

Fluoview

Scanning Laser Biological Microscope

Ver. 1.1

WARNING



Before using your microscope, the items in this manual identified by the mark shown to the left should be read carefully until completely understood to ensure safe operation.

Thank you for purchasing an Olympus Fluoview Microscope. Before using your microscope, read this manual thoroughly to make sure you obtain full performance from all of the functions provided by this system.

This manual is divided into the following five sections:

- For Safe Usage Introduction to Fluoview
- Operation
- Hardware
- Troubleshooting Q & A



August 1996

SAFETY GUIDE

-About This Manual

This manual explains potential risks and provide important safety information designed to ensure the safe use of the FLUOVIEW System. Thoroughly study this manual before operating the system.

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3 Precautions for Use of the System

1 Safety Precautions



Depending on the laser configuration, this laser product is classified as follows:

Ar laser configuration ····· CLASS II



Kr and Ar laser configuration ······ CLASS III a



Precautions for Use

Always pay careful attention to the following points.

- Avoid engaging mirror cubes into the light path in such a manner that the laser beam is directed outward. This is extremely dangerous as unprotected eyes may be exposed to the laser beam.
- If the cube turret of the reflected light fluorescence module, the DIC slider (optional) or the dummy slider is removed during laser output (during laser scanning), laser beam radiation will occur inside the microscope. This is extremely dangerous. Never remove the cube turret, the DIC slider, or the dummy slider.

The cube turret, the DIC slider (optional), or the dummy slider is to be removed only by trained personnel, and only when power has been removed from the laser system.

1. Safety Precautions

• If the objective revolving nosepiece or the objective lens is removed during laser output (during laser scanning), the laser beam will be emitted uncontrollably. This is extremely dangerous. Never remove the objective revolving nosepiece or the objective lens.

The nosepiece or the objective lens is to be removed only by trained personnel, and only when power has been removed from the laser system.

 If the transmitted light lamp housing or the lamp socket is removed during laser output (during laser scanning), the laser beam light will be emitted uncontrollably. This is extremely dangerous. Never remove the reflected light lamp housing or the lamp socket.

The transmitted light lamp housing or the lamp socket is to be removed only by trained personnel, and only when power has been removed from the laser system and from the microscope.

- If the scan unit's (2) detection mode selector slider is detached during laser output (during laser scanning), laser beam radiation will occur inside the microscope (beam reflected from the specimen). This is very dangerous. Never remove the scan unit's detection mode selector slider.
 The scan unit's (2) detection mode selector slider is to be detached only by trained personnel, and only when power has been removed from the laser system.
- Using tools to remove the protective housing, or otherwise dismantling/remodeling units, is
 extremely dangerous as this may lead to uncontrollable laser beam radiation inside the unit. Never
 attempt to dismantle/remodel the system.
- Never excessively bend, strain, or step on the optical fibers. Damaged optical fibers may result in laser beam leakage. This is extremely dangerous. Should the optical fibers be damaged, immediately turn OFF the laser power supply unit and contact your Olympus representative.
- Hot air is emitted from the laser cooling fan exhaust outlet. Keep flammable materials and materials adversely affected by heat at a safe distance of the outlet.
- The power consumption of both the power unit and the laser unit is very large. Ensure that each unit obtains power from separate electrical systems.
- To prevent shock hazards, always ground the equipment.
- Always unplug the power cord before replacing fuses.
- Since the mixture (ether (70%) and alcohol (30%), etc.) used to clean the optical components of the system is highly flammable, be careful to keep these chemicals away from open fire and potential sources of electrical sparks, such as when the main switches are moved to "|" (ON) or "O" (OFF).

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Please note that the laser beam is emitted when the display's function panel ([Acquire] panel) looks as shown below.



Disposal of Laser Tubes

Strict adherence to applicable industrial guidelines and laws is required when disposing of laser tubes. Before disposing of laser tubes, contact your Olympus representative for further information.

Safety Symbols

The following symbols are found on the equipment. Study the meaning of the symbols, and always use the equipment in the safest possible manner.

Symbol	Explanation
\triangle	Before use, carefully read the instruction manual. Improper handling could result in injury to the user and/or damage to the equipment.
	Indicates that the main switch is ON.
0	Indicates that the main switch is OFF.

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2 Warning Labels

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2 - 1 Warning Labels Concerning Laser Safety

2-1-1 Warning Labels

(1) Ar laser configuration





Attached position BX50 configuration (Similar in case of BXWI configuration)



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2-1-2 Aperture Label

(Indicates where the laser beam is emitted.)



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IX Configuration





The laser beam is emitted from the condenser lens.



When the transmitted light detector is not mounted (left) and when mounted (right)





When the transmitted detection unit is not mounted (left) and when mounted (right)



Common to all configurations

When the slider cover is removed



In addition to the locations shown in this manual, a protective housing label is also attached.

For replacement of soiled or peeling warning labels, please contact your Olympus representative.

3 Precautions for Use of the System

Installation

- The FLUOVIEW system will be assembled and setup by qualified technicians. Avoid moving the system as this may adversely affect the adjustment of the optical system. In case it becomes necessary to move the system, please consult your Olympus representative.
 Olympus shall not be liable for improper adjustments, damages, or other problems occurring as a result of the system being moved by the customer or other unauthorized personnel.
- Pulling the optical fiber in case of the KrAr laser configuration will change the laser output. Never modify by pulling the optical fiber.
- To prevent overheating, leave at least 30 cm of free space between the exhaust opening of the laser cooling fan and walls or other objects. Laser tube overheating will result in unstable laser oscillation or the laser oscillation may stop. Excessively high temperature inside the unit may also result in malfunction and/or damage.
- Openings for a cooling fan for ventilation are provided on the rear panel of the control unit, computer, and the magneto-optical disk unit. To prevent the risk of overheating and other damage, the openings should never be blocked. Leave at least 10 cm of free space between the openings and walls or other objects.

Avoid installation in places exposed to direct sunlight, high humidity, high temperatures, and dust. Operating environment: Ambient room temperature 10~35°C; relative humidity 30~80%.

 Avoid installation in places exposed to vibrations. Consult your Olympus representative if the location is subjected to vibrations.

Handling

- The FLUOVIEW system is a precision instrument. Handle it with care and avoid subjecting it to sudden or severe impact.
- Never excessively bend, strain, or subject the laser fiber to crushing force as this will result in reduced performance.
- The piezoelectric stage and the piezoelectric nosepiece (option) employ a piezoelectric element which is fragile. Never subject to impact and ensure that undue force is never applied to these units.

Precautions for Use

 When the built-in power supply for transmitted light is turned on for a long time, accumulated heat may cause metal to stretch and the focus to drift. To achieve highly precise image data, it is recommended to turn OFF the main switch on the microscope frame.

3. Precautions for Use of the System

- Standard objectives for biological use are employed for both the upright microscope system configuration (BX) and the inverted microscope system configuration (IX). Accordingly, use cover glass with a thickness of 0.17 mm or Petri dishes with a bottom thickness of 0.17 mm. It is recommended to use special cover glass for LSM water immersion objectives.
- When using the FLUOVIEW system, it is recommended to dim the light in the room to reduce the adverse effects of extraneous light.
- To ensure a stable laser beam output, allow at least 10 minutes for warm-up after the laser power supply is turned ON.
- After turning the reflected light power supply OFF, wait at least 10 minutes before turning ON again. Turning ON sooner will reduce the service life of the mercury burner.

About the Computer and Software

- Microsoft Windows are pre-installed on the computer for use with the FLUOVIEW system. Microsoft Windows are pre-installed on the computer for use with the FLUOVIEW system. The customer should make a back-up of the pre-installed program and store the back-up floppy disks carefully. (Olympus offers no support for back-up, missing back-up, etc.) For details on Microsoft Windows, refer to the User's Manual for Microsoft Windows.
- When the empty capacity of the hard disk becomes low, data processing will become very slow. Periodically erase unnecessary data files from the hard disk. For details on erasing data files, refer to the User's Manual for Microsoft Windows.
- When closing the software, always click the <Exit> button at the bottom of the [File I/O] panel. If you display Windows' [Task List] dialog box and click the <End Task> button to close the software, the file conditions, etc. of the last session will not be saved and subsequently not recalled next time the software is started.
- When using the Z-motor, bring the specimen into focus by operating the coarse (fine) adjustment knob on the microscope frame after clearing the check from the [Keep locked] check box on the [Z Stage] panel of the [Acquire] panel (refer to Section 4-2-3-1 in the Operation Manual). Operating the focus adjustment knobs on the microscope frame while the [Keep locked] check box remains checked may result in damage to the Z-motor.

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Introduction to Fluoview

Concerning This Section

This section provides an overview of the Fluoview System and should be read before the system is used. After reading and understanding this section, please read the Operation section.

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Cautions

- (1) This software and manual may not be reproduced in part or in their entirety without the express written permission of Olympus.
- (2) The contents of this manual are subject to change without notice.

Registered Trademarks

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All other company names and product names are the trademarks or the registered trademarks of the respective companies.

Care and Maintenance

- (1) When not in use, always use the accessory cover to protect your microscope from dust.
- (2) This microscope is a precision instrument, so do not disassemble any of the components.
- (3) Keep dirt, fingerprints off the lenses and filters. Remove any dirt by wiping lightly with soft gauze. Stubborn dirt can be removed by wetting the gauze with a mixture of alcohol and ether (3:7 ratio), or benzene.

Ether is highly combustible so keep away from open flame and use caution when turning the main switch on and off.

(4) Clean the various parts by wiping with a soft cloth moistened with diluted detergent. Do not use organic solvents because these can cause deterioration of the paint and plastic parts.

Moving the Fluoview

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Avoid moving this microscope since this can adversely affect adjustment of the optical system.

Please consult with your Olympus sales representative before moving this system.

Olympus is not responsible for any problems resulting from moving this system.

Section 1 Introduction

1-1 Fluoview Manual Configuration

There are two Fluoview manuals; the Users Manual and the On-screen Manual (on-line help). The Users Manual consists of five sections. The contents of these sections are described below.

1-1-1 Users Manual Configuration

• For Safe Usage

This section explains the requests, cautions and warnings related to usage of the Fluoview System.

• Introduction to Fluoview

This section provides an overview of the Fluoview System.

Operation

This section explains the method of operation, including the input of images and different types of image processing.

Hardware

This section provides detailed explanations of the Fluoview System hardware functions and specifications.

Troubleshooting Q & A

This section explains the various countermeasures that can be taken in case a problem should occur.

1-2 Conventions Used in This Manual

The following is an explanation of the various conventions used in this manual.

<u> </u>	Caution, remark ar	nd one-point advisory symbols.
	Symbol	Explanation
	*	Caution items are indicated by (\bigstar) .
	Ø	Remarks to be observed and one-point advisories are indicated by (\odot).

♦ Menu, command button and dialogue box conventions.

Usage	Explanation
[Config] panel	The names of panels, dialogue boxes, list boxes, check boxes, etc., are enclosed in square brackets.
<ok> button <open file=""> button</open></ok>	buttons are enclosed in triangular parentheses.

♦ Mouse Operation	
Convention	Explanation
Click	This means to press and immediately release the mouse button.
Double click	This means to rapidly press and release the mouse button twice.
Drag	This means to hold down the mouse button, move the pointer to the desired location and release the mouse button.

Note: Unless otherwise specified, the terms click, double click and drag are used in this manual in relation to the left mouse button.

♦ Key Operation	
Convention	Explanation
Enter	The names of keys are enclosed in rounded boxes .
Alt + F1	The plus (+) sign indicates combined key operations.
	For example, Alt + F1 means to hold down the Alt
	key and press the F1 key.
Direction keys	\rightarrow , \leftarrow , \uparrow and, \downarrow keys.

♦ Terms Unique to This System

Convention	Explanation
XY observation	This means using XY scan to observe.
(Other observation)	(The same is for XZ, Xt, XYt and XYZt observation.)

Section 2 System Overview

The Olympus Fluoview is a confocal scanning type laser fluorescent microscope that utilizes a common focal point optical system to realize high resolution and high contrast as well as a spectacular improvement in resolution in the optical axis.

This microscope provides researchers with the ability to perform automatic sectioning, three-dimensional structuring and time fluctuation observation as well as various types of image processing and analysis.

2-1 Principle of Operation

The scanning laser microscope focuses the laser beam into a tiny spot through the objective lens and scans specimens in the X and Y directions.

The scan unit detects fluorescent and transmitted light from specimens and outputs specimen images to the image monitor.

As shown in the diagram, the confocal optical system has a pinhole located at a position (confocal plane) which is optically conjugate with the in-focus position and which eliminates all light from outside the in-focus position. Consequently, these lights appears dark in images so thick systematized specimens can be sliced optically.

With ordinary optical microscopes, light from outside the in-focus position is superimposed over the image forming rays at the in-focus position, which results in images that are blurred.

The laser beam that passes through a specimen is detected by the transmitted light detector and a transmitted light image is formed, but this is not a confocal image.

However, extremely important data can be obtained by synthesizing the transmitted light image and the confocal fluorescent light image.



2-2 Fluoview Features

- 1. The detector has a resolution of 12 bits, so images are extremely clear.
- 2. 1 A high-resolution 1024 x 768 pixel system is used. Non-interlaced output signals are used for clear, flickerless images.
- 3. Transmitted light is detected by a photomultiplier detector, so transmitted light images are sharp and clear.
- 4. An auto-gain function eliminates any need for bothersome sensitivity adjustment.
- 5. Two image modes, 2-channel fluorescent images or fluorescent image + transmitted light image, can be selected with one touch.
- 6. The scan unit is corrected up to the infrared range to assure compatibility with a variety of lasers. Use is also possible with both erect image and inverted image microscopes.
- 7. The confocal aperture is a 5-position turret, so the correct confocal aperture for each objective lens can be selected with one touch.



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2-4 System Configuration

2-4-1 System Units and Their Roles



Photo 2-1 BX50 System



Laser unit Consists of a laser oscillator and fiber coupling (argon laser shown to the left).



Photo 2-2 BXWI System



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Compressor Supplies air to the vibration-free stand.



Photo 2-3 IX70 System



Transmitted light detector (option) This unit is used to obtain transmitted light images.



Compressor Supplies air to the vibration-free stand.

2-5 Software Function Configuration

This software uses panel type windows.

With conventional software, it is necessary to select a menu and then select the command to be executed. With panels, however, software functions can easily be executed by merely selecting the panel index for the function to be executed, like a system notebook or file folder.

2-5-1 Software Panel Configuration

The panel indexes for all of the functions cannot be displayed at one time but must be scrolled. The panel list is shown below for use as reference when scrolling.



2-5-2 Function Panels and Display Panels

The Fluoview software utilizes two types of panels: the function panel and the display panel.

The functions panel includes the [Acquire], [File I/O], [Tile], [Process], [Analyze], and [Visualize] panels.

The [Live] panel and [(File Name)] panel which are read in from a file are displayed in the display panel.



★ With this software, a panel is simply called the '0000 panel' instead of the '0000 panel of the function panel'.

For example, the [File I/O] panel of the function panel is simply called the [File I/O] panel.

2-5-3 Drag and Drop Function Execution Icon

This software uses the drag and drop method of selecting image files and observation methods (dye color name or transmitted light). With this intuitive method, selection requires only picking up [Item to Select (image file or observation method)] with the mouse and dragging it to the setting location, where it is dropped.







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2-6 System Setup

This system must be set up by a special technician. To maintain the performance and for safety, never disassemble or re-adjust this system.

2-6-1 Power Consumption

As shown below, some of the units consume considerable power; therefore, check the capacity of the power outlet before connecting this system.



2-6-2 Turning on the Power Supply

Turn on the power supply of each unit.

When only reading data, the laser power supply and illumination power supply are not necessary and need not be turned on.

To obtain a stable laser output, turn on the laser power supply and allow the system to warm up for 10 min. or more before using.

Once the illumination power supply (mercury vapor lamp power supply) has been turned off, do not turn it on again for at least 10 min. to avoid shortening the service life of the mercury vapor lamp.

- Turning on the laser power supply Argon laser :
 - (1) Turn on the power supply switch.
 - (2) Turn the key to the ON position.



Krypton/argon laser :

Please refer to the laser instruction manual.

- Please refer to the laser instruction manual.
 - (1) Turn on the power supply switch.



(1)

- Turning on the power controller power supply
 - Turn on the main switch.
 For details, refer to the Power Controller SSI-9501 instruction manual.
 - (2) Turning on the switches
 - If all of the switches are turned on, the power for the entire system can be turned on and off with the main switch as described in item (1).



The units are connected as shown below.



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- Turning on the control unit power supply
 - (1) Turn on the power supply switch.

This unit is connected to the power controller, so the power switch can be left turned on.



- Turning on the electromagnetic disk power supply
 (Please read the electromagnetic disk instruction manual.)
 This unit is connected to the power controller, so the power switch can be left turned on.
- Turning on the microscope power supply (Please read the instruction manual of the microscope used.) This unit is connected to the power controller, so the power switch can be left turned on.

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2-7 System Operational Procedure Overview

A flow chart is used here to explain the operational procedures so that operation of the entire system can be more easily understood. For reference, the relevant manual sections ($\begin{bmatrix} 1 \\ 1 \end{bmatrix}$ and numbers (()) are indicated at the right side of the flow chart for each procedure. Please refer to 2-7-1 Fluorescent Light Observation Procedure and 2-7-2 Transmitted Light Observation Procedure.


2-7-1 Fluorescent Light Observation Procedure





2-8 Identifying Images From Different Methods of Observation

Fluoview displays various image icons which can be used to identify the method of observation used

to read in images. (See the table to the right.)

When the [File I/O], [Tile], [Process], [Analyze] and [Visualize] panels are selected, the icon of the image of the selected [Display] panel is displayed inside the box at the top of the respective panel. Also, when the icon area of the [Files] list box of the [File I/O] panel or image file is dragged, the icon of the image is used in various aspects. This serves in the identification of the method of observation.

Image icon	Icon Meaning
	XZ observation
	XZ observation, 2-channel mode
$\overline{\bigcirc}$	XT observation
Ø	XT observation, 2-channel mode
	XZT observation
	XZT observation, 2-channel mode
	XY observation
	XY observation, 2-channel mode
	XYt observation
	XYt observation, 2-channel mode
	XYZ observation
	XYZ observation, 2-channel mode
	XYZt observation
	XYZt observation, 2-channel mode
	Animated image
	Binocular erect image/erect image viewed through colored glasses

HARDWARE MANUAL

This instruction manual describes functions, specifications, assembly (connections) and adjustment of the hardware. Before reading this instruction manual, please thoroughly review the preceding manual "INTRODUCTION TO FLUOVIEW" for an outline of the system.

LASER SAFETY PRECAUTION

Units indicated as "OPERATOR SERVICE" should be removed only by a person who has had laser safety training, and only after turning off the laser unit. This should not be done by any untrained persons.

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Removing any of the following units is considered to be OPERATOR SERVICE :

- Cube turret
- Objective revolving nosepiece
- Objective lens
- DIC slider or dummy slider
- Transmitted light lamphousing and / or lamp socket
- Detection mode selector slider (Scan unit)

See 2-1, 2-7, 2-10.

1 STANDARD CONFIGURATIONS

1-1 BX Upright Microscope System Configuration

Description	EC Code	BX50-Ar Set	BX50-KrAr Set
Scan Unit	FVX-SU	1	1
Excitation Cube	FVX-DM488	1	
Filter	FVX-BA565IF	1	
Dual Wavelength Excitation Cube	FVX-DM488/568		1
Barrier Filter	FVX-BA550RIF		1
Barrier Filter	FVX-BA585IF		1
Kr and Ar Laser Line Filter	FVX-LLF-KR		1
Polarizing Filter for Upright Microscope	FVX-PO-U	1	1
Control Unit	FVX-CU	1	1
Pupil Lens	FVX-PL-IBX50	<u> </u>	1
Laser Tube	FVX-LT	1	1
Extension Unit	FVX-EXTU	1	1
Stand for BX	FVX-ST	1	11
Rubber Feet Anti-Vibration Table for BX	FVX-UVT-BX	1	··· 1
Ar Laser Unit 2	FVX-LU-AR2	1	
Ar Power Supply Unit 100	FVX-PS-AR100	1	
Laser Combiner	FVX-COM-KRAR		1
Z-Motor Unit	FVX-ZM	1	1
Desk for Microscope Frame	FVX-DK8070	1	1
Computer Desk	FVX-DK6570	1	1
Microscope	BX50-FLA-FVX	1	1
Computer	PC-AT-P95	1	1
Monitor 17"	MONITOR17	1	11
Power Controller	SSI-9501	1	1
SCSI Kit	SCSI-1510-JPN	11	1
Polaroid Recorder	FVX-POLA	1	1

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5 SPECIFICATIONS

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1-2 IX Inverted Microscope System Configuration

Description	EC Code	IX70-Ar Set	IX70-KrAr Set
Scan Unit	FVX-SU	1	1
Excitation Cube	FVX-DM488	1	
Filter	FVX-BA565IF	1	
Dual Wavelength Excitation Cube	FVX-DM488/568		1
Barrier Filter	FVX-BA550RIF		1
Barrier Filter	FVX-BA585IF		1
Kr and Ar Laser Line Filter	FVX-LLF-KR		1
Polarizing Filter for Inverted Microscope	FVX-PO-U	1	1
Control Unit	FVX-CU	1	1
Pupil Lens	FVX-PL-IBX50	1	1
Air Anti-Vibration Table for IX	FVX-ST	· 1	1
Compressor	FVX-UVT-BX	1	1
Ar Laser Unit 2	FVX-LU-AR2	1	
Ar Power Supply Unit 100	FVX-PS-AR100	1	
Laser Combiner	FVX-COM-KRAR		1
Z-Motor Unit	FVX-ZM	11	1
Computer Desk	FVX-DK6570	1	1
Microscope	IX70-FLA-FVX	1	1
Computer	PC-AT-P95	1	1
Monitor 17"	MONITOR17	1	1
Power Controller	SSI-9501	1	1
SCSI Kit	SCSI-1510-JPN	1	1
Polaroid Recorder	FVX-POLA	1	1

1-3 Optional Accessories

Green HeNe Laser Unit	FVX-LU-HEG	Used in combination with the Ar laser. Suitable for TRITC and PI observation. 543 nm excitation wavelength. Can not be combined with the Kr laser.
Dual Wavelength Excitation - Cube	FVX-DM488/543	Dual wavelength excitation cube for Ar laser and HeNe green laser.
Barrier Filter	FVX-BA530RIF	Short-pass filter for blocking the 543 nm wavelength beam emitted by the HeNe green laser. Used for 488/543 nm dual wavelength excitation.
Transmitted Light Detector for BX	FVX-TD-BX	Transmitted Light Detector for BX50/BXWI for providing transmitted light to the scan unit via an optical fiber connection.
Transmitted Light Detector for IX	FVX-TD-IX	Transmitted Light Detector for IX for providing transmitted light to the scan unit via an optical fiber connection.
UCD Lens Unit	LSM-THI-UCD	Lens unit for use with the UCD condenser, mounted on the FVX-TD-IX.
LWUCD Lens Unit	LSM-THI-WUCD	Lens unit for use with the LWUCD condenser, mounted on the FVX-TD-IX.
Piezoelectric Z-Stage	FVX-PZT-BX1	When used with external sensor for full-cross control, this stage offers Z-movement with extremely high position reproducibility. Minimum step of 0.1 μ m; stroke length 100 μ m.
IX Frame (With Piezo)	IX70-FVXPZ-F	When used with external sensor for full-cross control, this IX frame offers Z-movement with extremely high position reproducibility. Minimum step of 0.1 μm; stroke length 100 μm.
Anti-Vibration Platform for IX	FVX-UVT-TA	Simple anti-vibration Platform for IX.
Barrier Filter	FVX-BA590	To minimize the fluorescence cross-over when observing double-stained specimens, such as FITC+TRITC, using the Ar laser (488 nm).
Barrier Filter	FVX-610IF	
Barrier Filter	FVX-BA510-540	Band pass filter for use with FITC.
Optional Barrier Filter Slider	FVX-BA-OPT	Optional barrier filter slider for customized filter placement.
		Compatible with a filter diameter of $13_{-0.3}$ mm and a filter thickness of 2-3 mm.
Optional Emission dichromatic mirror Cube	FVX-SDM-OPT	Optional Emission DM cube for mounting a custom dichromatic mirror to separate the fluorescence emission for detection by the Channel 1 and Channel 2 PMT. Compatible with 18mm × 11 mm, 1 mm thickness dichroic mirrors.

2 MAIN UNITS AND DESCRIPTION OF CONTROLS

Also refer to Section 2-4, SYSTEM LAYOUT in the "INTRODUCTION TO FLUOVIEW" manual.

2-1 Scan Unit



(1) Pinhole turret



The pinhole turret contains 5 pinholes, Allowing selection of an optimum pinhole for all objectives. Select the pinhole number displayed on the control panel ([Acquire] panel).

If the acquired image is sufficiently bright and noise is not aparent even when the photomultiplier HV is raised, an even better image may be obtained by operating the pinhole turret and selecting a smaller pinhole.

The pinhole sizes are as follows:

- 1: 60 µm 2: 100µm
- 3: 150µm
- 4: 200µm

Select either 1, 2 or 3 in accordance with the pinhole size indicated for a given objective (see below).

Use to obtain a brighter image, Confocality is compromised.

5: 300µm

2–1

2 MAIN UNITS AND DESCRIPTION OF CONTROLS

In general, selecting a smaller pinhole than the pinhole size recommended for a given objective will not increase the axial resolution. However, in some cases the lateral resolution may increase. The effect differs with the refractive index of the specimen and the dispersion of the light.

The following table shows the recommended pinhole number for a given objective.

	Objective	NA	Pinhole No.
	PLAPO 40X	0.95	2
A	PLAPO 60XO	1.40	2
	PLAPO 100XO	1.40	3
	PLAPO 40XWLSM	0.90	2
	PLAPO 60XWLSM	1.00	3
	PLAPO 60XOLSM	1.10	3
	UPLAPO 10X	0.40	1
	UPLAPO 20X	0.70	1
X	UPLAPO 20XO	0.80	1
	UPLAPO 40X	0.85	2
	UPLAPO 40XO	1.00	2
. •	UPLAPO 60X	0.90	3
	UPLAPO 60XWPSF	1.20	2
	UPLAPO 100XO	1.35	3
	UPLFL10X	0.30	1
	UPLFL20X	0.50	2
	UPLFL40X	0.75	3
A	UPLFL60XO	1.25	2
	UPLFL100XO	1.30	3
	UMPLFL10XW	0.30	1
	UMPLFL20XW	0.50	2
	LUMPLFL40XW	0.80	2
	LUMPLFL60XW	0.90	3

2–2

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(2) Detection mode selector slider

Slider for selecting either two fluorescence signals or one fluorescence signal + transmitted light. 3 settings. This slider is placed at the A-section shown in Fig. (I).

Slider at the pushed-in position

Select this mode when observing a transmitted light and / or a fluorescence with emission wavelength shorter than 570nm, such as FITC, GFP, DiO, etc. As shown in Fig. (I) on the following page, a double-sided mirror is engaged into the light path.

Consequently, fluorescence light is reflected to the CH1 (PM1). When the transmitted light detector (option) is attached, the transmitted light is reflected via the optical filter connection to the CH2 (PM2).

Slider at the middle position

Select this mode when observing a double-stained specimen, such as FITC+PI, etc. As shown in Fig. (II) on the following page, a beam splitting dichroic mirror is engaged into the light path. The characteristics of this dichroic mirror make it reflect light with wavelengths below 570 nm and pass the longer wavelengths. Consequently, the fluorescence light can be split into light for the 1CH (PM1) and the 2CH (PM2). With the slider at this position, the transmitted light from the optical fiber connection is blocked.

Slider at the pulled-out position

Select this mode when observing a transmitted light image and / or fluorescence specimen with emission wavelengths longer than 570 nm, such as PI, TRITC, etc. As shown in Fig. (III) on the following page, nothing is engaged into the light path. Consequently, fluorescence light is directed to the CH2 (PM2). When the transmitted light detector (option) is attached, the transmitted light is directed via the optical fiber connection to the CH1 (PM1).

2--3





(3) Barrier filter slider

Up to two filters can be placed for both CH1 and CH2. At the pushed-in position, the filter is engaged into the light path. At the pulled-out position, it is disengaged.

- * If erroneously placed at the middle position, no image will appear. Always stop at the correct position.
- (4) ND filter turret

There are five positions.

Transmission ratio: 0, 6, 20, 50, and 100%.

(5) Filter slider cover screws

Operated when the barrier filter slider or the detection mode selector slider is replaced.

When this screw is loosened, the covers can be pulled off. Then replace the sliders, return the cover to its original position, and tighten the screw again.

Detection mode selector slider should be removed only, by a person who has had laser safety training, and only after turning off the laser unit.

(6) KrAr laser line filter turret

(Only attachable with the Kr and Ar laser combination)

Unit with built-in 5-filter turret for selection in accordance with the 488 nm and 568 nm excitation wavelength of the Kr and Ar laser.

- (1) 488: The specimen is excited at 488 nm. Used for observation of single-stained specimens, such as FITC.
- (2) 568: The specimen is excited at 568 nm. Used for observation of single-stained specimens, such as TRITC, PI.
- (3) 568: The specimen is excited at 488 nm and 568 nm. However, the 488 nm output + only is attenuated to 6% of the normal excitation light. Used for preventing the PI
 - AT6 from eclipsing the FITC when observing double-stained specimens such as FITC+PI.
- (4) 568: The specimen is excited at 488 nm and 568 nm. However, the 488 nm output
 only is attenuated to 25% of the normal excitation light. Used for preventing the PI
 - AT25 from eclipsing the FITC when observing double-stained specimens such as FITC+PI.
- (5) 568: The specimen is excited at 488 nm and 568 nm. Used for observation of double stained specimens, such as FITC+PI.

2–5

FITC emission tails longer than 570 nm. Accordingly, FITC fluorescence may be detected on the CH2 where it overlaps the PI fluorescence. (See the figure below.) The problem may be remedied by cutting the FITC by adjusting the OFFSET on the operation panel ([Acquire] panel). If not, it will be necessary to balance the excitation light by reducing the intensity of the 488 nm excitation light used for exciting the FITC. When observing a double-labeled specimen, such as FITC+PI, attempt balancing the intensities of the emissions by engaging the (3), (4), (5) filters in the described order.



2-2 Microscope Frame

The illustration below shows the main controls of the microscope frame. The stage, revolving nosepiece, etc., may differ from those illustrated.

For details on operation of the microscope frame, refer to the instruction manual for the microscope frame.

BX50 Configuration



...

- (1) Light path selector
 - When the knob is pushed-in, visual observation is possible.
 - When the knob is pulled-out, laser microscopy is possible.

(2) Cube turret

- Engage the designated cube for visual fluorescence observation.
- When used as a laser microscope and for visual transmitted light observation, operate the turret to place the index at the [] position.
 (Set the cube turret so that no cube is engaged.)

(3) Analyzer U-AN

(Optional)

- Engage the analyzer into the light path for visual transmitted Nomarski observation and transmitted polarized light observation. The analyzer is engaged at the pushed-in position.
- Always remove the analyzer from the light path when used as a laser microscope. The analyzer is disengaged at the pulled-out position. If the analyzer is engaged, a good image cannot be achieved.
- (4) DIC prism U-DICT

(Optional)

Engaged into the light path for laser Nomarski observation and visual transmitted
 Nomarski observation.

An improved image is achieved if the U-DICT is removed from the light path in case of laser fluorescence observation. For fluorescence observation alone, disengage the U-DICT from the light path.

[Leaving the U-DICT engaged during laser fluorescence observation will degrade the image quality somewhat.]

- (1) Filter
 - Always disengage filters from the light path for laser transmitted observation. A good image will not be obtainable if a filter is left engaged.

(2) Universal condenser

For Nomarski observation, engage the Nomarski prism (optional) suitable for the objective in use. (Similar for both visual and laser Nomarski observation.)

Note that the polarizer should also be engaged in the case of laser Nomarski observation.

(Note that the analyzer U-AN should be removed from the light path in the case of laser

Nomarski observation.)

[Note that laser phase contrast observation is not possible due to construction limitations.]



- (1) Light path selector
 - When the knob is set at the mostion, visual observation is possible.
 - Set the knob at the SP position for laser microscope use.

(2) Cube turret

- Engage the designated cube for visual fluorescence observation.
- When used as a laser microscope and for visual transmitted light observation, operate the turret to place the index at the [O] position.
 (Set the cube turret so that no cube is engaged.)
- (3) Analyzer IX-AN

(Optional)

- Engage the analyzer into the light path for visual transmitted Nomarski observation and transmitted polarized light observation. The analyzer is engaged at the pushed-in position.
- Always remove the analyzer from the light path when used as a laser microscope. The analyzer is disengaged at the pulled-out position. If the analyzer is engaged, a good image cannot be achieved.
- (4) DIC prism U-DICT

(Optional)

- Engaged into the light path for laser Nomarski observation and visual transmitted Nomarski observation.
- A better image is achieved if the U-DICT is removed from the light path in case of laser fluorescence observation. For fluorescence observation alone, disengage the U-DICT from the light path.

[Leaving the U-DICT engaged during laser fluorescence observation will degrade the image quality somewhat.]

- (1) Filter
 - Always disengage filters from the light path for laser transmitted observation. A good image will not be obtainable if a filter is left engaged.

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(2) Condenser

For Nomarski observation, engage the Nomarski prism (optional) corresponding to the objective in use. (This applies to both visual and laser Nomarski observation.) Note that the polarizer should also be engaged for Nomarski observation. (Note that the analyzer IX-AN should be disengaged for laser Nomarski observation.)

(3) Magnification selector knob

For LSM use, always select 1X (knob pushed-in).

The 1.5X setting cannot be used.

[Note that laser phase contrast observation is not possible due to construction limitations.]

2-3 Transmitted Light Detector (Optional)

- FVX-TD-BX (for BX50, BXWI)
 - (1) Light path selector
 - When the knob is pushed-in, laser transmitted light observation is possible.
 - When the knob is pulled-out, visual transmitted light observation is possible.



- (1) Light path selector
- FVX-TD-IX (for IX)
 - When the knob is pushed-in, laser transmitted light observation is possible.
 - When the knob is pulled-out, visual transmitted light observation is possible.

(1) Light path selector



3 PREPARATIONS FOR OBSERVATION

This section explains the order of procedures for specimen observation.

3-1 Fluorescence Observation

3-1-1 Bringing the Specimen into Focus

3-1-1-1 BX50, BXWI Configuration



- Push in the light path selector knob (1) on the trinocular observation tube to select the 100% at binocular eyepieces setting.
- 2. Operate the cube turret to engage the cube corresponding to the specimen fluorochrome.
- While looking through the eyepieces, bring the specimen into focus. Make sure to adjust the eyepiece diopter correctly. (Refer to the BX instruction manual.)
- * When using the Z-motor, clear the check from the [Engage motor] check box (see Section 4-2-2-1 of the OPERATION MANUAL) on the [Z Stage] panel on the [Acquire] panel. Then operate the microscope frame's focusing adjustment knob (fine) to bring the specimen into focus. Damage to the Z-motor may occur if the microscope frame's focusing adjustment knob is operated while the [Engage motor] check box is checked.
- * The specimen may float during oil immersion observation.

While referring to the figure on the left, attach the stage clips provided with the microscope frame.



3-1-1-2 IX Configuration



- Turn the light path selector dial (1) on the right side of the microscope to the position. While looking through the eyepieces, bring the specimen into focus.
 Make sure to adjust the diopter adjustment ring of the eyepiece. (Refer to the IX50/70 instruction manual.)
- 2. Operate the cube turret to engage the cube corresponding to the specimen fluorochrome.
- While looking through the eyepieces, bring the specimen into focus. Make sure to adjust the eyepiece diopter correctly. (Refer to the IX instruction manual.)
- * When using the Z-motor, clear the check from the [Engage motor] check box (see Section 4-2-2-1 of the OPERATION MANUAL) on the [Z Stage] panel on the [Acquire] panel. Then operate the microscope frame's focusing adjustment knob (fine) to bring the specimen into focus. Damage to the Z-motor may occur if the microscope frame's focusing adjustment knob is operated while the [Engage motor] check box is checked.



* The specimen may float during oil immersion observation.

While referring to the figure on the left, attach the stage clips provided with the microscope frame.

3-1-2 Selecting the LSM Light Path

3-1-2-1 BX50, BXWI Configuration

- 1. Pull out the light path selector (1) on the trinocular observation tube to the stop position.
- 2. Operate the cube turret (2) on the vertical illuminator to align the index with the [O] position.
- If the analyzer U-AN (3) is mounted, disengage it by pulling it out to the pulled-out clickstop. (Leaving the U-DICT engaged during laser fluorescence observation will degrade the image quality somewhat.)



3-1-2-2 IX Configuration

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- 1. Turn the light path selector (1) to the [SP] position.
- 2. Set the magnification selector knob (7) to 1x.
- 3. Rotate the cube turret to select [
- If the analyzer IX-AN (3) is mounted, disengage it by pulling it out to the far clickstop. (Leaving the U-DICT engaged during laser fluorescence observation will degrade the image quality somewhat.)



3-1-3 Selecting the Pinhole

Operate the pinhole turret (1) to select the pinhole corresponding to the pinhole number indicated for each objective on the control panel. (Refer to Section 2-1, Scan Unit.)

3-1-4 Selecting the Detection Mode

Set the detection mode selector slider to the designated position in accordance with the fluochrome of the specimen to be observed. If in doubt about the setting, refer to Section 4-1-2, Adjusting the Scan Unit, in the Operation Manual and follow the prompts of the [Microscope Configuration] window.

Example for reference

Fluochrome	Detection Channel	Detection Mode Selector Slider Position
FITC Lucifer Yellow etc.	CH1	Pushed in
FITC + TRITC FITC + PI etc.	CH1/CH2	Middle position
PI TRITC	CH2	* Pulled out

(Refer to Section 2-1, Scan Unit.)

* In case of single-stained specimens, a brighter image can be observed if the slider is set at the pushed-in or out positions. Avoid the middle position.

3-1-5 Engaging the Barrier Filter

Depending on the fluochrome of the specimen to be observed, engage barrier filters as required.

The barrier filter slider is engaged into the light path at the pushed-in position, and disengaged at the pulled-out position.

* No image will appear if the slider is stopped between the positions. Always set at correct position.

If in doubt about the setting, refer to Section 4-1-2, Adjusting the Scan Unit, in the Operation Manual and follow the prompts of the [Microscope Configuration] window.

Example for reference

Fluorochrome	CH1		CI	H2
FITC	BA510IF	-	_	_
FITC + PI	BA510IF	(BA500-540)	BA565IF	(BA590)
TRITC	_	-	BA565IF	_

3--5



3-1-6 Selecting the ND Filter

Operate the ND filter turret (4) to select the appropriate ND filter. Select the ND filter in accordance with the specimen brightness and level of photobleaching. The ND20% filter may be appropriate to start with.

3-1-7 Selecting the Laser Line Filter (Kr and Ar Laser Combination)

Operate the laser filter turret (7) to select the laser line filter.

Examples for reference					
Fluochrome	Laser line filter				
FITC Lucifer Yellow etc.			488		
FITC + TRITC FITC + PI etc.	568 + 488	or	568 + 488 AT25	or	568 + 488 AT6
PI TRITC etc.			568		

(Refer to "(6) Laser line filter turret" in Section 2-1, Scan Unit.)

3-2 Transmitted Observation

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The procedures for transmitted observation are similar to those for fluorescence observation. Also refer to Section 3-1, Fluorescence observation.

3-2-1 Selecting the Transmitted Light Detector Light Path (Visual Setting)

3-2-1-1 BX50, BXWI Configuration

Pull out the light path selector (1).



(1) Light path selector

3-2-1-2 IX Configuration

Pull out the light path selector (1).



(1) Light path selector

3-2-2 Bringing the Specimen into Focus

(Refer to Section 3-1-1, Bringing the Specimen into Focus.)

When using Nomarski DIC attachment, refer to the instruction manuals for the respective microscope frames.

3 PREPARATIONS FOR OBSERVATION

3-2-3 Selecting the LSM Light Path

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3-2-3-1 BX50, BXWI Configuration

- 1. Pull out the light path selector (1) on the trinocular observation tube to the pulled-out clickstop.
- 2. Operate the cube turret (2) on the vertical illuminator to align the index with the
- 3. If the analyzer U-AN (3) is mounted, disengage it by pulling it out to the pulled-out clickstop. (It makes no difference if the DIC prism U-DICT (4) remains engaged.)
- 4. Disengage all the filters (5) from the light path.
- 5. Select the LSM light path on the transmitted light detector. (Push in the light path selector (1) on the transmitted light detector.)



3-2-3-2 IX Configuration

- 1. Turn the light path selector (1) to select the [SP] setting.
- 2. Set the magnification selector knob (7) to IX.
- 3. Operate the cube turret to select the [O] position.
- 4. If the analyzer U-AN (3) is mounted, disengage it by pulling it out to the far clickstop. (It makes no difference if the DIC prism U-DICT (4) remains engaged into the light path.)
- 5. Remove all the filters from the light path.
- 6. Select the LSM light path for the transmitted light detector. (The Light path selector of the transmitted light detector should be set at the pushed-in position.)



3-2-4 Selecting the Detection Mode

When the detection mode selector slider (2) is:

- Pushed in, transmitted light is detected by CH2.
- Pulled out, transmitted light is detected by CH1.

Consequently, when only transmitted observation is performed, the detection mode selector slider (2) may be set at either position.

When performing fluorescence observation simultaneously with transmitted observation, set the detection mode selector slider at the position specified for the specimen fluochrome. If in doubt about the setting, refer to Section 4-1-2, Adjusting the Scan Unit, in the Operation Manual and follow the prompts of the [Microscope Configuration] window.

(Refer to Section 2-1, Scan Unit in this manual.)

Examples for reference

Observation modes	CH1	CH2	Detection mode selector slider
FITC + TR(Transmitted)	FITC	TR(Transmitted)	Pushed in
TRITC + TR(Transmitted)	TR(Transmitted)	TRITC	Pulled out

The middle position of the detection mode selector slider is used for observation of double-stained specimens like FITC+PI.



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3-2-5 Disengaging the Barrier Filter

Remove all the barrier filters from the channel to detect transmitted light. (The barrier filter slider (3) should be at the pulled-out position.)

If in doubt about the setting, refer to Section 4-1-2, Adjusting the Scan Unit, in the Operation Manual and follow the prompts of the [Microscope Configuration] window.

3-2-6 Selecting the ND Filter

Operate the ND filter turret (4) to engage a suitable ND filter.

Select the ND filter in accordance with the specimen brightness and level of photobleaching . The ND20% filter may be appropriate to start with.

4 SPECTRAL CHARACTERISTICS OF FILTERS

- BA510IF BA565IF BA565IF BA550RIF BA550RIF BA550RIF BA510-540 BA510
- · Representative filter combinations for Ar laser use



- EDM570 is a dichroic mirror for splitting fluorescence light into CH1/CH2.
- DM488 is dichroic mirror for excitation.

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Barrier filters

- DM488/568 BA510IF BA550RIF 100 ٠ EDM570 50 · BA610IF BA585IF 600 650 700 450 500 550 Excitation Excitation wavelength wavelength 488nm 568nm
- · Representative filter combination for the Kr and Ar laser combination
 - EDM570 is a dichroic mirror for splitting fluorescence light into CH1/CH2.
 - DM488/568 is dichroic mirror for double excitation for use with the KrAr laser.

5 SPECIFICATIONS

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	Unit	Specifications
Laser unit	Ar laser -	5 mW output, random or linear polarization. Air cooled Argon ion laser (488 nm) Scan unit connection: Single mode optical fiber (3 m) Power requirements: 100V 10A (MAX)
	Kr laser	15 mW output, linear polarization. Air cooled Kr ion laser (568 nm) Scan unit connection: Single mode optical fiber (3 m) Power requirements: 200V single phase, 20A or more
Scan unit	Scan mode	Polarized light produced by dual scanning galvanometer mirrors.
	Laser input	Via fiber connection
	Shutter	Blocks the laser when not scanning
	ND filter	5-aperture switchable turret type: 100, 50, 20, 6, 0% transmission
	Scan area	16X12 rectangular area inscribed in the F.N. 20 boundary circle. ZOOM: 1 to 10.
	Scan center position	ZOOM 1.5 to 10: The center position may be moved within the X-Y direction within the ZOOM 1 scanning area.
	Scan mode	XY, XZ, XT, XYZ, XYT, XYZT, volume projection
	Detection mode setting	3-step switchable (CH1/CH2: FL/TR, FL/FL, TR/FL)
	Barrier filter	2 filters per channel. Sharp-cut filter (filters for laser use) Band pass filter (user-replaceable)
	Confocal pinhole	5-pinhole turret (60, 100, 150, 200, 300 μm)
	Light detector	Photomultiplier
	Applicable wavelengths	350 - 650 nm
	Dimensions (mm)	390(W) x 280(D) x 197(H)
	Weight	11kg
Control unit	Image input	2-channel input OFFSET: 0-100% GAIN: 1-10X A/D conversion. 4096 gradations (12 bit)
	Real-time processing	Kalman filter processing. Peak detection integration.
	Image memory Input memory	1024 pixel X 768 pixel X 4096 gradations
	Image display	LUT alteration, tiling, zooming, 3D display.
	Image processing	Filter processing, image processing.
	Image analysis	Line profile, intensity map, histogram, length, area measurement
	Image output	Gradations: R, G, B, 256 gradations each Output signal: R, G, B Sync non-interlace.
	Z-movement	Standard: Z-motor (resolution 0.1 μm) Optional: Piezoelectric stage (for BX), piezoelectric nosepiece (for IX). Stroke: 100 μm, resolution: 0.1 μm
	Dimensions (mm)	124 (W) x 342 (D) x 382 (H)
	Weight	9kg
	Power requirement	100 / 120 / 220 / 240V AC 1.6 / 1.6 / 0.8 / 0.8A 50/60Hz

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5 SPECIFICATIONS

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	AUnit 🧐 👘 🕬	Specifications
Laser unit	Ar laser	5 mW output, random or linear polarization. Air cooled Argon ion laser (488 nm) Scan unit connection: Single mode optical fiber (3 m) Power requirements: 100V 10A (MAX)
	Kr laser	15 mW output, linear polarization. Air cooled Kr ion laser (568 nm) Scan unit connection: Single mode optical fiber (3 m) Power requirements: 200V single phase, 20A or more
Scan unit	Scan mode	Polarized light produced by dual scanning galvanometer mirrors.
	Laser input	Via fiber connection
	Shutter	Blocks the laser when not scanning
	ND filter	5-aperture switchable turret type: 100, 50, 20, 6, 0% transmission
	Scan area	16X12 rectangular area inscribed in the F.N. 20 boundary circle. ZOOM: 1 to 10.
	Scan center position	ZOOM 1.5 to 10: The center position may be moved within the X-Y direction within the ZOOM 1 scanning area.
	Scan mode	XY, XZ, XT, XYZ, XYT, XYZT, volume projection
	Detection mode setting	3-step switchable (CH1/CH2: FL/TR, FL/FL, TR/FL)
	Barrier filter	2 filters per channel. Sharp-cut filter (filters for laser use) Band pass filter (user-replaceable).
	Confocal pinhole	5-pinhole turret (60, 100, 150, 200, 300 μm)
	Light detector	Photomultiplier
	Applicable wavelengths	350 - 650 nm
Control unit	Image input	2-channel input OFFSET: 0-100% GAIN: 1-10X A/D conversion. 4096 gradations (12 bit)
	Real-time processing	Kalman filter processing. Peak detection integration.
	Image memory Input memory	1024 pixel X 768 pixel X 4096 gradations
	Image display	LUT alteration, tiling, zooming, 3D display.
	Image processing	Filter processing, image processing.
	Image analysis	Line profile, intensity map, histogram, length, area measurement
	Image output	Gradations: R, G, B, 256 gradations each Output signal: R, G, B Sync non-interlace.
	Z-movement	Standard: Z-motor (resolution 0.1 μm) Optional: Piezoelectric stage (for BX), piezoelectric nosepiece (for IX). Stroke: 100 μm, resolution: 0.1 μm
	Dimensions (mm)	124 (W) x 342 (D) x 382 (H)
	Power requirement	100V 3A

. U	nit .	Specifications				
Microscope frame						
[Upright]	Microscope frame	BX50, BXWI				
BX50-FLA-FVX	Trinocular observation tube light path selection	2 settings (100% for binocular eyepieces; 100% for LSM)				
BXWI-FLA-FVX	Objective	UIS series				
[Inclined]	Microscope frame	IX70-FVX-F, IX70-FVXPZ-F				
IX70-FLA-FVX	Objective	UIS series				
Stand for BX		Scan unit, for support Specially designed for BX microscopes				
Computer system	Computer Monitor Magneto-optical disk	IBM PC-AT compatible, Windows 3.11 (English) 17" 230 MB				
Power controller	No. of outlets Capacity	6 / JP (not usable for the laser power supply unit, reflected light module power supply) 100V 15A (MAX)				
Z-motor		Microscope fine adjustment knob axis. One full turn: 100 μm Minimum resolution: 0.1 μm^{\star}				
Computer desk	Dimensions (mm)	650 (W) x 700 (D) x 670 (H)				
Stand for BX	Dimensions (mm)	800 (W) x 700 (D) x 670 (H)				
Anti-vibration table for BX	Anti-vibration system	Rubber feet				
	Dimensions (mm)	790 (W) x 667 (D) x 73 (H)				
Anti-vibration table for IX, BXWI	Anti-vibration system	Air				
	Dimensions (mm)	1000 (W) x 700 (D) x 750 (H)				
Options	Piezoelectric Z-stage	Cross-control by external sensor Stroke: 100 μ m Minimum resolution: 0.1 μ m Position reproducibility: ± 0.2 μ m				
	Transmitted light detector	2 light path settings; transmitted detection/transmitted illumination Connected to the scan unit via optical fiber connection.				
	Objective micrometer	1 mm 100 degrees, for reflected light				
	Green HeNe laser unit	Random polarization 1.5 mW (543 nm) Direct connection, ND filter turret provided				
	Rubber feet anti- vibration table for IX	1000 (W) x 680 (D) x 73 (H) (mm)				

	Specifications
Operating environment	Indoor use.
	Altitude : Max. 2000m
	 Ambient temperature : 5°C to 40°C (41° F to 109° F)
-	10°C to 35°C (Performance guarantees)
	 Maximum relative humidity 80% for temperatures up to 31°C (88° F) decreasing linearly through 70% at 34°C (93° F), 60% at 37°C(99° F), to 50% relative humidity at 40°C (109° F)
	 Main supply voltage fluctuations not to exceed ±10% of the nominal voltage.
	Pollution Degree 2 (in accordance with IEC 664)
	Installation / Over voltage Category II (in accordance with IEC 664)

Troubleshooting Q & A

Concerning

This section explains countermeasures to be taken in the event that a problem occurs.

Please read this section and try the countermeasures before contacting Olympus. If the problem persists, then contact Olympus.

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Due to the usage, the functions of the system sometimes are not fully effective, although there has been no failure; therefore, when a problem occurs, refer to the following chart and apply the appropriate countermeasures.

If the same phenomenon continues to occur after the countermeasures have been taken, contact your local Olympus dealer.

Symptom	Cause	Countermeasure	Manual Reference:
1. No fluorescent light image.	The laser is not oscillating.	Make sure the laser unit power supply is turned on and the emission key is on.	Introduction to Fluoview, 2-6-2.
	The light path selector lever is not set to the LSM light path.	Set the light path selector lever to the LSM light path.	Hardware, 2-2, 3-1-2.
	The visual observation dichroic mirror and analyzer are on the microscope side.	Remove the visual observation dichroic mirror and analyzer from the light path.	
	The detector photomultiplier voltage is low.	Use the PMT scale of the [Acquire] panel to increase the photomultiplier voltage.	Operation, 4-1-3-10
	The excitation light is weak.	Change to a high transmission ratio ND filter.	Hardware, 2-1, 3-1-6.
	The fluorescent light dye color and excitation wavelength are not matched (krypton/argon laser combination).	Select a laser line filter that matches the fluorescent light dye color. Refer to Operation, 4-1-2 Scan Unit Setting. Follow the instructions in the [Microscope Configuration] window.	Operation, 4-1-2. Hardware, 3-1-7.
	The fluorescent light dye color and absorption filter are not matched.	Select an absorption filter that matches the fluorescent light dye color. Refer to Operation, 4-1-2 Scan Unit Setting. r Follow the instructions in the [Microscope Configuration] window.	Operation, 4-1-2. Hardware, 3-1-5 and 4.
	The confocal aperture is too small.	Increase the aperture.	Hardware, 2-1.
	The offset value is too large.	Lower the offset to an appropriate value.	Operation, 4-1-3-10.

Symptom	Cause		Manual Reference.
1. No fluorescent light image.	The photomultiplier of the channel set with the detection mode setting knob is not set.	Check the [Ch1] and [Ch2] check boxes of the [Acquire] panel.	Hardware, 2-1. Operation, 4-1-3-3.
2. No transmitted light image.	The transmitted light detector is not correctly set.	Correctly set the transmitted light path selector knob of the transmitted light detection unit.	Hardware, 3-2-3.
		Correctly set the detection mode setting slider of the scan unit.	Hardware, 2-1, 3-2-4
	The transmitted light filter at the microscope base is in the light path.	Remove the filter from the light path.	Hardware, 2-2, 3-2-3.
	The absorption filter is in the light path.	Remove the absorption filter in the transmitted light detection channel of the scan unit from the light path.	Hardware, 3-2-5.
3. Images are disrupted.	Too much vibration at the setting location.	Consult your Olympus dealer.	_
· · · · · · · · · · · · · · · · · · ·	Fluorescent or other external light is being detected.	Darken the room and read the image again.	· · . —
4. Visibility is poor.	The analyzer is in the light path.	Remove the analyzer from the light path.	Hardware, 3-1-2, 3-2-3.
	Scanning speed is fast.	Lower the scanning speed to an appropriate speed or integrate the images.	Operation, 4-1-3-11, 4-1-5.
	The fluorescent light dye color and absorption filter are not matched.	Select an absorption filter that matches the fluorescent light dye color. Refer to Operation, 4-1-2 Scan Unit Setting. Follow the instructions in the [Microscope Configuration] window.	Operation, 4-1-2. Hardware, 3-1-5 and 4.
	The excitation light is too weak.	Change to a high transmission ratio ND filter.	Hardware, 2-1, 3-1-6.
	Too much vibration at the setting location.	Consult your Olympus dealer.	
5. Blurring and uneven clarity.	The specimen is mounted inclined.	Set the specimen correctly.	Refer to the microscope instruction manual.
6. Flare is visible.	Non-fluorescent glass is not being used.	Use non-fluorescent glass.	_

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Symptom - La	Cause	to Countermeasure	Manual Reference
6. Flare is visible.	The cover glass thickness is not suitable.	Use a cover glass with a thickness of 0.17mm.	
	The specimen is over- stained.	Use an appropriate dye color or increase the offset value.	Operation, 4-1-3-10.
7. Circular flares appear in the center of the image.	The excitation wavelength and absorption filter type are not matched.	Select an absorption filter that matches the excitation wavelength.	Operation, 4-1-2. Hardware, 3-1-5 and 4.
	An absorption filter has not been inserted.	Refer to Operation, 4-1-2. Follow the instructions in the [Microscope Configuration] window.	
8. Blurring is visible.	The focus is not correctly adjusted.	Re-focus using visual observation.	Refer to the microscope instruction manual.
	The correct pinhole has not been selected.	Select the correct pinhole.	Hardware, 2-1.
9. Images are dark with excessive noise.	The dye color is too weak.	Set an appropriate dye color.	-
	The pinhole is too small.	Adjust to an appropriate position.	Hardware, 2-1.
	The photomultiplier HV exceeds -800.	Use an HV of less than -800. If dark, adjust the gain.	Operation, 4-1-3-10.
	Scanning speed is too fast.	Lower the scanning speed to an appropriate speed or integrate the images.	Operation, 4-1-3-11.
	The excitation light is too weak.	Change to a high transmission ratio ND filter.	Hardware, 2-1, 3-1-6.
10. Poor reproduction of Z motor positions.	The Z position slips because the tension of the microscope coarse movement handle is too light.	Increase the tension by turning the adjustment ring and adjusting until the coarse movement handle tension increases suitably.	Refer to the microscope instruction manual.
11. The Fluoview software will not start.	The Fluoview software has already been started.	Press the keyboard (<u>Alt</u>) and (<u>Tab</u>) keys to switch to Fluoview.	,
	Another application software is operating.	Shut down the other application software and start the Fluoview software.	Operation, 2-1-1.
12. The [Acquire] panel is not displayed.	The control unit is not recognized.	Check the connection between the computer and control unit. Turn on the control unit power supply. Turn on the AUX2 switch of the power controller.	Introduction to Fluoview, 2-6-2.

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	Symptom	Cause	Countermeasure	Manual Reference
13.	The scale of the [Z Stage] in the [Acquire] panel is not working.	The Z stage (Z revolver) motor is not being excited.	Check the [Engage motor] check box of the [Z Stage] panel.	Operation, 4-1-3-8.
14.	The fine movement handle of the microscope will not move or moves with difficulty.	The Z stage (Z revolver) motor is being excited.	Clear the [Engage motor] check box of the [Z motor] panel in the [Acquire] panel.	Operation, 4-1-3-8.
15.	Images cannot be saved to disk.	The disk has not been formatted (in the case of a floppy disk or photoelectric disk).	Format the disk.	Operation, Appendix A.
		The photomagnetic disk is not recognized.	Make sure the photoelectric disk power supply is on.	Refer to the photoelectric disk instruction manual.
ŀ			Check the cable connections.	
		Insufficient empty space on the disk.	Increase the available space by deleting unnecessary data or use a new disk (formatting is necessary).	— Operation, Appendix A.
16.	Images are not output to the printer.	The printer is not being recognized.	Make sure the printer power supply is on.	Refer to the printer instruction manual.
			Check the cable connections.	
17.	The mouse pointer does not move when the mouse is moved.	The software is misoperating.	Press the keyboard + <u>Ctrl</u> <u>Alt</u> + <u>Dellete</u> keys and observe the messages that appear. Shut down Fluoview. Shut down Windows and restart the computer.	Operation, 2-1.