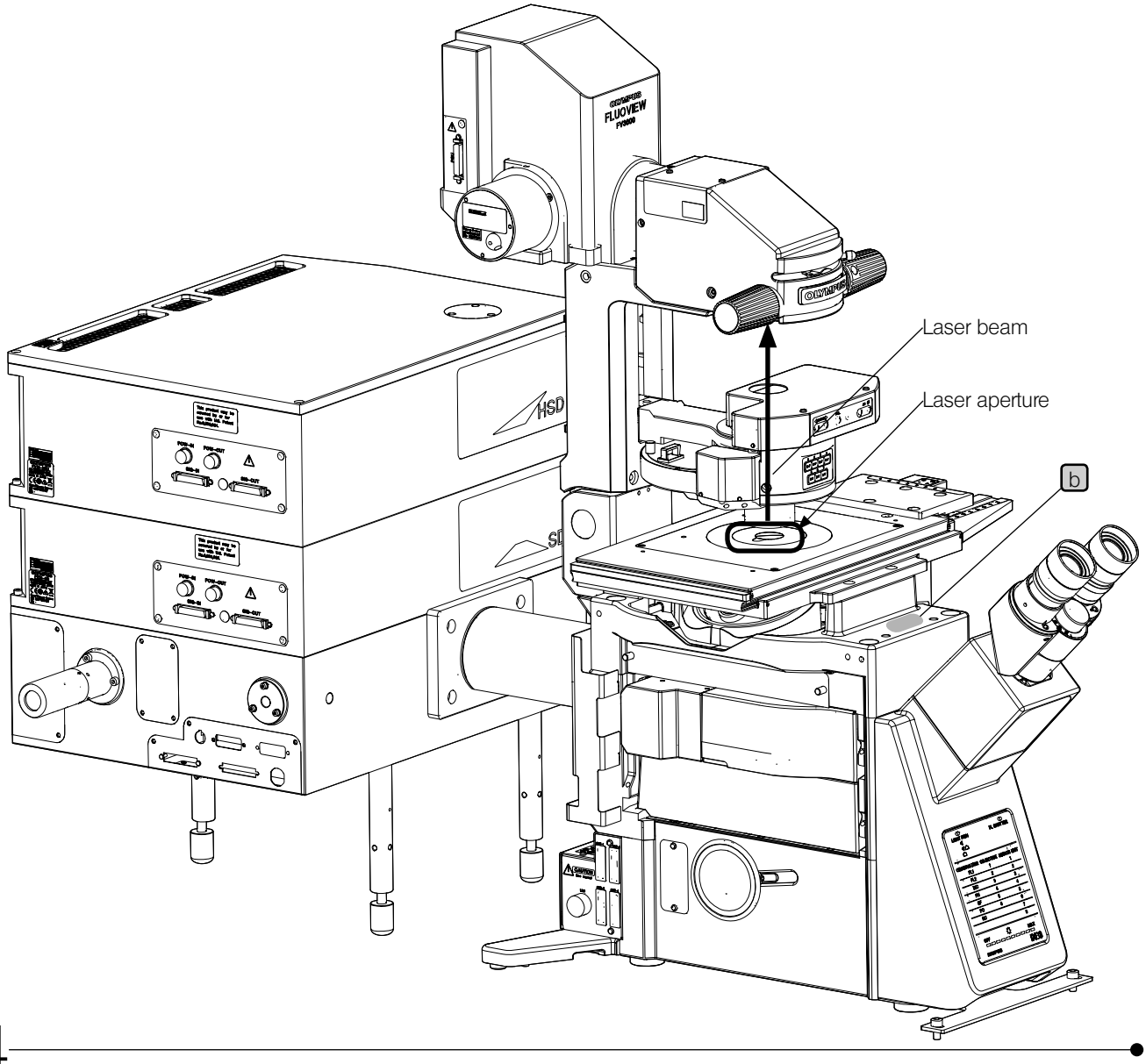
b

AVOID EXPOSURE -
LASER RADIATION IS EMITTED
FROM THIS APERTURE

Position to attach labels

· IX83 2 port frame IX83P2ZF

The laser beam emits from the position indicated by the arrow.



3 Protective housing label

⚠CAUTION **If the laser unit is damaged, the invisible laser beam may be emitted from the emission exit of the laser unit.**

c

CAUTION - CLASS 4 VISIBLE AND
INVISIBLE LASER RADIATION WHEN OPEN
AVOID EYE OR SKIN EXPOSURE TO
DIRECT OR SCATTERED RADIATION

d

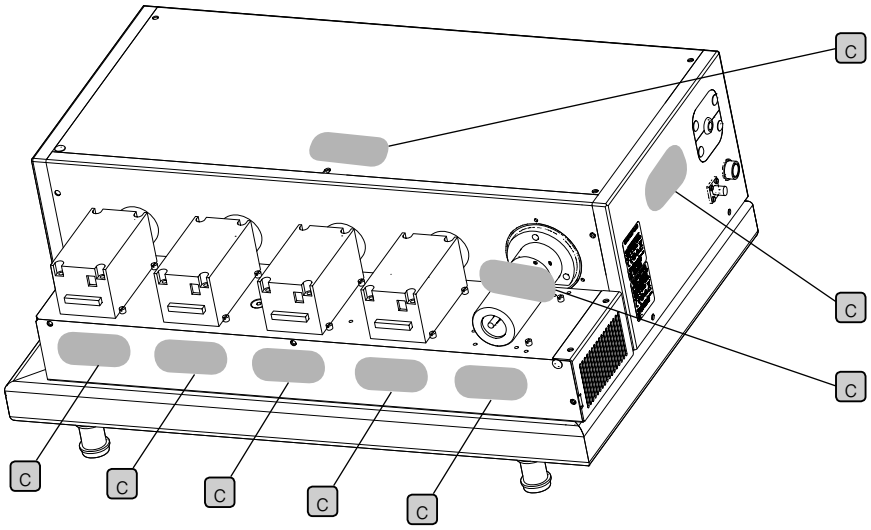
CAUTION - CLASS 3B LASER RADIATION
WHEN OPEN
AVOID EXPOSURE TO THE BEAM

e

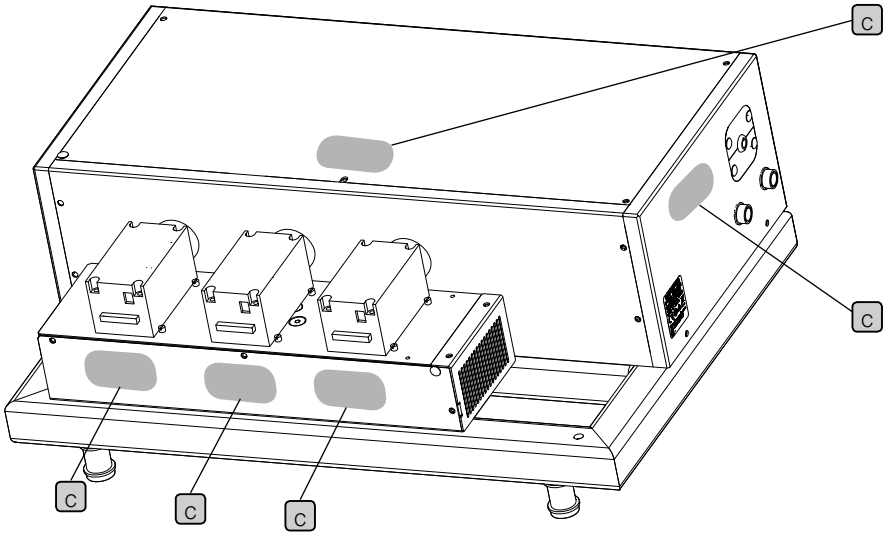
CAUTION - CLASS 2M LASER
RADIATION WHEN OPEN
DO NOT STARE INTO THE BEAM OR VIEW
DIRECTLY WITH OPTICAL INSTRUMENTS

Position to attach labels

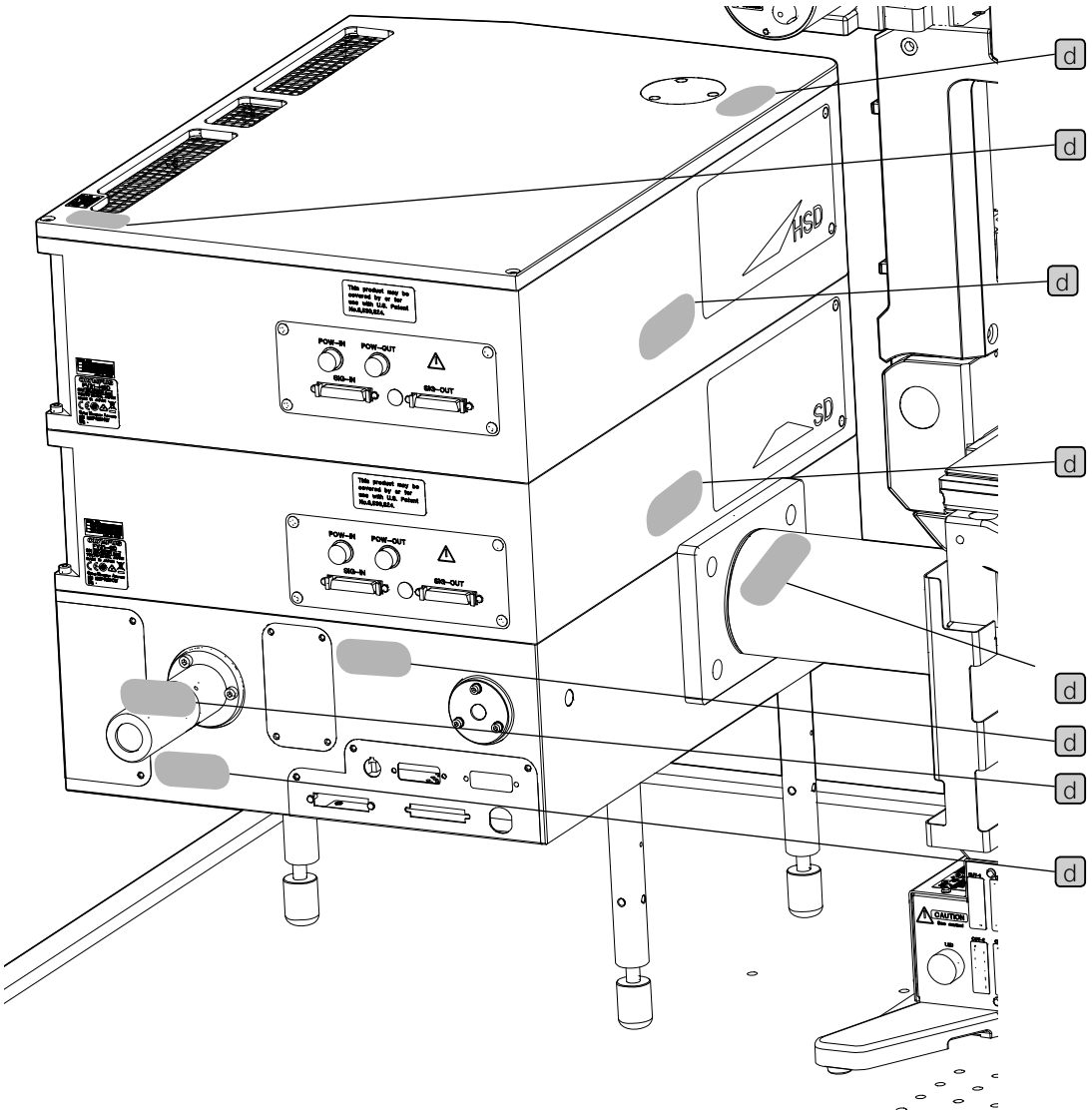
- Main laser combiner FV31-MCOMB
- Fiber unit between laser combiners FV31-CCFUR



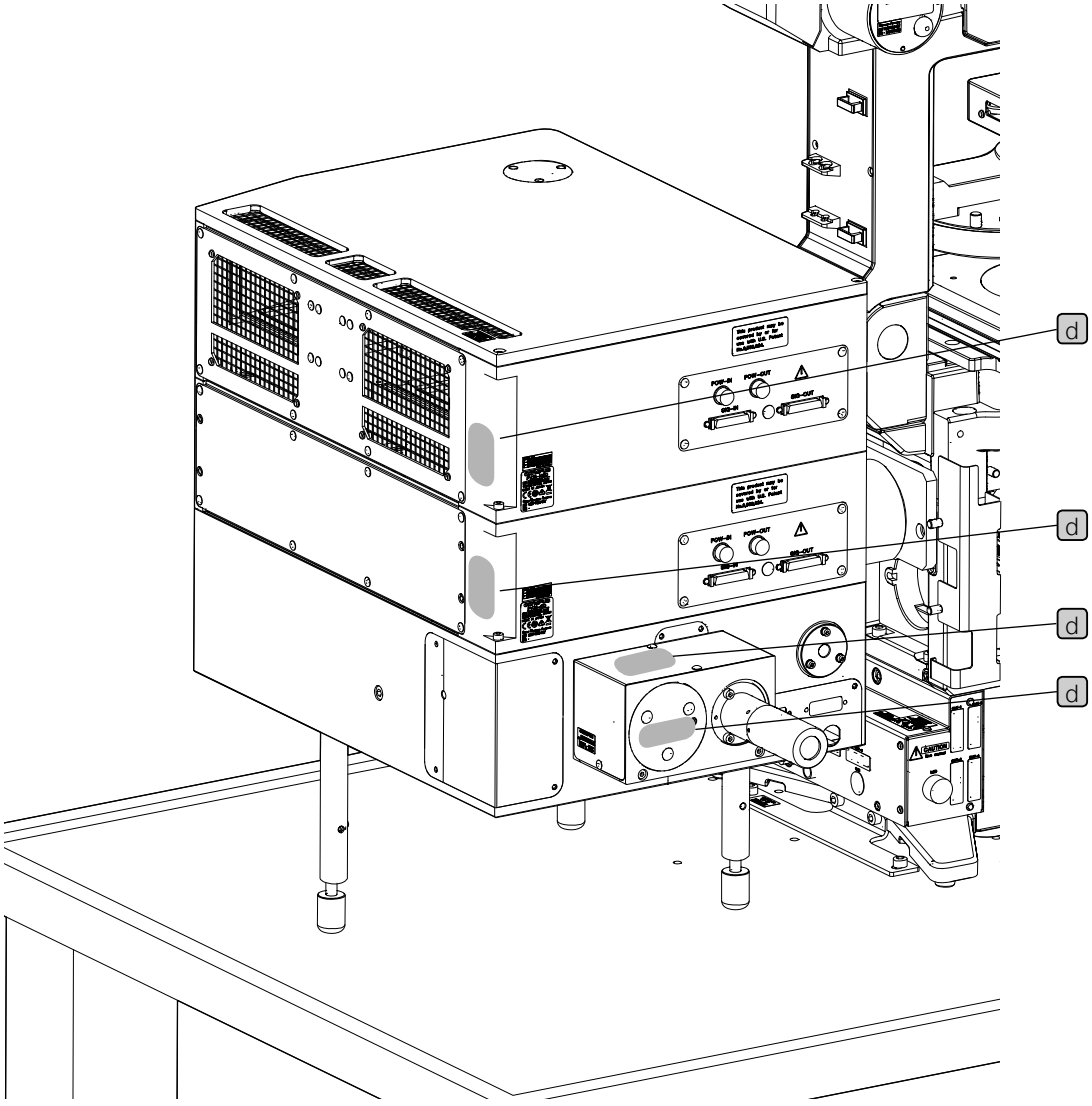
- Sub laser combiner FV31-SCOMB



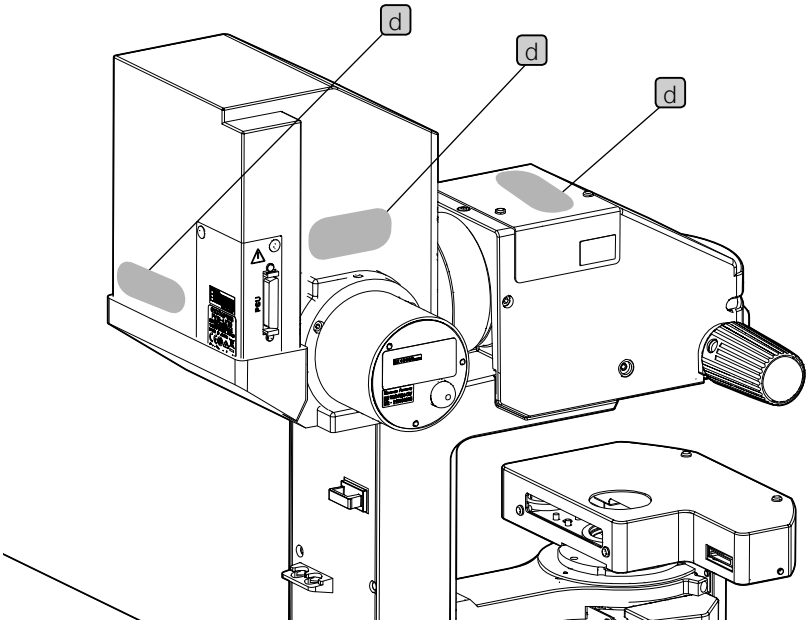
- Top cover FV31-TCOV
- Spectral detector FV31-SD or high sensitivity spectral detector FV31-HSD
- Scan unit FV31-SU or hybrid scan unit FV31-HSU
- Fiber unit between laser combiner and scanner FV31-FURBB
- Stand of scanning unit for IX FV30-STIMP



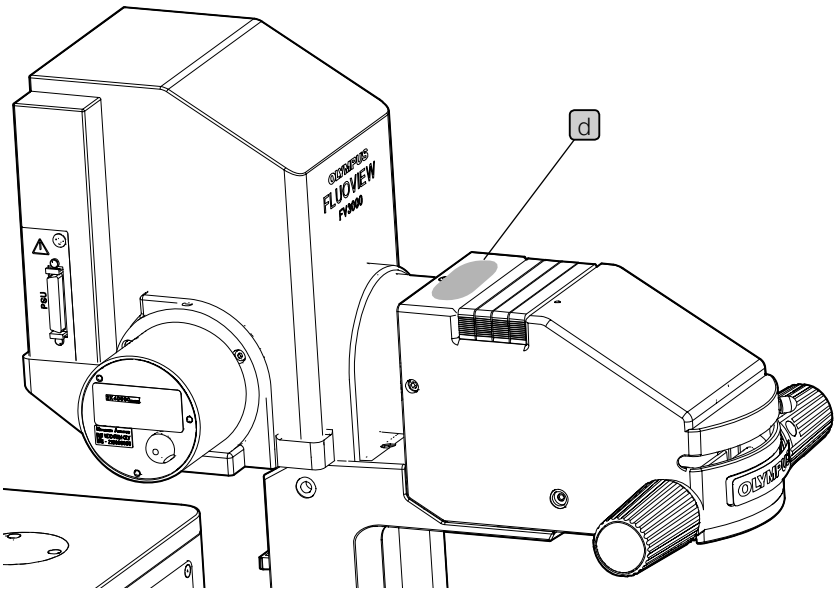
- Spectral detector FV31-SD or high sensitivity spectral detector FV31-HSD
- Beam axis combiner FV31-BCOMB



· Transmitted detector FV31-LETD
· Transmitted filter cover FV12-IXCOV

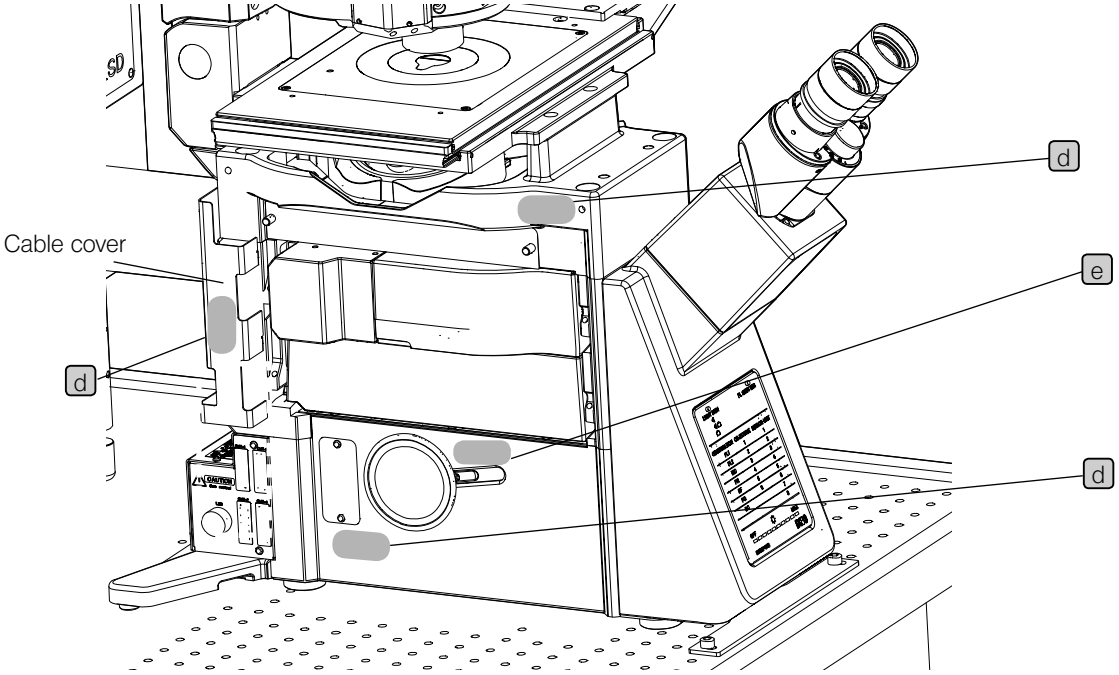


· Transmitted illuminator column IX3-ILL
·When the transmitted filter cover FV12-IXCOV is not attached :

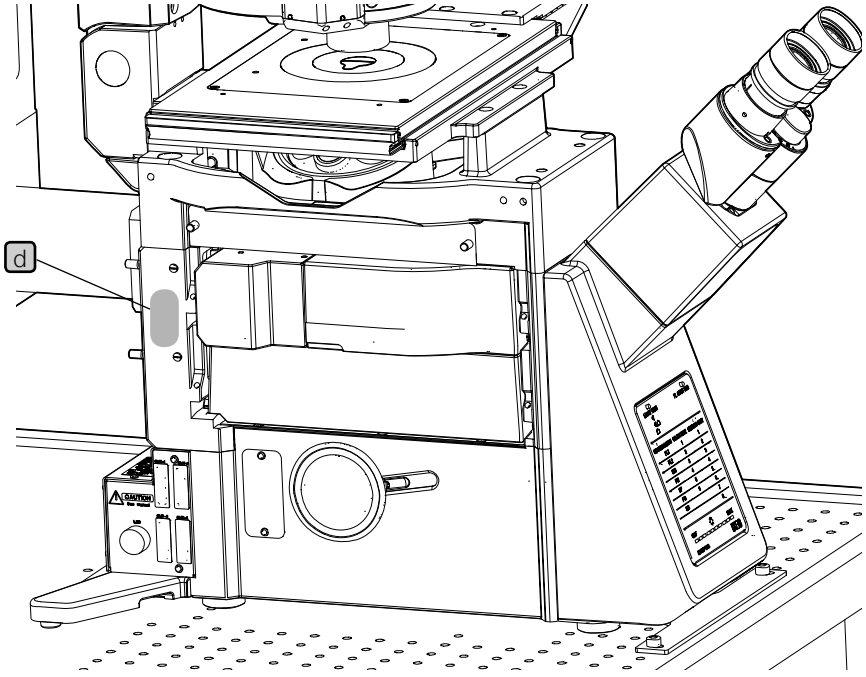


· IX83 2 port frame IX83P2ZF

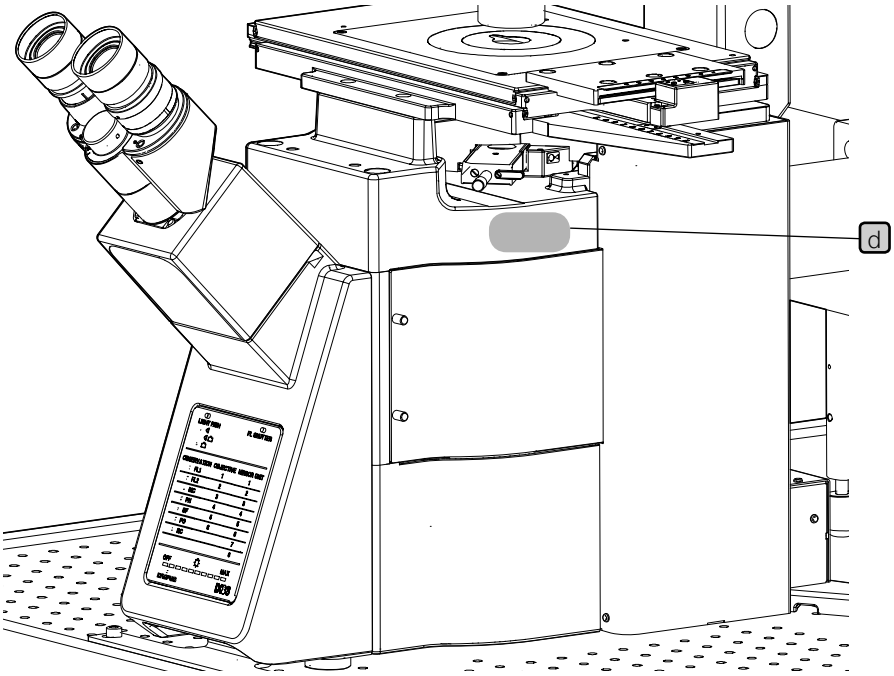
When the cable cover is attached :



When the cable cover is not attached :



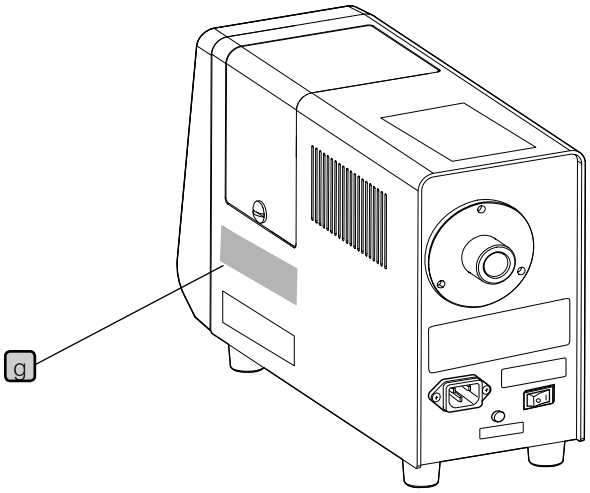
· IX83 2 port frame IX83P2ZF



2-2 Warning labels for heat safety

g

Light source U-HGLGPS



Though protective housing labels are attached to other places than the places indicated in this manual, the said labels are intended for Olympus technical and service personnel.

⚠CAUTION **When warning labels are dirty or peeled off, contact Olympus for replacement or inquiries.**

2-3 Immersion oil labels

Read carefully the descriptions on the labels of the immersion oil you purchased.

Immersion oil	Descriptions
IMMOIL-8CC IMMOIL-500CC IMMOIL-F30CC	The cautions and the handling procedures of the immersion oil are described.

3 Specifications

TIP For products not listed in the following table, refer to the instruction manuals provided with respective products.

3-1 Specifications of units

· Dimensions and weights

Unit		Specifications
Main laser combiner FV31-MCOMB*3	Dimensions (mm)	285 (D) x 420 (W) x 173 (H)
	Weight	12.7 kg
Sub laser combiner FV31-SCOMB	Dimensions (mm)	285 (D) x 420 (W) x 173 (H)
	Weight	11.0 kg
Fiber unit between laser combiners FV31-CCFUR	Dimensions (mm)	Coupling lens : Φ24 x 67 (H) Fiber : Φ5 x 3000 (Length) Collimating lens : Φ48 x 109 (H) Mount : 109 (D) x 59 (W) x 62 (H)
	Weight	In total of coupling lens, fiber, collimating lens, and mount : 0.54 kg
Beam axis combiner FV31-BCOMB	Dimensions (mm)	102 (D) x 129 (W) x 76 (H)
	Weight	0.86 kg
Polarization beamsplitter cube for FV31-BCOMB FV31-PBSC	Dimensions (mm)	40 (D) x 24 (W) x 25 (H)
	Weight	0.04 kg
IX83 2 port frame IX83P2ZF	Dimensions (mm)	Microscope frame : 323 (D) x 474.7 (W) x 360 (H) Touch panel : 211 (D) x 190 (W) x 105 (H)
	Weight	Microscope frame : 18.7 kg Touch panel : 2.6 kg
Control box IX3-CBH	Dimensions (mm)	294.5 (D) x 100 (W) x 214 (H)
	Weight	4.2 kg
Controller U-MCZ	Dimensions (mm)	110.5 (D) x 203.1 (W) x 97.2 (H)
	Weight	2.1 kg
Ultrasonic stage for IX3 IX3-SSU	Dimensions (mm)	Ultrasonic stage for IX3 : 333.4 (D) x 366 (W) x 33.5 (H) XY controller : 54 (D) x 223 (W) x 88 (H) Controller for ultrasonic stage : 280 (D) x 100 (W) x 215 (H)
	Weight	Ultrasonic stage for IX3 : 3.8 kg XY controller : 1 kg Controller for ultrasonic stage : 3.9 kg
Z drift compensator IX3-ZDC2	Dimensions (mm)	272 (D) x 287 (W) x 63 (H)
	Weight	2.5 kg
PCI interface board FV30-PCIB	Dimensions (mm)	224 (D) x 127 (W) x 22 (H)
	Weight	0.185 kg
IO interface box FV30-ANALOG	Dimensions (mm)	258.2 (D) x 426 (W) x 137.9 (H)
	Weight	5.6 kg
Motorized fluorescent mirror turret FV30-RFACA	Dimensions (mm)	190.5 (D) x 258.7 (W) x 69.7 (H)
	Weight	2.5 kg
Laser safety unit for IX3-ILL FV31-ILLSW	Dimensions (mm)	Box : 72 (D) x 129 (W) x 121 (H) Arm : 54 (D) x 127 (W) x 30 (H)
	Weight	In total of box and arm : 0.5 kg

Unit		Specifications
Laser interlock switch FV30-ILSW	Dimensions (mm)	64 (D) x 30 (W) x 42 (H)
	Weight	0.15 kg
Simple anti vibration plate FV31-AVP	Dimensions (mm)	900 (D) x 450 (W) x 34 (H)
	Weight	15 kg
Reverse spectrum DM at 560nm FV31-RSDM560	Dimensions (mm)	Φ12.7 x 2 (H)
	Weight	0.001 kg
ExDM turret set for standard 4LD system FV31-EXDM-M	Dimensions (mm)	52 (D) x 137 (W) x 192 (H)
	Weight	0.7 kg
ExDM turret set for optional 6LD system FV31-EXDM-MS	Dimensions (mm)	52 (D) x 137 (W) x 192 (H)
	Weight	0.7 kg
Laser power monitor FV31-LPM	Dimensions (mm)	Spectrum glass : Φ28 x 25 (H) Base plate : 65 (D) x 46 (W) x 77 (H)
	Weight	In total of spectrum glass and base plate : 0.11 kg
Transmitted detector FV31-LETD	Dimensions (mm)	197 (D) x 131 (W) x 188 (H)
	Weight	2.7 kg
Spectral detector FV31-SD	Dimensions (mm)	320 (D) x 425 (W) x 100 (H)
	Weight	12.7 kg
High sensitivity spectral detector FV31-HSD	Dimensions (mm)	320 (D) x 425 (W) x 120 (H)
	Weight	15.4 kg
Excitation DM for VBY lasers FV31-DM-VBY	Dimensions (mm)	Φ23 x 7 (H)
	Weight	0.02 kg
Excitation DM for NE lasers FV31-DM-NE	Dimensions (mm)	Φ23 x 7 (H)
	Weight	0.02 kg
Excitation DM for NEO lasers FV31-DM-NEO	Dimensions (mm)	Φ23 x 7 (H)
	Weight	0.02 kg
Excitation DM for VBO lasers FV31-DM-VBO	Dimensions (mm)	Φ23 x 7 (H)
	Weight	0.02 kg
Excitation DM for VEO lasers FV31-DM-VEO	Dimensions (mm)	Φ23 x 7 (H)
	Weight	0.02 kg
Fixing collor for optional excitation DM FV31-DM-OPT	Dimensions (mm)	Φ23 x 7 (H)
	Weight	0.02 kg
Top cover FV31-TCOV	Dimensions (mm)	320 (D) x 425 (W) x 10 (H)
	Weight	3.5 kg
Fiber unit between laser combiner and scanner FV31-FURBB*3	Dimensions (mm)	Coupling lens unit : Φ24 x 67 (H) Fiber unit : Φ5.6 x 3000 (Length) Collimate lens unit : Φ48 x 123 (H)
	Weight	In total of coupling lens, fiber, and collimating lens : 0.46 kg
Transmitted DIC filter unit IX3-FDICT	Dimensions (mm)	45.5 (D) x 41.6 (W) x 39 (H)
	Weight	0.04 kg

Unit		Specifications
Stand of scanning unit for IX FV30-STIMP	Dimensions (mm)	Adapter : 140 (D) x 178 (W) x 98 (H) Legs : Φ20 x 165 (H)
	Weight	Adapter : 1.4 kg Legs : 0.6 kg
Shift DIC prism for transmitted observation U-DICTS High contrast DIC prism for transmitted observation U-DICTHC High resolution DIC prism for transmitted observation U-DICTHR	Dimensions (mm)	34 (D) x 135.9 (W) x 13.5 (H)
	Weight	0.08 kg
Motorized DIC slider IX3-DICTA	Dimensions (mm)	Relay box : 23 (D) x 94.5 (W) x 57 (H) Prism slider : 71.6 (D) x 264.8 (W) x 46.5 (H)
	Weight	0.8 kg
Transmitted illuminator column IX3-ILL	Dimensions (mm)	269.8 (D) x 215.8 (W) x 399.5 (H)
	Weight	3.5 kg
Frame fix plate IX3-FP	Dimensions (mm)	46.5 (D) x 221 (W) x 19 (H)
	Weight	0.4 kg
Transmitted filter cover FV12-IXCOV	Dimensions (mm)	90 (D) x 68 (W) x 31 (H)
	Weight	0.08 kg
Adapter kit for LD445 FV31-ADP445	Dimensions (mm)	82 (D) x 96 (W) x 76 (H)
	Weight	0.34 kg
Adapter kit for LD514 FV31-ADP514	Dimensions (mm)	82 (D) x 96 (W) x 76 (H)
	Weight	0.34 kg
Adapter Kit for LD594 FV31-ADP594	Dimensions (mm)	82 (D) x 96 (W) x 76 (H)
	Weight	0.34 kg
Adaptor kit of mirror FV31-ADPM	Dimensions (mm)	82 (D) x 96 (W) x 76 (H)
	Weight	0.34 kg
Umbra shield for IX83 FV31-SPCOV	Dimensions (mm)	Condenser cover : Φ65 x 88 (H) Specimen cover : 140 (D) x 180 (W) x 24 (H)
	Weight	Condenser cover : 0.2 kg Specimen cover : 0.21 kg
Light source U-HGLGPS	Dimensions (mm)	317 (D) x 111 (W) x 195 (H)
	Weight	3.5 kg
LED Lamp house IX3-LHLEDC	Dimensions (mm)	Φ66 x 67.5 (H)
	Weight	0.44 kg
Scan unit FV31-SU*1	Dimensions (mm)	320 (D) x 425 (W) x 125 (H)
	Weight	16 kg

Unit		Specifications
Hybrid scan unit FV31-HSU*2	Dimensions (mm)	320 (D) x 425 (W) x 125 (H)
	Weight	16 kg
Power supply for system FV31-PSU*1,*2	Dimensions (mm)	400 (D) x 426 (W) x 242 (H)
	Weight	15 kg

*1 : The combination of the scan unit FV31-SU and the power supply for system FV31-PSU is called the galvo scan unit with power supply FV31-SU-P.

*2 : The combination of the hybrid scan unit FV31-HSU and the power supply for system FV31-PSU is called the hybrid scan unit with power supply FV31-HSU-P.

*3 : The combination of main laser combiner FV31-MCOMB, fiber unit between laser combiner and scanner FV31-FURBB described in [3-1 Specifications of units], and OBIS405-50LX, OBIS488-20LS, OBIS561-20LS, and OBIS640-40LX described in [3-2 Specifications of combinable laser systems] is called main laser combiner with 4 LDs FV31-MCOMB-P.

· Rating list of this power supply for system

Name	Rating
Power supply for system FV31-PSU	AC 100 - 120 V / 220 - 240 V 3.2 A / 1.5 A 50 / 60 Hz
Control box IX3-CBH	AC 100 - 120 V / 220 - 240 V 4.6 A / 2.8 A 50 / 60 Hz
IX83 2 port frame IX83P2ZF	AC 100 - 240 V 1.7 A 50 / 60 Hz (Rating of touch panel controller)
Light source U-HGLGPS	AC 100 - 240 V 2.0 A 50 / 60 Hz
Ultrasonic stage for IX3 IX3-SSU	AC 100 - 120 V / 220 - 240 V 0.35 A / 0.2 A 50 / 60 Hz

Items	Specifications
Laser radiation from Z drift compensator IX3-ZDC2	Laser diode Laser wavelength : 790 nm (Class 1 IEC60825-1 : 2007) Laser pulse duration : 2.1 ms, Frequency : 200 Hz Laser radiation (instantaneous maximum power) : 240 μW Laser diode power Beam divergence angle : 0.1 to 0.49 rad Maximum power : 20 mW
Laser radiation from the system	· Wavelength : 400 to 700 nm · Maximum power : 500 mW · Pulse amplitude CW · Beam divergence angle : 0.08 to 2.5 rad (when the objective is attached)
Operating environment	· Indoor use · Altitude : Max. 2,000 meters · Ambient temperature : 18 to 28 °C Fluctuation range : ±2.5 °C · Relative humidity : 30 to 75 % · Supply voltage fluctuation : ±10 % · Pollution degree : 2 (in accordance with IEC60664-1) · Installation (Overvoltage) category : II (in accordance with IEC60664-1)

Note **To extend the service life of this system, avoid using it in the following environments.**

- **In a place with excessive dust or dirt :**
  Dust and dirt taken into the cooling fan may lead to failure. Clean this system and peripherals periodically to avoid accumulation of dust.
- **In a place where the temperature is low or high, or where the humidity is high :**
  This includes, for example, a place that is near a water tap, hot-water heater, room humidifier, heater, stove and a place subject to water drops.
- **In a place where humidity changes suddenly :**
  When a cold room is heated quickly, moisture may condense on the equipment (condensation) and could irreparably damage the optical systems.
- **In a place subject to direct sunlight**
- **Outdoors**
- **In a place where a flammable gas is produced**
- **In a place with excessive vibrations**
- **On an unstable surface of floor or platform**

3-2 Specifications of combinable laser systems

Unit		Specifications
OBIS405-50LX*3	Information to describe	Wavelength : 405 ± 5 nm Power : 50 mW Beam divergence (full angle) : < 1 mrad
	Dimensions (mm)	70 (D) x 40 (W) x 38 ((H)
	Weight	0.16 kg
OBIS445-75LX	Information to describe	Wavelength : 445 ± 5 nm Power : 75 mW Beam divergence (full angle) : < 1.1 mrad
	Dimensions (mm)	70 (D) x 40 (W) x 38 ((H)
	Weight	0.16 kg
OBIS488-20LS*3	Information to describe	Wavelength : 488±2nm Power : 20mW Beam divergence (full angle) : < 1.2 mrad
	Dimensions (mm)	70 (D) x 40 (W) x 38 ((H)
	Weight	0.16 kg
OBIS514-40LX	Information to describe	Wavelength : 514±5 nm Power : 40 mW Beam divergence (full angle) : < 1.2 mrad
	Dimensions (mm)	70 (D) x 40 (W) x 38 ((H)
	Weight	0.16 kg
OBIS561-20LS*3	Information to describe	Wavelength : 561±2nm Power : 20mW Beam divergence (full angle) : < 1.2 mrad
	Dimensions (mm)	70 (D) x 40 (W) x 38 ((H)
	Weight	0.16 kg
OBIS594-20LS	Information to describe	Wavelength : 594±2nm Power : 20mW Beam divergence (full angle) : < 1.3 mrad
	Dimensions (mm)	70 (D) x 40 (W) x 38 ((H)
	Weight	0.16 kg
OBIS640-40LX*3	Information to describe	Wavelength : 635-644 nm Power : 40 mW Beam divergence (full angle) : < 1.3 mrad
	Dimensions (mm)	70 (D) x 40 (W) x 38 ((H)
	Weight	0.16 kg
Power supply for LD OBIS 6 Laser Remote	Dimensions (mm)	Power supply unit : 67.5 (D) x 104.9 (W) x 35.9 (H) AC adapter : 189 (D) x 89.4 (W) x 47.1 (H) (excluding cables)
	Weight	Power supply unit: 0.24 kg AC adapter : 0.36 kg

· List of rating of power supply unit of this laser system

Name		Rating		
Power supply for LD	OBIS 6 Laser Remote	AC 100-240V	1.58-0.64A	47-63Hz

This laser system is a Class A product used for basic environments defined in EMC International Standards IEC61326-1.

■ Proper selection of the power supply cord

If no power supply cord is provided, please select the proper power supply cord for the equipment by referring to “Specifications” and “Certified Cord” below;

CAUTION : In case you use a non-approved power supply cord for Olympus products, Olympus can no longer warrant the electrical safety of the equipment.

Specifications

Voltage Rating	125 V AC (for 100-120 V AC area) or , 250 V AC (for 220 - 240 V AC area)
Current Rating	6 A minimum
Temperature Rating	60 °C minimum
Length	3.05 m maximum
Fittings Configuration	Grounding type attachment plug cap. Opposite terminates in molded-on IEC configuration appliance coupling.

Table 1 Certified cord

A power supply cord should be certified by one of the agencies listed in Table 1 , or comprised of cordage marked with an agency marking per Table 1 or marked per Table 2. The fittings are to be marked with at least one of the agencies listed in Table 1. In case you are unable to buy locally the power supply cord which is approved by one of the agencies mentioned in Table 1, please use replacements approved by any other equivalent and authorized agencies in your country.

Country	Agency	Certification Mark	Country	Agency	Certification Mark
Argentina	IRAM		Italy	IMQ	
Australia	SAA		Japan	JET, JQA,	
Austria	ÖVE		Netherlands	KEMA	
Belgium	CEBEC		Norway	NEMKO	
Canada	CSA		Spain	AEE	
Denmark	DEMKO		Sweden	SEMKO	
Finland	FEI		Switzerland	SEV	
France	UTE		United Kingdom	ASTA BSI	,
Germany	VDE		U.S.A.	UL	
Ireland	NSAI				

Table 2 HAR flexible cord

Approval organizations and cordage harmonization marking methods

Approval Organization	Printed or Embossed Harmonization Marking (May be located on jacket or insulation of internal wiring)		Alternative Marking Utilizing Black-Red-Yellow Thread (Length of color section in mm)		
			Black	Red	Yellow
Comite Electrotechnique Belge (CEBEC)	CEBEC	‹HAR›	10	30	10
Verband Deutscher Elektrotechniker (VDE) e.V. Prüfstelle	‹VDE›	‹HAR›	30	10	10
Union Technique de l´Electricité (UTE)	USE	‹HAR›	30	30	10
Instituto Italiano del Marchio di Qualita´ (IMQ)	IEMMEQU	‹HAR›	10	30	50
British Approvals Service for Electric Cables (BASEC)	BASEC	‹HAR›	10	10	30
N.V. KEMA	KEMA-KEUR	‹HAR›	10	30	30
SEMKO AB Svenska Elektriska Materielkontrollanstalter	SEMKO	‹HAR›	10	10	50
Österreichischer Verband für Elektrotechnik (ÖVE)	‹ÖVE›	‹HAR›	30	10	50
Danmarks Elektriske Materialkontroll (DEMKO)	‹DEMKO›	‹HAR›	30	10	30
National Standards Authority of Ireland (NSAI)	‹NSAI›	‹HAR›	30	30	50
Norges Elektriske Materiellkontroll (NEMKO)	NEMKO	‹HAR›	10	10	70
Asociacion Electrotecnica Y Electronica Espanola (AEE)	‹UNED›	‹HAR›	30	10	70
Hellenic Organization for Standardization (ELOT)	ELOT	‹HAR›	30	30	70
Instituto Portages da Qualidade (IPQ)	I np I	‹HAR›	10	10	90
Schweizerischer Elektro Technischer Verein (SEV)	SEV	‹HAR›	10	30	90
Elektriska Inspektoratet	SETI	‹HAR›	10	30	90

Underwriters Laboratories Inc. (UL)	SV, SVT, SJ or SJT, 3 X 18AWG
Canadian Standards Association (CSA)	SV, SVT, SJ or SJT, 3 X 18AWG

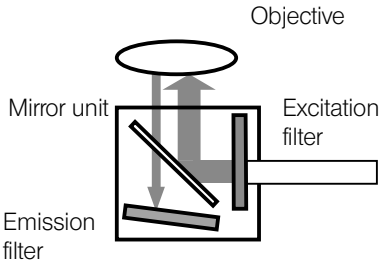
Operation / Maintenance Manual

Precautions and notes for glare prevention

When the excitation filter is removed from the fluorescence mirror unit

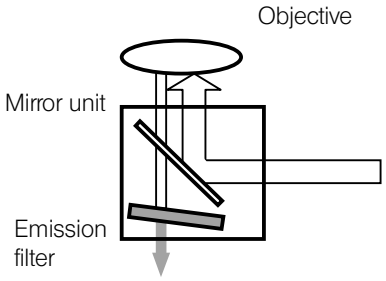
If the excitation filter is removed from the fluorescence mirror unit and attached to the excitation filter slider or the excitation filter wheel on the white lamp side, **the intense glaring light (*1) may enter the eyepieces** in the following cases.

1. When the illumination light from the white light lamp (*2) enters the light path **without passing through an excitation filter**.
2. When the illumination light from the white light lamp enters the light path **through an excitation filter that does not match the mirror unit type.**



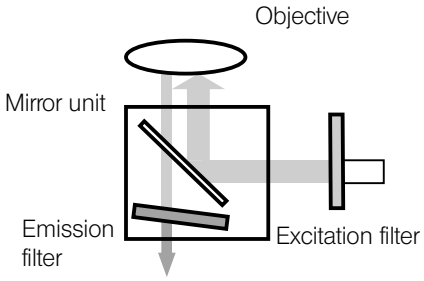
Ordinary mirror unit

The light transmitted through the excitation filter is blocked completely by the emission filter.



Case 1 described above

Among the white light, only the light transmitted through the emission filter enters the observation light path.



Case 2 described above

The light transmitted through the excitation filter may not be blocked completely by the emission filter in some cases.

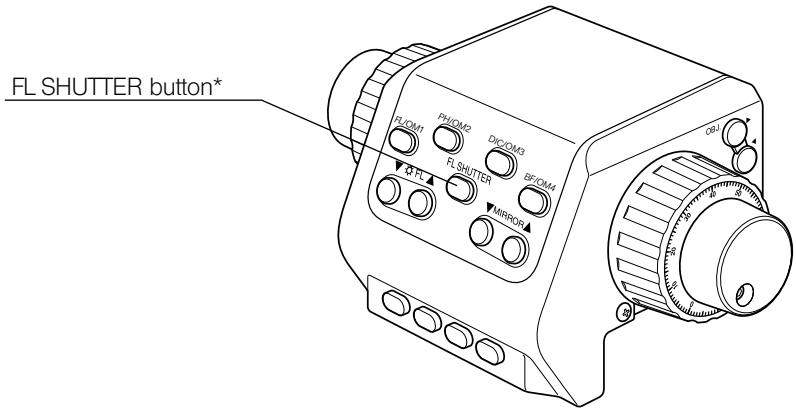
(*1) This light will not injure your eyes even if it enters your eyes. However, be sure to stop observation through the eyepieces, and engage the mirror unit and the excitation filter of appropriate combination in the light path and restart observation.

(*2) The white light lamp refers to all lamps for reflected light illumination including mercury and xenon burners.

⚠CAUTION **Never use the mirror units described in Case 1 and 2 for LSM (laser scanning microscope) observation.**

Opening and closing the shutter to block the light of the white light lamp.

Press the FL SHUTTER button of the controller U-MCZ to switch between irradiation and blocking of the illumination light.



* The FL SHUTTER button opens/closes the reflected shutter only.

Contents

1 System outline

1-1 Features of FV3000

- Using the high sensitivity spectral detector FV31-HSD allows you to acquire the image with high S/N ratio.
- Setting the arbitrary acquisition wavelength to both the high sensitivity spectral detector FV31-HSD and the spectral detector FV31-SD allows you to acquire the images of different wavelengths simultaneously.
- Using the hybrid scan unit FV31-HSU allows you to observe the image in high speed.
- Even in a long hour time-lapse observation, you can acquire the image keeping the accuracy of repetition time.

1-2 Features of each system

1 Detector unit

Types of detector units

TIP The following table shows the three types of detector units.

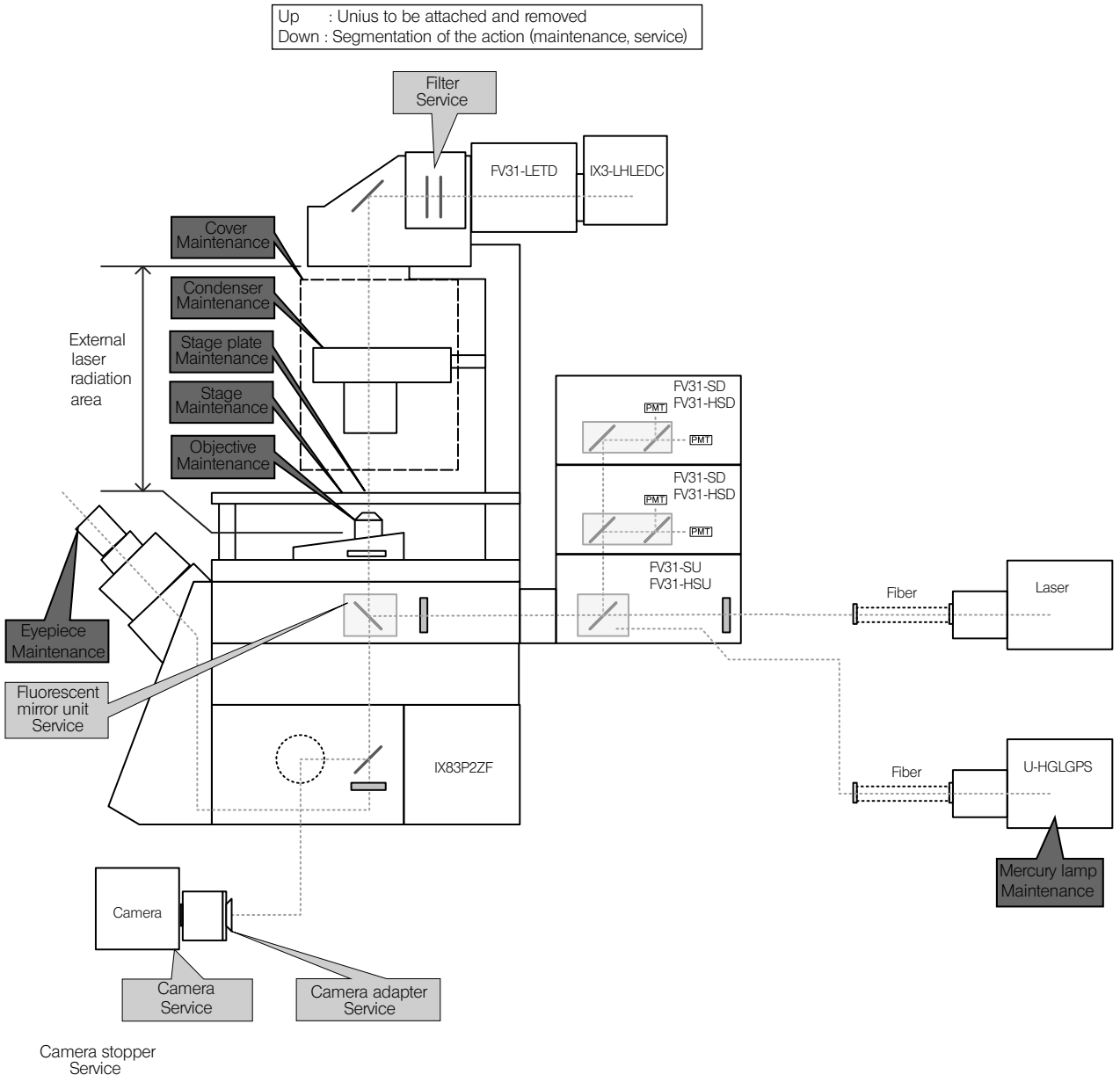
Detector unit
Spectral detector FV31-SD
High sensitivity spectral detector FV31-HSD
Transmitted detector FV31-LETD

Spectral detector FV31-SD and high sensitivity spectral detector FV31-HSD

TIP The spectral detector FV31-SD and the high sensitivity spectral detector FV31-HSD can be attached up to 2 units by combining each other as shown in the table below.

	Combination of detector					
	I	II	III	IV	V	VI
Second unit	-	-	Spectral detector FV31-SD	High sensitivity spectral detector FV31-HSD	Spectral detector FV31-SD	High sensitivity spectral detector FV31-HSD
First unit	Spectral detector FV31-SD	High sensitivity spectral detector FV31-HSD	High sensitivity spectral detector FV31-HSD	Spectral detector FV31-SD	Spectral detector FV31-SD	High sensitivity spectral detector FV31-HSD

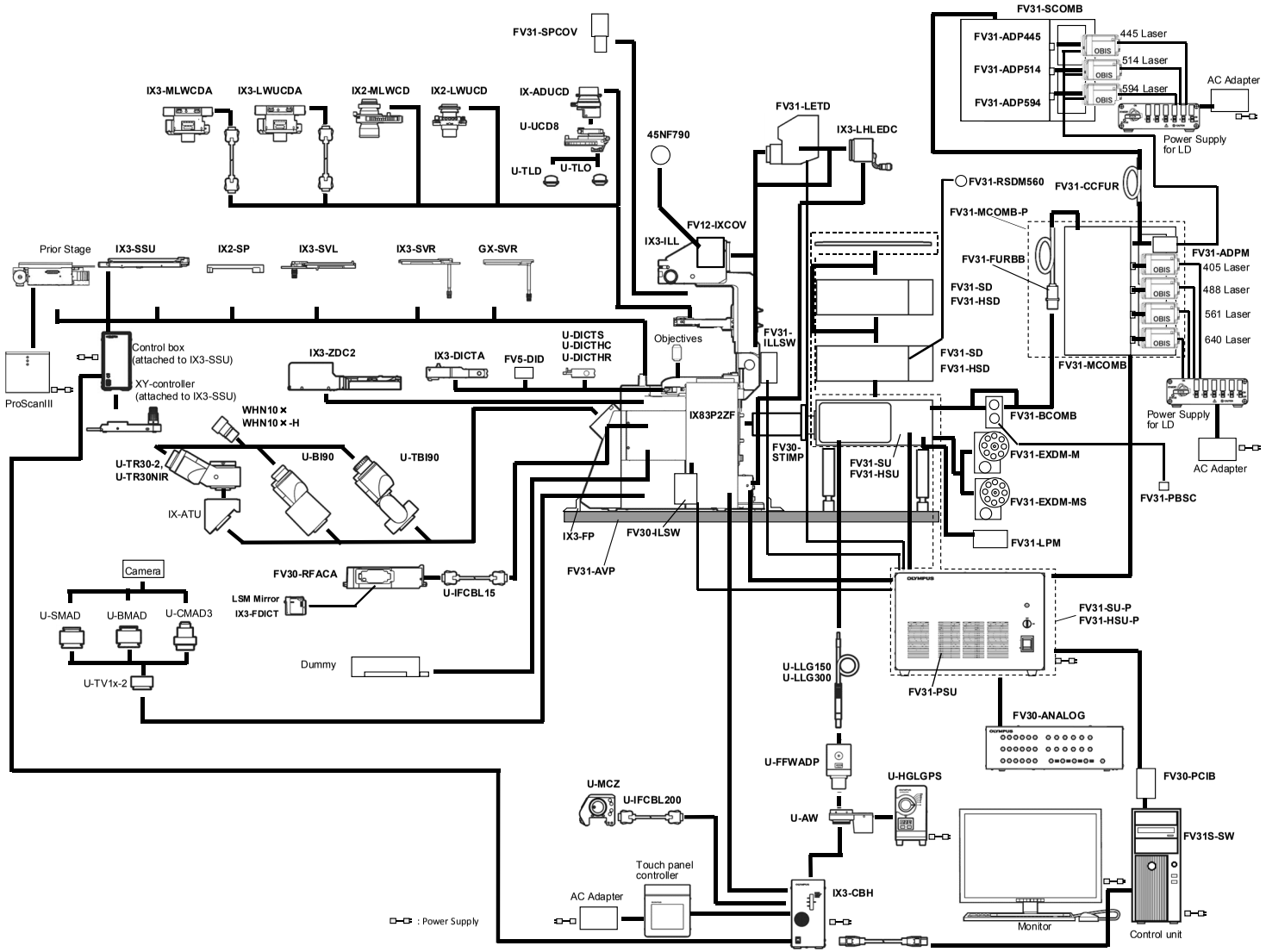
1-3 Allowable work area when attaching / detaching units



⚠CAUTION

- **The maintenance works may be conducted in the area of laser apertures necessary for LSM observation. Be sure to stop emitting the laser before working in this area.**
- **Olympus service / technical personnel or engineers who received the service training provided by Olympus will carry out the attaching/detaching work categorized as the service in accordance with FV3000 Service Manual. Personnel other than those described above are not allowed to conduct the service work.**

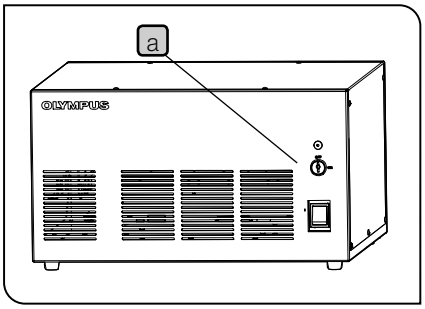
1 System diagram



TIP When additional units are required to this system, Olympus service engineers will perform the installation.

1-5 Confirmation when working with FV3000

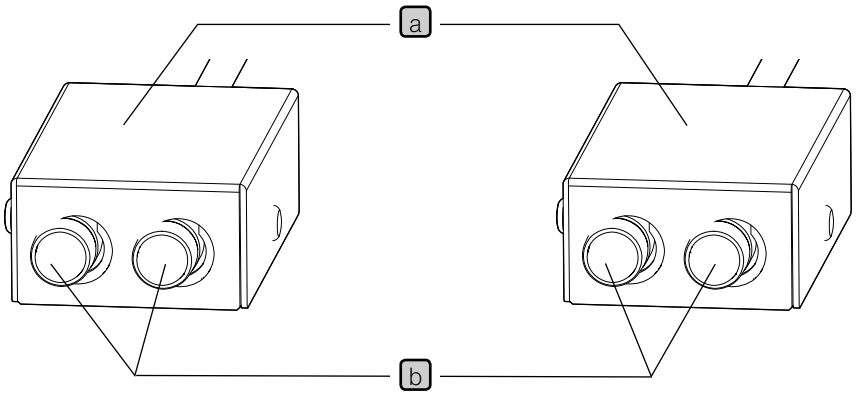
1 Key operation



The power supply for system FV31-PSU is equipped with a start key [a].
Before starting the maintenance work, set the key to OFF and remove it.

2 Checking the emission indicator

There are two lamp boxes [a] connected to the power supply for system (FV31-PSU) .
When the LED lamp [b] is ON, the laser is ready to be emitted.



TIP One of two lamp boxes [a] is installed within 2 m from the tip of the objective, and the other is installed within 2 m from the monitor for the standard image acquisition / analysis software for FV3000 FV31S-SW.

⚠CAUTION
- **Before starting the maintenance work, make sure that the LED lamps [b] are turned OFF.**
- **Before or during the maintenance work, always confirm whether the LED lamps [b] are turned ON or OFF.**
- **Among four LED lamps [b], if some lamps are turned ON and some lamps are turned OFF, stop using the system and contact Olympus.**
- **Do not remove the connection cable of the lamp box [a] or hide the lamp box [a] by covering the lamp box [a], etc. Doing so falls under the remodeling, and is not in conformance with Laser Safety Standards.**

TIP If the units prepared by customers except this system are installed after the lamp box [a] is installed by Olympus personnel, be sure to keep the lamp box [a] in view.

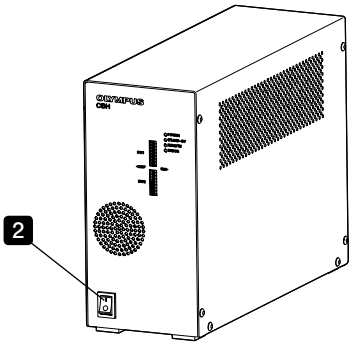
2 Precautions for use ⚠

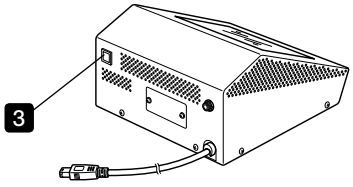
⚠CAUTION
- Be sure to wear the laser protective glasses appropriate for the laser you use.
- During laser-scanning, a laser is emitted from the objective or an area where the objective is mounted on the revolving nosepiece.
- Cover the hole on the revolving nosepiece with the cap when the objective lens is not mounted on the revolving nosepiece, since a high-power laser is emitted from this hole.
- Never place a highly reflective object over the objective or look directly at the tip of the objective.
- In case of darkening the area around the IX83 2 port frame IX83P22F for experiments, be careful not to catch on cables or collide with the desk.

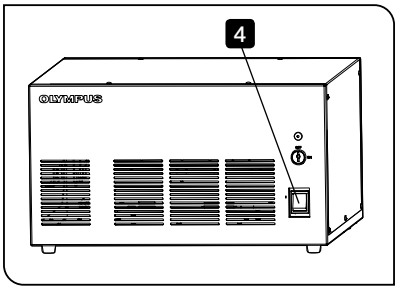
Note
- Since the GaAsP-PMT used with the high sensitivity spectral detector FV31-HSD has an extremely high light-receiving sensitivity, the element may be deteriorated if subjected to the high-intensity ambient light. For that reason, GaAsP-PMT is a consumable item and its lifetime, etc. is not covered by warranty. If the observation becomes interfered while using this product continuously, such as the image becomes dark, etc., GaAsP-PMT will be replaced or adjusted by Olympus technical personnel. This work is a paid service even if it is performed within one year after delivery.
- The control unit data may be unexpectedly corrupted. Make frequent backups of your data.
- Olympus shall have no liability for any damage (including compensation for the corrupted control unit data) from the use or misuse of this product.
- Microsoft Windows has been installed on the control unit of this system.
  Backup and keep the system data in a safe place. (Olympus does not provide support for backing up your system data.)
  For more information about the control unit and Microsoft Windows, refer to respective manuals provided.
- The quality assurance for this system is provided at factory default settings.
  Olympus shall have no liability for any operating failure or disordered function due to changes of environment settings (BIOS change) of the control unit or installation of other software by users.
- When the remaining available hard disk space becomes small, the data processing rate may drop drastically or errors may occur. To prevent this, delete unnecessary data files frequently. For procedures to delete the data file, refer to the manuals provided with Microsoft Windows.
  The free space of hard disk should be at least 1 Gbyte.
- Disable the screen saver (by setting it to None) before using the system.
  The screen saver is set to (None) in advance at delivery.
- Never delete the folder or modify its names which are in the hard disk of the control unit at delivery. Otherwise, the software may not start.
- Never execute other software while executing the standard image acquisition / analysis software for FV3000 FV31S-SW. Otherwise, the software may not work properly.
- Do not connect the USB unit which is not provided by Olympus to the control unit. Connecting the USB unit which is not provided by Olympus may cause a malfunction to the control unit.
- If you want to connect the control unit to the network, install the antivirus software.
- If the product is connected to the network, the system may not be operated properly due to following problems.
  ① Virus infection through the network
  ② Malfunction caused by applying OS service pack, security patch, or updating third party software including runtime libraries
  ③ The acquired images are missed or the acquisition process is delayed or stopped due to the overloaded system which is caused by running the antivirus software, etc.

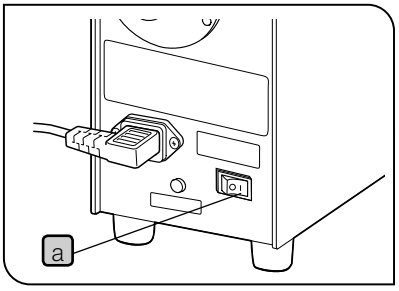
3 Turning ON / OFF the power and starting / exiting the software

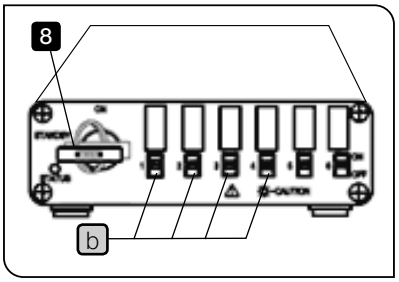
3-1 Turning ON the power (Operation)











TIP Be sure to follow the order to turn ON the power described in Step 2 and Step 3.

1 Set the main switches of the control unit and the monitor to I (ON) .

2 Set the main switch of the control box IX3-CBH to I (ON) .

3 Press the main switch of the touch panel controller to set it to ON.

4 Set the main switch of the power supply for system FV31-PSU [a] to I (ON) .

5 Set the main switch of the AC adapter of the Power supply for LD OBIS 6 Laser Remote connected to the main laser combiner FV31-MCOMB to I (ON) .

6 Set the main switch of the AC adapter of the Power supply for LD OBIS 6 Laser Remote OBIS 6 Laser Remote connected to the sub laser combiner FV31-SCOMB to I (ON) .

7 Set the main switches of rest of units in this system to I (ON) .

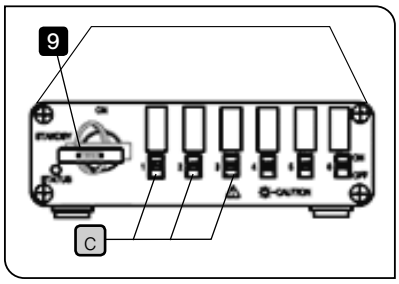
- Light source U-HGLGPS [a]
- Power supply for LD OBIS 6 Laser Remote connected to the main laser combiner FV31-MCOMB [b]
- Power supply for LD OBIS 6 Laser Remote connected to the sub laser combiner FV31-SCOMB [c]

TIP The order to turn ON the power in Step 4 does not matter.

8 Rotate the start key of the Power supply for LD OBIS 6 Laser Remote connected to the main laser combiner FV31-MCOMB clockwise to set it to ON.

TIP The laser installed on the main laser combiner FV31-MCOMB starts.

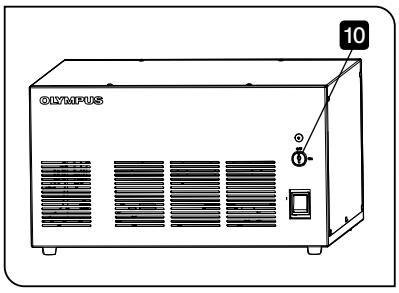
⚠CAUTION **The instruction manual for laser device is included, but do not perform any operation described in this user's manual other than starting and exiting the laser device.**



9 Rotate the start key of the Power supply for LD OBIS 6 Laser Remote connected to the sub laser combiner FV31-SCOMB clockwise to set it to ON.

TIP The laser installed on the sub laser combiner FV31-SCOMB starts.

⚠CAUTION **The instruction manual for laser device is included, but do not perform any operation described in this user's manual other than starting and exiting the laser device.**



10 Set the start key of the power supply for system FV31-PSU to ON

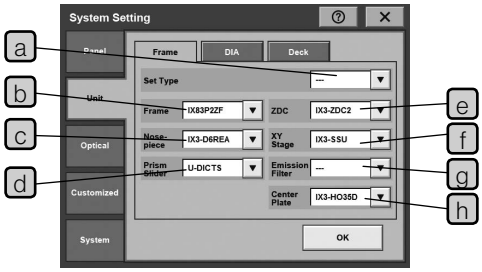
11 .Logon Windows with your own user ID.

TIP If you have not yet registered your user ID, logon with the factory default ID.
Factory default ID : olympus
Password : olympus

3-2 Setting on the touch panel controller (Maintenance)

Before starting the standard image acquisition / analysis software for FV3000 FV31S-SW, check that settings on the [Frame]tab, [DIA] tab and [Deck] tab of the touch panel controller are made as follows.
If settings are different from followings, the error occurs when starting the standard image acquisition / analysis software for FV3000 FV31S-SW.

Settings on the [Frame] tab

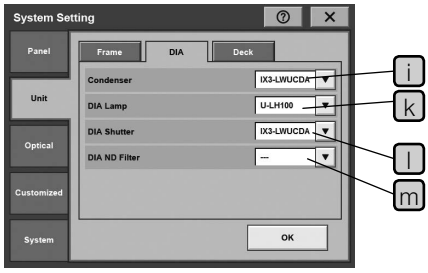


	Unit / Frame	Selection item
a	Set Type	[---]
b	Frame	[IX83P2ZF]
c	Nosepiece	[IX3-REA]
d	Prism Slider	Select from followings. [U-DICT] [U-DICTS] [U-DICHC][U-DICHR] [IX3-DICTA] [Other] [---]
e	ZDC	Select from followings. [IX3-ZDC2] [---]
f	XY Stage	Select from followings. [IX3-SSU] [---]*
g	Emission Filter	[---]
h	Center Plate	**

* If you use the stage other than IX3-SSU, select [---].

** If you select [IX3-SSU] in f, select the center plate you use.

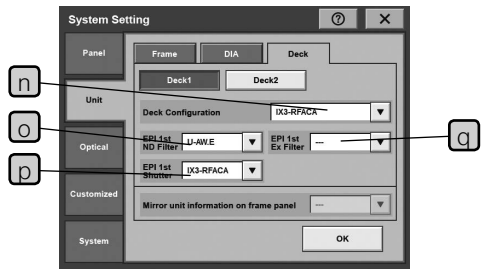
Settings on the [DIA] tab



	Unit / DIA	Selection item
i	Condenser	Select from followings. [IX3-LWUCDA] [Other] [---]
k	DIA Lamp	Select from followings. [IX3-LHLEDC] [U-LH100] [Other]
l	DIA Shutter	Select from followings. [IX3-LWUCDA] [Other]*** [---]***
m	DIA ND Filter	[---]

***If you use the condenser other than IX3-LWUCDA, select [Other] or [---].

Settings on the [DIA] tab



	Unit / Deck	Selection item
n	Deck Configuration	[FV30-RFACA]
o	EPI 1st ND Filter	Select from followings. [U-AW.E] [---]
p	EPI 1st Shutter	[FV30-RFACA]
q	EPI 1st Ex Filter	[---]

3-3 Starting the standard image acquisition / analysis software for FV3000 FV31S-SW (Operation)

1 Start the standard image acquisition / analysis software for FV3000 FV31S-SW.

2 Double-click the [FV31S-SW] icon on the desktop.

[FV31S-SW] icon

3 Depending on option units you purchased, the stage setting dialog box may appear. Select the followings and click the [OK] button.

- Selection whether or not to drive the resonant scanner
- Selection whether or not to control the XY stage by the software
- XY stage type
- Selection whether or not to perform the centering in XY direction.

TIP
- It takes approx. 20 to 30 seconds until the software starts after the [OK] button is clicked.
- If the ultrasonic stage for IX3 IX3-SSU is selected as a XY stage, the message to select whether or not to perform the auto-cleaning appears. (If the 20X or higher objective is engaged in the light path, the auto-cleaning is not available.)
- While executing the standard image acquisition / analysis software for FV3000 FV31S-SW, the XY correction value of the ultrasonic stage for IX3 IX3-SSU which was set by the touch panel controller is disabled.

4 Select [Configuration] in the [Tools] menu of the standard image acquisition / analysis software for FV3000 FV31S-SW.

TIP For details, refer to Online Help of the standard image acquisition / analysis software for FV3000 FV31S-SW.

3-3 Completing the observation with the system (Operation)

1 Uncheck the checkbox of the laser by the standard image acquisition / analysis software for FV3000 FV31S-SW to stop the laser emission and exit the software.

TIP For details, refer to Online Help of the standard image acquisition / analysis software for FV3000 FV31S-SW.

2 Set the start key of the power supply for system FV31-PSU to OFF.

3 Rotate the start key of the Power supply for LD OBIS 6 Laser Remote connected to the main laser combiner FV31-MCOMB counterclockwise to set it to STAND BY.

TIP The laser installed on the main laser combiner FV31-MCOMB stops.

⚠CAUTION **The instruction manual for laser device is included, but do not perform any operation described in this user's manual other than starting and exiting the laser device.**

4 Rotate the start key of the Power supply for LD OBIS 6 Laser Remote connected to the sub laser combiner FV31-SCOMB counterclockwise to set it to STAND BY.

TIP The laser installed on the sub laser combiner FV31-SCOMB stops.

⚠CAUTION **The instruction manual for laser device is included, but do not perform any operation described in this user's manual other than starting and exiting the laser device.**

5 Set the main switches of units described below to O (OFF).

- Light source U-HGLGPS
- Power supply for LD OBIS 6 Laser Remote connected to the main laser combiner FV31-SCOMB
- Power supply for LD OBIS 6 Laser Remote connected to the sub laser combiner FV31-SCOMB

TIP The order to turn OFF the power in Step 5 does not matter.

6 Set the main switch of the AC adapter of the Power supply for LD OBIS 6 Laser Remote connected to the main laser combiner FV31-MCOMB to O (OFF).

7 Set the main switch of the AC adapter of the Power supply for LD OBIS 6 Laser Remote connected to the sub laser combiner FV31-SCOMB to O (OFF).

8 Set the main switch of the power supply for system FV31-PSU to O (OFF).

TIP Be sure to follow the order to turn OFF the power described in Step 9 and Step 10.

9 Press the main switch of the touch panel controller to set it to OFF.

Note **Do not long-press the main switch of the touch panel controller.**

10 Set the main switch of the control box IX3-CBH to O (OFF).

11 Shutdown the Windows.

12 Set the main switches of the control unit and the monitor to O (OFF).

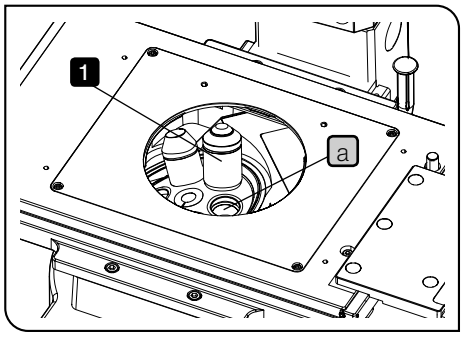
4 Operating procedures of each part

CAUTION From [4-1 Objective (Operation / Maintenance)] to [4-7 Umbra shield for IX83 FV31-SPCOV (Operation / Maintenance)] in this chapter, the maintenance works are included in the necessary laser aperture during LSM observation. Be sure to stop emitting the laser before starting the applicable maintenance works.

4-1 Objective (Operation / Maintenance)

CAUTION
- Be sure to check the laser emission status before starting to work. For details, see page 4. Working with the objective is dangerous because you work near the laser aperture.
- Be sure to attach or detach the objective to or from the revolving nosepiece while the laser is not emitted.

1 Removing and mounting the objective (Maintenance)



1 Remove the stage center plate and remove the objective to be replaced from the revolving nosepiece.

Then, mount the necessary objectives.

TIP
- It is convenient to mount objectives in the order from lower to higher magnifications.
- Use the touch panel controller or the controller U-MCZ to rotate the revolving nosepiece.

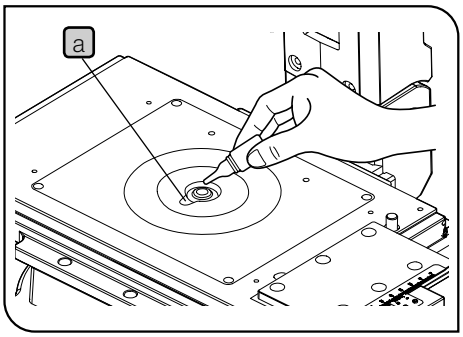
Note
- **Do not rotate the motorized revolving nosepiece manually. Otherwise, it will cause damage to the revolving nosepiece.**
- **Handle the objectives carefully, and screw them completely until they stop.**
- **Keep the removed objectives in the dedicated case.**

CAUTION **Put the provided caps to the screw holes [a] of the revolving nosepiece where no objective is mounted.**

2 Motorized revolving nosepiece (Operation)

The motorized revolving nosepiece can be rotated to switch the objective by pressing the buttons assigned by the touch panel controller or the controller U-MCZ. The motorized revolving nosepiece has a function that the revolving nosepiece is lowered automatically to prevent the objective from colliding with the stage when changing the objectives. The distance to lower the revolving nosepiece can be set by the touch panel controller or the controller U-MCZ.

3 Using oil, silicon or water immersion objective (Operation)



TIP If you use an oil-immersion objective, use the immersion oil.

Note **Always use the immersion oil supplied by Olympus.**

IMMOIL-8CC
IMMOIL-500CC
IMMOIL-F30CC

⚠CAUTION **Follow the cautions described on the label of the immersion oil.**

TIP Be sure to attach the cap to the objective if the oil-proof cap can be attached.

1 Bring the specimen into focus with the low magnification objective.

2 Rotate the revolving nosepiece to engage the oil immersion objective in the light path.

3 Remove the specimen or move the notch a near the tip of the objective. Apply a drop of the provided immersion oil to the tip of the objective. Place the specimen on the stage and rotate the focusing knob to bring the specimen into focus.

Note
- **Use as little oil as possible. Wipe the remaining oil on the oil-proof cap lightly after use. Remove the cap and clean the tip of the objective and around and the cap.**
- **If the oil contains air bubbles, the image will be degraded and the Z drift compensation cannot be performed properly. Make sure that the oil is free of air bubbles.**

TIP The presence of air bubbles can be checked by viewing the pupil of the objective (viewed as a bright circular shape) in the tube after removing the eyepiece and opening the field diaphragm and the aperture diaphragm completely.

4 After use, remove the immersion oil from the tip of the objective by wiping with the gauze slightly moistened with absolute alcohol.

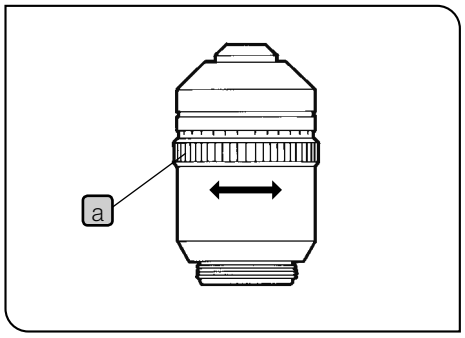
TIP
- The same procedures apply when using the silicon or water immersion objective.
- If you use a silicon immersion objective, use the silicon oil.

Note **Always use the silicon oil supplied by Olympus.**

SIL300CS-30CC

4 Using objectives with correction collar (Operation)

TIP If the cover glass thickness does not match the thickness scale of an objective with correction collar, the objective cannot deliver its performance. When using an objective with correction collar, perform the following adjustment as required.



Adjustment procedure

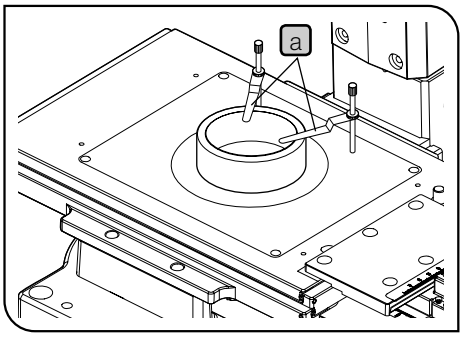
1. If the cover glass thickness is known, rotate the correction collar a to match the value on the scale.
2. If the cover glass thickness is unknown, rotate the correction collar a and the fine focusing knob of the controller U-MCZ alternately to find the highest contrast position.

4-2 Stage (Operation / Maintenance)

⚠CAUTION • **Be sure to check the laser emission status before starting to work. For details, see page 4. Working with the stage is dangerous because you work near the laser aperture.**

• **If you want to use other stage holders than those provided with this system, never use the stage holders which reflect the light or are flammable.**

1 Placing the specimen (Operation)



1 Place the specimen on the center of the stage.

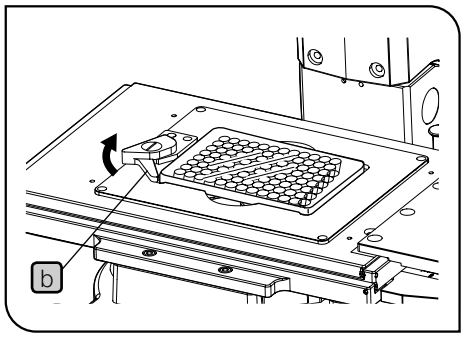
TIP If the specimen tends to slide on the stage, you can clamp down the specimen with the stage clips (IX-SCL) a.

Mechanical stage with right handle IX3-SVR

For the mechanical stage with right handle IX3-SVR, in addition to the holder for round stage center plate, following specimen holders corresponding to each specimen can be attached.

- IX3-HOW : Microplate holder
- IX3-HOS : Slide holder
- IX3-HO35D : Dish holder

⚠CAUTION **The specimen holder fixes the specimen to reproduce the specimen position. Do not push up the specimen by the objective. The specimen may be popped out.**



Microplate holder IX3-HOW

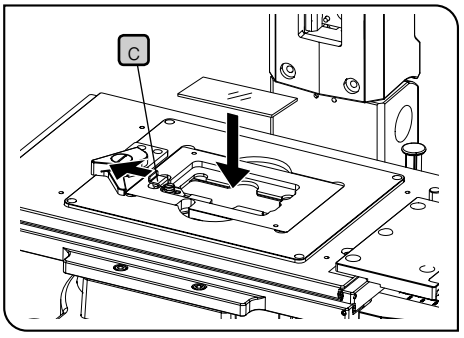
Open the specimen holder of the microplate holder IX3-HOW b, place the microplate in the center, push it toward the right diagonal direction, and return the specimen holder back to the original position.

«Mountable microplate»

Microplate in compliance with SLAS (ANSI/SBS Microplate Standards issued on Jan. 9, 2004)

Size : 127.76 (plus or minus 0.5) x 85.48 (plus or minus 0.5) mm

Specimen holder : Slide holder IX3-HOS, dish holder IX3-HO35D

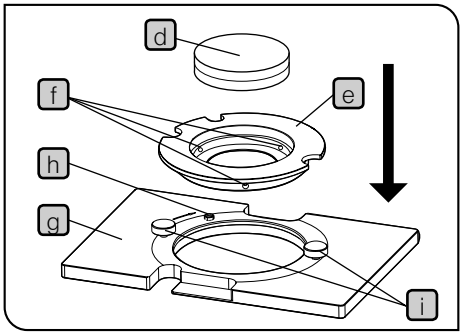


Slide holder IX3-HOS

Open the specimen fixing part c of the slide holder IX3-HOS, place the specimen in the center, push it toward the right diagonal direction, and return the specimen fixing part back to the original position.

«Chamber slide (recommended) »

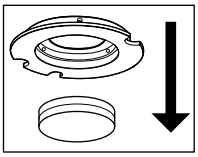
- IWAKI Chamber Slide II (76 x 26 x 0.8 to 1.0 mm)
- Nunc Lab-Tek II Chamber Slide System (25 x 75 x 1.2 mm)
- BD Falcon Culture Slide (25 x 75 x 1.2 mm)



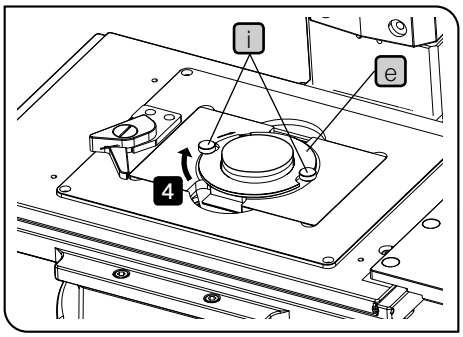
Dish holder IX3-HO35D

1 Place the 35 mm dish [d] on the 35 mm dish fixing holder [e]. Tighten the clamping screws [f] (3 screws) on the side with the Allen screwdriver provided with the dish holder IX3-HO35D to secure the 35 mm dish.

TIP The 35 mm dish can be secured easily by tightening the clamping screws after flipping over the 35 mm dish in advance.



⚠CAUTION **Do not tighten the clamping screws too firmly. The dish may be damaged.**



2 Set the fixing holder [e] in the center of the dish holder IX3-HO35D [g] so that the notch meets the holder fixing knob [i].

3 Loosen the holder fixing knobs [i].

4 Rotate the fixing holder [e] clockwise to push it completely to the rotation stopper [h].

5 Tighten the holder fixing knobs [i].

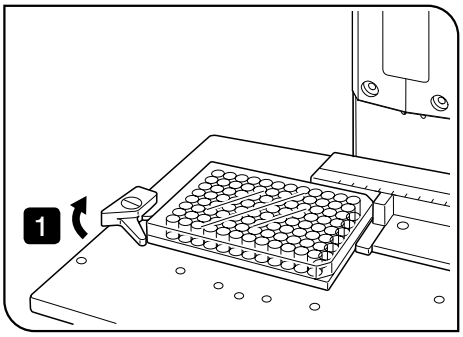
TIP The 35 mm dish fixing holder [e] can be sterilized by using the autoclave.

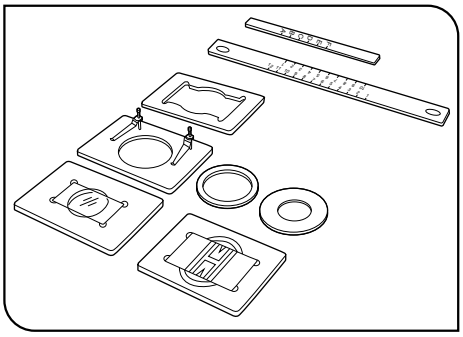
«35 mm glass bottom dish (recommended) »

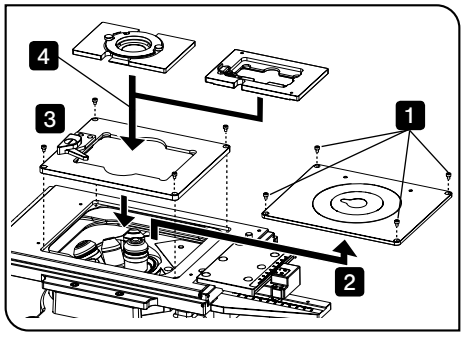
- Matsunami Glass D111310
- MatTek P35GC-1.5-14-C

«35 mm dish (recommended) »

- BD Falcon 351008







Mechanical stage IX-MVR and plain stage IX2-SP

TIP 96-well or 24-well microplates, etc. are held in place by the specimen holder.

Microplates with dimensions of max. 136 mm x 92 mm can be accommodated in this way.

1 Open the specimen holder of the stage, slide the microplate into the holder frame and return the specimen holder back to the original position.

TIP To hold other vessels than microplates, various optional holders are available. When using a Terasaki plate holder for holding Terasaki plates (72-well, 60-well) , it is necessary to replace the stage scales with those provided with the plate holder. Dish holders are available for Ø35 mm, Ø54 mm and Ø65 mm dishes, a slide glass holder is available for holding slide glass, and IX2-BCTP* is available as a blood cell test plate holder.

* A blood cell test plate or other calculating chamber for bacteria and eosinophil which are suitable for dimensions 77 (W) x 35 (D) x 2 (H) mm can be used for attaching the IX2-BCTP. Ø60 mm dish can also be used.

Specimen holder (mechanical stage with right handle IX3-SVR)

1 Remove the holder clamping screws (4 screws) using the Allen wrench provided with the mechanical stage with right handle IX3-SVR.

2 Remove the round stage center plate holder.

3 Set the microplate holder IX3-HOW in the center of the stage, and attach the holder clamping screws.

4 If you are using the slide holder IX3-HOS or the dish holder IX3-HO35D, set the specimen holder in the center of the microplate holder IX3-HOW.

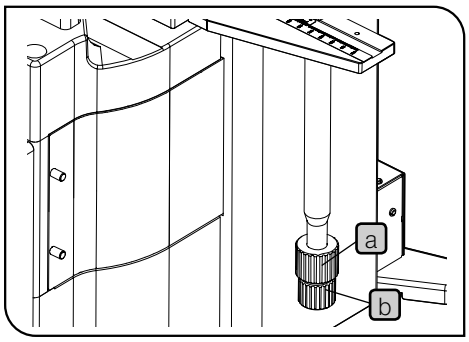
2 Moving the specimen (Operation)

Note • **Do not attempt to rotate the stage knob forcedly exceeding the stage movable range. The stage may be damaged.**

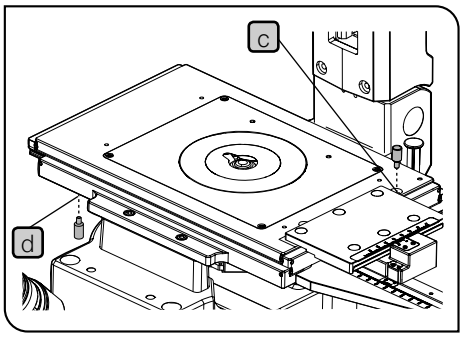
• **As the objective may interfere with the stage depending on the focus position, be sure to operate carefully.**

Mechanical stage with right handle IX3-SVR

The specimen can be moved to a desired position by rotating the X-axis knob a and Y-axis knob b.



TIP If the stage is used for a long time period, the stage movement range may be narrower rarely. In this case, move the stage several times within the full movement range toward the front/back or right/left direction while holding the top surface of the stage with both hands.



Movement control knob

Attaching the movement control knob provided with the mechanical stage with right handle IX3-SVR makes it difficult to move the stage even though you may touch the stage accidentally during observation so that the observation position can be secured.

Attaching the movement control knob to the hole of c makes the movement in Y-axis direction heavier. Attaching it to the hole of d (backside of the stage) makes the movement in Y-axis direction heavier.

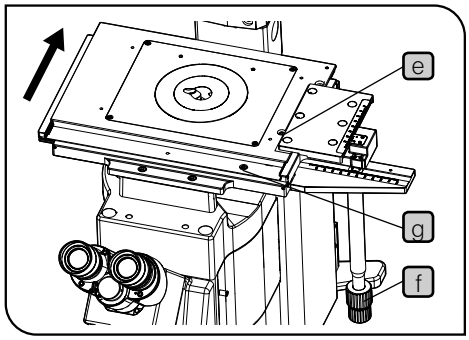
Note **Do not tighten the movement control knob too firmly. The stage may be damaged.**

TIP If you tighten the movement control knob while focusing on the specimen, it will be defocused.

Movement range limit screw

As a factory default, the mechanical stage with right handle IX3-SVR is equipped with the movement range limit screw which limits the stage movable range in the vertical or horizontal direction.

To enlarge the movable range to observe microplates, etc., remove the movement range limit screw.



1. Remove the movement range limit screw [e] in the vertical direction with the Allen screwdriver provided with the system.
2. Rotate the Y-axis knob [f] to move the stage in Y direction.
3. Remove the movement range limit screw [g] in the horizontal direction with the Allen screwdriver provided with the system.

Stage movable range

With movement range limit screw : 50 mm in vertical direction, 50 mm in horizontal direction

Without movement range limit screw : 75 mm in vertical direction, 114 mm in horizontal direction

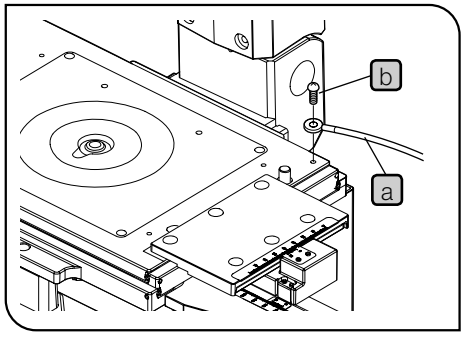
Note **When using the center plate provided with the mechanical stage with right handle IX3-SVR, attach the movement range limit screw.**
If the movement range limit screw is not attached, the objective may collide with the stage.

Mechanical stage IX-MVR

The specimen can be moved to a desired position by rotating the X-axis knob and Y-axis knob in the same manner as the mechanical stage with right handle IX3-SVR.

TIP The stage movement range is 130 mm in X direction and 85 mm in Y direction.

3 Connecting the grounding wire (Maintenance)



Mechanical stage with right handle IX3-SVR

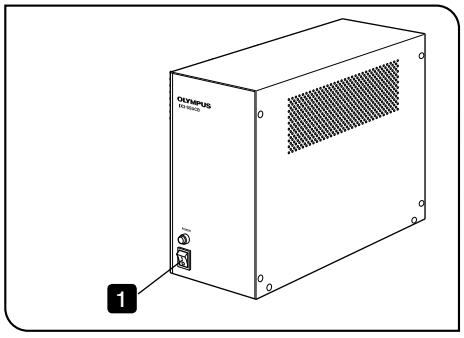
TIP A grounding wire can be attached to the stage for electrophysiological experiments, etc.

Prepare a grounding wire [a] and one M4 screw [b] and attach the grounding wire to a screw hole on the stage surface.

⚠CAUTION **The screw hole may sometimes be stuck by paint, etc. In such a case, screw in the M4 screw a few times to expose the metallic thread inside the screw hole and improve the contact before attaching the grounding wire firmly.**

4-3 Using the ultrasonic stage for IX3 IX3-SSU (Operation / Maintenance)

1 Turning ON the power (Operation)

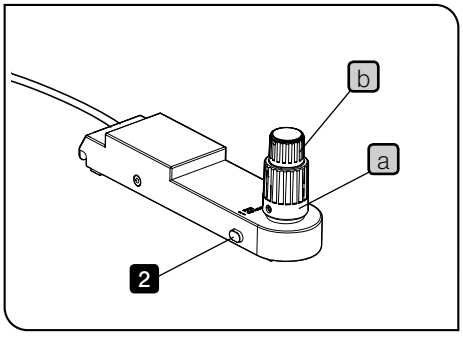


Note **Make sure that the power cords and connection cables are connected properly and securely.**

1 Set the main switch of the controller for ultrasonic stage to ▌(ON) .

Note
- **To control the standard image acquisition / analysis software for FV3000 FV31S-SW, turn ON the controller for ultrasonic stage first and then start the standard image acquisition / analysis software for FV3000 FV31S-SW.**
- **When the operation is finished, exit the standard image acquisition / analysis software for FV3000 FV31S-SW first and then turn OFF the power of the controller for ultrasonic stage.**
- **When using multiple controllers, there are some controllers where the order to turn ON or OFF the power supplies may be specified. For details, see page 6.**

2 Moving the stage (Operation)



1 Rotate the X-axis knob [a] and the Y-axis knob [b] to move the stage.

2 Press the coarse/fine adjustment selection button to switch between the coarse and fine adjustment.

- Pressed : Coarse adjustment
- Release press : Fine adjustment

Sound heard when the stage moves : You may hear the relatively large sound when the stage starts to move, but this is not a malfunction.

The stage may shake slightly when it moves, but this is not a failure.

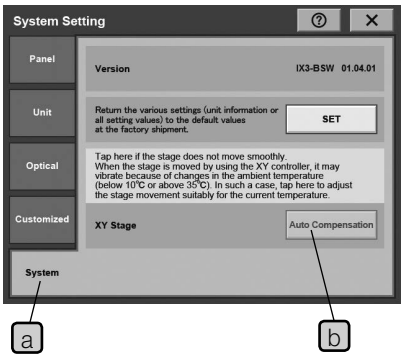
Note
- **Depending on the objective types or focusing positions, the objective may interfere the stage or the specimen holder.**
- **Particularly, when using the round center plate holder provided with the system, if you move the stage until the objective is placed outside the round center plate holder, be careful that the objective will interfere the stage.**

3 Compensating the operating parameters (Maintenance)

You can compensate the stage operating parameters. If the stage operating parameters are shifted after transportation or depending on the use environments, the movement may be unstable, such as a stage may not move smoothly, etc. In such a case, compensate the stage operating parameters.

Note **Perform the auto compensation while the specimen, etc. is not on the stage.**

TIP While executing the standard image acquisition / analysis software for FV3000 FV31S-SW, the XY compensated value of the ultrasonic stage for IX3 which was set by the touch panel controller is disabled.



1. Operate the touch panel controller to open the [MENU] screen.
2. Tap the [System Setting] button to open the [System Setting] screen.
3. Tap the [System] tab a and tap the [Auto Compensation] button b.
4. The screen to confirm the auto compensation appears. Tap [OK] to start the auto compensation.

Caution **During auto compensation, the stage moves automatically (for several minutes) . Do not touch the stage while the stage is moving.**

5. When the auto compensation is completed, the completion message appears on the touch panel screen.

Note **If a foreign substance is in the stage drive unit, the phenomenon may not be improved by the auto compensation. In this case, set the main switch of the controller for ultrasonic stage to ○ (OFF) . Then, move the stage manually in front/back/right/left directions for 10 reciprocations each within the full movable range and perform the auto compensation again.**

TIP Setting the center plate to be combined to the touch panel controller allows you to set the YX movement range according to the center plate. For detail setting procedures, see Help of the touch panel.

4 Cleaning the drive units (Maintenance)

When using this stage for a long time period, the stage movement may be deteriorated due to the wear of the ultrasonic drive unit. Clean the units as follows on regular basis.

Cleaning method :

· Set the main switch of the controller for ultrasonic stage to O (OFF) . Then, move the stage manually in front/back/right/left directions for 10 reciprocations each within the full stage movable range taking one second for one reciprocation.

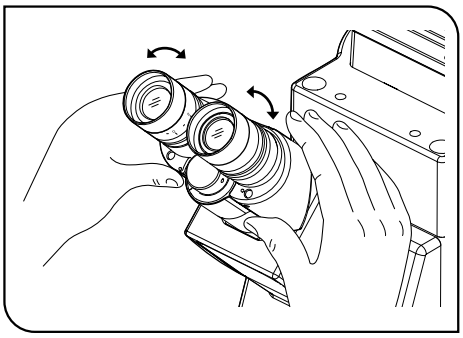
Cleaning interval :

· Approx. once a year (depends on frequency of use)

TIP You can perform the auto cleaning on the standard image acquisition / analysis software for FV3000 FV31S-SW. For details, refer to Online Help of the standard image acquisition / analysis software for FV3000 FV31S-SW.

4-4 Observation tube (Operation)

1 Adjusting the interpupillary distance



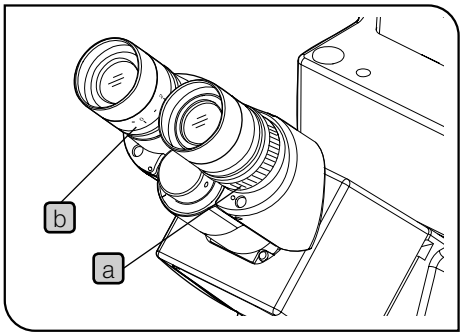
While looking through the eyepieces, move the binocular section until the left and right fields of view coincide completely. The index · indicates the interpupillary distance.

TIP Take a note of your interpupillary distance so that it can be quickly adjusted next time.

2 Adjusting the diopter

TIP The diopter adjustment makes it possible to reduce the specimen focusing error even after the objective is switched. As the diopter differs depending on individuals, the diopter adjustment is required for each person.

Note **The eyepiece with diopter adjustment ring should always be inserted into the observation tube without the diopter adjustment ring.**



1. Set the both diopter adjustment rings to the scale "0".
2. Engage a high-magnification objective (e.g. 40X) in the light path, look into the right eyepiece with your right eye, and bring the specimen into focus using the FOCUS button/slider of the touch panel controller.

TIP Do not use an immersion objective.

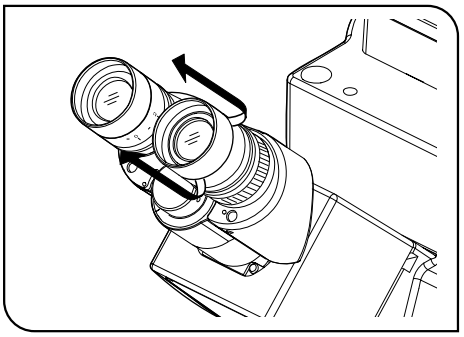
3. Engage a low-magnification objective (e.g. 10X) in the light path, rotate only the right diopter adjustment ring [a] to bring the specimen into focus. At this time, do not touch the FOCUS button/slider.
4. Looking into the left eyepiece with your left eye, rotate only the left diopter adjustment ring [b] to bring the specimen into focus.

TIP The above procedure adjusts the diopter with reference to the right eye, but it is also possible to adjust with reference to the left eye. In this case, follow the above procedure by inverting "right" and "left".

Using an eyepiece with micrometer

1. Looking through the eyepiece with micrometer, rotate the diopter adjustment ring [b] so that the micrometer in the field of view is sharply visible.
2. Looking through the eyepiece with micrometer, bring the specimen into focus using the FOCUS button/slider of the touch panel controller so that both the micrometer and specimen are sharply visible.
3. Looking through the other eyepiece, rotate only the diopter adjustment ring [a] to bring the specimen into focus.

3 Using the eye shades



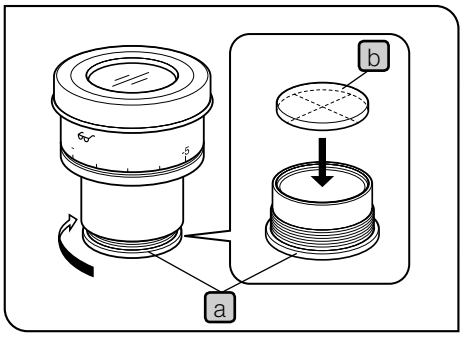
When wearing eyeglasses

Use the eye shades in the folded-down position.
(This will prevent the eyepieces from getting scratched by the contact with eyeglasses.)

When not wearing eyeglasses

Extend the folded eye shades in the arrow direction to prevent the extraneous light from entering between the eyepieces and eyes.

4 Using the eyepiece micrometer



The eyepiece micrometer can be attached to the eyepiece for UIS2 observation WHN10X-H. Use the Ø24 mm, 1.5 mm thick micrometer.

To attach the micrometer, rotate the built-in micrometer-mounting frame [a] in the arrow direction (see the picture) to remove it from the eyepiece, and place a micrometer [b] into the micrometer-mounting frame so that the surface with the model indication faces down.

Re-attach the micrometer-mounting frame in the original position.

TIP
- The micrometer-mounting frame may be too tight for some micrometers. In this case, if you grasp the micrometer-mounting frame with a strong force, the frame may be deformed and harder to be removed. Therefore, rotate the frame by holding the circumference with a light and uniform force or by applying the frame against a rubber sheet to remove it.
- Be careful not to touch the lens or micrometer surface with your fingers.

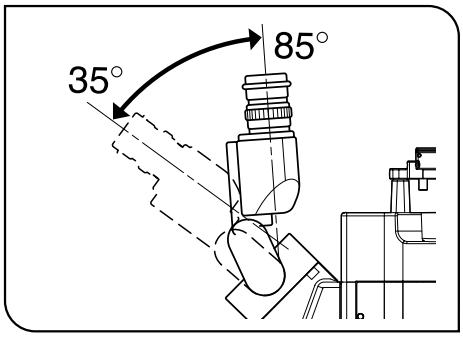
5 Selecting the light path of the trinocular tube

Slide the light path selector knob to select the desired light path.

(ex.) Trinocular tube U-TR30-2

Light path selector knob position		
Pushed in	Middle position	Pulled out
Eyepiece 100%	Eyepiece 20% Camera port 80%	Camera port 100%

6 Adjusting the tilt (Tilting widefield observation tube U-TBI90 only)



TIP Adjust the height and tilt of the eyepieces to obtain the most comfortable viewing position.

Holding the binocular section with both hands, adjust it to the desired position.

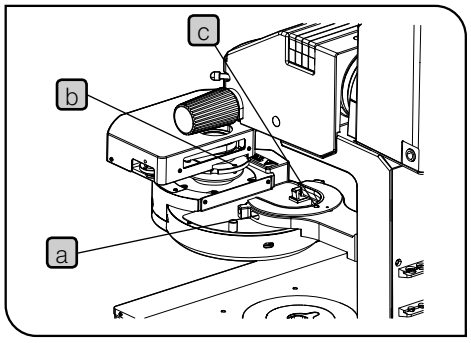
Note **Never attempt to apply excessive force to the binocular section beyond the upper or lower limit position. Applying the excessive force could damage the limiting mechanism.**

TIP If the U-TBI90 is used together with any type of intermediate attachment, the vignetting appears around the field of view.

4-5 Condenser (Operation / Maintenance)

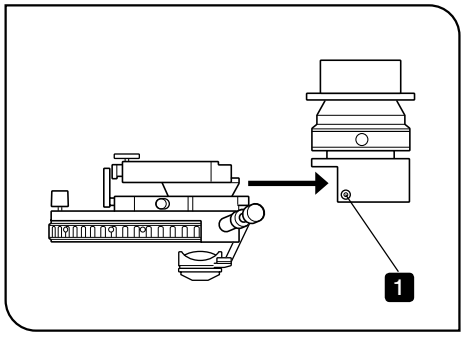
⚠CAUTION **Be sure to check the laser emission status before starting to work. For details, see page 4. Working with the condenser is dangerous because you work near the laser aperture.**

1 Attaching the condenser (Maintenance)



Motorized LWD condenser IX3-LWUCDA
ULWCD condenser IX2-LWUCD
ULWCD condenser IX-ULWCD
Medium distance condenser IX2-MLWCD

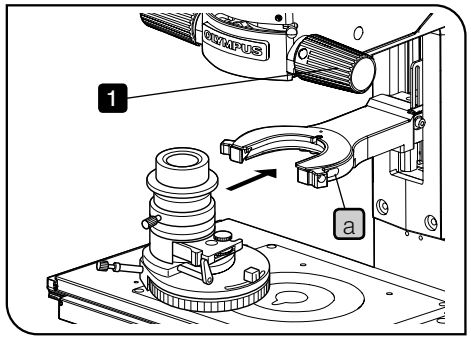
1. Loosen the condenser clamping screw [a] with the Allen screwdriver provided with the system.
2. Insert the condenser into the mount dovetail on the condenser holder, and push it horizontally until its positioning pin [b] fits into the positioning groove [c] on the mount dovetail.
3. Tighten the condenser clamping screw [a] with the Allen screwdriver provided with the system.



Universal condenser U-UCD8

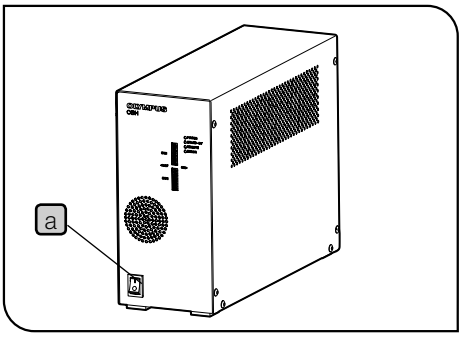
[Attaching to the UCD adapter IX-ADUCD]

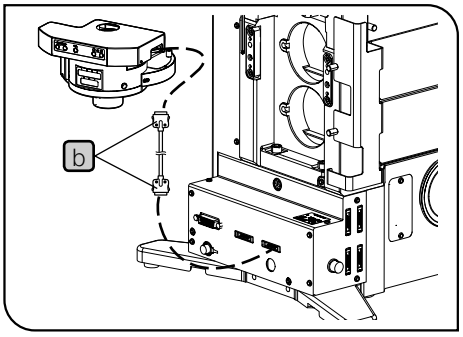
1. Loosen two setscrews on the UCD adapter IX-ADUCD using the Allen screwdriver provided with the system.
2. Push in the universal condenser U-UCD8 horizontally into the adapter until the condenser's positioning pin fits into the positioning groove on the mount dovetail.
3. Tighten two setscrews on the UCD adapter IX-ADUCD using the Allen screwdriver provided with the system.



[Attaching to the transmitted illuminator column IX3-ILL]

1. Raise the condenser height adjustment knob to the upper limit position.
2. Loosen the condenser clamping screw [a] with the Allen screwdriver provided with the system.
3. Insert the mount section of the UCD adapter IX-ADUCD into the mount dovetail on the condenser holder (the condenser turret is positioned on the right side) . Push the UCD adapter horizontally into the condenser holder until the condenser's positioning pin fits into the positioning groove on the mount dovetail.
4. Tighten the condenser clamping screw [a] with the Allen screwdriver provided with the system.





Motorized LWD condenser IX3-LWUCDA

[Connecting to the IX83 2 port frame IX83P2ZF]

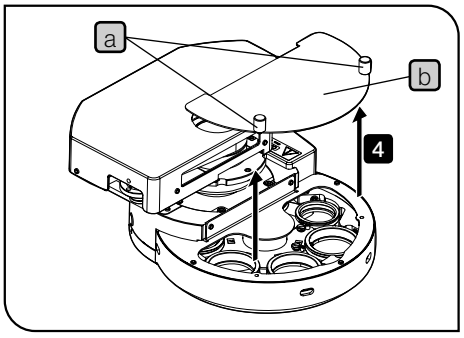
Note
- **Be sure to set the main switch a of the control box IX3-CBH to O (OFF) before connecting the condenser.**
- **Do not connect the connector b to those other than the IX83 2 port frame IX83P2ZF and the motorized LWD condenser IX3-LWUCDA.**
  When connecting the connectors, align the orientation of the connectors and insert them securely.

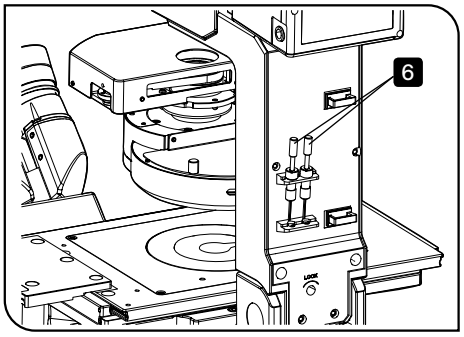
2 Attaching optical elements (Maintenance)

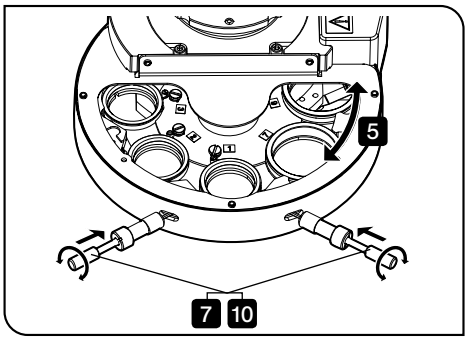
l Attaching phase contrast optical elements

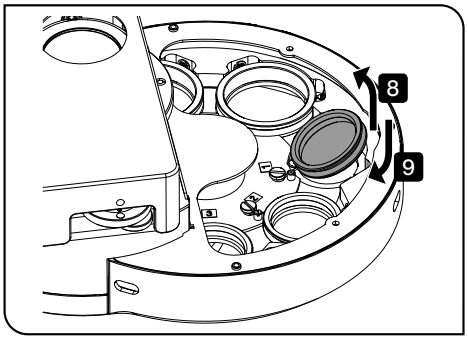
Motorized LWD condenser IX3-LWUCDA

TIP
- As a factory default, the dummy element is in the turret hole. Keep the dummy element in a safe place because it will be used again when the microscope is transported next time.
- In general, the turret No.[7] is defined as the bright field (BF) position, and the dummy element remains in the turret hole.









1 Turn OFF the power in the sequence of the touch panel controller, control box IX3-CBH and control unit.

2 Remove the connector of the condenser from the connector of the back side of the IX83 2 port frame IX83P2ZF.

3 Remove the condenser from the column. (page 24)

4 Loosen two clamping screws a and remove the top cover b.

5 Rotate the turret so that the turret holes to insert the optical elements come to the center.

6 Take out the condenser centering knobs (2 pcs) from the storage area on the back side of the transmitted illuminator column IX3-ILL.

7 Insert the condenser centering knobs, and loosen the optical element clamping screw while pushing the knob.

8 Hold a phase contrast ring (the dummy element when using this system for the first time) , and while lightly pushing it toward the direction where the spring inside the turret is attached, hold the ring upward slowly and remove it from the opposite side of the spring.

9 Attach the phase contrast ring to be used to the turret hole by pushing it to the spring inside the turret.

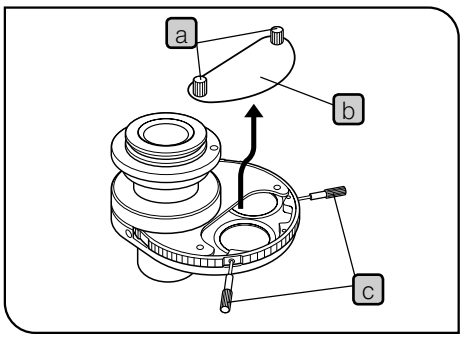
10 Tighten the optical element clamping screw using the condenser centering knobs.

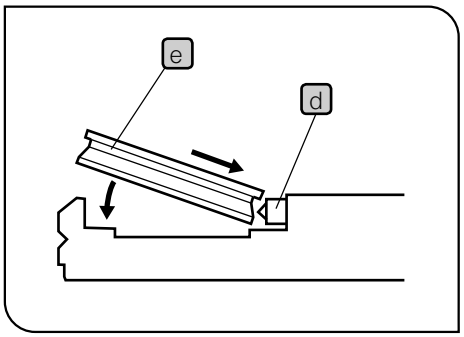
Note
- **Be careful not to apply pressure to the ring slit inside the frame.**
- **If the optical element centering knobs are attached, the turret cannot be rotated.**
- **If the optical element clamping screws are loosened, the clamping screws will interfere while rotating the turret. Be sure to tighten the screws firmly.**
  Do not tighten the optical element centering knobs too firmly. The frames of optical elements may be deformed.
- **When using the motorized LWD condenser IX3-LWUCDA, make sure to insert the dummy elements in the turret holes where no optical elements are engaged. If the dummy elements are not inserted, the centering screws in the turret holes may drop off inside of the turret by the vibration of continuous rotation of the turret.**

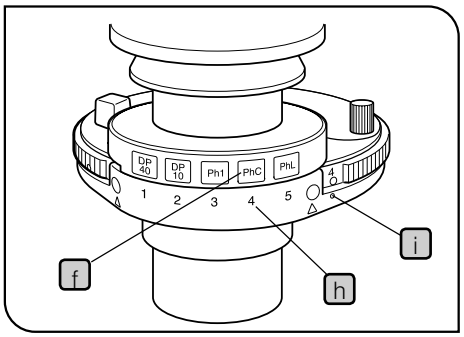
11 When all of the required optical elements have been mounted, attach the cover and tighten the detachable screws.

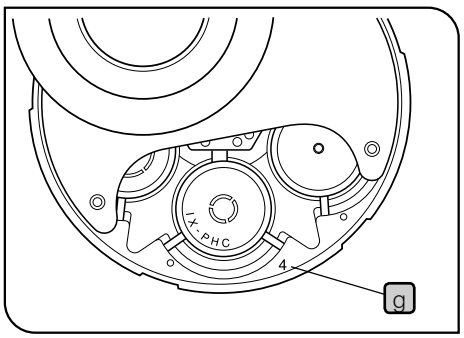
12 Attach the condenser to the column and connect the connector of the condenser to the connector on the back side of the IX83 2 port frame IX83P2ZF.

13 Turn ON the powers in the order of the control unit, control box IX3-CBH and touch panel controller. (The turret position of the condenser will be initialized.)









ULWCD condenser IX3-LWUCDA
ULWCD condenser IX LWD

TIP Do not engage any optical element in the brightfield (BF) light path.

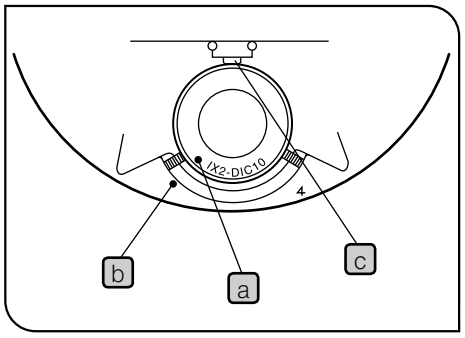
1. Place the condenser in a direction as shown in the picture, loosen two clamping screws [a] and remove the top cover [b].
2. Rotate the turret so that the number of the next optical element to be inserted in the uncovered position is visible.
3. Loosen the optical element position centering screws by rotating it counterclockwise using the optical element centering knobs [c].
4. While pushing the spring [d] inside the turret with the edge of the phase contrast ring [e], insert the ring completely until the ring contacts the bottom of the turret hole.
5. Rotate the optical element centering knobs clockwise to tighten the centering screws lightly.
6. Place the index [f] provided with each optical element in the index insertion hole having the same number [h] as the number [g] of the hole in which the corresponding optical element is mounted. (The ULWCD condenser IX-ULWCD does not have the index.)

TIP The optical element with the number indicated by the mark [i] is engaged in the light path.

7. When all of the required optical elements have been mounted, attach the cover and tighten the detachable screws.

Note
- **Be careful not to apply the pressure to the ring slit inside the frame.**
- **If the optical element centering knobs are attached, the turret cannot be rotated.**
- **If the optical element clamping screws are loosened, the clamping screws will interfere while rotating the turret. Be sure to tighten the screws firmly.**
  Do not tighten the optical element centering knobs too firmly. The frames of optical elements may be deformed.
- **To remove an optical element index, use the tip of a ball-point pen or mechanical pencil.**

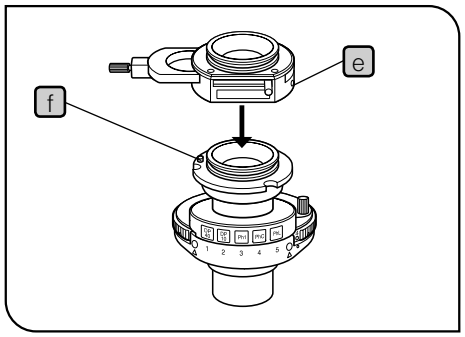
ii Attaching DIC optical elements



TIP
- The attaching method is same as that for the phase contrast optical elements (page 26) , except that the positioning pin and positioning groove should be aligned when attaching the DIC optical element.
- Align the positioning index [a] on the DIC prism with the positioning index [b] on a turret hole. And insert the DIC prism completely until it touches the bottom of the turret hole so that the positioning pin of the DIC prism fits into the pin hole securely. When inserting the DIC prism , push the spring [c] inside the turret slightly sideward.

Note
- **Be careful not to touch the DIC prism inside the frame.**
- **Do not tighten the optical element centering knobs too firmly. The frame of optical elements may be deformed.**
- **Before replacing the optical element of the motorized condenser, be sure to set the power of the control box IX3-CBH to ○ (OFF) . If the optical element is replaced while the power is set to | (ON) , restart the microscope system.**

III Attaching the polarizer IX-LWPO

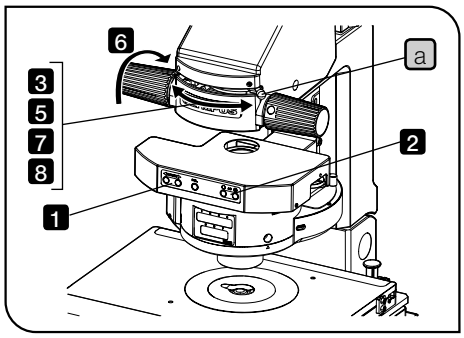


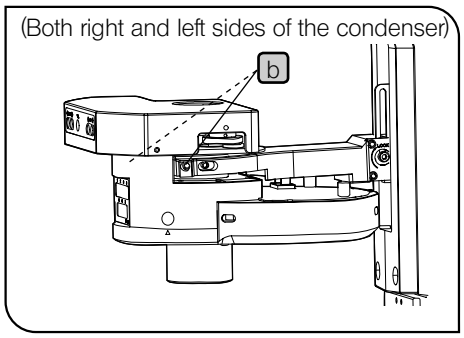
Note **This polarizer can be mounted only on the ULWCD condenser IX2-LWUCD.**

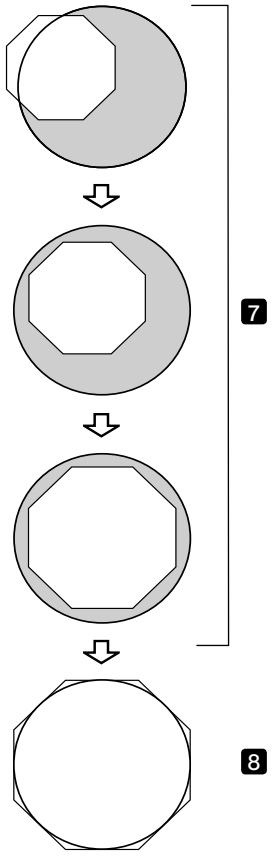
TIP This polarizer is used in combination with the ULWCD condenser IX2-LWUCD for the DIC observation or simplified polarizer light observation.

1. Loosen the polarizer clamping screw [e] using the Allen screwdriver provided with the system.
2. Align the pin hole on the polarizer with the positioning pin [f] on the condenser, and insert the polarizer unit into the upper part of the condenser.
3. Tighten the polarizer clamping screw firmly.

3 Centering the condenser (Operation)







Motorized LWD condenser IX3-LWUCDA
ULWCD condenser IX3-LWUCDA
ULWCD condenser IX LWD
Medium distance condenser IX2-MLWCD

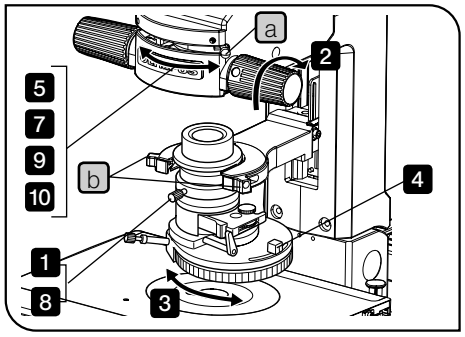
1. Rotate the turret to select the brightfield observation "BF" (where no optical element is engaged in the light path) .
2. Open the aperture diaphragm fully.
3. Set the field diaphragm lever [a] to the fully open position (◎→○) .
4. Use the 10X objective to bring the specimen into focus.
5. Use the field diaphragm lever [a] to narrow down the diaphragm until the field diaphragm image comes into the field of view.
6. Slowly move the condenser height fine adjustment knob to bring the field diaphragm image into focus.
7. While gradually moving the field diaphragm lever to open the field diaphragm [a], insert the Allen screwdriver provided with the system in the adjustment hole [b] and rotate it so that the field diaphragm image comes to the center of the field of view of the eyepieces.
8. Open the field diaphragm gradually until its image inscribes the field of view. Now the condenser is centered.

TIP In actual observation, open the field diaphragm until its image circumscribes the field of view.

Effect of field diaphragm

This is a diaphragm to adjust the area to be illuminated. By narrowing down the diaphragm to the level circumscribing the field of view depending on objectives, the excess light can be blocked to acquire the image with good contrast.

TIP Depending on the condenser to be used, the field diaphragm may not be visible with the 40X or higher objective.



Universal condenser U-UCD8

1 Rotate the condenser height fine adjustment knob counterclockwise to loosen it, and push the knob all the way toward the rear side.

2 Rotate the condenser height adjustment knob in the arrow direction to lower the condenser to the level not to contact the specimen.

3 Rotate the turret to select the brightfield observation "BF" (where no optical element is engaged in the light path) .

4 Use the aperture diaphragm lever to open the aperture diaphragm fully.

5 Set the field diaphragm lever [a] to the fully open position (⊗→○) .

6 Use the 10X objective to bring the specimen into focus.

7 Use the field diaphragm lever [a] to narrow down the diaphragm until the field diaphragm image comes into the field of view.

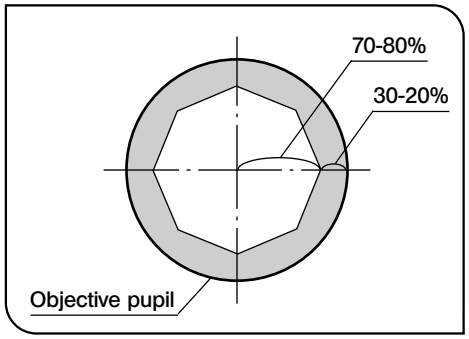
8 Slowly move the condenser height fine adjustment knob to bring the field diaphragm image into focus. When the image is brought into focus, rotate the knob clockwise to clamp it.

9 While gradually moving the field diaphragm lever to open the field diaphragm [a], insert the Allen screwdriver provided with the system in the adjustment hole [b] and rotate it so that the field diaphragm image comes to the center of the field of view of the eyepieces.

10 Move the field diaphragm lever to open the field diaphragm [a] gradually until its image inscribes the field of view. Now the condenser is centered.

TIP
- In actual observation, open the field diaphragm until its image circumscribes the field of view.
- Before replacing the specimen or dropping the immersion oil, use the condenser height adjustment knob to raise the condenser first. When it is finished, lower the condenser to its lowest position.

4 Using the aperture diaphragm (Operation)

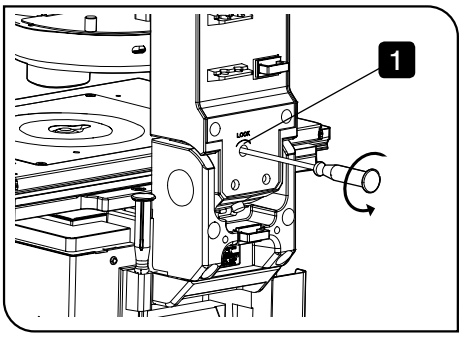


TIP
- In general, the resolving power of an objective is fully utilized if the diaphragm is narrowed down to the diameter corresponding to the numerical aperture (NA) of the objective.
- Depending on specimens, the aperture diaphragm is narrowed down to increase the image contrast or deepen the focal depth in observation or acquisition of images.
  With a general specimen, it is appropriate to narrow down the aperture diaphragm to approximately between 70% and 80% of the numerical aperture of the objective, but you can narrow it down furthermore as needed.
- To check the perimeter of the aperture diaphragm, remove the eyepieces and look into the eyepiece sleeves to view the aperture diaphragm image and the objective's exit pupil.

4-6 Transmitted illuminator column IX3-ILL (Operation / Maintenance)

1 Tilting the transmitted illuminator column IX3-ILL (Maintenance)

TIP
- When replacing the large specimen, setting the manipulator or replacing the patch electrode, tilting the transmitted illuminator column IX3-ILL secures the wider work space.
- Even though the transmitted illuminator column IX3-ILL is tilted, the LED lamp house IX3-LHLEDC can illuminate the specimen surface. Therefore, it is convenient for checking the specimen roughly or identifying the position to place the specimen.



1. Rotate the Allen wrench provided with the system to loosen the tilting clamping screw.
2. Hold the upper front part of the illumination unit and tilt the column slowly to the backmost position.

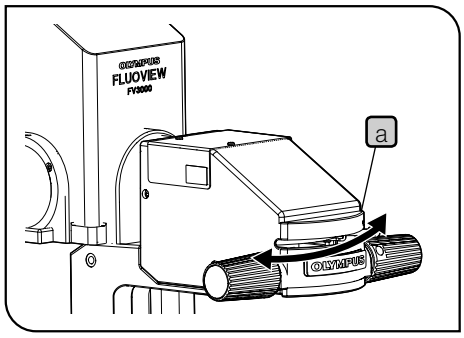
Note **Be sure to hold the column with your hand and tilt it slowly and carefully. When returning the column to the original position, do this in the same manner.**

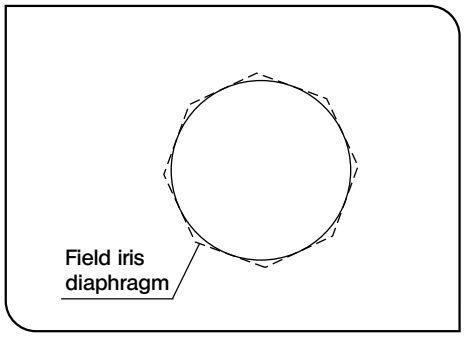
TIP Unless the transmitted illuminator column IX3-ILL is returned to the original position, the laser beam won't be emitted from the objective even though the laser unit is running.

⚠CAUTION
- **When tilting or returning the transmitted illuminator column IX3-ILL, be careful not to pinch your fingers between the hinge.**
- **Be sure to tighten the tilting clamping screws during observation.**
- **In general, keep the tilting clamping screws tightened during operation. If you need to loosen the clamping screw in using micriscope system, be careful not to allow the transmitted illuminator column IX3-ILL fall down unexpectedly.**
- **Do not stop tilting the transmitted illuminator column IX3-ILL in the middle of tilting. The transmitted illuminator column IX3-ILL may fall down to the front side or back side.**
- **Before moving or transporting the microscope system, be sure to tighten the tilting clamping screws in advance.**

Note **Do not turn ON the light source U-HGLGPS while the transmitted illuminator column IX3-ILL is being tilted.**

2 Using the field diaphragm (Operation)



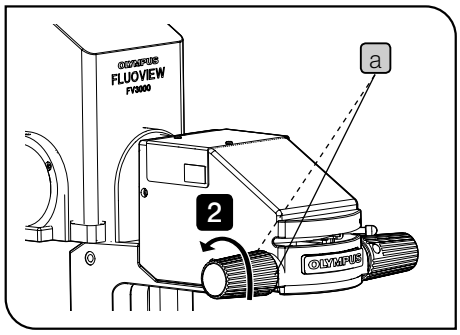


TIP The field diaphragm is a diaphragm to adjust the area to be illuminated. By narrowing down the diaphragm to the level circumscribing the field of view depending on objectives, the excess light can be blocked to acquire the image with good contrast.

1 Move the field diaphragm lever [a] to left or right to close or open the field diaphragm.

○ : Direction for opening the diaphragm
⊗ : Direction for closing the diaphragm

3 Adjusting the tension of the condenser height adjustment knob (Operation)

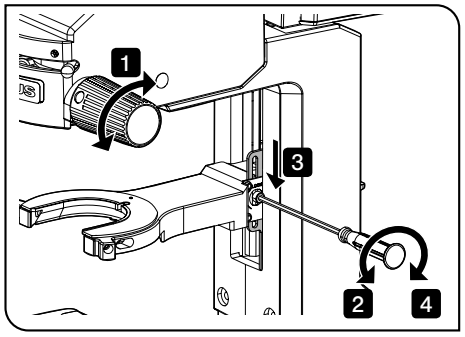


1 Loosen the two knob clamping screws [a] on the left adjustment knob using the Allen screwdriver provided with the system.

2 While holding the right adjustment knob not to rotate it, rotating the left adjustment knob in the arrow direction decreases the rotation tension and in opposite direction increases it respectively. Rotating the right adjustment knob allows adjusting the tension of the condenser height adjustment knob while checking it.

3 After adjustment, tighten the two knob clamping screws [a] securely.

4 Condenser refocusing stopper (Operation)



TIP The condenser refocusing stopper is a mechanism to return the condenser back to the original positon easily after moving the condenser.

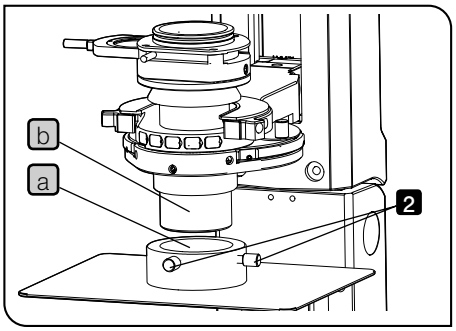
1. Rotate the condenser height adjustment knob to bring the field diaphragm image into focus.
2. Loosen the stopper clamping screw using the Allen screwdriver provided with the system.
3. Push the bottom of the stopper to the column securely.
4. Tighten the clamping screws of the stopper using the Allen screwdriver provided with the system.

Note **Rotating the condenser height adjustment knob beyond the condenser height adjustment area with an excessive force could damage the microscope system. Pay careful attention when rotating it.**

TIP If the manipulator is assembled to the column, the Allen screwdriver provided with the system may not be used in some cases.

4-7 Umbra shield for IX83 FV31-SPCOV (Operation / Maintenance)

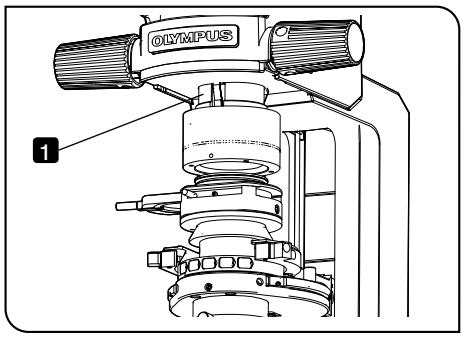
1 Attaching the specimen light cover (Operation)



1. Put the hole [a] of the specimen light cover into the condenser [b].
2. Tighten two fixing knobs of the specimen light cover.

Note **Be sure to tighten two fixing knobs completely.**

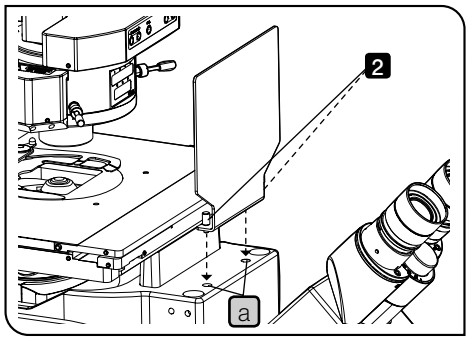
2 Attaching the condenser cover (Maintenance)



1. Put the condenser cover in the hole of the transmitted illuminator column IX3-ILL.

4-8 Antiglare plate (Maintenance)

1 Attaching the antiglare plate

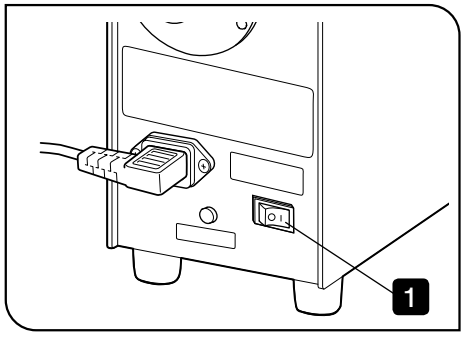


1 Remove the sticker attached to the antiglare plate mount hole a on the front side of the IX83 2 port frame IX83P2ZF.

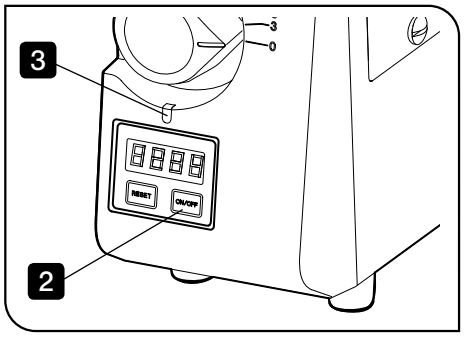
2 Screw in two fixing knobs to the antiglare plate mount hole a to attach the antiglare plate.

4-9 Mercury burner for the light source U-HGLGPS (Operation)

1 Turning ON the mercury burner



1 Set the main switch on the back side of the light source to / (ON) . The software version appears in the counter, and then (after approximately 3 seconds) , the accumulated lighting time of the mercury burner appears. When the accumulated lighting time of the mercury burner appears, each switch becomes operable.



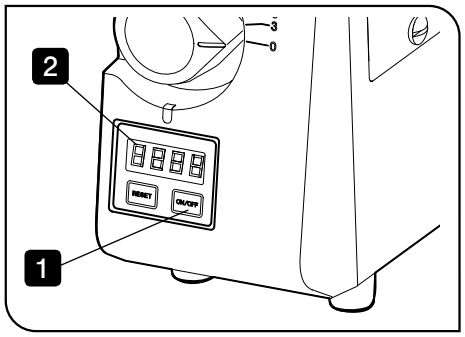
2 Press the lamp switch on the front side of the light source U-HGLGPS.

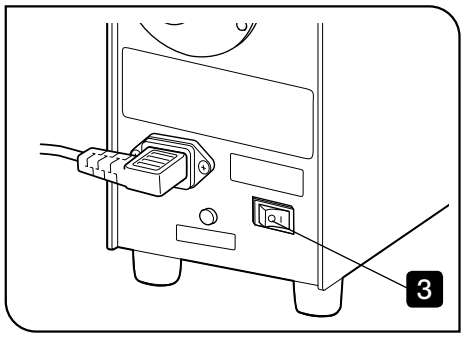
3 The pilot lamp turns ON in blue. If the mercury burner is not turned ON, the pilot lamp turns ON in red. In this case, see page 37.

TIP

- The light volume of the mercury burner stabilizes in approximately 90 seconds after it is turned ON. If you want to stabilize the light volume furthermore, wait approximately 5 minutes after it is turned ON.
- If you attempt to turn OFF the mercury burner within 2 minutes (by long-pressing the lamp switch) after it was turned ON , the pilot lamp blinks in blue. The mercury burner turns OFF passing 2 minutes after it was turned ON, and the pilot lamp stops blinking in blue.

2 Turning OFF the mercury burner





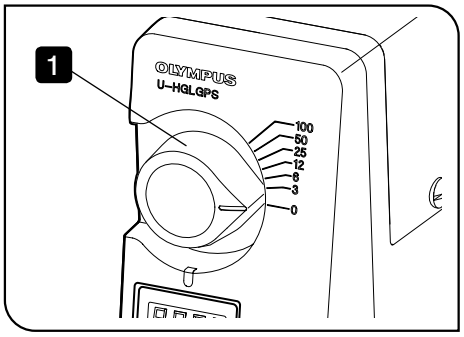
1 Long-press (0.5 seconds) the lamp switch on the front side of the light source U-HGLGPS to turn OFF the mercury burner.

2 After the mercury burner is turned OFF, the countdown of the cooling time (300 seconds) is displayed in the counter. Any operations by the buttons are disabled until the indication of the countdown becomes "0".

3 When the indication of the countdown becomes "0", set the main switch of the light source to O (OFF) .

TIP
- If you want to turn ON the mercury burner again, wait approximately 10 minutes after it was turned OFF. The mercury burner is heated immediately after it is turned OFF. If the mercury burner is turned ON while heated, the lifetime of the mercury burner may be shortened.
- The fan of the light source U-HGLGPS stops working automatically when passing 60 seconds after the lamp switch is long-pressed (0.5 seconds) .

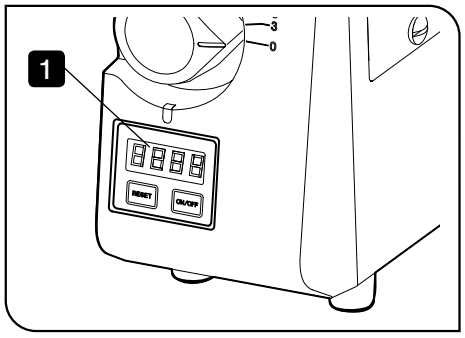
3 Adjusting the brightness



1 Rotate the light volume adjustment dial to adjust the light volume. The light volume can be adjusted in 7 levels. Each number indicates the percentage (0–100%) of each light volume where the maximum light volume is 100%.

TIP Rotate the light volume adjustment dial slowly by each level.

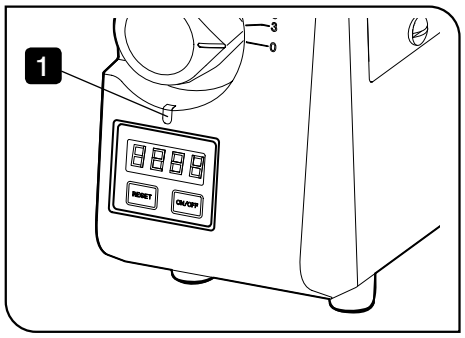
4 Counter display



1 When the main switch is turned ON, the accumulated lighting time of the mercury burner appears in the counter. For details, see the table below.

Operation	Display in counter
Immediately after the main switch is turned ON	The software version appears. (For 3 seconds)
When the mercury burner is in stand-by status (3 seconds after the main switch is turned ON)	The accumulated lighting time of the mercury burner appears. (Unit : 1 hour)
When the mercury burner is turned OFF (when the lamp switch is long-pressed)	The countdown of the cooling time (300 seconds) appears. (Unit : 1 second)
When the end of the lifetime of the mercury burner is coming closer.	When the accumulated lighting time of the mercury burner exceeds 1900 hours, the counter blinks every second while the mercury burner is being turned ON.
When the display is disabled due to abnormal EEPROM data	Err is displayed.

5 Pilot lamp

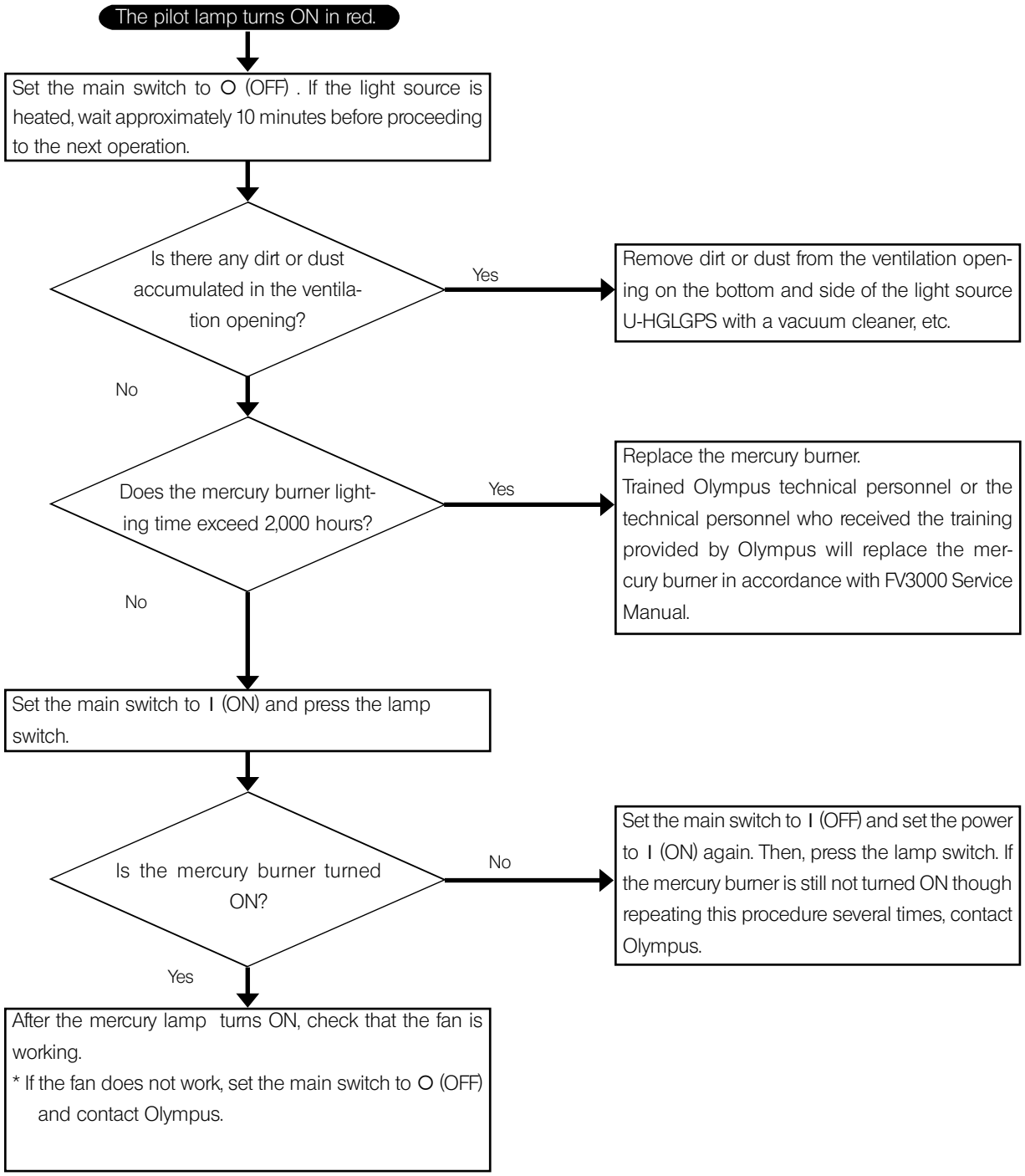


1 The mercury burner status appears on the pilot lamp. For details, see the table below.

Mercury lamp status	Pilot lamp display
In stand-by status	The pilot lamp turns OFF.
When the lamp switch is pressed and the mercury burner is turned ON properly.	The pilot lamp turns ON in blue.
The lamp switch is pressed, but the mercury burner is not turned ON.	The pilot lamp turns ON in red.
When the mercury burner is turned OFF.	The pilot lamp turns OFF.
When the lamp switch is long-pressed (0.5 seconds) within 2 minutes after the mercury burner was turned ON.	The pilot lamp blinks in blue. (When passing 2 minutes after the mercury burner was turned ON, the mercury burner is turned OFF and the pilot lamp also turns OFF.)
When the mercury burner is turned OFF abnormally while the mercury burner is being turned ON.	The pilot lamp turns ON in red.
When the temperature of the microscope frame is raised abnormally due to the deterioration of the cooling status, etc.	The pilot lamp turns ON in red.

When the pilot lamp turns ON in red

If the system does not work properly, e.g. the mercury burner is not turned ON, etc., the pilot lamp turns ON in red. Follow the procedures described below.



4-10 Compensation function of the Z drift compensator IX3-ZDC2 (Operation)

TIP
- Various settings of the Z drift compensation function are made by the touch panel controller or the standard image acquisition / analysis software for FV3000 FV31S-SW. For details, see respective Help.
- This manual describes the setting procedures using the touch panel controller.
- Among compensation functions of the Z drift compensator IX3-ZDC2 set by the touch panel controller, following three information are overwritten by the information of the standard image acquisition / analysis software for FV3000 FV31S-SW.

  [Focus Limit Setting]

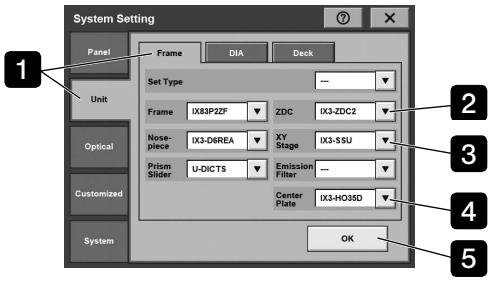
  [Coverslip Type]

  [Coverslip Thickness]
- For main switches in this system, the order to turn ON and OFF is specified. For details, see page 6.

1 Starting the system

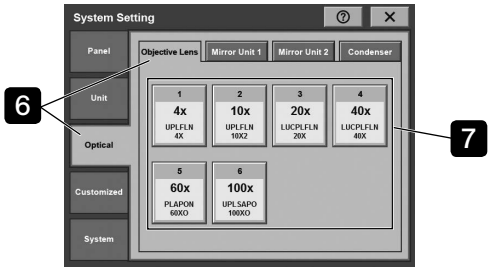
1. Set the main switch of the control box IX3-CBH to ON.
2. Set the main switch on the back side of the touch panel controller to ON. The [MENU] screen appears on the touch panel controller.
3. Tap [System Setting]on the [MENU] screen. The [System Setting] screen appears.

2 Setting the units



1. Tap the [Unit] tab and tap the [Frame] tab.
2. Tap the ▼ button in [ZDC] to select [IX3-ZDC2] from the list and tap it.
3. Tap the ▼ button in [XY Stage] to select the stage in use from the list and tap it.
4. Tap the ▼ button in [Center Plate] to select the specimen holder in use from the list and tap it.
5. Set other necessary units and tap the [OK] button.

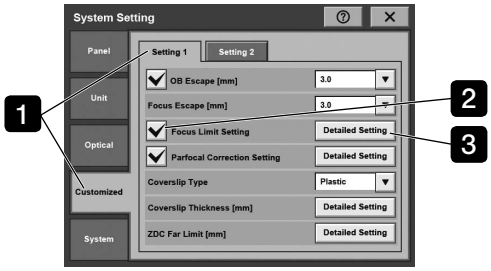
TIP If [XY Stage] and [Center Plate] are not set, you cannot use the recommended settings of [Focus Limit Setting] described later.

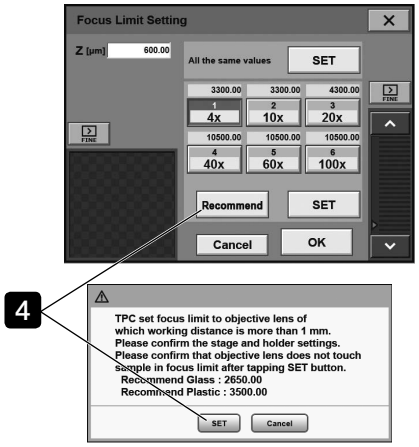


6. Tap the [Optical] tab and tap the [Objective Lens] tab.
7. Set the objective in use by each position of the revolving nosepiece.

TIP If the objectives in use are not set properly, you cannot use the Z drift compensation function properly.

3 Setting [Focus Limit Setting]





TIP The focus limit (upper limit position of the focus movement area) must be set by each objective in order to prevent the objective from touching the specimen during Z drift compensation.

1 Tap the [Customized] tab and tap the [Setting 1] tab.

2 Tap the [Focus Limit Setting] checkbox to tick it.

3 Tap the [Detailed Setting] button in [Focus Limit Setting]. The [Focus Limit Setting] screen appears.

Setting the objective using the recommended values

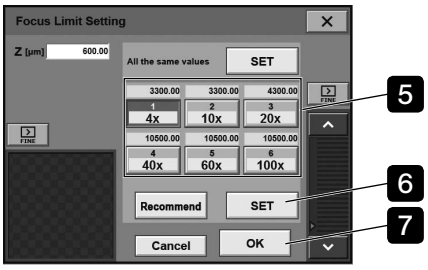
TIP When setting the units, if the ultrasonic stage for IX3 IX3-SSU in selected in [XY Stage] and holders other than the microplate holder IX3-HOW is selected in [Center Plate], you can set the focus limit of the objective with the working distance of 1mm or more to the recommended value easily. For details, see the table below.

4 Tap the [Recommend] button. Confirm the dialog box displayed and tap the [SET] button.
The focus limit of the objective with the working distance of 1mm or more is set to the recommended value.

Recommended focus limit values

Objective	Setting TPC [Center Plate]	Recommended focus limit values
UPLSAPO10X2 UPLFLN10X2 UPLFLN10X2PH UPLFLN20X UPLFLN20XPH UPLFLN40X UPLFLN40XPH	IX3-HO35D	2650µm
	IX3-HOS	2250µm
	---	3450µm
CPLFLN10XPH LUCPFLN20X LUCPLFLN20XPH LUCPFLN40X LUCPFLN40XPH LUCPFLN60X LUCPFLN60XPH	IX3-HO35D	3500µm
	IX3-HOS	2250µm
	---	4300µm

Setting the objective without using the recommended values



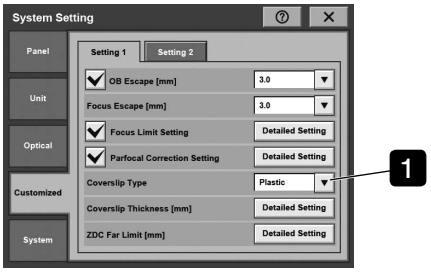
TIP For the objective to which the recommended value is not (cannot be) used, set the individual focus limit.

5 Tap the objective button.

6 Bring the specimen into focus. Then, move the focus position upward (direction in which the objective moves closer to the specimen) and tap the [SET] button. This focus position is defined as the focus limit.
Repeat from **5** to **6** for the number of times of objectives you want to set the focus limit.

7 When the focus limit is set, tap the [OK] button. The [Focus Limit Setting] screen will be closed.

4 Setting [Coverslip Type]

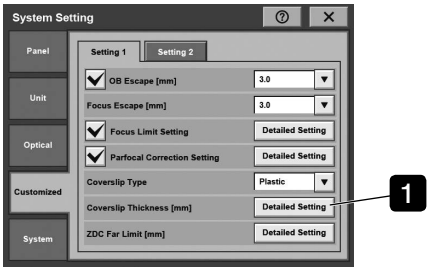


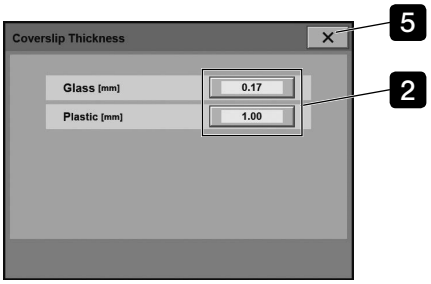
TIP This field is used to set the material (glass or plastic) of the coverslip of the specimen vessel you will use.

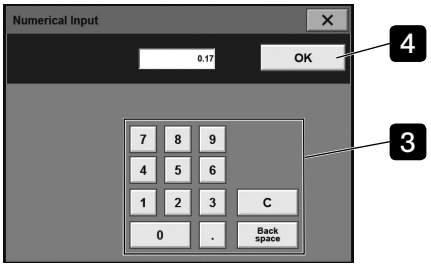
1 Tap the ▼ button of [Coverslip Type] to select [Plastic] or [Glass] from the list and tap it.

TIP If you select [Plastic] for the objective which is usable only with the glass coverslip of the specimen vessel, [Glass] is selected automatically.

5 Setting [Coverslip Thickness]







TIP This field is used to set the thickness of the coverslip of the specimen vessel you will use by each material (glass or plastic) .

1 Tap the [Detailed Setting] button in [Coverslip Thickness]. The [Coverslip Thickness] screen is displayed.

TIP On the [Coverslip Thickness] screen, the setting value (mm) of the thickness of the coverslip of the specimen vessel is displayed by each material (glass or plastic) .
The initial value has been set to 0.17 mm for glass and 1.00 mm for plastic as default. Change the setting values according to the specimen vessel you use.

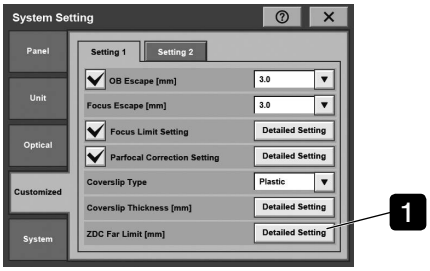
2 Tap the display area of the setting value you want to change. The [Numeric Input] screen appears.

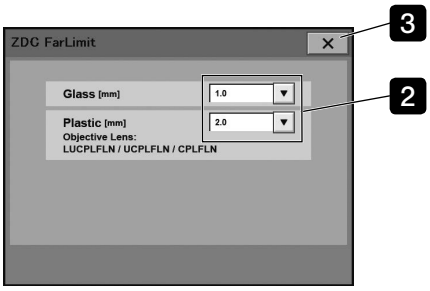
3 Tap the button to input the values and input the coverslip thickness. Tap the [C] button or the [Back space] button to cancel the input.

4 When values are input, tap the [OK] button. The [Numeric Input] screen is closed.

5 After the thickness of the glass and plastic coverslips are set, tap the [X] button on the [Coverslip Thickness] screen. The [Coverslip Thickness] screen is closed.

6 Setting [ZDC Far Limit]





TIP This screen is used to set the movement area to search the coverslip of the specimen vessel taking the focus limit as the upper limit position during Z drift compensation.
Setting this value smaller reduces the time required for the Z drift compensation, but the coverslip of the specimen vessel may not be found and the probability to fail in Z drift compensation may be higher.

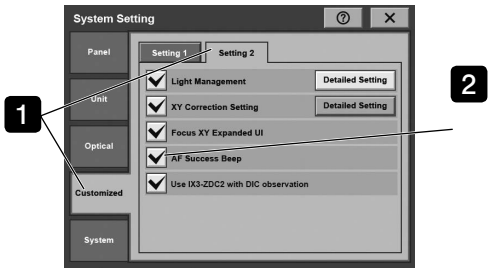
1 Tap the [Detailed Setting] button in [ZDC Far Limit]. The [ZDC Far Limit] screen appears.

TIP On the [ZDC Far Limit] screen, the setting value (mm) of the thickness of the coverslip of the specimen vessel is displayed by the coverslip material (glass or plastic) . The initial value has been set to 1.0 mm for glass and 2.0 mm for plastic as default.

2 Tap the ▼ button of the setting value you want to change, select the setting value and tap it.

3 After setting the values for glass and plastic, tap the [X] button on the [ZDC Far Limit] screen. The [ZDC Far Limit] screen is closed.

7 Setting [AF Success Beep]

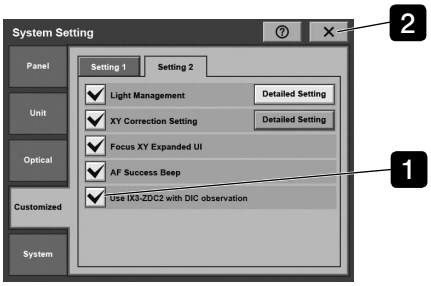


TIP This screen is used to set whether or not to beep the buzzer sound of the control box IX3-CBH when the Z drift compensation is successful.

1 Tap the [Customized] tab and tap the [Setting 2] tab.

2 Tap the [AF Success Beep] checkbox. Unchecking the checkbox will set not to beep the buzzer sound of the control box IX3-CBH when the Z drift compensation is successful.

8 Other settings and completing system settings



1 If you use the Z drift compensator IX3-ZDC2 for DIC observation, tap the [Use IX3-ZDC2 with DIC observation] checkbox to tick it.

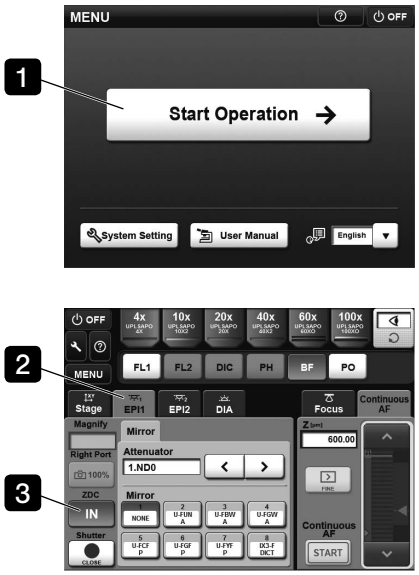
2 When all system settings are completed, tap the [X] button on the [System Setting] screen. The [System Setting] screen is closed and the [MENU] screen appears.

4-11 Observation using Z drift compensation (Operation)

⚠CAUTION

- **The Class1 laser beam is emitted from the objective. It is not dangerous, but do not look at the laser beam directly for a long time. (Even if the product is damaged, the strong laser beam will never be emitted.)**
- **Since the laser beam is an infrared light, it is not visible with your eyes. Do not look at the objective and around directly for a long time.**

1 Switching [ZDC IN]



1 Tap [Start Operation] on the [MENU] screen of the touch panel controller. The [Operation] screen appears.

2 Tap the [EPI1] tab or the [EPI2] tab.

3 Tap the [ZDC IN] button to change between ON/OFF of [ZDC IN].

TIP

- When [ZDC IN] is ON (the button color is blue and the text color is white) , the dichroic mirror of the Z drift compensator IX3-ZDC2 is engaged in the observation light path of the IX83 2 port frame IX83P2ZF and the Z drift compensation becomes available.
- When [ZDC IN] is OFF (the button color is white and the text color is gray) , the dichroic mirror of the Z drift compensator IX3-ZDC2 is removed from the observation light path of the IX83 2 port frame IX83P2ZF. If you do not use the Z drift compensation, set [ZDC IN] to OFF to enable the observation without the minor light volume loss of the observation light or the observation with the infrared light (790 nm or more) .

2 Adjusting the correction collar of objective

When using the objective with correction collar, adjust it as follows.

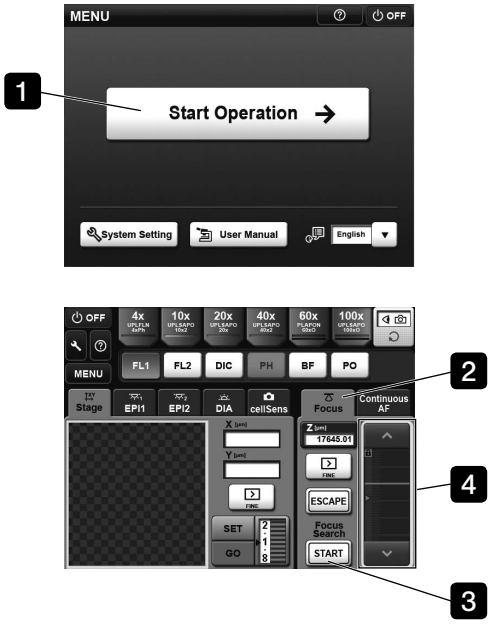
When the coverslip thickness of the specimen vessel is known :

Adjust the coverslip thickness value to the scale of the correction collar.

When the coverslip thickness of the specimen vessel is not known :

You can get the optimal position from the resolution contrast of the image. If the image is not clear even though it is focused, rotate the correction collar clockwise or counterclockwise to bring the image into focus in each direction and compare the image. Rotate the correction collar back to the direction where the image is clear. And rotate the correction collar clockwise or counterclockwise slightly to compare the image. Repeat this procedure a few times to get the optimal position.

3 Observation in focus search mode



1 Tap [Start Operation] on the [MENU] screen of the touch panel controller. The [Operation] screen appears.

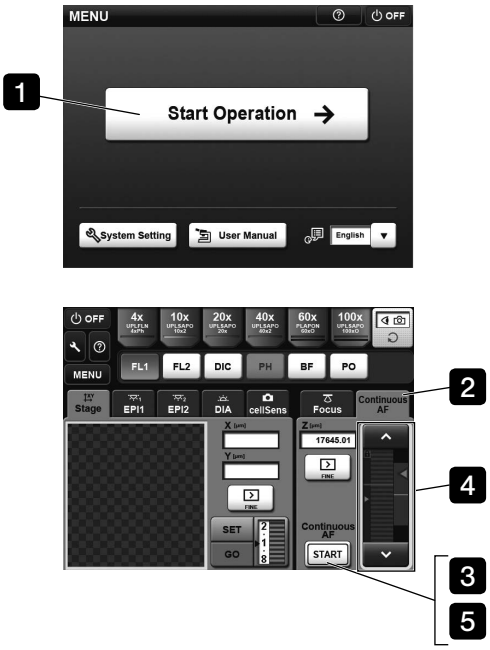
2 Tap the [Focus] tab.

3 Tap the [START] button. The Z drift compensation starts.

TIP
- In focus search mode, the Z drift compensation is completed when the coverslip of the specimen vessel is brought into focus.
- When the Z drift compensation is successful, the buzzer sound beeps once only if [AF Success Beep] is ticked. If the Z drift compensation is not successful, the buzzer sound beeps three times.

4 Move the focus position upward (direction in which the objective moves closer to the specimen) and bring the intended observation positon into focus.

4 Observation in continuous focus mode



1 Tap [Start Operation] on the [MENU] screen of the touch panel controller. The [Operation] screen appears.

2 Tap the [Continuous AF] tab.

3 Tap the [START] button. The Z drift compensation starts.

TIP
- In continuous focus mode, the Z drift compensation keeps working until the [START] button is tapped again.
- When the Z drift compensation is successful, the buzzer sound beeps once only if [AF Success Beep] is ticked. If the Z drift compensation is not successful, the buzzer sound beeps three times.

4 Move the focus position to bring the intended observation positon into focus.

5 Tap the [START] button. The Z drift compensation finishes.

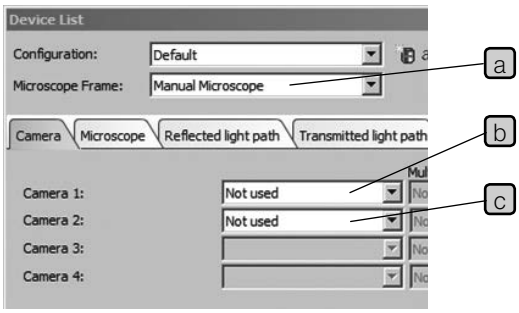
TIP In the next continuous focus mode, the focus position in the last continuous focus mode will be brought into focus. However, in the following cases, the coverslip of the specimen vessel is brought into focus.

- When the objective is changed
- When the focus search mode is executed
- When [Coverslip Type] is changed

4-12 Life science imaging software CellSens Dimension (Operation)

The life science imaging software CellSens Dimension and the standard image acquisition / analysis software for FV3000 FV31S-SW can be used simultaneously. In this case, make settings in the [Device List] dialog box of the life science imaging software CellSens Dimension as follows.

Reference For detail setting procedures, see Online Help of the life science imaging software CellSens Dimension.



	Device List	Selection item
a	Microscope Frame	[Manual Microscope]
b	Camera 1	[Not used]
c	Camera 2	[Not used]

Reference
- When using the life science imaging software CellSens Dimension alone, you can change some units configured with this system and can change the settings on [Frame] tab, [DIA]tab and [Deck] tab of the touch panel controller accordingly.
- When using the standard image acquisition / analysis software for FV3000 FV31S-SW if units are changed as above, return the changed units to the original configuration and change the settings on [Frame] tab, [DIA]tab and [Deck] tab of the touch panel controller accordingly. For details, see page i.

5 Optical units (by product group)

5-1 Optical units attached to the detector

CAUTION **The optical units must be attached and detached to and from the detector by Olympus technical personnel. Contact Olympus.**

TIP The optional dichroic mirror (DM) can be attached to the turrets in the spectral detector FV31-SD and the high sensitivity spectral detector FV31-HSD.

- FV31-RSDM560 : Reverse spectrum DM at 560nm

5-2 Optical units attached to the scan unit

CAUTION **The optical units must be attached and detached to and from the scan unit by Olympus technical personnel. Contact Olympus.**

TIP
- There are 2 types of the turret set of the dichroic mirror (DM) which can be attached to the scan unit FV31-SU and the hybrid scan unit FV31-HSU.
  - FV31-EXDM-M : ExDM turret set for standard 4LD system
  - FV31-EXDM-MS : ExDM turret set for optional 6LD system
- Only one turret set type can be attached to one scan unit.
- The following optional dichroic mirrors (DM) can be attached to both turret sets.
  - FV31-DM-VBY : Excitation DM for VBY lasers
  - FV31-DM-NE : Excitation DM for NE lasers
  - FV31-DM-NEO : Excitation DM for NEO lasers
  - FV31-DM-VBO : Excitation DM for VBO lasers
  - FV31-DM-VEO : Excitation DM for VEO lasers
  - FV31-DM-OPT : Fixing collor for optional excitation DM

5-3 Optical units attached to the mirror cassette

TIP You can create a fluorescent mirror unit with wavelength characteristics suitable for your needs using a commercially available dichroic mirror.

⚠CAUTION **The work to attach or detach the fluorescent mirror unit to or from this system falls under the service work.**
Trained Olympus technical personnel or the technical personnel who received the training provided by Olympus will attach or detach the fluorescence mirror unit to or from this system in accordance with FV3000 Service Manual.

	Optical unit name	Unit to which the created optical unit is attached
Fluorescent mirror unit	U-FF	Motorized fluorescent mirror turret FV30-RFACA

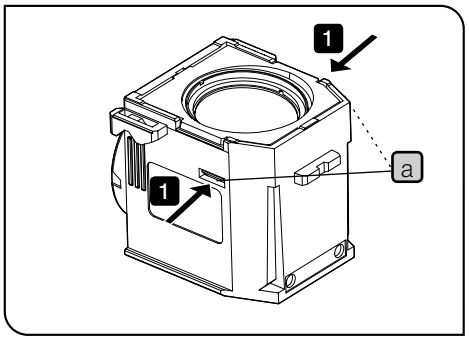
1 Assembling the fluorescent mirror unit U-FF (Maintenance)

TIP You can create an original fluorescent mirror unit by attaching the commercially available emission filter, excitation filter or dichroic mirror to the empty mirror unit frame U-FF.

⚠CAUTION
- **Combining with the incorrect filter may allow the UV light from the eyepiece to enter your eyes. Be careful when creating the original fluorescent mirror unit.**
- **Never use the original fluorescent mirror unit you created for LSM observation.**

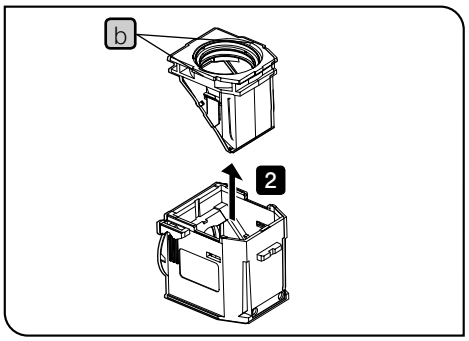
Dimensions of optical parts

	U-FF
Emission filter	Diameter : Ø24.8 to Ø25.0 mm Thickness : 2.5 to 6.0 mm
Excitation filter	Diameter : Ø24.8 to Ø25.0 mm Thickness : 2.5 to 6.0 mm
Dichroic mirror	Long side : 35.6 to 38.0 mm Short side : 25.2 to 26.0 mm Thickness : 0.9 to 1.4 mm

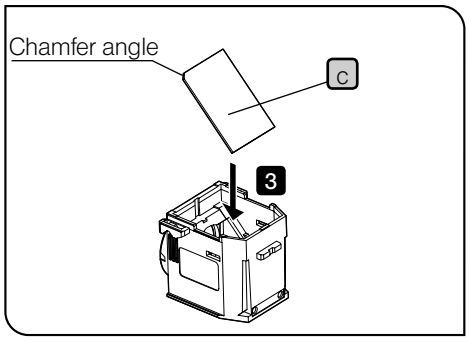


Attaching the dichroic mirror

1 Push in the tab [a] of the fluorescent mirror unit using the pointed object, e.g. tweezers, etc.

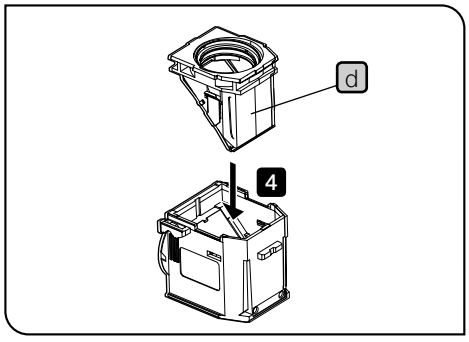


2 Hold the [b] part of the emission filter frame and pull it out upward.

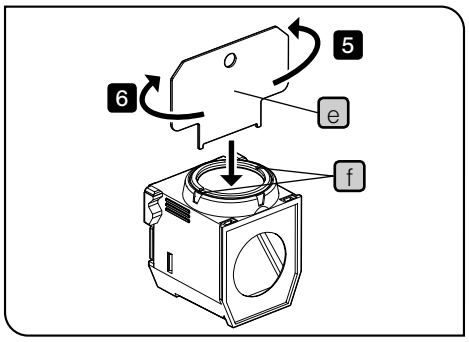


3 Place the dichroic mirror [c] on the dichroic mirror frame facing the reflective surface down (toward dichroic mirror frame) .

TIP
- When using an Olympus dichroic mirror, attach the dichroic mirror facing its chamfer angle toward the upper left (or lower right) direction.
- When using a dichroic mirror made by other companies, attach the dichroic mirror facing the reflective surface down.

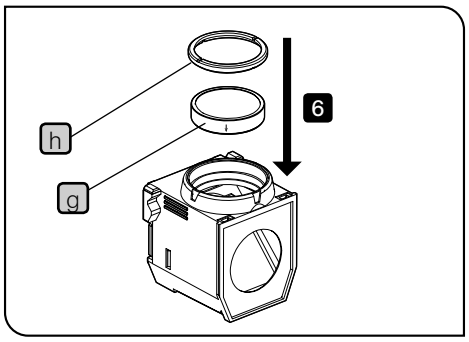


4 Return the emission filter frame [d].



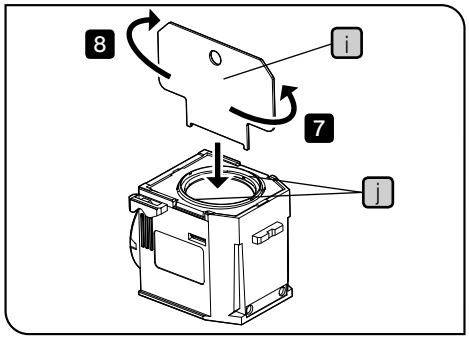
Attaching the excitation filter

5 Insert the holding ring screwdriver [e] in the groove [f] of the filter holding ring and remove the filter holding ring.



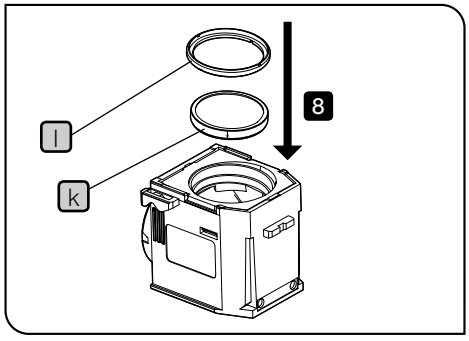
6 Attach the excitation filter [g] to the fluorescent mirror unit, return the filter holding ring [h], and attach it using the holding ring screwdriver [e].

TIP
- When using an Olympus excitation filter, place the filter so that the arrow mark (⇓) on the side faces the fluorescence mirror unit.
- When using an excitation filter made by other companies, follow the instruction manual of the respective excitation filter.



Attaching the emission filter

7 Insert the holding ring screwdriver [i] in the groove [j] of the filter holding ring and remove the filter holding ring.



8 Attach the emission filter [k] to the fluorescent mirror unit, return the filter holding ring [l], and attach it using the holding ring screwdriver [i].

TIP
- When using an Olympus emission filter, place the filter so that the arrow mark (⇓) on the side faces the fluorescence mirror unit.
- When using an emission filter made by other companies, follow the instruction manual of the respective emission filter.

6-1 Touch panel controller (Operation / Maintenance)

1 Basic operations of touch panel controller (Operation / Maintenance)

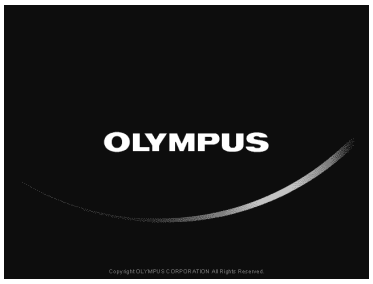
1 The touch panel controller can be operated by touching the displayed button directly with your fingertip.

TIP The touch panel controller uses a static capacitance type touch panel. Therefore, it may not react if it is touched by a finger wearing a rubber or vinyl glove. In this case, use a touch pen for a static capacitance type touch panel.

2 When you select the button, the selected button turns in blue to indicate that it is selected.

3 For the differences in the touch methods (tap, drag and hold) , refer to Online Help of the touch panel controller.
For procedures to display Online Help, see page 54.

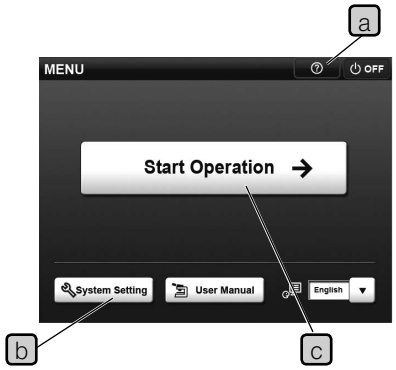
2 Starting the touch panel controller (Operation / Maintenance)



1 When the main switch of the touch panel controller is set to ON, the black startup screen appears (for approx. 20 seconds) and then, the blue startup screen appears.

TIP The order to turn ON the main switches of this system is specified. For details, see page 6.

TIP When the touch panel controller is in the stand-by mode (where the main switches of the control box IX3-CBH and touch panel controller are turned ON) . the touch panel controller starts in a blue screen.



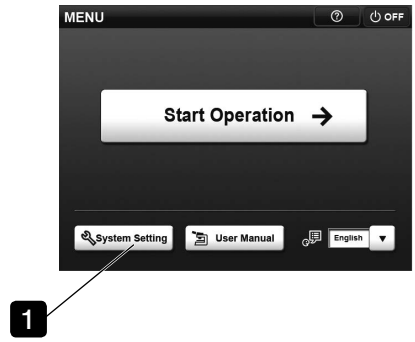
2 Tap the Online Help button a.
Before starting to operate the touch panel controller, read the following descriptions to understand how to operate the touch panel controller.

- Basic operations of the touch panel controller
- How to use this Online Help

Refer to the descriptions of functions as needed during actual operation.

TIP Only when you start the touch panel controller for the first time, Start Operation c cannot be selected unless System Setting b has been completed.

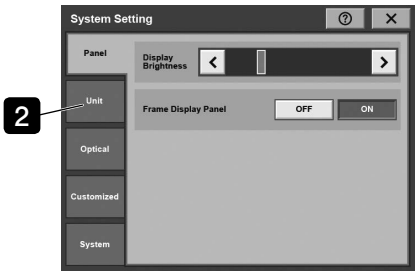
3 System setting (Maintenance)



The system setting is required when using the system for the first time or after replacing system units.

TIP The microscope system cannot be operated unless the unit setting and optical setting are completed. Be sure to set them.

1 Tap the System Setting button. The [System Setting] screen appears on the touch panel.



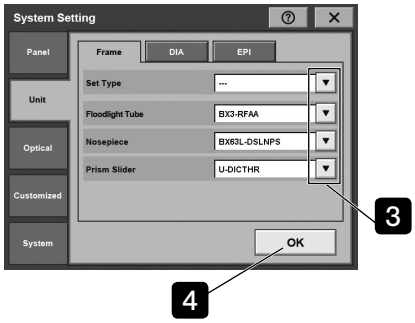
Unit setting

This tab is used to set the units connected to the IX83 2 port frame IX83P2ZF.

2 Tap the [Unit] tab on the [System Setting] screen to display the [Unit] screen.

3 Set the units connected to the IX83 2 port frame IX83P2ZF. Tapping ▼ marks of each field displays the unit names. Select the units in use by each field and tap them.

4 Tap the OK button to save the settings you made. If you want to make other settings, tap the tab for the desired setting. If you want to finish the setting, tap the [X] mark to close the [System Setting] screen.

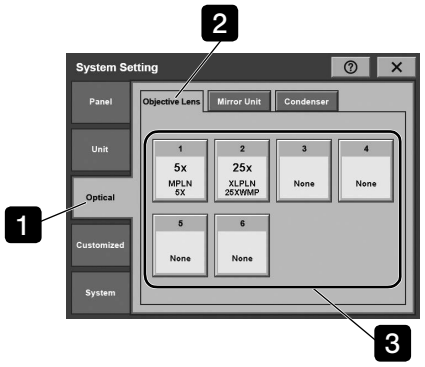


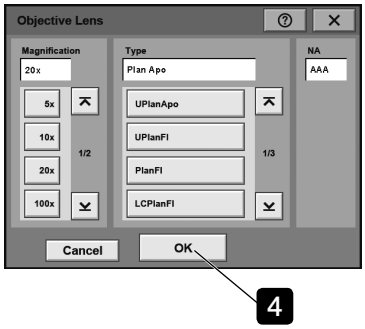
TIP Among settings made by the touch panel controller, following three information are overwritten by the information of the standard image acquisition / analysis software for FV3000 FV31S-SW.

Objective
Fluorescent mirror unit
Condenser lens

Optical setting

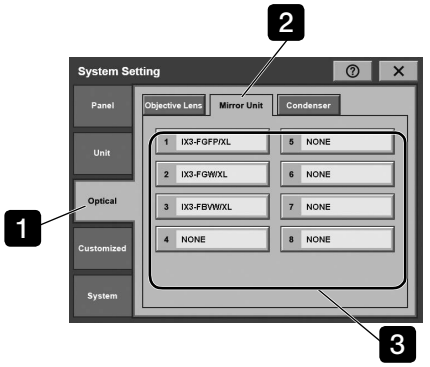
This tab is used to set the optical systems (objective, mirror unit and condenser) .

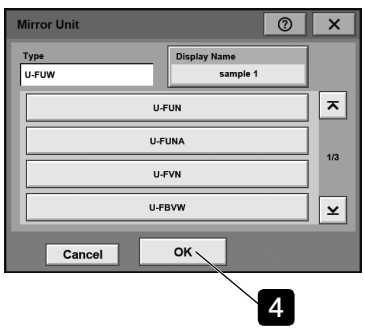




Setting the objective

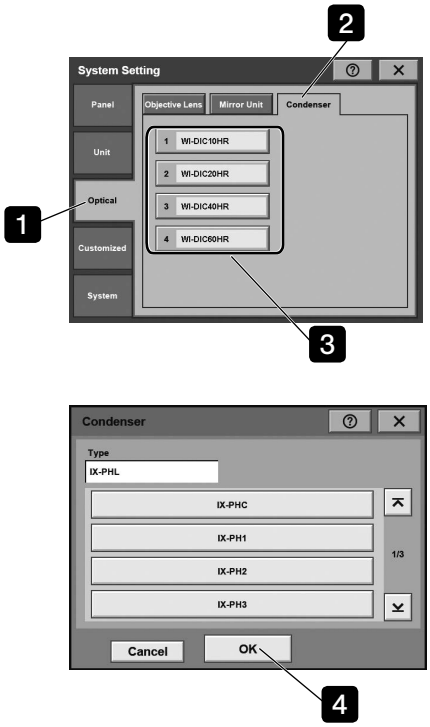
1. Tap the [Optical] tab on the [System Setting] screen to display the [Optical] screen.
2. Tap [Objective Lens] tab on the [Optical] screen.
3. Set the objective attached to the IX83 2 port frame IX83P2ZF for each revolving nosepiece hole.
   Tap the revolving nosepiece hole number in use to open the [Objective Lens] screen.
4. Select the magnification and type of the objective and tap the [OK] button to save the selected magnification and type.
5. In the same procedures, set the magnifications and types of objectives in other revolving nosepiece holes. No setting is required for the unused revolving nosepiece holes.
6. If you want to make other settings, tap the tab for the desired setting. If you want to finish the setting, tap the [X] mark to close the [System Setting] screen.





Setting the mirror unit

1. Tap the [Optical] tab on the [System Setting] screen to display the [Optical] screen.
2. Tap [Mirror Unit] tab on the [Optical] screen.
3. Set the mirror unit attached to the IX83 2 port frame IX83P2ZF for each mirror unit number. Tap the mirror unit number in use to open the [Mirror Unit] screen.
4. Select the mirror unit type and tap the [OK] button to save the selected mirror unit type.
5. In the same procedures, set other mirror unit numbers. No setting is required for the unused mirror unit numbers.
6. If you want to make other settings, tap the tab for the desired setting. If you want to finish the setting, tap the [X] mark to close the [System Setting] screen.



Setting the condenser

1. Tap the [Optical] tab on the [System Setting] screen to display the [Optical] screen.
2. Tap the [Condenser] tab on the [Optical] screen.
3. Set the optical elements attached to the condenser for each condenser turret number. Tap the turret number in use to open the [Condenser] screen.
4. Select the type of the optical elements and tap the [OK] button to save the selected type of the optical elements.
5. In the same procedures, set other turret numbers. No setting is required for the unused turret numbers.
6. If you want to make other settings, tap the tab for the desired setting. If you want to finish the setting, tap the [X] mark to close the [System Setting] screen.

Other system settings

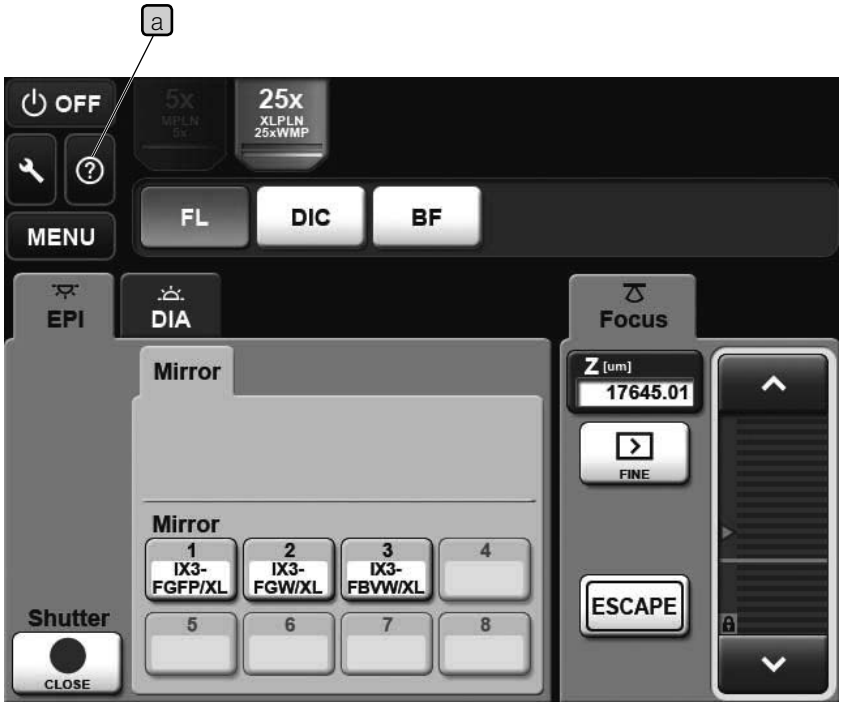
You can set the focus in System Setting. Set it if necessary.

For detail setting procedures, see Online Help of the touch panel controller.

4 Basic operations (Operation)

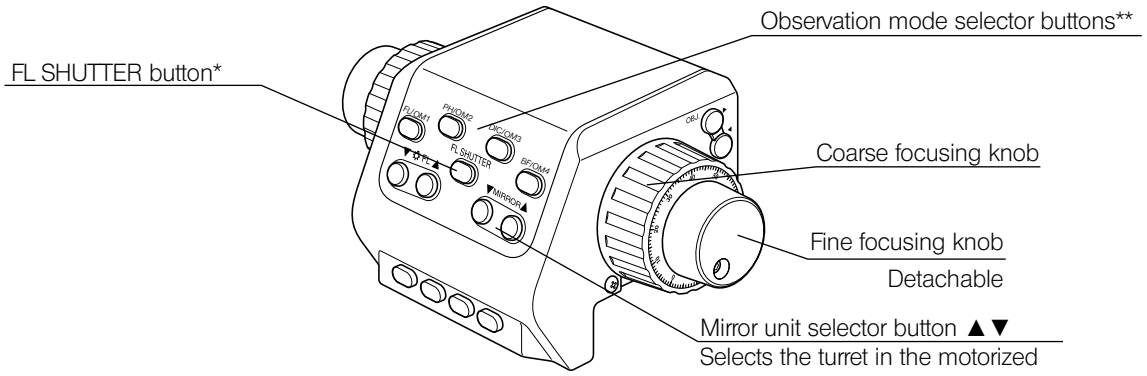
1 Tap [Start Operation] in the [MENU] screen to display the following screen.

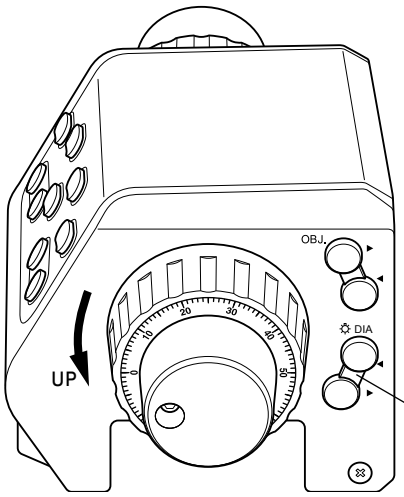
TIP
- For detail operating procedures of the touch panel controller during observation, see Online Help of the touch panel controller.
- To display the Online Help, tap Online Help button [a] on the touch panel.



6-2 Controller U-MCZ (Operation)

1 Nomenclature and functions





* The FL SHUTTER button opens/closes the reflected shutter only.

** Among 4 observation mode selection buttons, the FL button and the DIC button are enabled in conjunction with the standard image acquisition / analysis software for FV3000 FV31S-SW. If [Ocular] is selected in [Ocular] Tool Window on the standard image acquisition / analysis software for FV3000 FV31S-SW, you can select the transmitted light observation or the reflected light observation either by the controller U-MCZ or the standard image acquisition / analysis software for FV3000 FV31S-SW.

	Controller U-MCZ	Standard image acquisition / analysis software for FV3000 FV31S-SW
Transmitted light observation	DIC button	DIA button
Reflected light observation	FL button	EPI button

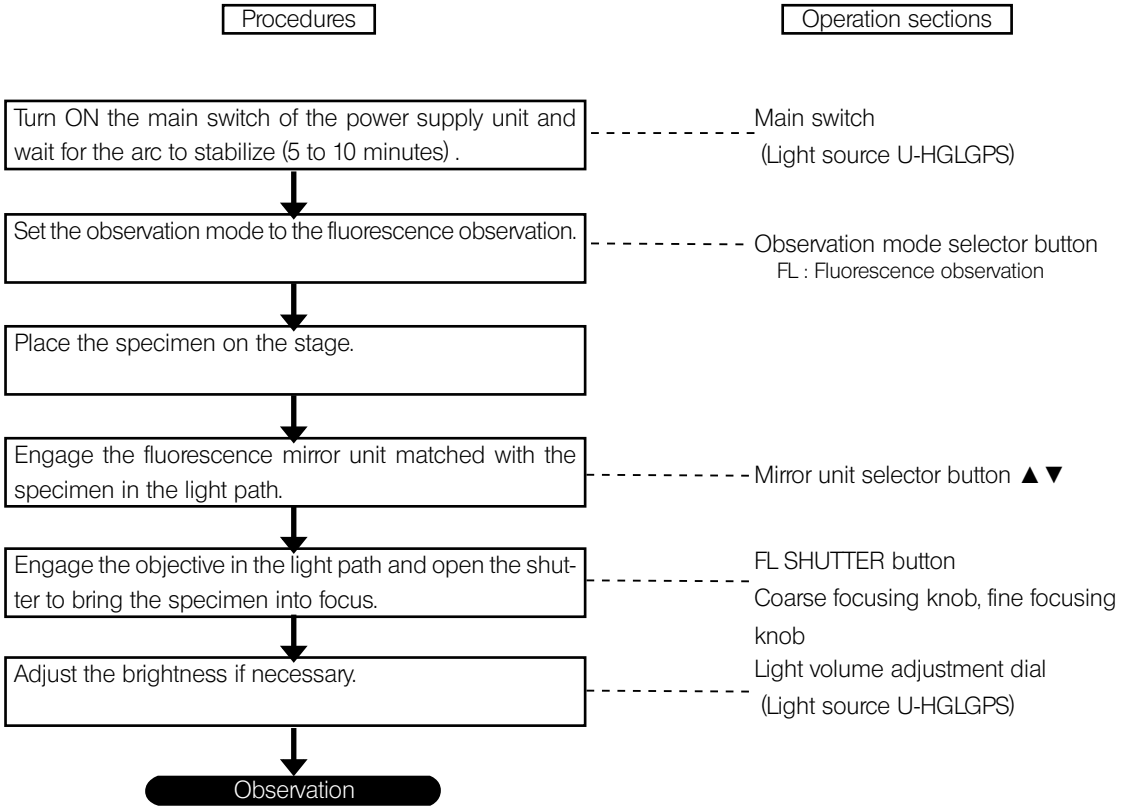
7 Other observation methods

7-1 Reflected fluorescence observation

TIP If you need the simultaneous observations of reflected fluorescence observation with the phase contrast observation or transmitted light differential interference contrast (DIC) observation, see [7-5 Simultaneous fluorescence observation] (page 70) .

1 Outline of reflected fluorescence observation procedures

TIP • Operation sections are explained using the controller U-MCZ.
• For detail operating procedures of the touch panel controller, see Online Help of the touch panel controller.



TIP Use the shutter if you stop observation for a short time. - - - - - - FL SHUTTER button

2 General precautions for observation

1) Make sure that the power cords and connection cables are connected properly and securely.
2) If you perform only the transmitted phase contrast observation or the transmitted DIC observation*, leave one turret position empty not to attach the fluorescent mirror unit. Doing so allows to reproduce the original colors.
   *This procedure is not required when using the transmitted DIC mirror unit IX3-FDICT with built-in analyzer.
3) Always use Olympus immersion oil for oil immersion objectives.
4) If you use an objective with correction collar, you can correct the contrast degradation caused by the variation in cover glass thickness by adjusting the correction collar.

   Compensation procedures

   If the cover glass thickness is known, match it with the correction collar scale. If the thickness is not known, while rotating the collection collar, use the fine focusing knob to bring the specimen into focus to find the best contrast position.

5) Utilize the shutter when you stop the observation for a short time.
6) Photobleaching of specimens

   This system features the high excitation light intensity to ensure the bright observation for dark fluorescence specimens. Therefore, performing the observation using high-magnification objectives for a long time period may cause photobleaching quicker than usual, which may deteriorate the visibility (contrast) of fluorescent images.

   In such a case, slightly reduce the excitation light intensity to slow photobleaching down and to improve the fluorescence images. Use the ND filters or the aperture diaphragm to reduce the excitation light intensity as far as the observation is not affected, or use the shutter to prevent the specimen from exposing to the light unnecessarily.

   Using the commercially-available photobleaching protection agent (DABCO, etc.) can also delay the photobleaching of specimens. The use of the photobleaching protection agent is recommended especially for the high-magnification observations.

TIP Be careful that the photobleaching protection agents cannot be used with certain kinds of specimens.

⚠CAUTION **Be careful, if you use this system by removing the excitation filter from the fluorescence mirror unit and attaching it to the excitation filter slider on the white lamp side, the very bright light may enter the eyepieces.**

3 Selecting the fluorescent mirror unit

Select the fluorescence mirror unit with the excitation filter which matches the fluorescent stain in use.

TIP Usage depending on the excitation light bandwidth :
Depending on excitation light types, several combinations of the excitation filters with different bandwidths are available. In general, the wide-bandwidth (W) set is used, but if the fluorescence light emitted from substances other than the fluorescent stain is strong, the narrow-bandwidth (N) is recommended (though the fluorescence brightness becomes slightly darker) .

Combinations of dichroic mirrors and filters of fluorescence mirror units

Excitation method	Mirror unit	Excitation filter	Emission filter	Dichroic mirror	Applications
V	U-FVN	BP400-410	BA460IF	DM455	· Catecholamine observation · Serotonin observation · Tetracyline : Bone, teeth
BV	U-FBVW	BP400-440	BA460IF	DM455	· Quinacrine, quinacrine mustard : Chromosome · Thioflavine S : Lymphocyte · Acriflavine : Nucleic acid · ECFP
	IX3-FBVWXL				
B	U-FBW	BP460-495	BA510IF	DM505	· FITC : Fluorescent antibody · Acridine orange : DNA, RNA · Auramine : Tubercle bacillus · EGFP, S65T, RSGFP
	U-FBN	BP470-495			
G	U-FGW	BP530-550	BA575IF	DM570	· Rhodamine, TRITC : Fluorescent antibody · Propidium iodide : DNA · RFP
	IX3-FGWXL				
Y	U-FYW	BP540-585	BA600IF	DM595	· Texas red : Fluorescent antibody

Combinations for color separation

B	U-FBWA	BP460-495	BA510-550	DM505	For observing only the B-excitation stain when using B-excitation stain with TRITC or Texas Red
	U-FBNA	BP470-495			
G	U-FGWA	BP530-550	BA575-625	DM570	For observing only the G-excitation stain when using G-excitation stain together with Cy5.
	U-FGNA	BP540-550			

Exclusive combinations for fluorescent proteins

CFP	U-FCFP	BP425-445CFP	BA460-510CFP	DM455CFP	For ECFP
	IX3-FCFPXL				
GFP	U-FGFP	BP460-480GFP	BA495-540GFP	DM490GFP	For EGFP
	IX3-FGFPXL				
YFP	U-FYFP	BP490-500YFP	BP515-560YFP	DM515YFP	For EYFP
	IX3-FYFPXL				
RFP	U-FRFP	BP535-555	BA570-625	DM565	For RFP
	IX3-FRFPXL				
mCherry	U-FMCHE	BP565-585	BA600-690	DM595	For mCherry
	IX3-FMCHEXL				

4 Combinations of objectives and various observation methods

Objective		Reflected fluorescence						Phase contrast (Phase contrast ring)	DIC		
									U-UCD8		IX3-LWUCDA IX2-LWUCD
		340	U	V	BV	B	G		U-TLO	U-TLD	
UPLSAPO	4X	△	○	○	○	○	○	—	—	—	—
	10X2	△	○	○	○	○	○	—	—	○	○
	20X	△	○	○	○	○	○	—	○	○	○
	20XO	△	○	○	○	○	○	—	○	○	○
	30XS	△	○	○	○	○	○	—	—	○	○
	40X2	△	○	○	○	○	○	—	○	○	○
	60XO	△	○	○	○	○	○	—	○	○	○
	60XW	△	○	○	○	○	○	—	○	○	○
	60XS	△	○	○	○	○	○	—	○	○	○
	100XO	△	○	○	○	○	○	—	○	○	○
UPLFLN	4X	△	○	○	○	○	○	○* (PhL)	—	—	—
	10X2	△	○	○	○	○	○	○* (Ph1)	—	○	○
	20X	△	○	○	○	○	○	○* (Ph1)	—	○	○
	40X	△	○	○	○	○	○	○* (Ph2)	○	○	○
	40XO	△	○	○	○	○	○	—	○	○	○
	60X	△	○	○	○	○	○	—	○	○	○
	60XOI	△	○	○	○	○	○	○* (Ph3)	○	○	○
	100XO2	△	○	○	○	○	○	○* (Ph3)	○	○	○
	100XOI2	△	○	○	○	○	○	—	○	○	○
PLAPON	60XO	—	△	○	○	○	○	—	○	○	○
	60XOSC	—	△	○	○	○	○	—	○	○	○
CPLFLN	10XPH	△	△	△	△	△	△	○* (PhC)	—	—	○
LCACHN	20X	—	△	△	△	○	○	○* (PhC)	—	—	—
	40X	—	—	—	—	○	○	○* (Ph2)	—	—	—
LUCPLFLN	20X	○	○	○	○	○	○	○* (Ph1)	○	○	○
	40X	○	○	○	○	○	○	○* (Ph2)	○	○	○
	60X	△	○	○	○	○	○	○* (Ph2)	○	○	○
UAPON	20XW340	○	○	○	○	○	○	—	○	○	○
	40XW340	○	○	○	○	○	○	—	○	○	○
	40XO340	○	○	○	○	○	○	—	○	○	○

○ : Available combination.
○* : A phase contrast objective is required for phase contrast observation.
△ : Usable but the image may be very dark.
— : Not available.

5 Igniting the mercury burner

Turn ON the main switch. The illumination light will stop flickering and stabilize in 5 to 10 minutes after ignition.

Note
- **Do not turn ON and OFF the main switch of light source U-HGLGPS in short interval in order to extend the life of the mercury burner. If you want to stop observation for a short time, do not turn OFF.**
- **The mercury burner cannot be reignited until the mercury vapor has cooled down and liquefied. Wait for approx. 10 minutes and reignite a mercury burner.**

TIP
- The mercury burner may not be ignited by the one-time operation on rare occasions due to its characteristics. In this case, turn OFF the main switch, wait for 5 to 10 seconds, then turn it ON again.
- When resetting the hour counter, hold the reset button until "0.0" is displayed.

Brightness control of mercury burner using the light source U-HGLGPS

For details, see page 35.

Brightness control of LED lamp using the touch panel controller

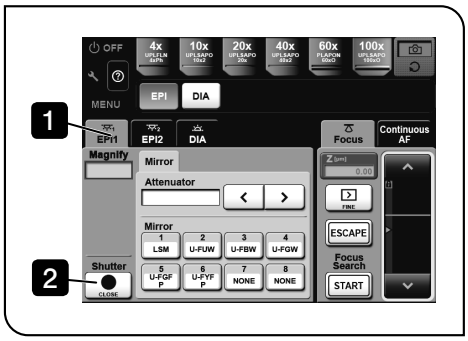
1 Tap DIA.
DIA : For transmitted light observation

2 Adjust the brightness using the brightness adjustment button.

6 Opening / closing the shutter

The shutter can be opened / closed by the FL SHUTTER button on the touch panel controller or the controller U-MCZ. The open/close status of the shutter can be checked by the touch panel controller.

TIP For detail operating procedures, see Online Help of the touch panel controller.



When using the touch panel controller

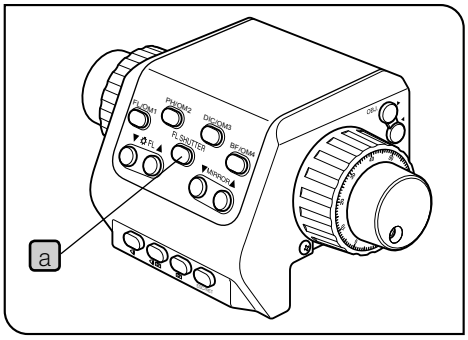
1 Tap EPI1 .

EPI1 : For observation using the motorized fluorescent mirror turret FV30-RFACA

2 Tap Shutter .

Blue : The shutter is closed.

White : The shutter is opened.



When using the controller U-MCZ

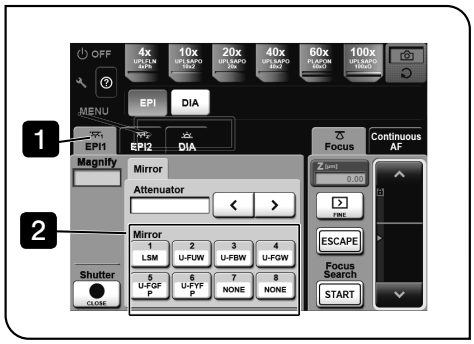
Press the FL SHUTTER button a of the controller U-MCZ.

TIP The FL SHUTTER button a opens / closes the reflected shutter only. The transmitted shutter opens / closes automatically depending on the observation modes.

7 Selecting the turret in motorized fluorescent mirror turret FV30-RFACA

The mirror unit to be engaged in the light path can be selected using the touch panel controller or the controller U-MCZ.

TIP
- The display panel shows the turret number engaged in the light path of the motorized fluorescent mirror turret FV30-RFACA.
- For detail operating procedures, see Online Help of the touch panel controller.

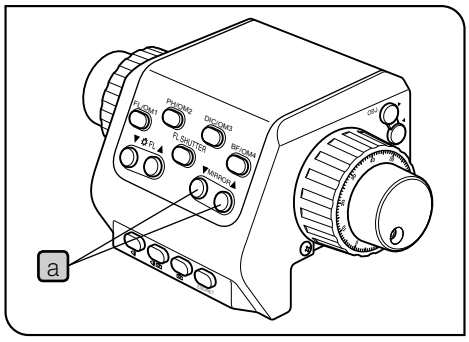


When using the touch panel controller

1 Tap EPI1 .

EPI1 : For observation using the motorized fluorescent mirror turret FV30-RFACA

2 Tap the turret number to be used.



When using the controller U-MCZ

Press the MIRROR button a of the controller U-MCZ.

TIP You can select the turret in the motorized fluorescent mirror turret FV30-RFACA using the controller U-MCZ.

7-2 Phase contrast observation

TIP • A phase contrast objective, phase contrast optical element and centering telescope U-CT30-2 are required for the phase contrast observation.

• If a DIC slider, analyzer or polarizer is engaged in the light path, remove it from the light path.

1 Phase contrast optical elements and applicable objectives

Motorized LWD condenser IX3-LWUCDA, ULWCD condenser IX2-LWUCD

TIP Insert the optical element (small) in the 30 mm mount hole and the optical element (large) in the 38 mm mount hole.
When observing the specimens in wells, it is recommended to use the IX-PHC to obtain the phase contrast effect in a wide range of field of view.

Optical element	Indication	Applicable objectives
IX-PHL (small)	PhL	UPLFLN4XPH
IX-PHC (small)	PhC	CPLN10XPH, LCACHN20XPH, CPLFLN10XPH
IX-PH1 (small)	Ph1	UPLFLN10X2PH, UPLFLN20XPH, LUCPLFLN20XPH
IX-PH2 (small)	Ph2	UCPLFLN20XPH, UPLFLN40XPH, LUCPLFLN40XPH, LUCPLFLN60XPH, LCACHN40XPH
IX-PH3 (large)	Ph3	PLAPON60XOPH, UPLFLN60XOIPH, UPLSAPO100XOPH, UPLFLN100XO2PH

Example of attaching optical elements (Motorized LWD condenser IX3-LWUCDA)

Small diameter : PH1, PHL, free (or PH2, C, free)

Large diameter : PH3, DIC40, DIC60, DIC100

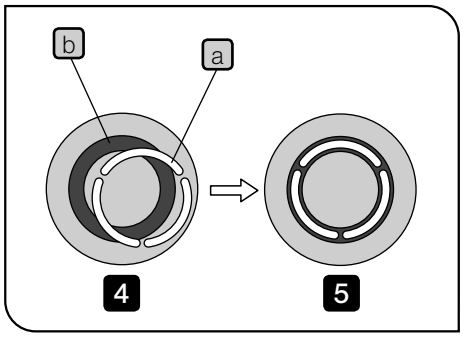
ULWCD condenser IX-ULWCD

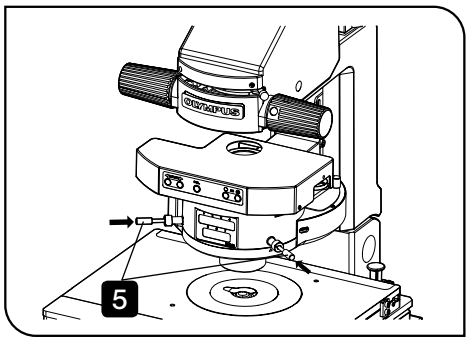
TIP IX-PHCU or IX-PH1U can be attached only in Ph1 and PhC. (Do not remove the built-in elements.)

Optical element	Indication	Applicable objectives
PHL (built-in)	PhL	UPLFLN4XPH
IX-PHCU	PhC	CPLN10XPH, LCACHN20XPH, CPLFLN10XPH
IX-PH1U	Ph1	UPLFLN10XPH, UPLFLN20XPH, LUCPLFLN20XPH,
PH2 (built-in)	Ph2	UCPLFLN20XPH, UPLFLN40XPH, LUCPLFLN40XPH, LUCPLFLN60XPH, LCACHN40XPH

TIP When using the universal condenser U-UCD8 or the medium distance condenser IX2-MLWCD, refer to the instructions provided with the respective condenser.

2 Centering the phase contrast ring slit





TIP Open the aperture diaphragm during phase contrast observation.

1. Engage the phase contrast objective in the light path to bring the specimen into focus.
2. Remove an eyepiece and attach the centering telescope U-CT30-2 in place.
3. Engage the ring slit of the condenser which matches the phase contrast objective in the light path.
4. Rotate the knurled section of the centering telescope to focus on the ring slit a and the phase plate b of the objective.
5. Pushing the optical element centering knobs, rotate the phase contrast ring slit centering screws so that the ring slit image overlaps on the phase plate of the objective.

TIP Do not release the hand suddenly while the optical element centering knobs are being pushed in. The optical element centering knobs may be popped out.

6. Remove the centering telescope U-CT30-2 and attach an eyepiece in place.

TIP If the vessel is not completely flat, it may become necessary to adjust the centering again to obtain the optimum contrast. Adjust the centering for each magnification.

7. Adjust the field diaphragm so that its image circumscribes the field of view. and perform the phase contrast observation.

TIP
- Engaging the green filter in the light path will improve the contrast.
- The thin shading may occur in the periphery of the field of view during visual observation.

7-3 Differential interference contrast observation

TIP
- If you use a plastic dish, the DIC effect cannot be obtained due to the polarization characteristic of the dish. Use a glass bottom dish.
- The DIC optical elements, DIC slider, analyzer, and polarizer are required for DIC observation.

1 DIC optical elements and applicable objectives / DIC sliders

Motorized LWD condenser IX3-LWUCDA, ULWCD condenser IX2-LWUCD

Insert the optical elements (small) shown in parenthesis into the 30 mm mount hole and other optical elements (large) into the 38 mm mount hole.

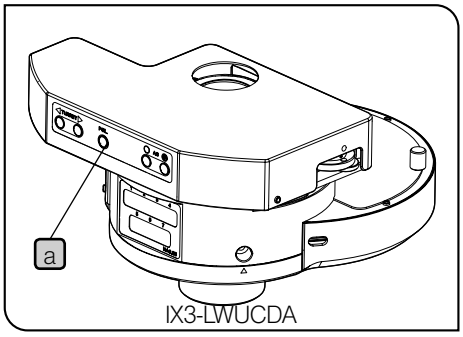
Applicable objectives \ DIC slider		U-DICTS	U-DICTHC	U-DICTHR
UPLSAPO	10X2	(IX2-DIC10)	–	–
	20X	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	20XO	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	30XS	IX2-DIC30	–	–
	40X2	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	60XO	IX2-DIC60	–	–
	60XW	IX2-DIC60	–	–
	60XS	IX2-DIC60	–	–
	100XO	IX2-DIC100	–	–
	100XOPH	IX2-DIC100	–	–
PLAPON	60XO	IX2-DIC60	–	–
	60XOPH	IX2-DIC60	–	–
UPLFLN	10X2	(IX2-DIC10)	–	–
	20X	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	40X	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	40XO	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	60X	IX2-DIC60	–	–
	60XOI	IX2-DIC60	–	–
	100XO2	IX2-DIC100	–	–
	100XOI2	IX2-DIC100	–	–
LUCPLFLN	20X	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	40X	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	60X	IX2-DIC60	–	–
UAPON	20XW340	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	40XW340	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	40XO340	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	100XOTIRF	IX2-DIC100	–	–
	150XOTIRF	IX2-DIC100	–	–
UCPLFLN	20X	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	20XPH	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
APON	60XOTIRF	IX2-DIC60	–	–
	100XHOTIRF	IX2-DIC100	–	–

When using the universal condenser U-UCD8, refer to the provided instructions.

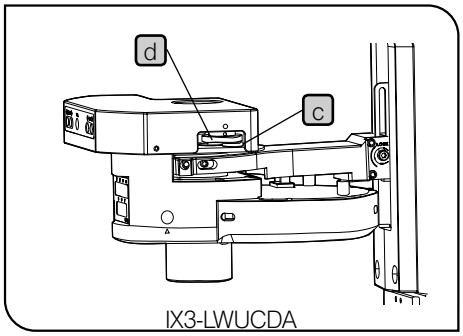
2 Attaching the analyzer and DIC slider

TIP Trained Olympus technical personnel or the technical personnel who received the service training provided by Olympus attach the analyzer and the DIC slider in accordance with FV3000 Service Manual.

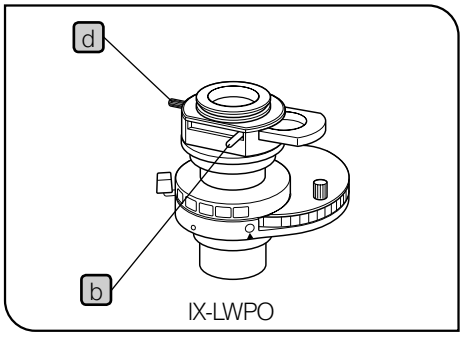
3 Adjusting the cross-Nicol



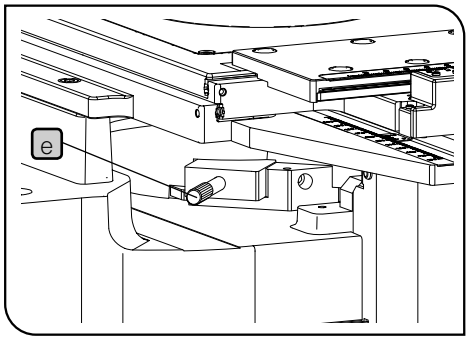
IX3-LWUCDA

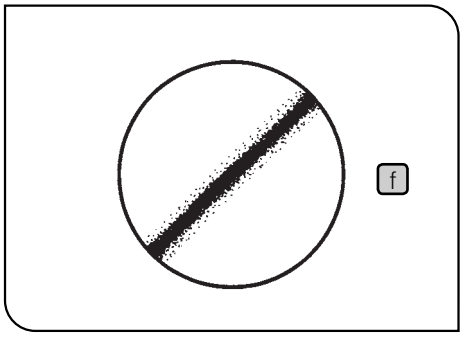


IX3-LWUCDA



IX-LWPO





1. Rotate the turret of the condenser to set the brightfield observation (BF) light path (where no optical element is engaged in the light path) .
2. When using the motorized LWD condenser IX3-LWUCDA, press the motorized section operating button [a] to engage the polarizer in the light path.

   When using the polarizer IX-LWPO, move the polarizer attaching/detaching lever [b] in the right direction to engage the polarizer in the light path.
3. Engage the 10X objective in the light path, place an specimen applicable for brightfield observation on the stage, bring the specimen into approximate focus and remove the specimen from the light path.
4. Refer to page 66, and engage the DIC slider and the analyzer in the light path.
5. Remove the eyepieces from the observation tube and look into the observation tube.
6. Rotate the prism movement knob [e] of the DIC slider clockwise until the knob stops. A black interference stripe and a rainbow-colored interference stripe appears alternatively. Stop the knob at the position in which the black interference stripe can be seen. (State of [f])
7. When using the motorized LWD condenser X3-LWUCDA, rotate the clamping knob [c] clockwise until the knob stops to loosen the polarizer rotation knob [d].

   When using the polarizer IX-LWPO, rotate the polarizer rotation/clamping knob [d] counterclockwise slightly to loosen the polarizer rotation/clamping knob.
8. While looking into the observation tube, rotate the polarizer rotation/clamping knob [d] horizontally until the black interference stripe becomes darkest. This is the cross-Nicol position.
9. When the position is determined, clamp the polarizer.

   When using the motorized LWD condenser IX3-LWUCDA, rotate the clamping knob [c] counterclockwise until the knob stops.

   When using the polarizer IX-LWPO, rotate the polarizer rotation/clamping knob [d] clockwise until the knob stops.

4 Observation method

1. Rotate the condenser turret to engage the optical element suitable for the objective in use in the light path.
2. Engage the objective to be used in the light path.
3. Place the specimen on the stage and bring the specimen into focus by moving the objective up or down.
4. Adjust the field diaphragm so that its image circumscribes the field of view.
5. Narrow down the aperture diaphragm to enhance the contrast.
6. Engage the DIC slider in the light path.
7. Rotate the prism movement knob of the DIC slider to select the interference color that can provide the optimum contrast in accordance with the specimen.

Shift DIC prism for transmitted observation U-DICTS :
High contrast DIC prism for transmitted observation U-DICTHC :
High resolution DIC prism for transmitted observation U-DICTHR
} The background interference color is continuously variable from black to light gray.

TIP Only when using the universal condenser U-UCD8 for the sensitive color observation, engage the 1 λ plate (sensitive color plate) UUCDTP530 in the light path.

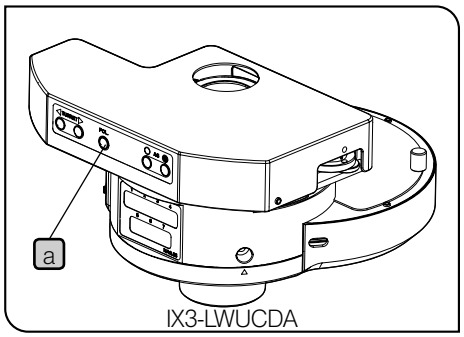
- Setting the background color to dark provides observation similar to the darkfield observation.
- Setting the background color to gray provides observation with high contrast in 3D feeling and the highest sensitivity in gray sensitive color.
- Setting the background color to magenta sensitive color allows very small change in phase to be observed as a change in color.
- The detection sensitivity has a directional characteristic due to the configuration of the DIC prism. Therefore, the contrast may sometimes be improved by rotating the specimen on the stage.

7-4 Simplified polarized light observation

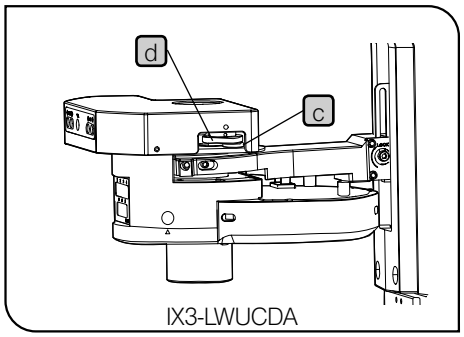
1 Attaching the analyzer and polarizer

TIP Trained Olympus technical personnel or the technical personnel who received the service training provided by Olympus attach the analyzer and the slider in accordance with FV3000 Service Manual.

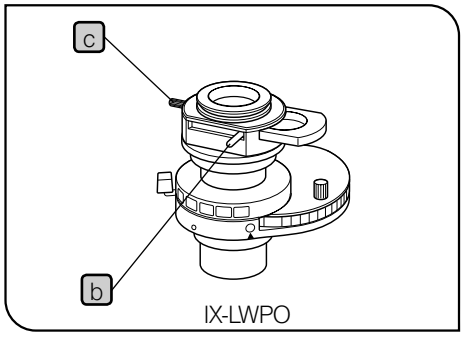
2 Observation method



IX3-LWUCDA



IX3-LWUCDA



IX-LWPO

1. Rotate the turret to set the brightfield observation (BF) light path (where no optical element is engaged in the light path) .
2. When using the motorized LWD condenser IX3-LWUCDA, press the motorized section operating button [a] to engage the polarizer in the light path.

   When using the polarizer IX-LWPO, move the polarizer attaching/detaching lever [b] in the right direction to engage the polarizer in the light path.
3. Rotate the revolving nosepiece to engage the objective in the light path.
4. Refer to page 66, and engage the analyzer in the light path.
5. When using the motorized LWD condenser X3-LWUCDA, rotate the clamping knob [c] clockwise until the knob stops to loosen the polarizer rotation/clamping knob [d].

   When using the polarizer IX-LWPO, rotate the polarizer rotation/clamping knob [d] counterclockwise slightly to loosen the polarizer rotation/clamping knob.
6. Rotate the polarizer rotation/clamping knob [d] and stop at the position where the field of view is darkest.
7. When the position is determined, clamp the polarizer.

   When using the motorized LWD condenser IX3-LWUCDA, rotate the clamping knob [c] counterclockwise until the knob stops.

   When using the polarizer IX-LWPO, rotate the polarizer rotation/clamping knob [d] clockwise until the knob stops.
8. Place a specimen on the stage and bring the specimen into focus. Now the specimen can be observed with the simplified polarized light observation.
9. Adjust the field diaphragm so that its image circumscribes the field of view.
10. Narrow down the aperture diaphragm to enhance the contrast.

7-5 Simultaneous fluorescence observation

TIP By properly combining equipment, this system can be used in transmitted light brightfield observation, transmitted light phase contrast observation and transmitted light DIC (Differential Interference Contrast) observation in addition to the reflected fluorescence observation. With specimens which bleach quickly, you can reduce photobleaching to the minimum by determining the position to observe the specimen at first using the phase contrast observation or the DIC observation. The reflected fluorescence observation can also be executed simultaneously with the phase contrast or the DIC observation.

1 Simultaneous reflected fluorescence / phase contrast observations

TIP The phase contrast observation requires the phase contrast objective and the condenser.

Condenser
· Motorized LWD condenser IX3-LWUCDA
· ULWCD condenser IX2-LWUCD
· Medium distance condenser IX2-MLWCD
· Universal condenser U-UCD8
· ULWCD condenser IX-ULWCD

1. Rotate the turret of the motorized fluorescent mirror turret FV30-RFACA to engage the position where the fluorescence mirror unit is not attached in the light path.
2. Insert the ring slit which is the same type of the Ph display of the objective in the turret of the condenser, and engage it in the transmitted light illumination light path.
3. Center the ring slit and phase plate.
4. Engage the mirror unit corresponding to the desired excitation in the light path and open the shutter.
5. Adjust the transmitted light volume for the best balance of fluorescence and phase contrast brightness.

2 Simultaneous reflected fluorescence / DIC (transmitted) observations

TIP The DIC observation requires the condenser, DIC prism and filters.

Condenser	DIC prism	Filter
· Motorized LWD condenser IX3-LWUCDA · ULWCD condenser IX2-LWUCD · Medium distance condenser IX2-MLWCD · Universal condenser U-UCD8	· Shift DIC prism for transmitted observation U-DICTS · High contrast DIC prism for transmitted observation U-DICTHC · High resolution DIC prism for transmitted observation U-DICTHR	· Transmitted DIC filter unit IX3-FDICT

Note **Do not insert the analyzer in the DIC slider. The fluorescence observation image may be darker and the analyzer may be burnt.**

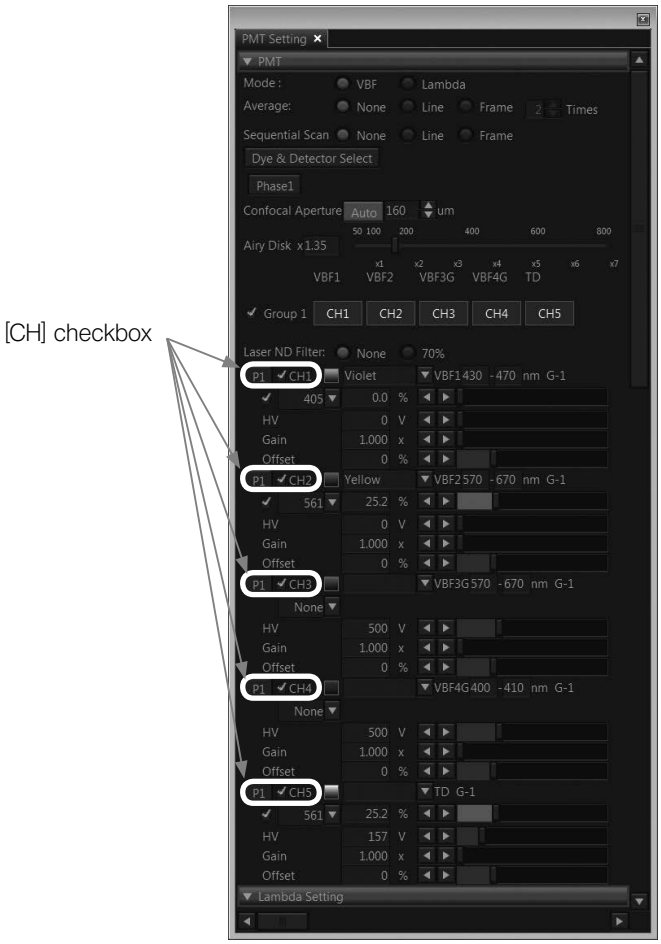
1. Engage the position where the fluorescence mirror unit is not attached in the light path.
2. Adjust the polarizer on the DIC condenser to the “Crossed Nicol” position.
3. Insert the DIC slider into the revolving nosepiece.
4. Rotate the turret on the condenser to select the Nomarski prism turret which matches the objective to be used for observation.
5. Engage the objective to be used in the light path.
6. Place the specimen on the stage and bring the specimen into focus.
7. Adjust the field diaphragm and the aperture diaphragm of the condenser.
8. Rotate the prism movement knob on the DIC slider to adjust the contrast of the DIC image.
9. Engage the mirror unit corresponding to the desired excitation in the light path and open the shutter.
10. Adjust the transmitted light volume to get the optimum fluorescence and DIC image brightness.

TIP Each motorized module can be switched to the simultaneous observation setting status by operating the button on touch panel controller.
For details, refer to Online Help of touch panel controller.

TIP
- Refer to Online Help of the standard image acquisition / analysis software for FV3000 FV31S-SW.
- When using this system, we recommend you to darken the room light to reduce the ambient light that affects observations. (Particularly, the room light affects the differential interference observation images.)
- Do not apply cool air to this system from the air conditioner or so. This system may be unstable.
- Do not bend or extend the optical fiber excessively, or do not step on it. The performance may be deteriorated significantly.
- Do not put any object on top of the laser unit. The laser unit may be overheated, causing the protection circuit activated to stop the laser oscillation.
- Do not put any object on top of the main laser combiner FV31-MCOMB or the sub laser combiner FV31-SCOMB. The laser power to be emitted may fluctuate to affect the observation image.

Spectral detector FV31-SD, high sensitivity spectral detector FV31-HSD, transmitted detector FV31-LETD

Note
- **When performing the normal fluorescence observation, PMT or GaAsP-PMT becomes deteriorated. Therefore, adjust HV and the laser power during observation in order not to saturate the image intensity.**
- **When you are not acquiring the image, be sure to uncheck all items in the [CH] checkbox in the [PMT Setting] tab of the standard image acquisition / analysis software for FV3000 FV31S-SW.**



9 Signal connection with external devices

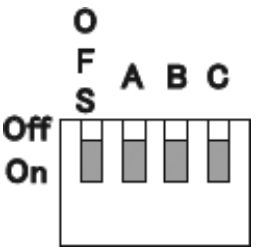
Using the IO interface box FV30-ANALOG, this system is able to transmit and receive electric signals to and from external devices.

9-1 Features of the IO interface box FV30-ANALOG

- Capable of receiving electric signals transmitted from external devices as an image data equivalent to that acquired via four channels.
- Capable of processing a variety of electric signals, such as action potentials, current, temperature, pressure and flow rate, depending on the type of external device.
- Since the intensity data of the specimen and the electric signal transmitted from the external devices can be treated as a data of the same time axis, the correlation between the light stimulation and the electrophysiological reaction associated with the light stimulation can be analyzed, and also the high synchronous data can be acquired easily in the application which observes the fluorescence variation with applying the electric stimulation.
- Capable of transmitting scan timing signals (e.g., scan start, horizontal/vertical synchronization, sampling clock) to external devices.
- Allowing the user to select the measurement range from six different levels depending on the signal output value of the external device
- Trigger inputs and outputs can be controlled by synchronizing them with operations of this system.

9-2 Specifications of the IO interface box FV30-ANALOG

Items	Specifications	
Analog voltage input specifications	Input range and gradation 1) -10 V to +10 V One gradation = 4.88 mV 2) 0 V to +10 V One gradation = 2.44 mV 3) -5 V to +5 V One gradation = 2.44 mV 4) 0 V to +5 V One gradation = 1.22 mV 5) -1 V to +1 V One gradation = 0.49 mV 6) 0 V to +1 V One gradation = 0.25 mV Select the input voltage range from these six levels using the DIP switch. Input impedance : Approximately 10 kΩ Maximum input voltage range : - 10 V to + 10 V Signal ground To be connected to the chassis ground.	"1) " is used as a standard. The voltage per each gradation shown on the left is a standard value when the gradation is 4096. Do not add signals exceeding the input voltage range described on the left. This unit will be damaged.
Analog voltage Sampling specifications	Sampling rate Galvano scan mode : 1 Msps Resonant scan mode : 3.3 Msps	This information will be the data for acquiring the latest sampling value at each pixel timing in each scan mode.

<table>
<tr><th>Items</th><th>Specifications</th></tr>
<tr><td>Dip switch for changing the analog voltage input range</td><td>


The input range settings by the dip switch are shown below.
<table>
<tr><th>Input mode</th><th>SW OFS</th><th>SW A</th><th>SW B</th><th>SW C</th></tr>
<tr><td>−10 — +10V</td><td>OFF</td><td>ON</td><td>OFF</td><td>OFF</td></tr>
<tr><td>0 — +10V</td><td>ON</td><td>ON</td><td>OFF</td><td>OFF</td></tr>
<tr><td>−5 — +5V</td><td>OFF</td><td>OFF</td><td>ON</td><td>OFF</td></tr>
<tr><td>0 — +5V</td><td>ON</td><td>OFF</td><td>ON</td><td>OFF</td></tr>
<tr><td>−1 — +1V</td><td>OFF</td><td>OFF</td><td>OFF</td><td>ON</td></tr>
<tr><td>0 — +1V</td><td>ON</td><td>OFF</td><td>OFF</td><td>ON</td></tr>
</table>
Do not perform the switch settings other than those described above. This unit may malfunction.
</td></tr>
<tr><td>Synchronization signal output specifications</td><td>
Output channel number : 18ch
<table>
<tr><th rowspan="2">Name</th><th rowspan="2">Content</th><th>TRAC1 (MAIN)</th><th>TRAC2 (SIM)</th><th>TRAC3</th><th>TRAC4</th></tr>
<tr><th>Observation side</th><th>Option</th><th>Option</th><th>Option</th></tr>
<tr><td>SCAN</td><td>Scan in progress</td><td>○</td><td>○</td><td>○</td><td>○</td></tr>
<tr><td>LOOP</td><td>Repeat process in progress</td><td>○</td><td>○</td><td>○</td><td>○</td></tr>
<tr><td>DE</td><td>Effective pixels (VD and HD)</td><td>○</td><td>○</td><td>–</td><td>–</td></tr>
<tr><td>VD</td><td>Vertical synchronization</td><td>○</td><td>○</td><td>○</td><td>○</td></tr>
<tr><td>HD</td><td>Horizontal synchronization</td><td>○</td><td>○</td><td>–</td><td>–</td></tr>
<tr><td>PCLK</td><td>Pixel clock</td><td>○</td><td>○</td><td>–</td><td>–</td></tr>
</table>
For details of synchronization signals, refer to 7-4 Synchronization signal output timing specifications.
As TRAC3 and 4 are optional outputs, they cannot be used with this system.

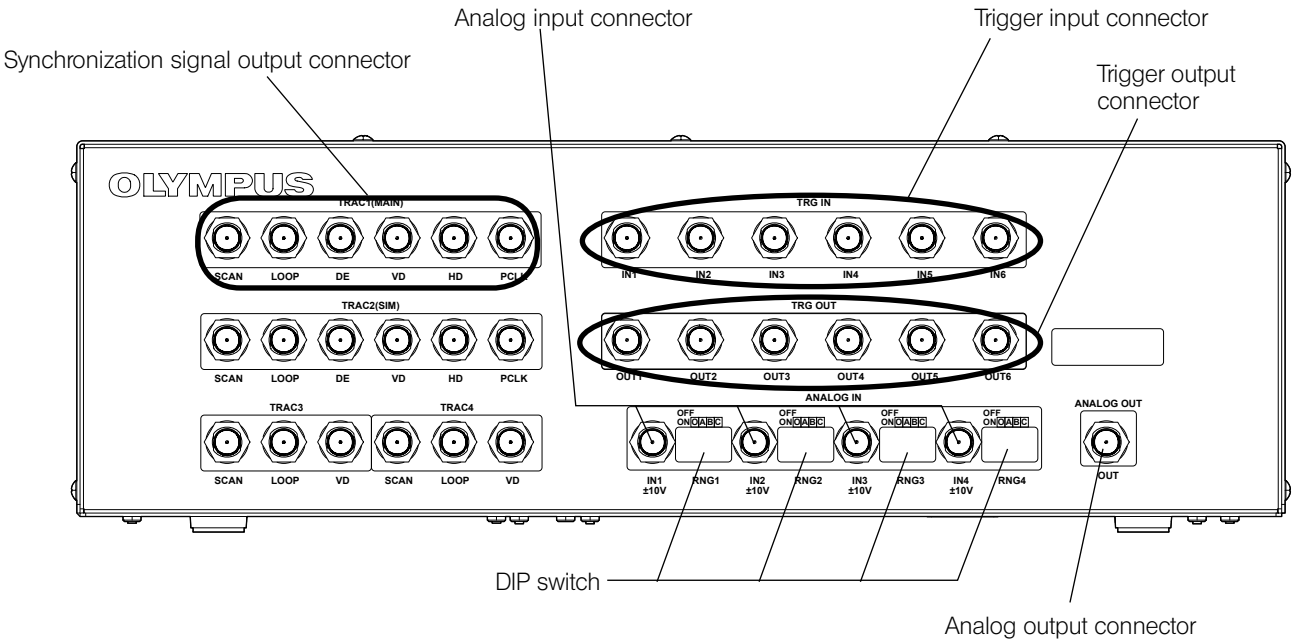
Output signal level
TTL signal level
Output voltage L : 0.4 V or lower
Output voltage H : 2.7 V or higher

Signal ground
To be connected to the chassis ground.
</td></tr>
</table>

Items	Specifications
	Connection of this output to external mechanism In order to prevent the system from malfunctions caused by noises, etc., use the Schmitt trigger type input IC for the logic IC, etc. to which this signal is inputted. Input impedance of the external mechanism connected to this output 10kΩ or more TIP In order to give a higher priority on the accuracy of this signal, shorten the BNC cable used for connection as much as possible. Note **Do not short-circuit the output or add the signals from outside. This unit may be damaged.**
Trigger input specifications	Input channel number : 6ch Input signal level TTL signal level Input voltage L : 0.8 V or lower Input voltage L : 2.0 V or higher Input voltage range -0.5 V to +5.5 V Input impedance Pull up to 5V with approximately 3.3 kΩ. Signal ground To be connected to the chassis ground. Output impedance of the external mechanism connected to this input 75 Ω Note **• Do not add signals exceeding the input voltage range described above. This unit may be damaged.** **• If the output impedance of the external mechanism described above is not 75 Ω, this unit and external devices may be damaged.**

Items	Specifications
Trigger output specifications	Output channel number : 6ch As OUT6 is a reserved port, it cannot be used with this system. Output signal level TTL signal level Output voltage L : 0.4 V or lower Output voltage H : 2.7 V or higher Signal ground To be connected to the chassis ground. Connection of this output to external mechanism In order to prevent the system from malfunctions caused by noises, etc., use the Schmitt trigger type input IC for the logic IC, etc. to which this signal is inputted. Input impedance of the external mechanism connected to this output 10 kΩ or more Note **Do not short-circuit the output or add the signals from outside. This unit may be damaged.**

9-3 Setting procedures (Maintenance)



Note

- Make sure that the analog voltage output of the external devices connected to the analog input is within the range between -10 V and +10 V. If it exceeds this range, use an attenuator, etc. Adding the signal exceeding the input voltage range described above will damage the IO interface box FV30-ANALOG.
- Be sure to turn OFF the powers of the IO interface box FV30-ANALOG and external devices before connecting devices. When turning ON the power, turn ON the external devices first and then turn ON IO interface box FV30-ANALOG.
  Before turning OFF the powers, turn OFF the power of the IO interface box FV30-ANALOG first and then turn OFF the powers of the external devices.
- The power of the IO interface box FV30-ANALOG is automatically turned OFF when the power supply for system FV31-PSU is turned OFF.

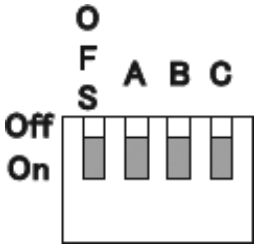
1 Setting the analog input range from external devices

TIP The power of the IO interface box FV30-ANALOG is automatically turned OFF when the power supply for system FV31-PSU is turned OFF.

1 Set the analog input range after turning OFF the powers of the IO interface box FV30-ANALOG and external devices.

2 Remove the sticker of the dip switch (RNG 1 to 4) on the IO interface box FV30-ANALOG.

3 Use a sharp tool such as tweezers, etc. to change the DIP switches to the range according to the analog voltage output value of the external devices.



Input mode	SW OFS	SW A	SW B	SW C
−10 — +10V	OFF	ON	OFF	OFF
0 — +10V	ON	ON	OFF	OFF
−5 — +5V	OFF	OFF	ON	OFF
0 — +5V	ON	OFF	ON	OFF
−1 — +1V	OFF	OFF	OFF	ON
0 — +1V	ON	OFF	OFF	ON

Note **Do not use this unit with the combinations other than those described above. This unit may malfunction.**

4 Reattach the switch stickers.

2 Connection

1 Set the analog input range after turning OFF the powers of the IO interface box FV30-ANALOG and external devices.

TIP The power of the IO interface box FV30-ANALOG is automatically turned OFF when the power supply for system FV31-PSU is turned OFF.

2 Be sure to use the connection cables which meet following specifications.

- Characteristic impedance 75 Ω
- Length of connection cable Maximum length : 3 m

3 Acquiring the analog voltage input signal

The analog signal can be acquired by selecting Current Detector on the standard image acquisition / analysis software for FV3000 FV30S-SW as a channel for acquiring the data.

TIP For details, refer to Online Help of the standard image acquisition / analysis software for FV3000 FV31S-SW.

9-4 Specifications of synchronizing signal output timing (Operation)

1 Synchronizing signal type

There are 6 types of synchronizing signals outputted from the IO interface box FV30-ANALOG.

SCAN

This signal indicates that the scan is in progress. This is a TTL signal where Scanning = H level and Being stopped = L level.
The initial status when the power is turned ON is L level.

LOOP

This signal indicates a repetitive cycle timing in the series scan. The output timing differs depending on the scan mode. Refer to "LOOP signal output timing" for details.
The initial status when the power is turned ON is L level.

VD

This signal indicates a vertical synchronizing signal. This is a TTL signal where Enabled period = L level and Disabled period = H level.
The initial status when the power is turned ON is H level.

HD

This signal indicates a horizontal synchronizing signal. This is a TTL signal where Enabled period = L level and Disabled period = H level.
The initial status when the power is turned ON is H level.

DE

This signal indicates a data enable signal. This is a TTL signal which becomes L level during the period when both VD signals and HD signals are enabled. (Equivalent to the logical sum (OR) of VD signals and HD signals.)
The initial status when the power is turned ON is H level.

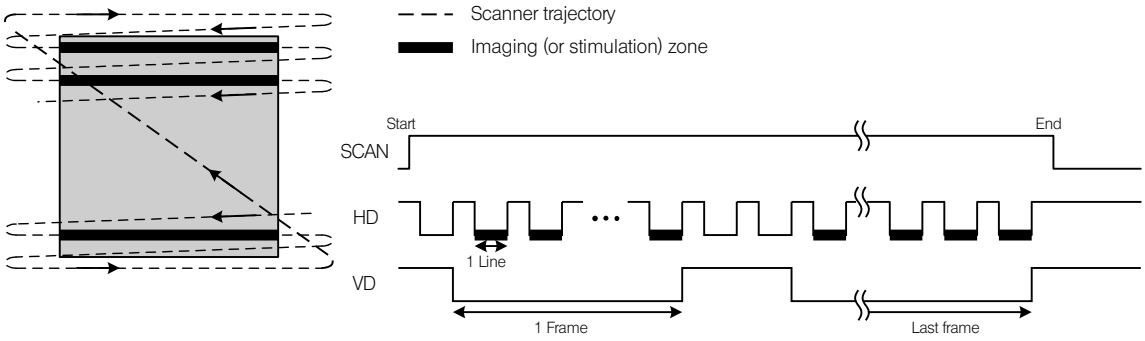
PCLK

This signal indicates a pixel clock signal. The clock frequency corresponding to the scan speed specified by the application software is outputted.
In the initial status when the power is turned ON, the 500 kHz clock signal is outputted.

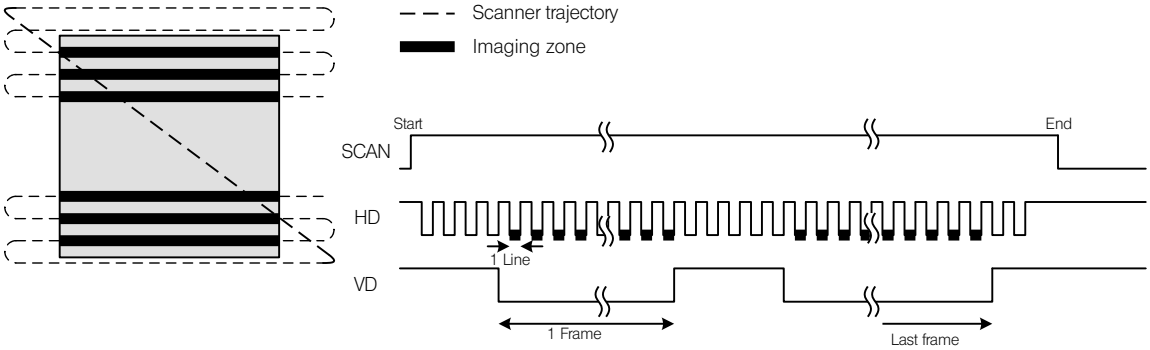
2 Synchronizing signal output timing by scan mode

The relation between the scan operation and the synchronizing signal output in each mode is illustrated below.

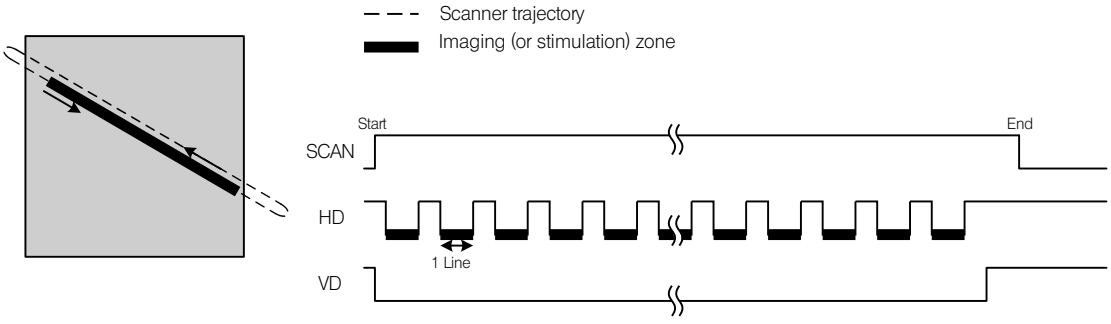
XY (Region) scan



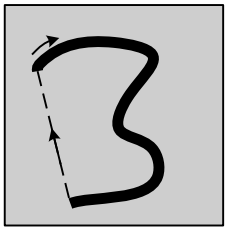
Resonant XY scan

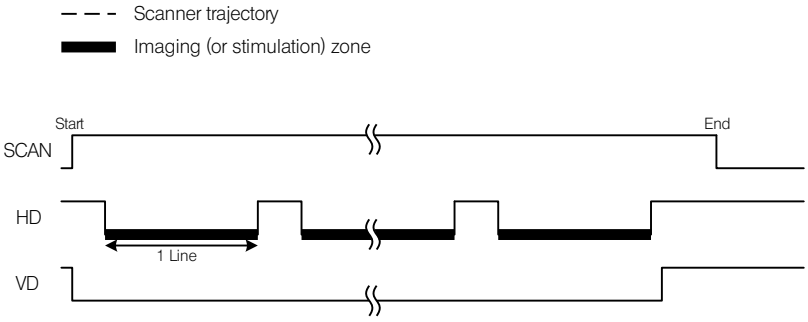


Line scan

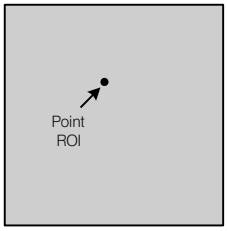


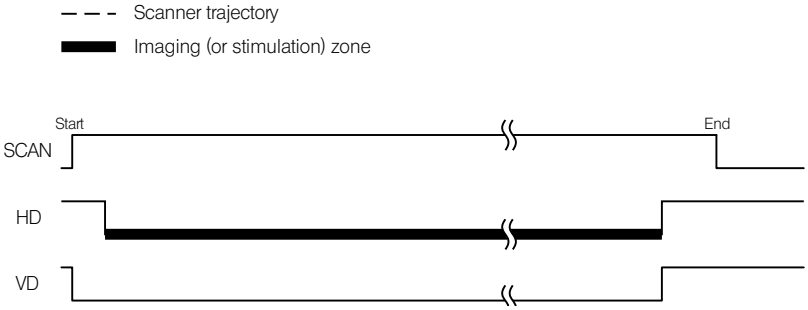
Free line scan



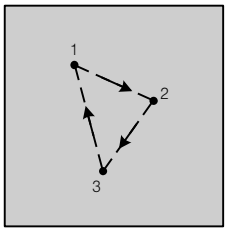


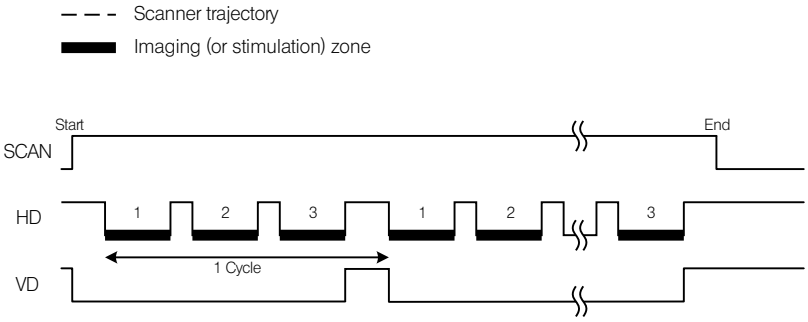
Point scan



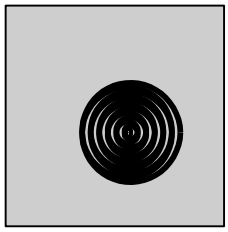


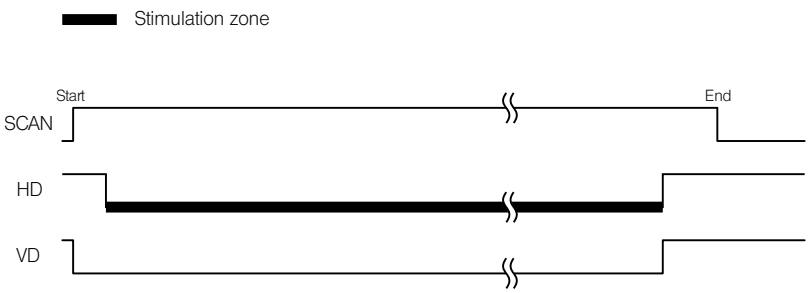
Multi point scan





Tornado scan



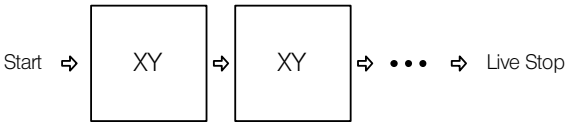


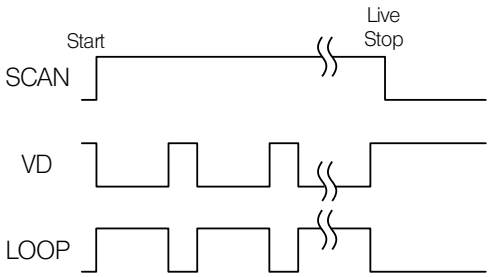
3 LOOP signal output timing

The LOOP signal output timing in each series scan is illustrated below with operation examples.

Live scan

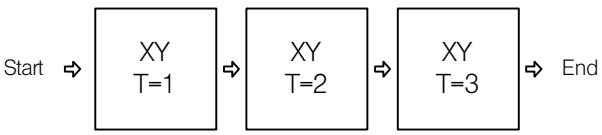
Note that the waveshape of Live scan in Line-Z is same as that in Line ZT illustrated below.

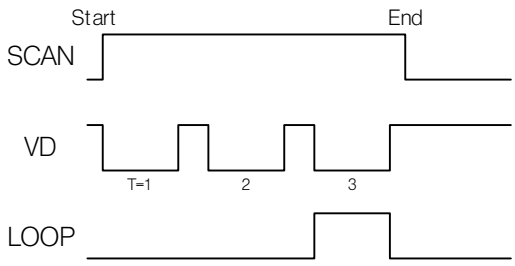




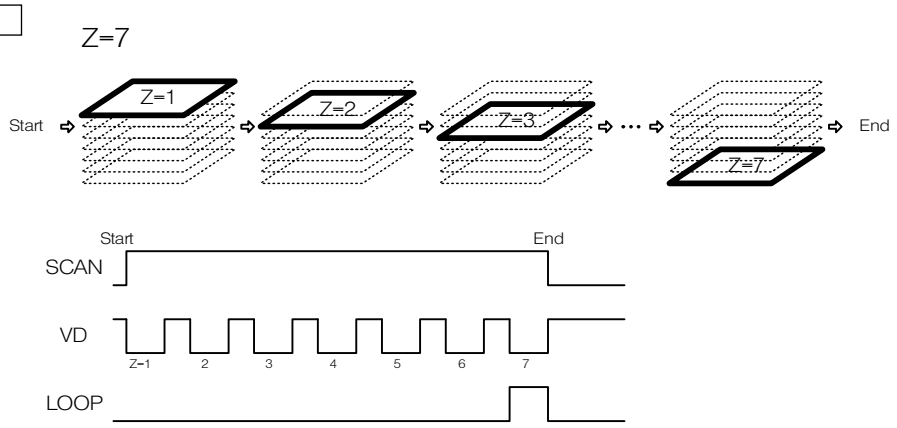
XYT scan

T=3

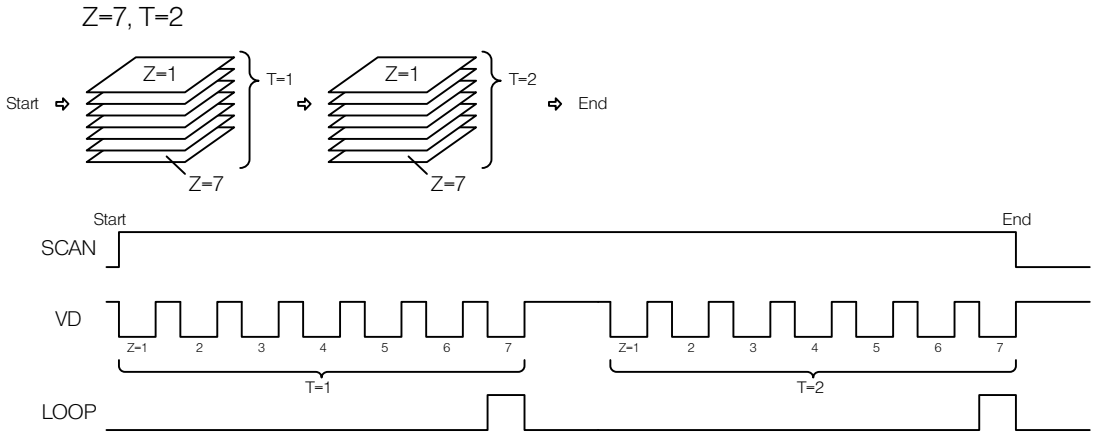




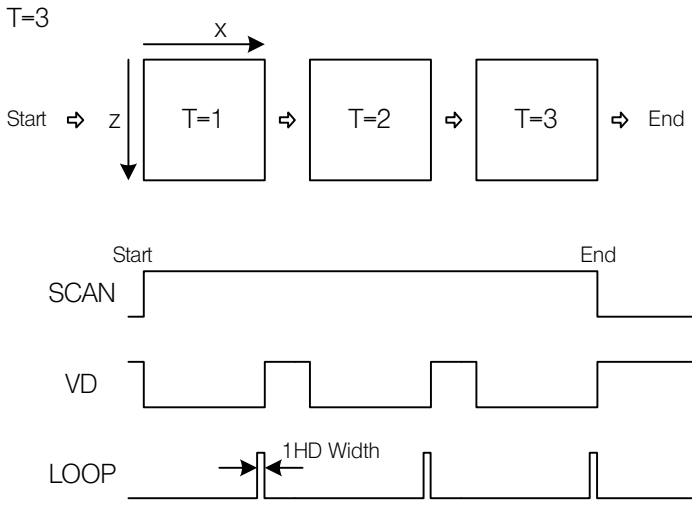
XYZ scan



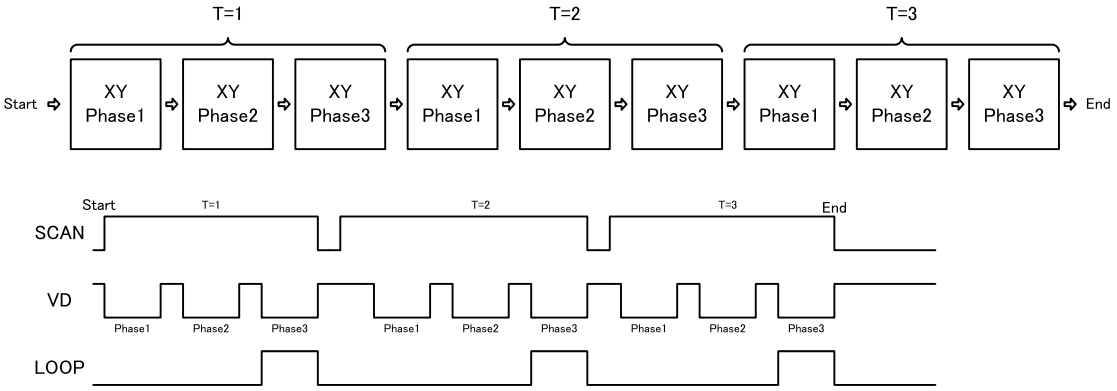
XYZT scan



Line ZT scan

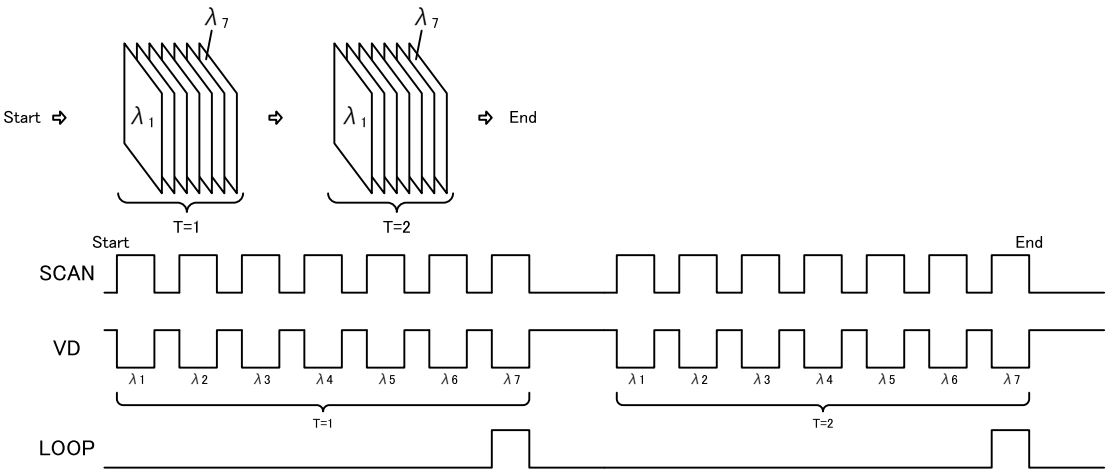


XYT scan (When using the VirtualChannel)



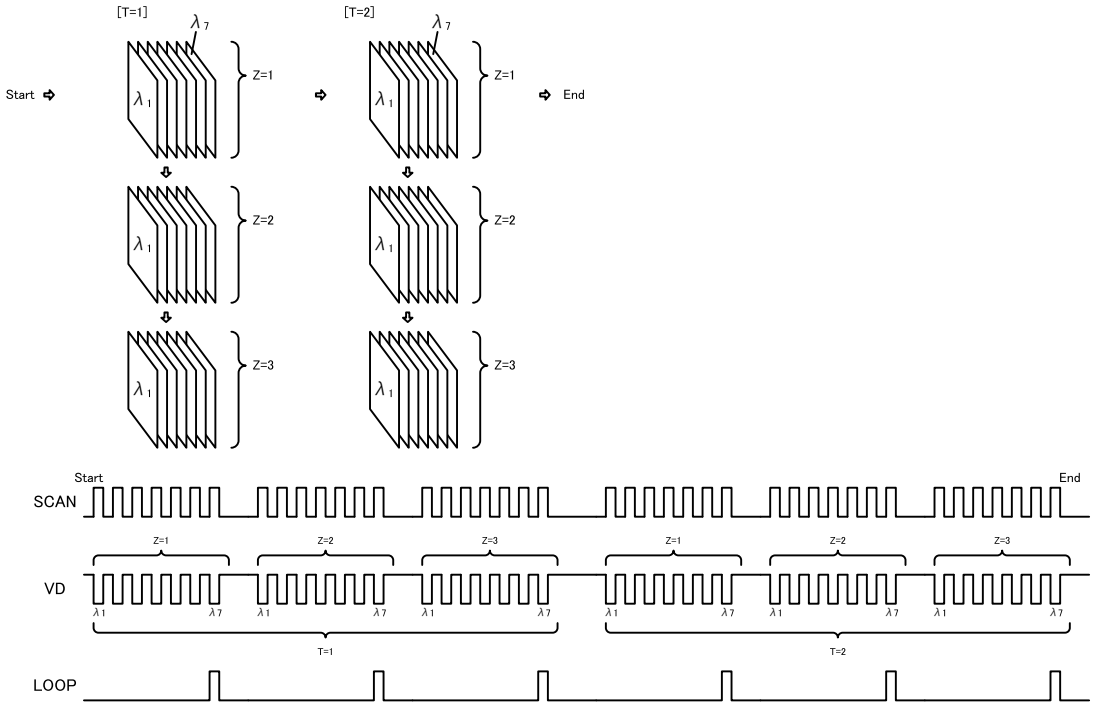
XYλT scan

λ=7 step (from λ_1 to λ_7), T=2



XYλZT scan

λ=7 step (from λ_1 to λ_7), Z=3, T=2



10 Preventive inspection sheet for illumination devices

- We recommend performing “Preventive Inspections“ periodically (every time you replace mercury burners and at least once every 6 months) .
- The table below identifies the check items to be observed. Put (X) if not applicable or (○) if applicable.
- If there are any check marks ( ) , immediately stop using the product and request inspection to Olympus or replace with new illumination device (s) .
- If you detect an abnormality other than those listed below or Olympus products other than illumination devices, also stop using the product and request inspection to Olympus.
- The repair, replacement and inspection that passed the warranty period are paid-services.

If you have any questions, please contact Olympus.

	Check results (Date)			
Check items	/	/	/	/
1. More than 8 years have passed since original purchase, or exceeds 20,000 hours of use.				
2. Light flickers when you move a lamp cable or illumination devices.				
3. A lamp cable is unusually hot to touch.				
4. Burning or smoke odor.				
5. Signs of deformation, backlash, or looseness, etc. when you assemble / disassemble the illumination device. (I.e. it is hard to open/ close the lid during lamp-replacement.)				
6. Illumination device / housing has become deformed, cracked or tarnished in any way.				
7. Lamp cables or wiring parts have become deformed, cracked or tarnished in any way.				
8. Frequent repairs to similar devices put into use at the same time as the unit being checked.				

* If the spaces are not enough for check, copy this sheet.

11 Care and storage (Maintenance)

- Never leave stains or fingerprints on the tip of lenses and filters. Should a glass component be stained, simply blow dirt away using a commercially available blower and wipe gently using a piece of cleaning paper (or clean gauze) . If a lens is stained with fingerprints or oil smudges, wipe it gently with gauze slightly moistened with commercially available absolute alcohol.
- Since the absolute alcohol is highly flammable, the system must be handled carefully. Be sure to keep it away from open flames or potential sources of electrical sparks – for example, electrical equipment that is being switched ON or OFF. Also remember to always use the system only in a well-ventilated room.
- To clean a part, wipe with a soft cloth moistened with a little drop of diluted neutral detergent. Do not use organic solvents that will deteriorate the surface coating and plastic parts.
- In case dirt such as blood or body fluid adheres to this system, soak a piece of soft cloth into diluted mild detergent and use the sterile cloth to wipe out this system for prevention of infection and, after removing all the dirt visible, wipe it out for 15 seconds or more, using Ethanol for disinfection. Meanwhile, make it certain that the power of this system is turned off at disinfection. Disinfection method described in this manual does not insure the disinfection level. However, the Ethanol for disinfection is a disinfectant solution that is sorted as mild level disinfectant solution. In case microbes that cannot be disinfected with use of Ethanol or 70V/V% Isopropanol are used, contamination or carryover by objective lens may occur and; in such a case, the user is requested to use his or her own judgment whether the applicable is appropriate or not.
- In case maintenance operation is performed on this system which was contacted by the specimen with possible infection, wear the protective equipments such as gloves, or clean this system before starting maintenance operation.
- In high-humidity environment, condensation may occur in this system. So follow the environmental condition not only in use but also in storage for a long term.

12 Troubleshooting guide (Operation / Maintenance)

Depending on usage, this system may not deliver the expected performance despite not being at fault. Should any problem occur, refer to the following list to take the appropriate corrective action.
If a problem persists, contact Olympus.

12-1 LSM observation

Problem	Cause	Remedy
1. The laser beam is not emitted from the tip of the objective.	The laser system is not turned ON.	Turn ON the laser system. Make sure that the start key of the laser system is ON.
	The start key of the laser system was turned ON immediately after turning ON the power of the laser system.	If the start key of the laser system is turned ON immediately after turning ON the power of the laser system, the laser may not be oscillated. Return the start key of the laser system to STAND-BY and turn it ON again.
	The laser wavelength is not selected.	Check the laser wavelength you use.
	The objective is not engaged in the light path.	Engage the objective in the light path. When using a manual revolving nosepiece, stop the objective at the click position.
	The laser beam is weak.	Increase the laser intensity.
	Since an object is placed on the laser system, the sufficient laser light is not emitted.	Remove the object from the laser system and restart the laser that is not emitted.
	The laser unit is ON while the cooling fan is stopped.	Turn OFF the laser unit and set the power supply for system FV31-PSU to ON to start the cooling fan. Confirm that the laser unit is cooled down and then, start the laser unit.
2. The fluorescence image is not displayed.	The selected excitation dichroic mirror is not appropriate for the observed fluorescence wavelength and excitation laser wavelength.	Engage the excitation dichroic mirror appropriate for the observed fluorescence wavelength and excitation laser wavelength in the optical path.
	The selected spectral dichroic mirror is not appropriate for the observed fluorescence wavelength.	Insert the spectral dichroic mirror appropriate for the observed fluorescence into the optical path.
	The spectral part appropriate for the fluorescence wavelength to be observed is not set.	Set the wavelength which matches the fluorescence wavelength.
	The selected excitation wavelength is not appropriate for the fluorescent staining method.	Select the laser wavelength appropriate for the fluorescent staining method.
	The focus is not properly adjusted.	Adjust the focus.
	The HV value for the detection channel is too low.	Increase the HV value.
	The OFFSET value is too large.	Decrease the OFFSET value.
	The detector for the channel to be detected is not selected.	Select the detector.
3. The transmitted light image is not displayed.	The channel for transmitted light observation is not selected.	Select the channel for transmitted light detection.

Problem	Cause	Remedy
	The transmitted light illumination filter, etc. which is not appropriate for LSM observation is inserted into the optical path.	Remove the transmitted light illumination filter which is not appropriate for LSM observation from the optical path.
	The HV value for the transmitted light detection channel is too low.	Increase the HV value.
	The OFFSET value for the transmitted light detection channel is too large.	Decrease the OFFSET value.
4. The image is disturbed.	There is too much vibration at the location where this system is installed.	Contact Olympus.
	The external light such as that from a fluorescent lamp is detected.	Darken the room before acquiring the image.
5. The reflected light (laser beam) appears in the fluorescence image.	The spectral filter is not set properly or it is not set.	Insert the spectral dichroic mirror that can block the excitation laser wavelength into the optical path.
	The spectral part appropriate for the laser wavelength to be used is not set.	Set the wavelength which matches the laser wavelength to be used.
6. The fluorescence image is not clearly visible.	The tip of the objective lens is dirty.	Wipe the tip of the objective lens with gauze.
	When using an objective with a correction collar, the correction collar is not adjusted properly.	Adjust the correction collar.
	The thickness of the cover glass is not appropriate.	Use the cover glass that is 0.17-mm thick.
7. The fluorescence image is dark and contains much noise.	The PMT is deteriorated.	The GaAsP-PMT must be replaced with a new one, since it is a consumable product. Contact Olympus.
	The laser beam is weak.	Increase the laser intensity.
	The selected excitation wavelength is not appropriate for the fluorescent staining method.	Select the laser wavelength appropriate for the fluorescent staining method.
	The selected excitation dichroic mirror is not appropriate for the observed fluorescence wavelength and excitation laser wavelength.	Engage the excitation dichroic mirror appropriate for the observed fluorescence wavelength and excitation laser wavelength in the optical path.
	The selected spectral dichroic mirror is not appropriate for the observed fluorescence wavelength.	Insert the spectral dichroic mirror appropriate for the observed fluorescence into the optical path.
	The scanning speed is too fast.	Set the scanning speed to a lower level.
	The HV value is too high.	Decrease the HV value and increase the Gain value, or decrease the scanning speed to decrease the HV value.
	The wavelength width of the acquisition wavelength range is too small.	Increase the wavelength width of the acquisition wavelength range.
	The image is stained too lightly.	Select the optimal fluorescence staining method.
8. The image is irregularly blurred. The brightness is uneven.	The specimen or stage is installed at an angle.	Properly set the specimen or stage.

Problem	Cause	Remedy
9. The image blurs.	The focus is not properly adjusted.	Adjust the focus by visual observation.
10. Flare is observed.	The specimen is overly stained.	Stain the specimen properly or increase the OFFSET amount.
11. The visual fluorescence observation is not available.	The optical path selector inside the scanner is not set as a visual observation optical path.	Insert the selector into the optical path for visual observation.
	The shutter for the mercury burner is closed.	Open the shutter for the mercury burner.
	The power supply unit of the mercury burner is not turned ON.	Turn ON the power supply unit of the mercury burner.
	The mirror unit with a dichroic mirror is not set in the illuminator turret.	Insert the mirror unit with a dichroic mirror into the optical path.

12-2 Visual observation

Problem	Cause	Remedy
1. The LED lamp does not turn ON.	The power cord of the control box IX3-CBH is not connected securely.	Connect the power cord to a power outlet securely.
	The main switch of the control box IX3-CBH is not set to ON.	Set the main switch to ▌(ON) .
	The light intensity is set to the minimum.	Adjust the brightness using the controller U-MCZ.
2. The field of view is dark even though the LED lamp turns ON.	The brightness is not adjusted.	Adjust the brightness using the controller U-MCZ.
	The centering of the condenser is not performed.	Perform the centering of the condenser so that the field diaphragm image is positioned at the center of the field of view.
	The light path is set for camera.	Change the light path.
	Too many filters are used.	Reduce the number of filters to the minimum required.
	The stage center plate is engaged in the light path.	Move the stage and place the specimen again.
	The field diaphragm is not opened wide enough.	Open the field diaphragm sufficiently.
3. The field of view is obscured or not evenly illuminated.	An objective that falls outside the condenser's illumination range is used.	Use a condenser that matches the objective.
	The centering of the condenser is not performed.	Perform the centering of the condenser so that the field diaphragm image is positioned at the center of the field of view.
	The field diaphragm is narrowed down too far.	Open the field diaphragm sufficiently.
	A filter is stopped in an intermediate position.	Set the filter at the appropriate position.

Problem	Cause	Remedy
4. Dirt or dust is visible in the field of view.	Dirt / dust on the specimen.	Clean thoroughly.
	Dirt / dust on the eyepieces.	
	Dirt / dust on a mirror unit.	
	Dirt / dust on the optical element.	
	Condenser is not correctly positioned and the frost filter Ø45 mm (45FR) or the filter is focused.	Move the condenser vertically until the field diaphragm image is formed on the specimen surface.
5. The image glares.	The condenser is raised too high.	Lower the condenser to the proper position.
	The aperture diaphragm is narrowed down too far.	Open the aperture diaphragm.
6. The error occurs when starting this system.	The power supply units were turned ON in a different order.	Turn OFF all power supply units once and turn ON the power supply units again in a correct order. For the order to turn ON the power supply units, see page 6.
7. The visibility of observed image is poor. · The image is not sharp. · The contrast is poor. · Details are poorly visible.	The objective in use is not designed for UIS2 series.	Replace with an objective designed for UIS2 series.
	When using an objective with a correction collar, the correction collar is not adjusted properly.	Adjust the correction collar to obtain the optimal contrast.
	The tip of the objective lens is dirty.	Clean thoroughly.
	The immersion oil appropriate with an oil immersion objective is not used.	Use Olympus immersion oil for the oil immersion objective, Olympus silicone oil for the silicone immersion objective and water for the water immersion objective.
	The immersion oil contains bubbles.	Remove bubbles.
	The immersion oil is dried up.	Apply the immersion oil.
	Inappropriate slide or cover glass thickness.	Replace with glasses of appropriate thickness.
	Lenses (condenser, objective, eyepieces, culture vessels) are dirty.	Clean thoroughly.
	The ring slit and phase plate are not centered.	Center them correctly.
	A plastic culture vessel is used.	Replace the plastic culture vessel with a glass vessel.
8. A part or one side of the field of view is blurred.	The specimen is tilted with respect to the stage.	Place the specimen correctly on the stage and secure it with the stage clip.
9. Field of view of one eye does not match with that of the other.	The interpupillary distance is incorrect.	Adjust the interpupillary distance.
	The diopter is not adjusted correctly.	Adjust the diopter correctly.
	You are not accustomed to parallel optical axis.	When looking into the eyepieces, do not stare at image from the beginning, but see the overall field of view. It is sometimes recommended to turn your eyes away from the eyepieces, look far off and look into the eyepieces again.

Quick reference guide

The following symbols are used in this quick reference guide.

: Indicates a potentially hazardous situation which, if not avoided, may result in damage to the equipment or other property.

: Indicates commentary for ease of operation.

For details, refer to Online Help of the standard image acquisition / analysis software for FV3000 FV31S-SW.

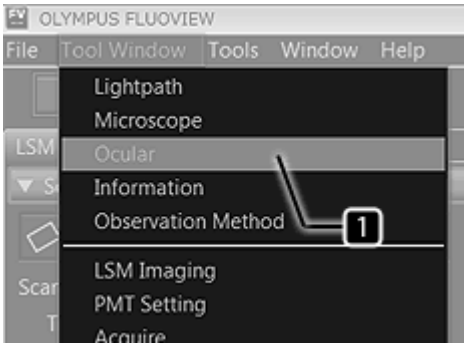
Table of contents

Observation procedures

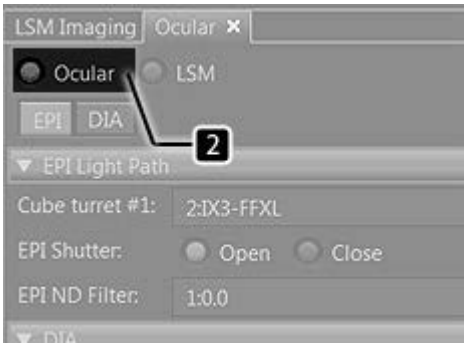
1. Visual observation with microscope

1.1. Transmitted DIC observation

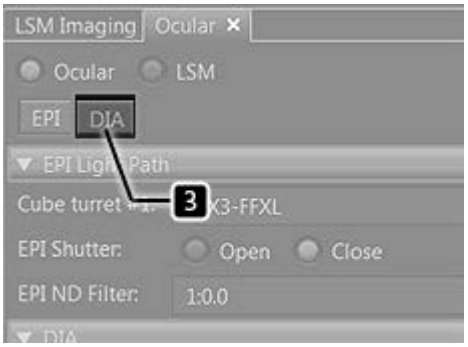
1 Select [Ocular] in the [Tool Window] menu on the software screen. [Ocular] Tool Window appears.



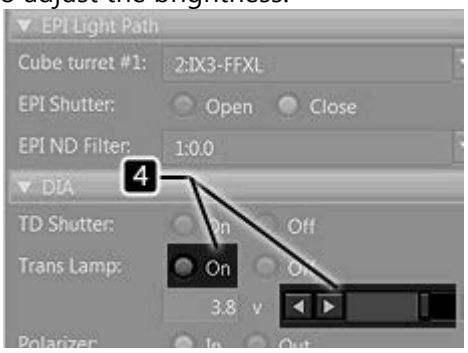
2 Select "Ocular" in [Ocular] Tool Window.



3 Tap DIA button.



4 Select "On" in [Trans Lamp], and use the slider to adjust the brightness.



5 Place the specimen on the stage.

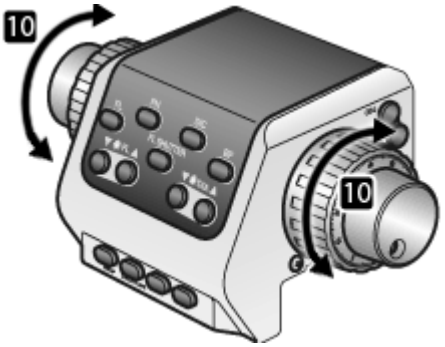
6 Tap the objective button  of the touch panel controller to engage the objective used for observation in the light path.

7 Tap the DIC button of the touch panel controller to engage the DIC prism and the objective used for observation in the light path.

8 Tap the light path selection button  of the touch panel controller to set the display of the [Light path selection] button to  .

9 Operate the condenser you are using to engage the polarizer in the light path.

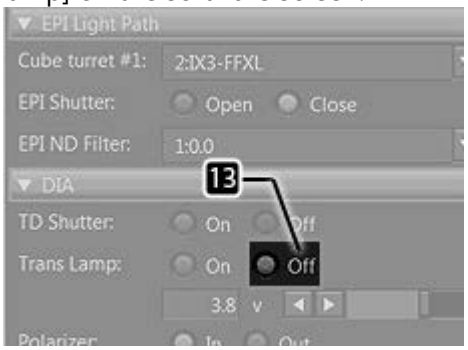
10 With looking into the eyepiece, rotate the focusing knob of U-MCZ to bring the specimen into focus.



11 Tap the [DIA] tab and the [Brightness] tab of the touch panel controller to display the screen, and adjust the brightness.
Tapping the [<<] or [>>] button changes the lamp voltage in increments of 10.
Tapping the [<] or [>] button changes the lamp voltage in increments of 1.

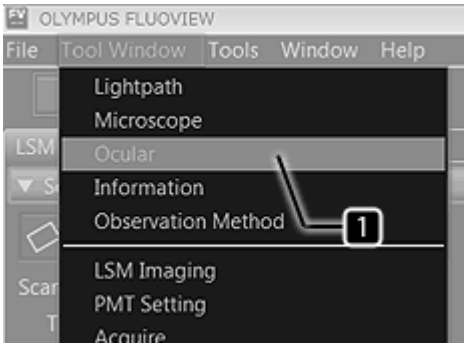
12 Tap the [DIA] tab and the [Contrast] tab of the touch panel controller to display the screen, and adjust the contrast of the DIC observation image.
Tapping the [<<] or [>>] button changes the contrast coarsely.
Tapping the [<] or [>] button changes the contrast finely.

13 To finish the observation, select "Off" of [Trans Lamp] on the software screen.

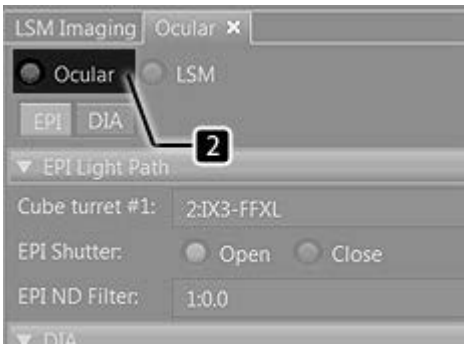


1.2. Fluorescence observation

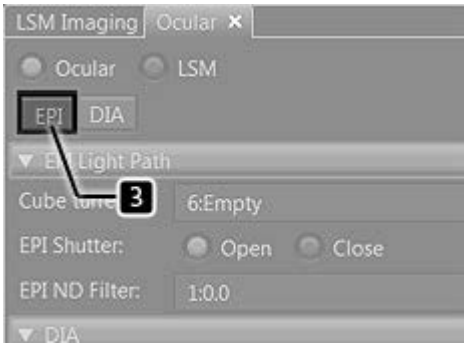
1 Select [Ocular] in the [Tool Window] menu on the software screen. [Ocular] Tool Window appears.



2 Select "Ocular" in [Ocular] Tool Window.



3 Tap EPI button.



4 Place the specimen on the stage.

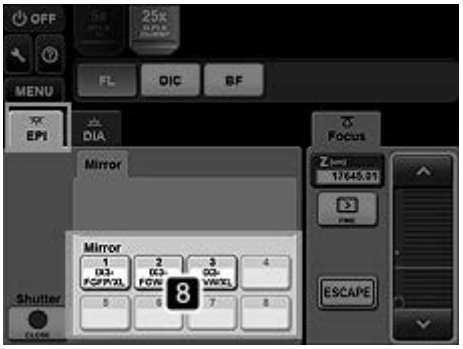
5 Tap the objective button  of the touch panel controller to engage the objective used for observation in the light path.

6 Tap the FL button of the touch panel controller.

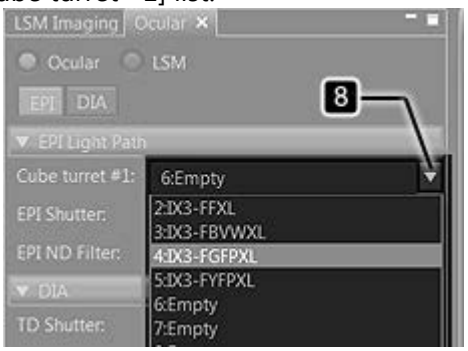
7 Tap the [Light path selection] button  of the touch panel controller to set the display of the [Light path selection] button to  .

8 Select the mirror unit suitable for the fluorescent probe for observation on either the touch panel controller or the software screen.

Touch Panel Controller: Tap the  tab to display the screen, and tap the button of the mirror unit.



Software screen: Select the mirror unit in the [Cube turret #1] list.



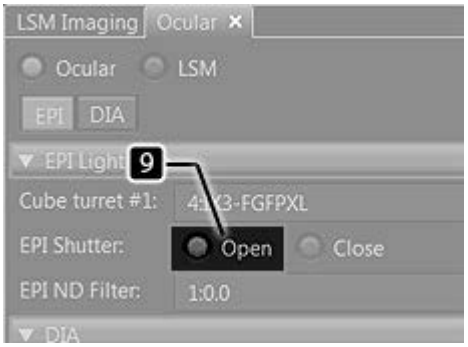
9 Open the shutter of the reflected illumination light on either the touch panel controller or the software screen.

Touch Panel Controller: Tap the 

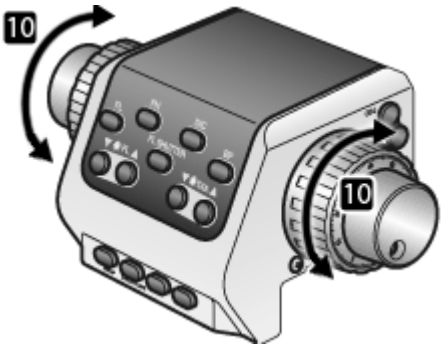
button to open (

) the shutter.

Software screen: Select "Open" in [EPI shutter].



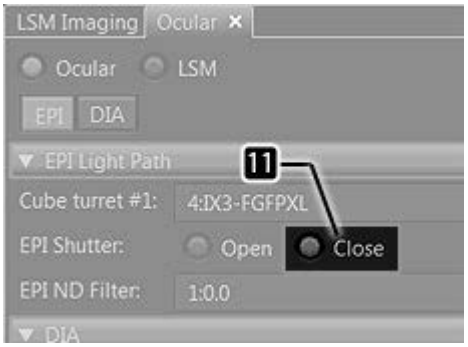
10 With looking into the eyepiece, rotate the focusing knob of U-MCZ to bring the specimen into focus.



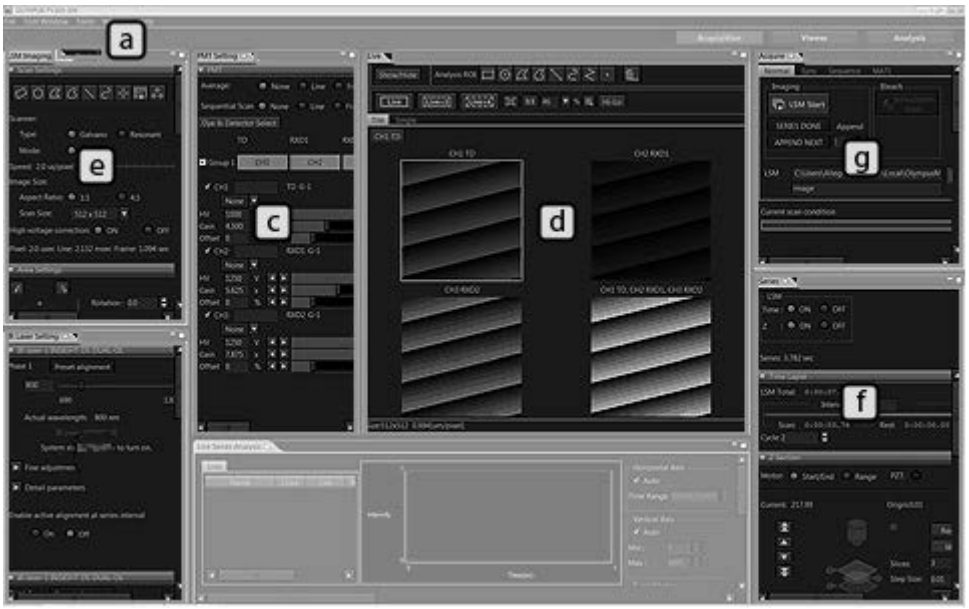
11 To finish the observation, close the shutter of the reflected illumination.

Touch Panel Controller: Tap the CLOSE button to close (CLOSE) the shutter.

Software screen: Select "Close" in [EPI shutter].



2. LSM observation structure of software screen



This section describes structure of the software screen for LSM observation.
By default, Tool Windows are arranged as shown above.
Tool Window can be arranged freely. To return to the default structure, select [Default workspace] in the [Window] menu.

a Ocular Tool Window

This field is used to select either visual observation or LSM observation.
Various settings for visual observation are also made in this field.
By default structure, this Tool Window is hidden behind [LSM Imaging] Tool Window. Click the [Ocular] tab to display this Tool Window.

c PMT Setting Tool Window

This field is used to set the image channels.
The laser intensity or the detector sensitivity is adjusted per each channel.

d Live Window

This field displays the images by repeat scan.

e LSM Imaging Tool Window

This field is used to set the scan method for image acquisition.
Scanner selection, scan area, scan speed or etc. is set in this field.

f Series Tool Window

This field is used to set the Z series and T series image acquisition.

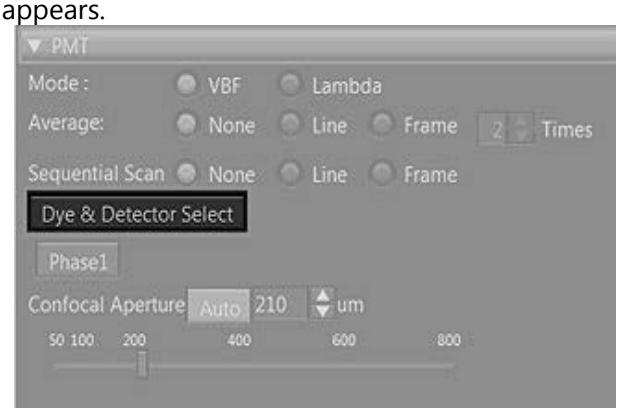
g Acquire Tool Window

This field is used to acquire the images.

3. Acquiring image

3.1. Assigning the detector to channel

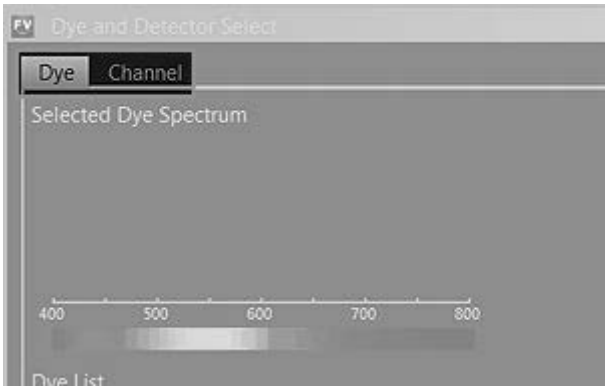
1 Press the Dye & Detector Select button on [PMT Setting] Tool Window. The [Dye & Detector Select] dialog box appears.



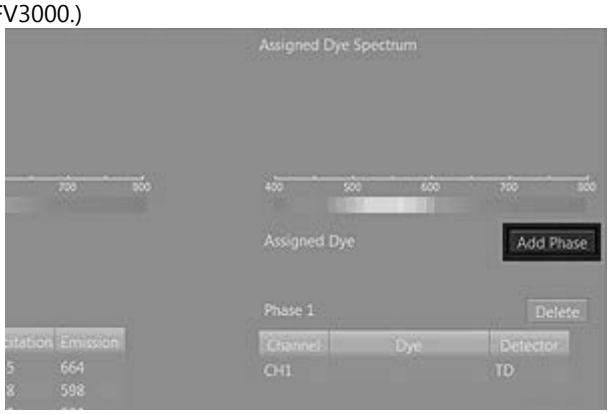
2 Select the [Dye] or the [Channel] tab in the [Dye & Detector Select] dialog box.

Dye Specify the fluorescent reagent (Dye) and assign the observation channel.

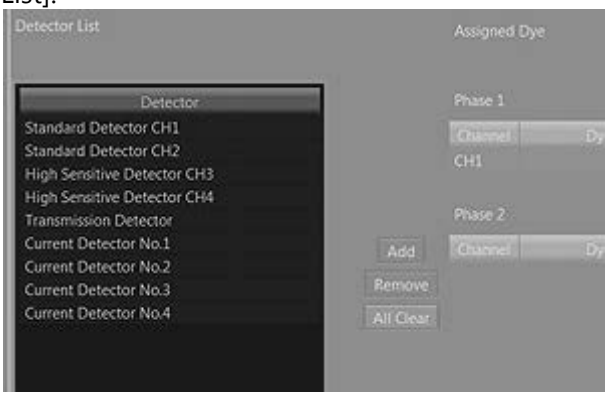
Channel Specify the detector and assign the observation channel.



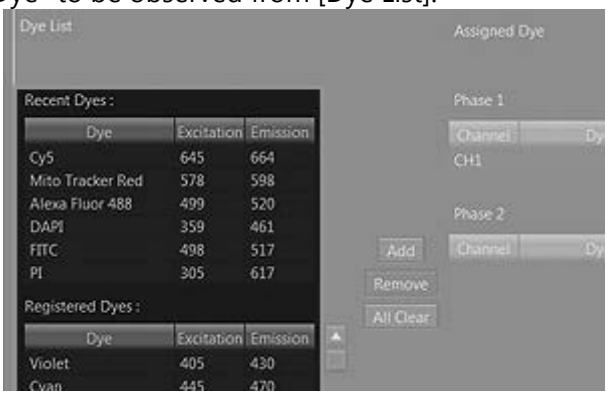
3 If you want to observe in multiple phases using the virtual channel scan, press the Add Phase button to add the phase.
(The virtual channel scan is the option of FV3000.)



4 If you selected [Channel] in 2, select the detector to be assigned to the observation channel from [Detector List].



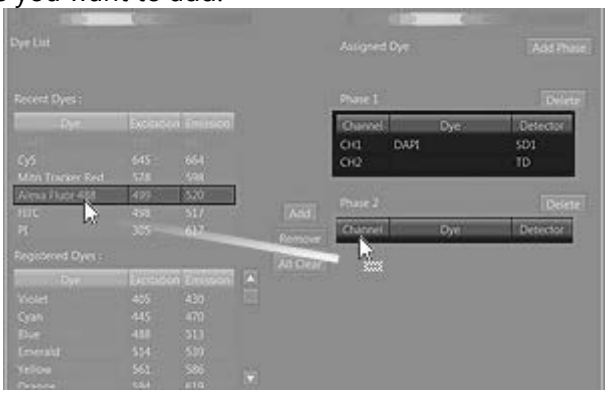
If you selected [Dye] in 2, select "Dye" to be observed from [Dye List].



If "Dye" you want to observe is not in the list, create "Dye" or select "Dye" with similar "Excitation" and "Emission" wavelengths instead.
You can create "Dye" in [Dye Editor] dialog box displayed when you select [Dye Editor] in the [Tool] menu.

5 If you did not add the phase in 3, press the Add button.

If you added the phase in 3, drag&drop the selected detector or "Dye" to the observation channel list of the phase you want to add.



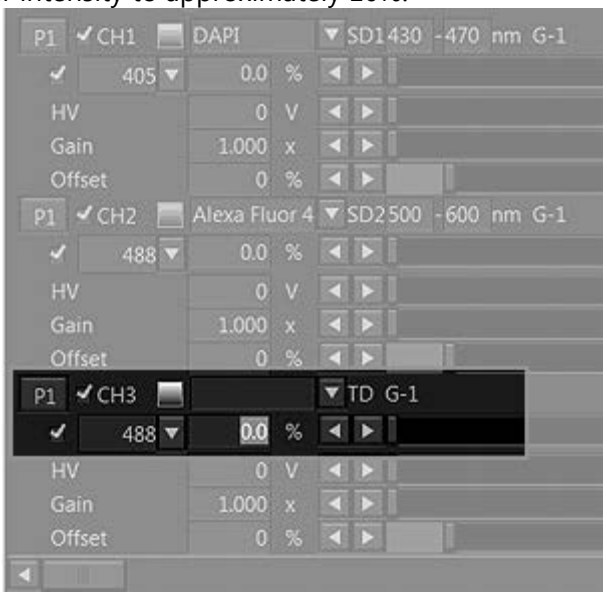
The observation channel number and the abbreviation (physical channel name) of the assigned detector are displayed in the observation channel list.

6 After setting all channels, press the OK button.

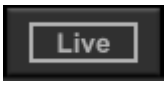
3.2. Adjusting the live image

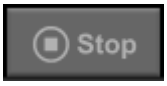
1 Select "LSM" in [Ocular] Tool Window.

2 On [PMT Setting] Tool Window, tick the checkboxes of the channel to acquire the image and the laser, and set the laser intensity to approximately 10%.



3 Set Image Size.

4 Press the 

button on the [Live] window. (The button view is changed to 

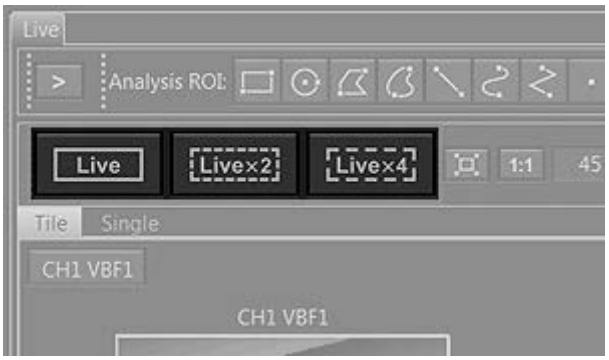
.)

The live image (repeat scan image) is displayed on the LSM live area.

Pressing the 

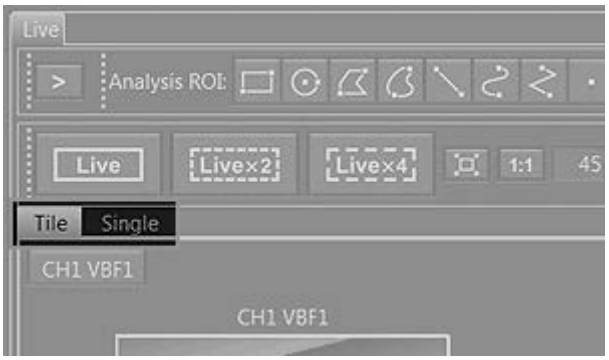
button or the 

button will display the live image whose repeat interval is reduced by subtractive scan. This function is useful when searching the cells or observing with moving the focus.



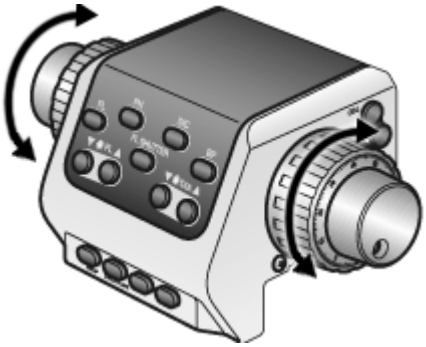
5 Select the display method of the live image.

Tile	The LSM live area is divided, and the image of each channel and the image composing all channels are displayed side by side.
Single	The image composing all channels is displayed over the entire LSM live area.

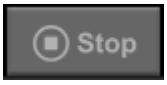


6 Place the specimen on the stage.

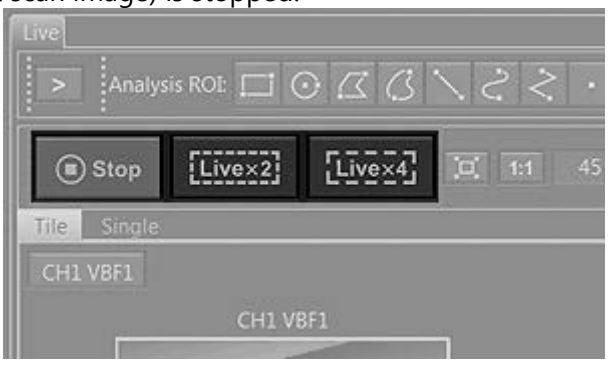
7 With viewing the live image, rotate the focusing knob of U-MCZ to bring the specimen into focus.



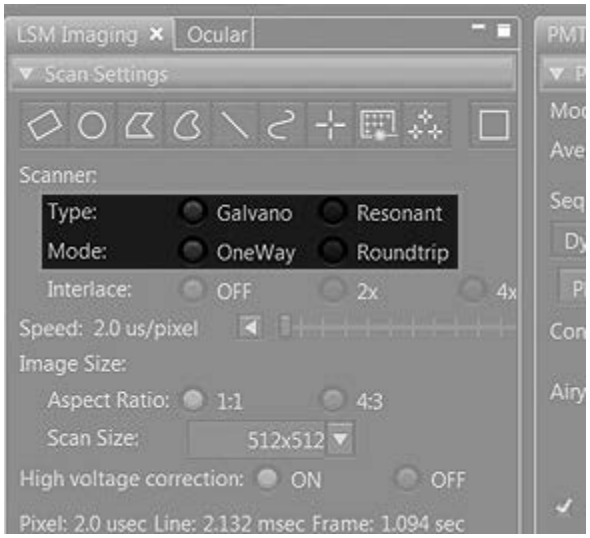
8 On [PMT Setting] Tool Window, set Laser Intensity (%), Sensitivity (HV), Gain and Offset to adjust the live image.

9 Press the 

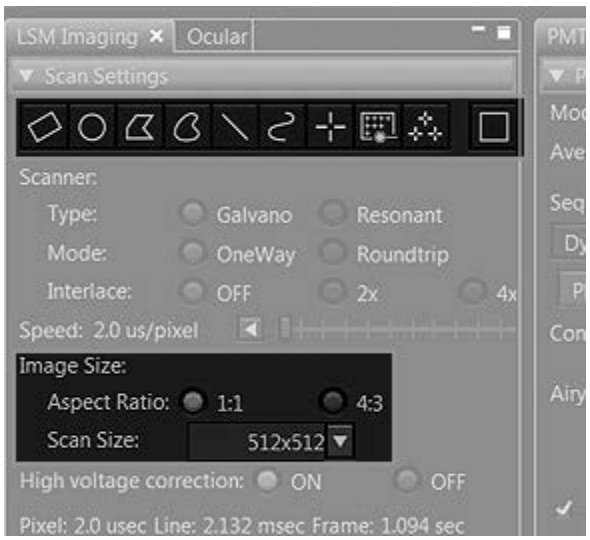
button on the [Live] window.
The display of the live image (repeat scan image) is stopped.



10 On [LSM Imaging] Tool Window, select the scan method in [Type] and [Mode].
Type "Galvano" (High definition scan) or "Resonant" (High speed scan)
Mode "OneWay" (Scan in one direction) or "Roundtrip" (Scan in both directions)



11 Specify ROI or [Image Size] to set the scan area.



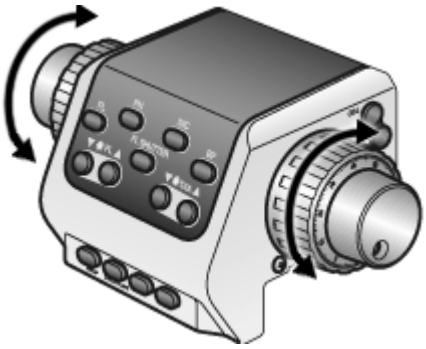
3.3. Acquiring the series image

3.3.1. Setting Z series (Specifying start/end positions)

1 Select "ON" in [Z] on [Series] Tool Window.

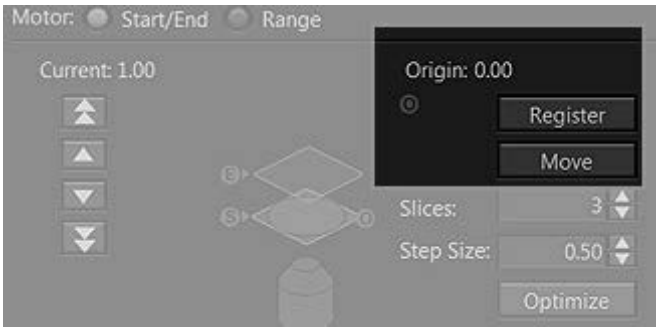
2 Select "Start/End" in [Motor] on [Z Section].

As described below, set Z series with changing the Z position by rotating the focusing knob of U-MCZ.



3 Press the Register button in [Origin] at the Z position which is the reference position to acquire the images.

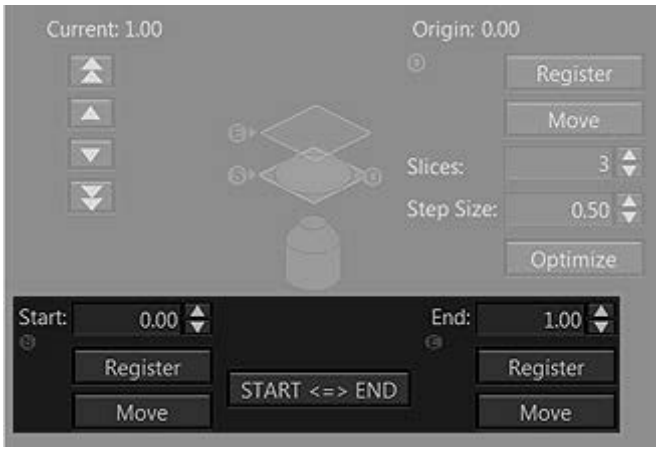
The distance from the Z position registered in [Origin] to the current Z position is displayed in [Current].
If you register the top surface of the specimen in [Origin], the depth from the top surface to the observation position is always displayed in [Current], which is very useful.



4 Press the Register button in [Start] at the Z position to start acquiring the image.

Then, go on to press the Register button in [End] at the Z position to end acquiring the image.

Press the START <=> END button to reverse the start position and the end position.



5 Set either [Slices] or [Step Size]. Setting one will set the other automatically.

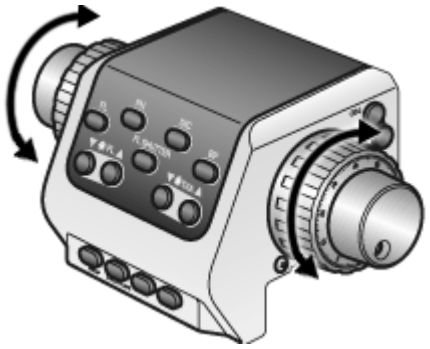
3.3.2. Setting Z series (Specifying the center position and the range)

1 Select "ON" in [Z] on [Series] Tool Window.

2 Select "Range" in [Motor] on [Z Section].

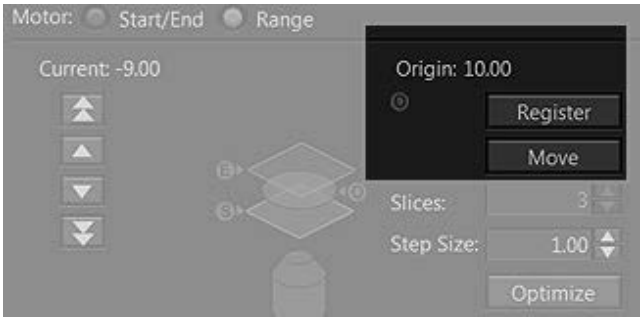
"Range" is suitable when you set the acquisition area in the Z direction assuming the displacement of the focus position during acquiring T series images.

As described below, set Z series with changing the Z position by rotating the focusing knob of U-MCZ.



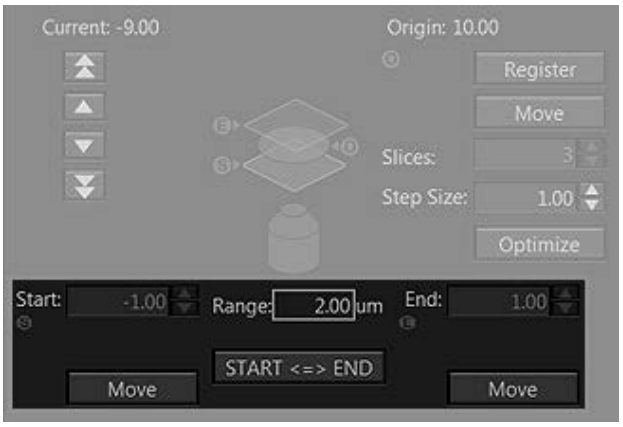
3 Press the Register button in [Origin] at the Z position which is the reference position to acquire the images.

The distance from the Z position registered in [Origin] to the current Z position is displayed in [Current].
If you register the top surface of the specimen in [Origin], the depth from the top surface to the observation position is always displayed in [Current], which is very useful.



4 Set the range to acquire the image in [Range]. The start position and the end position to acquire the image are set automatically.

Press the START <=> END button to reverse the start position and the end position.



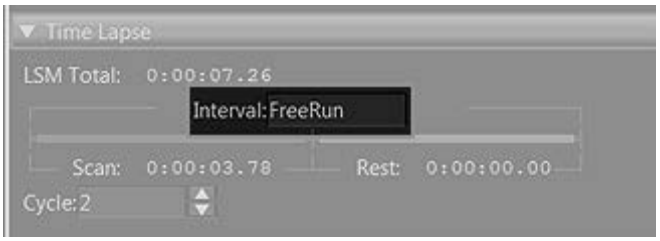
5 Set either [Slices] or [Step Size]. Setting one will set the other automatically.

3.3.3. Setting T series

1 Select "ON" in [Time] on [Series] Tool Window.

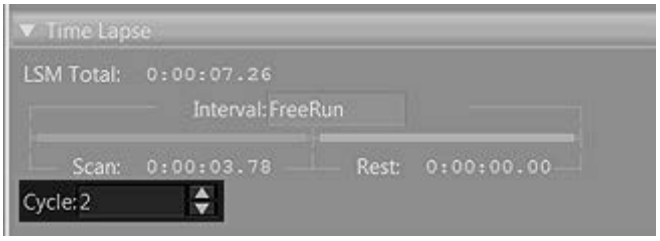
2 Set the interval to acquire the image in [Interval] on [Time Lapse].

If you attempt to set the time shorter than the time displayed in [Scan] (time taken for acquiring 1 cycle image) in [Interval], "FreeRun" appears. In this case, the interval to acquire the image is the time displayed in [Scan].



3 Set the number of image acquisitions in [Cycle].

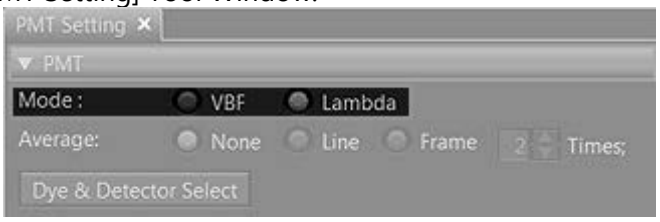
The time from the start of the T series image acquisition to the end of the T series image acquisition is calculated and displayed in [LSM Total]. Set [Cycle] so that [LSM Total] becomes an appropriate time.



3.3.4. Setting Lambda series (acquiring by a single channel)

Changing to Lambda mode

1 Select "Lambda" in [Mode] in [PMT Setting] Tool Window.



Selecting one channel to be used

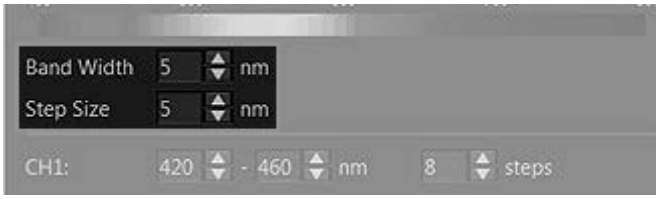
2 Press the Dye & Detector Select button. The [Dye & Detector Select] dialog box appears.

3 Select the [Channel] tab in [Dye & Detector Select] dialog box.
Select the detector to be assigned to the observation channel in [Detector List], and press the Add button. The observation channel number and the abbreviation (physical channel name) of the assigned detector are displayed in the observation channel list.

4 Press the OK button to close the [Dye & Detector Select] dialog box.

Setting the width of the wavelength for which the photometry is performed and the interval between wavelengths to start acquisition

5 In [PMT Setting] Tool Window, set the width of the wavelength for which the photometry is performed per each step in [Band Width], and set the interval between the wavelength to start acquisition and the wavelength to start acquisition in the next step in [Step Size].

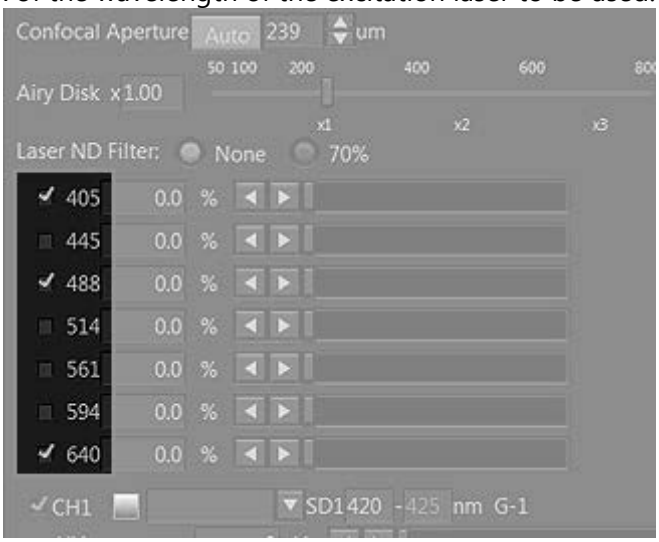


Setting the wavelength to start photometry and the wavelength to end photometry

6 Set them by dragging the mouse on the profile display area or set the value directly to [CH1].

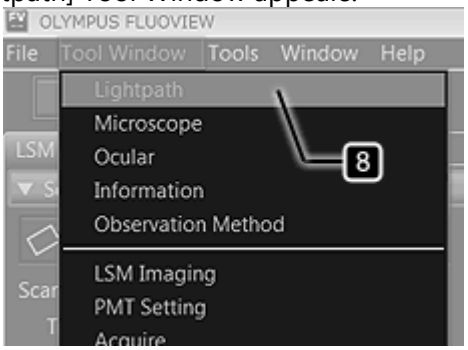
Selecting the excitation laser to be used

7 Tick the checkbox of the wavelength of the excitation laser to be used.

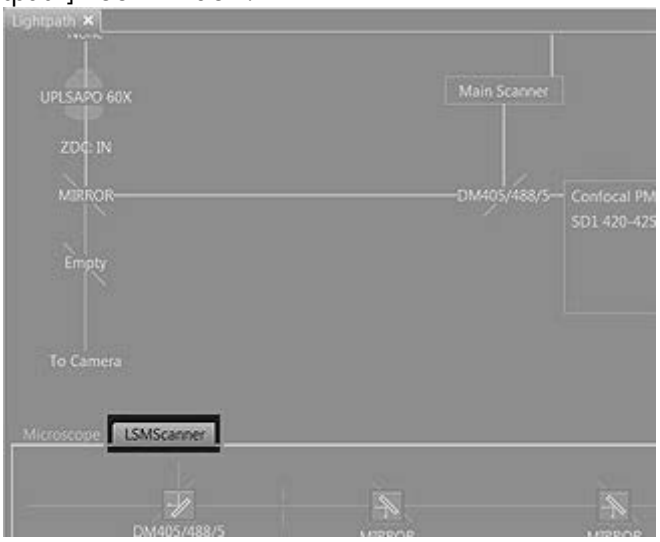


Selecting DM and SDM so that the intended fluorescence wavelength reaches the detector

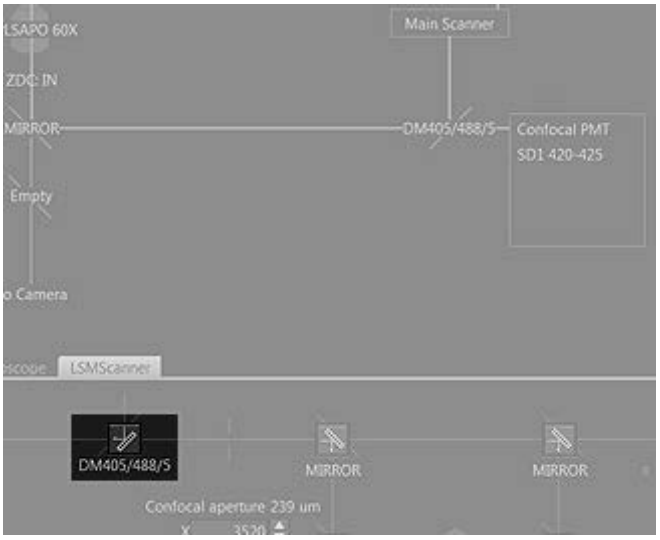
8 Select [Lightpath] in [Tool Window] menu. [Lightpath] Tool Window appears.



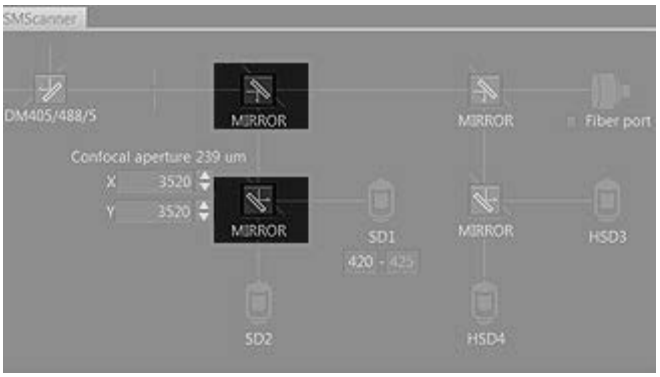
9 Select [LSMScanner] tab in [Lightpath] Tool Window.



10 Press the DM button to display the dichroic mirror (DM) list. Select "DM" which reflects the wavelength of the excitation laser selected in 7.



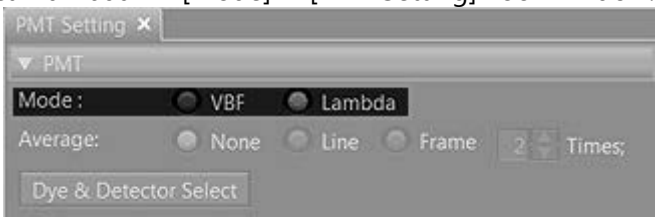
11 Press the SDM button to display the photometry dichroic mirror (SDM) list. Select "SDM", "Mirror" or "Glass" so that the intended fluorescence wavelength reaches the detector of CH1.



3.3.5. Setting Lambda series (acquiring by multiple channels)

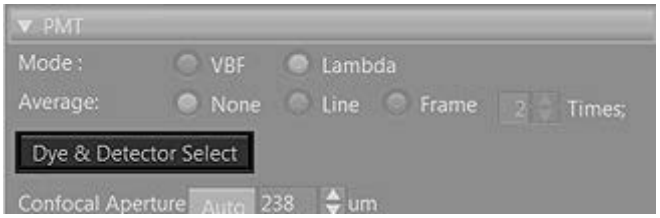
Changing to Lambda mode

1 Select "Lambda" in [Mode] in [PMT Setting] Tool Window.

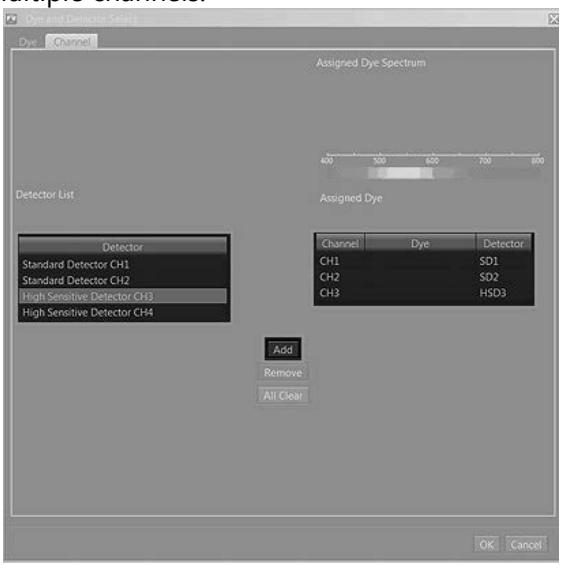


Selecting multiple channels to be used

2 Press the Dye & Detector Select button. The [Dye & Detector Select] dialog box appears.



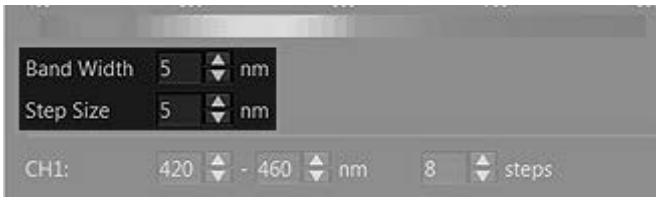
3 Select the [Channel] tab in [Dye & Detector Select] dialog box.
Select the detector to be assigned to the observation channel in [Detector List], and press the Add button. The observation channel number and the abbreviation (physical channel name) of the assigned detector are displayed in the observation channel list.
Repeat this operation for a number of multiple channels.



4 Press the OK button to close the [Dye & Detector Select] dialog box.

Setting the width of the wavelength for which the photometry is performed and the interval between wavelengths to start acquisition

5 In [PMT Setting] Tool Window, set the width of the wavelength for which the photometry is performed per each step in [Band Width], and set the interval between the wavelength to start acquisition and the wavelength to start acquisition in the next step in [Step Size].

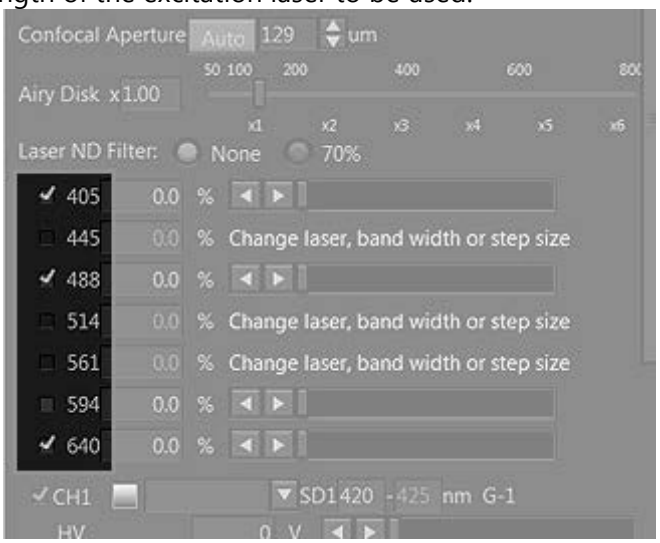


Setting the wavelength to start photometry and the wavelength to end photometry by each channel

6 Set them by dragging the mouse on the profile display area or set the value directly to each channel ([CH*x*]) .

Selecting the excitation laser to be used

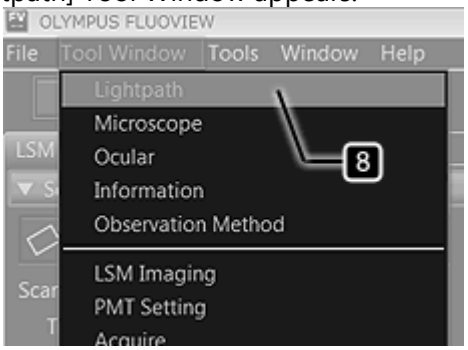
7 Tick the checkbox of the wavelength of the excitation laser to be used.



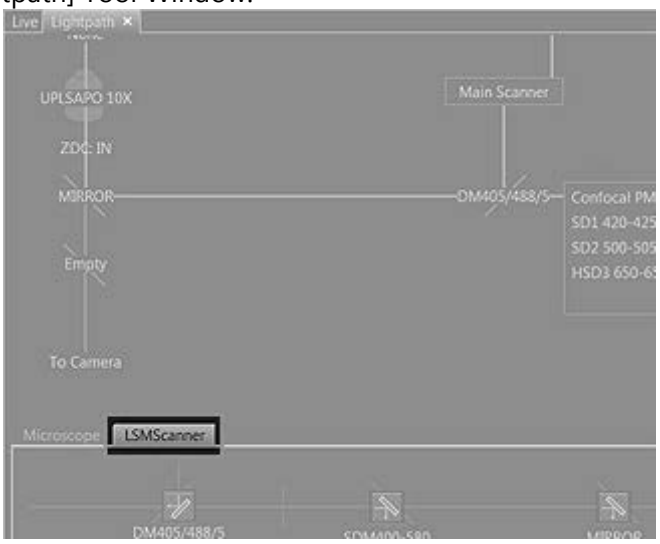
You cannot select the laser which includes ±5nm of the excitation wavelength in the photometry wavelength range of each channel.

Selecting DM and SDM so that the intended fluorescence wavelength reaches the detector

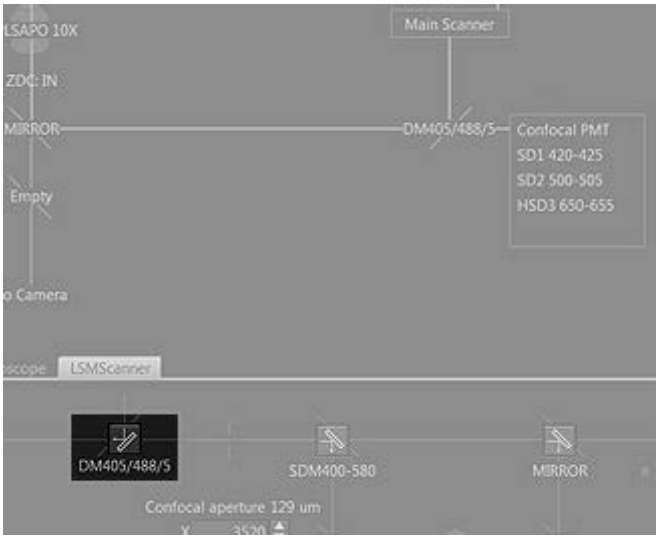
8 Select [Lightpath] in [Tool Window] menu. [Lightpath] Tool Window appears.



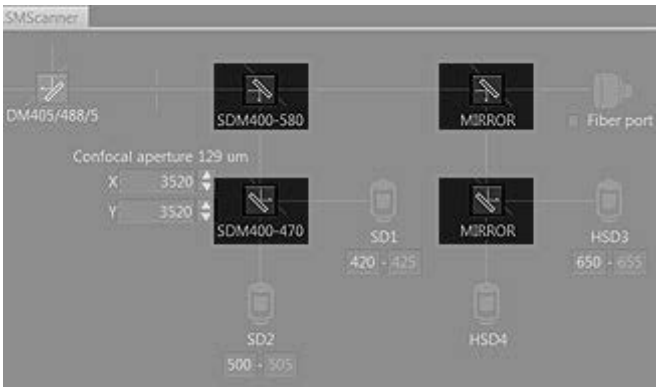
9 Select [LSMScanner] tab in [Lightpath] Tool Window.



10 Press the DM button to display the dichroic mirror (DM) list. Select "DM" which reflects the wavelength of the excitation laser selected in 7.



11 Press the SDM button to display the photometry dichroic mirror (SDM) list. Select "SDM", "Mirror" or "Glass" so that the intended fluorescence wavelength reaches each detector.



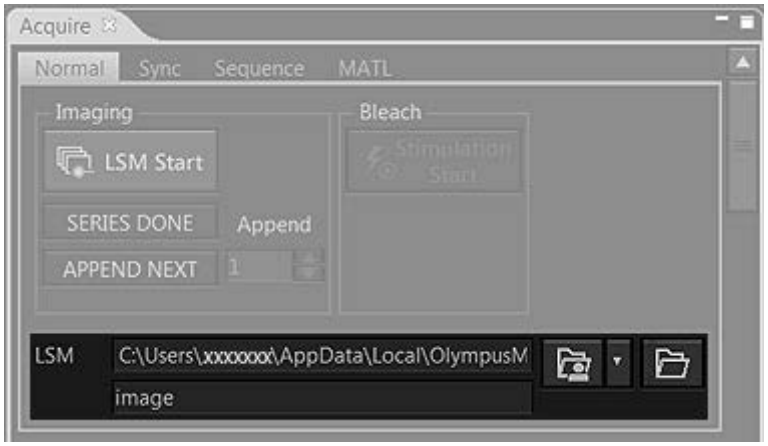
3.3.6. Starting acquisition

1 Select [Normal] tab in [Acquire] Tool Window.

2 Press the  button to display the dialog box, and select the folder to save the images.

In order to organize files easily after acquiring the images, it is recommended to create a new folder before acquiring the images and specify that folder as the save destination of the images.

The acquired images are saved automatically.

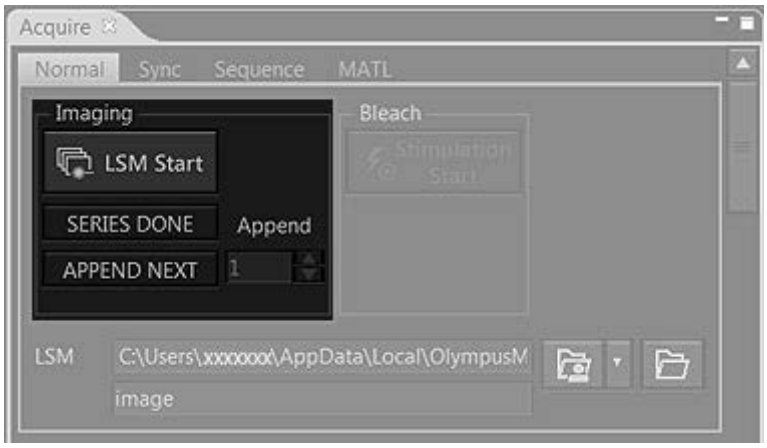


3

Press the 

button to start acquiring the image.

When the image acquisition starts, the [Image] window opens and the image in process of acquisition is displayed.



4

After the image is acquired, pressing the 

button allows you to perform the image acquisition repetitively under the same condition. Press the 

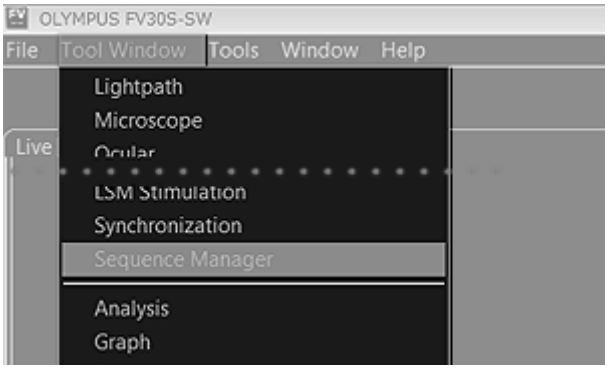
button to compete the image acquisition.

3.4. Automation of acquisition

3.4.1. Planning acquisition (Creating protocols)

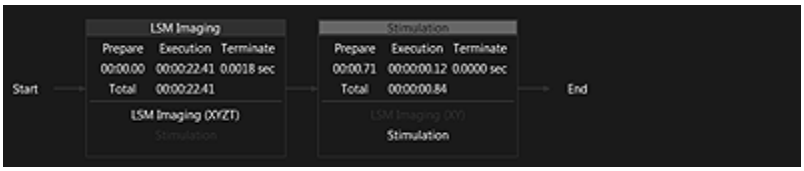
The tasks, such as to acquire, stop, loop images or etc. are registered as a series of flow. This series of flow is called Protocols. The protocols are created in [Sequence Manager] Tool Window .

1. Select [Sequence Manager] from the [Tool Window] menu. [Sequence Manager] Tool Window appears.



Registering Acquisition task

1. Press the [Add task] button to register tasks.
   If there are tasks which were registered, the new task is added to the last task.



Registering Wait task or Pause task

1 Press the Wait/Pause button in [Add task] to display the [Wait/Pause Setting] dialog box.

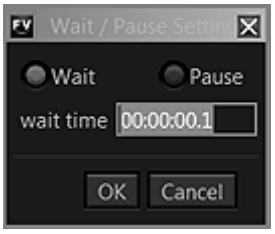
2 Select "Wait" or "Pause".

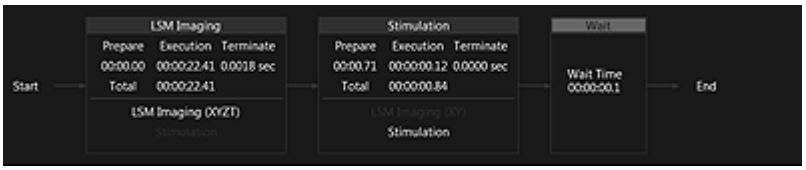
Wait The execution of protocols is paused until the time specified in [wait time] elapses.

Pause

The execution of protocols is paused until the Resume button in [Acquire] Tool Window is pressed.

Press the OK button to register the Wait task or the Pause task.





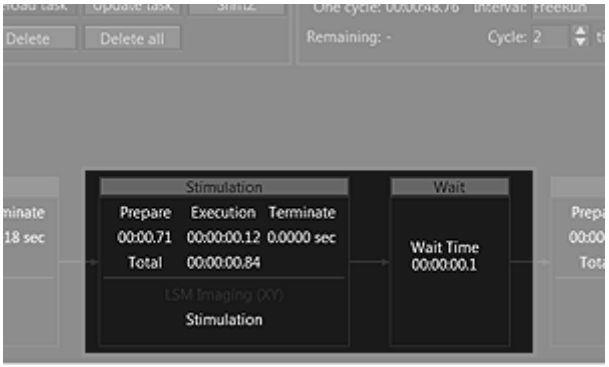
If the tasks configured as follows are registered, the protocol may be stopped in the middle.

[Configuration]

- **Wait task of 30 seconds or more immediately after Stimulation task**
- **Cycle Rest time of 30 seconds or more after Stimulation task (Cycle Rest time means the Rest time from the time when one cycle is completed to the time when the next cycle starts and it is the time subtracting [One cycle] from [Interval].)**

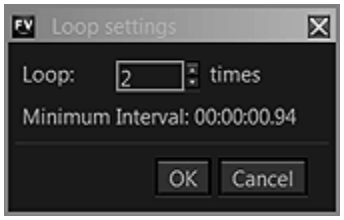
Registering Loop task

1 Click the task to be repeated. If you want to select multiple tasks, click them with pressing Ctrl key or Shift key.



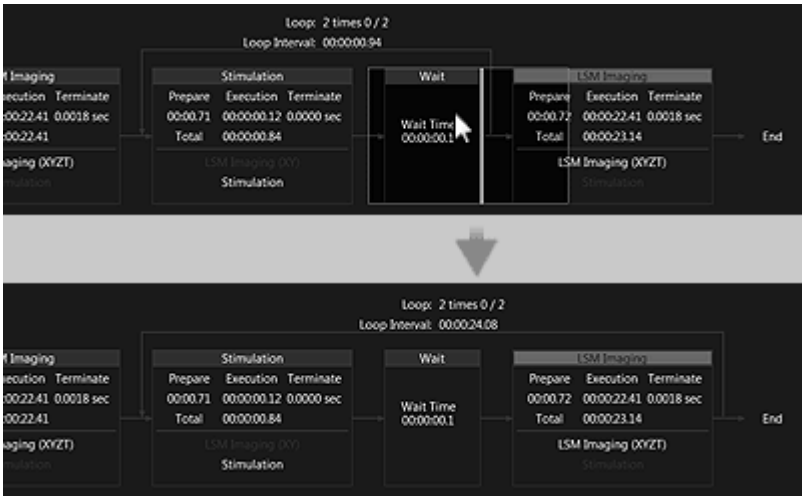
2 Press the Loop button in [Add task] to display the [Loop Setting] dialog box.

3 Set the number of repetitions in [Loop], and press the OK button. The Loop task is registered.



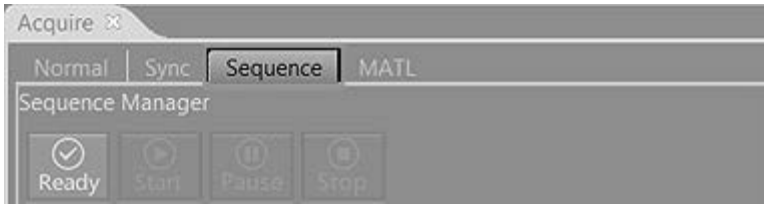
Moving the task position

1. When you drag the task you want to move, the vertical line appears to show the position to insert the task. Drag the task so that this line comes to the destination.



3.4.2. Executing and registering protocol

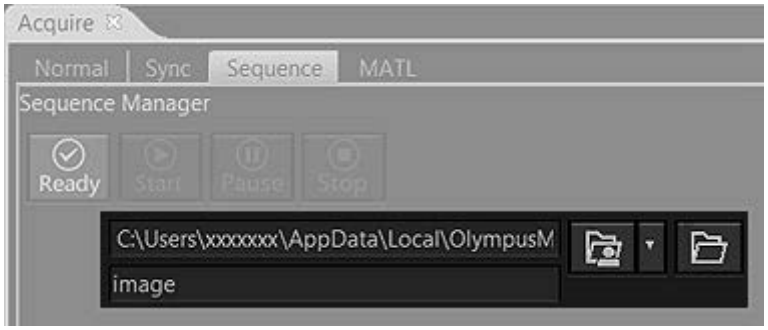
1 Select [Sequence] tab in [Acquire] Tool Window.



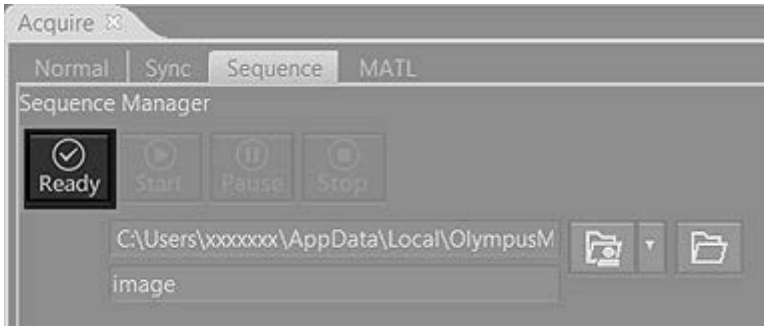
2 Press the  button to display the dialog box, and select the folder to save the images.

In order to organize files easily after acquiring the images, it is recommended to create a new folder before acquiring the images and specify that folder as the save destination of the images.

The acquired images are saved automatically.

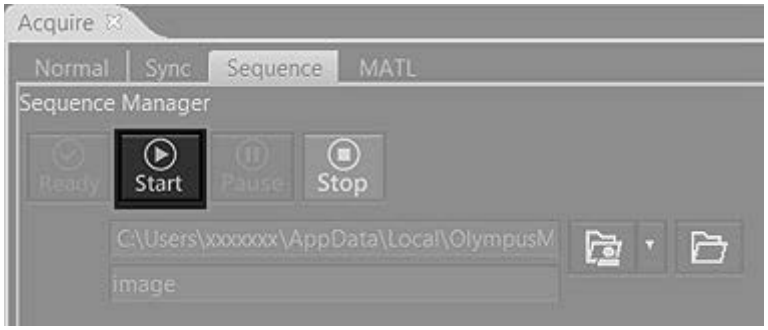


3 Press the  button. The protocols set in [Sequence Manager] Tool Window are registered to the system of the laser microscope.



4

Press the Start button. The protocols start (are executed).



Pause: The running protocols are paused.

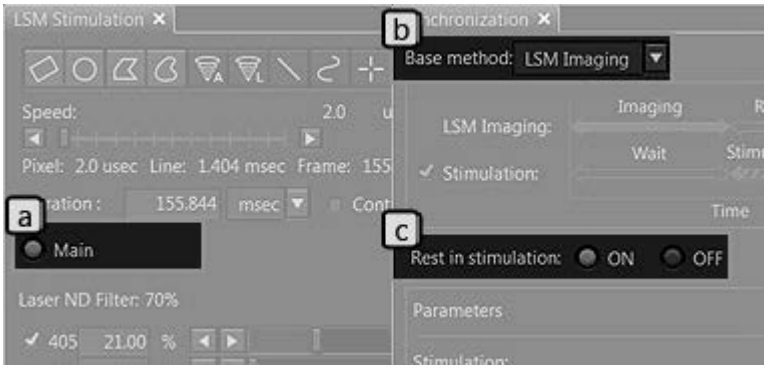
Stop: The running protocols are stopped.

If you started the protocol by ticking [append task as Ch/λ data] or [append each task in each cycle as T series data] in [Sequence Manager] Tool Window, do not close the window of the acquired image until the protocol finishes.
If the window of the acquired image is closed during executing the protocol, the append process is not executed.

3.5. Timing of image acquisition and light stimulation

3.5.1. Creating timeline

This function allows you to create the timeline of the image acquisition and the light stimulation.
The image acquisition is set in [Series] Tool Window and the light stimulation in [LSM Stimulation] Tool Window respectively in advance.
The timeline is created in [Synchronization] Tool Window, but the setting items differ depending on the combination of a, b or c to be selected in the following picture.

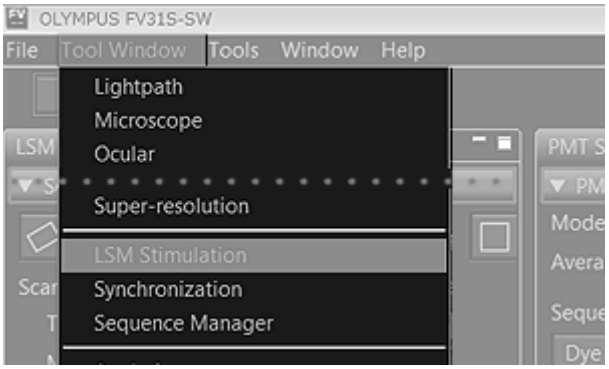


This section describes the operating procedures by using the following selection as an example.

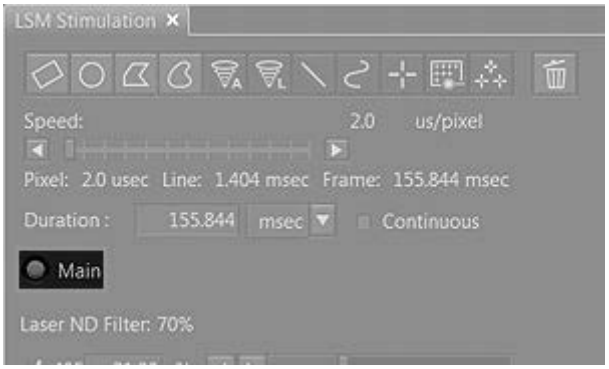
a: "Main" (The main scanner is used for the light stimulation.)
b: "LSM Imaging" (The image acquisition becomes the reference of the start time.)
c: "ON" (The image acquisition is stopped during the light stimulation.)

Setting the light stimulation

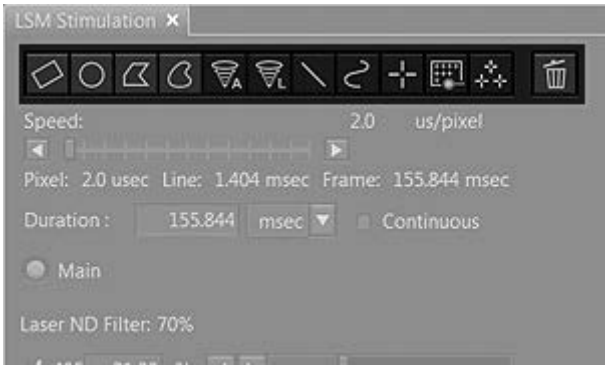
1. Select [LSM Stimulation] in [Tool Window] menu. [LSM Stimulation] Tool Window appears.



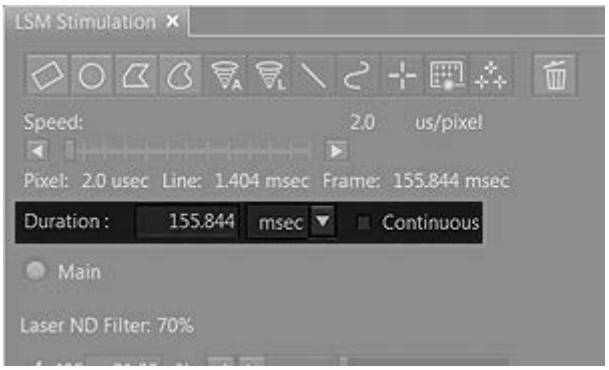
2. Set the scanner to be used for the light stimulation to "Main". (If only the main scanner is installed on the system, confirm that it is selected.)



3. Select ROI, and specify the light stimulation range in the LSM live range on [Live] Window.



4 Uncheck [Continuous] in [Duration], and enter the duration of the light stimulation in the box.

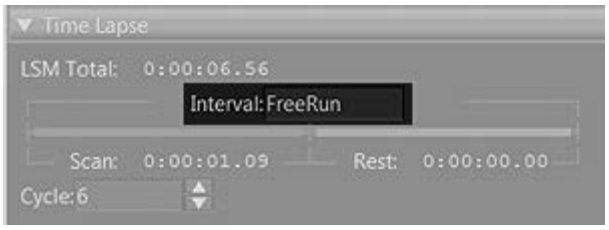


Setting the T series image acquisition

1. Set [Time] to "ON" on [Series] Tool Window.

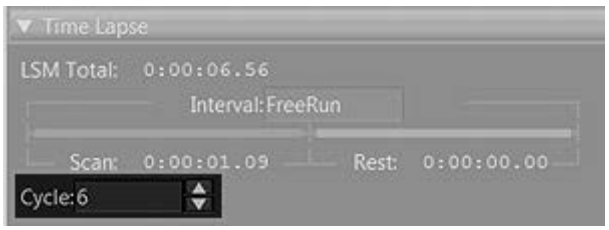
2. Set the interval to acquire the image in [Interval] on [Time Lapse].

   If you attempt to set the time shorter than the time displayed in [Scan] (time taken for acquiring 1 cycle image) in [Interval], "FreeRun" appears. In this case, the interval to acquire the image is the time displayed in [Scan].



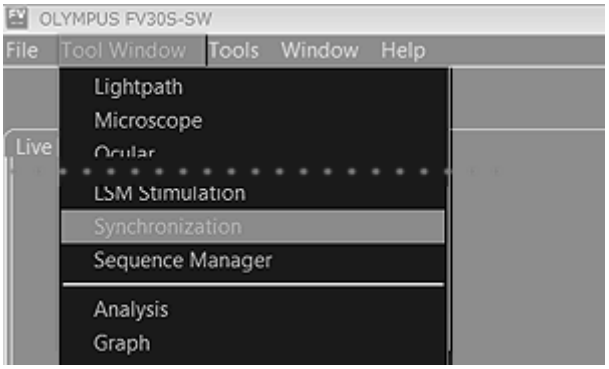
3. Set the number of image acquisitions in [Cycle].

   The time from the start of the T series image acquisition to the end of the T series image acquisition is calculated and displayed in [LSM Total]. Set [Cycle] so that [LSM Total] becomes an appropriate time.

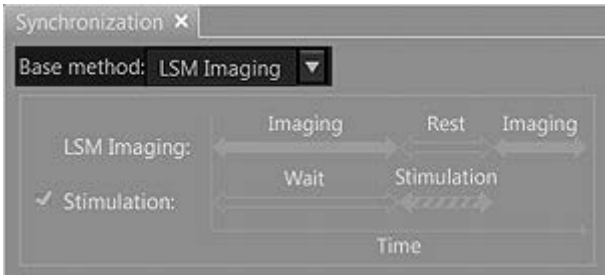


Creating the timeline

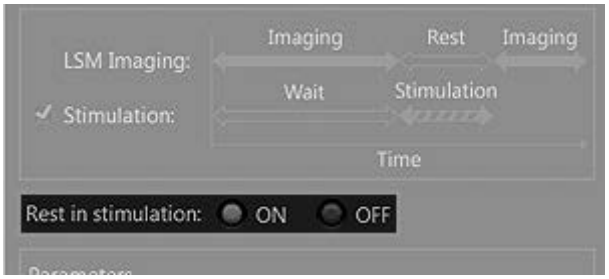
1. Select [Synchronization] in the [Tool Window] menu. [Synchronization] Tool Window appears.



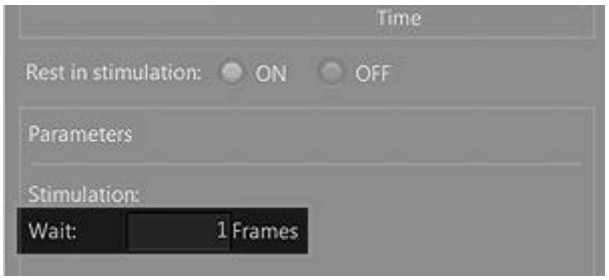
2. Select "LSM Imaging" in [Base method]. The image acquisition becomes the reference of the start time.



3. Select "ON" in [Rest in stimulation]. The image acquisition stops during the light stimulation.



4 Set the timing to start the light stimulation by the number of imaging frames.
Double-click the [Wait] box and enter the number of imaging frames.



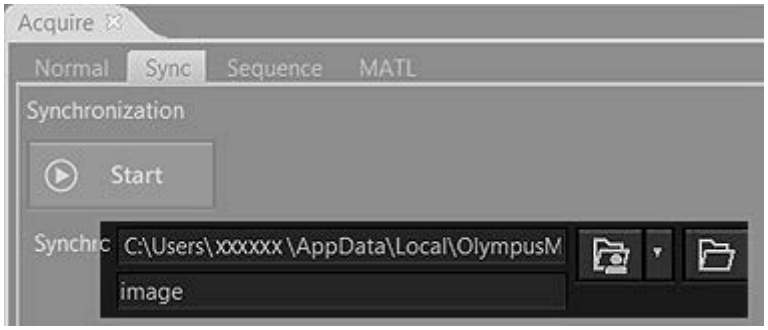
3.5.2. Executing timeline

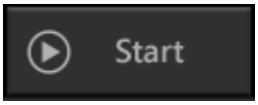
1 Select [Sync] tab in [Acquire] Tool Window.

2 Press the  button to display the dialog box, and select the folder for saving the image.

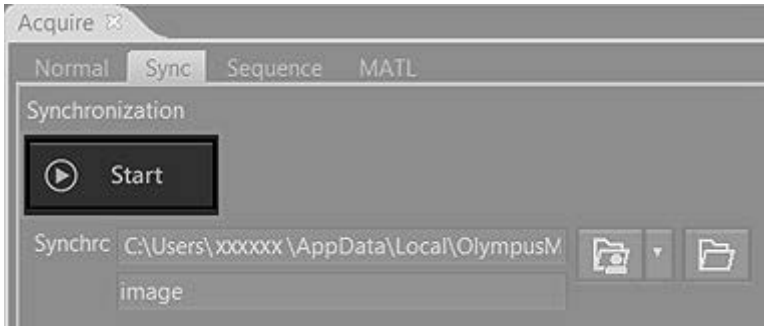
In order to organize files easily after acquiring the images, it is recommended to create a new folder before acquiring the images and specify that folder as the save destination of the images.

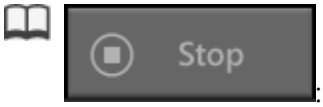
The acquired images are saved automatically.



3 Press the 

button. The image acquisition or the light stimulation starts according to the timeline set in [Synchronization] Tool Window.





: Stops the image acquisition or the light stimulation.

For advanced observation

4. Acquiring the super-resolution image

4.1. Outline of acquisition procedures

GaAsP-PMT is required to use this function. FV31-HSD must be installed on the system.

1 Attach the objective lens applicable for super-resolution to the microscope and register this objective lens in the [Microscope] tab in the [Configuration] dialog box.

Objective lens applicable for super-resolution
UPLSAPO60XW
UPLSAPO60XO
UPLSAPO60XS
UPLSAPO60XS2
UPLSAPO100XO
UPLSAPO100XS
PLAPON60XO
PLAPON60XOSC
PLAPON60XOSC2
APON60XOTIRF
APON100XHOTIRF
UAPON100XOTIRF
UPLSAPO30XS
UPLSAPO40XS
UPLSAPO40X2

2 Set the super-resolution mode to OFF, and adjust the live image and set the basic observation conditions.

- Assigning the detector to channel
- Adjusting the live image

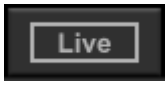
Adjust the position so that the observation target comes to the center of the live image.
When the super-resolution mode is set to ON according to the following procedures, the live image is zoomed-in (enlarged). Therefore, if the observation target deviates from the center of the live image significantly, the observation target is not visible when the live image is zoomed-in.

3 Set the super-resolution mode to ON.

4 Adjust the scan conditions while performing the repeat scan [*1].

5 Set for acquiring the image in the super-resolution mode.

6 Acquire the super-resolution image.

*1 Repeat Scan means to acquire the live image continuously by pressing the 

, 

or 

button in the [Live] window.

4.2. Setting the super-resolution mode to ON

1 Select "ON" in [Super-resolution imaging] in the [Super-resolution] tool window to set the super-resolution mode to ON.

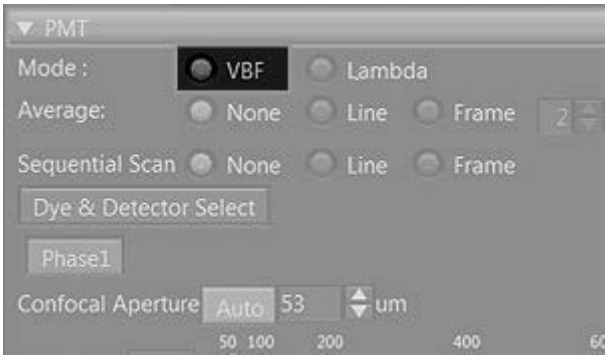
When the super-resolution mode is set to ON, following functions are set automatically.

Tool window name	Function	Setting contents
[LSM Imaging] [LSM Stimulation]	ROI	All ROIs are deleted. While the super-resolution mode is ON, only one ROI for imaging the rectangle can be specified.
[LSM Imaging]	[Type:]/[Mode:] in [Scanner:]	"Galvano"/"OneWay" is set.
[LSM Imaging]	[Scan Size:] in [Image Size:]	The maximum image size acquirable by the super-resolution is 4096*4096. The size close to the image size which is set before setting the super-resolution mode to "ON" is set.
[LSM Imaging]	[Zoom:]	Based on the magnification of the current objective lens and the image size, the zoom magnification is calculated so that the pixel pitch (pixel resolution) becomes the specified pixel pitch, and this zoom magnification is set.
[PMT Setting]	[Average:]	The accumulation (Averaging process when acquiring the image) is set to "None".
[LSM Imaging]	[Speed:]	"8.0 us/Pixel" is set.
[PMT Setting]	[HV] of GaAsP channel	The value close to the HV value which is set before setting the super-resolution image to "ON" is set in the range between 350 and 500 V.
[PMT Setting]	[Confocal Aperture]	The value optimal for acquiring the image in the super-resolution mode is set.

When acquiring the image in the super-resolution mode, it is recommended to set [Rotation] in [Area Settings] in the [LSM Imaging] tool window to 0°.

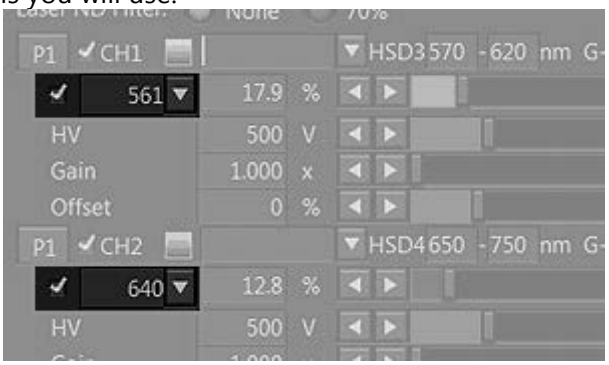
4.3. Adjusting scan conditions

1 Select "VBF" in the [PMT Setting] Tool Window.



2 Select the GaAsP channel you will use in the [PMT Setting] Tool Window.

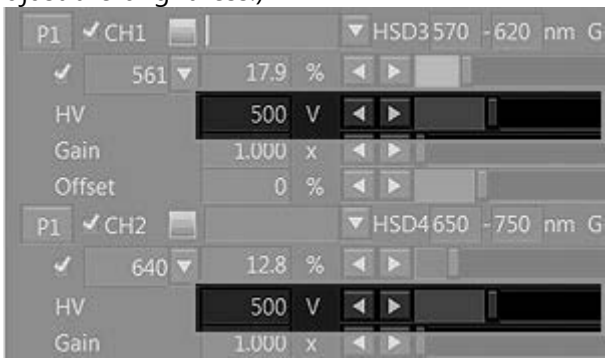
3 Assign the laser to all GaAsP channels you will use.



Even though "ON" is selected in [Super-resolution imaging] in [Super-resolution] Tool Window, if the laser is not assigned to the GaAsP channel, the super-resolution image cannot be acquired.

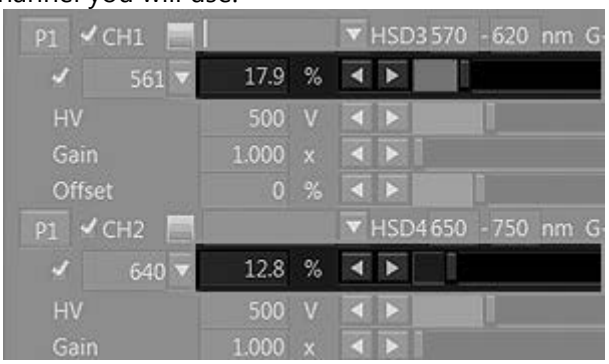
4 Adjust [HV] of the GaAsP channel you will use according to the purpose.

- 500 V: The discoloring is prevented. (The laser power is reduced, but the noise elements may be increased.)
- 350 V: The super-resolution is prioritized. (The noise elements are decreased. Increase the laser power to adjust the brightness.)



For acquisition of the super-resolution image, HV can be set in the range between 350 and 500 V.

5 Adjust the laser level of the GaAsP channel you will use.



It is recommended to increase the intensity value of the image to the following target intensity values.

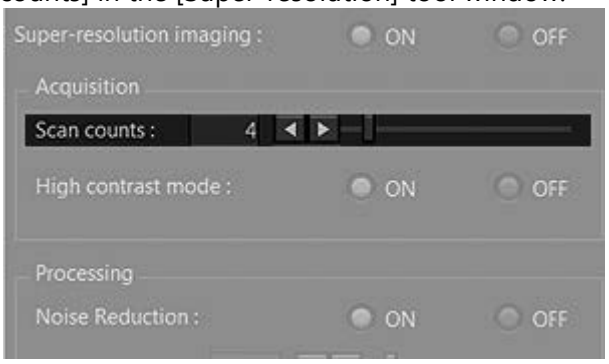
Standard mode: Approx. 2400

High Contrast mode: Approx. 1800

The intensity value can be confirmed by moving the mouse pointer on the image in the [Live] window. The intensity value indicated by the mouse pointer is displayed in "Int:" in the lower area of the window. Use this method to confirm the intensity value, and adjust the laser level so that the most of intensity values of the image become the target intensity values.

4.4. Setting the super-resolution

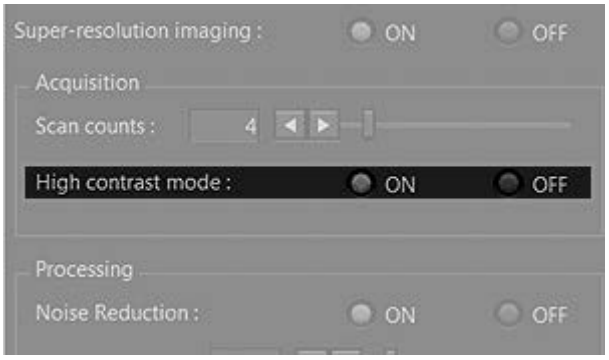
1 Set the cumulative number in [Scan counts] in the [Super-resolution] tool window.



2 Select the super-resolution processing mode in [High contrast mode].

ON High Contrast mode

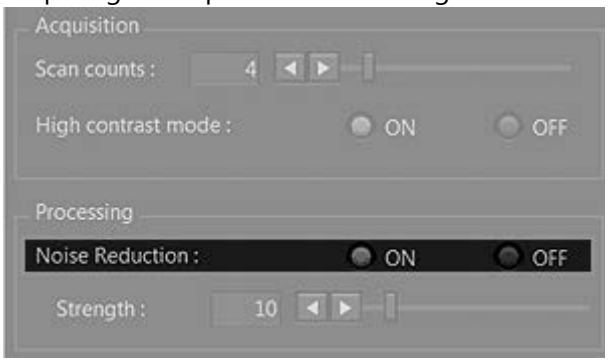
OFF Standard mode



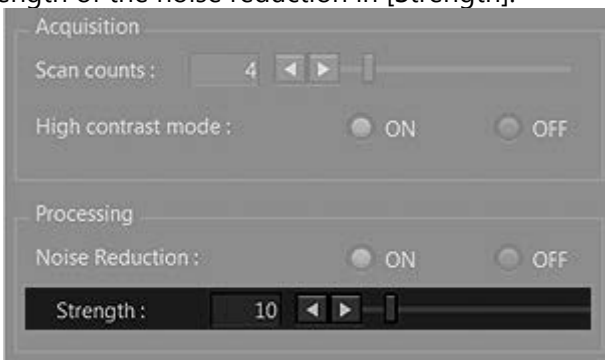
3 In [Noise Reduction], set whether or not to reduce the noise concurrently with acquiring the image.

ON The noise is reduced while acquiring the super-resolution image and it is saved in an image different from the super-resolution image. This is used when the noise is noticeable on the super-resolution image.

OFF The noise is not reduced while acquiring the super-resolution image.



4 If "ON" is selected in 3, set the strength of the noise reduction in [Strength].



Even though you acquire the super-resolution image after "OFF" is selected in 3 to set not to reduce the noise concurrently with acquiring the image, you can reduce the noise from the super-resolution image afterwards. In this case, set each parameter in the [Processing] tab in the [Analysis] tool window after the super-resolution image is acquired and click the [Process] button. The noise is reduced.

4.5. Acquiring the super-resolution image

1 Click 

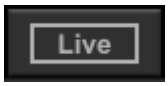
or 

in the [Acquire] tool window to acquire the super-resolution image. When the image is acquired, the super-resolution image is displayed in the [Image] window and the live image in the [Live] window is cleared. At the same time, the super-resolution image is saved to the hard disk. (Images before processing the super-resolution are not saved.)

The acquisition of the super-resolution image may discolor the specimens quickly because the time to irradiate the laser to the specimens is longer than that of the normal acquisition.

During the acquisition in the super-resolution mode, the accumulation is performed regardless of the settings in [Average] in the [PMT Setting] tool window. However, during the repeat scan[*1], the integration is not performed.

While acquiring the super-resolution image, the light stimulation is not available.
During the Rest time (waiting time) while acquiring the T series image, the repeat scan[*1] is not available.

[*1] Repeat Scan means to acquire the live image continuously by pressing the 

, 

or 

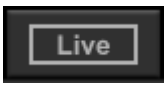
button in the [Live] window.

[*2] MALT is an abbreviation of Multi Area Time Lapse and it means to observe the chronological changes in the multiple regions.

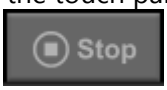
5. Parfocal adjustment

This function is used to adjust the parfocal difference when switching over the objective lens. The parfocal adjustment value is set according to the following procedures.

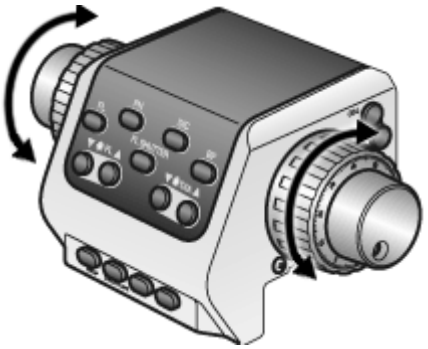
1 Bring the image into focus using the low magnification objective lens once so that you can configure following settings easily. Among the objective lenses attached, place the low magnification objective lens in the light path.

2 Press the 

button on the [Live] window. Rotate the focusing knob of U-MCZ to bring the specimen into focus while viewing the live image. (The specimen can also be brought into focus by the [Focus] tab of the touch panel controller.)

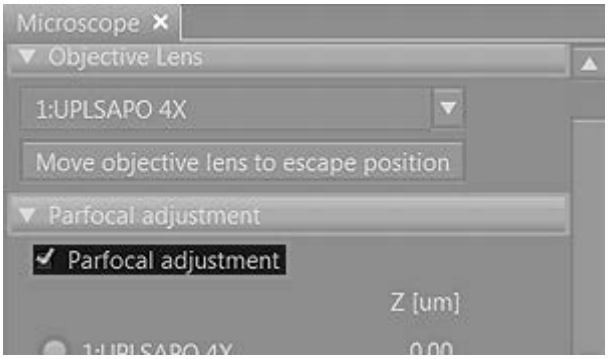
When the specimen is brought into focus, press the 

button on the [Live] window to stop the live.

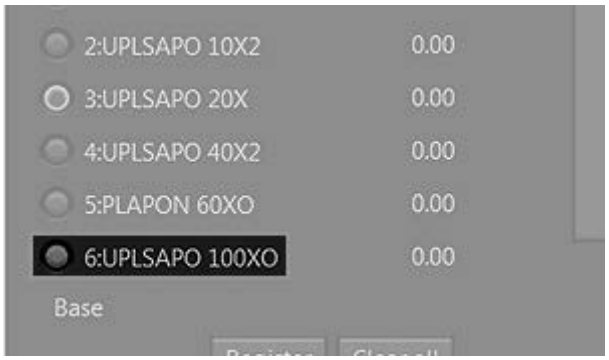


3 Among the objective lenses attached, place the highest magnification objective lens in the light path. Bring the specimen into focus in the same way as 2.

4 Tick [Parfocal adjustment] on the [Microscope] tool window.

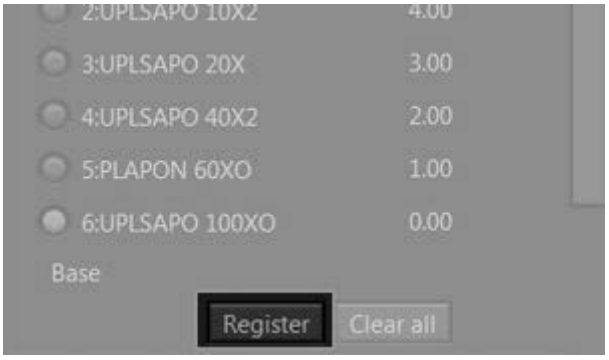


5 On the [Microscope] tool window, select the hole number of the revolving nosepiece where the highest magnification objective lens is attached. The highest magnification objective lens is set as a reference objective lens.

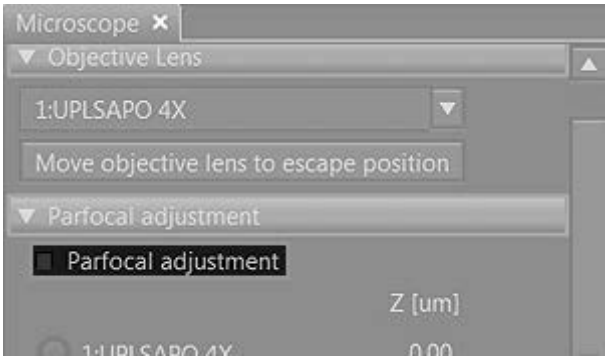


6 Place the objective lens with second highest magnification in the light path and bring the specimen into focus in the same way as 2. The relative value from the Z position of the reference objective lens is displayed in a. Set the same to all objective lenses.

7 Press the Register button. The relative values of all objective lenses are registered as parfocal adjustment values.



8 Uncheck [Parfocal adjustment].

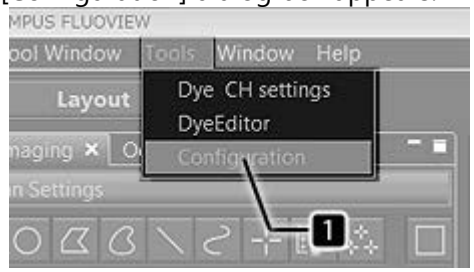


6. Z drift compensation

6.1. Advance preparations

6.1.1. Checking that the objective lens corresponds to the Z drift compensation (ZDC).

1 Select [Configuration] in the [Tools] menu. The [Configuration] dialog box appears.



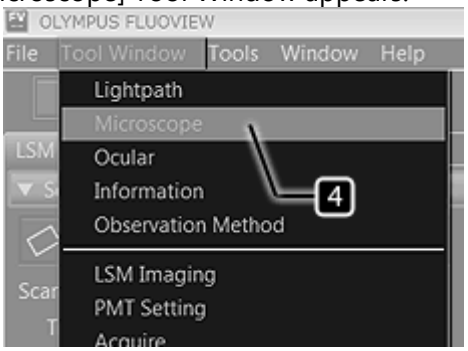
2 In [Objective Lens] in [Microscope] tab in the [Configuration] dialog box, check whether the objective lens installed on the system corresponds to ZDC. You cannot use ZDC with the objective lens which does not correspond to ZDC.

6.1.2. Setting the coverslip materials and coverslip thickness

3 In [ZDC] in [Microscope] tab in the [Configuration] dialog box, set the coverslip materials and the coverslip thickness. If you select [Plastic] for the objective lens which is usable only with the glass coverslip, the glass is selected automatically and the autofocus is executed.

6.1.3. Setting the Near limit to the objective lens

4 Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.



5 In [Z Limit Setting] in [Microscope] Tool Window, set the Near limit to all objective lenses. If the Near limit is not set properly, the autofocus may be failed.

6.1.4. Acquiring the live image for observation

6 Acquire the live image for observation according to "Assigning the detector to channel" and "Adjusting the live image" in "Acquiring the image".

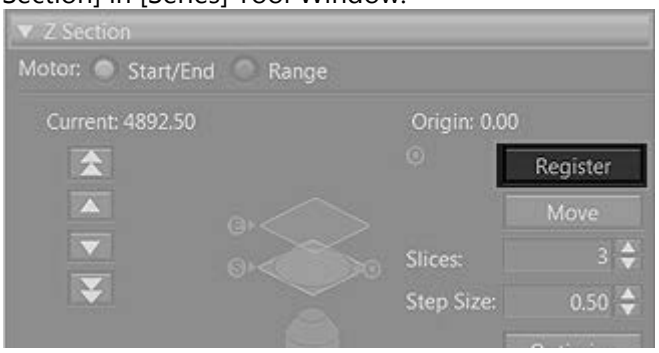
6.2. When performing One Shot AF

6.2.1. Performing One Shot AF

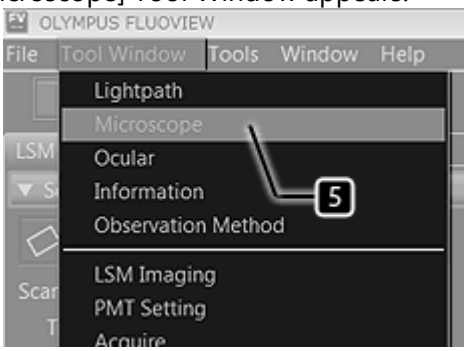
When using specimens where the distance between the coverslip position (top surface of the coverslip)*1 and the intended focusing position is similar, if you repeat imaging by changing specimens, this function allows you to save the effort and time to perform the manual focusing each time you change specimens.

Preparing for focusing

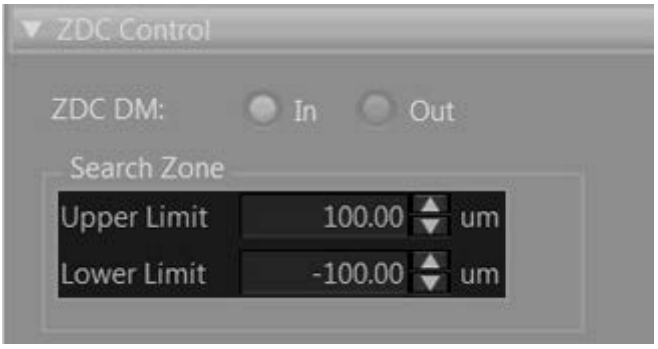
1. Open the [EPI] tab or the [DIA] tab on the touch panel controller and press the IN button of [ZDC] to place the dichroic mirror of ZDC in the light path.
2. Open the [Focus] tab on the touch panel controller and press the START button of [Focus Search] to start the autofocus.
3. Press the [Register] button in [Z Section] in [Series] Tool Window.



4. Move the Z position to the intended focusing position using the focusing knob of U-MCZ or the / / / buttons in [Z Section] in [Series] Tool Window.
5. Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.

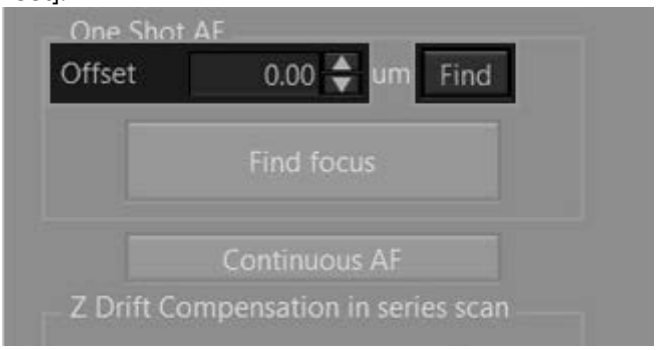


6 In [Upper Limit] / [Lower Limit] in [Search Zone] in [ZDC Control] in [Microscope] Tool Window, specify the search zone of the coverslip position[*1] using the Origin coordinate[*2] of Z as a base point.



When using either the immersion liquid objective lens or the silicon oil immersion objective lens, specify the zone including the bottom surface of the cover glass to prevent the fail in detecting the coverslip position[*1].

7 Press the [Find] button of [One Shot AF] to acquire the offset value between the coverslip position[*1] and the specimen position currently being observed. The acquired offset value is set in [Offset].

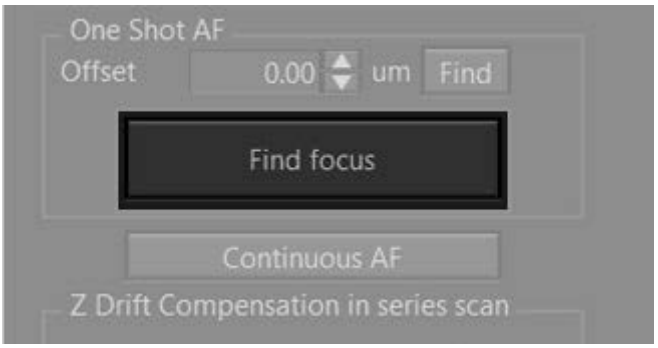


If you fail in acquiring the offset value, the following causes may be assumed. Make the necessary settings again and press the [Find] button again.

- The coverslip position[*1] may not be in the search zone. Specify the Origin coordinate[*2] of Z closer to the top surface of the coverslip or widen the search zone.
- The thickness or materials of the coverslip may not be set properly. Set the thickness or materials of the coverslip properly in [ZDC] in [Microscope] tab in the [Configuration] dialog.
  If you are using the coverslip whose thickness is not supported (less than 0.15 mm), use the coverslip whose thickness is supported (0.15 mm or more).

Moving the Z position to the intended focusing position

8 Press the [Find focus] button.
If the coverslip position[*1] is detected successfully, the Z stage moves from the coverslip position to the position where the offset value acquired in 7 is added to he coverslip position.



If you fail in detecting the coverslip position, the following causes may be assumed. Make the settings again and press the [Find focus] button again.

- The coverslip position[*1] may not be in the search zone. Specify the Origin coordinate[*2] of Z closer to the top surface of the coverslip or widen the search zone.
- The thickness or materials of the coverslip may not be set properly. Set the thickness or materials of the coverslip properly in [ZDC] in [Microscope] tab in the [Configuration] dialog.
  If you are using the coverslip whose thickness is not supported (less than 0.15 mm), use the coverslip whose thickness is supported (0.15 mm or more).

[*1] The coverslip position is the position focused on the top surface of the coverslip.

[*2] Origin coordinate is the reference position of Z registered in [Origin] in the [Series] Tool Window.

6.3. When combining series scan with the Z drift compensation

6.3.1. Performing the Z drift compensation continuously during series scan

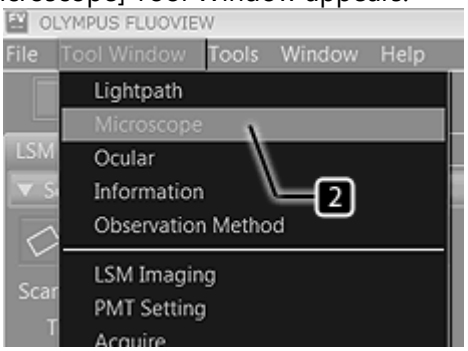
As this function performs the drift compensation in real-time during series scan, the focusing can be followed at a high-speed.

Setting for acquiring the series image

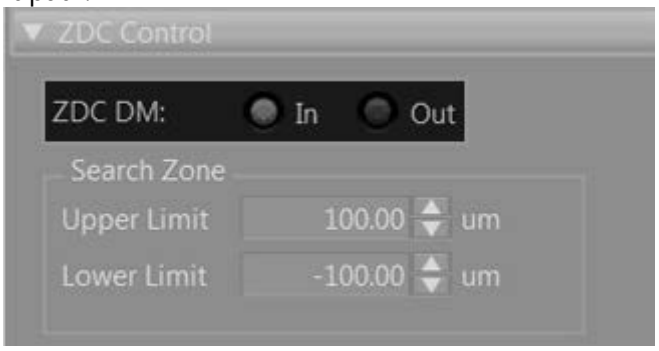
1 Set for acquiring the series image according to "Acquiring the series image" in "Acquiring the image".

Performing Continuous AF

2 Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.



3 Set [ZDC DM] in [ZDC Control] on [Microscope] Tool Window to "In" and place the dichroic mirror of ZDC in the light path.



With Continuous AF, the dichroic mirror of ZDC must be placed in the light path. Therefore, the wavelength range where the dichroic mirror of ZDC reflects (790 nm) cannot be used as a excitation wavelength or a photometry wavelength.

4 Press the [Continuous AF] button in [ZDC Control] in [Microscope] Tool Window. The Z position is adjusted automatically so that it is focused continuously at the focused Z position when this button was pressed.

Continuous AF cannot be performed for the series scan which includes the Z-axis.

If you fail in Continuous AF, the following causes may be assumed. Make the necessary settings again and press the [Continuous AF] button again.

- The coverslip position[*1] may not be in the search zone. Specify the Origin coordinate[*2] of Z closer to the top surface of the coverslip or widen the search zone.
- The thickness or materials of the coverslip may not be set properly. Set the thickness or materials of the coverslip properly in [ZDC] in [Microscope] tab in the [Configuration] dialog.
  If you are using the coverslip whose thickness is not supported (less than 0.15 mm), use the coverslip whose thickness is supported (0.15 mm or more).

Starting to acquire the series image

5 Press 

in [Normal] tab in [Acquire] Tool Window to start acquisition.

[*1] The coverslip position is the position focused on the top surface of the coverslip.

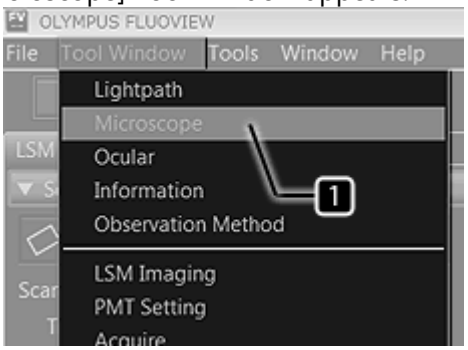
[*2] Origin coordinate is the reference position of Z registered in [Origin] in the [Series] Tool Window.

6.3.2. Performing the Z drift compensation when starting series scan

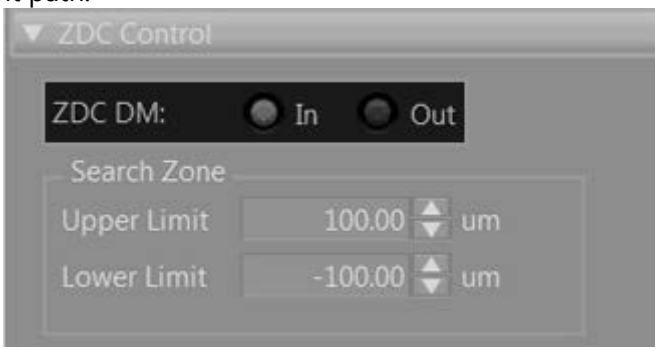
This function performs the Z drift compensation before starting the series scan. If the Rest time[*1] longer than 30 seconds is set for acquiring T series images, the Z drift compensation is performed before starting to acquire the next frame. If the coverslip position[*2] is changed by drift, the Origin coordinate[*3] of Z is compensated to offset that variation to retain the focus positon.

Setting for acquiring the series image

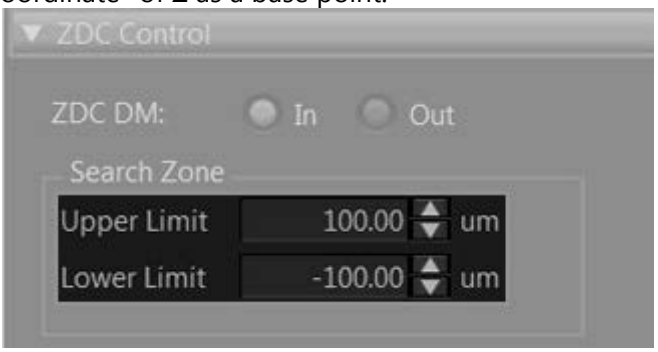
1 Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.



2 Set [ZDC DM] in [ZDC Control] in [Microscope] Tool Window to "In" and place the dichroic mirror of ZDC in the light path.



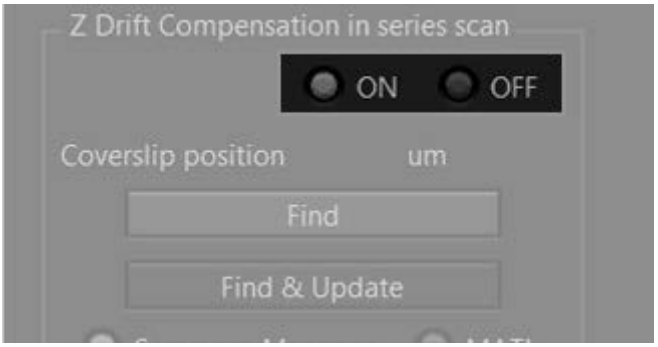
3 Specify the search zone of the coverslip position[*2] in [Upper Limit] / [Lower Limit] in [Search Zone] using the Origin coordinate[*3] of Z as a base point.



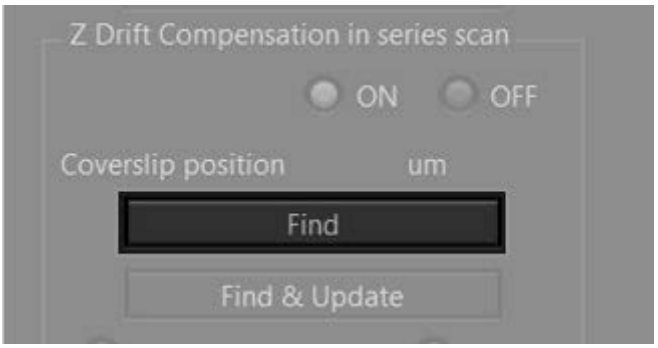
4 Set for acquiring the series image according to "Acquiring the series image" in "Acquiring the image".

Acquiring the coverslip positon

5 Set [Z Drift Compensation in series scan] in [ZDC Control] in [Microscope] Tool Window to "ON".



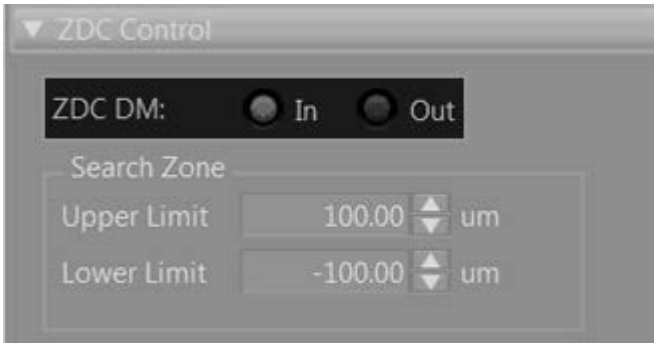
6 Press the [Find] button of [Coverslip position]. The coverslip top surface position is acquired.



Selecting the status of the dichroic mirror of ZDC

7 Select the status of the dichroic mirror of ZDC whether it is in the light path or not in [ZDC DM] in [ZDC Control] on [Microscope] Tool Window.

ZDC DM	Status of dichroic mirror of ZDC	Features
In	Placed in the light path	Since the dichroic mirror of ZDC will not be inserted or removed, the scan starts early, but the observation using the wavelength range where the dichroic mirror of ZDC reflects (790 nm) as a excitation wavelength or a photometry wavelength is not available.
Out	Removed from the light path	The dichroic mirror of ZDC is placed in the light path only when performing the Z drift compensation. Therefore, it takes longer time to place or remove the dichroic mirror in or from the light path comparing to the case when [ZDC DM] is set to "In". However, since the dichroic mirror is removed from the light path during the scan, the observation using the wavelength range where the dichroic mirror of ZDC reflects (790 nm) as a excitation wavelength or the photometry wavelength is available.



Starting to acquire the series image

8 Press the LSM Start button in [Normal] tab in [Acquire] Tool Window to start acquisition.

The drift compensation value is not recorded to the Z position information of the acquired image.

*1 The Rest time is the time between finishing acquisition of the first cycle and starting the acquisition of the second cycle.

*2 The coverslip position is the position focused on the top surface of the coverslip.

*3 Origin coordinate is the reference position of Z registered in [Origin] in the [Series] Tool Window.

6.4. When combining Sequence Manager with the Z drift compensation

6.4.1. Performing the Z drift compensation per each task of Sequence Manager

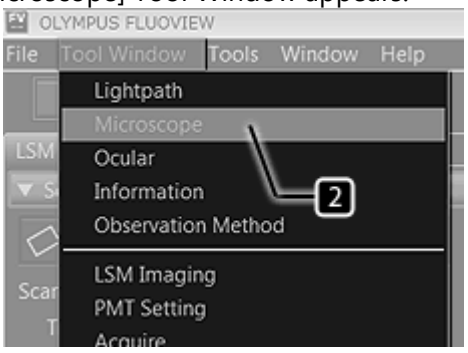
This function performs the Z drift compensation per each task registered to Sequence Manager when the relevant task starts. If the coverslip position*1 is changed by drift, the Origin coordinates*2 of Z of all tasks registered are compensated to offset that variation to retain the focus positon.

Setting for acquiring the series image

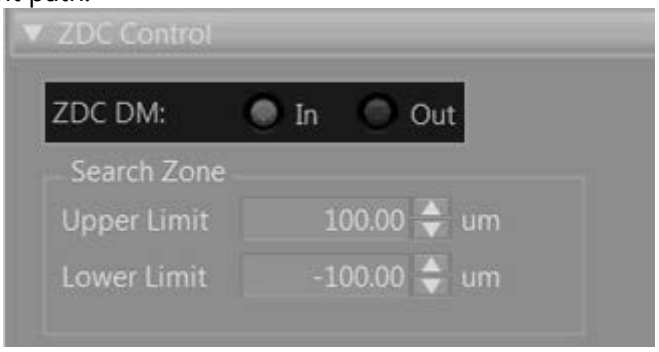
1 Set for acquiring the series image according to "Acquiring the series image" in "Acquiring the image".

Setting for performing the Z drift compensation

2 Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.

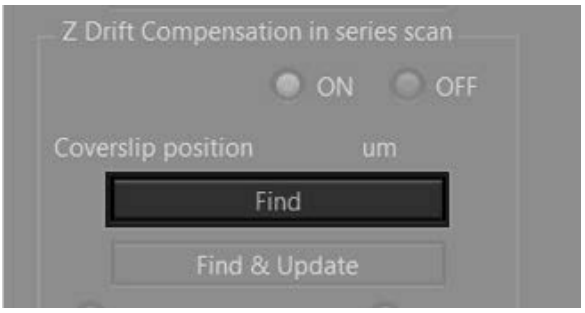


3 Set [ZDC DM] in [ZDC Control] on [Microscope] Tool Window to "In" and place the dichroic mirror of ZDC in the light path.



Acquiring the coverslip positon

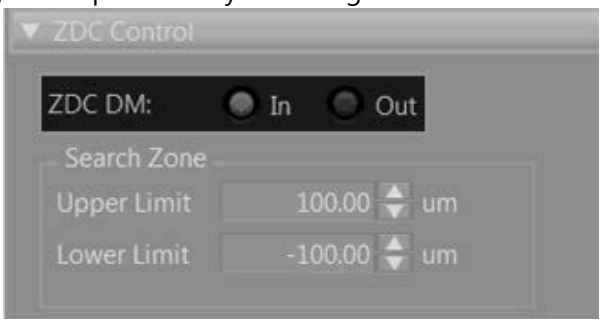
4 Press the [Find] button of [Coverslip position]. The coverslip top surface position is acquired.



Selecting the status of the dichroic mirror of ZDC

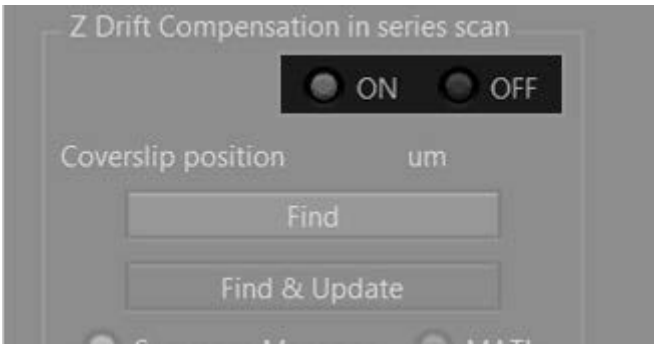
5 Select the status of the dichroic mirror of ZDC whether it is in the light path or not in [ZDC DM] in [ZDC Control] on [Microscope] Tool Window.

ZDC DM	Status of dichroic mirror of ZDC	Features
In	Placed in the light path	Since the dichroic mirror of ZDC will not be inserted or removed, the scan starts early, but the observation using the wavelength range where the dichroic mirror of ZDC reflects (790 nm) as a excitation wavelength or a photometry wavelength is not available.
Out	Removed from the light path	The dichroic mirror of ZDC is placed in the light path only when performing the Z drift compensation. Therefore, it takes longer time to place or remove the dichroic mirror in or from the light path comparing to the case when [ZDC DM] is set to "In". However, since the dichroic mirror is removed from the light path during the scan, the observation using the wavelength range where the dichroic mirror of ZDC reflects (790 nm) as a excitation wavelength or a photometry wavelength is available.



Setting ON/OFF of the Z drift compensation

6 Set [Z Drift Compensation in series scan] in [ZDC Control] in [Microscope] Tool Window to "ON".



Registering tasks

7 Register the tasks according to "Planning acquisition (Creating protocols)" in "Automation of acquisition" in "Acquiring the image".

📖 When registering multiple tasks to Sequence Manager, the In/Out status of the dichroic mirror of ZDC and ON/OFF of the Z drift compensation can be set per each task. In order to set these, perform 3 and 6 per each task.

*1 The coverslip position is the position focused on the top surface of the coverslip.

*2 Origin coordinate is the reference position of Z registered in [Origin] in the [Series] Tool Window.

6.4.2. Updating the coverslip positon of the task

This function updates the coverslip position[*1] per each task registered to Sequence Manager.

Reflecting the image acquisition conditions set to the task to the relevant Tool Windows

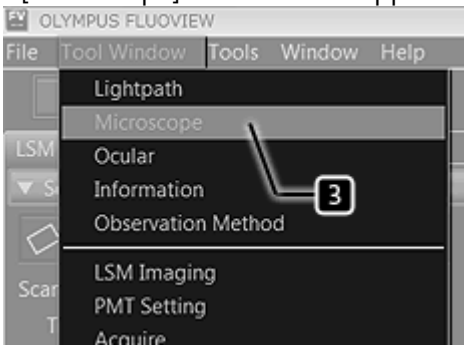
1. Select the task you want to update the coverslip positon in [Sequence Manager] Tool Window, and press the Reload task button in [Task]. The image acquisition conditions of the selected task are reflected to the acquisition conditions of the relevant Tool Windows.

Adjusting the Z position to update the Origin coordinate[*2] of Z

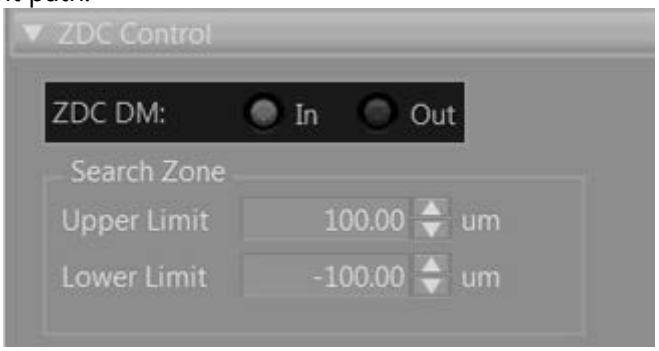
2. Adjust the Z position using the focusing knob of U-MCZ or the / / / buttons in [Z Section] in [Series] Tool Window, and press the Register button.

Setting for performing the Z drift compensation

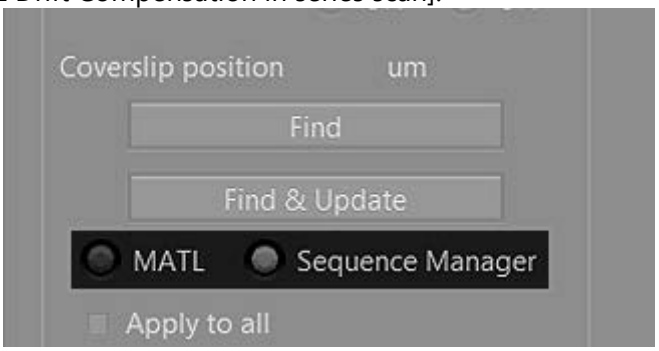
3. Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.



4 Set [ZDC DM] in [ZDC Control] on [Microscope] Tool Window to "In" and place the dichroic mirror of ZDC in the light path.

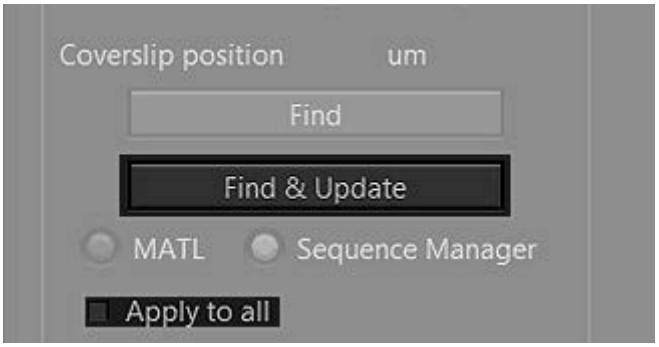


5 Select "Sequence Manager" in [Z Drift Compensation in series scan].



Updating the coverslip positon of the task

6 Press the [Find & Update] button. The coverslip position is updated for the selected task.



If you want to reflect this update to all tasks registered to Sequence Manager at a time, check [Apply to all] and press the [Find & Update] button.

Updating the image acquisition conditions of the task

7 Press the Update task button in [Task] in [Sequence Manager] Tool Window. The image acquisition conditions set in the relevant Tool Windows are updated as the image acquisition conditions of the selected task.

[*1] The coverslip position is the position focused on the top surface of the coverslip.

[*2] Origin coordinate is the reference position of Z registered in [Origin] in the [Series] Tool Window.

6.5. When combining MATL with the Z drift compensation

6.5.1. Combining MATL with the Z drift compensation

The Z drift compensation is performed by group or area registered when acquiring MATL[*1] ([Map] Sub Pane in [Live] Window). If the coverslip position[*2] is changed by drift, the Origin coordinate[*3] of Z of each area registered is compensated to offset that variation to retain the focus positon.

Selecting the timing to execute the Z drift compensation

1 Select [Configuration] in the [Tools] menu. The [Configuration] dialog box appears.

2 In [Z Drift Compensation in MATL] in [ZDC] in the [Microscope] tab, select the timing to execute the Z drift compensation.

Each group	Executes the Z drift compensation in the center of the relevant group before acquiring each group[*4].
Each area	Executes the drift compensation in the center of the relevant area before acquiring each area.

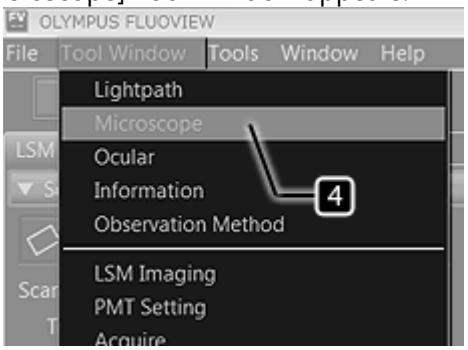
If "Each area" is selected, the focus map [*5] function cannot be used.

Setting for acquiring the series image

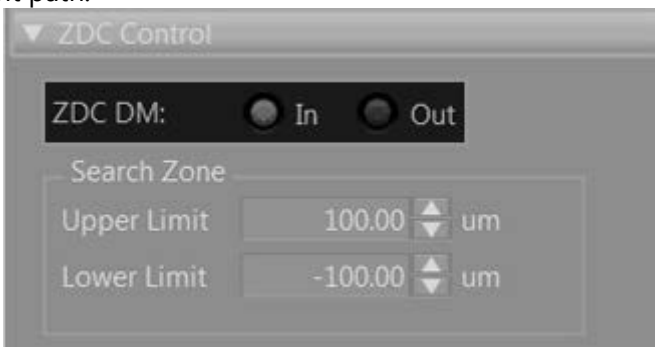
3 Set for acquiring the series image according to "Acquiring the series image" in "Acquiring the image".

Setting for performing the Z drift compensation

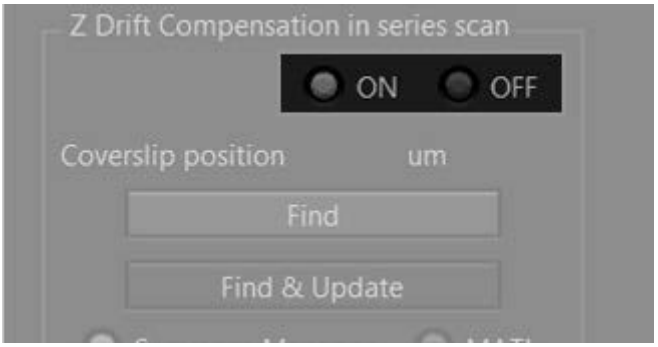
4 Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.



5 Set [ZDC DM] in [ZDC Control] in [Microscope] Tool Window to "In" and place the dichroic mirror of ZDC in the light path.

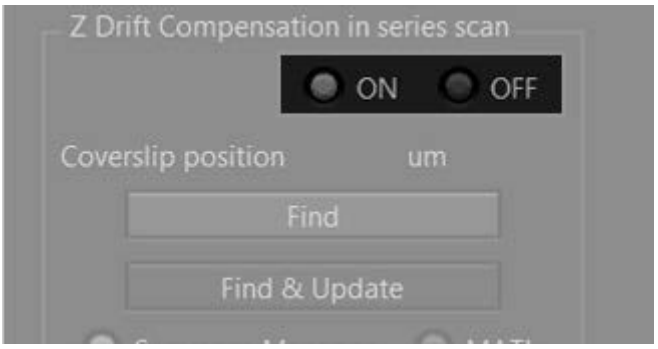


6 Set [Z Drift Compensation in series scan] in [ZDC Control] in [Microscope] Tool Window to "ON".



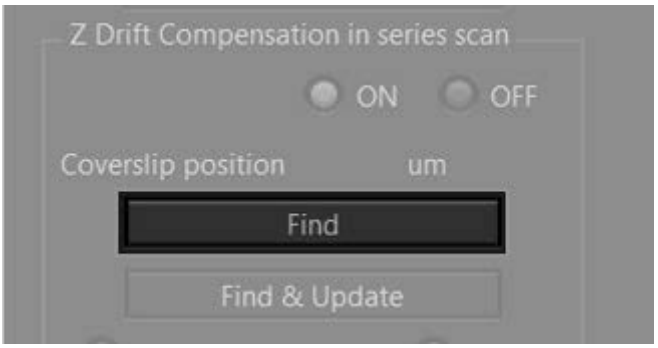
Setting ON/OFF of the Z drift compensation

7 Set [Z Drift Compensation in series scan] in [ZDC Control] in [Microscope] Tool Window to "ON".



Acquiring the coverslip positon

8 Press the [Find] button of [Coverslip position]. The stage moves to the center position of the group[*4] of MATL[*1] registered, and the coverslip top surface position is acquired.



The Z drift compensation is applied differently depending on whether the coverslip position is acquired by group or only by the first group.
If you acquire the coverslip position only by the first group, the coverslip position acquired is reflected to all groups. Therefore, the drift and the tilt of the cover glass are compensated for all subsequent groups at the same time. In this case, the acquisition area of Z is set only with the first group.
If you acquire the coverslip position by group, the coverslip position is retained by group. Therefore, compensate the tilt of the cover glass manually when setting Z of that group and also compensate the drift only for the Z drift compensation. In this case, you need to set the acquisition area of Z by group.

6.5.2. Registering the group of MATL

9 In [Map] Sub Pane in [Live] Window, register the group[*4] of MATL[*1].

[*1] MATL is an abbreviation of Multi Area Time Lapse and it means to observe chronological changes in the multiple regions.

[*2] The coverslip position is the position focused on the top surface of the coverslip.

[*3] Origin coordinate is the reference position of Z registered in [Origin] in the [Series] Tool Window.

[*4] A group means an area created by pressing the button once in [Register] in [Map] Sub Pane in [Live] Window.

[*5] This means the function to correct the tilt using 3 reference points specified by group when acquiring MATL[*1] including stitching. This function is used when the focus position is shifted by the tilt amount in each field of view even at the same Z position (number of images), such as the XY stage or the specimen is tilted, etc.

Updating the coverslip position of the group

This function updates the coverslip position[*3] of the relevant group[*2] of MATL[*1].

Reflecting the image acquisition conditions set to the group to the relevant Tool Windows

1 Select the group[*2] you want to update the coverslip position in [Map] Sub Pane in [Live] Window, and press the  button in [Registered Area List]. The image acquisition conditions of the selected group are reflected to the acquisition conditions of the relevant Tool Windows.

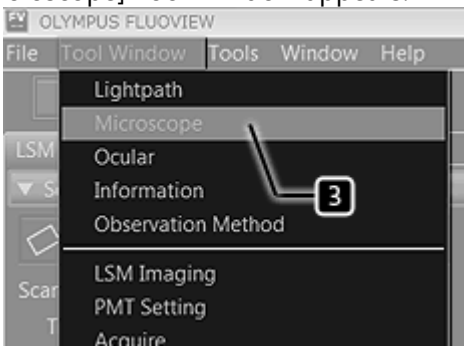
Adjusting the Z position to update the Origin coordinate[*4] of Z

2 Adjust the Z position using the focusing knob of U-MCZ or the  buttons in [Z Section] in [Series] Tool Window, and press the 

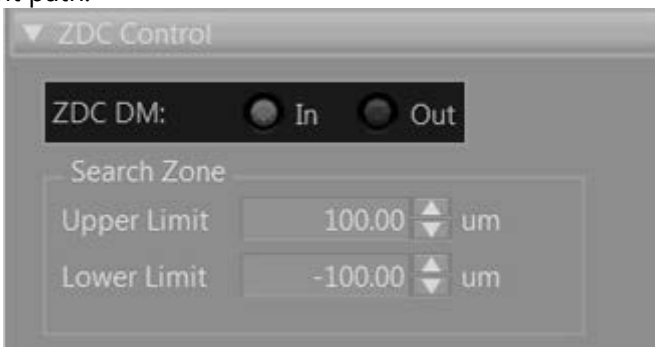
button.

Setting for performing the Z drift compensation

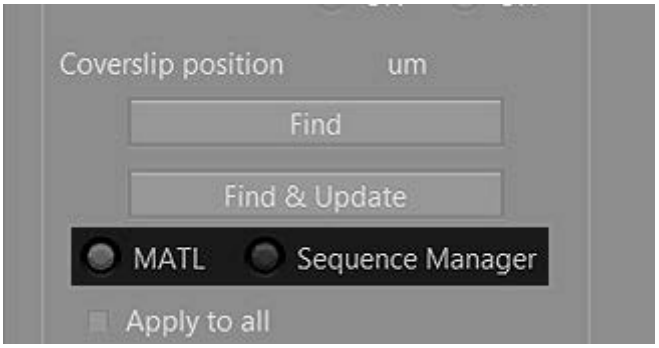
3 Select [Microscope] in [Tool Window] menu. [Microscope] Tool Window appears.



4 Set [ZDC DM] in [ZDC Control] on [Microscope] Tool Window to "In" and place the dichroic mirror of ZDC in the light path.

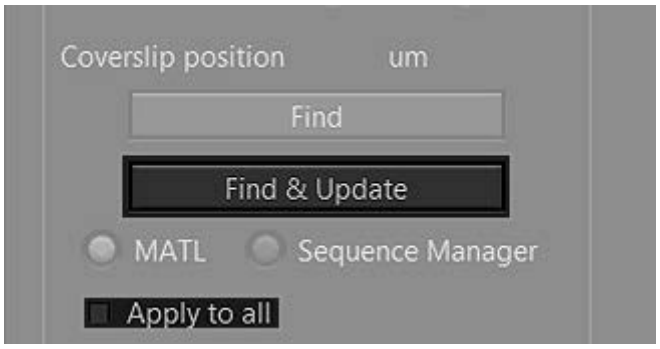


5 Select "MATL" in [Z Drift Compensation in series scan] in [ZDC Control] in [Microscope] Tool Window.



Updating the coverslip position of the group

6 Press the [Find & Update] button. The coverslip position is updated for the selected group.



If you want to reflect this update to all groups[*2] registered to MATL[*1] at a time, check [Apply to all] and press the [Find & Update] button.

Updating the image acquisition conditions of the task

7 Press the button in [Registered Area List] in [Map] Sub Pane in [Live] Window. The image acquisition conditions set to the relevant Tool Windows are updated as the image acquisition conditions of the selected group.

[*1] MATL is an abbreviation of Multi Area Time Lapse and it means to observe chronological changes in the multiple regions.

[*2] A group means an area created by pressing the button once in [Register] in [Map] Sub Pane in [Live] Window.

[*3] The coverslip position is the position focused on the top surface of the coverslip.

[*4] Origin coordinate is the reference position of Z registered in [Origin] in the [Series] Tool Window.

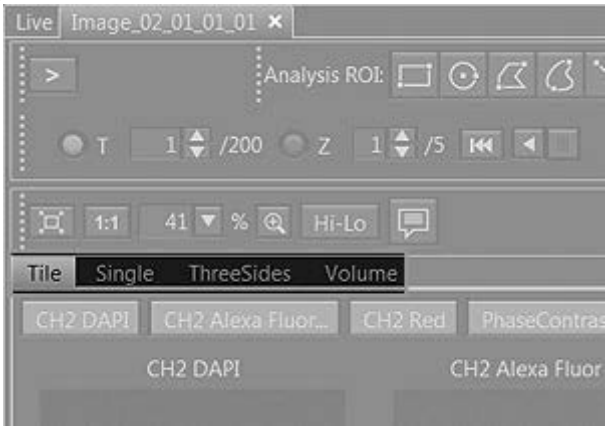
7. Viewing image

7.1. Opening the image file

1 Select [Open] - [Image] in the [File] menu on the software screen to display the dialog box, and select the image to view.
The [Image] window opens and the image appears.

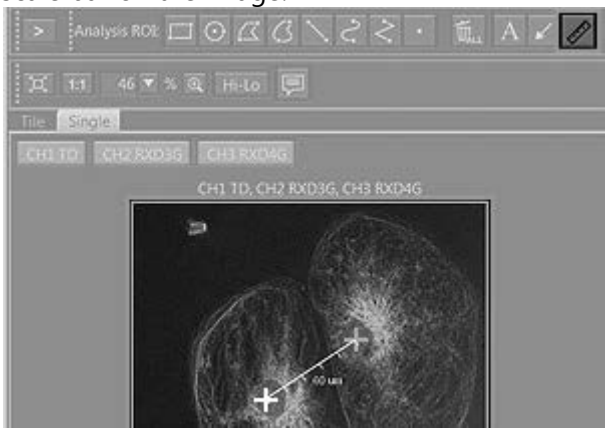
2 Select the display method of the images.

[Tile]	The image display area is divided and the image of each channel and the image composing all channels are displayed side by side.
[Single]	The image composing all channels is displayed over the entire image display area.
[Three Slides]	XY image, XZ cross sectional image, YZ cross sectional image, XT cross sectional image and YT cross sectional image are displayed.
[Volume]	The 3D image is displayed.



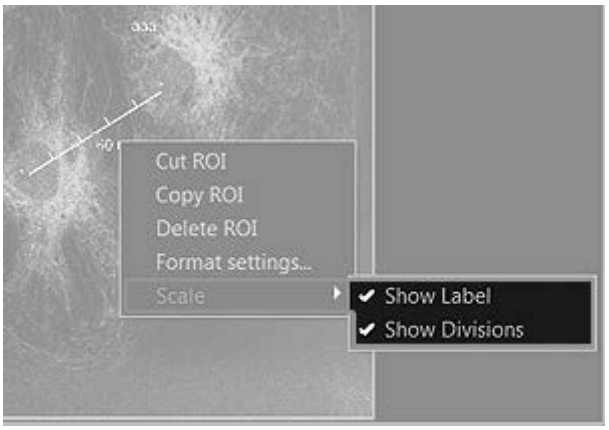
7.2. Drawing the scale bar

1. Press the  button in the [Image] window and move the mouse pointer onto the image. The mouse pointer is changed to +.
2. Drag the area you want to draw the scale bar on the image.



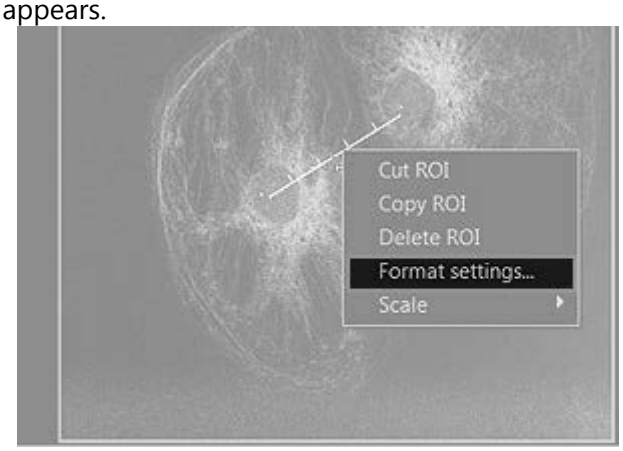
7.2.1. Selecting Show/Hide of label and scale divisions

1. Right-click on the scale bar drawn on the image. Select [Scale] in the menu displayed and select Show or Hide in the sub menu.

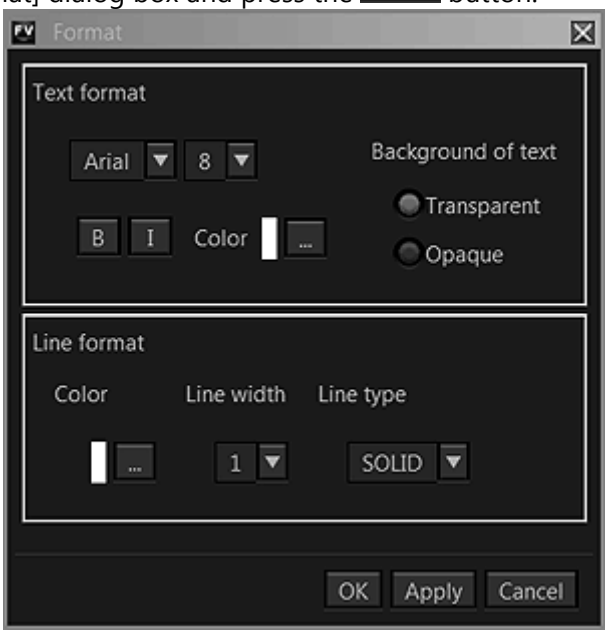


7.2.2. Changing lines of the scale bar or text formats

1 Right-click on the scale bar drawn on the image. Select [Format settings] in the menu displayed. The [Format] dialog box appears.

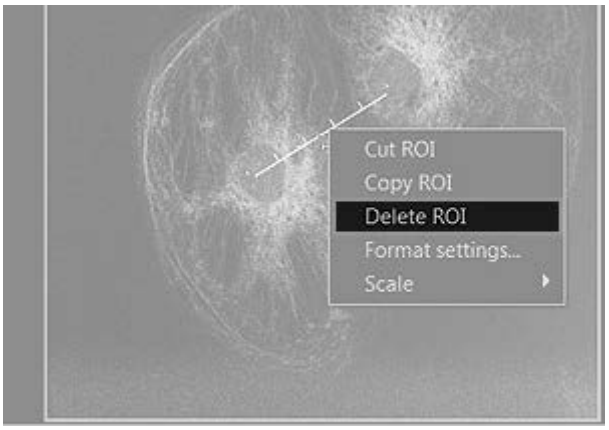


2 Change necessary items in the [Format] dialog box and press the OK button.



7.2.3. Deleting the scale bar

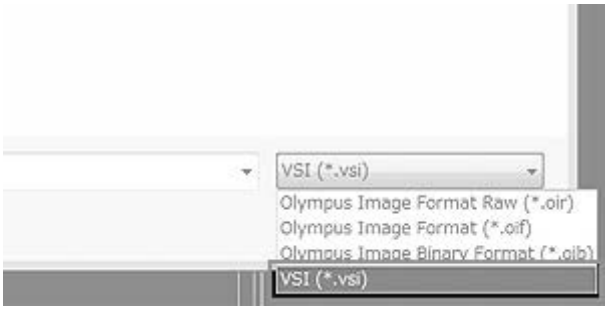
1 Right-click on the scale bar drawn on the image. Select [Delete ROI] in the menu displayed. The scale bar is deleted.



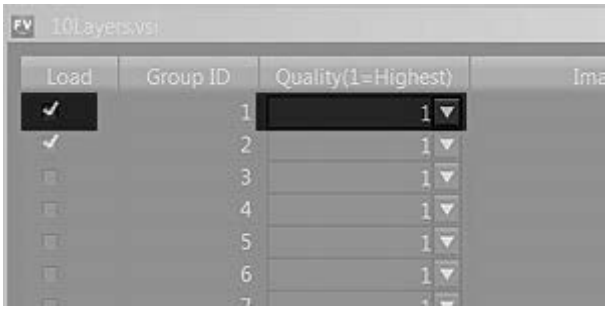
7.3. Opening the VSI file

1 Select [Open] - [Image] in the [File] menu on the software screen.
The dialog box for selecting the file is displayed.

2 Specify the file format to "VSI". Select the VSI file and open it.
The group for loading images and the dialog box for selecting "Quality" are displayed.



3 Tick [Load] of the group for loading images and select [Quality].
Depending on [Quality] selected, the image size to be displayed varies. The mage size is displayed in [Image Size].



"Group ID"
Group is equivalent to the layer on cellSens.
In cellSens, one image file is composed of multiple layers, and each layer is the image acquired under the different observation conditions. With this software, when the VSI file is opened, each layer included in the VSI file is assigned to the group.

"Quality"
“Quality” is used to select the image size you want when opening the selected VSI file.
Selecting "1" displays the image in the largest size and with the higher quality, but consumes the memory. The larger the value is, the smaller the image size becomes and the lower the image quality becomes, but the memory consumption can be reduced.

4 Press the OK button. The image of the selected group(layer) is displayed.

7.4. Opening the position information file acquired by MATL

If the image is acquired by MATL, the following files are saved in the folder where images are saved.

- Image file in the multiple areas which are registered in [Map] Sub Pane (***.oir file)
- File which relates each image to the position information where such image is acquired (***.omp2info, ***_forVSIImages.omp2info file)

Opening the omp2info file displays the position information on [Map] Sub Pane in [Image] window.

1 Select [Open] - [MATL] in the [File] menu.
The dialog box for selecting the file appears.

2 Select the "***.omp2info" file or the "***_forVSIImages.omp2info" file and open the selected file.
The position information is displayed on [Map] Sub Pane in the [Image] window.

> **If you process the image acquired by MATL with cellSens and create the stitched image using this software, open the "***_forVSIImages.omp2info" file.**

3 Clicking in the area on [Map] Sub Pane in the [Image] window displays the image acquired at that position in the [Image] window.
(If you opened the "***_forVSIImages.omp2info" file in 2, the VSI file is displayed.)

> **Follow the cautions described below when loading the "***.vsi" file from the "***_forVSIImages.omp2info" file properly. Otherwise, the VSI file cannot be loaded properly.**
>
> - **If you process the image ("***.oir" file) in each area acquired by MATL with cellSens, the name of the image file (.vsi file) to be created after processing should be same as the name of the .oir file before processing.**
> - **Be sure to save the .vsi file to be created after processing the image with cellSens in the same location as the "***_forVSIImages.omp2info" file.**

8. Exporting the image

8.1. Exporting a single image

The image can be exported (saved) in the file format which can be used by other software. The formats which can be exported are shown below.

- TIFF
- Bitmap
- PNG
- JPEG
- WMV

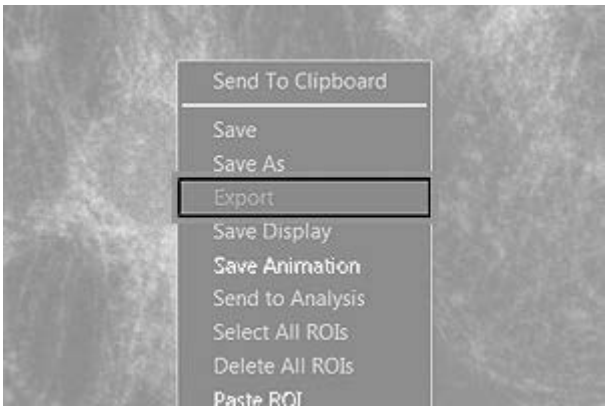
} general purpose format

- Olympus Image Format
- Olympus Image Binary Format
- Fluoview Multi-TIFF Compatible

} dedicated format

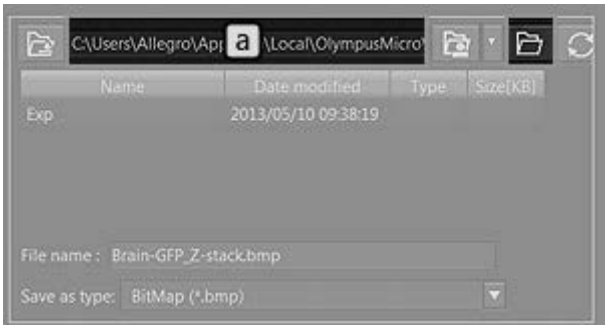
The WMV format can be exported from the series image.

1 Right-click on the image to be exported. Select [Export] in the menu displayed. The [Export] dialog box appears.

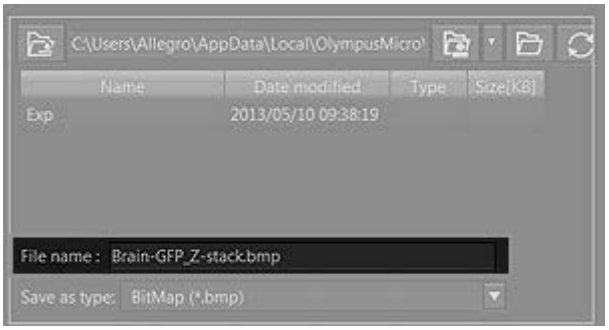


2 The save destination of the image to be exported is displayed in a. If you want to change the save destination, press the button to display the dialog box and select the folder of the save destination.

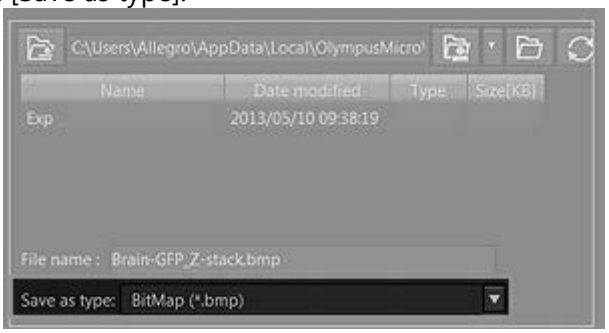
In order to organize image files easily, it is recommended to specify the save destination.



3 The file name of the image to be exported is displayed in [File Name]. If you want to change it, enter the file name directly.



4 Select the file type to be exported in [Save as type].



5 Select the channel to be exported in [CH/Range].

6 This procedure is necessary when exporting the series images.
Set the range to be exported and the number of steps (export per how many number of frames) in c.

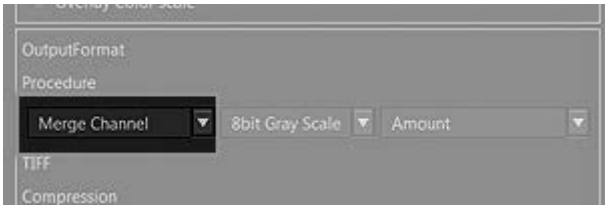
The following table shows the settings in c when the b button is pressed.

Current Frame	The frames currently displayed are set in [Image] Window.
Selected Frame	The frame ranges set in  and  are set in [Image] Window.
Reset	The settings are reset and the ranges including all frames are set.

7 This procedure is necessary when general purpose format is selected in [Save as type] in 4.
In [ROI overlay], set whether or not to overlay the ROI and the color bar displayed on the image over the image to be exported.

8 This procedure is necessary when general purpose format is selected in [Save as type] in 4.
Select the method to export channels.

Merge Channel	Multiple channels are merged to one image.
RGB Color	Each of multiple channels is made to one image.
RGB Color with Merge	One image merged from multiple channels and a number of channels of images made from each of multiple channels are created.
Raw Data Extracted	Each of multiple channels is made to one gray scale image (monochrome image without color information).



If you select "TIFF" in [Save as type] and select "Raw Data Extracted", the 16bit image (same format as this software) can be exported, but it is available only with the software equipped with following functions.

- **The 16bit gray scale can be displayed.**
- **The image level can be corrected. (The image becomes completely dark unless the data range displayed in the level correction can be adjusted.)**

9 This procedure is necessary when general purpose format is selected in [Save as type] in 4.
Select the bit color.

8bit Gray Scale	Color information in gray scale with 8 bit per each pixel.
16bit Gray Scale	Color information in gray scale with 16 bit per each pixel. (This item can be selected when "Raw Data Extracted" is selected in 8.)
24 bit Full Color	Color information in full color with 24 bit per each pixel.

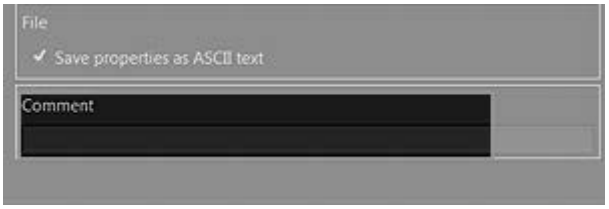
10 This procedure is necessary when the dedicated format (Olympus Image Format or Olympus Image Binary Format) is selected in [Save as type] in 4.
Select whether or not to compress the image when exporting.

11 This procedure is necessary when "JPEG" is selected in [Save as type] in 4.
Select the compression ratio of the image when exporting. The larger the value, the higher the compression ratio and the lower the image quality.

12 This procedure is necessary when "WMV" is selected in [Save as type] in 4.
Set the frame rate of the movie.

13 Ticking this checkbox will output the properties (acquisition conditions when acquiring the image) in text.

14 This procedure is necessary when "Olympus Image Format" or "Olympus Image Binary Format" is selected in [Save as type] in 4.
If you enter comments, they will be saved in the image properties.



15 Press the Save button. The image will be exported.

8.2. Exporting multiple images

The image can be exported (saved) in the file format which can be used by other software. The formats which can be exported are shown below.

- TIFF
- Bitmap
- PNG
- JPEG
- CSV

} general purpose format

- Olympus Image Format
- Olympus Image Binary Format
- Fluoview Multi-TIFF Compatible

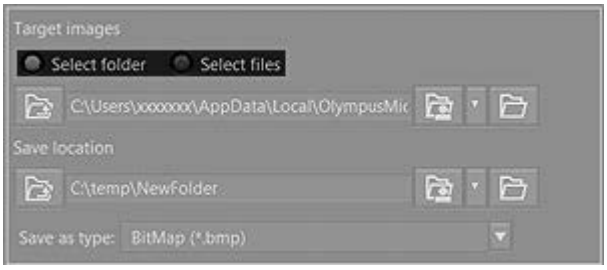
} dedicated format

The CSV format is selected when exporting the intensity data from the multipoint scan[*1] image.

1 In the [File] menu on the software screen, select [Export multiple files].
The [Export multiple files] dialog box appears.

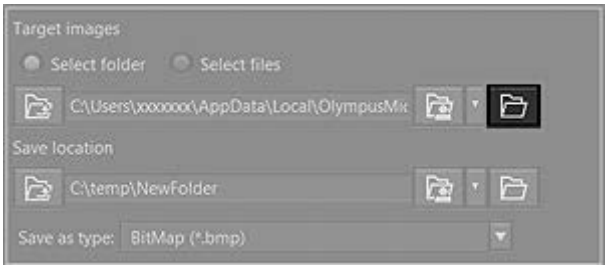
2 Select the method to export images.

Select folder All files in the specified folder are exported.
Select files One or multiple files specified are exported.



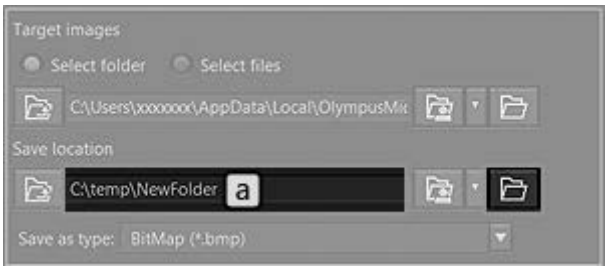
3 Press the button to display the dialog box. Select the folder or the file to be exported.

You can select multiple files. The method to select files is same as the method to select multiple files in Windows® Explorer.

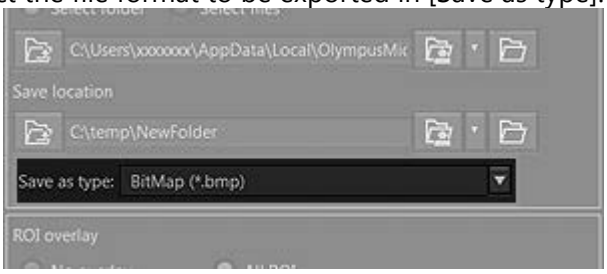


4 The save destination of the image to be exported is displayed in a. If you want to change the save destination, press the button to display the dialog box and select the folder of the save destination.

In order to organize image files easily, it is recommended to specify the save destination.



5 Select the file format to be exported in [Save as type].



6 This procedure is necessary when the general purpose format is selected in [Save as type] in 5.
In [ROI overlay], set whether or not to overlay the ROI and the color bar displayed on the image over the image to be exported.

7 This procedure is necessary when general purpose format is selected in [Save as type] in 5.
Select the method to export channels.

Merge Channel	Multiple channels are merged to one image.
RGB Color	Each of multiple channels is made to one image.
RGB Color with Merge	One image merged from multiple channels and a number of channels of images made from each of multiple channels are created.
Raw Data Extracted	Each of multiple channels is made to one gray scale image (monochrome image without color information).

If you select "TIFF" in [Save as type] and select "Raw Data Extracted", the 16bit image (same format as this software) can be exported, but it is available only with the software equipped with following functions.

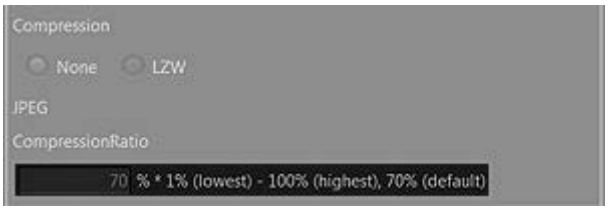
- **The 16bit gray scale can be displayed.**
- **The image level can be corrected. (The image becomes completely dark unless the data range displayed in the level correction can be adjusted.)**

8 This procedure is necessary when general purpose format is selected in [Save as type] in 5.
Select the bit color.

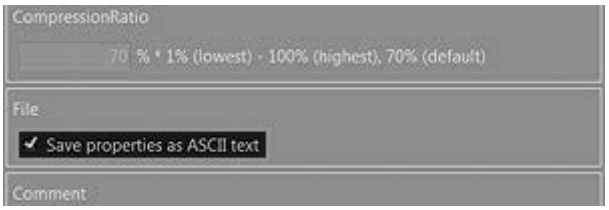
8bit Gray Scale	Color information in gray scale with 8 bit per each pixel.
16bit Gray Scale	Color information in gray scale with 16 bit per each pixel. (This item can be selected when "Raw Data Extracted" is selected in 7.)
24 bit Full Color	Color information in full color with 24 bit per each pixel.

9 This procedure is necessary when the dedicated format (Olympus Image Format or Olympus Image Binary Format) is selected in [Save as type] in 5.
Select whether or not to compress the image when exporting.

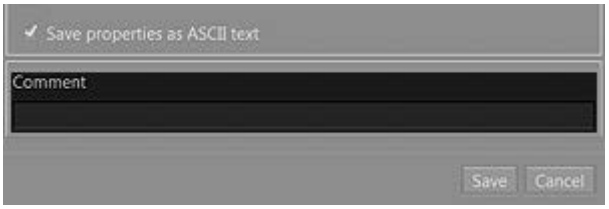
10 This procedure is necessary when "JPEG" is selected in [Save as type] in 5.
Select the compression ratio of the image when exporting. The larger the value, the higher the compression ratio and the lower the image quality.



11 Ticking this checkbox will output the properties (acquisition conditions when acquiring the image) in text.



12 This procedure is necessary when "Olympus Image Format" or "Olympus Image Binary Format" is selected in [Save as type] in **5**.
If you enter comments, they will be saved in the image properties.



13 Press the Save button. The image will be exported.

*1 A mode for consecutive light irradiation and sampling of multiple points.

9. 3D view and operation

9.1. Image in 3D view

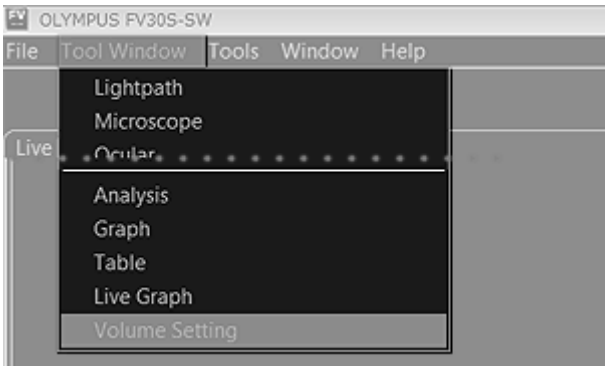
The Z series image can be viewed in 3D.

1. Activate [Image] window of the Z series image.
2. Select the [Volume] tab in [Image] window. The 3D construction starts and the 3D image is displayed gradually.

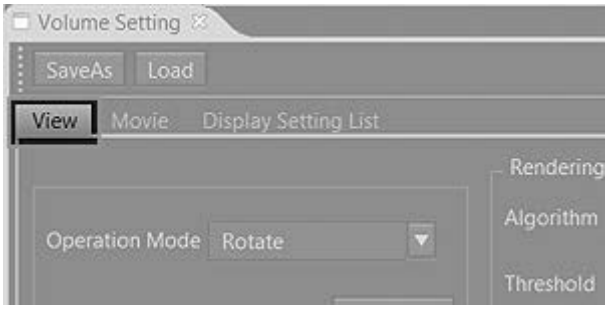
9.2. Operating 3D image

The 3D image can be operated with [View] tab in [Volume Setting] Tool Window.

1. Select [Volume Setting] in the [Tool Window] menu. [Volume Setting] Tool Window is displayed.

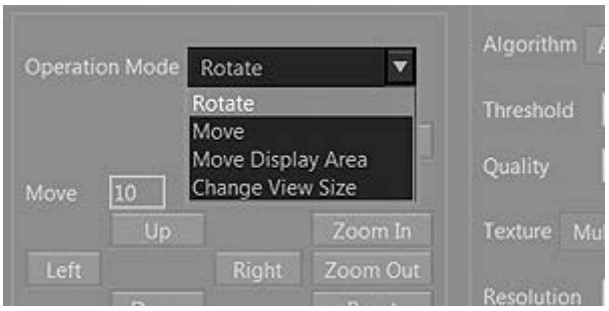


2 Select [View] tab in [Volume Setting] Tool Window.

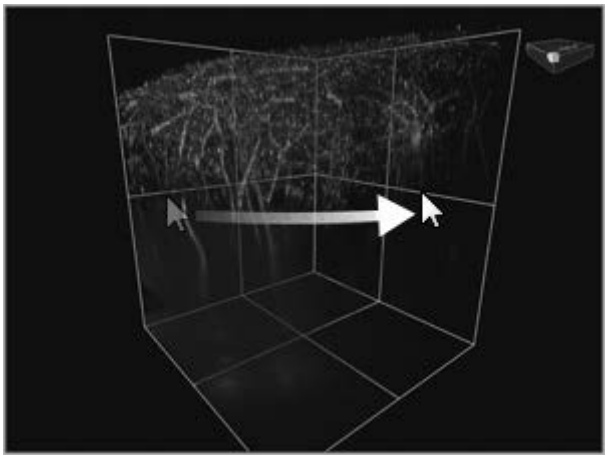


9.2.1. Rotating 3D Image

1 Select "Rotate" in [Operation Mode].



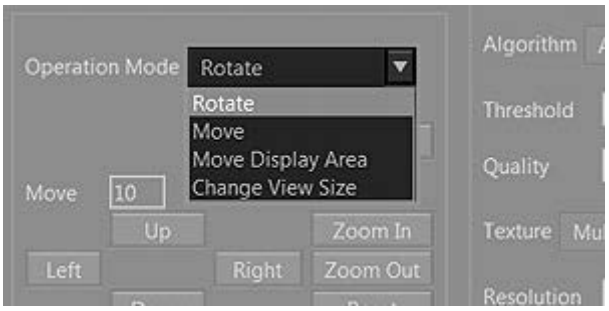
2 Drag the mouse on the 3D image in the direction you want to rotate. The 3D image rotates.
Click on the 3D image to stop rotation.



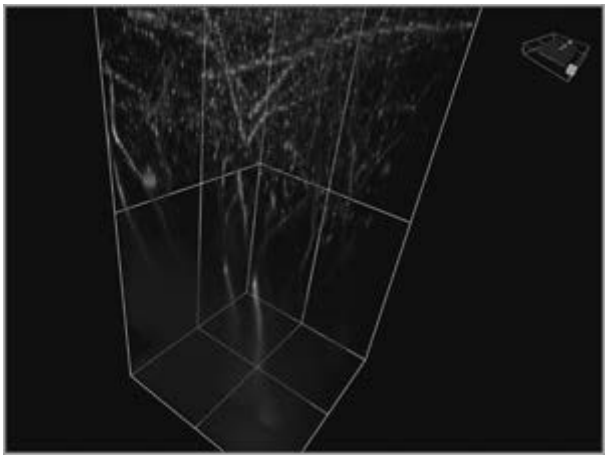
9.2.2. Zooming 3D Image

If the 3D image is displayed by the data acquired with the high resolution, this function enables to display the more detailed structure than when the entire image is displayed.

1 Select "Rotate" in [Operation Mode].



2 Rotate the mouse wheel backward on the 3D image. The 3D image is zoomed in. The zooming is such that the view field is moved toward the center of the image displayed.

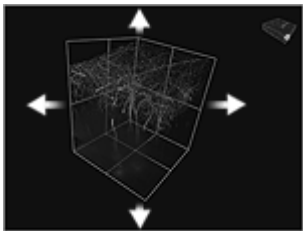


Moving and zooming the display area

You are also allowed to move and zoom in the display area while "Rotate" is selected in [Operation Mode].

Shift key + dragging mouse : Moves the display area

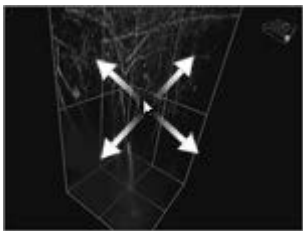
Shift key + mouse wheel : Zooms in the display area



Moving the 3D image display area during zooming

You can move the display area (visible area) of the 3D image during zooming even though "Rotate" is selected in [Operation Mode].

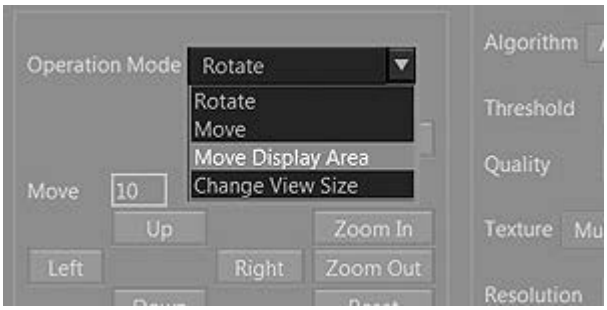
Ctrl key + dragging mouse : Moves the display area.



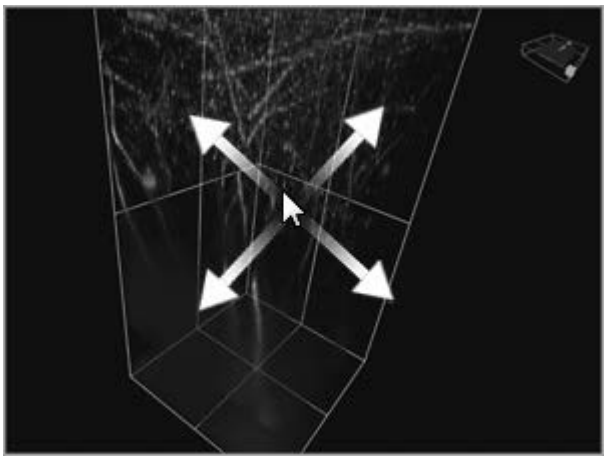
9.2.3. Changing the visible area of 3D image

When you zoom in the image in "Rotate" mode, the area cut out from of the whole 3D image is viewed as the visible area in the display area.
The visible area in the display area can be changed by moving the 3D image up/down/right/left.

1. Select "Move Display Area" in [Operation Mode].

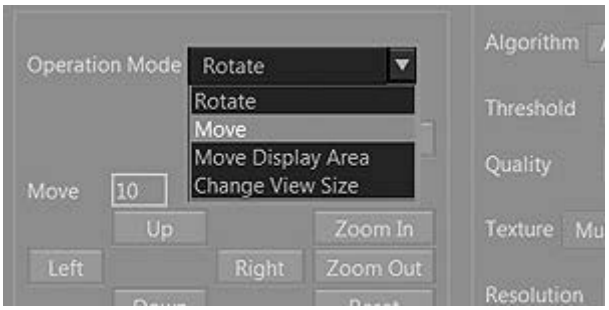


2. Drag the mouse on the 3D image to move the 3D image.

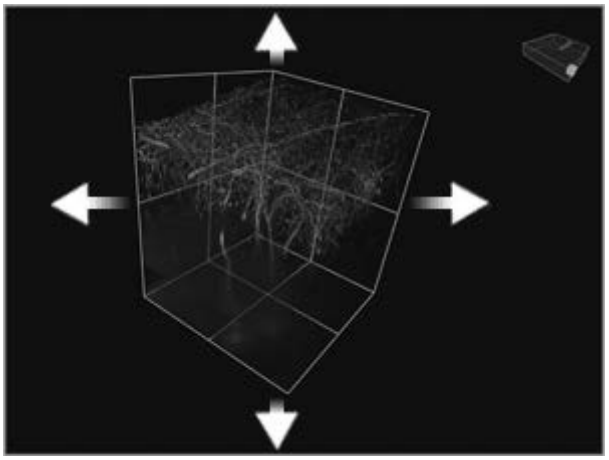


9.2.4. Moving and zooming in the display area

1 Select "Move" in [Operation Mode].



2 Drag the mouse on the 3D image in the direction you want to move. The whole display area is moved.
Rotate the mouse wheel toward front or back to zoom out or zoom in the whole display area.

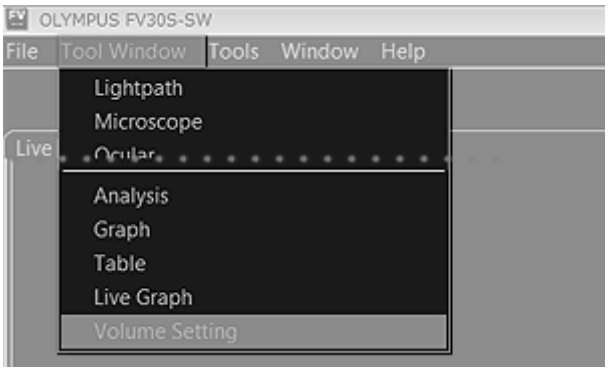


9.3. Creating movie

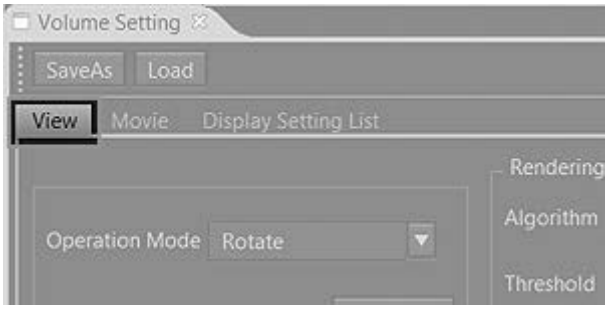
9.3.1. Recording 3D image movement

The movie can be created by moving the 3D image by dragging the mouse.

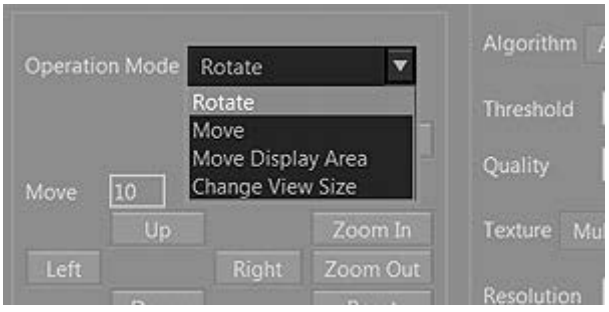
1 Select [Volume Setting] in the [Tool Window] menu. [Volume Setting] Tool Window is displayed.



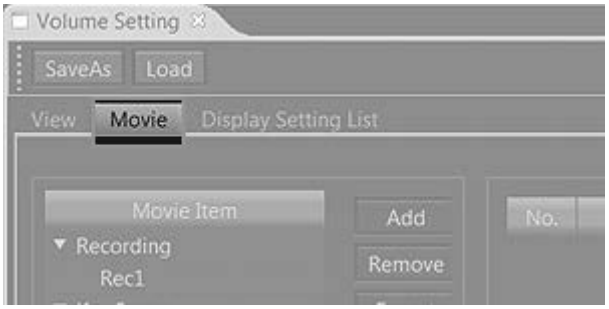
2 Select [View] tab in [Volume Setting] Tool Window.
(2 and 3 are the procedures necessary for moving the 3D image by dragging the mouse.)



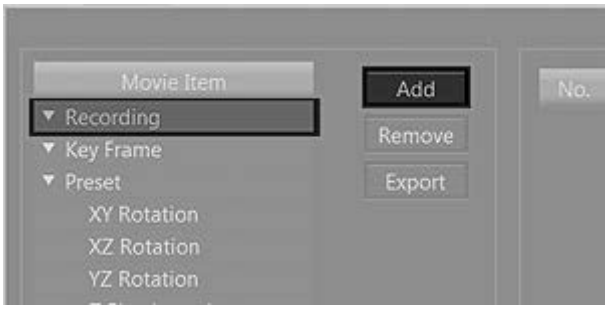
3 Select "Rotate" in [Operation Mode].



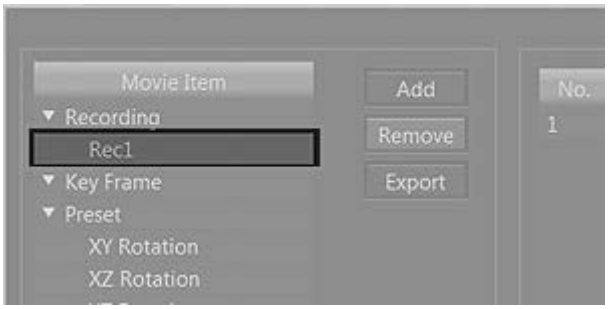
4 Select [Movie] tab in [Volume Setting] Tool Window.



5 Select "Recording" in [Movie Item] and press the Add button. "Rec1" is shown below "Recording".

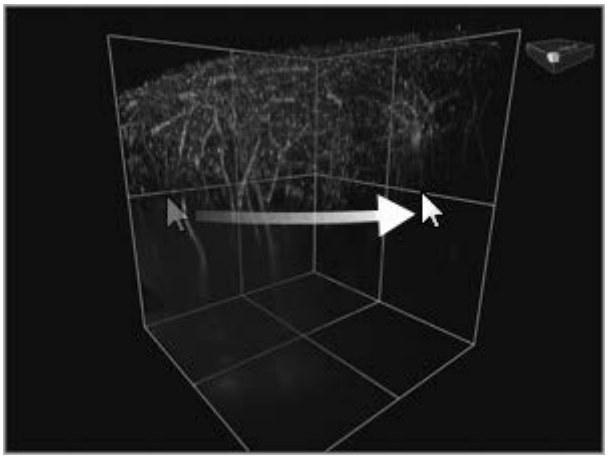


6 Select "Rec1" in [Movie Item].

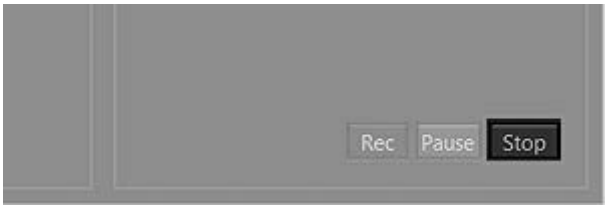


7 Double-click on the value of [FrameRate] to display the button. Press the button to select the frame rate.

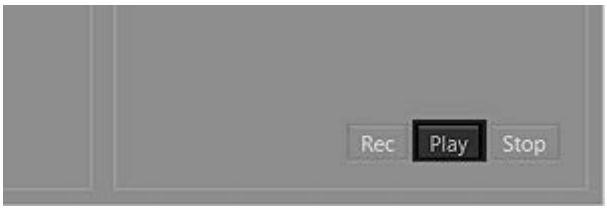
8 Press the Rec button to start recording, and move the 3D image in [Image] window by dragging.



9 Press the Stop button to stop recording.



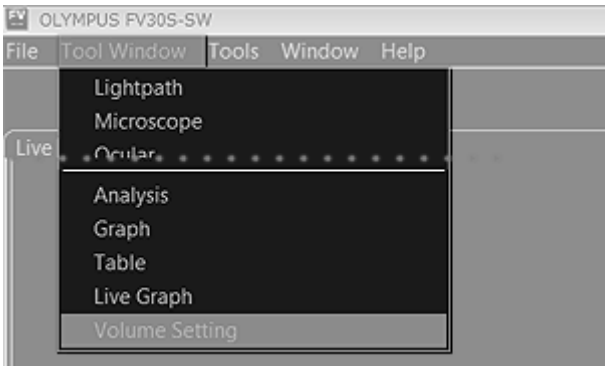
10 Press the Play button to play back the movie.



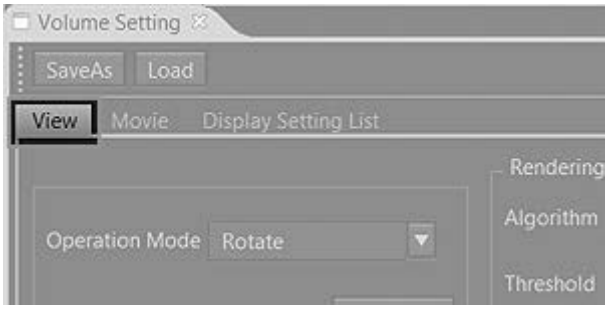
9.3.2. Creating the movie by registering Key Frame

The statuses you want to display are registered as Key Frames with moving the 3D image by dragging the mouse. The movie between Key Frames is interpolated automatically when you play back the movie.

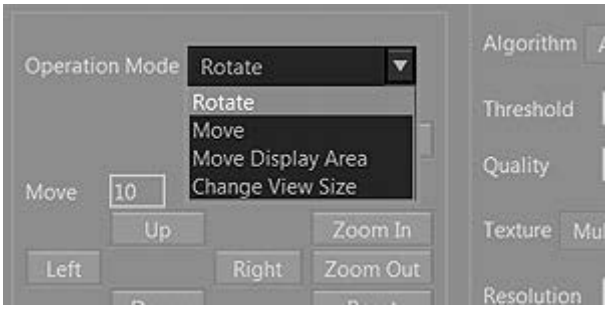
1 Select [Volume Setting] in the [Tool Window] menu. [Volume Setting] Tool Window is displayed.



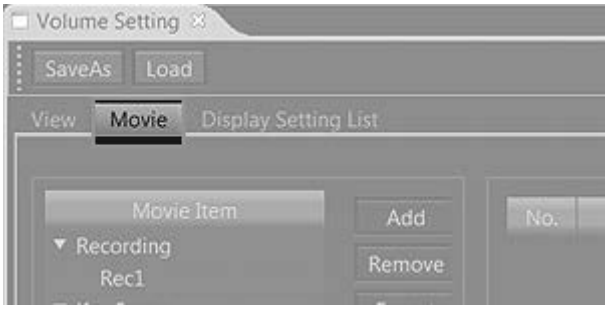
2 Select [View] tab in [Volume Setting] Tool Window.
(2 and 3 are the procedures necessary for moving the 3D image by dragging the mouse.)



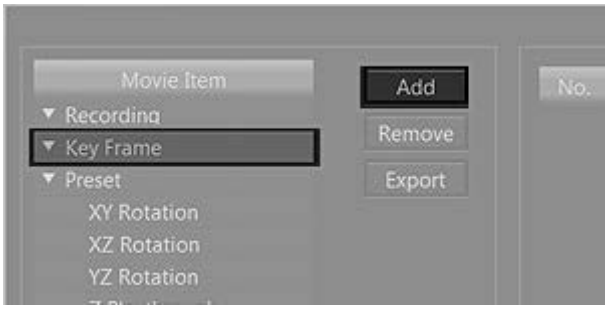
3 Select "Rotate" in [Operation Mode].



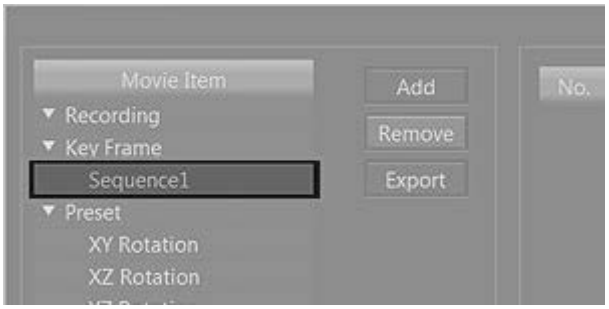
4 Select [Movie] tab in [Volume Setting] Tool Window.



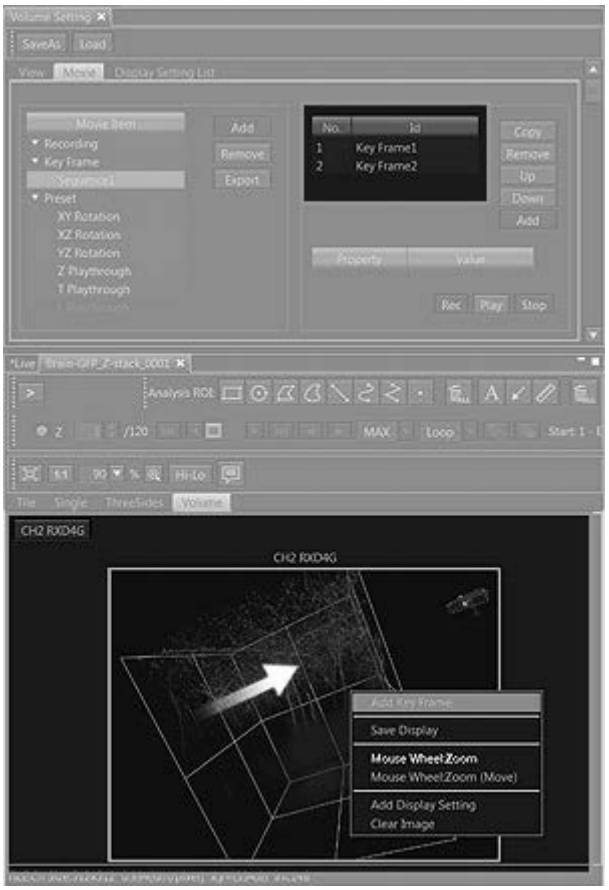
5 Select "Key Frame" in [Movie Item] and press the Add button. "Sequence1" is shown below "Key Frame".



6 Select "Sequence1" in [Movie Item].

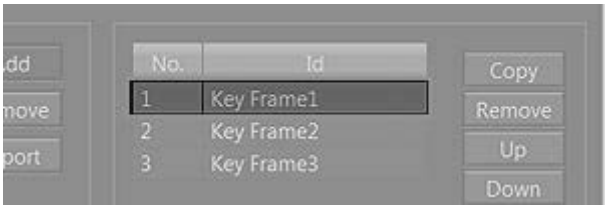


7 Move the 3D image in [Image] window by dragging and right-click at the desired status. When you select [Add Key Frame] in the menu displayed, the display status is registered and "Key Frame x" is displayed in [Id] in [Volume Setting] Tool Window.

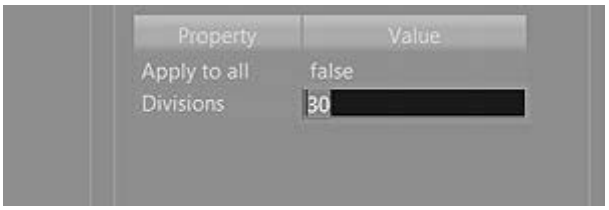


8 Repeat 7 and register the statuses you want to display as Key Frames.

9 Select "Key Frame1" in [Id].



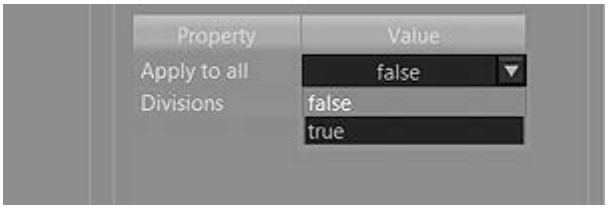
10 Double-click on the value in [Interval] to set the number of frames which interpolate between Key Frames. The larger the number of frames, the smoother the movie runs, but the slower the movie plays back.



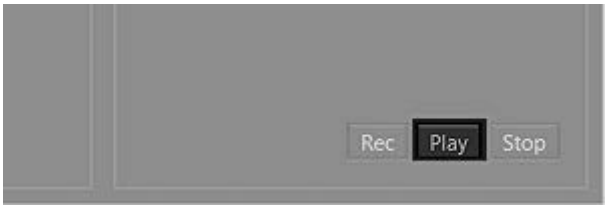
11 Double-click on "True" or "False" in [ApplySame] to display the button. Press the button to select "True" or "False".

"True" : The number of frames between all Key Frames will be same.
"False" : The number of frames between each Key Frame will be different.

If you select "False", repeat 9 and 10 for all Key Frames registered.



12 When you press the Play button, the data between Key Frames is interpolated automatically to play back the movie.



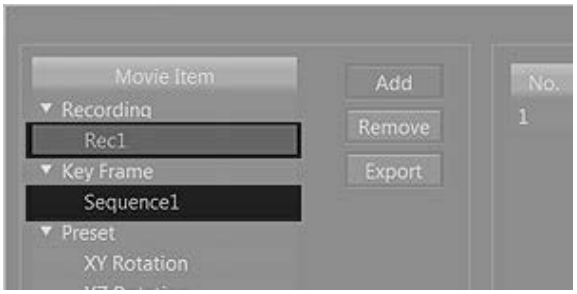
9.4. Exporting movie

The movie can be exported (saved) in WMV type.

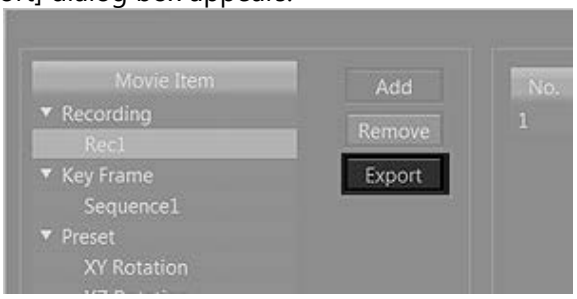
1 Select "Rec xx" or "Sequence xx" in [Movie Item] and select the movie you want to export.

Rec xx : This is a movie recorded with moving the 3D image by dragging the mouse.

Sequence xx : This is a movie created by interpolating between Key Frames after Key Frames are registered with moving the 3D image by dragging the mouse.



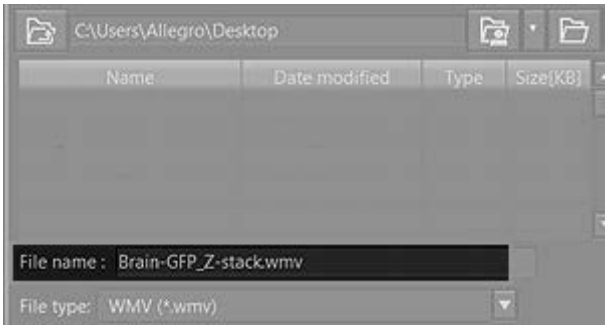
2 Press the Export button. The [Export] dialog box appears.



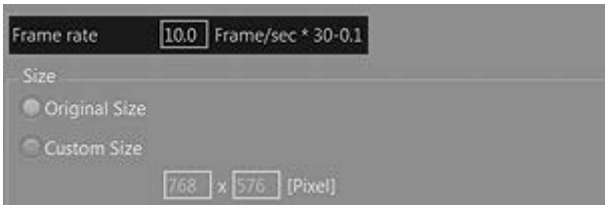
3 The save destination of the movie to be exported is displayed in a. If you want to change it, press the button to display the dialog box and select the folder of the save destination.

In order to organize movie files with ease, it is recommended to specify the save destination.

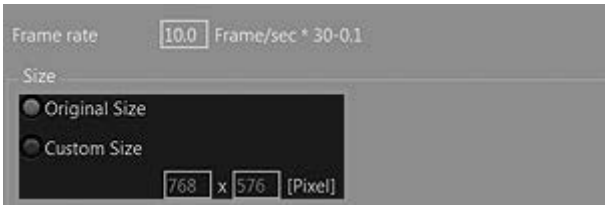
4 The file name of the movie to be exported is displayed in [File name]. If you want to change it, enter the file name directly.



5 The default value is displayed in [Frame rate]. If you want to change it, enter the value directly.



6 If you export the image by changing the original image size, select "Custom Size" in [Size] and enter the value directly.



7 Press the Save button. The move is exported.

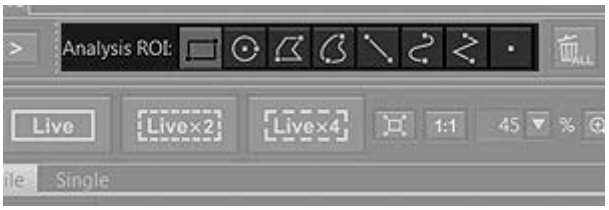
10. Analyzing image

10.1. Drawing average intensity profile in ROI of live image

This function allows you to confirm the intensity variation during LIVE.
Specifying the ROI on the live image will draw the average intensity profile in the ROI in synchronization with acquisition.

1. Display the live image in the LSM live range on [Live] window in advance.

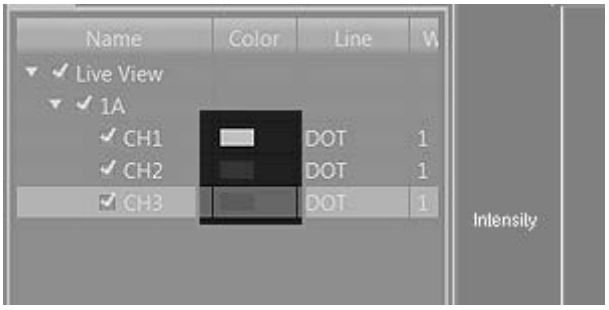
2. Select the ROI tool in [Analysis ROI], and specify the area you want to confirm the intensity on the live image.



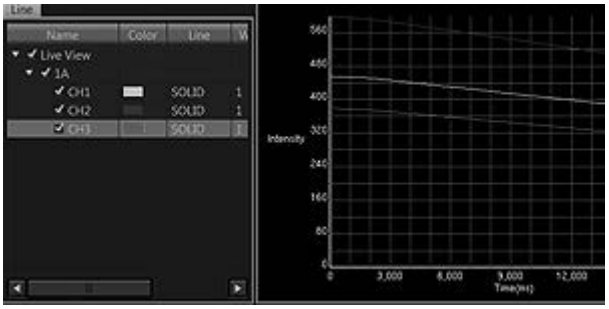
3. When "Live"/"ROI ID"/"Channel" checkboxes appear on [Live Graph] Tool Window, tick each of them. (Uncheck the checkbox of the information of which you don't want to draw the average intensity profile.)

4. To identify the average intensity profile of each channel, set the color of the profile.

   Double-click the color display area, and click the  button displayed. When the dialog box appears, select the color.



5 Press the Live button on [Live] window to start acquiring the live image. The average intensity profile is drawn in synchronization with acquisition.



Time lapse of the average intensity profile

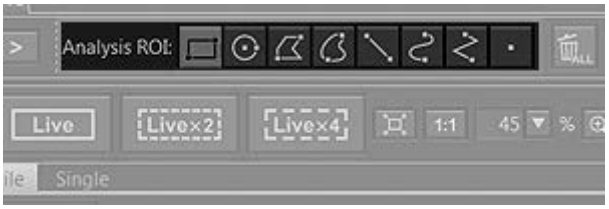
The time lapse of the average intensity profile starts counting when the acquisition starts. The counting continues even though the acquisition stops. If the acquisition starts again, the time in the average intensity profile is counted at a continuous basis.

If you press Clear Graph to delete the average intensity profile, the average intensity profile will be displayed with that time as a start time when you start the acquisition next time.

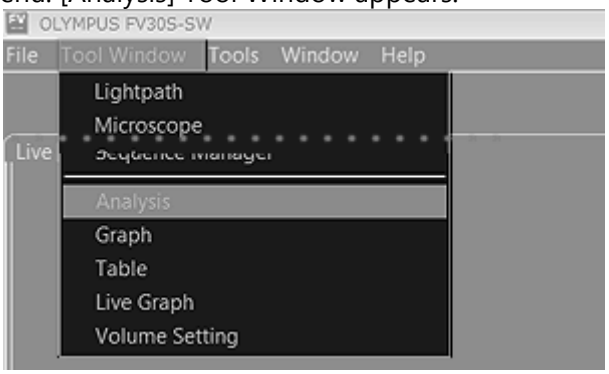
10.2. Drawing average intensity profile in ROI of series images

This function allows you to confirm the intensity variation of the series image.
Specifying ROI on the series image will draw the average intensity profile in ROI of the arbitrary line viewing the series image in the T axis direction or the arbitrary line viewing the series image in the Z axis direction.

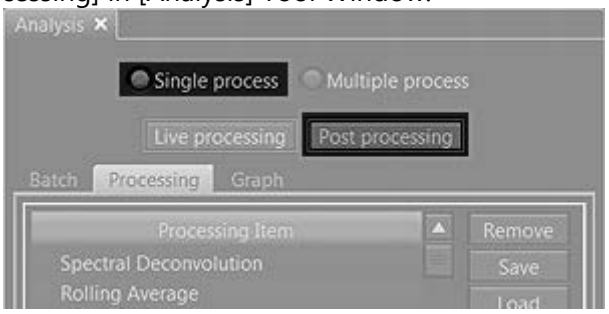
1 Select the ROI tool in [Analysis ROI] on [Image] Window, and specify the area you want to confirm the intensity on the image.



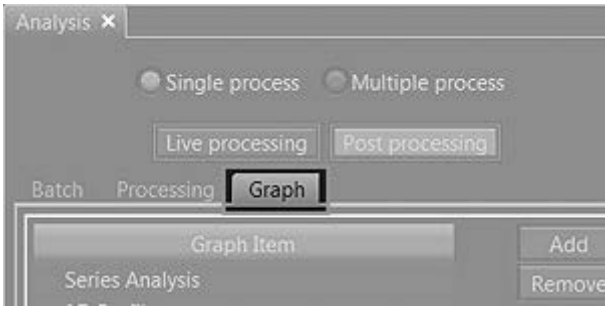
2 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



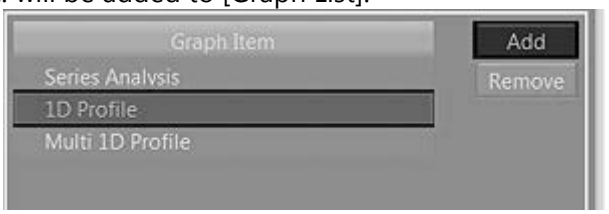
3 Select [Single process] and [Post processing] in [Analysis] Tool Window.



4 Select the [Graph] tab.

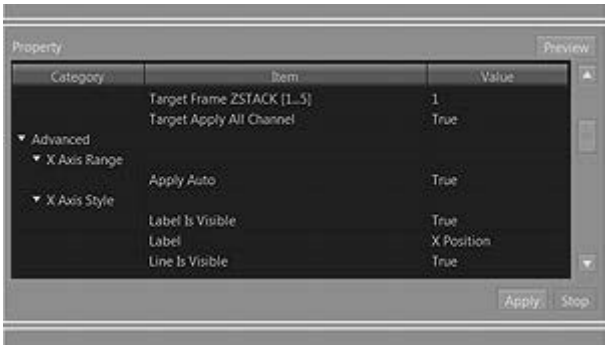


5 Select "1D Profile" in [Graph Item], and press the Add button. The information, e.g. types of graphs to be drawn, etc. will be added to [Graph List].



Multiple graphs can be registered to [Graph List] to allow you to draw multiple graphs at a time.

6 Set the required items in [Property].



7 Press the Apply button. All graphs registered to [Graph List] are drawn on [Graph] Tool Window.

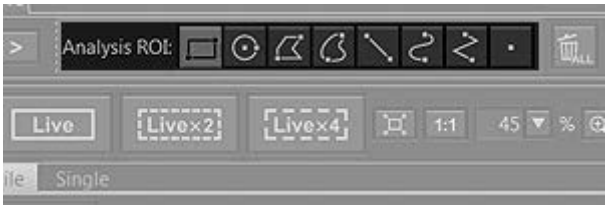
If you draw multiple graphs at a time, the graph drawn last is displayed on [Graph] Tool Window.

To change the graph to be displayed, press the > button to open [Gallery], and select the thumbnail.

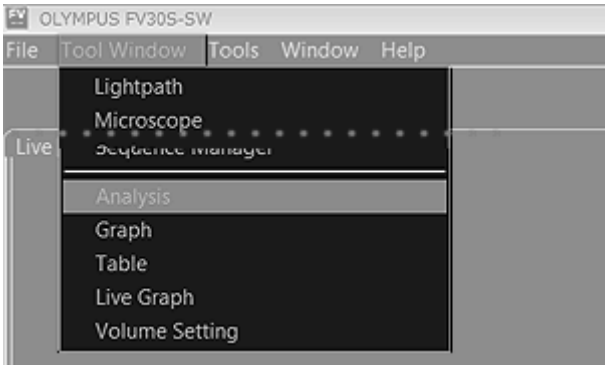
10.3. Measuring and drawing the graph of measured results

Specifying ROI on the series image enables to measure the intensity in ROI and to output results in the table. The average intensity profile in ROI is also displayed.

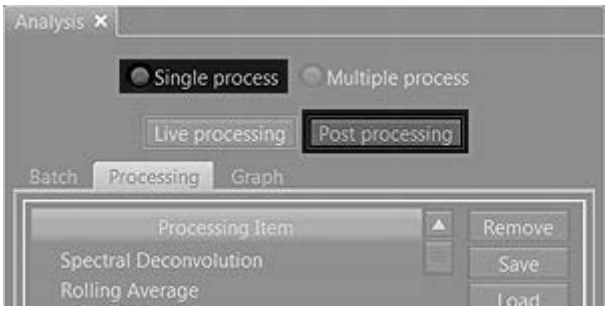
1. Select the ROI tool in [Analysis ROI] on [Image] Window, and specify the area you want to measure on the image.



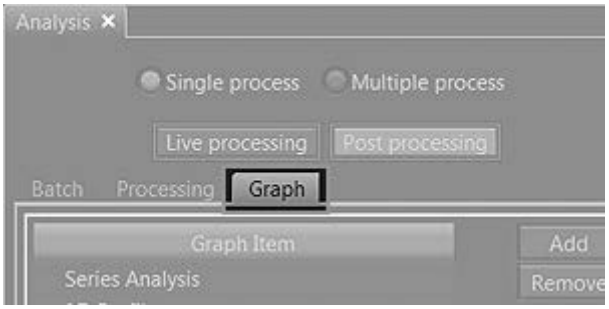
2. Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



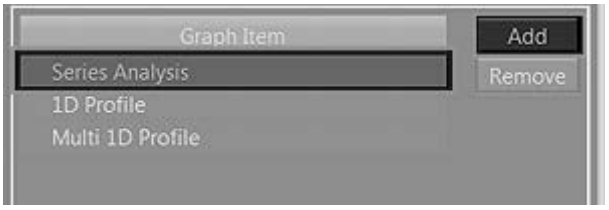
3. Select [Single process] and [Post processing] in [Analysis] Tool Window.



4 Select the [Graph] tab.



5 Select "Series Analysis" in [Graph Item], and press the Add button. The information, e.g. types of graphs to be drawn, etc. will be added to [Graph List].



Multiple graphs can be registered to [Graph List] to allow you to draw multiple graphs at a time.

6 Set the required items in [Property].

7 Press the Apply button. The average intensity profile in ROI is drawn on [Graph] Tool Window.
Also, the measured results in ROI and statistics of measured results are displayed on [Table] Tool Window.

If you draw multiple graphs at a time, the graph drawn last is displayed on [Graph] Tool Window.

To change the graph to be displayed, press the > button to open [Gallery], and select the thumbnail.

11. Processing image

11.1. Unmixing

11.1.1. Performing Spectral Image Unmixing during scanning

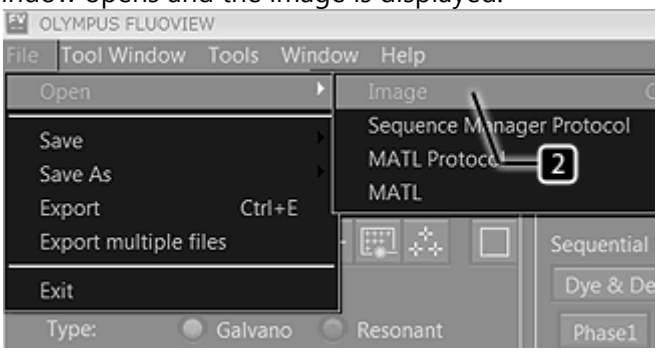
By opening the image for acquiring the spectral data required for fluorescent separation and specifying the area (ROI), this method performs the fluorescent separation process based on the spectral data of the specified area.
This section describes the procedures to perform Spectral Image Unmixing when acquiring the image from the double-dyed specimen.

Setting for acquiring the Lambda series image

1 Set for acquiring the Lambda series image according to "Setting Lambda series (acquiring by a single channel) or "Setting Lambda series (acquiring by multiple channels)".

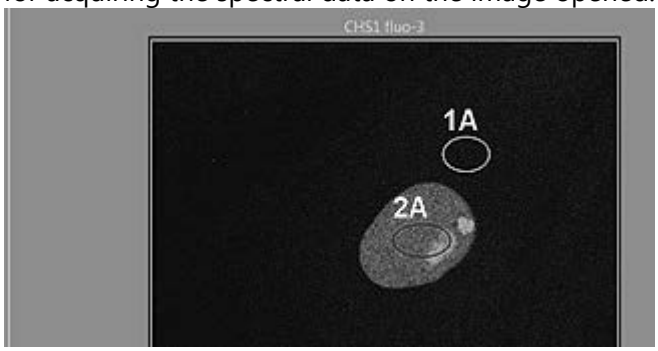
Opening the image for acquiring the spectral data required for fluorescent separation

2 Select [Open] - [Image] in [File] menu to display the dialog box and select the image in the dialog box. The [Image] window opens and the image is displayed.



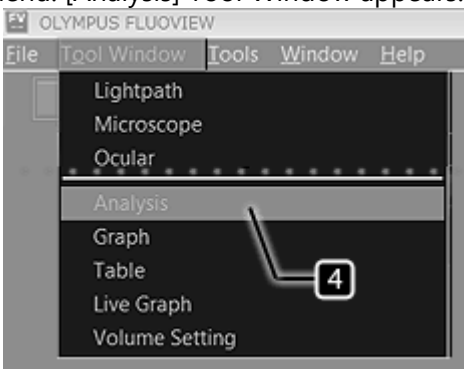
Specifying ROI for acquiring the spectral data

3 Specify two ROIs for acquiring the spectral data on the image opened.

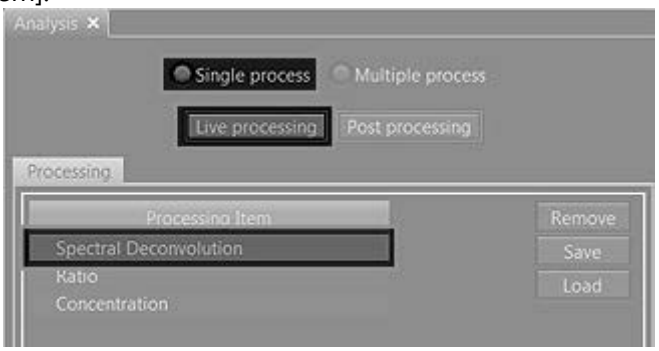


Performing Spectral Deconvolution

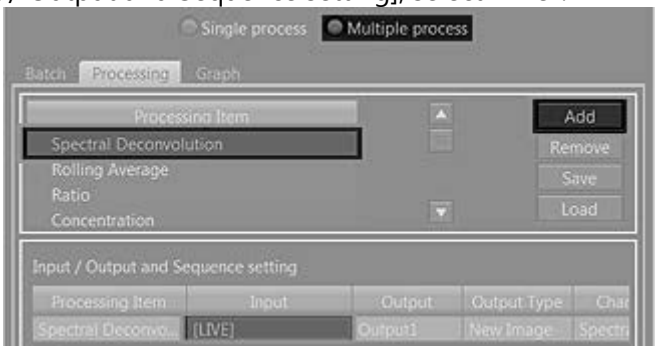
4 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



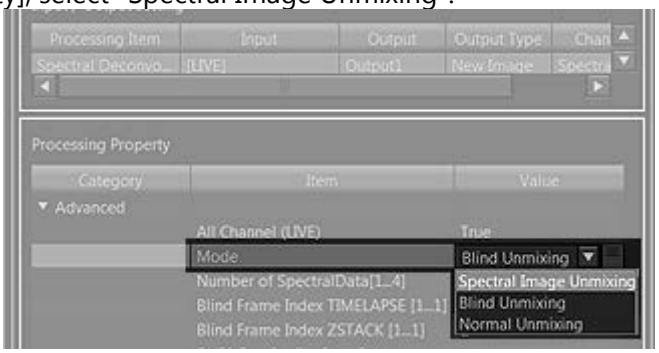
5 Select [Single process] mode, press the [LiveProcessing] button and select [Spectral Deconvolution] in [Processing Item].



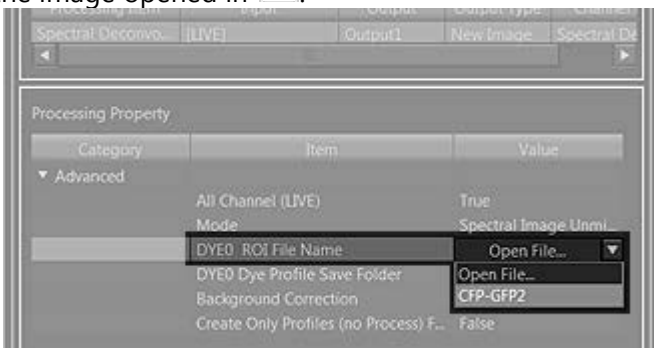
Or, if you perform Spectral Deconvolution as one of sequential processes, select [Multiple process], select [Spectral Deconvolution] in [Processing Item] and press the [Add] button. In [Input] in [Input / Output and Sequence setting], select "Live".



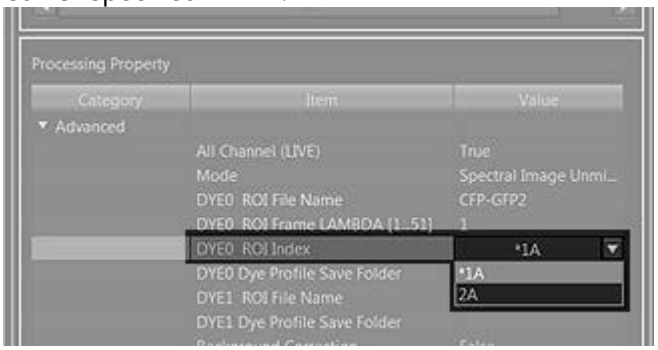
6 In [Mode] in [Processing Property], select "Spectral Image Unmixing".



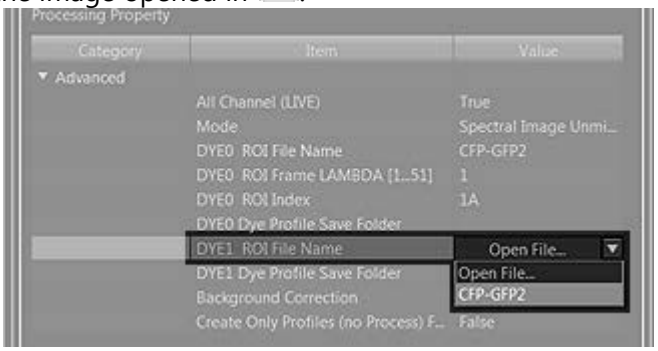
7 In [DYE0 ROI File Name], select the image opened in 2.



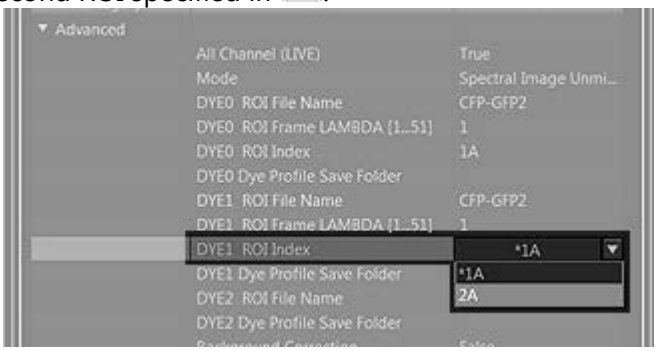
8 In [DYE0 ROI Index], select the first ROI specified in 3.



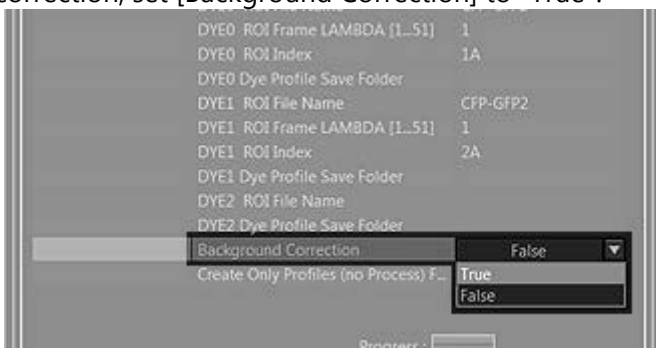
9 In [DYE1 ROI File Name], select the image opened in 2.



10 In [DYE1 ROI Index], select the second ROI specified in 3.

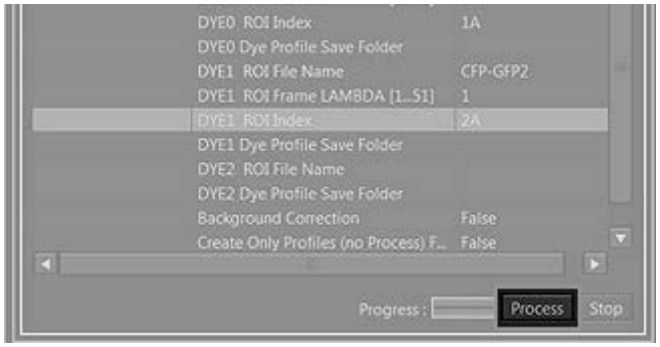


11 If you perform the background correction, set [Background Correction] to "True".



If the background correction is set to "True", the background spectrum is calculated from the target image automatically and the background is deleted.

12 If you select [Multiple process] in 5, press the [Process] button. Now, you are in the waiting status to start the scan. If you select [Single process] in 5, the fluorescent separation process starts in synchronization with the start of scan.



Starting acquisition of the Lambda series image

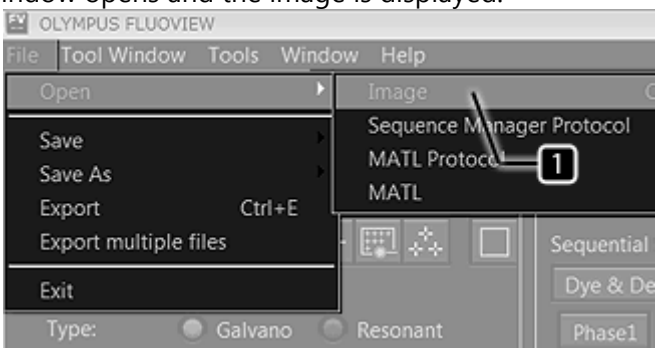
13 Press LSM Start in [Acquire] Tool Window to start acquisition.
When acquiring the XYL series image, the fluorescent separation process is performed after the scan is completed and the image after separation is displayed in [Image] window automatically.
When acquiring the XYLT series image, XYLZ series image or XYLZT series image, the fluorescent separation process is performed each time when one λ scan is completed and the image after separation is displayed in [Image] window automatically.

11.1.2. Performing Spectral Image Unmixing for acquired images

By opening the image for acquiring the spectral data required for fluorescent separation and specifying the area (ROI), this method performs the fluorescent separation process based on the spectral data of the specified area.
This section describes the procedures to perform Spectral Image Unmixing for the image acquired from the double-dyed specimen.

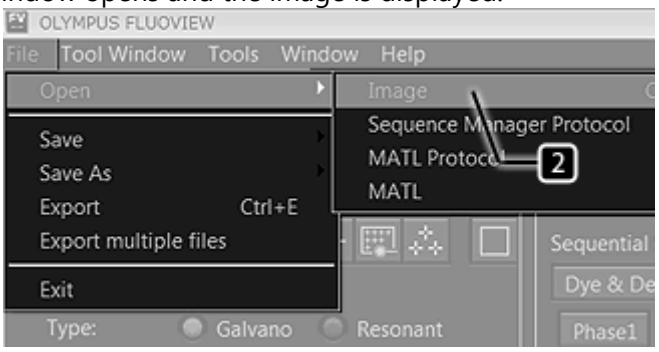
Opening the target image for image processing

1 Select [Open] - [Image] in [File] menu to display the dialog box and select the image in the dialog box. The [Image] window opens and the image is displayed.



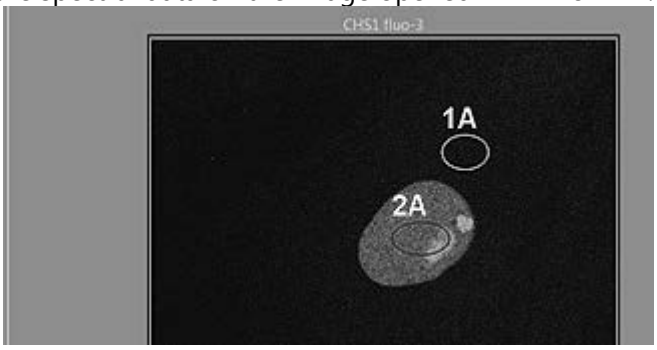
Opening the image for acquiring the spectral data required for fluorescent separation

2 If you want to get the spectral data to be used for the fluorescent separation from the image different from the target image for processing, open that image. (If you want to get the spectral data to be used for the fluorescent separation from the target image for processing, this procedures is not necessary.)
Select [Open] - [Image] in [File] menu to display the dialog box and select the image in the dialog box. The [Image] window opens and the image is displayed.



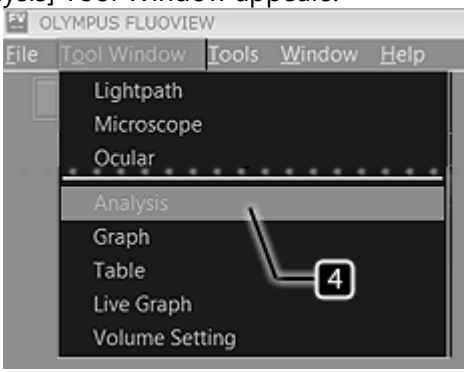
Specifying ROI for acquiring the spectral data

3 Specify two ROIs for acquiring the spectral data on the image opened in 1 or 2.

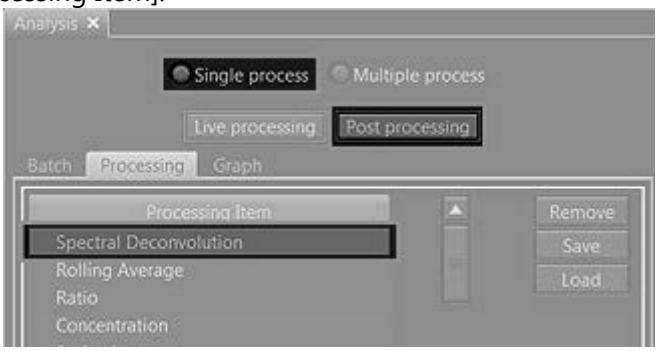


Performing Spectral Deconvolution

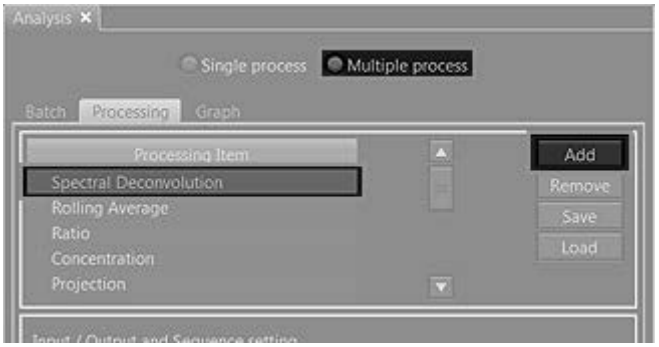
4 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



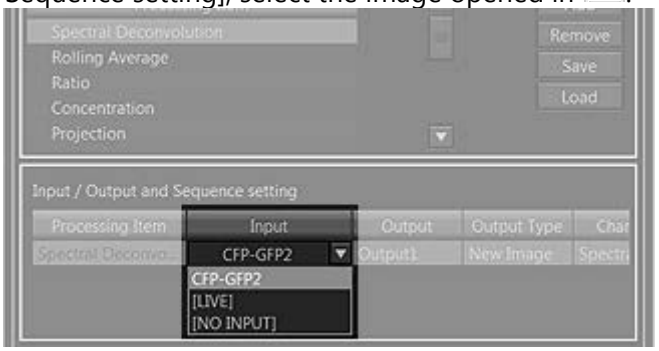
5 Select the [Single process] mode, press the [Post Processing] button and select [Spectral Deconvolution] in [Processing Item].



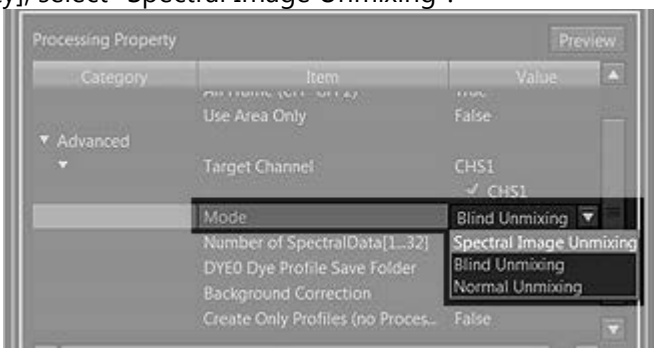
Or, if you perform Spectral Deconvolution as one of sequential processes, select [Multiple process], select [Spectral Deconvolution] in [Processing Item] and press the [Add] button.



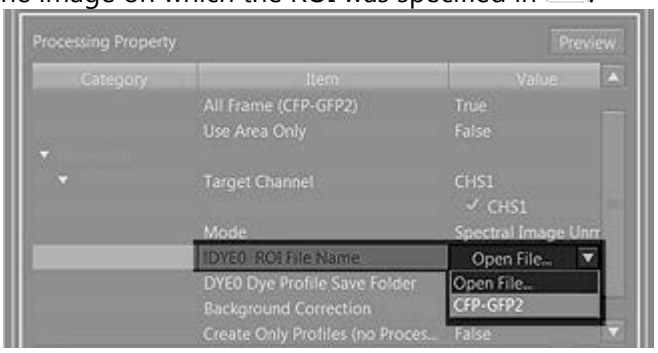
6 In [Input] in [Input / Output and Sequence setting], select the image opened in 1.



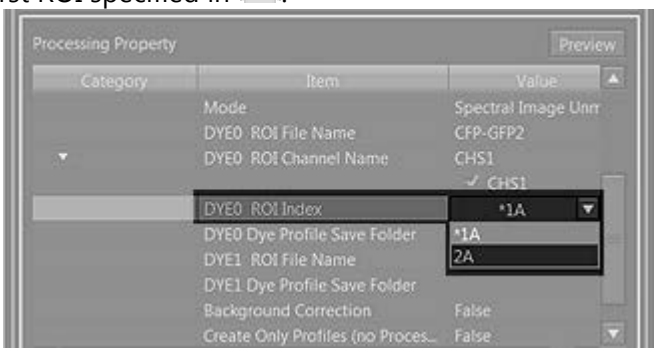
7 In [Mode] in [Processing Property], select "Spectral Image Unmixing".



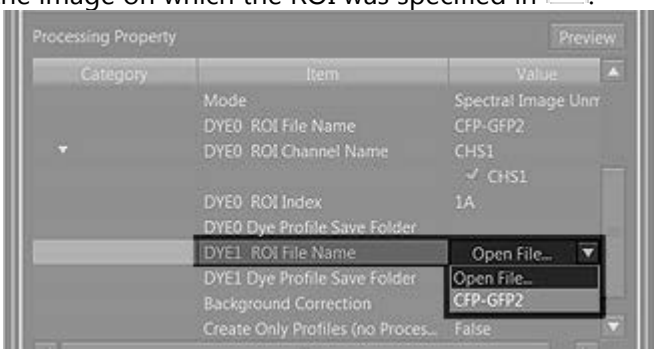
8 In [DYE0 ROI File Name], select the image on which the ROI was specified in 3.



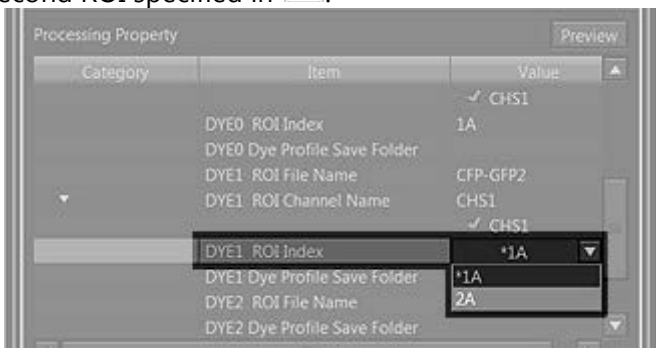
9 In [DYE0 ROI Index], select the first ROI specified in 3.



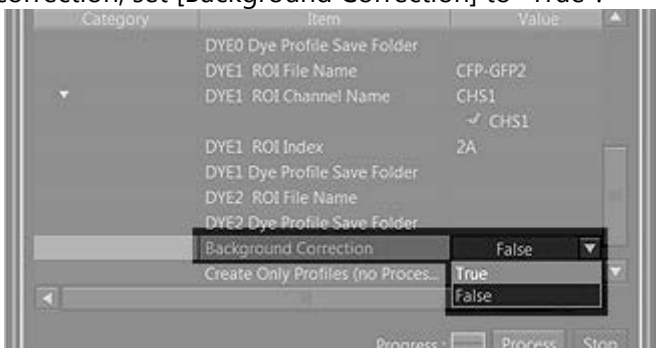
10 In [DYE1 ROI File Name], select the image on which the ROI was specified in 3.



11 In [DYE1 ROI Index], select the second ROI specified in 3.

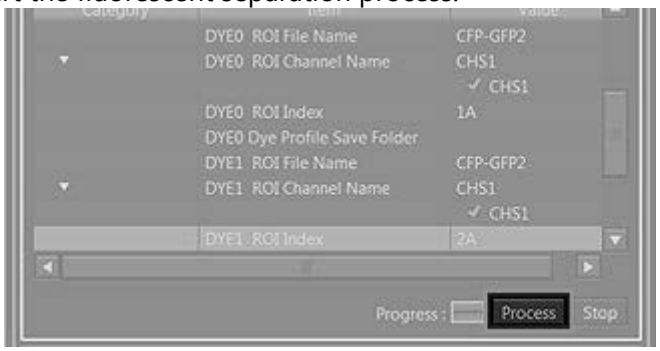


12 If you perform the background correction, set [Background Correction] to "True".



If the background correction is set to "True", the background spectrum is calculated from the target image automatically and the background is deleted.

13 Press the [Process] button to start the fluorescent separation process.



11.1.3. Performing Blind Unmixing during scanning

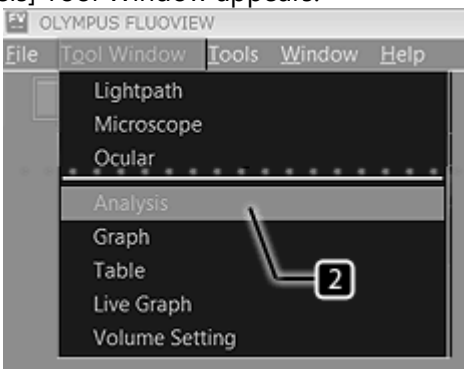
This method performs the automatic fluorescent separation process by specifying the arbitrary number of fluorochrome without specifying any image for acquiring the spectral data required for fluorescent separation or any area.
This section describes the procedures to perform Blind Unmixing when acquiring the image from the double-dyed specimen.

Setting for acquiring the Lambda series image

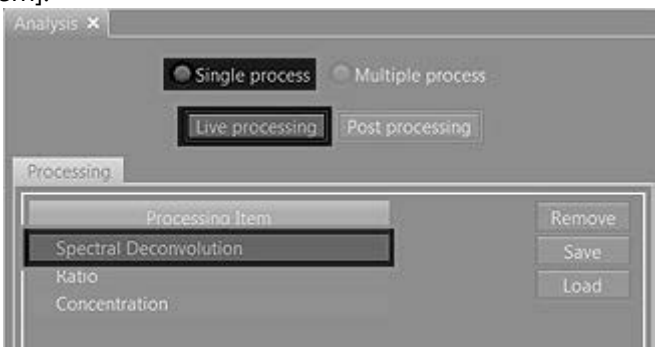
1. Set for acquiring the Lambda series image according to "Setting Lambda series (acquiring by a single channel) or "Setting Lambda series (acquiring by multiple channels)".

Performing Spectral Deconvolution

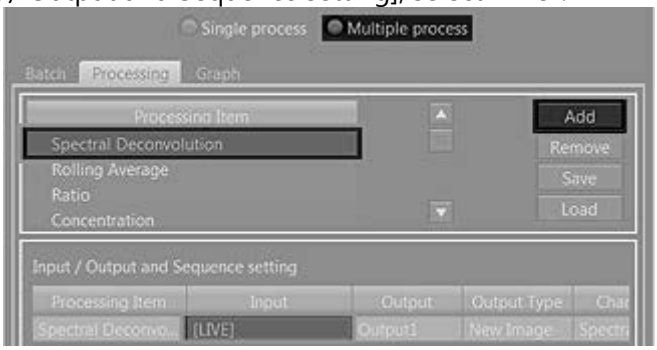
2. Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



3 Select [Single process] mode, press the [LiveProcessing] button and select [Spectral Deconvolution] in [Processing Item].

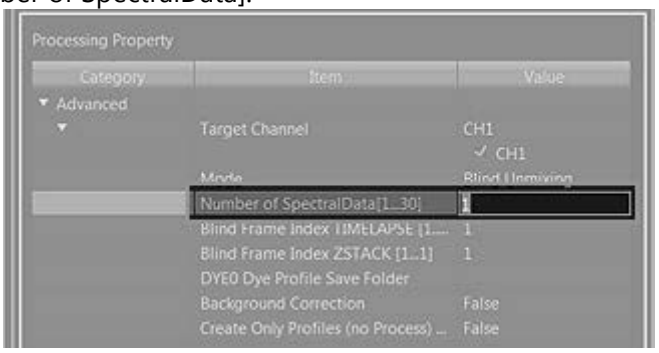


Or, if you perform Spectral Deconvolution as one of sequential processes, select [Multiple process], select [Spectral Deconvolution] in [Processing Item] and press the [Add] button. In [Input] in [Input / Output and Sequence setting], select "Live".

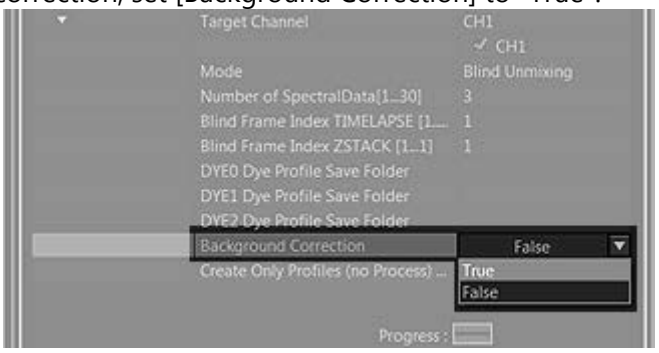


4 In [Mode] in [Processing Property], select "Blind Unmixing".

5 Set the number of dyes in [Number of SpectralData].

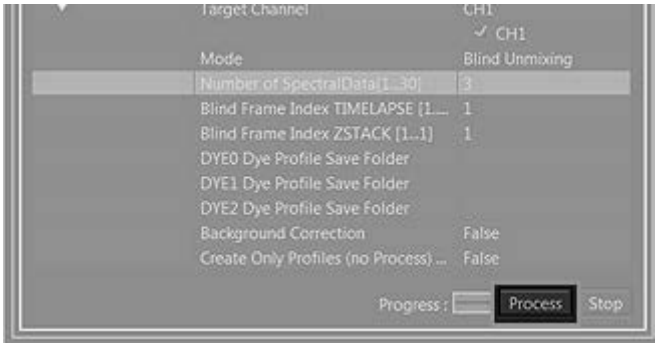


6 If you perform the background correction, set [Background Correction] to "True".



📖 If the background correction is set to "True", the background spectrum is calculated from the target image automatically and the background is deleted.

7 If you select [Multiple process] in 3, press the [Process] button. Now, you are in the waiting status to start the scan. If you select [Single process] in 3, the fluorescent separation process starts in synchronization with the start of scan.



Starting acquisition of the Lambda series image

8 Press 

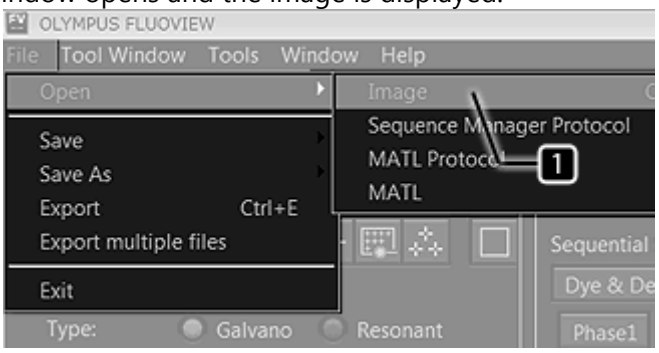
in [Acquire] Tool Window to start acquisition.
When acquiring the XYL series image, the fluorescent separation process is performed after the scan is completed and the image after separation is displayed in [Image] window automatically.
When acquiring the XYLT series image, XYLZ series image or XYLZT series image, the fluorescent separation process is performed each time when one λ scan is completed and the image after separation is displayed in [Image] window automatically.

11.1.4. Performing Blind Unmixing for acquired images

This method performs the automatic fluorescent separation process by specifying the arbitrary number of fluorochrome without specifying any image for acquiring the spectral data required for fluorescent separation or any area.
This section describes the procedures to perform Blind Unmixing for the image acquired from the double-dyed specimen.

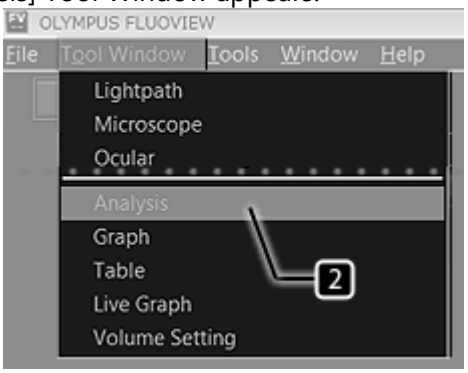
Opening the target image for image processing

1 Select [Open] - [Image] in [File] menu to display the dialog box and select the image in the dialog box. The [Image] window opens and the image is displayed.

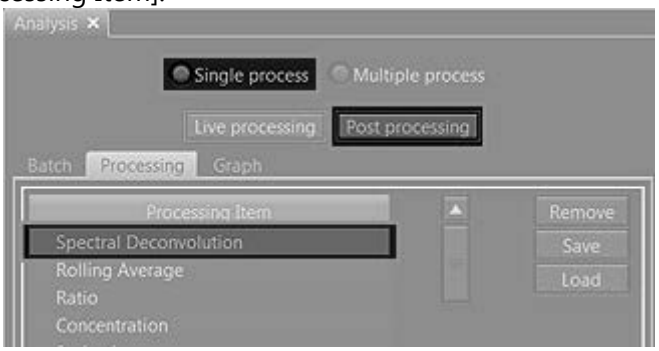


Performing Spectral Deconvolution

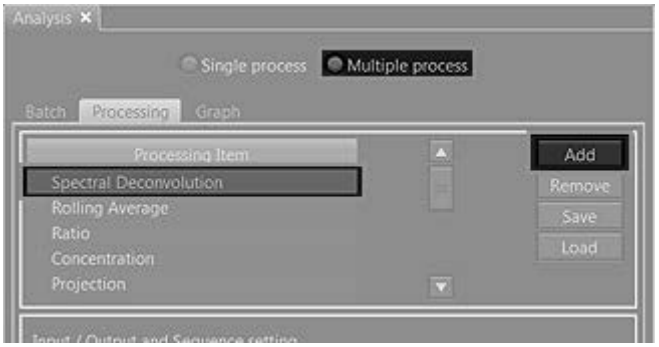
2 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



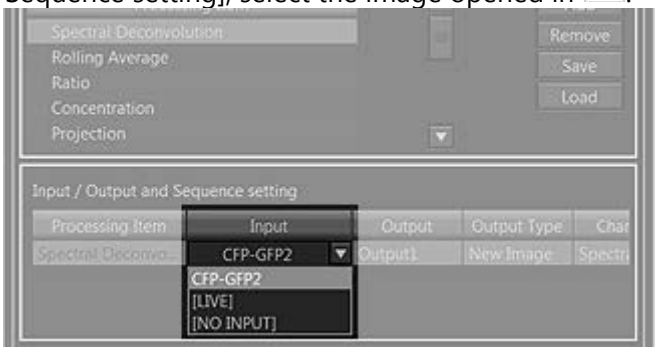
3 Select the [Single process] mode, press the [Post Processing] button and select [Spectral Deconvolution] in [Processing Item].



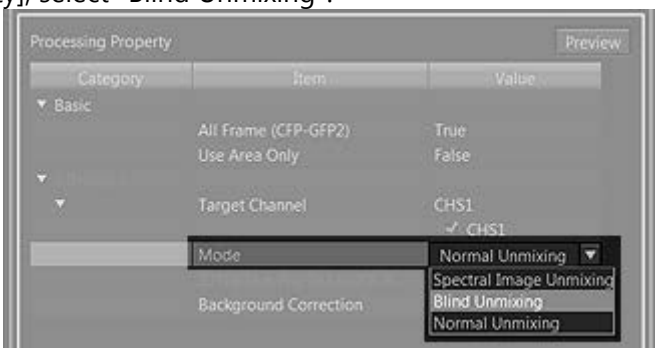
Or, if you perform Spectral Deconvolution as one of sequential processes, select [Multiple process], select [Spectral Deconvolution] in [Processing Item] and press the [Add] button.



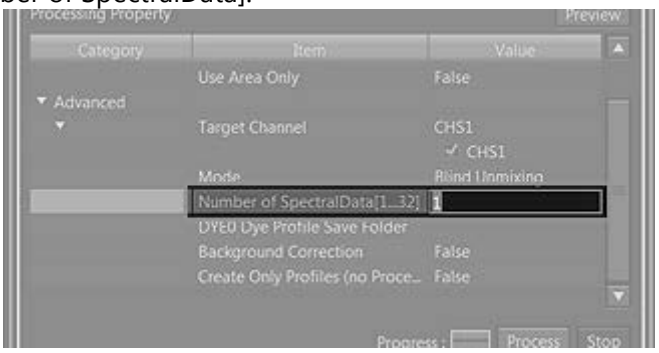
4 In [Input] in [Input / Output and Sequence setting], select the image opened in 1.



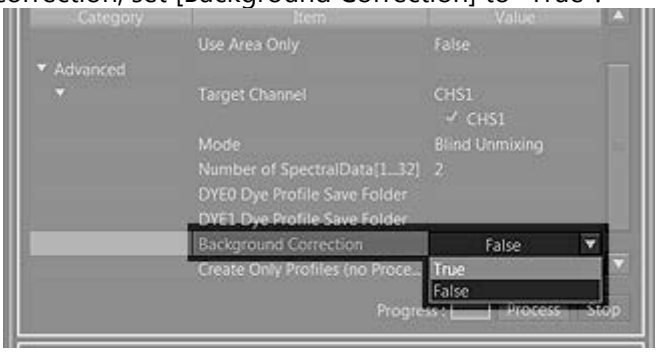
5 In [Mode] in [Processing Property], select "Blind Unmixing".



6 Set the number of dyes in [Number of SpectralData].

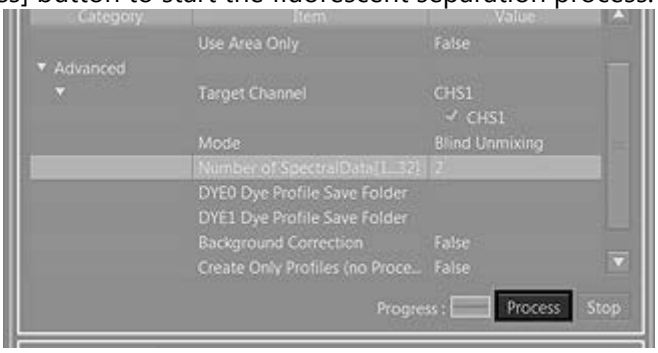


7 If you perform the background correction, set [Background Correction] to "True".



If the background correction is set to "True", the background spectrum is calculated from the target image automatically and the background is deleted.

8 Press the [Process] button to start the fluorescent separation process.



11.1.5. Performing Normal Unmixing during scanning

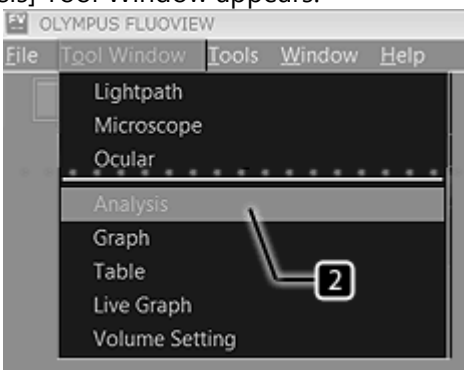
This method performs the fluorescent separation process using the spectral data of the fluorochrome which is preset in the system.
This section describes the procedures to perform Normal Unmixing when acquiring the image from the double-dyed specimen.

Setting for acquiring the Lambda series image

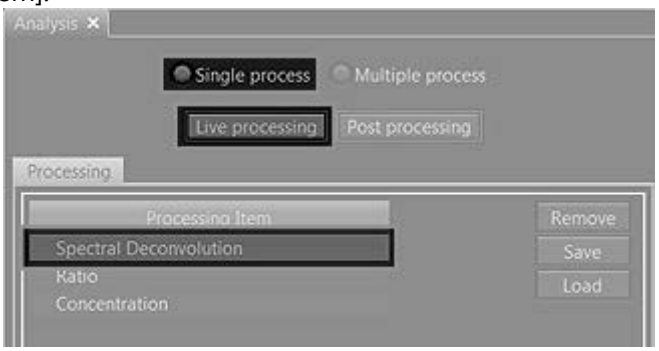
1 Set for acquiring the Lambda series image according to "Setting Lambda series (acquiring by a single channel) or "Setting Lambda series (acquiring by multiple channels)".

Performing Spectral Deconvolution

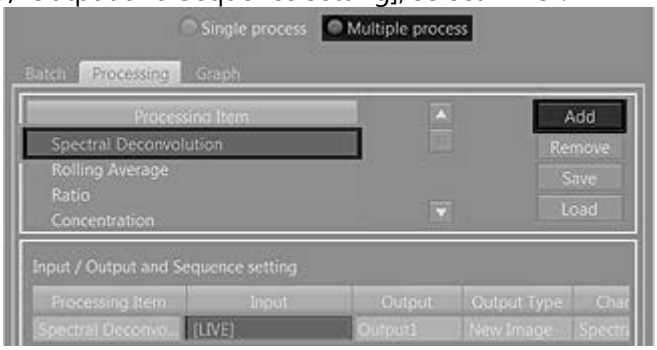
2 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



3 Select [Single process] mode, press the [LiveProcessing] button and select [Spectral Deconvolution] in [Processing Item].

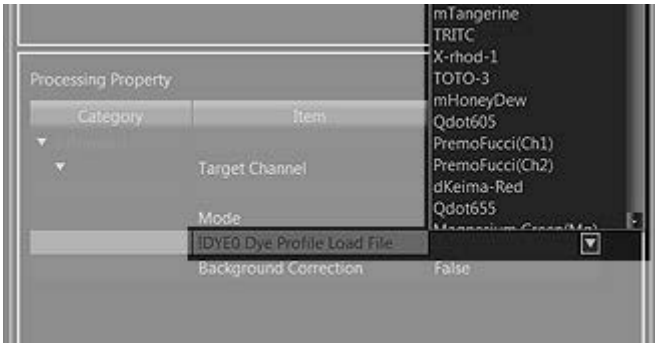


Or, if you perform Spectral Deconvolution as one of sequential processes, select [Multiple process], select [Spectral Deconvolution] in [Processing Item] and press the [Add] button. In [Input] in [Input / Output and Sequence setting], select "Live".

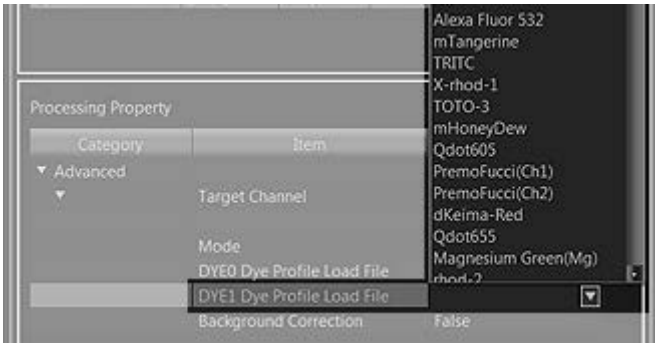


4 In [Mode] in [Processing Property], select "Normal Unmixing".

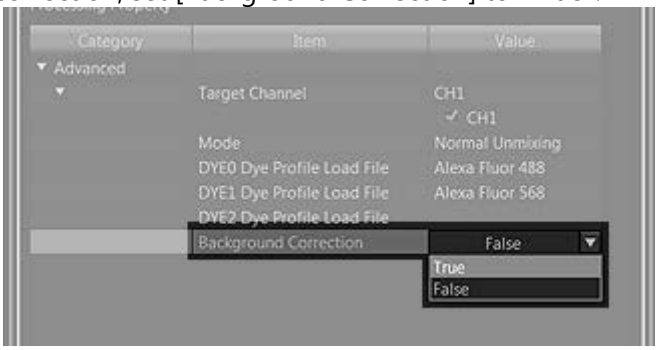
5 In [DYE0 Dye Profile Load File], select the first dyeing method among the double-dye.



6 In [DYE1 Dye Profile Load File], select the second dyeing method among the double-dye.

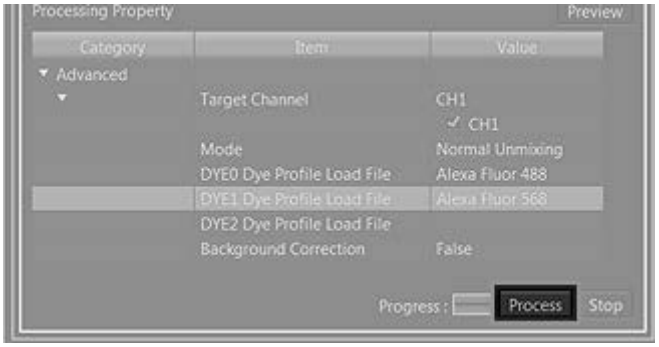


7 If you perform the background correction, set [Background Correction] to "True".



If the background correction is set to "True", the background spectrum is calculated from the target image automatically and the background is deleted.

8 If you select [Multiple process] in 3, press the [Process] button. Now, you are in the waiting status to start the scan. If you select [Single process] in 3, the fluorescent separation process starts in synchronization with the start of scan.



Starting acquisition of the Lambda series image

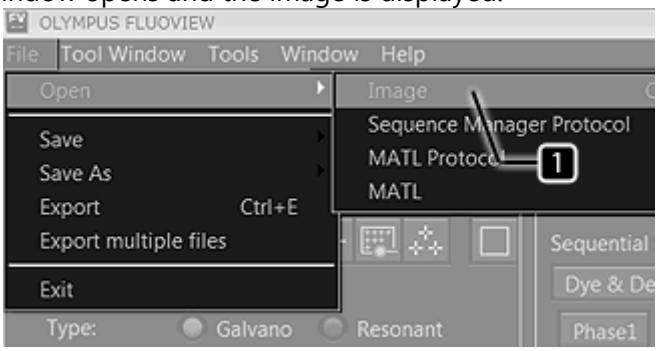
9 Press LSM Start in [Acquire] Tool Window to start acquisition.
When acquiring the XYL series image, the fluorescent separation process is performed after the scan is completed and the image after separation is displayed in [Image] window automatically.
When acquiring the XYLT series image, XYLZ series image or XYLZT series image, the fluorescent separation process is performed each time when one λ scan is completed and the image after separation is displayed in [Image] window automatically.

11.1.6. Performing Normal Unmixing for acquired images

This method performs the fluorescent separation process using the spectral data of the fluorochrome which is preset in the system.
This section describes the procedures to perform Normal Unmixing for the image acquired from the double-dyed specimen.

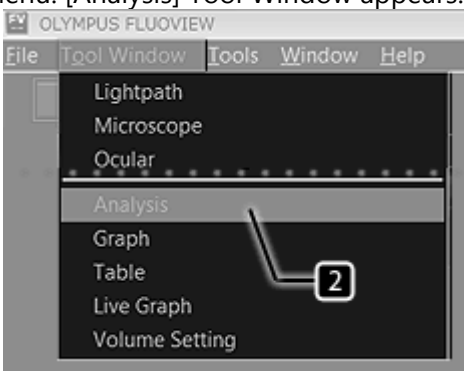
Opening the target image for image processing

1 Select [Open] - [Image] in [File] menu to display the dialog box and select the image in the dialog box. The [Image] window opens and the image is displayed.

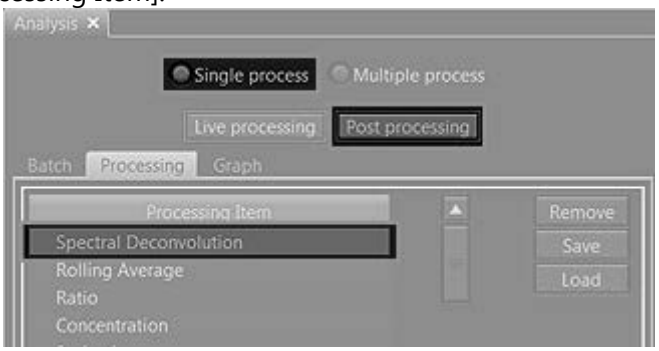


Performing Spectral Deconvolution

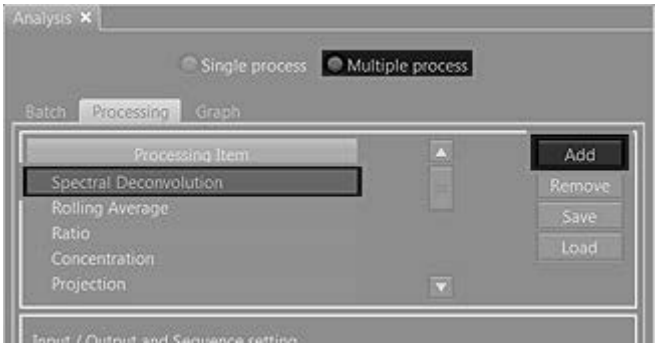
2 Select [Analysis] in [Tool Window] menu. [Analysis] Tool Window appears.



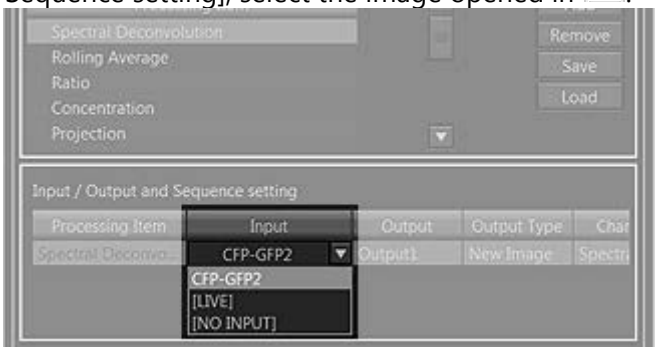
3 Select the [Single process] mode, press the [Post Processing] button and select [Spectral Deconvolution] in [Processing Item].



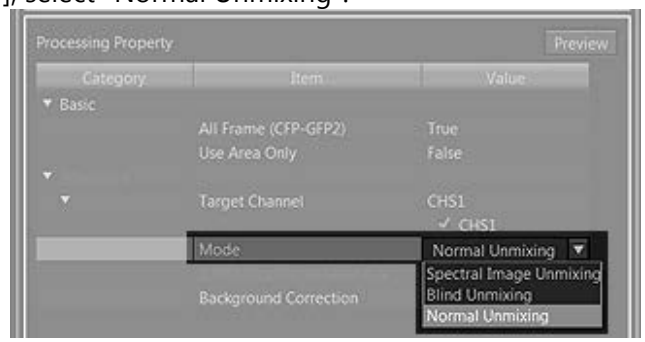
Or, if you perform Spectral Deconvolution as one of sequential processes, select [Multiple process], select [Spectral Deconvolution] in [Processing Item] and press the [Add] button.



4 In [Input] in [Input / Output and Sequence setting], select the image opened in 1.



5 In [Mode] in [Processing Property], select "Normal Unmixing".



6 In [DYE0 Dye Profile Load File], select the first dyeing method among the double-dye.

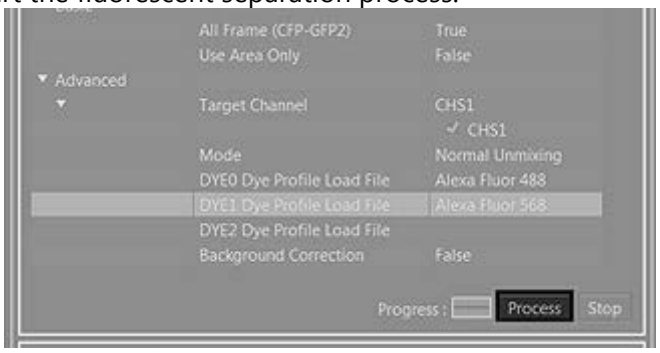
7 In [DYE1 Dye Profile Load File], select the second dyeing method among the double-dye.

8 If you perform the background correction, set [Background Correction] to "True".

📖 If the background correction is set to "True", the background spectrum is

calculated from the target image automatically and the background is deleted.

9 Press the [Process] button to start the fluorescent separation process.



12. Exiting the system

1. Select [Configuration] in the [Tools] menu on the software screen. The [Configuration] dialog box appears.
2. Select [Exit] in the [File] menu to exit the software.

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